

1 The authoritative version of this manuscript can be found in the Wiley journal *Water*  
2 *and Environment Journal* [here](#). Full citation details:  
3 White, K. J., Mayes, W. M. and Petrovan, S. O. (2017), Identifying pathways of  
4 exposure to highway pollutants in great crested newt (*Triturus cristatus*) road  
5 mitigation tunnels. *Water and Environment Journal*. DOI: [10.1111/wej.12244](https://doi.org/10.1111/wej.12244)  
6  
7

This is the peer reviewed version of the following article: White, Katie J.; Mayes, William M.; Petrovan, Silviu O. Identifying pathways of exposure to highway pollutants in great crested newt (*Triturus cristatus*) road mitigation tunnels: Exposure pathways of highway pollutants to *Triturus cristatus*. *Water and environment journal : the journal / the Chartered Institution of Water and Environmental Management*, 31(3), (310-316), which has been published in final form at <https://doi.org/10.1111/wej.12244>. This article may be used for non-commercial purposes in accordance With Wiley Terms and Conditions for self-archiving.

8  
9 **Identifying pathways of exposure to highway pollutants in great crested**  
10 **newt (*Triturus cristatus*) road mitigation tunnels**

11  
12 **AUTHORS**

13 Katie J. White BSc., School of Environmental Sciences, University of Hull, UK

14 William M. Mayes PhD., CEnv., FRGS., School of Environmental Sciences, University  
15 of Hull, UK, corresponding author: [w.mayes@hull.ac.uk](mailto:w.mayes@hull.ac.uk), +44(0)1723357292.

16 Silviu O. Petrovan PhD. Conservation Coordinator, Froglife, Peterborough, UK and  
17 Research Associate, Conservation Science Group, Department of Zoology,  
18 University of Cambridge, Cambridge CB2 3QZ, UK.

19  
20 **ABSTRACT**

21 Road mitigation tunnels are increasingly deployed for amphibians but very little is  
22 known about chemical pollution in such schemes. We assessed pollution pressures  
23 associated with road runoff at a major great crested newt mitigation scheme in  
24 England. Sediments and waters in the mitigation system were analysed for major  
25 physico-chemical parameters, trace metals and total petroleum hydrocarbons and  
26 compared to a nearby reference site. Seven out of eight tested metals including  
27 copper, zinc, lead and iron were in significantly greater concentrations in the tunnels  
28 than at a reference site and at environmentally-significant concentrations. Water  
29 samples also exhibited elevated concentrations of aluminium and chromium and  
30 occasionally extreme alkaline pH associated with leaching of portlandite in tunnel  
31 cements. High conductivity values in waters and sediments corresponding with  
32 seasonal de-icing salt application were also apparent. The study highlights the  
33 potential pollutant pressures for amphibians associated with large-scale urban  
34 development and road mitigation schemes.

35  
36 Keywords: urban pollutants, road runoff, road mitigation; trace metals, de-icing salts,  
37 amphibian

## INTRODUCTION

Road networks and road traffic have increased substantially across the globe and are significantly impacting amphibian populations (Beebee, 2013; Petrovan and Schmidt, 2016). In Europe, populations of great crested newts (*Triturus cristatus*) have suffered severe declines due to habitat loss and fragmentation as a result of urbanisation and intensification of agricultural practices (Langton *et al.*, 2001). Some amphibian declines are also attributed globally to environmental pollution (Egea-Serrano *et al.*, 2012). Pollutants of particular concern include chemical pesticides and fertilisers (Langton *et al.*, 2001), as well as trace metals, road de-icing salts and hydrocarbons (Duff *et al.* 2010; Sparling *et al.*, 2010).

In the UK, *T. cristatus* are protected by national and European legislation including the EU Habitats Directive (European Council Directive 92/43/EEC) and the Wildlife and Countryside Act 1981. Like other amphibians, *T. cristatus* require a combination of aquatic and terrestrial habitat for foraging, overwintering and breeding. Also, *T. cristatus* typically form metapopulations where there are pond clusters and it is essential for their long-term conservation that they can navigate between habitat patches and ponds to forage for resources, breed and prevent narrowing of the gene pool (Edgar and Bird, 2006). Short-distance migrations of newts towards breeding sites (ponds) normally occur in early spring (March-April), while the longer distance dispersal movements mostly occur during autumn months (September-November). As part of ecological mitigation, road underpasses may be installed to allow newt movements between ponds or between aquatic and terrestrial habitat, especially when newly-constructed roads fragment their habitats. Such road underpasses are increasingly implemented in Europe as connectivity mitigation around urban and suburban developments. However, given the resultant proximity of the amphibian habitat to a source of potential aquatic pollutants- roads (Van Boheman and Van De Laak, 2003), and the sensitivity of amphibians to many common pollutants (due to their permeable skin), there are concerns that road tunnels may act as a pathway to exposure of amphibians to road-related pollutants. Typical pollutants associated with road runoff consist of trace metals, de-icing salts in winter and petroleum and diesel hydrocarbons (Bäckström *et al.*, 2003). Metal issues usually focus on those sourced from general vehicle wear, e.g. copper and zinc from brake pads, and the latter additionally from tyre wear and corrosion of galvanized safety barriers (Legret and Pagotto, 1999). In addition, there may be stark seasonal and event-based changes in pollutants associated with seasonal application of deicing salts and first flush episodes respectively (Legret and Pagotto, 1999; Lee *et al.*, 2002). Contaminants may reach the tunnels and aquatic habitat through runoff, road splash or through dry

79 deposition in particulate form (Bäckström et al, 2003); thus there are several  
80 pathways by which the pollutants could accumulate in the habitat and impact the  
81 population. This study aims to provide an initial assessment of potential pollutant  
82 exposure in newt mitigation tunnels at a major urban development in England and to  
83 use this as a case study.

84 We hypothesised that there would be no significant difference in contaminant  
85 concentrations or major physico-chemical parameters between sediment samples  
86 collected from amphibian mitigation tunnels and those from reference sites.

## 88 **METHODS**

### 89 *Study site*

90 The study site was a mitigation scheme constructed in 2014 in England adjacent to a  
91 major retail development. As part of the ecological mitigation, given the discovery of  
92 a medium-sized population of *T. cristatus*, a new wetland habitat was created away  
93 from the construction site and exclusion from the development was ensured with  
94 amphibian fencing according to standard practices (Natural England, 2015). Three  
95 other amphibian species were also present and use the tunnels, plus the protected  
96 European otter (*Lutra lutra*). Two pairs of polymer-concrete amphibian climate tunnels  
97 (ACO, Germany) were installed underneath a newly constructed main access road to  
98 enable the newts to safely navigate between ponds to forage and breed. These  
99 widely-used tunnels have open slots into the road surface, allowing free air and  
100 humidity circulation in an effort to minimise microclimate differences which could  
101 reduce amphibian usage. The western entrances to the tunnels are adjacent to areas  
102 of terrestrial habitat and pondscapes, with the eastern side adjoining terrestrial habitat  
103 and a newly-constructed Sustainable Urban Drainage System (SUDS; Figure 1). The  
104 area is mostly suburban and the site lies on clays and silts of Quaternary age. The  
105 reference site was 6 km from the development, in a similar lowland area with a mix of  
106 both terrestrial and aquatic habitats, similar topography and similar superficial  
107 geology; the key difference being that it was away from any potential road pollutant  
108 sources.

### 110 *Sampling and analysis*

111 Between 12 and 18 bulk sediment samples were taken from tunnel entrances and  
112 road surfaces in January, March and November 2015 and March 2016 depending on  
113 availability of material to sample. Samples were taken from the concrete aprons of  
114 the 4 different tunnel entrances at the site and consisted of up to 500g bulk sample

115 and placed in polythene sample bags. Six reference samples were taken on each  
116 occasion from the nearby reference site. Sediment conductivity and pH were  
117 determined in the laboratory using standard soil paste methods (1:5 dilution with  
118 deionised water: ISO, 2005) and measurement on a frequently-calibrated Myron  
119 Company Ultrameter. Organic content of sediments was determined by loss on  
120 ignition at 450°C until a constant weight was achieved. Air-dried sediment samples  
121 were homogenised using a pestle and mortar, disaggregated then sieved (<2mm)  
122 prior to elemental analysis using a Niton XL3t XRF analyser (Thermo Scientific,  
123 2007). Standard certified reference materials were utilised to ensure accuracy of  
124 readings, with all readings within 10% of prescribed values. Five tunnel and 5  
125 reference replicates collected in spring 2016 were analysed for total petroleum  
126 hydrocarbons (TPH) using gas chromatography with a flame ionisation detector (GC-  
127 FID) by ESG Ltd.

128 Where ponded water was apparent by the tunnel entrances in November 2015 and  
129 March 2016, major physico-chemical parameters (pH, conductivity, temperature)  
130 were analysed in the field using a Myron Ultrameter calibrated on day of sampling.  
131 Filtered (0.45µm) and total dissolved water samples were taken from selected  
132 locations for elemental content using a Perkin Elmer Optima 5300 DV Inductively  
133 Coupled Plasma-Optical Emission Spectrometer.

134 Data were not normal even after log transformation (Kolmogorov Smirnov  $p > 0.05$ ) so  
135 non-parametric methods were used to test differences in median contaminant  
136 concentrations and major physico-chemical parameters between tunnel and  
137 reference sites and between seasons at the tunnel sites (Mann-Whitney U-test). The  
138 geochemical code Phreeqc v. 3.3.3. was used to determine saturation indices for a  
139 range of mineral phases in water samples (Parkhurst and Appelo, 1999).

## 142 **RESULTS**

### 143 *Sediments*

144 Median sediment pH in the tunnels was 8.4; significantly greater than at the reference  
145 site which had a median of 6.2 (Mann-Whitney U,  $W = 1258$ ,  $df = 51$ ,  $P = < 0.001$ ).  
146 Electrical conductivity values were significantly greater in the tunnel sediments than  
147 at the reference site (Mann-Whitney U,  $W = 1192$   $df = 51$ ,  $P = 0.001$ ) (Figure 2). The  
148 conductivity data was the only parameter that demonstrated a significant seasonal  
149 fluctuation, with significantly higher conductivity values in the tunnels in winter months  
150 (January and March: median of 630µS/cm) than autumn months (November: median  
151 of 270µS/cm), prior to the main period of deicing salt application (Figure 3). Organic

152 content of the sediments did not differ between reference and tunnel sites (Mann  
153 Whitney:  $P > 0.05$ ). However, significant differences were apparent in elemental  
154 composition of the sediments (Figure 4). Tunnel sediments were typically more  
155 mineral rich, with significantly greater concentrations of Ca, K, Ti and Fe than  
156 reference sites ( $P: < 0.001$  to  $0.005$ , Table 1, Figure 2). Of the trace elements of  
157 potential concern that were above detection limits, Cu, Mn, Pb and Zn were all  
158 apparent at significantly higher concentrations in the tunnel sediments than at  
159 reference sites ( $P < 0.001$ , Figure 4). Cd, Cr and V were below detection limits (10, 30  
160 and 40 ppm respectively) in all sediment samples. Replicate samples of loose  
161 sediment on the road surface in the vicinity of the tunnels showed a very similar  
162 composition to the tunnel sediments with high mineral content (Ca, K, Fe, Ti),  
163 electrical conductivity and elevated metal content (Cu, Mn, Pb, Zn).

164 Of the sub-samples analysed for Total Petroleum Hydrocarbons, similar patterns  
165 were apparent to the metals with a significantly higher TPH content in tunnels than  
166 the reference site (Mann-Whitney U,  $W = 40$ ,  $df = 5$ ,  $P = 0.01$ ; Figure 4). The tunnels  
167 had a median TPH content of 406mg/kg (range 136-2220mg/kg) and most of these  
168 carbon molecules were longer-chained ( $>C_{21} - C_{35}$ ) (Supplementary Information).  
169 The reference site had a median TPH content of 41mg/kg (range 35 to 53 mg/kg).

### 171 *Water quality*

172 The water quality data revealed some notable patterns with regard to high sodium  
173 levels (particularly in winter) in road surface samples and elevated pH at tunnel sites  
174 which is coincident with metal enrichment (notably Al, Cr and Se: Table 2). The  
175 tunnels also showed Na concentrations above surrounding SUDS samples and  
176 consistent with mixing of road surface waters with SUDS/pondscape waters (Table  
177 2). The higher dissolved metal concentrations of oxyanion-forming metals would be  
178 anticipated with the high pH. These highly alkaline tunnel sites were also  
179 characterised by white secondary precipitates on the substrate. Very high calcium  
180 concentrations were apparent (Table 2), while geochemical modelling of the waters  
181 suggest that this site was supersaturated with respect to calcite ( $CaCO_3$ ; Table 2).  
182 The surrounding SUDS water quality samples did not reveal any notable pollutant  
183 pressures and are general suitable for aquatic life and typical of lowland settings  
184 (Oldham et al. 2002).

## 186 **DISCUSSION**

187 The sediment and water analyses revealed that the null hypothesis of no significant  
188 difference in potential contaminants between mitigation tunnels and reference  
189 conditions can be rejected. A series of pollution pressures were apparent, including  
190 metal enrichment, salinity, extremely alkaline pH and potential enrichment of  
191 petroleum-related organics.

192 There were significantly higher concentrations ( $P < 0.001$  to  $0.005$ ) of trace metals in  
193 the tunnels for 7 of the 8 tested elements (Cu, Zn, Pb, Fe, Mn, Sr and Ti) compared  
194 to the reference site, which was a more rural area with a greater distance from major  
195 roads, unlike the tunnels which were underneath a main access road. It is probable  
196 that the road was the primary source of contamination as these metals are associated  
197 with road runoff (Ward, 1990). Moreover, the elements found in elevated  
198 concentrations in the tunnels were found in even greater concentrations in road  
199 surface dusts (Table 1), which suggests a clear pathway of movement. This is most  
200 likely due to surface runoff via the vents in the roof of the mitigation tunnels, or dry  
201 deposition of particulates (Bäckström *et al.*, 2003). The build-up of surface  
202 particulates at kerb edges close to tunnel vents was apparent on sample visits.

203  
204 The road-affiliated pollutants were likely sourced from the motor vehicles themselves;  
205 Cu and Zn particulates may be deposited with brake disc and tyre wear (Ward, 1990;  
206 Legret and Pagotto, 1999) and others, such as aluminium, may have petrogenic  
207 sources (Brown and Peake, 2006). Some elements may additionally be sourced from  
208 wear of the concrete tunnels themselves or road surfaces, where industrial residues  
209 such as blast furnace slags are often used in surfacing (Mahieux *et al.*, 2009). The  
210 markedly higher Ca concentrations and pH at tunnel sites are consistent with  
211 weathering of alkaline construction materials (Figure 2).

212  
213 Metals of concern that appear to be present in the new tunnels and surface water  
214 include Cu, Zn, Pb, Fe and Mn. Guidance for toxicity thresholds of metals in  
215 sediments are not well established, although the Threshold and Predicted Effects  
216 Level (TEL and PEL) guidance have been informally used as a screen in many UK  
217 settings (UKTAG, 2006). Most metals of interest (e.g. Cu, Pb and Zn) were above the  
218 lower Threshold Effects Level (above which negative effects on sediment dwelling  
219 organisms may be expected) and generally within the higher PEL values (above  
220 which negative impacts on aquatic biota may be expected: Figure 4). While some  
221 reference sites were also above the lower TEL value, possibly owing to mineral  
222 enrichment in superficial deposits given glacial and fluvial transport of sediments from  
223 adjacent areas of mineralisation (Dunham & Wilson, 1990), metal concentrations  
224 were significantly higher in the tunnels (Figure 4) than reference sites. More refined  
225 analyses on metal bioavailability would be desirable in the future to formulate more

226 robust risk assessments. The water samples suggest enrichment of Al and Cr in  
227 particular where there is a very high pH (Table 2). Encouragingly, Zn and Cu did not  
228 exceed quality standards in freshwater samples although negative effects of  
229 dissolved zinc on amphibians at levels below quality standards have been observed  
230 (Lefcort *et al.*, 1998). Amphibians are particularly sensitive to trace metals due to  
231 their permeable skin and thin epidermis (Hopkins *et al.*, 2013).

232 The pH of both the sediment and water samples from tunnels were high; with one  
233 extreme reading of 11.3 reported at one of the tunnel entrances. Such alkalization of  
234 surface waters is uncommon naturally, but is widely reported as a product of  
235 weathering of alkalinity generating minerals such as portlandite (Ca(OH)<sub>2</sub>) in concrete  
236 (Gomes *et al.*, 2016). In this case, leaching of such minerals from tunnels and road  
237 ballast is likely (Nodvin *et al.*, 1986) and is consistent with the supersaturation with  
238 respect to calcite (Table 2). *T. cristatus* are generally found in pH ranges of 4.4-9.5  
239 (Langton *et al.*, 2001); thus the extreme pH of 11.3 is much greater (100 times higher)  
240 than the documented *T. cristatus* range of tolerance. Such extreme pH could be of  
241 significance to amphibians due to elevated ionic strength, elevated hydroxide  
242 concentrations (Fominykh, 2008) as well as potential indirect effects of increased  
243 mobility of oxyanion-forming contaminants, such as Cr (Table 2), which would be  
244 expected in hexavalent form at such a pH (Takeno, 1996). The area of high pH water  
245 at this site was relatively small, which should limit potential exposure, however,  
246 relatively little is known about the tolerance of amphibians to extremely alkaline  
247 waters (Fominykh, 2008).

248  
249 Overall, conductivity was much greater in the tunnels than at the reference site  
250 (Figure 2). The proximity to the road suggests that the elevated conductivity is a result  
251 of seasonal de-icing salt application on the road; a notion supported by the  
252 significantly higher conductivity in March (post de-icing salt application) than in  
253 November (pre de-icing salt application) (Figure 3). Water samples had higher  
254 conductivities than the sediment (Table 2). The greatest was water at the roadside  
255 (S3) in March 2016 which was an extreme of 10,200µS/cm and extreme Na  
256 concentrations (1189mg/L); indicative of the presence of de-icing salts. Deicing salts  
257 pose serious ecological risks to amphibians due to the salinity increases and direct  
258 toxicity of chloride (Hopkins *et al.*, 2012; Duff *et al.*, 2010). Salts can have extreme  
259 adverse effects on amphibians at all life stages (Hopkins *et al.*, 2012); though  
260 embryonic and larval life stages are more sensitive than adults (Turtle, 2000).

261 Total petroleum hydrocarbon concentrations were significantly greater in the tunnels  
262 than the reference site (P = 0.01, Figure 4). Though the presence of more  
263 hydrocarbons in the tunnels does not categorically show that they are of petroleum



264 origin (as analysis is subject to interference from organic matter and chlorinated  
265 solvents: Villalobos *et al.*, 2008), the existence of many long chain molecules is  
266 indicative of this. However, as a preliminary screen it warrants further attention, given  
267 the obvious pollution pathway in this case. Very little research has been done on the  
268 impacts of petroleum hydrocarbons on amphibians, although there is evidence that  
269 petroleum contamination of freshwater habitats has negative impacts on tadpole  
270 growth and unsuccessful metamorphosis of the anuran *Hyla cinerea* (Mahaney,  
271 1994).

## 272

### 273 **CONCLUSIONS AND MANAGEMENT CONSIDERATIONS**

- 274 1. A series of pollutant pressures on amphibian populations using road mitigation  
275 tunnels were identified.
- 276 2. These include trace metals, hydrocarbons, de-icing salts and extreme alkaline pH  
277 contaminating different features of the habitat including the tunnels themselves  
278 and surface water.
- 279 3. The exact risks posed by these potential pathways remain unclear, but the  
280 relatively limited research conducted in this area suggests that impacts could be  
281 adverse and need highlighting in planning and design of mitigation schemes for  
282 amphibians, and great crested newts in particular.
- 283 4. Further research is needed to assess the exact exposure of amphibians to the  
284 contaminants at this and other sites and should incorporate more refined  
285 assessments of metal bioavailability (e.g. metal speciation) integrated with  
286 amphibian tunnel usage data (i.e. seasonality and exposure times).

### 287

### 288 **ACKNOWLEDGEMENTS**

289 Laurence Jarvis is thanked for assisting in sample collection. SOP is grateful for  
290 funding at Froglife by the Esmée Fairbairn Foundation under grant NR 14-0534.

### 291

### 292 **REFERENCES**

- 293 Bäckström, M., Nilsson, U., Håkansson, K., Allard, B. and Karlsson, S. (2003).  
294 Speciation of heavy metals in road runoff and roadside total deposition. *Water, Air,  
295 and Soil Pollution*, 147 (1-4), pp.343-366.
- 296 Beebee, T. J. (2013). Effects of road mortality and mitigation measures on amphibian  
297 populations. *Conservation Biology*, 27(4), 657-668.

- 298 Brown, J.N. and Peake, B.M. (2006). Sources of heavy metals and polycyclic  
299 aromatic hydrocarbons in urban stormwater runoff. *Science of the Total*  
300 *Environment*, 359 (1), pp.145-155.
- 301 Duff, J. P., Colville, K., Foster, J. and Dumphreys, N. (2011). Mass mortality of great  
302 crested newts (*Triturus cristatus*) on ground treated with road salt. *Veterinary*  
303 *Record*, 168(10), 282-282.
- 304 Dunham, K.C., Wilson AA. (1985) *Geology of the Northern Pennine Orefield,*  
305 *Economic Memoir of the British Geological Survey, 1<sup>st</sup> Ed.* HMSO, London. 247  
306 pp.
- 307 Edgar, P. and Bird, D.R. (2006). Action plan for the conservation of the crested newt  
308 *Triturus cristatus* species complex in Europe. *Strasbourg, France.*
- 309 Egea-Serrano, A., Relyea, R.A., Tejedó, M. and Torralva, M. (2012). Understanding  
310 of the impact of chemicals on amphibians: a meta-analytic review. *Ecology and*  
311 *evolution*, 2 (7), pp.1382-1397.
- 312 Fominykh, A.S. (2008) An experimental study on the effect of alkaline water pH on  
313 the dynamics of amphibian larval development, *Russian Journal of Ecology*, 39:  
314 145-147.
- 315 European Commission (1992) Directive 92/43/EEC on the conservation of natural  
316 habitats and of wild fauna and flora.
- 317 Gomes, H.I., Mayes, W.M., Rogerson, M.R., Stewart, D.I., Burke, I.T. (2016) Alkaline  
318 residues and the environment: a review of impacts, management practices and  
319 opportunities. *Journal of Cleaner Production*, 112: 3571-3582
- 320 Hopkins, G.R., French, S.S. and Brodie, E.D. (2012). Potential for local adaptation in  
321 response to an anthropogenic agent of selection: effects of road deicing salts on  
322 amphibian embryonic survival and development. *Evolutionary applications*, 6 (2),  
323 pp.384-392.
- 324 Hopkins, G.R., French, S.S. and Brodie, E.D. (2013). Increased frequency and  
325 severity of developmental deformities in rough-skinned newt (*Taricha granulosa*)  
326 embryos exposed to road deicing salts (NaCl & MgCl<sub>2</sub>). *Environmental pollution*,  
327 173, pp.264-269.
- 328 ISO (2005) *Soil Quality: pH determination. ISO 10390:2005.* International Standards  
329 Organisation.
- 330 Langton, T.E.S., Beckett, C.L., and Foster, J.P. (2001). Great Crested Newt  
331 Conservation Handbook, Froglife, Halesworth.
- 332 Lee, J.H., Bang, K.W., Ketchum, L.H., Choe, J.S. and Yu, M.J. (2002). First flush  
333 analysis of urban storm runoff. *Science of the Total Environment*, 293 (1), pp.163-  
334 175.
- 335 Lefcort, H., Meguire, R.A., Wilson, L.H. and Ettinger, W.F. (1998). Heavy metals alter  
336 the survival, growth, metamorphosis, and antipredatory behavior of Columbia

337 spotted macgreg frog (*Rana luteiventris*) tadpoles. *Archives of Environmental*  
338 *Contamination and Toxicology*, 35 (3), pp.447-456.

339 Legret, M. and Pagotto, C. (1999). Evaluation of pollutant loadings in the runoff waters  
340 from a major rural highway. *Science of the Total Environment*. 235 (1), pp.143-  
341 150.

342 Mahaney, P.A. (1994). Effects of freshwater petroleum contamination on amphibian  
343 hatching and metamorphosis. *Environmental Toxicology and Chemistry*, 13 (2),  
344 pp.259-265.

345 Mahieux, P.Y., Aubert, J.E. and Escadeillas, G. (2009). Utilization of weathered basic  
346 oxygen furnace slag in the production of hydraulic road binders. *Construction and*  
347 *Building Materials*, 23 (2), pp.742-747.

348 Natural England (2015) Guidance Great crested newts: surveys and mitigation for  
349 development projects; [https://www.gov.uk/guidance/great-crested-newts-surveys-](https://www.gov.uk/guidance/great-crested-newts-surveys-and-mitigation-for-development-projects)  
350 [and-mitigation-for-development-projects](https://www.gov.uk/guidance/great-crested-newts-surveys-and-mitigation-for-development-projects) (accessed Nov 2016)

351 Nodvin, S.C., Weeks, L.B., Thomas, E.P. and Lund, L.J. (1986). Alkalinization of a  
352 High-Elevation Sierra Nevada Stream. *Water Resources Research*, 22 (7),  
353 pp.1077-1082.

354 Oldham R.S., Keeble J., Swan M.J.S., Jeffcote M. (2000). Evaluating the suitability of  
355 habitat for the great crested newt (*Triturus cristatus*). *Herpetological Journal*. 10  
356 (4): 143-155

357 Parkhurst, D.L.; Appelo, C.A.J. (1999) User's guide to PHREEQC. U.S. Geological  
358 Survey Water-Resources Investigations Report 99-4259.

359 Petrovan, S.O. and Schmidt, B.R. (2016) Volunteer Conservation Action Data  
360 Reveals Large-Scale and Long-Term Negative Population Trends of a Widespread  
361 Amphibian, the Common Toad (*Bufo bufo*). PLoS ONE 11(10): e0161943.

362 Sparling, D.W., Linder, G., Bishop, C.A. and Krest, S. eds. (2010). *Ecotoxicology of*  
363 *amphibians and reptiles*. CRC Press.

364 Thermo Scientific. (2007). Thermo Scientific NITON XRF analysers. Available at:  
365 <<http://www.repamet.com/XL3%20Brosur.pdf> > [Last accessed 28/04/2016].

366 Turtle, S.L. (2000). Embryonic survivorship of the spotted salamander (*Ambystoma*  
367 *maculatum*) in roadside and woodland vernal pools in southeastern New  
368 Hampshire. *Journal of Herpetology*, pp.60-67.

369 UKTAG Sediment Subgroup (2006) *Assessment of sediment contaminants*. Report  
370 for Habitats Directive Technical Advisory Group on Water Quality. Environment  
371 Agency. 10pp.

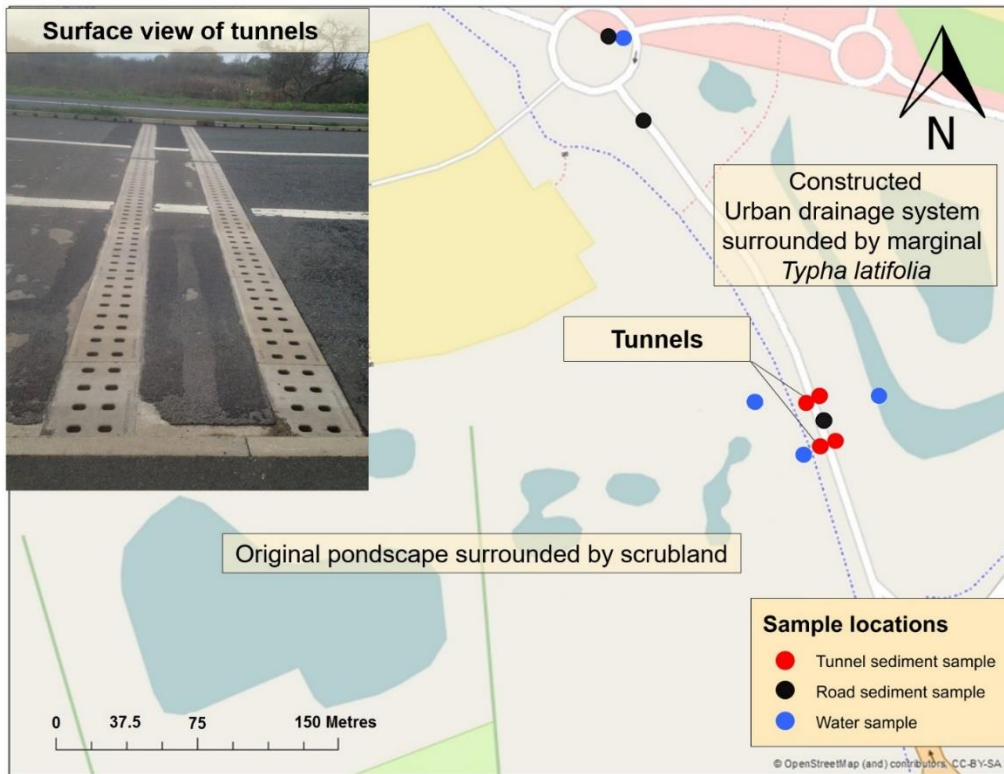
372 Van Bohemen, H.D. and Van De Laak, W.J. (2003). The influence of road  
373 infrastructure and traffic on soil, water, and air quality. *Environmental*  
374 *Management*, 31 (1), pp.50-68.

375  
376  
377  
378  
379  
380  
381  
382  
383  
384  
385  
386  
387  
388

Villalobos, M., Avila-Forcada, A.P. and Gutierrez-Ruiz, M.E. (2008). An improved gravimetric method to determine total petroleum hydrocarbons in contaminated soils. *Water, air, and soil pollution*, 194 (1-4), pp.151-161.

Ward, N.I. (1990). Multielement contamination of British motorway environments. *Science of the Total Environment*, 93 pp.393-401.

390 Figure 1. Location of the sample site showing sampling locations and the amphibian  
391 mitigation tunnels.

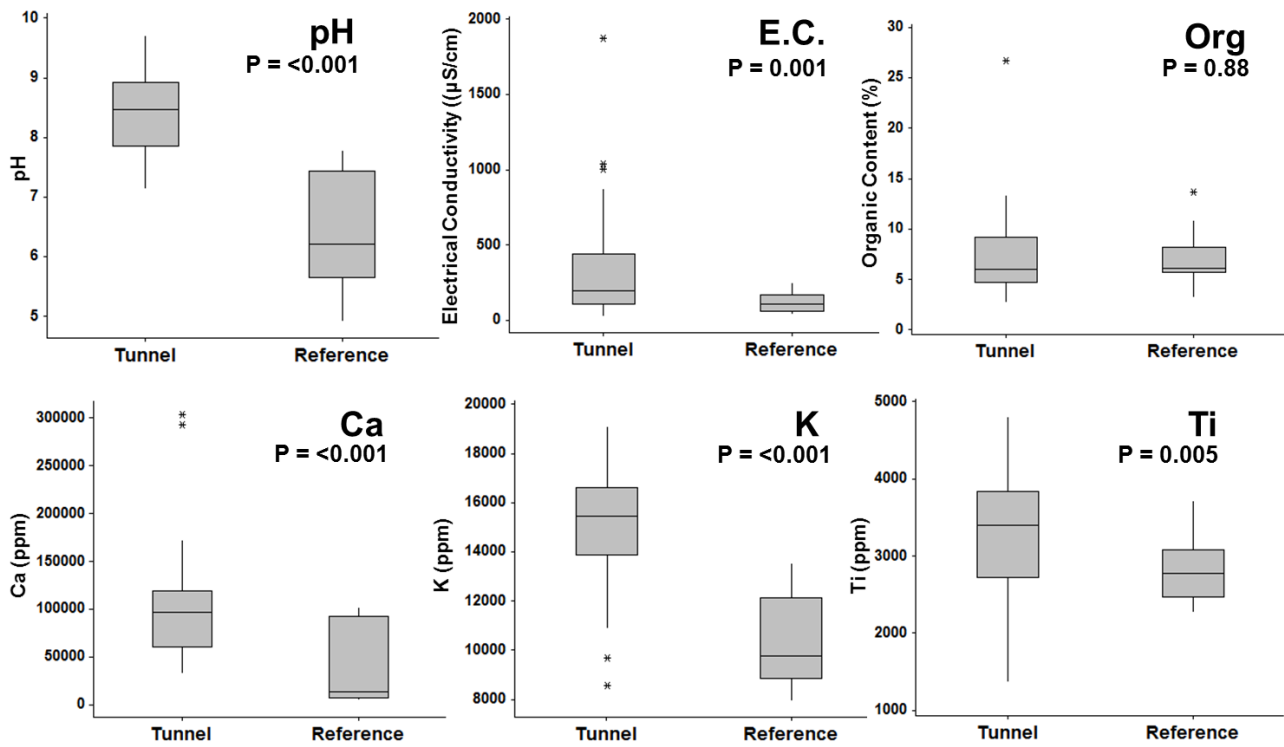


392

393

394  
395  
396

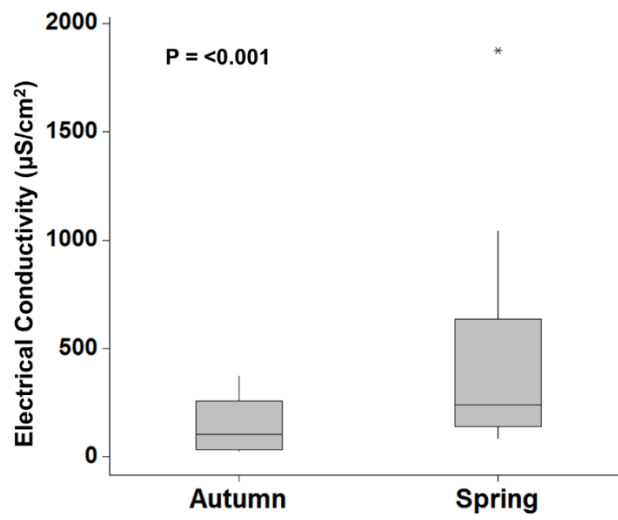
Figure 2. Comparison of major physico-chemical characteristics in sediments between tunnel and reference sites. Data aggregated from all sample months.  $n = 24$  for reference sites,  $n = 56$  for tunnel sites.



397

398  
399

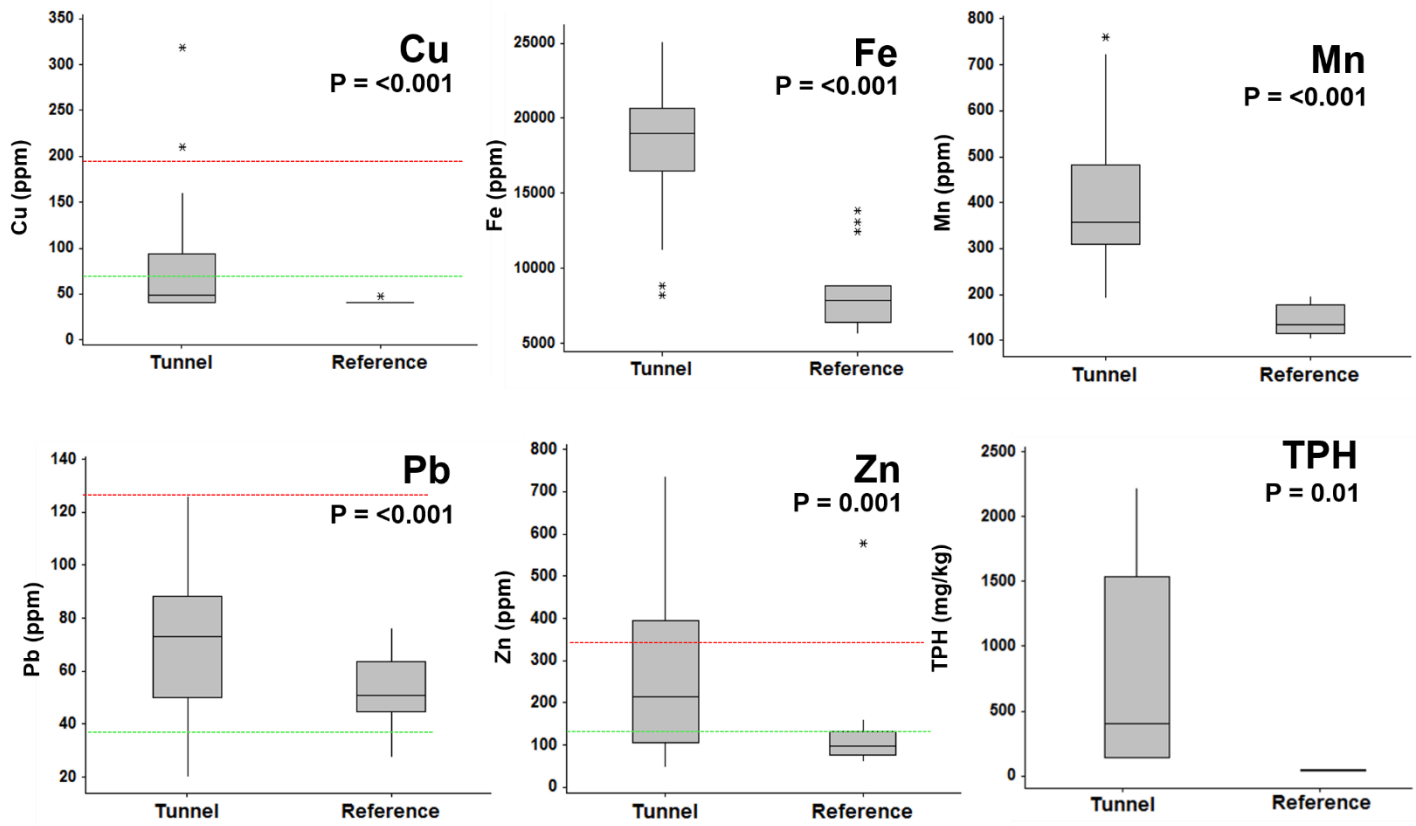
Figure 3. Comparison of sediment electrical conductivity in tunnels between autumn and spring sampling.  $n = 18$  for both autumn and spring.



400  
401  
402

403  
404  
405  
406

Figure 4. Comparison of major and minor elemental composition of sediments between tunnel and reference sites. Data aggregated from all sample months.  $n = 24$  for reference sites,  $n = 56$  for tunnel sites. Green line shows Threshold Effects Level; Red line shows Probable Effects Level (see text for description)



407

408  
409



410 Table 1: Range of filtered elemental concentrations in water samples. \*All elements  
 411 in  $\mu\text{g/L}$  excluding Ca, Mg, K and Na which are displayed in mg/L.  $n = 3$  for  
 412 Sustainable Urban Drainage System (SUDS) and Tunnel mouth. Pondered road  
 413 surface samples were only available for collection on one occasion (Jan 16).

	<b>SUDS</b>	<b>Tunnel entrances</b>	<b>Road Surface</b>
<b>pH</b>	7.1-8.7	9.6-11.4	8.2
<b>E.C. (<math>\mu\text{S/cm}</math>)</b>	507-1832	710-3471	10,200
<b>Ca</b>	58-116	132-311	30
<b>Mg</b>	30-85	21.4	20
<b>Na</b>	30-290	488-646	1190
<b>K</b>	4-9	5-13	4
<b>Al</b>	31-96	201-253	105
<b>Cr</b>	1-2	9-12	1
<b>Cu</b>	5-11	4-6	38
<b>Fe</b>	22-2585	10-16	33
<b>Mn</b>	12-120	6-7	30
<b>Pb</b>	<LOD	<LOD	<LOD
<b>Se</b>	7-19	6-41	16
<b>Sr</b>	270-358	280-474	128
<b>Zn</b>	5.8-5.8	1-2	10
<b><math>SI_{CaCO_3}</math></b>	-1.14 to +0.63	+1.45 to +1.97	-1.28 to -1.45

427  
428  
429

Table 2. Range of values for physicochemical parameters of sediment and elemental concentrations (ppm) taken for different sediment samples.

430

	<b>Reference</b>	<b>Tunnels</b>	<b>Road</b>
<b>pH</b>	4.9-7.8	7.3-9.6	7.1-9.7
<b>E.C. (<math>\mu\text{S/cm}</math>)</b>	40.7-249.6	44.9-1872	26.9-874.1
<b>Ca</b>	4819-101868	32400-172800	30300-88861
<b>Fe</b>	5602-13846	11194-23502	8157-24184
<b>K</b>	7941-13538	12388-19089	8561-13538
<b>Ti</b>	2277-3710	2550-4806	1363-4520
<b>Cu</b>	40-47	40-210	75-320
<b>Mn</b>	65-196	191-761	210-725
<b>Pb</b>	27-76	20-126	24-76
<b>Zn</b>	61-279	48-736	65-491