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Reduction of amphibian roadkill by one-side barriers

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Amphibians are the most affected vertebrates by roadkill. Often, to minimise this problem mitigation barriers are installed. While two-side barriers effectiveness is well described in the literature, one-side barriers are poorly studied. The present study aimed to evaluate the effectiveness of one-side barriers installed by LIFE LINES project along the EM535 road in south-east Portugal. Two types of one-side barriers (one permanent and another temporary one made from tarp) were monitored through road surveys. For each barrier, the influence of interaction between the period (before and after the barrier installation) and road sector (control-no barrier, permanent or temporary barrier installed) on the number of amphibians was evaluated. The result showed that, contrary to the temporary barrier, the permanent one-side barrier appeared to be effective. Thus, the ineffectiveness of the temporary barrier may be related to (i) the tarp material that allows some amphibians to escape, (ii) the presence of vegetation that goes over the top of the barrier, or (iii) increased fence-end effects due to its short length. Even though less effective than two-side barriers, one-side barriers could be installed when there is budget limitation in conservation projects or if land use conflicts exist. Given that our study was the first to evaluate the effectiveness of one-side barriers, we discuss the potential constrains that may have affected our results.

Key words: barriers; effectiveness; mitigation; mortality; road.

Road kills are one of the main conservation problems for amphibians (Collins & Storfer, 2003; Stuart *et al.*, 2004), and so the increase in the number of roads and vehicles makes necessary the development of actions to minimize their impacts (van der Ree *et al.*, 2015). The physiological constraints of amphibians compel them to move through different terrestrial and aquatic patches to complete their life cycle (Hamer *et al.*, 2015). Habitat fragmentation caused by roads reduces connectivity among those patches, which can result in a decrease of amphibian populations (Hamer *et al.*, 2015). The roads might also promote habitat loss, introduction of exotic species, and behavioural changes in amphibians, among other impacts (Colino-Rabanal & Lizana, 2012; Hamer *et al.*, 2015). Also, due to their slow movements, amphibians are extremely likely to be road-killed, this being one of the major impacts of road infrastructure to these animals (Colino-Rabanal & Lizana, 2012).

Even though the casualties depend on the amphibian local abundance (Martínez

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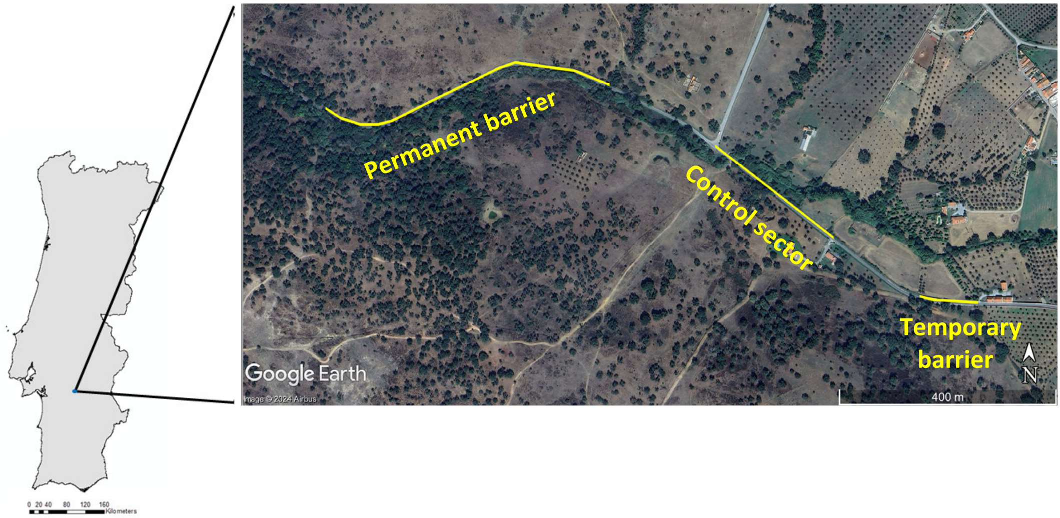

Figure 1: Location of the control and barrier sectors. The temporary barrier was 60 m long and made of tarp. The permanent barrier was 800 m long and made of concrete. The control sector was 200 m long, located between the two barriers and 200 m apart from each one. Image retrieved from Google Earth on 16 January 2024 (attributions are displayed in the image).

-Freiría & Brito, 2012), terrestrial species are more likely to be road killed than aquatic ones, and anurans are killed more often than urodeles (Carvalho & Mira, 2011; Martínez-Freiría & Brito, 2012; Beebee, 2013). Mediterranean regions show two seasonal peaks in road-killed amphibians, in autumn and spring, consistent with the amphibians' migratory periods (Seo *et al.*, 2015). In fact, besides structural reasons like road density, some of the factors that affect the number of amphibian carcasses on the road are also migration drivers like annual rainfall or moon phase (Carvalho & Mira, 2011; Martínez-Freiría & Brito, 2012; Jarvis *et al.*, 2021).

Among all methods to reduce road mortality, mitigation barriers are the most frequently used. The effectiveness of these barriers differs according to the type of barrier, with temporary barriers being less effective than permanent ones (Cunnington *et al.*, 2014; Helldin & Petrovan, 2019). However, most of the scientific literature only evaluates the effectiveness of two-side barriers (installed on both sides of the road). The only study focused on one-side barriers, with reptiles as target species, did not find differences between before and after the installation of the barriers (Markle *et al.*, 2017). This study aims to understand whether one-side barriers are effective in reducing the number of amphibians on the road, which would consequently reduce the risk of amphibians to be road-killed, and if so, understand if they have the potential to be used as mitigation measures in cases of lack of investment or land use constraints.

MATERIALS AND METHODS

Study area and amphibian species

The sampled road was located in the Alentejo region, near the village of Santia-

Figure 2: Pictures of the barriers and of a drainage underpass. A: Permanent (concrete) barrier; the barrier wall was located at road level. B: One of the three drainage underpasses associated with the permanent barrier, whose entry is covered by vegetation. C: Temporary (tarp) barriers, which did not have associated underpasses.

go do Escoural (Évora district, Portugal). The EM535 is a small municipal road with low traffic intensity, with less than five vehicles per hour. The landscape surrounding the road is mainly Mediterranean shrub-woodland dominated by *Quercus ilex* and *Quercus suber*, where the main activity is livestock farming. Next to the road there is a small river that flows parallel to it.

According to Loureiro *et al*. (2008) and Speybroeck *et al*. (2020), 13 amphibian species can be found in the study area: four urodeles (*Lissotritoon boscai, Pleurodeles waltl, Salamandra salamandra* and *Triturus pygameus*) and nine anurans (*Alytes cisternasii, Bufo spinosus, Discoglossus galganoi, Epidalea calamita, Hyla meridionalis, Hyla molleri, Pelodyctes ibericus, Pelobates cultripes* and *Pelophylax perezi*).

Mitigation barriers

We examined two types of one-side barriers with different characteristics, whose location can be seen in Figure 1. The first barrier (permanent barrier) was built on March 30th 2018. This barrier was made of concrete, was 800 m long and 35 cm high, and was associated with three drainage underpasses that allowed the movement of amphibians beneath the road (Fig. 2a,b). The second (temporary) barrier was made of linoleum (tarp) and was installed on December 14th 2018. This barrier was substantially shorter than the previous one (60 m), moderately higher (40 cm) and did not have drainage underpasses associated (Fig. 2c). The lower part of this temporary barrier was buried into the soil to prevent amphibians from escaping under it, although some parts of the barrier were lifted over the soil. In both structures, some vegetation grew over the barriers, especially during spring.

During the LIFE LINES project two-side barriers were installed in this road; however, these barriers are expensive, so the LIFE LINES team tested one-side barriers as a possible, cheaper alternative to the two-side barriers. The search of alternatives is vital, so that organizations with low financial support, as NGOs and municipalities, can implement mitigation measures to reduce amphibian's road mortality.

Data collection

All data were collected in a 2-km stretch of the road using road surveys, before and after the installation of the barriers. The carcasses were removed from the road to avoid counting the same dead amphibian twice. Live amphibians were translocated to the side of the road, according to their walking direction. All surveys were done at night, with temperatures above 10ºC and on rainy days or when the relative humidity was above 80%. The control sector (200 m long) was considered a stretch of road without the influence of any barriers, located 200 m apart from each barrier (Fig. 1). In each sampled night, we surveyed all studied sectors and so the sampling effort was homogenous for all sectors. We always started sampling in the temporary barrier sector, moved to the control sector and finished with the permanent barrier sector.

In the years 2015, 2016, 2017, 2018, and 2019, data were collected during the LIFE LINES project. These data resulted from single surveys per night through the road that were conducted at 20 km/h during the autumn and winter. In the subsequent years, 2020 and 2021, data were collected in road surveys with two surveys per night, separated by 20 minutes, at a speed of 10 km/h. This speed is slower than in most studies (e.g. GARRIGA *et al*., 2017; CANAL *et al*., 2018), which was possible due to the small size and low traffic intensity of the sampled road sectors and contributes to improve amphibian detection. The samplings between 2020 and 2021 were done during the four seasons. It is important to notice that these two last years of the study were affected by restrictions linked to the COVID-19 pandemics, which led to a decrease in traffic intensity, and consequently to a reduction of amphibians' roadkill (LECLAIR *et al*., 2021).

Road surveys were done in January (n = 2), February (n = 7), March (n = 6), April (n = 13), May (n = 6), June (n = 1), September (n = 1), October (n = 15), November (n = 25) and December (n = 5). Of the total number of road surveys, one was conducted in summer, 44 in autumn, 16 in winter and the remaining 20 were conducted in spring.

Statistical analyses

For each sampling and road sector, we calculated an index of abundance by dividing the number of observed amphibians in each night by the length of each sector. The abundance indexes (square root transformation of the number of amphibians per night per kilometre) corresponding to each barrier sector were compared with that of the control sector with a negative binomial generalized linear mixed model (GLMM)

Table 1: Number of alive (top numbers) and dead (bottom numbers) individuals per species at each sampled sector and period. Although control sector is the same for both barriers, it is shown twice because the installation of each barrier was not simultaneous (see text for details). The length of each sector and the number of samplings (N) are indicated. Ac: *Alytes cisternasii*; Bs: *Bufo spinosus*; Dg: *Discoglossus galganoi*; Ec: *Epidalea calamita*; Hm: *Hyla meridionalis*; Lb: *Lissotriton boscai*; Pc: *Pelobates cultripes*; Pi: *Pelodyctes ibericus*; Pp: *Pelophylax perezi*; Pw: *Pleurodeles waltl*; Ss: *Salamandra salamandra*; Tp: *Triturus pygmaeus*.

Mitigation type	**Sector**	**Period**	**N**	**Species**												**Total**
				Ac	**Bs**	**Dg**	**Ec**	**Hm**	**Lb**	**Pc**	**Pi**	**Pp**	**Pw**	**Ss**	**Tp**	
Permanent barrier	Control—no barrier (200 m)	Before installation	25	0	2	2	2	0	0	3	0	1	0	1	4	15
				0	0	0	0	1	0	0	0	0	0	1	1	3
		After installation	56	0	4	15	161	3	5	3	1	15	4	17	93	321
				0	2	15	171	0	1	1	0	16	4	6	24	240
	Permanent barrier (800 m)	Before installation	25	0	2	5	40	0	4	1	0	4	0	19	7	82
				0	2	2	16	0	0	0	0	0	0	7	1	28
		After Installation	56	2	5	8	45	1	6	3	0	25	2	24	31	152
				0	1	2	86	0	1	1	1	10	0	13	0	123
Temporary barrier	Control—no barrier (200 m)	Before installation	31	0	2	6	121	0	0	4	0	6	2	1	6	148
				0	2	5	146	1	0	0	0	3	2	1	1	161
		After installation	50	0	4	13	42	3	5	2	1	10	2	17	93	188
				0	0	11	25	0	1	1	0	13	2	6	22	82
	Temporary barrier (60 m)	Before installation	31	0	3	0	49	0	1	1	0	2	0	0	1	57
				0	1	0	66	0	1	0	0	1	0	0	0	69
		After installation	50	0	0	1	94	0	0	2	0	1	0	0	3	101
				0	0	1	30	0	2	0	0	2	0	2	1	38

using the Restricted Maximum Likelihood (RML), similar to the approach of MARKLE *et al.* (2017). We used this method because the response variable did not fulfil the parametric assumption of normality and homoscedasticity and there was not any suitable non-parametric method alternative to multifactorial ANOVA. Additionally, the GLMM is suitable for repeated samples, which is the case of this study. In the GLMM models we included 'Sector' (control and permanent barrier or temporary barrier) and 'Period' (before and after barrier installation) as fixed effects, and 'Day' (the day when each road survey was conducted), 'Observers' (the person/persons who collected the data) and 'Sample' (LIFE LINES and non-LIFE LINES) as random effects. These random effects were included to minimize the different speed of the road surveys, the different detection probabilities caused by different observers and the possible temporal effect of each sampling day on amphibian abundance. For each barrier, one GLMM was implemented. The best model was obtained by the combination of the random effects and was selected using the Akaike Information Criterion (AIC). Posterior to the GLMMs, we conducted Tukey tests to identify which interactions between 'Sector' and 'Period' were significant. Statistical analyses were performed within the R version 4.2.2.

RESULTS

We recorded a total of 1229 amphibians (dead and alive) (Table 1) from a total of 81 road surveys in the three road sectors between 2015 and 2021. Of the existent species in the area, only one (*Hyla molleri*) was not recorded. The natterjack toad, *Epidalea calamita*, was the most recorded species (760) while the least recorded ones were *Hyla meridionalis* (5) and *Pelodytes ibericus* (4) (Table 1).

The best fitted model for the temporary barrier was the one that included 'Day' and 'Observers' as random effects (Table 2). In the temporary barrier sector, no significant changes in the abundance of amphibians on the road were observed after the installation of the barrier ($P = 0.6997$). The presence of the temporary barrier reduced the number of amphibians on the road (Fig. 3) but the interaction between sector and period was not significant (Table 3). Also, there were no significant differences for the variables sector and period (Table 3).

For the permanent barrier, the best fitted model only included 'Day' as random effect (Table 2). There was a decrease of 72% (8.5 amphibians per night per km) in the average amphibian abundance on the road on the permanent barrier sector compared to the control one ($P < 0.001$). The model for the permanent barrier revealed a significant interaction between sector and period (Table 3). The result of the Tukey tests for this interaction showed a significant increase in amphibian abundance in the control sector after the installation of the barrier compared to the same sector before the barrier was installed or compared to the barrier sector either before or after the barrier installation (Fig. 4). In this model, the variable period was also

Table 2: Selection of the best-fit models, based on the Akaike Information Criterion (AIC), to explain variations in the number of amphibians per night and road kilometre associated with each mitigation option (installation of either a temporary or a permanent one-side barrier). Selected models are indicated with bold characters.

Mitigation type	Model	AIC
Temporary barrier	Sector * Period + (1∣Day) + (1∣Observers) + (1∣Sample)	1297.6
	Sector * Period + (1∣Day) + (1∣Observers)	**1295.6**
	Sector * Period + (1∣Day)	1304.1
Permanent barrier	Sector * Period + (1∣Day) + (1∣Observers) + (1∣Sample)	1103.1
	Sector * Period + (1∣Day) + (1∣Observers)	1113.6
	Sector * Period + (1∣Day)	**1099.1**

Table 3: Results of the selected GLMM to explain variations in the number of amphibians per night and road kilometre associated with each mitigation option (see Table 2 for details on model selection).

Model	Model term	Fixed effects				Random effects	
		Estimate	**SE**	**Z**	**P**	**Variance**	**SD**
Temporary barrier	Sector	0.08301	0.45812	0.181	0.856		
	Period	0.67484	0.60620	1.113	0.266		
	Sector x Period	-0.29505	0.59059	-0.500	0.617		
	Day					0.3662	0.6051
	Observers					1.1979	1.0945
Permanent barrier model	Sector	0.5746	0.3820	1.504	0.1325		
	Period	2.4271	0.3786	6.411	**1.44e-10**		
	Sector x Period	-2.4372	0.4486	-5.432	**5.57e-08**		
	Day					0.6551	0.8094

significant when considered without the interaction with the variable sector (Table 3).

Discussion

Our study is the first one to demonstrate the effectiveness of one-side barriers in reducing amphibians' roadkill, showing how the number of amphibians on the road was reduced because of the installation of the permanent one-side barrier. Research about mitigation of amphibian roadkill indicates that the combination of different mitigation measures tends to reduce roadkill frequency by about 40%; however, when only the mitigation barriers are considered, the effectiveness increases up to 54% (Rytwinski *et al.*, 2016). Nevertheless, most of the studies only consider two-side barriers, which in the case of amphibians can reduce the road-kills between 40 and 100%, depending on the type of barrier (temporary or permanent) (Dodd *et al.*, 2004; Cunnington *et al.*, 2014; Helldin & Petrovan, 2019). In one of the few studies that evaluate one-side barriers, Markle *et al.* (2017) showed that these barriers did not reduce the number of road-killed reptiles. This difference between taxa in effectiveness might be explained by the higher desiccation risk of amphibians compared to reptiles, which does not allow them to cover the same distances as reptiles (Russell *et al.*, 2005). Additionally, some of the barrier's features, such as their length and presence of underpasses, may contribute to this difference in effectiveness between amphibians and reptiles.

The presence of the temporary barrier did not reduce the number of amphibians on the road compared to the control (no barrier) sector. Although most studies focus on the permanent barriers, we have identified some explanations for the low effectiveness of temporary barriers. The first one is that these barriers are easily overpassed; usually the construction material of these barriers is impermeable plastic, easily climbed by amphibians that possess adhesive disks or those whose abdo-

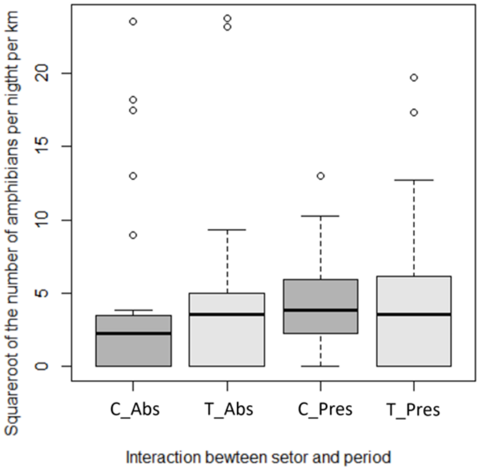

Figure 3: Median (black lines), inter-quartile range (boxes) and 95% confidence interval (external bars) of the square root of the number of amphibians per night per km for each road sector (C: control, T: temporary barrier, P: permanent barrier) and period (Abs: absence of the barrier, Pres: presence of the barrier). Open circles represent outlying data.

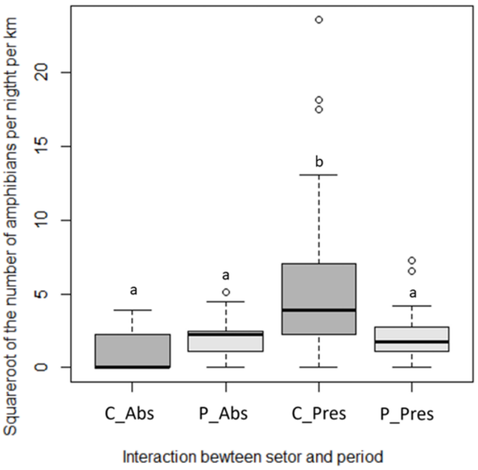

Figure 4: Median (black lines), inter-quartile range (boxes) and 95% confidence interval (external bars) of the square root of the number of amphibians per night per km for each road sector (C: control, P: permanent barrier) and period (Abs: absence of the barrier, Pres: presence of the barrier). Open circles represent outlying data. Different lower-case letter indicate different treatments at $P < 0.05$ level, as indicated by Tukey tests.

men adheres to the substrate, which are the cases of *Hyla* spp. or *Triturus pygmaeus,* respectively (ARESCO, 2005; SMITH *et al.*, 2006; SCHMIDT & ZUMBACH, 2008). The second possible explanation is that some amphibian species can either climb through the vegetation (HAMER *et al.*, 2015) or go under the barrier. The lower part of the barrier is often not properly buried in the ground, which allows amphibian to cross behind it (HAMER *et al.*, 2015). Since it is not common to perform a frequent maintenance of temporary barriers, the vegetation overgrowth may particularly affect their effectiveness. The third explanation would be that, due to its short size, the temporary barrier could be easily surrounded, leading to an increase of amphibians in the fence end; likewise, some of the amphibians crossing at the fence end could eventually enter the mitigated section, hence affecting the temporary barrier effectiveness (HELLDIN & PETROVAN, 2019). Finally, a last explanation could be the presence of tarp tears in the temporary barrier through which amphibians could cross (HAMER *et al.*, 2015).

The observed differences in the amphibian abundance in the control sector between before and after the installation of the barriers would suggest that amphibian population sizes were different between these two periods. The fluctuations of amphibian populations are a welldocumented phenomenon (see Green, 2005). This result would highlight the importance of BACI (Before-After-Control-Impact) studies, which compare the impacted site with the control site before and after the impact itself. This kind of analysis allows to identify if the differences are related to the effectiveness of the barriers or to natural fluctuations of populations (van der Grift *et al.*, 2015). The differences between before and after the installation of the barrier in the control sector illustrate an increase in the population size. The possible reasons for this increase may be related to climatic variables and go beyond the scope of this study.

The model for the permanent barrier had differences between the control and permanent barrier after its installation, so the presence of the barrier decreased the total abundance of amphibians on the road. The described effectiveness for permanent two-side barriers ranges between 63 and 100% (e.g. Dodd *et al.*, 2004; Helldin & Petrovan, 2019), which places the effectiveness of the permanent one-side barrier analysed in our study (72%) within the range of those permanent two-side barriers already studied. In the study by Dodd *et al.* (2004), the effectiveness of the permanent two-side barrier increased from 65% to 93% when the data from the species of the genus *Hyla* were removed. In our study, the absence of records *Hyla molleri* and the few records of *Hyla meridionalis* (five individuals) show that the observed effectiveness was not affected by the presence of individuals from this genus.

Some studies have found mass mortality events for juveniles in road segments without barriers (Petrovan & Schmidt, 2019). In the case of one-side barriers, the same might happen, particularly for amphibians that come from the side that has no barrier. Nevertheless, we believe that in our study this did not happen, in part because the low speed used in the road surveys allowed us to detect small sized juveniles (down to 2-3 cm). In addition, the fact that we sampled (in 2020 and 2021) during the four seasons, whenever the climatic conditions were suitable to amphibians, allowed us to cover possible juvenile dispersal events outside the conventional season (spring and autumn). Finally, it is known that high traffic intensity reduces the persistence of carcasses of small animals on the roads (Santos *et al.* 2011); however, the road sampled in the present study has a low traffic intensity (maximum of five vehicles per hour), hence the duration of juvenile carcasses might be high enough to not overlook their carcasses because of an eventual quick disappearance.

Although the presence of barriers might reduce the number of road-killed amphibians (Cunnington *et al.*, 2014; Rytwinski *et al.*, 2016), the barriers themselves may compromise amphibian movement and therefore their reproduction, dispersal and migrations (Schmidt & Zumbach, 2008; Colino-Rabanal & Lizana, 2012). To allow amphibian movement, at the time of the barrier construction specific underpasses for amphibians are installed, which adds to drainage underpasses that can also be used by animals (Colino-Rabanal & Lizana, 2012). These underpasses have an important role in ensuring the maintenance of amphibian life cycles when barriers are installed. We did not collect data on amphibian usage of underpasses for two reasons. First, during the LIFE LINES survey, the team did not collect that information. This is particularly important since the drainage underpasses already existed prior to the barrier installation, and some of the amphibians might have already gone through them. Therefore, we had no way to compare the use of the underpasses before and after the barrier installation. Second, it was very frequent that there was too much vegetation on the entries of the underpasses, which did not allow to get data on their use (see Fig. 2b).

Our results must be interpreted with caution since we had some methodological limitations. First, this study has several geographical constraints since only 2 km of road were sampled and only one replicate per barrier type was used, which may limit generalization to all one-side barriers. Second, the use of two different data collection methods (LIFE LINES data and data collected by us), particularly the different survey speed and number of surveys per night, may affect the results, specifically when comparing before and after the installation of barriers. Finally, the LIFE LINES road surveys were conducted during autumn and winter only, which could have caused the spring migration and the juvenile dispersal to have been missed. All these uncertainties may reflect in our data and mean that the permanent one-side barrier may not have the high observed effectiveness. On the other hand, we used long-term monitoring data (seven years), which allowed us to identify if the observed changes were linked to fluctuations in population. We found that one-side permanent barriers appeared to reduce the number of amphibians on the road, and consequently the number of road-killed amphibians. Despite that, we believe that, whenever possible, two-side barriers should be installed because one-side barriers leave one side of the road with open access to amphibians. Due to the lower effectiveness compared to the two-sided barriers and to the study constrains, one-side barriers should only be installed to preserve amphibian biodiversity in cases of funding shortage or land use constraints. This study is the first evidence of the effectiveness of one-side barriers to reduce road-killed amphibians; however, additional studies designed to deal with the limitations identified above should be conducted to confirm their effectiveness.

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