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#### From Effects of Linear Transport Infrastructures on Amphibians to Mitigation Measures

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#### **Abstract**

Linear transport infrastructures (e.g., roads, highways, railways) are affecting biodiversity by habitat loss and fragmentation, degraded or suppressed connectivity, and direct and indirect mortality. In response, planners try to propose mitigation or compensatory measures. Amphibians are particularly impacted by these infrastructures, in terms of habitat loss but also because their obligatory migration to breeding sites exposed them to the barrier effect of infrastructure (direct mortality and loss of connection among subpopulations). Several compensatory (e.g., creation of new ponds) and mitigation measures (construction of wildlife passage) have been proposed specifically for amphibians. This chapter aims to describe measures implemented for amphibian populations and tries to evaluate their efficiency in terms of frequentation (wildlife passage) and population persistence.

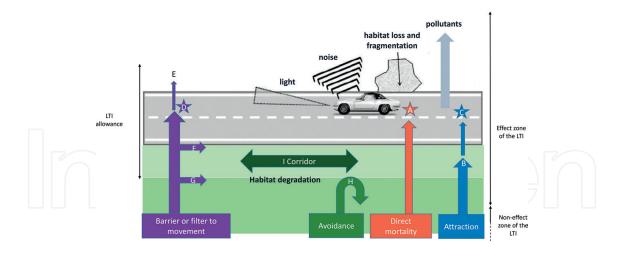
**Keywords:** roads, railways, wildlife passage, mortality, amphibians, habitat fragmentation, population persistence

#### 1. Introduction

The construction of linear transport infrastructure (LTI) such as roads and railways is one of the major anthropogenic alterations to the planet's ecosystems (e.g. see [1]). The effects of LTI such as roads (42 million km of roads around the world) and railways on wildlife were identified as early as the end of the nineteenth century [2]. Throughout the twentieth century, data on the effects of LTIs have accumulated, with a strong increase over the last 20 years [3, 4].

At the same time, planners are trying to use knowledge on species biology and the types of impacts identified to propose mitigation or compensatory measures. These actions have led to





**Figure 1.** Schematic view of environmental effects of a linear transport infrastructure. The building of the LTI and the traffic induced a set of direct and indirect impacts on the biodiversity, in the effect zone of the LTI. (A) Direct mortality via collision with vehicles; (B) attraction (e.g. scavengers for crushed fauna); (C) potential mortality of attracted animals; (D) direct mortality of crossing individuals; (E) successful crossing; (F) avoidance of the LTI; (G) avoidance of the LTI allowance degraded habitat; (H) avoidance of the LTI allowance; (I) corridor effect (adapted from [22, 37]).

the emergence of a theme called "road ecology" [5], which focuses on impacts (roads, but can be extended to all transport infrastructures) and mitigation measures [3, 5].

LTIs have different effects on biodiversity. The animal component of this biodiversity, and in particular the amphibian community, will be considered here, even if the other components are concerned, for example, by habitat loss. The main impacts of LTIs are habitat loss, habitat fragmentation, loss of connectivity, and direct and indirect mortality (**Figure 1**). Historically, amphibians were among the first animals reported as being heavily impacted by roads [6].

While railways represent ~1 million km and are expected to increase by 45% by 2050 [1], their impact in comparison with other infrastructures such as roads and highways is poorly studied [3, 7]. Railways are expected to cause the same types of effects as roads [8], that is, habitat loss, landscape fragmentation, direct mortality [9] and indirect disturbances such as noise pollution and light pollution [10, 11]. Two hundred and fifty-nine studies have been published in relation to road networks, compared with 17 studies about rail networks (of which 3 concern amphibians) in 2014 [3].

#### 2. Habitat loss and fragmentation

In amphibians, habitats destroyed by LTIs are breeding habitats (e.g., aquatic sites where egg and larvae development occur) and terrestrial habitats (transitional habitats for migration to and from aquatic sites, dispersal of juveniles, and growth/hibernation for juvenile and adult terrestrial phases). LTIs can also have a barrier effect, preventing colonization (dispersion) or movements between breeding sites and terrestrial habitat (migration). Habitat loss and the barrier effect (i.e., landscape fragmentation) are difficult to quantify separately because the composition and structuring of landscape elements affect the dynamics of the animal populations that frequent them [12].

Habitat loss and fragmentation can have measurable effects in terms of genetic richness (within the same species) or of specific richness. The genetic structure of a salamander species (*Plethodon cinereus*) is altered near a motorway, but not near secondary roads [13]. Habitat fragmentation leads to an increase in inbreeding, loss of heterozygosity, a decrease in adult numbers, loss of allelic richness and a greater differentiation among 'subpopulations', the agile frog *Rana dalmatina* [14, 15]. Roads are barriers to gene flow in the common frog *R. temporaria* in Germany and France, where genetic differentiation between populations is highest in the densest road network regions [16] or with a higher traffic [17]. Amphibian-specific richness is greater when moving away from roads [9, 18–20], but it is difficult to separate the effect of roads and the effect of landscape co-variables (e.g., forest cover, crops, urbanized area) [21].

The cumulative effects of ILTs delineate an 'impact zone' of various widths. The distance of effect can vary from 40 m to 1.5 km with an average of 500 m, depending on the species considered [22–26].

#### 3. Direct mortality

LTIs are a major source of vertebrate mortality, with consequences to local population dynamics [27]. Due to user safety issues or exploitation (traffic loss), LTI studies are primarily concerned with large mammals (e.g., ungulates and bears, [3]). Vehicle collision mortality can affect the demographics of many vertebrate and invertebrate species, among them are amphibians [25, 28, 29].

As with roads, the direct mortality of wildlife crossing railway tracks depends on species agility and their ability to cross the railway. Mammals, birds, reptiles and amphibians suffer railway deaths [3, 7, 30, 31]. The railway track has been noted to trap small vertebrates such as terrestrial turtles (*Terrapene carolina*) between the tracks [32].

The persistence of crashed small vertebrate bodies can be very short due to scavengers. Their identification can also be difficult because of the body degradation. Both effects can affect species' detectability, degrading the accuracy of the mortality effect evaluation (but see [33] and the use of genetic tracks to identify crushed animal carcasses).

Direct mortality may cumulate with the barrier effect by decreasing the abundance, occurrence and specific richness of amphibian communities [12, 18, 21, 26, 34–36].

#### 4. Mitigation measures

Increased knowledge of the effects of LTIs on biodiversity, biodiversity protection and legislation has led to the implementation of mitigation measures. The aim of these measures is to enable long-term population viability near the LTI [24, 35, 37]. The effect of habitat destruction is most often offset by measures to restore or create new habitats. A typical example is the creation of new amphibian-breeding ponds [38].

To reduce the barrier effect of LTIs, wildlife passages-devoted to a specific fauna of small vertebrates (including amphibians)-began to be implemented in the 1960s in Europe [39]. Wildlife passages (above or below the LTIs) aim to limit direct mortality and to maintain a level of connectivity, allowing gene flow between sub-populations on either side of the LTI [40], a key factor for the viability of amphibian metapopulation [9, 17].

Several approaches have been implemented to reduce direct amphibian mortality on LTIs during movements (migration and dispersal): the first one is to act on traffic, that is, vehicle speed reduction, signaling and temporary closure of roads [9, 25, 41]. The second one is by changing the main amphibian migration routes, for example, by creating breeding sites on the LTI side that avoids most breeding individuals having to cross it [38]. In Germany, 99% of the individual of a common toad *Bufo bufo* breeding population visited the new site the year after its creation [42].

The classical approach is to subtract migrating/dispersing individuals from the risk of crossing the LTIs, thanks to barrier systems (either permanent or temporary) and buckets that trap individuals, which are captured and released on the other side of the LTI.

In many cases, the direct mortality and barrier effects are reduced, thanks to a combination of devices that limit the access to the LTIs and allow its crossing. Wildlife passages above ILTs (viaducts) and barrier systems channel amphibians in their direction, drastically reducing mortality between wetlands but at a high construction cost [22, 43]. These viaducts are highly developed in different species of (large) vertebrates, but their attractiveness and efficiency for amphibians remain poorly documented [9, 44].

In most cases, the wildlife passage designed for amphibians is built to allow crossing below the LTI. It is a round or a square underpass (tunnel) of variable size (e.g., from 0.50 to 1.5 m in height). The entrances are connected to barriers (e.g., plastic or metallic fences). These barriers must be extended by at least 100 m on both sides of the tunnel and at least 0.6 m in height (e.g., [9, 28]).

In addition to the passage designed for wildlife, it is often possible to adapt the many hydraulic systems to make them usable by small vertebrates such as amphibians [22].

An important issue for wildlife passage efficiency is their location, that is, selected in order to maintain the main natural movements (e.g., migratory and dispersal of amphibians in terrestrial phases) [25, 28]. The choice can be dictated by the knowledge of migration routes (assessed by the distribution of crushing zone or the description of a landscape structure) to determine how the amphibian community functions in the landscapes impacted by the LTI. The location of high-crushing zone should not be used as the only indicator for placing wildlife passage: a crashed amphibian number can reflect local density, which can be strongly reduced near roads [28].

If the wildlife passages are effective in reducing adult-breeding mortality, they are often not suitable to animals returning from breeding sites such as juveniles [9]. This failure to account juvenile mortality could explain the declines and even the extinction of amphibian populations where adult mortality (*B. bufo*) has been strongly decreased, thanks to wildlife passages [9, 45].

On the other hand, a review of studies on 123 wildlife passages shows that they provide little information on the fact that they have decreased the barrier effects of the LTI on population connectivity [46].

#### 5. Factors influencing the use of wildlife passage by amphibians

There is little information on the behavior of amphibians nearby and inside wildlife passages (tunnels). Some experiments exist with amphibians, to test the size of the tunnel, the type of substrate, light and humidity.

#### 5.1. Type and size of wildlife passage

Three European anuran species (*B. bufo*, *R. esculenta* and *R. dalmatina*) were tested in an experimental design allowing counting the number of individual entering or not an artificial tunnel 2 m long. The proportion of crossing varied from 66, 65 and 27% in these three species [47]. The north American spotted salamander *Ambystoma maculatum* was tested with several tunnels varying in width (from 0.3 to 0.8 m) and used them whatever their width was [48].

#### 5.2. Substrate type

A tunnel with an earthy substrate is more clearly used than a tunnel with a bare concrete substrate in anuran (*R. esculenta*, *R. dalmatina*, *R. clamitans*, *R. pipiens*) [47, 49]. A marginal effect was observed with the spotted salamander (*A. maculatum*) and the common toad *B. bufo* where the two substrates were used indifferently [47, 48]. In this experimental tunnel device, the choice was also offered to stay on a grassy substrate, or to use one of the tunnels with earthy or bare concrete substrates [47]. The water frog *R. esculenta* and the common toad *B. bufo* used both tunnels, while the agile frog *R. dalmatina* was more frequently found outside the tunnel on the grassy substrate [47]. The use of a natural substrate in wildlife passage can provide habitat continuity and encourage animals to cross [25], and bare concrete, with high alkalinity, could be a repellent to amphibians [47].

#### 5.3. Other factors

Substrate moisture, air temperature, tunnel brightness and noise can influence the use of wildlife passages. A temperature difference between the outside and the inside of a tunnel can deter amphibians [25]. The presence of an opening on top of the tunnel, allowing ventilation and light entry, increased the crossing speed of the spotted salamander *A. maculatum* (cited in [25]). The green frog *R. clamitans* and the leopard frog *R. pipiens* prefer using artificial tunnel with some light entrance [49].

Microclimate (temperature, relative humidity, mean light) in the tunnel was recorded during movement and orientation of Australian frogs (striped marsh frog *Limnodynastes peronii*, the Golden bell frog *Litoria aurea* and the broad-palmed frog *L. latopalmata*) [52]. The tunnel usage was not related to air temperature, humidity or light level recorded inside the tunnel (**Figure 2**).



**Figure 2.** Experimental setup to study the tunnel-crossing behavior of amphibians. Experimental tunnel (ACO climate tunnel model KT 500) used to record amphibian-crossing movements, Photographs by Andrew Hamer (from ([52]). (a) Global view of the tunnel; (b) a view through the tunnel from the entrance arena; (c) view of the tunnel entrance with the infrared camera on the left and the temperature data logger next to the camera. The black plastic bucket is the raised acclimation chamber used for tested animals.

The soil moisture of the surrounding habitat influences the use of tunnels in the north American salamanders (the Santa Cruz long-toed salamander *A. macrodactylum* [51]).

The influence of environmental factors inside and in the surrounding habitats of tunnels on their use by amphibians definitively needs more experimental studies.

Amphibians use various information when migrating and dispersing. Anurans can use the reproductive call of their conspecific to locate the breeding site. This attraction was used to encourage the use of artificial tunnels [47, 52]. European newt species (the palmate newt, *Lissotriton helveticus*, the marbled newt *Triturus marmoratus*, the great crested newt *T. cristatus* and the smooth newt *L. vulgaris*), which do not have reproductive calls, can use the call of sympatric anuran species (the Perez's frog *Pelophylax perezi*, the common toad *B. bufo*, the green toad *Pseudoepidalea viridis*, and the Natterjack toad *Epidalea calamita*), to locate the breeding site [53, 61–63]. It is likely that many amphibians use this cue to orientate, but more studies are definitively needed, especially on the potential of improving the use of wildlife passages. Olfaction to detect the breeding site, thanks to its particular bench of odors, is well known in amphibians. It is also possible that amphibians use smelling traces (conspecific or other species) on the substrate to orientate [47, 54], but to our knowledge, this behavior has not been tested in a wildlife passage context.

#### 6. Evaluating mitigation measures

Mitigation measures to reduce the negative effects of LTIs and traffic on amphibians have varying degrees of success [40, 47, 52].

It is crucial to carry out the evaluation of these mitigation measures and to clearly distinguish the (simple) counting of individual using a wildlife passage and the (complex) global evaluation of the mitigating measures. A combination of methods is often required to achieve this goal.

#### 6.1. Compensatory pond and translocation

Compensatory measures in response to the destruction of aquatic sites include the creation of new ponds and possibly the translocation of the amphibian community that colonized the destroyed ponds [55]. Evaluation of these actions is very rare, most often without an estimate of the success of mitigation [38, 56]. New ponds can lead to an increase in amphibian biodiversity in highly altered habitats [27]. A return time of an amphibian community was 2–3 years in a compensatory pond, that is, the time to observe the same species as in the destroyed ponds [28].

Denton et al. [57] proposed several criteria to assess the success of mitigation based on the creation of compensatory ponds and translocation in the Natterjack toad *B. calamita*. The translocation was the transfer of spawns (5–6000 eggs) during two successive years. The first criterion is the initial success, that is, the emergence of metamorphosed individuals at least one of the 2 years of the transfer. The second criterion is the intermediate success, that is, the return of adults for reproduction before the third year after the transfer (age of sexual maturity is 3 years in this species). The third criteria is the complete success, that is, when reproduction continues in the new site for at least 5 years, with the number of adults remaining stable or increasing, and the production of a second generation of toadlets. The experiment has failed if no return or reproduction is observed for 10 years after the first transfer. These criteria can be adapted to different species to assess the success of mitigation measures. The assessment of compensatory pond measures should also take the maintenance of the quality of the newly created aquatic sites into account.

Evaluating the establishment of populations in translocated ponds remains a difficult task [38], due to large natural variations in amphibian populations and/or the long generation time, requiring long-term monitoring to verify the 'real' success of such operations.

#### 6.2. Ecopasses and barriers to dispersion

Many amphibian species are known to use wildlife passages (tunnels), sometimes with a high abundance: Up to 3000 common toad *B. bufo* are counted each year in a wildlife passage in France [58], and the monitoring of a tunnel for 12 years in Switzerland shows an increase of its use by the common toad *B. bufo* and the common frog *R. temporaria* [59].

The most effective devices—without real comparative studies having been carried out—consist of a guide barrier and funnel underpasses [27, 40, 49, 50] (**Figure 3**). Barriers and walls are



**Figure 3.** Ecopassage designed for the migration of amphibians along the road RD657 in NE France. This ecopassage allowed the migration of amphibians such as the common frog *Rana temporaria* and the common toad *Bufo bufo*. A concrete tunnel, with the floor covered by the soil, allows the animal crossing. A metallic barrier prevents the animals to access the road and directs them toward the tunnel entrance. This ecopassage is located below the local road joining Novéant-sur-Moselle to Arry in Northeastern France (photograph by Alain Morand, CEREMA-EST).

effective in reducing amphibian mortality around LTIs when they are sufficiently high and well installed [27, 39, 40]. They effectively reduce collision mortality but lead to landscape fragmentation [25], making migration and/or dispersion impossible, thereby isolating populations [27]. This ability to control the movement of amphibians by physical barriers (e.g. fences) is used to channel them toward the inlet of the tunnel, thus preventing their penetration on the LTI [25, 40], and reducing direct mortality [40, 51]. However, arboreal species such as *Hyla* sp. suffered direct mortality as they can easily cross fences [40] and their use of tunnel is rare [9]. The fence, with a 0.60-m high, blocked 100% of the individuals in the Green frog *R. clamitans* and the Leopard frog *R. pipiens* [49].

Assessments of mitigation measures often lack scientific quality (e.g. no or poor data before the implementation, no replication of situations and no experimental assessment of wildlife passage use). In a review of 22 studies describing LTIs wildlife passage, only three evaluate the effectiveness (positive in the three studies) [27, 38, 40, 41]. The proportion of individuals entering a wildlife passage (its use) was 13%, while only 5% crossed it completely (effectiveness). This difference may be due to the tunnel characteristics (e.g. tunnel length) and/or the exploratory behavior of adults and juveniles [52].

There are many methods to measure the use of wildlife passage by large mammals. With smaller animals such as small mammals and amphibians, fingerprint plates, pitfall traps and fences, and camera traps have been used [27, 40, 50, 51]. Capture-marking-recapture provides information on the use of, for example, tunnels [50], but also on the dynamics of the monitored populations [60].

Nonfunctional wildlife passage is also observed [49], mostly due to their bad location, poor designs and/or behavior of target species [49]. It should also be noted that wildlife passage

can potentially function as an ecological trap: Predators may use them as hunting grounds (e.g. carnivorous small mammal [9, 25, 41] and snake [29]).

#### 7. Conclusion

The effectiveness of wildlife passages varies by species, and its evaluation will need several steps: The first and very important one is to evaluate the potential effects of the planned LTI on the population functioning (e.g. habitat destruction and connectivity) for the targeted animal community. This will allow classifying the different projects of wildlife passage in terms of objectives (which can differ from one wildlife passage to another). The monitoring (if no data are already available) has to be performed in the proposed locations of wildlife passages and in control areas (outside the site of, e.g., tunnel construction and in areas without the influence of the LTI). These monitoring has to be carried out post construction on the same locations using the same methods. In amphibian species, a combination of methods can be proposed to allow this monitoring, such as population genetics, capture-marking-recapture and occupancy modeling.

The assessment of mitigation measures (barrier/wildlife passage) focusing on amphibian community thus needs to take into account (1) the spatial aspect, that is, the effect distance of the LTI in the landscape (the 'impact zone'), the functioning of this community in the area where mitigation measures are implemented and the functioning of the community in the surrounding landscape; (2) the temporal aspect, that is, biological characteristics of each species (e.g., demographic traits) and of the landscape elements (e.g., natural evolution of compensatory ponds, of tunnel substrate and surroundings); and (3) the knowledge of behavioral determinants and movement capacities of each species that lead to the use or avoidance of wildlife passages.

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