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

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

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Abstract:	<p>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 <i>Triturus cristatus</i>, marbled newt <i>Triturus marmoratus</i>, alpine newt <i>Ichthyosaura alpestris</i>, fire salamander <i>Salamandra salamandra</i>, spined toad <i>Bufo spinosus</i>, agile frog <i>Rana dalmatina</i> and edible/pool frog <i>Pelophylax kl. esculentus/lessonae</i>) 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. <i>R. dalmatina</i>, <i>S. salamandra</i> and <i>T. cristatus</i> (but not <i>B. spinosus</i>) 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 <i>S. Salamandra</i> and <i>T. cristatus</i> 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. <i>P. kl. esculentus/lessonae</i> exhibited a large increase in complete crossings and in speed. <i>T. cristatus</i> 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.</p>

1 Acoustic enrichment in wildlife passages under railways improves their
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4 2 use by amphibians

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28 14 ABSTRACT

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30 15 Linear transport infrastructure can impact wildlife directly or indirectly, and
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32 16 amphibians are particularly affected. In some cases, mitigation measures – such as
33 17 tunnels below the linear transport infrastructure – are implemented, but an

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18 evaluation of their effectiveness is often lacking, especially for small animals such as
19 amphibians. Few studies have focused on crossing behaviour in underpasses, yet this
20 information may be crucial to improve mitigation measures. In this study, we
21 conducted experiments in three tunnels below a high-speed railway with 40
22 individually marked amphibians (great crested newt *Triturus cristatus*, marbled newt
23 *Triturus marmoratus*, alpine newt *Ichthyosaura alpestris*, fire salamander *Salamandra
24 salamandra*, spined toad *Bufo spinosus*, agile frog *Rana dalmatina* and edible/pool
frog
25 *Pelophylax kl. esculentus/lessonae*) which were released at the entrance of tunnels 47
26 varying in length. Behaviours (e.g. completed crossing, U-turn, not moving, and stay)
27 and crossing speed were recorded. *R. dalmatina*, *S. salamandra* and *T. cristatus* (but not
28 *B. spinosus*) exhibited crossing rate differences between tunnels 21 m and 40 m in 51
29 length. More individuals performed “complete crossing” through the shorter tunnel.
30 The crossing speed of the *S. salamandra* and *T. cristatus* did not differ between the
31 tunnels of different lengths. In a second experiment, we evaluated the effect of 32
32 acoustic enrichment on the crossing behaviour of one frog and three newt species
33 by broadcasting a soundtrack of mating calls of syntopic anuran species in the
34 tunnels.
35 *Pelophylax kl* exhibited a large increase in complete crossings and in speed. *T. cristatus*
36 showed an increase in tunnel complete crossings (but not speed) in one of the
37 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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38 method could help to inform the design of wildlife passages as mitigation measures 39 for
impacted amphibian populations, though more experiments on the effect of 40 tunnel
characteristics on amphibian behaviour would be valuable.

10 41

11 42 **Keywords:** Transport infrastructure, Tunnels, Mitigation evaluation, Acoustic 12
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47 **1. Introduction**

48 Global passenger and freight travel is expected to double from its level in 2010 in
49 coming decades, and it is projected that around the world an additional 25 million

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11 50 kilometres of paved roads and 335,000 kilometres of railway tracks will be needed

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14 51 (Dulac, 2013). The effects of linear transport infrastructure (LTI) such as roads and

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17 52 railways on animals have long been known (Barbour, 1895) and have been

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20 53 extensively studied (e.g. Rytwinski and Fahrig, 2015). The construction of LTI in

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23 54 landscapes can directly alter the physical environment by changing the habitat

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26 55 surface as well as its connectivity, impacting animals through direct mortality and

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29 56 behaviour changes in land use and movements (Jackson and Griffin, 2000;

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31 57 Trombulak and Frissell, 2000). The resulting decrease in population size, landscape

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34 58 connectivity and dispersal can significantly alter the viability of populations

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37 59 (Eigenbrod et al., 2008; Forman and Alexander, 1998; Glista et al., 2009; Hamer et al.,

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40 60 2014; Lesbarrères and Fahrig, 2012).

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43 61 By affecting connectivity, landscape fragmentation caused by LTI can alter the

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46 62 two main types of animal movements: (1) migration (i.e. seasonal movements

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49 63 between different landscapes at different times of an individual's lifecycle), and (2)

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52 64 dispersal (i.e. an individual's movement away from its birth area to reproduce in a

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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

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67 the groups most affected by landscape fragmentation (Beebee, 2013; Cushman,
2006; Holderegger 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

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79 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.

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

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96 broadcasting vocalizations (Putman and Blumstein, 2019). In amphibians, acoustic
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25 26 enrichment with conspecific chorus calls has been shown to improve the colonization
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28 of new ponds by frogs and toads (Buxton et al., 2015; James et al., 2015). These
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30 findings suggest that acoustic enrichment might be an interesting avenue to explore
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33 to increase the effectiveness of wildlife passages.
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36 The aim of this study was to evaluate the use of wildlife passages by
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38 101 amphibians and investigate methods to improve their effectiveness. To this end we
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41 102 studied the movement of seven European amphibian species (spined toad *Bufo*
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43 103 *spinosus*, agile frog *Rana dalmatina*, edible/pool frogs *Pelophylax kl. esculentus/lessonae*,
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45 fire salamander *Salamandra salamandra*, alpine newt *Ichthyosaura alpestris*, marbled
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47 newt *T. marmoratus* and great crested newt *Triturus cristatus*), in underpasses below a
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49 105 high-speed railway track in France by: (1) performing homing experiments to
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53 106 compare their ability to cross tunnels of two lengths in the same location, and (2)
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evaluation by analysing different crossing behaviours and speed in tunnels with and without the broadcasting of anuran calls.

the

efficacy **2. Materials and methods**

y of *2.1 Study area and wildlife crossing structures*

acoustic The study area was located in the region of Pays-de-la-Loire in northwestern France.

ic Our focus was on three wildlife crossing structures constructed at different locations

enrich under the same high-speed railway track. Tunnels 1 and 2 are separated by a

109 distance of 800 m and are close to the town of Chantenay-Villedieu. Tunnel 3 is about

110 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

112 m in height (figure 1): in length, tunnel 1 is 21 m, tunnel 2 is 40 m and tunnel 3 is 18.5

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11 113 m. Tunnels 1 and 2 are dry fauna-crossing structures installed for amphibians, while

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14 114 tunnel 3 is a hydraulic structure that had a light trickle of water during the

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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 30

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

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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

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49 126 captured using methods that depended on the species. *B. spinosus* and *S. salamandra*
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52 127 were collected on land before reproduction (from February to early April in 2018 and
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128 2019) during nocturnal transects along railway track fences. Individuals of *R.*

129 *dalmatina* were caught in water at the beginning of the species' reproductive period
130 (from February to early March 2019) with minnow traps (mesh 4 mm). *P. kl.*

131 *esculentus/lessonae* (consisting of the edible frog *P. kl. esculentus* and the pool frog *P.*
132 *lessonae*: hereafter, *Pelophylax kl.*) were caught in ponds before egg-laying (mid-
May
133 2018 and 2019) manually or with a dip-net at night with the help of a flashlight. *I.*
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11 134 *alpestris* and *T. marmoratus*) were caught in ponds during their reproductive period
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14 135 (mid-May 2019) with minnow traps. *T. cristatus* individuals were caught in ponds
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17 136 during their reproductive period (from mid-February to mid-May 2018 and 2019)
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20 137 with minnow traps. A total of 1179 individuals were captured: the numbers for each
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23 138 species are provided in Table 1.

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26 139 The captured specimens were marked with transponders (PIT-tags, 1.4 × 8 mm,
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29 140 weight 34.2 mg, Biolog-Tiny REF 10268 [R02-0717]), consisting of electronic chips 30
31 141 packaged in a 'biocompatible' glass ampoule that meet international standards for
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34 142 animal identification (ISO 11784/11785). The animals were anaesthetized with 2%
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37 143 xylocaine (ointment). The PIT-tags were injected subcutaneously at the intersection
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40 144 of the belly and flank with a PIT-tag injector (Biolog-Tiny REF 10268 [R0 955]). They
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43 145 were housed in several opaque plastic tanks (0.80 × 0.40 × 0.25 m) with moist forest
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46 146 litter. They were kept outside, close to the capture site, in a shaded place with an
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49 147 ambient temperature ranging from 5°C (night) to 15°C (day). The time spent in 50
51 captivity between capture and the experiment was about 1–2 days for newts, toads 52 148
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54 149 and frogs. As the number of *S. salamandra* captured in a night could be low, some of
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63 150 these individuals were housed for up to 10 days. The results obtained with

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151 individuals housed 1 to 5 days and individuals housed 6-10 days were similar
152 (χ^2 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

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11 156 from the prohibition to capture and transport specimens of protected animal species,

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14 157 29 May 2017). The protocols used in this study were approved by the Ethical

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17 158 Committee of UMR 5175 CEFE (Montpellier, France), decision of the committee held

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20 159 on 27 June 2017.

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26 161 *2.3 Experiments*

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29 162 Before an experiment, each individual was identified by a hand-held RFID reader 30

31 163 (reader RS1-F1, Biolog-ID©, reading distance = 4 to 10 cm). One experimenter with a

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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

170 individual's number and the exact time of exit and then returned it to a new storage

171 tank.

172 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

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

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178 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.

182

2.4 *Crossing behaviour*

184 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

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49 189 crossed the tunnel to the exit. The proportions of each behaviour category were 50
51 calculated as the number of individuals displaying the behaviour divided by the 52 190
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54 191 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

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11 196 tunnels were compared with a Wilcoxon-Mann-Whitney test.

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17 198 2.5 *Acoustic enrichment*

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20 199 In 2019, an acoustic enrichment experiment was performed during the crossing

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23 200 experiments. In this test, we used a soundtrack of a mixed chorus of edible frog

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26 201 *Pelophylax kl esculentus* and European tree frog *Hyla arborea*, the two main species

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29 202 using mating calls in the ponds in our study area. The recordings were made with an 30

31 203 omnidirectional microphone (Sennheiser ME62/K6, flat frequency response between

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34 204 20 Hz and 20 kHz) coupled with a Fostex FR2LE recorder. The signal was sampled at

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37 205 44.1 kHz with a 16-bit dynamic. Files were saved in .wav format. The stereo

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40 206 soundtrack was emitted using a D-Jix M349BT MP3 audio player connected to two

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43 207 loudspeakers (BOOM 2, Ultimate Ears). The loudspeakers were installed outside the

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46 208 tunnel, 10m from the exit and space 5m apart. The average amplitude of the signal

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49 209 emitted was set at 74.8 ± 0.8 dB with a Lutron SL-4001 sound level meter (mean \pm SD, 50

51 1 m from the loudspeaker, C weighting, slow settings, re: 20μ Pa). The number of 52 210

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54 211 individuals and the species tested in these experiments are provided in Table 1.

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213 2.6. Statistical analysis

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

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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

220 **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*

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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 (figure 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 (figure 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, figure 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, figure 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.*

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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).

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).

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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, figure 3).⁵⁴

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, figure 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, χ^2 test p-value: 0.030). More ‘complete crossings’ and less ‘U-turns’ were observed in the acoustically enriched tunnel (figure 4b). However, the

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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 30

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,

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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-

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12 281 Freeman-Halton test, test p-value: 0.12, figure 4f). The number of individuals

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15 282 completely crossing the tunnels was too low to calculate crossing speed (Table 1).

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18 283 In *Pelophylax* kl. adults (tunnel 3), the proportion of behaviour categories

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21 284 significantly differed with and without acoustic enrichment (without enrichment N =

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24 285 228; with enrichment N = 35, χ^2 test, p-value: 1×10^{-13}). The four categories showed

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27 286 marked changes in the enriched tunnel: only one individual did not move, only one

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30 287 individual stayed in the tunnel, and only one did a U-turn. The majority, 91% (N =

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33 288 35), of the tested frogs completely crossed the enriched tunnel (Figure 4g). Moreover,

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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

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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 25

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 45

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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 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.,

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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 (Cayueta 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

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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

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11 344 were similar in all tunnels and tested conditions and so did not bias the comparisons.

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15 345 Studies have tested how tunnel characteristics influence the crossing of

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18 346 amphibians with 'experimental' tunnels (i.e. devices mimicking tunnels implemented

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21 347 below LTIs). Tested characteristics include diameter (Patrick et al., 2010), length

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23 348 (Patrick et al., 2010), substrate (Lesbarrères et al., 2004; Trochet et al., 2019), and light,

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26 349 humidity and temperature (Bain et al., 2017). Our study was performed in 'real'

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29 350 tunnels below a high-speed railway track; tunnels whose dimensions differed only in

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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

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

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11 365 and 1 m/min in the wet tunnel (Bain et al., 2017). In the (Hamer et al., 2014) study,

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14 366 Australian frogs had a mean speed of 0.8 m/min. In our experiments, adult *T.*

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17 367 *cristatus* newts crossed the 21-m tunnel at a mean speed of 0.39 ± 0.03 m/min (N=88),

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20 368 which was faster than the *S. salamandra* adults (mean speed = 0.29 ± 0.02 m/min,

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23 369 N=47). The length of the crossed tunnels did not affect the speed of either *S.*

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26 370 *salamandra* or *T. cristatus*.

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29 371 When amphibians migrate or disperse and seek to orient themselves when

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32 372 moving from point A to point B, they can use a redundant, multisensory orientation

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35 373 system (Sinsch, 2006). This may include celestial information (Diego-Rasilla and

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38 374 Luengo, 2002), magnetism (Diego-Rasilla, 2003; Diego-Rasilla et al., 2008, 2005;

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41 375 Fischer et al., 2001; Phillips, 1986), and olfactory cues (Joly and Miaud, 1993). The

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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 (*Bufo 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

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389 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

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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

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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

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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
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12 428 emitted calls, e.g. mixing heterospecific vocalizations with conspecific vocalizations of
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15 429 the target species (Putman and Blumstein, 2019), frequency and sound amplitude, the
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18 430 distance and position of loudspeakers in the tunnel, etc. As including loudspeakers in
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21 431 a wildlife passage could be costly (in terms of installation, risk of degradation, etc.),
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24 432 acoustic enrichment could be planned for specific periods (the best timing remains to 25
26 433 be evaluated): for example, to allow the establishment or reinforcement of a breeding
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29 434 migration path. Another option could be natural acoustic enrichment, e.g. by creating
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32 435 breeding sites colonized by a community of anurans close to the wildlife passage. As
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35 436 the effectiveness and durability of acoustic playback on attracting animals for wildlife

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38 437 management is poorly known, further investigation would be valuable in evaluating
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46 440 **Declaration of competing interest**

47
48 441 The authors declare that they have no known competing financial interests or
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50 442 personal relationships that could have appeared to influence the work reported in 51
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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)

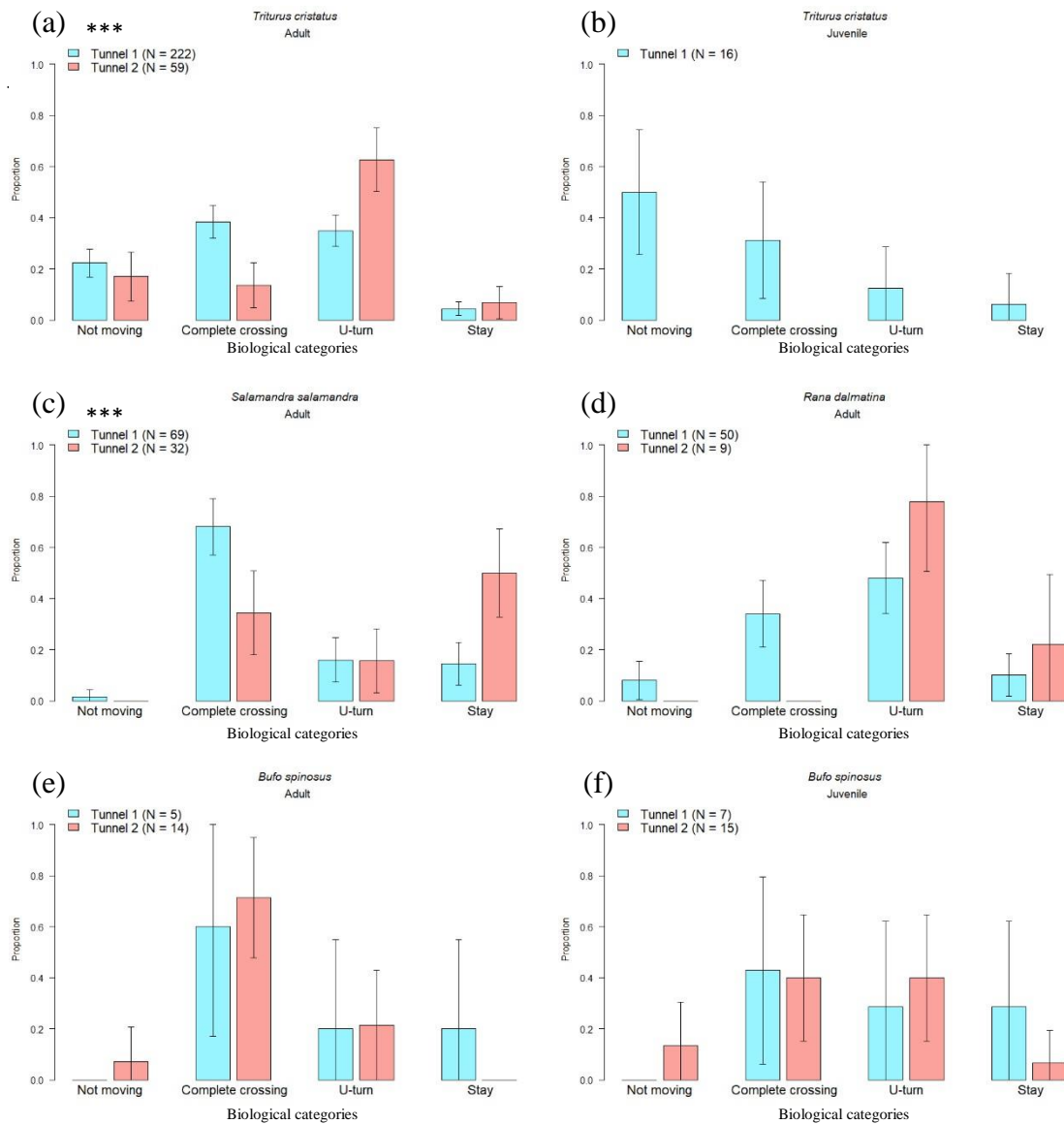


Figure 3: Mean speed of two amphibian species (*Salamandra salamandra* and *Triturus cristatus*, both adults) crossing two tunnels of different lengths. Blue bars: Tunnel 1 (21 m length, see figure 1) and light red bars: tunnel 2 (40 m length). N: sample size, vertical bars; confidence intervals at 0.05.

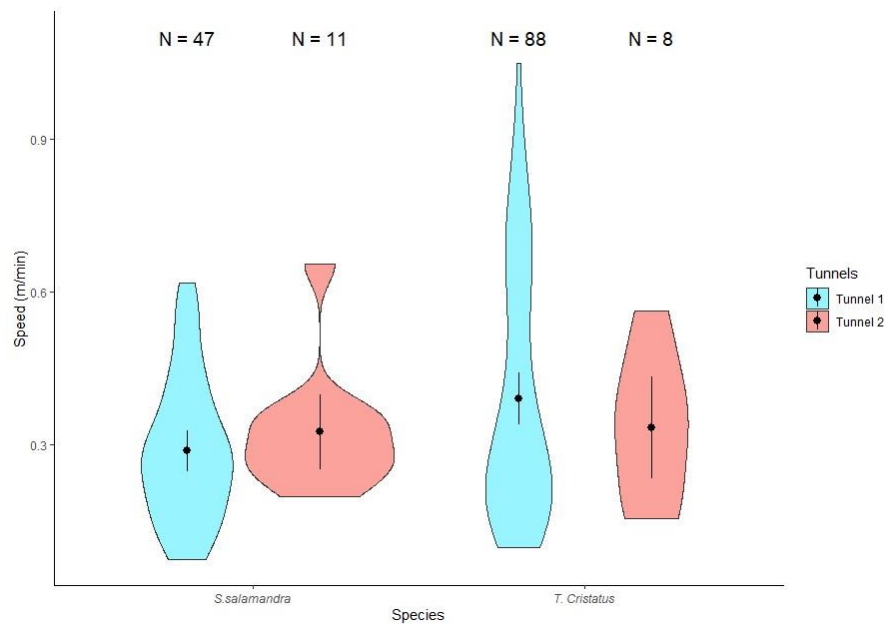
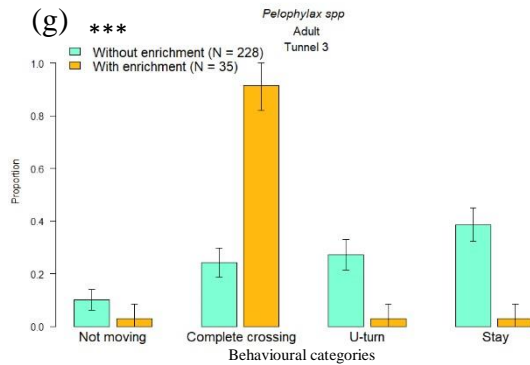
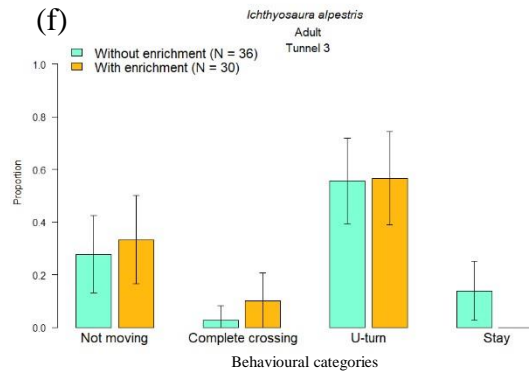
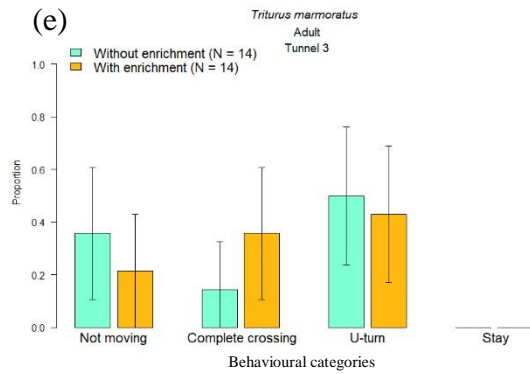
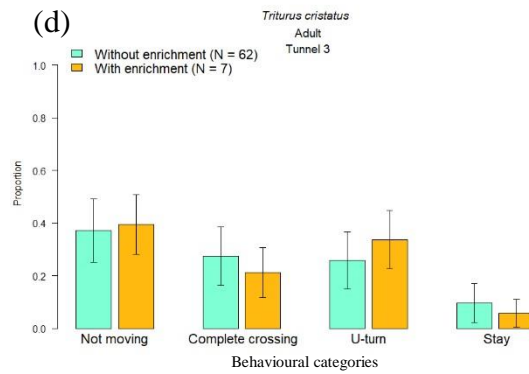
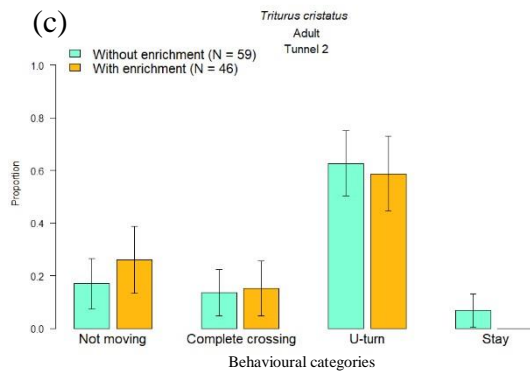
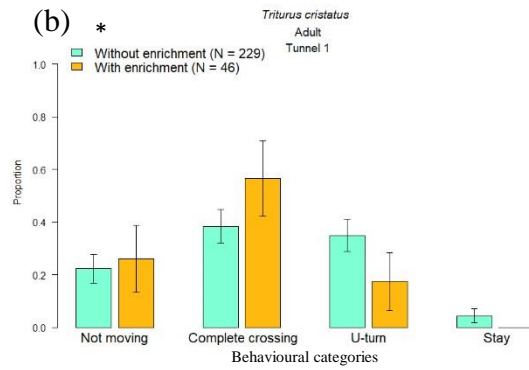
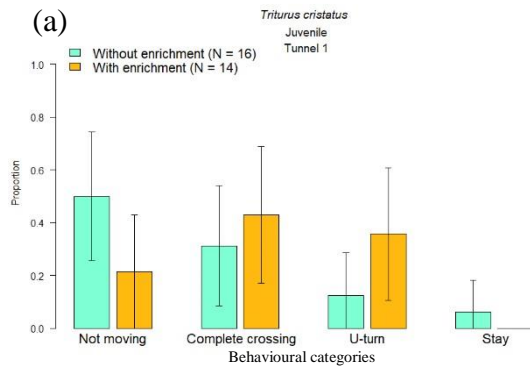


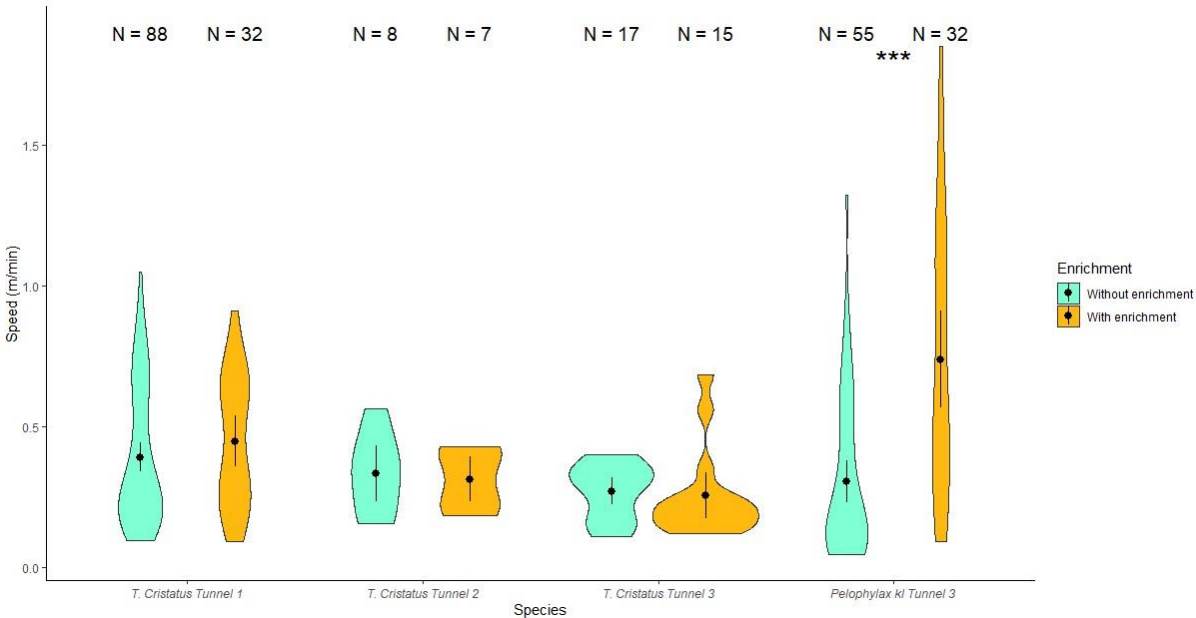
Figure 4: Proportion of different behavioural patterns for four amphibian species in three tunnels with and without acoustic enrichment. Patterns: ‘Not moving’, ‘Complete crossing’, ‘U-turn’ and ‘Stay’ (described in ‘Materials and methods’). Green bars: without acoustic enrichment, orange bars = with 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

1 **Figure 5:** Crossing speed of two amphibian species (*Triturus cristatus* and *Pelophylax kl.*, 2
3 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,
4 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: