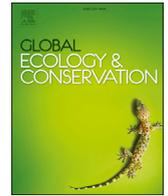




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## Original Research Article

## Acoustic enrichment in wildlife passages under railways improves their use by amphibians

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## ABSTRACT

Linear transport infrastructure can impact wildlife directly or indirectly, and amphibians are particularly affected. In some cases, mitigation measures – such as tunnels below the linear transport infrastructure – are implemented, but an evaluation of their effectiveness is often lacking, especially for small animals such as amphibians. Few studies have focused on crossing behaviour in underpasses, yet this information may be crucial to improve mitigation measures. In this study, we conducted experiments in three tunnels below a high-speed railway with individually marked amphibians (great crested newt *Triturus cristatus*, marbled newt *Triturus marmoratus*, alpine newt *Ichthyosaura alpestris*, fire salamander *Salamandra salamandra*, spined toad *Bufo spinosus*, agile frog *Rana dalmatina* and edible/pool frog *Pelophylax kl. esculentus/lessonae*) which were released at the entrance of tunnels varying in length. Behaviours (e.g. completed crossing, U-turn, not moving, and stay) and crossing speed were recorded. *R. dalmatina*, *S. salamandra* and *T. cristatus* (but not *B. spinosus*) exhibited crossing rate differences between tunnels 21 m and 40 m in length. More individuals performed “complete crossing” through the shorter tunnel. The crossing speed of the *S. salamandra* and *T. cristatus* did not differ between the tunnels of different lengths. In a second experiment, we evaluated the effect of acoustic enrichment on the crossing behaviour of one frog and three newt species by broadcasting a soundtrack of mating calls of syntopic anuran species in the tunnels. *Pelophylax kl* exhibited a large increase in complete crossings and in speed. *T. cristatus* showed an increase in tunnel complete crossings (but not speed) in one of the acoustically enhanced tunnels. These results indicate that acoustic enrichment can improve the crossing of underpasses. This low cost and easily implementable method could help to inform the design of wildlife passages as mitigation measures for impacted amphibian populations, though more experiments on the effect of tunnel characteristics on amphibian behaviour would be valuable.

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## 1. Introduction

Global passenger and freight travel is expected to double from its level in 2010 in coming decades, and it is projected that around the world an additional 25 million kilometres of paved roads and 335,000 km of railway tracks will be needed (Dulac, 2013). The effects of linear transport infrastructure (LTI) such as roads and railways on animals have long been known (Barbour, 1895) and have been extensively studied (e.g. Rytwinski and Fahrig, 2015). The construction of LTI in landscapes can directly alter the physical environment by changing the habitat surface as well as its connectivity, impacting animals through direct mortality and behaviour changes in land use and movements (Jackson and Griffin, 2000; Trombulak and Frissell, 2000). The resulting decrease in population size, landscape connectivity and dispersal can significantly alter the viability of populations (Eigenbrod et al., 2008; Forman and Alexander, 1998; Glista et al., 2009; Hamer et al., 2014; Lesbarrères and Fahrig, 2012).

By affecting connectivity, landscape fragmentation caused by LTI can alter the two main types of animal movements: (1) migration (i.e. seasonal movements between different landscapes at different times of an individual's lifecycle), and (2) dispersal (i.e. an individual's movement away from its birth area to reproduce in a different population) (Joly, 2019; Ronce, 2007). As a consequence, it is not surprising that amphibians, which have a biphasic lifecycle (terrestrial and aquatic), are one of the groups most affected by landscape fragmentation (Beebee, 2013; Cushman, 2006; Holderegger and Di Giulio, 2010).

Wildlife passages constructed for small vertebrates (often tunnels below railway tracks) aim to reduce the barrier effect and thus its alteration of population functioning (Beebee, 2013; Clevenger and Ford, 2010; Colino-Rabanal and Lizana, 2012; Dodd et al., 2004; Hamer et al., 2014; Jarvis et al., 2019). However, while evaluating the effectiveness of these structures for different species is crucial (Jarvis et al., 2019; Lesbarrères and Fahrig, 2012), amphibian behaviour near and inside the tunnels is rarely investigated (Matos et al., 2018; Testud and Miaud, 2018). Some experiments have tested abiotic tunnel characteristics such as diameter and length (Patrick et al., 2010), substrate (Lesbarrères et al., 2004; Trochet et al., 2019), and light, humidity and temperature (Bain et al., 2017). Biotic parameters (e.g. physiological condition of amphibians, vegetation colonization of the tunnels) or social information (e.g. presence of conspecific cues), on the other hand, have been little studied.

According to the chorus attraction hypothesis (Gerhardt and Huber, 2002), amphibians may use the calls of conspecifics or other species for orientation (Buxton et al., 2015; Diego-Rasilla and Luengo, 2007, 2004; James et al., 2015; Madden and Jehle, 2017; Pupin et al., 2007; Swanson et al., 2007). For example, adult of marbled newt *Triturus marmoratus* orientate to the calls of the natterjack toad *Epidalea calamita* in an experimental arena (Diego-Rasilla and Luengo, 2004). Wildlife managers have begun capitalizing on this acoustic attraction to lure animals to specific places by broadcasting vocalizations (Putman and Blumstein, 2019). In amphibians, acoustic enrichment with conspecific chorus calls has been shown to improve the colonization of new ponds by frogs and toads (Buxton et al., 2015; James et al., 2015). These findings suggest that acoustic enrichment might be an interesting avenue to explore to increase the effectiveness of wildlife passages.

The aim of this study was to evaluate the use of wildlife passages by amphibians and investigate methods to improve their effectiveness. To this end we studied the movement of seven European amphibian species (spined toad *Bufo spinosus*, agile frog *Rana dalmatina*, edible/pool frogs *Pelophylax kl. esculentus/lessonae*, fire salamander *Salamandra salamandra*, alpine newt *Ichthyosaura alpestris*, marbled newt *T. marmoratus* and great crested newt *Triturus cristatus*), in underpasses below a high-speed railway track in France by: (1) performing homing experiments to compare their ability to cross tunnels of two lengths in the same location, and (2) evaluating the efficacy of acoustic enrichment by analysing different crossing behaviours and speed in tunnels with and without the broadcasting of anuran calls.

## 2. Materials and methods

### 2.1. Study area and wildlife crossing structures

The study area was located in the region of Pays-de-la-Loire in northwestern France. Our focus was on three wildlife crossing structures constructed at different locations under the same high-speed railway track. Tunnels 1 and 2 are separated by a distance of 800 m and are close to the town of Chantenay-Villedieu. Tunnel 3 is about 70 km (direct distance) from the other tunnels and is close to the town of Beaulieu-sur-Oudon. The three tunnels consist of square concrete culvert of 1 m in width and 1 m in height (Fig. 1): in length, tunnel 1 is 21 m, tunnel 2 is 40 m and tunnel 3 is 18.5 m. Tunnels 1 and 2 are dry fauna-crossing structures installed for amphibians, while tunnel 3 is a hydraulic structure that had a light trickle of water during the experimental period.

The tunnels were implemented in areas where previous inventories showed the presence of many ponds and amphibian migration between these ponds and surrounding terrestrial habitats. The individuals used in the homing experiments were collected (in water and on land, see below) in the closed surrounding of the tunnels (e.g. less than 500 m).

### 2.2. Capture and marking of specimens

Specimens of seven species most representative of the local amphibian community were captured for the study (*B. spinosus*, *S. salamandra*, *R. dalmatina*, *P. kl. esculentus/lessonae*, *I. alpestris*, *T. marmoratus* and *T. cristatus*). The seven species were captured using methods that depended on the species. *B. spinosus* and *S. salamandra* were collected on land before

reproduction (from February to early April in 2018 and 2019) during nocturnal transects along railway track fences. Individuals of *R. dalmatina* were caught in water at the beginning of the species' reproductive period (from February to early March 2019) with minnow traps (mesh 4 mm). *P. kl. esculentus/lessonae* (consisting of the edible frog *P. kl. esculentus* and the pool frog *P. lessonae*: hereafter, *Pelophylax kl.*) were caught in ponds before egg-laying (mid-May 2018 and 2019) manually or with a dip-net at night with the help of a flashlight. *I. alpestris* and *T. marmoratus* were caught in ponds during their reproductive period (mid-May 2019) with minnow traps. *T. cristatus* individuals were caught in ponds during their reproductive period (from mid-February to mid-May 2018 and 2019) with minnow traps. A total of 1179 individuals were captured: the numbers for each species are provided in Table 1.

The captured specimens were marked with transponders (PIT-tags,  $1.4 \times 8$  mm, weight 34.2 mg, Biolog-Tiny REF 10268 [R02-0717]), consisting of electronic chips packaged in a 'biocompatible' glass ampoule that meet international standards for animal identification (ISO 11784/11785). The animals were anaesthetized with 2% xylocaine (ointment). The PIT-tags were injected subcutaneously at the intersection of the belly and flank with a PIT-tag injector (Biolog-Tiny REF 10268 [R0 955]). They were housed in several opaque plastic tanks ( $0.80 \times 0.40 \times 0.25$  m) with moist forest litter. They were kept outside, close to the capture site, in a shaded place with an ambient temperature ranging from 5 °C (night) to 15 °C (day). The time spent in captivity between capture and the experiment was about 1–2 days for newts, toads and frogs. As the number of *S. salamandra* captured in a night could be low, some of these individuals were housed for up to 10 days. The results obtained with individuals housed 1–5 days and individuals housed 6–10 days were similar ( $\chi^2$  test, P-value = 0.47). The individuals were released at the place of capture the day after the experiment. Authorization to catch amphibians was provided by the regional government of the study area (Regional Environment, Housing and Planning Agency of Sarthe, Mayenne and Ille-et-Vilaine, interprefectoral derogation from the prohibition to capture and transport specimens of protected animal species, May 29, 2017). The protocols used in this study were approved by the Ethical Committee of UMR 5175 CEFE (Montpellier, France), decision of the committee held on June 27, 2017.

### 2.3. Experiments

Before an experiment, each individual was identified by a hand-held RFID reader (reader RS1-F1, Biolog-ID©, reading distance = 4–10 cm). One experimenter with a reader was positioned at the entrance and another at the exit of the tunnel. The tested individual was released at the entrance of the tunnel and the exact time recorded (min). If the individual did not move in the tunnel after 10 min, it was returned to a new storage tank. Individuals that entered the tunnel but then did a U-turn and exited at the entrance were returned to a new storage tank after noting the exact time of exit. If the individual crossed the tunnel, the experimenter at the exit noted the individual's number and the exact time of exit and then returned it to a new storage tank.



Fig. 1. Tunnel implemented under a railway as mitigation for the amphibian community (square concrete culvert 21 m length, 1 m high, 1 m width).

**Table 1**

Number of individuals of the seven species tested in the different experiments. Life stage A = adult; J = juvenile. (1) caught on land before reproduction; (2) caught when entering water (before egg laying); (3) caught in water before egg laying; (4) caught in water during reproduction.

Species	Life stage	Without acoustic enrichment			With acoustic enrichment			Total		
		Tunnel 1	Tunnel 2	Tunnel 3	Tunnel 1	Tunnel 2	Tunnel 3	Tunnel 1	Tunnel 2	Tunnel 3
<i>Bufo spinosus</i> (1)	A	5	14					5	14	
	J	7	15					7	15	
<i>Rana dalmatina</i> (2)	A	50	9					50	9	
	J									
<i>Pelophylax kl.</i> (3)	A			228			35			263
	J			61						61
<i>Salamandra salamandra</i> (1)	A	69	32					69	32	
<i>Ichtyosaurus alpestris</i> (4)	A			36			30			66
<i>Triturus cristatus</i> (4)	A	229	59	62	46	46	71	275	105	133
	J	16	3	7	14	3	4	30	6	11
<i>Triturus marmoratus</i> (4)	A			14			14			28

The experiments were conducted over 22 nights from March 2018 to May 2018 and from February to May 2019 (tunnel 1 = 7 nights, tunnel 2 = 7 nights, tunnel 3 = 8 nights). They were performed after sunset (from about 7:00pm to 9:30pm). During a test, an individual was released at the tunnel entrance every 1 min. In the case of the presence of a returning individual (animals within 50 cm of the entrance and moving towards the tunnel entrance), the next release was stopped until the capture of this individual. The experiment was stopped 4 h 30 min after the last tested individual was released at the entrance to start its crossing. The individuals that did not exit the tunnel – either at the entrance or at the exit – were then collected by an experimenter, identified and their position in the tunnel was recorded.

#### 2.4. Crossing behaviour

The crossing behaviour of specimens in the tunnels was described by four main behavioural categories: (1) 'Not moving': individuals that did not move in the tunnel after 10 min, (2) 'U-turn': individuals that entered the tunnel but did a U-turn and exited at the entrance, (3) 'Stay': individuals that did not exit at the entrance or at the exit by the end of the experiment, and (4) 'Complete crossing': individuals that crossed the tunnel to the exit. The proportions of each behaviour category were calculated as the number of individuals displaying the behaviour divided by the number of individuals tested.

Additionally, in two Urodela species (*T. cristatus* and *S. salamandra*), the crossing speed in tunnels 1 and 2 was evaluated for individuals that entirely crossed the passage. This was estimated by the length of the tunnel (in metres) divided by the time taken to completely cross the tunnel (in minutes). The mean speeds between tunnels were compared with a Wilcoxon-Mann-Whitney test.

#### 2.5. Acoustic enrichment

In 2019, an acoustic enrichment experiment was performed during the crossing experiments. In this test, we used a soundtrack of a mixed chorus of edible frog *Pelophylax kl. esculentus* and European tree frog *Hyla arborea*, the two main species using mating calls in the ponds in our study area. The recordings were made with an omnidirectional microphone (Sennheiser ME62/K6, flat frequency response between 20 Hz and 20 kHz) coupled with a Fostex FR2LE recorder. The signal was sampled at 44.1 kHz with a 16-bit dynamic. Files were saved in .wav format. The stereo soundtrack was emitted using a D-Jix M349BT MP3 audio player connected to two loudspeakers (BOOM 2, Ultimate Ears). The loudspeakers were installed outside the tunnel, 10 m from the exit and space 5 m apart. The average amplitude of the signal emitted was set at  $74.8 \pm 0.8$  dB with a Lutron SL-4001 sound level meter (mean  $\pm$  SD, 1 m from the loudspeaker, C weighting, slow settings, re: 20  $\mu$  Pa). The number of individuals and the species tested in these experiments are provided in Table 1.

#### 2.6. Statistical analysis

The proportion of each behaviour category was compared between tunnels (of different lengths and with or without acoustic enrichment) using the Fisher-Freeman-Halton test or  $\chi^2$  test (depending on the sample size for each behaviour category). The mean speed of individuals that completely crossed a tunnel was compared between tunnels with a Wilcoxon-Mann-Whitney test.

### 3. Results

#### 3.1. Crossing behaviour and speed in tunnels of different lengths

In adult *T. cristatus* newts, the proportion of behaviour categories significantly differed between tunnel 1 and 2 (tunnel 1 N = 222; tunnel 2 N = 59,  $\chi^2$  test, p-value: 0.00091). More 'complete crossings' and less 'U-turns' were observed in the

shorter tunnel (Fig. 2a). In juvenile *T. cristatus* newts, the proportion of behaviour categories was not compared between tunnel 1 and 2 because of the low number of individuals in tunnel 2 (tunnel 1 N = 16; tunnel 2 N = 3, Fig. 2b). In *S. salamandra* adults, the proportion of behaviour categories also significantly differed between tunnel 1 and 2 (tunnel 1 N = 69; tunnel 2 N = 32, Fisher-Freeman-Halton test, p-value: 0.00091). More 'complete crossings' and fewer 'stays' were observed in the shorter tunnel (Fig. 2c). In adult *R. dalmatina* frogs, the proportion of behaviour categories did not differ between tunnel 1 and 2 (tunnel 1 N = 50; tunnel 2 N = 9, Fisher-Freeman-Halton test p-value: 0.07). However, no 'complete crossings' and more 'U-turns' were observed in the longer tunnel (Fig. 2d). In adult *B. spinosus* toads, the proportion of behaviour categories did not differ between tunnel 1 and 2 (tunnel 1 N = 5; tunnel 2 N = 14, Fisher-Freeman-Halton test, p-value: 0.5, Fig. 2e). This was also the case for juvenile *B. spinosus* toads (tunnel 1 N = 7; tunnel 2 N = 15, Fisher-Freeman-Halton test, p-value: 0.6, Fig. 2f).

According to the previous results, individuals (in *S. salamandra* and *T. cristatus*) that completely crossed a tunnel allowed estimating a mean crossing speed. In *S. salamandra* adults, the crossing speed did not differ between tunnel 1 and 2 (tunnel 1 N = 47; tunnel 2 N = 11, Wilcoxon-Mann-Whitney test, p-value: 0.71, Fig. 3). This was also the case in *T. cristatus* adults (tunnel 1 N = 88; tunnel 2 N = 8, Wilcoxon-Mann-Whitney test, p-value: 0.31, Fig. 3).

### 3.2. Comparisons between species

Behaviour categories observed in tunnels 1 and 2 in *T. cristatus* and *B. spinosus* adults and juveniles, and in *S. salamandra* and *R. dalmatina* adults are shown in Fig. 2. Of these species, we found that *T. cristatus* adults were the least likely to enter the tunnels (Fig. 2a), while *S. salamandra* completed the most crossings (Fig. 2c), and *R. dalmatina* did the most U-turns (Fig. 2c).

Concerning crossing speed, *T. cristatus* adults crossed tunnel 1 more quickly than *S. salamandra* adults (*T. cristatus* N = 88; *S. salamandra* N = 47, Wilcoxon-Mann-Whitney test, p-value: 0.043, Fig. 3).

### 3.3. Acoustic enrichment in tunnels

In juvenile *T. cristatus* newts (tunnel 1), the proportion of behaviour categories did not differ with or without acoustic enrichment (without N = 16; with N = 14, Fisher-Freeman-Halton test, p-value: 0.21, Fig. 4a). The number of juveniles that completed crossings was too low to calculate the crossing speed (Table 1). In *T. cristatus* adults (tunnel 1), the proportion of behaviour categories significantly differed with and without acoustic enrichment (without enrichment N = 229; with enrichment N = 46,  $\chi^2$  test p-value: 0.030). More 'complete crossings' and less 'U-turns' were observed in the acoustically enriched tunnel (Fig. 4b). However, the crossing speed did not change (without enrichment N = 88; with enrichment N = 32, Wilcoxon-Mann-Whitney test p-value: 0.63, Fig. 5). In tunnel 2, in adult *T. cristatus* newts, the proportion of behaviour categories did not differ with or without acoustic enrichment (without N = 59; with N = 46, Fisher-Freeman-Halton test, test p-value: 0.22, Fig. 4c), nor did enrichment change the crossing speed (without N = 8; with N = 7, Wilcoxon-Mann-Whitney p-value: 1.0, Fig. 5). In tunnel 3, the proportion of behaviour categories in adult *T. cristatus* newts did not differ with or without acoustic enrichment (without N = 62; with N = 71, Fisher-Freeman-Halton test, test p-value: 0.57, Fig. 4d), nor did enrichment change the crossing speed (without N = 17; with N = 15, Wilcoxon-Mann-Whitney test p-value: 0.31, Fig. 5).

In adult *T. marmoratus* newts (tunnel 3), the proportion of behaviour categories did not differ with or without acoustic enrichment (without N = 14; with N = 14, Fisher-Freeman-Halton test, test p-value: 0.45, Fig. 4e). The number of individuals completely crossing the tunnels was too low to calculate crossing speed (Table 1).

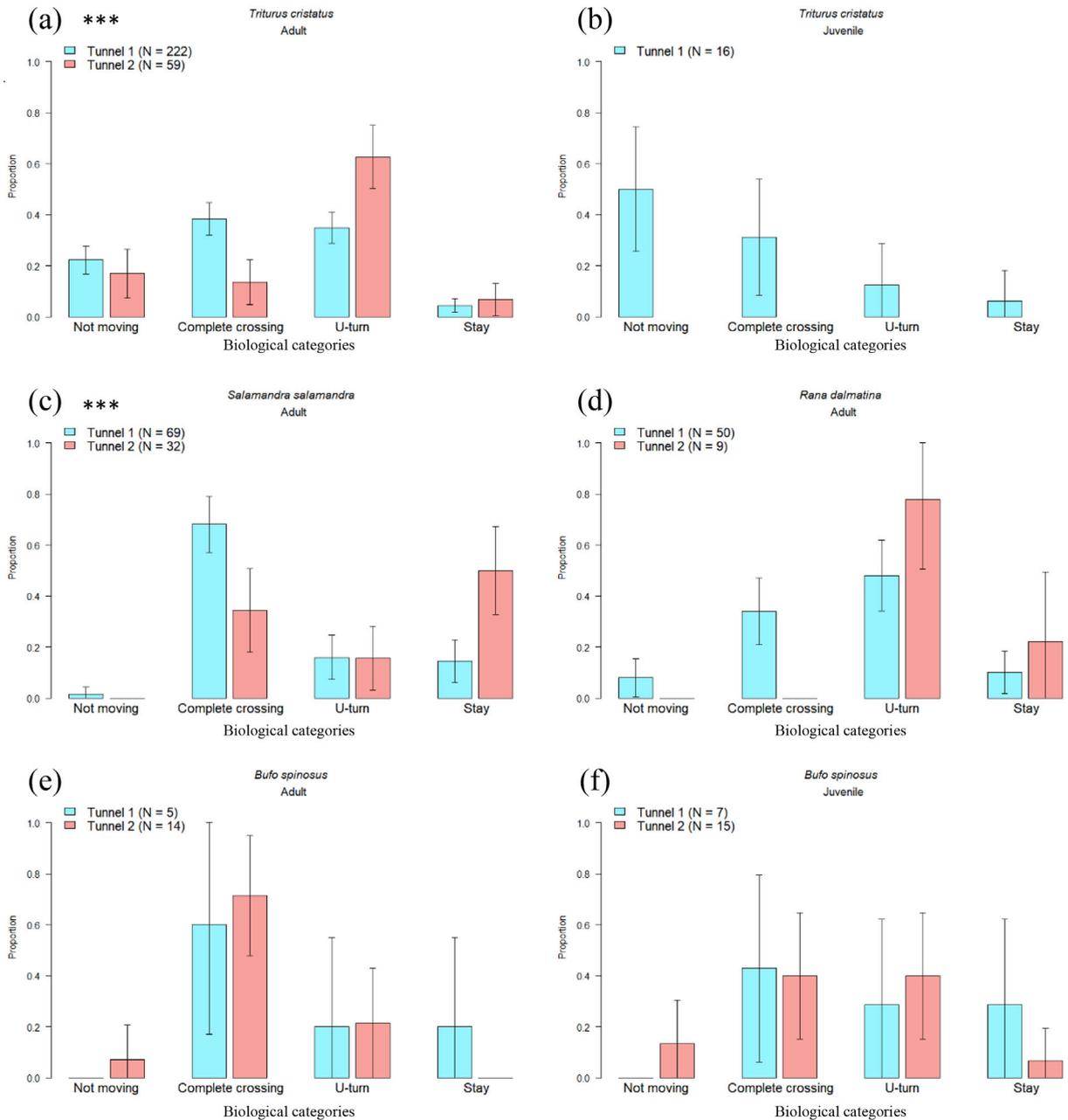
In *I. alpestris* adults (tunnel 3), the proportion of behaviour categories did not differ with or without acoustic enrichment (without N = 36; with N = 30, Fisher-Freeman-Halton test, test p-value: 0.12, Fig. 4f). The number of individuals completely crossing the tunnels was too low to calculate crossing speed (Table 1).

In *Pelophylax* kl. adults (tunnel 3), the proportion of behaviour categories significantly differed with and without acoustic enrichment (without enrichment N = 228; with enrichment N = 35,  $\chi^2$  test, p-value:  $1 \times 10^{-13}$ ). The four categories showed marked changes in the enriched tunnel: only one individual did not move, only one individual stayed in the tunnel, and only one did a U-turn. The majority, 91% (N = 35), of the tested frogs completely crossed the enriched tunnel (Fig. 4g). Moreover, the crossing speed in this tunnel was doubled (without N = 55; with N = 32, Wilcoxon-Mann-Whitney test, p-value:  $7.7 \times 10^{-7}$ , Fig. 5).

## 4. Discussion

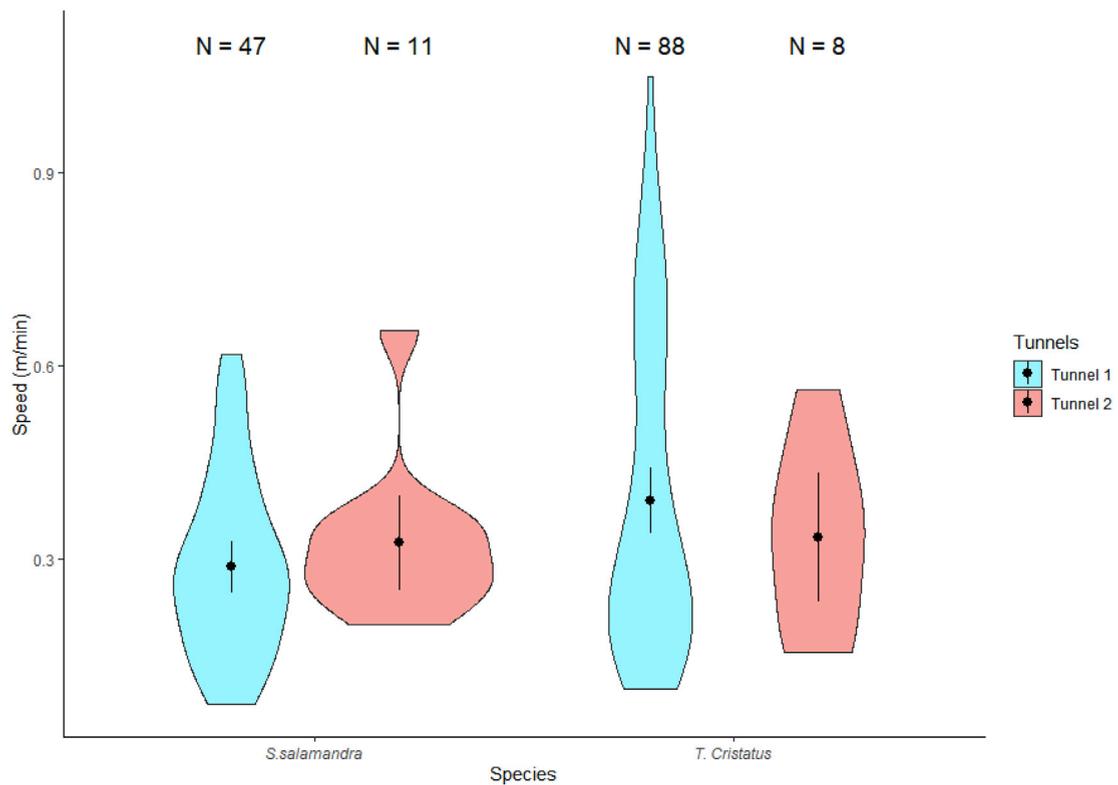
Various studies have demonstrated that linear transport infrastructure strongly alters animal land use behaviour and movements (Jackson and Griffin, 2000; Trombulak and Frissell, 2000). When LTI interrupts amphibian migration or dispersal, different mitigation methods have been implemented with varying efficacy (Colino-Rabanal and Lizana, 2012). Wildlife passages constructed specifically for small vertebrates such as amphibians can reduce direct mortality from LTI as well as its barrier effect, contributing to improve population viability (Beebe, 2013; Clevenger and Ford, 2010; Dodd et al., 2004; Hamer et al., 2014; Jarvis et al., 2019). However, the effectiveness of these mitigation measures has not been sufficiently assessed (Jarvis et al., 2019; Lesbarrères et al., 2004; Pagnucco et al., 2012; Puky et al., 2007; Testud and Miaud, 2018; Woltz et al., 2008), especially for European newts (Matos et al., 2017).

Evaluating the behaviour of amphibians in tunnels is challenging (see a review in Testud et al., 2019). The lack of behavioural data on small species crossing tunnels is partly due to methodological issues (Weber et al., 2019). For example,



**Fig. 2.** Proportion of different behavioural patterns for four amphibian species in two tunnels of different lengths. Patterns: 'Not moving', 'Complete crossing', 'U-turn' and 'Stay' (described in 'Materials and methods'). Blue bars: tunnel 1 (21 m length) and light red bars: tunnel 2 (40 m length). (a) *Triturus cristatus* adults, (b) *Triturus cristatus* juveniles, (c) *Salamandra salamandra* adults, (d) *Rana dalmatina* adults, (e) *Bufo spinosus* adults, (f) *Bufo spinosus* juveniles. N: sample size, Vertical bars; confidence intervals at 0.05. \* refers to test significance (\*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ ). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

camera traps are widely used for movement tracking in tunnels (reviewed in [Jumeau, 2017](#)). The detection rate can be low with amphibians ([Pagnucco et al., 2012](#)), but the current increase in the quality of video traps make them usable with amphibians ([Hobbs and Brehme, 2017](#); [Jarvis et al., 2019](#); [Pomezanski and Bennett, 2018](#)). Marking amphibians with fluorescent pigments was used as a method to record continuous tracks on the tunnel floor, but this method is limited in terms of the number of individuals that can be monitored and does not provide information on movement speed ([Matos et al., 2019](#)). A more promising method is PIT-tagging individuals and tracking their movements with detectors installed in tunnels ([Atkinson-Adams, 2015](#); [Testud et al., 2019](#)).

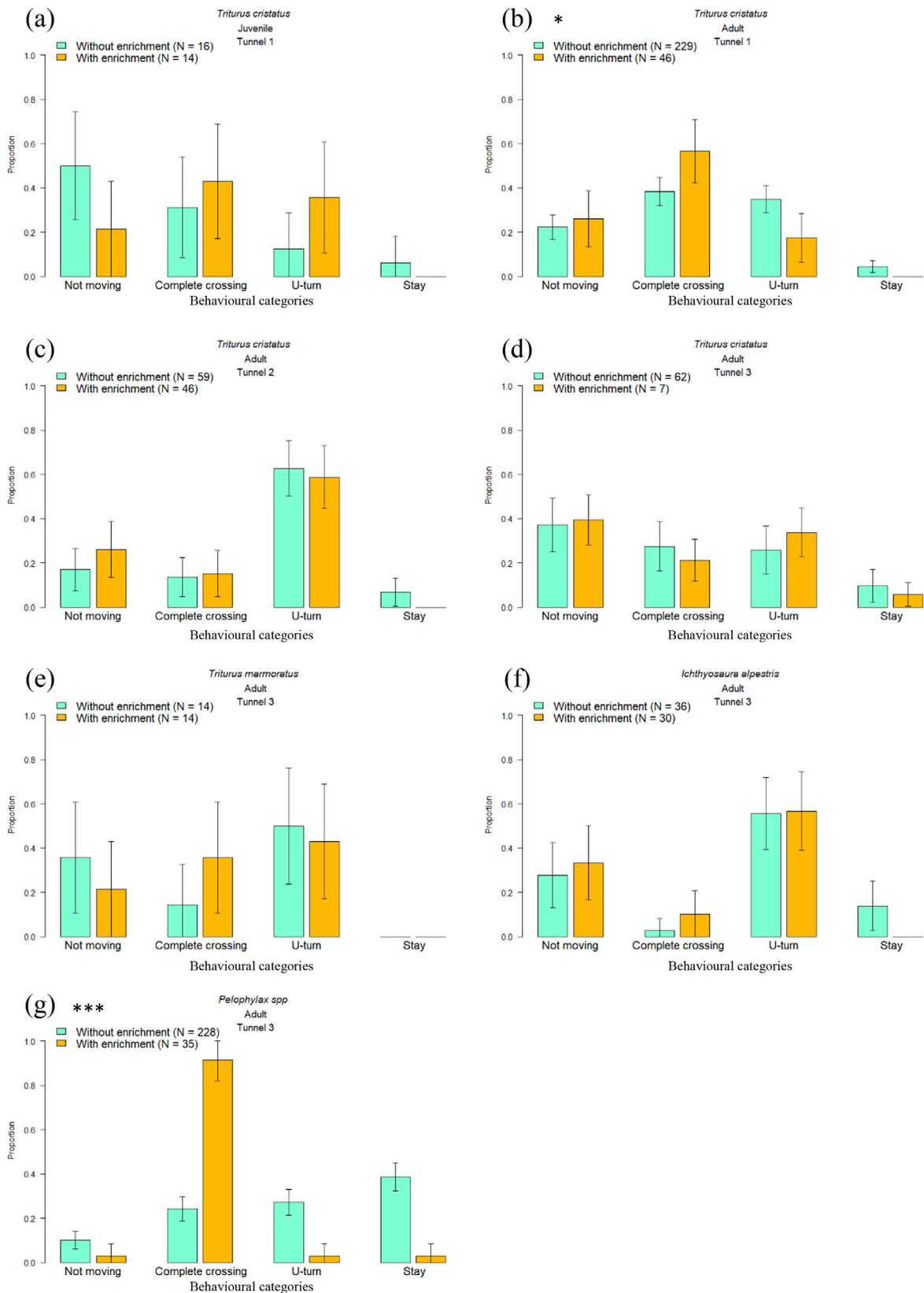


**Fig. 3.** Mean speed of two amphibian species (*Salamandra salamandra* and *Triturus cristatus*, both adults) crossing two tunnels of different lengths. Blue bars: Tunnel 1 (21 m length, see Fig. 1) and light red bars: tunnel 2 (40 m length). N: sample size, vertical bars; confidence intervals at 0.05. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

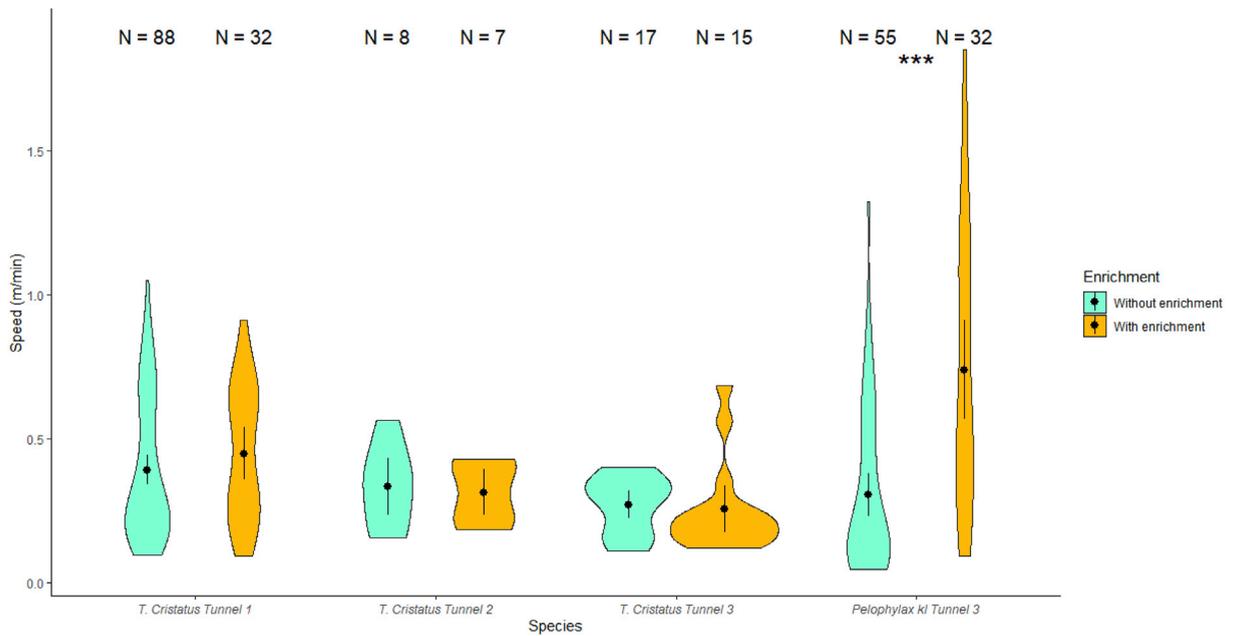
We used PIT-tagging and based our experiments on the concept of homing behaviour, i.e. the propensity of displaced amphibians to come back to the place of capture (Dole, 1972; Sinsch, 1987). We assumed that individuals captured on land while migrating to a breeding place (this was the case for *S. salamandra* and *B. spinosus*) were in a suitable physiological condition to evaluate their terrestrial movements in tunnels. In the case of the newts *T. cristatus*, *T. marmoratus* and *I. alpestris*, these were caught in ponds at dates when they were reproductively active. Newts caught in ponds can successfully return to the site of capture from displacement distances of more than 100 m (e.g. Joly and Miaud, 1989; Twitty et al., 1964). In future, it would also be interesting to perform similar homing experiments to evaluate tunnel use with newts caught on land (i.e. before they enter the aquatic phase).

Our experimental design raised another issue about movement behaviour. The specimens were released at the entrance of a tunnel every minute. We analysed the data considering each specimen trajectory as independent; however, several individuals (of the same species) were present in the tunnel and could potentially interact. Newts can use specific and heterospecific information (e.g. density) to emigrate from ponds (Cayuela et al., 2018). Laboratory experiments showed that juvenile *T. cristatus* can detect scents left on the substrate by conspecifics (Hayward et al., 2000). However, the use of conspecific cues during migration on land remains unknown. In anurans such as toads, intra- and inter-sexual interactions such as couple forming, can potentially change the movement of migrating adults (e.g. Arntzen, 1999). In our experiments, 4 female and 17 male *B. spinosus* were tested, but we do not have information on their interactions. Lastly, water frogs, which travel mainly by jumping, could also potentially influence conspecifics when crossing tunnels. Nonetheless, despite the fact that certain complex behavioural interactions may have occurred in the tunnels during the experiments, we can consider that these were similar in all tunnels and tested conditions and so did not bias the comparisons.

Studies have tested how tunnel characteristics influence the crossing of amphibians with 'experimental' tunnels (i.e. devices mimicking tunnels implemented below LTIs). Tested characteristics include diameter (Patrick et al., 2010), length (Patrick et al., 2010), substrate (Lesbarrères et al., 2004; Trochet et al., 2019), and light, humidity and temperature (Bain et al., 2017). Our study was performed in 'real' tunnels below a high-speed railway track; tunnels whose dimensions differed only in length. In experiments with two tunnels (21 m and 40 m long – both 1 m wide and 1 m high), the longer length was found to impact the proportion of complete crossings by *S. salamandra* (from 68% to 34%), adult *T. cristatus* (from 38% to 14%) and *R. dalmatina* (from 34% to 0%). No effect of length was observed for *B. spinosus*, but this result needs to be confirmed with a higher number of tested individuals. In other studies, a mean crossing rate of 74.5% for the crested newt and 80.6% for the smooth newt (*Lissotriton vulgaris*) were observed in a 'real' tunnel 24 m in length and 0.5 m in diameter (Jarvis et al., 2019).



**Fig. 4.** Proportion of different behavioural patterns for four amphibian species in three tunnels with and without acoustic enrichment. Patterns: 'Not moving', 'Complete crossing', 'U-turn' and 'Stay' (described in 'Materials and methods'). Green bars: without acoustic enrichment, orange bars = with acoustic enrichment. Tunnel 1 (21 m length, dry), tunnel 2 (40 m length, dry) and tunnel 3 (18.5 m length, wet). (a) *Triturus cristatus* juveniles in tunnel 1, (b) *Triturus cristatus* adults in tunnel 1, (c) *Triturus cristatus* adults in tunnel 2, (d) *Triturus cristatus* adults in tunnel 3, (e) *Triturus marmoratus* adults in tunnel 3, (f) *Ichthyosaura alpestris* adults in tunnel 3, (g) *Pelophylax* kl. adults in tunnel 3. N: sample size, vertical bars; confidence intervals at 0.05. \* refers to test significance (\*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ ). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)



**Fig. 5.** Crossing speed of two amphibian species (*Triturus cristatus* and *Pelophylax kl.*, both adults), in three tunnels with and without acoustic enrichment. Green bars: Tunnel 1 (21 m length, dry), and orange bars: tunnel 2 (40 m length, dry) and tunnel 3 (18.5 m length, wet). N: sample size, vertical bars; confidence intervals at 0.05. \* refers to test significance (\*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ ). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

About 38% of the individuals of three species of Australian frogs (*Limnodynastes peronii*, *Litoria aurea* and *L. latopalmata*) entering a tunnel 12 m long and 0.5 m wide crossed it completely (Hamer et al., 2014). In the California tiger salamander *Ambystoma californiense*, 51% of individuals completely crossed a 'real' tunnel 22 m in length and 0.25 m in diameter (Bain et al., 2017). In the same study, the crossing rate was not influenced by the relative humidity of the tunnel, though the mean speed of this salamander was 0.77 m/min in the dry tunnel and 1 m/min in the wet tunnel (Bain et al., 2017). In the (Hamer et al., 2014) study, Australian frogs had a mean speed of 0.8 m/min. In our experiments, adult *T. cristatus* newts crossed the 21-m tunnel at a mean speed of  $0.39 \pm 0.03$  m/min ( $N = 88$ ), which was faster than the *S. salamandra* adults (mean speed =  $0.29 \pm 0.02$  m/min,  $N = 47$ ). The length of the crossed tunnels did not affect the speed of either *S. salamandra* or *T. cristatus*.

When amphibians migrate or disperse and seek to orient themselves when moving from point A to point B, they can use a redundant, multisensory orientation system (Sinsch, 2006). This may include celestial information (Diego-Rasilla and Luengo, 2002), magnetism (Diego-Rasilla, 2003; Diego-Rasilla et al., 2008, 2005; Fischer et al., 2001; Phillips, 1986), and olfactory cues (Joly and Miaud, 1993). The chorus attraction hypothesis (Gerhardt and Huber, 2002) postulates that amphibians can also use conspecific and heterospecific amphibian calls to locate a site of interest (Buxton et al., 2015; Diego-Rasilla and Luengo, 2007, 2004; James et al., 2015; Madden and Jehle, 2017; Pupin et al., 2007; Swanson et al., 2007). Even non-calling species such as newts can exhibit positive phonotaxis for sympatric anuran songs: adult marbled newts (*T. marmoratus*) orientate to the calls of the *E. calamita* (Diego-Rasilla and Luengo, 2004). Adult palmate newts (*Lissotriton helveticus*) orientate to the calls of Iberian water frog (*Pelophylax perezi*) (Diego-Rasilla and Luengo, 2007). Adult *L. vulgaris* orientate to the calls of the common toad (*Bufo bufo*) and European green toad (*Bufoles viridis*) (Pupin et al., 2007). Adult *T. cristatus* orientate to the call of the *B. bufo*, but not to that of the sympatric common frog (*Rana temporaria*) (Madden and Jehle, 2017). Similarly, the palmate newt *Lissotriton helveticus* did not orientate to *R. temporaria* calls ((Diego-Rasilla and Luengo, 2007). This lack of response to the common frog call was explained by the fact that the newt's breeding migration did not overlap with the frog's calling period. These four cited studies were performed in experimental conditions using circular arenas. Very few studies have used acoustic enrichment to direct amphibians towards a target in nature. Of these, the colonization of new ponds by the green and golden bell frog (*Litoria aurea*) was encouraged using acoustic enrichment (speakers with conspecific frog calls, James et al., 2015). Similarly, the Cope's gray treefrog (*Hyla chrysoscelis*) and the American toad (*Anaxyrus americanus*) colonized new habitats where conspecific chorus calls were broadcasted (Buxton et al., 2015).

To our knowledge, no previous studies have been conducted on acoustic enrichment in natural environments with Urodela species, and particularly in the context of wildlife passages for both Anura and Urodela species. Enrichment increased the proportion and speed of *Pelophylax* spp. crossing the tunnel (conspecific call). In newts, broadcasting heterospecific calls resulted in *T. cristatus* adults completing more crossings and doing less U-turns in the 'enriched' tunnel 1 (the speed was unchanged). However, this difference was not observed in 'enriched' tunnels 2 and 3. For the marbled and alpine newts, no

effect of acoustic enrichment was observed on the proportion of crossings in tunnel 3. These divergent findings suggest the effect of acoustic enrichment on the behaviour of newts in wildlife passages needs to be confirmed by testing more individuals and using other calls and tunnel characteristics.

## 5. Conclusion

The length of the tunnel probably influenced the proportion of complete crossings by the fire salamander and crested newt; previous studies have recommended limiting the length of wildlife passages as much as possible (Patrick et al., 2010). Construction constraints can result in rather long tunnels, and increasing the width or diameter (while keeping costs reasonable) in order to increase the use by amphibians still remains to be tested (Colino-Rabanal and Lizana, 2012; Puky, 2003). The effects of other characteristics (humidity, light, substrate, etc.) also need more investigation, with careful consideration of interspecific differences (Baguette and Dyck, 2007; Lesbarrères et al., 2010; Puky et al., 2007).

Studies have shown that acoustic enrichment is successful at attracting amphibians to newly created aquatic sites. In this study, our experiments demonstrated the potential of this method in improving the effectiveness of wildlife passages: both the water frog (when conspecific calls were broadcast) and the crested newt (when heterospecific calls were broadcast) performed better in tunnel 1 (21 m) – however, they did not complete more crossings in the other two acoustically enriched tunnels.

These results could be extended with further studies focusing on the type of emitted calls, e.g. mixing heterospecific vocalizations with conspecific vocalizations of the target species (Putman and Blumstein, 2019), frequency and sound amplitude, the distance and position of loudspeakers in the tunnel, etc. As including loudspeakers in a wildlife passage could be costly (in terms of installation, risk of degradation, etc.), acoustic enrichment could be planned for specific periods (the best timing remains to be evaluated): for example, to allow the establishment or reinforcement of a breeding migration path. Another option could be natural acoustic enrichment, e.g. by creating breeding sites colonized by a community of anurans close to the wildlife passage. As the effectiveness and durability of acoustic playback on attracting animals for wildlife management is poorly known, further investigation would be valuable in evaluating this in wildlife passages for small fauna.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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