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Research article

Effectiveness of permanent drift fences in reducing roadkill risk of amphibians

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ABSTRACT

Roads are an important source of human economic progress, but also a threat to wildlife populations and natural habitats. Roads are responsible for the direct mortality of hundreds of millions of animals worldwide, with special negative effects for amphibians. Since the middle of the twentieth century, various types of mitigation measures have been constructed to reduce the negative effects of roads. However, despite the large availability of potential solutions designed for this purpose, there is still a knowledge gap about their effectiveness for amphibians. This study analysed whether permanent concrete drift fences reduced the roadkill risk for amphibians. We applied a before-after-control-impact (BACI) design in two road segments with concrete drift fences for amphibians. We recorded amphibians on these road segments three years before and three years after the fence installation. We further tested whether the presence of these mitigation measures transferred the animals to sites adjacent to the drift fences, creating new potential mortality aggregation sites (fence-end effect). Our results show a significant reduction in the number of amphibians reaching the sites with the drift fences. We were, however, unable to demonstrate the potential movement route transference, as our results were inconclusive. Despite the increase in amphibian numbers at the control sites in the first year after fence installation, the following two years presented similar amphibian numbers as the pre-fence years. We recognise the importance of permanent drift fences in reducing the mortality of amphibian populations; however, we encourage future studies to include tunnel-crossing data as well, to truly unveil the roadkill reduction power of amphibian mitigation measures, while maintaining or increasing connectivity between roadside habitats.

1. Introduction

Every year, hundreds of millions of animals die on the roads, victims of vehicle collisions (Forman and Alexander, 1998; Loss et al., 2015; Hill et al., 2019). This is frequently the primary source of mortality for many species, increasing the risk of extinction in many parts of the world (Forman et al., 2003; Grilo et al., 2021). Currently, amphibians are the most threatened vertebrate group on the planet (Houlahan et al., 2000; Stuart et al., 2004; IUCN, 2023), with roads as one of the major causes of population decline (Carr and Fahrig, 2001; Glista et al., 2008; Beebee, 2013; D'Amico et al., 2015) along with habitat loss and consequent fragmentation (Houlahan and Findlay, 2003; Cushman, 2006). Amphibians are extremely prone to death on roads, especially because of their complex life cycles, with specific movement routes due to distinct terrestrial and aquatic phases (Richter et al., 2001; Joly, 2019). A higher amount of roadkill occurs when roads cross the amphibian movement routes between the terrestrial and aquatic territories (coincident with hibernation/estivation and reproduction habitats) (Orłowski, 2007; Eigenbrod et al., 2008; Sillero, 2008; Beebee, 2013; Pinto et al., 2023).

Understanding the effectiveness of roadkill mitigation measures in wildlife mortality is crucial to developing new methods to reduce the negative effects of roadkill on populations. The general aim of a roadkill mitigation structure is to impede animals from reaching the road, while (in most cases) providing a safe alternative to cross it: hence, the connectivity between habitats is improved on each side of the road (Forman et al., 2003; Dodd Jr et al., 2004; Hamer et al., 2015). The first roadkill

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mitigation measures for amphibians were built in Europe in the 1960s (Puky, 2003), and currently, different types of measures are used, including: 1) road signs, used to alert drivers to a particular segment of the road that is frequently crossed by amphibians; 2) temporary road closure, usually during amphibian movement peaks; 3) temporary or permanent drift fences, built of canvas or plastic (temporary) or made from concrete, metal or UV-resistant polymers (permanent) to prevent animals from reaching the road surface; and 4) underpasses or road tunnels, with one entry on each side of the road, to allow animals to move safely between both sides of the road (reviewed in: Jochimsen et al., 2004; Schmidt and Zumbach, 2008). Based on the available data, the use of drift fences together with underneath road tunnels is considered the most favourable solution for reducing mortality in amphibians (Puky, 2003; Schmidt and Zumbach, 2008; Glista et al., 2009; Hamer et al., 2015). However, the susceptibility of animals to bypass the fence ends and access the road, may compromise management efforts to reduce road mortality, potentially undermining the overall effectiveness of the mitigation measure (Rytwinski et al., 2016; Markle et al., 2017).

The construction of these structures frequently has high costs, limiting their implementation in the field (Lesbarrères and Fahrig, 2012); therefore, temporary drift fences may be employed more frequently than permanent ones. However, permanent mitigation measures require less maintenance than temporary ones (Dodd Jr et al., 2004), which ultimately may represent a benefit in the medium/long term (Hamer et al., 2015) and consequently, an increased efficiency. Although several studies attempt to identify the success of these measures, many fail due to insufficient monitoring (Van der Ree et al., 2007), or lack the temporal scale with sampling both before and after the installation of the mitigation measure, which may result in uncertain or misleading conclusions (Lesbarrères and Fahrig, 2012).

A before-after-control-impact (BACI) sampling design is often considered the best approach to assess the impact of a stressor in the environment (Underwood, 1991; McDonald et al., 2000), producing useful information in road management studies (Roedenbeck et al., 2007; Lesbarrères and Fahrig, 2012). Therefore, it is necessary to assess mitigation measures' effectiveness, so that resources can be directed to the most cost-effective ones. The success of allowing amphibians to safely cross roads during their seasonal movements is crucial for ensuring the long-term viability of their populations (Rytwinski and Fahrig, 2012).

In this study, we aim to assess the effectiveness of specific permanent amphibian mitigation measures to reduce road mortality risk in a region with historically high mortality records (Pinto et al., 2023). Specifically, we implemented a BACI design over six years, to test whether permanent concrete drift fences effectively reduce amphibian mortality risk. We also tested whether the presence of these mitigation structures resulted in new roadkill aggregations in adjacent non-mitigated road sections. For this, we established two hypotheses: 1) the concrete drift fences significantly prevent amphibians from reaching the roads, reducing consequentially their roadkill risk; and 2) the presence of amphibians on the road does not increase in road sections adjacent to the drift fences (absence of fence-end effect), not affecting amphibian movement routes.

2. Methodology

2.1. Study area

This study was conducted in southern Portugal (29N 599606E, 4285394N), in an area with one of the ecosystems with the highest biodiversity in the western Mediterranean Basin, also known as '*montado*' (Pinto-Correia et al., 2011). The area is dominated by a mixture of Mediterranean cork oak (*Quercus suber*) and holm oak (*Quercus rotundifolia*) forests with varying tree density and agricultural areas in equal proportions. The topography is generally flat, ranging between 100 m and 400 m a.s.l. (Correia, 1993). The area is characterised by a Mediterranean climate, with mild and wet winters (mean temperatures between 5.8 °C to 12.8 °C) and hot and dry summers (mean temperatures between 16.5 °C to 30.2 °C). The average annual rainfall ranges between 500 and 650 mm (IPMA, 2021). The study area is intersected by the main transportation corridor connecting Lisbon to Madrid, comprising several roads, including one highway as well as some national and municipal roads. For this study, we surveyed two municipal road segments: EM529 and EM535.

2.2. Mitigation measures

Five permanent concrete drift fences were implemented in two road segments of the study area with high amphibian mortality, in late spring (April–May) of 2018: two in road EM529 and three in road EM535 (Fig. 1). The fences were built within a LIFE Nature and Biodiversity Program of the European Commission (LIFE LINES – LIFE14-NAT-PT001081). These structures were developed by a local contractor and were designed considering the characteristics of several models available in the market. The fences are made of 40 cm high concrete blocks with a smooth surface, to prevent most of the species from climbing the top (Conan et al., 2023), and its upper part is (whenever possible) levelled with the road/shoulder surface, to prevent the animals from becoming trapped on the road between fences. They are also "L" shaped, with a slope towards the opposite side of the road (Fig. 1A; more details on supplementary materials Fig. S1). The drift fences were installed on both sides of the roads and have on average 401 m of length (140–1000 m) (Table 1).

Each fence includes at least 2 tunnels: either existing ones (mostly drainage culverts; Fig. 1A) that were adapted to perfectly align their entrance with the drift fences (to prevent animals from reaching the road) or specific amphibian tunnels (ACO, Germany) that were also installed when culverts were absent.

2.3. Study design and data collection

The study was carried out using a BACI design (Underwood, 1991, 1994). For each of the five fenced road sections, we assigned four control sites: two 'proximal control' and two 'distal control' sites with equal lengths (half the length of the respective fence for each side). We defined the proximal control sites immediately after each fence end, and the distal control sites 100 m apart (Fig. 2). We selected this distance for two reasons: 1) to ensure some spatial independence between the two control types while maximizing the number of fences included in the study design; and 2) this distance is typically the maximum length travelled by some species along a drift fence before individuals give-up and turn back if they cannot cross a road (Ottburg and van der Grift, 2019; Brehme et al., 2021). Road section ID 5 presented only one proximal and one distal control site (with the same length as the respective fenced site) as it is located near a crossroad (Table 1; Fig. 1B). To test our hypotheses, we defined a Treatment predictor with three categories: fence, proximal control, and distal control sites. We also defined a Year predictor where the surveys performed in the years 2015, 2016 and 2017 represent the sampling before fence installation, and the ones performed in 2018, 2019 and 2020 the sampling after fence installation. Although our study included data before and after fence installation, it is worth mentioning that, since the fences were installed at sites with high amphibian roadkill, our control sites may still introduce some bias (Soanes et al., 2024).

Amphibian surveys followed a standardized protocol consisting of night-time surveys in autumn, conducted on rainy nights with minimum wind and average temperature ≥10 °C (conditions of maximized amphibian activity – Sillero, 2008; Matos et al., 2012). On each survey, two experienced observers drove a car at a constant speed (20–30 km/h), scouting both road lanes and registering every amphibian encountered on the roads. These procedures aimed to mitigate the potential low detection rates characteristic of roadkill surveys and small-bodied species (Barrientos et al., 2018). All detected amphibians were identified to the lowest possible taxonomic level and the dead

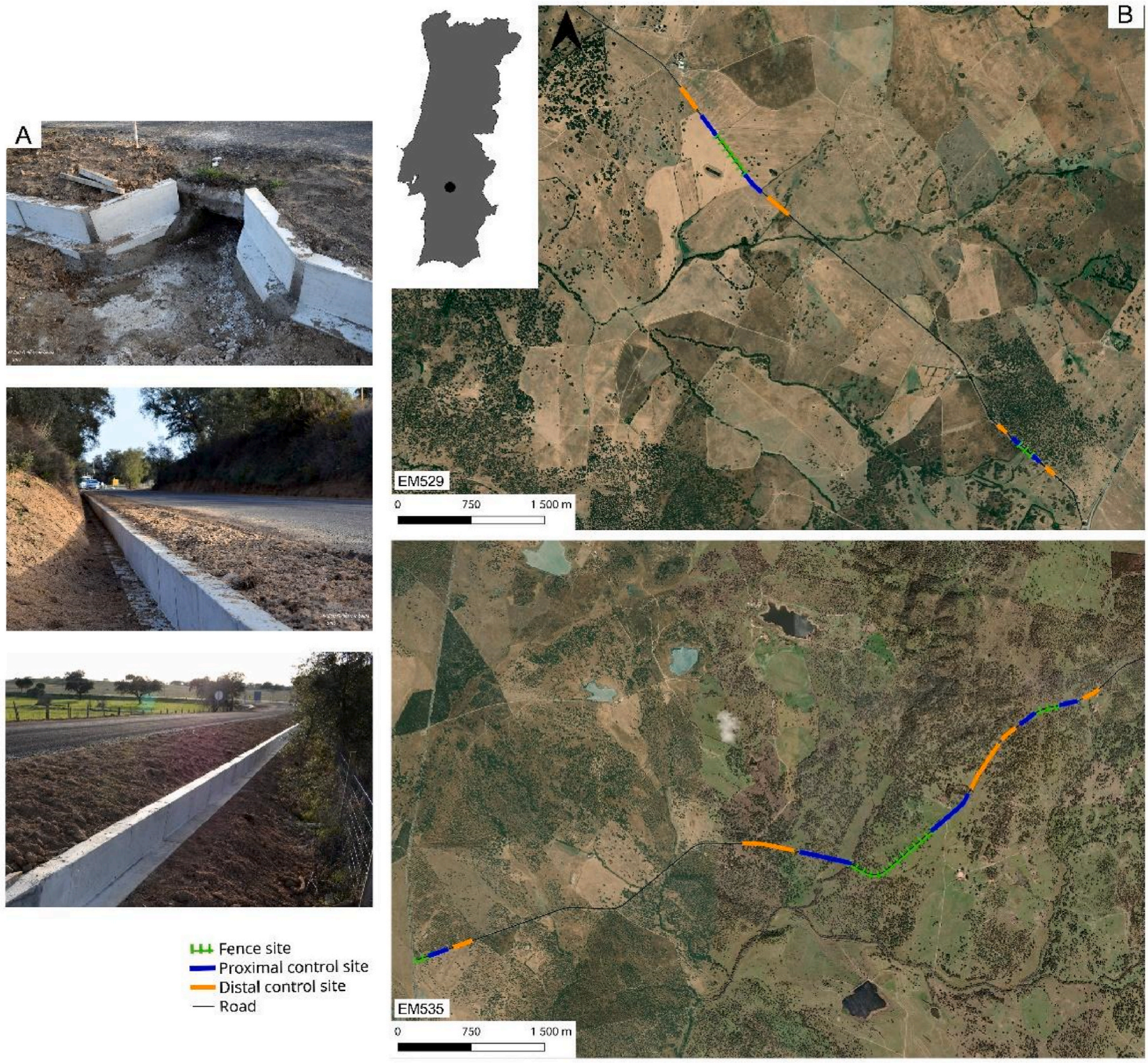

Fig. 1. – Specific amphibian mitigation measures installed on both surveyed road segments. (A) displays the different types of mitigation measures (permanent concrete drift fences and underneath tunnels), while (B) shows the location of roads EM529 and EM535 as well as defined treatment sites (fence, proximal control, and distal control).

animals were removed from the road to avoid double counting during later surveys. Live amphibians found crossing the road were also moved and placed on the road verges in the direction they were heading. The GPS position of each observation was also recorded: observations <1 m apart were considered as a single GPS point with as many observations as animals encountered.

2.4. Data analyses

We used the number of amphibians recorded on the roads (dead or alive) as a response variable and assumed that all live amphibians found on the road were at risk of roadkill. Besides Treatment and Year predictors, we also extracted the percentage of tree density within a buffer of 250 m for each site, from LANDSAT imagery (with 30 m pixel spatial resolution). We used tree density as an indicator of forest structure, where higher percentages represent a proxy of high-quality habitat for most amphibian species.

We built two different models, according to our hypotheses.

- Model H1 assessed the effectiveness of concrete drift fences in reducing the number of amphibians accessing the road (model H1) through a Generalised Linear Mixed Model (GLMM; Bolker et al., 2009) with "Treatment" ('fence' vs. 'distal control'), "Year" (2015–2020), and the interaction of "Treatment" and "Year" as main predictors; the percentage of tree density as a predictor to account for habitat differences and the length of each treatment site as an offset parameter (log scaled) to account for possible bias in our data; and the survey ("Visit") and treatment site ("Site code") as random effects to account for unbalanced sampling and possible correlations between successive visits at the same sites (see Table S1 on supplementary materials for a resume of the predictors).
- Model H2 assessed whether road sections adjacent to the concrete drift fences were subject to higher roadkill (model H2) through a second GLMM with "Treatment" ('proximal control' vs. 'distal control'), "Year" (2015–2020), and the interaction of "Treatment" and "Year" as main predictors. The model structure was identical to the previous one, with tree density as an additional predictor, the length of each treatment site as an offset parameter, and survey and treatment site as random effects (see Table S1 on supplementary materials for a resume of the predictors).

We built both models with a negative binomial distribution, as our data presented high values of overdispersion (Zuur et al., 2009). To test for potential autocorrelation in our data, we performed a Moran's I test

Table 1
Description of sampled treatment sites, the length, road, and percentage of tree density.

Section ID	Site code	Treatment	Length (m)	Road	Tree density (%)
ID 1	F1	Fence	400	EM529	0
	PC1_1	Proximal Control	200	EM529	0
	PC1_2	Proximal Control	200	EM529	0
	DC1_1	Distal Control	200	EM529	0
	DC1_2	Distal Control	200	EM529	1
ID 2	F2	Fence	140	EM529	17
	PC2_1	Proximal Control	70	EM529	11
	PC2_2	Proximal Control	70	EM529	24
	DC2_1	Distal Control	70	EM529	15
	DC2_2	Distal Control	70	EM529	7
ID 3	F3	Fence	300	EM535	0
	PC3_1	Proximal Control	150	EM535	20
	PC3_2	Proximal Control	150	EM535	19
	DC3_1	Distal Control	150	EM535	22
	DC3_2	Distal Control	150	EM535	10
ID 4	F4	Fence	1000	EM535	11
	PC4_1	Proximal Control	500	EM535	3
	PC4_2	Proximal Control	500	EM535	13
	DC4_1	Distal Control	500	EM535	28
	DC4_2	Distal Control	500	EM535	12
ID 5	F5	Fence	165	EM535	0
	PC5_1	Proximal Control	165	EM535	2
	DC5_1	Distal Control	165	EM535	1

(Moran, 1950) for spatial autocorrelation, and a Durbin-Watson test (Durbin and Watson, 1950) for temporal autocorrelation.

To evaluate drift fence impact (model H1), we calculated the BACI effect, representing the differential change between the fenced and the distal control sites, compared in the years before and after fence installation (Schwarz, 2015). We adopted the same procedure for model H2 for both control treatments.

We performed all the statistical analysis using the packages “glmmTMB” (Brooks et al., 2017), “DHARMa” (Hartig, 2022), “performance” (Lüdecke et al., 2021), “MuMin” (Barton, 2020) and “lsmeans” (Lenth, 2016) on software R (version 4.1.2; R Core Team, 2021). Tree density was extracted in QGIS software (version 3.24.1; QGIS Development Team, 2022).

3. Results

Between 2015 and 2020, we performed 83 surveys in both road segments (35 surveys in road EM529 and 48 surveys in road EM535), with 24 surveys before and 59 after fence installation. These surveys produced a database of 1593 amphibians reaching the road, belonging to 12 species (Table S2 – supplementary materials).

The two models showed a good fit to the data despite their R-squared values: model H1 explained 46% of the variance, while the model H2 explained 34% of the variance. Further model evaluation revealed that the residual plots exhibited no patterns, and both spatial and temporal autocorrelations had no significant values (Moran’s I – model H1: 0.13, $p = 0.25$; model H2: 0.29, $p = 0.15$; and Durbin-Watson – model H1: 1.62, $p = 0.17$; model H2: 1.98, $p = 0.15$).

Concerning the effectiveness of the concrete drift fences (model H1), although the “Treatment” showed no significance, the “Year” 2018 was significantly different (Model H1: $Z = -0.04$, $p > 0.05$; $Z = 1.257$, $p < 0.01$, respectively). The interaction term between “Treatment” and “Year” revealed that all the years representing post-fence installation were significantly different (2018: $Z = -1.947$, $p < 0.01$; 2019: $Z = -2.438$, $p < 0.01$; 2020: $Z = -1.082$, $p < 0.01$). The installed fences significantly decreased the number of amphibians reaching the road. The estimated mean number of amphibians per visit per site on the fenced sites declined from nearly 1 in 2015 to 0.42 in 2020, representing a reduction of more than half the number of amphibians. In contrast, on the proximal control sites, the mean number of amphibians per visit and site increased from nearly 1 in 2015, to 1.6 in 2020 (Fig. 3). The contrast analysis performed to determine the BACI effect estimated from the model H1 was significant: 5.68 ± 1.05 ($p < 0.001$).

Concerning the potential fence-end effect (model H2), the number of amphibians reaching the road between distal and proximal control sites was not significantly different ($Z = 0.271$, $p > 0.05$), but the year 2018 was significantly different ($Z = 1.657$, $p < 0.01$). None of the interactions “Treatment” x “Year” was significant (2016: 0.633, $p > 0.05$; 2017: 0.973, $p > 0.05$; 2018: $Z = -0.439$, $p > 0.05$; 2019: 0.285, $p > 0.05$; 2020: $Z = -0.252$, $p > 0.05$): the number of amphibians is not significantly different between road sections adjacent to the fences and the distal control sites (Fig. 4). The contrast analysis used to determine the BACI effect for the model H2 was also not significant: 0.12 ± 0.84 ($p > 0.05$).

Tree density was not significant for any of the models (Model H1: $Z = 0.02$, $p > 0.05$; Model H2: $Z = 0.01$, $p > 0.05$). Table 2 summarises model H1 and model H2 results.

4. Discussion

Our study measured the effectiveness of roadkill mitigation structures built specifically for amphibians. Our results support our first hypothesis that permanent concrete drift fences are effective in reducing the number of amphibians on the roads. The significance of the interaction terms reveals that the decrease in amphibian numbers was linked to the mitigation, with no reduction observed in the distal control treatment. The concrete drift fences acted as a barrier preventing amphibians from accessing the road, resulting in a roadkill decrease of more than half when compared with the years before fence installation (2015–2017). Several studies reported similar results, with drift fences reducing amphibian roadkill between 40% and 100% (Cunnington et al.,

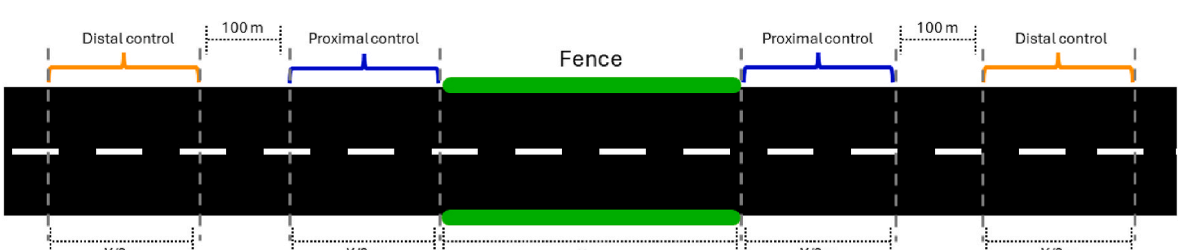

Fig. 2. – Schematic design applied for each drift fence (green line). The proximal (blue) and the distal (orange) control sites extend to both sides of the fence, with half the length of the respective fenced site. Distal control sites are placed 100 m apart from the proximal control sites (Road section ID 5 presented only one proximal and one distal control site).

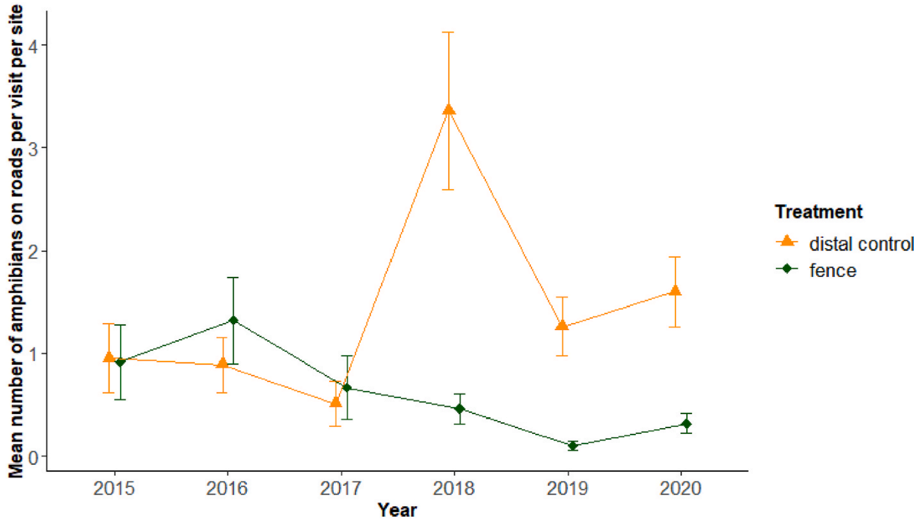

Fig. 3. – Least square means values (and respective SE) of amphibians per visit per site found on both roads in the years before (2015–2017) and after (2018–2020) drift fence installation (all distal control and fence sites). Fences significantly reduced the number of amphibians reaching the roads.

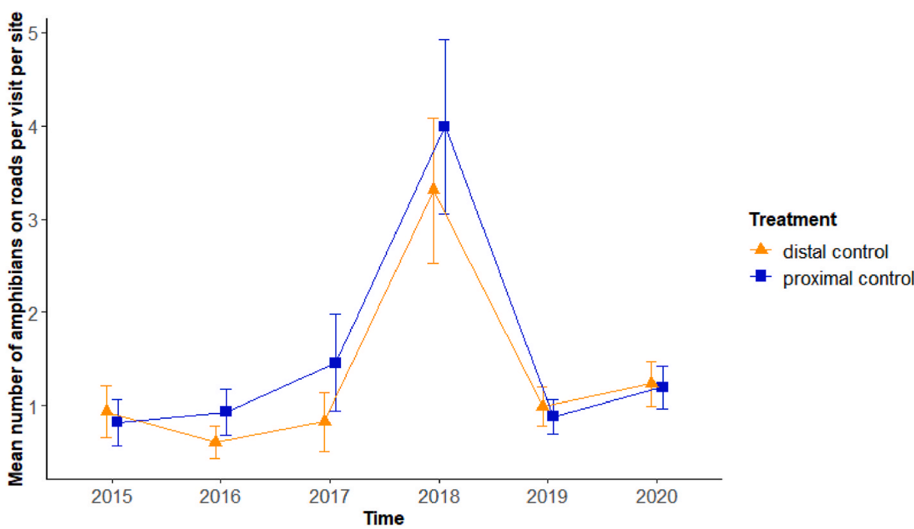

Fig. 4. – Least square means values (and respective SE) of amphibians per visit per site found on roads on both control treatments in the years before (2015–2017) and after (2018–2020) drift fence installation (all distal and proximal control sites). The year 2018 is significantly different in both treatments.

2014; Rytwinski et al., 2016; Helldin and Petrovan, 2019; Boyle et al., 2021). In fact, Cunnington et al. (2014) stated that fences are a far more efficient road mitigation measure than tunnels in reducing amphibian roadkill and that priority should be given to the installation of fences so that amphibians can be kept away from roads.

Despite our results on fence effectiveness, these were not 100% effective as we still found some amphibians on the roads in the fenced sites after fence installation. There is the possibility that some animals entered the fenced sites either by climbing the drift fences (Dodd Jr et al., 2004), or coming from the adjacent sites (moving along the road). Oppositely to Dodd Jr et al. (2004), who reported a decrease in drift fence effectiveness with tree frogs – overall mortality reduction from 93.5% to 65% - we only found two individuals of a species with climbing abilities (*Hyla meridionalis*) on the roads in fenced sites after fence installation, excluding this as the main reason. Also, a possible lack of maintenance of the vegetation surrounding the fences could have facilitated the access to the road (Hamer et al., 2015; van der Ree et al., 2015). Nevertheless, the concrete drift fences proved to be effective in impeding most of the amphibians from accessing the roads, with a reduction in amphibian sights of nearly 60%. Moreover, in a recent study, Conan et al. (2023) demonstrated – through a series of tests – that a permanent drift fence (e.g., concrete) with a minimum height of 40 cm and an overhang on top, was able to stop most amphibians from reaching the road. Our fences comprise most of the characteristics these authors detected to be essential for the effectiveness of these mitigation measures.

We were, however, unable to demonstrate that the permanent drift fences do not alter amphibian movement routes (second hypothesis), as our results were inconclusive. We did not detect significant differences between the two treatments (distal and proximal control) across the analysed years. Despite the mean number of amphibians on roads at these sites in 2019 and 2020 being equivalent to the years before fence installation, we cannot conclude for sure whether movement routes were modified, as we did not collect tunnel-crossing data. To our knowledge, Helldin and Petrovan (2019) is the only study that reported fence-end effects for amphibians. In this work in Sweden, the authors found that amphibian roadkill increased in an unfenced site adjacent to fences, probably linked with a movement route change and possible mortality transference. We found an increase in amphibians on the roads in 2018 for both proximal and distal control sites; however, this does not

Table 2
Models with respective coefficients and R^2. Significant results are in bolt.

	Estimate	Std. Error	z value	p-value	Confidence interval (95%)
Model H1					
Treatment	−0.039	0.404	−0.098	0.922	−0.83; 0.75
Year2016	−0.069	0.442	−0.158	0.875	−0.94; 0.79
Year2017	−0.625	0.538	−1.162	0.245	−1.68; 0.43
Year2018	1.257	0.400	3.142	**0.002**	**0.47; 2.04**
Year2019	0.278	0.398	0.696	0.486	−0.50; 1.06
Year2020	0.516	0.393	1.315	0.188	−0.25; 1.29
Tree density	0.019	0.010	1.903	0.060	−0.001; 0.03
Treatment*Year2016	0.432	0.477	0.907	0.365	−0.50; 1.37
Treatment*Year2017	0.307	0.588	0.522	0.602	−0.84; 1.46
Treatment*Year2018	−1.947	0.461	−4.227	< **0.001**	**−2.85; -1.04**
Treatment*Year2019	−2.438	0.541	−4.508	< **0.001**	**−3.49; -1.38**
Treatment*Year2020	−1.082	0.445	−2.432	**0.015**	**−1.95; -0.21**
R^2	0.46				
Model H2					
Treatment	0.271	0.339	0.798	0.425	−0.39; 0.94
Year2016	0.611	0.455	1.343	0.798	−0.28; 1.50
Year2017	0.420	0.531	0.791	0.429	−0.62; 1.46
Year2018	1.657	0.439	3.767	< **0.001**	**0.80; 2.52**
Year2019	0.758	0.423	1.795	0.073	−0.07; 1.59
Year2020	0.653	0.404	1.618	0.106	−0.14; 1.44
Tree density	0.010	0.005	1.779	0.075	−0.001; 0.02
Treatment*Year2016	−0.633	0.433	−1.464	0.143	−1.48; 0.21
Treatment*Year2017	−0.974	0.521	−1.871	0.061	−1.99; 0.05
Treatment*Year2018	−0.439	0.403	−1.089	0.276	−1.23; 0.35
Treatment*Year2019	−0.285	0.394	−0.724	0.469	−1.06; 0.49
Treatment*Year2020	−0.252	0.377	−0.668	0.504	−0.99; 0.49
R^2	0.34				

necessarily imply a fence-end effect (see below). Other studies have also reported an increase in road mortality at adjacent ends of the mitigation fences (Clevenger et al., 2001; Markle et al., 2017). Yet, these studies were conducted with different taxonomic groups (mammals and reptiles, respectively) and thus, cannot be comparable. Despite the lack of studies reporting fence-end effects for amphibians, potential solutions to reduce this problem can be found in the literature (and some may be adaptable to amphibians), such as: 1) increasing the length of the mitigation drift fences, as some authors reported roadkill reduction at longer fence lengths (Huijser et al., 2016); 2) installation of terminal fence segments – either perpendicular to the road or "V" shaped – to discourage animals from circumventing the fence and accessing the road, and guide them back to the fence and towards a safe underneath passage (Harman et al., 2023); and 3) increasing the number of tunnels along the fence to increase the likelihood of an animal to find and use them (Ottburg and van der Grift, 2019).

According to our models, the year 2018 was significantly different, as we recorded an exceptionally high number of amphibians on both control treatments, with numbers returning to fence pre-construction period in the following years. Amphibian activity is highly dependent on external factors, such as temperature and precipitation (Araújo et al., 2006; Glista et al., 2008), and this increase may have been triggered by environmental conditions present in the study area in that year (and not analysed in this study). For example, the year 2018 was unusually rainy for the region (IPMA, 2023), which could have prompted amphibian activity, justifying these higher numbers. In fact, this year was responsible for 25% of all the collected animals on sampled sites across the entire study period. Nevertheless, it could also mean a possible fence-end effect, with movement transference, as the number of amphibians on the roads increased at sites of both control types in that year. Still, the numbers of amphibian sights in the following years (2019 and 2020) are similar to the ones before fence installation, with even slightly lower numbers in proximal control sites when compared to 2017. This could eventually suggest no fence-end effect, but rather a response to an atypical year, or even a fence construction effect detected in 2018 but not in the following years. We minimised this potential construction effect, by choosing a sampling season (autumn) different from the construction period (late spring), though some effects may still have been detected. Unfortunately, the lack of tunnel-crossing data impedes a clearer conclusion, and the results from our second hypothesis should be interpreted with caution.

As the fences act as a barrier to amphibian movements towards the roads preventing them from being roadkilled, they can also have a counter effect, increasing the barrier effect (Jaeger and Fahrig, 2004). This behaviour has been previously reported by some studies where amphibians gave up and moved back after certain distances travelled along a fence (e.g., Matos et al., 2019; Ottburg and van der Grift, 2019; Brehme et al., 2021), returning to the original habitats without breeding (Schmidt and Zumbach, 2008). Our data does not allow us to measure this behaviour, although we do not discard it may occur. For species dispersing longer distances and subject to higher mortality, drift fences may provide substantial advantages in decreasing road fatalities (Lesbarrères et al., 2004), but for less mobile species, road crossings may be less frequent (Matos et al., 2019), resulting in genetically isolated populations (Cushman, 2006; Baguette et al., 2013).

Boyle et al. (2021) showed that not only did the fences reduce the number of amphibians on the roads, but underneath tunnels were likely to be used by local species assemblages promoting connectivity at a population level. Jarvis et al. (2019) reported similar results. Although we do not possess this type of data, we expect similar responses from the populations occurring in our study area. Still, more data are needed, especially tunnel-crossing data and data on local populations, to fully understand if these mitigation structures are beneficial for the long-term persistence of amphibian populations. In a 14-year duration study, Pinto et al. (2024) revealed a continuous decrease in roadkill numbers for some amphibian species in the same study area. The authors link this reduction to a possible depletion in local populations (among other reasons). If so, mitigation measures like permanent drift fences together with underneath road tunnels might help to restore connectivity and reduce amphibian road mortality, increasing the likelihood of population recovery.

5. Conclusions and recommendations

Our results highlight the importance of long-term monitoring studies in evaluating the effectiveness of measures to mitigate amphibian roadkill. Roadkill risk has been drastically reduced with the installation of concrete drift fences specifically designed for amphibians, even when environmental conditions are most suitable for this taxonomic group. Since permanent mitigation structures are usually very expensive and demand considerable management (e.g., periodic surrounding vegetation cut), priority should be given to areas with severe historical roadkill patterns, or where existing populations near roads face marked reductions. Within these areas, mitigation measures should be installed in road segments with roadkill hotspots. Drift fence effectiveness should be measured by continuous sampling (both before and after the structure installation). When budgets are constrained, some adaptative measures can be applied: for example, drainage culverts are known to be used by some species; and their adaptation, as was done in our study area (with concrete drift fences towards both culvert sides), may yield similar results for many species.

We also acknowledge that all aspects of the mitigation measures should be accounted for (drift fence and tunnel usage; fence-end sampling; among others) to fully detect possible movement changes and identify what achieves the best results. Most likely there will not be a one-size-fits-all measure but, by understanding what works best, it will be possible to adjust existing structures and implement additional ones that may benefit a wide range of species, at the least possible cost.

CRediT authorship contribution statement

Tiago Pinto: Writing – review & editing, Writing – original draft, Resources, Methodology, Formal analysis, Data curation, Conceptualization. **Neftalí Sillero:** Writing – review & editing, Supervision, Methodology, Conceptualization. **António Mira:** Writing – review & editing, Project administration, Methodology, Funding acquisition. **Luís G. Sousa:** Writing – review & editing, Resources. **André Oliveira:** Writing – review & editing, Resources. **Sara M. Santos:** Writing – review & editing, Supervision, Methodology, Conceptualization.

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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