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

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Global Ecology and Conservation
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--Manuscript Draft--

Manuscript Number:	GECCO-D-20-00039R2
Article Type:	Research Paper
Keywords:	Transport infrastructure; Tunnels; Mitigation evaluation; Acoustic orientation
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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. P. kl esculentus/lessonae 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.

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

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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.

Keywords: Transport infrastructure, Tunnels, Mitigation evaluation, Acoustic orientation

3

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 kilometres 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

4

56 behaviour changes in land use and movements (Jackson and Griffin, 2000;

57 Trombulak and Frissell, 2000). The resulting decrease in population size, landscape

58 connectivity and dispersal can significantly alter the viability of populations

59 (Eigenbrod et al., 2008; Forman and Alexander, 1998; Glista et al., 2009; Hamer et al.,

60 2014; Lesbarrères and Fahrig, 2012).

61   By affecting connectivity, landscape fragmentation caused by LTI can alter the

62 two main types of animal movements: (1) migration (i.e. seasonal movements

63 between different landscapes at different times of an individual's lifecycle), and (2)

64 dispersal (i.e. an individual's movement away from its birth area to reproduce in a

65 different population) (Joly, 2019; Ronce, 2007). As a consequence, it is not surprising

66 that amphibians, which have a biphasic lifecycle (terrestrial and aquatic), are one of

5

67 the groups most affected by landscape fragmentation (Beebee, 2013; Cushman, 2006; 68Holderegger and Di Giulio, 2010).

69 Wildlife passages constructed for small vertebrates (often tunnels below railway

70 tracks) aim to reduce the barrier effect and thus its alteration of population

71 functioning (Beebee, 2013; Clevenger and Ford, 2010; Colino-Rabanal and Lizana,

72 2012; Dodd et al., 2004; Hamer et al., 2014; Jarvis et al., 2019). However, while

73 evaluating the effectiveness of these structures for different species is crucial (Jarvis

74 et al., 2019; Lesbarrères and Fahrig, 2012), amphibian behaviour near and inside the

75 tunnels is rarely investigated (Matos et al., 2018; Testud and Miaud, 2018). Some

76 experiments have tested abiotic tunnel characteristics such as diameter and length

77 (Patrick et al., 2010), substrate (Lesbarrères et al., 2004; Trochet et al., 2019), and light,

78 humidity and temperature (Bain et al., 2017). Biotic parameters (e.g. physiological

6

79 condition of amphibians, vegetation colonization of the tunnels) or social information

80 (e.g. presence of conspecific cues), on the other hand, have been little studied.

81 According to the chorus attraction hypothesis (Gerhardt and Huber, 2002),

82 amphibians may use the calls of conspecifics or other species for orientation (Buxton

83 et al., 2015; Diego-Rasilla and Luengo, 2007, 2004; James et al., 2015; Madden and

84 Jehle, 2017; Pupin et al., 2007; Swanson et al., 2007). For example, adult of marbled

85 newt *Triturus marmoratus* orientate to the calls of the natterjack toad *Epidalea calamita*

86 an experimental arena (Diego-Rasilla and Luengo, 2004). Wildlife managers have

87 begun capitalizing on this acoustic attraction to lure animals to specific places by

7

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 Beaulieusur-Oudon. The three tunnels consist of square concrete culvert of 1 m in width and 1 m in height (figure 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

9

115   experimental period.

116   The tunnels were implemented in areas where previous inventories showed the

117   presence of many ponds and amphibian migration between these ponds and

118   surrounding terrestrial habitats. The individuals used in the homing experiments

119 were collected (in water and on land, see below) in the closed surrounding of the

120   tunnels (e.g. less than 500 m).

121

122   *2.2   Capture and marking of specimens*

123   Specimens of seven species most representative of the local amphibian community

124   were captured for the study (*B. spinosus*, *S. salamandra*, *R. dalmatina*, *P.* kl.

125   *esculentus/lessonae*, *I. alpestris*, *T. marmoratus* and *T. cristatus*). The seven species were

10

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.

11

139   The captured specimens were marked with transponders (PIT-tags, 1.4 x 8 mm,

140 weight 34.2 mg, Biolog-Tiny REF 10268 [R02-0717]), consisting of electronic chips

141   packaged in a 'biocompatible' glass ampoule that meet international standards for

142   animal identification (ISO 11784/11785). The animals were anaesthetized with 2%

143   xylocaine (ointment). The PIT-tags were injected subcutaneously at the intersection

144   of the belly and flank with a PIT-tag injector (Biolog-Tiny REF 10268 [R0 955]). They

145   were housed in several opaque plastic tanks (0.80 × 0.40 × 0.25 m) with moist forest

146   litter. They were kept outside, close to the capture site, in a shaded place with an

147 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   148

149   and frogs. As the number of *S. salamandra* captured in a night could be low, some of

150   these individuals were housed for up to 10 days. The results obtained with

12

151 individuals housed 1 to 5 days and individuals housed 6-10 days were similar

152 (χ2 test, P-value=0.47). The individuals were released at the place of capture the day

153 after the experiment. Authorization to catch amphibians was provided by the

154 regional government of the study area (Regional Environment, Housing and

155 Planning Agency of Sarthe, Mayenne and Ille-et-Vilaine, interprefectoral derogation

156 from the prohibition to capture and transport specimens of protected animal species,

157 29 May 2017). The protocols used in this study were approved by the Ethical

158 Committee of UMR 5175 CEFE (Montpellier, France), decision of the committee held

159 on 27 June 2017.

160

161 *2.3   Experiments*

162 Before an experiment, each individual was identified by a hand-held RFID reader

163 (reader RS1-F1, Biolog-ID©, reading distance = 4 to 10 cm). One experimenter with a

13

164   reader was positioned at the entrance and another at the exit of the tunnel. The tested

165   individual was released at the entrance of the tunnel and the exact time recorded

166   (min). If the individual did not move in the tunnel after 10 min, it was returned to a

167   new storage tank. Individuals that entered the tunnel but then did a U-turn and

168   exited at the entrance were returned to a new storage tank after noting the exact time

169 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   170

171   tank.

172 The experiments were conducted over 22 nights from March 2018 to May 2018 173

and from February to May 2019 (tunnel 1 =7 nights, tunnel 2 = 7 nights, tunnel 3 = 8

174   nights). They were performed after sunset (from about 7:00pm to 9:30pm). During a

175   test, an individual was released at the tunnel entrance every 1 min. In the case of the

176   presence of a returning individual (animals within 50 cm of the entrance and moving

177   towards the tunnel entrance), the next release was stopped until the capture of this

14

178   individual. The experiment was stopped 4 h 30 min after the last tested individual

179   was released at the entrance to start its crossing. The individuals that did not exit the

180   tunnel – either at the entrance or at the exit – were then collected by an experimenter,

181   identified and their position in the tunnel was recorded.

182

183   *2.4   Crossing behaviour*

184   The crossing behaviour of specimens in the tunnels was described by four main

185   behavioural categories: (1) 'Not moving': individuals that did not move in the tunnel

186   after 10 min, (2) 'U-turn': individuals that entered the tunnel but did a U-turn and

187   exited at the entrance, (3) 'Stay': individuals that did not exit at the entrance or at the

188   exit by the end of the experiment, and (4) 'Complete crossing': individuals that

189 crossed the tunnel to the exit. The proportions of each behaviour category were

190 calculated as the number of individuals displaying the behaviour divided by the

191 number of individuals tested.

192 Additionally, in two Urodela species (*T. cristatus* and *S. salamandra*), the

193 crossing speed in tunnels 1 and 2 was evaluated for individuals that entirely crossed

194 the passage. This was estimated by the length of the tunnel (in metres) divided by the

195 time taken to completely cross the tunnel (in minutes). The mean speeds between

196 tunnels were compared with a Wilcoxon-Mann-Whitney test.

197

198 *2.5 Acoustic enrichment*

199 In 2019, an acoustic enrichment experiment was performed during the crossing

200 experiments. In this test, we used a soundtrack of a mixed chorus of edible frog

16

201   *Pelophylax kl esculentus* and European tree frog *Hyla arborea*, the two main species

202 using mating calls in the ponds in our study area. The recordings were made with an

203   omnidirectional microphone (Sennheiser ME62/K6, flat frequency response between

204   20 Hz and 20 kHz) coupled with a Fostex FR2LE recorder. The signal was sampled at

205   44.1 kHz with a 16-bit dynamic. Files were saved in .wav format. The stereo

206   soundtrack was emitted using a D-Jix M349BT MP3 audio player connected to two

207   loudspeakers (BOOM 2, Ultimate Ears). The loudspeakers were installed outside the

208   tunnel, 10m from the exit and space 5m apart. The average amplitude of the signal

209 emitted was set at 74.8 ± 0.8 dB with a Lutron SL-4001 sound level meter (mean ± SD,

210 1 m from the loudspeaker, C weighting, slow settings, re: 20 µ Pa). The number of

211   individuals and the species tested in these experiments are provided in Table 1.

212

213   *2.6. Statistical analysis*

214   The proportion of each behaviour category was compared between tunnels (of

17

215 different lengths and with or without acoustic enrichment) using the Fisher216

Freeman-Halton test or χ^2 test (depending on the sample size for each behaviour

217 category). The mean speed of individuals that completely crossed a tunnel was

218 compared between tunnels with a Wilcoxon-Mann-Whitney test.

219

3. Results

221 *3.1 Crossing behaviour and speed in tunnels of different lengths*

222 In adult *T. cristatus* newts, the proportion of behaviour categories significantly

223 differed between tunnel 1 and 2 (tunnel 1 N = 222; tunnel 2 N = 59, χ^2 test, p-value:

224 0.00091). More 'complete crossings' and less 'U-turns' were observed in the shorter 30

225 tunnel (figure 2a). In juvenile *T. cristatus* newts, the proportion of behaviour

226 categories was not compared between tunnel 1 and 2 because of the low number of

227 individuals in tunnel 2 (tunnel 1 N = 16; tunnel 2 N = 3, figure 2b). In *S. salamandra*

18

228 adults, the proportion of behaviour categories also significantly differed between

229 tunnel 1 and 2 (tunnel 1 N = 69; tunnel 2 N = 32, Fisher-Freeman-Halton test, p-value:

230 0.00091). More 'complete crossings' and fewer 'stays' were observed in the shorter

231 tunnel (figure 2c). In adult *R. dalmatina* frogs, the proportion of behaviour categories

232 did not differ between tunnel 1 and 2 (tunnel 1 N = 50; tunnel 2 N = 9, Fisher

233 Freeman-Halton test p-value: 0.07). However, no 'complete crossings' and more 'U

234 turns' were observed in the longer tunnel (figure 2d). In adult *B. spinosus* toads, the

235 proportion of behaviour categories did not differ between tunnel 1 and 2 (tunnel 1 N

236 = 5; tunnel 2 N = 14, Fisher-Freeman-Halton test, p-value: 0.5, figure 2e). This was

237 also the case for juvenile *B. spinosus* toads (tunnel 1 N = 7; tunnel 2 N = 15, Fisher

238 Freeman-Halton test, p-value: 0.6, figure 2f).

239 According to the previous results, individuals (in *S. salamandra* and *T. cristatus*)

240 that completely crossed a tunnel allowed estimating a mean crossing speed. In *S.*

19

241 *salamandra* adults, the crossing speed did not differ between tunnel 1 and 2 (tunnel 1

242 N = 47; tunnel 2 N = 11, Wilcoxon-Mann-Whitney test, p-value: 0.71, figure 3). This

243 was also the case in *T. cristatus* adults (tunnel 1 N = 88; tunnel 2 N = 8, Wilcoxon-

244 Mann-Whitney test, p-value: 0.31, figure 3).

245

246 *3.2   Comparisons between species*

247 Behaviour categories observed in tunnels 1 and 2 in *T. cristatus* and *B. spinosus* adults

248 and juveniles, and in *S. salamandra* and *R. dalmatina* adults are shown in figure 2. Of

249 these species, we found that *T. cristatus* adults were the least likely to enter the

250 tunnels (figure 2a), while *S. salamandra* completed the most crossings (figure 2c), and

251 *R. dalmatina* did the most U-turns (figure 2c).

20

252   Concerning crossing speed, *T. cristatus* adults crossed tunnel 1 more quickly

253   than *S. salamandra* adults (*T. cristatus* N = 88; *S. salamandra* N = 47, Wilcoxon-Mann-

254 Whitney test, p-value: 0.043, figure 3).

255

256   *3.3   Acoustic enrichment in tunnels*

257   In juvenile *T. cristatus* newts (tunnel 1), the proportion of behaviour categories

258   did not differ with or without acoustic enrichment (without N = 16; with N = 14,

259   Fisher-Freeman-Halton test, p-value: 0.21, figure 4a). The number of juveniles that

260   completed crossings was too low to calculate the crossing speed (Table 1). In *T.*

261 *cristatus* adults (tunnel 1), the proportion of behaviour categories significantly

262 differed with and without acoustic enrichment (without enrichment N = 229; with

263 enrichment N = 46, χ^2 test p-value: 0.030). More 'complete crossings' and less 'U-

264 turns' were observed in the acoustically enriched tunnel (figure 4b). However, the

21

265   crossing speed did not change (without enrichment N = 88; with enrichment N = 32,

266   Wilcoxon-Mann-Whitney test p-value: 0.63, figure 5). In tunnel 2, in adult *T. cristatus*

267 newts, the proportion of behaviour categories did not differ with or without acoustic

268   enrichment (without N = 59; with N = 46, Fisher-Freeman-Halton test, test p-value:

269   0.22, figure 4c), nor did enrichment change the crossing speed (without N = 8; with N

270   = 7, Wilcoxon-Mann-Whitney p-value: 1.0, figure 5). In tunnel 3, the proportion of

271   behaviour categories in adult *T. cristatus* newts did not differ with or without

272   acoustic enrichment (without N = 62; with N = 71, Fisher-Freeman-Halton test, test p-

273   value: 0.57, figure 4d), nor did enrichment change the crossing speed (without N =

274   17; with N = 15, Wilcoxon-Mann-Whitney test p-value: 0.31, figure 5).

275   In adult *T. marmoratus* newts (tunnel 3), the proportion of behaviour categories

276   did not differ with or without acoustic enrichment (without N = 14; with N = 14,

22

277 Fisher-Freeman-Halton test, test p-value: 0.45, figure 4e). The number of individuals

278 completely crossing the tunnels was too low to calculate crossing speed (Table 1).

279 In *I. alpestris* adults (tunnel 3), the proportion of behaviour categories did not

280 differ with or without acoustic enrichment (without N = 36; with N = 30, Fisher-

281 Freeman-Halton test, test p-value: 0.12, figure 4f). The number of individuals

282 completely crossing the tunnels was too low to calculate crossing speed (Table 1).

283 In *Pelophylax* kl. adults (tunnel 3), the proportion of behaviour categories

284 significantly differed with and without acoustic enrichment (without enrichment N =

285 228; with enrichment N = 35, χ^2 test, p-value: 1×10^{-13}). The four categories showed

286 marked changes in the enriched tunnel: only one individual did not move, only one

287 individual stayed in the tunnel, and only one did a U-turn. The majority, 91% (N =

288 35), of the tested frogs completely crossed the enriched tunnel (Figure 4g). Moreover,

23

the crossing speed in this tunnel was doubled (without N = 55; with N = 32,

Wilcoxon-Mann-Whitney test, p-value: 7.7×10^{-7}, Figure 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 (Beebee, 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

24

303   Miaud, 2018; Woltz et al., 2008), especially for European newts (Matos et al., 2017).

304   Evaluating the behaviour of amphibians in tunnels is challenging (see a review

305   in Testud et al., 2019). The lack of behavioural data on small species crossing tunnels

306 is partly due to methodological issues (Weber et al., 2019). For example, camera traps

307   are widely used for movement tracking in tunnels (reviewed in Jumeau, 2017). The

308   detection rate can be low with amphibians (Pagnucco et al., 2012), but the current

309   increase in the quality of video traps make them usable with amphibians (Hobbs and

310   Brehme, 2017; Jarvis et al., 2019; Pomezanski and Bennett, 2018). Marking

311   amphibians with fluorescent pigments was used as a method to record continuous

312   tracks on the tunnel floor, but this method is limited in terms of the number of

313 individuals that can be monitored and does not provide information on movement

25

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).

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 320 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.,

326 1964). In future, it would also be interesting to perform similar homing experiments

327 to evaluate tunnel use with newts caught on land (i.e. before they enter the aquatic

328 phase).

329 Our experimental design raised another issue about movement behaviour. The

330 specimens were released at the entrance of a tunnel every minute. We analysed the

331 data considering each specimen trajectory as independent; however, several

332 individuals (of the same species) were present in the tunnel and could potentially

333 interact. Newts can use specific and heterospecific information (e.g. density) to

334 emigrate from ponds (Cayuela et al., 2018). Laboratory experiments showed that

335 juvenile *T. cristatus* can detect scents left on the substrate by conspecifics (Hayward et

336 al., 2000). However, the use of conspecific cues during migration on land remains

337 unknown. In anurans such as toads, intra- and inter-sexual interactions such as

27

338 couple forming, can potentially change the movement of migrating adults (e.g.

339 Arntzen, 1999). In our experiments, 4 female and 17 male *B. spinosus* were tested, but

340 we do not have information on their interactions. Lastly, water frogs, which travel

341 mainly by jumping, could also potentially influence conspecifics when crossing

342 tunnels. Nonetheless, despite the fact that certain complex behavioural interactions

343 may have occurred in the tunnels during the experiments, we can consider that these

344 were similar in all tunnels and tested conditions and so did not bias the comparisons.

345   Studies have tested how tunnel characteristics influence the crossing of

346 amphibians with 'experimental' tunnels (i.e. devices mimicking tunnels implemented

347 below LTIs). Tested characteristics include diameter (Patrick et al., 2010), length

348 (Patrick et al., 2010), substrate (Lesbarrères et al., 2004; Trochet et al., 2019), and light,

349 humidity and temperature (Bain et al., 2017). Our study was performed in 'real'

350 tunnels below a high-speed railway track; tunnels whose dimensions differed only in

351   length. In experiments with two tunnels (21 m and 40 m long – both 1 m wide and 1

352   m high), the longer length was found to impact the proportion of complete crossings

353   by *S. salamandra* (from 68% to 34%), adult *T. cristatus* (from 38% to 14%) and *R.*

354   *dalmatina* (from 34% to 0%). No effect of length was observed for *B. spinosus*, but this

355   result needs to be confirmed with a higher number of tested individuals. In other

356   studies, a mean crossing rate of 74.5% for the crested newt and 80.6% for the smooth

357   newt (*Lissotriton vulgaris*) were observed in a 'real' tunnel 24 m in length and 0.5 m in

358   diameter (Jarvis et al., 2019). About 38% of the individuals of three species of

359   Australian frogs (*Limnodynastes peronii, Litoria aurea* and *L. latopalmata*) entering a

360   tunnel 12 m long and 0.5 m wide crossed it completely (Hamer et al., 2014). In the

361   California tiger salamander *Ambystoma californiense*, 51% of individuals completely

362   crossed a 'real' tunnel 22 m in length and 0.25 m in diameter (Bain et al., 2017). In the

363   same study, the crossing rate was not influenced by the relative humidity of the

364 tunnel, though the mean speed of this salamander was 0.77 m/min in the dry tunnel

365 and 1 m/min in the wet tunnel (Bain et al., 2017). In the (Hamer et al., 2014) study,

366 Australian frogs had a mean speed of 0.8 m/min. In our experiments, adult *T.*

367 *cristatus* newts crossed the 21-m tunnel at a mean speed of 0.39 ± 0.03 m/min (N=88),

368 which was faster than the *S. salamandra* adults (mean speed = 0.29 ± 0.02 m/min,

369 N=47). The length of the crossed tunnels did not affect the speed of either *S.*

370 *salamandra* or *T. cristatus*.

371   When amphibians migrate or disperse and seek to orient themselves when

372 moving from point A to point B, they can use a redundant, multisensory orientation

373 system (Sinsch, 2006). This may include celestial information (Diego-Rasilla and

374 Luengo, 2002), magnetism (Diego-Rasilla, 2003; Diego-Rasilla et al., 2008, 2005;

375 Fischer et al., 2001; Phillips, 1986), and olfactory cues (Joly and Miaud, 1993). The

376   chorus attraction hypothesis (Gerhardt and Huber, 2002) postulates that amphibians

377   can also use conspecific and heterospecific amphibian calls to locate a site of interest

378   (Buxton et al., 2015; Diego-Rasilla and Luengo, 2007, 2004; James et al., 2015; Madden

379   and Jehle, 2017; Pupin et al., 2007; Swanson et al., 2007). Even non-calling species

380   such as newts can exhibit positive phonotaxis for sympatric anuran songs: adult

381   marbled newts (*T. marmoratus*) orientate to the calls of the *E. calamita*) (Diego-Rasilla

382   and Luengo, 2004). Adult palmate newts (*Lissotriton helveticus*) orientate to the calls

383   of Iberian water frog *Pelophylax perezi*) (Diego-Rasilla and Luengo, 2007). Adult *L.*

384   *vulgaris* orientate to the calls of the common toad (*Bufo bufo*) and European green

385   toad (*Bufotes viridis*) (Pupin et al., 2007). Adult *T. critatus* orientate to the call of the *B.*

386   *bufo*, but not to that of the sympatric common frog (*Rana temporaria*) (Madden and

387   Jehle, 2017).. Similarly, the palmate newt *Lissotriton helveticus* did not orientate to *R.*

388   *temporaria* calls ((Diego-Rasilla and Luengo, 2007). This lack of response to the

31

389 common frog call was explained by the fact that the newt's breeding migration did

390   not overlap with the frog's calling period. These four cited studies were performed in

391 experimental conditions using circular arenas. Very few studies have used acoustic

392 enrichment to direct amphibians towards a target in nature. Of these, the

393 colonization of new ponds by the green and golden bell frog (*Litoria aurea*) was

394   encouraged using acoustic enrichment (speakers with conspecific frog calls, James et

395 al., 2015). Similarly, the Cope's gray treefrog (*Hyla chrysoscelis*) and the American

396 toad (*Anaxyrus americanus*) colonized new habitats where conspecific chorus calls

397 were broadcasted (Buxton et al., 2015).

398   To our knowledge, no previous studies have been conducted on acoustic

399 enrichment in natural environments with Urodela species, and particularly in the

32

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

33

413   the fire salamander and crested newt; previous studies have recommended limiting

414   the length of wildlife passages as much as possible (Patrick et al., 2010). Construction

415   constraints can result in rather long tunnels, and increasing the width or diameter

416   (while keeping costs reasonable) in order to increase the use by amphibians still

417 remains to be tested (Colino-Rabanal and Lizana, 2012; Puky, 2003). The effects of other

418   characteristics (humidity, light, substrate, etc.) also need more investigation, with

419 careful consideration of interspecific differences (Baguette and Dyck, 2007; Lesbarrères

420 et al., 2010; Puky et al., 2007).

421 Studies have shown that acoustic enrichment is successful at attracting amphibians to

422 newly created aquatic sites. In this study, our experiments demonstrated the potential

423 of this method in improving the effectiveness of wildlife passages: both the water frog

424 (when conspecific calls were broadcast) and the crested newt (when heterospecific

34

425 calls were broadcast) performed better in tunnel 1 (21 m) – however, they did not 426

complete more crossings in the other two acoustically enriched tunnels.

427 These results could be extended with further studies focusing on the type of

428 emitted calls, e.g. mixing heterospecific vocalizations with conspecific vocalizations of

429 the target species (Putman and Blumstein, 2019), frequency and sound amplitude, the

430 distance and position of loudspeakers in the tunnel, etc. As including loudspeakers in

431 a wildlife passage could be costly (in terms of installation, risk of degradation, etc.),

432 acoustic enrichment could be planned for specific periods (the best timing remains to 25

433 be evaluated): for example, to allow the establishment or reinforcement of a breeding

434 migration path. Another option could be natural acoustic enrichment, e.g. by creating

435 breeding sites colonized by a community of anurans close to the wildlife passage. As

436 the effectiveness and durability of acoustic playback on attracting animals for wildlife

35

437   management is poorly known, further investigation would be valuable in evaluating

438   this in wildlife passages for small fauna.

439

Declaration of competing interest

441   The authors declare that they have no known competing financial interests or

442 personal relationships that could have appeared to influence the work reported in

443   this paper.

444

445   **Acknowledgements**

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Figure

Figure 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)
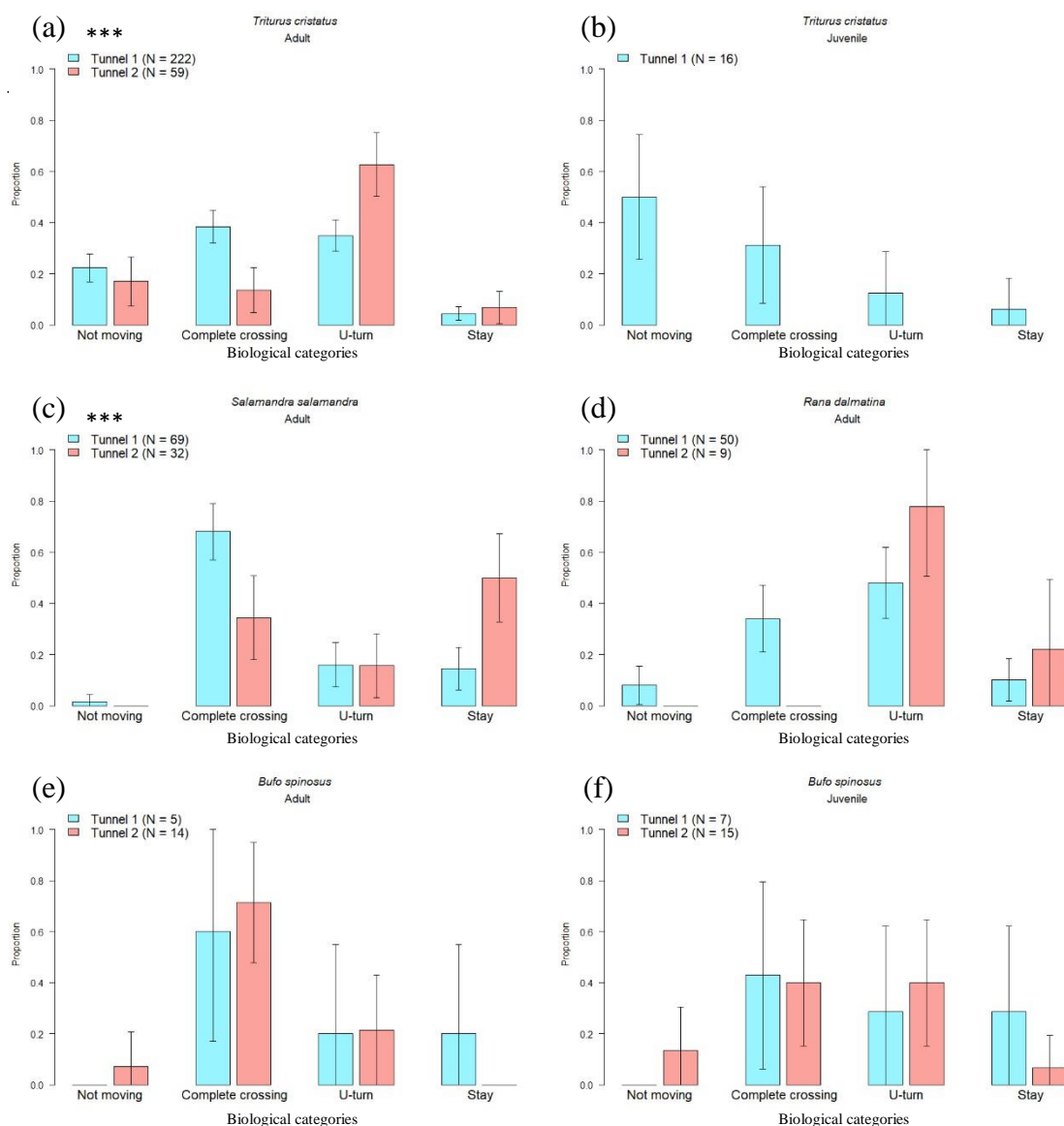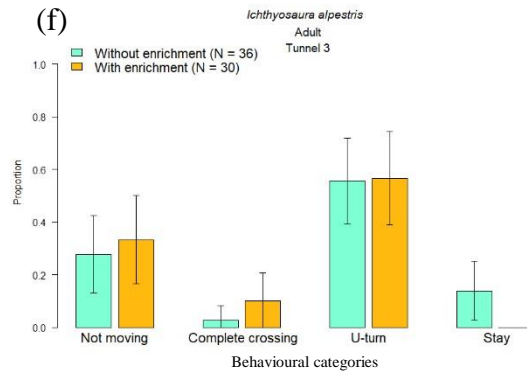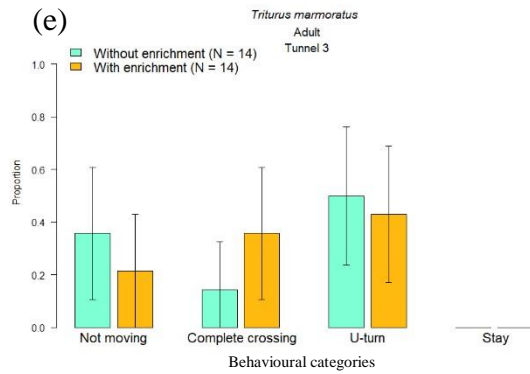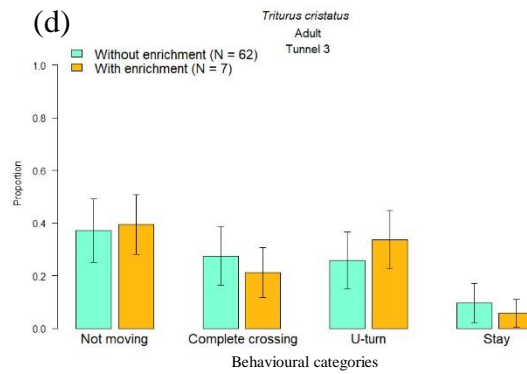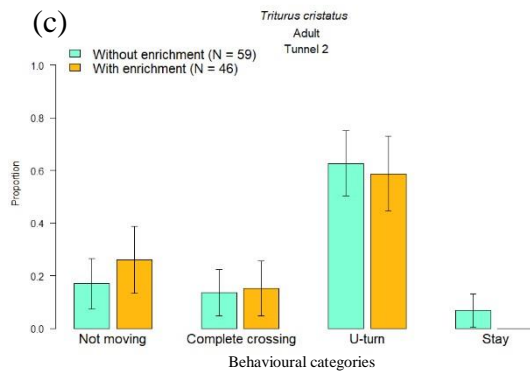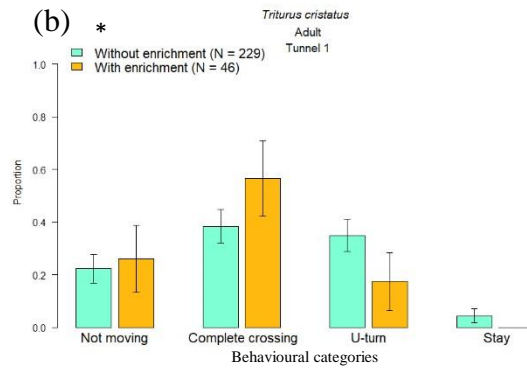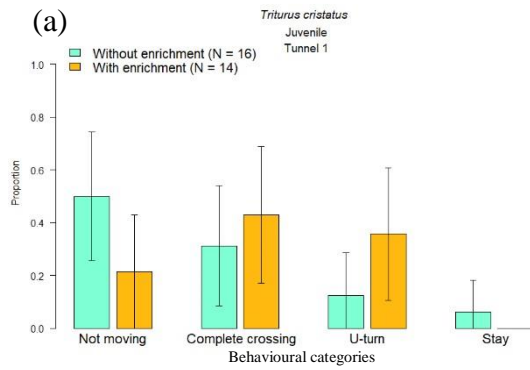

Figure 3: Mean speed of two amphibian species (*Salamandra salamandra* and *Triturus cristatus,* both adults) crossing two tunnels of different lengths. Blue bars: Tunnel 1 (21 m length, see figure 1) and light red bars: tunnel 2 (40 m length). N: sample size, vertical bars; confidence intervals at 0.05.
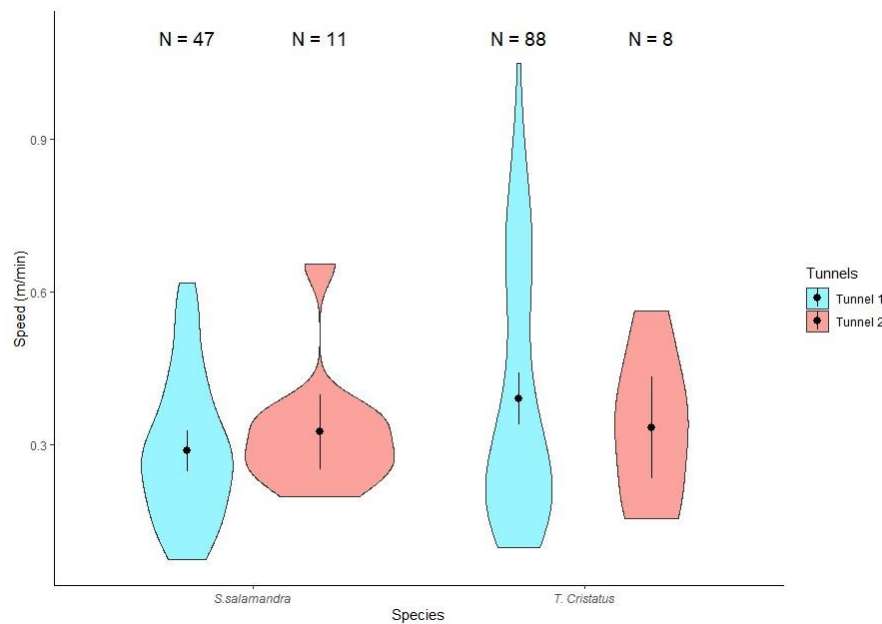
Figure 4: Proportion of different behavioural patterns for four amphibian species in three tunnels with and without acoustic enrichment. Patterns: 'Not moving', 'Complete crossing', 'U-turn' and 'Stay' (described in 'Materials and methods'). Green bars: without acoustic enrichment, orange bars = with 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) *Ichtyosaura 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)

Figure

(a) *Triturus cristatus* Juvenile Tunnel 1

(b) * *Triturus cristatus* Adult Tunnel 1

(c) *Triturus cristatus* Adult Tunnel 2

(d) *Triturus cristatus* Adult Tunnel 3

(e) *Triturus marmoratus* Adult Tunnel 3

(f) *Ichthyosaura alpestris* Adult Tunnel 3

(g) *** *Pelophylax spp* Adult Tunnel 3
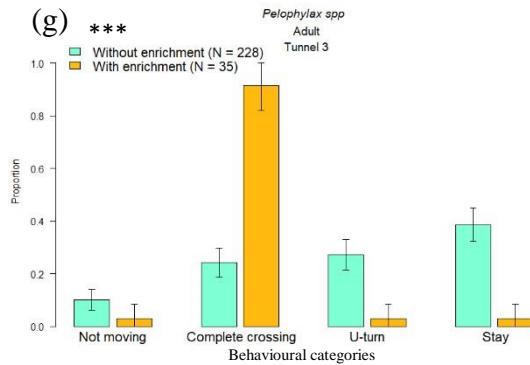
Figure

1   **Figure 5:** Crossing speed of two amphibian species (*Triturus cristatus* and *Pelophylax* kl., 2
     both adults), in three tunnels with and without acoustic enrichment. Green bars: Tunnel 1 (21
3   m length, dry), and orange bars: tunnel 2 (40 m length, dry) and tunnel 3 (18.5 m length,
wet).
4 N: sample size, vertical bars; confidence intervals at 0.05. * refers to test significance (*: 5
P<0.05; **: P<0.01; ***: P<0.001)

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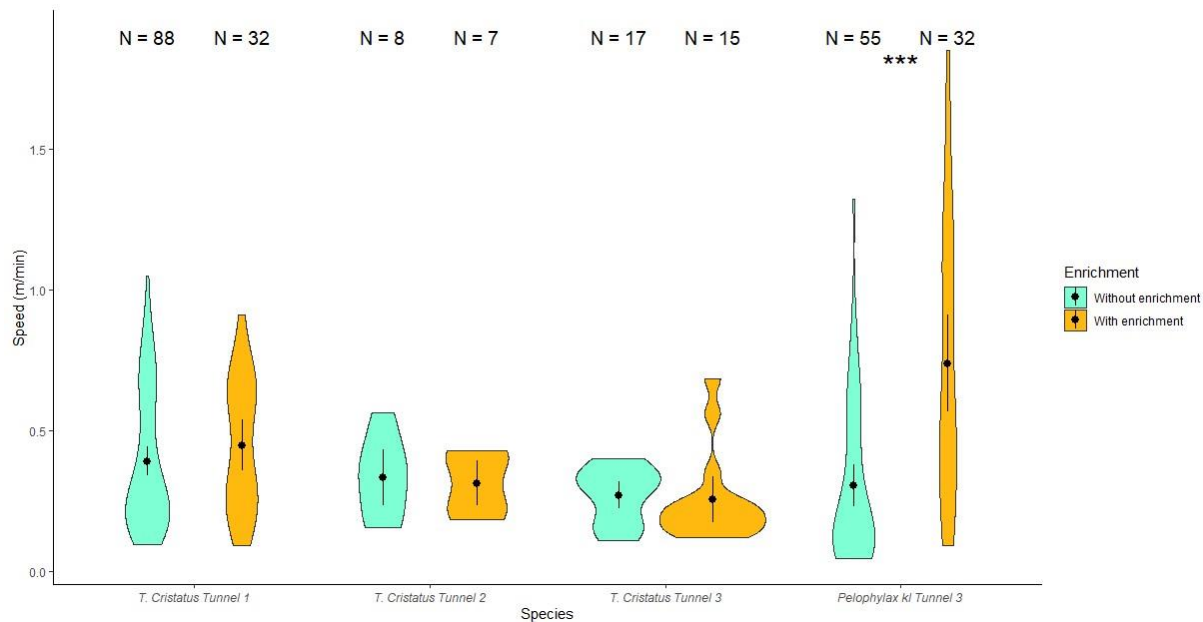
Figure

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

Conflict of Interest

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: