#### **Short Communication**

# What type of amphibian tunnel could reduce road kills?

David Lesbarrères, Thierry Lodé and Juha Merilä

**Abstract** Increased traffic volumes worldwide are contributing to amphibian declines, and measures to reduce the occurrence of road kills are needed. One possible measure is the construction of underpasses through which animals can pass under roads, but little is known about whether amphibians will choose tunnels if given a choice or about their preferences for different tunnel types. We tested the preferences of three anuran species for two kinds of concrete amphibian tunnels currently used in France. One was a tunnel lined with soil, the other a bare concrete pipe. The animals could use the tunnels or bypass them over a grassy area. Water frogs *Rana esculenta* and common toads *Bufo bufo* showed a

preference for the tunnels, whereas agile frogs *Rana dalmatina* avoided them. Among the individuals that chose either of the tunnels, all species showed a significant preference for the tunnel lined with soil. These results indicate that species differ in their preferences and in their likelihood of using underpasses when given a choice. This highlights the fact that there is no unique solution to the problem, and underpasses are only one of the possible mitigation measures that need to be assessed.

**Keywords** Amphibian tunnels, *Bufo bufo*, choice tests, highways, *Rana dalmatina*, *Rana esculenta*, road kills.

The decline of amphibian populations throughout the world is now a well established fact, although the debate about the causes continues (Alford & Richards, 1999). One factor contributing to these declines is traffic mortality: during their annual movements a large proportion of adults in a given population might perish in road kills (Vos & Chardon, 1998; Means, 1999; Carr & Fahrig, 2001; Hels & Buchwald, 2001). Kuhn (1987) estimated that a traffic volume of 24-40 vehicles per hour would result in 50% mortality among migrating common toads Bufo bufo, while 60 vehicles per hour would cause 90% (Percsy, 1994). There are comparable figures for other species (van Gelder, 1973; Fahrig et al., 1995; Hels & Buchwald, 2001, and references therein). Even if increased traffic volumes do not cause direct extinctions, they may have indirect negative effects on local populations by increasing their isolation through habitat fragmentation (Andrews, 1990; Vos & Chardon, 1998). This in turn may have negative consequences for populations, as

thereby reduce viability (Saccheri *et al.*, 1998). Furthermore, reduced population sizes caused by fragmentation will increase the stochastic risk of extinction (Bennett, 1990).

A potentially simple measure to reduce traffic mortality, and fragmentation in amphibian populations is

isolation reduces genetic diversity (Sjögren-Gulve, 1994),

increases inbreeding (Lesbarrères et al., 2003) and may

ity and fragmentation in amphibian populations is the construction of amphibian tunnels or bridges, allowing animals to pass under or over roads that have high traffic volumes (van Leeuwen, 1982; Langton, 1989; Tyning, 1989; Beier & Noss, 1998). In France the total highway network has increased by 88% in the two last decades (Association des Sociétés Françaises d'Autoroutes et d'ouvrages à péage, 2001). Public and scientific committees initiated the construction of highway underpasses for amphibians in 1984 (Mougey, 1996) but their efficiency for different amphibian species has seldom been assessed (but see Simonyi et al., 1999; Veenbaas & Brandjes, 1999). Our aim was to assess three anuran species (common toads, water frogs Rana esculenta and agile frogs Rana dalmatina) for their preference or avoidance of tunnels used in highway construction in western France. In particular, we were interested to know whether: (1) there are inter-species differences in their preference for utilizing tunnels, and (2) there is preference for a particular type of tunnel.

The choice experiments were carried out in a 15 m<sup>2</sup> outdoor enclosure near the University of Angers, France,

David Lesbarrères (Corresponding author) and Juha Merilä Ecological Genetics Research Unit, Department of Biological and Environmental Sciences, PO BOX 65, FIN-00014, University of Helsinki, Finland.

E-mail david.lesbarreres@helsinki.fi

**Thierry Lodé** Laboratoire d'Écologie Animale, Université d'Angers, Belle-Beille, F-49045, Angers cedex, France.

Received 15 November 2002. Revision requested 10 April 2003. Accepted 5 September 2003.

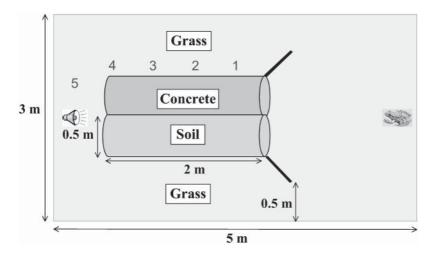


Fig. 1 Schematic representation of the experimental design for the choice experiments. Tape recorded male calls of each species, as appropriate, were played from the other end of the tunnels during the experiments to create a soothing environment for the animals. All tests were carried out at night (22.00–03.00). Numbers (1–5) refer to 'crossing success' scores: the distance each individual that entered into either of the tunnels had moved forward during the experiment.

between February and May 2001. The experimental design consisted of two concrete tubes (diameter = 0.5 m, length = 2 m) placed next to each other on a grass surface (Fig. 1). The bottom of one of the tunnels was lined with sand and humus and the other had a plain concrete surface. We placed two 0.5 m lengths of drift fence at a  $45^{\circ}$  angle with respect to the tunnel openings (Fig. 1) to simulate highway fences and provide the same probability of choices. Animals could also choose to bypass the tunnels and pass across the grass (Fig. 1).

Adult males and females (sexes were pooled in all analyses as they did not differ in their choice in any of the species) in breeding condition were used (42 water frogs, 32 agile frogs and 41 common toads). During the test period animals were maintained in individual vivariums and released back to their native ponds after the experiments.

The experiments began by placing a single test animal 1.2 m in front of the tunnels. Each experiment lasted for 10 minutes and was repeated 4 days later, using the same individuals, to test for consistency of choice amongst individuals. We first tested each species' preference for tunnels by comparing the number of individuals that chose either of the tunnels (2 alternatives; combined width 1 m) with the number of individuals that chose to bypass the tunnels across the lawn (2 alternatives; combined width 1 m; Fig. 1). After testing for potential preference for tunnels, we restricted the analyses to those individuals who chose either of the two tunnels, and tested for preference with respect to the substrate within the tunnel. In addition to testing the preference for a particular tunnel type, we also scored (on a scale of 1-5; Fig. 1) the distance that each individual that entered into either of the tunnels had moved forward during the experiment. We called this measure 'crossing success'.

There was no evidence for differential choice between first and second trials in any of the species (Log-linear model:  $\chi^2 = 0.23$ , P = 0.89; Table 1) but there

were significant inter-species differences in whether or not they entered the tunnels (Log-linear model:  $\chi^2 = 23.94$ , P < 0.001). Both water frogs and common toads preferred tunnels to the grass, whereas agile frogs generally preferred the grass (significant for only one of the two trials; Table 1). Among the pooled sample of individuals of all species that entered the tunnels during the first trial, 68.4% preferred the tunnel lined with soil to the bare concrete tunnel (Log-linear model:  $\chi^2 = 11.48$ ; P = 0.003). The choice was significant for water frogs and agile frogs in both trials, but not for common toads (Table 1). The average crossing success during the first trial was always higher for the tunnel lined with soil than for bare concrete tunnels but differences were never significant (Mann-Whitney U-tests:  $z \le -1.10$ ,  $P \ge 0.27$ for all species). The same was true for the second trial  $(z \le -0.57, P \ge 0.56)$  for all three tests).

These results suggest that while agile frogs seem to avoid tunnels when given a choice, the two other species prefer tunnels. Furthermore, agile frogs and water frogs entering the tunnels preferred the tunnel lined with soil over the one with a bare concrete surface, whereas toads did not discriminate between the tunnels' substrate. A possible explanation for this difference is that there is something in the concrete piping that acts as a deterrent for frogs, but does not influence toads as strongly. It has been suggested that the alkalinity of concrete deters frogs (Mougey, 1996), and it is possible that olfactory cues can influence movement towards tunnels (Dall'Antonia & Sinsch, 2001). During breeding migration amphibians are known to orientate by physico-chemical cues such as smell (Sjögren-Gulve, 1998). French engineers constructing underpasses for amphibians already take this into account: during the installation process tunnels are sprinkled with water from nearby ponds to encourage the amphibians to use them (Mougey, 1996). Whatever the reason for preference or avoidance of particular type

**Table 1** Results of the choice experiments using three species of anuran during two trials. Individuals (n = number) could either enter the tunnels (soil covered or bare) or bypass them by going around, over a lawn (see Fig. 1). Unsuccessful individuals either did not move or went backwards.

	Trial no.	Lawn (n)	Tunnels (n)	$\chi^2$	Unsuccessful individuals (n)	Soil-covered tunnel (n)	Bare tunnel (n)	$\chi^2$
Water frog Rana esculenta	1	14	26	3.60°	2	20	6	7.54**
	2	12	29	7.05**	1	22	7	7.76**
Common toad Bufo bufo	1	13	23	2.78°	5	12	11	0.04
	2	10	31	10.76***	0	15	16	0.03
Agile frog Rana dalmatina	1	23	9	6.13*	0	8	1	5.44*
	2	16	8	2.67	8	7	1	4.50*

 $<sup>^{\</sup>circ}P < 0.1$ ,  $^{*}P < 0.05$ ,  $^{**}P < 0.001$ ,  $^{***}P < 0.0001$ 

of tunnels, our results suggest that underpasses should be lined with soil substrate as suggested by Lesbarrères & Lodé (2000), and this has already been done in Switzerland where concrete pipes under roads have mud-lined interiors (Müller, 1992).

Apart from olfactory cues, other factors can also be involved in the choice or avoidance of particular tunnel types. These can include cues from magnetic fields (Fisher *et al.*, 2001) and the intensity of light at the tunnel exit (Epain-Henry, 1992). Furthermore, it is also likely that moisture has a significant effect on tunnel choice. For instance, frogs are more sensitive to desiccation than toads because of the differences in their skin structure, and so could prefer soil to concrete because the former is likely to better maintain moisture levels. It is therefore possible that moist concrete tunnels in the wild are not avoided to the degree indicated by our experiments.

In conclusion, the results of this study show that although the three species tested show differential preference for entering tunnels, they all seem to prefer soil-lined to bare concrete tunnels. The results suggest that, for maintaining population connectivity, concrete tunnels could be improved, and that highway constructors should use soil-lined underpasses rather than plain concrete ones. Given the preference of agile frogs to bypass tunnels, and the much wider habitat tolerance of *Bufo bufo* when approaching roads, effective underpass construction should probably also involve fencing, forcing animals towards tunnels despite their preference for avoiding them. The results also show that, because of a species-specific preference for tunnel use, it may be difficult to propose a protective measure that works equally well for all species.

### **Acknowledgements**

The Autoroutes du Sud de la France (DL), the academy of Finland (JM) and the CIMO (DL) supported this study. We are grateful to A. Pagano, D. Germain, C. Gicquel, S.

Mareau and R. Criou for their help with the fieldwork, and to C. Baudoin, M. Hellström-Phillips, R. O'Hara and three anonymous referees for valuable comments on earlier drafts.

#### References

Alford, R.A. & Richards, S.J. (1999) Global amphibian declines: a problem in applied ecology. *Annual Review of Ecology and Systematics*, **30**, 133–165.

Andrews, A. (1990) Fragmentation of habitat by roads and utility corridors: a review. *Australian Zoologist*, **26**, 130–141.

Association des Sociétés Françaises d'Autoroutes et d'ouvrages à péage (2001) *Chiffres Clés 2001*. Département Information-Communication de l'Association des Sociétés Françaises d'Autoroutes et d'ouvrages à péage, Paris, France.

Beier, P. & Noss, R.F. (1998) Do habitat corridors provide connectivity? *Conservation Biology*, **12**, 1241–1252.

Bennett, A.F. (1990) *Habitat Corridors: Their Role in Wildlife*Management and Conservation. Department of Conservation and Environment, Melbourne, Australia.

Carr, L.W. & Fahrig, L. (2001) Effect of road traffic on two amphibian species of differing agility. *Conservation Biology*, 15, 1071–1078.

Dall'Antonia, P. & Sinsch, U. (2001) In search of water: orientation behaviour of dehydrated natterjack toads, *Bufo calamita*. *Animal Behaviour*, 61, 617–629.

Epain-Henry, C. (1992) Les aménagements pour Batraciens de l'autoroute A71 en Sologne: l'avis du biologiste. In *Gestion et Protection des Amphibiens: de la Connaissance aux Aménagements* (ed. Association Française des Ingénieurs Ecologues), pp. 31–36. Ministère de l'Environement, Mulhouse, France.

Fahrig, L., Pedlar, J.H., Pope, S.E., Taylor, P.D. & Wegner, J.F. (1995) Effect of road traffic on amphibian diversity. *Biological Conservation*, 73, 177–182.

Fisher, J.H., Freake, M.J., Borland, S.C. & Phillips, J.B. (2001) Evidence for the use of magnetic map information by an amphibian. *Animal Behaviour*, **62**, 1–10.

Hels, T. & Buchwald, E. (2001) The effect of road kills on amphibian populations. *Biological Conservation*, **99**, 331–340.

Kuhn, J. (1987) Straßentod der Erdkröte (Bufo bufo L.): Verlustquoten und Verkehrsaufkommen, Verhalten auf der Staße. Naturschutz und Landschaftspflege in Baden-Württember, 41, 175–186.

- Langton, T.E.S. (ed.) (1989) Amphibians and Roads: Proceedings of the Toad Tunnel Conference. ACO Polymer Products Ltd, Shefford, UK.
- Lesbarrères, D. & Lodé, T. (2000) La conservation des amphibiens: exemple d'aménagements autoroutiers. *Bulletin de la Société des Sciences Naturelles de l'Ouest de la France*, **22**, 37–48
- Lesbarrères, D., Pagano, A. & Lodé, T. (2003) Inbreeding and road effect zone in a Ranidae: the case of Agile frog, *Rana dalmatina* Bonaparte, 1840. *Comptes-Rendus Biologie*, **326**, 68–72.
- Means, D.B. (1999) The effects of highway mortality on four species of amphibians at a small, temporary pond in northern Florida. In *ICOWET III, The International Conference on Wildlife Ecology and Transportation* (ed. David Ziegler).
  Http://www.dot.state.fl.us/emo/sched/ICOWET\_III.htm [accessed 11 February 2004]. Florida Department of Transportation, Missoula, USA.
- Mougey, T. (1996) Des tunnels pour batraciens. *Le Courrier de la Nature*, **155**, 22–28.
- Müller, S. (1992) Aménagements et mesures de protection en faveur des Batraciens en Suisse. In *Gestion et Protection des Amphibiens: de la Connaissance aux Aménagements* (ed. Association Française des Ingénieurs Ecologues), pp. 45–61. Ministère de l'Environement, Mulhouse, France.
- Percsy, C. (1994) A propos des migrations de batraciens. *Les cahiers des Réserves Naturelles. Réserves Naturelles Ornithologiques de Belgique*, **7**, 109–114.
- Sacccheri, I., Kuussaari, M., Kankare, M., Vikman, P., Fortelius, W. & Hanski, I. (1998) Inbreeding and extinction in a butterfly metapopulation. *Nature*, 392, 491–494.
- Simonyi, A., Puky, M., Tóth, T., Pásztor, L., Bakó, B. & Molnár,
   Z. (1999) Progress in protecting wildlife from transportation impacts in Hungary and other European countries.
   In ICOWET III, The International Conference on Wildlife Ecology and Transportation (ed. David Ziegler).
   Http://www.dot.state.fl.us/emo/sched/ICOWET\_III.htm

- [accessed 11 February 2004]. Florida Department of Transportation, Missoula, USA.
- Sjögren-Gulve, P. (1994) Distribution and extinction patterns within a northern metapopulation of the pool frog, *Rana lessonae*. *Ecology*, **75**, 1357–1367.
- Sjögren-Gulve, P. (1998) Spatial movement patterns in frogs: Target-oriented dispersal in the pool frog, *Rana lessonae*. *Ecoscience*, **5**, 31–38.
- Tyning, T. (1989) Amherst's tunneling amphibians. *Defenders*, **64**, 20–23.
- van Gelder, J.J. (1973) A quantitative approach to the mortality resulting from traffic in a population of *Bufo bufo L. Oecologia*, 13, 93–95.
- van Leeuwen, B.H. (1982) Protection of migrating common toad (*Bufo bufo*) against car traffic in The Netherlands. *Environmental Conservation*, **9**, 1.
- Veenbaas, G. & Brandjes, J. (1999) Use of fauna passages along waterways under highways. In *ICOWET III*, *The International Conference on Wildlife Ecology and Transportation* (ed. David Ziegler). Http://www.dot.state.fl.us/emo/sched/ICOWET\_III.htm [accessed 11 February 2004]. Florida Department of Transportation, Missoula, USA.
- Vos, C.C. & Chardon, J.P. (1998) Effects of habitat fragmentation and road density on the distribution pattern of the moor frog *Rana arvalis*. *Journal of Applied Ecology*, **35**, 44–56.

## **Biographical sketches**

D. Lesbarrères, T. Lodé and J. Merilä are evolutionary biologists who have been working with local adaptation, conservation and genetics of amphibians in France and Scandinavia over the past 5 years. They are particularly involved in conservation projects for the promotion of protected areas for amphibians near highways.