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2 Effectiveness of small road tunnels and fences in reducing amphibian
3 roadkill and barrier effects; case studies of retrofitted roads in Sweden
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17 Abstract

18 Schemes to reduce road impacts on amphibians have been implemented for decades in Europe,
19 yet, several aspects on the effectiveness of such schemes remain poorly understood. Particularly
20 in northern Europe, including Sweden, there is a lack of available information on road mitigation
21 for amphibians, which is hampering implementation progress and cost-effectiveness analyses of
22 mitigation options. Here we present data derived from systematic counts of amphibians during
23 spring migration at three previous hot-spots for amphibian roadkill in Sweden, where amphibian
24 tunnels with guiding fences have been installed. We used the data in combination with a risk
25 model to estimate the number of roadkills and successful crossings before vs. after mitigation and
26 mitigated vs. adjacent non-mitigated road sections. The estimated number of amphibians killed or
27 at risk of being killed by car traffic decreased by 91–100% and the estimated number successfully
28 crossing the road increased by 25–340% at mitigated road sections. Data however suggested
29 fence-end effects that may moderate the reduction in roadkill. We discuss possible explanations
30 for the observed differences between sites and construction types, and implications for amphibian
31 conservation. We show how effectiveness estimates can be used for prioritizing amphibian
32 passages along the existing road network. Finally, we emphasise the importance of careful
33 monitoring of amphibian roadkill and successful crossings before and after amphibian passages
34 are constructed.

35

36 1. Introduction

37 Amphibian populations may be severely impacted by road mortality and barrier effects of roads
38 and traffic (Hels & Buchwald, 2001; Jaeger & Fahrig, 2004; Nyström *et al.*, 2007; Beebee 2013).
39 Mass mortalities of amphibians often occur where roads cut across annual migration routes
40 between hibernation and breeding habitats. Roadkill, habitat loss and the generally harsh
41 environment for amphibians along roads can also lead to avoidance and barrier effects,
42 preventing them from reaching crucial habitats or resources. In attempts to reduce such negative
43 effects, road mitigation measures have been developed and implemented for over 40 years in
44 Europe (Langton 2015). However, monitoring of such measures is often lacking or insufficient
45 (e.g., focusing solely on usage) and previous studies have shown varying results (e.g., Brehm,
46 1989; Meinig, 1989; Zuiderwijk, 1989; Puky & Vogel, 2003; Mechura *et al.*, 2012; Faggyas &

47 Puky, 2012; Ottburg & van der Grift, 2019; Matos *et al.*, 2018). Consequently, numerous aspects
48 on the actual effectiveness of road mitigation schemes for amphibians remain poorly understood,
49 hampering cost-effective planning efforts and opportunities for improvements.

50 Well-functioning mitigating schemes for amphibians are strongly needed as populations of
51 amphibians continue to decline in Europe, including some of the main target species for road
52 mitigation, the common toad (*Bufo bufo*), the common frog (*Rana temporaria*) and the great
53 crested newt (*Triturus cristatus*) (Bonardi *et al.*, 2011; Beebee, 2013; Petrovan & Schmidt, 2016;
54 Kyek, Kaufmann & Lindner, 2017). In northern Europe, including Sweden, there is however a
55 widespread lack of available information on the effectiveness of road mitigation for amphibians.
56 This is particularly concerning due to the well developed road network and to the potentially
57 complex effects of the harsher climate on microclimatic conditions inside road tunnels or other
58 unforeseen aspects. The absence of structured information and evidence of effectiveness is
59 hampering implementation progress and much needed cost-effectiveness analyses of mitigation
60 options.

61 To minimise the road impacts on amphibians, road managers in and near Stockholm (the Swedish
62 Transport Administration and Stockholm Municipality) constructed passages for amphibians at
63 three hot-spots for amphibian roadkill, i.e., where large concentrations of amphibians were killed
64 on roads, particularly during spring migration, and thus were considered to be road sections in
65 critical need of ecological mitigation. The passages were in the form of permanent tunnels with
66 double-sided guiding fences intended to lead the amphibians safely under the road in both
67 directions. The constructions largely followed the European (Juell *et al.*, 2003) and Swedish
68 (Eriksson, Sjölund & Andrén, 2000; Banverket, 2005) guidelines for design and dimensions,
69 however with tunnels narrower than the recommended minimum diameter 0,6–1 m and with a
70 distance between neighboring tunnels in some cases longer than the recommended maximum of
71 30–60 m.

72 Before and after the construction of these passages, the number and location of amphibians on the
73 road as well as along the fences and in the tunnels were recorded, as the basis for planning of the
74 mitigation constructions and monitoring of their effectiveness. Here we summarise the results of
75 these counts, and discuss the implications in terms of reduced roadkill and barrier effect,
76 differences between constructions, and improved amphibian conservation. We propose a baseline

77 for prioritizing amphibian passages along the existing road network, and suggest some directions
78 for further studies that would support the planning of amphibian mitigation schemes.

79

80 2. Material and methods

81 2.1 Study sites and available field data

82 The three monitored sites – Skårby, Kyrksjölöten and Skeppdalsström – are similar in several
83 respects. The roads are all of intermediate size (7-8 m wide, ca 3,000-9,000 vehicles per average
84 day; Table 1), and mainly used for local and commuting traffic in Stockholm metropolitan area
85 (Fig. 1). The landscape is a small-scale valley terrain at 10–30 m elevation, with a mix of forest,
86 farmland and housing/garden areas. The mitigated road sections all have an important amphibian
87 breeding wetland of around 5–10 ha nearby and main overwintering habitat, typically woodland,
88 on the opposite side of the road (Fig. 2–4). Before mitigation, the road sections were well known
89 hot-spots for amphibian roadkill during spring migration. The amphibian species diversity in the
90 region is limited, with only five species occurring; common toad, common frog, moor frog (*Rana*
91 *arvalis*), smooth newt (*Lissotriton vulgaris*) and great crested newt.

92 The mitigation systems are roughly similar in terms of dimensions of tunnels and fences and
93 length of road section mitigated, while there are some differences in exact dimensions and
94 material of the constructions (Table 1). At all sites, tunnels were impacted by running or standing
95 water to a varying degree during the studies (Table 1).

96 Live and dead amphibians were counted along the road prior to construction of the passage,
97 aiming to identify the most critical road sections for mitigation and to locate major migration
98 routes where tunnels should be placed. Amphibians were also counted post-mitigation, along the
99 road, along fences and in tunnels, to assess the anticipated reduction in roadkill and evaluate the
100 use of the tunnels. While the field efforts varied between sites and periods (Table 2), all data
101 collection was conducted during peak spring migration, with methods that could be considered
102 comparable in terms of number of amphibians found per time and road interval. Data collection
103 methods included visual search on the road and along fences, pitfall trapping along temporary
104 fences, net trapping in tunnels and customised timelapse camera trapping in tunnels. Methods
105 applied at each site are described in more detail in Supplemental Article S1.

106 2.2 Data treatment and analyses

107 We standardised the available data on amphibian counts on and near the roads, along fences and
108 in tunnels to be able to compare, as far as possible, each site before and after mitigation and the
109 mitigated road section with adjacent non-mitigated sections. We summarised the number of
110 amphibians found on and near the road (including along temporary fences at site 2) per night (site
111 1) or evening (site 2–3) and 50m road interval, assuming that these data were collected with a
112 similar effort over the road section searched, and with a similar effort before and after mitigation,
113 within each site. We however acknowledge that the method used along the temporary fence at
114 site 2 was too different to allow a direct comparison with non-mitigated sections for that site.

115 To be able to tentatively compare the performance of different tunnels at a site, we calculated the
116 number through each tunnel per night (at site 1) or number of movements (in + out) and the net
117 number through each tunnel per 24h-period (at site 2–3). To assess the number of amphibians
118 successfully crossing a mitigated road section through the tunnels we summarised the net number
119 through all tunnels at the site.

120 To assess the number of amphibians killed and the number successfully crossing a non-mitigated
121 road section, we used the relationship presented by Hels & Buchwald (2001) on the risk of
122 getting killed for an amphibian on the road depending on average traffic intensity and species
123 (Fig. 5). According to this relationship, a proportion of the amphibians found alive on and near
124 the road attempting to cross it should have made it successfully to the other side even without
125 being rescued, and concomitantly, the number of amphibians found dead on the road should
126 represent also a certain number that survived and managed to cross.

127 In site 1, newts made up ca 98% of amphibians observed, so we analysed only data on newts
128 from this site, and pooled the two newt species in the analyses. Most of the newts found when
129 searching the road were dead (ca 72%). Using the information presented Hels & Buchwald
130 (2001) in combination with average traffic intensity and species analysed, we estimated that 62%
131 of newts trying to cross the road surface at the site would get killed by traffic (as read in Fig. 5),
132 and accordingly assumed that each newt found dead represented $(1/0.62)-1 = 0.61$ newt that had
133 managed to cross.

134 In site 2, common toads made up ca 99% of amphibians observed, and accordingly we analysed
135 only data on common toad. Most of the toads found when searching the road were dead (ca 82%),

136 while all toads found or captured along the temporary fence were alive. We estimated a 70% risk
137 of getting traffic killed for toads trying to cross the road surface at the site (Fig. 5), and assumed
138 that each toad found roadkilled represented $(1/0.70)-1 = 0.43$ toads that had crossed successfully
139 and that each toad found along the temporary fence represented $1-0.70 = 0.30$ toad that would
140 have managed to cross the road, had the fence not been in place.

141 In site 3, significant numbers were found of 4 species (all except great crested newt), so we
142 included all amphibians in the analyses for that site. Most amphibians found on or approaching
143 the road were alive (ca 83%). We assumed that on average 75% (newts 79%, toads and frogs
144 72%; Fig. 5) of amphibians trying to cross the road surface would get killed by traffic and that
145 each amphibian rescued represented $1-0.75 = 0.25$ amphibian that would have managed to cross
146 the road, had the rescue not taken place.

147

148 3. Results

149 The number of amphibians found on or heading for the road, i.e. animals killed or at risk of being
150 killed by car traffic, during spring migration decreased at mitigated road sections at all three sites
151 (Fig. 6). The estimated number of individual amphibians saved by the mitigation measures
152 ranged from 25 to >200 per night at the three sites (Table 3), corresponding to a 91–100%
153 decrease in roadkilled amphibians along mitigated road sections. Outside mitigated road sections
154 the changes from before to after mitigation were smaller and more variable; the number of
155 amphibians on the road decreased by 33% at site 1, increased by over 300% at site 2, while there
156 was virtually no change at site 3. At site 2, the number of amphibians on the road peaked just
157 outside of the fence-ends (intervals 8 and 15–17; see Fig. 6). At site 1 and 2, some individuals
158 were found on the road just inside the fence-ends (east end at site 1, both ends at site 2; Fig. 6).
159 No amphibians were found on a fenced road section >100 m from a fence-end.

160 The number of amphibians passing through the tunnels varied greatly between sites (3000%
161 difference; Table 4), largely following the number that was killed before mitigation, i.e., many
162 more at site 1. The estimated number of amphibians successfully crossing the road increased at
163 mitigated sections, ranging from 2–180 more individuals per night (Table 5), corresponding to a
164 25–340% increase compared to the situation before mitigation. In addition, the estimated number

165 successfully crossing along non-mitigated sections differed between before and after mitigation,
166 and over the entire site (mitigated + non-mitigated road sections combined) the mitigation
167 implementation resulted in 2–162 more individuals crossing the road per night (Table 5), or a 16–
168 340% increase.

169 The number of amphibians passing through the tunnels also varied greatly among the tunnels at
170 sites 1 and 3 (Table 4). Tunnel no. 2 at site 3 stood out by the large discrepancy between the high
171 number of amphibians moving in and out of the tunnel entrance and the low net number passing
172 through. This tunnel had a shallow pool in the northern (entrance) side, while the southern (exit)
173 side was completely submerged due to a construction fault.

174

175 4. Discussion

176 The compiled results from the monitoring of amphibian passages at the three sites (Skårby,
177 Kyrksjölöten, Skeppdalsström) indicate that the passages were effective in reducing the number
178 of roadkilled amphibians during spring migration, compared to a situation before mitigation
179 measures were implemented. None or very few amphibians were found on the fenced road
180 sections, where prior to mitigation amphibians had been killed in the hundreds or thousands each
181 spring. These results are well in line with those from many other studies, showing significant
182 reductions in amphibian roadkill after the construction of adequate road fences (e.g., Meinig,
183 1989; Dodd, Barichivich & Smith, 2004; Jochimsen *et al.*, 2004; Stenberg & Nyström, 2009;
184 Malt, 2011; Matos *et al.*, 2017; Matos *et al.*, 2018; Hill *et al.*, 2018; Jarvis, Hartup & Petrovan,
185 2019).

186 However, the data from at least two of our sites suggested the presence of fence-end effects
187 (Huijser *et al.*, 2016) which may influence the overall reduction in amphibian roadkill. Peaks in
188 numbers of amphibians on the road just outside fence-ends at site 2 suggest that some individuals
189 following the fence by-passed the final portions of fencing, despite the angled design, and that
190 part of the mortality was merely transferred from fenced to unfenced road sections. The increase
191 in amphibians on the entire unfenced part of the road at site 2 may also be explained by
192 individuals finding new migration routes when the previous ones have been occupied by fences,
193 while tunnels are avoided or simply not encountered (though we also see several alternative

194 explanations to that pattern; see below). Furthermore, at site 1 and site 2 some amphibians cut
195 into the mitigated road section near the fence-ends. This may be an effect of animals moving
196 diagonally over the road, not being strictly directional in their movements, or following the road
197 along curbs or other minor structures into the fenced section. Nearer to the middle of the fenced
198 sections no amphibians were found on the road, and accordingly, in the central parts of the
199 mitigated road sections the decrease in roadkilled amphibians was 100% at all three sites.

200 These fence-end effects, and the fact that many amphibians crossed and were killed on the road
201 outside the fenced sections, imply that longer fences are likely to result in a larger reduction in
202 roadkill (Buck-Dobrick & Dobrick, 1989; Huijser *et al.*, 2016). While this notion may seem trivial,
203 it has important implications for management (see below).

204 It is imperative that the effectiveness of amphibian passages in the form of under-road tunnels
205 with associated guiding fences are not only assessed on the basis of the reduction in roadkill but
206 also on the number of animals making it successfully to the other side of the road (Jochimsen *et*
207 *al.*, 2004; Schmidt & Zumbach, 2008). Previous studies have indicated that many amphibians
208 reaching the fences do not find their way through the tunnels, either because the tunnels are too
209 widely separated or the tunnels or guiding structures are inadequate, and as a consequence
210 amphibians may return to the terrestrial habitats without breeding (Allaback & Laabs, 2003;
211 Jochimsen *et al.*, 2004; Schmidt & Zumbach, 2008; Pagnucco *et al.*, 2012). Several European
212 studies have reported the overall rates of individual toads or newts using tunnels ranging from 3%
213 to 98% of those encountering the guiding fences (Brehm, 1989; Buck-Dobrick & Dobrick, 1989;
214 Langton, 1989; Meinig, 1989; Zuiderwijk, 1989; Mechura *et al.*, 2012; Matos *et al.*, 2017; Matos
215 *et al.*, 2018; Ottburg & van der Grift, 2019, Jarvis, Hartup & Petrovan, 2019).

216 The results from our three sites indicated that the mitigation schemes likely reduced the barrier
217 effects of the roads. We assumed that even without mitigation in place, a certain proportion of
218 amphibians manage to cross a road without getting killed by traffic, that most amphibians survive
219 where the traffic intensity is very low, but that the proportion surviving decreases exponentially
220 with increasing traffic (Hels & Buchwald 2001; Jacobson *et al.*, 2016). Importantly however, on
221 all three sites studied, the number of individuals passing through the tunnels in spring exceeded
222 the number estimated to have crossed the road surface successfully over the mitigated section
223 before the mitigation was in place.

224 Several factors in the technical construction of amphibian passages may affect their effectiveness:
225 width, shape and length of tunnels, distance between tunnels, height and shape of guiding
226 barriers, substrate in tunnels and along barriers, construction material, moisture, vegetation and
227 drainage in and around the passages, and special features such as cover objects, guiding
228 structures at entrances and slotted tops (reviews in Jochimsen *et al.*, 2004; Hamer, Langton &
229 Lesbarrères, 2015; Jackson, Smith & Gunson, 2015). Our data did not allow a systematic analysis
230 of how these factors relate to the passage effectiveness. With the information at hand, we can
231 only speculate about the differences observed. At site 1, many newts were carried through the
232 tunnels by the water running in direction towards the wetland, and at site 3, standing water in one
233 of the tunnels appeared to attract many amphibians to the tunnel entrance but blocked the tunnel
234 for actual crossings. Shallow standing or running water in and around tunnels can attract
235 amphibians and help them finding their way through (Rosell *et al.*, 1997; Eriksson, Sjölund &
236 Andrén, 2000; Jochimsen *et al.*, 2004; Schmidt & Zumbach, 2008, Jarvis, Hartup & Petrovan,
237 2019), but high water levels make tunnels impassable (Buck-Dobrick & Dobrick, 1989; Rosell *et al.*,
238 1997; Jochimsen *et al.*, 2004). Water levels may thus have significant but complex impact on
239 amphibian passage effectiveness. Additionally, the water and soil inside and adjacent to
240 amphibian tunnels can suffer high pollution levels with road surface contaminants including salt
241 used for deicing roads as well as various metals and other substances (White, Mayes & Petrovan,
242 2017). At site 2, both the tunnels and the distance between them were longer, which may explain
243 a bypass effect, i.e., peaks in animals on the road just outside fence-ends. Previous studies
244 suggest that long tunnels and long fences without tunnels make amphibians give up and turn back
245 (Zuiderwijk, 1989; Jochimsen *et al.*, 2004; Jackson, Smith & Gunson, 2015; Hill *et al.*, 2018;
246 Ottburg & van der Grift, 2019; Matos *et al.*, 2018); these individuals may eventually try crossing
247 the road on another spot. There were significant movements in and out of the tunnels at this site,
248 which may indicate that animals hesitated to pass through. However, the total numbers actually
249 crossing through the tunnels were broadly similar to the estimated number killed or crossing the
250 fenced section before mitigation (58.8/24h vs. $32.1+13.8=45.9$ /night).

251 There are several plausible explanations for the changes in the number of amphibians on the road
252 outside mitigated sections (most pronounced at site 1 and 2), other than the potential bypass
253 effect described above. The most obvious is that the field effort at some sites and time periods
254 was insufficient (three nights or less for data collection) and the data therefore were influenced by

255 random events. Another is that the fieldwork methods were in fact not similar enough with regard
256 to how the basic method was applied in practice to allow the data standardisation and
257 comparisons. The changes observed may also depend on annual differences in population
258 numbers or temporal migration patterns. In this case, the effect sizes on mitigated sections can be
259 adjusted according to the changes on non-mitigated sections. It is however important to note that
260 the non-mitigated sections studied were not true controls (comparators), as they were not
261 unaffected by the mitigation measure (the intervention).

262 The standardisation of data required a number of assumptions and simplifications that may have
263 introduced errors. We adopted an approach where we tried finding the unifying patterns in studies
264 of amphibian passages conducted with slightly different aims, budgets, staffing and time frames.
265 Despite these limitations, we believe that the general picture given by these studies, before vs.
266 after mitigation and along vs. outside the mitigated road section, contributes significantly to the
267 knowledge of how amphibian passages at roads can reduce roadkill and barrier effects on
268 amphibians during spring migration.

269 5. Implications for management

270 There is scant evidence in literature that the construction of amphibian passages will lead to long-
271 term conservation of amphibian populations (Beebee, 2013; Smith, Meredith & Sutherland, 2018,
272 Jarvis, Hartup & Petrovan, 2019), and also for our three sites it is difficult to be certain to what
273 degree the observed reductions in roadkill and barrier effect will have a significant and long-
274 lasting effect on the population level. However, the estimated number of newts saved by the
275 mitigation system (>200 individuals per peak migration night) and the number of newts crossing
276 through the tunnels (ca 180 per peak migration night) at site 1 (Skårby) are each in the same
277 order of magnitude as the total estimated number of breeding newts at the site (2,000-2,300
278 individuals, assuming that there are around 10 peak migration nights per season; Peterson &
279 Collinder, 2006). It is reasonable to believe that such an improvement in survival significantly
280 benefits the conservation of the local newt populations.

281 As a contrast, the low number of amphibians successfully crossing through the tunnels at site 3
282 (Skeppdalsström) – ca 10 individuals per night, an increase with only 2 per night compared to
283 what may have crossed the road successfully without any mitigation – may appear discouraging.
284 Neither the reduction in the number killed (some 25 per peak migration night) can sum up to

285 anywhere near the total estimated number of amphibians breeding at the site (ca 1,300
286 individuals; Andersson & Lundberg 2015). The results from site 2 (Kyrksjöloten) indicate that
287 many more toads manage to cross the road alive using the tunnels compared to before mitigation,
288 but these results cannot be put in relation to any estimated population size, and the conclusion
289 regarding the benefit to conservation is confused by the possible bypass effects (see above).

290 However, it is important to point out that there should be a minimal level of road traffic where
291 amphibian passages of the kind described here need to be considered, as implied by the
292 relationship between traffic intensity and risk of getting killed described by Hels & Buchwald
293 (2001; Fig. 5). On roads with low traffic many amphibians are likely to cross the road without
294 being killed, and an amphibian passage with fences that hinders some of these movements may
295 lead to a decrease in the number of successful crossings, and cause more harm than good (Jaeger
296 & Fahrig, 2004; Jochimsen *et al.*, 2004; Schmidt & Zumbach, 2008; Pagnucco *et al.*, 2012). The
297 cut-off point depends on the combination of traffic intensity and effectiveness of passages.

298 Using the data from the present cases, and assuming a constant passage rate through tunnels, the
299 breakeven point for site 1 would be at a risk of around 45%, corresponding to a hypothetical
300 average daily traffic of ca 1,000 vehicles. In other words, had the traffic been <1,000 vehicles per
301 day, the construction of the tunnels with fences would have led to fewer amphibians reaching the
302 breeding pond, i.e., an increased barrier effect. For site 3, where the increase in successful
303 crossings was small, the breakeven would be at ca 70% risk, or a hypothetical traffic of ca 6,000
304 vehicles per day. The data from site 2 did not allow a similar assessment due to the increase in
305 amphibians killed on non-mitigated sections.

306 These calculations, as well as all data treatment in our work, rely heavily on Hels & Buchwald's
307 risk model for amphibians. While their study is well conducted, the results are based on few
308 species and limited observations, and as far as we know has not been replicated or the model
309 predictions empirically tested. Given the need for road managers to know under what
310 circumstances the construction of amphibian passages is motivated, and when not, we strongly
311 recommend further study of the relation between road characteristics (traffic, width etc.) and the
312 roadkill risk for amphibians when attempting to cross.

313 At all three sites the mitigation was restricted solely to the most critical road sections (see Fig. 6),
314 despite recommendations in ecological assessments from all sites to include also contiguous

315 sections (Collinder, 2007; Helldin, 2015; Lundberg, 2015). Our results suggest that mitigation
316 (guiding fences and additional tunnels) extending at least some 100 m outside of the most critical
317 road section could minimise fence-end effects and further improve the passage effectiveness.

318 An alternative approach to decrease fence-end effect could be to fortify fence-ends, for example
319 by modifying the angles or extending fences perpendicularly from the road. Amphibians could
320 potentially be helped in finding and entering tunnels with relatively simple means by installing
321 guiding structures at the tunnel entrances where these are not already in place (site 3). It is
322 however unclear to what degree such adaptations would improve the effectiveness of existing
323 passages.

324 Amphibian passages tend to be costly, not least when constructed on existing roads, and it is
325 therefore crucial for road managers to know where passages may be critical for amphibian
326 conservation and how passages can best be designed. To build up the knowledge of amphibian
327 passages at roads, the reduction in roadkill and barrier effects should be monitored when new
328 amphibian passages are constructed, or when existing passages are adapted (Hamer, Langton &
329 Lesbarrères, 2015; Helldin, 2017). The monitoring should use comparable methods before and
330 after mitigation, include the quantification of amphibians killed and amphibians successfully
331 crossing, over a long enough road section to cover bypass effects. Quality data should be secured
332 by a field effort spanning over multiple years before and after mitigation, and multiple times each
333 year. Results from such studies could be combined in global analyses (e.g., meta-analyses) to
334 explore differences between construction types and trade-offs between the economic investment
335 and expected effect size (cost-efficiency), thereby helping to point out where passages along
336 existing roads are warranted.

337 Finally, it is important to note that our results only focused on adult breeding migrations in
338 spring, without including the summer and autumn migrations of juveniles away from the
339 breeding ponds. Recent population models indicate that the survival of post-metamorphic
340 juveniles is of fundamental importance for the persistence of amphibian populations (Schmidt &
341 Zumbach, 2008; Petrovan & Schmidt, *in press*). Adults and juveniles using the passages later in
342 the season for leaving the breeding areas may experience dryer tunnels or even water
343 counterflow. Juvenile amphibians may be particularly sensitive to the design of underpasses and
344 associated barrier fences (Schmidt & Zumbach, 2008) given their higher desiccation risk.

345 However, due to their very small size and unpredictable migration timing, juveniles remain very
346 rarely quantified in terms of both road mortality impacts and usage of mitigation systems, despite
347 their crucial role in population dynamics (Petrovan & Schmidt, *in press*). Future studies should
348 prioritise incorporating juveniles in mitigation assessments.

349

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361

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- 518

519 Tables

520

521 *Table 1. Characteristics of the roads and the amphibian mitigation measures at the three study*
 522 *sites near Stockholm, Sweden. Data on individual tunnels are listed from east to west (see Fig. 2–*
 523 *4).*

Site	1. Skårby					2. Kyrksjölöten		3. Skeppdalsström				
Location	59°13'34N 17°43'55E					59°20'53N 17°55'35E		59°18'16N 18°29'32E				
Construction year of mitigation measure	2005, additional tunnels in 2008					2014		2015				
Road												
Name/no	Road 584					Spångavägen		Road 222				
Owner/manager	Swedish Transport Administration					Stockholm Municipality		Swedish Transport Administration				
Mitigated section (m)	300					315		190+110				
Traffic (daily average) ^a	3,000					7,800		8,600				
Width (m)	7					16 ^b		7				
Guiding fences (barriers)												
Hight	40					45		40				
Material	Cement concrete					Polymer concrete		Metal				
Sides	Double sided					Double sided		Double sided				
Location	Parallel to road					Parallel to road		Parallel to road				
End	Wide V-shape					U-shape		Narrow U-shape				
Top	Straight					Angled		Angled				
Tunnels												
Type	Closed top circular					Closed top dome		Closed top circular				
Guiding structure	(T-shape with roof) ^c					I-shape		None				
Number	5					2		5				
Diameter (cm)	40	50	40	40	40	50x32 (both)		30 (all)				
Length (m)	11	?	11	16	12	25	19	10 (all)				
Material ^d	M	Cc	M	M	M	Pc	Pc	P	P	P	M	P
Water ^e	R	R	D	R	R	S	R	D	S	S	D	R
Max water depth (cm)	10	5	–	5	5	5	1	–	30	25	–	5
Distance between (m)	55	55	70	75	180		47	55	215 ^f	115		

524 *a: Data from 2007-2015*

525 *b: Including pedestrian and bike lanes*

526 *c: Not clear whether these were in place during monitoring*

527 *d: M = metal, Cc = cement concrete, Pc = polymer concrete, P = plastic*

528 *e: R = running, D = dry, S = standing (at the time for fieldwork)*

529 *f: Including distance between mitigated sections*

530

531

532 *Table 2. Amphibian data collection methods and efforts at the three study sites near Stockholm,*
 533 *Sweden.*

Site	1. Skårby		2. Kyrksjölöten		3. Skeppdalsström	
	Before	After	Before	After	Before	After
Visual search						
Section searched (m)	520		ca 1000		ca 950	
No. of nights	1	4	17	3	7	4
Time period	15–16 April 2004	6–22 April 2008	27 March –9 May 2012	8–15 April 2015	7–19 April 2015	7–18 April 2016
Pitfall trapping along temporary fences						
Section trapped (m)	–		350	–	–	
No. of nights	–		17	–	–	
Time period	–		27 March–9 May 2012	–	–	
Net trapping						
No. of tunnels	–	4	–	–		
No. of nights	–	5	–	–		
Time period	–	9–11 April 2010, 15– 18 April 2013	–	–		
Camera trapping						
No. of tunnels	–	–	–	2	–	4
No. of nights	–	–	–	32	–	7–11 ^a
Time period	–	–	–	1 April–3 May 2015	–	5–23 April 2016

534 *a: Differed between tunnels; see table 4.*

535

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537

538 *Table 3. Estimated number of amphibians killed per night along the studied road sections before*
 539 *and after mitigation, separated between mitigated and adjacent non-mitigated sections. Data*
 540 *were standardised to allow comparisons within and among sites; see text for further explanation.*

Site 1. Skårby			541
Section	Before	After	Δ
Mitigated	228	10	-218
Non-mitigated	91	60	-31
<i>Total</i>	<i>319</i>	<i>70</i>	<i>-249</i>
Site 2. Kyrksjölöten			
Section	Before	After	Δ
Mitigated	32.1	2.8	-29.3
Non-mitigated	10	47.4	+37.4
<i>Total</i>	<i>42.1</i>	<i>50.2</i>	<i>+8.1</i>
Site 3. Skeppdalsström			
Section	Before	After	Δ
Mitigated	25.2	0	-25.2
Non-mitigated	10.3	9.8	-0.5
<i>Total</i>	<i>35.5</i>	<i>9.8</i>	<i>-25.7</i>

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544

545 *Table 4. Number of amphibian recordings in the tunnels, and the net number passing through per*
 546 *night or 24h-period. For site 2–3 (cameras) data are separated between animals moving into the*
 547 *tunnel (i.e. in direction toward the breeding wetland) and those moving out (direction from the*
 548 *wetland). At site 1 (traps), only animals moving toward the wetland could be counted, as net*
 549 *traps blocked the tunnels in the other direction. Tunnels that were not monitored are indicated by*
 550 *lack of data.*

Site 1. Skårby (only newts, 5 nights during peak migration period)					
Tunnel no.	S newt	GC newt	Both sp.		Net no./night
1	555	145	700		140.0
2	–	–	–		–
3	21	28	49		9.8
4	612	90	702		140.4
5	111	5	116		23.2
<i>Sum</i>	<i>1299</i>	<i>268</i>	<i>1567</i>		<i>313.4</i>
Site 2. Kyrksjölöten (only common toad, 14 significant migration days)					
Tunnel no.	In	Out	Net no.	In+out/24h	Net no./24h
1	871	389	482	90.0	34.4
2	544	214	330	54.1	23.6
<i>Sum</i>	<i>1415</i>	<i>603</i>	<i>812</i>	<i>144.1</i>	<i>58.8</i>
Site 3. Skeppdalsström (all amphibians, 7-11 days during peak migration period)					
Tunnel no.	In	Out	Net no.	In+out/24h	Net no./24h
1 (9 days)	41	17	24	6.4	2.7
2 (11 days)	258	254	4	46.5	0.4
3 (7 days)	70	38	32	15.4	4.6
4 (7 days)	20	0	20	2.9	2.9
5	–	–	–	–	–
<i>Sum</i>	<i>389</i>	<i>309</i>	<i>80</i>	<i>71.2</i>	<i>10.5</i>

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Table 5. Estimated number of amphibians successfully crossing the road per night along the studied road sections before and after mitigation, separated between mitigated and adjacent non-mitigated sections. Data were standardised to allow comparisons within and among sites; see text for further explanation.

Site 1. Skårby			554
Section	Before	After	Δ
Mitigated	139.1	319.5 ^a	+180.4
Non-mitigated	55.5	36.6	-18.9
<i>Total</i>	<i>194.6</i>	<i>356.1</i>	<i>+161.5</i>
Site 2. Kyrksjölöten			
Section	Before	After	Δ
Mitigated	13.8	60.1 ^a	+47.1
Non-mitigated	4.3	19.4	+15.1
<i>Total</i>	<i>18.1</i>	<i>80.4</i>	<i>+62.3</i>
Site 3. Skeppdalsström			
Section	Before	After	Δ
Mitigated	8.4	10.5 ^a	+2.1
Non-mitigated	3.4	3.3	-0.1
<i>Total</i>	<i>11.8</i>	<i>13.7</i>	<i>+1.9</i>

556 *a*: Including the number passing through tunnels; see table 4.

557

558

559 Figures

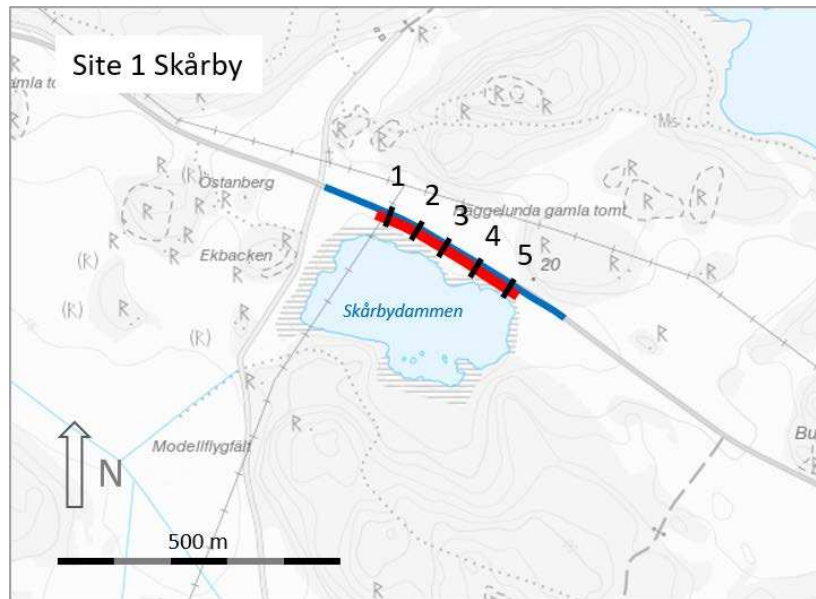
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561

562 *Figure 1: Overview of the three study sites in Stockholms larger metropolitan area. Map image*
563 *credit: Lantmäteriet.*

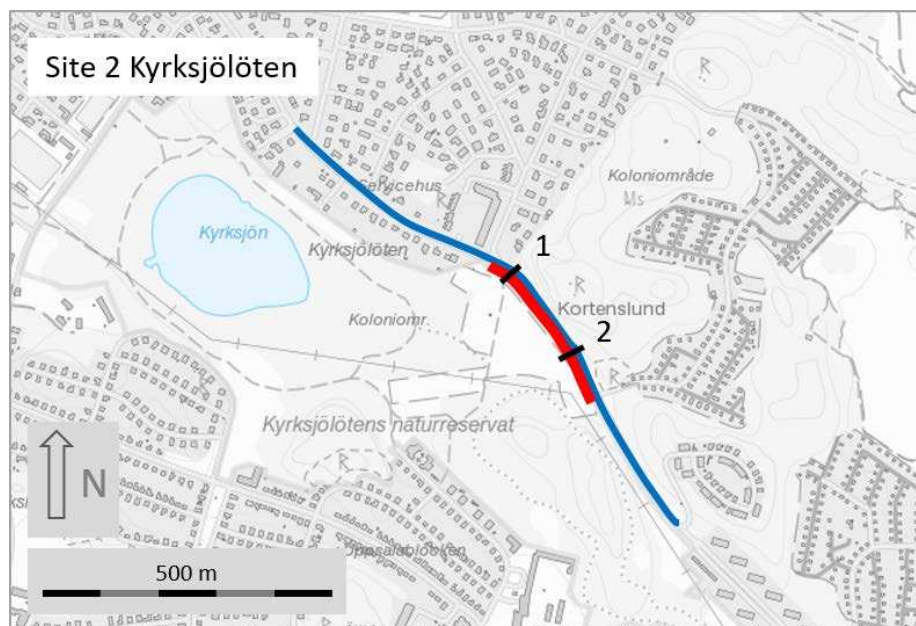
564



565
566 *Figure 2: Map of Skårby (site 1) and the wetland Skårbydammen. Red line denote mitigated*
567 *(fenced) section, black lines are the tunnels, and blue line is the road section where amphibians*
568 *were counted before and after mitigation. Map image credit: Lantmäteriet.*
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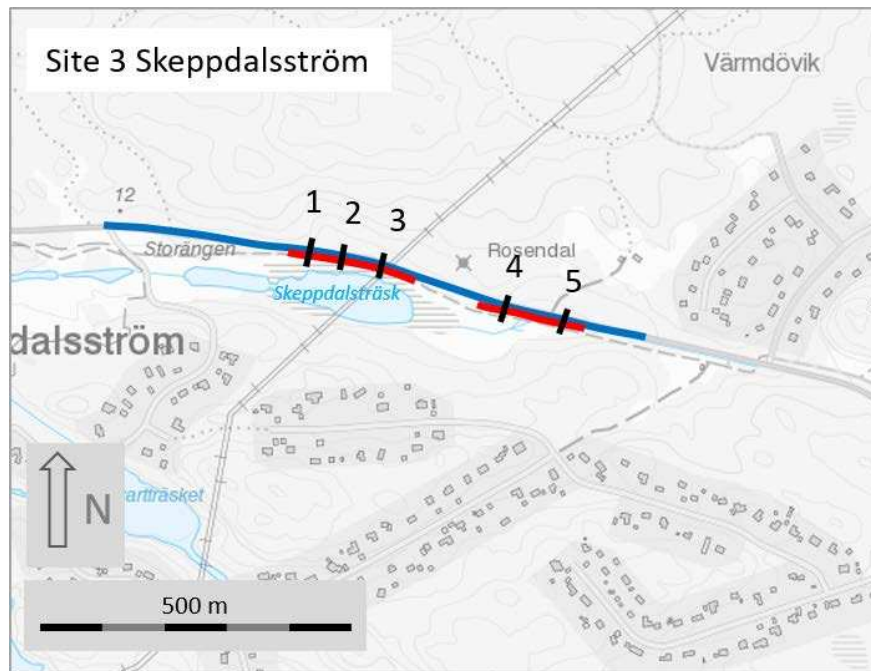


572

573 *Figure 3: Map of Kyrksjölöten (site 2) and lake Kyrksjön. Red line denote mitigated (fenced)*
574 *section, black lines are the tunnels, and blue line is the road section where amphibians were*
575 *counted before and after mitigation. Map image credit: Lantmäteriet.*

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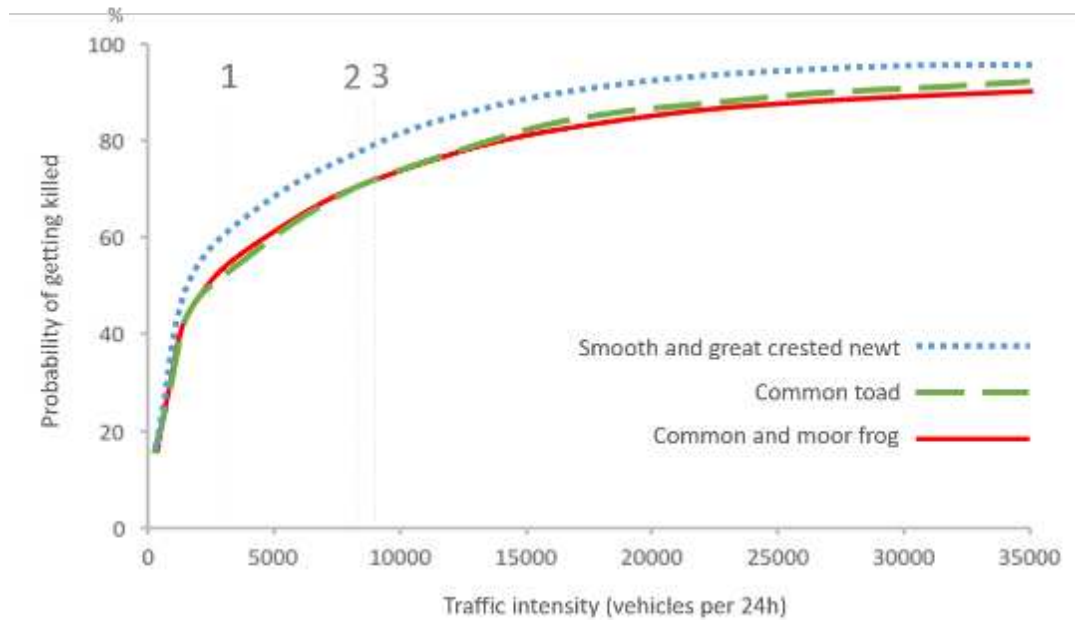


578

579 *Figure 4: Map of Skeppdalsström (site 3) and the wetland Skeppdalsträsk. Red line denote*
580 *mitigated (fenced) section, black lines are the tunnels, and blue line is the road section where*
581 *amphibians were counted before and after mitigation. Map image credit: Lantmäteriet.*

582

583

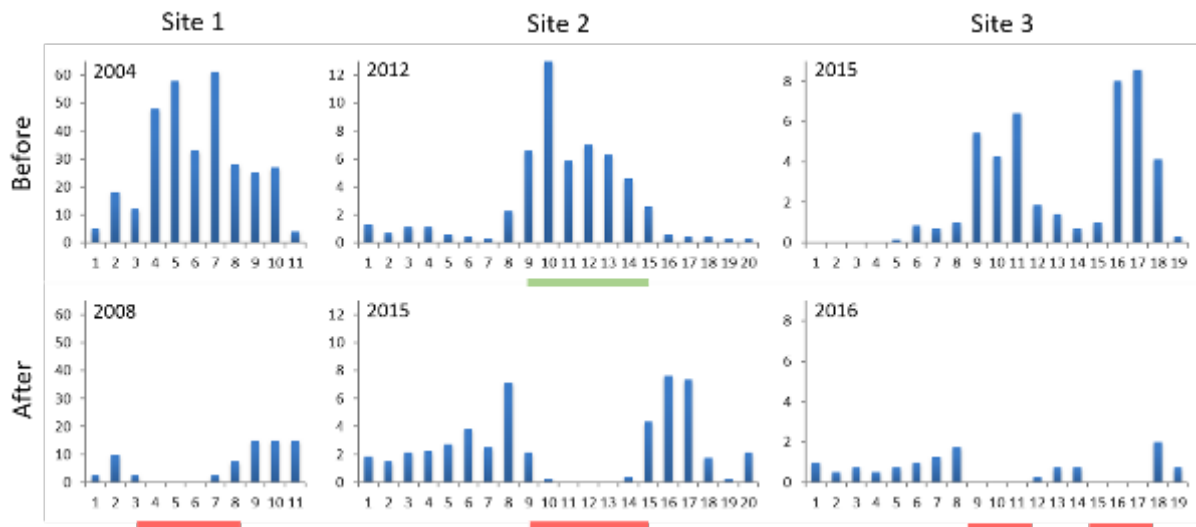


584

585 *Figure 5. Probability of getting killed for an individual of different amphibian species at different*
 586 *traffic intensities, as described by Hels & Buchwald (2001). The probability of getting killed is*
 587 *weighted by amphibian behaviour (velocity and diurnal activity) and diurnal variation in traffic*
 588 *intensity, and assuming that amphibians are crossing perpendicular to the road. Traffic intensity*
 589 *of the three study sites are indicated by vertical dashed lines.*

590

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593

594 *Figure 6. The number of amphibians found along the studied road sections, divided per*
 595 *evening/night and 50m road interval starting from northwest. Upper graphs are before*
 596 *mitigation, lower graphs are with mitigation in place. Site 1: Number of dead newts (smooth +*
 597 *great crested) found per night; Site 2: Number of live and dead common toads found per night;*
 598 *Site 3: Number of live and dead amphibians (four species) found per evening. Red lines denote*
 599 *mitigated sections (permanent amphibian fencing), green line at site 2 denotes temporary fenced*
 600 *section. Due to the difference in method, the data from counts along the temporary fence at site 2*
 601 *cannot be directly compared to the other data.*

602

603