

Investigating the Effectiveness of Road-related Mitigation Measures under Semi-controlled Conditions: A Case Study on Asian Amphibians

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Abstract Road traffic is the main factor causing the decline in amphibian populations worldwide. The proper design of an amphibian tunnel is one of the most efficient measures to mitigate the negative impacts of road traffic on amphibians. However, no study has investigated the effectiveness of amphibian tunnels under semi-controlled conditions in Asian amphibians. Here, we selected two representative amphibian species, the Chinese brown frog, *Rana chensinensis*, and the Asiatic toad, *Bufo gargarizans*, which suffer the most severe road mortality along the roads in Northeast China. We placed experimental arrays of culverts of various sizes (diameters of 1.5, 1, and 0.5 m for circular culverts; side lengths of 1.5, 1, and 0.5 m for box culverts), and substrate type (soil, concrete, and metal) to examine the preferences of both species during the migratory season between May and September in 2016 and 2017. The results revealed that the Chinese brown frog preferred mid- and large-sized culverts as well as soil culverts. We concluded that culverts with a side length ≥ 1 m, lined with soil, and accompanied by a ≥ 0.4 m high guide drift fence and $\leq 45^\circ$ gradient on the roadside ditch wall would best facilitate road crossings for both species and likely for other amphibian species in Northeast China.

Keywords amphibian tunnels, crossing structure, drift fence, highway, roadside ditch, road ecology

1. Introduction

The amphibian population is decreasing worldwide (Trombulak and Frissell, 2000; Alford *et al.*, 2001; Taylor and Goldingay, 2010). Road traffic is one of the most critical factors causing the decline in amphibian populations (Gibbs and Shriver, 2005; Eigenbrod *et al.*,

2008; Hamer *et al.*, 2015; Heigl *et al.*, 2017; Pereira *et al.*, 2018). To prevent this problem, some amphibian crossing structures have been built in several countries (Clevenger and Huijser, 2011; Danunciacao *et al.*, 2013; Bennett, 2017). However, the effectiveness of design parameters for amphibian crossing structures has been rarely investigated by road ecologists (Ward *et al.*, 2015), and only a few studies have been performed in North America, France, the Netherlands, and China (Lesbarreres *et al.*, 2004; Woltz *et al.*, 2008; Patrick *et al.*, 2010; Fu, 2012).

The amphibian population in Northeast China migrates

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seasonally between pond and forest habitats from May to September, and many amphibians cross roads during this migration period and are killed by vehicles (Wang *et al.*, 2013). However, no study has focused on the design of amphibian crossing structures in this region. According to the guidelines for green road construction, wildlife crossing structures should be constructed along roads to better protect the wildlife resources of China; however, there is a lack of detailed guidelines and a design standard for culverts in China (Ministry of Transport, 2016).

Only a few experimental studies have provided design parameters for amphibian crossings in the field (Lesbarreres *et al.*, 2004; Woltz *et al.*, 2008; Patrick *et al.*, 2010; Zhang *et al.*, 2010), but deficiencies exist that hinder application of the results to practice. First, significant differences have been reported between the size of experimental culverts and the actual culverts; the length of most experimental culverts was ≤ 3 m (Lesbarreres *et al.*, 2004; Woltz *et al.*, 2008; Patrick *et al.*, 2010), while the length of actual culverts is ≥ 10 m for common highways in China. The diameter of most experimental culverts was ≤ 0.6 m (Lesbarreres *et al.*, 2004; Woltz *et al.*, 2008; Patrick *et al.*, 2010), but should be at least 0.75 m based on the design standard in China and most were more than 1 m. Second, no study has focused on drift fences as amphibian passage ways in China. A drift fence is vital to guide amphibians to cross passages and prevent them from directly entering the roadways (Clevenger and Huijser, 2011; Rytwinski *et al.*, 2016). Third, the substrate of almost all culverts in China is concrete or metal, but whether or not amphibians have a preference has not been studied (Ministry of Transport, 2007). Fourth, the drainage ditches are ladder-shaped or square, so if an amphibian falls into a ditch, it is difficult to escape. Thus, ditch angle is an important parameter to consider, so amphibians can escape from the ditch during the migration period (Zhang *et al.*, 2010). Fifth, no study has investigated the effectiveness of road-related mitigation measures under semi-controlled conditions in Asian amphibians. The Chinese brown frog and the Asiatic toad are the most road-killed wildlife in Northeast China (Wang *et al.*, 2013). Therefore, this study will be the first to focus on the preference of two amphibian species for typical highway culverts under semi-controlled conditions.

The present study was designed under semi-controlled conditions to evaluate: 1) preferences for culverts of different sizes and substrate types for these two species; 2) the ability to escape different drainage ditch slope angles; and 3) to provide a sufficiently high drift fence

associated with the culverts to prevent amphibians from entering the roadways and to guide them safely across the passage.

2. Materials and Methods

2.1. Study area The Changbai Mountain National Nature Reserve is a typical nature reserve located in southeast Jilin Province, adjacent to North Korea. Nine amphibian species inhabit this reserve, including the Chinese brown frog and the Asiatic toad (Luo *et al.*, 2015). Amphibian species migrate from May to September, which is the same time traffic volume increases due to peak tourist activity; thus, roadkill is unavoidable (Wang *et al.*, 2013). We selected a field experimental area to perform our trials under semi-controlled conditions during May and September in 2016 and 2017, which was close to the Ring Changbai Mountain Scenic highway. This road cuts through the traditional migration route of amphibians, so our field experimental area represented realistic conditions.

2.2. Experimental design

Amphibian species for the field experiment We caught about 300 Chinese brown frogs and 100 Asiatic toads from the nearby forest or river beside the field experiment area to fulfill the requirements for experiment 1. We placed them in a temporary artificial pool. We selected 30 individuals randomly each time for experiment 1, and did not reuse any individuals, so each amphibian was tested once in experiment 1 to avoid the influence of repeated exposure to stimuli (Martin and Bateson, 1986). After experiment 1, all amphibians were placed in the temporary pool to recover for at least 2 hours. Then, we used them to carry out experiments 2 and 3; like experiment 1, we did not reuse any individuals. After all of the experiments were terminated, all of the amphibians were released into the natural forest and rivers nearby.

Experiment 1: Amphibian preference for different culverts We designed six culverts 10 m long each and located them in a radiating pattern (Figure 1A). The process of the experiment was as follows: the substrate type for the pipe culvert was metal and that for the box culvert was concrete. Thirty amphibians were placed in the middle of the field experiment arena ("M" in Figure 1A), where the test amphibians could easily see all of the experimental culverts. The experiment lasted about 40 min, and we performed the experiment three times consecutively. We checked the number of amphibians in the pitfall trap at the exit of each culvert and also checked the number that entered the culverts but remained inside.

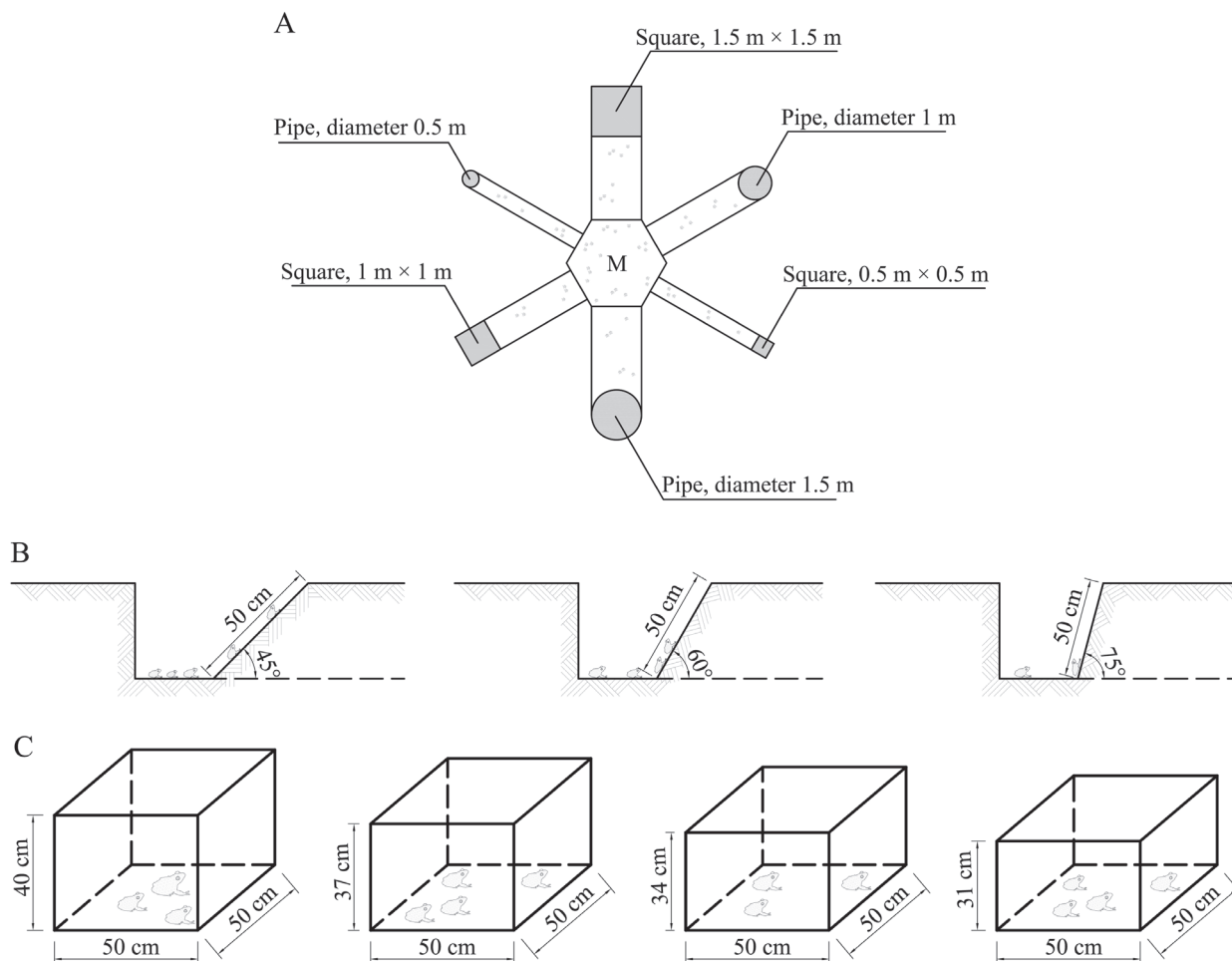


Figure 1 Design schematic of the arenas used in experiments 1–3. (A) We used an iron ring (diameter 1 cm) to construct the frame for the box culverts, and iron sheeting (thickness 0.5 cm) to build the pipe culvert structures, with black shade screen attached to the outside to simulate the actual culvert environment. We designed the three diameters of the pipe culverts or side lengths for the box culvert, which were 1.5, 1, and 0.5 m. We simulated the three substrate types, including soil from the nearby forest, concrete from quick-drying cement, and metal made from iron sheeting. We used white transparent plastic to connect the exit of every culvert to a pitfall trap with about 1 m between them. The depth of each pitfall was about 0.6 m with almost perpendicular walls to prevent the amphibians from escaping. (B) We used concrete brick from a roadside ditch to construct the drainage ditches with slopes of 45°, 60°, and 75°, all with slope lengths of about 50 cm. (C) We used concrete brick similar to experiment 2 to construct a square arena with side wall heights of 40, 37, 34, and 31 cm separately.

We kept the substrate type of the pipe and box culverts as concrete, and repeated the same experiment three times. We kept the substrate type in all culverts as soil and repeated the experiments three times. We performed the experiment nine times separately during May and September in 2016 and 2017. Altogether, we carried out 21 experiments for Chinese brown frogs and six experiments for the Asiatic toad because of the relatively small sample sizes in 2016 and 2017.

Experiment 2: Ability to escape a drainage ditch with different slope angles We put 10 amphibians into each ditch to observe the number that escaped within 15 min. We carried out eight experiments at 45°, 12 experiments at 60°, and eight experiments at 75° with the Chinese

brown frog. We carried out three experiments at 45°, six experiments at 60°, and three experiments at 75° with the Asiatic toad (Figure 1B).

Experiment 3: Suitable drift fence height for the amphibian culverts Because of their physiology, Chinese brown frogs are better jumpers than Asiatic toads (Liu and Zheng, 2009; Luo *et al.*, 2015), which was demonstrated by our field observations. Therefore, we only tested the Chinese brown frog in this experiment. We placed 10 frogs in the center of the arena and observed the number that jumped out within 15 min. We carried out 10 experiments at heights of 40 and 37 cm separately, and nine experiments at heights of 34 and 31 cm separately (Figure 1C).

2.3. Data analysis

Experiment 1: Amphibian preference for different culverts The length of all experimental culverts was 10 m, which was the approximate actual length of secondary class road culverts but was longer than any culverts used previously. Therefore, the time required for amphibians to cross the culverts was much longer than that reported previously (Lesbarreres *et al.*, 2004; Woltz *et al.*, 2008; Patrick *et al.*, 2010; Fu, 2012). Although we allotted 40 min for each experiment, many of the amphibians remained inside the culverts. We expected that the amphibians would cross the culvert once they entered.

Therefore, we combined the number that entered the culvert inside and fell into the pitfalls traps as the “crossing number” for each experimental culvert. Because the crossing number was measured every 40 min and was a Poisson distribution, we used a generalized linear model with the crossing number for each 40 min as the dependent variable and size and substrate type as the independent variables for the fixed factors. Experiment round was a random factor, because the amphibian response may vary during the day or depending on climatic conditions. This model also included the impact of the fixed effect of the two factors and the random effect of one factor and the interacting effect among the fixed effect factors.

Experiment 2: Ability to escape a drainage ditch with different slope angles The data were normally distributed (K-S test, $P > 0.05$), so analysis of variance was used to test the difference in escape ability among the 45°, 60°, and 75° slope angles.

Experiment 3: Suitable drift fence height for the amphibian culverts The data were not normally distributed (K-S test, $P < 0.05$), so the Kruskal–Wallis test was used to analyze the differences in jumping ability of frogs among the 40, 37, 34, and 31 cm culverts, and the Mann–Whitney U -test was used for the comparisons.

A P -value < 0.05 was considered significantly for all statistical tests. All statistical analyses were conducted using SPSS 19.0 for Windows software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Preference for different experimental culverts

According to the generalized linear model, size and substrate type influenced the number of amphibians crossing the experimental culverts, while experiment round had no effect (Table 1). The number of Chinese brown frogs crossing the largest (12.73 ± 1.10) and mid-

sized (11.63 ± 1.02) culverts was significantly higher than the number crossing the small-sized culverts (8.92 ± 0.92) (Figure 2A). The number of Chinese brown frogs crossing the soil substrate type of culvert (12.78 ± 0.95) was significantly higher than the number crossing the concrete type of culvert (9.67 ± 0.95) (Figure 2B).

3.2. Escape test The escape ability of the Chinese brown frog was significantly different among the three drainage ditch slope angles ($F = 27.961$, $P < 0.001$, Figure 3). The escape ability of the Chinese brown frog for the 45°

Table 1 Variables retained in the generalized linear model.

Source	Wald	df	P -value
Chinese brown frog			
(Intercept)	629.694	1	< 0.001
Size	9.258	2	0.01
Substrate type	6.968	2	0.031
experiment round	2.269	2	0.322
Size * Substrate type	5.502	4	0.24
Asiatic toad			
(Intercept)	353.633	1	< 0.001
Size	1.866	2	0.393
experiment round	0.329	2	0.848

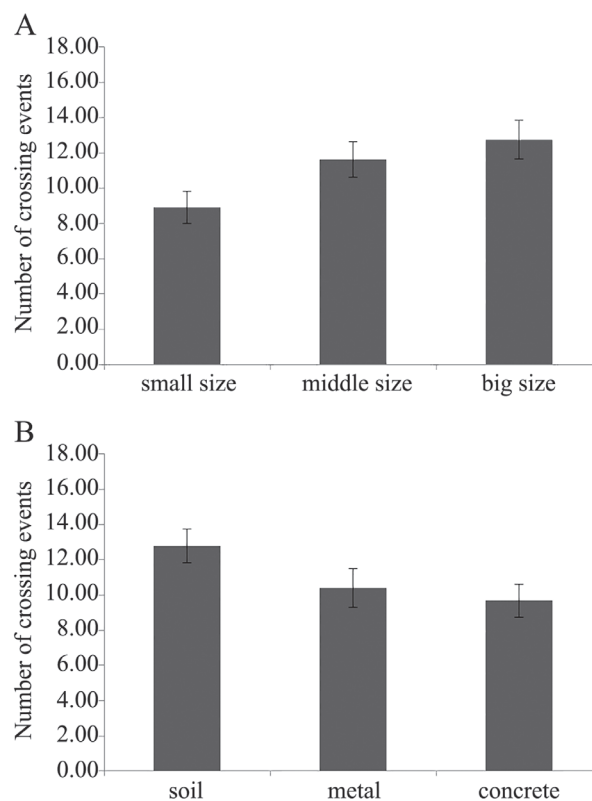


Figure 2 Number of Chinese brown frogs crossing events for experimental culverts. (A) Number of Chinese brown frog crossing events for the three sizes of culverts; (B) Number of Chinese brown frog crossing events the three substrate types.

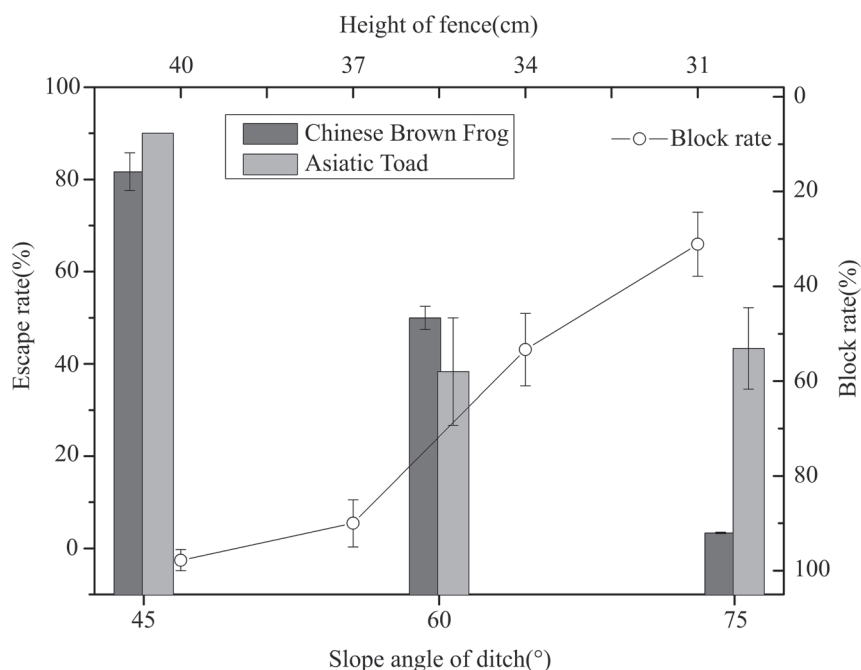


Figure 3 Escape rate of Chinese brown frog and Asiatic toad among the 45°, 60°, and 75° ditch slope angles and the block rate of the Chinese brown frog among the different heights of experimental drift fences.

slope ($86.25\% \pm 5.96\%$) was significantly higher than that of the 60° ($43.85\% \pm 8.36\%$) and 75° ($2.5\% \pm 1.64\%$) sloped culverts. Escape ability was also significantly different among the three slope angles for the Asiatic toad ($F = 5.613$, $P = 0.026$, Figure 3); escape ability from the slope angle of 45° ($90\% \pm 0\%$) was significantly higher than that of the 60° ($38.33\% \pm 11.67\%$) and 75° ($43.33\% \pm 8.82\%$) sloped culverts. Therefore, $\leq 45^\circ$ slope angle would greatly benefit the escape of both amphibian species from a drainage ditch.

3.3. Chinese brown frog drift fence test The block rate increased with increasing drift fence height (Figure 3). The block rate was only $31.11\% \pm 6.76\%$ at the 31 cm height, which was significantly lower than that at the 34 cm height ($53.33\% \pm 7.64\%$) ($Z = -2.992$, $P < 0.005$), which was also significantly lower than that of the 37 cm height ($90\% \pm 5\%$) ($P < 0.005$). The block rate at the 40 cm height ($97.78\% \pm 2.22\%$) was not significantly different from that of the 37 cm height ($Z = -1.278$, $P = 0.201$).

4. Discussion

Large tunnels provide greater airflow and natural light conditions; however, smaller tunnels with grated slots for ambient light and moisture can be effective for amphibians crossing the tunnels (Clevenger and Huijser,

2011). During the migratory season, spotted salamanders showed no preference for culverts of varying aperture size, length, or substrate (Patrick *et al.*, 2010). Snapping turtles (*Chelydra serpentina*), green frogs (*Rana clamitans*), and leopard frogs (*Rana pipiens*) prefer larger diameter tunnels (> 0.5 m), whereas painted turtles (*Chrysemys picta*) prefer tunnels of intermediate diameter (0.5–0.6 m); the narrow sides and low roofs of these tunnels may make it impossible for anurans to use their characteristic salutatory locomotion while traversing the tunnels (Woltz *et al.*, 2008). Frogs in Mangshan National Nature Reserve prefer culverts with widths of 0.3–0.6 m (Fu, 2012). The present study demonstrated that Chinese brown frogs preferred large (diameter or side length = 1.5 m) and mid-sized culverts (diameter or side length = 1 m), whereas Asiatic toads had no preference. We believed that Chinese brown frogs move in a salutatory locomotion manner, while Asiatic toads move in a crawling manner, which may lead to differences in their preference for culverts.

The frogs preferred the soil substrate type but have a strong preference not to move across concrete, whereas the toads have no preference between the soil and metal substrate types of culverts (Queensland Department of Main Roads, 2000; Lesbarreres *et al.*, 2004; Woltz *et al.*, 2008). We observed this situation in our research area. We found that culverts of the soil substrate type were preferred by Chinese brown frogs. The skin of amphibians

was more prone to desiccation than that of many other vertebrates (Mazerolle and Desrochers, 2005). The dehydration rate experienced by Chinese brown frogs was higher than that of Asiatic toads and perhaps desiccation risk negatively affected the preference of the Chinese brown frog for the concrete and metal types of culverts (Luo *et al.*, 2015). Square culverts and pipe culverts are both popular in Northeast China. Furthermore, the bottom of the culvert contains soil and sand from seasonal rainfall, which is beneficial for the amphibian crossings. However, rapid water flow or flooding of the culverts prevents amphibians from moving through the structures (Patrick *et al.*, 2010; Clevenger and Huijser, 2011).

Almost all roadside drainage ditches are rectangular or ladder-shaped in China; amphibians find it difficult to escape from the culvert and it becomes a death trap (Zhang *et al.*, 2010). Our results showed that when the slope angle of the drainage ditch was 45° , approximately 90% of the Chinese brown frogs and Asiatic toads escaped successfully. In addition, plant growth can mitigate isolation in the amphibian habitat by enabling the frogs to traverse roadside ditches (Zhang *et al.*, 2010). Most drainage ditches are made of concrete in China (Ministry of Transport, 2012). The water flow velocity in the concrete drainage ditch is fast and easy to dry, which is not conducive to the movement of amphibians (Luo *et al.*, 2015). Drainage ditches of an ecological category have been recommended to replace concrete ditches to improve the quality of the roadside habitat (Goosem *et al.*, 2010) and help amphibians and other wildlife move along the highway corridor to wildlife passages (Wang *et al.*, 2017). The vegetation coverage in the ecological drainage ditch was high, so it played a role shading and reducing the evaporation of water; on the other hand, it kept the bottom of the ditch moist, which was conducive to amphibious activities.

A wildlife crossing structure combined with a drift fence is one of the most effective measures to mitigate road kill of amphibians (Ward *et al.*, 2015; Rytwinski *et al.*, 2016). Drift fences with a height of 0.6 m effectively prevented frogs from entering the roadway, while 0.4 m high drift fences are useful for other amphibian species (Woltz *et al.*, 2008; Clevenger and Huijser, 2011). The present results indicate that a 0.4 m high drift fence might prevent Chinese brown frogs from crossing the drift fence line, possibly because of their smaller body size, as jumping ability of amphibians is affected much more by morphology than physiology (Woltz *et al.*, 2008). In addition, drift fence material must be entirely opaque, of smooth fabric (rigid plastic, polythene, or canvas),

with a lip design at the top to keep the amphibians from climbing or jumping over (Woltz *et al.*, 2008; Clevenger and Huijser, 2011).

China has abundant amphibian resources, and most of them are endemic. The amphibian population has been declining in recent years due to habitat reduction and fragmentation (Yu *et al.*, 2006). The total length of China's highways ranks second in the world, and that of the expressway ranks first in the world; however, there is a lack of research on the extent of the highway network near amphibian populations. Furthermore, it is necessary and urgent to protect amphibians from highway construction (Wang *et al.*, 2013). The latest version of the industry standard called "Highway Engineering Technical Standards" clearly states that highways should combine the need for grazing and wildlife migration, and the appropriate location should be chosen to locate wildlife crossing structures (Ministry of Transport, 2014). However, there are no relevant specifications for amphibian tunnel designs or methods, such as size, substrate, and facilities (Ministry of Transport, 2007). Therefore, this study has important reference value for future standardization of amphibian tunnels for the Ministry of Transport of China.

5. Conclusion

Although this study only tested two amphibian species, the representative characteristics are obvious in Northeast China (Luo *et al.*, 2015). The mitigation measures used for these two species should be applied to other amphibian species in this area. This study suggests that large mid-sized culverts (diameter or side length ≥ 1 m) of the soil substrate type helped amphibians safely cross the road; the angle of the drainage ditch slope should be $\leq 45^\circ$ to help the amphibians escape, and the drift fence should reach a height of 40 cm to keep amphibians from entering the roadway to effectively decrease the frequency of roadkill.

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