

Title

Short-term movements and behaviour govern the use of road mitigation measures by a protected amphibian

Authors

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4 1 • **Lay Summary**
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7 2 Can amphibians' perception of barriers determine the
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9 3 success or failure of road mitigation schemes? By analysing
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11 4 short-term movements of *Triturus cristatus* at a tunnel and
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13 5 fence system we show these movements were limited in
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15 6 distance and most adult newts returned to the original area,
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17 7 without crossing the road through tunnels. Mitigation
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19 8 fences, installed to guide amphibians towards tunnels, were
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21 9 not followed and mostly represented movement barriers. *T.*
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23 10 *cristatus* behaviour changed significantly inside tunnels,
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25 11 with longer distances travelled and a straighter orientation.
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4 12 **Abstract**

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6 13 Road mitigation infrastructure for pond-breeding amphibians aims to
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8 14 provide a safe and sustainable passage for individuals between critical
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10 15 habitat patches. However, relatively little is known about how
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12 16 amphibians interact with mitigation systems because of the challenges of
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14 17 documenting movements at sufficiently large sample sizes. The effect of
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16 18 real or perceived barriers to short-term movement could ultimately
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18 19 determine the success or failure of road mitigation schemes. We
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20 20 quantified behavioural responses of the protected great crested newt
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22 21 (*Triturus cristatus*) in a complex road mitigation system in the UK. We
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24 22 used fluorescent paint to mark individuals in order to measure distance
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26 23 travelled and trajectory orientation over two seasons (spring when adults
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28 24 migrate to breeding ponds and autumn when newts disperse) and in three
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30 25 components of the mitigation system (fences, tunnel entrances and inside
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32 26 the tunnels). A total of 250 juveniles and 137 adult great crested newts
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34 27 were marked and tracked during 38 survey nights. Adults were
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36 28 individually identified using belly-pattern recognition. There was
37
38 29 substantially greater activity along the fences during autumn (82% of
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40 30 newt captures) compared to spring. *Triturus cristatus* typically moved
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42 31 short distances each night (3.21 m night⁻¹ in spring and 6.72 m night⁻¹ in
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44 32 autumn), with a maximum of 25.6 m travelled inside a tunnel. Adult
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46 33 recapture rates were low (9.7%) and only 3% of the newts found along
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48 34 the fences reached the tunnel entrances. Movements were straighter in
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50 35 spring and inside the tunnels and newts had higher crossing rates in
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4 36 autumn compared to spring. Overall, behaviour and seasonal movement
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6 37 patterns significantly influenced the use of the mitigation system, in a
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8 38 way that could impact landscape connectivity for *T. cristatus* over the
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10 39 long-term. Adequate incorporation of fine-scale movement dynamics
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12 40 could help develop new behavioural models, inform our understanding of
13
14 41 amphibian ecology and substantially improve future road mitigation
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16 42 projects.
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21 44 **Keywords:** connectivity, dispersal, *Triturus cristatus*, individual
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23 45 behaviour, migration, underpasses.
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46 **Introduction**

47 Roads cause multiple and diverse ecological impacts on habitat and
48 wildlife populations by directly contributing to habitat loss and reducing
49 the quality of surrounding habitats (Forman and Alexander, 1998;
50 Spellerberg, 1998; Trombulak and Frissel, 2000). The negative influence
51 of roads threatens population viability through wildlife traffic-related
52 mortality and by acting as a barrier to animal movements (Forman et al.,
53 2003), therefore increasing habitat fragmentation and isolation.

54 For amphibian populations, habitat loss and consequent fragmentation
55 represent major threats (Cushman, 2006). Road construction in particular
56 reduces habitat availability and increases the distance between habitat
57 patches that are crucial for seasonal movements and maintenance of
58 population dynamics for amphibians (Fahrig et al., 1994; Hels and
59 Buchwald, 2001). Moreover, high amphibian mortality rates on roads and
60 road avoidance behaviour compound these impacts, leading to worldwide
61 concern about the potential effects of roads on amphibian population
62 viability (Fahrig et al., 1994; Jaeger and Fahrig et al., 2004; Glista et al.,
63 2007; Petrovan and Schmidt, 2016).

64 Road mitigation measures such as tunnels and fences have been
65 implemented for decades, aiming to safely guide amphibians between
66 habitat patches in order to maintain landscape-scale connectivity within
67 and between populations (Beebee, 2013). Knowledge of terrestrial
68 movement patterns is typically used to guide decisions on the locations
69 of mitigation systems (Clevenger and Waltho, 2005). The protected great

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4 70 crested newt (*Triturus cristatus*) is one of the main road mitigation
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6 71 target species in Europe (Ward et al. 2015; Matos et al. 2017), and
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8 72 landscape permeability is essential for the species' dispersal and
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10 73 migration (Halley et al., 1996; Griffiths et al., 2010). After
11
12 74 implementation of road mitigation, great crested newts can be
13
14 75 particularly active around tunnels and fences, especially after emigration
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16 76 from ponds to hibernation and refuge sites during autumn (adults and
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18 77 juveniles) and during spring migration when adults move towards the
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20 78 ponds to reproduce (Matos et al., 2017) yet detailed aspects of their
21
22 79 interactions with the mitigation system remain unknown.
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25 80 Previous studies on amphibians suggest that fences and tunnels alter
26
27 81 typical behaviour and that responses to mitigation infrastructure are
28
29 82 species specific (Jackson and Tynning, 1989; Allaback and Laads, 2003;
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31 83 Pagnucco et al., 2011). Post-mitigation monitoring often inadequately
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33 84 assesses mitigation effectiveness and typically does not account for the
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35 85 behaviour of target species at different points along the mitigation
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37 86 system (Woltz et al., 2008, Pagnucco et al., 2012). For example, no
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39 87 evidence is available to explain whether pond-breeding amphibians use
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41 88 mitigation systems as part of their home ranges or whether they simply
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43 89 use them for transit (Langton, 1989; Hamer et al., 2015). Similarly, it
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45 90 remains unclear whether amphibian fences actively guide amphibians
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47 91 towards tunnels or rather deflect their movements at random. Mitigation
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49 92 advice assumes that amphibians will follow fences for considerable
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51 93 distances (30-50 m or more) in order to reach tunnel entrances (Schmidt
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4 94 and Zumbach, 2008) yet this is largely untested, especially for newt
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6 95 species. Even when amphibians encounter tunnels, they may be reluctant
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8 96 to enter and cross them (Jackson and Tynning, 1989; Allaback and Laads,
9
10 97 2003; Matos et al. 2017).

11
12 98 Quantitative analyses of behavioural changes in movement patterns in
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14 99 response to road mitigation (eg. attraction/rejection, distances moved
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16 100 and trajectory orientation) are extremely limited. Studies describing
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18 101 encounter and transit of each part of the mitigation system by newts
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20 102 during critical phases of the annual cycle are lacking (Schmidt and
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22 103 Zumbach, 2008; Hamer et al., 2015), limiting opportunities for evidence-
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24 104 based improvements to mitigation systems. Understanding the type and
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26 105 the consequence of movements performed by individuals could inform
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28 106 individual-based models for predicting population-scale movements
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30 107 relative to mitigation systems and thus enable more effective advice on
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32 108 fence and tunnel placement (Pontoppidan and Nachman, 2013). However,
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34 109 individual behaviour among amphibians guides their dispersal success
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36 110 and can influence populations' distribution patterns in the landscape
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38 111 (Baguette and Dyck, 2007, Sinsch, 2014). Newts display a range of
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40 112 behaviours and motivations while moving on land (e.g. foraging,
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42 113 searching for refuge, use of underground shelters), and slight changes of
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44 114 direction for each individual can lead to different conclusions on the
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46 115 intent of the movement (i.e. dispersers or residents) (Sinsch, 2014).
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48 116 Traditional techniques for studying terrestrial movements of urodeles
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50 117 can directly influence their behaviour thus confounding conclusions
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4 118 about individual choices. For instance, radio-tracking has been used to
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6 119 study terrestrial movements of *Triturus* species (Jehle, 2010, Jehle and
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8 120 Arntzen, 2000, Schabetsberger et al., 2004; Jehle et al., 2011) providing
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10 121 information on total distance travelled (30 – 400 m) and direction of
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12 122 movement after and before the aquatic phase. Yet, transmitters must be
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14 123 internally implanted, force-swallowed or externally mounted, with
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16 124 consequences for behaviour (Schabetsberger et al., 2004). Moreover, the
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18 125 number of newts that can be efficiently followed over a short period of
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20 126 time using this technique is restricted and limited in spatial scale (1-20
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22 127 m) (Jehle, 2000).

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25 128 This study aimed to investigate the short-term terrestrial movements of
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27 129 individual *T. cristatus* within a road mitigation system. We tested the
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29 130 following assumptions about the great crested newt's use of the
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31 131 mitigation system: (1) On contact with a fence, newts adjust their
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33 132 movement to follow it; (2) On encountering a tunnel entrance newts
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35 133 move into and cross through the tunnel; (3) Activity peaks in the annual
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37 134 cycle in spring (migration) and autumn (dispersal) are reflected
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39 135 differently in the use of the mitigation system.

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42 136 For this, we measured distance travelled and trajectory orientation of
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44 137 individual *T. cristatus* per night during two periods of field surveys, in
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46 138 autumn and spring. In addition, we quantified the proportion of
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48 139 recaptured *T. cristatus* and their final positions in the system using
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50 140 photographs of adult belly-patterns.

141 **Materials and Methods**

142 **Study site**

143 The study was conducted at Orton Pit/Hampton Nature Reserve,
144 Cambridgeshire, UK (52° 32'24N, 0°16'53W) (**Fig 1a**), a 145ha Site of
145 Special Scientific Interest, a Special Area of Conservation and a Natura
146 2000 site. The area is characterised by a complex of over 340 ponds,
147 between 15-50 years old. The site is currently home to the largest known
148 single population of great crested newts in the UK and possibly Europe,
149 estimated at around 30,000 individuals (JNCC, 2015) as well as a very
150 large population of smooth newt (*Lissotriton vulgaris*), and small
151 numbers of common frog (*Rana temporaria*) and common toad (*Bufo*
152 *bufo*).

153 Between 1990 and 2000 a large-scale habitat restoration programme was
154 implemented to protect the great crested newts, including pond
155 modification and fish eradications. In 2006, a new housing development
156 was built towards the north of the reserve, serviced by a 10-12m wide
157 road designed to support 1000 to 10 000 vehicles per day. Concrete
158 'newt barriers' were installed adjacent to this road (**Fig. 1b**). During
159 road construction some large ponds were re-profiled and some were in-
160 filled to accommodate the road. A mitigation system was installed to
161 facilitate movement of amphibians under the road between the eastern
162 and western parts of the reserve (**Fig. 1b**).

163 The road mitigation system was composed of one 0.5 m diameter, 30 m
164 long polymer concrete 'amphibian tunnel' with open slots at the top

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4 165 (ACO, Germany) in the centre, two large ARCO concrete and metal sheet
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6 166 underpasses (5.5 m wide x 2 m high, 40 m long) spaced 100 m apart and
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8 167 two 200 to 300 m long heavy duty plastic fence systems (Herpetosure
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10 168 UK), placed 10-50 m away from the road and partly angled (~45°) to
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12 169 guide amphibians towards the tunnels. The distance from the tunnels to
13
14 170 the nearest great crested newt breeding pond is at least 31-74 m
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16 171 depending on tunnel and side of the road. In an effort to minimise human
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18 172 disturbance the entrances of the large tunnels were protected with a bar
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20 173 fence and gate system.
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25 175 **Data collection**

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27 176 *T. cristatus* movement data were collected during two periods of peak
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29 177 activity: autumn (between 17 September and 26 October 2014 covering
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31 178 the period of post-breeding dispersal) and spring (between 6 March and 3
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33 179 April 2015, covering the period of adult migration towards breeding
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35 180 sites). For both seasons, night surveys (marking) started 2-3 hours after
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37 181 the sunset and had a mean duration of 2 hours. Morning surveys
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39 182 (recaptures) started 1 hour before sunrise and mean duration depended
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41 183 on the number of *T. cristatus* captured (2-3 hours). Days with favourable
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43 184 weather (rain in the last 3 days and temperatures above 6°C) were
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45 185 specifically selected to maximise the number of *T. cristatus* monitored
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47 186 (**Table 1**).
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53 188 *Tracking method*
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4 189 We used multiple fluorescent pigment applications to track the short-
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6 190 term movements of *T. cristatus* throughout the road mitigation system.
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8 191 Fluorescent pigments have been proven a useful technique in
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10 192 microhabitat studies for amphibians (Eggert, 2002; Ramirez et al., 2012).
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12 193 The extent of daily movements might be over-estimated by home range
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14 194 analysis (Wells, 2007); however, fluorescent marking can provide
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16 195 detailed information on daily patterns, even under wet conditions, and
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18 196 especially for newts because of their relative short movements compared
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20 197 to other amphibians (Jehle, 2000). Although it has been used in
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22 198 combination with transmitters to improve detectability, pigments alone,
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24 199 when rapidly applied, do not seem to influence behaviour (Eggert, 2002).
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26 200 Once painted and released, newts leave a discrete fluorescent trail that
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28 201 can be observed for several hours or even days, whilst avoiding the need
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30 202 for more disruptive tracking methods. Using this method, a single
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32 203 observer can monitor the movements of hundreds of individuals,
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34 204 collecting detailed information on trajectory orientation and type of
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36 205 movement displayed. This method has been tested on a range of
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38 206 amphibians at different life stages and is considered safe and less
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40 207 invasive than radio tracking for assessing short-term movements
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42 208 (Nishikawa, 1990; Eggert, 2002; Roe and Grayson, 2009; Pittman and
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44 209 Semlitsch, 2013).

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51 211 *T. cristatus* capture-mark-recapture
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4 212 Newts were surveyed by following fences along both sides of the road
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6 213 during each survey with the start point alternating between surveys (A in
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8 214 **Fig. 1b**). Newts were also surveyed at tunnel entrances (within 2-3 m
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10 215 radius around tunnel openings: **Fig. 1b**) and inside the tunnels (north and
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12 216 south tunnels: C in **Fig. 1b**). Only the two large tunnels permitted entry
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14 217 to capture individuals and measure behaviour inside the tunnel.

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17 218 At initial observation, individual activity and position in the system (at
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19 219 the fence, tunnel entrance and inside the tunnel) were recorded.

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21 220 Orientation and microhabitat use at the start and end of movement, as
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23 221 well as activity in three specific categories (moving, foraging, and
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25 222 stationary) were also documented. Adult great crested newts were
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27 223 captured by hand, given a unique identification number, and a
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29 224 photograph was taken of the ventral pattern for future identification.
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31 225 Life stage (adult/juvenile) and sex (female/male) were recorded, and a
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33 226 colour was allocated for marking. Marking consisted of dipping newts'
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35 227 tails, hind legs and ventral surface in fluorescent powder (DayGlo Color
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37 228 Corp. fluorescent pigments) and pressing gently to ensure adhesion of
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39 229 sufficient powder. Newts were marked with four different colours (Z-18-
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41 230 3 Green, Z-15-3 Orange, Z-17-N Yellow and Z-11-3 Pink) in order to
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43 231 differentiate paths within and between surveys (**Fig. 2a**).

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46 232 To minimize stress, animal processing was completed in less than 1
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48 233 minute and no body measurements were collected. Newts were released
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50 234 at their exact point of capture and left undisturbed for at least 5 hours.

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53 235 We used different colours to mark individuals in close proximity and
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4 236 removed paint tracks from previous nights with a brush or by disturbing
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6 237 the soil before each survey.

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8 238 To evaluate minimum long-term movements between seasons and
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10 239 positions in the system we used ventral patterns to identify recaptured
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12 240 adults. Recaptures were identified automatically from photographs using
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14 241 I3S software (pattern software) (Hartog and Reijns, 2014). Recapture
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16 242 rates were calculated by season, position in the system and between
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18 243 initial and final positions.

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22 23 245 *Position recording*

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25 246 *T. cristatus* trails were monitored using an ultraviolet light (UV WF-
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27 247 501B LED 365NM) to detect the fluorescent powder. Trail start/end
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29 248 positions and points at each directional change were temporarily marked
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31 249 with small flags and distances were recorded using a tape measure (to
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33 250 the nearest 0.01m). Track trajectory was measured using a compass
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35 251 (using a range of 0-180°) in relation to the position in the system (see
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37 252 below Data analysis). Final location was recorded to the nearest 3m
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39 253 using a portable GPS (GPS Essentials 4.4.8, 2015). If the entire path was
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41 254 less than 30-40 cm between the initial capture point and the final point,
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43 255 movement was recorded as zero (similar to Ramirez et al., 2012).

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48 49 257 **Data analysis**

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51 258 Short-term movements were analysed using data on distance travelled,
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53 259 track trajectory and frequency of movement performed by adults and
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4 260 juvenile *T. cristatus* in three positions along the road mitigation system
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6 261 (fence, tunnel entrance and inside the tunnels) and between seasons
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8 262 (autumn and spring). For simplification, only two age classes were used:
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10 263 adults and juveniles. The latter comprised both young-of-the-year in
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12 264 autumn as well as individuals hatched in the previous year.
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266 *Movement data: distance travelled and trajectory orientation*

267 Movement data for each individual were analysed as total distance
268 travelled per night (track length) and track trajectory. Track trajectory
269 was summarised as straightness and orientation relative to the mitigation
270 system. Straightness was calculated from the ratio between displacement
271 and total distance travelled (**Fig. 2b**). Displacement distance was
272 determined by dividing the straight-line distance between the start to the
273 end of track with total distance travelled (Sinsch, 1990; Roe and
274 Grayson, 2009). Straightness is an index that varies between 0-1; values
275 close to 0 indicate a curved route and a value of 1 indicates a straight
276 path. This analysis can distinguish between rambling behaviour and
277 directed movements. Orientation was calculated using the deflection
278 angle at three capture points (**Fig. 2b**). When individuals were captured
279 at the fences or tunnel entrances, angles ranged between 0° and 180°.
280 When captured inside the tunnels, angles ranged between 0° and 90°.
281 Inside the tunnels and along the fences, values of 0° indicated
282 individuals moving towards the exit/entrance of the tunnel and values of

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4 283 90°-180° indicated individuals moving away from entrance/exit of the
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6 284 tunnel.

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10 286 *Season and location effects*

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12 287 Movement data of adults and juveniles that moved > 40 cm per night
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14 288 were summarised by season (autumn and spring), location inside the
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16 289 mitigation system and life stage. To quantify differences in behaviour in
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18 290 different parts of the system, we calculated: (1) the number of
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20 291 individuals moving from the three positions in the system (fence, tunnel
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22 292 entrance and inside the tunnel) and final positions (surrounding
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24 293 environment or remaining in the system) and (2) changes in distance
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26 294 travelled and orientation relative to the three positions in the system.

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29 295 We used a generalized linear mixed model (GLMM) to calculate the
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31 296 effects of season, position in the system and life stage on movements of
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33 297 *T. cristatus*. Season, position and life stage were fixed effects and
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35 298 position and life stage were nested within season, which was entered as a
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37 299 random effect. Three null models containing the most significant
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39 300 variables and intercept were included for comparison (season +
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41 301 mitigation + life stage + (1|season)). We then tested three models with
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43 302 the most significant variables, with no test for interactions. We
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45 303 compared model fit using Akaike information criterion (AIC) to optimize
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47 304 goodness-of-fit but avoided overfitting of the candidate models
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49 305 (Burnham and Anderson, 2002). After selecting the most parsimonious
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51 306 model, we determined the significance of fixed factors by analysis of
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4 307 deviance (Burnham and Anderson, 2002). Models were fitted using a ML
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6 308 distribution and lmer function in package lm4 (Bates et al., 2014) in R
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8 309 software (R Core Team, 2016).

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Review Copy

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4 311 **Results**

5
6 312 In total, 38 surveys were carried out: 24 during autumn and 14 during
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8 313 spring. Cold and dry weather meant that additional surveys in spring had
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10 314 to be abandoned as no newts could be found. A total of 387 *T. cristatus*
11
12 315 were caught and paint-marked (280 and 107 per season, respectively); of
13
14 316 these, 250 were juveniles and 137 were adults (73 females and 64 males)
15
16 317 (**Table 1; Table S1**).

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18 318 Among the 270 (70%) *T. cristatus* that moved >40 cm, 44 (16.3%) were
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20 319 adult males, 35 (13%) adult females and 191 (70.7%), were juveniles), a
21
22 320 higher percentage moved in autumn (82%) than in spring (18%, **Table 1**).
23
24 321 Most movements were performed along the fences during autumn (58%)
25
26 322 with no *T. cristatus* found inside the tunnels in spring. Final positions
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28 323 were mainly recorded in the mitigation system (n = 215, 80%) while 55
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30 324 (20%) were found moving in the direction of the surrounding
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32 325 environment (**Table 1**).

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38 327 *Final positions and seasonal movements*

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40 328 For both seasons combined, only 3% of newts moving along the fences
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42 329 encountered the tunnels (3 adults and 3 juveniles), 25% moved to the
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44 330 surrounding environment (23 adults and 26 juveniles), and approximately
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46 331 70% remained at the fences (53 adults and 89 juveniles). When captured
47
48 332 at the tunnel entrances, the majority of *T. cristatus* moved towards the
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50 333 fences (70%), 11% moved in the direction of the surrounding
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52 334 environment and only 19% stayed at the entrance or entered the tunnel.
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4 335 A high percentage of *T. cristatus* captured inside the tunnels remained
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6 336 inside or at the entrance (78%) while 22% found the tunnel exit towards
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8 337 the surrounding environment (4%) or reached the fences on the opposite
9
10 338 side of the road (18%) (**Fig. 3**).

11
12 339 The adult recapture rate was small and not significantly different
13
14 340 between seasons, averaging at 9.7% (**Table 2**). Recapture rates were
15
16 341 borderline significantly different between locations in the system ($\chi^2=$
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18 342 5.94, df = 2, P = 0.051). Most recaptured *T. cristatus* were located
19
20 343 around the fences (11%) and 3% were captured in the tunnels (entrance
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22 344 and inside). The majority of recaptures was at the fence (4%), followed
23
24 345 by a small percentage in the tunnels (1%) and surrounding environment
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26 346 (0.5%).

31 347 32 348 *Distance travelled*

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34 349 The vast majority of *T. cristatus* moved very short distances, with 77%
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36 350 of those tracked during autumn and 97% in spring moving less than 5m
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38 351 per night (**Fig. 4; Fig. 5**). Distances of more than 10 m per night were
39
40 352 only recorded in autumn (5% of juveniles and 1% of adults).

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42 353 Variation in distance travelled per night was dependent on the initial
43
44 354 capture position in the system (**Table 3**). There was a higher number of
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46 355 *T. cristatus* at the fences (n= 197) with shorter movements (mean.night⁻¹
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48 356 = 3.21 m), a low number of captures at the tunnel entrances (n= 45)
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51 357 with short movements (mean.night⁻¹ = 3.41 m), and a small number of

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4 358 captures inside the tunnel (n=28) with long movements (mean.night⁻¹=
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6 359 6.72 m) (**Fig. 5; Table 3**).

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8 360 The maximum distance travelled in a night was 25.6 m (adult male)
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10 361 inside a tunnel. For adult recaptures, the greatest distance between
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12 362 capture points was 20.3 m by a female over 38 nights.

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17 364 *Trajectory orientation*

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19 365 Newts were observed moving in different directions in relation to the
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21 366 fences; no particular angles were preferred (**Fig. 5**). There was a
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23 367 significant effect of season on directionality (straightness of 1), with *T.*
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25 368 *cristatus* paths being straighter during spring (**Table 4**). Orientation of
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27 369 trajectories was dependent on individuals' position in the system.
28
29 370 Trajectory orientation was significantly different for newts moving along
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31 371 the fences compared to the newts moving around the tunnel entrances
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33 372 and inside the tunnels, with a clear orientation towards the tunnel
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35 373 entrance or exit apparent inside the tunnels (**Fig. 5; Table 4**).

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4 374 **Discussion**

5
6 375 Using fluorescent marking to investigate fine-scale movement of *T.*
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8 376 *cristatus* we showed that: (1) distances travelled per night were
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10 377 generally small; (2) very few *T. cristatus* moving along mitigation fences
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12 378 reached and entered the road tunnels; (3) *T. cristatus* moving into
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14 379 tunnels remained there at least overnight but their movements were
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16 380 straighter than outside; (3) the orientation of movements at the fences
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18 381 was mostly away from the tunnels (4) there were large seasonal
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20 382 differences in movements (longer movements during autumn compared to
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22 383 spring).

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27 385 *Behaviour along the fences*

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29 386 Despite the high number of *T. cristatus* encountered at the fences during
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31 387 the night, the observed low adult recapture rate between seasons and the
32
33 388 small capture numbers in spring suggests that individuals do not cross
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35 389 the road as part of annual breeding migrations. Additionally, few
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37 390 encounters with the tunnel entrances and the straight paths at a range of
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39 391 angles away from the fences are consistent with *T. cristatus* using the
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41 392 fences as part of their normal foraging environment (Oldham et al.
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43 393 2000). Fences may constitute barriers, keeping newts away from the
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45 394 road, hence preventing road mortality, but do not appear to guide *T.*
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47 395 *cristatus* directly towards tunnels. This is consistent with previous
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49 396 results that showed salamanders moving in different directions along the
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51 397 fence and not specifically towards the tunnel entrances (Allaback and
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4 398 Laads, 2003). Effective tunnel crossings by newts may therefore be
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6 399 improved by positioning more tunnels close together. Allaback and
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8 400 Laads, (2003) suggested a maximum of 30m intervals between tunnels
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10 401 for salamanders, yet our results suggest that such distances are too great
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12 402 for *T. cristatus*.

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14 403 For amphibians that can suffer high road mortality such as the common
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16 404 toad (*Bufo bufo*), fences offer important benefits by reducing mortality
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18 405 and directly guiding individuals towards tunnels (Lesbarrerès et al.,
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20 406 2004). Given the lower terrestrial movements of newts (Kovar et al.,
21
22 407 2009) along with our results showing a tendency to reflect off the fence
23
24 408 in random directions, this may mean that fences result in less frequent
25
26 409 road crossings and hence lower environmental connectivity.
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28 410 Furthermore, the fence area offered good refuge conditions; newts use
29
30 411 small mammal burrows (Jehle and Arntzen, 2000), which were abundant
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32 412 along the fences. Active feeding and refuge use was observed during this
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34 413 study, especially in areas with dense vegetation.
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40 415 *Behaviour in the tunnels*

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42 416 Our results indicated differences in distances travelled and orientation of
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44 417 *T. cristatus* at the entrance and inside the tunnels. At tunnel entrances,
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46 418 distances travelled by *T. cristatus* were shorter than inside the tunnels. A
47
48 419 higher proportion of animals might be guided towards the tunnels if
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50 420 fences adjacent to the entrance were angled by 45° in relation to the
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52 421 tunnel and shorter than 20m (Jackson, 2003). Entrances in this study
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4 422 presented the same angle (45°, see Fig. 5.1) and were 3 m long.
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6 423 Nevertheless, *T. cristatus* seemed to change path where the fence angle
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8 424 at the tunnel entrance changed to direct them towards the inside of the
9
10 425 tunnel and numbers of individuals attempting to cross were still very low
11
12 426 (20%). Experimental studies could test the optimal length of fence at the
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14 427 tunnel entrances in order to maximise opportunities for encountering the
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16 428 tunnel entrance.

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18 429 The majority of *T. cristatus* found at the tunnel entrance moved away
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20 430 from the tunnel and towards the fence or the surrounding environment.
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22 431 Changes in orientation in tunnel entrances have been reported for
23
24 432 *Ambystoma* species (Jackson and Tynning, 1989; Allaback and Laabs,
25
26 433 2003). Microhabitat conditions at tunnel entrances can impact the way in
27
28 434 which amphibians move through this area to get to the tunnel (Glista et
29
30 435 al., 2009) and amphibian tunnels can suffer significant pollution from
31
32 436 the road surface (White et al. 2017). Usually tunnel entrances are cleared
33
34 437 of vegetation and debris to facilitate entry by amphibians, but that can
35
36 438 create areas denuded of vegetation (Langton, 1989). Newts may hesitate
37
38 439 to enter tunnels due the changes in microhabitat and environment (e.g.
39
40 440 temperature and moisture) associated with vegetation removal (Allaback
41
42 441 and Laabs, 2003). Cover may determine if a tunnel may be more
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44 442 “attractive” and natural vegetation could provide a continuity of habitat
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46 443 towards and inside the tunnel (Glista et al., 2009). It is still unclear
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48 444 whether *T. cristatus* choose a particular environment in these systems or
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4 445 if they return to known refugia or other environments while moving on
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6 446 land during the night.

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8 447 Once inside the tunnel, *T. cristatus* tended to move in straight paths
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10 448 towards the tunnel exit but percentages of individuals making a full or
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12 449 partial (if the individual was found already inside the tunnel) crossing in
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14 450 one night were low. In past *ex situ* experiments, low rates of tunnel
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16 451 crossing rates were recorded - 0.68 - 0.77, 0.27-0.66 and 0.07–0.21
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18 452 (Lesbarrerès et al., 2004; Woltz et al., 2008, Hamer et al., 2014,
19
20 453 respectively). Tunnel use in this study was higher for *T. cristatus* over
21
22 454 both seasons (0.8 captures.night⁻¹). Numerous factors appear to influence
23
24 455 the decision of an amphibian to enter and cross a tunnel (Glista et al.,
25
26 456 2009), e.g. position of system, moisture, temperature, light, substrate
27
28 457 and noise and the understanding of how tunnels can be adapted to meet
29
30 458 the best environmental conditions for full successful crossings is still
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32 459 improving (Patrick et al., 2010; Lesbarrerès et al., 2004).

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34 460 The low number of adult *T. cristatus* moving towards the tunnels
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36 461 combined with changes of orientation and the small number of recaptures
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38 462 suggest that responses towards human-made infrastructure may be
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40 463 negative (Jehle and Arntzen, 2000; Knowlton and Graham, 2010). Adult
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42 464 *T. cristatus* prefer areas well covered with vegetation around breeding
43
44 465 ponds and show a strong sense of fidelity towards breeding sites (Jehle,
45
46 466 2000). This fidelity may be extended to the terrestrial sites around
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48 467 ponds, causing adults to remain near the pond between breeding seasons
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50 468 (Jehle et al., 2011; Sinsch, 1990; Sinsch, 2014). Due to homing ability,
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4 469 adults perform shorter and less random/explorative movements when
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6 470 encountering a fence or a tunnel, which may play an important role in
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8 471 the changes of orientation and non-permanence in the system (Sinsch and
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10 472 Kirst, 2015). However, information on amphibian habituation and
11
12 473 evidence on use of navigation cues and homing to explain behaviour at
13
14 474 the entrance and inside the tunnels is still lacking (Pagnucco et al.,
15
16 475 2012). Overall the effectiveness of tunnels in facilitating successful
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18 476 crossing by amphibians may be linked to how such infrastructure is
19
20 477 sensitive to behavioural changes and inter-species differences (Baguette
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22 478 and Dyck, 2007).

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26 27 480 *Seasonal effect in movements*

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29 481 Differences in frequencies of use of the mitigation system and movement
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31 482 length between the two seasons may illustrate the type of movement that
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33 483 the system facilitates (Sinsch, 2014). The longer and more frequent
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35 484 movements in autumn compared to spring are consistent with dispersal
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37 485 being the main type of movement facilitated by the system in the present
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39 486 study (Matos et al., 2017).

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41 487 Although distances travelled were shorter in spring, there was an even
42
43 488 stronger effect of season on the path straightness of *T. cristatus*. Spring
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45 489 migration is typically considered to involve more direct, straight-line
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47 490 movements (Jehle and Arntzen, 2000; Wells, 2007) which was the case in
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49 491 our study. The number of spring days with precipitation was lower than
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4 492 in autumn, which might have influenced the straightness of movement
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6 493 patterns (Kovar et al., 2009).

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10 495 **Conclusions and future research**

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12 496 Our findings highlight three important aspects of road mitigation
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14 497 systems that may influence *T. cristatus* behaviour and consequently the
15
16 498 effectiveness of these systems: 1) mitigation systems need to support the
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18 499 longer-distance and more frequent movements in autumn by facilitating
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20 500 tunnel crossing and metapopulation connectivity; 2) fences generally
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22 501 acted as barriers to movement, directing *T. cristatus* away from the road
23
24 502 and rarely towards the tunnels; 3) overall tunnel use was low and *T.*
25
26 503 *cristatus*' responses to encountering the tunnel (change in orientation)
27
28 504 rarely resulted in tunnel crossing. A better understanding of the
29
30 505 consequences of these results for metapopulation dynamics and
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32 506 persistence, as well as behavioural responses of *T. cristatus* to
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34 507 infrastructure could help determine if improvements to tunnel design are
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36 508 required.

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40 509 Behavioural data collection is time consuming but once compiled it can
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42 510 be used to parameterise individual-based or random walk models to
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44 511 simulate long-term amphibian movements (Pontoppidan and Nachman,
45
46 512 2013; Pittman et al., 2014; Sinsch, 2014). In combination with data on
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48 513 population dynamics, individual-based modelling could provide insights
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50 514 into the minimal number of successful crossings per year for population
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52 515 persistence over the long-term (30-50 years). The adequate inclusion of
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4 516 behavioural data in road ecology studies has the potential to generate
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6 517 critical evidence for improving the effectiveness of such schemes as well
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8 518 as increasing our understanding of limits and motivations in movement
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10 519 ecology for different species in a changing environment.

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762 Table 1 – Movements of adult and juvenile *T. cristatus* marked with
 763 fluorescent powder in autumn 2014 and spring 2015. Maximum (max.),
 764 minimum (min.) and median values for measured behavioural traits
 765 (distance travelled, straightness and orientation per night) by season.
 766 Data represent 24 days of surveys over 6 weeks in autumn and 14 days
 767 over 5 weeks in spring.

768

	Autumn			Spring		
Distance (m)	max	min	median	max	min	median
females	16.4	0.6	2.72	6.5	1.0	3.0
males	25.6	0.5	2.52	7.7	1.5	2.9
juveniles	19.4	0.6	3.1	5.3	1.3	2.2
Straightness (0-1)						
females	1.0	0.0	1.0	1.0	0.65	1.0
males	1.0	0.33	1.0	1.0	0.64	1.0
juveniles	1.0	0.0	1.0	1.0	0.51	1.0
Orientation (0°-180°)						
females	180.0	0.0	84.38	180.0	0.0	30.0
males	180.0	0.0	90.00	180.0	0.0	180.0
juveniles	180.0	0.0	90.00	180.0	0.0	180.0

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4 771 Table 2 – Movement parameters for adult *T. cristatus* recaptured by sex
5
6 772 (females and males). Number of position recapture points, number of
7
8 773 individuals that moved, and range of values for distance travelled,
9
10 774 straightness and orientation per night.

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Recaptures	Females	Males
n° points	31	25
n° indiv	14	10
moved	11	9
distance (m)	0.8-16.4	1.5-11.8
straightness	0.33-1.0	0.33-1.0
orientation (0°-180°)	17.2-112.5	22.5-157.5

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780 Table 3 – Mean value and range of observed behaviour per season and position in the mitigation system

Variables	Season		Position in mitigation		
	mean (range)		mean (range)		
	Autumn (n=144)	Spring (n=86)	Fence (n=76)	Tunnel entrance (n=76)	Tunnel (n=76)
Distance travelled per night (m)	4.20 (0.50-25.60)	2.81 (1.30-4.90)	3.21 (0.5-15.3)	3.41 (1.1-8.65)	6.72 (0.75-25.60)
Straightness (0-1)	0.84 (0-1)	0.95 (0.77-1.0)	0.89 (0.17-1.0)	0.83 (0.42-1.0)	0.78 (0.0-1.0)
Orientation (0-180)	77.02 (0-180)	109.5 (0.0-180.0)	93.81 (0.0-180.0)	96.48(0.0-180)	20.84 (0.0-67.50)

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782 Table 4 – Parameter estimates from GLMMs for behavioural movement changes of *T. cristatus* in a road mitigation
783 system. For each behavioural prediction (distance travelled per night (m), straightness (0-1) and orientation (0-180)) we
784 present: significant factors, estimate (β), standard error (SE), Chi-square Wald test II (W), p-values (bold if significant)
785 and value of AIC.

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Behaviour	Variables	Model parameters					AIC
		β	SE	Chisq	df	P-value	
Distance travelled per night (m)	Intercept	3.42	0.59				
	mitigation.tunnel	3.60	0.93	17.55	2	<0.001	556.44
Straightness (0-1)	Intercept	0.87	0.03				
	season.spring	0.08	0.05	4.18	1	0.04	24.66
Orientation (0-180)	Intercept	88.79	7.56				
	mitigation.tunnel	-67.95	14.77	23.69	2	<0.001	1130

788

789 Table S1 – Counts for adults and juvenile newts per capture position, movement and final position

N (Total)		Capture position						Movement						Final position			
Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring
		Fences		Tunnels		Moved		Mean distance (m)		Mean straightness (0-1)		Mean angle (o)		Mitigation	Habitat	Mitigation	Habitat
65	12	13	11	3	1	14	1	3.01	0.4	0.68	0.16	113.99	16.19	19	35	2	1
38	34	4	33	5	1	9	7	1.81	1.3	0.55	0.33	49.66	31.06	10	23	11	1
37	8	9	8	2	0	8	0	3.4	0.14	0.65	0.08	67.49	20.83	8	21	2	0
46	36	9	34	2	2	9	12	2.83	1.78	0.71	0.54	55.57	55.07	9	26	13	6
50	17	15	14	5	3	13	9	4.92	1.89	0.72	0.65	58.4	68.33	15	23	11	1
44	0	12	0	4	0	13	0	4.48	0	0.54	0	114.81	0	17	17	0	0
Total	280	107	62	100	21	7	66	29									
N (juveniles)		Capture position						Movement						Final position			
Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring
		Fences		Tunnels		Moved		Mean distance (m)		Mean straightness (0-1)		Mean angle (o)		Mitigation	Habitat	Mitigation	Habitat
49	7	34	6	15	1	40	2	4.04	0.79	0.63	0.27	89.16	48.57	9	31	1	1
29	17	23	16	6	1	23	5	2.86	0.57	0.68	0.29	74.18	21.18	2	22	5	0
26	8	18	8	8	0	21	2	3.6	0.43	0.69	0.25	92.86	45	3	18	2	0
35	17	23	15	12	2	26	7	3.99	1.37	0.77	0.36	81.31	39.71	5	21	6	1
30	5	16	3	14	2	25	3	5.86	0.78	0.48	0.55	72.9	90	4	21	3	0
28	0	20	0	8	0	21	0	4.06	0	0.72	0	116.03	0	6	15	0	0
Total	197	54	134	48	63	6	156	19									
	790																
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4 792 **Figure legends**

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8 794 Fig. 1 - Study area and field work transects. (a) Location of the study
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10 795 area in Peterborough, UK (b) transects surveyed along the mitigation
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12 796 system (black arrow lines with black arrows indicating direction) with
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14 797 location of fences (east/west sides) and tunnel entrances (symbols) (c)
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16 798 southern underpass entrance and fence at Hampton Nature Reserve.
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21 800 Fig. 2 – *Triturus cristatus* movement data collection. (a) Individual newt
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23 801 dipped in yellow fluorescent powder; (b) deflection angle ranged from 0
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25 802 to 180 for three positions in the system, with 0 indicating movement
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27 803 directly towards the tunnel and 180 indicating movement directly away
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29 804 from the tunnel. Smaller dots on the pathway diagram represent change
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31 805 of direction.
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36 807 Fig. 3 – Percentages of *T. cristatus* (adults and juveniles) by final
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38 808 position (fences, tunnels or habitat) for each initial point of capture in
39
40 809 the system.
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45 811 Fig. 4 – Seasonal distribution of estimated distance travelled per night
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47 812 by *T. cristatus*.
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51 814 Fig. 5 – Movement behaviours of adult ($n_{\text{females}} = 35$ and $n_{\text{males}} = 44$;
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53 815 colour: light grey) and juvenile ($n = 191$; colour: dark grey) *T. cristatus*
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4 816 captured at three points along the mitigation system ($n_{\text{fence}} = 197$;
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6 817 $n_{\text{entrance}} = 45$ and $n_{\text{tunnel}} = 28$). Distance travelled, straightness index (0-1)
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8 818 and orientation relative to the tunnel were estimated per life stage class
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10 819 for both survey seasons.
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Acknowledgements

Data collection and logistics were supported by the University of Hull. M. Goodman, S. Allain, P. Furnborough and G. Dubois assisted with data collection. C. M. was funded by a postgraduate grant from the University of Hull and funding from Natural England. SP was supported by an Esmée Fairbairn Foundation grant to Froglife.

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		Season					
		Autumn			Spring		
Distance (t)		max	min	median	max	min	median
	females	16.4	0.6	2.72	6.5	1.0	3.0
	males	25.6	0.5	2.52	7.7	1.5	2.9
	juveniles	19.4	0.6	3.1	5.3	1.3	2.2
Straightness (0-1)							
	females	1.0	0.0	1.0	1.0	0.65	1.0
	males	1.0	0.33	1.0	1.0	0.64	1.0
	juveniles	1.0	0.0	1.0	1.0	0.51	1.0
Orientation (0°-180°)							
	females	180.0	0.0	84.38	180.0	0.0	30.0
	males	180.0	0.0	90.00	180.0	0.0	180.0
	juveniles	180.0	0.0	90.00	180.0	0.0	180.0

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Recaptures	Females	Males
n° points	31	25
n° indiv	14	10
moved	11	9
distance (m)	0.8-16.4	1.5-11.8
straightness	0.33-1.0	0.33-1.0
orientation (0°-180°)	17.2-112.5	22.5-157.5

Review Copy

Variables	Season		
	mean (range)		
	Autumn (n=144)	Spring (n=86)	Fence (n=76)
Distance travelled per night (m)	4.20 (0.50-25.60)	2.81 (1.30-4.90)	3.21 (0.5-15.3)
Straightness (0-1)	0.84 (0-1)	0.95 (0.77-1.0)	0.89 (0.17-1.0)
Orientation (0-180)	77.02 (0-180)	109.5 (0.0-180.0)	93.81 (0.0-180.0)

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Position in mitigation	
mean (range)	
Tunnel entrance (n=76)	Tunnel (n=76)
3.41 (1.1-8.65)	6.72 (0.75-25.60)
0.83 (0.42-1.0)	0.78 (0.0-1.0)
96.48(0.0-180)	20.84 (0.0-67.50)

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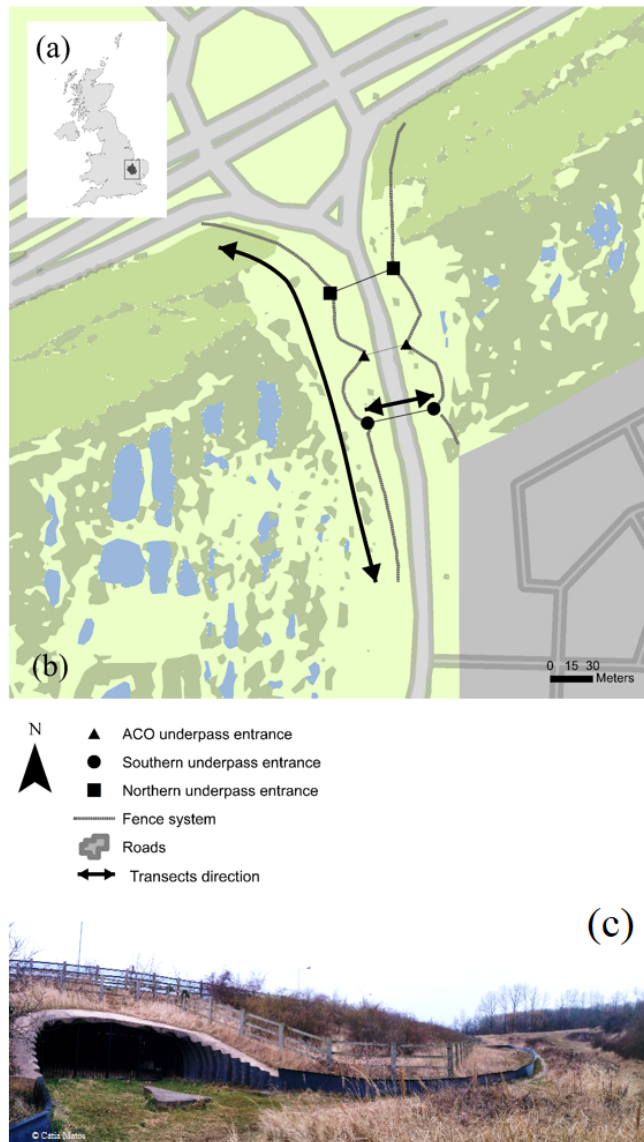
Behaviour Variables	Model parameters					
	β	SE	Chisq	df	P-value	AIC
ravelled per night (m)						
Intercept	3.42	0.59				
mitigation.1	3.60	0.93	17.55		2 <0.001	556.44
aightness (0-1)						
Intercept	0.87	0.03				
season.spri	0.08	0.05	4.18		1 0.04	24.66
entation (0-180)						
Intercept	88.79	7.56				
mitigation.1	-67.95	14.77	23.69		2 <0.001	1130

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N (Total)		Capture position							
Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	
		Fences		Tunnels		Moved		Mean dis	
65	12	13	11	3	1	14	1	3.01	
38	34	4	33	5	1	9	7	1.81	
37	8	9	8	2	0	8	0	3.4	
46	36	9	34	2	2	9	12	2.83	
50	17	15	14	5	3	13	9	4.92	
44	0	12	0	4	0	13	0	4.48	
280	107	62	100	21	7	66	29		
N (juveniles)		Capture position							
Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	
		Fences		Tunnels		Moved		Mean dis	
49	7	34	6	15	1	40	2	4.04	
29	17	23	16	6	1	23	5	2.86	
26	8	18	8	8	0	21	2	3.6	
35	17	23	15	12	2	26	7	3.99	
30	5	16	3	14	2	25	3	5.86	
28	0	20	0	8	0	21	0	4.06	
197	54	134	48	63	6	156	19		

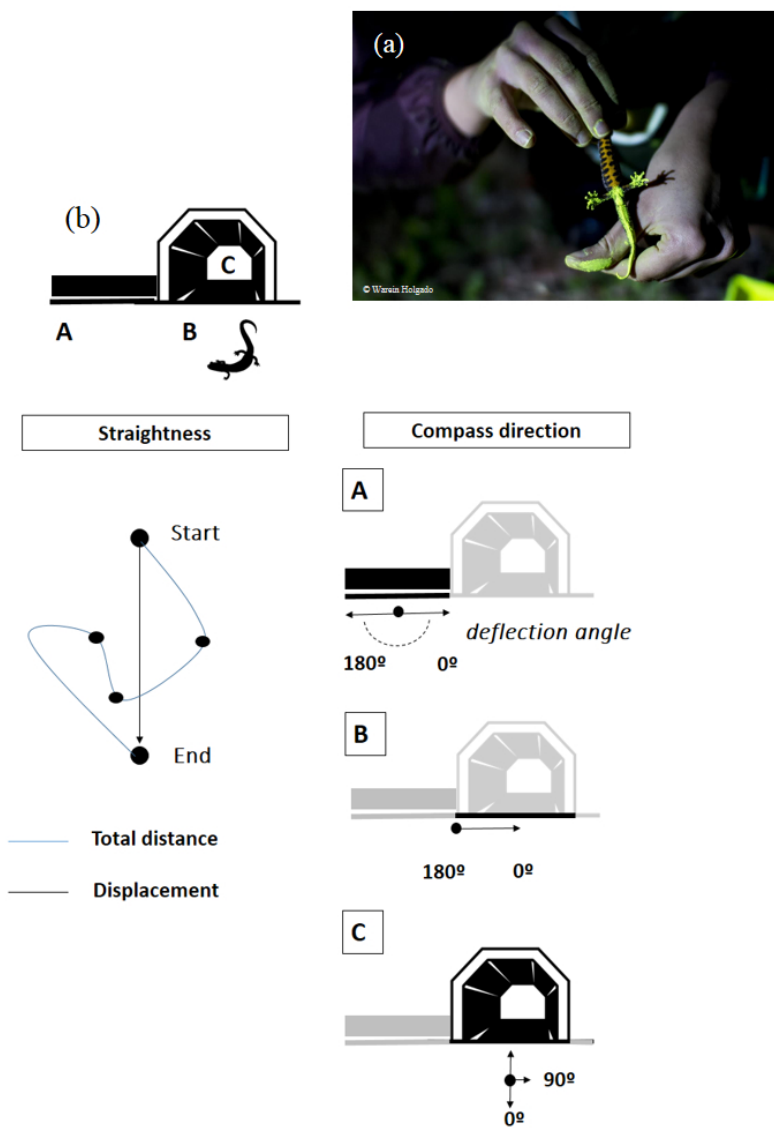
	Movement					Final position			
	Spring tance (m)	Autumn Mean straightness (0-1)	Spring Mean straightness (0-1)	Autumn Mean angle (o)	Spring Mean angle (o)	Autumn Mitigation Habitat		Spring Mitigation Habitat	
5	0.4	0.68	0.16	113.99	16.19	19	35	2	1
6	1.3	0.55	0.33	49.66	31.06	10	23	11	1
7	0.14	0.65	0.08	67.49	20.83	8	21	2	0
8	1.78	0.71	0.54	55.57	55.07	9	26	13	6
9	1.89	0.72	0.65	58.4	68.33	15	23	11	1
11	0	0.54	0	114.81	0	17	17	0	0

	Movement					Final position			
	Spring tance (m)	Autumn Mean straightness (0-1)	Spring Mean straightness (0-1)	Autumn Mean angle (o)	Spring Mean angle (o)	Autumn Mitigation Habitat		Spring Mitigation Habitat	
16	0.79	0.63	0.27	89.16	48.57	9	31	1	1
17	0.57	0.68	0.29	74.18	21.18	2	22	5	0
18	0.43	0.69	0.25	92.86	45	3	18	2	0
19	1.37	0.77	0.36	81.31	39.71	5	21	6	1
21	0.78	0.48	0.55	72.9	90	4	21	3	0
22	0	0.72	0	116.03	0	6	15	0	0



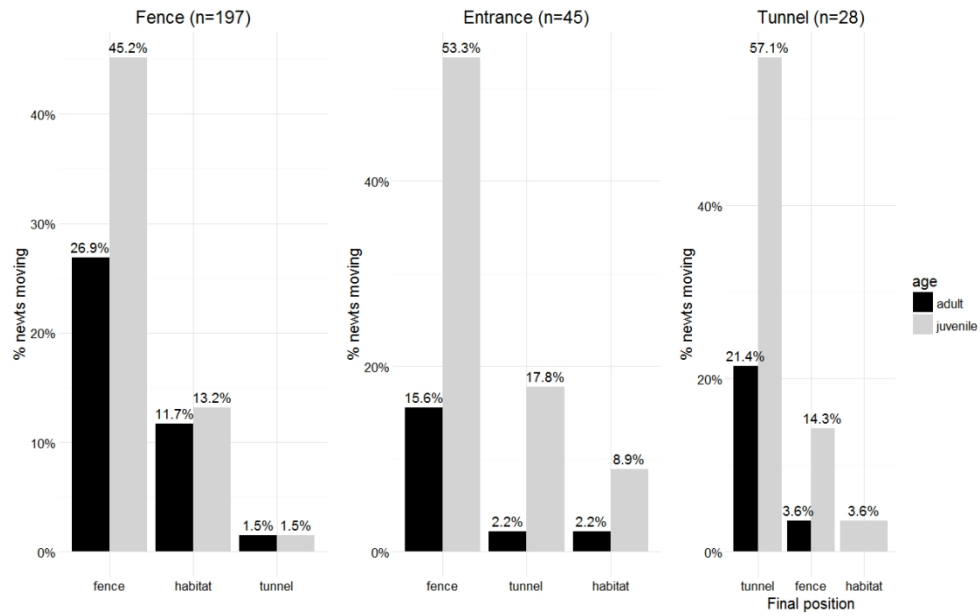
45 Study area and field work transects. (a) Location of the study area in Peterborough, UK (b) transects
46 surveyed along the mitigation system (black arrow lines with black arrows indicating direction) with location
47 of fences (east/west sides) and tunnel entrances (symbols) (c) southern underpass entrance and fence at
48 Hampton Nature Reserve.

49 190x275mm (96 x 96 DPI)



45 Triturus cristatus movement data collection. (a) Individual newt dipped in yellow fluorescent powder; (b)
46 deflection angle ranged from 0 to 180 for three positions in the system, with 0 indicating movement directly
47 towards the tunnel and 180 indicating movement directly away from the tunnel. Smaller dots on the
48 pathway diagram represent change of direction.

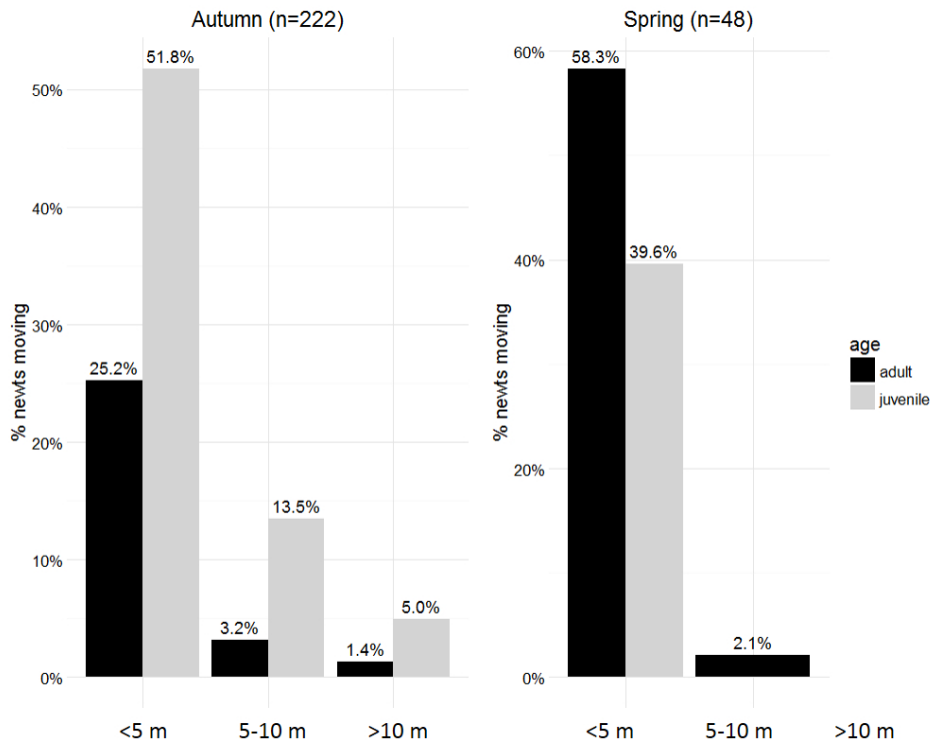
49 190x275mm (96 x 96 DPI)



– Percentages of *T. cristatus* (adults and juveniles) by final position (fences, tunnels or habitat) for each initial point of capture in the system.

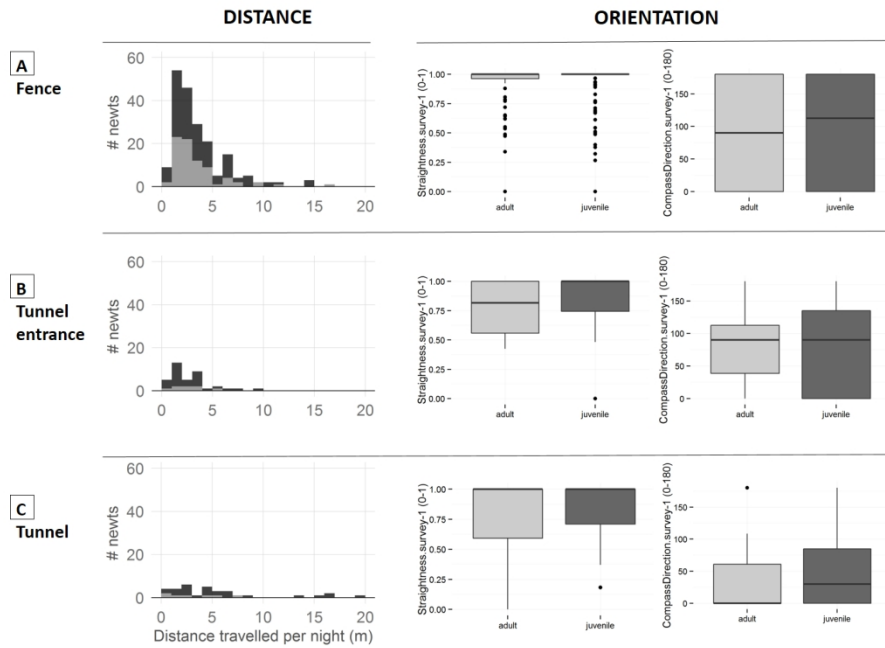
459x282mm (72 x 72 DPI)

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Seasonal distribution of estimated distance travelled per night by *T. cristatus*.

279x220mm (96 x 96 DPI)



Movement behaviours of adult (nfemales= 35 and nmales = 44; colour: light grey) and juvenile (n= 191; colour: dark grey) *T. cristatus* captured at three points along the mitigation system (nfence= 197; nentrance= 45 and ntunnel= 28). Distance travelled, straightness index (0-1) and orientation relative to the tunnel were estimated per life stage class for both survey seasons.

450x300mm (96 x 96 DPI)