Herpetofauna and roads: a review

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Roads and traffic are tightly related to some of the mains threats for biodiversity. Road network affects wildlife populations due to, among other effects, partial occupation and transformation of landscape, alteration of surrounding habitat, dispersal of physicochemical pollutants, fragmentation and loss of connectivity, or direct road-kills. Because of their ecological characteristics, amphibians and reptiles are very exposed to road effects. In this article we review the relationship between these faunal groups and the road network. Amphibians exhibit high road-kill rates that can condition viability of some populations, and are vulnerable to pollution of road margins. Reptiles also suffer casualties because of road-kills when they move to paved roads for thermoregulation. Roads, especially those with high traffic load, act as barriers that difficult movements and contribute to population isolation in both groups. However, road impacts do not have equal intensity over space and time, and consequently some spatio-temporal patterns can be defined. Not all species show the same degree of exposure to road impacts, which depend on specific ecological requirements in each case. Mobile species are generally more vulnerable than sedentary ones. There are also intra-specific differences as a function of gender and age. All these considerations must be taken into account when designing and implementing the corresponding mitigation measures necessary to reduce the negative effects of roads on herpetofauna populations.

Key words: fragmentation; mitigation measures; road ecology; road-effect zone; road-kills; road pollution.

Herpetofauna y carreteras: revisión. Las carreteras y el tráfico rodado están estrechamente relacionados con varias de las principales amenazas para la biodiversidad. La red viaria impacta en las poblaciones de fauna silvestre, entre otros, mediante la ocupación y transformación de parte del territorio, la alteración del hábitat circundante, la dispersión de contaminantes físico-químicos, la fragmentación y pérdida de conectividad, o la mortalidad directa por atropello. Debido a sus características ecológicas, tanto los anfibios como los reptiles presentan una gran exposición a los efectos de las carreteras. En este artículo hacemos una revisión de la relación entre estos grupos faunísticos y la red viaria. Los anfibios experimentan elevadas tasas de atropello que pueden condicionar la viabilidad de algunas poblaciones y son vulnerables a la contaminación ligada a los márgenes de las carreteras. Los reptiles también sufren bajas por atropello al acudir a la calzada a termorregular. Para ambos grupos, las carreteras, sobre todo las de alta capacidad, constituyen barreras que dificultan sus movimientos y aíslan sus poblaciones. Sin embargo, los impactos no revisten la misma intensidad ni en el espacio ni en el tiempo, siendo posible definir una serie de patrones espacio-temporales. No todas las especies presentan el mismo grado de exposición a los impactos de las carreteras sino que éste depende de los requerimientos ecológicos específicos de cada una de ellas. En general, las especies más móviles son las más vulnerables. También hay diferencias intraespecíficas en función del sexo y la edad. Todas estas consideraciones deben ser tenidas en cuenta a la hora de diseñar e implementar las medidas de mitigación correspondientes para tratar de reducir los efectos negativos de las carreteras sobre las poblaciones de herpetofauna.

Key words: atropellos; contaminación; ecología de carreteras; fragmentación; medidas de mitigación; zona efectiva de la carretera.

The increment of both transport networks and social concern about environmental issues has led to an increase in the interest for studying the relationships between wildlife and roads. This has attracted the attention of many researchers, resulting in the emergence of a new discipline known as road ecology, whose main goal is the study of the interactions between organisms and the environment linked to road networks and traffic (FORMAN *et al.*, 2003). Roads impact wildlife by means of causing habitat loss, population fragmentation, direct mortality, changes in animal behaviour, physical and chemical alterations of the environment, and dispersal of exotic species (MALO *et al.*, 2004; JAARSMA *et al.*, 2006; FAHRIG & RYTWINSKY, 2009). Furthermore, the construction of new roads facilitates the use and modification of adjacent habitats by humans (FORMAN & ALEXANDER, 1998; TROMBULAK & FRISSELL, 2000), which indirectly contributes to additional impacts on wildlife.

Amphibians and reptiles show certain ecological characteristics that make them highly vulnerable to roads impacts. For example, they show a low vagility in comparison to other vertebrates, being especially susceptible to habitat fragmentation by linear infrastructures. Moreover, in reptiles, roads constitute an important heat source for thermoregulation. In the case of amphibians, many species show complex life cycles, usually involving periodical migrations among the various complementing habitats in order to complete their annual cycle. Mortality rates during migrations associated with direct road-kills are in some cases high enough to cause effects at the population level. Moreover, their permeable skin, with osmoregulatory and respiratory functions, makes them sensitive to road pollution. These direct and indirect effects of roads are among the main causes contributing to the global amphibian decline (BLAUSTEIN & WAKE, 1990; HOULAHAN *et al.*, 2000; COLLINS & STORFER, 2003; NYSTRÖM *et al.*, 2007). In this review, we summarize the progress made in the study of the effects of roads on the herpetofauna at multiple scales. Furthermore, we also analyse the mitigation measures proposed to reduce these road impacts.

HERPETOFAUNA IN ROAD MORTALITY STUDIES

The first studies in road ecology at the beginning of the 20th century alerted about the direct mortality of wildlife caused by vehicles on roads. The first specific studies about herpetofauna focused mostly on snake mortality (BUGBEE, 1945; FITCH, 1949; CAMPBELL, 1956), but also on amphibians (CARPENTER & DELZELL, 1951). HODSON (1960) made regular counts in a 3.2 km road stretch near Northamptonshire (England) and found 683 vertebrates of 42 species killed on the road. The species with the highest mortality rate was the European common frog (*Rana temporaria*) with 191 casualties.

Most of the studies have been carried out in Europe (e.g. Belgium: BALLASINA, 1989; Germany: PODLOUCKY, 1989; the Netherlands: ZUIDERWIJK, 1989; Switzerland: RYSER & GROSSENBACHER, 1989; Portugal: BRITO & ÁLVARES, 2004; France: LESBARRÈRES *et al.*, 2006, Poland: BRZEZIŃSKI *et al.*, 2012), North America (PALIS, 1994; ASHLEY & ROBINSON, 1996; RAY *et al.*, 2006) and Australia (SEABROOK & DETTMAN, 1996; HOSKIN & GOOSEM, 2010), but in recent times, new studies have been conducted in South America (CAIRO & ZALBA, 2007;

HARTMANN *et al.*, 2011; COELHO *et al.*, 2012; QUINTERO-ÁNGEL *et al.*, 2012) and Asia (SESHADRI *et al.*, 2009; ZHANG *et al.*, 2010; GU *et al.*, 2011; TOK *et al.*, 2011).

If the herpetofauna is especially vulnerable to road effects, road surveys will be expected to obtain a high rate of casualties in comparison to other vertebrate groups. Table 1 shows the results of several studies that have quantified road mortality for all vertebrate groups in different countries, and herpetofauna, especially amphibians, appears in most cases as the group with the highest road-kill rates. The percentage distribution among the different groups varies

both with the type of landscape present in the study area and the methodology followed in the road surveys. Thus, in some cases the number of amphibian and reptile casualties is low due to the low suitability of the prospected areas for these groups, or because the census technique underestimates the real number of casualties of these small-sized animals (MONTORI *et al.*, 2003). Nevertheless, GRYZ & KRAUZE (2008) found in a two-year monitoring study of a local road across Poland's Biebrza River Valley that 90.7% of all the reported casualties were amphibians, especially anurans like the common toad (*Bufo bufo*), the moor frog (*Rana*

Table 1: Percentage of amphibian and reptile casualties in road surveys aimed to study road mortality for all vertebrate groups. Specific surveys for a certain group were not included in the table.

Study	Amphibians (%)	Reptiles (%)	Survey methodology	Location and landscape description	Traffic volume (vehicles/day)
GONZÁLEZ-PRIETO *et al.* (1993)	89.2	5.0	Walking. Weekly surveys.	Road stretch parallel to Miño River, Ourense (Spain). Oak forest with some eucalyptus and pine spots, cultivated areas, scrublands and sub-urban habitat.	n/a
ASHLEY & ROBINSON (1996)	93.8	2.7	Walking and bicycling. Three times per week during spring-summer.	Wetlands in the Long Point Causeway, Lake Erie (Canada).	~ 3000
CLEVENGER (1999)	23.3	-	Vehicle and walking. Methodologies adapted to different specimen sizes.	Bow River Valley, along the Trans-Canada highway corridor in Banff National Park.	> 14000
LODÉ (2000)	29.2	1.1	Vehicle.	Department of Vendée (France).	19320
CLEVENGER *et al.* (2003)	7.1		Vehicle. From April to November.	Central Rocky Mountains (Canada).	~ 14000
TAYLOR & GOLDINGAY (2004)	0.4	5.9	Vehicle.	New South Wales (Australia). Coastal lowlands and volcanic plateau. Open pastures, eucalypt forest, plantations, scattered areas of rainforest.	5000-20000
DÁVILA BLANES *et al.* (2007)	9.0	48.0	Vehicle. Daily surveys.	Road of access to Carrascal de la Font Roja Natural Park (SE Spain).	n/a
GLISTA *et al.* (2008)	93.3	1.3	Vehicle. Twice per week.	Indiana (USA). Wetlands and ditches surrounded by agricultural lands and hardwood.	1900-6300
GRYZ & KRAUZE (2008)	90.7	2.0	Walking. 3-7 days per month.	Biebrza River Valley (Poland). River, oxbow lakes, meadows and flooded pastures.	~ 850
HOBDAY & MINSTRELL (2008)	-	0.8	Vehicle. Five regions, one survey per season.	Tasmania (Australia). Native and regenerated forest, woodland, grasslands and farming regions.	n/a
GEROW *et al.* (2010)	41.5	41.7	Vehicle and walking. Once per week.	Saguaro National Park in south-eastern Arizona (USA).	2200-6000
CARVALHO & MIRA (2011)	24.1-66.1	4.4-6.5	Vehicle. Every two weeks.	Mediterranean agro-silvo-pastoral system (Portugal).	3000-7000
GARRIGA *et al.* (2012)	62.0	12.2	Vehicle. Three times in spring and three times in autumn.	Road stretches across Catalonia (Spain).	126-10466

arvalis) and other *Rana* spp. that were killed during their migration to the breeding ponds. GLISTA *et al.* (2008) reported a percentage of amphibians even higher (95%) for four road surveys conducted in Indiana, USA.

Unfortunately, despite these evidences, there are not too many studies addressing long-term trends in herpetofauna road-kills. The direction of these trends is difficult to foresee and may vary among areas. In some parts, road-kills have increased in recent decades in line with the road network expansion and the increasing number of vehicles and displacements (CARVALHO & MIRA, 2011). On the other hand, road-kills in other areas have decreased as a consequence of parallel population declines associated with the own road impacts (BROCKIE *et al.*, 2009). Considerable variations among years are common due to population fluctuations related to environmental conditions or stochastic phenomena (COOKE, 1995). Thus, long-term studies are needed to detect accurate trends.

As mentioned above, amphibian road-kills can reach very high rates. GOLDINGAY & TAYLOR (2006) estimated a mortality of more than 40 000 frogs per year in a 4-km stretch road in north-eastern New South Wales, Australia. Among reptiles, snakes are also commonly killed on roads due to their use as thermoregulation sites (ROSEN & LOWE, 1994). Freshwater turtles are another of the most vulnerable reptile groups to road mortality (GOODMAN *et al.*, 1994; HAXTON, 2000). Data about lizard road-kills are scarce, but this group does not usually seem to suffer an elevated rate of road mortality; for example, in a study about the impact of off-highway recreation in southern California desert lands, GRANT & DOHERTY (2009) found no direct

mortality of the flat-tailed horned lizard (*Phrynosoma mcallii*) caused by off-road vehicles, although they caused other disturbances and indirect impacts that reduced habitat quality for the species. However, some saurian species can reach high road-kill rates on a local scale. In a road near Barcelona (Spain), the common wall gecko (*Tarentola mauritanica*) accounted for 20.2% of all herpetofauna road-kills, being the second most killed species (MONTORI *et al.*, 2003). Nevertheless, the comparison of road mortality rates among all these studies is very complex and the results are difficult to interpret due to differences in study areas, population abundance, species richness, types of road, traffic densities, and species surveyed (JAKOB *et al.*, 2003; PINOWSKI, 2005; GLISTA *et al.*, 2008; SILLERO, 2008; ELZANOWSKI *et al.*, 2009).

Just as with other issues in conservation, social perception of wildlife road-kills varies in relation to species size. In general little public awareness is given to small size faunal groups such as amphibians and reptiles. However, they are suffering numerous casualties on the roads that can compromise population viability at a local scale (LANGTON, 1989; FAHRIG *et al.*, 1995). This situation is even more puzzling considering that the implementation of effective solutions would be economically viable, at least for the main hotspots of herpetofauna road-kills.

METHODOLOGIES TO STUDY ROAD MORTALITY

Road mortality is generally recorded by direct counting of both dead and alive specimens on roads (VAN GELDER, 1973). However, when detecting all casualties by counting is

not viable because, for example, a high extension of territory is aimed to be covered, a combination of counting and road fencing can be used (GIBBS & SHRIVER, 2005). In these cases there is always a discrepancy between the actual and the quantified numbers. Apart from species' anatomical and ecological characteristics, detection probability varies with the number of surveys, methodology used, and experience and skills of the staff involved in the census. Other important factors to take into account are the presence of scavengers that can remove the corpses, the topography and type of vegetation on the edges of the road, the weather and the time of the day (ANTWORTH et al., 2005). Thus, it is estimated that the actual number of road-kills can be up to 12-16 times higher than the estimated by a daily census (SLATER, 2002).

Amphibian carcasses remain just a few hours on the road (SANTOS et al., 2011). Given that most road-kills occur at night, many carcasses may have disappeared in the morning, especially in roads with elevated traffic volumes. Furthermore, because of their small size and their fragility they are difficult to detect. Some species like the common fire salamander (Salamandra salamandra) may remain for longer on the road due to their tough skin and unpalatability (SANTOS et al., 2011). The season is also important to get accurate estimations; for example, to adequately study the road mortality problem on amphibian populations, sampling should be conducted preferably during migration days.

The method of sampling depends on the goals of the study. Quantifying mortality and assessing potential population consequences require a method that allows the detection of as many individuals as possible so that the closer

we get to the real number, the better. These studies require walking surveys because during vehicle surveys many corpses remain undetected, and detection probabilities vary with the species. Some herpetofaunal studies consider that the maximum vehicle speed allowing for detection of all road-killed specimens is 20 km/h (SANTOS et al., 2007). However, if the aim is to locate hotspots where mitigation measures have to be implemented, both walking and vehicle surveys are valid. While walking surveys increase detection probabilities, vehicle surveys allow covering greater distances in less time, which is important considering the short time of residence of the corpses on the road (LANGEN et al., 2007).

ROAD-KILL SPATIO-TEMPORAL DISTRIBUTION

Herpetofauna road-kills are not randomly distributed in either space or time, but are concentrated in certain road segments during certain periods of time throughout the year. As amphibians and reptiles perform relatively localized displacements, it is possible to identify the variables that explain the spatial distribution of the hotspots, as well as the places where priority to the installation of mitigation measures should be given. The availability of suitable habitats (closeness to ponds and rivers, natural vegetation, absence of anthropogenic disturbance) is a factor commonly identified in road-kill spatial models (SILLERO, 2008). LANGEN et al. (2009) found that, for amphibian and reptile species in the north of New York State, road-mortality hotspots were located within a distance of 100 m from a wetland, especially in elevated road sections that presented wetlands on both

sides. SANTOS *et al.* (2007), working in the road network of Catalonia (NE Spain), identified the presence of streams crossing the road, little steep roadsides, and semi-natural vegetation as the main explanatory factors of *B. bufo* road-kill locations. ORŁOWSKI *et al.* (2008) found that the proportion of habitat occupied by forests and the presence of ponds were the main determinants of the distribution of amphibian road-kills in south-western Poland. Not only the presence but also the size of water bodies in the road vicinity was also relevant (ORŁOWSKI, 2007). In other cases, wet grasslands instead of forest are the landscape types most associated with amphibian road-kills (GU *et al.*, 2011). For freshwater turtles in New York State, hotspots were located at causeways that were larger than 200 m length, in close proximity to water, and with high forest coverage (LANGEN *et al.*, 2012). It is important to take into account that the spatial distribution of road-kills is correlated with species local abundance (ORŁOWSKI, 2007), which in turn depends on habitat features and varies throughout the year and among years.

Apart from landscape variables, road parameters are also important to locate herpetofauna road-kills. For example, the probability of crossing highways is practically null (HELS & BUCHWALD, 2001, but see CARRETERO & ROSELL, 2000). Also, road-kill rate can be lower in main roads than in secondary ones, which could be explained by the previous population decline caused by roads with high traffic intensity (ORŁOWSKI, 2007). In general terms, it is supposed that the number of road-kills increases with traffic volume. Due to their small sizes and relatively low speed of movements, amphibians and reptiles suffer high number of casualties even with very low traffic volumes. Thus, VAN GELDER (1973) estimated that a traffic frequency of 10 vehicles per hour caused the loss of 30% *B. bufo* females that tried to cross the road to and from a breeding pond in the Netherlands. Furthermore, roads with low traffic volume can be more attractive for basking and feeding, as demonstrated by LEBBORONI & CORTI (2006) in lizards from central Italy, thus involving a higher risk of road-kill than roads with high traffic density. Nevertheless, the relationship between road-kills and traffic volumes varies with the species considered. MAZEROLLE (2004a) found that increased traffic intensities elevated the number of casualties in the American toad (*Anaxyrus americanus*) but decreased it in the spring peeper (*Pseudacris crucifer*). For frogs of the genus *Lithobates* the maximum number of road-kills was obtained at medium traffic volumes. For a given species, it is possible to calculate the probability of an individual being road-killed by considering the speed, the angle of intersection, and the traffic intensity. However, as explained below, some animals show specific behaviours in relation to traffic that reduce accuracy of this kind of estimates.

At regional scale, habitat variables are better indicators to define areas with high road mortality rates than traffic volume, since they determine local distribution of the species (ORŁOWSKI *et al.*, 2008). For this reason, it is essential to understand the different spatial scales at which threat processes operate. In this sense, road segment and population scales are more suitable than regional and species distribution scales to identify the best location for implementation of the mitigation measures (BEAUDRY *et al.*, 2008).

The temporal distribution of herpetofauna road-kills is related to species' activity patterns (BERNARDINO & DALRYMPLE, 1992; BONNET *et al.*, 1999). Inter-specific differences can also be partially explained by differences in species' ecological requirements. For amphibians, high risk exists during breeding migrations, which show high inter-specific variation in terms of distance (MONTORI *et al.*, 2003; KOVAR *et al.*, 2009). In the Lozoya Valley (Madrid, Spain) the peaks of road mortality for the natterjack toad (*Bufo calamita*) and *B. bufo* occurred between the last two weeks of March and the first two weeks of May, during the seasonal migrations to the breeding sites (SCV, 2003). As migration distances increase, the probability of crossing roads also increases. Moreover, the breeding habitat requirements condition road-kill locations. Thus, species that can breed in any accumulation of shallow water will have less defined hotspots than those species that breed in ponds or streams.

In turtles and snakes, breeding season also concentrates most road-kills (BONNET *et al.*, 1999; CURETON & DEATON, 2012). Furthermore, daily variations in temperature and precipitation may also influence road mortality rates, as observed by SHEPARD *et al.* (2008a) in these two groups of reptiles, whose frequency of road-kills is positively correlated with minimum daily temperatures.

The behaviour in response to roads and traffic is another important factor determining the spatio-temporal distribution of road-kills, not only at the inter-specific but also at the intra-specific level. Many individuals remain immobile when sensing the arrival of a vehicle, which involves an increase in the crossing time, and consequently in the probability of being killed (MAZEROLLE *et al.*, 2005). For example, northern leopard frogs (*Lithobates pipiens*) move at a slower speed and following more tortuous paths as they approach roads. Furthermore, movement speed is also reduced as traffic volume grows, two synergistic factors that reduce the probability of cross success in this species (BOUCHARD *et al.*, 2009).

In the case of snakes and other reptiles as the snapping turtle (*Chelydra serpentina*), the number of road-kills increases as a consequence of the intentional action of the drivers. In a study conducted in Canada, up to 2.5% of the drivers positively selected to hit reptiles (ASHLEY *et al.*, 2007). In other Australian study, 25% of drivers reported that they intentionally ran over invasive cane toads (*Rhinella marina*). However, field experiments did not confirm this behaviour, but found a rate of collisions not different from random (BECKMANN & SHINE, 2012).

IMPACTS OF ROADS ON HERPETOFAUNA

The effects of road pollution

The impact of the emission of pollutants by vehicles and the use of certain chemicals in road maintenance (herbicides, de-icing salts) on herpetofauna (Fig. 1) has been studied almost exclusively in amphibians, and most examples point to a negative effects on their populations. For example, the proximity to the road correlates with an increased probability of suffering skeletal malformations and a smaller body size in larval wood frogs (*Lithobates sylvaticus*) (REEVES *et al.*, 2008). Although the causes are not entirely clear, an increased risk of injury caused by predation due to a small size, a change in the composi-
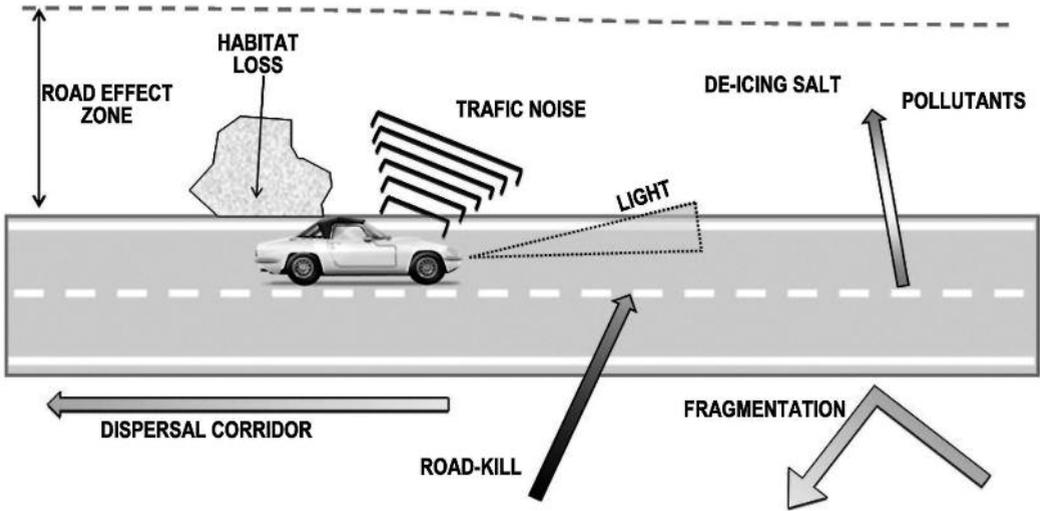
Figure 1: Main road effects on herpetofauna populations. Construction of new roads can imply the loss of high quality habitats for herpetofauna. Moreover, amphibians and reptiles can be road-killed when trying to cross. Roads difficult their movements by increasing fragmentation and exerting a barrier effect; however, road ditches sometimes act as corridors. Traffic flow also impacts on populations through dispersal of chemical pollutants or other types of pollution as noise or lighting. Some practices like the use of de-icing salt in winter maintenance can also impact amphibian or reptile populations. All these road impacts extend tens or hundreds of meters away from the road edge delimiting the road-effect zone.

tion of predator community, or the presence of chemical contaminants from vehicle traffic could explain this phenomenon (REEVES *et al.*, 2008). Also, amphibian larvae near roads with high traffic volume bioaccumulate lead from fossil fuels at doses that may have physiological and reproductive effects (BIRDSALL *et al.*, 1986). However, this problem has been reduced after removal of this heavy metal from fuels.

A widely extended maintenance practice is the use of de-icing salt to prevent the formation of ice and snow on the road. This affects large regions at high latitudes and mountainous areas where frost and snow precipitation are common during winter. Winds or the water runoff can carry salt tens to hundreds of meters away from the roadside, increasing salinity in rivers and ponds nearby. High concen-

trations of salt in the water act as an environmental stressor affecting aquatic fauna with low tolerance to salinity. On amphibians, high salt concentrations can induce death by dehydration. Increased water salinity in road surroundings produced a reduction in survival rate, metamorphosis time, activity, and weight, as well as an increase in the number of malformations in larval *L. sylvaticus* (SANZO & HECNAR, 2006). For this species and for the spotted salamander (*Ambystoma maculatum*), KARRAKER *et al.* (2008) also found a reduction in the survival rate of embryos and larvae along with an up to 50% decrease in the number of egg masses in the vicinity of roads. They concluded that these effects could lead to local extinctions, especially of *A. maculatum* populations, given its high sensitivity to salinity. Laboratory experiments conducted in micro-

cosms with water collected from basins receiving runoff from high-capacity roads showed total mortality of exposed *L. sylvaticus* larvae, while *A. americanus* larvae did not suffer lethal effects (SNODGRASS *et al.*, 2008). In this sense, because of the inter-specific differences in sensitivity, the salt used on roads could act as a stressor capable of changing the structure of amphibian communities (COLLINS & RUSSELL, 2009).

The impact of traffic noise and light

High levels of environmental noise caused by traffic pose a great challenge for species that use acoustic communications (Fig. 1). Thus, traffic noise reduces the ability of female Cope's gray treefrogs (*Hyla chrysoscelis*) to detect male calls (BEE & SWANSON, 2007). In an attempt to avoid masking by high levels of environmental background sound, terrestrial animals can introduce changes in the features of their acoustic signals. This capacity could be a key factor for reproductive success in noisy environments. In amphibians, some well-documented cases for this phenomenon show that the increases in signal frequencies in order to avoid masking (i.e. whistling tree frog, *Litoria ewingii*) are less marked than those identified for birds, but also sufficient to improve communication capacity (PARRIS *et al.*, 2009). Another species, the Neotropical treefrog (*Dendropsophus triangulum*), is capable to increase its song rate (KAISER & HAMMERS, 2009). In an experiment exposing anurans inhabiting far away from roads to traffic noise recordings, CUNNINGTON & FAHRIG (2010) showed that animals immediately altered their vocalization characteristics in a similar way as indivi-

duals living permanently in locations with high traffic noise. This plasticity is essential to maintain acoustic communication in environments with traffic noise. However, LENGAGNE (2008) did not detect the ability to modify the frequencies or temporal structure of the call in the European tree frog (*Hyla arborea*), which suggests that those species capable to acclimatize to traffic noise may have a competitive advantage respect to less plastic species. In this regard, SUN & NARINS (2005) found that in a community exposed to environmental noise, individuals of the two-striped grass frog (*Hylarana taipehensis*) took advantage of this factor by increasing its song rate while the other three studies species decreased it.

Light is another element associated with roads and traffic that may impact herpetofaunal populations. As many species are nocturnal, car lights may lessen their ability to prevent being killed by vehicles, since such lights saturate their retinas leaving them blind and disoriented for a few seconds. Light pollution also affects the ability of some species to detect and capture prey (BUCHANAN, 1993).

Habitat fragmentation and loss of connectivity

Roads act as barriers and/or filters, causing habitat fragmentation and population isolation (Fig. 1). Fragmentation is among the largest threats for amphibian and reptile populations (BECKER *et al.*, 2007). Several studies have demonstrated the huge fragmentation effect caused by roads on herpetofaunal populations. For instance, in an experiment carried out in six paved roads in Virginia and West Virginia (USA) with translocated individuals of redback salamander

(*Plethodon cinereus*) the rate of return for those who had to cross a road was about a 50% lower than for those who just had to pass through the forest (MARSH *et al.*, 2005). In this species, genetic differences are visible in those populations bisected by a major highway but not in those separated by secondary roads (MARSH *et al.*, 2008).

As expected, road fragmentation does not affect equally to all species. Short-term impact of fragmentation correlates positively with species' dispersal ability, although those species with low dispersal abilities may be equally affected over long time periods (CUSHMAN, 2006). Most frog species in a wooded area showed no rejection to the presence of roads and forest roads, and even some species positively selected areas close to roads at certain stages of development. The opposite happened to urodeles as salamanders, whose populations were bigger in remote areas than in zones close to roads (DEMAYNADIER & HUNTER, 2000). For forest salamanders, the edge effect of forest roads due to reduced moisture and vegetation cover is comparable to recently cleared areas (MARSH & BECKMAN, 2004). Roads also alter selection patterns of the spawning sites in urodeles, especially in those species with little tolerance to alteration, as CHAMBERS (2008) observed for the Jefferson salamander (*Ambystoma jeffersonianum*) or the marbled salamander (*Ambystoma opacum*), whereas the more tolerant eastern newt (*Notophthalmus viridescens*) suffered less severe alterations of spawning site due to road impacts.

Fire-roads can also act as barriers to herpetofauna movements, although there is important variation among species. CARTHEW *et al.* (2009) found that for most species of terrestrial fauna in south-eastern South Australia, including the bold-striped cool-skink (*Bassiana duperreyi*), fire-roads were not an obstacle. However, for the painted spadefoot toad (*Neobatrachus pictus*) no crosses of the fire-road were detected.

Snake responses to habitat fragmentation by roads can vary substantially depending on the species' ecological and anatomical features, the smaller being generally more reluctant to cross (ANDREWS & GIBBONS, 2005). The massasauga (*Sistrurus catenatus*) shows a clear rejection to traverse roads. In Manitoba (Canada) the red-sided garter snake (*Thamnophis sirtalis parietalis*) avoided gravel roads (SHINE *et al.*, 2004). The same results were found for the eastern box turtle (*Terrapene carolina*) and the western box turtle (*Terrapene ornata*). If this rejection was heritable, road-kills would decrease over time because those individuals who tended not to cross the roads would be selected, which in turn would increase the degree of isolation between populations (SHEPARD *et al.*, 2008b). The interruption of seasonal migrations caused by roads reduced genetic diversity and increased genetic differentiation in timber rattlesnakes (*Crotalus horridus*), even in a short period of time (CLARK *et al.*, 2010).

Some species can use roads as corridors (Fig. 1). To move between favorable habitats, green frogs (*Lithobates clamitans*) frequently used, with high survival rates, road drainage ditches inside a matrix of peat fields where they rarely ventured (MAZEROLLE, 2004b). Furthermore, road ditches are suitable insolation areas in forested ecosystems. Roads can also constitute a route of access to habitats with high vegetation cover for termophile species (HEDEEN & HEDEEN, 1999).

However, the fact that roads can act as corridors may also have negative effects because, similarly, they may favour the spread of invasive species. Road surroundings are habitats with a substantial level of alteration that are favourable to invasive species. For example, the progressive spread of *R. marina* in Australia has been especially fast in areas with high road density and high connectivity (URBAN *et al.*, 2008), which is an expected outcome considering that this invasive toad is more abundant in the roads or tracks than in the surrounding habitats (SEABROOK & DETTMAN, 1996).

Effects of roads on herpetofauna populations

A good review of the effects of roads on herpetofauna abundance (and also for the rest of the vertebrate groups) can be found in FAHRIG & RYTWINSKI (2009). They found in the literature 22 amphibians and six reptiles for which road effects had been evaluated. The responses were negative for more than 70% of amphibians and 80% of reptiles, although the results were sometimes contradictory. Population declines of herpetofauna species have been attributed, at least partially, to road mortality (FAHRIG *et al.*, 1995; GIBBS & SHRIVER, 2002, 2005; MARCHAND & LITVAITIS, 2004; PUKY, 2006). FAHRIG *et al.* (1995) or SUTHERLAND *et al.* (2010) showed that amphibian density was lower in roads with high traffic volumes than in roads with little traffic. Moreover, the ratio between dead and alive individuals was higher as traffic intensity increased. This fact supports the hypothesis that road-kills can cause the decline of amphibian populations near roads, especially in those

roads with high traffic volumes. These studies were carried out with anurans but the results are similar for urodeles (SEMLITSCH *et al.*, 2007). Mortality rates can be higher in new roads and tend to diminish through the time, which would be related to the fact that road-kills contribute to progressively decrease population size, thus causing population declines (CARRETERO & ROSELL, 2000). In general, amphibian species richness decreases in presence of roads (FINDLAY & HOULAHAN, 1997; GARRIGA *et al.*, 2012). It was estimated for *A. maculatum* in several breeding pools in New York State that under the average displacement performed during migration to breeding sites, one to three-quarters of the population could be affected by road mortality. In those populations, road-kills involved the addition of more than 10% to natural mortality, which would jeopardize their long-term viability (GIBBS & SHRIVER, 2005).

Thresholds of both road density and traffic volume over which the implementation of mitigation measures is justified can be defined using life tables and migration distances of each species (GIBBS & SHRIVER, 2005). However, differences in behaviour may make some species more susceptible than others to road impact. Thus, the level of vulnerability depends on the mobility of each species. A research carried out in the Ottawa-Carleton region (Ontario, Canada) revealed that, while a mobile species like *L. pipiens* showed a negative correlation between their population abundances and traffic density on nearby roads, in another species with shorter displacements like *L. clamitans* there was no relationship between population size and traffic intensity (CARR & FAHRIG, 2001).

Reptile populations are also vulnerable to roads impacts. A study in Ontario, Canada, with radio-marked individuals of eastern rat snake (*Pantherophis obsoletus*) showed that the species did not avoid the road and, although the probability of collision was low (less than 1%), mortality was big enough to compromise the long-term population viability (ROW *et al.*, 2007). For snakes, mobility is also a key factor to explain the inter-specific differences found in population vulnerability to road impacts (BONNET *et al.*, 1999). Thus, forager snakes show higher vulnerability to roads than sedentary ones (MEEK, 2009). A model including road network, traffic volume and mobility of two species estimated an annual road mortality rate about three times higher for mobile species than for sedentary ones (14-21 % for the plain-bellied watersnake, *Nerodia erythrogaster*, vs. 3-5% in the Lake Erie water snake, *Nerodia sipedon*) (ROE *et al.*, 2006).

It is necessary to point out that road mortality can affect populations as long as these populations are limited by mechanisms independent from population density, in which case road-kills have an additive effect. Moreover, the distribution of casualties in relation to sex and age is also very important to assess the real impact of road-kills. For example, the mortality of females of oviparous species on their egg-laying migrations is likely to be more damaging to population viability than the same mortality rate affecting males or neonates (BONNET *et al.*, 1999). These sex- or age-related differences in magnitude of road impacts can modify the demographic structure of herpetofauna populations. In northern Portugal, males of Lataste's viper (*Vipera latastei*) and Seoane's viper (*Vipera seoanei*) were more frequently found road-killed than females, and

the same happened to adults relative to immature individuals. Moreover, a peak in the number of road-kills was found during the spring (BRITO & ÁLVARES, 2004), and the same happened for populations of those two species in northern Spain (MARTÍNEZ-FREIRÍA & BRITO, 2012). On the other hand, MONTORI *et al.* (2003) suggested that for most snake species in Catalonia, including the horseshoe whip snake (*Hemorrhois hippocrepis*), the Montpellier snake (*Malpolon monspessulanus*), the ladder snake (*Rhinechis scalaris*) and the southern smooth snake (*Coronella girondica*), immature individuals would be more frequently road-killed than adults, being the peak of road mortality coincident with juvenile dispersal in late summer and fall. However, because of the low detectability of small individuals the real impact of road-kills on immature specimens would be underestimated.

Male-biased road mortality has also been reported for other snake species like *S. catenatus*, with a peak at the end of the summer (SHEPARD *et al.*, 2008a), or *C. horridus*, with males showing a road mortality rate 13 times higher than females (ALDRIDGE & BROWN, 1995). This bias could be explained by the high mobility of males, especially during the mating season when they increase their movements in search of females. Common green iguana (*Iguana iguana*) males suffer more road-kills than females, and populations near roads show a female-biased sex ratio of the adult population (RODDA, 1990). For certain freshwater turtle species, road-kills constitute a serious threat for their populations (GIBBS & SHRIVER, 2002). A well-documented phenomenon in these species is the disproportionately high number of females road-killed during their displacements to

overland nesting locations, an effect that is not so pronounced in semi-aquatic and terrestrial turtles. As a result, freshwater turtle populations near roads are biased towards males (STEEN & GIBBS, 2004; STEEN *et al.*, 2006) and this bias has increased linearly during last decades (GIBBS & STEEN, 2005). Moreover, the edges of the road may become attractive places to establish nests, which attracts females to the vicinity of the road (ARESCO, 2005a). Thus, in many aquatic turtles the nesting period coincides with the maximum rate of road-kills (BEAUDRY *et al.*, 2010). On the contrary, for other species like the diamondback terrapin (*Malaclemys terrapin*) there is no relationship between the proximity to roads and variations in individual density or sex ratio (GROSSE *et al.*, 2011).

Road impacts do not act only at a local scale. On a regional scale, the presence of roads is one of the main elements that explain the presence of various species of salamanders, but with different results depending on the specific tolerance to altered environments: the tolerant species are benefited while the abundance of the sensitive ones decreases (WARD *et al.*, 2008). Road density is negatively correlated to the presence of *R. arvalis* whose probability of occurrence in habitats adjacent to roads is halved compared to what happens in non-fragmented areas. This fact shows that habitat fragmentation is one of the factors that explain the species' spatial distribution patterns (VOS & CHARDON, 1998). The viability of a common spadefoot (*Pelobates fuscus*) metapopulation isolated by roads is quickly compromised by a slight decrease of individuals dispersing from source populations (HELS & NACHMAN, 2002). In fact, the magnitude of the impact of the road network on

forest anurans can be as high as the impact of habitat loss, as demonstrated by EIGENBROD *et al.* (2008), who observed in Ontario (Canada) that the species richness and abundance of three of the six species studied were more correlated to traffic density than to the absence of forest.

As a consequence of the fragmentation caused by linear infrastructures there is a reduction in genetic exchange (VOS *et al.*, 2001). Fragmented populations are very vulnerable to inbreeding processes. Low heterozygosity values were found in agile frog (*Rana dalmatina*) populations living in ponds near roads. The likely cause of this genetic homogenization is the reduction of the number of adult individuals, either by road-kills, noise or pollution (LESBARRÈRES *et al.*, 2003). Beyond lower allelic richness, populations fragmented by roads have a higher degree of genetic differentiation than populations from non-fragmented habitats (LESBARRÈRES *et al.*, 2006).

Road-effect zone for herpetofauna

The combined effect of all road impacts on herpetofauna populations delimits a "road-effect zone" (Fig. 1) that can be defined as the area in which ecological effects extend outward from a road (FORMAN & ALEXANDER, 1998). In the Galapagos Islands, lava lizards (*Microlophus albemarlensis*) have a lower density in the upper 100 m from the road edge, and show a probability of having experienced tail loss up to 30 times higher than individuals from other populations (TANNER & PERRY, 2007). The effect on the Tenerife lizard (*Gallotia galloti*) varies in relation to the adjacent habitat; while roads can play their usual role as barriers or source of

mortality, in those cases where roads cross habitats unsuitable for the species, like the laurel forest, they may act as corridors (DELGADO GARCÍA *et al.*, 2007). Lungless salamanders avoid even the forest roads created for the timber industry, especially the oldest ones, within a band of about 35 m (SEMLITSCH *et al.*, 2007). BOARMAN & SAZAKI (2006) found that the population density of desert tortoises (*Gopherus agassizii*) increased with the distance to the road edge, being especially small within a band of about 800 m around the road. VON SECKENDORFF HOFF & MARLOW (2002) extended this road-effect zone to a 4000 m bandwidth.

The decrease in population density may not have linear relationship with the distance to the road and it is possible to detect thresholds; for example, EIGENBROD *et al.* (2009) identified in Ontario a threshold in population abundance at 250-1000 m from road edge for more than half of the studied species, as well as for the species richness itself. Finally, off-road vehicles may add an adverse effect on populations inhabiting road proximities (BURY & LUCKENBACH, 2002).

Mitigation measures to reduce road impacts on herpetofauna

Various mitigation measures with different efficacies and costs have been proposed in order to solve, or at least reduce the impact of road-kills and habitat fragmentation on amphibians and reptiles. The construction of fences or walls that impede access to the road has been effective in reducing mortality of amphibians (RYSER & GROSSENBACHER, 1989; DODD *et al.*, 2004). The installation of a temporary fence reduced the mortality in

freshwater turtles by more than 99%, effectively preventing the crossing attempts, which had only a 2% of chance of success (ARESCO, 2005b). However, although the installation of fences decreases the mortality rate due to collisions, it also reduces the connectivity with the other side of the road, making the migration to breeding sites difficult and enhancing population isolation.

In order to promote habitat defragmentation, the adaptation of drainages and the construction of wildlife crossings designed for herpetofauna have been proposed. The effectiveness of this measure is supported by the fact that amphibian migration is facilitated by tunnels constructed for drainage under roads (HARTEL *et al.*, 2009). The design and construction features that increase the effectiveness of herpetofauna passages vary among species, although there is considerable flexibility that facilitates decision-making to find a compromise considering the species present in the area. WOLTZ *et al.* (2008) found that all the studied species – two freshwater turtles, *C. serpentina* and the painted turtle (*Chrysemys picta*), and two anurans (*L. clamitans* and *L. pipiens*) – preferred tunnels with diameter widths above 50 cm and sand or gravel firm, together with a fence of about 60 cm. The species showed a strong dislike for structures with diameters below 30 cm. In this sense, longer tunnels require larger diameters (PUKY, 2003). LESBARRÈRES *et al.* (2004) found that while *B. bufo* and the edible frog (*Pelophylax esculentus*) used the tunnels, *R. dalmatina* rejected them. All species preferred soil beds rather than bare concrete and corrugated steel. A viaduct construction together with the installation of a fence designed for amphibians and reptiles drastically reduced mortality in a road that run between two

wetlands (SCOCCIANTI, 2006). The accessibility must be guaranteed in order to keep the tunnels or other kind of herpetofauna crossings functional (PUKY, 2003).

The location of herpetofauna passages should be selected following the knowledge acquired in the studies about the spatio-temporal distribution of road-kills as well as about the herpetofauna use of the space (PATRICK *et al.*, 2010). Decision-making must take into account those routes positively selected by animals and the amplitude of their movement ranges (VAN GELDER *et al.*, 1986; HARTEL *et al.*, 2009). Several methodologies have been used to quantify the effectiveness of the mitigation measures: track plates (not specific for herpetofauna) (e.g. MATA *et al.*, 2005), funnel traps or pitfall traps at tunnel exits (DODD *et al.*, 2004) or cameras to monitor tunnel use (PAGNUCCO *et al.*, 2011).

Additionally, the structures associated with main roads, like ditches for drainage of rain water, seemingly harmless, can become real traps for amphibians during their migration. ZHANG *et al.* (2010) described this problem for juveniles and sub-adults of Asian common toad (*Duttaphrynus melanostictus*). A design with sloping side walls with a maximum tilt of 66°, with a firm edge of stone and gravel with a cement grout and vegetation would minimize the impact of these structures on amphibian populations.

Another suggested measure to reduce road impacts on fauna is the maintenance of clear, unvegetated margins to allow drivers for seeing animals before they begin to cross the road (ROSELL & VELASCO, 1999). While this is a measure basically though for large mammals, it can indirectly benefit reptiles; especially in forested areas, clear margins would constitute an area of insolation, out of the

zone of collision risk, for most reptiles that tend to use roads for thermoregulation.

During road construction, habitat destruction can affect negatively to herpetofauna populations. GUYOT & CLOBERT (1997) proposed to minimize the impact on a population of Hermann's tortoise (*Testudo hermanni*) by means of capturing and maintaining them in fenced spaces during construction of the road, and then releasing them after completion of the works. It has also been proposed that, when aquatic habitats used by amphibians for breeding are eliminated as part of the road construction process, the creation of replacement ponds close to where the destroyed ones were located would minimize the impact for populations (LESBARRÈRES *et al.*, 2010).

Cost-benefit analysis is a possible approach to facilitate decision-making about where to locate mitigation measures. This approach follows economic criteria and tends to maximize the return in measure investments. SHWIFF *et al.* (2007), following the principles of ecological economics, quantified the economic losses associated with the herpetofauna road-kills in a Florida wilderness. Contrarily to these estimations including, for example, large ungulates, where it is possible to assess vehicle or human damages related to collisions, in the case of herpetofauna the authors selected the penalty that would be imposed by law in the state of Florida for the collection of an individual of the species found killed on roads in order to calculate the economic value of the losses.

Most mitigation measures are usually thought and designed for their implementation at a local scale, following the recommendations of the environmental impact assess-

ments. However, this scale fails to ensure adequate consideration of the potentially serious cumulative, indirect and synergistic ecological effects of roads. An adequate plan to minimize road impacts should start at the regional scale by spatial planning and strategic environmental assessment of the road network (TREWEEK et al., 1998), including the conservation of the herpetofauna species as another goal to achieve. For example, certain species such as the common snake-necked turtle (*Chelodina longicollis*) use different types of water points as a function of hydroperiods and seasonal movements. Therefore, their conservation requires not only the protection of water points and a land area around them, but should consider the diversity of habitats they use and ensure connectivity between them (ROE & GEORGES, 2007). The first step could be the identification of areas with high herpetofauna diversity. These would be the priority areas to install mitigation measures in the existing roads, and at the same time, they would be the areas protected from new infrastructure projects (BENAYAS et al., 2006). The same treatment should be applied to protected areas, where road-kill rates can be elevated due to the reception of visitors (GARRIGA et al., 2012).

CONCLUSION

According to the results of dozens of studies about the relationship between herpetofauna and roads, the increasing vehicular traffic is widely suspected to compromise herpetofauna conservation and to play a role in population declines. Nevertheless, the research about the road impacts on the herpetofauna is fragmented, limited for comparisons among zones, with methodological problems,

and inconclusive (ELZANOWSKI et al., 2009). Moreover, research results may sometimes be unexpected. For example, highway water ponds may surprisingly contribute to increase amphibian biodiversity in altered landscapes (LE VIOL et al., 2012). As we stated, the results of the road effects on herpetofauna populations can be contradictory (see FAHRIG & RYTWINSKI, 2009). We should deepen in the origins of these contradictions and the role that the differences in methodology play on them. An important effort to integrate all the information available is required.

To increase our understanding about this topic we should exploit any technique or methodology available. Particularly promising are the advances in genetic analyses that allow the detection of small differences between populations that have partially lost their connectivity. At this respect, genetic results reveal that traffic intensity reduction could be a good solution for certain species (e.g. the palmate newt, *Lissotriton helveticus*) but not for others for which even the secondary roads with low traffic volumes act as barriers (e.g. the common midwife toad, *Alytes obstetricans*) (GARCIA-GONZALEZ et al., 2012). Therefore, DNA analysis becomes a powerful tool to facilitate road planners and environmental managers the decision-making about the most effective mitigation measures. This decision is not always easy since the species present can respond differently to the same mitigation measure. Moreover, population consequences also vary among species both quantitatively and qualitatively. In this context it becomes necessary to develop mechanisms to reach a consensus solution.

Road effects have been quantified only for a few number of herpetofauna species. More research on unstudied species and in different

habitats is required, especially in those areas comprising both high biodiversity levels and an increasing expansion of the road networks (e.g. rainforests, HOSKIN & GOOSEM, 2010). Moreover, other possible road effects or mechanisms of affection should be considered. Thus, the loss of older individuals by road-related mortality could lead long-lived species to a depression in population reproduction rates (KARRAKER & GIBBS, 2011).

Much more effort should focus on the increase of public awareness about this conservation issue. Indirect impacts of roads are not visible and, unlike with large mammals, direct effects such as road mortality on herpetofauna often go unnoticed for the society. It is necessary to help people take consciousness of the problem by implementing educational initiatives for transport and spatial planners and designers, drivers, environmental managers, and other people involved in transport and conservation issues, as well as for the public in general. The aim should be to achieve the routine incorporation of mitigation measures at a local scale and an adequate spatial planning to minimize road impacts on herpetofauna populations at a regional scale.

Acknowledgement

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