Road ecology and Neotropical amphibians: contributions for future studies

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Abstract. Many species of amphibians have suffered serious population declines. Several factors contribute separately or jointly to these declines. However, the reduction of an available habitat due to human expansion is still the main cause, and roads are a major mean for this expansion. Both the construction phase and the subsequent use of roads have negative consequences for amphibians. We reviewed the literature on the subject within the Neotropical context. To this end, the paper begins with a summary of recent reviews and proceeds through an analysis of sampling methods used in roadkill studies, mitigation measures and the Neotropical scenario and concludes with several suggestions to guide future studies. More attention will be given to roadkills, which is one of the primary impacts on wildlife that is caused by roads. Even in the Neotropical zone most studies are foot-based, the richness and abundance of amphibians affected are higher in regions outside the Neotropics. One possible explanation is that in the other regions, the proportion of studies exclusively on amphibians is bigger. Regarding mitigation measures, most studies only indicates what should be used, but do not implement or evaluate their effectiveness.

Keywords. Neotropical amphibians, roads, roadkill, mitigation, review.

INTRODUCTION

Currently, amphibians have attracted the attention of researchers and conservationists due to population declines that have been recorded worldwide (43% of species), with 168 species being considered extinct (Global Amphibian Assessment, 2012), the highest values than any other group of vertebrates (Stuart et al., 2004). Several factors appear to contribute separately or jointly to these declines, such as fragmentation, loss or destruction of habitat, diseases such as chytridiomycosis, overexploitation, reduction of the ozone layer and acidic rain (Weygoldt, 1989; Young et al., 2001; Eterovick et al., 2005). The primary cause, however, appears to be the reduction of suitable habitats due to an expansion of human activities on natural areas, which is largely enhanced by roads (Glista et al., 2008). There are several recent reviews on the environmental impacts of roads (e.g. Fahrig and Rytwinski, 2009; Rytwinski and Fahrig, 2012) and how they affect populations and communities of amphibians (e.g. Colino-Rabanal and Lizana, 2012; Beebee, 2013). However, there is no study with emphasis on Neotropical proposing the standardization of methods that facilitate future comparisons between different studies. The Neotropical region includes countries with the largest absolute richness of amphibians and with the largest number of endangered species in the world (Global Amphibian Assessment, 2012). Many of the measures that would help reducing the impact of roads on amphibians can be used for other animal groups and geographic regions.

Both the construction phase and the subsequent use of the roads have negative consequences for amphib-

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ians. Several indirect effects can be mentioned: chemical pollution, for example, the application of salt to remove ice (Denoël et al., 2010), sound pollution with the traffic noise interfering with male vocalization near roads (Hoskin and Goosem, 2010), light pollution that is known to mainly promote instant immobilization, which makes amphibians more susceptible to vehicle collisions when crossing roads (Mazerolle et al., 2005) and genetic effetcs, with roads reducing gene flow and decreasing genetic diversity in amphibians (Lesbarrères et al., 2003). However, death caused by vehicle-wildlife collisions is considered to be the greatest cause of non-natural deaths of vertebrates (Forman and Alexander, 1998), and amphibians are the most frequently killed vertebrates on roads (Puky, 2004).

Even though vehicle-animals collisions are considered one of the greatest impacts, the road mortality rates still is underestimated, since most studies do not account the carcass removal and detection rate. Properly defining road mortality rates is important to identify road stretches where concentrating mitigation measures and to determine the effectiveness of these measures (Teixeira et al., 2013). In the Neotropics there are no studies on the implementation of mitigation measures, or assessing their effectiveness to amphibians.

In this context, the present paper aims to: i) compare the methods that have been used to evaluate roadkills impacts on neotropical amphibians with studies from other regions, and ii) assess what mitigation measures are the most frequently advocated by researchers.

MATERIAL AND METHODS

We conducted a literature review using eight major databases (Isi Web of Knowledge, Scopus, Elsevier, JSTOR, ScienceDirect, SpringerLink, Wiley Inter Science and Scielo). We also consulted the references identified in the articles resulting from the database search. For the search, we used the following keywords in various combinations: road, amphibians, vertebrates, roadkill, road effect, road impact, road mortality and mitigation measures.

For the comparison of methods were selected only amphibian roadkill studies, regardless of criteria. Possible problems in the methodology were identified and discussed. For the analysis of mitigation measures we selected studies with some mitigation proposals or studies that tested the measures for amphibians.

The number of species of the neotropical zone and other regions was compared by Mann-Whitney test, since sample normality was rejected by Shapiro-Wilk test ($P < 0.05$), as well as the traffic intensity in both regions. The relationship between the number of vehicles per day on the roads and the number of individuals roadkilled was tested by simple linear regression (only studies that have provided this information). The proportion of studies that has provided the information or used given method was compared between Neotropics and other regions by chi-2 test. The parameters used in this test were number of studies exclusively about amphibians, survey methods, paving, sampling period, landscape description, sampling time and roadkill rates. For all tests the level of significance was 0.05.

RESULTS

Methodological Analysis

In the Neotropics we found 19 studies, being 12 in Brazil (63.2%), three in Mexico (15.8%), two in Argentina (10.5%) and two in Colombia (10.5%). For the studies conducted outside of Neotropical zone (N = 26), 13 (50%) were performed in the United States, nine (34.6%) in Europe, two (7.7%) in Australia, two (7.7%) in Canada, one (2.6%) in China, and one (2.6%) in Turkey. The parameters studied are described in Table 1.

The number of amphibian species affected was higher in studies conducted in other regions than in the Neotropics ($U = 115.5$, $Z(U) = 2.25$, $P = 0.023$). The average number of individuals roadkilled was about 10 times greater in studies conducted outside of Neotropical zone. There was no relationship between the number of individuals roadkilled and the number of vehicles per day on the roads, both for studies of neotropical zone ($F_{1,6} = 0.32$, $R^2 = 0.05$ $P = 0.595$) as for the other regions ($F_{1,6} = 3.19$, $R^2 = 0.34$, $P = 0.122$) and for the regions pooled together ($F_{1,14} = 0.0003$, $R^2 = 0$ $P = 0.987$). There was no difference in traffic volume between Neotropics and the other regions either ($U = 18$, $Z(U) = 1.91$, $P = 0.056$).

The proportion of studies that exclusively surveyed amphibians in the neotropics (21%) was lower than other regions (55.2%, $\chi^2 = 15.35$, df = 1, $P < 0.001$). The species most affected by roadkills in the Neotropics are represented by the genera *Leptodactylus* and *Rhinella*.

The usual survey methods used in the studies inside and outside of Neotropical zone differ. The proportion of studies using patrolling by car was different ($\chi^2 = 11.87$, df= 1, $P < 0.001$); outside Neotropical zone approximately 42% were conducted by car, while in the Neotropics were 15.8%. Foot-based counts showed similar proportions ($\chi^2 = 0.016$, df= 1, $P = 0.991$), 36.8% for the Neotropics e 37.9% for the other regions. Paved roads were the major target of these studies ($\chi^2 = 0.223$, df= 1, $P = 0.691$), both in the Neotropics (92%) and outside (85.7%).

The sampling period usually encompassed more than one season in both regions ($\chi^2 = 1.10$, df = 1, $P = 0.335$) and the proportion of studies was 61.5% in the Neotropical zone and 73.7% in the other regions. Furthermore, the percentage of Neotropical studies (36.8%) that performed a detailed description of the studied area was

Table 1. Information available in studies of road ecology conducted inside and outside of Neotropics, including amphibians, but not exclusively.

	Reference	Country	Total distance sampled (Km)	Paving	Traffic intensity (number of vehicles per day)	Sampling methods	Sampling duration (days)	Amphibian richness	Amphibian abundance	Sampling time	Period (different seasons= df, point sampling= ps*)	Landscape description	Studies exclusively about amphibians	Road-kill rate (number of roadkill amphibians per km)
OUTSIDE NEOTROPICS	Aresco, 2005	USA	0.7	paved	21500	on foot	1315	12	840	-	df	-	-	0.87
	Carvalho and Mira, 2011	Portugal	26	paved	4957.5	car	52	14	1093	-	df	yes	-	0.80
	Clevenger et al., 2003	Canada	117	paved	19115	car	-	2	48	morning	df	-	-	7.355 e-4
	Coleman et al., 2008	USA	14.8	unpaved	-	car	104	5	58	morning and evening	df	-	-	0.04
	Dodd Jr et al., 2004	USA	3.2	paved	11000	on foot	72	8	1647	all day	df	yes	-	7.14
	Estes-Zumpf et al., 2010	USA	8	-	-	on foot	-	4	20	-	df	yes	-	-
	Fahrig et al., 1995	Canada	84.3	paved	8500	on foot	6	-	1856	evening	ps	yes	yes	3.67
	Gibbs and Shriver, 2005	USA	1	-	-	on foot	6	1	66	-	ps	-	yes	11
	Glista et al., 2008	USA	12	-	-	car	124	9	9809	-	df	yes	yes	6.59
	Gryz and Krauze, 2008	Poland	2.51	paved	35	on foot	51	5	1046	morning	df	-	-	81.7
	Goldingay and Taylor 2006	Australia	0.2	-	-	on foot	13	8	1029	morning	ps	-	yes	395.8
	Gu et al., 2011	China	11	-	-	on foot	60	3	494	morning	ps	yes	yes	21.95
	Hartel et al., 2009	Romenia	83.3	-	-	car	26	2	1631	evening	ps	-	yes	0.75
	Hoskin and Goosem, 2010	Australia	0.4	paved	6950	on foot	-	7	-	evening and morning	df	yes	yes	-
	Kobylarz, 2001	USA	4	paved + unpaved	-	on foot	10	10	185	evening	ps	-	yes	4.62
	Langen et al., 2007	USA	355	paved	7209	on foot + car	96	10	-	morning and evening	ps	-	-	19.33
	Langen et al., 2009	USA	438.4	-	-	on foot	10	10	394	morning	ps	yes	-	0.9
	Mazerolle, 2004	Canada	20	paved	18	car	37	10	3657	-	df	-	yes	4.9
	Meek, 2012	França	10	paved	-	bicycle	360	7	539	afternoon	df	-	yes	-
	Orlowski, 2007	Poland	48.8	-	-	car	172	1	957	afternoon	df	yes	yes	-
	Orlowski et al., 2008	Poland	52.3	-	5545	on foot + car	216	10	3742	morning and evening	df	yes	yes	82.69
	Santos et al., 2007	Spain	28	-	-	car	3	3	115	-	ps	yes	yes	-
	Shwiff et al., 2007	USA	10	paved	-	car	1095	3	39	morning	df	-	-	0.003
	Sillero et al., 2008	Spain	-	-	-	car	17	14	312	evening	ps	-	yes	0.23
	Smith and Dodd Jr, 2003	USA	3.2	paved	-	on foot	156	7	833	-	df	-	-	1.67
	Tok et al., 2011	Turkey	144	-	-	car	-	8	82	-	df	-	-	0.004
	Mean ± standard deviation		59.12±109.4		8482±7124.9		181.9±343.9	7±4.2	1276±2036.1					30.7±87.05

(Continued)

Table 1. Continued.

	Reference	Country	Total distance sampled (Km)	Paving	Traffic intensity (number of vehicles per day)	Sampling methods	Sampling duration (days)	Amphibian richness	Amphibian abundance	Sampling time	Period (different seasons= df, point sampling= ps*)	Landscape description	Studies exclusively about amphibians	Road-kill rate (number of roadkill amphibians per km)
Neotropics	Andrade and Moura, 2011	Brazil	4.5	paved + unpaved	-	car	-	6	20	-	df	-	yes	-
	Attademo et al., 2011	Argentina	6	paved	6025	on foot	30	7	352	morning	df	-	-	1.63
	Cairo and Zalba, 2007	Argentina	2	paved	1200	on foot	-	1	25	afternoon	df	yes	yes	-
	Coelho et al., 2012	Brazil	4.41	paved	2132	on foot	16	13	1433	morning	df	yes	yes	20.3
	Gonzalez-Gallina et al., 2013	México	112	paved	64	on foot + car	34	1	1	afternoon	df	yes	-	0
	Grosselet et al., 2008	México	1.2	-	-	bicycle	36	2	100	morning and afternoon	ps	-	-	2.31
	Hengemühle and Cademartori, 2008	Brazil	12	paved	-	on foot	21	-	24	-	df	-	-	0.006
	Melo and Santos-Filho, 2007	Brazil	63	paved	-	car	25	4	11	morning	df	yes	-	0.006
	Milli and Passamani, 2006	Brazil	28	paved	-	motorcycle	70	2	4	morning	ps	-	-	0.002
	Morales-Mávil et al., 1997	México	8	paved	-	on foot	515	9	139	-	df	yes	-	0.03
	Prado et al., 2006	Brazil	19.2	-	-	car	12	-	11	morning	df	-	-	0.04
	Quintéro-Ángel et al., 2012	Colômbia	6.4	paved	1968	on foot	153	2	7	evening	df	yes	-	0.007
	Santana, 2012	Brazil	400	-	-	motorcycle	12	5	39	morning	df	yes	-	0.04
	Santos et al., 2012	Brazil	13	-	250	bicycle	36	3	3	morning	df	-	-	0.006
	Silva et al., 2007	Brazil	28	-	60	on foot + car + bicycle	120	9	51	morning and evening	df	-	-	0.003
	Silva et al., 2011	Brazil	35	paved	-	motorcycle	32	-	103	-	ps	yes	yes	0.09
	Souza et al., 2010	Brazil	4.45	paved	3650	-	2190	1	1	morning	df	-	-	-
	Turci and Bernarde, 2009	Brazil	110	-	-	motorcycle	30	2	68	-	ps	-	-	0.02
	Vargas-Salinas et al., 2011	Colombia	96	-	-	on foot	40	1	5	-	ps	-	-	0.05
	Mean ± standard deviation		50.2±92.4		1918.5±2073.7		198.3±527.1	4.3±3.6	126±327.1					1.53±5.14

* Sampling performed in a given period, not including more than one season.
- = information was not available in the study cited.

similar to studies from outside (about 42.3%, $\chi^2 = 0.38$, df = 1, P = 0.613). In most studies sampling was performed during morning ($\chi^2 = 2.38$, df = 1, P = 0.145), both for the Neotropics (77%) and for outside (59%).

The calculation of roadkill rates of amphibians in the Neotropics was uncommon and was only performed by four studies (21%), as well as in the other regions (nine studies; 34.5%, $\chi^2 = 3.28$, df = 1, P = 0.093).

Mitigation Measures

Out of the 22 studies analyzed, only three evaluated the effectiveness of proposed mitigation measures and two tested the preferences for specific attributes of crossing structures. Among these studies, none is located in the Neotropics. All studies that tested the efficiency, considered that the measure is effective to reduce roadkill. A large majority of studies performed in the Neotropics (77%) only indicated measures without testing them. Wildlife crossings (amphibian tunnels and culverts) associated with drift fences or barrier walls are among the most adopted and frequently advocated mitigation measures (Table 2).

DISCUSSION

Methodological Analysis

The greatest richness of amphibians in the world, which is found in the Neotropics, mainly Brazil (Segalla et al., 2012), is not represented in the studies on the Neotropical species killed by traffic. The expectation that this low number of species and individuals could be the

Table 2. Studies that have proposed or analyzed the effectiveness of mitigation measures.

	Reference	Country	Mitigation measure(s)	Monitoring duration	Was effectiveness tested?	Are measures effective?	Was the mitigation only proposed?
Other regions	Aresco, 2005	USA	Temporary drift fence + drainage culvert	2.5 years	yes	yes	no
	Clevenger et al., 2003	Canada	Culverts	-	no	-	yes
	Dodd Jr et al., 2004	USA	Barrier wall + culvert	2 years	yes	yes	no
	Fahrig et al., 1995	Canada	Barrier wall + underpasses	-	no	-	yes
	Gibbs and Shriver., 2005	USA	Barrier wall, tunnels, transporting individuals	-	no	-	yes
	Gu et al., 2011	China	Underpasses and traffic control measures	-	no	-	yes
	Hoskin and Goosem, 2010	Australia	Bridges, concrete barriers	-	no	-	yes
	Kobylarz, 2001	USA	Barriers, underpasses, traffic signs	-	no	-	yes
	Lesbarrères et al., 2004	França	Amphibian tunnels	1 month	*	-	no
	Lesbarrères et al., 2010	França	Habitat replacement	4 years	yes	yes	no
	Mata et al., 2005	Espanha	Culverts, underpasses and overpasses	2 months	no	-	no
	Patrick et al., 2010	USA	drift fence + culvert	3 months	no	-	no
	Woltz et al., 2004	USA	Several road crossing structures	2 months	*	-	no
Neotropical Zone	Andrade and Moura, 2011	Brazil	Drift fence + culvert	-	no	-	yes
	Attademo et al., 2011	Argentina	Underpasses or culverts + barrier wall	-	no	-	yes
	Cairo and Zalba, 2007	Argentina	Underpasses	-	no	-	yes
	Coelho et al., 2012	Brazil	Passages + drift and barrier fences	-	no	-	yes
	González-Gallina et al., 2013	México	Drainage culverts and overpasses	-	no	-	yes
	Hengemühle and Cademartori, 2008	Brazil	Traffic signs, speed bumps, passages, Barrier wall	-	no	-	yes
	Melo and Santos-Filho, 2007	Brazil	Traffic signs, speed bumps, fences and Tunnels	-	no	-	yes
	Santana, 2012	Brazil	Unspecified	-	no	-	yes
	Souza et al., 2010	Brazil	Traffic signs, speed bumps, passages, Barrier wall	-	no	-	yes

* Studies that tested the preferences for specific attributes of crossing structures.
- = information was not available in the study cited.

result of a lower number of vehicles traveling per day on roads in the neotropics was not confirmed, because the traffic volume in the neotropics and other regions was equal. However, one of the major characteristics of roads that have an influence on the mortality of amphibians and also of other animals is the volume of traffic. A higher number of vehicles traveling on the roads per day increases the chance of small animals such as amphibians to be roadkill (Fahrig et al., 1995; Hels and Buchwald, 2001). The low number of roadkilled amphibians in the Neotropics may also be due to samplings that included wild vertebrates in general, that is, studies not specific for quantifying roadkilled amphibians. In these studies amphibians are considered the most underestimated group (Milli and Passamani, 2006; Coelho et al., 2008; Hengemühle and Cademartori, 2008; Souza et al., 2010; Attademo et al., 2011; Santos et al., 2012; González-Gallina et al., 2013). The inclusion of more than one group of vertebrates in the survey objective is not often accompanied by people trained to identify all animal groups, which skews the results.

Although most Neotropics studies have been conducted on foot, the amphibian richness affected is higher outside Neotropical zone. Monitoring performed at lower speeds, such as on foot or by bicycle, facilitates the finding of small specimens (Silva et al., 2007; Hengemühle and Cademartori, 2008). At higher speed, the visualization is limited. For example, Langen et al. (2007) showed that the number of animals detected by on foot surveys was approximately 50 times higher than a survey made by car. Moreover, the carcasses of small animals do not remain on the road for long, either due to predators or because they deteriorate faster (Slater, 2002; Taylor and Goldingay, 2004; Antworth et al., 2005).

Certain behaviors of some amphibian species also affect the roadkill rates. These animals are mostly active during rainy periods and/or after rainfalls, while the ground is still wet (Cairo and Zalba, 2007). However, activity peak may be different among species. For example, in a study conducted in Denmark, *Rana temporaria* and *R. arvalis* were active soon after sunset, while *Bufo bufo* was active between 10 and 11 p.m. (Hels and Buchwald, 2001). The speed and daytime movement patterns of species are important characteristics of vulnerability, as species that are slow and diurnal are more likely to be roadkilled are those (Cairo and Zalba, 2007). Nevertheless, the migration of amphibians occurs mainly at night, when traffic volume is reduced (approximately 80% of traffic volume occurs during the day; Festin, 1996). In the Neotropics only one study was made at night, this may have reduced detection rate.

Spatio-temporal factors can influence amphibian roadkills, Coelho et al. (2012) demonstrated a significant concentration of amphibian roadkills in summer and showed that variables such as average daily temperature, precipitation and photoperiod (variables strictly related to amphibian activities) were the most important factors related to the temporal distribution of roadkills of amphibians in general and of particular species, such as *L. latrans*, *R. icterica* and *Hypsiboas faber*. The spatial distribution of roadkills was not random. Amphibian mortality was related to types of land cover, distance from water bodies, artificial light and roadside ditches, which indicated the importance of local characteristics for the occurrence of mortality hotspots (Coelho et al., 2012).

With the increase of the impact of the roadkills and the need to understand the factors that influence them, more complex studies began to be developed in the Neotropics, which considered the influence of the sampling effort in estimating wildlife roadkills (Bager and Rosa, 2011) and the spatial-temporal approaches linked to these traffic-induced mortalities (Rosa and Bager, 2012; Coelho et al., 2012).

Mitigation Measures

There is consensus that a system including construction of tunnels and barriers is better to anurans, as it would allow all seasonal migrations, including those of juveniles (Jochimsen et al., 2004; Puky and Vogel, 2004; Puky, 2006; Andrews et al., 2008; Schmidt and Zumbach, 2008; Glista et al., 2009; Lesbarrères and Fahrig, 2012). The quantification of the animals that use these structures, not only by the simple counting of individuals but by methods that include capture-mark-recapture, can assist in the estimation of the real proportion of amphibian populations that benefit from this measure (Schmidt and Zumback, 2008). Hylids and other arboreal amphibians, however, rarely use these passages. Specific tests for these groups are rare and necessary, as well as alternative methods of passing as rope bridges used by primates and other arboreal mammals (Weston et al., 2011).

Although many of the problems can be related to inappropriate design or the lack of planning for these structures (Puky and Vogel, 2004; Andrews et al., 2008; Lesbarrères and Fahrig, 2012), perhaps the lack of scientific rigor and in the efficiency assessment is one of the major causes (Lesbarrères and Fahrig, 2012). Additional issues are related to wildlife crossings, such as studies without replications, lack of information between pre- and post-construction and relatively short monitoring periods.

Studies testing the effectiveness of given mitigation methods is essential for planning strategies for the most affected taxa. Some of these studies can also increase the ability to anticipate and prevent large number of roadkills, such as those using collision models applied them for mitigation projects (Gunson et al., 2011). Nevertheless, the implementation of wildlife crossings does not always benefit the amphibian populations. When the implementation of mitigation measure is in roadkill hotspots, must be directed to safe crossing sites by extensive barrier fencing. However, tunnels may focus predation on high local concentrations of individuals. Occasional mass mortality by flooding or oil seepage is also possible. Tunnel and fence systems also require continuous maintenance to work well, and this is often not sustainable (Allaback and Laabs, 2003, Beebee, 2013).

Another point to be considered in the determination of mitigation measures is that amphibians have some preference regarding the design features of the tunnels. For instance *Rana esculenta*, *R. dalmatina*, *R. pipiens* and *R. clamitans* prefer tunnels with specific type of substrate, for example, soil (Lesbarrères et al., 2004; Woltz et al., 2008), that are brighter and have larger diameter openings (Woltz et al., 2008). As for the tunnel length, *R. pipiens* and *R. clamitans* prefer tunnels that are 6.1-9.1 m in length (Woltz et al., 2008).

Alternative measures that have worked well for amphibians include the temporary closure of roads, usually at night. Although amphibian death on the roads could occur before the daily closure time, this method appears to be efficient because it protects different age structures and life stages within the population (Schmidt and Zumback, 2008), and can be reasonably applied, for example, to roads that cut through protected areas. However, in many situations, roads are not closed to traffic because people are not willing to use alternative routes (Jochimsen et al., 2004; Schmidt and Zumback, 2008).

The Road Ahead

Regardless of a long- or short-term sampling, studies demonstrate the high amphibian mortality among vertebrates (Fahrig et al., 1995; Hels and Buchwald, 2001; Smith and Dodd Jr, 2003; Semlitsch et al., 2007). However, as many contradictory studies are published due to differences in the sampling protocols, the definition of the methodology of amphibian roadkill studies must be supported by several points, certain of which are controllable, and others of which are not. Actual roadkills depend on variables that are not often considered in the studies, such as: features of the surrounding landscape, species traits (type and breeding season, population density, seasonal activity and movement speed), characteristics of the road (traffic intensity and number of lanes) (Balkenhol and Waits, 2009; Garcia-Gonzales et al., 2012; Santana, 2012) and even the intention of drivers to kill wild animals or certain species that are considered somehow less charismatic, such as the cane toad (*Rhinella marina*) (Beckmann and Shine, 2012).

However, variables that influence road mortality can be controlled to optimize the results. Among those variable are the following: type of sampling (on foot or by car), period of the day during which the data are collected and number of daily collections, survey of species in the vicinity of the road, sampling effort, training of the team (experience of the observers), detection and removal rates. That is, the result of the sampling will depend on the techniques used, on the experience and skills of those conducting the job of detecting and identifying the organisms, the time spent and the faunal composition in each location (Silveira et al., 2010). Therefore, defining a practical and accurate methodology to enable comparisons between different studies and, consequently, conclusions that can be more generalizable has a paramount importance. The use of appropriate methodology will enable an even more accurate location of priority areas for mitigation or areas that should be avoided in the planning of new roads (Lesbarrères and Fahrig, 2012).

Characteristics of the surroundings of the road can influence the roadkill rates, such as the occurrence of specific habitats and potential breeding areas (Malo et al., 2004). The composition, configuration and quality of water bodies (Findlay et al., 2001; Mazerolle and Desrochers, 2005; Orlowski, 2007) and also the presence of forested areas near the roads (Carvalho and Mira, 2011) are indicative of areas with high mortality rates for amphibians. In a study conducted in China, the mortality was higher for the stretches of road with a greater proportion of wet grasslands within a 1-km radius (Gu et al., 2011).

The comparison of amphibian roadkills is difficult because of the different methods used and conditions of the local population (Elzanowski et al., 2009). In general, samplings conducted on foot allow the detection of a greater number of live or dead species and also of small animals and juveniles compared to samplings performed by car (Taylor and Goldingay, 2004, Langen et al., 2007). The monitoring of the wildlife of the surroundings is essential because the effects of roads on different species are related to their biological, ecological and behavioral characteristics (Eigenbrod et al., 2008). In addition, there are many local factors (population density and landscape, among others) that can affect the richness and abundance of roadkilled species (Coffin, 2007). Therefore, a survey performed exclusively on the road should not be considered to be representative of the total area, precisely because many species avoid the roads. Moreover, there are several studies criticizing the use of roads as a method of species survey (Case, 1978; Enge and Wood, 2002;

Steen and Smith, 2006). However, this criticism does not imply that data collected on roads cannot be used to extrapolate the potential distribution of species recorded in the area (McCarthy et al., 2012).

The sampling effort of the study may also interfere in the quantification of roadkilled animals. As amphibians experience a high temporal variation in population, long-term studies are ideal to identify these fluctuations. However, studies of this magnitude are not always feasible, and there is also the need for a quick response. An alternative is to assess many areas with different degrees of impact (Fahrig et al., 1995). Amphibians also show seasonal variation of activity; the periods of greatest activity are the wettest months when reproduction peaks (Smith and Dodd Jr, 2003; Glista et al., 2008; Attademo et al., 2011). Clearly, roads near or crossing water bodies will exhibit a greater incidence of mortality (Langen et al., 2009). The ideal sampling effort should vary according to the size and heterogeneity of the area, and one of the methods to assess the effort is through rarefaction curves (Bager and Rosa, 2011).

A comparison of mortalities between roads is impractical if only raw data are considered due to differences in the characteristics of roads (e.g., extent) and sampling design (Rosa et al., 2012). An alternative that facilitates the comparison is to analyze roadkill rates (ratio between roadkill number and time or space unit) (Gummier-Costa and Speber, 2009; Turci and Bernarde, 2009). Moreover, the addition of factors determining observation error into population models increases the accuracy of results: the incorporation of sampling effort into analyses may improve the reliability of estimates (Bonardi et al., 2011).

Human resources directly affect the detection of roadkilled animals. The team must be properly trained, qualified and competent for carcass detection and species identification. The effectiveness of sampling will depend on the skill and experience of the team because trained personnel can find more species and individuals (Silveira et al., 2010). Another factor that should be taken into account is the position of the observer on the road. Samplings in which the team walks in all directions and lanes during all collections ensure a greater accuracy of the results.

If the detection and persistence rates are not considered, the number of individuals is underestimated. Carcass persistence on road can be affected by scavengers activity, weather (Slater, 2002) and traffic flow, whereas detectability can be influenced by carcass size (Morrison, 2002), amount of roadside vegetation, number of surveys, methodology used and skills and experience of the team involved in the census (Colino-Rabanal and Lizana, 2012).

Based on the abovementioned information, we could focus on the following points for reflection by taking into account the goal of obtaining a roadkill rate closer to the reality of the road effect and the development of a sampling protocol. The aim of this protocol is to improve the measurement of roadkill rate, allowing comparison between landscapes. We suggest the following:

i. Sampling performed on foot. If the area is too large, we suggest a combination of two sampling methods (on foot and by car) to produce a more accurate measure of the roadkill rate, as well as a greater representativeness of the species (Silveira et al., 2010). The selection of stretches of road where the sampling will be performed on foot can be made through draws, selection of points located at regular intervals, random points distributed throughout the road and points purposely selected due to their features (e.g., areas that cut through water bodies). Consider all direction and all lanes that compose the road during the sampling is important too.

ii. Conducting a pilot study to define the sampling method according to the species, such as because the best time of the day and months for sampling. If a pilot study is not feasible, the sampling should be performed during multiple periods of the day to include species with different behaviors and to prevent the loss of carcasses.

iii. Determining specific characteristics of the surrounding landscape to verify correlation with stretches of road near water bodies, forested areas or certain land uses.

iv. Quantify the traffic volume (Fahrig et al., 1995; Hels and Buchwald, 2001; Mazerolle, 2004).

v. Evaluate the persistence rates of carcasses and the detection rate (Teixeira et al., 2013).

vi. Whenever possible, the research team should collect individuals or tissue samples and include them in zoological collections (Balkenhol and Waits, 2009).

vii. Concomitantly with the surveys, the team should also perform an inventory of the fauna in the area surrounding the studied road (Rosa et al., 2012).

viii. Prioritize, whenever possible, long-term (Andrews et al., 2008) and comparative studies with controls replication. When there is a need for quick response, the minimum sampling effort for surveys of more than one year should be weekly or even more frequent collection. In areas exhibiting high environmental seasonality, we suggest twice a week collection for at least one year. However, to include the most affected species, a bi-weekly monitoring is sufficient (Bager and Rosa, 2011).

ix. Use time series analyses to determine which mitigation measure is more appropriate.

x. To monitor the mitigation measures, we recommend comparisons of roadkills before and after the methods are applied.

xi. Use the geographic information system GIS tools and visit the site before implementing new roads (Andrews et al., 2008).

xiii. Form multidisciplinary teams, involving different social actors (e.g., government members, engineers, conservationists and local residents). Multidisciplinary teams may better evaluate / limit issues before the construction of the road, and implement monitoring and mitication after road constructions (Andrews et al., 2008; Lesbarrères and Fahrig, 2012).

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