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ORIGINAL RESEARCH

Importance of water availability for amphibian roadkill in a mediterranean landscape

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Abstract

Roads can negatively impact ecosystems by fragmenting habitats and affecting animal movements and behaviour. One of the major noticeable effects of roads is animal mortality by vehicle collisions, a paramount threat to wildlife, especially for amphibians. In the context of reduced water availability, particularly in the Mediterranean region, amphibians are projected to be one of the most negatively affected animal groups. In this study, we used 14-year road mortality data collected along 120 km of roads, combined with landscape and remote sensing variables, to identify the drivers of amphibian mortality spatial patterns in a Mediterranean landscape, in Southern Portugal. We recorded 5116 carcasses belonging to five amphibian species. Generalised linear models showed that for most of the species, roadkill numbers increased with the decrease in water availability in water bodies. Also, the distance of water bodies to the roads was important in understanding amphibian roadkill patterns, with a general increase in mortality at reduced distances. Land use variables were also significant drivers for amphibian road mortality, with species-specific responses. Roadkill numbers decreased for the Iberian ribbed newt and the fire salamander in agricultural dominated areas. Our results also show an increase in roadkill numbers for the natterjack toad in areas with higher percentage of olive groves, and an opposite response for the fire salamander. We recognize the importance of long-term studies in assessing roadkill patterns, and their value for amphibian monitoring and conservation.

Keywords Roadkill mortality · Spatial patterns · Remote sensing · Land surface temperature · Conservation

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Introduction

Roads and highways are one of the most ubiquitous man-made features across the landscape. They aim to facilitate connectivity among humans and economic progress (Meijer et al. 2018), but they also pose a varied range of negative effects, including habitat loss, degradation, and fragmentation; shifts in biophysical flows; spreading of invasive species; changes in the availability of resources such as water and nutrients; and disturbance caused by chemical, light and noise pollution (Coffin 2007; van der Ree et al. 2015). Roads also create a barrier to wildlife movement, isolating populations, and modifying animal behaviour such as migration or dispersal, ultimately jeopardising the long-term viability of populations (Jongsma et al. 2014; Ceia-Hasse et al. 2017; Howell and Seigel 2019). However, one of the major effects of roads on wildlife is the direct mortality by roadkill, whose magnitude depends on ecological requirements and life history traits, making some species more prone than others (Forman et al. 2003; D'Amico et al. 2015; Santos et al. 2016). Among vertebrates, the amphibians are the taxonomic group with the highest mortality rates (Glista et al. 2008) due to their biological traits, such as low vagility, narrow habitat tolerances and seasonal migrations to and from reproduction areas (Carr and Fahrig 2001; Sillero 2008). Higher mortality is often reported on roads close to water bodies used for reproduction (Ascensão and Mira 2005; Orlowski 2007; Santos et al. 2007; Cooke 2011), and on migration routes between high-quality habitats (Sillero 2008). Behavioural traits also make this taxon exceptionally susceptible to death on roads, including immobility when approaching vehicles (Mazerolle et al. 2005); not avoiding roads during their migrations, moving slower on the asphalt, than in surrounding areas (Bouchard et al. 2009); attraction of some species to roads in foraging excursions, to hunt or in search for a potential mate (Speybroeck et al. 2016). In addition, some amphibian species depend on an intricate landscape structure to complete their complex life cycles that allow movement from feeding and aestivation/hibernation to reproduction habitats (Joly 2019). These traits make this group particularly vulnerable to genetic structuring and potential population declines due to roads (Holderegger and Di Giulio 2010).

The Mediterranean Basin is considered the second largest biodiversity hotspot in the world (Médail and Quezel 1999), and is unique because of its long history of coexistence between wildlife and humans. Nevertheless, since the middle of the twentieth-century human pressures have been strongly intensified including the construction of large road networks crossing or close to important natural areas (Blondel et al. 2010). As a result, this proximity could potentially place wildlife at a higher risk (Fahrig et al. 1995), justifying the need for further studies aiming to understand the role of landscape features in promoting or impeding roadkill (e.g. Sillero 2008; Carvalho and Mira 2011). The Mediterranean is also considered one of the most vulnerable regions in the world to the impacts of global warming (Tuel and Eltahir 2020). During the last century, the air temperature has risen in all regions of the Mediterranean, particularly in the Iberian Peninsula and southern France, with an increase of almost 2 °C (UNEP/MAP 2017). The predictions for this region also include a substantial reduction in rainfall, contributing to desertification (UNEP/MAP 2017; Tuel and Eltahir 2020). This is even more alarming since the Iberian Peninsula comprises almost 50% of European fauna and flora, with more than 30% of endemic species (Araújo et al. 2007) and within these, twenty-eight amphibian species including twelve endemisms (Carretero et al. 2018). Ectotherms are considered especially vulnerable to climate change (Gibbons et al. 2000; Araújo et al. 2006) and for many amphibian species, future predictions point to a contraction of their distribution ranges (Araújo et al. 2006;

Carvalho et al. 2010). The increase in temperature and rainfall reduction are expected to decrease the availability of water resources and enhance the spread of infectious diseases (Pounds et al. 2006), two factors that are negatively affecting amphibian populations worldwide. The known water dependency for most amphibian species to complete their life cycles, places this group at exceptional risk, especially in semi-arid areas (Henle et al. 2008; Carvalho and Mira 2011). These effects, coupled with the costs of the roadkill, may produce catastrophic consequences for amphibians, thus, it is urgent to address the drivers of amphibian roadkill in the Mediterranean region to mitigate this threat more efficiently.

Several studies have addressed amphibians' roadkill patterns in conjunction with the use of new technology and methods (Elzanowski et al. 2009; Glista et al. 2008; Sillero 2008; Matos et al. 2012; Mestre et al. 2019); nevertheless, the influence of landscape factors on roadkill patterns, and the extent to which they can be predicted, is yet to be unveiled (Cushman 2006; Beebee 2013; Petrovan and Schmidt 2019). Most studies that address this issue are conducted in short-term periods, with occasional records, and report mostly seasonal variations in roadkill, which could lead to misleading results (Sillero 2008). Long-term studies, however, incorporate data with variations between years, providing more robust inferences that allow to better understand the possible impact on the persistence of populations (Pinto et al. 2020). Coupled with this, the use of free spatial high-resolution remote sensing data that provides consistent long-term Earth observation from local to global scales (Wang et al. 2010), can yield innovative valuable data (such as vegetation productivity and landscape structure) at very small spatial scales and periodicity. Since amphibians respond to small scales, these products are an important tool to assess the landscape and climatic drivers of amphibian roadkill. There are several studies that infer that remote sensing data such as vegetation productivity, water availability and land surface temperature have great potential in mapping land use change trends at small scales (Ehsan and Kazem 2013; Campos et al. 2012; Muro et al. 2018). In combination with other factors that previous research demonstrated to be related to amphibian road mortality (e.g. distance to ponds; habitat quality) (Sillero 2008; Carvalho and Mira 2011; Coelho et al. 2012; Matos et al. 2012; Heigl et al. 2017), these tools should improve the efficiency and feasibility of conservation and mitigation measures, often highly expensive and taxa specific (Ascensão et al. 2019).

In this study, we identify the drivers of amphibian mortality spatial patterns in a Mediterranean landscape in Southern Portugal, by combining landscape and remote sensing predictors. Specifically, we explore water availability drivers across different amphibian species, to better understand the mortality patterns of most roadkilled amphibians. We hypothesise that low water availability in the landscape will be correlated with lower probabilities of amphibian road mortality since these conditions reduce suitable habitats for amphibians (Araújo et al. 2006). Moreover, water bodies closer to roads will be correlated with higher levels of amphibian mortality because these places are common aggregation sites for most amphibian species (D'Amico et al. 2015).

Methodology

Study area

The study was conducted in an area of approx. 210.000 ha, located in Alentejo, Southern Portugal (29 N 599606E, 4285394 N, WGS84) (Fig. 1). The topography is flat (ranging

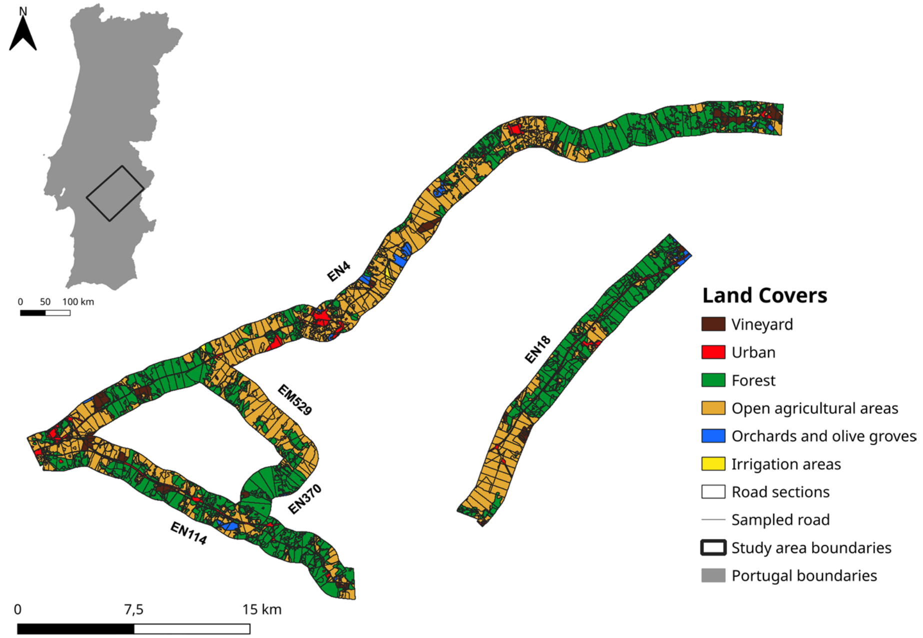

Fig. 1 Map of the study area in Southern Portugal, with the sampled roads and land cover in the 1000 m buffer for each 500 m road segment

from 100 to 400 m a.s.l.), and the landscape is mainly composed of Mediterranean cork (*Quercus suber*)/holm (*Quercus rotundifolia*) forests, with varying tree cover (between 20 and 80 trees per hectare; Pinto-Correia and Mascarenhas 1999) composing the complex agrosilvopastoral system called *montado* (Pinto-Correia 1993), and agricultural areas in equal proportions. Production activities like cereal crops combined with extensive livestock grazing are also present (Pinto-Correia and Mascarenhas 1999), as well as open agricultural areas, orchards and permanently irrigated agricultural areas. This landscape mosaic structure is considered one of the ecosystems with the highest biodiversity in the western Mediterranean Basin (Pinto-Correia et al. 2011). The climate is typically Mediterranean, with hot and dry summers (where temperatures can exceed 40 °C), mild and wet winters (5.8–12.8 °C), and annual precipitation ranging between 500 and 650 mm (IPMA 2021). The study area is crossed by the main transportation corridor between Lisbon and Madrid and a network of linear infrastructures. Roadkill surveys were carried out along four National Road sectors (EN4, EN114, EN370 and EN18; > 4000 vehicles/day, IP 2005) and one Municipal Road sector (EM529; < 4000 vehicles/day, IP 2005). All roads are two-lane wide.

Roadkill data

Amphibian roadkill data were compiled from the University of Évora database, for a period of 14 years (2006 to 2020), corresponding to a total of ca. 120 km of sampled roads (distributed across 5 roads; Table S1 – Supplementary materials). Over this period, an experienced observer drove a car at 20–40 km/h during the first morning hours (to reduce the impact of traffic and scavengers in carcass removal), checking both sides of the road (including lanes and shoulders), and collecting and registering roadkill animals (for further details on monitoring procedures, see Santos et al. 2011; Pinto et al. 2020). All amphibian carcasses were identified to the lowest possible taxonomic level and removed from the road to avoid double counting. Due to budget constraints over the years, the sampled roads and sampling periodicity varied across the study time frame (STF); some years were sampled daily, others weekly, and others had mixed sampling (daily in Spring and Summer months, and weekly sampling in Winter months; Table S1 - Supplementary materials). To standardise carcass persistence errors and maximise temporal and spatial variability, we retrieved only weekly roadkill data. For this study, we selected carcasses that were identified to the species level and retained five species with high numbers of roadkill (> 400): fire salamander (*Salamandra salamandra*), Iberian ribbed newt (*Pleurodeles waltl*), natterjack toad (*Epidalea calamita*), spiny common toad (*Bufo spinosus*), and Iberian spadefoot toad (*Pelobates cultripes*). We used the taxonomy and nomenclature according to Carretero et al. (2018).

Environmental predictors

For analysis purposes, we split each road into 500 m-length contiguous segments and because species perceive landscape at different scales, sometimes due to a different spatial use (Bennet et al. 2006; Ingham and Samways 1996), we defined four buffer widths at each road section (100 m, 250 m, 500 m, and 1000 m) to characterise landscape traits around each segment. The road segment size was based on the mean distances regularly covered by the most roadkilled amphibian species (Wells 2007; Joly 2019), and the buffer sizes were based on the average road effects on previous studies (Langen et al. 2009; Carvalho and Mira 2011; Joly 2019).

We selected several environmental predictors based on the species ecology and aims of the study (Sillero 2008; Carvalho and Mira 2010; Matos et al. 2012; D’Amico et al. 2015). To provide our models with the most updated information on environmental changes, and variations of local dynamics across the study time frame in our study area, we used remote sensing-derived predictors, revealing a set of local dynamics, together with land cover, topography and distance to nearest highway and water bodies. A total of 21 environmental predictors describing different categories were measured (Table 1): land cover ($n = 6$), water availability ($n = 5$), vegetation productivity ($n = 6$), land surface temperature ($n = 2$), road proximity ($n = 1$), and topography ($n = 1$). For the land cover predictors, we used CORINE Land Cover (CLC; EEA) 2006, 2012 and 2018 to characterise the land use during the 2006–2020 period. We merged some land cover classes to reflect the main land uses in the study area (Table S2—Supplementary materials). At each buffer, we extracted the mean land cover class from the three CORINE years to a 30 m pixel resolution using QGIS Software (QGIS Development Team 2022).

Water is a critical element for amphibians, regulating their activity and reproduction (Speybroeck et al. 2016); for this reason, we calculated predictors that represent water availability in the landscape. We computed the mean Normalised Difference Moisture Index (M_NDMI; Gao 1995), sensitive to changes in water content on vegetation, and its standard deviation (SD_NDMI), representing the variation throughout the STF (2006–2020); and the mean Normalised Difference Water Index (M_NDWI; McFeeters 1996) and the respective standard deviation (SD_NDWI), to monitor changes related to water content in water bodies and its variability, respectively. These indexes were calculated from

Table 1 List of predictors included in the analyses, with respective code, description, and source

Predictor class	Name	Code	Description and measure unit	Source	References
Land cover	Irrigation areas	IRRIG	Percentage of irrigated area (%)	CORINE 2006, 2012, 2018	EEA
	Forest areas	FOREST	Percentage of forested area (%)	CORINE 2006, 2012, 2018	EEA
	Olive groves and orchards	OLIVE	Percentage of olive grove and general orchard area (%)	CORINE 2006, 2012, 2018	EEA
	Urban areas	URBAN	Percentage of urban area (%)	CORINE 2006, 2012, 2018	EEA
	Open agricultural areas	AGRIC	Percentage of agricultural area (%)	CORINE 2006, 2012, 2018	EEA
	Vineyards	VINE	Percentage of vineyard area (%)	CORINE 2006, 2012, 2018	EEA
Wateravailability	Mean Normalized Difference Moisture Index	M_NDMI	Mean water content in vegetation. − 1 (indicating no moisture in vegetation) to 1 (indicating high moisture in vegetation)	USGS	Gao ((1995)
	Standard deviation of Normalized Difference Moisture Index	SD_NDMI	Inter-annual variation in changes in moisture content of leaves	USGS	Gao (1995)
	Mean Normalized Difference Water Index	M_NDWI	Mean changes in water content of water bodies. − 1 (indicating no water) to 1 (indicating water)	USGS	McFeeters (1996)
	Standard deviation of Normalized Difference Water Index	SD_NDWI	Inter-annual variation in changes in water content of water bodies	USGS	McFeeters (1996)
	Distance to ponds	DIST_PNDS	Distance to the nearest water body (meters)	QGIS	N/A

Table 1 (continued)

Predictor class	Name	Code	Description and measure unit	Source	References
Vegetationproductivity	Mean Enhanced Vegetation Index	M_EVI	Mean changes in primary production content index. − 1 (indicating stressed vegetation) to 1 (indicating healthier vegetation)	USGS	Liu and Huete (1995)
	Standard Deviation of Enhanced Vegetation Index	SD_EVI	Inter-annual variation in primary production content index	USGS	Liu and Huete (1995)
	Mean Normalized Vegetation Index	M_NDVI	Mean changes in primary production content index. − 1 (indicating stressed vegetation) to 1 (indicating healthier vegetation)	USGS	Rouse et al. (1973)
	Standard Deviation of Normalized Vegetation Index	SD_NDVI	Inter-annual variation in primary production content index	USGS	Rouse et al. (1973)
	Mean Soil-Adjusted Vegetation Index	M_SAVI	Mean changes in primary production content index. − 1 (indicating stressed vegetation) to 1 (indicating healthier vegetation)	USGS	Huete (1988)
	Standard Deviation of Soil-Adjusted Vegetation Index	SD_SAVI	Inter-annual variation in primary production content index	USGS	Huete (1988)
Land surfacetemperature	Mean Land Surface Temperature	M_LST	Mean radiative temperature of land surface (°C)	Google Earth Engine (source code – Ermida et al. 2020)	Ermida et al. (2020)
	Standard Deviation of Land Surface Temperature	SD_LST	Inter-annual variation in radiative temperature of land surface (°C)	N/A	Ermida et al. (2020)
Roadproximity	Distance to highway	DIST_HGHW	Distance to highway ‘A6’ (meters)	QGIS	N/A
Topography	Elevation	ELEV	Elevation retrieved from digital elevation model (meters)	Direção Geral do Território (DGT)	DGT 2008

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 a brief description of the LANDSAT program please see Supplementary materials] accounting for the STF. The time frame included full years of the study period, and we only retained high-quality images with 0% cloud cover for the whole study area (WRS-2 scene: path 203, row 33). We also calculated 'distance to ponds' predictor by applying Euclidean distance to a 30 m spatial resolution raster (derived from existing cartography) to measure the influence of the water bodies on roadkill (range of water bodies area = 26–395 m^2). We performed the calculation of this last predictor using QGIS Software (QGIS Development Team 2022). In addition, we computed several predictors of vegetation productivity that have been successfully used in previous amphibian studies and explain species occurrence (e.g. Qian et al. 2007), namely: mean and SD of the Enhanced Vegetation Index (M_EVI and SD_EVI; Liu and Huete 1995); the mean and SD of the Normalised Difference Vegetation Index (M_NDVI and SD_NDVI; Rouse et al. 1973); and the mean and SD of the Soil-Adjusted Vegetation Index (M_SAVI and SD_SAVI; Huete 1988). All these indexes quantify vegetation greenness and their variability across the STF, however, EVI corrects for some atmospheric conditions and canopy background noise, being more sensitive in dense vegetation areas (Liu and Huete 1995), and SAVI attempts to minimise soil brightness influence using a correction factor (Huete 1988). Since amphibians are ectothermic, the temperature is also a major determinant of their activity (Araújo et al. 2006; Speybroeck et al. 2016). Therefore, we calculated the mean and SD Land Surface Temperature (M_LST and SD_LST; Hulley et al. 2019) and used it as the air temperature surrogate. These indexes (vegetation productivity and land surface temperature) were obtained following the same procedures as the remote sensing water availability predictors (NDMI and NDWI). We combined all the images into composites and calculated the mean (M) and standard deviation (SD) for each index, across the STF. Higher values for the SD denote greater heterogeneity, while lower SD values represent more consistent similar values throughout all years. Overall, we obtained a total of 84 images (Table S3—Supplementary materials), with an average of 5.6 images per year (min = 0 for 2012; max = 11 for 2017; SD = 2.87); the year 2007 contains two images, one for June and another for August, the year 2012 had no quality images available, and therefore was excluded from the analyses. We processed all composite images and calculated all previously mentioned remote sensing indexes in Google Earth Engine (Gorelick et al. 2017).

Roads can exacerbate a barrier effect on amphibians (Fahrig et al. 1995), so we obtained the road proximity predictor ('distance to highway') using the same procedure as the 'distance to ponds'; also using QGIS Software (QGIS Development Team 2022) we extracted elevation from a digital elevation model (DGT 2018) since water typically concentrates in lower topographies, where it can create ponds and water bodies, of extreme importance for amphibians (Santos et al. 2007). We stacked all environmental predictors in a 30 m spatial resolution multi-raster layer (see Table 1 for a resume of the predictors) for the entire study time frame using R software (R Core Team 2021).

Data analysis

We used the number of roadkills per road segment of each species as a response variable. To account for possible bias in our roadkill count data, we included an offset parameter in the models (log scaled). This offset corresponds to the total number of weeks each road segment was sampled (sampling effort) and converts the raw count roadkill data to period standardised rates (Zuur et al. 2007). For each environmental predictor (except for distances) and each species, we selected the best response scale (100 m, 200 m, 500 m, and 1000 m) by applying univariate Poisson Generalised Linear Models (GLM) (Salgueiro et al. 2018) and extracting the predictor scale showing the lowest Akaike's Information Criterion (AIC; Akaike 1974). We then used the selected variables to assemble our models.

Our modelling procedure for each species was based on GLMs with a negative binomial distribution (as our data presented high values of overdispersion, Zuur et al. 2009), and we modelled the count data with all the selected predictors from the four scales, while using 'sampling effort' as an offset parameter. We started our analysis by assessing multicollinearity, using the Variance Inflation Factor (VIF) (Zuur et al. 2010; Dormann et al. 2013), excluding all predictors with a VIF higher than 5 (Dormann et al. 2013; Sillero et al. 2021). The removed predictors included: 'M_NDVI', 'SD_NDVI'; 'M_EVI'; 'M_SAVI' and 'SD_SAVI'. Prior to modelling, we standardised all predictors to zero mean and unit variance, so model coefficients could be comparable (Zuur et al. 2009). We then randomly selected 20% of available zeros for each species dataset as absences, to avoid zero-inflated problems and divided the data into training (70%) and test (30%) data (Field et al. 2012), to evaluate the explanatory power of each model. Following, we performed backwards stepwise selection using AIC to assess the relative likelihood of each model (Akaike 1974) between the stepwise output and the full model (the model constructed with all predictors that we considered after VIF analysis). We selected the models with the lowest AIC, plotted the Pearson residuals to assess normality and evaluated goodness of fit with the percentage of deviance explained by the model. To account for potential autocorrelation in our data, we performed a Moran's I test (Moran 1950) on each model residuals and calculated a spatial autocovariate as the distance weighted average of neighbouring response values (Dormann et al. 2007). While Moran's I measures the global spatial autocorrelation, the autocovariate identifies the local spatial structure of our roadkill data (Anselin 1995). We then rerun the models with the autocovariate as an additional predictor. We compared the AIC of the previously run models, with the ones accounting for the spatial autocorrelation and selected the models with the lowest AIC values. If $\Delta AICc < 2$, the models were considered equally supported (Burnham and Anderson 2002), and the one without the autocovariate was retained. Then we performed all the previously mentioned steps for model evaluation. The predictive performance of models was assessed with Pearson correlation between observed and predicted roadkill.

We performed all the analyses using R software packages raster, rgdal, performance, spdep and MASS (R Core Team 2021).

Results

We recorded a total of 5116 amphibian carcasses, belonging to the five selected species, between 2006 and 2020, most of which belonged to the natterjack toad (47%). The second most roadkilled amphibian species was the fire salamander accounting for 18% of total carcasses (Table 2). In total, we registered approximately three amphibian carcasses per road kilometre per year (Table 2).

Table 2 Amphibian roadkill recorded in the sampled roads during the 14-year study time frame (2006–2020)

Road	*S. salamandra*	*P. waltl*	*P. cultripes*	*E. calamita*	*B. spinosus*	Total
EN4	150	129	307	678	342	1606
EN18	47	24	17	55	42	185
EN114	262	227	200	1553	270	2512
EN370/EM529	455	118	74	132	34	813
Total	914	498	598	2418	688	5116

Main drivers of amphibian mortality

Overall, models show fair-to-good levels of data fit and predictive performance. The residual plots show randomly scattered residuals around zero, the Pearson correlations averaged 0.37 (0.29–0.58; Table S4—Supplementary materials), and the amount of explained variance averaged 20% (min $R2=0.112$; max $R2=0.341$). Spatial autocorrelation presented minimal values, with Moran's I averaging 0.08 (min = 0; max = 0.16; Table S5—Supplementary materials). Three models improved after inclusion of the autocovariate, although its coefficient was only significant (and positive) for urodeles (fire salamander: coef = 0.149, CI = 0.086, 0.213; Iberian ribbed newt: coef = 0.155, CI = 0.071, 0.239).

Concerning the land cover predictors, the natterjack toad was the only species to show increased mortality in segments with low abundance of irrigation areas ('IRRIG'; coef= −0.484; CI= −0.83, −0.24) and forest ('FOREST'; coef= −0.534; CI= −0.78, −0.29). The mortality of the fire salamander increased in road segments with a lower cover of olive groves and orchards ('OLIVE'; coef = −0.116; CI= −0.23, −0.02) (Fig. 2), while mortality of natterjack toad increased with a higher cover of this land use class ('OLIVE'; coef = 0.168; CI = 0.04, 0.30). Lower proportions of open agricultural areas ('AGRIC') had more roadkills for both the Iberian ribbed newt (coef= −0.197; CI= −0.34, −0.06) (Fig. 3) and the fire salamander (coef= −0.117; CI= −0.23, −0.005), and lower proportions of vineyards ('VINE') also contributed to increased fire salamander roadkill (coef= −0.233; CI= −042., −0.09). In the water availability predictors category, our models show that for all species except the Iberian ribbed newt, roadkill increased in road segments with low mean values of water content in water bodies ('M_NDWI'; Table 3). Shorter distances of water bodies to roads ('DIST_PNDS') were associated with higher mortality for the Iberian ribbed newt and for the Iberian spadefoot toad (coef= −0.166; CI= −0.27, −0.06; coef= −0.229; CI= −0.39, −0.07, respectively), but represented less mortality for the natterjack toad (coef = 0.223; CI = 0.05, 0.40) (Fig. 4). Higher variation in moisture content in vegetation was responsible for an increase in the roadkill for the Iberian ribbed newt ('SD_NDMI'; coef = 0.20; CI = 0.06, 0.35). A lower variation in primary production ('SD_EVI') caused an increase in the roadkill for the fire salamander (coef= −0.146; CI= −0.24, −0.06) and for the Iberian ribbed newt (coef= −0.157; CI= −0.28, −0.03), and a decrease in the roadkill of the natterjack toad (coef = 0.392; CI = 0.19, 0.61). Road segments with higher mean land surface temperature ('M_LST') had higher mortality values for the natterjack toad, the Iberian spadefoot toad, and the Iberian ribbed newt (coef = 0.698; CI = 0.38, 1.04; coef = 0.284; CI = 0.05, 0.52; coef = 0.175; CI = 0.02, 0.33, respectively), and lower mortality values for the spiny common toad (coef= −0.112; CI= −0.22, −0.01) (Fig. 5). A higher variation in the land surface temperature ('SD_LST') had the same effect for the Iberian spadefoot toad and the Iberian ribbed newt (coef = 0.347;

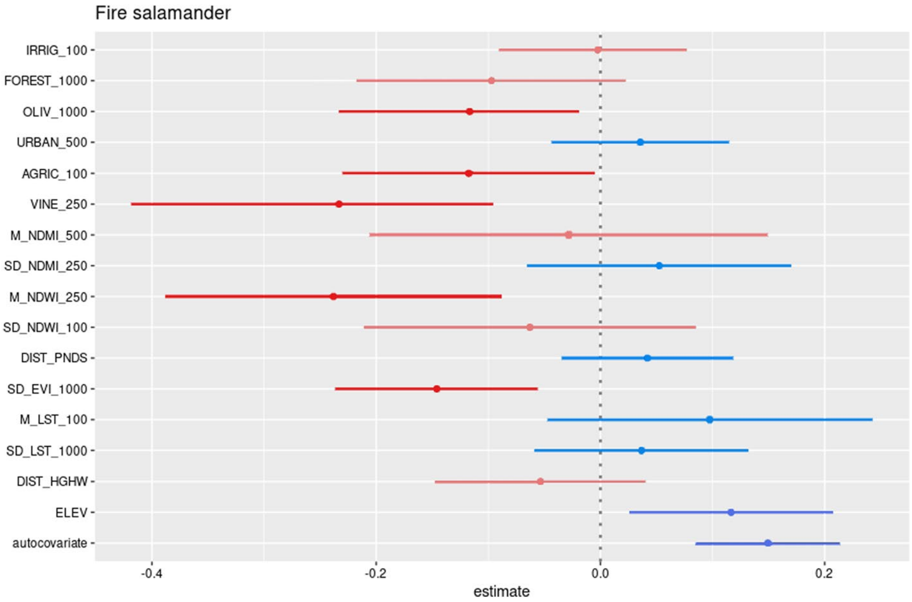

Fig. 2 Model results for the fire salamander (estimates and 95% confidence intervals). Dark blue lines represent statistically significant positive values; light-blue lines represent non-statistically significant positive values; light-red lines represent non-statistically significant negative values; and red lines represent statistically significant negative values

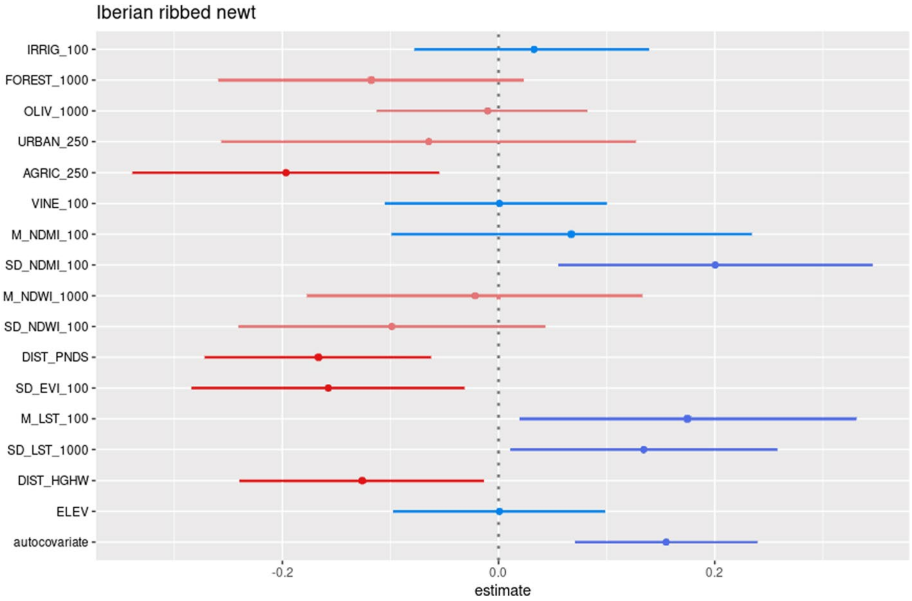

Fig. 3 Model results for the Iberian ribbed newt (estimates and 95% confidence intervals). Dark blue lines represent statistically significant positive values; light-blue lines represent non-statistically significant positive values; light-red lines represent non-statistically significant negative values; and red lines represent statistically significant negative values

Table 3 Models for the analysed species with coefficients, AIC and R^2. Significant results are in bold

	Estimate	Std. Error	t Value	p-Value	Confidence interval (95%)
Fire salamander (*Salamandra salamandra*)					
IRRIG_100	−0.002	0.042	−0.056	0.955	−0.090; 0.077
FOREST_1000	−0.096	0.060	−1.269	0.205	−0.219; 0.019
OLIVE _1000	−0.116	0.055	−2.128	**0.033**	**−0.233; −0.019**
URBAN_500	0.036	0.039	0.914	0.361	−0.043; 0.114
AGRIC_100	−0.117	0.057	−2.051	**0.041**	**−0.229; −0.005**
VINE_250	−0.233	0.081	−2.878	**0.004**	**−0.418; −0.096**
M_NDMI_500	−0.028	0.089	−0.316	0.752	−0.205; 0.149
SD_NDMI_250	0.052	0.059	0.878	0.381	−0.065; 0.170
M_NDWI_250	−0.238	0.074	−3.201	**0.001**	**−0.387; −0.089**
SD_NDWI_100	−0.063	0.073	−0.860	0.390	−0.211; 0.085
DIST_PNDS	0.042	0.039	1.085	0.278	−0.034; 0.118
SD_EVI_1000	−0.146	0.046	−3.207	**0.001**	**−0.236; −0.056**
M_LST_100	0.097	0.073	1.345	0.179	−0.047; 0.242
SD_LST_1000	0.037	0.047	0.771	0.441	−0.059; 0.132
DIST_HGHW	−0.053	0.047	−1.144	0.253	−0.147; 0.040
ELEV	0.117	0.046	2.550	**0.011**	**0.026; 0.207**
Autocovariate	0.149	0.032	4.672	**< 0.001**	**0.086; 0.213**
Model AIC	1540				
R^2	0.26871				
Iberian ribbed newt (*Pleurodeles waltl*)					
IRRIG_100	0.033	0.055	0.601	0.548	−0.077; 0.139
FOREST_1000	−0.118	0.071	−1.655	0.099	−0.259; 0.023
OLIVE_1000	−0.010	0.050	−0.200	0.841	−0.112; 0.082
URBAN_250	−0.0646	0.095	−0.683	0.495	−0.256; 0.013
AGRIC_250	−0.197	0.071	−2.751	**0.006**	**−0.339; −0.0557**

Table 3 (continued)

	Estimate	Std. Error	t Value	p-Value	Confidence interval (95%)
VINE_100	0.0007	0.052	0.015	0.988	−0.105; 0.099
M_NDMI_100	0.067	0.083	0.806	0.421	−0.099; 0.234
SD_NDMI_100	0.200	0.074	2.721	**0.007**	**0.056; 0.346**
M_NDWI_1000	−0.022	0.077	−0.280	0.779	−0.177; 0.133
SD_NDWI_100	−0.099	0.072	−1.380	0.169	−0.240; 0.043
DIST_PNDS	−0.166	0.052	−3.150	**0.002**	**−0.272; −0.063**
SD_EVI_100	−0.157	0.063	−2.494	**0.013**	**−0.284; −0.032**
M_LST_100	0.175	0.078	2.252	**0.025**	**0.019; 0.331**
SD_LST_1000	0.134	0.061	2.208	**0.028**	**0.011; 0.258**
DIST HGHW	−0.126	0.053	−2.251	**0.025**	**−0.239; −0.014**
ELEV	0.0007	0.049	0.015	0.989	−0.097; 0.098
Autocovariate	0.155	0.042	3.729	**< 0.001**	**0.071; 0.239**
Model AIC	880				
$\mathbf{R}^2$	0.15453				
Natterjack toad (*Epidalea calamita*)					
IRRIG_100	−0.483	0.136	−3.565	**< 0.001**	**−0.828; −0.236**
FOREST_100	−0.534	0.118	−4.524	**< 0.001**	**−0.780; −0.289**
OLIVE _100	0.168	0.065	2.604	**0.009**	**0.040; 0.301**
URBAN_1000	−0.076	0.084	−0.913	0.362	−0.22; 0.095
AGRIC_250	−0.209	0.112	−1.868	0.062	−0.430; 0.011
VINE_1000	−0.179	0.101	−1.768	0.078	−0.422; −0.013
M_NDMI_100	−0.124	0.109	−1.141	0.255	−0.369; 0.121
SD_NDMI_500	−0.265	0.128	−2.076	0.384	−0.586; 0.045
M_NDWI_500	−1.004	0.134	−7.486	**< 0.001**	**−1.282; −0.734**
SD_NDWI_500	0.026	0.128	0.200	0.842	−0.255; 0.306

Table 3 (continued)

	Estimate	Std. Error	t Value	p-Value	Confidence interval (95%)
DIST_PNDS	0.2232	0.083	2.677	**0.008**	**0.048; 0.397**
SD_EVI_1000	0.392	0.096	4.076	**< 0.001**	**0.182; 0.612**
M_LST_100	0.698	0.137	5.064	**< 0.001**	**0.375; 1.035**
SD_LST_500	0.174	0.099	1.763	0.079	−0.049; 0.402
DIST_HGHW	0.467	0.094	4.970	**< 0.001**	**0.267; 0.671**
ELEV	−0.292	0.109	−2.667	**0.008**	**−0.525; −0.062**
Model AIC	1900				
R^2	0.34087				
Spiny common toad (*Bufo spinosus*)					
IRRIG_100	−0.017	0.058	0.293	0.769	−0.097; 0.129
FOREST_250	−0.075	0.067	−1.121	0.263	−0.209; 0.059
OLIVE _1000	−0.099	0.062	−1.589	0.113	−0.237; −0.017
URBAN_100	0.012	0.046	0.268	0.789	−0.083; 0.107
AGRIC_1000	0.003	0.066	0.044	0.965	−0.129; 0.135
VINE_100	0.049	0.051	0.978	0.329	−0.051; 0.149
M_NDMI_1000	0.029	0.084	0.343	0.732	−0.139; 0.195
SD_NDMI_500	0.068	0.106	0.637	0.524	0.142 0.279
M_NDWI_500	−0.205	0.079	−2.585	**0.010**	**−0.366; −0.051**
SD_NDWI_500	−0.108	0.105	−1.029	0.304	−0.318; 0.101
DIST_PNDS	−0.079	0.052	−1.522	0.129	−0.183; 0.023
SD_EVI_100	−0.031	0.057	−0.543	0.588	−0.144; 0.079
M_LST_1000	−0.112	0.050	−2.230	**0.026**	**−0.216; −0.009**
SD_LST_500	0.015	0.071	0.207	0.836	−0.128; 0.157
DIST_HGHW	−0.036	0.061	−0.600	0.549	−0.160; 0.086
ELEV	−0.125	0.052	−2.389	**0.017**	**−0.229; −0.021**

Table 3 (continued)

	Estimate	Std. Error	t Value	p-Value	Confidence interval (95%)
Autocovariate	0.090	0.046	1.953	0.052	−0.0005; 0.181
Model AIC	1258				
R^2	0.11229				
Iberian spadefoot toad (*Pelobates cultripes*)					
IRRIG_100	−0.088	0.095	−0.926	0.358	−0.277; 0.100
FOREST_500	−0.115	0.121	−0.945	0.345	−0.358; 0.127
OLIVE _1000	−0.117	0.092	−1.273	0.203	−0.314; 0.043
URBAN_100	−0.0007	0.079	−0.009	0.993	−0.163; 0.160
AGRIC_1000	−0.041	0.118	−0.350	0.727	−0.282; 0.197
VINE_100	−0.121	0.109	−1.102	0.271	−0.362; 0.074
M_NDMI_1000	0.282	0.167	1.687	0.093	−0.049; 0.615
SD_NDMI_500	−0.052	0.106	−0.489	0.625	−0.262; 0.159
M_NDWI_250	−0.309	0.120	−2.570	**0.011**	**−0.552; −0.069**
SD_NDWI_1000	−0.092	0.111	−0.832	0.406	−0.337; 0.146
DIST_PNDS	−0.229	0.084	−2.704	**0.007**	**−0.393; −0.069**
SD_EVI_100	−0.012	0.101	0.121	0.904	−0.197; 0.220
M_LST_100	0.284	0.117	2.405	**0.017**	**0.049; 0.523**
SD_LST_1000	0.347	0.119	2.929	**0.004**	**0.122; 0.577**
DIST_HGHW	0.309	0.094	3.270	**0.001**	**0.122; 0.498**
ELEV	0.035	0.084	0.411	0.681	−0.136; 0.205
Model AIC	943				
R^2	0.11011				

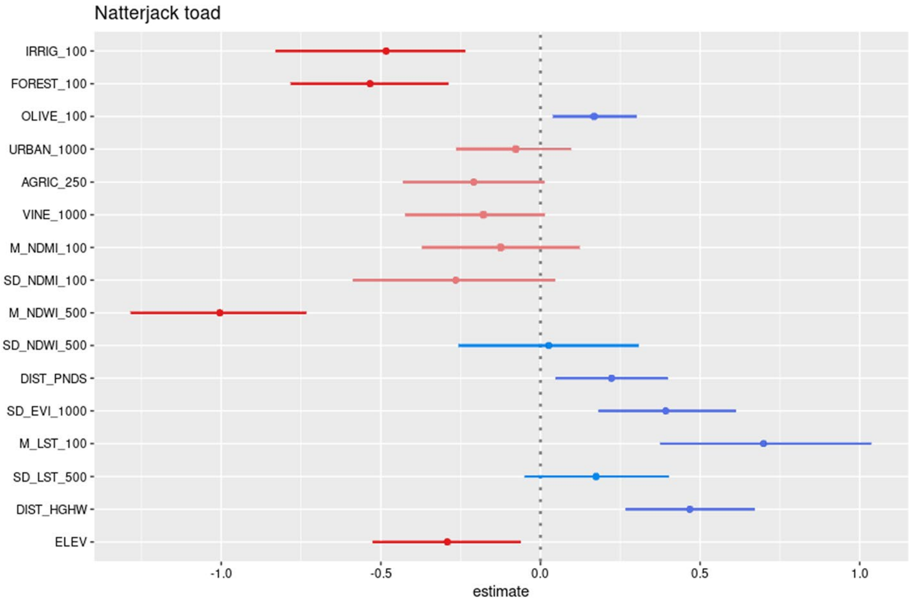

Fig. 4 Model results for the Natterjack toad (estimates and 95% confidence intervals). Dark blue lines represent statistically significant positive values; light-blue lines represent non-statistically significant positive values; light-red lines represent non-statistically significant negative values; and red lines represent statistically significant negative values

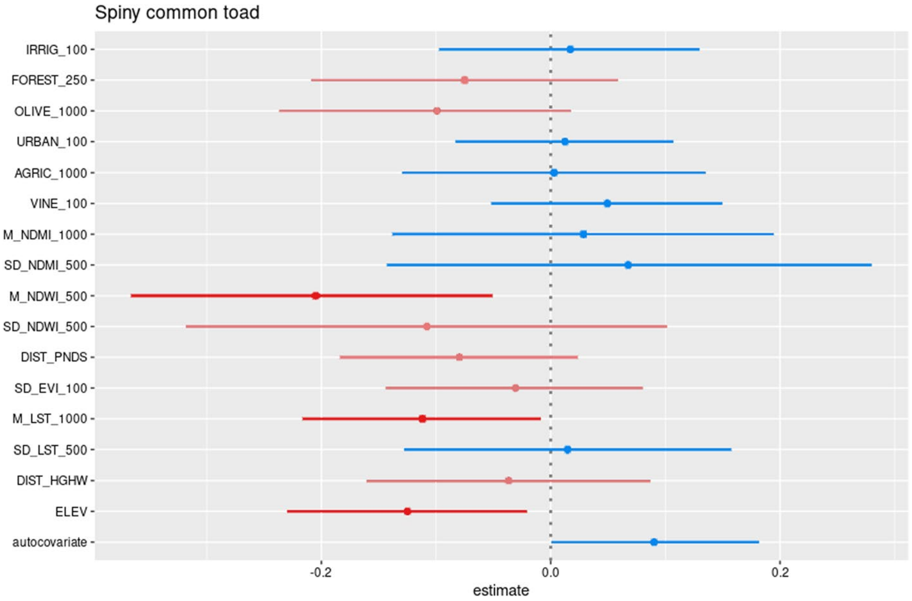

Fig. 5 Model results for the spiny common toad (estimates and 95% confidence intervals). Dark blue lines represent statistically significant positive values; light-blue lines represent non-statistically significant positive values; light-red lines represent non-statistically significant negative values; and red lines represent statistically significant negative values

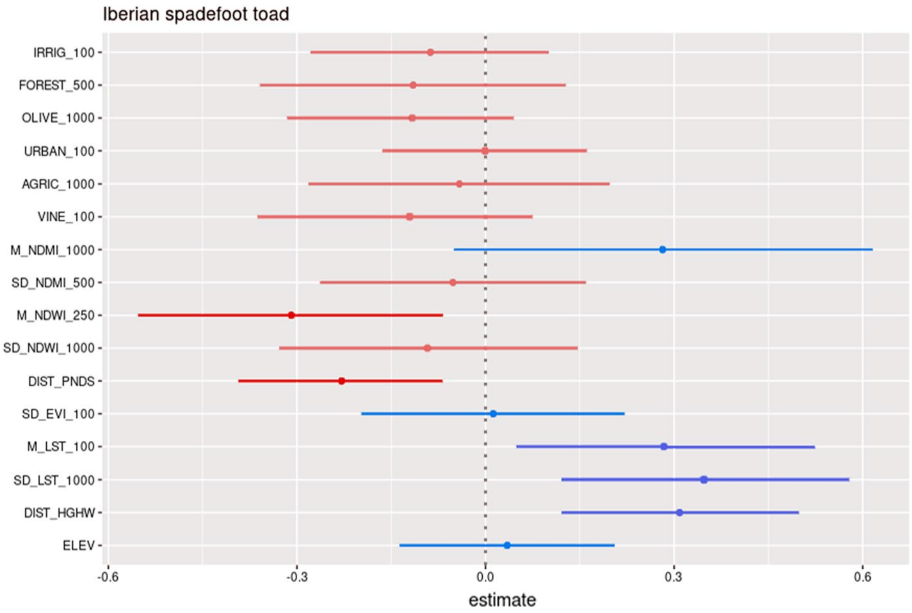

Fig. 6 Model results for the Iberian spadefoot toad (estimates and 95% confidence intervals). Dark blue lines represent statistically significant positive values; light-blue lines represent non-statistically significant positive values; light-red lines represent non-statistically significant negative values; and red lines represent statistically significant negative values

CI = 0.12, 0.58 (Fig. 6); coef = 0.134; CI = 0.01, 0.26, respectively). Roadkills of the Iberian spadefoot toad and the natterjack toad were higher for roads at longer distances to the highway ('DIST_HGHW'; coef = 0.309; CI = 0.12, 0.50; coef = 0.467; CI = 0.27, 0.67, respectively), while the Iberian ribbed newt had an opposite response (coef= −0.126; CI= −0.24, −0.01). Lastly, lower elevations ('ELEV') were associated with higher mortality for the natterjack toad (coef= −0.292; CI= −0.53, −0.06) and the spiny common toad (coef= −0.125, CI= −0.23, −0.02), whereas the fire salamander presented higher road mortality in higher elevations (coef = 0.117, CI = 0.03, 0.21).

In four of the five studied amphibian species, roadkill spatial patterns were driven by water availability and land surface temperature predictors. Land cover predictors, vegetation productivity, road proximity and topography were each significant for three species.

Discussion

Our study analysed whether water-related predictors, together with other important environmental variables for amphibians, would influence their mortality patterns. Our models show contrasting results, supporting the hypothesis that roadkill drivers are species-specific, as previously reported in other studies (e.g. Sillero 2008; Matos et al. 2012; Mestre et al. 2019). Overall roadkill risk increases when water availability in surrounding water bodies is low; however, our models also show that other predictors also have a significant contribution to amphibian roadkill.

Contrary to other studies (e.g. Sillero 2008; Carvalho and Mira 2011) our models show that few land cover classes were statistically important in explaining amphibians' roadkill, suggesting they may not act singly, but related to other important drivers. We found reduced amphibian mortality associated with agricultural intensification (namely olive groves and orchards, vineyards, and irrigation areas). Beja and Alcazar (2003) registered a decrease in amphibian richness in a protected area in southwest Portugal, mainly due to agricultural intensification. Also, Carpio et al. (2016) addressed the herptile composition in olive groves in Southern Spain and reported a deficit for both amphibians and reptiles in large areas covered by this monoculture. There, fire salamanders were most associated with woodlands and pastures, and less with olive, whereas more generalist species like the natterjack toad, the spiny common toad and the Iberian ribbed newt were more commonly associated with olive groves. Olive groves are generally homogeneous, highly human-intervened (e.g., ploughing) and offer no natural vegetation cover or shelter. The lower roadkill risk in areas with a higher proportion of these land covers may be related to the species' incapacity to reproduce and occur there. In fact, we only detected an increase in road mortality for the natterjack toad in one land cover class associated with agricultural intensification (olive groves). This could be related to the species' generalist characteristics, but also to the water availability in these cultures. Many of the olive groves are drip irrigated, and the presence of puddles could potentially attract this species, since the natterjack toad seeks these shallow temporary waters for reproduction (Gómez-Mestre 2014). Nevertheless, many of these systems are also associated with high levels of agrochemicals, known to be harmful to amphibian survival and growth (Baker et al. 2013). Although there is no abundance data (Loureiro et al. 2008), the natterjack toad is probably the most frequent species in the study area and the one with the highest numbers of roadkill. Orlowski (2007) and D'Amico (2009) found that high levels of amphibian roadkill are related to higher local abundances. Thus, the higher natterjack toad mortality in land use classes where other species are not so frequent could also reveal its higher abundances. Nevertheless, the natterjack toad presents high plasticity and adaptability (Gómez-Mestre 2014), and these results should be interpreted with caution, since other predictors (e.g. dispersal capacity; terrestrial site fidelity; Sanuy et al. 2000; Miaud and Sunay 2005) may exacerbate an effect on the roadkill risk, far more than the land cover. Also, the Iberian ribbed newt and the fire salamander had lower roadkill in segments with a higher cover of open agricultural areas. For the Iberian ribbed newt, this is probably due to its highly aquatic behaviour, which apparently reduces its abundance in agricultural areas with high levels of grazing, as in our study area. Nonetheless, the terrestrial habits of the Iberian ribbed newt are almost unknown (Salvador 2014), which limits our conclusions about this species. The lower abundances of fire salamander roadkill in agricultural areas are probably related to its more specialist habitat preferences, since this species tends to occur in more forested areas (Velo-Antón and Buckley 2015; Speybroeck et al. 2016).

Our models highlight that water content in water bodies mediates amphibian roadkill for most of the analysed species (four of them). Although we expected higher quantities of water to attract more amphibians, increasing roadkill risk, our models show the opposite result. All studied species rely on water at least for reproduction and larval development and some of them present high water body fidelity, and aestivation/hibernation areas often occur in the surroundings of the water bodies (Joly 2019). Years of lower water availability would require individuals to travel greater distances to find suitable sites for reproduction, posing a higher road mortality risk; or even to cease reproduction, which in turn could drastically reduce populations, due to lower recruitment from juveniles (Cushman 2006) Segev et al. (2010) found that stable ponds support larger populations of fire salamander (*S.*

infraimmaculata) in Israel. Ribeiro et al. (2011) studied the importance of pond networks for amphibian populations in a Mediterranean region and found that the presence of certain species is highly correlated with the structural connectivity of the pond network. These studies highlight the importance of water (ponds) for the stability of amphibian populations in human-intervened landscapes. Future projections predict a strong reduction in water availability for the Mediterranean region (Tuel and Eltahir 2020). The depletion of suitable water bodies as a consequence of water scarcity, together with the triggered movement in search for potential reproduction areas will increase roadkill. Previous findings describe a positive relationship between water availability and amphibian roadkill (Glista et al. 2008; Sillero et al. 2019). Those studies report to areas with higher rainfall and humidity than ours, where water availability in water bodies should not be a limiting factor for amphibian reproduction, thus reducing their need to move longer distances to complete their life cycle.

The proximity of roads to water bodies seems to exert different responses on our species. The natterjack toad was the only species showing a higher risk of mortality in road segments further away from water bodies. This is expected, as in the study area, this species reproduces mostly in ephemeral ponds with an area lower than 10 m^2 (Speybroeck et al. 2016), too small and too seasonal to be mapped in CORINE land cover products. Thus, these small ponds were not accounted for in the water availability predictor class. Another explanation for this result could be the ability of this species to disperse longer than the others (Sinsch et al. 2012), enabling it to search for water at a larger distance from roads (less disturbed areas). For two other species (Iberian spadefoot toad and Iberian ribbed newt) the roadkill is higher when the distance from water bodies to roads is shorter. This response could be explained by the regular distances covered by these species, reflecting the higher abundance and movements of amphibians near water bodies, since they reproduce on these sites and tend to shelter in surrounding areas, particularly for species with poor capacities for dispersal, such as the Iberian spadefoot toad and the Iberian ribbed newt (Recuero 2014; Salvador 2014).

Our models show that for urodeles (the fire salamander and the Iberian ribbed newt), the lower the inter-annual variation in humidity content in vegetation (SD_EVI), the higher the roadkill. Road segments with lower SD_EVI reveal more stable and predictable local conditions across the years, thus being more attractive for amphibians to occur there, increasing their abundance in those places. Moreover, in our study area, these stable sites correspond to less human-intervened dense forests, and these road segments are more prone to amphibian movement, justifying the increase in road mortality for these two species. For the natterjack toad, our models predicted a higher mortality with greater variation of humidity content in vegetation; a response that can be associated with the more generalist behaviour of this species regarding habitat sensitivity and occurrence (Gómez-Mestre 2014). The Iberian ribbed newt roadkill was also associated with road segments that have higher inter-annual variation in moisture content in vegetation (SD_NDMI). This response was not expected since higher moisture levels typically promote amphibian movement (Mestre et al. 2019). As little is known from Iberian ribbed newt movements in their terrestrial phase, it is possible that this species tolerates a wider range of environmental shifts than other amphibian species.

Amphibians are ectothermic and their activity is extremely dependent on the temperature (Speybroeck et al. 2016); our models confirm a decrease in roadkill for the spiny common toad, and an increase in roadkill for two anuran species (the natterjack toad and the Iberian spadefoot toad) and one urodele (the Iberian ribbed newt) in road segments embedded in areas with high land surface temperature. Muro et al. (2018) found that land surface temperature is an indicator of changes in wetlands in Tanzania, with overall temperature increasing with farmland expansion; the same trend has also been observed in changes in hydrological regimes. Although we did not test it, this association could eventually explain the increase in roadkill with higher temperatures (except for the spiny common toad): in the context of future predictions of increased temperatures, the hydrological periods could be shortened, increasing amphibian movements in search for suitable conditions, which would represent potential roadkill risk for these species. Likewise, the inter-annual variation in the temperature produced similar results, with peaks in mortality occurring in road segments with higher variation in temperature for the Iberian ribbed newt and the Iberian spadefoot toad. Higher values of this predictor represent road segments with a greater local variance in temperature, which in turn could force amphibian displacements, increasing the likelihood of roadkill. This positive link between climatic factors such as temperature and roadkill has already been suggested (Puky 2005; Glista et al. 2008).

The proximity of the highway that crosses the study area appears to produce different effects on the roadkill of the studied species. For the natterjack and the Iberian spadefoot toads, roadkill is higher on road segments further away from the highway. This may reveal an avoidance effect due to the disturbance caused by the highway's higher traffic and velocity, or previous roadkill that may have extirpated local populations aroud this infrastructure (Jackson and Fahrig 2011). Highways are much wider than national roads, and typically concentrate more traffic (the known major determinant of the barrier effect); moreover, amphibians commonly show a reduced tendency for crossing wide roads with these characteristics (Fahrig et al. 1995). Orlowski (2007) also observed higher amphibian mortality rates on roads with low or moderate traffic density, than on roads with higher traffic. Additionally, anurans rely on auditory cues to communicate, which may lead them to avoid noisy locations and concentrate in areas away from them (Nelson et al. 2017). We found the opposite result for the Iberian ribbed newt, with higher mortality close to the highway. Urodeles are poorer dispersers than anurans, known to remain in the same small area for longer periods of time (Segev et al. 2010; Bani et al. 2015; Sinai et al. 2020); also, the noise produced by the highway traffic does not interfere in their communication since they communicate via chemical cues (Speybroeck et al. 2016). This may suggest that other negative effects such as pollutant emission by vehicles, chemical transportation from road runoff, or even herbicides used in road verge maintenance could contribute to the higher mortality of the Iberian ribbed newt close to highways (Forman and Alexander 1998; Colino-Rabanal and Lizana 2012). However, as stated before, terrestrial movements of the Iberian ribbed newt are poorly studied, which only allows us to speculate on this matter.

For the spiny common toad and the natterjack toad, our results forecast higher mortality in lower altitudes, where the land surface is flatter propitiating water retention. This is in accordance with other studies (Santos et al. 2007; Matos et al. 2012), where the authors suggest that amphibians move towards lower altitudes where breeding sites are more common. However, we detected the opposite relation with the fire salamander, with higher roadkill density at upper elevations. The fire salamander is considered a forest species, with a preference for wet and shaded environments (Velo-Antón and Buckley 2015). Despite the low altitudinal range in our study area, forest patches with high tree density are most concentrated in higher and steeper areas also characterised by high humidity and less human intervention. Nonetheless, our results should be interpreted with caution especially because the altitudinal range in the study area is low (about 300 m). Higher amphibian roadkill densities in high altitudinal areas have, however, been observed in other areas of the Iberian Peninsula (e.g., Espinosa et al. 2012; Garriga et al. 2017).

Conclusions

Our study confirmed that water-related predictors are important drivers of amphibian roadkill. In the context of predicted reduced water availability in the future, amphibians are likely to be severely affected, especially in semi-arid regions such as the Alentejo. Rain irregularity, with possible lower precipitation, may aggravate the scenario of drought, leading to less water availability, necessary for amphibian reproduction and development; this will force the animals to move longer in search of alternative water bodies suitable for reproduction, increasing their roadkill risk. Future studies should combine roadkill data with population data to better access populations' status and evaluate the effect of mortality induced by roads on population trends.

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Declarations

Conflict of interest The authors declare no conflict of interests.

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