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Movement of pond-breeding amphibians in fragmented landscapes:

Responses of Great Crested Newt (*Triturus cristatus*) to road mitigation

a Thesis submitted for the Degree of Doctor of Philosophy

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Movement of pond-breeding amphibians in fragmented landscapes:
Responses of Great Crested Newt (Triturus cristatus) to road mitigation

Submitted by Cátia S. Santos Matos in fulfilment of the requirements of the degree Ph.D in Biological Sciences, on the _/_/_.

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I. Abstract

With the increase of transportation system in the world, roads facilitate opportunities for human social and economic development. Roads are also the primary cause of multiple and diverse negative ecological effects. Habitat and wildlife populations are directly disturbed as roads contribute to habitat loss, habitat fragmentation and reduction of the quality of surrounding habitats. Barrier effects and traffic mortality are amongst the principal factors impacting species that need to move among important habitats to complete their life cycle leading to fragmentation, isolation and local population extinctions. Pond-breeding amphibians can be particularly impacted in this way, with mortality rates of 60-90% imposed by roads in some circumstances. Road mitigation measures, such as tunnels and associated fences, are implemented to manage this problem and restore connectivity at the landscape level in order to sustain migration and dispersal movements for amphibians and maintain metapopulation dynamics over the long-term.

In the UK, the demand for the implementation of these mitigation infrastructures has increased in the past decade as urban development reached a detrimental point for the European Protected Species, *Triturus cristatus,* the Great Crested Newt. Road mitigation measures for newt species are notoriously difficult to implement efficiently due to the behavioural characteristics of this group and the poor understanding of how it influences road mitigation effectiveness. Their ability to climb vertical surfaces, the poor capacity for crossing large distances over land and general avoidance of small, narrow structures such as tunnels are some examples of responses that may influence how planning and design of mitigation can support and facilitate patterns of movements for the species. There is no clear understanding of how these responses and patterns influence successful crossings and dispersal in the long-term in the UK or the rest of Europe. Therefore, it is challenging to predict mitigation long term effectiveness, provide evidence-based guidance to developers despite their substantial costs and potentially crucial importance for maintaining connectivity and dispersal for this European protected species.

The main aim of this study was to evaluate *T. cristatus* movement patterns in areas impacted by roads and at which road mitigation measures had been deployed in order to develop evidence-based improvements for the strategic planning and design of dispersal corridors for

future mitigation. From a pitfall data monitoring scheme, I investigated the species' behavioural traits at a road mitigation site to understand spatial and temporal patterns of movement. Also, I calculated regional connectivity indexes in a sub-urban area to understand the importance of spatial scale for movement when collecting species presence and absence data from local ponds. I measured short-term behavioural responses to a road mitigation system during two diferent seasons using non-invasive marking techiniques. And finally, I relate how local climatic factors affect successful crossings in tunnels and overall use of a mitigation system using standard monitoring data from previous chapter pitfall data. These results showed seasonality and yearly movements having an important role in calculating successful use of mitigation and directionality of movement. Newts' movements were higher in the course of autumn dispersal than at other times of year, and movement between patches varied greatly among years. Fences operated as a barrier to dispersing newts, potentially preventing road mortality but also reducing dispersal. Landscape analysis showed how annual home-range position and size affects connectivity at regional level for newts when considering roads as barriers. Predicted dispersal patches increased with landscape permeability, which was associated with road type; minor roads were more permeable. Behaviour analysis towards responses in a road mitigation system showed distance of short-term movements changing significantly around the fences. These responses were independent of newts' age. And finally, local weather patterns influenced newts' successful crossings among years.

These results showed the multi-scalar perspective of responses of *T. cristatus* to road mitigation systems and indicate the need to include consideration of multiple spatial and temporal scales when predicting the consequences of road construction and mitigation on *T. cristatus* metapopulation dynamics and hence conservation status. Identification of patterns of responses according to individual circumstance (age, sex) and climatic conditions facilitated calculation of potential landscape connectivity at distinctive spatial and temporal scales, and could inform improved advice for system design. Clear definitions of mitigation effectiveness have been repeatedly called for, but rarely offered. Here we have shown how spatial and temporal scales of newt responses need to be incorporated into these definitions, but also how variable they can be. Consequently, during and following road mitigation we recommend that newt responses should be monitored at these multiple scales to inform an adaptive approach to *T. cristatus* conservation when they are threatened by roads. The ultimate goal would be to include response patterns, scales and variability in a new iterative and innovative management tool that enables estimation of mitigation effectiveness for *T. cristatus* landscape connectivity over the short- to long-term.

To human-nature-species interaction,

"the more "connected" we (humans) become, non-human life with which we share this planet becomes increasingly disconnected."

Conservation Connectivity, Crooks and Sanjayan (2006)

II. Acknowledgements

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III. Table of contents

VI. List of Figures

V. List of Tables

1. General introduction

Habitat fragmentation and loss is a global threat to biological diversity (EEA/FOEN, 2011). The primary vehicle for this is the construction of roads, making it the first form of human communities' colonization worldwide (Iuell et al., 2003). About 80% of Earths terrestrial surface remains roadless, with a role in the rapid transportation of humans and goods, roads provide connectivity and opportunities for social and economic development (Ibisch et al., 2016). Roads are also a cause of multiple and diverse ecological effects, mostly at habitat and wildlife population levels by directly contributing to habitat loss and reducing the quality of surrounding habitats (Forman and Alexander, 1998; Spellerberg, 1998; Trombulak and Frissel, 2000). Road construction and development represent critical threats to wildlife populations (Forman and Alexander, 1998; Forman et al., 2003). The negative influence of roads threaten population viability through wildlife traffic-related mortality and also by acting as a barrier to animals' movement (Forman et al., 2003) and therefore increasing habitat fragmentation and isolation.

Amphibians appear to be exceptionally vulnerable to the effects of roads, demonstrating the highest rate of road kills of any group of vertebrates and with long-term disruptions of their important seasonal movements required to complete life cycles (Glista et al., 2007). Their movement patterns differ according to the species and season, varying between reproductive, over-winter refuge and feeding sites, and depending on the quality of the habitat matrix (Joly et al., 2001, Andrews et al., 2008). If a road bisects these habitats, the likelihood of road mortality increases, dispersal becomes more limited and barrier effects for species movements become apparent (Jaeger et al., 2005; Andrews et al., 2008). Consequently, roads can affect population dynamics, reduce gene flow and potentially compromise long term population survival (Trombulak and Frissel, 2000; Andrews et al., 2008). Additionally, roads also introduce significant chemical, light and sound pollution, both in the immediate area and over a larger distance, creating a "road zone" that can substantially influence species movement and habitat quality (Forman and Alexander, 1998).

Due to increasing environmental and safety concern, over the past decades, wildlife road crossing structures have been implemented in many countries in order to reverse these problems by linking habitat patches in an attempt to reestablish animal movement routes and minimize crossings over the road surface. However, for pond-breeding amphibians there is no clear evidence explaining which impacts road mitigation is most effective against. There is little empirical evidence of road mitigation supporting different types of movement, such as foraging, breeding migration or juvenile dispersal. With the increase of urban expansion and consequently, road networks, without adequately-supported evidence mitigation may be used as a quick solution but prove ineffective in the long-term. Additionally, as behavioural responses to roads and road mitigation determine landscape connectivity at different points in time and under different environmental conditions, the lack of evidence on the role of mitigation system design in determining these patterns limits the ability to predict how impacts on and how responses of pond breeding amphibians will change in response to changing climate and landscapes. Spatial (fence or tunnels) and temporal (seasonality, years) factors influence amphibian responses to tunnel crossing, and a framework to disassemble and recognize scales of behavioural responses to these systems does not currently exist, but is required, especially for threatened and protected species.

The great crested newt (*Triturus cristatus*) is a European protected species which has declined substantially in the UK and Europe over recent decades, largely due to habitat loss and degradation (Langton et al. 2001; Jehle et al. 2011). Nevertheless, the species remains relatively widespread in the UK, both in rural and semi-urban environments and is therefore regularly the subject of road mitigation schemes with tunnels and fences. Such schemes can carry considerable costs and cause substantial delays for infrastructure projects but there is little indication to upkeep their effectiveness. Where schemes have failed this has been attributed to incorrect placement and poor design in relation to the behaviour of the target species (Puky, 2003; Woltz et al., 2008). Evaluation of the effectiveness of mitigation measures is therefore required to ensure that only sound practices are employed. This thesis aims to clarify the role of road mitigation in promoting landscape connectivity for great crested newt populations impacted by roads.

1.1. Roads effects on pond-breeding amphibian population dynamics and landscape connectivity

In urbanized landscapes the spatial arrangement is heterogeneous and characterized by the presence of isolated natural habitat patches delimited by a matrix of residential, commercial, industrial, agricultural land uses and linear infrastructures, such as railways and roads (Hamer and McDonnell, 2008; Bauer and Swallow, 2013). In this *roadscape* scenario, maintaining metapopulation processes through landscape connectivity is essential to secure populations' survival and gene flow (Semlitsch, 2000; Smith and Green, 2005; Baguette et al., 2013). Usually this is achieved by preserving or restoring natural terrestrial migration or dispersal routes. As maintaining or improving landscape connectivity becomes a common goal for practitioners and road ecologists, its components and applicability require a more extended explanation.

Landscape connectivity is "the degree to which the landscape facilitates animal movement and other ecological flows" (Taylor et al. 1993). It is species-specific and comprises two attributes: structural connectivity, which encompasses the composition and configuration of the landscape and functional connectivity, which is how the organism perceives and responds to landscape structure (Crooks and Sanjayan, 2006). Both of these components are measured separately in order to understand habitat fragmentation effects on biodiversity (Fahrig, 2003). Increases in fragmentation caused by roads disrupt landscape connectivity, metapopulation mechanisms and impedes colonization of newly created habitat patches through terrestrial corridors (Hale et al., 2012).

Amphibians are particularly vulnerable to roads and suffer the highest road-kill rates compared with other vertebrate groups (Glista et al., 2007). When roads intersect migration paths or landscape permeability is interrupted, amphibian population dynamics can be impacted, reducing gene flow and compromising their survival (Glista et al., 2007). Worldwide, mitigation measures have been designed and applied to reduce road-kills and to re-establish connectivity (Forman et al., 2003; Clevenger and Wierzchowski, 2006). This can be achieved with different combinations of solutions, which are implemented hierarchically from local to regional scales (Iuell et al., 2003; Forman et al., 2003).

Movements on land and consequently the amount and quality of terrestrial habitat determine population presence and persistence in fragmented landscapes for pond-dwelling amphibians (Ficetola and Bernardi, 2004). Consequently, high levels of patch isolation and low-quality of the wetland surroundings caused by roads represent critical obstacles for more sensitive amphibians, including newts (Ficetola and Bernardi, 2004). To improve landscape connectivity, road mitigation should ultimately ensure and allow individuals to move between multiple habitats at local scales and reduce barrier effects, therefore supporting population persistence at regional scales (Forman et al., 2003).

Pond-breeding amphibian movements are supported when connectivity that has been disrupted by road construction is replaced and maintained between terrestrial habitats (Rothermel, 2004; Matos et al. 2017). Results on the matter show that a diverse matrix positively influences amphibian presence, suggesting that modeling distributions at different terrestrial spatial scales beyond the pond patch could explain how populations persist in fragmented landscapes (Joly et al., 2001; Ribero, 2011). In this way the terrestrial habitat becomes an integral part of the pond-breeding amphibian patch (home-range), when in fact much of species' life cycle is spent on land after the reproductive season (Ficetola and Bernardi, 2004; Rittenhouse and Semlitsch, 2007; Pontoppidan and Nachman, 2013).

Generally, pond-breeding amphibians are isolated in wetland patches across this landscape and successful colonization of patches is achieved by more mobile and resilient species (Ficetola et al., 2004). For a European Protected Species (EPS) such as *Triturus cristatus*, that exhibits clear selection for specific habitat features and has relatively low mobility, effects of isolation on populations caused by roads are high (Joly et al., 2001; Ficetola and Bernardi, 2004). Road effects can influence populations of this species at multiple scales: modification on displayed behavioural responses (individual level), interruption of movement patterns with decrease of connectivity causing reduction in recruitment of individuals to local populations (local effects) and consequently affecting metapopulation dynamics (regional effects) (**Fig. 1.1**).

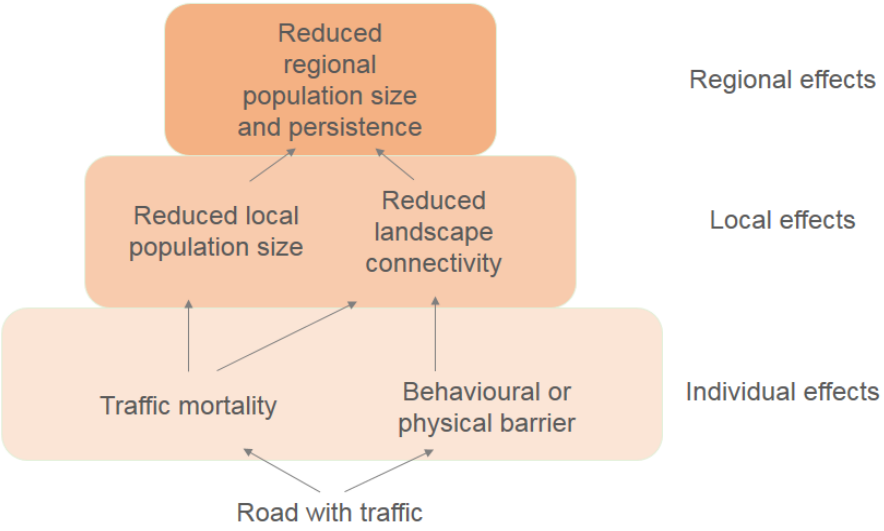


Fig. 1.1 – Road system effects on individual animals and populations at three spatial scales (adapted from Forman et al., 2003)

1.2 Metapopulation dynamics and pond-breeding amphibian movement behaviour

When applying successful conservation measures for declining amphibian species, sufficient knowledge of population mechanisms and associated variables such as size, life stage, sex ratio, survival, recruitment and dispersal is required for further strategic planning (Griffiths et al, 2010). Road mitigation systems are no exception and the metapopulation model is considered the basis for such applications (Pontoppidan and Nacham, 2013). A metapopulation can be described as, a “set of discrete populations of the same species, in the same general geographical area, that may exchange individuals through migration, dispersal, or human-mediated movement” (from Akçakaya et al., 2006). Furthermore, metapopulation studies involve the outlining of variance on such variables for pursuing specific conservation actions and its applicability has been discussed widely in recent decades in relation reversing amphibian population declines (Storfer, 2003).

For instance, for pond-breeding amphibians metapopulations to persist they require connections between ponds via suitable terrestrial habitat for dispersal contributing to high rates of colonization to compensate losses (Arntzen and Teunis, 1993; Semlitsch, 2000; Smith and Green, 2005). Source or sink patches are the dichotomous outcomes of the degree of connectedness and population persistence (Hanski, 1998). Information on rates of colonization is especially important for species conservation in highly fragmented landscapes where there is a high risk of isolation and population extinction (Baguette et al., 2013).

Hence, a review of 53 amphibian metapopulation case studies concluded that these events outside the ponds must be considered in tandem with events inside ponds (“ponds-as-patches” approach), since most amphibian species include the use of terrestrial habitats in their life cycles when moving between patches (Smith and Green, 2005). Described as short distance dispersers, pond-breeding amphibian’s seasonal long distance movements can reach more than 10 km for particularly mobile species (Smith and Green, 2005). *T. cristatus* can exceptionally move up to 1km during annual dispersal (Kupfer and Kneitz, 2000), although typical distances are generally much shorter. From a conservation perspective, the applicability of the metapopulation model to pond-breeding amphibian species is dependent on dispersal ability (Baguette et al., 2012). Movement metrics (e.g. distances and frequency) during seasonal dispersal and breeding migration, hence patch colonization, are required for effective planning of habitat management (Griffiths et al, 2010).

Dispersal is critical for the maintenance of metapopulations and is considered “the ecological process determining connectivity” (Palmer et al., 2014). Two types of dispersal can be defined: (1) natal or pre-breeding dispersal is the movement of individuals from the site of birth to the site of reproduction and (2) movement between successive sites of reproduction (Matthysen, 2012). Van Dyck and Baguette (2005) suggested a high variation in individual behaviour within these movements that interferes with estimates of functional connectivity.

Individuals’ behavioural patterns change between populations but also within populations (Baguette and Van Dyck, 2007). Individuals participating beyond routine movements, where there is evidence of long-distance movements between patches, represent a non-random fraction of a population (Baguette and Van Dyck, 2007). This leads to differences in dispersal abilities being variable between species but also among individuals. These differences may influence the potential degree of functional connectivity for a particular species in a determined landscape context. For instance, in a landscape where patches are close together and within the maximum dispersal distance of a specific population, colonization/exchange of individuals will be positively influenced, therefore increasing local explorative movements and consequently connectivity at regional scale (Baguette and Van Dyck, 2007).

However, not all amphibians may persist as metapopulations due to the different mobility during different life stages (Hanski, 1994) and this can have important consequences in fragmented landscapes, where isolation and quality of terrestrial habitat will influence individuals’ space use (Baguette et al., 2012). In these cases, at the evolutionary level two spatial strategies are adopted: (1) more sedentary individuals remain within their home-range where all the life cycle resources can be found and (2) nomadic individuals frequently explore their environment searching for resources outside their home-range (Baguette et al., 2012). Nomadic individuals are likely to be more adaptable in fragmented landscapes and in frequently changing environments (Baguette et al., 2012).

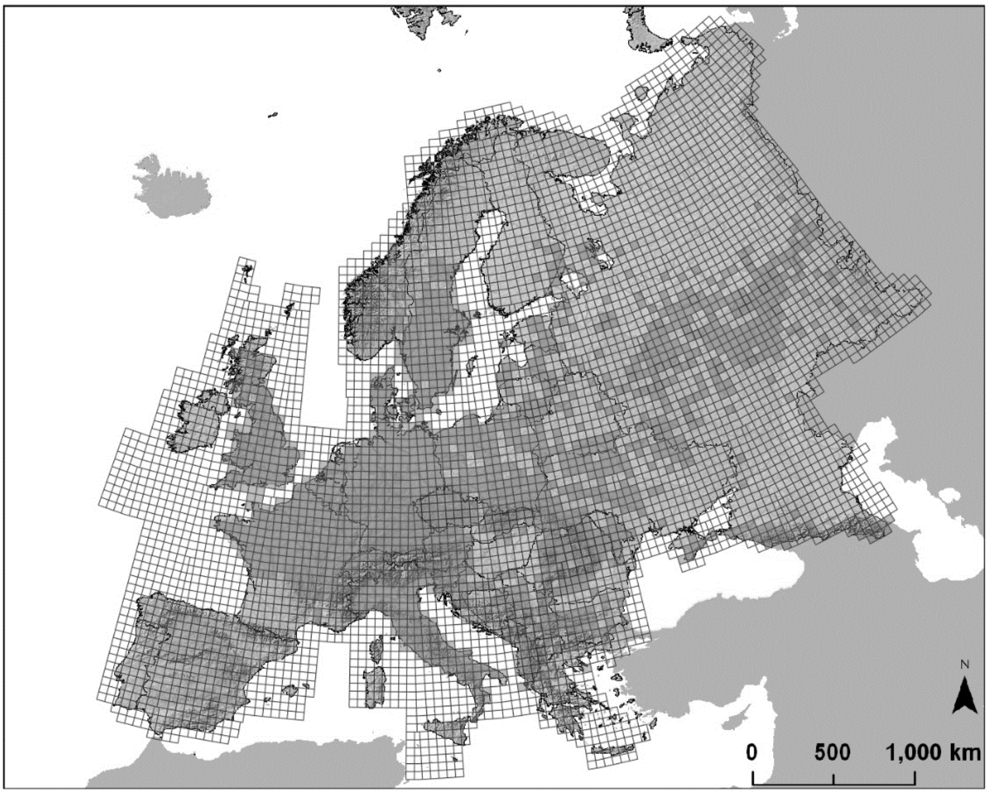
Hereby, functional connectivity will then present different degrees accordingly to the dispersal cost in specific regions and permeability would differ locally in any application of road mitigation measures (Zetterberg et al., 2010). Connectivity will be dependent not only on patterns of seasonal migration but also on individuals' preferences for dispersal depending on the degree of landscape fragmentation (Baguette and Van Dyck, 2007). The local adaptation of populations will influence individual behaviour variations and consequently connectivity in heterogeneous landscapes (Sinsch, 2014). These rare long-distance movements present advantages for colonization of new patches and provide better understanding of the annual movement capacity of a population and can be highly valuable in planning linear infrastructures implementation settings (Sinsch, 2014). Knowledge of local amphibian population dynamics and individual responses have then the potential to better inform temporal and spatial scales at which functional connectivity must be maintained at regional scale in order to promote population persistence in the long-term. Therefore, the contribution of dispersal abilities and individual variation becomes an integral part for measuring effectiveness of road mitigation in landscapes impacted by roads.

1.3 *T. cristatus* movement behaviour and landscape ecology

This section includes a review of terrestrial habitat use, dispersal and landscape-scale patterns of distribution of *T. cristatus* and how consideration of different spatial scales can contribute to newt conservation.

T. cristatus was the selected candidate to use as a model to understand patterns of movement for pond-breeding amphibians in a road mitigation system because: (1) its declining global conservation status, althought it is a relatively common species, its presence in semi-urban and urban environments in the UK make the species vulnerable to road development (e.g. Beebe and Griffiths, 2005); (2) it exhibits low dispersal abilities with restricted movement in the landscape and therefore is likely to respond to even modest barrier effects (e.g. Kupfer and Kneitz, 2000); (3) at the population level, *T. cristatus* shows a strong homing tendency with clearly defined seasonal movements between habitat patches, which enables a clear distinction of spatial and temporal movement patterns (e.g. Jehle and Arntzen, 2000; Denöel et al., 2018).

Newst are tailed amphibians (Amphibia: Caudata) and belong to order Urudela and the family Salamandridade. Normally newts are often separated from salamanders by having a well-defined aquatic phase as breeding adults (Griffiths, 1996; Malmgren, 2001). With 14 species of newts present in Europe and divided into four genera, the distribution range of *T. cristatus* extends from northern and central Europe to east to the Ural Mountains (Griffiths, 1996; Arntzen, 2003; Sillero et al., 2014) (**Fig.1.2**).



Triturus cristatus complex

Fig. 1.2 – Updated distribution of *T. cristatus* complex* (Laurenti, 1768) on the new Atlas of Europe. Map resolution of 50 × 50 km grid, quadrats in dark grey contains the presence of *T. cristatus*. Map adapted from Sillero et al., 2014. (*Species complex is a group of closely related species that are very similar in appearance to the point that the boundaries between them are often unclear. For *T. cristatus* this includes the Italian crested newt (*Triturus carnifex*), the Danube crested newt (*Triturus dobrogicus*) and the southern crested newt (*Triturus karelinii*).

In the UK, *T. cristatus* is one of the six native amphibians (the list includes *Bufo bufo*, *Bufo calamita*, *Rana temporaria*, *Lissotriton helveticus* and *Lissotriton vulgaris*). The Great Crested Newt (*Triturus cristatus*) is the largest newt in the UK (and Europe) reaching a maximum adult length of 170mm (Langton et al., 2001). Mature males range between 85-120mm and females may mature at a length of 90mm typically reaching 110-130. They are distinguished from the other two UK newts by size and colouring (Langton et al., 2001).

The appearance of an adult crested newt is granular, having a black or dark brown colour with darker spots (**Fig. 1.3**).

Fig. 1.3 – Female *T. cristatus* in Hampton Nature Reserve, Spring 2015

Juveniles are very similar to adults in appearance, although the belly patterns are indistinguished until they reach a mature stage. A full developed juveniles (young of the year) is about 18 weeks and normally it takes another 12-18 months to fully become an adult (Langton et al., 2001).

Like most amphibian species, *T. cristatus* needs two habitat types to complete its life cycle: an aquatic habitat for reproduction and larval development and a terrestrial habitat necessary for post-breeding activity and juvenile maturation (Langton et al., 2001; Malmgren, 2001; Gustafson, 2011). Different movements are performed at different spatio-temporal scales between aquatic and terrestrial habitats: seasonal migration (twice/year and longer) and dispersal (over two years) (Semlitsch, 2008; **Fig. 1.4)**.

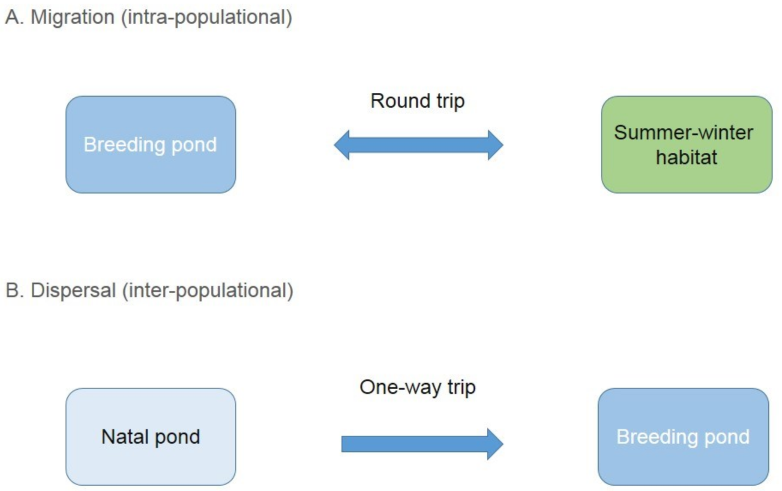


Fig. 1.4 – (A) Migration and (B) dispersal in pond-breeding amphibians with arrows representing movement. A local population is characterized by the group of individuals of a single breeding pond or cluster of ponds in close vicinity (adapted from Semlitsch, 2008 and Sinsch, 2014).

As soon as the hibernation phase ends, *T. cristatus* move to aquatic breeding sites from terrestrial over-wintering sites (**Table 1.1**). This seasonal migration is triggered by temperature rise and rainfall, and takes place mostly at night (Langton et al., 2001). In the UK it occurs between February and April, when temperatures are above 4-5ºC (Langton et al., 2001; Jehle et al., 2011), and adults can remain in the pond from between 10 days and 7 months (Jehle et al., 2011) but most breeding-age adults leave the ponds by June (Langton et al. 2001). Short movements between refugia and foraging areas and other ponds can be performed by newts during the breeding season (Jehle and Artzen, 2000; Jarvis, 2012). If conditions get too dry, *T. cristatus* adults may undergo an estivation phase during summer (Jehle and Artzen, 2000) during which activity is reduced. In the UK, between August and October juvenile newts start to leave the pond to travel to over-winter refuge sites (Jehle et al., 2011).

Table 1.1– Timing in months of the main annual activities of *T. cristatus* (adapted from The Great Crested Newt Conservation Handbook, Langton et al., 2001)

Activity/Month	**JAN**	**FEB**	**MAR**	**APR**	**MAY**	**JUN**	**JUL**	**AUG**	**SEP**	**OCT**	**NOV**	**DEC**
Hibernation	▨	▨								▨	▨	▨
Migration to pond		▨	▨									
Breeding season			▨	▨	▨	▨						
Migration from pond (Adults)					▨	▨	▨	▨				
Migration from pond (Juveniles)								▨	▨	▨		

1.3.1 T. cristatus use of terrestrial habitat

T. cristatus terrestrial habitat is essential for foraging, winter-summer refuge, migration and dispersal movements (Jehle, 2000; Jehle and Arntzen, 2000; Gustafson, 2011). Deciduous woodlands, forest patches, agricultural fields and pastures can be considered suitable habitats for the species in different stages of the annual cycle (Duff, 1989; Müllner, 2001; Edgar and Bird, 2006; Hartel et al., 2010). As suggested by Gustafson (2011) the main requirement for an environment to be suitable as a terrestrial habitat is the existence of adequate and sufficient microhabitats for foraging and hibernation. Specifically, patches of woodland with damp meadows, grassland and other dense ground-level vegetation around (or close) to breeding ponds (Duff, 1989; Jehle and Arntzen, 2000; Edgar and Bird, 2006; Gustafson, 2011; Jehle et al., 2011).

Forest patches have been shown to be extremely important for providing refuge to this species. The presence of deciduous or coniferous forests within 50 m of ponds situated in diverse landscapes across Denmark had a positive influence on *T. cristatus* occupancy within those ponds especially during summer (Rannap and Briggs, 2006). *T. cristatus* can also occur in grasslands and agricultural fields if they separate a pond area from a forest (Joly et al., 2001; Rannap and Briggs, 2006). However, these habitats can negatively influence *T. cristatus* presence if they are within 50-100 m of a pond (Joly et al., 2001; Rannap and Briggs, 2006). During their terrestrial life stage,newts, including great crested newts, can be found under planks, large stones, in tree root hollows, under litter, dead wood, inside rotting stumps and under grassy vegetation (Jehle, 2011; Gustafson, 2011). Small mammal burrows also seem to be an important refuge for this species, especially during summer (Jehle and Artzen, 2000), with differences in terrestrial microhabitat use by *T. cristatus* observed in different areas. This can be related to habitat availability and suitability of refuges in the different study areas. However, this information only provides a snapshot of the actual terrestrial resources that can be used in several points of the annual cycle of newts. The patterns of terrestrial distribution are however, highly defined during migration and dispersal movements.

1.3.1.1 Migration and dispersal patterns

The patterns of migration and dispersal in pond-breeding amphibians vary spatially and temporally as both movements occur between different habitat patches during different phases of great crested newt life cycle. Thus, this fact affects the scale at which conservation actions can be directed to support one or both types of movements (Smith and Green, 2005; Semlitsch, 2008). Behavioural analysis of movement can include bias due to the species' wide distribution, as patterns vary substantially between geographic regions, and knowledge on movements still lack an understanding of the main resource use during dispersal, timing and spatial factors affecting dispersal; and how both movements affect species distribution and dynamics in the landscape (Gustafson et al., 2006; Hartel et al., 2010; Gustafson, 2011; Jehle et al., 2011).

Migration

First, for pond-breeding amphibians, migration can be separated into two types: primary and secondary movements. Primary movements are performed by adults between summer-winter terrestrial habitats and breeding ponds. Secondary movements include those between foraging sites and between hibernation and estivation refugia (Semlitsch, 2008). They are considered 'intra-population' migration (Semlitsch, 2008). Semlitsch (2008) classified the occurrence of these movements to be within a local population, which means that migratory movements are undertaken by one breeding group of an amphibian species in one or a cluster of several reproduction sites. Migratory movements are important for exploration of the local habitat, maintaining the capacity for recolonization of unoccupied patches and ensuring the migration of individuals within the population (Sinsch, 2014).

The timing of *T. cristatus* adult migratory movements to breeding ponds can vary with geographical location and individual characteristics (Jehle et al., 2011). After hibernation, *T. cristatus* migrate to breeding ponds in early spring (Kupfer and Kneitz 2000; Müllner, 2001; Malmgren, 2002; Jehle et al., 2011) and such movements to ponds were recorded from forest cover areas (especially deciduous woodland) and grassland (Kupfer and Kneitz 2000; Müllner, 2001; Malmgren, 2002).

Differences between sexes are also found in the timing of *T. cristatus* migration (Semlitsch, 2008). For example, to maximize reproductive success with females, males are the first to arrive in the pond early spring followed by females during a few weeks later. This increases the probability of finding a mate and reduces the risks of eggs freezing after being deposited (Sinsch, 2014). The time spent in the aquatic phase may vary depending on the weather conditions, life stage and individual behaviour (Artzen and Teunis, 1993; Jehle et al., 2011). Weather conditions (e.g. levels of precipitation and temperature) may affect water levels in the breeding pond. If the pond is permanent or temporary, the density of newts in the pond may fluctuate (Jehle et al., 2011). If there is a high level of competition between adults in a population, the time of residency in ponds may decrease. Also, the timing of arrivals between immature and adult newts vary and the duration of the period spent in the pond for these two life stages depends on resources, reproductive success and exploratory behaviour (Jehle et al., 2011). The duration of the aquatic phase may affect terrestrial habitat use as it influences the frequency and number of newts emerging and foraging around the pond (Kovar et al., 2009).

A number of radio-tracking studies during spring migration and summer foraging have been performed for this species (e.g. Jehle, 2000; Jehle and Arntzen, 2000; Gustafson, 2011). However, most observations of newt foraging and refugia use are within short distances of the breeding pond (Müllner, 2001). Philopatry (site fidelity) appears to be an intrinsic characteristic of *T. cristatus*, which shows high fidelity for breeding ponds (Jehle, 2000). Distances covered by night are short and can range from 10-50 m, reaching 100 m on certain occasions (Jehle, 2000; Jehle and Arntzen, 2000). Jehle and Artzen (2000) registered that 70% of observed adult movement towards summer habitats were within 20 m of the breeding ponds. Gustafson (2011), observed that there were differences in length of stay in refuges that were located within 75 m (minimum) and 200 m (maximum) from breeding ponds. Some studies with displaced *T. cristatus* showed that at distances greater than 500 m from breeding ponds, adults may colonize other areas (Griffiths et al, 2010; Jehle et al., 2011).

In contrast to migration movement, pond-breeding amphibian dispersal is defined as movements between different ponds, normally differentiated as natal ponds (birth sites) and breeding ponds (reproduction sites) (Semlitsch, 2008). Juvenile dispersal movements are defined as unidirectional and can reach longer distances than those of adults and occur at between-population scale (Semlitsch, 2008).

In the case of *T. cristatus*, juveniles may be primarily responsible for gene flow between sub-populations (Jehle and Artzen, 2000; Malmgren, 2002). For instance, Malmgren (2002) studied post-breeding movement patterns of *T. cristatus* for adults, juveniles and metamorphs between ponds and the adjacent terrestrial habitats over two years. Orientation of dispersing juveniles was considered to be random compared to adults and movements from the natal pond to forest patches had maximum distances of 120 m. Adult data suggested a different directionality from the juveniles, and repeat movements to the same terrestrial habitat were consistent over the years (Malmgren, 2002), as seen in previous studies (Jehle and Artzen, 2000). In some experiments, juveniles can detect and track chemical cues left by adults after leaving the pond, following them to favored terrestrial habitats (Edgar and Bird, 2006). However, metamorphs avoided following adults if the emergence phase from the pond overlapped. In this way competition with adults can be reduced (Malmgren, 2002). Nevertheless, it is not clear if these differences in directionality are due to climate conditions, water levels within ponds or habitat availability for dispersal.

T. cristatus juveniles can also migrate over longer distances (Kupfer and Kneitz 2000, Malmgren 2002). For example, the maximum recorded dispersal distance for a newt was observed by Kupfer (1998) from an immature individual that migrated 1,300 m in 7 weeks. It is also suggested that dispersing *T. cristatus* require a variety of land uses and habitats within 500 m from the breeding pond. Differences between dispersal distances from 100 m, 500 m to 1000 m indicate that *T. cristatus* needs a complex of suitable habitats interconnected with breeding ponds (Jehle, 2000; Gustafson, 2011; Jarvis, 2012). Nevertheless, metamorphs and juveniles may vary their movement patterns because of physiological and morphological restrictions and reproductive maturation (Semlitsch, 2008). As a general theory for amphibian juvenile dispersal, Semlitsch (2008) proposed that these movements might be divided into several discrete events that allow individuals to move longer distances in different stages of the life cycle. Juveniles would move on land until reaching reproductive maturity in a new pond (Semlitsch, 2008). This model showed a time scale of 3 years for a metamorph to find a new pond in an area of 1 km^2. Thus, differences in registered dispersal distances could be related to different phases in juvenile migration (Semlitsch, 2008).

Uncertainties about the role of dispersal movements emerge when trying to understand if they are sufficient to secure and establish new populations over time and overall connectivity (Jarvis, 2012). Jarvis (2012) calculated inter-pond movements in order to analyze pond dynamics and *T. cristatus* population survival. Eleven movements performed by adults were registered during this study. This number was shown to be sufficient for population persistence in this situation. These results indicated that this population had a high degree of independence in pond dynamics, however how landscape and terrestrial habitat changes influence these movements is not yet clear (Rothermel, 2004). Conversely, to differentiate intra-population migration and inter-population dispersal, knowledge on the spatial patterns of movements within the population is necessary over the long-term (Sinsch, 2014).

Dispersal between populations of *T. cristatus* is mainly the reserve of juveniles, sometimes during mass migration movements (Kupfer; 1998; Kupfer and Kneitz 2000, Malmgren, 2002). An understanding of the drivers of short and large-scale movements in terrestrial habitats could explain species responses in fragmented landscapes (Karlsson et al., 2007). Additionally, an improved understanding of inter-pond movements could aid the prediction of new areas of colonization, and hence an improved assessment of metapopulation persistance (Griffiths et al., 2010).

This section reviewed the main purpose of migration and dispersal for pond-breeding amphibian life cycles, distances measured and orientation of movement, how it varies within populations for age and sex, main environmental factors registered influencing patterns and its importance for landscape connectivity.

1.3.2 Landscape patterns of *T. cristatus* distribution

Movements of pond-breeding amphibians are strongly influenced by landscape components and qualities of the surrounding landscape (e.g. Ficetola et al., 2007). As *T. cristatus* depends on both terrestrial and aquatic habitats to complete their life cycle and the amount, the availability (landscape composition) and the connection (landscape configuration) between these landscape features influences their semiaquatic communities' distribution, abundance and movement patterns (Ficetola et al., 2007; Gustafson et al., 2011).

Landscape composition

The home-range size and the habitat use necessary for a species to be able to perform their life cycle is based on resource abundance and distribution (Semlitsch, 1998; Sinsch, 2014). In the case of pond-breeding amphibians, evaluation of the area of land necessary around ponds for conservation purposes typically involves the setting of buffers or distance thresholds (Semlitsch, 1998; Joly et al., 2003). Numerous studies specify that the amphibian suitable terrestrial habitat needed to accomplish their annual life cycle (home-range) should be between 100-400 m around ponds (Semlitsch, 1998; Semlitsch and Bodie, 2003; Ficetola et al., 2008). Most semiaquatic amphibians perform movements between ponds and terrestrial habitats within these distances (Sinsch, 2014). In the case of *T. cristatus*, buffers can go from 5-400 m around ponds and this measures are taken into account when analyzing their distribution and occupancy (Jehle, 2000; Joly et al., 2001; Rannap and Briggs, 2006). These thresholds were based on the percentage measured of newt abundance at local scale in some cases around a single or a group of ponds (e.g. Jehle, 2000). In a study performed in Denmark, between 60-80% of ponds were occupied with *T. cristatus* if the buffer radius was set at 50 m (Rannap and Briggs, 2006). Jehle (2000) demonstrated that 95% of *T. cristatus* were moving within 63 m of the breeding ponds in agricultural area in France. A higher percentage of newts used less than 100m around ponds during the spring season adding to the importance of these buffer areas around ponds for the conservation of newts within the landscape.

However, the terrestrial habitat within these buffers might not be enough for the species long-term survival as dispersal movements may encompass unsuitable habitats as well as require larger spatial scales. For example, Denöel and Ficetola (2007) tried to assess threshold incidence for different landscape attributes for three different *Triturus* species. A threshold in landscape composition (from 200 m to 400 m, depending on the specie) was found when considering the percentage of both forest (suitable habitat) and crops (unsuitable). This means that the amount of suitable and unsuitable habitat within the pond buffer will influence newts' occupancy in ponds. This is important when considering populations that are present in human-altered landscapes (urban and agricultural), where suitable habitat is confined to small patches within the landscape and are essential for long-distance movements such as dispersal (Ficetola et al., 2004; Denöel and Ficetola, 2007).

Landscape configuration

The availability of suitable terrestrial habitat and the connection between the suitable patches are primarily considered when analyzing pond-breeding amphibians' distribution and abundance patterns (Denöel and Ficetola, 2007; Ficetola et al., 2008; Gustafson et al., 2011). The distribution of terrestrial habitat patches facilitates or impedes amphibian terrestrial movements and dispersal through the landscape (Joly et al., 2003).

In the case of *T. cristatus*, pond density, distance to forests and open areas location (plus width) influence occupancy and distribution across the landscape (Joly et al., 2001; Denöel and Ficetola, 2007). Areas with groups of ponds, close to forests edges connected by open areas, seem to favor species movements between patches and maintain high levels of *T. cristatus* abundance (Joly et al., 2001; Ficetola and Bernardi, 2004; Denöel and Ficetola, 2007; Ficetola et al., 2008). Landscape configurations with an intermediate pond frequency (for *T. cristatus*, a maximum of five ponds in 50 ha), show high levels of newt abundance. Also, a positive association has been found between the width of uncultivated areas and newt abundance (Joly et al., 2001). Joly et al. (2001) suggest that connectivity between the newt terrestrial and the aquatic habitat is related to the area of uncultivated land and this area influences the extent of movements and dispersal between populations. These metrics between patches also vary between other pond-breeding amphibian species and are dependent on site specifications (Joly et al., 2001). Denöel and Ficetola (2007) showed that the calculation of the threshold distance from breeding ponds to suitable terrestrial habitats (e.g. forests) is crucial and they proposed that for each pond area a quantitative estimate of the maximum distance between these patches should be calculated.

Nevertheless, there is a contrast concerning the habitat matrix configuration for newt movement, especially in human-altered landscapes. In such degraded environments, movements can increase in order to explore new territory and resources, or decrease to avoid exposure to unsuitable habitats or threats (Ficetola et al., 2008). *T. cristatus* are known to use hedgerows and ditches as corridors between pond patches, but evidence of the most important landscape components that constitute corridors for newt movements and dispersal in fragmented landscapes is still limited (Joly et al., 2001). In the UK; Habitat Suitability Index (HSI) is a tool used in monitoring assessments for *T. cristatus* terrestrial habitat quality around ponds (Oldham et al., 2000). Its classifications lacks a more specific approach to classify the matrix as a landscape factor if ecologists want to use it to calculate connectivity metrics between suitable patches (e.g Wilkinson and Arnell, 2012). The use of this tool is relatively easy when it tries to evaluate the state of quality of ponds for net presence of newts. It is commonly used during development projects to classify if receptor ponds in mitigation schemes hold sufficient quality for the presence of high numbers of newts. However, this classification must be carefully analyzed when considering the suitability of individual ponds for the overall connectivity of newts in the landscape. This is crucial when considering HIS index only takes the extend area of evaluation of $50m^2$ (Oldham et al., 2000).

Suitable reproduction areas are an important integrant part of landscape configuration for *T. cristatus* and it needs to be revaluated within the regional threshold for long-term connectivity when assessing potential mitigation schemes. Individual evaluation of the matrix can lead to misuse of landscape connectivity metrics. Equally, proposed scales to evaluate connectivity metrics from the pond and terrestrial perspective within the matrix are still lacking an integrative approach within the development projects.

1.4 Linking pond-breeding amphibians' movement behaviour, landscape connectivity and road mitigation systems

In temperate regions, the main terrestrial movements of pond-breeding amphibians are classified in: (1) migration with primary movements towards reproductive sites and secondary daily movements for feeding and exploration nearly ponds and (2) dispersal, intra-population movements with movement between ponds, towards hibernation sites and also inter-population movements of both adult and juveniles. Habitat composition within the pond area (100-400m) and matrix configuration (forest cover) influences the presence of species in the landscape and the responses of individuals while moving. Higher the availability and presence of suitable habitat for completion of migration and dispersal, the higher the degree of landscape connectivity (Ficetola et al., 2007; Gustafson et al., 2011). Worldwide, mitigation measures have been designed and applied to maintain or re-establish connectivity in fragmented landscapes (Forman et al., 2003; Clevenger and Wierzchowski, 2006). With different combinations of solutions, which are implemented hierarchically in several steps, landscape connectivity is the primary focus which can be achieved (from local to regional scales) through wildlife crossing structures (Iuell et al., 2003; Forman et al., 2003). Solutions include the implementation of passages connecting habitat patches below or above the road (Iuell et al., 2003; Forman et al., 2003). For pond-breeding amphibians, a variety of crossings and fencing have been proposed, built and tested for different species in order to assess their effectiveness both at new developments and existing roads (Fahrig et al., 1995; Gibbs and Shriver, 2002; Andrews et al. 2008; Langen et al., 2009).

The types of mitigation measures for pond-breeding amphibians can vary according to: (1) conservation target (species or habitat), (2) time of implementation, (3) function of the structure and associated systems and (4) species movement attributes (Iuell et al., 2003; Clevenger and Wierzchowski, 2006; Schmidt and Zumbach, 2008; Bissonette and Cramer, 2008). The different types of crossings are categorized by their position in relation to the roads, building material and size (length and width).A list of main examples is included below:

Table 1.2 - Examples of commonly used wildlife crossing structure types, materials, dimensions (Cramer et al., 2011).

Type	Crossing structure	Materials	Dimensions (width (road length) x height
Underpasses	Small underpass	Metal pipe, concrete, small box culverts	1.5 m span or less
	Medium underpass	Concrete	larger than 1.5 m span, to 2.4 m span x 2.4 m rise
	Larger underpass	Concrete	6.1 m span x 2.4 rise or 3.1 m span x 3.1 m rise and open span bridges
	Specialized Culverts	Currents designs are small culverts, exclusive for amphibians and reptiles	0.5 m span
Overpasses	Extensive bridge	Concrete	Designed for each site. Dimensions vary.
	Wildlife Overpasses	Concrete	As small as 6.7 m wide, > 50 m wide preferably

For amphibians, underpasses designed by specialist companies are typically the most common measures applied locally (Iuell et al., 2003). Small underpasses are always combined with fencing systems which direct individuals towards the tunnels entrances (Hamer et al., 2015). There is a variety of different fence designs that not only take into consideration the engineering process of implementation but also the ecological information for a specific mitigation target, normally based on the features of species' movement ecology traits (Clevenger and Huijser, 2011).

The process of strategic planning and design of road mitigation systems differ greatly from study to study as each mitigation measure scheme has distinctive programming features and goals (Hamer et al., 2015). A few problems seem to have been persistent: (1) differences in passage implementation processes mean the evaluation of the proper location is site-species-dependent and solutions differ greatly in different studies with no integration of new knowledge; (2) environmental and species-related factors are not always included in planning as it depends on the main objective of implementation and (3) evaluation of the mitigation measure effectiveness is of variable quality and usually undertaken over very short time frames (1-2 years) (Lesbarères and Fahrig, 2012).

For pond breeding amphibians it is necessary to address these problems specifically:

1 – Planning process: passage implementation, appropriate location and future solution integration

For new roads, recently calculated sites of movement crossings can provide valuable information for landscape connectivity maintenance. Movement information enables researchers to calculate the best corridors at landscape level (Lesbarères and Fahrig, 2012).However, several problems seem to persist when trying to incorporate regional information into local scale conservation actions (Hamer et al., 2015).

Planning measures on existing roads (retrofitting) can be achieved while calculating clustered distribution of movements (Sillero, 2008; Glista et al, 2009; Matos et al, 2012). Due to road density and length, locating specific sites for movement corridors presents a cost-efficient mitigation application (Langen et al, 2009). However, at regional levels, critical sites can include other important features such as (1) high-quality habitat areas where amphibians are most likely to cross roads, (2) crossing rates resulting in road mortality which are more probable between ponds areas, and (3) locations where physical or behavioural blockages might prevent amphibians from crossing roads (Langen et al, 2009; Bager and Rosa, 2010).

Currently, road projects continue to be developed and measures are implemented with insufficient understanding of individual behaviour and movement patterns once mitigation is in place (Schmidt and Zumbach, 2008; Lesbarères and Fahrig, 2012; Hamer et al., 2015). An understanding of individual behaviour and movement patterns in road mitigation systems would improve the adaptation of new projects at local scales in order to maintain functional connectivity. Maintenance of local-scale connectivity should scale up to maintain connectivity at the regional-scale, thus promoting metapopulation persistence.

2 – Design process: environmental and species-related factors in planning and goal-driven measures

Behavioural responses from species and populations to the implementation of underpasses differ greatly between studies (Puky, 2003; Puky and Vogel, 2003). The majority of studies have evaluated the frequency of amphibians encountering drift fences and passing through tunnels during peaks of activity (e.g Pagnucco et al., 2012). These metrics were shown to be inadequate for evaluating the effectiveness of mitigation for promoting long-term connectivity because they do not demonstrate how infrastructures support dispersal of individuals in the long-term (Jackson and Tyning, 1989; Jenkins, 1996; Puky and Vogel, 2003; Malt, 2012; Pagnucco et al., 2012).

Specifically, road mitigation effectiveness studies for pond-breeding amphibians typically report :1) spatial and temporal patterns of movements (location in the system or hourly/weekly data), (e.g. Patrick et al., 2010; Lesbarrères et al., 2004), 2) an understanding of local and regional environmental processes affecting species behaviour (e.g. Woltz et al., 2008) and large scale plans for long-term dispersal (Patrick et al., 2010) and3) quantitative behavioural information (distances, turning angles and avoidance behaviour), (e.g. Pagnucco et al., 2012).

However, amphibian movements take place at different spatial, seasonal and temporal scales depending on the movement type and motivation. For instance, feeding is undertaken at the local scale by individuals during daily foraging movements. In contrast, dispersal activity requires wider movements during distinct seasons (Zetterberg et al., 2010), for example, dispersal from ponds to hibernation sites in autumn by adult and juvenile *T. cristatus* (Zetterberg et al., 2010). These latter events are classified as movements between "metapatches" and considered functional units at an inter-generational temporal scale (Zetterberg et al., 2010).

Behavioural information is currently used to develop accurate results and explore potential bias on connectivity metrics in mitigation scenarios (Zetterberg et al., 2010). Connectivity metrics are best evaluated using a general approach to species-specific spatial and temporal use of the landscape gradient (**Fig. 1.5**; e.g. Zetterberg et al., 2010). The incorporation of the most significant parts of a species' life cycle can support evidence on the degree of connectivity offered by a corridor at multiple spatial and temporal scales (Zetterberg et al., 2010).

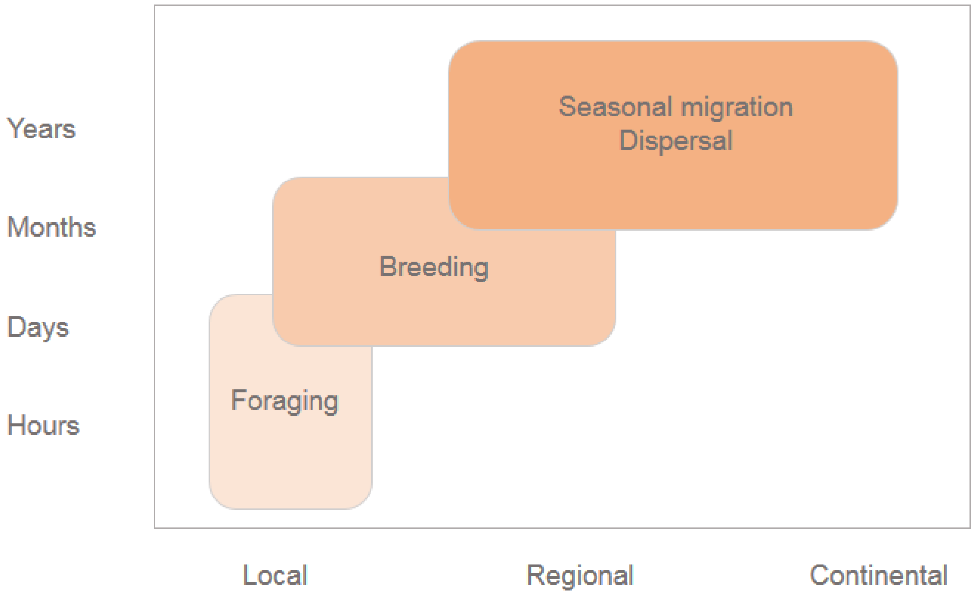


Fig. 1.5 Spatial and temporal levels for three main movement behaviors vital for conservation connectivity (adapted from Crooks and Sanjayan (eds), 2006)

3- Monitoring process: mitigation measures effectiveness evaluation

To resume, lack of aims, achievable objectives and translation to actual road mitigation effectiveness calculation by incorporating both individual and population levels are the top "need solving" problems when considering road effects on amphibian populations and landscape connectivity (Langton and Petrovan, 2013). For amphibians in particular, there is not a single study on *T. cristatus* road impacts or mitigation in the UK (Smith and Sutherland, 2014). Clearly, effectiveness needs strong insights on the mechanics and functional use of the mitigation measures and it is drawn from the comparison of monitoring protocols as goals are set up from different results are obtained from different strategies. Measuring effectiveness is dependent on 3 essential elements: 1) setting measurable goals, 2) adequate measurements of the effects of structure in time and space and 3) parameters need to be related to main concern and integrated in future projects for comparison (Van der Ree and Jaeger, 2015).

Monitoring programmes aiming to assess effective functioning of road mitigation systems have usually sought to evaluate the system's capacity for preventing amphibian road mortality (Beebee, 2013; Hamer et al., 2015). However, improved objectives for monitoring effective connectivity from road mitigation would have to identify at which scales are habitat resources being used by pond-breeding amphibians and how do mitigation actions affect movements that are critical to population persistence and landscape connectivity. Also, the longevity of connectivity should be assessed in order to deduce whether mitigation is sufficient for long-term persistence of pond-breeding amphibians (Andrews et al., 2008). Consistency in approach between monitoring studies would allow comparisons between studies and subsequent development of new updated guidelines (Hamer et al., 2015). Hereby, we propose a multi-scale effectiveness analysis necessary to address the different scales and variance of movement essential for *T. cristatus* landscape connectivity.

1.5 *T. cristatus* road mitigation planning and long-term conservation in England

As described previously, specific habitat and environmental conditions are important for *T. cristatus* movements during both the terrestrial and aquatic phases. The spatial scales involved differ among the use of terrestrial habitat and the species seasonal movements across multiple landscape elements (Ficetola et al., 2008). Terrestrial habitat can influence the migratory movements of newts at a smaller scale, affecting species distribution and presence at the landscape scale. The regional scale of species distribution influences dispersal patterns between populations at a larger scale in the long-term (Ficetola et al., 2008; Denöel et al., 2013). For example, Ficetola et al. (2008), describes the importance of considering different scales for semiaquatic amphibian conservation. From the breeding-pond initial point forests presence and distance are significant at the local scale (> 500 m) especially during autumn/dispersal seasonal and road length (density) and hydrography network density are influential at a larger scale (1000-1500 m) for both spring/migration and autumn/dispersal movements.

In England, the regulatory authority responsible for nature protection (Natural England, NE) provides standard advice for local planning authorities who independently assess the impact of new infrastructures on target species, such as *T. cristatus*. Surveys and mitigation are implemented when new developments are in place and these are generally necessary (1) if a pond is located within 250m and (2) if any suitable refuges for newts, such as grassland, scrubland, woodland or hedgerows are included in the development area (Natural England, 2015a). The first approach consists in a consultant ecologist, from independent organisations, providing advice and detailed planning for future surveys and mitigation measures. These institutions are awarded any licenses supplied for the interference with the habitat and newts handling (Natural England, 2015a). These plans normally indicate how to "avoid, reduce or manage any negative effects to protected species", which include *T. cristatus* (Natural England, 2015a). Detailed guidance on how to apply for a licence is provided at https://www.gov.uk/government/publications/great-crested-newts-apply-for-a-mitigation-licence.

The stated guidance for the long-term impacts of isolation by fragmentation and post-development interference on the target specie shows impacts on newt breeding and terrestrial habitats scored as “high, medium and low” (**Table 1.1**). For breeding ponds, the impacts are high if development fragments or isolates the breeding pond and has a medium impact on neighboring ponds. For the terrestrial habitat, the impact of isolation and fragmentation decreases from high to low as the distance of development from the breeding pond increases (from 50 m from the breeding pond to 50-250 m and then more than 250 m). The impact of a post-construction development on breeding ponds and neighbouring ponds is classified as high and low, respectively. The development impact on terrestrial habitat from 50m of the breeding pond is medium and from 50 to 250 m and more than 250 m is low (Natural England, 2015b). The guidance document states that the further away is the development from the breeding-pond where the species is first detected the impact from isolation by fragmentation decreases.

Table 1.3 – Scale of impacts from isolation by fragmentation and post-development intervention on *T. cristatus* populations in breeding ponds and terrestrial habitat (adapted from https://www.gov.uk/guidance/great-crested-newts-surveys-and-mitigation-for-development-projects, Natural England, 2015b). The document suggests that even low impacts need mitigation measures.

Isolation by fragmentation,	**Impact**
Effect on breeding ponds	*High*
Effect on the other ponds newts use	*Medium*
Effect on terrestrial habitat less than 50 meters from breeding	*High*
Effect on terrestrial habitat 50-250 meters from breeding pond	*Medium*
Effect on terrestrial habitat more than 250 meters from breeding pond	*Low*

Post-development interference	**Impact**
Effect on breeding ponds	*High*
Effect on the other ponds newts use	*Low*
Effect on terrestrial habitat less than 50 meters from breeding pond	*Medium*
Effect on terrestrial habitat 50-250 meters from breeding pond	*Low*
Effect on terrestrial habitat more than 250 meters from breeding pond	*Low*

For road mitigation design, national guidelines suggest (1) the creation, restoration and improvement of breeding sites and terrestrial habitats; (2) the establishment of corridors and stepping stones between habitats to link populations; (3) altering road routes and (4) installing green bridges or underpasses (Natural England, 2015b). New ponds should be placed within 250 m of each other and new terrestrial habitat must be included 500 m around the mitigation ponds. To keep individual newts away from development areas population translocations are performed to move newts found within 1 km of the site, facilitated by permanent amphibian fencing to prevent migration back to the point of capture. Tunnels or culverts combined with a fencing system can be deployed at the local scale as an alternative to maintaining dispersal routes. Post-mitigation monitoring schemes to compare population trends may be required for 5 or more rarely, 10 years, if the impacts of development is scored as high and the site importance for the population is high as well. No monitoring is required if impacts are judged to be low or for small population size or low site importance (Natural England, 2015b).

Currently, the advice for local planning authorities is based on simplistic scales of impact with no indication for assessing the overall landscape connectivity metrics for dispersal corridors and migration movements. Additionally, there is no indication on the impact of the classification of these developments in population numbers and long-term persistence. This study sought to explore natural and simulated responses of movements from newts in order to identify practical improvements for road mitigation planning and design based on new insights on landscape connectivity for great crested newts at multiple spatial scales.

2. Aims and Hypothesis

Research problem

The impact of roads on the movement ecology of *T. cristatus* is still inadequately characterized and hence poorly incorporated into road mitigation planning, potentially jeopardizing its long-term conservation status.

Research aims and hyphothesis

The main aim of this study was to evaluate *T. cristatus* movement patterns in areas impacted by roads and where road mitigation measures had been deployed in order to develop evidence-based improvements for the strategic planning and design of dispersal corridors for future mitigation for this species.

Specifically, I sought:

a) **to quantify newt movement spatial and temporal patterns between habitat patches in a road mitigation system,** since functional connectivity is spatially variable over time. Patterns of connectivity occurring in a road mitigation system for *T. cristatus* may change over time due to temporal changes in behavioural responses. I hypothesised that use of the mitigation scheme by newts would be greatest during seasonal peaks of activity (autumn and spring) and would increase over time as the vegetation around the tunnels became better established.

b) **to measure scales of terrestrial habitat connectivity for newts in a complex, human-altered landscape.** Structural and functional connectivity are negatively affected by fragmentation at the landscape level. The impact of roads affects *T. cristatus* seasonal movements at local scales. *T. cristatus* persist in a metapopulation structure dependent on local maintenance of migration and dispersal movements. Effects of roads are then multi-scalar impacting long-term dispersal patterns for the species. I hypothesised that roads would affect landscape connectivity for newts depending on the type of road impact and the type of movement performed during seasonal peaks of activity (autumn and spring). Identification of these impacts and their seasonality could help prioritize local mitigation actions based on functional connectivity metrics.

c) **to evaluate spatial and temporal variation of newt movements and dispersal responses in a road mitigation system.** Landscape connectivity is dependent on dispersal movements between populations. Small scale patterns of resource use may reflect long-term individual dispersal patterns. Do dispersal patterns of species behaviour vary in different spatial and temporal scales in the road mitigation system? I hypothesised that responses of newts would change as they moved along a road mitigation during activity peaks (spring and autumn), on approach towards a road, newts re-orientate travelling along the fence and when encountering a tunnel.

d) **to determine the influence of environmental factors on newt use of road mitigation structures.** Environmental factors affect the timing of newt behaviour changes since their life cycles are highly dependent on environmental conditions. Temperature and precipitation influence pond-breeding amphibian phenology. Hence, newts are highly sensitive to weather and climate changes, yet these are not typically accounted for during road mitigation planning. Does temperature, precipitation and humidity affect spatial and temporal patterns of movements in a road mitigation system? Do local environmental factors affect microclimate in tunnels and fences? I hypothesized that responses of newts and use of fences and tunnels would change if local ambient temperature, humidity levels and precipitation changed.

This thesis includes a general introduction, a literature review, four empirical chapters, each addressing one of the objectives stated above formatted for suitable submission to a peer-reviewed scientific journal, a general discussion with main conclusions and attachments of secondary results and respective statistical code used for data analysis.

General introduction and literature review (CHAPTER 1)

The literature review was based on the main research components to establish how behavioural responses to road mitigation can help understand landscape connectivity for pond-breeding amphibians in fragmented landscapes. First, I explored how roads can be detrimental to terrestrial movement mechanisms for amphibian population persistence was explored. Second, I investigated how pond-breeding amphibian metapopulation dynamics is dependent on patterns of movement in fragmented landscapes was investigated. Third, different aspects of movement behaviour from amphibians that can potentially help identify landscape connectivity degrees or being achieved in road mitigation scenarios were combined. Next, by using *T. cristatus* as a model species, information on its spatial ecology and landscape distribution patterns was enumerated and described. Finally, some aspects of *T. cristatus* road mitigation planning and design that are in place at the moment in Europe and UK were summarised and two study cases are here introduced as examples to answer the research problem, built general goals and study hypothesis.

Empirical chapters and research articles (CHAPTER 3 – 6)

Facilitating permeability of landscapes impacted by roads for protected amphibians: patterns of movement for the great crested newt (CHAPTER 3) (Matos **et al.***, 2017, PeerJ);*

I monitored a multi-tunnel and fence system over five years and investigated the impact of the scheme on movement patterns of two newt species, including the largest known UK population of the great crested newt (*Triturus cristatus*), a European Protected Species. I used a stage descriptive approach to quantify newt movement spatial and temporal patterns to calculate scales of movement for diferent points in the system for a local population. This chapter presents numerical evidence of seasonal and yearly variation for attempts and successful tunnel crossings. Results were valuable to recognize response dimensions in a non-natural system to next analyse regional patterns of movements for landscape connectivity.

Modelling roads as barriers to landscape connectivity in a threatened pond-breeding amphibian: a network approach (CHAPTER 4);

I explored how structural and functional connectivity changed when prioritizing pond-breeding amphibian movement corridors at different spatial scales across a large study are. Responses of *T. cristatus* were modelled in a variety of stages of urban and rural development and where linear infrastructures acted as potential barrier to movement. This chapter aimed to present potential terrestrial dispersal/colonization corridors and investigate linkages between patches for diferent movement scales. Results show significant differences for the landscape-scale population dispersal corridors. Dispersal movement showed to be crucial to analyse best location and prioritize mitigation efforts for newt movement at long-term.

Short-term movements and behaviour of T. cristatus in a road mitigation system (CHAPTER 5);

I aimed to quantify the short-term individual variation of behavioural responses of *T. cristatus* at the local level. I used fluorescent paint as a marking technique to measure distance travelled and trajectory orientation per night of individual newts across two seasons. This study presents data on reluctance of individuals to enter tunnels and re-orientation from the tunnels while traveling along fences. These local responses may determine if a complete crossing is successful and therefore contribute to calculate bias in planning effectiveness for dispersal movements at long-term.

Effects of environmental factors and seasonality on newt movement in a road mitigation system (CHAPTER 6);

I investigated how newt movement was influenced by climate and weather factors in a mitigation system in order to assess potential differences in condition between ambient and mitigation systems. Also, I aimed to understand the possible influence of changes in climate that could alter species movements in these systems with potential effects in long-term effectiveness. The frequency of newt captures at fences and within tunnels increased with weekly mean air temperature, total precipitation, high humidity at ground level and local seasonality. Results support indication of newt dispersal through the system and its effectiveness being influenced by both local climatic factors and the tunnel microclimate.

General discussion, conclusions and future research (CHAPTER 7)

I discussed how the improved understanding of newt behaviour and movement ecology in road mitigation systems and human-altered labdscapes can provide new insights to improve (with respect to appropriateness and efficiency) monitoring and to improve (with respect to effectiveness) mitigation design and planning.

CHAPTER 3

Facilitating permeability of landscapes impacted by roads for protected amphibians: patterns of movement and future directions

Matos, C., Petrovan, S., Ward, A., Wheeler, P. (2017). Facilitating permeability of landscapes impacted by roads for protected amphibians: patterns of movement for the great crested newt. *PeerJ* 5:e2922; **DOI 10.7717/peerj.2922**

Abstract

Amphibian populations are highly vulnerable to road mortality and habitat fragmentation caused by road networks. Wildlife road tunnels are considered the most promising road mitigation measure for amphibians yet generally remain inadequately monitored, resulting in mixed success rates in the short-term and uncertain conservation benefits in the long-term. We monitored a complex multi-tunnel and fence system over five years and investigated the impact of the scheme on movement patterns of two newt species, including the largest known UK population of the great crested newt (*Triturus cristatus*), a European Protected Species. We used a stage descriptive approach based on capture positions to quantify newt movement patterns. Newt species successfully used the mitigation but the system constituted a bottleneck to movements from the fences to the tunnels. Crossing rates varied widely among years and were skewed towards autumn dispersal rather than spring breeding migration. There was a substantial negative bias against adult male great crested newts using the system. This study indicates that road tunnels could partially mitigate wider connectivity loss and fragmentation at the landscape scale for newt species. However, the observed bottleneck effects and seasonal bias could have population-level effects which must be better understood, especially for small populations, so that improvements can be made. Current requirements for monitoring mitigation schemes post-implementation are probably too short to assess their effectiveness in maintaining connectivity and to adequately understand their population-level impacts.

Keywords: connectivity, great crested newt, migration, dispersal, smooth newt, roads, underpass, wildlife crossing

3.1 Introdution

Worldwide, road networks represent a major threat to amphibian population viability. Roads restrict and interrupt amphibian movements and cause high mortality through road kills during seasonal migration and dispersal (Fahrig et al., 1994; Hels and Buchwald, 2001; Glista et al., 2007; Matos et al., 2012; Petrovan and Schmidt, 2016).

Road crossing structures for amphibians, typically small diameter tunnels or underpasses and associated fence systems, have been implemented for over 30 years along spring migration routes in Europe and are currently considered the most promising road mitigation solution for amphibians (Brehm, 1989; Iuell et al. 2003; Lesbarrères and Fahrig, 2012). However, while some studies report reductions in road mortality rates, at least in the short term, few have performed a detailed comparative analysis of tunnel- and fence-use by amphibians (Jochimsen et al., 2004; Pagnucco et al., 2012) and virtually none for newts (Schmidt and Zumbach, 2008; Beebee, 2013).

Successful and robust mitigation is especially relevant for declining or threatened species where road networks could severely impact on the population connectivity and ultimately long term population survival. The great crested newt (*Triturus cristatus*) is a European Protected Species which has declined substantially over recent decades, largely due to habitat loss and habitat degradation (Langton et al., 2001; Jehle et al., 2011). However, the species remains relatively widespread in the UK, including in semi-urban environments, and is therefore regularly the subject of road mitigation schemes in an attempt to maintain habitat and population connectivity between the two sides of the road. Such schemes can involve populations of hundreds or even thousands of individuals, carrying substantial financial costs and cause significant delays for infrastructure projects. Evaluation of mitigation success is therefore required to ensure that only sound practices are employed (Ward et al. 2015).

Road connectivity schemes typically adopt linkage strategies which target species dispersal as the main process determining landscape-scale connectivity (Baguette and Dyck, 2007; Baguette et al., 2013). For pond-breeding amphibians, such as *T. cristatus*, metapopulation dynamics are highly dependent on connectivity and consequently dispersal as determining fundamental processes for long term population viability (Halley et al., 1996; Semlitsch, 2008;

Griffiths et al., 2010). In addition, barriers to movement may limit individuals' ability to secure specific habitat requirements at different stages of maturity (Sinsch, 1990). Adult movements (migration) between aquatic and terrestrial habitats are defined as short-term migration movements because of their duration and distance (Pittman et al., 2014). Long-term, wide-ranging movement (dispersal) is primarily performed by juveniles, which move significantly more among sub-populations and through landscapes than adults (Rothermel, 2004). Therefore, temporal and spatial variation in amphibian movements should be incorporated into assessments of the effectiveness of road crossing structures and mitigation schemes (Clevenger and Waltho, 2005). Equally, most published road mitigation studies have only presented use-frequency over short time periods (1-2 years), and lacked comparisons regarding seasons and trends over several years (Jackson and Tyning, 1989; Allaback and Laads, 2003; Pagnucco et al., 2011).

We performed a 5-year monitoring study aiming to assess potential functional connectivity of a road mitigation scheme for *T. cristatus* and other amphibian species in the UK. We investigated whether newts successfully crossed the road using the mitigation scheme and if crossing rates differed between species, sexes and age classes. We hypothesised that use of the mitigation scheme by newts would be greatest during seasonal peaks of activity (autumn and spring), independently of age, and would increase over time as the vegetation around the tunnels became better established. Finally, we investigated if newt movement was facilitated by the tunnels between the two parts of the population separated by the road. The main objectives were to: (1) characterise different types of newt movement for age and sex class in relation to the mitigation system, (2) assess annual, seasonal and spatial differences in movement patterns and (3) evaluate if movement through the tunnels was maintained over time by determining which variables explain seasonal and directional movement variance among years of monitoring.

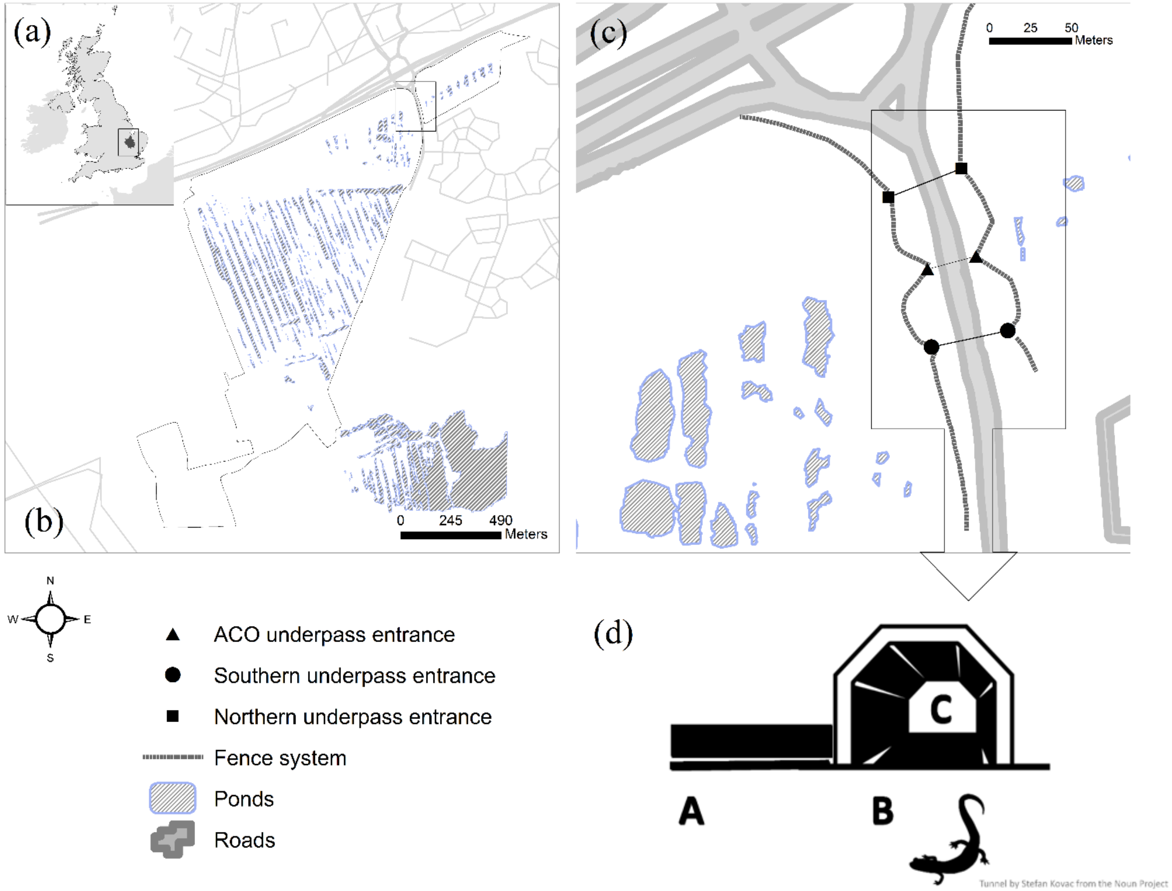
Ultimately, our goal was to understand how the mitigation scheme supported the movements and connectivity of the newt population and therefore draw conclusions on its effectiveness for the maintenance of the wider population in the long term. Given that no published data exist on road mitigation systems for *T. cristatus* this study could inform other current and future mitigation schemes for this protected species and newt species in general.

3.2 Material and Methods

3.2.1 Study area

The study was conducted in Orton Pit/Hampton Nature Reserve (52º 32'24N, 0º16'53W), a designated Special Site of Scientific Interest, Special Area of Conservation and Natura 2000 site, located south of Peterborough, Cambridgeshire (UK) (**Fig. 3.1a; Fig. 3.2**). This 145 ha reserve mainly comprises a section of former industrial brick clay extraction site but also includes woodland and patches of scrub. The main site is characterised by a complex of over 340 ponds, ranging from 15-50 years old. Between 1990 and 2000 a large-scale habitat restoration took place including pond modification and fish eradications. Concomitantly, an extensive amphibian translocation programme took place with 54,000 adult amphibians and 66,000 juveniles moved to the reserve from the nearby brickpit area. Of these, 24,000 were adult great crested newts and 9,000 were adult smooth newts (*Lissotriton vulgaris*) with the rest represented by common toads (*Bufo bufo*) and common frogs (*Rana temporaria*) (HCI, 2000). Following translocation, concrete 'newt barriers' were installed on sections of the reserve along the border of the new development land and associated road. The site is currently home to potentially the largest single population of great crested newts in the UK and possibly Europe, estimated at around 30,000 individuals, as well as a very large population of smooth newts, but common frogs and common toads have become exceedingly rare (Froglife, 2012).

The northern reserve area is divided by a 10-12m wide, high-traffic-volume (1 000 – 10 000 vehicles/day) road constructed in 2006 which serves the new housing development (**Fig. 3.1b; Fig. 3.2a**). Construction of this road involved the reprofiling of some large ponds and filling in of others. Consequently, a comparison of pre-road amphibian movement rates across the pre-road and post-road was not possible because of changes in the landscape and the lack of specific monitoring data before the road was built. The road mitigation system was composed of: one polymer concrete 'amphibian tunnel', with open slots at the top, manufactured by ACO Germany (0.5 m diameter, 30m long), two large ARCO concrete and metal sheet underpasses (5.5m wide x 2 m high, 40m long) spaced 100 m apart, and two heavy duty plastic fence systems (Herpetosure UK, 200-300 m in length), placed 10-50 m away from the road, angled to guide amphibians towards the tunnels. In an effort to minimise human disturbance the entrances of the large tunnels were protected with a locked bar fence and gate system. The three underpasses (northern, central or ACO, and southern tunnel) connect the two main sections (west and east sides of the road) in the north of the reserve (**Fig. 3.1c; Fig.3.2b**).



Icons: Tunnel by Stefan Kovac and Newt by Andrea Novoa for Noun Project (www.nounproject.com)

Fig. 3.1 – (a) Location of the study area in Peterborough, UK (b) west (large) and east (small) sections of Hampton Nature Reserve (SSSI) (c) monitored sections of the mitigation system (in bold) (d) For each capture point a code was defined: movements along the fence system (A); movements in tunnel/attempted crossing (B); movement in tunnel/successful crossing (C).

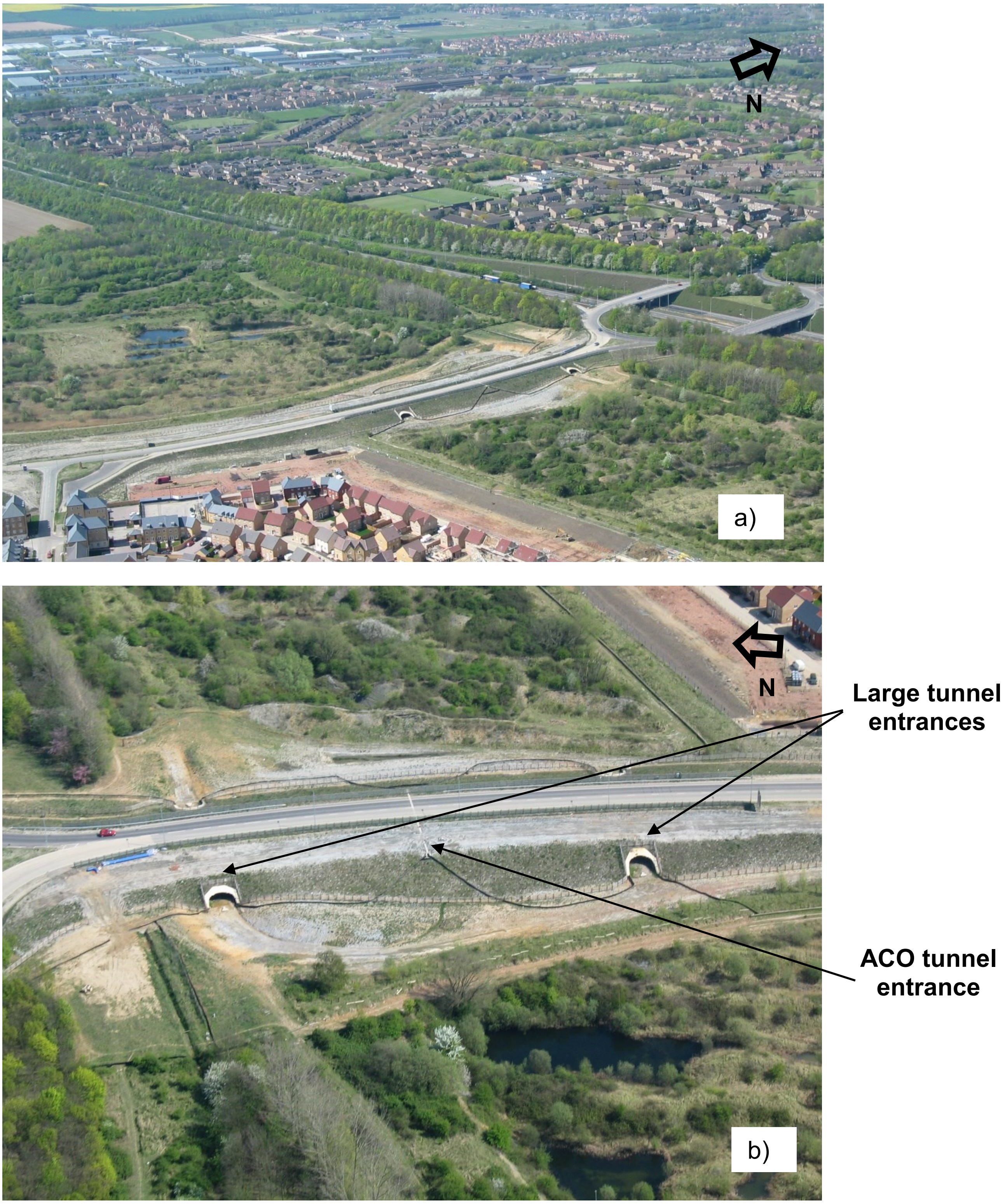


Fig. 3.2 – View over the road mitigation scheme in Hampton Nature Reserve: a) tunnels entances from the east side, view over the west part of the reserve and north and south urban settlements; b) tunnels entances and ponds from the west side.

3.2.2 Field methods and amphibian movement patterns

Surveys were conducted between April 2007 - October 2012 with no data collected in 2009. Data were collected by Froglife workers and volunteers (Froglife.com) and granted to be used in this thesis research. Monitoring averaged 7 months per year and 8-12 days per month, starting in spring (typically March) and ending in autumn (mid-late October), with no monitoring during winter due to amphibian inactivity. Tunnel usage was monitored using pitfall traps at both entrances of the north and south tunnels. Tunnel pitfalls, extended across the tunnel width, had double (inner and outer) 25cm deep concrete and metal sheet trenches, each with an inverted top edge. This allowed the recording of complete and attempted crossings in these two tunnels: newts trapped in the inner trench travelled through the tunnel (completed), those in the outer trench just entered the tunnel (attempted). In addition, bucket pitfall traps and a temporary plastic fence were used for monitoring the central ACO tunnel. Inner/outer trenches were opened for the same number of times on each side, rotating every 4 days. The total number of trapping days varied in the first two years as the methodology was tested (**Table 3.1**). Trapping focused on spring and autumn, the main periods of amphibian movements, but included at least 4 days of trapping per month during the summer months. From 2008 until the end of the study the fence system was surveyed on trapping nights using night-time torch surveys. Additionally, in 2008 only, short drift fences and three 10-l plastic bucket pitfall traps were placed on each side of the road in front of the tunnel fences (east and west). Traps were checked daily, in early morning and again in the evening along with fence checks.

Amphibian species, number, sex and age class (adult or juvenile) were recorded together with the position in the mitigation system (tunnel/fence) and side of the road. No individual marking was performed and trapped amphibians were released in vegetation near the capture point. All trapping and handling was done under a Natural England licence (Ref: 04/01204/REM).

Although the tunnels were primarily put in place for the protected *T. cristatus*, which was also the main focus of the monitoring, *L. vulgaris* data were included at all stages during the analysis.

Table 3.1 – Response and predictor variables used for the GLMM analysis

Code	**Variable description**	**Values (mean ± SE)**
MIs	Movement index for season. Number of observations (amphibian captures), per capture point (A, B and C) per time period (number of night surveys) in each season (autumn, spring and summer). Continuous variable.	0.29±0.06 (0-3.78)
MId	Movement index for direction. Number of observations (amphibian captures), per capture point (A, B and C) per time period (number of night surveys) in each side of the road (East and West). Continuous variable.	0.12±0.03 (0-1.72)
Age	Amphibian age classes. Factor.	Adults, Juveniles
Season	Monitoring seasons. Factor.	autumn, spring and summer
Side	Side of road where amphibians were observed moving/captured. Factor	east, west
Type	Type of movement in the mitigation system. Capture points. Factor.	Moving along the fence (A), Attempt cross at tunnel entrance (B) and sucessful crossing in the tunnel (C).
Species	Newt species. Factor	*Triturus cristatus* (TC) and *Lissotriton vulgaris* (LV)
Year	Years of monitoring. Factor	2008, 2010, 2011, 2012

3.2.3 Variables and Data Analysis

We coded newt movement on the basis of capture location within the mitigation scheme and their position in relation to the road (**Fig. 3.1d**). Captures along the fences were coded ‘A’, captures at tunnel entrances coded ‘B’, and captures of animals which had successfully crossed the road through tunnels coded ‘C’.

In 2007 the northern tunnel was mostly flooded and the additional fence system monitoring (to assess ‘A’ movements) was only started in 2008. Consequently, we only used data from 2008-2012 for this part of the analysis. Data were grouped into seasons: ‘spring’ (March, April, May); ‘summer’ (June, July, August); ‘autumn’ (September, October). Direction classes (‘east’ and ‘west’) describe (1) the position of capture with respect to the road such that animals captured at fences and tunnel entrances (A, B) and (2) for those that successfully crossed from east to west (C) would be classified as ‘West’ and similarly those that moved through tunnels from west to east were classified as ‘East’.

In order to standardise measures of movements among years, we developed an index (MI) that represents the amphibian use of the mitigation scheme at different observation points (A, B and C) and allows data to be compared separately for season and direction without bias due to differences in trapping effort each year:

$$MI_{ij} = n_i/CN_j$$

where ni is the number of observations (amphibian captures separated by age class) of a species for each capture point i and CNj is the number of capture nights per time period j (which varied for years (MIy), season (MIs) and direction (MId)).

We estimated differences in captures between age (adults/juveniles), sex (male/female) classes and among capture points (A, B and C) using Pearson chi-square test ($\chi2$). We examined the relative importance of age, season, side of capture, movement type and species for two movement patterns (seasonal and direction) along the years of monitoring (see **Table 3.1** for details on variables). A generalized linear mixed-effect model (GLMM) with a zero-inflated Poisson (ZIP) error structure with log link was fitted for two response variables (*MIs* and *MId*), ZIP were used due to high presence of zeros in response variable distribution, this way potential overdispersion and bias is avoided in parameter estimation (Bolker et al., 2012). We separated the analysis into four models to clarify the role of each independent variable, considering years as a random effect.

Two null models (one for season and another for directionality) containing the most significant variables and intercept was included for comparison (age + season/side + type + species (1|year)). From here we tested three models with the most significant variables, with no test for interactions. We compared model parsimony using Akaike information criterion (AIC) to optimize goodness-of-fit but avoid overfitting of the candidate models (Burnham and Anderson, 2002). After selecting the most parsimonious model, we determined the significance of fixed factors by analysis of deviance (Burnham and Anderson, 2002).

All statistical procedures were carried out using R 3.2.2 (R Development Core Team, 2004). ZIGLMM models were fitted using glmmADMB package (Bolker at al., 2012).

3.3 Results

A total of 831 amphibian captures were recorded over 353 trapping nights during the five years (**Table 3.2**). Fence and tunnel captures were highest in autumn (57.3%), spring (34.4%) and summer (8.3%). All four amphibian species found in the study area were recorded during surveys with the two anurans forming less than 1% of captures (R. temporaria 0.84%, B. bufo 0.12%). *T. cristatus* was the most frequently caught species (87.6% of captures) alongside *L. vulgaris* (11.4% of captures) (**Table 3.2**).

Age class was determined for 821 newt (98.8%) observations (**Table 3.S1** in Supplemental Material). Adult *T. cristatus* represented 60.4% of the species captures versus 39.5% juveniles. By contrast, for *L. vulgaris* 69.9% of captures were juveniles and 30.1% adults. Sex was determined for almost all adult newts (i.e. 464 newts, **Table 3.S1**) with *T. cristatus* adult females outnumbering adult males by over three to one (78.5% of captures).

There were higher numbers of detections along the fences than inside the tunnels (64.8% and 35.2% of captures, respectively) (**Table 3.S1**). The short drift fences deployed in 2008 only captured 24 individuals (4.8% of the total individuals at the fence. The southern tunnel produced the highest number of newt captures (142 observations, 49%) followed by 125 in the northern tunnel and 23 in the central ACO tunnel (respectively with 43% and 8% of the records). More newts were captured on the main reserve side ('West', 60%) than on the east side of the road (40%).

Table 3.2 – Survey effort: Number of survey days per year of monitoring and number and percentage of amphibian species recorded in the system per year.

	Monitoring				Species				
Year	**Months**	**Seasons**	**Days**	**Mean (days per month)**	**N**	***T. cristatus***	***L. vulgaris***	***R. temporaria***	***B. bufo***
2007	5	2	48	9.6	10	6	3	0	1
2008	9	3	113	12.5	234	197	36	1	0
2010	8	3	64	8.0	248	209	36	3	0
2011	6	3	64	10.67	48	41	4	3	0
2012	8	3	64	8.00	291	275	16	0	0
Total	36	14	353	9.76	831	728	95	7	1
%					100	87.61	11.43	0.84	0.12

3.3.1 Newt movement patterns in the road mitigation system

More *T. cristatus* and a higher proportion of adults were caught at the fence (A) than at tunnel entrances (B) (**Fig. 3.3a; Table 3.S1**) (χ^2ageA/B = 21.39, df=1 P<0.001). Although fewer animals and a higher proportion of adults were captured having crossed the tunnels (C) than those that reached tunnel entrances (B), these differences were not significant indicating that any movement bottleneck effect took place between fence and tunnel entrance movements but not subsequently (**Fig. 3.3**).

Overall, capture rates for *T. cristatus* adults and juveniles were not consistent through the mitigation system (**Fig. 3.3a; Table 3.S1**). More adults were recorded after successful tunnel crossings (C) (55.7%) while juveniles were most frequently caught at the tunnel entrances prior to crossing (B) 55.4% (χ^2adults = 76.44, df=2 P<0.001) (**Fig. 3.3a; Table 3.S1**).

L. vulgaris displayed a different pattern to *T. cristatus*, although the low number of captures at all three locations makes it difficult to draw clear inferences (**Fig. 3.3b; Table 3.S1**). Captures of both adults and juveniles of this species differed significantly among the three capture points in the system (χ^2ageB/C = 4.90, df=1, P=0.03; χ^2ageB/C = 6.60, df=1, P=0.01) with fewest adults and most juveniles caught at stage B (χ^2adults = 10.97, df=2 P<0.01).

There were no significant differences in *T. cristatus* sex-ratios concerning points A, B and C. However, the proportion of *T. cristatus* females captured was much higher than males overall (80.5% females) and at each point in the system (**Fig. 3.3a; Table 3.S1**): 78.6% (A), 87.5% (B) and 85.2% (C). In contrast, *L. vulgaris* males were more frequently caught than females overall although this pattern was not consistent across the different capture points in the mitigation system: 66.7% males (A), 20.0% (B) and 60.0% (C) (χ^2males = 34.6, df=2, P<0.001) (**Fig. 3.3b; Table 3.S1**).

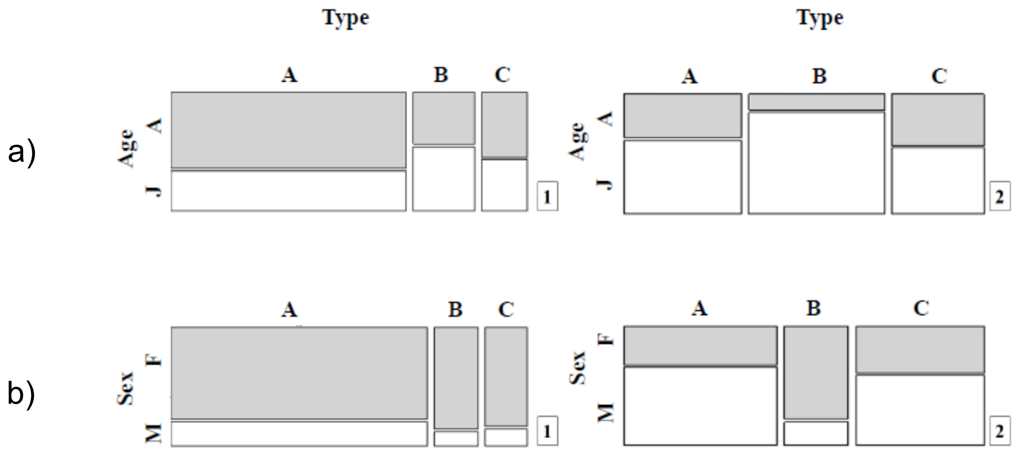


Fig. 3.3 – Population proportions for the different movement types (A, B and C) by a) age and b) sex for (1) *T. cristatus* and (2) *L. vulgaris*

3.3.2 Temporal and directional patterns of newt movements

T. cristatus captures at the fence (A) and tunnel (B, C) varied considerably among years (H=117.75, df=2, p<0.001). MIy values for A ranged from 0.33-4.00 captures per night, for B from 0.13-0.58 captures per night and for C from 0.08-0.77 (Table 3). *L. vulgaris* capture rates differed significantly among years (H= 26.17, df=2, P<0.001) although variance was relatively consistent between capture points (**Table 3.3**).

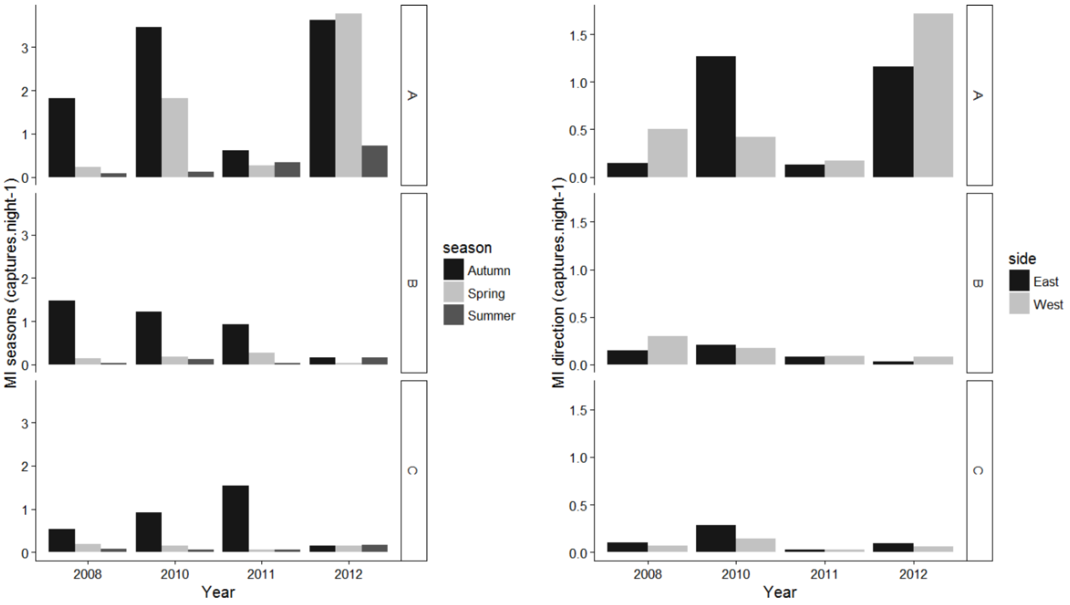
From 2010 overall mean values for successful tunnel crossings (numbers of newts caught at B relative to C) dropped for both newt species (**Table 3.3**). This pattern was particularly evident for *T. cristatus* captures despite an increase in B values during 2011 (**Table 3.3**). Overall, MIy values for successful crossings (C) were relatively low for with the exception of 2012, remaining below 0.77 captures per night for *T. cristatus* and below 0.22 captures per night for *L. vulgaris*, with zero crossings for the last two monitoring years for *L. vulgaris* (**Table 3.3**).

More newts were captured during autumn than in any other season (**Fig. 3.4**). Overall, higher numbers of successful crossings were also recorded during autumn over the years. Whereas spring and summer mitigation use was low (**Fig. 3.4**). GLMM analysis showed that seasonality had an effect on attempting and successful crossings for both species with no effect from age (**Table 3.4**).

During the study period, movement of newts was recorded on both sides of the road in two directions (**Fig. 3.4b**). MId varied significantly between sides along the years, but with no prevalence of movement from any particular direction (**Fig. 3.4b**; **Table 3.4**). However, models showed potential effect of species in movement direction between sides (**Table 3.4**).

Table 3.3 – Captures, year movement index (MI_y) at each point in the mitigation system over the study period for two newt species.

***Triturus cristatus* (Great crested newt)**												
	A				**B**				**C**			
Years	**N**	***MI***	**Mean±SD**	***s*²**	**N**	***MI***	**Mean±SD**	***s*²**	**N**	***MI***	**Mean±SD**	***s*²**
2008	99	0.88	1.79±1.62	2.62	66	0.58	0.43±0.30	0.09	32	0.28	0.27±0.20	0.05
2010	125	1.95			35	0.55			49	0.77		
2011	21	0.33			15	0.23			5	0.08		
2012	256	4.0			8	0.13			11	0.17		
***Lissotriton vulgaris* (Smooth newt)**												
2008	5	0.04	0.12±0.09	0.01	20	0.18	0.10±0.09	0.01	11	0.10	0.08±0.10	0.01
2010	10	0.16			12	0.19			14	0.22		
2011	3	0.05			1	0.02			0	0		
2012	14	0.21			2	0.03			0	0		



a) b)

Fig. 3.4 – a) Season (*MIs*) and b) direction (*MId*) patterns of MI values (captures.night^{-1}) for newt capture points (A, B and C) observed per year for both newt species

Table 3.4 – Parameter estimates for the generalized linear mixed-effect model (GLMM) of seasonal and directional movement indexes ((*MIs* = 144 and *MId*= 96) for both newts species (*T. cristatus* and *L. vulgaris*) with coefficient (β); standard error (SE); t statistics (t-value) and corresponding significance (P-value). Null deviance (ND) and residual deviance (RD) include information on predicted response by the null and by all predictors model, respectively.

Response		**Intercept**	**SE**	***Z***	***P***	**AIC**	**Likehood ration**
Seasonality							
	Null	-1.35	0.53	-2.54	0.01		
	Global	-1.49	0.58	-2.55	0.01	146.6	-64.30
	TypeB	-1.23	0.40	-3.09	0.002		
	TypeC	-1.55	0.45	-3.43	<0.001		
	Spring	-0.99	0.36	-2.75	0.01		
	Summer	-2.30	0.61	-3.72	<0.001		
	Species	2.22	0.51	4.29	<0.001		
	type + season + species	-1.55	0.56	-2.75	0.006	**144.8**	-64.38
	type + season	0.08	0.32	0.26	0.79	174.2	-80.12
	season + species	-2.24	0.55	-4.04	<0.001	160.3	-74.16
Direction							
	Null	-2.24	0.59	-3.78	<0.001		
	Global	-2.79	1.01	-2.76	0.005	68.1	-26.06
	TypeB	-1.38	0.80	-1.71	0.08		
	TypeC	-1.56	0.87	-1.80	0.07		
	Species	2.11	0.96	2.19	0.02		
	type + side + species	-2.99	0.98	-3.04	0.002	**66.7**	-26.34
	type + side	-1.46	0.47	-3.07	0.002	72.5	-30.25
	side + species	-3.71	0.96	-3.86	<0.001	68.4	-29.20

3.4 Discussion

By quantifying the different types of movements (A, B and C) this study has shown how two pond-breeding amphibian species used road mitigation tunnels, demonstrating differences in attempted and successful crossings and movement along the system, while highlighting substantial temporal and directional variability.

3.4.1 Newt movement patterns at the road mitigation system

The observed lower rates of successful and attempted crossings relative to fence movement suggest a movement bottleneck effect for dispersers. This is in line with other studies showing amphibian numbers reducing along the mitigation system (Patrick et al., 2010; Pagnucco et al., 2012).

Newts require well-kept and well-designed fences to prevent them from climbing onto the road (Schmidt and Zumbach, 2008). The substantially higher capture rates at fences compared to the tunnels could be explained by the “fence effect”: Allaback and Laads (2003) showed that salamanders attempted to avoid road mitigation fences and once avoided they appeared disoriented and moved in other directions, not necessarily towards mitigation tunnels. In this respect, fences can mimic the barrier effects of roads (Hels and Buchwald, 2001; Jaeger and Fahrig, 2004; Mazerolle et al., 2005), especially for newts, including *T. cristatus*, which are relatively poor dispersers overland (Jehle and Arntzen, 2000). Future research should investigate optimising fence and tunnel placement in order to minimise such potential barrier effects.

Sex ratios differed between capture points along the mitigation system for both newt species but overall far more females than males were observed for *T. cristatus*, a pattern previously recorded for some salamander species (Aresco, 2005; Pagnucco et al., 2012). This may be due to differences in: (1) population sex ratio, (2) sex differences in time spent in the pond and (3) migration distances to and from the ponds (Latham and Knowles, 1996; Hayward, 2002; Schabetsberger et al., 2004; Jarvis, 2013). Newt home ranges are generally small (with linear movements away from the pond between 30-400 m) (Jehle, 2000; Jehle and Arntzen, 2000; Müllner, 2001) but adult females undertake longer distance movements in autumn compared to males in the closely related Italian crested newt - Triturus carnifex (Schabetsberger et al., 2004). The differences in migratory behaviour between male and female newts and both pond position and distance to the tunnel may influence amphibian cues and motivation to move and consequently, the capture rates along the system (Buck-Dobrick and Dobrick, 1989; Sinsch, 1990). Our results suggest that the sex-biased migratory behaviour and the considerable distance from trapping points to the nearest ponds (30-74 m) favoured females over males, at least for *T. cristatus*.

The potential impact on the reproductive success of the population from the low adult male crossing rates observed in this study remains unknown but might be compensated by juvenile dispersal assuming no sex bias in crossing rates for this category. However, while the high juvenile movement in autumn indicates these movements as dispersal, the ultimate reasons for adult female long distance travel in autumn, including crossing through the road tunnels, remain unclear and somewhat contradict studies showing high breeding site fidelity for adults of this species (Jarvis, 2012).

Moreover, variability in movement is also linked to differences in behaviour of individuals, and this is true for different populations (Sinsch, 2014). The spatial context of the individual will define and trigger its decision to move or to stay (Baguette and Dyck, 2007). However, studies on individual-based spatial behaviour of newts to improve mitigation systems are still inexistent. Results could clarify how mitigation structures influence temporal and permanent residency of newts in the vicinity of the mitigation, fundamental for quantifying patterns of terrestrial movement and connectivity for broader scales (Baguette and Dyck, 2007, Baguette, 2013).

3.4.2 Annual patterns of newt movements

Usage rates by newts of the mitigation system varied considerably among years. Captures at the fence increased over time while captures at the tunnel entrances and subsequently, the actual crossings, decreased. This could reflect how environmental variables influence movements of newts throughout the system. Favourable environmental conditions will not only facilitate amphibian dispersal across the landscape but also influence frequency of migrations (Sinsch, 1990; Sinsch, 2014). We suspect that the prolonged dry weather conditions in 2011 could have contributed to the decline in fence captures in 2011 and attempts and successful crossings in 2012. However, studies showing evidence of unfavourable weather conditions influencing tunnel environments and consequently newt behaviour have not been documented to our knowledge.

Low rates of successful crossings in tunnels were also reported before for *Salamandridae* family: *L. vulgaris* in Germany (12% of the attempted crossings) (Brehm, 1989), *Ambystoma macrodactylum* and *Taricha granulosa* (4% each) (Malt, 2012), *Ambystoma macrodactylum croceum* (9% of those detected at the fence; Allaback and Laads, 2003) and 1%-23% for the same species in Canada (Pagnucco et al., 2012) over a 2-3 year period. The higher crossing success rate in our study was potentially caused by the very large diameter of the tunnels compared to other studies, although at 30 and 40m these tunnels are amongst the longest ever used for amphibians.

3.4.3 Seasonal movements

Tunnels were mostly used for autumn movements, which for *T. cristatus* are typically long-distance (Jehle and Artzen, 2000). An increase in tunnel use by juveniles during this season might be linked to the start of the postmetamorphic phase and emergence from ponds (Duff, 1989; Hayward, 2002) as well as juvenile dispersal attempts. Spring movement rates for adults were low, indicating that breeding migration (from terrestrial hibernation sites to aquatic breeding habitats) through tunnels was very limited. This may be due to a combination of factors such shorter-distance movements by adults during spring migration to breeding sites (Griffiths et al., 2010; Jehle, 2000) and potential overwinter mortality.

As part of the ongoing site monitoring the 200m road section above and near the mitigation tunnels was surveyed intensively on foot every two early mornings for 220 days in the maximum activity period for amphibians, between September 2013 and October 2014. No amphibian road kill was ever recorded despite the fact that newts, especially *T. cristatus*, were occasionally seen near the road surface at night. The lack of observed amphibians road use could indicate the effectiveness of the fence system for mitigating road mortality (Cunnington et al., 2014). However, the road may also represent a significant barrier to movement contributing to a possible display of avoidance behaviour (Mazerolle et al., 2005).

Prior to the construction of the road and mitigation structures, a two year study was conducted to better understand connectivity and movement behavior of *T. cristatus* in the southern part of the same site (HCI, 2006). The results indicated a very similar pattern compared to the newt movement observed in our study. The vast majority of the newts were trapped in autumn, with very little adult pond migration movement in spring for *T. cristatus*. Equally, overall there was a smaller number of males compared to females and large differences in the number of individuals between the two years of surveys (HCI, 2006). The similar patterns with pre-road construction movement data for this species suggest that the tunnel mitigation system may partially influence the newt movements but the general patterns remain unchanged.

3.4.4 Movement directionality in the mitigation system

Directionality of movements differed between the two species and years of monitoring. *T. cristatus* showed higher movement rates from the large area of habitat in the west to the smaller area in the east while *L. vulgaris* mostly moved from east to west, balancing the use of the mitigation between the two sides during years of captures. Although the precise drivers of differential direction of movement are unclear, the extensive suitable habitat on both sides of the road makes it unlikely that movement through tunnels is driven by habitat availability. It is possible that density-dependent dispersal from the larger *T. cristatus* population in the west is responsible for the observed pattern in this species, but the opposite pattern in *L. vulgaris* is less easily explained. However, our results are consistent with the importance of winter and breeding habitats on both sides of the mitigation system for intra-population movements (Oldham et al., 2000; Malmgren, 2002; Hartel et al., 2010). Studies from mitigation schemes with unidirectional movement between seasons (breeding ponds on one side of the mitigation scheme, terrestrial, non-breeding habitat on the other) indicated lower, adult-biased numbers of amphibians crossing (Pagnucco et al, 2012; Allaback and Laads, 2003). In order to provide adequate connectivity over sub-populations over time, tunnels should facilitate movements of amphibians in both directions and for both adults and juveniles. Nevertheless, the dynamics of *T. cristatus* sub-populations, including adult survival, are driven mainly by juvenile dispersal, and effective recruitment can increase the probability of successful breeding (Griffiths et al., 2010). In this case, the larger population can be considered the source and the mitigation measure may play an important role in maintaining population viability.

3.5 Implications for conservation and conclusions

Understanding how functional connectivity and population movements are influenced by road mitigation infrastructure could underpin the development of improved mitigation schemes.

The very low adult tunnel crossing rate by newts in spring raises fundamental questions about how such mitigation systems should be implemented for newt species. Road tunnels for newts may maintain landscape connectivity through facilitating autumn dispersal but whether or not it supports spring migration to breeding sites where a road separates terrestrial and aquatic habitat remains unclear.

Although road mitigation projects can be focused on single species, as in this case for *T. cristatus*, wider species impact monitoring would be required for a better understanding of the mitigation impacts. This should include potential predators, competitors, other protected species or pest species.

We observed considerable annual variation in captures and successful crossing rates, highlighting the need for long-term monitoring both to assess the effectiveness of individual mitigation schemes in maintaining connectivity. The 5 years of monitoring carried out in our study, and which are typically required in the UK, are probably an absolute minimum to adequately do this. Moreover, our results underline the value of improving the evaluation of terrestrial movements for newt species in order to successfully mitigate the negative population impacts of road networks.

3.6 Attachments

Table 3.S1 – *T. cristatus* and *L. vulgaris* annual captures and percentage by age, sex and season in the mitigation system. March-May ('Spring'), June-August ('Summer') and September-October ('Autumn').

Years		**Age**		**Sex**		**Mitigation system (% Tunnel/Fence; Side)**						**Season**		
***Triturus cristatus* (Great crested newt)**														
	N	**Adults**	**Juveniles**	**Males**	**Females**	**ACO**	**North**	**South**	**Fence**	**East**	**West**	**Spring**	**Summer**	**Autumn**
2007	6	6	0	2	4	4	0	2	0	2	4	3	1	2
2008	197	53	144	14	39	7	32	59	99	64	133	42	5	150
2010	209	159	50	31	127	8	29	47	125	93	116	77	6	126
2011	41	21	20	8	13	0	15	5	21	18	23	11	17	13
2012	275	201	74	30	169	0	14	5	256	96	179	122	23	130
Total	728	440	288	85	352	19	90	118	501	273	455	255	52	421
%	100	60.44	39.56	19.45	80.54	2.61	12.36	16.20	68.82	37.5	62.5	35.03	7.14	57.82
***Lissotriton vulgaris* (Smooth newt)**														
	N	**Adults**	**Juveniles**	**Males**	**Females**	**ACO**	**North**	**South**	**Fence**	**East**	**West**	**Spring**	**Summer**	**Autumn**
2007	3	1	0	0	1	0	0	3	0	3	0	0	3	0
2008	36	9	27	3	6	4	13	14	5	14	22	12	3	21
2010	36	8	28	6	1	0	20	6	10	24	12	9	4	23
2011	4	0	4	0	0	0	1	0	3	0	4	0	4	0
2012	16	10	6	6	4	0	1	1	14	13	3	7	0	9
Total	95	28	65	15	12	4	35	24	32	54	41	28	14	53
%	100	30.12	69.89	55.55	44.44	4.21	36.84	25.26	33.68	56.84	43.15	29.47	14.73	55.78

Table 3.S2 - A summary of a generalized mixed linear model analysis of movement index within years of monitoring (MI_s = 144 and MI_d= 96), which was used as a random effect in our model. SD is the standard deviation.

	Seasonality		**Direction**	
Random effects	**Variance**	**SD**	**Variance**	**SD**
Year (2008;2010-2012)	0.1877	0.4332	8.12e-07	9.0e-07
	total=144, year=4		total=96, year=4	

CHAPTER 4

Modelling roads as barriers to landscape connectivity in a threatened pond-breeding amphibian: a network approach

Matos, C., Petrovan S., Wheeler, P. Ward A. (in prep.). Modelling roads as barriers to landscape connectivity in a threatened pond-breeding amphibian: a network approach.

Abstract

Habitat fragmentation affects pond-breeding amphibian populations worldwide where urban expansion combined with the development of linear infrastructure (eg. roads) causes loss of landscape-scale connectivity. Mitigation measures including road tunnels and fence systems have all been implemented in an effort to reduce the impact of development, especially where protected species are concerned. However, these measures are usually applied from a local perspective and in the absence of adequate understanding of their role in maintaining population and habitat connectivity at a landscape scale. I explored how structural and functional connectivity changed when prioritizing pond-breeding amphibian movement corridors. Corridors were modelled at different dispersal scales in a region with a variety of stages of urban and rural development and where linear infrastructure acts as barriers to movement. I used recent regional great crested newt (*Triturus cristatus)* pond survey data from the South-East UK to develop a framework using annual home-range patches (AHR) as terrestrial and wetland units. Then I calculated potential terrestrial dispersal/colonization corridors and investigate linkages between patches. I analysed how landscape components, such as habitat quality, amount and scale of movement would vary in two scenarios representing degrees of road permeability for minor roads. I found significant positive differences for population dispersal corridors were minor roads were permeable, indicating the crucial importance of prioritizing these areas for newt movement at long-term. Adequate regional planning incorporating population surveys and habitat patch modelling can direct conservation and mitigation action where it is most needed by maximising population connectivity at the landscape scale.

Keywords: connectivity, dispersal, graph theory; great crested newt, home-range patches; pond network; road mitigation,

4.1 Introduction

Pond systems suffer from huge development pressure in densely populated regions in Europe including much of the UK, with many amphibian populations translocated or lost as the ponds are drained or destroyed as part of this process (Edgar et al., 2005). Population translocation for pond-breeding amphibians in Europe has varying degrees of success and can significantly alter the population connectivity across the wider landscape (Lewis et al. 2016). However, both conservation and mitigation efforts are often still applied at local, site-scale without properly taking into account the intrinsic significance of the pond network in the landscape and its importance for species movement capacity and long-term conservation (Denöel and Ficetola, 2007).

The great crested newt (*Triturus cristatus*) is a European protected species which has declined substantially in the UK and Europe over recent decades (Langton et al. 2001; Jehle et al. 2011) mainly due to the destruction and degradation of pond habitats. The species remains locally common or even abundant in parts of England and as a consequence is often the subject of mitigation projects including translocations of populations and creation of new habitats. As a pond-breeding amphibian, this species needs to move between aquatic and terrestrial habitats during their annual seasonal migrations but equally, individuals can move between interconnected subsets of different populations over a wider area (Jehle, 2000; Jehle and Artzen, 2000; Jehle et al., 2001; Malmgren, 2001; Gustafson, 2011).

At the individual level, we can consider different spatio-temporal scales for juvenile and adult stages where seasonal migration is performed within the patch twice per year and potential long-distance dispersal between patches can go over two years (Semlitsch, 2008; Sinsch, 2014). Most mobile individuals will shape population processes at the landscape level (Sinsch, 2014). Therefore, inter-patch movements are essential to maintain metapopulation dynamics (Griffiths and Williams, 2001; Griffiths et al., 2010) and movements of *T. cristatus* between ponds ensure colonization of new sites and genetic exchange between populations. As a consequence, understanding the potential for intrapopulation migration and interpopulation dispersal is needed when assessing functional connectivity at landscape scale for this species (Denöel and Ficetola, 2007; Ficetola et al., 2008).

Being a protected species highly dependent on specific environmental conditions and sensitive to landscape changes and human pressure, especially linear infrastructures (Langton et al., 2001; Malmgren, 2001; Gustafson, 2011), *T. cristatus* is regularly the subject of road mitigation schemes involving tunnels and fences (Lewis et al., 2016). These have been especially designed and applied to prevent road-kills and to re-establish connectivity (Matos et al., 2017). Usually, such schemes are implemented with reference only to local amphibian habitat patches (Matos et al., 2017). Consequently, it is questionable whether current road mitigation practices contribute to the long-term conservation of the species (Matos *et al*., *2017*). Road networks development represent high pressure on amphibian populations' persistence especially in urban areas (Hamer and McDonnell, 2008). In the UK, road mitigation gradually became an integrated part of environmental management for *T. cristatus* populations' connectivity in fragmented landscapes (Ward et al., 2015). A landscape-scale approach, which accommodates both life stages and movement patterns is needed when planning mitigation efforts and habitat management for this species (Denöel et al., 2013).

Pond-breeding amphibians' persistence in urban and suburban environments will depend on their life history traits and landscape attributes to sustain their populations (Hamer and MacDonnel, 2008). *T. cristatus* seasonal migration (adult annual migration and intra-population movements) and dispersal (juvenile inter-population movements) in the landscape occur in different spatial and temporal scales (e.g. Jehle, 2000; Jehle and Artzen, 2000). Terrestrial habitat use during dispersal events is greater when compared to spring migration from the hibernation sites (Jehle, 2000). Therefore, when analysing corridors for this species, the area of terrestrial habitat will vary between habitat patches. Consequently, home-range areas and dispersal corridors may be dependable on habitat availability around the pond (Hamer and MacDonnel, 2008). Landscape connectivity for *T. cristatus* in urban areas would then be highly dependent on habitat availability, habitat quality and species seasonal responses (Hamer and MacDonnel, 2008).

Network (graph-theory) analysis can be used to understand and prioritize conservation efforts for amphibian patch networks (Ribeiro et al., 2011; Decout et al., 2012; Clauzel et al., 2014). Graph-theory provides a straight forward solution to calculate levels of connectivity in the landscape. Results incorporate and combine habitat patch dynamics, distribution, suitability and a bibliographic analysis on species movement highly dependable on landscape permeability. For *T. cristatus*, a graph theoretical approach allowed to re-focus the corridor results at appropriate spatial scales depending on the life cycle phase and specific seasonal movement of the species. In this way this analysis aims to prioritize patches that contributed substantially to interpopulation dispersal and hence could represent significant locations for road mitigation at local scale to promote long-term viability of *T. cristatus*.

I explored how structural and functional connectivity changed when prioritizing pond-breeding amphibian movement corridors at different dispersal scales in a region with a variety of stages of urban and rural development and where linear infrastructures act as barriers to amphibian movement. I first modelled a terrestrial and wetland unit for *T. cristatus* movement which incorporated the potential annual home-range (AHR) of the species. Secondly, I calculated possible terrestrial/colonization corridors by analysing species presence and movement resistance in the landscape using species distribution models (SDM) and least-cost surface modelling. Finally, in the interests of prioritizing conservation efforts, patch importance for maintaining the overall connectivity of the network of corridors previously calculated system was assessed using graph analysis.

4.2 Materials and Methods

Potential annual home-range (AHR) patches for population of newts were defined around each pond in order to create a terrestrial and wetland unit (variable in size and shape) for the population annual movement capacity. Then, these patches were used to calculate potential terrestrial dispersal and colonizing corridors under two scenarios where linear infrastructures acted as barriers - scenario I (SI) and scenario II (SII). High cost values of permeability for all linear infrastructures were attributed in SI and low cost values only for minor roads in SII (see below in *Data analysis*). Species presence and movement resistance in the landscape was analysed using presence/absence species distribution models (SDM) and least-cost surface modelling. Finally, in order to account for the two scales of species movement capacity (400m and 1000m), the importance of each AHR patch for maintaining the overall connectivity of the pond system was calculated using graph analysis.

4.2.1 Study area

The study was conducted around Marston Vale (52º05’37.65N, 0º28’45.77W), Bedfordshire, South East England (**Fig. 4.1**). This is a large area of circa 100 km², of mixed urban, suburban, agricultural and restored brownfield land. The area has been intensively used for over 100 years for extractive industries related to brick making and as a result the landscape is both relatively urbanized and also recovering from a long period of industrial activity. The landscape is crossed by a network of several important linear infrastructure elements, largely oriented north-south, including motorways, dual carriageway roads and minor roads, as well as an active rail track. There are large zones where former brick pits have been flooded and are now colonized by a range of amphibians and other freshwater species.

4.2.2 Field surveys and topographical data

Target areas encompassed all known populations of *T. cristatus* obtained from the local Biodiversity Recording and Monitoring Centre and a recent large scale pond survey project Natura International. Data were collected by Daniel Pier and volunteers and granted to be used in this thesis research.

In total, 149 ponds were surveyed in 2013 and 2014 (**Fig. 4.1**). In 2013, the effort focused on egg searches and Habitat Suitability Index (HSI) assessment to identify extant populations and opportunities for habitat restoration. For selected ponds, where habitat work was planned, further night surveys were undertaken using torches to confirm species presence or absence. Each pond was visited at least twice during the breeding season of 2013 and 2014.

For the study area, we collected information on linear infrastructures, land cover and pond location (**Table 4.1**). Information on roads and railways was obtained from digitized data provided by UK Department for Transport, 17 land cover variables were extracted from Ordnance Survey (2012) (Meridian™ 2 v1.2 Release 2) in raster format at 10 m resolution, and pond spatial information was obtained from Bedfordshire and Luton Biodiversity Recording and Monitoring Centre (**Table 4.1**). This set of variables was used to calculate potential annual home-range patches and as potential predictors of presence/absence of *T. cristatus* within species distribution models (SDM, see below).

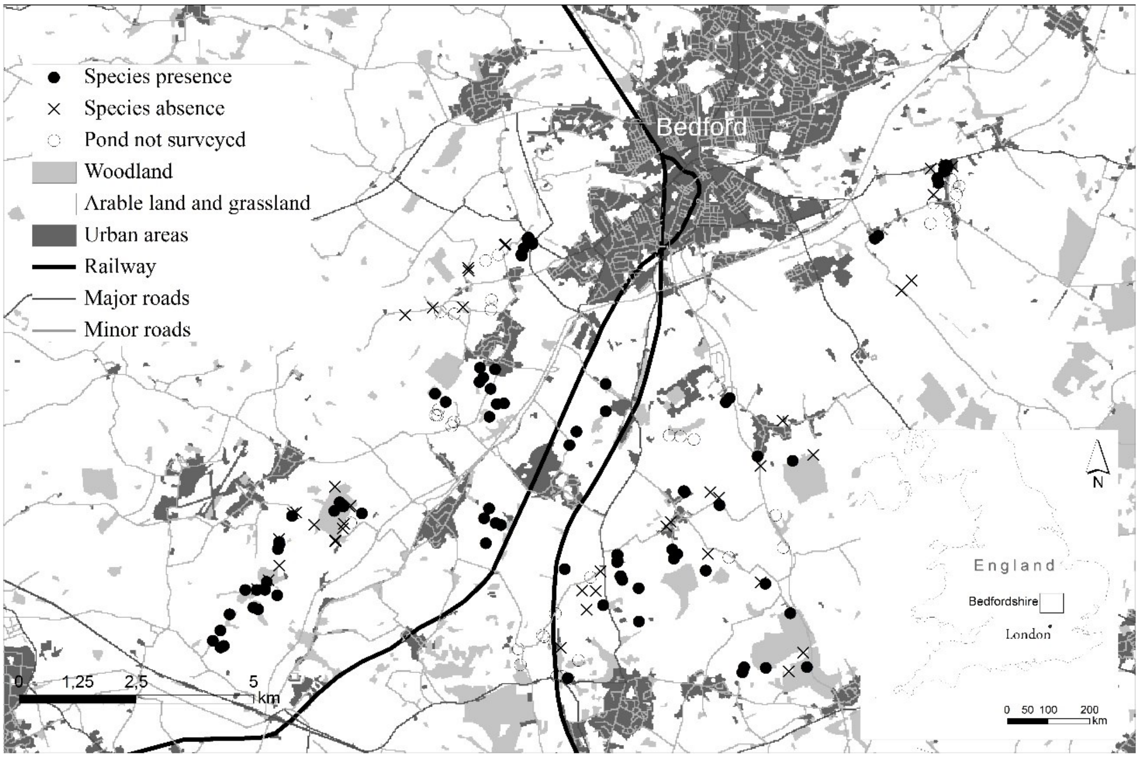


Fig. 4.1 – Map of the study area located in Bedfordshire, England showing the main land-use features and artificial linear infrastructures distribution. Ponds location with results on species presence/absence and ponds not surveyed are also represented.

Table 4.1 - Description and respective code, format, unit and source of variables used to develop the habitat suitability with correspondent cost values for the AHR (annual home-range) patch analysis (cost-surface models).

Variable	Unit	Source	Resistance cost value
Linear Instrastrutures (including railways)*			
Motorway	Presence/absence and distance from	Department for Transport (2012)	4000
Primary	Presence/absence and distance from		4000
A road	Presence/absence and distance from		4000
B road	Presence/absence and distance from		4000
Railway	Presence/absence and distance from		20
Land cover			
Major river	Presence/absence and distance from	Ordnance Survey. (2012). Meridian™ 2 v1.2 Release 2	10
Minor river	Presence/absence and distance from		4
Canal	Presence/absence		10
Unclassified	Presence/absence		4000
Broadleaved, mixed and yew woodland	Presence/absence and distance from		1
Coniferous woodland	Presence/absence		1
Arable and horticulture	Presence/absence		4
Improved grassland	Presence/absence		3
Rough grassland	Presence/absence		3
Neutral grassland	Presence/absence		3
Calcareous grassland	Presence/absence		3
Acid grassland	Presence/absence		3
Heather grassland	Presence/absence		3
Inland rock	Presence/absence		5
Freshwater	Presence/absence		1
Urban	Presence/absence and distance from		4000
Suburban	Presence/absence and distance from		4000
Ponds	Presence/absence and distance from	Bedfordshire and Luton BRMC	1

***Great Britain road numbering scheme.** Motorway, primary and A road: major roads, separate carriageways for the two directions of traffic, separated from each other, either by a dividing strip not intended for traffic, or exceptionally by other means; B roads: minor roads, dual carriageways to single track roads with passing places.

4.2.3 Newt movement data

Quantitative knowledge of *T. cristatus* movement distances at landscape scales is limited and because of the wide species distribution, information on spatial patterns varies substantially between studies from different geographic regions (Hartel et al., 2010; Gustafson et al., 2011; Jehle et al., 2011). For the purpose of this study, the scale values for movement capacity modelling (migration and dispersal) were based on mean estimates from published radio-tracking and translocation studies (Kupfer and Kneitz 2000; Jehle, 2000; Oldham et al., 2000; Malmgren 2002; Gustafson, 2011; Jarvis, 2012). Average movement distances ranged from 30 m to 400 m (migration and dispersal) and up to 1000 m (dispersal only) requiring *T. cristatus* to use a complex of terrestrial habitats interconnected with breeding ponds at various distances (Jehle, 2000; Gustafson, 2011; Jarvis, 2012).

4.2.4 Data analysis

Potential annual home-range patches (AHR)

Positions of all known ponds in the study region, irrespective of whether they had been surveyed or not, were used to calculate potential annual home-range (AHR) patches. Patches were generated on the basis of typical newt annual movements recorded in the literature (Kupfer and Kneitz 2000; Jehle, 2000; Oldham et al., 2000; Malmgren 2002; Gustafson, 2011; Jarvis, 2012). The limits of each patch were defined using least-cost distance analysis radiating out from the central point of each pond polygon on the basis of a 10m resolution friction surface generated by assigning different costs of movement ('resistance') to different habitats (**Table 4.1**). We set the maximum possible movement distance through optimum (i.e. resistance = 1) habitat at 400m with movement through other habitats scaled according to their defined resistance. Impermeable barriers were given resistance values of 4000. Analysis was carried out using ArcGIS 10.x Spatial Analyst (ESRI Inc. USA, 2008).

Species distribution models (SDM) for potential high-quality habitat areas (HQH) considering two barrier scenarios

Two SDMs (SI and SII) were developed in order to first infer on potential high-quality habitat areas (HQH e.g. terrestrial corridors and colonization areas) for *T. cristatus* and then to feed into the least-cost surface models for corridor analysis (see below).

Guisan and Thuiller (2005) define species distribution models (SDM) as “emprirical models relating field observations to environmental predictor variables, based on statistically or theoretically derived response surfaces”. One application that can derive from SDM is the development of a habitat suitability index. Habitat suitability indexes are often used to predict species occurrence through the modelling of appropriate environmental variables (Hirtzel et al., 2006). If empirical data is lacking, or difficult to collect in time-costly effective way or if data comes from a degraded database collection, expert knowledge or literature review can be used to quantify species-environment relationships. In this context, habitat suitability models (HSM) are modelled surfaces where each cell or grid is classified with a quality of habitat value characteristic for a species. Least-cost path analysis is a modelling practice that calculates efficient distances and good pathways based on resistance values that are assigned to cells in a grid (Joly et al., 2003; Baldwin et al., 2007; Jones, 2012). Values of resistance can be high and based on obvious barriers (buildings) or can vary in more subtle gradients (Joly et al., 2003; Baldwin et al., 2007). As a result, in a least-cost path analysis, it is considered that an animal seeks the path with the least cumulative cost for movement from one path to another (Joly et al., 2003; Baldwin et al., 2007).

Habitat cost scores were assigned to each variable in a raster land cover map (10m resolution) and later transformed into probability of habitat selection (habitat suitability) and use for the species (**Table 4.1**). As a result, suitability/cost scores within the SDM were based on the relative preference/cost associated with species movements and life history traits in different habitat types (Oldham et al., 2000; Rohweder et al., 2012). For each factor a weight was assigned in order to define a degree of importance on the basis of *T. cristatus* habitat use and occupancy extracted from the literature (Kupfer and Kneitz 2000; Jehle, 2000; Oldham et al., 2000; Malmgren 2002; Gustafson, 2011; Jarvis, 2012).

Low cost values were attributed to woodland, scrubland and grassland areas, near other forests and wetlands (ponds) (Oldham et al., 2000; Jarvis, 2012). High cost were attributed to urbanized areas, agricultural areas and linear infrastructures. Separately, for scenario I (SI) high cost values were attributed to all the linear infrastructures (all roads types and railway) and for scenario II (SII) only for major roads and railway leaving minor roads with low cost values representing permeability for newts (Oldham et al., 2000; Jarvis, 2012). (**Table S1** and **S2** in supplementary material). Cost scores were calculated using the geometric mean for each landscape factor. These were combined into one SDM for each barrier scenario with cell statistics using ArcGIS 10.x Spatial Analyst.

The final SDM, probability of habitat selection, was calculated by converting cost scores. Cost is the inverse of suitability, such that the highest cost has the lowest suitability. Costs were then reclassified and scaled from 0 to 100 (a cost of 100 translated into suitability 0 and a cost of 0 translated into suitability of 100). High-quality habitat areas (HQH) were described as those areas that had a habitat suitability score of ≥90. This analysis was performed using Corridor Design (Jenness et al., 2007) for ArcGIS 10.x.

Least-cost surface modelling for terrestrial dispersal and colonization corridors

Least-cost corridors were calculated between (1) AHR (those where *T. cristatus* presence was confirmed in field surveys) and HQH (derived from SDM) to create terrestrial dispersal corridors and (2) *T. cristatus* AHR with presence and AHR with absence or presence not confirmed (not surveyed) to generate potential colonization areas through habitat stepping-stones. I considered not surveyed ponds to have no breeding newts in order to model a worst-case scenario.

HQH areas and the suitability grid (SDM) were used in the calculation of the least-cost corridor model (**Table 4.S1** and 4**.S2** in supplementary material). By combining the aquatic and terrestrial sources of movement (AHR and HQH) and habitat suitability grid for two scenarios (SI and SII), two cost-distance surface maps were produced. Results estimated cumulative costs associated with movements between aquatic and terrestrial patches (simulating spring migration and dispersal) for this species. The cost-distance surface was reclassified into permeability grids using Corridor Design (Jenness et al., 2007) for ArcGIS 10.x. Cost-distance surface was reclassified into permeability grids, areas with the lowest 10% of cost-distance values were selected as these represent areas with high permeability of movement, approximately 90% of permeability (where there is the most efficient travel corridor) (Rohweder et al., 2012).

Ultimately resulting corridors were then overlapped with AHR patches and HQH areas to determine: (1) HQH areas and AHR patches per corridor (2) HQH areas and core area extent and (3) colonization corridors extent for each barrier scenario. Cost distance and the least-cost path were calculated using the “Create corridor model” tool from the Corridor Designer Toolbox in ArcGIS 10.1 (Jenness et al., 2007; Rohweder et al., 2012).

Spatial graph connectivity analysis for AHR patch prioritization

In graph theory, when applied to landscape ecology a graph is composed of a set of nodes (patches) and links (edges): nodes are the individual components within the network and links represent connectivity between nodes (Minor and Urban, 2008). Links can be binary or contain additional information about the level of connectivity (flux of individuals moving between nodes) (Minor and Urban, 2008). In our study, the landscape network was represented by AHR patches connected by dispersing individuals.

Graph-theory analysis was performed in three steps: (1) AHR patches were modelled as nodes. These patches were represented by the annual mean dispersal distance with cost distance using a friction map obtained from AHR analysis; (2) patches attribute was assessed based on the habitat suitability grid obtained from analysis II; (3) the connectivity index and number of links per AHR patch was calculated using graph analysis to prioritize most important patches within the pond network for conservation efforts.

To identify the most important AHR patches (presence) for connectivity, the Integral Index of Connectivity (IIC) was used (Pascual-Hortal and Saura, 2006). This index is based on patch attributes (calculated for each patch) and weighted links (least-cost path distance). The IIC index is a measure of patch permeability within the landscape matrix since it incorporates the habitat suitability in the patch and presence of links between patches into a single value. Eliminating each patch sequentially and assessing IIC gives a score (dIIC) for relative importance to landscape connectivity of each individual patch in a landscape.

We simulated dIIC calculations for two barrier scenarios using the permeability grids previously calculated in analysis III. Two scales were used as minimum and maximum thresholds for Newt Movement in spring and autumn (400m and 1000m, see *Newt Movement data* Methods section). The probability of occurrence calculated in the habitat suitability analysis (terrestrial corridors for *T. cristatus*) was used to classify individual annual home-range patches (only presence). For this, each patch was given a single habitat suitability score equal to the mean of all cells in that patch. Inter-patch links were analysed for individual patches using least-cost path distance, calculations were made from the centre of the AHR patch. If within a cluster a priority patch was identified this cluster holds the patch whose disappearance would have a stronger effect on the overall patch network connectivity (Minor and Urban, 2008). Following this pattern, medium and low classified patches and their connections can be tested to identify the suitable patches to improve landscape connectivity (Minor and Urban, 2008, Decout et al., 2012). This analysis was performed using Conefor 26 (Saura and Torné, 2009) and MatrixGreen 1.7 (Bodin and Zetterberg, 2012).

4.3 Results

A total of 302 ponds were identified in the study area. Of these, 173 were surveyed. Presence of breeding newts was confirmed in 148 of these; no evidence of newts was found in 154 ponds.

4.3.1 Annual home-range (AHR) as patches

A total of 176 AHR patches around ponds were calculated. Because many ponds were within our estimated movement distance of each other, the number of distinct patches generated was smaller than the number of ponds. Overall, 80 distinct AHR patches where newt presence had been confirmed were generated with a further 96 not surveyed or where surveys had not recorded newts (**Fig. 4.2**).

4.3.2 High-quality habitat areas (HQH) considering two barrier scenarios

The distribution pattern of HQH at the landscape level showed discontinuous areas suitable for presence (e.g. forests) surrounded by agricultural fields (**Fig. 4.3**). For scenario **I** (S**I**), all linear infrastructures were classified as barriers and for scenario **II** (S**II**), minor roads were characterized as permeable (**Fig. 4.3**). From the tenth percentile of the highly suitable areas, 229 patches with high-quality were obtained (**Fig. 4.3**).

Fig. 4.2 – Location of annual home-range (AHR) patches for *T. cristatus* records with (a) presence and (b) absence and ponds not surveyed in the study area. Main artificial linear infrastructures are also represented.

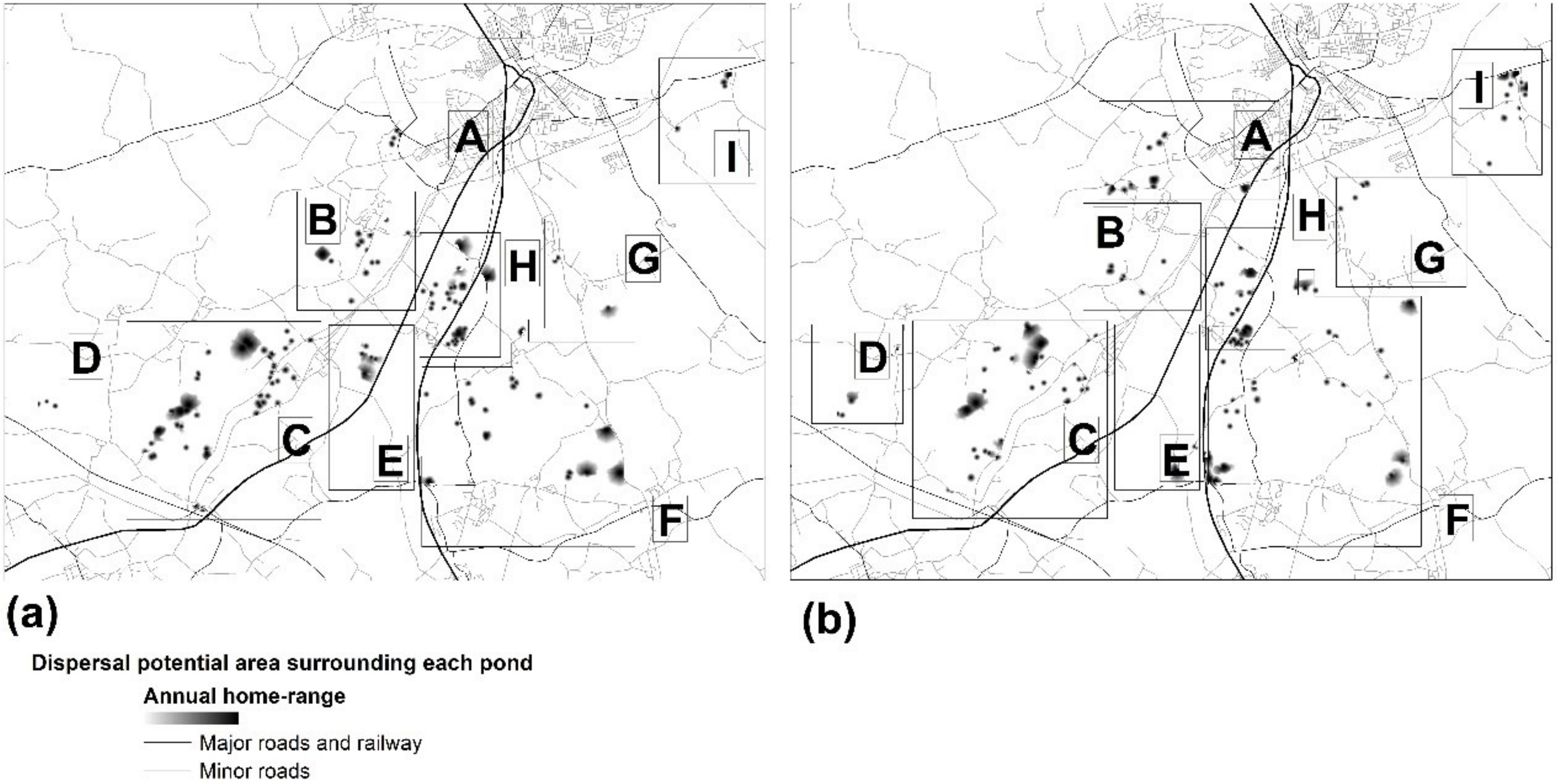
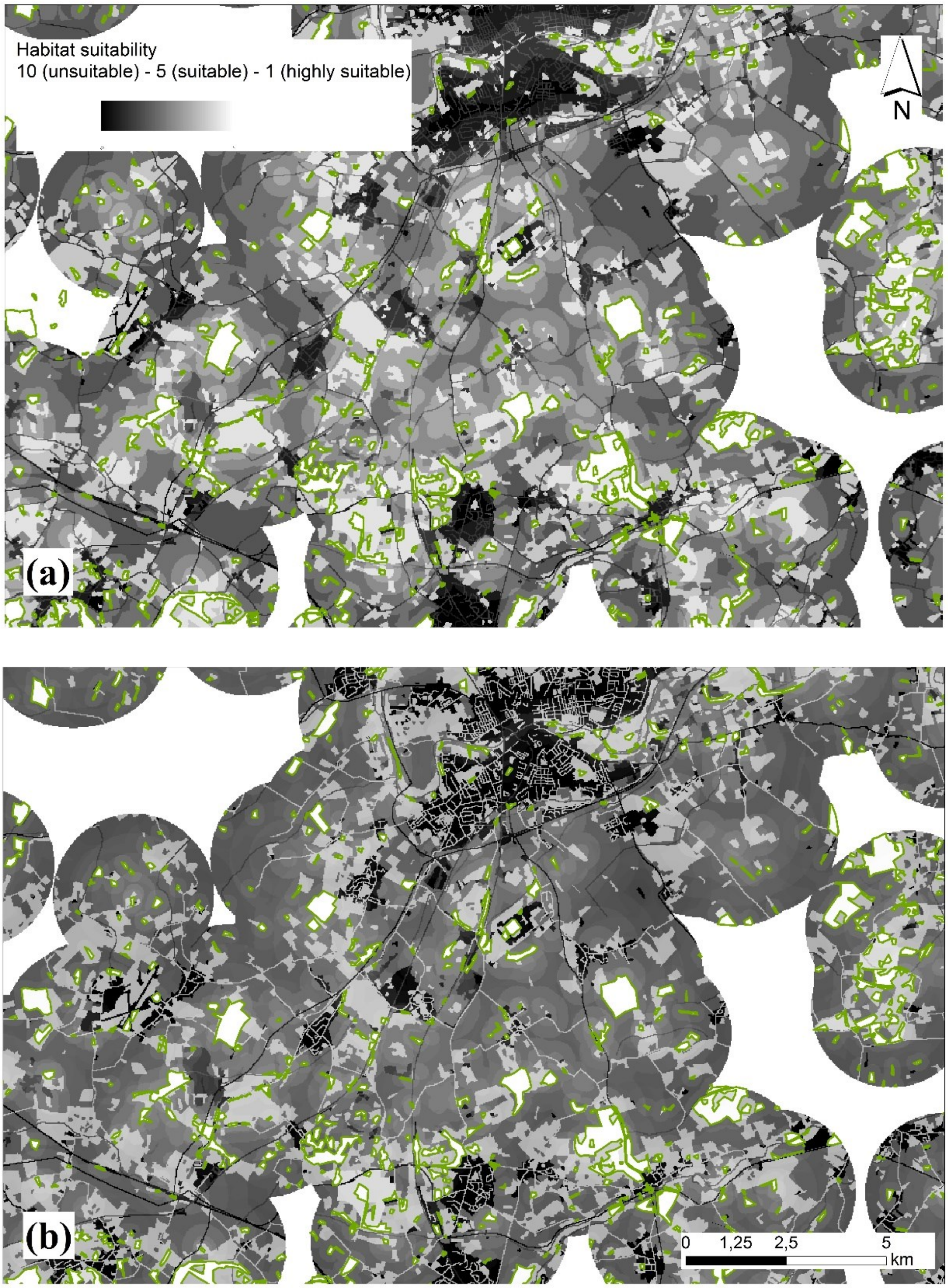


Fig 4.3 – Potential species distribution models (SDM) results for (a) SI (all linear infrastructures as barriers) and (b) SII (minor roads permeable for movement) cost surfaces for *T. cristatus.* Location of high-quality habitat (HQH) patches (>90%) in green. Habitat suitability ranges between 10 (unsuitable areas for movement) and 1 (highly suitable areas for movement).



4.3.3 Terrestrial dispersal corridors and colonization areas

From least-cost surface analysis between HQH areas and AHR patches with presence, nine terrestrial corridor clusters were classified (**Fig. 4.4**). SI showed that of 80 AHR patches, 46 were connected to 69 HQH areas resulting in 25 areas with 90% of permeability (core areas) while 13 AHR patches were unconnected to HQH areas. Clusters with the highest number of patches were C and F, which included 16 and 11 home-range patches, respectively (**Table 4.2**). SII showed substantially better connectivity: of 80 AHR patches, 72 were connected to 151 HQH areas resulting in 19 core areas. All AHR patches were connected to HQH areas for this scenario. Terrestrial corridor clusters with the highest number of core areas were C and F, each one covering 20 and 16 AHR patches, respectively (**Table 4.2**). When comparing both scenarios (SI and SII) for the main structural connectivity variables, core area varied significantly (ANOVA F= 3.804, df=1, p=0.05) (**Fig. 4.4; Fig. 4.S1**).

Large colonization corridors were represented as areas of higher density of absence/not surveyed newt AHR patches in the proximity of AHR patches with presence (**Fig. 4.4**). Colonization areas significantly different when comparing both barrier scenarios (ANOVA F= 6.198, df=1, p=0.02) (**Fig. 4.4; Fig. 4.S1**).

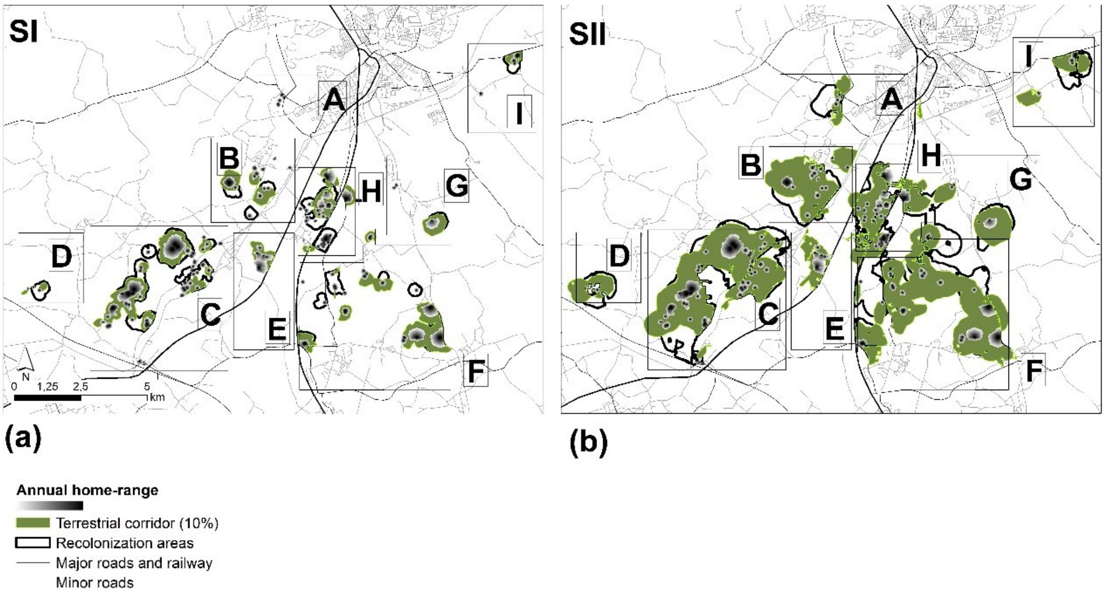


Fig. 4.4 - Terrestrial dispersal corridors (10th percentile of the least-cost path modelling) calculated from (a) SI (all linear infrastructures as barriers) and (b) SII (minor roads permeable for movement) for *T. cristatus*. AHR patches are included in each terrestrial dispersal corridor representing 9 groups of clusters. Potential colonization corridors between AHR patches (presence) and AHR patches (absence/not surveyed) are represented by the black line areas. Main artificial linear infrastructures are also represented.

Table 4.2 - Landscape metrics for AHR patches with GCN presence obtained from the least-cost modelling. For clusters in SI and SII, number of high-quality patches and respective area (km^2), results for each core area between high-quality patches area and AHR.

SI - All roads and railway as barriers								
Cluster	**High-quality patches (>90%)**				**Core area (Cluster)**			
	Patches	**%**	**HQ$_{area}$ (km^2)**	**%**	**AHR (GCN presence)**	**Corridors**	**C$_{area}$ (km^2)**	**%**
A	0	0	0	**0**	0	0	0	**0**
B	6	8.7	0.59	**11.41**	5	3	1.05	**9.7**
C	22	31.88	1.2	**23.21**	16	7	3.73	**34.44**
D	2	2.9	0.15	**2.9**	2	2	0.1	**0.92**
E	4	5.8	0.11	**2.13**	2	1	0.59	**5.45**
F	21	30.43	2.14	**41.39**	11	6	3.22	**29.73**
G	1	1.45	0.68	**13.15**	1	1	0.55	**5.08**
H	11	15.94	0.26	**5.03**	8	4	1.39	**12.83**
I	2	2.9	0.04	**0.77**	1	1	0.2	**1.85**
Total	**69**	**100**	**5.17**	**100**	**46**	**25**	**10.83**	**100**
SII - Permeable minor roads								
A	3	1.99	0.04	**0.34**	3	1	0.69	**1.87**
B	12	7.95	0.66	**5.63**	9	2	4.4	**11.91**
C	48	31.79	6.5	**55.46**	20	3	10.59	**28.67**
D	3	1.99	0.06	**0.51**	3	1	1.07	**2.9**
E	8	5.3	0.16	**1.37**	2	1	1.04	**2.82**
F	48	31.79	2.78	**23.72**	16	3	11.77	**31.86**
G	2	1.32	0.79	**6.74**	2	1	2.06	**5.58**
H	22	14.57	0.6	**5.12**	14	5	4.21	**11.4**
I	5	3.31	0.13	**1.11**	3	2	1.11	**3**
Total	**151**	**100**	**11.72**	**100**	**72**	**19**	**36.94**	**100**

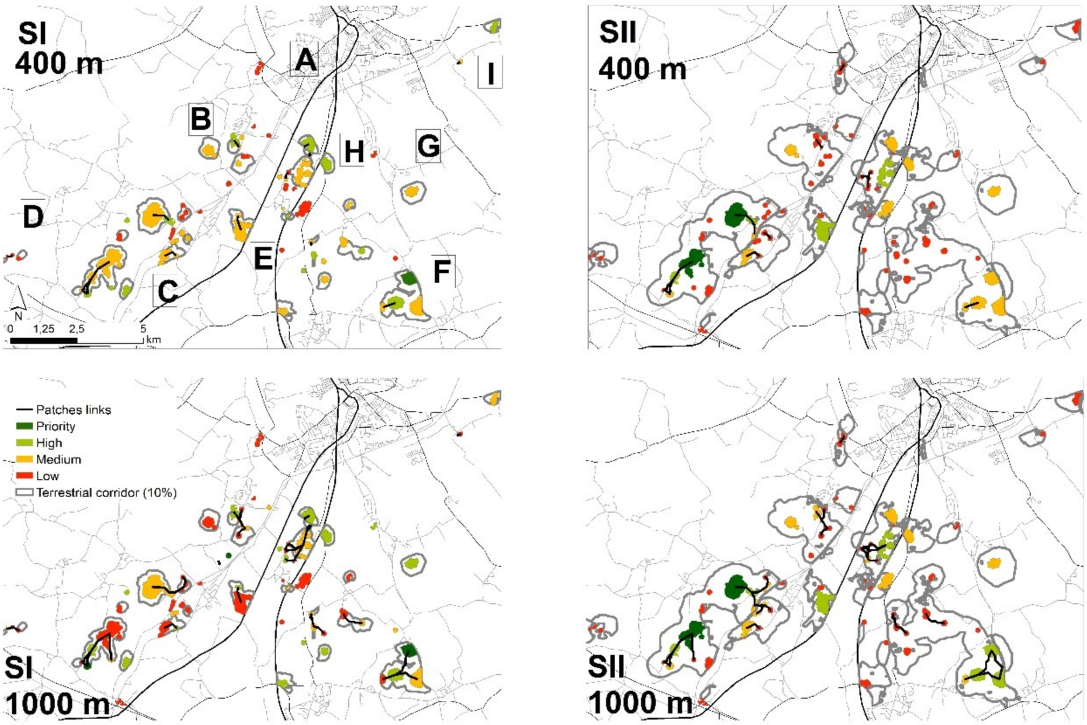
4.3.4 Connectivity index and characterization of the importance of AHR patches

The dIIC index ranked each AHR patch according to their attributes and topological distances. Highest cumulative dIIC values are present in dispersal corridors areas where: (1) there is higher number of patches, (2) patches distances are shorter and (3) when minor roads are permeable (**Fig. 4.5**; **Table 4.3**).

For both 400 and 1000m scale analyses, clusters C, F and H presented the highest cumulative connectivity (sum of the dIIC values for each patch in the cluster), suggesting that the relative importance of these three clusters for overall connectivity is maintained at the broader scale (**Fig. 4.5**). Isolated clusters D and G showed the lowest values of dIIC at both scales, including cluster I at 1000 m. Link analysis registered a high number of links for clusters C and H for both scales and cluster I for 1000m (**Fig. 4.5; Table 4.3**).

SI and SII showed an overall dIIC index mean of 1.49 and 1.80 for 400 and 1000 m, respectively. Within scenarios, dIIC mean improved with larges scales for connectivity (1000 m). Similarly, there was an increase in the sum of dIIC value when considering minor roads permeable (SII) (**Table 4.4**). Highest sum of dIIC was 141 for SII at 1000 m, improving overall network connectivity by 70% when compared to SI at 400m (**Table 4.4**).

a) b)



c) d)

Fig. 4.5 – Graph connectivity analysis results in SI and SII with ranked dIIC index at 400m and 1000 m dispersal scales (distance thresholds) for the 9 groups of AHR patches clusters. dIIC value ranges for each group of AHR patches cluster is available in Table S3 (supplementary material). Terrestrial dispersal corridors are represented with light grey areas for both scenarios.

Table 4.3 - Connectivity index results for patches in SI and SII corridors at two scales (400m and 1000 m) - number of patches, links, priority patches, sum and mean of dIIC index for each cluster.

	Connectivity index - SI corridors									
	dIIC (400 m)					**dIIC (1000 m)**				
	Patches	**Links**	**Priority**	**Sum**	**Mean**	**Nodes**	**Links**	**Priority**	**Sum**	**Mean**
A	0	0	0	0	0	0	0	0	0	0
B	5	1	0	7.31	1.46	5	4	1	9.91	1.98
C	16	6	0	28.23	1.76	16	9	1	32.11	2
D	2	1	0	1.17	0.58	2	2	0	1.85	0.92
E	2	1	0	1.17	1.23	2	1	0	5.27	2.63
F	11	1	1	26.22	2.38	11	4	1	20.76	2.65
G	1	0	0	1.91	1.91	1	0	0	3.58	3.58
H	8	5	0	14.88	1.86	8	10	0	20.76	2.59
I	1	0	0	2.54	2.54	1	0	0	1.79	1.79
Total	46	15	1	83.43	13.72	46	30	3	96.03	18.14
	Connectivity index - SII corridors									
A	3	1	0	0.5	0.16	3	1	0	0.42	0.14
B	9	1	0	3.33	0.37	9	4	0	4.86	0.54
C	20	8	2	73.5	3.67	20	13	2	79	3.95
D	3	0	0	0.07	0.02	3	0	0	0.06	0.02
E	2	0	0	9.01	4.5	2	0	0	4.18	2.09
F	16	1	0	15.05	0.94	16	8	0	33.28	2.08
G	2	0	0	1.56	0.78	2	0	0	1.26	0.63
H	14	3	0	15.34	1.09	14	8	0	17.22	1.23
I	3	0	0	0.62	0.21	3	0	0	0.72	0.24
Total	72	14	2	118.98	11.74	72	34	2	141	10.92

4.4 Discussion

By integrating recent information on *T. cristatus* pond occupancy at the landscape scale and knowledge of movement distance I identified potential terrestrial dispersal corridors under two barrier scenarios. I also incorporated local patch permeability (AHR) and a connectivity index to prioritise areas for improving permeability.

Through the use of AHR patches as units for least-cost modelling, both terrestrial and aquatic habitats were combined in the analysis to identify permeable buffer zones around the breeding ponds. Terrestrial dispersal corridors showed differences between core areas extension for both barrier scenarios and the use of a connectivity index (dIIC) provided a suitable tool to select which is best dispersal corridor in maintaining wider landscape connectivity for future planning at local scale (AHR patch).

4.4.1 AHR patches as units for modelling *T. cristatus* dispersal movements

Terrestrial habitats adjacent to wetland areas are critical for pond-breeding amphibian life cycles (Semlitsch, 1998). The analysis of AHR patches for *T. cristatus* indicated the presence of around 80 terrestrial patches containing both breeding ponds and terrestrial habitat with high landscape permeability at the study site. Patch size varied across the study area landscape, with bigger patches located within woodland areas and near (within 400m) higher numbers of ponds. As buffer size can vary depending on the habitat where the breeding site is located (Semlitsch, 1998; Crawford and Semlitsch, 2007) making terrestrial habitat availability a significant component for permeability between local ponds (Crawford and Semlitsch, 2007).

I also identified distinct clusters of merged AHR where there is high potential habitat suitability. In other studies a similar approach has enabled the identification of areas containing multiple breeding sites that sustain metapopulations (Ray et al., 2002; Joly et al., 2003; Safner et al., 2010). Additionally, it is suggested that these AHR should be considered for conservation priority given that they serve as "stepping stones" in the wider landscape (Decout et al., 2012).

4.4.2 Road barrier scenarios for the assessment of dispersal corridors

Model simulations indicated the most important areas for the species when migrating to and from hibernation sites or when dispersing to high-quality habitat areas. Here we calculated the landscape-based structural and functional connectivity by incorporating both landscape metrics and specific-species behaviour (Kindlmann and Burel, 2008; Decout et al., 2012).

Testing two scenarios with a 10% of threshold permitted to determine in what way two levels of habitat fragmentation influence landscape connectivity for the species at long-term. Core area showed to increase if minor roads are consider permeable in the landscape. Core area is the extent of movements on land between AHR and high-quality habitat patches (dispersal at long-term). This demonstrates how dispersal abilities across the landscape for the species can increase if terrestrial corridors are adapted at regional level.

For the UK, identification of high-quality habitat for *T. cristatus* management purposes has been limited to local assessments of ponds with little attention paid to terrestrial corridors between ponds (Wilkinson and Arnell, 2012; Arnell and Wilkinson, 2013). Pond density has been assumed as the basic measure of connectivity at the regional scale connectivity without actively taking account of seasonal migration over land by *T. cristatus* (Arnell and Wilkinson, 2013). Within population networks, species survival is maintained when patches are connected by dispersing individuals (Fahrig and Merriam, 1985). For pond-breeding amphibians, emigration from ponds patches is frequently in the direction of high-quality patches that include forests and scrubland (Denöel and Lehmann, 2006). Nevertheless, the importance of nearby ponds for *T. cristatus* is consistent with maintaining sufficient connectivity with other breeding ponds already connected by terrestrial habitat (Harper et al., 2008).

The probability of regional persistence and the decrease of isolation is enhanced by opportunities for recolonization of vacant patches by individuals (Harper et al., 2008). In heterogeneous environments dispersal is highly dependent on the spatial scale, where populations that are closer will receive more individuals than populations that are further away (Baguette et al., 2012). Therefore, identify dispersal corridors indicate where possible movement is facilitated and also new areas for potential population settlements.

Where a newt populations are likely isolated from the surrounding patches were also identified. These areas can represent challenges for regional planning and conservation. Occupied isolated patches may benefit from improvement of connectivity between other ponds or even the addition of new ponds to existing patches (the “rescue effect”, Karlsson et al., 2007). However, in the case of *T. cristatus*, population persistence can be higher when small ponds are interconnected with a single isolated large pond (Griffiths, 2004). Other studies, suggest that population abundance of other species of newts may decrease with pond density (Denöel and Lehmann, 2006). In the end, at the landscape level, the number of ponds combined with enough suitable terrestrial habitat for dispersal at long-term may offer the best condition for pond-breeding amphibians’ population maintenance (Denöel and Lehmann, 2006).

I also analysed both types of dispersal corridors in two different fragmentation scenarios. SI (low road permeability) simulations predicted 50% of AHR patches were connected to HQH areas, and 90% were connected for the SII scenario (higher road permeability). Here, I specifically tested the impact of minor roads for *T. cristatus* connectivity intra-population movements. For amphibians, minor roads present the highest rates of mortality and usually in these traffic-calmed areas individuals barrier effect decreases (Sillero, 2008; Langevelde et al., 2008). Therefore, from the planning perspective, these results showed that both major and minor roads effects can be included when analysing regional corridors, and it can also display the local impacts of roads for *T. cristatus* movements.

Resistance within the landscape matrix is one of the three main factors stated in this study affecting movement behaviour of this species (Kindlmann and Burel, 2008). Results showed that when increasing connectivity at the landscape scale, mitigation efforts can be predictably planned to create new regional corridors between habitat patches. Results showed larger local patches ensuring and withstand both adequate dispersal and colonization processes in longer terrestrial corridors. From a planning perspective, creating and enhancing high-quality habitat in areas where newts are present can secure and maintain local populations (Baguette et al., 2012), however new habitat in colonization areas where the species is not detected or is abcent will likely benefit the long-distance dispersal of individuals by providing stepping-stone habitats (Baguette et al., 2012).

4.4.3 Patch prioritization as an operational tool for conservation

The dIIC index identified important patches by measuring the topology within the network (number of links) as a structural attribute and taking habitat suitability into account as a functional attribute (Pascual-Hortal and Saura, 2006; Pereira et al., 2011). This allowed the identification of priority terrestrial corridors for newt long-term dispersal in heterogeneous landscapes.

I identified differences in spatial ranking for AHR patches at two scales under two barrier scenarios. This approached showed which dispersal corridors would maintain the most valuable patches for inter-population movements within the network at two dispersal scales (400 and 1000 m). High quality AHR patches located near each other in areas with low degree of fragmentation are positioned in higher ranking within the terrestrial corridors. For pond-breeding amphibians, the network approach supports the metapopulation theory as it comprises the terrestrial habitat surround the pond and the potential extended mobility of individuals ("dispersers") between ponds (Marsh and Trenham 2001; Fortuna et al., 2006). Therefore, AHR prioritization as a group within dispersal corridors can be used as a more meaningful method for overall landscape connectivity conservation than using individual ponds (Fortuna et al., 2006).

AHR patches importance changed with both scale and barrier scenarios showing the high variability of management options when considering a regional-scale pond network. SII with dispersal at 1000m is the considered optimal design for *T. cristatus* at regional scale – where corridors are prioritized when minor roads are highly permeable. These results showed that by changing dispersal distances and adding permeable roads the overall connectivity of the network for *T. cristatus* would improve. However, the network connectivity and gene flow between sub-populations is dependent on the stability of local conditions for dispersal to take place (Schön et al., 2011). Permeability at the regional scale is dependent on the local home-range habitat conditions and the possibility of individuals' choice to search for new areas and initiate the dispersal process (Doerr and Doerr, et al., 2004). Information on local patches that would benefit from implementing minor road mitigation showed to be valuable for this species movement predations at long-term.

Graph-theory analysis revealed to be a useful tool to understand the effects of roads for management of *T. cristatus* dispersal corridors. It enables the analysis of best and most important locations where potential movements can be restored and maintained. At the same time it incorporates both structural and functional connectivity into a network structure approach by using information on life cycle traits and real landscape features. This is translated into an optimal spatial representation of a complex model that can be metapopulation dynamics at regional-level in heterogeneous landscapes (Fortuna et al., 2006).

4.5 Habitat management and conclusions

The integrative approach used here showed how corridor assessment techniques is helpful to the evaluation of structural and functional connectivity analysis at the landscape scale when joined together with species presence records, behaviour and ecology. I was able to produce spatial representation which indicated the potential terrestrial corridors where long-term dispersal and consequently long-term connectivity would be beneficial.

Despite the coarse resolution of input data (presence/absence and not-surveyed pond locations) I derived projection scenarios for the visualization of connected and isolated habitat patches for *T. cristatus*. Results clearly indicated a variety of corridors that can be regarded as planning and design priorities for road mitigation efforts. Especially directed to local patches where ecological conservation projects are needed and are carefully considered.

However, ecological uncertainties at the population level and local environment are inherent to the modelling process and need to be considered: (1) stochastic effects and temporal scales were not included in our models, (2) changes in land use after our data were collected may modify the overall network and hence conclusions regarding the relative importance of habitat patches; and (3) terrestrial corridors and the connectivity index were specifically calculated for the patch network at our study site. If new ponds are added or removed the pond network will change with consequent change to the relative importance of patches and corridors. Due to these uncertainties it is not appropriate to conclude that that the worst connected or least important habitat patches at the study site could or should be lost. All patches likely incorporate some benefits for *T. cristatus* at the landscape scale.

Finally, I suggest the incorporation of this protocol as a preliminary assessment of the conservation status and connectivity degree at regional-level for the species (Neel et al., 2014). Once population abundances and movement patterns are not accessible to complement further analysis, the quantitate aspect of this method ensures a well-established aim and measureable standard when data are limited.

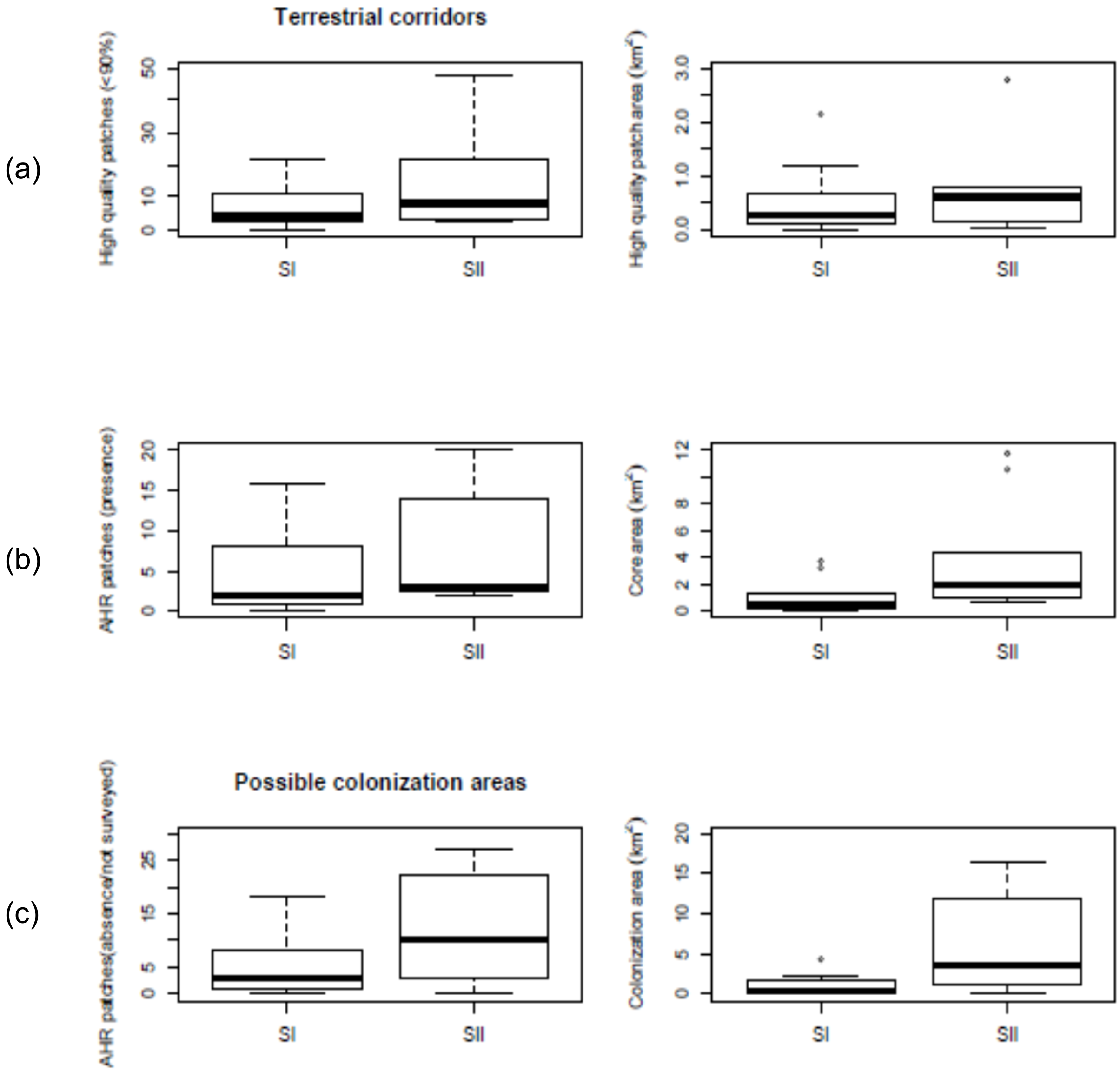


Fig. 4.S1 - Boxplots showing differences between the two barrier scenarios (SI and SII) for each AHR patches cluster (n=9). We compared (a) HQH areas and AHR patch numbers; (b) HQH area and core area extension (km^2); (c) AHM patches number and colonization area extension (km^2);

Table 4.S1 - Habitat-suitability scores attributed to land cover included into building the habitat-suitability model. In scoring land cover, 1-3: strongly preferred (1 being best); 4-5: usable but suboptimal habitat; 6-7: not breeding habitat, but perhaps occasionally used; 8-10: strongly avoided (with 10 being worst) (Jenness et al., 2007).

Land cover	**Value**	**:**	**Cost**
Unclassified	0	:	10
Broadleaved, mixed and yew wodland	1	:	1
Coniferous woodland	2	:	2
Arable and horticulture	3	:	6
Improved grassland	4	:	3
Rough grassland	5	:	3
Neutral grassland	6	:	3
Calcareous grassland	7	:	4
Acid grasssland	8	:	4
Heather grassland	11	:	4
Inland rock	14	:	8
Freshwater	16	:	3
Urban	22	:	10
Suburban	23	:	10
Railway	24	:	8
Motorway	25	:	10
Primary road	26	:	10
A road	27	:	10
B road	28	:	10
Minor road	29	:	8
Canal	30	:	7
Main river	31	:	7
Minor river	32	:	7

Table 4.S2 - Habitat-suitability scores attributed to distances from woodland, ponds and urban areas introduced to build the habitat-suitability model. All variables comprise distances from 0 to 2000 meters. Scoring distances from 0-200; 200-500; 500-1000 and 1500-2000: 1-3: strongly preferred (1 being best); 4-5: usable but suboptimal habitat; 6-7: not breeding habitat, but perhaps occasionally used; 8-10: strongly avoided (with 10 being worst) (Jenness et al., 2007).

	Lower	**Upper**	**Use value**
Distance from forests (meters)	0	200	3
	200	500	3
	500	1000	4
	1000	1500	4
	1500	2000	6
Distance from lakes (meters)	0	200	1
	200	500	2
	500	1000	4
	1000	1500	6
	1500	2000	8
Distance from urban areas (meters)	0	200	10
	200	500	9
	500	1000	7
	1000	1500	5
	1500	2000	3

Table 4.S3 – Results for the patches connectivity index analysis. dIIC value rank calculated for both scenarios (SI and SII) at two thresholds for species dispersal (400 and 1000 m).

		dIIC intervals	
Scenario	**Category**	**400 m**	**1000m**
SI	Priority	6.9 - 4.3	10.93 - 5.3
	High	4.31 - 2.0	5.31 - 2.9
	Medium	2.01 - 1.0	2.91 - 1.3
	Low	1.01 - 0.18	1.31 - 0.13
SII	Priority	25.06 - 9.9	21.56 - 10.7
	High	9.91 - 4.7	10.71 - 4.3
	Medium	4.71 - 0.9	4.3 - 1.0
	Low	0.91 - 0.001	1.01 - 0.001

CHAPTER 5

Short-term movements and behaviour of *Triturus cristatus* in a road mitigation system

Matos, C., Petrovan S., Wheeler, P. Ward A. (submitted). Short-term movements and behaviour govern road mitigation usage for a protected amphibian, the great crested newt. *Animal Conservation*

Abstract

Road mitigation tools for pond-breeding amphibians are strategically placed to link important habitat patches to restore and maintain landscape connectivity. Monitoring studies aiming to evaluate mitigation effectiveness usually report changes in amphibian behaviour when individuals encounter a tunnel or a fence. Reluctance of individuals to enter tunnels and re-orientation away from tunnels while traveling along fences are responses determining if a complete crossing can be successful. However, it is still unclear how spatial configuration of the road mitigation system is linked to behaviours and how this affects species dispersal' patterns. Quantitative information of fine-scale movement patterns in response to road mitigation structures could inform improved road planning and deployment of road mitigation. I aimed to quantify behavioural responses of *T. cristatus* to a road mitigation system located near Peterborough, UK. I used fluorescent paint to mark individuals in order to measure distance travelled and trajectory orientation in two seasons. I also assessed frequency of use and immovability of individuals in three parts of the systems (fences, tunnel entrances and inside the tunnels) and the number of newts that moved towards the surrounded environment. From 38 survey nights (24 in autumn and 14 in spring) a total of 250 juveniles and 137 adults were marked and tracked. More than 70% of newts found the mitigation system moved during the night, with greater activity along the fence during autumn (82%). Newts moved short distances per night (3.21 m.night^{-1} in spring and 6.72 m.night^{-1} in autumn) with a maximum of 25.6m recorded by an adult inside a tunnel. Straighter paths were recorded in spring with trajectory orientation changes being dependent on the position in the system. Paths were straighter inside the tunnel compared to the entrance. Season and position in the system were associated with whether a newt successfully crossed the road using the tunnel. Dispersal during autumn influenced responses from newts to road mitigation. Data on quantitative use are essential to develop new behavioural models. These would assist the progress on the definition of goals for monitoring effectiveness of tunnels across regional-scales.

Keywords: connectivity, dispersal, great crested newt, individual behaviour, migration, underpasses.

5.1 Introduction

Habitat loss and consequent fragmentation represent a major threat to amphibian populations (Cushman, 2006). Road construction in particular reduces habitat availability and increases distance between habitat patches that are crucial for seasonal movements and maintenance of population dynamics (Fahrig et al., 1994; Hels and Buchwald, 2001). Moreover, high amphibian mortality rates on roads and road avoidance behaviour compound the consequences of these impacts leading to worldwide concern about the potential effects of roads on amphibian populations' viability, and how to reverse it (Fahrig et al., 1994; Jaeger et al., 2005; Glista et al., 2007).

In the UK, mitigation measures such as tunnels and fences have been implemented to guide amphibians between habitat patches in order to maintain landscape-scale connectivity between populations (Beebee, 2013). Knowledge of terrestrial movement patterns is typically used to guide decision on the locations for road mitigation (Clevenger and Waltho, 2005). The great crested newt (*Triturus cristatus*) is one of the target species (Ward et al. 2015), as landscape permeability is essential for dispersal and migration movements (Halley et al., 1996; Semlitsch, 2008; Griffiths et al., 2010). After implementation of mitigation measures, newts can be particularly active around tunnels and fences, especially after emigration from ponds to hibernation and refuge sites during autumn (adults and juveniles) and during spring migration when adults move back to the ponds to reproduce (Matos et al., 2017).

The extent of studies on amphibians suggest changes in movement patterns in fences and tunnels and present differences for adaptive behaviour for different species (Jackson and Tyning, 1989; Allaback and Laads, 2003; Pagnucco et al., 2011). Post-mitigation monitoring often inadequately implies mitigation effectiveness without taking into account the behaviour of target species at different points along the mitigation system (Woltz et al., 2008, Pagnucco et al., 2012). For example, no evidence is available explaining whether pond-breeding amphibians can inhabit the system or whether they simply use them for transit (Langton, 1989; Hamer et al., 2015). Similarly, it remains unclear whether amphibian fences actively guide amphibians towards the tunnels or redirect them back into the environment at random. Even when amphibians encounter tunnels, there is some suggestion that they may be reluctant to enter and cross them (Jackson and Tyning, 1989; Allaback and Laads, 2003).

However, quantitative analyses of changes in movement patterns in response to road mitigation (eg. moving towards, away, distances and trajectory orientation) are limited. Finally, accounts describing encounter and transit of each part of the mitigation system by newts during activity peaks is lacking (Schmidt and Zumbach, 2008; Hamer et al., 2015), limiting opportunities for evidence-based improvements. Road mitigation design and deployment could be better informed by quantitative data on individual behaviour of amphibians with respect to different parts of mitigation systems. Knowledge on the type and consequence of movements performed by individuals could also inform individual-based models to predict population-scale movements relative to mitigation systems and thus provide more effective advice on fence and tunnel placement in order to reinstate or maintain landscape connectivity. Individual behaviour among amphibians guides their dispersal success and can influence populations' distribution patterns in the landscape (Baguette and Dyck, 2007, Sinsch, 2014). Newts display a range of behaviours while moving on land (e.g. foraging, searching for refuge, use of underground shelters), and slight changes of direction for each individual can lead to different conclusions on the intent of the movement (i.e. dispersers or residents) (Sinsch, 2014). Traditional techniques for studying terrestrial movements of newts can directly influence their behaviour thus confounding conclusions about individual choices. For instance, radio-tracking has been used to study terrestrial movements of *Triturus* species (Jehle, 2000, Jehle and Arntzen, 2000, Schabetsberger et al., 2004; Jehle et al., 2011) providing information on distance travelled (30 – 400m) and direction of movement after and before the aquatic phase (Jehle, 2000, Jehle and Arntzen, 2000; Schabetsberger et al., 2004). However, transmitters must be internally implanted, swallowed or externally mounted with consequences for behaviour (Schabetsberger et al., 2004). Moreover, the number of newts that can be efficiently followed over a short period of time using this technique is restricted and limited in spatial scale (1-20 m) (Jehle, 2000).

For this study, I used fluorescent pigment to track the short-term movements of *T. cristatus* throughout a road mitigation system. Fluorescent pigments have been proven to be a useful technique in microhabitat studies for amphibians (Eggert, 2002; Ramirez et al., 2012). The extent of daily movements might be over-estimated by home range analysis (Wells, 2007), however fluorescent marking can provide detailed information on daily patterns, even under wet conditions, especially for newts because of their relative short movements (Jehle, 2000).

Although it has been used in combination with transmitters to improve detectability, pigments alone when rapidly applied, seem not to influence behaviour (Eggert, 2002). Once painted and released, newts leave a fluorescent trail that can be observed for several hours whilst avoiding the need for more invasive and disruptive tracking methods. Using this method, a single observer can monitor the movements of hundreds of individuals providing detailed information on trajectory orientation and type of movement displayed by individuals. This method has been tested on a range of amphibians at different life stages including *Bufo bufo* (Eggert, 2002), *Limnodynastes peronii* (Martin and Murray, 2011), adult salamanders (*Plethodon. jordani*; Nishikawa, 1988; *P. cinereus*; Roberts and Liebgold, 2008), juvenile *Ambystoma maculatum* (Pittman et al., 2013; Pittman et al., 2013b) and adult *Notophthalmus viridescens* (Roe and Grayson, 2009) and is considered to be safe and more humane than radio tracking for assessing short-term movements.

This study aimed to investigate the terrestrial movements of individual newts within a road mitigation system. We hypothesized that responses of newts would change as they moved along the system during peaks of activity: (1) on approach towards a road, newts re-orientate themselves to travel along the fence (2) on encountering a tunnel entrance newts turn into the tunnel and cross it (3) responses are likely to vary between seasons and position in the system to account for peaks in movement activity during spring and autumn. For this I measured distance travelled and trajectory orientation of individual newts per night during two field seasons in autumn and spring. Also, I quantified the proportion of newts recaptured and final positions in the system.

5.2 Materials and Methods

5.2.1 Study site

The study was conducted at Orton Pit/Hampton Nature Reserve south of Peterborough, Cambridgeshire, UK (52º 32’24N, 0º16’53W) (**Fig 5.1a**). This site is designated as a Site of Special Scientific Interest, a Special Area of Conservation and as a Natura 2000 site. It covers an area of 145 ha comprising a water-logged section of former industrial brick clay extraction with some woodland and patches of scrub. The main site is characterised by a complex of over 340 ponds, between 15-50 years old. The site is currently home to the largest known single population of great crested newts in the UK and possibly Europe, estimated at around 30,000 individuals as well as a very large population of *Lissotriton vulgaris*, but *Rana temporaria* and *Bufo bufo* have become exceedingly rare (*please see Chapter 3*).

Between 1990 and 2000 a large-scale habitat restoration programme was implemented to protect the newts, including pond modification and fish eradications. However, in 2006 a new housing development was built towards the north of the reserve, serviced by a 10-12m wide road designed to support 1000 to 10 000 vehicles per day. Concrete ‘newt barriers’ were installed adjacent to this road (**Fig. 5.1b**). During road construction some large ponds were re-profiled and some were in-filled to accommodate the road. A mitigation system was installed to facilitate movement of amphibians under the road between the eastern and western parts of the reserve (**Fig. 5.1b**).

The road mitigation system was composed of one 0.5 m diameter, 30 m long polymer concrete ‘amphibian tunnel’ with open slots at the top (ACO Germany) in the centre, two large ARCO concrete and metal sheet underpasses (5.5m wide x 2 m high, 40m long) spaced 100 m apart in the north and south, and two 200 to 300 m long heavy duty plastic fence systems (Herpetosure UK), placed 10-50 m away from the road and angled (~45º) to guide amphibians towards the tunnels. In an effort to minimise human disturbance the entrances of the large tunnels were protected with a bar fence and gate system.

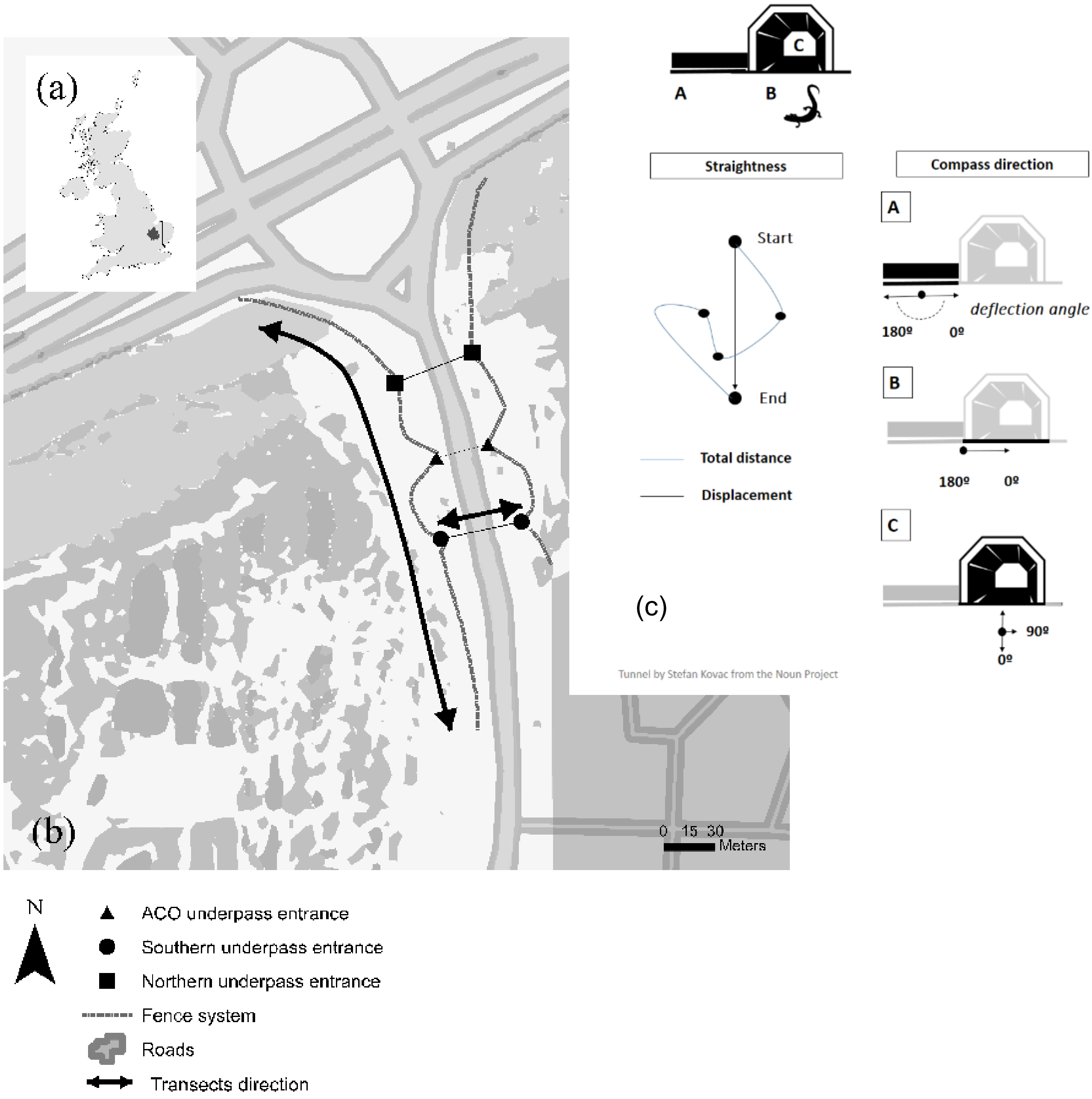


Fig. 5.1 - Study area and field work transects. (a) Location of the study area in Peterborough, UK (b) transects surveyed along the mitigation system (green lines with black arrows indicating direction) with location of fences (east/west sides) and tunnel entrances (symbols) (c) Scheme explaining how straightness and compass direction were measured at three points in the system (A, B and C). Straightness is the ratio between displacement and total distance. Compass direction was measured by calculating the deflection angle. This ranged from 0 to 180 for three positions in the system, with 0 indicating movement directly towards the tunnel and 180 indicating movement directly away from the tunnel. Smaller dots on the pathway diagram represent individuals' change of direction.

5.2.2 Data collection

Newt movement data were collected by me with the help of three volunteers (Mark Goodman, Steven Allain and Gilles Dubois) during two periods of peak activity: autumn (between 17 September and 26 October 2014 covering the period of juvenile dispersal) and spring (between 6 March and 3 April 2015 covering the period of adult migration). For both seasons, night surveys (marking) started 2-3 hours after the sunset and had a mean duration of 2 hours. Morning surveys (recaptures) started 1 hour before sunrise and mean duration depended on the number of newts captured (2-3 hours). Days with favourable weather (rain in the last 3 days and temperatures above 6ºC) were purposively selected to maximise the number of newts monitored (**Table 5.1**).

Newt tracking and capture-mark-recapture

Transects were surveyed for newts along both sides of the fence during each survey with the start point alternating between surveys (A in **Fig. 5.1b**). Newts were also surveyed at tunnel entrances (within 2-3 m radius around tunnel opening: **Fig. 5.1b**) and inside the tunnel (north and south tunnels: C in **Fig. 5.1b**). Only the large tunnels permitted entry to capture individuals and measure behaviour inside the tunnel.

At initial observation, each newt's activity and position in the system (at the fence, tunnel entrance and inside the tunnel) were recorded. The short-movement focus in determining the distance, orientation and microhabitat use of the start and end of movement, as well as register particular types of activity, namely moving, foraging, or any social/reproductive behaviour. The newt was then captured by hand and a photograph was taken of the ventral pattern for future identification. Newts were given a unique identification number, age (adults/juveniles) and sex (female/males) was observed, and a colour was allocated for marking. Marking consisted of dipping newts' tails, hind legs and ventral surface in fluorescent powder (DayGlo Color Corp. fluorescent pigments) and pressing gently to ensure adhesion of sufficient powder. Four different colours (Z-18-3 Green, Z-15-3 Orange, Z-17-N Yellow and Z-11-3 Pink) were used to mark newts in order to differentiate paths within and between surveys. Juveniles were coloured if their position was more than 5m from the nearest adult.

Powder painting is a non-invasive techinique and to minimize stress, animal processing was completed in less than 1 minute. There was no collection of tissues samples or measurement of snout-vent length. Newts were released after colouring at their point of capture and left undisturbed for at least 5 hours. In order to distinguish trails of individuals' whose paths coincided we used different colours to paint different individuals in close proximity and removed pathways from previous nights with a brush or by disturbing the soil before each nights' survey.

Position recording

Newt trails were monitored using an ultraviolet light to detect the fluorescent powder. Trail start/end positions and points at each directional change were temporarily marked with small flags and respective distances were assessed using a tape measure (to the nearest 0.1m). Track trajectory was measured using a compass (using a range of 0-180º) in relation to the position in the system (see below *Data analysis*) Final location was recorded to the nearest 3m using a portable GPS (GPS Essentials 4.4.8, 2015). For newts found inside the tunnel during night surveys, it was not possible to distinguish between those turning back to the entrance from which they came and those successfully exiting on the opposite side. If the path was less than 30-40 cm, zero movement was recorded (similar to Ramirez et al., 2012).

5.2.3 Data analysis

Short-term movements were analysed using data on distance travelled, track trajectory and frequency of movement performed by adults and juvenile newts in three positions along the road mitigation system (fence, tunnel entrance and inside the tunnel) between seasons (autumn and spring).

Movement data: distance travelled and trajectory orientation

Movement data for each newt were analysed as total distance travelled per night (track length) and track trajectory. Track trajectory was summarised as straightness and orientation relative to the mitigation system. Straightness was calculated from the ratio between displacement and total distance travelled (**Fig. 5.1c**). Displacement distance was determined by dividing the straight-line distance between the start to the end of track with total distance travelled (Roe and Grayson, 2009). Straightness is an index that varies between 0-1, values close to 0 indicate a curved route and a value of 1 indicates a straight path. This analysis was used to distinguish between rambling behaviour and directed movements (Roe and Grayson, 2009). Orientation was calculated using the deflection angle at three capture points **(Fig. 5.1c**). When newts were captured in the fence or tunnel entrance angles ranged between 0º and 180º. When captured inside the tunnel angles ranged between 0º and 90º. Inside the tunnel and along the fence, values of 0º indicated newts moving towards the exit/entrance of the tunnel and values of 90º-180º indicated newts moving away from entrance/exit of the tunnel.

Season and location effects

Movement data on adults and juveniles that moved > 40 cm per night were summarised by season (autumn and spring), part of the mitigation system (fence, tunnel entrance and tunnel) and by age. To quantify changes in behaviour along the system calculations were made of (1) the number of newts moving from the three positions in the system (fence, tunnel entrance and inside the tunnel) and final positions (surrounding environment or remaining in the system), and (2) changes in distance travelled and orientation relative to the three positions along the system.

We used a generalized linear mixed model (GLMM) to calculate the effects of season, position in the system and age on movements of newts. Season, position and age were fixed effects position and age were nested within season, which was entered as a random effect. Three null models containing the most significant variables and intercept was included for comparison (season + mitigation + age + (1|season)). From here we tested three models with the most significant variables, with no test for interactions. We compared model parsimony using Akaike information criterion (AIC) to optimize goodness-of-fit but avoid overfitting of the candidate models (Burnham and Anderson, 2002). After selecting the most parsimonious model, we determined the significance of fixed factors by analysis of deviance (Burnham and Anderson, 2002). Models were fitted using a Maximum Likelihood (ML) distribution and lmer function in package lm4 (Bates et al., 2014) in R software (R Core Team, 2016).

To evaluate minimum long-term movements between seasons and positions in the system we used ventral patterns to identify recaptured adults. Recaptures were identified automatically from photographs using I3S software (pattern software) (Hartog and Reijns, 2014). Recapture rates were calculated per season and position in the system and between initial and final positions.

5.3 Results

From a total of 38 surveys, 24 were during autumn and 14 during spring. A total of 387 newts were caught and coloured (280 and 107 per season, respectively) from these 250 were juveniles and 137 were adult newts (73 females and 64 males) (**Table 5.1**).

Among the 270 (70%) newts that moved >40 cm, a higher percentage moved in autumn (82%) than in spring (18%, **Table 5.1**). Most movements were performed along the fences during autumn (58%) with no newts found inside the tunnels in spring. Final positions were mainly recorded in the mitigation system (215 newts, 80%) while 55 (20%) were found moving in the direction of the surrounding environment (**Table 5.1**).

5.3.1 Final positions and seasonal movements

For both seasons combined, 3% of newts moving along the fence encountered the tunnel, 25% moved to the surrounding environment and approximately 70% remained at the fence. When captured at the tunnel entrance, a high percentage of newts moved towards the fence (70%), 11% of newts moved in the direction of the surrounding environment and 20% stayed at the entrance or entered the tunnel. A high percentage of newts captured inside the tunnel remained inside or at the entrance (80%) while 20% found the tunnel exit towards the surrounding environment (4%) or stayed at the fence (18%) on the opposite side of the road (**Fig. 5.2**).

The adult recapture rate was not significantly different between seasons, averaging at 9.7% (**Table 5.2**). Number of recaptures were borderline significantly different between locations in the system ($\chi^2= 5.94$, df = 2, P = 0.051). Most recaptured newts were located around the fence (11%) and 3% were captured in the tunnel (entrance + inside). From total number of captured newts, the majority of recaptures was at the fence (4%), followed by a small percentage in the tunnels (1%) and surrounding environment (0.5%).

Table 5.1 – 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	
		Fence		Tunnel		Move		Mean distance (m)		Mean straightness (0-1)		Mean angle (o)		Mitigation	Habitat	Mitigation	Habitat
65	12	13	11	3	1	14	1	3,01	0,40	0,68	0,16	113,99	16,19	19	35	2	1
38	34	4	33	5	1	9	7	1,81	1,30	0,55	0,33	49,66	31,06	10	23	11	1
37	8	9	8	2	0	8	0	3,40	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	58,40	68,33	15	23	11	1
44	0	12	0	4	0	13	0	4,48	0,00	0,54	0,00	114,81	0,00	17	17	0	0
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	
		Fence		Tunnel		Move		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

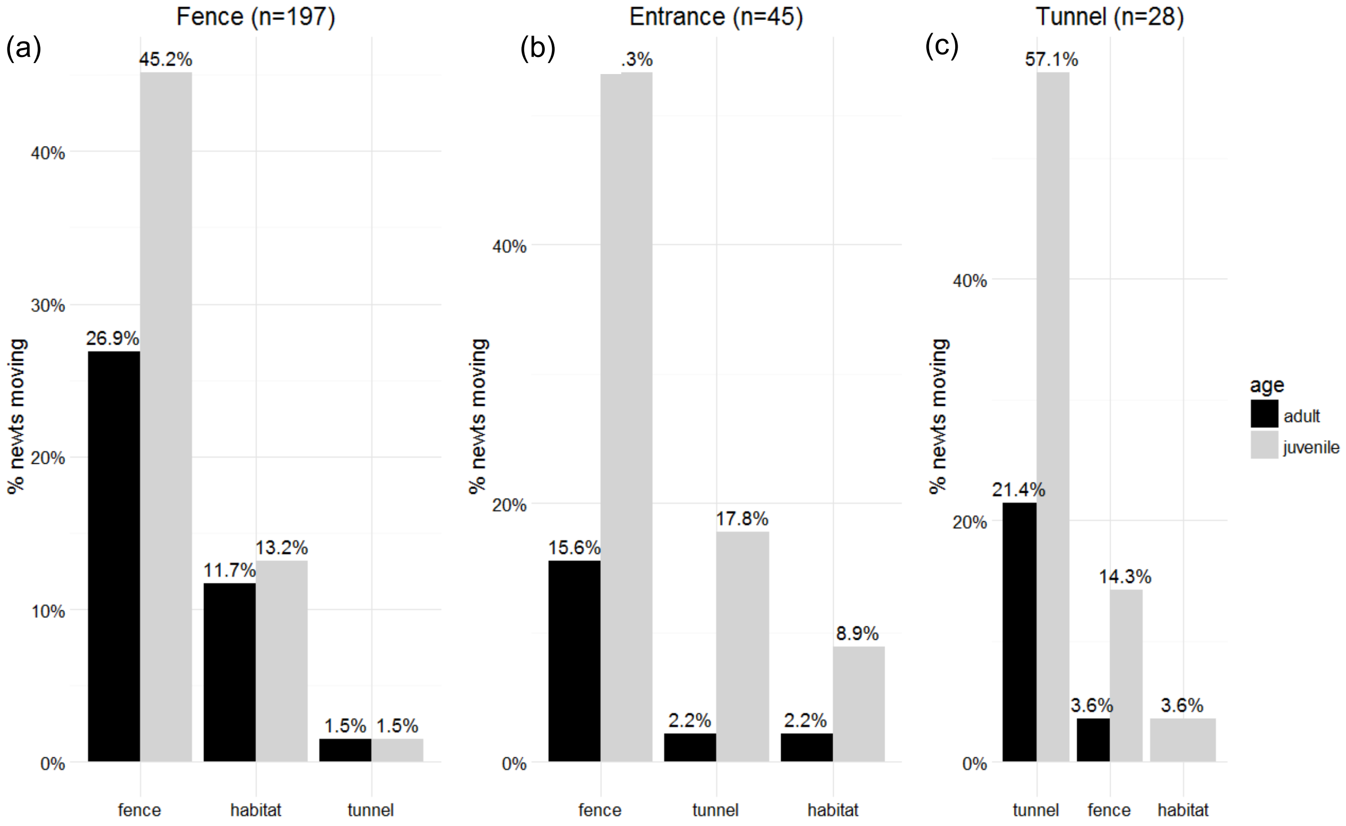
Table 5.2 – Movements of adult and juvenile *T. cristatus* coloured with fluorescent powder in autumn 2014 and spring 2015. Number of individuals that moved for each week (6 weeks/survey) per season, maximum (max.), minimum (min.) and median values values for measured behavioural traits - distance travelled (m), straightness (0-1) and orientation per night (0-180º).

Descriptive stats	**Season**							
	Autumn (24 days/6weeks))				**Spring (14 days/5 weeks)**			
	N indiv. that moved	**Máx.**	**Min.**	**Median**	**move**	**max**	**min**	**median**
Distance (m)								
females	32	16.4	0.6	2.72	16	6.5	1.0	3.0
males	34	25.6	0.5	2.52	13	7.7	1.5	2.9
juveniles	156	19.4	0.6	3.1	19	5.3	1.3	2.2
Straightness (0-1)								
females	32	1.0	0.0	1.0	16	1.0	0.65	1.0
males	34	1.0	0.33	1.0	13	1.0	0.64	1.0
juveniles	156	1.0	0.0	1.0	19	1.0	0.51	1.0
Orientation (0º-180º)								
females	32	180.0	0.0	84.38	16	180.0	0.0	30.0
males	34	180.0	0.0	90.00	13	180.0	0.0	180.0
juveniles	156	180.0	0.0	90.00	19	180.0	0.0	180.0

Table 5.3 – Number of adult recaptures of *T. cristatus* by sex for both seasons combined. Number of points captured for analysis, number of individuals, number of individuals that moved, maximum (max.), minimum (min.) and median values for measured behavioural traits - distance travelled (m), straightness (0-1) and orientation per night (0-180º).

Recaptures	**N points**	**N indiv.**	**N move**	**Max.**	**Min.**	**Total**
females						
Distance (m)	31	14	11	16.4	0.8	20.6
Straithgness				1.0	0.33	
Orientation (0º-180º)				112.5	17.2	
males						
Distance (m)	25	10	9	11.8	1.5	13.3
Straithgness				1.0	0.33	
Orientation (0º-180º)				157.5	22.5	

Fig. 5.2 – Percentages of newts (adults and juveniles) by final position (fence, tunnel or surrounding environment) for each initial point of capture in the system (a) fence (b) tunnel entrance and (c) inside the tunnels.



5.3.2 Distance travelled

The vast majority of newts moved very short distances with 77% of those coloured during autumn and 97% in spring moving less than 5m per night (**Fig. 5.3; Fig. 5.4**). Distances of more than 10 m per night were only recorded in autumn (5% of juveniles and 1% of adults).

Variation in distance travelled per night was dependent on the position in the system at which newts were caught (**Table 5.3**). There was a higher number of newts in the fence (n= 197) with shorter movements (mean.night^{-1}= 3.21m), low number of captures at the tunnel entrances (n=45) with short movements (mean.night^{-1}= 3.41m), and a small number of captures inside the tunnel (n=28) with long movements (mean.night^{-1}= 6.72m) (**Fig. 5.4; Table 5.2**).

The maximum distance travelled per night was 25.6m (adult male) inside the tunnel and minimum among those considered to have moved was 0.5m (adult male) in the fence. For adult recaptures, longest minimum distance travelled was 20.3m by a female.

5.3.3 Trajectory orientation

Higher number of newts were observed moving in diferent directions in relation to the fence, no particular angles was preferable (**Fig. 5.4)**. There was a significant effect of season on directionality (straightness of 1), where newt paths were straighter during spring (**Table 5.3**). Orientation of newt trajectories was dependent on their position in the system. Trajectory orientation was significantly different for newts moving along the fence from the newts moving around the tunnel entrance and the newts moving inside the tunnel (**Fig. 5.4; Table 5.3**).

Table 5.2 – Mean value and range of observed behaviour per season and position mitigation and position in the system

	Season		Position in mitigation		
	mean (range)		mean (range)		
Variables	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)

Table 5.3 – Parameter estimates from GLMMs for behavioural changes of *T. cristatus* in a road mitigation system. For each behavioural prediction (distance travelled per night (m), straightness (0-1) and Orientation (0-180) we present: significant factors, parameter estimate (ß), standard error (SE), Chi-square Wald test II (*W*), p-values (only significant) and value of AIC.

Behaviour	**Variables**	**Model parameters**					
		β	**SE**	**Chisq**	**df**	**P-value**	**AIC**
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

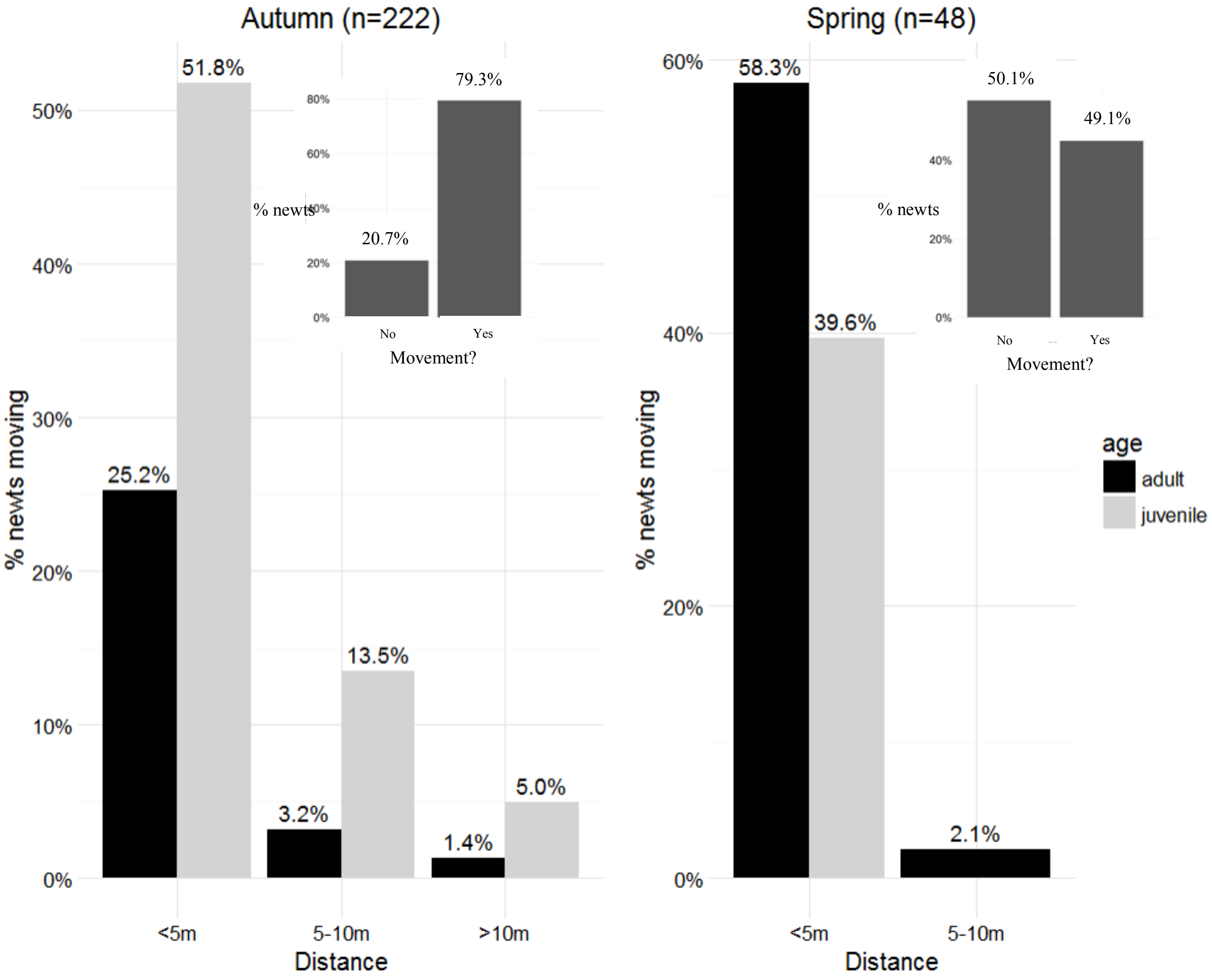


Fig. 5.3 – Season distribution of estimated distance travelled per night by newts during two seasons (autumn/spring). Percentages of newts (adults and juveniles) by final position (fence, tunnel or habitat) for each initial point of capture in the system (fence, tunnel entrance and inside the tunnels). Small graphics represent the percenetage of newts that moed during the specific season and were accountable to calculate distances.

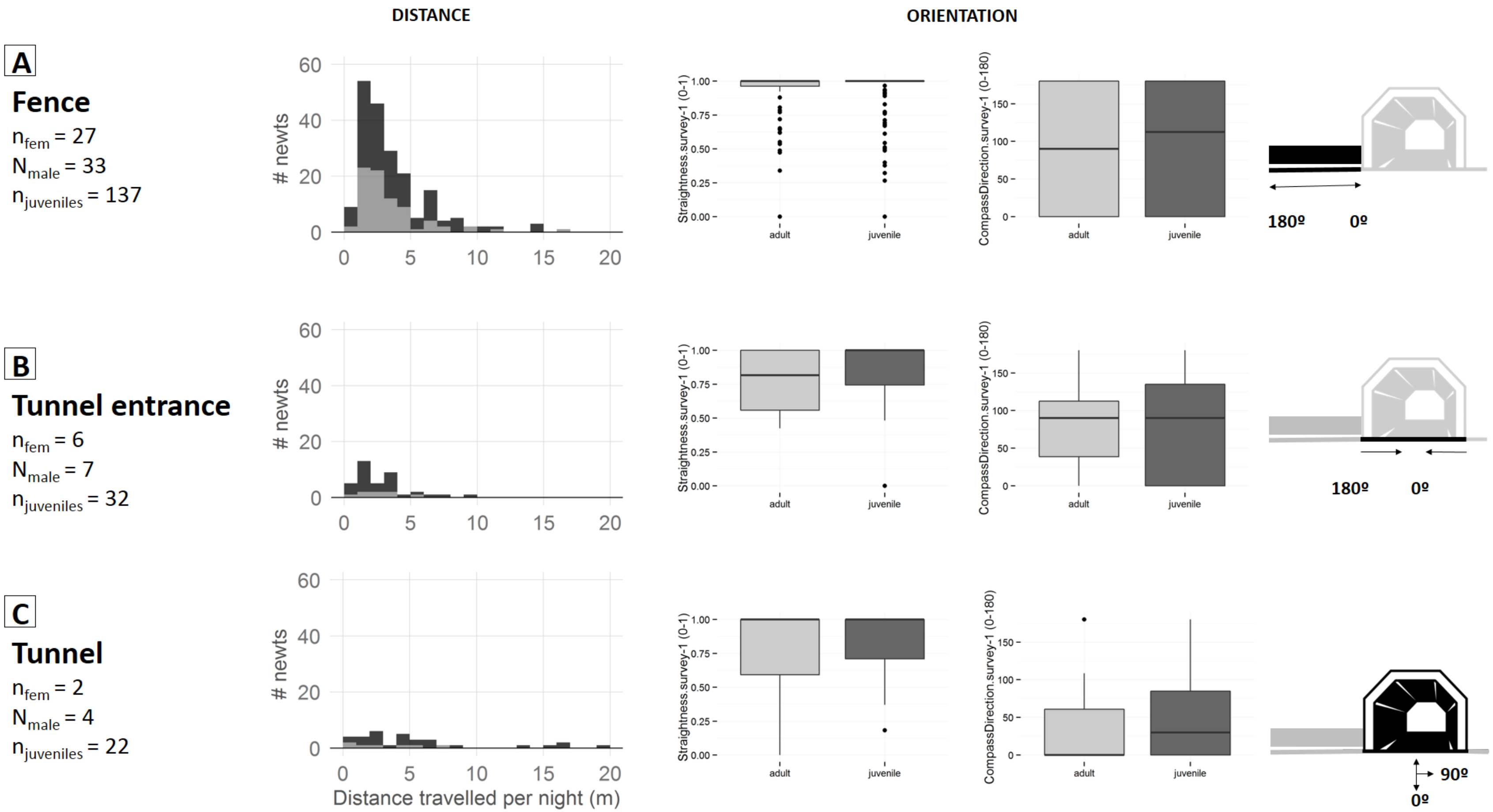


Fig. 5.4 –Descriptive statistics for movement behaviour of T. cristatus captured at three points along the mitigation system. Fist column shows number of females, males and juveniles capture in each part of the road mitigation (fence, tunnel entrance and inside the tunnel). Second columns shows frequency of newts for maximum distance travelled per night (m.survey-1), second and third column show boxplots for orientation. Here I estimated variation for straightness (index.survey-1) and compass direction (degree.survey-1) both relative to the fence, tunnel entrance and inside the tunnel. Light grey represent juvenile data and dark grey adult data.

5.4 Discussion

To our knowledge, this is the first study on the fine-scale movement of *T. cristatus* reflecting insights on the individual behaviour variation in a road mitigation system. Results from behaviour at the fences and tunnels are discussed separately to understand how newts respond differently to different parts of the system. Results on final positions, distance moved and trajectory orientation are related together to adress the effect of seasonality on these responses.

Our findings show: (1) newts moving along the fence tended to move back to the environment and with few entering the tunnel during the night, (2) most newts that encountered the tunnel moved towards the fence or the surrounding environment, but remained in the tunnel, at least overnight, if they moved into it; (3) the behavioural nature of movements (orientation) changed when newts encountered the tunnel and also changed inside the tunnel and (4) differences in responses between seasons (long movements during autumn compared to spring).

5.4.1 Behaviour along the fence

Despite higher number of newts remaining at the fence during the night, the observed low recapture rate between seasons is consistent with these individuals being dispersers and not residents. Additionally, few newts encountering the tunnel per night and straight paths at a range of angles away from the fence following release are consistent with newts using the fence as part of their normal foraging environment (Oldham et al. 2000). Fences may constitute barriers, keeping newts away from the road, hence preventing road mortality, but do not appear to guide them directly towards tunnels. This is consistent with other studies that showed salamanders moving in different directions along the fence and not specifically towards the tunnel entrances (Allaback and Laads, 2003). Effective tunnel crossings by newts may be improved by positioning tunnels close together. Allaback and Laads, (2003) suggest a maximum of 30m intervals between tunnels for salamanders, however more research is needed to understand the optimal density of tunnels (number per unit length of road) for *T. cristatus*.

For more terrestrial amphibians that can suffer high road mortality, such as *Bufo bufo*, fences can offer greater benefits by reducing mortality and directly guiding individuals towards tunnels (Lesbarrères et al., 2004). The reduced terrestrial mobility of newts along with their tendency to reflect off the fence in random directions may mean that fences facilitate less frequent road crossing and hence lower population connectivity. Also, the fence area offered good refuge conditions; newts use mammal burrows (Jehle and Arntzen, 2000), which were abundant along the fence. Use of the fence may be temporary, as evinced by the low number of recaptures, but some adults remained or returned to the fence between seasons (10% of captures). Active feeding and refuge use was observed during this study, especially in areas with more vegetation. Vegetation may allow newts to climb the fence, hence reducing its barrier effect and potentially reducing efficacy of road mortality mitigation. This hypothesis requires testing. Conversely, exploration of the potential benefits of abundant vegetation and other types of refuge close to the fence with respect to promoting movement along the fence and towards tunnels over longer periods of time could help further inform appropriate fence management.

5.4.2 Tunnel behaviour

Our results indicate diferences in distance travelled and orientation of newts at the entrance and inside of the small and larger tunnels. When considering the tunnel entrance, distances were shorter than inside the tunnel. A higher proportion of animals might be guided towards the tunnels if fences adjacent to the entrance are angled by 45º in relation to the tunnel (Jackson, 2003), and shorter than 20m (Jackson, 2003). Entrances in this study presented the same angle (45º, please see **Fig. 5.1**) and were 3m wide. Nevertheless, newts seemed to change path where the fence angle at the tunnel entrance changes towards inside the tunnel and numbers of individuals attempting to cross were still low (20%) compared to the number in the fence (80%). Experimental studies are needed to understand the best length of fence at the tunnel entrances in order to maximise opportunities for encountering the tunnel entrance.

The majority of newts found at the tunnel entrance moved away from the tunnel and towards the fence or the surrounding environment. Changes in orientation in tunnel entrances have been reported for *Ambystoma* species (Jackson and Tyning, 1989; Allaback and Laabs, 2003). Microhabitat at tunnel entrances can impact the way in which amphibians move through this area to get to the tunnel (Glista et al., 2009). Usually tunnel entrances are cleared of vegetation and debris by humans, presumably to facilitate entry by amphibians, but result in areas denuded of vegetation. Newts may hesitate to enter tunnels due the changes in microhabitat and environment (e.g. temperature and moisture) associated with vegetation removal (Allaback and Laabs, 2003). Cover may determine if a tunnel may be more “attractive” and natural vegetation could provide a continuity of habitat towards and inside the tunnel (Glista et al., 2009). It is still unclear whether newts choose a particular environment in these systems or if they get back to pre-known refuge or environments while moving on land during the night.

Once inside the tunnel, newts tended to move in straight paths towards the tunnel exit but percentages of individuals making a full or partial (if the individual was found inside the tunnel) crossing in one night were low. In past *ex situ* experiments, low rates of tunnel usage were recorded - 0.68 - 0.77, 0.27-0.66 and 0.07–0.21 – these are ranges for amphibian individual full tunnel crossing per study (Lesbarrères *et al*., 2004; Woltz *et al*., 2008, Hamer *et al*., 2014, respectively). Tunnel usage in this study was higher for newts across both seasons (0.8 captures.night^{-1}). Rates of tunnel usage are mainly influenced by the interaction of tunnel environment and animal behaviour (Glista et al., 2009). Numerous factors appear to influence the decision of an amphibian to enter and cross the tunnel (Glista et al., 2009), e.g. (position of system, moisture, temperature, light, substrate and noise) and the understanding of how tunnels can be adapted to meet the best environmental conditions for full successful crossings is still improving (Patrick et al., 2010; Lesbarrères et al., 2004).

For adults, the low number of individuals moving towards the tunnel combined with changes of orientation of small number of recaptures suggested usage of the system in short periods time (one night). Here adult adaptation behaviour towards human-made infrastructure may be negative (Jehle and Arntzen, 2000; Knowlton and Graham, 2010). Adults prefer areas well covered with vegetation around breeding ponds, showing a strong sense of fidelity towards breeding sites (Jehle, 2000). It is also suggested that this fidelity may be extended to the terrestrial sites around ponds causing adults to remain near the pond between breeding seasons (Jehle et al., 2011; Sinsch, 1990; Sinsch, 2014). Due to homing ability adults perform shorter and less random/explorative movements when encountering a fence or a tunnel, play an important role on the changes of orientation and non permanence in the system. Due to the short period of time over which studies are typically performed, information on amphibian habituation and evidence on use of navigation cues and homing to explain behaviour at the entrance and inside the tunnels is still lacking (Pagnucco et al., 2012). Overall effectiveness of tunnel successful crossing may be attached to how infrastructures are adaptable to behavioural changes and differences (Baguette and Dyck, 2007). How the behaviour of an individual in road mitigation systems is affected externally by a chain of events linked to population dynamics, time/season, habitat structure and composition could al so improve our understanding of calculating tunnel effectiveness (Baguette et al., 2013).

5.4.3 Seasonal effect in movements

Differences in frequencies of use of the mitigation system and movement length between the two seasons can allow conclusions on the type of movement that the system is supporting (Sinsch, 2014). The longer and more frequent movements in autumn compared to spring are consistent with dispersal being the main type of movement supported by the system in the present study (already described in Matos et al., 2017). Dispersal movements are mainly performed from breeding ponds in autumn by juveniles seeking resources (food and shelter) (Pittman et al., 2014). Dispersers usually move towards new areas and the remaining population returns to the natal pond (Schabetsberger et al., 2004, Wells, 2007, Sinsch, 2014). This explains the higher number of individuals moving in the system in autumn in comparison to spring.

Although distances travelled were shorter in spring, there was a stronger effect of season in path straightness of newts. Spring migration is considered to involve more direct, straight-line movements (Jehle and Arntzen, 2000; Wells, 2007). The number of spring days with precipitation was lower than in autumn, which might have influenced the straightness of movement patterns (Kovar et al., 2009). Nevertheless, spring is the season when newts emerge from hibernation; low body condition combined with instinctive homing may restrict movements to short distance as well (Kovar et al., 2009).

5.5 Conclusions and future research

Our findings highlight three important aspects of road mitigation systems that influence their use by newts: 1) mitigation systems need to support movement in autumn and spring by facilitating autumn tunnel crossing and preventing crossing over the road surface during spring; 2) fences generally acted as barriers to movement, directing newts away from the road, but rarely towards the tunnel; 3) use of tunnels was low, even during autumn, and newts' responses to encountering the tunnel (change in orientation) infrequently resulted in tunnel crossing. During movement seasons, amphibians may be deterred from crossing the tunnel, and since the fence acts as a barrier, regional connectivity will be reduced, with negative implications for metapopulation dynamics (Pontoppidan and Nacham, 2013). We need to better understand the consequences of these results for metapopulation dynamics and persistence, and whether improvements to tunnel design are required. By using individual-based modelling different scenarios of tunnel crossings between sub-populations could be testedto predict how road mitigation can support migration and dispersal over the long-term for *T. cristatus* (Pontoppidan and Nacham, 2013).

Behavioural data collection is time consuming but once compiled can be used to predict amphibian movements under future road developments. Such predictions could be generated using individual-based or random walk models to simulate long-term amphibian movements (Pontoppidan and Nachman, 2013; Pittman et al., 2014; Sinsch, 2014). In combination with data on population dynamics, individual-based modelling could provide insights into the minimal number of successful crossings per year for population persistence over the long-term (50-100 years) Application of local information on newt populations from local study cases before road construction could result in increased accuracy of predictions (Pontoppidan and Nachman, 2013). I recommend the replication of this study in other areas; those disturbed by road construction and also un-disturbed sites. Data on climate, surrounding habitat (number of ponds, hibernation sites) and local population features (abundance, age and sex distribution) over the two terrestrial seasons (migration and dispersal) would provide additional information to understand overall population dynamics and species connectivity over the long-term (Palmer et al., 2014; Pe'er et al., 2011). Also, future work needs a new improved understanding of what environmental factors influence a newt's decision to use the tunnel in order to optimise tunnel design.

CHAPTER 6

Effects of environmental factors and seasonality on newt movement in a road mitigation system

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Abstract

In Europe, including the UK, road mitigation systems, such as fences and tunnels, are used to assist great crested newt (*Triturus cristatus)* seasonal movements across *roadscapes* and to maintain habitat connectivity for the species. Newts are highly sensitive to environmental changes and abiotic factors associated with tunnels might influence their usage by newts. We investigated how newt movement was influenced by climate and weather factors in a mitigation system in order to assess potential ways of improving landscape connectivity for newt dispersal. Also, we aimed to understand the possible influence of changes in climate that could alter species movements in these systems with potential consequences for long-term effectiveness. We used data collected during a 4-year tunnel monitoring scheme for *T. cristatus* from Chapter 3 to evaluate how movement varied in relation to weather and tunnel environmental factors, such as temperature and precipitation within seasons and between years. We explored the relationship between weather and tunnel environment factors measured on site using a linear regression model (LM) and their influence on newt movement using generalized linear mixed models (GLMM).

LM results showed a strong positive relationship between weather patterns and the tunnel microenvironment. The frequency of newt captures at fences and within tunnels increased with weekly mean air temperature, total precipitation, high humidity at ground level and seasonality. Increases in maximum temperature between years negatively influenced movement at the fences and tunnel crossing attempts. Attempted and successful crossings of the tunnel increased with air humidity at ground level in the middle of the tunnel. Our findings support suggestions that newt dispersal in these systems is influenced by both local climatic factors and the tunnel microclimate. These results indicate the importance of considering seasonality and newt movement patterns in the landscape under climate change scenarios when implementing road mitigation schemes. Tunnel microclimate was directly associated with tunnel usage by newts. Substract type may influence levels of humidity and successful crossings. Research is required to assess whether tunnel microclimate can be manipulated independently of external climate to favour tunnel use and mitigate the negative effects of roads.

Keywords: road ecology, weather; climate, amphibian, underpasses, dispersal

6.1 Introduction

Amphibian populations are highly vulnerable to road mortality and habitat fragmentation caused by road networks (Fahrig et al., 1994; Hels and Buchwald, 2001; Glista et al., 2007). Tunnels combined with fence systems are typically implemented to minimize the negative consequences of roads on pond-breeding amphibian populations. By using information on spatial patterns of dispersal in autumn and from spring migration, tunnels are strategically located at points along the road with the highest probability of amphibian crossings (Matos et al., 2017; Hamer et al, 2015).

Seasonal movements between breeding and non-breeding habitat are fundamental to many amphibian lifecycles. Environmental variables strongly influence the timing of amphibian movements across landscapes, thus affecting their reproductive phenology and population dynamics (Timm et al., 2007). Consequently, climate change, such as milder winters and earlier springs, is likely to have direct effects on amphibians in temperate regions (Blaustein et al., 2010). These events in urbanised, fragmented landscapes can present a double threat for amphibians (Hamer et al., 2008) as the increase of linear infrastructures act as additional barrier to seasonal movements triggered by changes in weather patterns (Timm et al., 2007).

For highly aquatic amphibian species, such as the great crested newt (*Triturus cristatus*), which is dependent on ground moisture and air humidity, tunnel conditions may play an important role in their ability or choice to move and successfully cross the road (Schmidt and Zumbach, 2008; Beebee, 2013). Mass movements on land to reproduction and hibernation sites are triggered by a combination of weather factors that have an influence on amphibian physiology (Ficetola and Maiorano, 2016). At a much smaller scale, temperature (Langton, 1989), humidity (Merrow, 2007; Schmidt and Zumbach, 2008), rainfall (Pagnucco et al., 2012) and the presence of water inside the tunnel (Langton, 1989; Lesbarrères et al., 2004) can enhance or reduce the frequency of movements and influence the choice of entering the tunnel or not. However, the influence of regional- and micro-climate on the frequency of movements along the fence and through tunnels is still poorly understood.

Moreover, as climate windows influence timing of pond-amphibian movements the investigation of regional environmental variables affecting tunnel microclimate could help explain how stochastic events affect mitigation system effectiveness. It may also, offer insight into some of the consequences of climate change for newts (Lister et al., 2015). In a world of constant change this is particularly important for the species because it is not currently understood whether the effectiveness of road mitigation systems will remain constant, will improve or will worsen under various climate change scenarios (Lister et al., 2015). Moreover, the combination(s) of factors linked to variation in tunnel-use frequency by amphibians between seasons and years remains unclear (Schmidt and Zumbach, 2008; Hamer et al, 2015), but this information would be most valuable for integration within UK climate change policies for biodiversity protection (Hulme et al., 2002; Ward et al. 2015).

The aim of this study was to determine which weather factors and tunnel environmental conditions were associated with the frequency of road mitigation system use by great crested newts. I sought to: 1) understand if weather variables (regional) were related to the microclimate conditions within amphibian tunnels by testing the relationship between temperature, precipitation and humidity levels inside and outside underpasses; 2) analyse the potential effects of climate on seasonal newt movements with reference to different parts of the mitigation system (fence and tunnel) using a combination of generalized linear mixed models to explore relationships between annual maximum temperature, weekly mean temperature, precipitation and humidity levels and newt capture rates, and; 3) evaluate the effects of the internal tunnel microenvironment on the frequency of successful crossings by newts between seasons by testing relationships between ground air temperature and air humidity within tunnels and the weekly frequency of successful crossings.

6.2 Methods and Materials

6.2.1 Study area and data collection

The study was conducted in Orton Pit/Hampton Nature Reserve (52º 32’24N, 0º16’53W), a designated Site of Special Scientific Interest, Special Area of Conservation and Natura 2000 site, located south of Peterborough, Cambridgeshire (UK) (see **Chapter 3** for more details **Fig. 6.1a**). This 145 ha reserve mainly comprises a section of former industrial brick clay extraction site but also includes woodland and patches of scrub. The study was conducted in the northern area of the reserve which is divided by a 10-12m wide, high-traffic-volume (1 000 – 10 000 vehicles/day) road constructed in 2006, which serves a new housing development (**Fig. 6.1a**). This northern area encompasses 33 permanent ponds within 400m from the main road. Vegetation cover comprises patches of semi-natural scrubland (e.g. *Salix sp.*), semi-natural/plantation woodland (e.g. *Fagus sp.*, *Betula pendula*, *Acer pseudoplatanus*) (~250m from the road) with natural grassland/herbaceous understorey (~50m from the road).

The road mitigation system was composed of: one polymer concrete ‘amphibian tunnel’, with open slots at the top, manufactured by ACO Germany (0.5 m diameter, 30m long), two large ARCO concrete and metal sheet underpasses (5.5m wide x 2 m high, 40m long) spaced 100 m apart, and two heavy duty plastic fence systems (Herpetosure UK, 200-300 m in length), placed 10-50 m away from the road, angled to guide amphibians towards the tunnels (**Fig. 6.1b**). In an effort to minimise human disturbance the entrances of the large tunnels were protected with a bar fence and gate system. The three underpasses (northern, central or ACO, and southern tunnel) connect the two main sections (west and east sides of the road) in the north of the reserve (**Fig. 6.1b**).

The site hosts what is believed to be the largest single population of great crested newts in the UK and possibly Europe, estimated at around 30,000 individuals as well as a very large population of smooth newts, but common frogs and common toads are rare (Froglife, 2012)

For tunnel and fence monitoring, consult *Methods and Material* **Chapter 3** (Matos et al., 2017). Surveys were conducted primarily during spring and autumn (the main periods of amphibian movements), between April 2008 - October 2012 with no data collected in 2009, but included at least 4 days of trapping per month during the summer months. Monitoring averaged 7 months per year and 8-12 days per month, starting in spring (typically March) and ending in autumn (mid-late

October), with no monitoring during winter due to amphibian inactivity. Total number of trapping days varied in the first two years as the methodology was tested (**Table 6.2**). From 2008 until the end of the study the fence system was surveyed by torch during the hours of darkness. Additionally in 2008 only, short drift fences and three 10-l plastic bucket pitfall traps were placed on each side of the road in front of the tunnel fences (east and west). Traps and fences were checked daily, shortly after dawn and again before dusk.

Amphibian species, sex and age class (adult or juvenile) were recorded for each animal caught together with the position in the mitigation system (tunnel or fence) and side of the road. No individual marking was performed and trapped amphibians were released in vegetation near the capture point. All trapping and handling was done under licence from Natural England (Ref: 2014-1917-SCI-SC) and was approved following review by the University of Hull's Ethical Review process.

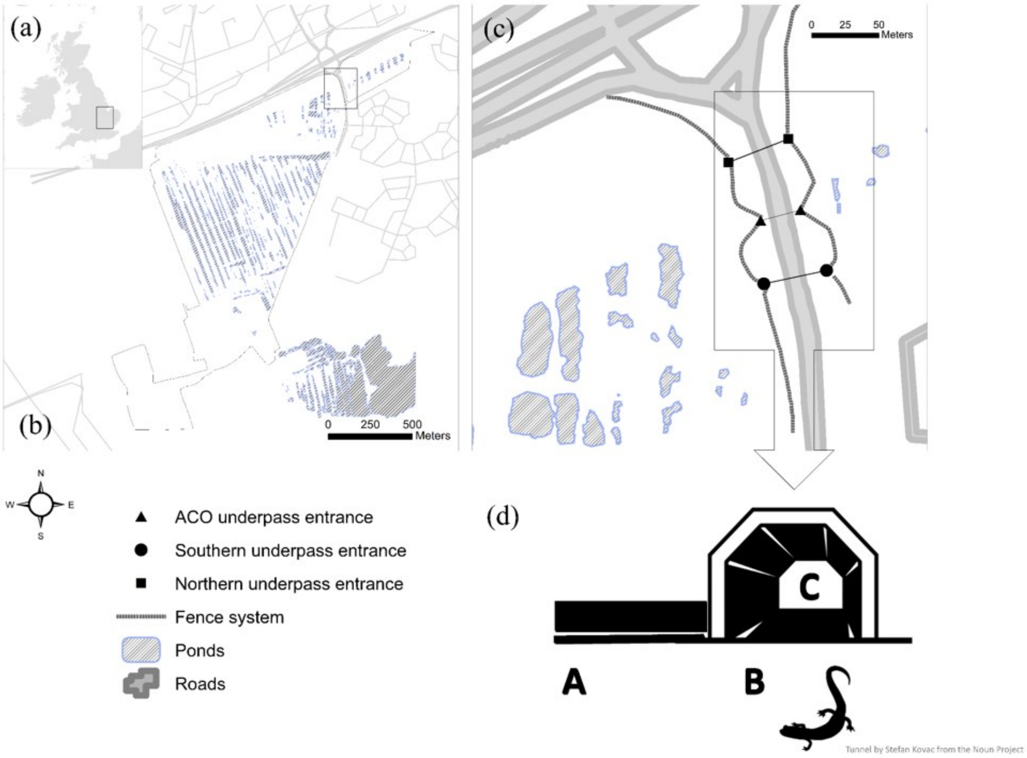

Fig. 6.1 – (a) Location of the study area in Peterborough, UK (b) west (large) and east (small) sections of Hampton Nature Reserve (SSSI) (c) monitored sections of the mitigation system (in bold) (d) For each capture point a code was defined: movements along the fence system (A); movements in tunnel/attempted crossing (B); movement in tunnel/successful crossing (C) (*see Chapter 3 for more details*).

6.2.2 Estimation of newt movement

For each capture we coded movement on the basis of capture location within the mitigation scheme (*see Chapter 3 for more details* **Fig. 6.1c**). Captures along the fences were coded ‘A’, captures at tunnel entrances coded ‘B’, and captures of animals which had successfully crossed the road through tunnels coded ‘C’. Here, we classify movement hierarchically, as captures of amphibians along mitigation systems generally decrease from the fence to the tunnel (Matos et al., 2017). A movement and B movement are considered “wider newt movement” in the system, representing individuals coming from the landscape, residents or dispersers (Matos et al., 2017). B movement and C movement are represented as “tunnel newt movement”, these are individuals actively using the tunnel. We estimated movement for A, B and C, separately, as the mean per week of number of newts captured by night at each location. We averaged nightly movement per week because the number of surveys (trapping nights) varied between weeks throughout the study period (2008-2012). Also, some weather factor measured from the Peterborough weather station were at weekly scale.

6.2.3 Environmental data

Daily average, maximum and minimum air temperature in ºC, and daily precipitation in mm were all averaged for each survey week. Weather data were collected from a weather station located in the immediate proximity of the reserve (less than 2 miles) in Peterborough, Cambridgeshire). Weather data were obtained from (http://www.peterboroughweatherwatch.com/). Cloud cover, wind speed and presence of water inside the tunnel were recorded during evening surveys (**Table 6.1**).

Air temperature (ºC) and humidity (%) were measured at ground level at the open traps (entrance) and in the middle of the tunnel: 2m for the middle ACO tunnel and 15m inside the north and south tunnels. The three tunnels were monitored for temperature and humidity during each survey, with values averaged for each week during analysis.

Table 6.1 – Summary of the different environmental variables used to build the LM and GLMM models for capture variation. Variables are categorised as relating to 'weather' or the tunnel environment.

Variable	Code	Description	Range	Units	Source
Year	YEAR	Year of survey	2008-2012		
Month	MONTH	Month of survey	3-10		
Survey	SURVEY	Survey week	1-69		
Weather					
Air temperature	TAIR	Mean daily air temperature over survey week	5.20 - 22.90	ºC	Peterborough weather station
Maximum temperature	TMAX	Maximum daily temperature over survey week	10.1 - 30.4	ºC	
Minimum temperature	TMIN	Minimum daily temperature over survey week	-1.60 - 17.0	ºC	
Humidity	GHUM	Mean daily humidity at ground level over survey week	48.60 - 93.00	%	During survey checks/nights, Froglife
Accumulated precipitation	ACPREC	Sum of daily precipitation values over survey week	0.00 - 24.00	mm	Peterborough weather station
Mean precipitation	AVPREC	Mean daily precipitation over survey week	0.00 - 1.75	mm	
Week precipitation	WPREC	Sum precipitation values from start/end of the previous survey week	0.00 - 1.75	mm	
Cloud cover	CLOUD	Mean survey cloud cover over survey week	0.00 - 100.00	%	During survey checks/nights, Froglife
Wind speed	WIND	Mean survey wind speed over survey week	0.00 - 2.50	km/h	
Tunnels					
Temperature entrance	TETUNNEL	Mean survey air temperature at ground level in tunnel entrances over survey week	5.48 - 19.40	ºC	During survey checks/nights, Froglife
Temperature middle	TMTUNNEL	Mean survey air temperature at ground level in middle of tunnel over survey week	5.60 - 18.60	ºC	
Humidity entrance	HETUNNEL	Mean survey humidity at ground level in tunnel entrance over survey week	57.70 - 90.20	%	
Humidity middle	HMTUNNEL	Mean survey humidity at ground level in middle of	61.00 - 90.10	%	

		tunnel over survey week		
Presence of water	FLOOD	Presence of water inside the tunnel	1-0	Present/absent

6.2.4 Statistical analysis

Weather patterns affecting tunnel conditions

To determine if weather patterns have a direct influence on tunnel environmental conditions we expected an increase in mean weekly air temperature and humidity at ground level inside the tunnels as external air temperature and average weekly precipitation increased.

For this, we fitted two simple linear models, between mean weekly air temperature (TAIR) as dependent variable and tunnel ground temperature at entrance and middle (TETUNNEL and TMTUNNEL) and between ground humidity (GHUM) as dependent variable and tunnel ground humidity at entrance and middle (HETUNNEL and HMTUNNEL).

Weather and tunnel environment factors affecting seasonal movements at the fence and tunnel

To understand how number of wider newt movement are affected by changes in weather patterns, for both adults and juveniles combined, we expected: (1) an increase in the number of captures at the fence (A) and consequently in tunnel attempts (B) with average daily rainfall per week. Also, we expected (2) lower numbers of captures along fences (A) and fewer attempts to cross (B) with higher minimum/maximum daily temperatures per week during late autumn and early spring (years with mild winters and mild springs). Finally, as tunnel conditions may influence amphibian behaviour and motivation to cross (Langton, 1989; Lesbarrères et al., 2004), (3) we expected an increase in attempts (B) and successful crossings (C) with tunnel humidity.

For the three hypothesis stated above we tested all possible variable combinations and interactions. Hypothesis 1, to find which weather factors were associated with wider newt movement, we fitted weather variables (TAIR, GHUM, ACPREC, WPREC, CLOUD, WIND, and SEASON) to A and B weekly captures, separately. Hypothesis 2, to understand if fluctuations in weekly mean air temperature between seasons affected wider newt movement, we fitted minimum/maximum temperature variables with season and year (TMIN, TMAX, SEASON and YEAR) to A and B weekly captures, separately. Hypothesis 3, to calculate which tunnel factors were associated with tunnel newt movement, we fitted tunnel environmental variables (TMTUNNEL, HMTUNNEL, FLOOD and SEASON) to B and C weekly captures, separately.

I used generalised linear mixed models (GLMMs) of average nightly number of captures per week using a restricted maximum likelihood (REML) method with Laplace approximation in order to avoid bias for the (co)variance parameters, as suggested by Wikle and Royle (2002) I constructed a Spearman's rank correlation matrix to explore relationships between all weather and tunnel environment factors. Highly correlated variables (r^2>0.70) with less biological meaning to test our hypothesis were excluded from the GLMM analysis. In each model we entered YEAR (n=4 years) as a random effect. I tested the interaction of SEASON on all variables in order to understand if the effect of environmental conditions on the number of newts caught was homogeneous between seasons. I selected the most parsimonious model by including only significant variables at 0.05 level of significance from the models with lowest AIC values. Models needed to be refitted to ML (maximum likelihood) in order to compare AIC values (Wikle and Royle, 2002). GLMM fitting was performed using package lme4 (Bates et al. 2012) for R 3.3.1 (R Core Team, 2016).

6.3 Results

The total number of trapping weeks was 69. From 2008, 2010-2012, 501 newts were caught at the fence (A), 124 during tunnel crossing attempts (B) and 97 newts crossed the tunnels successfully (C) in 305 survey nights (**Table 6.2**). Observed mean captures at fences, tunnel attempts and successful crossings was 1.65, 0.40 and 0.30 captures.night^{-1}, respectively and varied among years from 0.08-4.0 captures.night^{-1} (**Table 6.2**). In 2010 mean air temperature (17.4 ºC) and average accumulated precipitation (3.47 mm) during the survey period were the highest. Lowest values of precipitation were recorded in 2011 (0.75mm) (**Table 6.2**).

6.3.1 Weather patterns affecting tunnel conditions

Environmental and microclimate data from the tunnel between 2008-2012 were very similar, providing linear models with good fit to the data for both mean temperature and humidity for outside, entrance and middle of tunnels (**Fig. 6.S1 and 6.S2**). Mean air temperature outside was strongly correlated to ground temperature at the entrance and inside the tunnel (Spearman's rank correlation r^2=0.87, r^2=0.87, $p<0.001$, respectively). Equally, for outside humidity values, there was a highly significant linear relationship with ground humidity levels at the entrance and in the middle of the tunnel (Spearman's rank correlation r^2=0.72, r^2=0.71, $p<0.001$, respectively).

6.3.2 Weather factors affecting seasonal movements at the fence and tunnel attempts

Weekly captures away from the tunnels (A and B movements) varied significantly with external environmental factors (**Fig. 6.2**; **Table 6.3; Table 6.4**). Consistent with our hypothesis (2), captures at the fence (A) varied significantly with accumulated precipitation (Chi-square Wald test II W_A= 41.17, P<0.001) and season (Chi-square Wald test II W_A= 4.10, P<0.001). Crossing attempts in the tunnels (B) varied significantly with mean air temperature (Chi-square Wald test II W_B= 4.39, P=0.03), ground humidity levels (Chi-square Wald test II W_B= 8.38, P<0.001) and season (Chi-square Wald test II W_B= 11.24, P<0.001). For A and B there was a significant effect of the interaction between season and mean temperature (Chi-square Wald test II $W_{tair*season}$= 7.69, P= 0.02) (**Table 6.3; Table 6.4)**. In contrast, the previous week's precipitation, cloud cover, and wind did not significantly influence either fence or crossing attempt numbers of newts over the study period. When compared to the effects of maximum temperature, a negative influence of warming weather was found for newt captures at the fence along years (A movement) (**Table 6.3; Table 6.4**).

6.3.3 Tunnel factors affecting seasonal attempts and successful tunnel use

Overall, the number of attempted and successful crossings were positively associated with humidity levels in the middle of the tunnels (**Fig. 6.3**; **Table 6.3**). Results for hypothesis (4) suggest a positive association with ground humidity levels (Chi-square Wald test II W_B= 3.72, P=0.05; Chi-square Wald test II W_C= 13.67, P<0.001) and season (Chi-square Wald test II W_B= 13.60, P= 0.001; Chi-square Wald test II W_C= 18.41, P<0.001) for attempts and successful tunnel crossings (**Table 6.3**). A significant interaction was found between these two factors, indicating the importance of humidity in tunnel permeability, especially during autumn (**Fig. 6.3; Table 6.3**). Nevertheless, the effects of the different tunnel environmental factors presented a strong correlation between variables (**Fig. 6.S1 and 6.S2**).

Table 6.2 – Number of newts captured at the fence, attempting to cross the tunnel and successful crossing the tunnel (A, B and C) per year with number of nights of trapping, mean, maximum and minimum values for air temperature, humidity levels and precipitation.

Monitoring					***T. cristatus***						**Temperature (ºC)**			**Accumulated Precipitation (mm)**		
Years	**Months**	**Nights**	**Mean (days per month)**	**N (total)**	**A**		**B**		**C**		**Mean**	**Max**	**Min**	**Mean**	**Max**	**Min**
2008	9	113	12.5	197	99	0.88	66	0.58	32	0.28	11.73	25.70	-0.9	1.59	12.50	0.0
2010	8	64	8.0	209	125	1.95	35	0.55	49	0.77	17.40	30.40	0.80	3.47	16.00	0.0
2011	6	64	10.67	41	21	0.33	15	0.23	5	0.08	17.02	28.60	1.30	0.75	3.00	0.0
2012	8	64	8.00	275	256	4.0	8	0.13	11	0.17	15.23	30.40	-1.60	7.81	24.00	0.0
Total	31	305	9.76	722												

Table 6.3 – Model summaries for univariate (GLMM) relationships between weather and tunnel environmental conditions and wider and tunnel movements (A, B and C).

Hypothesis	**Mitigation**	**Model/significant variables**	**Estimate**	**SE**	**AIC**
Weather (2)	A	TAIR + GHUM + ACPREC + WPREC + CLOUD + WIND * SEASON + 1 | YEAR	-4.40	3.20	213.80
	B	TAIR + GHUM + ACPREC + WPREC + CLOUD + WIND * SEASON + 1 | YEAR	-1.46	0.52	28.42
Temperature (3)	A	TMAX + YEAR + 1 | YEAR	3.98	1.45	314.72
Tunnel (4)	B	TMTUNNEL + HMTUNNEL + FLOOD * SEASON + 1 | YEAR	-3.33	2.50	192.89
	C	TMTUNNEL + HMTUNNEL + FLOOD * SEASON + 1 | YEAR	-3.99	1.47	128.56

Table 6.4 - Model summaries for relationships of weather and tunnel environment factors with captures in the fence (A), tunnel attempts (B) and successful crossings (C)): significant factors and interactions, estimate (ß), standard error (SE), Chi-square Wald test II (*W*) and p-values (bold if significant).

Hyphotesis	**NM**	**Fixed effects**	**Estimate**	**SE**	***W***	**p**
(a) Relationship wide NM and weather variables						
	A	ACPREC	0.32	0.04	41.17	**<0.001**
		SEASON	3.11	0.80	4.10	**<0.001**
	B	TAIR	0.03	0.01	4.39	**0.03**
		GHUM	0.02	0.005	8.38	**<0.001**
		SEASON	-0.03	0.14	11.24	**<0.001**
		TAIR * SEASON	-0.14	0.31	7.69	**0.02**
(b) Relationship wide NM and maximum/minimum temperature						
	A	TMAX	-0.12	0.06	4.11	**0.04**
(c) Relationship tunnel NM and weather variables						
		HMTUNNEL	0.04	0.03	3.72	**0.05**
	B	SEASON	-0.83	0.46	13.60	**0.001**
		HMTUNNEL * SEASON	-0.09	0.04	5.87	**0.05**
		HMTUNNEL	0.05	0.01	13.67	**<0.001**
	C	SEASON	-0.37	0.27	18.41	**<0.001**
		HMTUNNEL * SEASON	-0.09	0.02	16.50	**<0.001**

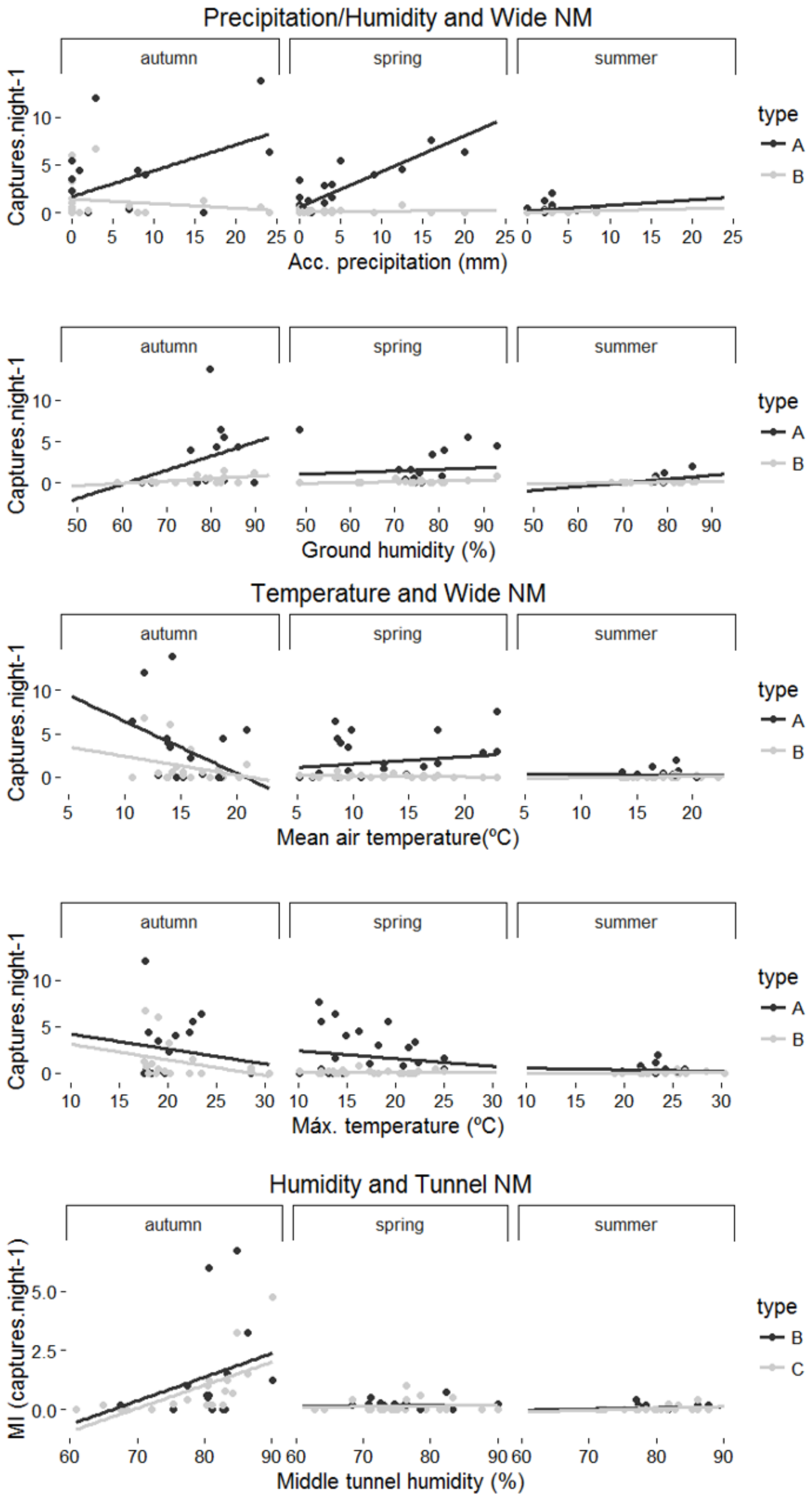

Fig. 6.2 – Weekly captures per night along the fence (A) and tunnel entrances (B) in relation to weather significant factors (GLMM) accumulated precipitation, average precipitation, ground humidity and maximum temperature between seasons and weekly captures per night at the tunnel entrances (B) and successful crossings (C) in relation to middle tunnel humidity between seasons.

6.4 Discussion

Amphibian movement is determined by complex environmental interactions which may change within and between seasons and over years (Timm et al., 2007). However, research on amphibian tunnels as mitigation for roads rarely considers time periods of more than a year and so has failed to investigate these effects on amphibian tunnel use. Our data showed that mean air temperature, accumulated precipitation, humidity levels and maximum temperature at local and regional scales influenced wider and tunnel newt movements during seasonal migration and dispersal in a road mitigation system.

These observations may have implications for planning, design and implementation of road mitigation systems when connecting habitat patches that are vital for dispersal. In this case, variation in weather patterns due to climate change are still under-considered when planning underpasses for amphibian movements (Van Teeffelen et al., 2015). Moreover, this is the first study to present more than two years of data on environmental factors affecting the movement of great crested newts in a road mitigation system.

6.4.1 Wide newt movement and effects of weather patterns

Wider newt movement in the mitigation system (A) was associated with accumulated precipitation, humidity levels, mean air temperature and maximum temperature. As a pond-breeding amphibian, *T. cristatus* migration patterns are highly dependent on precipitation (Dervo et al., 2016). Longer movements in the landscape are maximized by long periods of rainfall (Dervo et al., 2016). Also, precipitation is linked to population size as drier years are associated with declines of amphibian populations (Ficetola and Maiorano, 2016). Consequently, how far individuals travel on land may increase with moisture and could potentially lead to higher numbers of newts in fences and attempting to cross the tunnel.

Although capture rates in other systems were found to increase with precipitation levels (Pagnucco et al, 2012), mean air temperature and maximum temperature were associated with wider newt movements in this study. Temperature is considered the main driver of changes in amphibian phenology (Ficetola and Maiornano, 2016). An explanation for the negative effect of maximum temperature on A newt movements could be related to timing of movements to and from ponds. The migration phenology of *T. cristatus* can change between years with changes in maximum temperature, with individuals arriving later to the pond if maximum temperature increases within 3 months prior migration or dispersal movements (Simm, 2016). Additionally, maximum temperature may affect the survival rate of *T. cristatus* and negatively influence A newt movement between years if winters are warmer (Griffiths *et al*. 2010). In years of more stable temperatures individuals can start moving later which might impact numbers found at the fence later in autumn and early spring.

In contrast, the positive significant effect of mean air temperature on B newt movement is potentially linked to differences in microclimate within the environment between seasons. Warmer temperatures combined with precipitation might prolong newts' activity in the tunnel vicinity increasing the number of attempts. Air temperature has not been directly linked to tunnel usage rates until this study (Langton et al., 1989; Hamer et al., 2014). Here microclimate at the entrance may have an important role when considering crossing attempts.

Temperatures inside the tunnel are more stable throughout the day compared to the outside environment, although they were broadly consistent. As ectothermic animals, newts moving during the night may follow the continuum of warmer and moist areas. If tunnels maintain the same conditions as the outside, attempts during nights of good weather conditions are likely to be higher. The interaction of suitable weather patterns (temperature and precipitation) was not only associated with the seasonal movement of newts along the landscape but also in the road mitigation system at the local scale. Both regional and local effects on newt mitigation use are highly related to species life history traits. Therefore, road mitigation effectiveness becomes highly dependable on seasonal movement variation. Significant effect of precipitation and temperature may be important to consider when planning and design the road mitigation monitoring programs as it influences frequency of detection. During effectiveness assessment the probability of movement in fences and, therefore probability of finding the tunnel, require the integration of climate factors variability as explanatory variables affecting use further explaining the last section of this chapter (Lesbarrères et al., 2004).

6.4.2 Tunnel movement and tunnel environment effect

Our results showed tunnel micro-environmental factors directly associated with tunnel usage by newts. Higher levels of humidity were associated with a greater number of seasonal movement attempts and successful crossings. The few studies on road crossing structures have failed to produce a clear picture of the importance of tunnel abiotic environment. Temperature, light availability, moisture and presence of water have not been found to be associated with successful crossings (Hamer et al., 2014).

Whatever the case, minimum standards for tunnel physical attributes such as length, diameter, position and substrate are recommended to improve tunnel abiotic conditions for amphibians' (Woltz et al., 2008). An *ex situ* study showed frog species preference for soil substrate in tunnels over bare concrete tunnels and suggested that moist soils were particularly preferred due to amphibians' susceptibility to desiccation (Lesbarrères et al., 2004). Another study established that dehydration rates in tunnels were highly correlated with substrate type (Woltz et al., 2008). Tunnels in our study were of the soil-covered type which may help retain moisture during weeks of high accumulation of precipitation and encourage movement of a greater number of newts. Humidity can enhance individual movement as it improve surface conditions enhancing amphibian crossings over long distances through tunnels (Patrick et al., 2010). A more natural habitat inside the tunnel can help maintain the ideal humidity conditions for amphibian presence. A substrate type that facilitates plant growth and local vegetation to adapt may increase successful crossings for newts (Lesbarrères et al., 2004).

However, these studies focused on behaviour of animals translocated to an experimental site outside normal movement seasons (Woltz et al., 2008; Patrick et al., 2010). We suggest that further investigation is needed to understand how cost-effective and whether maintenance of moist environments inside amphibian underpasses can increase successful crossings. In some sites, amphibian tunnels are designed around a permanent flowing water feature, such as a stream, going through the middle and this might offer a suitable solution but would be difficult to implement if no running water is available on site.

6.4.3 Practical implications: monitoring weather conditions and tunnel environment

During the four years of monitoring there was a strong positive relationship between the environment outside and the environment in the entrance and middle of the tunnels. Per week, mean daily temperature and humidity levels fluctuated similarly between the two environments. Other studies have particularly mentioned that these differences may hinder or enable tunnel use for amphibian species (Langton, 1989; Jackson, 1996). However, our study tunnels were especially designed to permit air to flow through the underpasses and minimise temperature and moisture differences. Furthermore, temperature and humidity in the microenvironment inside the tunnel may not be same as the outside at some point during the day (Langton, 1989). This may have resulted in our observation of the positive association between temperature, humidity and tunnel crossing attempts (B movements). As the road can provide insulation and tunnels may conserve a warmer temperature and humidity levels during the night.

I consider our results a first step to understand if improved passages *in situ* enable amphibian crossings even if the tunnel environment is similar to conditions outside. The important point would be to translate attempted crossings to successful crossings, and determining whether tunnel environment might influence success. An improved passage design which can be adaptable for locations and new routes of migration may enable amphibian successful crossings over long periods of time (years and decades) (Van Teeffelen et al., 2015). This hypothesis needs testing however, as most populations are constinuasly changing their local requirements, size and habitat quality. As weather patterns influence tunnel microenvironment, a changing climate could alter dispersal and movement in the landscape if conditions do not meet newts' abiotic needs (e.g. Reading, 2007).

6.5 Conclusions and future research

Nevertheless, our results emphasize that changes in weather patterns may influence the presence and successful crossings of newts through tunnels in different seasons. Newts may face an additional challenge when moving between habitat patches using tunnels in case of extreme weather episodes, such as drought and high temperatures. The consequences of mitigation infrastructure for long-term population viability of this species under these circumstances are still poorly understood (Van Teeffelen et al., 2015).

Warmer temperatures and drier years as shown by our results can influence *T. cristatus* captures in the fence and tunnels during the main activity seasons (spring and autumn). From an annual scale, survival/extinction dynamics within sub-populations may be reduced in the presence of milder winters (Griffiths et al., 2010) Changes in weather patterns in different seasons (e.g. milder winters) associated with climate change are potential negative factors affecting landscape connectivity for newts under green infrastructure implementation (Van Teeffelen et al., 2015). As pond-breeding amphibian phenology changes seasonaly affecting reproductive success, juvenile recruitment and consequently metapopulation dynamics (Griffiths et al., 2010). In the long-term, seasonality may unpredictably influence patterns of movements, as these may contract or expand if the climate niche necessary for the species to perform its life cycle changes at the local scale (Van Teeffelen et al., 2015). Road mitigation planning may face a challenge when considering long-term landscape connectivity. Nevertheless, to recognize the role of road mitigation in facilitating or hindering dispersal in a changing climate for *T. cristatus* and which design alterations may be needed in the future at the regional-scale (Van Teeffelen et al., 2015; Lister et al., 2015). The revision of general recommendations of spatial planning (eg. habitat variables related to hotspots of mortality) could be complemented by inclusion of information on likely abiotic stochastic effects for newts (eg. mild winter or early springs) and integrated in a context-specific planning (Van Teeffelen et al., 2015).

In conclusion, the abiotic factors revealed to be important to monitor and evaluate effective usage and successful crossings in tunnels for *T. cristatus*. As environmental conditions affect individual responses and patterns of dispersal, the abiotic environment is a crucial factor influencing functional connectivity in the landscape for this species (e.g. Pontoppidan and Nachman, 2013). New improved research on the long-term effects of abiotic factors is needed in order to understand how road mitigation effectiveness changes in diferent regional climatic environments and for tunnels *in situ*. Our models can not be used to draw general conclusions for the general local planning of all *T. cristatus* populations, however it provides information on how information on climatic effects on fence and tunnel usage can help identify the response nature of successful crossings at the local level. At long-term, the localition and adaptability of the insfrastrutures to the abiotic environment will or will not provide the basic mechanism for functional connectivity at regional-scale. Nevertheless, the monitoring of climatic factors proves necessary to develop an integrated and adaptive design approach to predict best locations for tunnel placement (Hulme et al., 2002; Lister et al., 2015).

6.6 Attachments

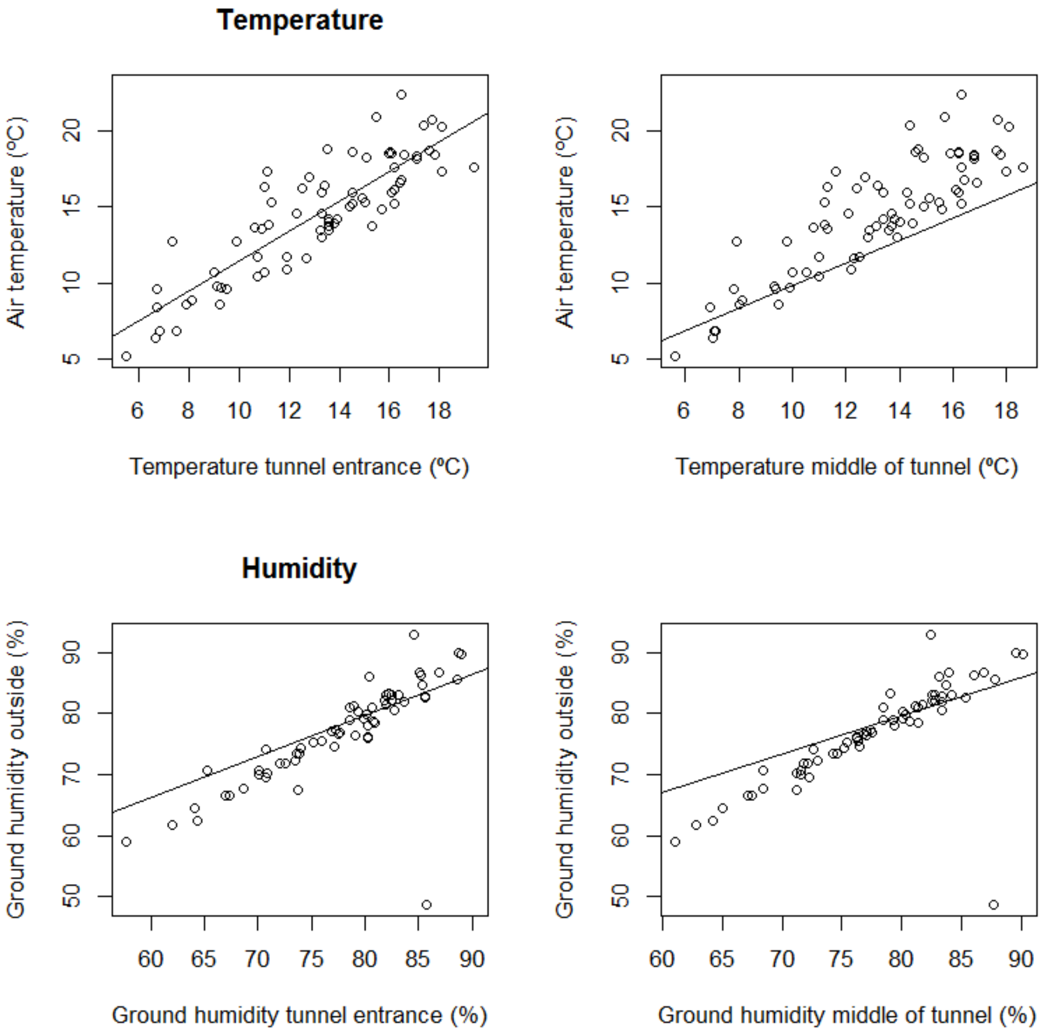

Fig. 6.S1 – Linear regression plots (with regression lines) of the relationship of temperature and humidity outside the tunnel and tunnel temperature and humidity at the entrance in the middle.

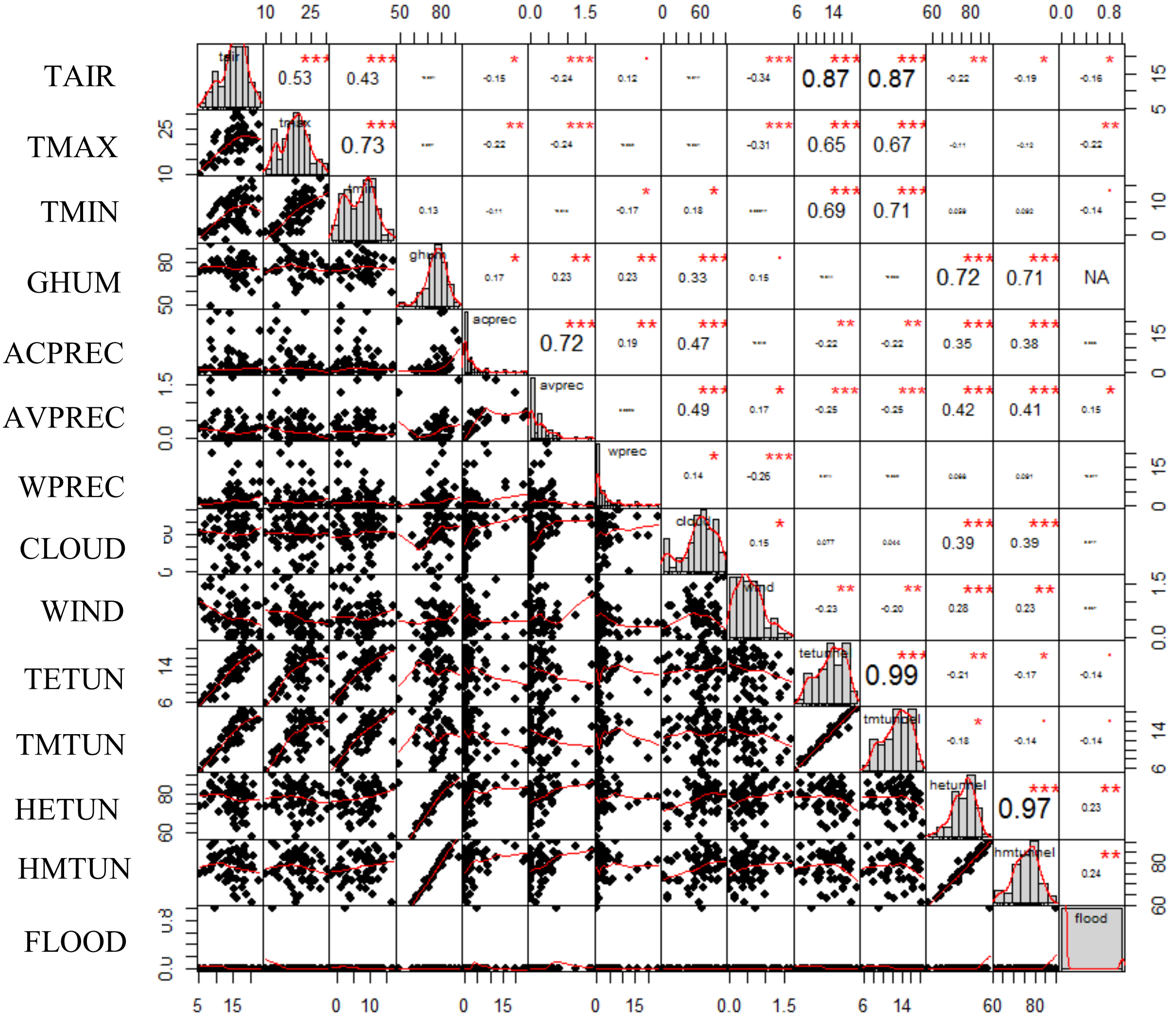

Fig. 6.S2 - Spearman’s rank correlation matrix for relationships between all weather and tunnel environment factors

CHAPTER 7

General discussion, conclusions and future research

7. General discussion

The overall aim of this thesis was to evaluate *T. cristatus* responses to roads and respective mitigation to propose new insights to improve strategic planning and detailed design of dispersal corridors for the maintenance of landscape connectivity for newts. The quantitative nature of this study illustrates to road ecologists and developers the spatio-temporal scales at which patterns of responses can be expected with respect to the effectiveness of road mitigation in relation to landscape connectivity for *T. cristatus*. Also, the incorporation of behavioral patterns into local and regional road mitigation planning offers the ability to assess landscape connectivity using biologically-relevant information during design development and post-implementation. Similarly, it introduces the potential influence of external factors on newt response patterns to road mitigation; factors that have as yet to be incorporated into road mitigation planning, but which could improve mitigation system use and increase its importance under climate change scenarios.

Responses were classified at different spatial and temporal scales in a landscape fragmented by roads and urban development. The use of reference knowledge described in **Chapter 2** on pond-breeding amphibian behaviour was used (1) to outline patterns of migratory and dispersal responses in a particular road mitigation system (**Chapter 3**), (2) to determine degrees of connectivity at the landscape-scale in a sub-urban area (**Chapter 4**), (3) to quantify spatial and temporal variability of individual behaviour in a road mitigation system (**Chapter 5**) and (3) to assess the role of external factors in determining temporal and spatial patterns of road mitigation system usage (**Chapter 6**).

First, we used a stage descriptive approach based on capture positions to quantify newt movement patterns. Annual and seasonal patterns of movement were examined at the local-scale. Movement responses varied significantly among individuals and age classes between fence and tunnel areas with peak activity during autumn dispersal. Spatial and temporal patterns of movement were present although effectiveness of the system, with respect to the frequency of successful tunnel crossing by newts, was difficult to assess as annual movement was highly variable.

Second, I calculated the potential terrestrial dispersal/colonization corridors and investigated linkages between newts' home-range patches using presence/abcense data. We also analysed how landscape components, such as habitat quality, amount and scale of movement would vary in two scenarios representing degrees of road permeability for minor roads. Seasonal movement patterns were used to determine structural and functional connectivity and habitat patches were prioritized using graph-theory analysis as a tool for road mitigation planning. I found that at landscape scale, high degree of connectivity for newts dispersal at long-term was determined by the extent of the available habitat when roads acted as barriers. These results indicated the crucial importance of directing mitigation efforts towards minor roads when there is a significant difference between population dispersal corridors at long-term for newt movements.

Third, I aimed to quantify variation in individual behavioural responses from *T. cristatus* to a road mitigation system. Spatial and temporal patterns in frequency of use/immovability in the system were calculated to provide quantitative data on responses for improving understanding on behavioural variability in effectiveness of use. Results showed newts moving short distances per night, with straighter paths recorded in spring and with trajectory orientation changes being dependent from the position in the system. In this study both seasons and position in the system have an influence on the choice of a newt to successful use the system. Individual response variability (distances and trajectory orientation) had an effect on the choice of entering and crossing the tunnel and shows the importance of including this variable in calculating effectiveness of systems over the long-term. Also, I investigated how newt responses were affected by climate and weather factors in a mitigation system in order to assess which external factors were most associated with increased movement frequency and the successful use of the mitigation system Precipitation and temperature positively influenced the frequency of use of fences and tunnels and humidity levels were linked to the frequency of successful crossings. These results showed the importance of considering seasonality and environmental factors variation when planning moniroting protocols for assessing future effectiveness in road mitigatio.

The overall results reported in this thesis suggest that monitoring variation in the spatial and temporal scales over which individual newts and their populations operate and factors that may influence this variation could contribute to improved practices with respect to road mitigation planning and effectiveness. The ultimate goal of road mitigation application for newts is to maintain metapopulation dynamics and landscape connectivity over the long-term

(Lewis et al., 2007, Lesbarrères and Fahrig, 2012). Metapopulation dynamics maintenance is normally associated with colonization rates of new patches in the landscape, maintaining the flow of individuals between new sites reversing patch isolation in the landscape. One of main movements supporting these dynamics is dispersal. Responses to road systems and landscape feautures give insights on the mechanics of how the system and the landscape are supporting newts' population dynamics. This thesis shows responses changing from the local perpective to a landscape perpective, describing the effects of mitigation systems and roads to functional connectivity.

Finally, I further discuss how road impacts in responses can be be measured before-after construction to describe how *T. cristatus* behavioural changes are useful to define clear landscape connectivity goals. Changes in responses before development can be predicted by using spatial explicit models and individual-based models which both can be used to indicate degrees of functional connectivity. From landscape to local level and where and how the species perform dispersal routes. Changes in responses after the construction can be measured by identifying the spatial and temporal use of the system and adapting the recognition of response patterns methodology. Before-after comparisons of spatial and temporal patterns of responses may help achieve goals towards the understanding of effective dispersal movements in mitigation systems. As explained, this evaluation needs clear insights on the mechanics and functional use of the mitigation system. Therefore, (1) I discuss how monitoring data can be used to calculate and include goals to analyse spatial and temporal parameters of newt responses and (2) I suggest how this data can be included among two specific stages of road development projects (strategic planning and design).

7.1 Patterns and variability in movement of newts in road mitigation systems: spatial and temporal analysis

Monitoring data at a road mitigation system were used to draw general conclusions on the spatial and temporal patterns of movements of newts in the system. Results showed a high degree of variability in use among individuals along the fence and tunnels (**Chapter 3**; **Chapter 5**). A high variation in annual captures and skewed numbers of attempted and successful crossings, influenced by seasonality and direction from both sides of the road (**Chapter 3**). Variation in annual patterns help draw improvements towards effective definition of goals to maintain connectivity at landscape scale by establishing and implying measured local scales of movements taking place for a particular population (**Chapter 3**; **Chapter 4**).

Nevertheless, patterns of connectivity assessed as small scale movements were analysed by verifying captures per day and months (seasonally) and larger scale movement patterns from the population were analysed by years (Crooks and Sanjayan (eds), 2006). Small-scale behavioural responses varied between spatial and temporal scales in a road mitigation system. Seasonal captures revealed behavioural nature of movement being dependent on the position in the system. The fence was effective at stopping road mortality by keeping individuals away from the road, however it worked as an additional barrier to movement and did not guide animals towards the tunnel. It acted like a barrier preventing road mortality, but also preventing movement of the majority of individuals. This low spatial permeability of road mitigation as already been detected for amphibians (Feldmann and Geiger, 1989, Buck-Dobrick and Dobrick, 1989).

Further studies have found this variance in numbers of captures over time at different parts of the system is related to individual patterns of species behaviour towards anthropogenic infrastructures (Buck-Dobrick and Dobrick, 1989). From a behavioural perspective, as individuals choose to not follow the fence or enter the tunnel, the possible presence of a semi-permeability to movement of the system is suggested. In the long-term, fluctuation in system usage does not necessarily translate to an inadequate performance of the system for amphibians, because it may simply reflect dynamics of the population and inter-annual variation in juvenile emergence (e.g. Jolivet, 2008), weather patterns (e.g. Jochimsen et al., 2004), years when individuals do not breed (e.g. Jolivet, 2008) and juvenile dispersal (e.g. Jolivet, 2008), all of which have influence in the numbers of animals available to use the system. Flutuation in diferent parts of the system, from the fence to successful tunnel crossings, can indicate number of migrants/dispersers that encountered the fence and successfully crossed the tunnel. For example, in **Chapter 3**, the system supported the successful crossing of 10.8% of moving individuals for the four years of monitoring in total. For planning purposes, this information advises on the sucessful stream of individuals between habitat patches and sub-populations, directionality of movement can then be calculated for further to understand the number of individuals expected to cross between the two sides (Knowlton and Graham, 2010; Hamer et al., 2014).

The temporal analysis indicated tunnels being mostly used during autumn movements. Long-distanced and more explorative behaviour occurred during autumn (especially for juveniles) relative to spring movements (Sinsch, 2014). During spring, crossing rates for adults were low, which could indicate breeding migration through tunnels was limited and could also be influenced by number of individuals surviving winter hibernation (Griffiths et al., 2010). The autumnal distribution pattern of road use is also evident for other newt species (Garriga et al., 2015). Usage rates of the mitigation system varied between years. Although, rates were especially low during 2011, captures at the fence increased over time while captures at the tunnel entrances and subsequently the actual frequency of crossings decreased. For herpetofauna species, fluctuations in usage between years are common in road systems contributing to uncertainty when evaluating effectiveness (Aresco, 2005; Smith et al., 2014). Temporal pattern results can indicate how the system is maintaining flow of dispersers over the year for this specific population.

As described in **Chapter 5,** this study showed seasonality playing an important role in the movements of individual newts. During autumn, higher numbers of individuals were found moving along the fence in both directions (towards and away from the tunnels) and movements inside the tunnel were *observed* versus *no observed* individuals in spring. The nature of movement behaviours (straightness of path) associated with foraging and dispersal was the main trait differing between seasons. Contrary to other studies, tortuous paths have been associated with fences and tunnel areas in other in situ mitigation systems (Pagnucco et al., 2011). Here, tortuosity of paths were likely to be associated with the phase of a newt's life cycle within a given season. For example, more juveniles were found moving through the terrestrial matrix during autumn than during other seasons (Pagnucco et al., 2011). It is clear that effective road mitigation for great crested newts must facilitate movement of newts between appropriate resource patches during each part of the newt life cycle, which vary between seasons. Variability is important to understand where the threshold in patterns of movement lie. For example, variation in seasonal movements' patterns may show amphibians using the fence and tunnels while foraging, travelling/dispersing and hibernating at different rates depending if it is autumn or spring. In this study, the highest extreme of variability occurs during autumn where individuals' extent their movement distances and express higher variability of behavioural traits compared to spring after hibernation. Multiple purposes corresponding to different components of newt seasonal cycles to the use of the infrastructure could help better-focus mitigation design goals and hence enhance rates of use and successful flow of individuals between habitats patches (Abrahms et al., 2015).

In **Chapter 6**, the influence of environmental factors on spatial and temporal patterns of newt movements in road mitigation systems indicated the likelihood of an overall increase of variability in use in the future due to climate change. First, a strong positive relationship between some climatic factors outside the tunnels and those inside the tunnels was observed. No significant variability was determined for air temperature and humidity levels between these two environments, however, there were slight changes in the air temperature between the middle of tunnels and the environment outside over the course of a night, which has also been observed before (Hamer et al., 2014). These changes are not considered to have a direct influence on amphibian behaviour while crossing tunnels (Hamer et al., 2014) as they might in open environments, such as on roads (Gravel et al., 2012). However, at the weekly scale, environmental conditions outside influenced rates of amphibian road mitigation use and this has been recorded in other cases since the timing of migration onset is dictated by temperature (ºC) and total precipitation (mm) (Pagnucco et al., 2012). Predicting weekly variation from future weather patterns may indicate the potential use rate for amphibians and include this factor within strategic road plans to asses' successful crossings for road mitigation designs for newts (e.g. Van Teeffelen et al., 2015). As mentioned above, plasticity in behaviour is directly correlated to local climatic conditions, the continuous analysis of weather factors in road mitigation systems at this scale can help calculate estimates of landscape connectivity in planning stages. In this stage changes in weather patterns such as temperature increase in winter or low precipitation levels during seasonal movements can be used to predict changes in use rates as it may influence the effectiveness of a mitigation system over time at landscape level (Van Teeffelen et al., 2015).

Accumulated precipitation, humidity levels, mean air temperature and maximum temperature varied between seasons and some (max. temperature) influenced the overall capture rate of *T. cristatus* in the system. Both precipitation and temperature are decisive factors driving amphibian phenology and abundance (Ficetola and Maiorano, 2016). These factors determine the timing of seasonal movements and survival before migration through road mitigation systems (Griffiths et al., 2010), such that their effectiveness is conditionally determined by seasonal weather patterns at the local-scale (Pagnucco et al., 2012). Consequently, local weather conditions may modify the overall microclimate and microhabitat at the tunnel entrance. This is the first study describing a potential link between attempts at road crossings and tunnel entrance microclimate *in situ*. However, the overall role of microclimate influencing the decision to cross is poorly understood. Whether a forced increase in humidity at tunnel would encourage crossings or system use remains unclear, despite the belief that moist habitats will provide better conditions for the presence of amphibians in these systems (Veenbaas and Brandjes, 1999). From the planning perspective the best tunnel floor substrate, distance to the nearest wetlands and tunnel microclimate still need to be identified if tunnel use is to be optimised.

Previous studies have reported inter-individual variability and local climatic factors influence road mitigation use rates and this is supported by a strong seasonal effect (Garriga et al., 2016). Temporal variability in newt dispersal patterns in autumn and spring are determined by regional weather patterns. For example, in existing roads amphibian road-kill during dispersal increases with ambient humidity making the consideration of weather variation within seasons a relevant position when planning road mitigation (Garriga et al., 2016). These observations on the influence of weather patterns on local newt movements are still missing from protocols to optimize road mitigation design (Rytwinski et al., 2015), despite their significance for understanding temporal patterns of mitigation infrastructure use. To facilitate this, minimum, maximum and optimum threshold values of temperature, precipitation and humidity during activity peaks of amphibians inside and outside the tunnels need to be established to promote mitigation infrastructure use.

The use of information on behavioural responses to mitigation may support the understanding of patterns of movements in future planning and design when data from other systems is lacking. For planning purposes, temporal and spatial patterns goals can integrate the number of dispersers' successful crossing seasonal/yearly for a specific example and compare it later on with patterns of dispersers of other populations (Smit et al., 2006). Integrating spatial and temporal analysis across monitoring studies, variation of usage rates between fence/tunnels, years and seasons will contribute to the regularization of data collected for pattern search after road implementation (Smit et al., 2006; Smith et al., 2014).

7.2 Maintenance of landscape connectivity at multiple scales: habitat composition and availability

In **Chapter 3**, local scale directionality analysis showed newt movements being bidirectional between habitat patches when a road acts as barrier and road mitigation is in place. From our case study, habitat on both sides of the road was similar, with areas of woodland and ponds, however the size of the habitat areas between sides was different. *T. cristatus* capture numbers were higher at the bigger patch compared to the smaller sized patch. Other studies have determined directionality of movement through road mitigation systems to be related to habitat type on both sides of the road. However, these studies have shown most movements being unidirectional or following a non-random pattern with a well travelled route, with individuals moving from hibernation sites located on one side towards reproductive ponds on the other side (Pagnucco et al, 2012; Crosby, 2014). Tunnel systems must enable two-way movements of amphibians as it permits migration in both directions with no need to install them in pairs (Zuiderwick, 1989). If habitat composition and quality is the same on both sides, movement might be driven without habitat selection constraining migration directionality. For this reason, low friction landscape patches on both sides present the best option for efficient local corridors enabling amphibians to achieve their life cycles and connect isolated populations (Joly et al., 2003). Directionality between habitat patches can provide information on the strength of the connection (Peterman et al., 2013).

However, during juvenile dispersal this may not work in the same way. For example, several studies have found terrestrial habitat quality surrounding ponds influencing juvenile survival and the duration and directionality of juvenile movements (deMaynadier and Hunter 1999; Rittenhouse and Semlitsch 2006; Popescu and Hunter 2011). In temperate regions, juvenile movements during dispersal phase are explorative and may not necessary be driven towards a specific direction, especially if plenty of refuge areas are found within the range of movements (Sinsch, 2014). Therefore, analysing directionality in tunnels for juveniles can provide knowledge on the degree of dispersal rate and percentage of dispersers during a determined year. A dispersal rate could function as an index to calculate the effective connection between sides of the road. This index would include number of actual dispersers in the fence versus actual dispersers' successful crossings. For this however, more information is needed on the mechanics of flow of individuals along systems would help determine what type of goals for the direction of gene flow and hence understand the influence of the source-sink status of habitat patches at local-levels for the long-term role of mitigation in the landscape (Grift et al., 2012).

In **Chapter 5**, annual home-range (AHR) patch sizes were used to analyse potential dispersal corridors representing best located sites for inter-population movements at the landscape-level when roads acted as barriers. These AHR patch sizes varied across the study area; we found bigger patches located within woodland and near (within 400m) higher numbers of ponds. This threshold of 400m was tested before to calculate the importance of terrestrial availability and landscape connectivity for pond-breeding amphibians (Denöel and Ficetola, 2007). Different species of newts tolerate different maximum distance thresholds among suitable landscape components such as forest forest and unsuitable landscape components crops (Denöel and Ficetola, 2007). Despite telemetry studies showing 95% of *T. cristatus* movements concentrated in a maximum 70m around a pond patch per season (e.g. Jehle, 2000) 250 m is the maximum threshold around breeding ponds where development impact analysis is applicable at local level in England (Natural England, 2015b). This means that long-term maximum accumulative movement for pond-breeding amphibians may be underestimated when measuring total distance of movement in a life of an amphibian based only on telemetry data (Sinsch, 2014).

The maximum distance threshold for AHR patches is highly dependable on the variability of the terrain given a determined area of development. The optimum maximum threshold can be calculated during the planning phases of a project where landscape features such as pond distribution, land use cover, altitude, etc. can be embedded in spatial-explicit models. By including these type of models in the strategic planning the best threshold for AHR patches and the appropriate scale of action can be calculated for a certain area accordingly with the availability of dispersal corridors. This type of analysis can also provide baseline information not only for road mitigation planning but for long-term newt conservation by defining high-quality habitat of hibernation/refuge sites and hence optimal placement of dispersal corridors. When applying a buffer with distance of 400m to calculate AHR patches, the overall connection between these were low even when considering the worst case scenario of fragmentation caused by all linear infrastructures acting as barriers. The use and analysis of maximum distance threshold around breeding ponds impacted by development is valuable for landscape-scale planning to ensure that subpopulations remain connected in order to support the persistence of the metapopulation in an otherwise fragmented landscape (Denöel and Ficetola 2007). At the same time, at the local level, woodland and nearby (within 400m) higher density of ponds are crucial over the long-term for amphibian species, such as newts. (Harper et al., 2008). For example, high probability of local newt population persistence requires sufficient composition and structure of terrestrial habitat within a threshold distance of the annual home-range. This way, the risk of local extinction can be reduced by habitat availability and vice-versa, as local dynamics are highly dependent on preservation of ponds at the landscape level (Harper et al., 2008; Ficetola et al., 2015).

Both of these findings suggest the modelling of landscape metrics (composition and availability) could help predict road effects on *T. cristatus* movements when planning and designing roads and road mitigation, both including pond and terrestrial areas at the landscape scale. Another landscape feature to include in this analysis is the location of uninhabited ponds. We showed that uninhabited ponds where *T. cristatus* presence is not confirmed can amplify habitat availability in sub-urban areas. The importance of the quality of the terrestrial habitat surrounding inhabited ponds and the abundance of pond-breeding amphibian species at regional scale are influenced by the presence of inhabited ponds (Denöel and Lehman, 2006). The abundance of newts at the landscape scale is linked to the number and distribution of inhabited ponds, which act as stepping-stones for recolonization and dispersal and having an additional role on stabilizing amphibians movement in the landscape at long-term (Denöel and Lehman, 2006; Peterman et al., 2013). The overall balance of the metapopulation structure can thus be maintained by this “rescue effect” when including the presence of uninhabited ponds in conservation efforts (e.g. Denöel and Lehman, 2006).

This adds an interesting point for planning and project development schemes for road projects where ponds with no recorded newt presence and which are in the path of planned road development may normally be destroyed or substituted by terrestrial habitat (Gustafson et al., 2016). This study illustrates the potential importance of apparently uninhabited ponds when evaluating landscape connectivity for newts since their presence can decrease inhabited pond isolation and hence promote metapopulation persistence (Denöel and Lehman, 2006). Concerning planning and designing road mitigation systems, any development and action concerning ponds at which *T. cristatus* presence is not confirmed should be evaluated at the landscape scale and their potential role in promoting long-term connectivity should be considered. Ponds concluded as uninhabited, using traditional techniques, may actually be inhabited, but newts may not have been detected, or may be temporarily inhabited. If so, environmental DNA monitoring based on the analysis of individual migrants or breeding evidence in ponds can confirm the absence of *T.* cristatus in these areas (Biggs et al., 2015). This technique has been recommended to use as confirmation of false negative for the presence of *T. cristatus* in ponds in large scale studies as it represents a cheaper, more effective and easier technique to apply when compared with bottle trapping and egg counting (Biggs et al., 2015). A study in the UK using data from more than 250 ponds showed eDNA monitoring confirming presence of *T. cristatus* correctly in 91.5% of the cases (Biggs et al., 2015). This monitoring tool can help the inclusion of uninhabited ponds as potential newt habitat within the context of the broader landscape at the planning stage. In any case, if the newts are present in the pond the pond must be proptected. This is crucial to help prioritise development and mitigation actions in order to better conserve newt metapopulations and maintain potential connectivity (Betts et al., 2015).

7.3 Why integrating patterns and scales of movement into road mitigation effectiveness assessment?

Resource requirements change across spatial scales due to variation in connectivity between the local and landscape scale (Pittman et al., 2014). At the local scale, most behavioural studies showed spatial variability in the direction of newts in relation to the mitigation infrastructure. This has usually been translated in terms of rates of infrastructure use or in terms of selection (or avoidance) based on the rejection/attraction towards fences and tunnels (e.g. Smith and Sutherland, 2014). A series of behavioural measurements (such as distance travelled and path straightness) can be drawn from these results to describe the main responses to mitigation infrastructure in more detail since they are essential to understand the mechanistic use of mitigation systems (Pagnucco et al., 2011). Accordingly, it is proposed that the identification of resource selection attributed to a behavioural response can help identify the role of an anthropogenic infrastructure (e.g. roads) in supporting those responses (Abrahms et al., 2015). Therefore, the role of the system's constituent parts in supporting multiple aspects of newt behaviours at the local-scale is significant for landscape connectivity. Our results support the conclusion that different parts of a system assist expression of distinctive behavioural traits of newts while moving: foraging, refuge-use and migration/dispersal. This idea has been discussed previously (Hamer et al., 2015), however the incorporation of behavioural measures as part of effectiveness monitoring has not been advocated. Future systems could be improved if resource selection is considered prior to implementation to promote attraction towards fences and tunnel crossings (Woltz et al., 2008, Hamer et al., 2014).

At the landscape level, other studies have found that incorporating inter-individual variability in dispersal (plasticity in behaviour) often leads variable estimates of landscape connectivity (Palmer et al., 2014). Inter-individual variability is bimodal as dispersal behaviour is highly influenced by two important factors (1) **local pressure to individual selection** as we previously stated in **sections 7.1 and 7.2**, where differences in population dynamics and landscape structure exert dispersal pressure on individuals and (2) **plasticity in dispersal strategies** can also be condition-dependent (Palmer et al., 2014). Both factors can be used to understand the nature of behavioural variability in measuring goals for effective road mitigation. For instance, local climatic conditions influence pond-breeding amphibians dispersal propensity of an individual as it controls its phenology (Timm et al., 2007). If weather conditions are not suitable for yearly movement, survival can be compromised (Timm et al., 2007). Local climatic conditions may determine the sedentariness and nomadic profile of a given individual during seasonal movements (Baguette et al., 2012). More sedentary individuals remain within their home-range where all the life cycle resources can be found and (2) nomadic individuals frequently explore their environment searching for resources outside their home-range (Baguette et al., 2012).

At the population level, recent studies showed *T. cristatus* displaying highly side phonotype (e.g. homing) and a low site faithful phenotype (Denöel et al., 2018). Results showed high infidelity rate not only during autumn but including breeding season. Dispersal behaviour occurred within intra-annual movements and be biphasic during the breeding season. Individuals would move to diferent ponds within the reproductive site multiples times in a year and the probability would increase with the consistency of movements. Individuals that would move over a specific time frame would have the tendency to move again (Denöel et al., 2018). The coexistence of both dispersal strategies behaviours within a population adds to the probability of dispersal movement between ponds at the landscape level multiple times per year given a specific population. This study showed the special heteregeneity of newts' intra-population and inter-population movements being driven in addiction to natal sites. It adds a valuable support for the analysis of landscape connectivity at multiple scales for diferent dispersal strategies (**Chapter 4; Chapter 5**).

Following monitoring of climate variables and infrastructure effectiveness across a range of systems and circumstances, simulations of the potential local and landscape responses of newts to a mitigation system under different weather patterns could estimation of likely mitigation effectiveness of new or planned systems (Van der Grift et al., 2015). Additionally, inclusion of detailed behavioural responses (e.g. orientation of newts/rates of rejection in parts of the system per season) could help optimise mitigation system placement within a landscape to promote spring migration and dispersal via the tunnels across years. Tunnel rates preditions for both seasons must be complemented with climatic factors potential disturbance in numbers expected for a given population (Pontoppidan and Nachman, 2013). Information can be collected for several affected local populations and then be extrapolated to understand how at a landscape scale road effects are more prominent. Moreover, results from behavioural studies in local sites have the potential to supply a platform for new understanding on the barrier effects in movement to regional population dynamics (Pontoppidan and Nachman, 2013). How this information can be collected and analysed is explained in the following section.

In conclusion, the identification regional scales and respective spatial and temporal patterns of behaviour at local scale with associated variability (individual and climatic factors) can provide an initial structure that determine planning goals supporting multiple aspects of newt behaviours significant for landscape connectivity.

7.4 How to include patterns and scales of movement to calculate potential landscape connectivity?

This study has highlighted the importance of including information on spatial and temporal patterns and scales of connectivity in newt movements for planning and designing road mitigation. The aim of these projects must be to maintain conservation status by maintaining metapopulation dynamics. This may be achieved by maintaining/improving landscape connectivity to facilitatie dispersal and migration. It is not feasible for road engineers to measure population dynamics as long-term measurement of population flux at landscapes scales (including before-after comparisons), such as newt tracking, mark-recapture, genetic studies are time and cost prohibitive, and hence potential connectivity analysis may offer a proxy prior to mitigation implementation.

Connectivity analysis can be used to define the aim of a road project and to set a value standard against which scale effectiveness can be assessed (Schmidt and Zumbach, 2008; Lesbarères and Fahrig, 2012; Hamer et al., 2015). Our results do not confirm that mitigation effectiveness is maintened at long-term only by calculating potential connectivity at a regional or scale level. However, it showed how potential connectivity can be calculated to prioritize areas where roads may have an impact in population dynamics and identify main newts terrestrial corridors.

Potential connectivity of a landscape is therefore a tool henced to assess structural connectivity in combination with data on a species' presence and movement patterns in relation to landscape structures (Ernst, 2014). In this way, spatial and temporal connectivity patterns are estimated to help define the most appropriate scale to maintain connectivity for a certain region or population. Within these spatial scales, road mitigation planning has to account for the possible variability in movement and climatic factors that influence newt responses (**Fig. 7.1**).

Within a connectivity analysis for newts in a landscape with road mitigation infrastructure the first response property is scale newt movements can be summarised at local and landscape scales separately. Local scale movements can correspond to yearly movements, seasonal migration to and from reproductive ponds, within home-range areas/intrapopulational.

Landscape level scale correspond to maximum accumulated distance to individual dispersers, outside home-range areas/interpopulational. Scale analysis example: road barrier effects on annual home-range patches/dispersal corridors. The second property is patterns; the spatial and temporal patterns of newt movements – in response to landscape features, including mitigation systems, measured over time (between seasons or years). The third property is variability of behavioural responses to seasonality, where plasticity in individual behaviour is conditioned by weather factors in newts. For each property, we describe how responses can be analysed and determined (**Fig 7.1**).

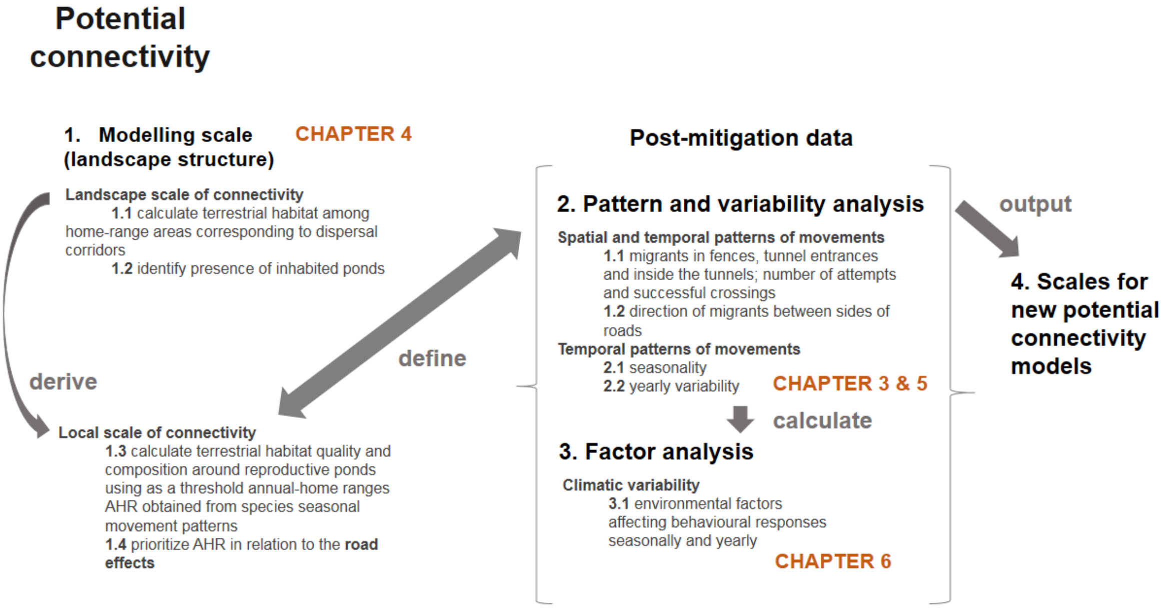

Fig. 7.1 Response proprieties and examples of respective variables that can be used to calculate potential connectivity for *T. cristatus.*

For example, to calculate potential local and landscape connectivity the project planning must have into account patterns of response movements within annual home-ranges and movements outside annual home-ranges. This must be achieved by considering responses for two scales and for two scenarios: 1) various degrees fragmentation from linear infrastructures (regional scale) and (2) potential mitigation (prioritize at local-scale). Patterns of behaviour along fences during a certain season post-mitigation will determined the most appropriated scale of analysis for connectivity for future mitigation models. Here, other behavioural traits could be identified during migration and dispersal season at local-scale and be used for the new models as well.

7.5 Measuring effectiveness by stages

> "If road mitigation experiments became a standard part of any new or existing road project, how will the next 20-30 years look? Quite simply, improvements in road mitigation would increase rapidly. Each new mitigation experiment would build on the insights from past experiments (even those in other regions or on other species) and be incorporated in the design of new road mitigation experiments to ensure more effective mitigation." – Rytwinski et. al., 2015

Rytwinski et al. (2015) argued the definition of effectiveness is still under development as studies in road ecology improve knowledge on the benefits of mitigation deployment for landscape connectivity and wildlife movements. Effectiveness with respect to facilitation of dispersal through a road mitigation system is governed by a complex combination of factors influencing spatial and temporal patterns of movement for pond-breeding amphibians (Lister et al., 2015). Effective road mitigation for pond-breeding amphibians would have to maintain/improve landscape connectivity, by facilitating dispersal and migration (Jackson and Tyning, 1989, Schmidt and Zumbach, 2008; Beebee, 2013; Hamer et al., 2015). The first definition o and the introduction of the term "tunnel efficiency", is stated as "high" number of individuals successfully crossing the tunnels and movements of individual amphibians from and to hibernation sites in road systems combined with the influence of abiotic factors in the use of mitigation (Jackson and Tyning, 1989). However, from a metapopulation dynamics perspetive effective road mitigation measures for *T. cristatus* would have to guarantee that population processes (including movement) for a certain population, in a certain area, would ocurr as if the road did not exist (Rytwinski et. al., 2015).

This study has shown that movement patterns of *T. cristatus* are associated with their position in the system, the age of individuals, and the season. However, the long-term consequences of variation in mitigation system use between years for this species for metapopulation dynamics is still not fully explained. It remains unclear whether current road mitigation practices for newts adequately account for the temporal and spatial scales at which newt metapopulations operate, and hence their long-term effectiveness cannot yet be predicted.

Additional evidence for defining appropriate goals by which effectiveness can be assessed and hence measurements taken are still required (Hamer et al., 2015). Therefore, is vital to road mitigation effectiveness assessment to track and accumulate new data to operate effectively in new management pratices (Williams, 2011; Rytwinski et. al., 2015).

There are four sources of uncertainties when dealing with management of ecological systems: (1) partial observability, refers to uncertainty about population status and individual behaviours; (2) environmental uncertainty, corresponds to the influence of stochastic events, such as variability in climate; (3) partial controllability, accounts for the differences between the actions directed by the decision makers and the actions that are actually applied; (4) structural or process uncertainty, concerns the lack of understanding of ecological relationships and population dynamics (Williams, 2011). For *T. cristatus*, all of these uncertainties limit the capacity to make informed decisions about road mitigation and will influence the effect of development results on the population, therefore influencing system effectiveness (Williams, 2011; Williams and Brown, 2012).

The majority of current advice from planning authorities is based on fixed distance buffers around breeding ponds (Lesbarères and Fahrig, 2012), but does not indicate how to assess the overall indirect impact of roads on dispersal corridors and amphibian movements among local and regional populations (Schmidt and Zumbach, 2008; Lesbarères and Fahrig, 2012; Hamer et al., 2015). Neither does it take account of environmental effects on amphibian use of mitigation structres (Hamer et al., 2015), nor does it include guidance on sound monitoring practices for effectiveness estimation (Schmidt and Zumbach, 2008; Smith et al., 2014; Van der Ree and Jaeger, 2015). The current guidance is prescriptive and hence inflexible, and the considerable uncertainties associated with predicting effectiveness mean that an adaptive approach is required. Before moving to monitoring of effectiveness decision making needs to be adapted for future protocol comparison by including (1) **formulation of goals** based on actual spatial and temporal **scales** of movement from mitigation sites, (2) **detection of management opportunities and predictions of management** outcomes of road effects on populations in previous defined seasonal and annual movements and (3) **distinguish key uncertainties accordingly to species populations**, spatial and environmental knowledge available (Williams, 2011).

Adaptive management can be used to set instructive action towards previous implemented goals, “as it occurs through the informative practice of management itself” (Williams, 2011). It relies on the premise that resource management effectiveness is not based on trial and error, but a learning process. Decision making is adapted with formulation of goals, detection of management opportunities, predictions of management outcomes and distinguishing of key uncertainties (Williams, 2011). An adaptive approach to decision making in road mitigation for pond-breeding amphibians would draw inference from the iterative addition of evidence on mitigation effectiveness without having to engage in the extremely challenging and costly measurement of metapopulation dynamics. The learning process would incorporate a strategic planning, a careful design and adaptable testing (Williams, 2011). A setup up and iterative phase can be delimited in order to involve the key components of adaptive management. First the setup phase (strategic planning and project development) includes the framing of the population problems in relation to the development and stakeholders, goals, management alternatives, prediction models and monitoring protocols. Second, the iterative phase uses the previous stipulated elements in an ongoing cycle of learning about the systems case (management intervention, monitoring, assesment and adaptation) (**Fig. 7.2**)

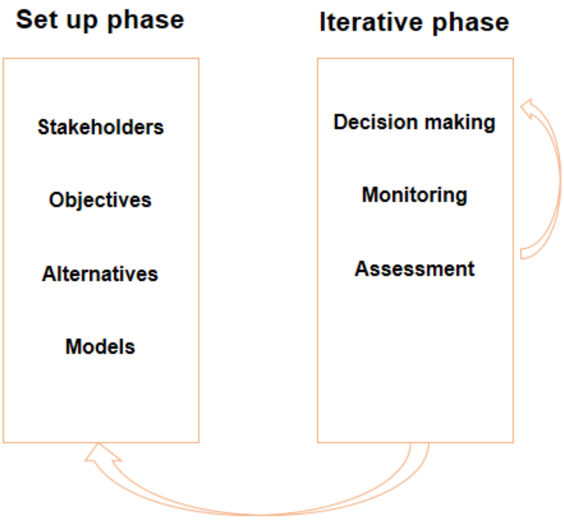

Fig. 7.2 Adaptive management phases used for incorporating adaptive learning in management of ecological resources (adapted from Williams, 2011)

Further on we explain how the design phase is adaptable to the previous phase's results and adaptable to the local/regional needs of each study case. The incorporation of appropriate ecological scales of landscape connectivity for road mitigation planning and design provides new insights on potential connectivity assessment prior to development and allows identification of the presence of barriers to movements from local to landscape scales (Rytwinski et. al., 2015). The addition of predictive models to the planning stage of road mitigation would represent an advancement within the UK and much of Europe for amphibians to standardized potential connectivity protocols (Rytwinski et. al., 2015). For the setup up and iterative phase, we suggest how important results from the chapters can be used as new research lines to test effectiveness for long-term for pond-breeding amphibians and how the methodology and results can be included to calculate connectivity in protocols.

A. SETUP PHASE: strategic planning and project development:

The setup phase would include strategic planning and project development of road construction, land acquisition and mitigation design. This phase must consider the inclusion of main spatial scales of species population dynamics, from local to landscape, including seasonal variation in order to **estimate potential landscape connectivity** and **acknowledging intra- and inter- annual variation in movements**. Here the main objective is to calculate potential connectivity for a species region and metapopulation, including connectivity analysis from landscape to local scale. This can be achieved by defining actual reference state of the system at local/regional level and build models with connectivity prediction and individual variation (**Fig. 7.3**):

A.1 POTENTIAL CONNECTIVITY

Local and landscape scales: Predicting connectivity at local and regional scales (**Chapter 5**) analyse and calculate landscape resistance and effects of fragmentation on overall habitat quality and availability at the landscape-scale. This analysis takes into account landscape scales of dispersal movements at medium-term (**Table 7.1**).

1. Identify and prioritize local and critical areas requiring an increase in connectivity by means of incorporating in regional development planning home range patches, (ponds with and without newts), resource selection, high-quality habitat areas and minor roads.

2. Calculate potential dispersal corridors and connectivity indexes at regional level by applying resource selection and the presence of high-quality habitat areas and features for the species.

Table 7.1 – <u>SCALES:</u> data collection, analysis and results for local and landscape scales

DATA COLLECTION AND ANALYSIS:	
Define spatial and temporal patterns of movements for species	***Prediction for future development:***
Presence/absence species map distribution in regional context	Habitat suitability index analysis for resource selection at regional scale Dispersal corridor analysis with road effects scenarios
Calculate aquatic/terrestrial areas for species life cycle in regional context: annual home-range patches	Connectivity indexes and prioritization of annual-home range patches
RESULTS:	
Local and landscape scales defining **potential spatial areas of long-term connectivity** of movements in the local/regional context. Prioritizing areas of mitigation application before moving into project development. **Measurable objectives:** Dispersal corridors at landscape level and prioritization of annual home-range patches. Example: Number of X annual home-range patches located in region Y need to be prioritized for road mitigation to increase	

potential connectivity at landscape scale in the following order: a, b, c for the following dispersal corridors: a1, b1, c1.

B. ITERATIVE PHASE: management intervention, monitoring, assesment and adaptation:

The iterative phase targets the adaptation of habitat management at the landscape-scale and the implementation of road mitigation systems for newts (tunnels and fences) at the local level. Management intervation phase aims to apply the information on newt movements at landscape and local scales from the setup stage for **potential connectivity** in reference to local monitoring protocols of road developments and engineering processes. Monitoring and assessment aim **test measured scales for effectiveness** goals to the adaptive management protocol. Adaptation phase aims to review of preliminary objectives to ensure they accommodate the multiple spatial scales at which newt populations operate. Measurable objectives from **A.1** are integrated, justified and adaptable to the management intervention **B.1** phase to decide on the solution. Measurable objectives from **B.2** are tested using the first set of monitoring data collected following intervention (**Fig. 7.3**):

B.1 MANAGEMENT INTERVENTION

Intervention applied at local level: from potential connectivity analysis in setup phase the annual home-range patches located in region Y need to road mitigation **which increase potential connectivity** at landscape scale are prioritized for intervention.

B.2 MONITORING AND ASSESSMENT

Spatial and temporal patterns: Includes analysis of spatial and temporal scales of movement from monitoring data from previous mitigation schemes (**Chapter 4**), including information on system components and individual/population responses (**Table 7.2**).

1. **Spatial scale:** Description and analysis of impending proportions of use of fences and tunnels on local systems through calculation of overall frequencies for both age and sex. Comparison to neighbour pond adult abundance and number of annual dispersers (juveniles). Set up numerical aims (e.g. number of individual captures per night/number of individuals in the population) based on spatial movement potential in before-control protocols for later comparison with after-impact monitoring results in order to infer on systems spatial use.

2. **Temporal scale**, annual and seasonal patterns of movements: describe and analyse proportions of use per night and season for the local system through calculating overall frequencies for both age and sex and compare it to the neighbour pond adult abundance and number of annual dispersers (juveniles). Set up numerical aims based on annual and seasonal movement potential in before-control protocols for later comparison with after-impact monitoring results in order to infer on annual variation of use.

Table 7.2 – SPATIAL AND TEMPORAL PATTERNS: data collection, analysis and results

DATA COLLECTION AND ANALYSIS:	
Meta-analysis of previous protocols and monitoring data from the region (100km^2):	***Prediction for future development from results of previous protocols and monitoring data from the region (100km^2):***
Fence width Tunnel entrance width Tunnel height and width Number of individual captures per night/seasonal/year at different points before/after application of the system in two directions	Potential response (movement index) to predictor variables (season, year, systems tunnels and fences position) at different local scales including habitat type on both sides of the roads (100, 250,400m buffer)).

RESULTS:
Spatial and temporal patterns defining movement indexes of potential use from short to long-term (migration to dispersal) of movements in the local context. Definition of spatial and temporal scales for connectivity analysis at regional level. Measurable objectives: spatial and temporal variables for potential responses in X road mitigation sites located in region Y. <u>Example:</u> movement in fences increased during autumn. Dispersal movements prevail over spring movements.

<u>Inter-individual behaviour and local climatic factors:</u> include behavioural diversity and climatic variation in estimates of local connectivity (**Chapter 6**). This analysis takes into account the long-term local scale of dispersal.

1. Description of variation in individual behaviours in response to roads and mitgation infrastructures in the landscape, and data on weather patterns (precipitation, temperature and humidity levels) during activity peak seasons (autumn and spring).

Table 7.3 – <u>VARIABILITY:</u> data collection, analysis and results for inter-individual behaviour and local climatic factors

DATA COLLECTION AND ANALYSIS:	
New/first monitoring data (before project development)	***Prediction and development of <u>individual-based model</u> (IBM) using previous calculated numerical goals***
Fence width	Potential response (movement index) to predictor variables (season, year, systems tunnels and

Tunnel entrances width Tunnel height and with Number of individual captures per night/seasonal/year at different points before/after application of the system in two directions Local climatic patterns (precipitation, temperature and humidity)	fences position) at different local scales including habitat type on both sides of the roads (100, 250,400m buffer)). Behavioural variation and simulations of tunnel effectiveness with local climatic patterns variation (precipitation, temperature and humidity) for seasonal migration and dispersal at long-term.

RESULTS:

Iterative model showing individual movement variation prediction in the setup local context at long term. Weather patterns can be defined for potential scenarios of increasing/decreasing dispersal movements with variation in weather patterns among years. Models updated for local setups and finally compared with BACI monitoring data. **Measurable objectives:** Dispersal rates and number of potential successful crossings for a specific local setting and population. Example: SETTING LOCAL GOALS: For main activity peak seasons with variation in weather patterns scenarios among years. Number of potential number of individuals dispersing in population X. Before development number of individuals migration/dispersal at the local setting for population X. After development number of individuals using different sizes of fence, tunnel entrances and successful crossings for population X. MONITORING DATA: Compare predicted results and variation of number of individuals from before/after development to before-after-control-intervention (BACI) monitoring data collected for population X (habitat variables and weather data included).

B.3 ADAPTATION

New models for potential connectivity: adding variation in individual behaviour (e.g. successful crossings and avoidance) and weather patterns data (precipitation, temperature and humidity levels) to build individual-based model (IBM) predictions to analyse potential upper limits of local systems supporting dispersal movements for the population at long-term as it accounts for uncertainty in the system effectiveness.

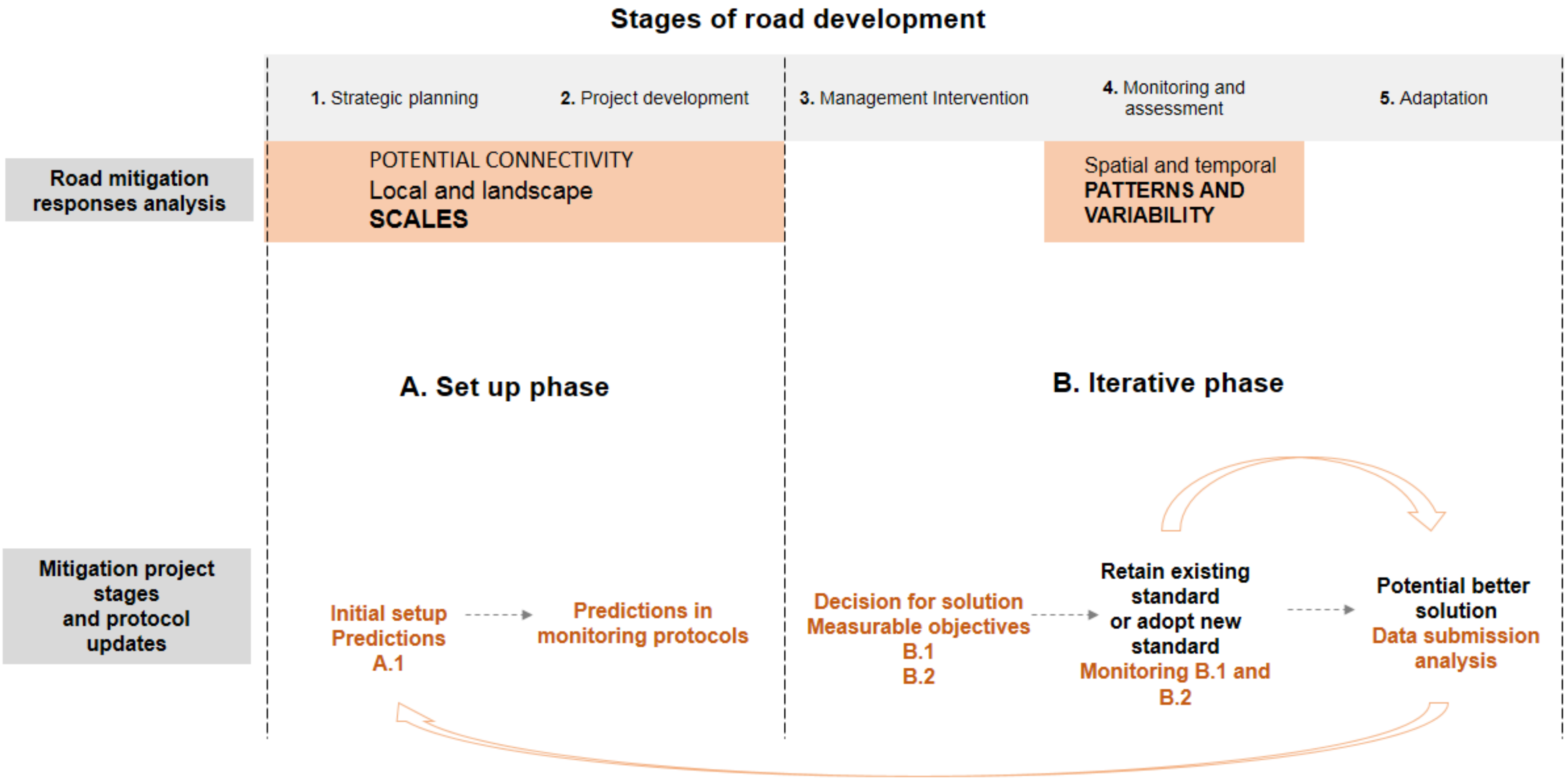

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Fig 7.3 – Proposal for the inclusion of road mitigation objectives and response analysis to current stages of road development using adaptive management practices. Road mitigation responses analysis are represented with orange boxes. Mitigation project stages and protocol updates for (A) Set-up phase and (B) Iterative phase. Arrows represent the learning adapation of monitoring results of patterns and variability in the system (Stages of road development adapted from Rytwinski et al. (2015)).

A road-impacted landscape is more likely to sustain viable newt populations if regional population processes and local behavioural patterns governing them are incorporated into road mitigation planning. Effective planning can learn valuable lessons from systems already implemented if quality monitoring data are available.

Road mitigation system monitoring techniques have increasingly become automated (e.g automatic cameras, Pagnucco et al, 2012), the automatic collection of data on attempted and successful crossings provides an opportunity to better predict outcomes under different planning strategies, although the large volumes of data collected in this way can present challenges for data storage and management. Ths could profitably be solved by forming new digital communities with support of online platforms to help organize information for landscape connectivity analysis and analysis of newt responses to mitigation infrastructure (Pontoppidan and Nachman, 2013). Provision of such data as open-source could help predict patterns of movement for different road mitigation systems (Rytwinski et. al, 2015).

The first “Amphibians and Roads” workshop held in 1989 initiated the beginning of observational data gathering on road mitigation systems and behavioural patterns of amphibians (Langton, 1989). This book comprises a series of monitoring and research studies across Europe and includes the first published account of migrant amphibians’ reactions to tunnel entrances. Thirty years later the inclusion of multiple spatial and temporal scales to newt behaviour and ecology in monitoring and planning is still lacking (Allaback and Laads, 2003). The increase of development projects in areas with Great Crested Newt populations in the UK demands improved best practice and guidance based on up-to-date knowledge of the species ecology at multiple scales. In 2012 a new network was born from the need to introduce new perspectives on mitigating linear infrastructure effects on amphibian and reptile populations. As a national charity aiming to help protect amphibians and reptiles in Britain, Froglife organized a workshop on “amphibians and Roads” in Peterborough, UK in the same year (Langton and Petrovan, 2013). From this workshop, the new network, ENPARTS (European Network for the Protection of Amphibians and Roads from Transport Systems) gathered expertise from all over Europe to discuss the urgent need to develop and promote best practice and research on road effects and road mitigation effectiveness for herpetofauna. ENPARTS is one of the networks which this study can help.

For ENPARTS, the failure to develop “defined targets” for comparing mitigation schemes seemed to be a root problem for the large number of projects that suffered “bad design and poorly implemented solutions” (Langton and Petrovan, 2013). Networks like ENPARTS seek to ensure promulgation of best practices such as implementing objectives towards connectivity at larger scales and test road mitigation effectiveness by working closely with ecological consultants. One goal would be to advocate the implementation of potential connectivity analysis and reach further from research in landscape ecology results providing a platform for practical strategic solutions settled in basic landscape and local needs for *T. cristatus*. Potential connectivity analysis can be implemented as a standard solution for a set up phase delivering a regional framework for local resolutions. This approach may take off part of the pressure from development organizations to come with the best solution for diferent mitigation locations (Langton and Petrovan, 2013). As mainy wildlife crossing structures are implemented without “being linked to protection of habitat linkages that are a part of a secure network of appropriate habitats on both sides of the transport corridor is better understood” (Langton and Petrovan, 2013).

7.6 Conclusions

Roads impact newts at local and landscape scales, threatening their status globally. Road mitigation is compulsory where *T. cristatus* populations are to be impacted by road development, but little is known about the scale of road impacts on *T. cristatus* metapopulations, or the spatial and temporal scales at which the landscape needs to be managed to preserve species populations. Moreover, 'effectiveness', while not adequately defined for road mitigation for newts, is taken as simply providing a means for safely crossing the road structure, supported by evidence of its use at some point in time, without understanding the temporal pattern of use or whether the frequency of use is sufficient to maintain metapopulation dynamics.

We found that movements of newts varied in road mitigation systems depending on the spatial structure and temporal context of the landscape. These patterns revealed the effects on newt movements of minor road development and road mitigation structures (fences bottleneck of individuals, tunnels can facilitate road crossing) and important seasonal and yearly variation. Seasonal variation needs to be considered to ensure that all parts of newt life cycles are supported by planned mitigation at appropriate spatial scales. However, inter-annual variation may be very challenging to predict so we need to be aware that data on a single year's monitoring is unlikely to be representative of movement in all years. Inter-annual variation can be used as basic data to analyse the population status before-after the development. Mitigation needs to support population processes during years of highest and lowest movement rates. This includes mitigation of the negative consequences of local climatic factors and variation in responses between newts for numbers of newts in road mitigation systems.

Nevertheless, we conclude that incorporation of Patterns/Variability and Scales of movement responses into road mitigation planning and design phases is important for improving the calculation of potential connectivity and for evaluating mitigation effectiveness, using biologically-relevant measurements for biologically relevant scales. Current methodology for the evaluation of effectiveness need to shift to a more detailed consideration of the species' behaviour and ecology if metapopulations are to be maintained in perpetuity. When developers only focus their attention on evidence of crossings or the number of animals that cross the tunnel full evaluation of mitigation effectiveness is not achieved because a detailed understanding of movement frequency over time and space and its contribution to metapopulation dynamics is precluded. However, before overall spatial and temporal patterns for road mitigation effectiveness can be optimised, is important to understand where populations of newts may have been under pressure from road development. I suggest the need to adapt future potential connectivity protocols with an actualized and present state for the national situation of *T. cristatus* and other pond-breeding amphibians.

At the national level, the need to adapt landscape modeling for great crested newt long-term conservation protocols is under necessary changes (Bormpoudakis et al., 2015). Developments will impact species populations in the next decade (Bormpoudakis et al., 2015), consequently metapopulation dynamics will change with the possibility of local exctinctions. Is necessary to strategically defined at what extent are these changes acceptable for *T. cristatus* in the UK and divert the effort towards key sites and subpopulations. This way we gain a better understanding of where to conserve at local level and take note of key priority landscape patches required for maintain metapopulation dynamics.

To truly protect the long term conservation of *T. cristatus* impacted by linear infrastructures, would need a) a clear "population, long-term-driven" definition of effectiveness by understanding scale of action of a road mitigation and what patterns occur before-after intervention and b) unambiguous, insightful yet fairly simple method for predicting and evaluating mitigation effectiveness by standardized methodologies for spatial features (tunnels vs. fences) and temporal lines (seasonal vs. yearly). The monitoring and comparison of results are just the beginning to improve the tracking of the overwhelming impact of urbanisation in pond-breeding amphibians and other migratory species (Chapter 3 and 5). The introduction of potential connectivity predictions in planning phase during road development projects would enable monitoring protocols to renew and adapt measurable objectives (Chapter 4) as automated tools can be used to improve the track of mitigation environment throughout development (Chapter 6). Quantitative information held an important role while tracking and inform long-term conservation efforts as many natural habitats are disappearing and connectivity is decreasing at larger-scales (from countries to continents). When habitat fragmentation becomes impossible to stop, mitigation measures and strategies are nature's allies to restore species movement and maintain population persistence. Roads are the first stage of urban sprawl and the initial destabilizer of natural ecosystems. Maintaining connectivity at the landscape level is the main conservation action promoting resilience and structure for the protection of biodiversity (Selva et al., 2011). I finally state that - as roads hold such importance as natural process disruptors, the ecological benefits of areas with no roads ("roadless areas") are the primary concept to include in any permanent solution delivered for road developers and road ecologists hold such responsibility to address this need for future road mitigation (Selva et al., 2011).

8. References

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