The distribution and dispersion of herpetofauna in lowland farmland: with a focus on the common toad (*Bufo bufo*)

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"The clever men at Oxford, know all that there is to be knowed. But they none of them know one half as much, as intelligent Mr. Toad. "

Kenneth Grahame

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Abstract

Concern over unexplained population declines in the common toad (*Bufo bufo*) has led to it being recognised as a priority species for conservation research. The general consensus among herpetologists is that the most important cause of the declines seen as part of a global amphibian extinction crisis is habitat loss and degradation.

The aim of thesis is to investigate the effect of habitat availability and quality on common toad populations in the United Kingdom, with a particular emphasis on the effect of land under agricultural use. I use occupancy modelling, resource selection function modelling and genetic techniques to determine the effects of both terrestrial and aquatic habitat on common toads. Based on my findings, I consider the impacts of habitat at local, landscape and national scales.

My research revealed the importance of pond density and presence of woodland in increasing relative probability of toad occurrence in the terrestrial habitat (Chapters 2 and 3) and the importance of water quality, woodland coverage and available terrestrial habitat coverage in determining common toad presence in ponds. The isolating effect of urban areas is demonstrated in Chapter 4 where Isolation By Barriers (IBB) explains genetic distance between common toad breeding populations better than Isolation By Distance (IBD). In Chapter 5 I again use the resource selection function for terrestrial habitat use developed in Chapter 3, to investigate the importance of terrestrial habitat availability in determining pond use by common toads at a national scale.

In my concluding chapter, I make recommendations for management and further research including consideration of potential interactions between the effect of habitat loss, fragmentation and degradation with other potential causes of common toad decline.

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Over the three field seasons we trained and were helped by 18 volunteers - the volume of data collected would not have been possible without these people. Thanks are due in particular to Sarah Thresher, Sonia Eynard, Laura Evans, Tom Hager, Mark Bradfield, Andrew Buxton, Holly Harrison, Ben Carver, Joe Platt, Sally Le Page, Nick Krol and Jack Browne.

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Chapter 5 would have been impossible without the expertise, experience and enthusiasm of Sonia who was responsible for all laboratory work. Sonia came to the project as a Masters student who'd I'd never met but left as my very dear friend. She did an excellent job with the genetics work and was just good fun to have around anyway. Dr Rob Coles, of Salford University, was a great help to Sonia and I in choosing the microsatellites we worked with and designing the protocol. In addition I would like to thank him for his continued support and providing us with data that significantly improved the impact of the genetics chapter.

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List of Original Publications

Below is a list of the original publications produced during this project. Contributions of authors for those appearing as main chapters are given on the title page of each chapter.

Chapter 2:

Salazar, R.D. Shaw, R.F. & Macdonald, D.W. (submitted) Factors affecting the use of uncultivated field margins by common toad, grass snake and common lizard in UK lowland farmland. Biological Conservation

Chapter 3:

Salazar, R.D., Montgomery, R.A., Thresher, S.E. & Macdonald, D.W. (under review) Mapping the relative probability of common toad occurrence in terrestrial lowland farm habitat in the United Kingdom. PLOS ONE

Chapter 4:

Salazar, R.D., Eynard, S.E., Coles, R.S., Montgomery, R.A., Reading, C.J., Rogers, A. & Macdonald, D.W. (submitted) The effect of isolation by distance and isolation by barriers on common toad *Bufo bufo* genetic distance. Biological Conservation

Chapter 5:

Salazar, R.D., Wilkinson, J.W. & Macdonald, D.W. (in prep) Determinants of pond occupancy for the UK common toad *Bufo bufo.* Biological Conservation

Appendix I:

Evans, L.C., Salazar, R.D. & Macdonald, D.W. (in prep) The effect of refuge material on the probability of detecting three widespread herptile species in UK lowland farmland. Journal of Herpetology.

Appendix II:

Eynard, S.E., Salazar, R.D., Rogers, A. & Macdonald, D.W. (submitted) Genetic structure of the common toad (*Bufo bufo*) population in Oxfordshire. Herpetology, Conservation and Biology.

Chapter 1: Introduction

Toads in a hole

Introduction

Over the past 100 years it has become evident that we are experiencing an amphibian extinction crisis (Houlahan et al. 2000; Wake 1991) which is being echoed across reptile populations (Gibbons et al. 2000). According to the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species, 32% of amphibians worldwide are threatened, with 21% of these recognised as Endangered or Critically Endangered (Baillie et al. 2004). The State of Nature report (Burns et al. 2013) revealed that over 60% of species assessed in the UK have declined in the past 50 years.

Potential causes of the mass decline in amphibians include habitat loss and degradation (including pollution), disease and the effects of climate change.

Of these, habitat change and the associated loss, degradation or fragmentation of areas used by amphibians is considered the greatest contributing factor to the declines we are witnessing as part of the Global Amphibian Extinction Crisis (Bishop et al. 2012; Gallant et al. 2007). A review by Gardner et al. (2007) showed lack of consensus and geographical bias in studies concerning herpetofauna, suggesting that further research into habitat change would be useful. Population declines are expected where habitat fragmentation necessitates risky migrations through unsuitable habitat (Becker et al. 2010). Ficetola and De Bernardi (2004) found community structure to be related to both quality of habitat and the degree of isolation: locally rarer species were those sensitive to habitat change and isolation, particularly newts and toads.

Globally, land conversion for agriculture has been identified as the factor contributing most to loss of habitat for lowland amphibians (Gallant et al. 2007).

In England approximately 60% of terrestrial land cover is classified as enclosed farmland (Natural England 2008), of which the greatest proportion is arable and horticultural land, with a slightly smaller proportion under improved grassland and rough pasture.

Conversion to arable farmland essentially replaces the complex vegetation of the original natural system with a structurally simplified monoculture. Deforestation changes water chemistry in nearby water bodies, reduces shade and reduces the lag time between rainfall and water level rise, potentially altering the hydroperiod. Conversion to pasture farmland can affect amphibians by increasing nutrient inputs to farmland ponds and poaching of the pond bank due to livestock going to the water to drink.

Other examples of habitat degradation through land use change include the increase of pesticides and fertilisers in the natural environment through agricultural conversion. Pesticides such as endosulfan in ecologically relevant concentrations are known to have neurotoxic effects on amphibians, causing impaired development in the common toad (Brunelli et al. 2009). The effect of agricultural chemicals on amphibians include reduced growth, developmental abnormalities and endocrine disruption, leading to reduced diversity and abundance of amphibians (Mann et al. 2009). Land conversion also leads to habitat fragmentation by isolating pockets of suitable habitat through converting land in between. The ability of amphibians (and reptiles) to move between habitats is also reduced by the presence of roads.

The United Kingdom is home to 13 herpetofauna species of varying conservation status. Of these species, 8 appear commonly in UK lowland farmland (common toad *B.bufo*, common frog *Rana temporaria*, common lizard *Zootoca vivipara*, slow worm *Anguis fragilis*, smooth newt *Lissotriton vulgaris*, palmate newt *Lissotriton helveticus*, grass snake *Natrix natrix* and Great crested newt *Triturus cristatus*). Declines in the 7 native amphibian species have been substantially attributed to effect of agricultural intensification (Beebee 2014).

Despite what its name may suggest, the common toad (*Bufo bufo* L.) is not nearly as common as it used to be. There have recently been dramatic declines recorded in many populations and often these declines are largely unexplained (Carrier and Beebee 2003; Young and Beebee 2004). Increasing concern for the species prompted its recognition as a UK Biodiversity Action Plan (UK BAP) priority species in 2007, indicating that it requires conservation action to prevent further declines. There are a few potential factors explaining the population declines noted by Carrier and Beebee (2003) and Bonardi et al. (2011) which include habitat loss (through land conversion for agriculture and development) and degradation, for example use of agricultural pesticides and fertilisers (Boone and James 2003; Boone and Semlitsch 2002; Brunelli et al. 2009), habitat fragmentation, perhaps through habitat loss or dissection by roads (Cushman 2006) and direct mortality through traffic on roads (Andrews et al. 2008b; Beebee 2013; Cooke and Sparks 2004). Emerging diseases; in particular, chytridiomycosis (Garner et al. 2005) and ranavirus (Daszak et al. 1999) also have the potential to exacerbate the effects of other causes of amphibian population declines occurring in concert with them.

Like many other amphibians, toads are particularly sensitive to the effects of land-use change and reduced quality and availability of habitat due to their dual phases requiring both aquatic and terrestrial environments. They are also only moderately mobile (Sinsch 1988) and highly philopatric (Reading 1991) and so vulnerable to isolation of their breeding populations by barriers such as roads or areas of unsuitable habitat. As ectotherms they are sensitive to temperature extremes and likely to be closely associated with habitats that have a more stable microclimate (such as dense vegetation, woodland) where they can protect themselves from freezing or desiccation. Though protected from some predators by toxins in their skin which make them unpalatable, avoidance of predation relies mainly on hiding in dense vegetation or underground as they are neither fast nor strong enough to defend themselves (Lemckert et al. 2012).

When we consider that almost 66% of the agricultural land in England falls under management with an agri-environment scheme (AES) (government funded schemes under which farmers receive payments for employing environmentally sensitive management practices, Natural England 2009), it becomes clear that we may be able to address habitat loss and degradation issues through sensitive management under these schemes. To achieve this we need to know which management options are likely to provide the greatest remediation for amphibians in the agricultural environment.

More than €24 billion were spent on AES in the European Union (EU) in the decade following 1994 and relatively few studies have tested the effectiveness of these schemes in delivering biodiversity conservation goals (Kleijn and Sutherland 2003). Studies concerning amphibians and reptiles are particularly under-represented (Kleijn and Sutherland 2003).

Land taken out of conventional production enhances biodiversity (review across Europe and N.America: Van Buskirk and Willi 2004). AES options increase habitat heterogeneity at the field scale and therefore should be expected to increase biodiversity by providing benefits to a suite of species (Benton et al. 2003; Macdonald et al. 2000). The Amphibian and Reptile Conservation Trust (ARC Trust) has produced guidance which suggests which AES management options have the greatest potential to provide good habitat for herpetofauna species (Baker et al. 2011; Edgar et al. 2010). For example, management and creation of linear features such as hedgerows, drainage ditches and uncultivated field margins may alleviate problems with habitat fragmentation by providing corridors through which herpetofauna are able to migrate between suitable habitat patches. Similarly, buffer strips surrounding ponds and other freshwater bodies may offer some protection from pesticide and fertiliser run off from crops, protecting water quality.

Uncultivated field margins, hedgerows and drainage ditches may also increase the food resource by increasing diversity and abundance of invertebrates. For example, field margins, hedgerows and hedgerow trees along boundaries of arable fields have been shown to increase the abundance and diversity of moths (Merckx et al. 2009; Merckx et al. 2010). Hedgerows have also been identified as important habitats for Great crested newts (Oldham et al. 2000) and grass snakes (Reading and Jofre 2009). Uncultivated margins showed increased abundance of many invertebrate groups when compared with cropped margins (Hof and Bright 2010; Meek et al. 2002) and these margins can be managed for improved provision of food for farmland birds (Vickery et al.

2009). Ditch banks adjacent to newly established field margins have also shown marked increase in plant biodiversity (Musters et al. 2009), increasing habitat heterogeneity which is linked to greater biodiversity in farmland (Benton et al. 2003). Ditches provide shade and shelter from weather extremes and thereby protect amphibians from desiccation (Blann et al. 2002). They can also provide important breeding habitat for toads and other amphibians (Beebee 2012; Hartel et al. 2011) and can be improved by AES management: Maes et al. (2008) found a higher abundance of amphibian species in AES managed ditches compared with that in control ditches.

These management options are also some of the most popular; e.g. 41% of hedgerows in England are managed as part of AES (Natural England 2009) and 6m uncultivated field margins are selected in 18% of Entry Level Stewardship (ELS: the lower AES tier open to all farmers).

Due to their dependency on different habitat types in order to breed, feed and hibernate, toads are likely to be sensitive to effects of agriculture in both the terrestrial and aquatic phases. Studies of toads in agricultural areas have shown avoidance of cropped areas (Janin et al. 2009) and a preference for woodland (particularly broadleaved) in the terrestrial environment (Latham 1997).

With proposed changes in countryside management policy it is crucial that we first understand the value of AES currently in place in order to prioritise which elements to feed into new schemes. The planned Countryside Stewardship scheme will change the options available for farmers and may lead to removal of semi-natural habitats no-longer supported with potentially catastrophic consequences for those species using them.

Creation and management of farmland ponds provide breeding habitat for amphibians and could help to redress the serious decline of pond numbers and quality noted in the Countryside Survey pond report (Williams et al. 2010). Selection of ponds for breeding by amphibians is influenced by both biotic and abiotic factors which may relate to pond quality (Indermaur et al. 2010a). Understanding the conditions that make a pond suitable for breeding amphibians is crucial if we are to be effective in our conservation action. Factors affecting suitability of potential breeding ponds for toads are largely unknown, or may be based on personal observation (e.g. Beebee 1981). There is limited information available in the literature on the likely preferences of toads for breeding ponds. Occupancy and abundance of toads in ponds may be better predicted by the terrestrial habitat than by pond quality (Hartel et al. 2008; Janin et al. 2009; Scribner et al. 2001).

Beebee (1981) suggests that ponds suitable for toads in lowland agricultural areas should be larger, older, contain fish and have a higher ionic score. However, common toad was absent from the 60 farmland ponds studied (Beebee 1981), and the expected suitable conditions are therefore not based on those data. Toads selected larger ponds and fewer of the available ponds in farmland than the *R.temporaria* (Cooke 1975). Halley et al. (1996) also suggested that there is a minimum pond size needed to establish a successful breeding toad population, related to carrying capacity of females, but was unable to provide an exact size.

Traffic poses a threat to all amphibians and reptiles, though it is most often recognised for the toad due to their breeding behaviour which leads to thousands of toads crossing roads to reach their breeding ponds each spring. In lowland central Europe, a recent synthesis of amphibian road traffic mortality data found common toad to be the most frequent victim of traffic in suburban landscapes, with common frog and newts more frequent victims of traffic in rural landscapes (Elzanowski et al. 2009). In moor frogs (*Rana arvalis*), it has been shown that there is a negative relationship between the density of roads in an area and the probability of ponds being occupied (Vos and Chardon 1998). Type of road and traffic load also alters the level of effect, with motorways producing greater chance of direct mortality than lesser roads (e.g. Hels and Buchwald 2001). Though small roads can have a larger than expected associated mortality of toads where they are used and crossed more often than motorways, which are regarded as impenetrable barriers (Matos et al. 2012). Roads are also recognised to pose an indirect threat where they isolate amphibians from large areas of otherwise suitable habitat (Beebee 2012) and where ponds are located near enough to receive pollutants in runoff and the potential pollution level is heightened when roads are salted; salt intolerant amphibians may then be excluded from these ponds (e.g. Collins and Russell 2009). Roads are also likely to represent risky habitats as they are coated in toxins (Forman 2003) and provide no cover from predators and reach temperature extremes (Lemckert et al. 2012).

Habitat fragmentation has been demonstrated as an important factor determining use of sites by toads. For example, the configuration of land use types is a more powerful predictor of toad occupancy than composition (Janin et al. 2009). Furthermore, beyond occupancy habitat quality and availability affect toads at the individual level, Janin et al. (2011b) also discovered that toad stress hormone levels and body condition were indicative of habitat availability and degree of fragmentation.

Arable farmland with its pockets of semi-natural habitats forms a landscape mosaic; the complexity and spatial extent of semi-natural habitats within which will substantially influence the biodiversity supported in the area (Bennett et al. 2006). Proportion of cultivated land is negatively associated with common toad presence (Ray et al. 2002).

When considering the effect of habitat change on a species it is essential to also look at the effect of reduced movement and therefore gene flow on genetic diversity of populations. The role of genetics in conservation research is increasingly recognised (Hedrick 2001). However, study of the conservation genetics of amphibians is under-represented in vertebrate research (Jehle 2010).

Due to philopatry (Reading et al. 1991) and moderate dispersal ability (Sinsch 1988) the common toad is potentially vulnerable to genetic isolation. In studies of toads the effect of isolation of urban environments has been apparent where toads in urban areas showed less genetic diversity than those in rural environments (Hitchings and Beebee 1998). Common toads on Jersey were usually found to be breeding in urban ponds and showed little gene flow between breeding populations, perhaps due to the isolating effect of the urban environment surrounding them (Wilkinson et al. 2007).

Isolation also affects the persistence of populations in pristine habitats; if these populations decline or become locally extinct they cannot be replaced or replenished where the intermittent habitat is so unsuitable as to be impermeable.

As a species which spends the majority of its adult life in the terrestrial environment (Beebee 1985) a comprehensive study of common toad habitat requirements must include habitat beyond the pond to capture the importance of those habitats used for migration, foraging, shelter and hibernation. Habitat use is likely to be different for these different activities as seen in the Western toad (Browne and Paszkowski 2014).

However, it is also important to note that conservation efforts for a species should not focus on habitat related measures alone. A complete approach would consider the potentially exacerbating effect of disease on already vulnerable populations (e.g. Voordouw et al. 2010). Chytridiomycosis (chytrid fungus) has only recently emerged as a significant threat to amphibians worldwide (Garner et al. 2005) and is of particular concern as the nature of the fungus having both hosted and free-swimming forms, makes it difficult to treat and easy to transfer to other water bodies (on gear, boots etc). In addition there may be interactions between habitat change and other sources of decline; disease, climate change, pollution that should not be ignored.

The aim of this thesis will be to assess the importance of terrestrial habitat for the common toad in farmland at a range of scales. Beginning in Chapter 2 we will move from a local ‘within field’ scale investigating the features that affect common toad occupancy of uncultivated field margins, to a larger farm scale developing a resource selection function (RSF) for common toad terrestrial habitat use in Chapter 3. We then go on to a landscape scale in Chapter 4 to determine the effect of barriers between breeding ponds on the genetic structure of common toads in Oxfordshire and in Chapter 5 apply the findings of previous chapters to investigate the role of terrestrial habitat availability in determining common toad pond use across Great Britain. The objective of this assessment of importance of habitat for the common toad is to identify key habitats that should be considered conservation priorities for the species and to inform policy, particularly in farmland where there is an opportunity to action effective conservation through the use of agri-environment schemes. The local, landscape and national scales included in the assessment are important to demonstrate that these lessons can be applied at a range of scales, in farmland and beyond.

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Chapter 2: Factors affecting the use of uncultivated field margins by common toad, grass snake and common lizard in UK lowland farmland

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Title:

Factors affecting the use of uncultivated field margins by common toad, grass snake and common lizard in UK lowland farmland

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Abstract

We monitored three widespread species of conservation concern, the common toad (*Bufo bufo*), grass snake (*Natrix natrix*) and common lizard (*Zootoca vivipara*), in uncultivated field margins on lowland arable farmland in the UK using artificial refuges and visual searches, to assess the importance of different margin characteristics. We used PRESENCE software to model occupancy, colonisation and extinction of the field margins for each species with covariates including distance to the nearest pond, field margin width, presence of a hedgerow, drainage ditch or both in combination.

Common toad colonisation was negatively associated with the increasing distance from the nearest pond. Grass snake detection probability was positively affected by soil temperature at the time of survey and negatively associated with increasing field margin width. Common lizard occupancy was positively associated with greater field margin width.

These results highlight potential actions that could be incorporated within agri-environment schemes on arable farmland in order to improve habitat availability and connectivity for these species. We recommend 1) management of ditches to hold water where they can be used as alternative ponds 2) creation of ponds and planning of new field margins close to existing ponds and ditches 3) extending the width and extent of field margins to increase the uncultivated areas that benefit less mobile species with smaller home ranges, such as the common lizard.

Introduction

The global amphibian extinction crisis has been well documented (Houlahan et al. 2000; Wake 1991). It is echoed by emerging reptile declines (Gibbons et al. 2000) and both are substantially attributed to habitat destruction and degradation (e.g. Gibbons et al. 2000; Stuart et al. 2004b). Reduced quality and quantity of natural and semi-natural habitats are often associated with agricultural conversion and intensification (Robinson and Sutherland 2002). For example, in the UK the 2007 countryside survey highlighted dramatic losses of ponds in the past century due to drainage (Williams et al. 2010). In England the agricultural landscape, accounts for approximately 60% of land cover (Natural England 2008), and also encompasses semi-natural habitats, such as woodland and scrub (12% of total land cover, Burns et al. 2013), ponds and wetlands and linear field boundaries and their associated features, such as uncultivated field margins, ditches and hedgerows. These habitats form a mosaic of different land uses (Bennett et al. 2006) and their persistence is dependent on sensitive management. Agricultural intensification has increased field size and reduced diversity of the farmed landscape (Robinson and Sutherland 2002) though there are opportunities to improve the biodiversity value of these landscapes through appropriate management.

Of England’s enclosed farmland, 66% is managed under agri-environment schemes (AES) (Natural England 2009), which are government funded schemes under which farmers receive payments for environmentally sensitive land management according to specific options. Field edge management options (such as management of hedges and uncultivated field margins in arable fields) are amongst those most frequently selected; e.g. 41% of England’s hedgerows are under AES management (Natural England 2009). The creation of uncultivated field margins around the field edge (henceforth field margins) in varying widths are also a popular option; e.g. 6m field margins found in 18% of Entry Level Stewardship (ELS) agreements (the lower AES tier open to all farmers; Natural England, 2009). Field margins and hedgerows improve the quality and availability of habitat for a suite of farmland species (e.g. Macdonald et al. 2000; Merckx et al. 2009; Vickery et al. 2009).

Loss or lowering in quality of uncultivated habitat features has been implicated in the declines of a range of taxonomic groups on farmland (e.g. pollinators and plants; Biesmeijer et al. 2006; mammals; Harris et al. 1995; birds; Krebs et al. 1999; odonates; Raebel et al. 2012).

In the UK, substantial declines in amphibians and reptiles have been witnessed over the last century, coinciding with changes in agricultural practices, increasing urban development (Cooke and Scorgie 1983) and reduced quantity and quality of ponds (Williams et al. 2010). In light of significant population declines (e.g. Carrier and Beebee 2003) common toad (*Bufo bufo* L.), grass snake (*Natrix natrix* L.) and common lizard (*Zootoca vivipara,* von Jacquin) were designated as UK Biodiversity Action Plan (UK BAP) priority species in 2007 in recognition of the need for conservation action for these species to prevent future declines (UK Biodiversity Partnership 2010).

Some agricultural management options have the potential to provide good habitat for amphibians and reptiles by creating a mosaic of refuges and areas to forage and bask (Baker et al. 2011; Edgar et al. 2010). For example, edge habitats are known to be preferred as habitat corridors by the grass snake (Reading and Jofre 2009) as they provide dense vegetation as a refuge adjacent to open areas for basking. Drainage ditches can be important habitats for the common toad in the absence of other breeding habitat (Beebee 2012; Hartel et al. 2011) and ditches are important movement corridors for other species of amphibians (Mazerolle 2005). The quality and value of ditches for amphibians can be improved by targeted management (e.g. Beebee 2012), and in the Netherlands, ditches managed under AES have greater amphibian species richness and abundance (Maes et al. 2008). The common lizard has a relatively small home range (Clobert et al. 1994) and therefore may be more responsive to habitat changes at a more localised scale, such as within the field margin.

These semi-natural habitats existing within farmland may also offer vital food resources through a greater abundance of invertebrates than in cultivated habitats (e.g. for field margins; Hof and Bright 2010; Meek et al. 2002).

Agricultural landscapes show increased taxonomic richness with increased habitat heterogeneity (Bennett et al. 2006; Benton et al. 2003), including increased species diversity for amphibians and reptiles (Atauri and de Lucio 2001). Ditches, hedgerows and field margins increase habitat heterogeneity, and may therefore be expected to be associated with greater species diversity, though this may depend on the scale at which species perceive heterogeneity of habitat (Wiens 2000).

To assess the value of different uncultivated field margins to common toad, common lizard and grass snake we modelled species occupancy, colonisation and extinction among field margins using covariates such as presence of hedges and ditches, distance from ponds and field margin width. We also modelled the effect of observer and environmental variables such as soil temperature and relative humidity on detection probability. As detection probability can be affected by heterogeneity in abundance of the species between sites (Royle and Nichols

2003) we included covariates also used in modelling occupancy, colonisation and extinction in the global model for detection probability to capture their effect on abundance. For example, distance from pond is highly correlated with pond density, which is an environmental correlate of toad abundance (Scribner et al. 2001). Occupancy is defined as the proportion of sites occupied in a given area (MacKenzie et al. 2002). Sampling and site covariates are included in the models to account for heterogeneity between sites and so allow their effect on the estimates of occupancy, colonisation, extinction and detection probability to be modelled.

We hypothesised that the common toad occupancy and colonisation would be more closely associated with drainage ditches than with hedgerows, particularly when these drainage ditches hold water for most of the year and would be negatively associated with increasing distance from ponds. We predicted that grass snakes would be more closely associated with hedgerows than drainage ditches. We expected common lizard occupancy to be positively associated with wider field margins and hedge and ditch combination sites as they may provide greater level of resources over the species small home range due to increased local habitat heterogeneity.

Methods

Study area and artificial refuges

The study was undertaken on arable farmland around the villages of Garford (51°39'44.49"N, 1°23'6.49"W), Marcham (51°40'3.19"N, 1°20'31.88"W) and East Hanney (51°38'5.49"N, 1°23'37.69"W) in Oxfordshire, UK. The landscape is best described as a mosaic of large arable fields with some areas of pasture and smaller patches of semi-natural habitats (ponds, woodland patches). Most arable fields in the landscape are bordered by uncultivated field margins of rough grassland/tall herb vegetation between 2-20m in width. Fifty one sampling units (transects) were set in five blocks on two farms under ELS management (Figure 1). Transects were in arable fields where the 6m uncultivated field margin option had been applied (uncultivated width of margins often exceeded 6m, varying from 6m to 21m), avoiding North facing boundaries.

Artificial refuges (henceforth refugia) were used to improve the likelihood of recording herptiles (e.g. Scheffers et al. 2009) as the species are difficult to detect using visual surveys alone (e.g. for a small grass snake population detection probability is 0.11 using visual searches, Kery 2002). Refugia were set in pairs, each consisting of one piece of carpet and one piece of roofing felt, each of approximately 50cm x 50cm. All roofing felt was of the same type, from the same manufacturer. Carpets were darkly coloured, to maximise heat absorption (pilot studies demonstrated no significant difference in temperature between the eight types of carpet used (Friedman chi-squared = 2.578, p = 0.9211, df = 7)). Five pairs of refugia, were set per transect, with one pair set every 20m. Each transect was set within the uncultivated field margin at <1m from the linear feature (hedge, ditch or combination), and was a minimum of 20m from any field corners. Each transect was a minimum of 100m from any other set transects to try to ensure independence of transects, unfortunately it would have been impractical to increase distance between transects to reflect maximum home range size of all species (e.g. common toad, 3.6km maximum migration distance, Sinsch 1988).

Transects were set in three treatments/combinations of linear feature: 16 transects set along field margins with a hedge (H), 16 along field margins with a ditch (D) and 19 along field margins where both hedge and ditch were present (HD) (Figure 1). Field margin width was measured and whether ditches were holding water was recorded at the beginning and end of each season. The distance to the nearest pond was measured using ArcGIS (ESRI 2011) from the centre of the transect to the nearest edge of the water body. Relative humidity and soil temperature data were obtained from the local weather station at Wytham (www.ceh.gov.uk). Transect check order was randomised to avoid repeated visits at the same time of day.

Refuge checks and animal handling

Fieldwork followed guidelines set out by the National Amphibian and Reptile Recording Scheme (NARRS, www.narrs.org.uk). Refugia were checked over two seasons (May - October 2011, March - October 2012), in conditions that maximised likelihood of animals using them as described by NARRS. These conditions required temperatures between 10 and 20°C, light/no wind and dry weather. At each check we recorded presence/absence of common toad, grass snake and common lizard. Surveyors competent in identification of the target species also completed a visual search while walking between refuge pairs in each transect and recorded species sightings.

All refuges were set 2 weeks (as recommended in NARRS guidance) before the first visit to allow the animals time to begin finding and using them. Each transect was visited on average 13.68 times (SE = 0.25) over two seasons (minimum visits = 8, maximum visits = 16). Visits were not included when refuges were discovered to be damaged or missing.

Analysis in PRESENCE

We used PRESENCE v7.3 (Hines 2006) occupancy modelling software, based on the single-species, multi-season model by MacKenzie et al. (2006) to estimate the probability of occurrence, colonisation and extinction of each species at each of the 51 sites (transects). The multi-season model assumes closure of sites within a survey season (if occupied/unoccupied it remains such for the season) and sites are open between seasons allowing, for example, an unoccupied site to be colonized (probability of colonization = γ) or an occupied site to go locally extinct (probability of extinction = ε). PRESENCE allows for different survey effort at each site.

Covariates improve the models by accounting for the variation present between sites. Covariates (and abbreviations) modelled for their effect on rates of occupancy (ψ), colonization (γ), extinction (ε) and detection probability (p) appear in Table 1. Continuous covariates (dPond, Temp, Mwidth) were scaled according to Gelman (2013) to be directly comparable with coded categorical variables. The effect of a covariate on occupancy can be described as the change in α, where $\psi(\alpha_1)$ is the estimated proportion of sites occupied by the model and $\psi(\alpha_2)$ is occupancy explained by the first covariate.

For each species, we developed a global model (with all possible covariates) for occupancy, colonization, extinction and detection probability. We then removed each covariate in turn to determine whether they improved the global model. Covariates were retained where ΔAICc >2. We then used the covariates from the simplified model to develop a set of models with those covariates in different combinations. Following standard practice (MacKenzie et al. 2002), models that failed to reach numerical convergence were removed from the analysis.

We used AICc (Akaike Information Criterion corrected for sample size; number of transects) to rank and select the best models for each species. Model-averaging (weighted according to AIC weight) was used to estimate occupancy, colonisation, extinction and detection probability using models with substantial support (ΔAICc <5).

Results

Naïve occupancy (proportion of transects where the species was detected on at least one visit), and model averaged estimated occupancy, colonisation, extinction and probability of detection for each species are presented in Table 2. Detections of all species were patchily distributed over the five survey blocks with observed proportions of sightings significantly different from those expected according to survey effort (Figure 2, G-test, $p<0.001$).

Common toad

Models 1 and 2 (Table 2) are inseparable (ΔAICc < 2) and so the simplest model (model 1) is selected as the most important. Colonization was negatively associated with increasing distance from the nearest pond ($\hat{\boldsymbol{\alpha}}_2 = -3.21$, SE*1.96 = 2.94). Supported models also include terms abundance driven change in detection probability associated with dPond and effect of Mwidth on colonization. However, as $\hat{\boldsymbol{\alpha}}_2 <$SE*1.96 in each case we cannot infer the effect of either of these covariates on either colonization or detection probability. Models that included covariates for occupancy or extinction did not improve the global model and were not selected. The constant model (no covariates) achieved reasonable support (ΔAICc < 5).

Grass snake

Both the selected models include Temp as a covariate for detection probability. Detection probability increased with Temp ($\hat{\alpha}_2$= 1.52, SE*1.96 = 0.84). Detection probability driven by heterogeneity in abundance was negatively associated with Mwidth ($\hat{\alpha}_2$= -2.22, SE*1.96 = 1.98). Models that included covariates for occupancy, colonization or extinction did not improve the global model and were not selected.

Common lizard

Both of the selected models include field margin width (Mwidth) as a covariate for occupancy. Common lizard occupancy is positively associated with Mwidth ($\hat{\alpha}_2$= 4.51, SE*1.96 = 3.48). Model 2 includes the covariate dPond for occupancy but as $\hat{\alpha}_2$<SE*1.96 we cannot make any inference regarding this relationship. Models that included covariates for colonization, extinction or detection probability did not improve the global model and were not selected.

Discussion

For the common toad, colonization of field margins was negatively associated with increasing distance to the nearest pond. Presence of a hedgerow or ditch did not influence toad occupancy or colonisation. There may be some redundancy in the benefits that either hedges or ditches can both provide e.g. even dry ditch banks also provide shade and so protect from desiccation (Blann et al. 2002). Both hedgerows and ditches increase the heterogeneity of field margin habitat and so are both expected to provide increased benefits to toads in terms of improved abundance of invertebrate prey (Benton et al. 2003).

Though there was also no detected effect of wet ditches in this study, management for improved water retention and open water areas may still improve suitability of ditches for toads as potential breeding sites (Beebee 2012). Where ditches reliably hold water for the crucial period of the year (tadpole development) they have the potential to act as ponds as well as providing valuable water sources, distance from which is recognised as being negatively associated with relative probability of toad occurrence in the terrestrial environment (Chapter 3: Salazar et al. in review).

The effect of distance to the nearest pond on colonisation is an intuitive result and expected given the species' dependence on ponds for breeding and known high levels of philopatry (Reading et al. 1991). The results accord with the findings of Chapter 3: Salazar et al. (in review) where the relative probability of toad occurrence increased closer to water bodies including ponds and wet ditches. However, as toads do not use all available ponds for breeding (e.g. a preference for larger breeding sites, Beebee 1985) then distance to any pond may not be as useful a covariate compared with distance to a known breeding site. This would especially be true if toads use wet ditches to navigate through a desiccating environment, the absence of which may force use of ponds as stepping stones. Relocation of toads between breeding ponds has also been shown to be negatively correlated with distance between ponds (Reading et al. 1991).

We had expected the grass snake to be positively associated with hedgerows as found by Reading and Jofre (2009). However, the grass snake did not appear to have distinct relationships with any of the covariates modelled here, only showing a negative effect of field margin width on detection probability (due to lower abundance). This lack of distinct association with habitat features may be due to grass snakes being a more generalist and mobile species (home ranges in excess of 1ha, Reading and Jofre 2009). Ditches could provide much the same benefits as hedgerows as edge habitats, both offering areas to bask adjacent to dense vegetation available as cover (Wisler et al. 2008). Ditches may also be equally preferred as they are likely to present more amphibian prey, as shown here with positive association to common toad occupancy and colonisation. Alternatively, grass snakes may be more affected by landscape scale features, more relevant to their large home range size.

For common lizard, the positive relationship detected between occupancy and field margin width could be explained by this species' very small home range which varies between 20m and 35m in diameter, making populations easy to isolate by inhospitable habitats (Clobert et al. 1994). Maximum field margin width where common lizard was detected in our study was approximately 20m, and so the species home range could fit within these field margins without needing to distort in shape to avoid cultivated areas. The minimum width of field margin where this species was detected was 9m. It would be interesting to discover whether this represents a threshold width under which common lizards are less likely to use field margins. Common lizards occupying narrower field margins (e.g. 6m) would need a much elongated home range to cover the same area whilst still avoiding cultivated ground. As such, common lizards in narrow buffer strips would need to traverse much greater distances to travel between each end of their home range. The findings support the case for improving habitat availability at the field scale by increasing the extent and width of field margins.

Increasing heterogeneity within the field margin habitat through presence of both a hedge and ditch together did not increase the occupancy of any of the three species studied here. For each of the species, either feature on its own seem sufficient, as together the level of redundancy may be enough to outweigh the effect of increased within habitat heterogeneity.

Further research is required to reveal the mechanisms underlying these species occupancy patterns. The effect of field margin width on less mobile species suggests that it could also be informative to investigate the effect of AES options for narrow field margins of only two or four metres in width as well as presence/absence of the field margin itself and the absence of both a ditch and hedgerow.

Field margins and edge habitats (ditches, hedges) managed alongside them should not be considered in isolation. Presence of amphibians in farmland may also be related to presence of scrub and woodland in the terrestrial environment (Beebee 1981, 2012). It would therefore be worth considering the effect of proximity to other landscape features such as roads, woodland/scrub, rough grassland (e.g. potential common toad hibernation sites Bosman et al. 1996) and urban areas on the likelihood of amphibians and reptiles occupying these field margins. Pond creation is an AES management option which could improve habitat availability for common toads, not only in providing much needed breeding habitat and redressing losses in the last century (Williams et al. 2010) but in increasing pond density in the wider landscape and thereby improving rates of colonisation of semi-natural habitats such as uncultivated field margins. This may facilitate movement of the species across the wider landscape to encourage greater levels of mixing between breeding populations.

This study demonstrated herpetofauna use of field margins in arable farmland. Creation of field margins around arable fields is a management option that could be encouraged to a greater extent across European agricultural systems. Insofar as these field margins are thought to improve connectivity at a landscape scale and provide habitat for a suite of species, the degree to which viable populations are able to persist may depend on sensitive placement and management of these field margin strips and their associated features.

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Tables

Table 1: Covariates used to estimate occupancy (*Ψ),* local colonization (*γ*), extinction (*ε*) and detection probability (p) for each species. Covariates (and abbreviations) for each model are described here.

Occupancy/Colonisation/Extinction	Detection probability
Constant (.)	Constant (.)
Site category (H/D/HD)	Site category (H/D/HD)
Presence of wet ditch (Dwet)	Presence of wet ditch (Dwet)
Distance to pond (dPond)	Distance to pond (dPond)
Field margin width (Mwidth)	Field margin width (Mwidth)
	Observer (Obs)
	Soil temperature (Temp)
	Survey (t)

Table 2: Model averaging for supported (ΔAICc <7) for common toad, grass snake and common lizard capture histories. Constant models also included for reference. The models are placed in AICc order. ΔAICc shows the change in AICc when compared with the top-most model. Model averaged estimates of occupancy, colonisation, extinction and detection probability and their associated standard error (SE) are given in italics. N= number of parameters.

Model	AICc	ΔAICc	AIC weight	N	-2*loglike	Ψ	SE Ψ	γ	SE γ	ε	SE ε	P	SE P
Common toad													
Naive Ψ = 0.06													
1. Ψ(.),γ(dPond),ε(.),p(.)	216.44	0	0.3987	5	205.11	0.09	0.05	0.45	0.13	0.41	0.49	0.14	0.04
2. Ψ(.),γ(dPond),ε(.),p(dPond)	217.01	0.57	0.2998	6	203.10	0.11	0.07	0.46	0.16	0.00	0.88	0.12	0.04
3. Ψ(.),γ(Mwidth+dPond),ε(.),p(.)	218.97	2.53	0.1125	6	205.06	0.09	0.05	0.44	0.15	0.41	0.49	0.14	0.04
4. Ψ(.),γ(Mwidth+dPond),ε(.),p(dPond)	219.65	3.21	0.0801	7	203.05	0.11	0.07	0.46	0.18	0.00	0.00	0.12	0.04
5. Ψ(.),γ(Mwidth),ε(.),p(dPond)	219.8	3.36	0.0743	6	205.89	0.13	0.08	0.65	0.24	0.00	0.00	0.09	0.03
6. Ψ(.),γ(.),ε(.),p(.)	221.33	4.89	0.0346	4	212.46	0.10	0.06	0.48	0.14	0.38	0.52	0.13	0.04
Model averaged						0.10	0.06	0.47	0.16	0.22	0.59	0.13	0.04
Total sum weights			1										
Grass snake													
Naive Ψ = 0.29													
1. Ψ(.),γ(.),ε(.),p(Mwidth+Temp)	390.37	0	0.8077	6	376.46	0.38	0.08	0.30	0.11	0.14	0.14	0.23	0.04
2. Ψ(.),γ(.),ε(.),p(Temp)	393.24	2.87	0.1923	5	381.91	0.34	0.08	0.27	0.09	0.14	0.14	0.24	0.04
Model averaged						0.37	0.08	0.29	0.10	0.14	0.14	0.23	0.04
Total sum weights			1										
Common lizard													
Naive Ψ = 0.12													
1. Ψ(Mwidth+dPond),γ(.),ε(.),p(.)	148.34	0	0.8594	6	136.34	0.12	0.06	0.05	0.03	0.32	0.20	0.32	0.05
2. Ψ(Mwidth),γ(.),ε(.),p(.)	151.96	3.62	0.1406	5	141.96	0.14	0.03	0.05	0.03	0.32	0.20	0.32	0.05
Model averaged						0.12	0.05	0.05	0.03	0.32	0.20	0.32	0.05
Total sum weights			1										

Figures

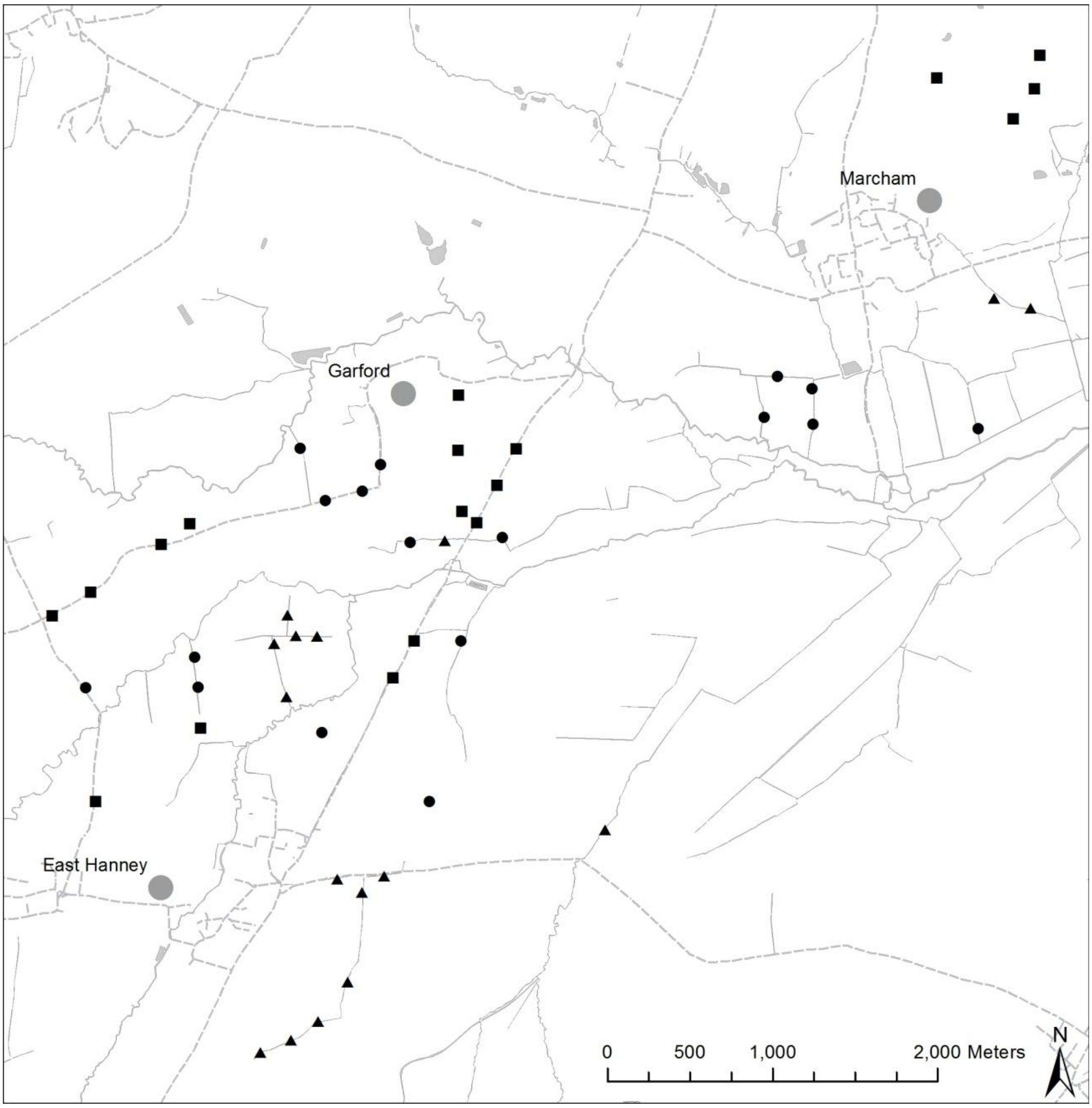

Figure 1: Locations of the 51 refugia transects: hedge + field margin (H, black square), ditch + field margin (D, black triangle), hedge + ditch + field margin (HD, black circle). Road (grey dashed line), named place (grey circle) and surface water (grey polygons) layers from OS Open Data www.ordnancesurvey.co.uk (Crown Copyright).

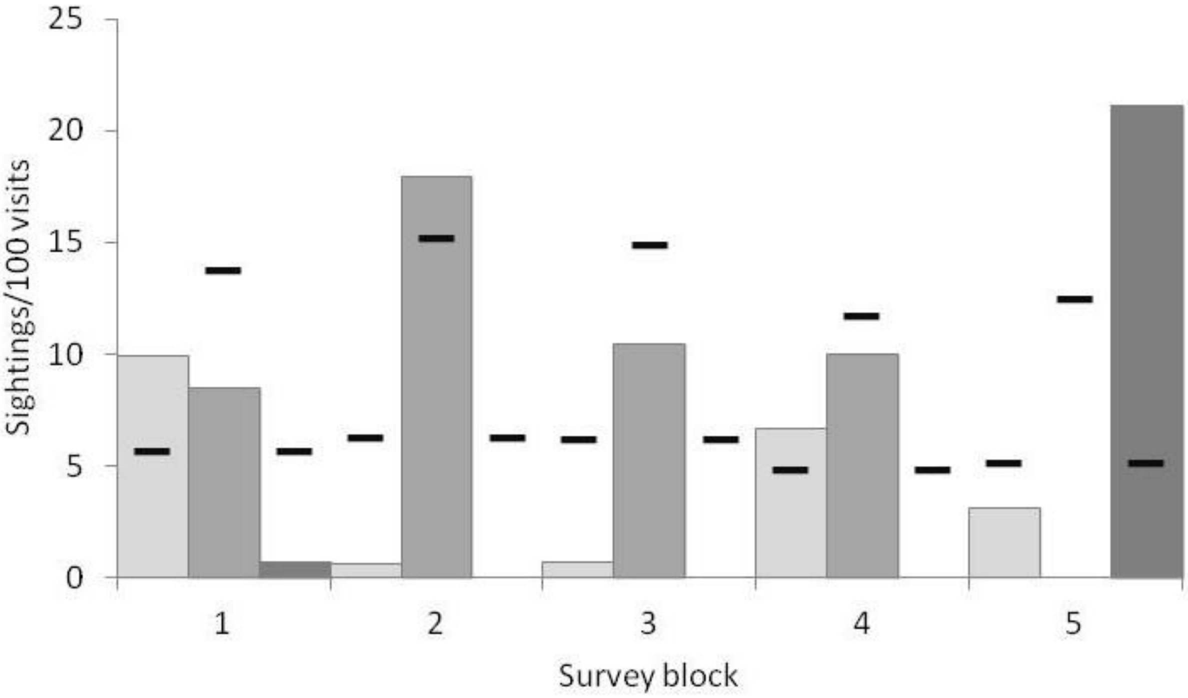

Figure 2: Frequency of sightings of common toad (light grey), grass snake (medium grey) and common lizard (dark grey) per 100 transect visits in each of the 5 survey blocks. Black lines show the expected frequencies based on survey effort for each block and species.

Chapter 3: Mapping the relative probability of common toad occurrence in terrestrial lowland farm habitat in the United Kingdom

Author contributions: Experimental design: RDS, DWM, Data collection: RDS, SET, Data analysis: RAM, RDS, Writing 1st draft of manuscript: RDS, Improvements to manuscript: RDS, RAM, SET, DWM

Chapter 3

Title:

Mapping the relative probability of common toad occurrence in terrestrial lowland farm habitat in the United Kingdom

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Abstract

The common toad (*Bufo bufo*) is of increasing conservation concern in the United Kingdom (UK) due to dramatic declines observed in populations in the past century. Many of these population declines coincided with reduction in both terrestrial and aquatic habitat availability and quality and have been primarily attributed to the effect of agricultural land conversion (of natural and semi-natural habitats to arable and pasture fields) and pond drainage. However, there is little evidence available to link habitat availability with common toad population declines, especially when examined at a broader landscape scale. Assessing such patterns of population decline at the landscape scale require an understanding of how this species uses terrestrial habitat.

We intensively studied the terrestrial resource selection of a large population of common toads in Oxfordshire, England, UK. Adult common toads were fitted with passive integrated transponder (PIT) tags at a breeding pond to allow detection in the terrestrial environment using a portable PIT antenna once toads left the pond and before going into hibernation (April/May-October 2012 and 2013). We developed a population-level resource selection function to assess the relative probability of toad occurrence in the terrestrial environment by collecting location data for 90 recaptured toads.

The predicted relative probability of toad occurrence was greatest in wooded habitat near to water bodies; relative probability of occurrence declined dramatically > 50 m from these habitats. Toads also tended to select habitat near to their breeding pond and toad occurrence was negatively related to urban environments.

Introduction

The global amphibian extinction crisis has been well documented and species declines are primarily attributed to habitat loss and degradation (Houlahan et al. 2000; Stuart et al. 2004a; Wake 1991). According to the IUCN Red List, 32% of amphibians worldwide are threatened, with 21% of those recognised as Endangered or Critically Endangered (Baillie et al. 2004). Despite what its name may suggest, the common toad (*Bufo bufo L.*) is not nearly as common as it used to be. In light of the dramatic and largely unexplained population crashes (Carrier and Beebee 2003), the UK Biodiversity Action Plan (UK BAP) recognized common toads as a priority species in 2007. Since designation, the decline of this species has not abated (e.g. Bonardi et al. 2011) requiring renewed conservation action to prevent further declines. For effective conservation action it is critical that we identify the causes of common toad population declines, in particular the effect of the dramatic landscape changes and pond losses associated with agricultural conversion and intensification in the UK over the past century (Blaxter and Robertson 1995; Williams et al. 2010).

Specifically, a detailed understanding of how common toads select resources in the landscape, is needed. Common toads have excellent potential for use as a model species for determining the effect of land use change on habitat suitability and connectivity. This is because they are moderately mobile (e.g. reported maximum migration of up to 3621m, Smith and Green 2005) and crucially are reliant on both terrestrial and aquatic environments. They are however, particularly difficult to study in the terrestrial phase being largely nocturnal and spending resting periods in hiding (Beebee 1985). Despite this, because toads spend a large proportion of their lives out of the water and must use the terrestrial environment to travel between breeding ponds and foraging or hibernation sites (Beebee 1981), any conservation management options must consider the effect of terrestrial habitat availability. Indeed, it has been demonstrated that toads' presence in breeding ponds is more dependent on terrestrial habitat quality than pond quality (Hartel et al. 2008; Scribner et al. 2001).

Common toad terrestrial habitat selection has primarily been assessed via radio-tracking (Daversa et al. 2012; Denton and Beebee 1994; Sztatecsny and Schabetsberger 2005) and manual searching. Both techniques pose problems for resource selection studies because radio-tracking is restricted to the number of toads it is possible to track at any one time, the tags themselves are finite (i.e. limited battery life), and the potential detrimental impact of the tag on each individual toad's movement behaviour and feeding remains under-evaluated (Madison et al. 2010). Though radio-tracking produces a wealth of data per individual (many fixes) these points tend not to be independent so that autocorrelation must be incorporated for accurate parameter estimation. Manual searching is also problematic as it is unlikely to return many finds and could easily be biased toward habitat types in which animals are more visible, or habitats that are associated with observer experience. For natterjack toads (*Bufo calamita*), Denton and Beebee (1992) found that searching for amphibians in the terrestrial environment can have highly variable levels of success, and may be disruptive to other fauna and destructive for the habitats being searched.

Passive Integrated Transponder (PIT) tags are used in this study, to both individually identify toads and to aid detection of individuals in the terrestrial environment. PIT tags are relatively inexpensive when compared with radio-tags, and the number of individuals that can be marked is limited only by population size and the ability of surveyors to catch animals. As use of PIT tags allows the study of many more individuals, problems with spatial autocorrelation common in radio-tracking studies (Harris et al. 1990) are largely avoided as recaptures are of different identifiable individuals. The deleterious effects of PIT tags on both common toad and common frog (*Rana temporaria*) have been found to be negligible when compared with dye marking (Brown 1997) and no effect of PIT tags has been shown on breeding or survival of frog species (e.g. golden bell frog, Pyke 2005). In other anuran species (spadefoot toad, *Pelobates fuscus*), PIT tags have been shown to have no significant effect on recapture rates or body condition (Jehle and Hodl 1998). Similarly, no effect on growth or survival of Plethodontid salamanders was found when PIT tags were used to aid detection of animals below ground, with a detection efficiency of 44%, far above detection rates when employing hand-capture methods (with a detection efficiency of 44%, far above rates when employing hand capture methods, Connette and Semlitsch 2012).

We deployed PIT tags on common toads in a terrestrial landscape to develop a population-level resource selection function (RSF) during the breeding season. To our knowledge this is the first study to use PIT detector technology on amphibians in a large terrestrial system (see Cucherousset et al. 2008 for a study of Pyrenean brook salamanders in streams; Hamed et al. 2008 for subterranean detection of ambystomatid salamanders within 36m of the breeding pond). The objective of this analysis was to determine the environmental features selected by common toads and the importance of proximity to these features by developing a map detailing the predicted relative probability of occurrence throughout the landscape.

Materials and Methods

Study area and PIT tagging

We selected a constructed farmland pond known to have a large breeding population of common toads in Garford, Oxfordshire (Latitude, Longitude: 51.6633,-1.3919; Fig. 1). The area surrounding this pond consists mainly of arable and pasture farmland, with patches of woodland, rough grassland, scrub, natural and constructed ponds, a small river and homes and gardens in the village of Garford.

During the breeding season (approximately 3 weeks from the beginning of March 2012 and 2 weeks from the beginning of April 2013) we captured toads at the Garford irrigation pond using visual search and hand nets. Passive Integrated Transponder (PIT) tags (Trovan ID-100 FDX bio-compatible glass encapsulated tags, 128 kHz frequency, 11.5 x 2.12mm, 90mg) were injected subcutaneously (using a syringe implanter, each tag pre-sterilised and loaded in a disposable needle) into the pinched dorsum of 1040 adult toads in which snout to urostyle length (SUL) exceeded 50mm. As the injection site closed quickly and without intervention (we noted closed wounds in toads recaptured the day after tagging) we did not use either a suture or veterinary tissue adhesive following injection. Tag retention in common toads has been reported at 100% (1997) and similarly we had no incidence of capturing toads previously marked that had lost their tags (toads were checked for an injection site prior to tagging). The bio-compatible glass casing of these tags prevents tissue irritation (Gibbons and Andrews 2004). We recorded sex, weight, SUL (to the nearest mm) and PIT tag number, as well as the tag number of the other toad when toads were captured in amplexus (breeding position - female clasped around back by male). Following tagging, we released toads within 10m of capture locations. Toad capture and tagging continued until no toads were found in the pond after searches on three consecutive days.

Permission to conduct this study on private land was given by the landowners (Millets Farm, Garford and Garford village homeowners). Tagging and handling of common toads was assessed and approved by the Oxford Local Ethical Research Panel and did not require a home office licence. We exceeded the minimum SUL (40mm) recommended by Pyke (2005) to only tag toads with SUL>50mm. Experienced surveyors handled toads for the minimum length of time possible and kept them cool and shaded while awaiting tagging or measurement.

Detection of toads in the terrestrial environment

Once tagging was complete (late March 2012, late April 2013), detection of toads began in the terrestrial environment using the portable PIT antenna and continued until first frost (October 2012 and 2013) when toads were expected to commence with hibernation. The PIT antenna used was a customised LID650 antenna and decoder box mounted on a carbon fibre pole. The antenna was passed over the ground, keeping it as close as possible to the base of the vegetation to allow reading through the vegetation and into the soil. Field workers worked in teams of two or more, with one operating the detector and the other ensuring full coverage of the area, changing operators every 15 minutes to avoid fatigue. The antenna has a read range of between 17 and 36cm (dependent on tag orientation) and reads through water, soil, vegetation and air at the same rate (Cucherousset et al. 2005). We used ArcMap v10 to divide the study area into 100m by 100m numbered squares (Fig. 1).

We next evaluated whether detection of the PIT tags was biased by habitat type (particularly vegetation height and density). We deployed 234 PIT tags over the 23 trials (minimum 9, maximum 15 tags per trial) within the study area and our detection teams then attempted to relocate the tags. We repeated these trials 6-9 times per habitat type (short grassland (<20cm height); tall grass/herb (<1.5m height); woodland/scrub (ground vegetation <1.5m, woody vegetation/trees 2-20m). We found that detection rates across the three vegetation height classes were not significantly different (Appendix 1: ANOVA, F = 0.57, p>0.5, df = 2), thus we did not detect sampling bias associated with habitat type.

We searched a total of 40 squares in 2012 and 39 squares in 2013; each square took up to 6hrs to search thoroughly, depending on how easy each area was to access. We searched within 500m of the breeding pond, as it was the maximum distance practically possible while maintaining a good probability of recapturing sufficient toads for modelling. The other consideration for this cut off distance was the ecology the common toad. For instance, the maximum migration distance (from the breeding pond to foraging area) reported for the common toad is approximately 3.6 km (see review, Smith and Green 2005) though studies have recorded migration much closer to the breeding pond, for example between 55m and 1600m in pine forest and pasture landscape of Bavaria, Germany (Sinsch 1988), a maximum of 470m from the pond in upland Spain (Daversa et al. 2012) and with maximum distances of between 170m and 1835m (Kovar et al. 2009) away from the breeding pond in a mixture of agricultural and forest habitats in the Czech republic. We therefore secured access to the farmland and many gardens in the habitat at a 500m radius from the breeding pond. We operated the detector in all weather conditions possible as during the day toads are largely inactive (Beebee 1985) and so weather should not affect their behaviour during that period. We avoided stormy or highly windy conditions which considerably diminished our ability to hear the beep when a tag was detected. Once detected, toads were again sexed, weighed and measured. We then recorded the exact location using a hand held GPS unit. Loose tags were recovered but location data not included in the analysis.

Environmental features

We developed a Geographic Information System (GIS) to describe the study area. This GIS consisted of a suite of covariates involving both aquatic and terrestrial features relevant to common toads during the breeding season. We calculated all covariates as distance metrics (in meters) from every portion of the study area to the nearest feature. We considered distance to breeding pond because all individuals were initially captured from this point and so were expected to remain close to this origin and return to the same pond each breeding season. Distance to water features (including ponds, rivers, and drainage ditches) was expected to be important as toads would need to maintain access to water during the terrestrial phase to avoid desiccation. Wooded habitat offers dense vegetation with opportunities to protect from desiccation and hide from predators. We considered urban areas as the bare ground of manmade surfaces (e.g. tarmac, concrete) would be risky to use as they offer no cover and so would have an increased level of exposure to drying or freezing weather, predation and risk of injury/death by traffic and so were expected to reduce the relative probability of toad occurrence. Edge habitats (the interface between two different land use types) were expected to be used as they were often characterised by a two different vegetation heights. Short vegetation or bare ground may have advantages in being easy to move through and offering warmer areas exposed to greater solar insolation for thermoregulation but have a higher risk of ill-effects of unsuitable weather and greater vulnerability to predation. An edge habitat mitigates these risks with the proximity of denser vegetation where a toad could quickly take refuge from unsuitable weather and hide from predators. Finally, we considered the effect of proximity to arable farmland as toads would be exposed to chemicals such as pesticides and fertilisers in these areas, lower food availability than in adjacent field margins (Hof and Bright 2010) and greater predation risk due to reduced cover. Occurrence of toads in breeding ponds has been shown to be negatively associated with arable farmland (Piha et al. 2007).

Population-level resource selection function

We developed a population-level Resource Selection Function (RSF) to estimate the proportional probability of common toad occurrence throughout the study area given a used-available design (Boyce and McDonald 1999; Hebblewhite et al. 2011; Manly et al. 1992; Millspaugh et al. in press). In this case, the locations returned from our PIT tagged toads functioned as our ‘used’ locations. We then needed to estimate ‘available’ locations in the landscape. To do so we randomly generated 4 locations per 100 m^2 within the squares surveyed in each year of the study. We chose 4 locations per $100m^2$ because this procedure generated a sample of available locations at a ~3:1 ratio to used locations, which is a conventional ratio in RSF modelling (e.g., Dzialak et al. 2011; Long et al. 2009). We ensured that no available location was generated within 20 m of a used location so that we did not confound the estimation of the probability of occurrence (e.g., Gervasi et al. 2013). Twenty meters is the extent of daily movements for common toads in the post-breeding season once they reach their foraging habitat (Sinsch 1988; Sztatecsny and Schabetsberger 2005). These used and available locations became our binary response variable in a mixed-effects logistic regression model which we fit as a function of the environmental features in STATA 10 (StataCorp LP, College Station, TX).

Given that we studied these toads over 2 consecutive breeding seasons (2012-2013) we modelled year of the study as a random intercept.

Our model selection procedures were based on established techniques for multivariate logistic regression models (Hosmer and Lemeshow 2000). We initiated these procedures by calculating univariate models for each of the environmental features. We ranked variable importance using Wald statistics and retained those covariates that were deemed influential i.e., $P < 0.25$ (Hosmer and Lemeshow 2000). We next tested for colinearity using Pearson correlation coefficients. In cases where two covariates were correlated ($|r| > 0.50$), we eliminated the weakest predictor as determined by Wald statistics. This process resulted in a candidate covariate set without colinearity. Starting with the strongest predictor we developed a multivariate model in a stepwise fashion. The additional parameter was retained when a significant ($\alpha < 0.05$) likelihood ratio test identified that the model log-likelihood was improved. We stopped this procedure when additional parameters failed to result in a significant likelihood ratio test (Hosmer and Lemeshow 2000). This procedure gave us our final model for evaluation.

We evaluated the predictive potential of this final model through receiver operating curves (ROC) and k-fold cross validation test (Boyce et al. 2002; Hosmer and Lemeshow 2000). From the ROC we calculated the area under the curve (AUC). The AUC value ranges from 0.5-1 where 0.5 is random and 1 indicates perfect discrimination. Models with AUC values greater than 0.7 are considered fair and those with AUC values exceeding 0.9 are considered highly accurate (Hosmer and Lemeshow 2000). For the k-fold cross validation test we generated 5 random subsets of the database each maintaining an 80:20 ratio of training to testing data. In this fashion we fit models using 80% of the dataset and validated the model using the remaining 20%. For each subset we developed a prediction, categorized it into 32 classes, and then simplified them into 10 bins (Boyce et al. 2002). We compared frequencies for each RSF bin between the training and the testing data for each subset and calculated Spearman's rank correlation.

Results

Toad detection

Between 2012 and 2013, we detected 91 toads in the terrestrial landscape during the breeding season. These toads were captured in hand, so that we were sure that they were toads and not loose PIT tags. Of these, one toad was recaptured in both years and so we removed the second recapture from the analysis leaving 41 relocated in 2012 and 49 in 2013. Of the 90 individuals, 13 were females, 77 were males, which was consistent with the sex ratio at tagging in the breeding pond (approximately 6 males per female). Our documented common toad recapture rate approached 9%. The residual body condition index was calculated using residuals of the linear regression of weight versus SUL (Bancila et al. 2010). The residual index of recaptured males did not differ significantly between capture in the pond versus later in the terrestrial environment (paired t-test, $t<0.001$, $p=1$, $df=67$), implying no loss of condition since tagging (pairs with missing values were excluded, females were excluded due to fluctuating weight dependent on whether or not they were carrying eggs).

Additionally we detected and collected 93 loose PIT tags which are suspected to have been passed in the faeces of common toad predators (e.g. grass snake and hedgehog).

Resource selection function modelling

In relation to the 90 used locations that we identified via PIT tag relocation, we generated a total of 275 available locations (124 in 2012 and 152 in 2013). Our collinearity matrix revealed that distance to edge habitat was highly correlated with both distance to wood ($|r| = 0.69$, $P < .001$) and distance to water ($|r| = 0.71$, $P < .001$). We removed distance to edge habitat from consideration because it was the weakest predictor of the three. Distance to arable land did not significantly improve model fit ($X^2 = 1.63$, $P = 0.20$) and was not retained for analysis. The final model predicting the relative probability of common toad occurrence featured distance to wood, distance to water bodies, distance to pond, and distance to urban areas. This model yielded a prediction (Fig. 3) that performed far better than average (area under the ROC curve = 0.891) with k-fold cross validation tests revealing that the training and testing data were strongly correlated (average $r_s = 0.638$). The predictive map developed in ArcMap from the final model revealed that the predicted relative probability of toad occurrence was greatest in habitat bordering the western portion of the breeding pond (Fig. 2). The lowest predicted relative probability of toad occurrence was associated with habitat in the human-dominated landscapes surrounding the breeding pond (Fig. 2). Specifically, the predicted relative probability of toad occurrence decreased with distance away from wooded areas (Fig. 3a), water bodies (Fig. 3b), and the breeding pond (Fig. 3c). Relative probability of toad occurrence declines similarly quickly with distance to water bodies and wooded habitats (approximately zero probability at 245m and 200m respectively) and more steadily for distance to the breeding pond. Conversely, the predicted relative probability of toad occurrence increased in habitat at a greater distance from urban areas (Fig. 3d).

Discussion

Common toad probability of occurrence was positively associated with the presence of nearby wooded habitat, declining with increasing distance. This accords with previous research where toad population size was positively associated with presence of woodland, and hedgerows within the terrestrial habitat matrix (Sztatecsny and Schabetsberger 2005) and terrestrial distribution was often positively associated with forest cover (Hartel et al. 2008; Romero and Real 1996; Sztatecsny and Schabetsberger 2005). Within and nearby wooded habitats, common toads would have easy access to dense vegetation which would be more stable in temperature and so offer protection from desiccation or freezing, and may offer reduced predation pressure with more opportunities to hide. Positive selection for woodland (Denton and Beebee 1994; Romero and Real 1996; Sztatecsny and Schabetsberger 2005) (particularly broadleaved) and rough grassland in the terrestrial environment (Baker et al. 2011; Latham 1997) is well documented in other studies. The western toad (*Anaxyrus boreas*) similarly used forest, wet scrubland and grasslands as foraging habitat in Canada (Browne and Paszkowski 2010a).

Distance from water bodies had a marked effect on the relative probability of occurrence of common toads in this terrestrial environment. This is likely due to the need for these amphibians to avoid desiccation. The results agree with Salazar et al. (unpublished results) where occupancy of toads in field margins in Oxfordshire was positively associated with the presence of an adjacent ditch and with Atauri and de Lucio (2001) who demonstrated importance of damp habitats in determining amphibian presence in the region of Madrid, Spain. This finding highlights the potential effect of reduced availability of wet habitats, such as ponds (Williams et al. 2010), on the occurrence of water dependent species.

Our results indicate that increased density of both wooded and wet habitats (e.g. through pond and ditch creation) in farmland would improve the utility of farmed landscapes for the common toad during the terrestrial phase by reducing potential distance to these positively selected habitats. The resulting increased habitat heterogeneity is also likely to enhance species diversity in farmland habitats for a suite of other taxa (Atauri and de Lucio 2001; Macdonald et al. 2000; Vickery et al. 2001). Wooded habitat and water bodies important during the foraging period are also key in providing hibernacula for the winter, for example in western toads, hibernation sites were located in woodland stands and in vegetated buffers along watercourses (Browne and Paszkowski 2010b). Other temperate amphibian species will have similar requirements for the habitat in which they move through and forage during the summer as they will also need to thermoregulate and avoid desiccation and predation. Increasing habitat heterogeneity would also increase landscape permeability by reducing the risk associated with larger movements through increasing the availability of favourable habitats. A review of the effect of habitat loss and fragmentation on amphibians by Cushman (2006) highlighted the crucial role of connectivity for persistence of populations.

We also found that common toads selected habitat in the vicinity of the breeding pond. This effect of distance from the breeding pond on toad probability of occurrence was expected as toads are only a moderately mobile species (Sinsch 1988) and are highly philopatric (Reading et al. 1991). This does, however, highlight the importance of increasing pond density to facilitate movement between breeding populations (of this and other water dependent species) at the landscape scale and to redress the dramatic loss of ponds witnessed in the last century (Williams et al. 2010).

The predicted relative probability of toad occurrence was negatively associated with urban areas, suggesting higher resistance of these habitats and corroborating the findings of Hitchings and Beebee (1998) and Janin et al. (2009). The corresponding increased relative probability of occurrence on the western side of the breeding pond (Fig. 1) highlights the importance of considering the configuration of the landscape, e.g. Janin et al. (2009) rather than just the composition. Our results suggest that spatial variation in the urban footprint can affect the distribution of common toads in the terrestrial landscape. For instance, wooded habitat near to a water body was not widely selected by toads in our study when those habitats were located close to an urban area. Though distance to arable farmland was not identified as an important predictor of toad occurrence, we did not recapture a single toad in cultivated fields, which accords with the negative association with arable farmland reported by Piha et al. (2007) and avoidance of ploughed land described by Janin et al. (2011a).

This is the first instance in which a portable PIT antenna has been used to aid detection of a species in an open system over a large area. The method was successful, facilitating collection of sufficient presence data points for detailed modelling without compromising the welfare of the study species and could be promising for the study of anurans in other systems. We recommend that conservation action for the common toad be centred on the creation of suitable habitat to increase the density of wooded habitats and water bodies in the farmed landscape. In particular, it is important to provide suitable breeding ponds to reduce distance between breeding populations and so encourage a higher degree of mixing. Furthermore, design of habitat corridors to connect isolated populations should consider diverting them from urban areas where possible and using woody vegetation and linear water bodies (such as ditches and streams), which are likely to be more attractive to moving common toads.

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Figures

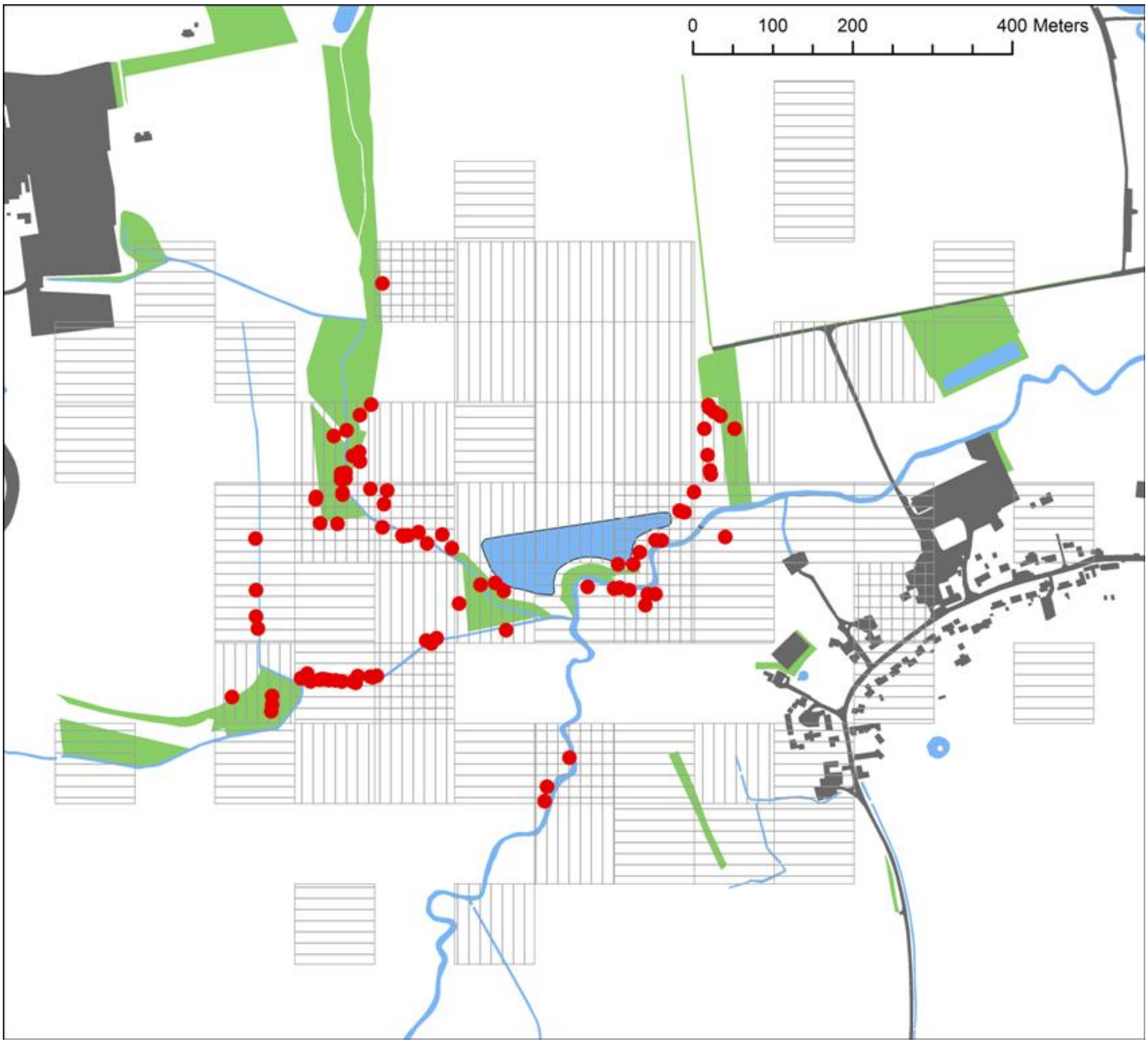

Figure 1: Characteristics of the landscape (green = wooded habitat, grey = urban areas, blue = water bodies) and PIT tag locations of common toads (red circles) in Garford, Oxfordshire (2012-2013).The breeding pond is outlined in black. Surveyed squares are shown in light grey (vertical hatch = 2012, horizontal hatch = 2013, cross hatch = surveyed both years).

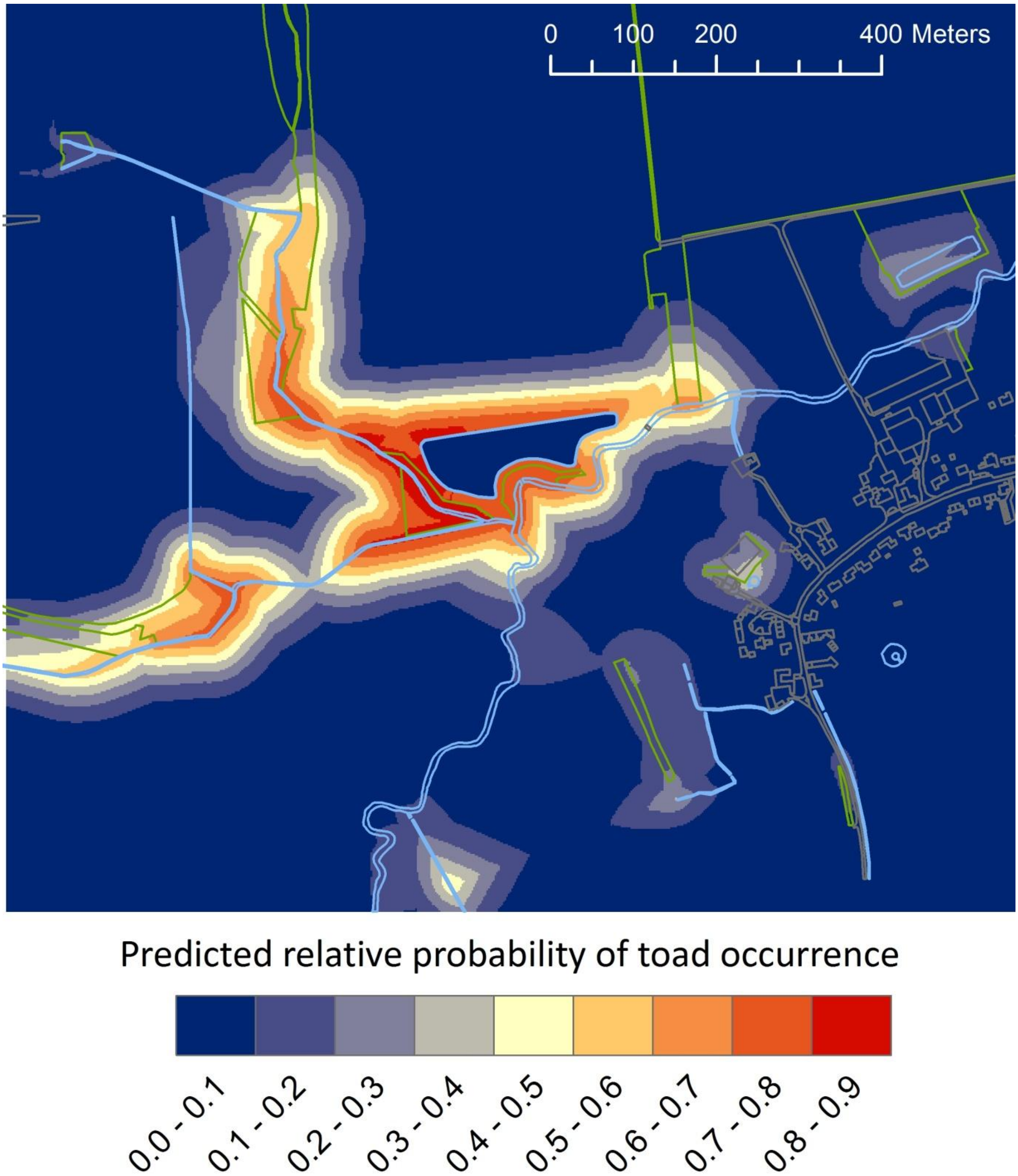

Figure 2: The predicted relative probability of common toad occurrence in Garford, Oxfordshire based on PIT tag data collected between 2012 and 2013.

Habitat characteristics outlined in green = wooded habitat, grey = urban, blue = water bodies.

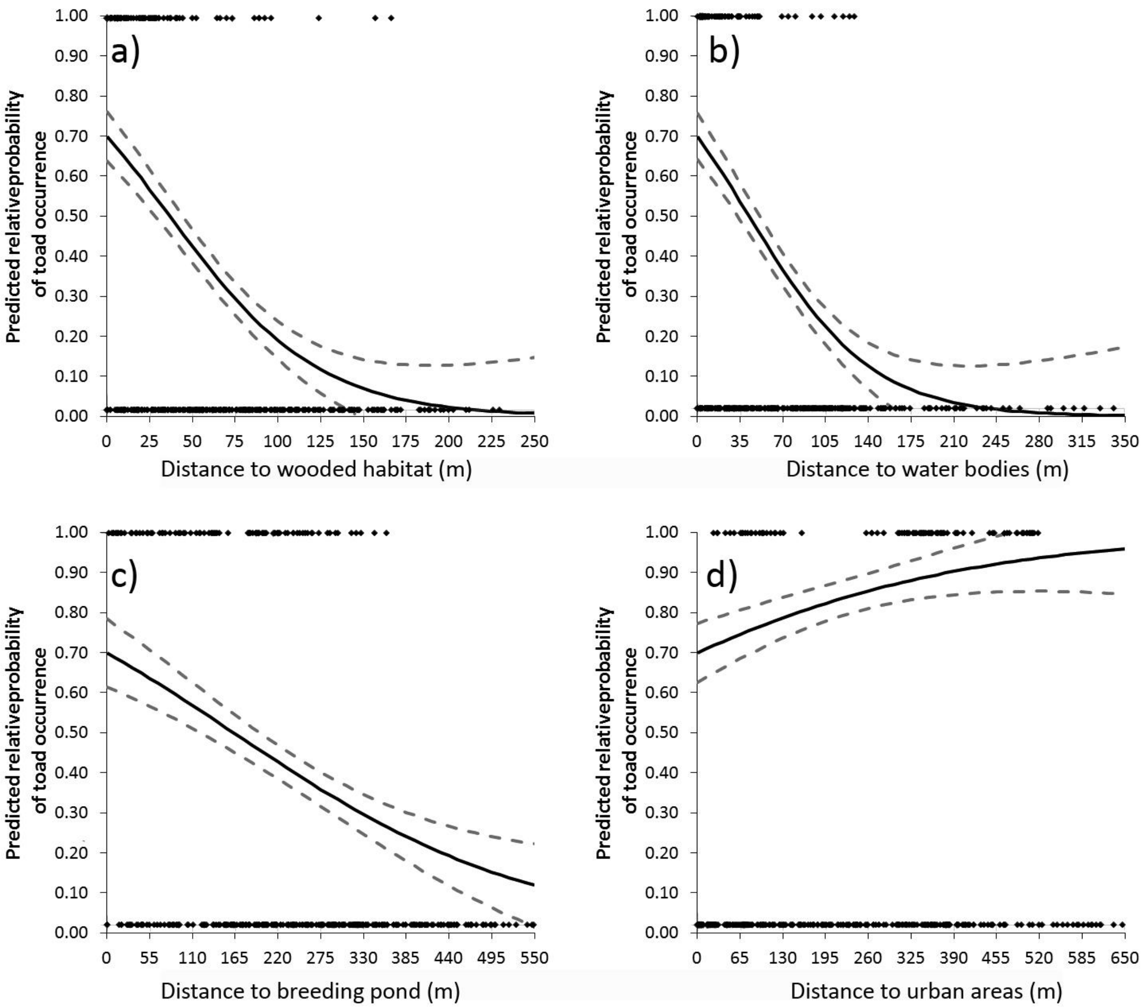

Figure 3: Mixed logistic regression functions for the environmental features in the final model predicting the relative probability of toad occurrence in Garford, Oxfordshire based on PIT tag data collected between 2012 and 2013. Dashed lines show the 95% confidence intervals of each estimate.

Appendices

Appendix 1: Vegetation PIT detection trials.

Vegetation type	Tags out	Detected	Rate
Short grassland	15	15	1.00
Short grassland	10	7	0.70
Short grassland	10	3	0.30
Short grassland	10	6	0.60
Short grassland	10	6	0.60
Short grassland	10	9	0.90
Tall grass/herb	10	9	0.90
Tall grass/herb	10	2	0.20
Tall grass/herb	10	8	0.80
Tall grass/herb	10	4	0.40
Tall grass/herb	10	4	0.40
Woodland/scrub	10	10	1.00
Woodland/scrub	10	3	0.30
Woodland/scrub	10	3	0.30
Woodland/scrub	10	6	0.60
Woodland/scrub	10	5	0.50
Short grassland	10	9	0.90
Tall grass/herb	9	7	0.78
Short grassland	10	7	0.70
Tall grass/herb	10	10	1.00
Short grassland	10	9	0.90
Tall grass/herb	10	10	1.00
Woodland/scrub	10	8	0.80

Chapter 4: The effect of Euclidean distance and isolation by barriers on common toad *Bufo bufo* genetic distance

Author contributions:

RS, DM conceived the experiment. RS, SE, CR were responsible for collection of samples. SE, RC were responsible for all laboratory work. RS, RM completed all GIS analyses. RS, SE, RC, RM analysed the data. RS wrote 1st manuscript draft. RS, SE, RC, RM, AR, DM, CR improved the manuscript.

The effect of Euclidean distance and isolation by barriers on common toad *Bufo bufo* genetic distance

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Abstract

Genetic diversity plays an integral role in species persistence. However, it remains challenging to quantify how degree of isolation affects animal populations. There are two types of isolation that are relevant for the common toad (*Bufo bufo*), a species of conservation concern: *i*) isolation by distance (IBD), where populations that are further apart are more isolated, and *ii*) isolation by barrier (IBB), where the presence of barriers to movement isolates populations.

Common toads experience IBB primarily in the context of breeding populations. Barriers include inhospitable habitats, roads, and urban areas. Additionally, the common toad has only moderate dispersal abilities and is highly philopatric and so may also be sensitive to IBD.

We collected common toad genetic material in rural landscapes of Oxfordshire, England to test the effect of IBD and IBB on toad genetic distance. We identified eight different breeding populations in this landscape. We detected a significant and positive relationship between IBB and genetic distance among these breeding populations. In contrast, no effect of IBD was detected at the local landscape scale (Oxfordshire, 2-22km between ponds) and it was only when we included a pond more than 100km from the main study area that we identified an effect of increasing Euclidean distance on genetic distance between ponds. The lack of a relationship between IBD and toad genetic distance might suggest that common toads are not as philopatric as previously thought; with reduced availability of suitable breeding ponds driving more migrants to disperse greater distances and thereby improving genetic mixing of the metapopulation.

Introduction

Genetic diversity is recognized as a vital component of long-term species persistence as evidenced by the incorporation of genetic research techniques to conservation initiatives (Hedrick 2001). Genetic research now commonly assesses how habitats and landscape configurations may restrict migration, and therefore gene flow (Sarre and Georges 2009). Such approaches are particularly relevant for amphibian species that tend to use disparate habitats over large areas, potentially magnifying effects of isolation (Ficetola et al. 2008). Two common mechanisms of isolation for amphibian species include isolation by distance (IBD), where populations further apart are more genetically dissimilar, and isolation by barrier (IBB), where populations separated by barriers are more dissimilar, with stronger and larger barriers acting as isolating factors due to the increased distance necessary for circumventing the barrier. Identification of the influence of IBD or IBB can be used to better allocate conservation attention toward isolated populations and determine the appropriate course of mitigation action (e.g. translocation or habitat improvement).

Common toads (*Bufo bufo* L.) are a highly philopatric species (e.g. Reading et al. 1991) with limited mobility (e.g. Sinsch 1988) and so, are likely to be susceptible to isolation of their breeding populations by distance. Further, where the species has undergone population declines (e.g. Beebee 2012) common toads tend to be rare and particularly vulnerable to the effects of habitat change and fragmentation which can increase isolation (Ficetola and De Bernardi 2004). Large urban areas and roads, for instance, can be particularly costly for migrating common toads (*Bufo bufo*), presenting barriers to movement and leading to direct mortality from vehicle traffic (Cooke 1995; Elzanowski et al. 2009). Populations that are more isolated have reduced exchange of individuals, and thereby genetic material, making them more genetically dissimilar (i.e., increasing the genetic distance). Genetic distance is a measure of genetic divergence between populations, in this case, between breeding ponds of common toads. Smaller genetic distances are indicative of populations that are more genetically-similar while larger distances show greater genetic divergence.

Common toads are now a species of conservation concern in the United Kingdom (UK) following the large and unexplained local population declines occurring over the last 30 years (e.g. Carrier and Beebee 2003). Toads tend to have lower genetic diversity in small urban ponds compared with larger, more rural ponds (Hitchings and Beebee 1998). Further, toads inhabiting more fragmented landscapes have higher levels of stress hormones and poorer body condition than toads in more contiguous habitats (Janin et al. 2011b). Despite these conservation issues, the principles of IBD and IBB, assessed in many anuran species, have not yet been applied to the study of common toads. For instance, Allentoft et al. (2009) showed the natterjack toad (*Bufo calamita*) in Denmark to have no detectable pattern of IBD, supporting their findings that the remaining distinct populations are genetically isolated by a different mechanism. Studies of the common frog (*Rana temporaria*) showed reduced population size, genetic diversity and fitness of populations in fragmented habitats when compared with those from continuous habitats (Johansson et al. 2007). Moor frogs (*Rana arvalis*) are especially sensitive to road and railway barriers, presence of which better explained genetic distance than isolation by distance alone (Vos et al. 2001).

To efficiently apply conservation action to prevent future declines it is crucial that we determine the current threats to species persistence. This must include threats to the species' genetic diversity. Here we compare measures of genetic distance, IBB and IBD to identify the mechanism for isolation of common toad breeding populations in Oxfordshire.

Methods

Study area and collection of genetic samples

We collected genetic samples, in the form of common toad tadpoles, from a sample of 8 breeding ponds distributed across west Oxfordshire (Fig. 1) between 18th June and 4th July 2012. Among our sample, ponds averaged 4484 m^2 (range 795 m^2 – 11436 m^2) and were between 3.8 km and 21.5 km (Euclidean distance) apart.

Using records of common toad breeding ponds, held by the Thames Valley Environmental Records Centre (186 locations; TVERC, www.tverc.org), we visited ponds to detect evidence of breeding in 2011 and collected 28 tadpoles for screening and optimisation of the genetic extraction and amplification protocol. Ponds were selected where toad tadpoles were considered to be present in sufficient numbers so that collection of a sample (26-48 tadpoles, Appendix 1) would not detrimentally impact the breeding success of that pond in the year of collection (i.e. sample size <5% population size). We collected a minimum of 26 samples per pond, as F_{ST} values (fixation index, measure of genetic differentiation) were expected to be greater than 0.05 (e.g. Wilkinson et al. 2007) when a sample size of 20 should be sufficient (Kalinowski 2005).

Sibling tadpoles from *Bufo spp*. tend to shoal together (Blaustein et al. 1990), so we stratified our sampling by shoals to avoid sampling closely related individuals. Where shoals were not easily visible due to dense vegetation or poor water clarity, we spaced collections a minimum of two metres apart. This meant that samples would be less likely to be genetically-related. Via these sampling efforts we minimized the potential negative impacts of our collection efforts on the breeding success of any one adult breeding pair and maximized the potential genetic diversity of the samples collected from each pond. We controlled sampling effort between ponds by completing each collection during a single visit to each pond, with two surveyors completing a circuit of the pond and collecting tadpoles from each shoal or net sweep at 2m intervals as appropriate.

We euthanized tadpoles using an approved Schedule 1 technique (not a regulated procedure under the Animals Scientific Procedures Act 1986): we administered a lethal dose of anaesthetic using Benzocaine (Orajel®) (Cecala et al. 2007), followed by pithing before storing in the freezer in 90% ethanol prior to genetic analysis.

To contextualise the genetic variation found within Oxfordshire we obtained genetic data for 116 adult common toads from a breeding pond in Dorset, UK (Lat 50.647645, Long -2.117100) which were sampled (by toe clip) in 2009. The Dorset pond was a minimum of 123.8km and maximum of 142.3km from the Oxfordshire ponds and could therefore reasonably be expected to be genetically isolated from our study ponds.

Ethical considerations

All field work conducted in the Oxfordshire pond cluster was reviewed and approved by the Oxford Local Ethical Review Committee. The project protocols for the Dorset pond were approved by the CEH Ethical Review Panel and completed under licence from the Home Office. Access to private land for collection of samples was granted by each landowner.

Genetic distance

We calculated genetic distance among all possible combinations (28 pairs) of the breeding pond pairs from the 8 Oxfordshire ponds using 7 microsatellite loci. Genetic distance between the Oxfordshire and Dorset ponds was calculated using the five microsatellite loci in common between the two studies (Coles 2013). As microsatellites are neutral markers (not under selection) we can assume that genetic differentiation (change in these traits) between populations has occurred through genetic drift.

DNA Extraction:

We extracted DNA from tadpole tails (remainder of the tissue retained for future use) using QIAGEN DNeasy blood and tissues extraction kit.

We amplified the Oxfordshire samples by multiplex PCR (partially following QIAGEN Type it kit protocol, see Appendix 2 for full details). We randomly selected three individuals for use in the PCR protocol optimisation: Ga21, Hi23 and St4. We used primers for seven microsatellite loci (see Appendix 3) previously developed for the species (Brede et al. 2001). Due to overlapping size of the loci analysed we created two separate multiplex associations (see Appendix 2) and labelled using 6FAM: blue, PET: red, NED: yellow and VIC: green. The loci Bbuf11 was amplified in a single PCR. Amplification and sequencing of samples from the Dorset pond were completed as described in Coles (2013).

F_{ST} calculations:

We assessed genetic distance using the Peak Scanner 1.0 (Applied Biosystems) software to score alleles. For consistency, scoring of the five loci in common (see Appendix 3) with those used for the Dorset pond was repeated in order to use the same methods as Coles (2013) using Tandem software (Matschiner and Salzburger 2009) to automate allele binning across the combined data set.

We used FSTAT (Goudet 1995) to develop F_{ST} values as the measure of genetic distance between each pair of breeding ponds (for detailed methods, see Eynard 2013). F_{ST} was chosen as our measure of genetic distance as it is most commonly used and is a more important measure of genetic population structure than G'_{ST} or D (Whitlock 2011).

Euclidean distance

We quantified Euclidean distance in ArcMap v10.0 (ESRI 2011) using the Point Distance tool, with each pond paired with all others to produce 28 distances in the Oxfordshire group and 36 distances including the Dorset pond (Table 2).

Cost distance

We also developed estimates of Cost Distance, where distance (in metres) was calculated around natural barriers to toad movement. We described these barriers by mapping the predicted relative probability of toad occurrence in the Oxfordshire pond cluster based on a resource selection function (RSF) (Chapter 3: Salazar et al. in review). We fit the RSF as a function of environmental features including proximity to wooded habitat, urban areas, and water bodies. The prediction, based on the environmental parameter estimates of the RSF, displayed the habitat within the study area that was suitable for toads. The cost distance tool then calculated distance between ponds (Table 1) within this RSF prediction (Appendix 4).

Statistical analyses

Matrices generated from Euclidean, cost distance, and total landscape resistance calculations were compared with simple and partial Mantel tests in zt software (Bonnet and Van de Peer 2002; Table 3).

Results

We extracted biological material from a total of 259 tadpoles collected from the 8 studied ponds (plus a further 28 in the previous year collected for screening and optimisation of the protocol).

Genetic distance (F_{ST}) between ponds was not significantly different from zero for three pairs of ponds in both sets of calculations using either seven or five microsatellite loci: Fawler-Bagley Wood, Cothill-Garford, Ducklington-Standlake (Table 1) and therefore these pairs are considered genetically identical.

We detected no significant relationship between Euclidean distance and cost distance detected for the Oxfordshire ponds (Mantel test: $r = -0.17$, $p = 0.237$).

The relationship between cost distance and genetic distance was positive and significant both with and without control of Euclidean distance (simple Mantel test: $r = 0.48$, $p < 0.05$, partial Mantel test (Euclidean controlled): $r = 0.48$, $p < 0.05$).

The relationship between Euclidean and genetic distance was positive and significant when pairs including the Dorset pond were incorporated into the matrices (simple Mantel test: $r = 0.98$, $p < 0.05$).

Discussion

We found a range of genetic distance among the 8 different common toad breeding ponds in Oxfordshire. The genetic distance between toad breeding ponds was best explained by IBB. Genetic distance increased with increasing cost distance (as determined by barriers to toad movement in this landscape) between ponds. The cost distance values used in this study were based on an RSF developed in previous work (Chapter 3: Salazar et al. in review) where habitats which increased relative probability of toad presence were those closer to woody vegetation and water bodies and further from urban areas. Barriers in the cost map (Appendix 4) were areas of zero percent relative probability of toad occurrence, further from woodland and water bodies and closer to urban areas. The size and strength of these barriers determined how much the cost distance would need to increase in order to circumvent the barrier. This result demonstrates the effect of urban areas with reduced density of more hospitable habitats (woodland, water bodies) as barriers to migration; affecting the ability of toads to move around the landscape to breed away from their natal pond. These results are congruent with established studies that identified lower genetic diversity in toad ponds in urban areas (Hitchings and Beebee 1998). The breeding pond pairs that were most genetically similar (Standlake-Ducklington) were ~6 km apart. Despite being separated by this distance, these two ponds were genetically identical based on the 7 microsatellite markers. This result suggests a greater level of movement of individuals between these ponds than expected considering their philopatry (e.g. Reading et al. 1991) and reported maximum migration distance of 3.6 km (Moore 1954). Common toads can colonise new ponds when the nearest breeding pond is within 950 m (Baker and Halliday 1999) and when we consider the high density of ponds between Standlake and Ducklington (Fig. 1) we hypothesize that the variety of ponds in this landscape matrix function as stepping stones, facilitating movement of individuals or their genetic material over several generations. Though it is impossible to know the extent to which toad translocation (for example, moving toad spawn between ponds) has affected the genetic structure of these breeding populations it is unlikely to have been commonplace for most of the ponds in this study as they were relatively isolated. The exception were the ponds at Fawler (closely monitored by the public as part of a toad crossing scheme) and Bagley Wood (on the grounds of a school) which were also genetically identical based on the 7 microsatellites despite the relatively large Euclidean and cost distances separating them.

We failed to detect a relationship between common toad genetic distance and Euclidean distance at the local landscape scale. When the Dorset pond was included as an outlier pond we did identify a significant positive (simple Mantel's test, $r = 0.98$, $p < 0.05$) relationship between genetic distance and Euclidean distance at this much greater scale (100+ km). This is interesting as it suggests that the effect of Euclidean distance in isolating rural common toad breeding populations operates at a much greater scale than we may have initially expected. It is only when we move beyond the local landscape scale (up to 22km in this study) that Euclidean distance begins to become truly isolating. However, future research is necessary to evaluate whether a threshold distance exists beyond which toad populations can be considered cut-off from each other with some degree of certainty. These findings suggest that despite concern over recent dramatic population level declines (Carrier and Beebee 2003), connectivity between rural common toad populations at the local landscape scale can be maintained with appropriate management. To encourage a greater level of movement between breeding populations we suggest the creation of more ponds and woody habitat. Creation of ponds would go some way to redress the losses experienced in the last century (Williams et al. 2010) and creation of nearby woody habitat would improve the relative probability of toads using these habitats during the terrestrial phase. Increasing the number of ponds increases pond density and so reduces distances between ponds which, though not shown to be limiting at the local landscape scale does become important at a larger scale.

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Tables

Table 1: Below diagonal: Genetic distances (F_{ST} values) between the 8 study ponds calculated in FSTAT using results from 7 loci.

Values in bold are have F_{ST} values not significantly different from zero and are considered identical (p-value threshold of significance is 0.001389). Above diagonal: Pairwise Cost distance (in meters) between Oxfordshire ponds.

	Bagley Wood	Combe	Cothill	Cumnor	Ducklington	Fawler	Garford	Standlake
Bagley Wood	-	14605	6935.5	22245.2	16844.1	11482.8	23288.1	19257.4
Combe	0.028	-	17257.3	14832.5	10238.5	10248.6	14584.9	10554.2
Cothill	0.0389	0.0305	-	25892.8	16721.5	11222.3	24755.6	21034.6
Cumnor	0.0639	0.0298	0.0576	-	16471	20062.8	11923	10754.8
Ducklington	0.0605	0.0182	0.041	0.0305	-	6269.56	8752.61	6305.14
Fawler	**0.0137**	0.0122	0.0343	0.0453	0.0206	-	14340.8	10757.4
Garford	0.0364	0.0146	**0.0078**	0.0416	0.0264	0.0229	-	5145.79
Standlake	0.0373	0.0181	0.0387	0.0442	**0.0033**	0.0107	0.0237	-

Table 2: Below diagonal: Euclidean distances (in metres) between the 9 study ponds. Above diagonal: Genetic distances (F_{ST} values) between the 9 study ponds calculated in FSTAT using results from 5 loci. Values in bold are have F_{ST} values not significantly different from zero and are considered identical.

	Bagley Wood	Combe	Cothill	Cumnor	Ducklington	Fawler	Garford	Standlake	Dorset
Bagley Wood	-	0.024	0.0397	0.0249	0.0382	**0.0064**	0.0313	0.0201	0.1415
Combe	18231	-	0.0277	0.0272	0.0133	0.0138	0.0158	0.0145	0.1309
Cothill	5752	17478	-	0.0248	0.0154	0.0354	**0.0065**	0.0234	0.1512
Cumnor	5542	13010	5388	-	0.016	0.0278	0.039	0.0206	0.1596
Ducklington	13759	9491	10302	8496	-	0.0152	0.0244	**-0.0017**	0.1543
Fawler	20827	3815	19135	15361	9658	-	0.028	0.0066	0.1437
Garford	10023	20516	4695	9813	11895	21471	-	0.0237	0.1344
Standlake	10996	14940	5828	7504	5874	15519	6068	-	0.1483
Dorset	132145	142315	128314	133618	132868	141417	123814	128014	-

Table 3: Results of Mantel tests comparing triangular matrices produced for Euclidean distance (Euclidean), genetic distance (F_{ST}) and landscape resistance (Cost distance). Significant results are in bold. Italics denote the control matrix for the partial Mantel tests.

Type	Matrix A	Matrix B	r	p
Simple	Oxon ponds F_{ST}	Oxon ponds Euclidean	-0.17	0.237
Simple	Oxon ponds F_{ST}	Oxon ponds Cost distance	0.48	**0.012**
Partial (*Euclidean*)	Oxon ponds F_{ST}	Oxon ponds Cost distance	0.48	**0.013**
Simple	All ponds F_{ST}	All ponds Euclidean	0.98	**0.048**

Figures

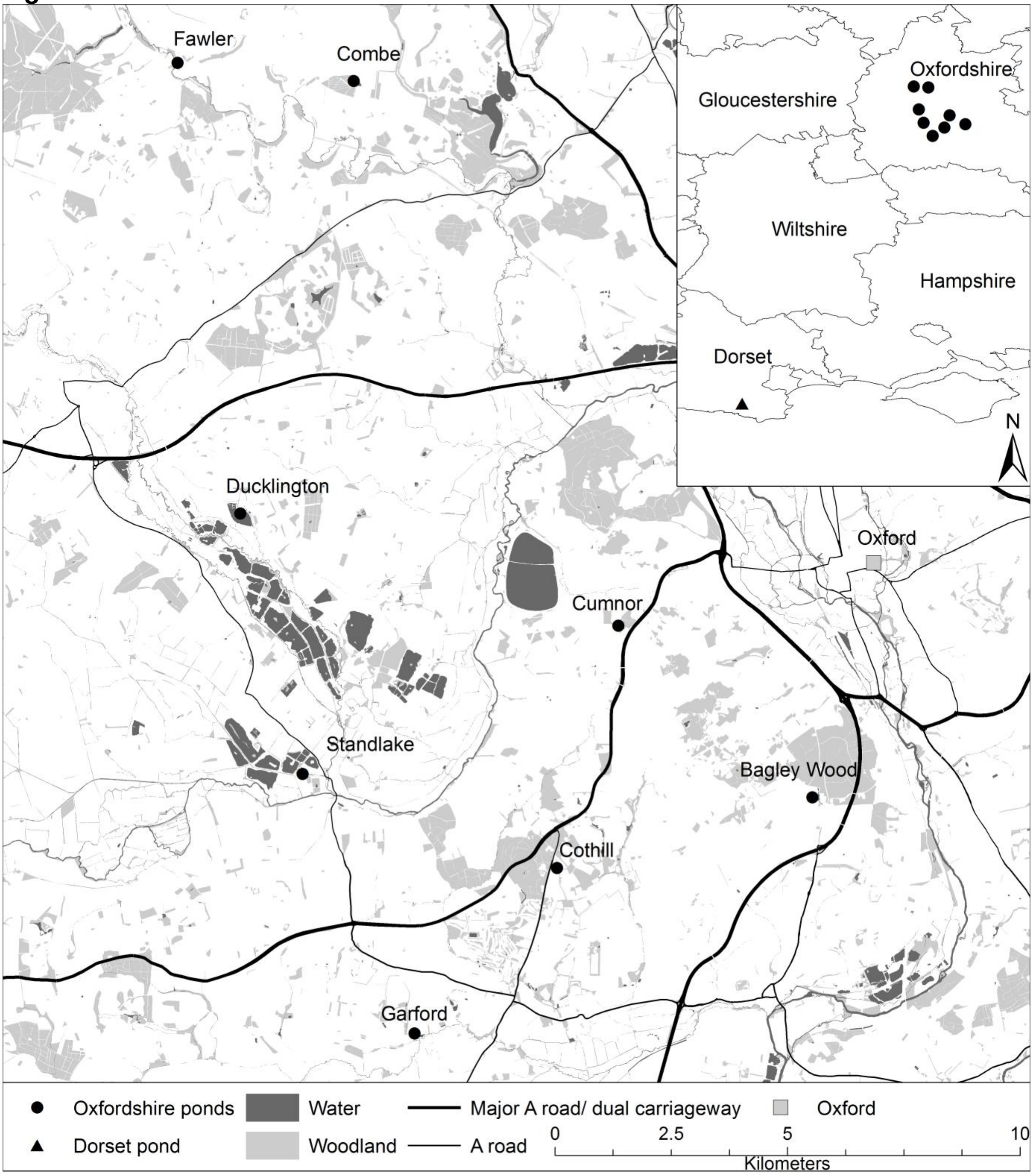


Figure 1: Locations of eight ponds in West Oxfordshire from which common toad genetic material was collected. Broad habitat types have been mapped between ponds. Inset map shows location of Dorset pond in relation to the Oxfordshire cluster. Habitat layers from OS Mastermap (Ordnance Survey 2011) and OS Open Data www.ordnancesurvey.co.uk (Crown Copyright).

Appendices

Appendix 1: Tissue sample numbers, source ponds and sampling dates.

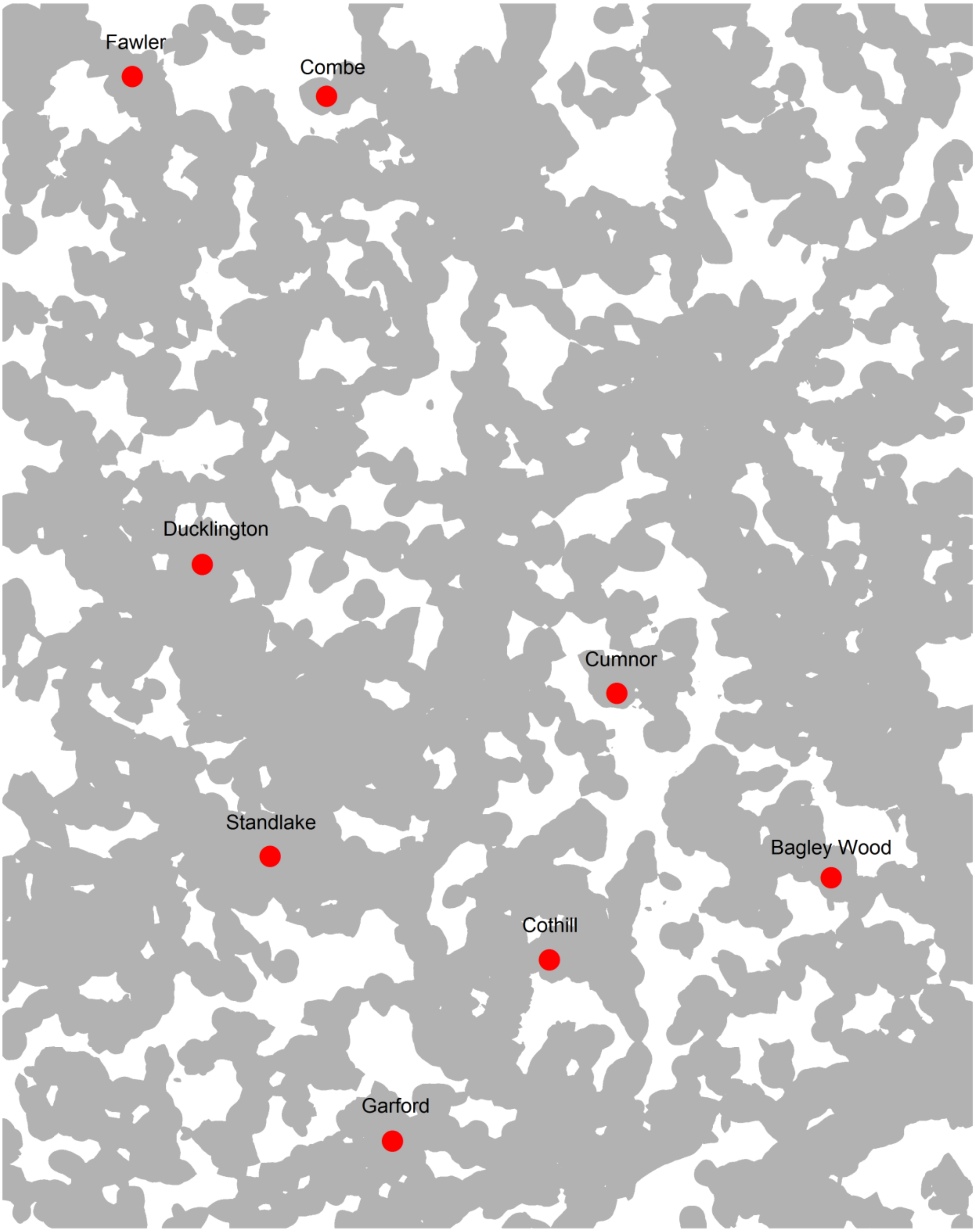
Pond name	No. tadpoles	Code	Sampling date
Bagley Wood	26	BW 1-26	25/06/12
Combe	48	Cb 1-48	18/06/12
Cumnor	30	Cu 1-30	27/06/12
Fawler	45	Fa 1-45	27/06/12
Garford	26	Ga 1-26	22/06/12
Standlake	31	St 1-31	29/06/12
Cothill	27	Hi 1-27	04/07/12
Ducklington	26	RC 1-26	26/06/12

Appendix 2: Multiplex design, labelling and Polymerase Chain Reaction conditions for microsatellites amplification.

PCR Name	Gene amplified/ Flourescent label	Reaction cycles
Multiplex4	Bbufμ15/Blue, Bbufμ14/Yellow, Bbufμ47/Red, Bbufμ65/Green	95°C for 5min denaturation, 94°C 30sec, 55°C 30sec and 70°C 30sec x25 cycles, 70°C for 30min final elongation step.
Multiplex6	Bbufμ54/Blue, Bbufμ62/Red	95°C for 5min denaturation, 94°C 30sec, 57°C 30sec and 70°C 30sec x23 cycles, 70°C for 45min final elongation step.
Singleplex11	Bbufμ11	94°C for 3min denaturation, 94°C 30sec, 51°C 1min and 72°C 1min x25 cycles, 72°C for 30min final elongation step.

Appendix 3: 7 Microsatellite loci and their associated primers used for amplification (Brede et al. 2001). Bold type indicates loci also used by Coles (2013) for the Dorset pond study.

Microsatellite loci (GenBank code)	Primer sequences (5'-3')
Bbuf54 (AY037820)	FAM-CATTGCGCTGCTGTCAGATTACAC TTAGGGATTGCCGTCCAGTTGTC
Bbuf14 (AY037811)	NED-CGTGCATGCAAGTGTACCTAACC ATGGAGAGTGAAGGGGAAAGAGTG
Bbuf47 (AY037818)	VIC-GGATCAAGCCCTCAGACAACTC CACAGCAGCAGAAATTTTGACCAG
Bbuf62 (AY037821)	PET-GCACATTCCTGTGTCCGTGTATAG ATTCCGAAAACGAAAAGAAAAGAG
Bbuf15 (AY037812)	FAM-TCAATATAGGAGTCCCAGAATGTC AATCCCCTAGCGTACACAAGATAC
Bbuf11 (AY037809)	GTCACATGGATAATAAATGAGACC TCTAATATTGATGACCAGACAACC
Bbuf65 (AY037823)	PET-GGATCTAAGCGCTGTGAGAGTGA CGGTCCGTGTTACCACTGATGC



Appendix 4: Cost map between 8 Oxfordshire ponds (red dots). White areas have 0% probability of toad occurrence.

Chapter 5: Determinants of pond use by the common toad (*Bufo bufo*)

Author contributions: Experimental design: RDS, DWM, Data collection, creation and management: RDS, JWW, RAM, Data analysis: RDS, Writing 1st draft of manuscript: RDS, Comments and improvements to the 1st draft: RDS, RAM, JWW, DWM

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Keywords: Common toad, Bufo, pond, terrestrial habitat suitability

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Abstract

The common toad *Bufo bufo* is of increasing conservation concern having experienced many dramatic and largely inexplicable population declines. The species' life history traits may make it more susceptible to decline as a result of changes in both landscape and breeding pond habitat suitability: it is philopatric, can move only moderate distances during its terrestrial phase and appears selective about the ponds in which it breeds. Efforts to increase pond density in rural areas could provide much needed habitat to encourage movement of toads between ponds and ensure mixing of breeding populations. For such efforts to be successful, it is first essential to determine what makes ponds suitable for use by toads so that efforts for pond creation and management can be sensitive to these factors.

We used pond survey data collected by volunteers through a national survey scheme for 368 ponds and added quantitative terrestrial habitat data derived using Ordnance Survey Open Data and a resource selection function. We used principal component analysis and logistic regression to determine the importance of various abiotic and biotic factors in predicting common toad presence in ponds.

The results suggest water quality, presence of other amphibian species and availability of suitable terrestrial habitat are important determinants of common toad presence in ponds.

Introduction

The number of ponds in the UK has declined dramatically over the last 100 years and this has been coupled with reduced water quality of ponds (Williams et al. 2010). Despite a more recent increase in the number of ponds through widespread pond creation projects, this has only gone a small way to redress the losses. With reduced number and quality of ponds in the landscape it is important to understand the factors that make a pond of sufficient quality to sustain amphibian populations. It is also increasingly necessary that our ability to assess the quality of breeding ponds is rapid as well as quantitative and should ideally be possible with only minimal resources and expertise. The standard method for assessing the biological quality of still freshwater bodies in the UK is PSYM (Predictive SYstem for Multimetrics), which requires a significant level of expertise in both aquatic plant and invertebrate identification.

One species likely to be affected by the decrease in quality and quantity of ponds in the rural landscapes is the common toad (*Bufo bufo* L.). Designation of the common toad as a UK Biodiversity Action Plan priority species demonstrates the increasing concern for the species in view of its recent and unexplained declines (Carrier and Beebee 2003; Young and Beebee 2004).

Amphibians are often considered good indicators of overall environmental quality of a habitat. Abiotic and biotic factors related to pond quality influence the likelihood of amphibians occupying and breeding in ponds successfully (Indermaur et al. 2010a), and can even influence individual growth rates of larvae (Indermaur et al. 2010b).

It is therefore possible to analyse the effect of these biotic and abiotic factors to develop a measure of the probability of occurrence of a species.

The Habitat Suitability Index (HSI) developed by Oldham et al. (2000) for the Great crested newt (*Triturus cristatus*) remains widely used by conservationists and ecological consultants alike to produce a score for the probability of the species using the pond of interest. It is part of the standard methodology used by the National Amphibian and Reptile Recording Scheme (NARRS) to survey for amphibians. The method requires little expertise or equipment and can be completed rapidly in a single visit with only limited supporting desk work required. However, a high score for the Great crested newt HSI, which indicates a high probability of presence of Great crested newts in the pond, does not necessarily indicate high overall quality of the pond or suitability for other amphibians.

Beebee's study of pond suitability for amphbians (1981) makes the recommendation that ponds suitable for common toads in lowland agricultural areas should be larger, older, contain fish and have a higher ionic score. However, common toad was absent from all of the 60 farmland ponds studied (Beebee 1981), and the expected suitable conditions are therefore not derived from those survey data. Cooke (1975) found that common toad selected larger ponds and fewer of the available ponds in farmland than common frog (*Rana temporaria*). This may be related to carrying capacity, as persistence of common toad breeding ponds was unrelated to proximity to a source pond but was related to extinction effects when pond carrying capacities were below 30 adult females (Halley et al. 1996). This suggests that there is a minimum pond size needed to establish a successful breeding toad population.

If common toads have more requirements than other amphibians for their choice of breeding ponds, it follows that there will be fewer suitable ponds available to them in any given area. This will hinder inter-site migration and gene flow, leading to breeding assemblages acting as discrete populations (e.g. Brede and Beebee 2004).

Common toads spend the majority of their adult life in the terrestrial habitat; migrating, foraging and hibernating (Beebee 1985) and so it is essential to assess the importance of habitat availability out of the pond as well. One component of the Great crested newt HSI looks at the effect of terrestrial habitat (Oldham et al. 2000). However, this subjective score is based on the surveyor's opinion of the availability of suitable habitat. Furthermore, a review by Gardner et al. (2007) revealed that there was a lack of consensus concerning the role of habitat change in driving population declines in herpetofauna species. Thus the importance of the availability of suitable habitat merits further scrutiny. Most declines of common toad investigated by Young and Beebee (2004) were attributed by respondents to changes in habitat, including the effect of roads and increased traffic. Any attempt to develop a habitat suitability index for a species that spends the majority of its life in the terrestrial environment (Beebee 1985) must consider both the terrestrial and the aquatic environment and this should be a quantitative assessment wherever possible. Indeed, toad occupancy is often better predicted by the terrestrial habitat than pond characteristics (Hartel et al. 2008; Janin et al. 2009) and the importance of landscape over local scale factors has similarly been demonstrated for other amphibian species (Scherer et al. 2012).

To address this need for a toad-specific habitat suitability index we combined NARRS amphibian survey data (including data on pond character) with a quantitative assessment of the coverage of major land-use types (woodland, surface water and urban areas) and "available habitat" as defined by a resource selection function from previous work (Chapter 3: Salazar et al. in review) to look at the key parameters to determine pond occupancy by common toad. We discuss the results with a view towards developing a rapid assessment method to determine both the suitability of ponds for common toads and the biological quality of ponds.

Methods

Data collection: Pond surveys by NARRS volunteers

For the purposes of this study, a pond is defined as between 1m^2 and 2ha in area, holding water for a minimum of 4 months a year (Biggs et al. 1994).

We analysed data collected as part of the National Amphibian and Reptile Recording Scheme (NARRS). This allowed us to use data from ponds selected for survey at random and at a greater geographical scale than we would have been able to achieve alone.

Data from NARRS presented in this paper have been collected by volunteers. Bonardi et al. (2011) discuss the value of using volunteer data in order to assess the conservation status of common toad in Italy. The authors raise the problem of heterogeneity in sampling strategy and volunteer skills and experience. These problems have been addressed in this case by using standardised methods set out by NARRS, where surveys have been designed to be undertaken with very little training required.

NARRS pond surveys include up to four visits to the pond closest to the bottom left corner of the surveyor’s designated survey square. During the first visit the surveyor records pond location and completes a GCN HSI (Oldham et al. 2000) which includes recording information on pond size, vegetation cover, shading, terrestrial habitat, water quality and presence of fish and water fowl. Surveys of amphibians at each visit record the survey methods used and numbers of each species and life stage. Incomplete survey records were removed from the NARRS data as were any records where pond location was uncertain. We used 368 pond survey records from NARRS for surveys undertaken between 2007 and 2012 (see Figure 1 for locations of survey ponds).

GIS analysis of terrestrial habitat

We quantitatively assessed the availability of suitable habitat in the terrestrial environment at a range of scales. Buffers from each pond edge were created in ArcMap v.10 (ESRI 2011) at distances of 500m, 1km, 2km and 3km. We created national coverage shapefiles for woodland, surface water and urban habitats and used the clip tool to determine percentage coverage of each habitat type in each of the buffers (Ordnance Survey Open Data, www.ordnancesurvey.co.uk).

Based on a resource selection function (RSF) developed in previous work (Chapter 3: Salazar et al. in review) we also created a surface of available habitat (with greater than 0% relative probability of toad occurrence) which was also clipped to the buffers to determine percentage coverage. The RSF was originally developed using data collected in Oxfordshire. We feel comfortable applying this RSF on a national scale as the land use types used in its development are simple, universal and agree with basic land use types used in similar work (Hartel et al. 2008; Janin et al. 2009; Joly et al. 2003). The RSF was developed at 20 m resolution because this was in keeping with similar work (15m, Janin et al. 2009) and biologically relevant as a maximum daily distance travelled for toads in their summer foraging habitat (Sztatecsny and Schabetsberger 2005).

Logistic regression for selection of terrestrial habitat scale

As the terrestrial habitat variables (percentage coverage of woodland, water, urban and available habitat) were highly correlated (see Appendix Table 1) we performed a series of logistic regressions in R (R Development Core Team 2011) to determine at which scale each of the variables affected pond occupancy most significantly. Those variables that significantly predicted toad presence in ponds at the scale (500m, 1000m, 2000m, 3000m) with the lowest p-value, were retained for further analyses.

Principal component analysis and logistic regression

Using R software (R Development Core Team 2011), we completed a principal component analysis on the selected variables (see Table 1) and used the vegan package (Oksanen et al. 2015) to extract scores for each component for use as predictors in a logistic regression for common toad presence in ponds.

Logistic regression of selected variables

We used R (R Development Core Team 2011) to perform individual logistic regressions for each of the variables from the first two components of the PCA (Appendix 1) to create coefficient plots for the significant variables (Figure 2).

Results

Common toad was present in 33% of the ponds surveyed in the NARRS data set.

Logistic regression for selection of terrestrial habitat scale

Common toad presence was not significantly explained by coverage of urban or water habitats at any scale of buffer. Habitat available (as calculated by RSF) was significant at 500m ($est = 0.039$, $SE = 0.009$, $z = 4.428$, $p < 0.001$), 1km ($est = -0.066$, $SE = 0.018$, $z = -3.72$, $p < 0.001$) and 2km ($est = 0.057$, $SE = 0.029$, $z = 1.963$, $p < 0.05$) and woodland coverage was significant at 500m ($est = 0.039$, $SE = 0.016$, $z = 2.379$, $p < 0.01$). We therefore retained woodland at 500m (wood500) and available habitat at 500m (RSF500) for use in the PCA (Table 1).

Principal component analysis

Scores for the first and second components were significant in predicting common toad presence in ponds ($est1 = -0.395$ $SE1= 0.09$ $z1 = -4.369$, $p1 <$

0.001, est2 = 0.326 SE2 = 0.089 z2 = 3.673, p2 < 0.001). These components explained approximately 31% of the variance in the data set. Loadings were highest for water quality, presence of each of the newt species and vegetation coverage for component one and presence of frogs, coverage of available habitat and woodland and pond surface area for component two (Table 2).

Logistic regression of variables with high loadings

We performed individual logistic regressions for each of the variables from the first two components of the PCA (see Table 3 for results). Presence of common frog was significantly positively associated with common toad presence. Higher water quality also had a significant positive effect. Presence of smooth and palmate newts was positively associated with common toad presence, though presence of Great crested newt was not significantly associated. A positive association between common toad presence and coverage of woodland and available habitat (RSF500) in the 500m pond buffer was detected. Coefficient plots are in Figure 2.

Discussion

Common toad was present in one third of ponds selected for survey by NARRS. This suggests that the presence of toads in ponds is in some way ecologically constrained. The first and second principal components were both significant in predicting common toad pond use. The first component of the PCA mainly related to features within the pond, such as water quality and amphibian species presence.

Likelihood of toad presence improves with improving water quality. However, it is unclear whether the increased likelihood of toad presence with other amphibians is due to the other species having similar habitat requirements or a true association; coefficient plots for common frog, palmate and smooth newt are very similar to that for water quality. It has previously been shown that common toad, common frog and other anuran species are able to overcome competition in breeding sites through differential habitat use, promoting high species diversity in ponds (Indermaur et al. 2010a).

Water quality was measured subjectively by surveyors and it would therefore be beneficial to employ a more objective and quantitative method in estimating water quality in these ponds. We suggest use of the rapid assessment method developed by the Freshwater Habitats Trust based on PSYM, the national standard for assessing biological quality of inland freshwater (Pond Conservation 2010).

Unlike other studies (Hartel et al. 2008), we found no evidence that presence of fish is a significant predictor of toad presence. As toad larvae are generally unpalatable to fish the presence of fish was expected to give toads a competitive advantage over other amphibians, however this effect was not detected here. Similarly we found no evidence to suggest that pond size was important in this study, despite previous work indicating it to be the case (Beebee 1981). A minimum pond size is still likely to apply, related to a carrying capacity of at least 30 adult females (Halley et al. 1996) and larger ponds are likely to have a longer and more stable hydroperiod to allow sufficient time for tadpoles to develop.

The second component of the PCA was more strongly loaded towards terrestrial habitat variables. Logistic regression of variables from the principal components revealed a positive association between common toad occupancy and that of other amphibian species, particularly common frog, smooth newt and palmate newt.

In agreement with previous studies (Hartel et al. 2008; Janin et al. 2009; Ray et al. 2002), we found terrestrial habitat variables were important in determining probability of common toad presence in ponds. The available habitat as defined by the RSF from previous work (Salazar et al. in review) was significant in predicting toads, and takes into account configuration of habitat rather than simple coverage. However, in this study, coverage of woodland was approximately equal in its potential for predicting toad use of ponds and does not rely on complex computation of the relative probability of toad occurrence. As such, we propose that for predicting toad use of ponds, coverage of woodland within a 500m buffer of the pond location is a more practical measure to use in developing a habitat suitability index. The effect of landscape configuration rather than simple coverage still merits further consideration however; potential movement zones computed over a resistance surface around ponds and barrier-based buffers have both been shown to be better predictors of common toad presence than composition alone (Janin et al. 2009; Zanini et al. 2008 respectively).

Water coverage was not significant in predicting toad presence, although pond density has been shown to be important in other studies (Scribner et al. 2001). It would perhaps be informative to investigate whether the aspect of water coverage most likely to be relevant to toads, pond density, predicts toad presence. This would remove the effect of other water bodies such as rivers, which may depending on their size act as barriers and so reduce available habitat surrounding a pond (Zanini et al. 2008). Similarly, although we found no evidence that urban area coverage predicts toad use of ponds the effects of urban areas in isolating toad populations are clearly important (Hitchings and Beebee 1998). Direct mortality on roads is an issue of great concern (Beebee 2013), especially in the UK where mass killings of toads on roads have prompted volunteers to set-up toad crossing schemes to help vulnerable populations during their migration. Therefore, further work should also consider quantifying the potential effects of roads in the area surrounding ponds.

This study of national level data demonstrated common toad occupancy of ponds is determined by a combination of habitat suitability characteristics both biotic (presence of other amphibians) and abiotic (water quality) and relating to both the aquatic and terrestrial environment. Further work is suggested to expand this study to separate effects of amphibian presence from those of water quality, and effects of the land use type coverage from those of landscape configuration and the presence of barriers.

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Tables

Table 1: Variables retained for PCA

Variable (scale)	Code
Pond surface area (m2)	Area
Water quality (1 = poor, 2 = average, 3 = good, 4 = excellent)	Quality
Percentage of pond bank perimeter shaded to at least 1m	Shade
Presence/absence of fish (0/1)	Fish
Presence/absence of smooth newt (0/1)	Smooth
Presence/absence of Great crested newt (0/1)	Crested
Presence/absence of palmate newt (0/1)	Palm
Percentage coverage of woodland within 500m pond buffer	wood500
Percentage coverage of available habitat within 500m pond buffer	RSF500
Presence/absence of common frog (0/1)	Frogs
Percentage coverage of pond sufarce with aquatic vegetation	Veg

Table 2: Principal component analysis results. Highest factor loadings for each component in bold.

Variable	Component 1	Component 2
Area	0.136	0.226
Shade		
Veg	-0.292	-0.155
Quality	-0.494	0.103
Fish		
Frogs	-0.141	0.314
Crest	-0.421	-0.332
Smooth	-0.425	-0.36
Palm	-0.451	0.233
wood500	-0.215	0.53
RSF500	-0.119	0.488

Table 3: Results of individual logistic regressions.

Toads (Variable)	Estimate	SE	Z	P
Common frog	1.1069	0.2458	4.503	<0.001
Water quality	0.4582	0.1389	3.299	<0.001
RSF 500m	0.013942	0.0043	3.24	<0.01
Palmate newt	0.7471	0.2388	3.128	<0.01
Wood 500m	0.015861	0.00586	2.708	<0.01
Smooth newt	0.559	0.2358	2.371	<0.05
Pond area	2.81E-05	2.33E-05	1.206	0.228
Great crested newt	0.2444	0.3228	0.757	0.449
Pond vegetation coverage	-0.0001254	0.00367	-0.034	0.973

Figures

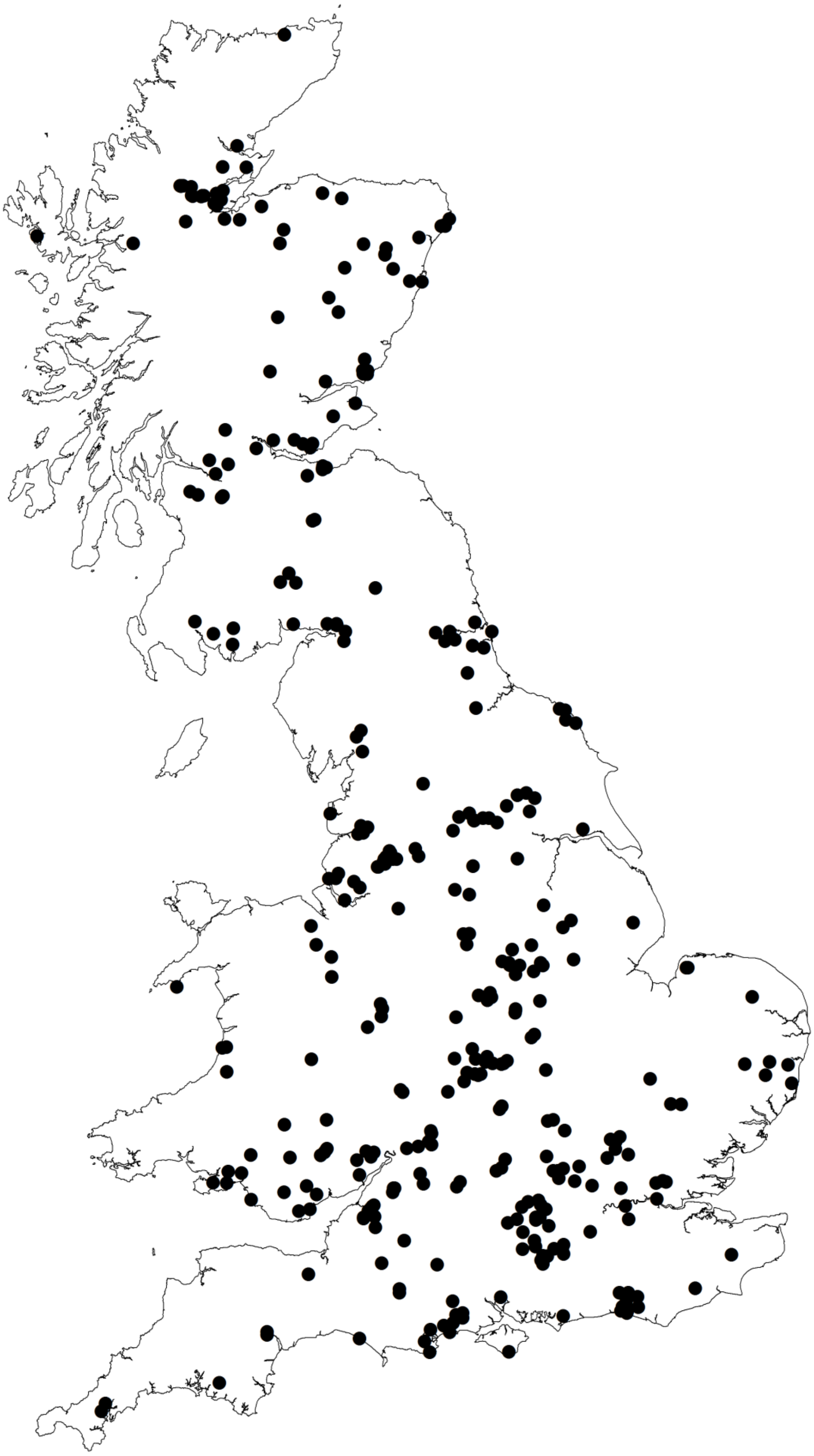

Figure 1: Distribution of the NARRS survey ponds.

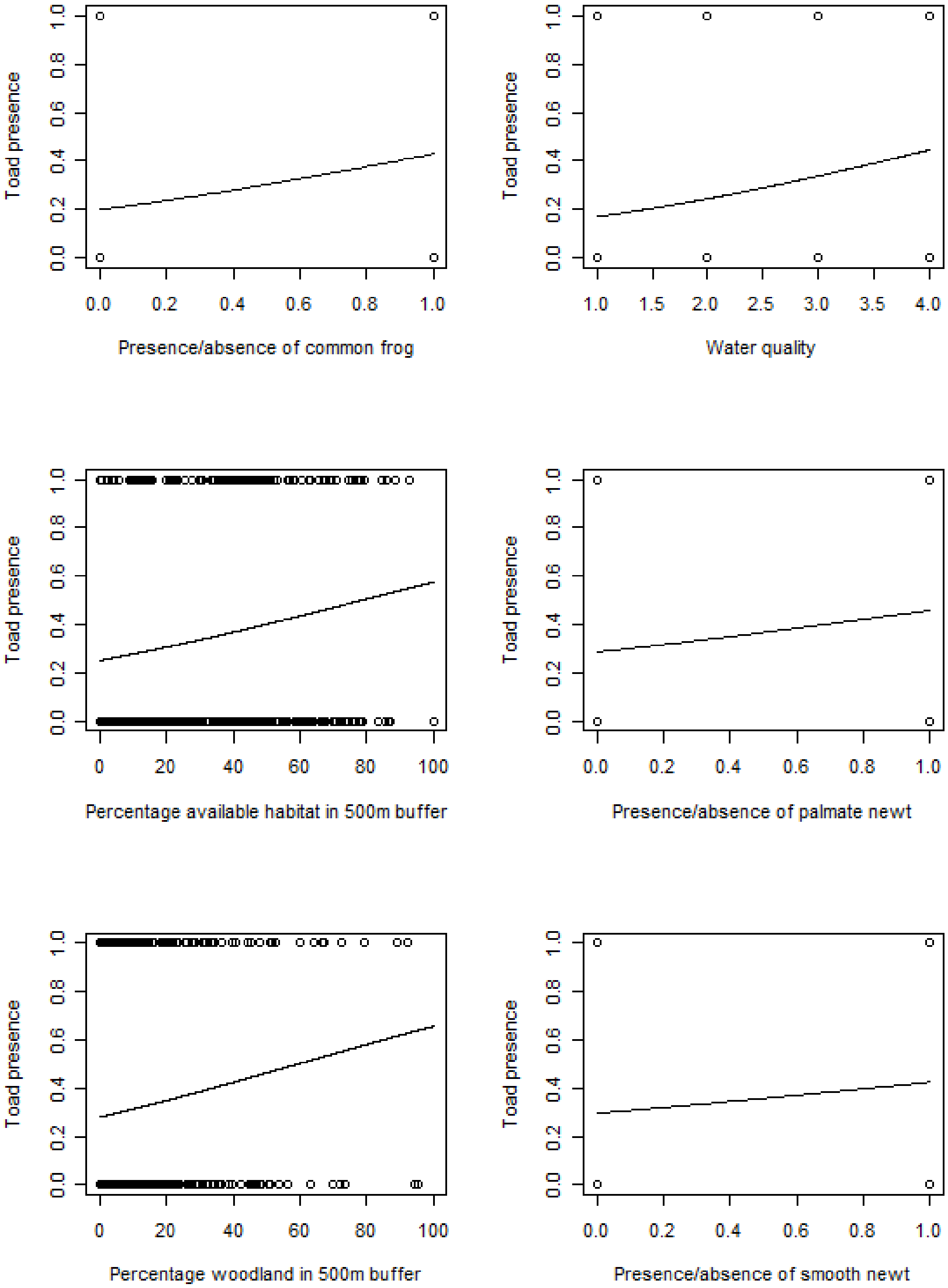

Figure 2: Coefficient plots for each of the variables contributing to common toad presence in ponds.

Appendices: Appendix 1: Correlation matrix of terrestrial habitat variables. Values >0.6 are highlighted.

	Toads	wood500	RSF500	urb500	wat500	urb1	wat1	wood1	RSF1	urb2	wat2	wood2	RSF2	urb3	wat3	wood3	RSF3
Toads	1	0.14	0.17	-0.01	-0.02	0.00	0.01	0.09	0.07	0.03	0.08	0.05	0.10	0.05	0.08	0.03	0.07
wood500	0.14	1	0.39	-0.26	-0.02	-0.25	-0.04	0.91	0.37	-0.23	-0.01	0.77	0.34	-0.23	0.01	0.69	0.29
RSF500	0.17	0	1	-0.16	0.36	-0.11	0.23	0.36	0.83	-0.07	0.16	0.30	0.56	-0.06	0.17	0.27	0.45
urb500	-0.01	-0.26	-0.16	1	-0.04	0.92	-0.02	-0.29	-0.17	0.77	-0.05	-0.28	-0.17	0.70	-0.06	-0.27	-0.15
wat500	-0.02	-0.02	0.36	-0.04	1	0.02	0.78	-0.04	0.28	0.08	0.47	-0.07	0.15	0.10	0.38	-0.05	0.11
urb1	0.00	-0.25	-0.11	0.92	0.02	1	0.01	-0.32	-0.16	0.93	-0.04	-0.34	-0.19	0.87	-0.04	-0.33	-0.17
wat1	0.01	-0.04	0.23	-0.02	0.78	0.01	1	-0.04	0.29	0.05	0.80	-0.04	0.26	0.08	0.65	-0.04	0.20
wood1	0.09	0.91	0.36	-0.29	-0.04	-0.32	-0.04	1	0.42	-0.32	0.02	0.91	0.43	-0.32	0.07	0.84	0.38
RSF1	0.07	0.37	0.83	-0.17	0.28	-0.16	0.29	0.42	1	-0.13	0.28	0.39	0.80	-0.12	0.31	0.35	0.68
urb2	0.03	-0.23	-0.07	0.77	0.08	0.93	0.05	-0.32	-0.13	1	-0.01	-0.36	-0.20	0.98	-0.01	-0.36	-0.20
wat2	0.08	-0.01	0.16	-0.05	0.47	-0.04	0.80	0.02	0.28	-0.01	1	0.03	0.39	0.02	0.92	0.05	0.36
wood2	0.05	0.77	0.30	-0.28	-0.07	-0.34	-0.04	0.91	0.39	-0.36	0.03	1	0.47	-0.36	0.09	0.97	0.43
RSF2	0.10	0.34	0.56	-0.17	0.15	-0.19	0.26	0.43	0.80	-0.20	0.39	0.47	1	-0.18	0.45	0.44	0.92
urb3	0.05	-0.23	-0.06	0.70	0.10	0.87	0.08	-0.32	-0.12	0.98	0.02	-0.36	-0.18	1	0.00	-0.37	-0.18
wat3	0.08	0.01	0.17	-0.06	0.38	-0.04	0.65	0.07	0.31	-0.01	0.92	0.09	0.45	0.00	1	0.10	0.47
wood3	0.03	0.69	0.27	-0.27	-0.05	-0.33	-0.04	0.84	0.35	-0.36	0.05	0.97	0.44	-0.37	0.10	1	0.42
RSF3	0.07	0.29	0.45	-0.15	0.11	-0.17	0.20	0.38	0.68	-0.20	0.36	0.43	0.92	-0.18	0.47	0.42	1

Chapter 6: Discussion

The toad to recovery

Discussion

Aims and Motivations

The global amphibian decline crisis has received much attention (Houlahan et al. 2000; Wake 1991), with 32% of amphibians worldwide threatened according to the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species (Baillie et al. 2004). There is a general consensus among herpetologists that the most important cause of these widespread amphibian declines is related to habitat loss and degradation (Bishop et al. 2012; Gallant et al. 2007; Lemckert et al. 2012). In the United Kingdom, declines in the 7 native amphibian species has substantially been attributed to effects of habitat loss due to agricultural conversion (Browne and Paszkowski 2014). The common toad (*Bufo bufo* L.) is widespread in Europe but has experienced dramatic and unexplained population declines (Bonardi et al. 2011; Carrier and Beebee 2003).

With this in mind, the focus of my thesis was to study common toad populations in response to habitat change. In particular I focused on toad populations where that habitat change occurred in, or was the result of conversion to, the most extensive landscape in England, which is farmland (Natural England 2008), the impact of which I discuss below.

The common toad is expected to be particularly vulnerable to the effects of habitat change and isolation of its breeding populations due to its reliance on both aquatic and terrestrial environments, its limited dispersal capacity (Sinsch 1988) and renowned philopatry (Reading et al. 1991). This means that individual toad breeding populations across an agricultural landscape are likely to become isolated, with little to no gene flow. Isolation of populations means that they cannot be replaced or replenished in the event of extinction or decline. Reduced genetic diversity in populations also poses threats with reduced fitness and adaptability as seen in the common frog *Rana temporaria* (Johansson et al. 2005). Though a species of conservation concern in the United Kingdom (e.g. desginated as a UK BAP priority species in 2007, UK Biodiversity Partnership 2010), it is however, widespread in Europe and therefore any recommendations for its conservation can be applied over a large scale.

Agriculture has the potential to effect the quality and availability of habitat suitable for common toads; conversion to arable farmland replaces a complex vegetation structure with monoculture. There is potential, however, for the semi natural habitats that remain in the UK’s agricultural landscape mosaic to harbour wildlife and biodiversity (Bennett et al. 2006). Furthermore, agri-environment schemes (AES) in Europe offer the prospect of sensitive management of farmland to promote and protect biodiversity in targeted habitats (Kleijn et al. 2006; MacDonald et al. 2007; Maes et al. 2008).

Summary and discussion of principal findings

Herpetofauna species are under-represented in research into the effectiveness of AES management (Kleijn and Sutherland 2003) and so in Chapter 2, I aimed to address this gap in current knowledge regarding the importance of uncultivated field margins for amphibians and reptiles. Common toad occupancy of field margins was positively associated with proximity to ponds. The finding agrees with (Scribner et al. 2001), where pond density was shown to be an environmental correlate of toad abundance, and with the findings of Chapter 3, which showed water bodies have a positive effect on the probability of toad occurrence. The results of Chapter 2 provided evidence of use of field margins by common toad, grass snake and common lizard. We made recommendations for improvement of field margin management in increasing the extent and width to benefit common lizard. Based on the findings, we also recommended an increase in the density of ponds to facilitate movement of common toads through the landscape. This is in agreement with Chapter 3 where we found that the distance to water bodies is a key factor determining the probability of toad occurrence in the terrestrial environment.

The work in chapters 2 and 3 focused on arable farmland. For a comprehensive view of the effectiveness of AES and value of management options for common toad and other herpetofauna species we would need to consider other management options, as well as look at the specific challenges posed by other farm types. Pasture farmland for example, will pose specific threats to common toad, such as poaching of pond banks, increased nutrient inputs and eutrophication of water bodies from livestock waste. Plans for future work should also consider the proposed changes in agri-environment schemes which are to be replaced by the countryside stewardship scheme. It is essential to identify which management options offer the greatest value for biodiversity to influence which should continue to be supported under the new scheme and to this aim, further research should include the study of field margins, hedgerow and ditch management and other AES options targeted at biodiversity conservation.

Common toads spend much of their adult life beyond the pond in the terrestrial environment (Beebee 1985) and so I deemed it an important part of my research into habitat change impact on toads to assess their use of the terrestrial environment. The Resource Selection Function (RSF) developed in Chapter 3 was used extensively through the remainder of the thesis; to develop a surface of barriers at the landscape scale between ponds in Oxfordshire to test for the effect of isolation by barriers in genetic distance between ponds (Chapter 4) and to determine proportion of available habitat in buffers around ponds at the national scale (Chapter 5). The RSF highlighted the importance of woodland and water bodies in increasing the likelihood of toad presence and the effect of urban areas in reducing toad probability of occurrence. It would be interesting, although beyond the scope of this thesis, to apply these findings to the occupancy modelling of Chapter 2 to determine whether distance to woodland and urban areas/roads improve occupancy models for common toad in field margins.

Chapters 2 and 3 focus on the study of toads in lowland farmland. However, it is also important to assess the potential effects of other land use types, and changes therein. For example, deforestation causes changes in the water chemistry of nearby water bodies, in the amount of shade, in lag-time (from rain to water level rise) and in hydroperiod of water bodies (Jackson and Jackson 2000). Presence of woodland in the first 50m from the river bank benefits aquatic invertebrate communities of rivers (Poole et al. 2013) and so may reasonably be expected to also affect aquatic invertebrates in ponds.

The negative impact of urban areas on common toads was demonstrated in Chapter 3 and supports the findings of (Hitchings and Beebee 1998), who found reduced genetic diversity of toads in urban areas due to their isolation. Urban areas are also highly associated with road density however, and it is difficult to separate these effects. Common toads have been identified as being particularly vulnerable to the effects of road, especially through direct mortality of migrating adults which can be significant in reducing toad population size (Andrews et al. 2008a; Beebee 2013; Cooke and Sparks 2004; Kuhn 1987; Matos et al. 2012). In the UK, concern for the species has prompted volunteer groups to set up toad crossings to rescue amphibians crossing busy roads near their breeding pond (e.g. Froglife’s Toads on Roads project: www.froglife.org). Data on the effectiveness of these toad crossings in protecting toad populations should be incorporated in the design of future road mitigation projects and will aid the study of the effect of roads on toad populations through direct mortality and indirectly as barriers to migration (Zanini et al. 2008).

Habitat requirements in the terrestrial phase are likely to be specific to the life phase and activity of toads; first migration as newly emerged metamorphs, seasonal migration of adults, foraging grounds, daytime shelters and hibernation sites. In Chapter 3 we focused on adults, and searched for PIT tagged adults during daylight hours when toads would be in their daytime shelter habitats for which they likely require specific microclimate (e.g. cane toad, Seebacher and Alford 2002). These shelters may be close to where they were foraging in the previous evening but may be subtly different in structure and microclimate. There is also likely to be a degree of seasonality associated with the toad records during PIT detecting e.g. Western toads were more likely to be found near breeding sites in the Spring and hibernation sites in the Autumn (Browne and Paszkowski 2010b).

Use of PIT tags as a search aid for common toads in the terrestrial environment is a fairly novel method and had not been used for amphibians at such a large scale in an open environment prior to my research (Connette and Semlitsch 2012; Cucherousset et al. 2008). It was preferred method, however, as it allowed me to gather data for more individuals than, for example, radio tracking, and the data would not be spatially autocorrelated. Furthermore, there are fewer welfare concerns with PIT tagging toads than other invasive marking techniques (e.g. see review of marking techniques, Ferner 2010). Toads tagged as part of my study were also photographed and future work in this project will involve developing a technique for photographic individual identification of toads from their belly patterns, as already commonly used for Great crested newts (Hagström 1973). This technique would involve even fewer welfare concerns for toads as it is non-invasive.

Isolation by barriers was more important in explaining genetic distance in toads than Euclidean distance in West Oxfordshire (Chapter 4). Common toads have substantial capacity for dispersal, as demonstrated by the lack of association with Isolation By Distance (IBD) and limited genetic structuring (Appendix II: Eynard 2013). This should not be taken for granted when considering habitat modification, as there may be a lag time in the effect of habitat change or isolation before they become apparent within populations (Piha et al. 2007). Safner et al. (2011) demonstrated the need to combine genetic and demographic approaches when considering the effects of rapid habitat change. In a case study of common frog, Johansson et al. (2007) highlighted the potential impact of reduced gene flow due to habitat fragmentation in the reduced fitness and adaptability of isolated populations. Because of the multiplicative effect of these impacts, populations may persist for long periods before the effects of habitat change become apparent (Piha et al. 2007).

Euclidean distance was associated with genetic distance at a much larger scale (over hundreds of kilometres, Chapter 4). We consider it is vital to continue research in this area to find the threshold distance at which isolation by distance becomes limiting to gene flow between toad breeding populations.

At the national scale common toads occupy one third of ponds, which suggests that their presence in ponds is in some way ecologically constrained. Water quality was shown to be important in this study, though the way it is measured in NARRS is not quantitative or robust. We suggest that by adding the freshwater habitat trust rapid assessment method to the NARRS survey protocol we would achieve a more quantitative measure of the biological quality of ponds.

Available habitat (as calculated by RSF: Chapter 3) was an important predictor of toad presence in ponds (Chapter 5) and barriers calculated by RSF explained genetic distance in toads better than Euclidean distance (Chapter 4). The RSF (Chapter 3) combined the effects of proximity to different land-use types and is a good example of the importance of considering configuration of habitat types within the landscape rather than just coverage of each type when assessing habitat suitability.

However, for the purposes of developing a tool which could be used to rapid assess probability of toads using ponds then coverage of woodland is likely to be a more practical approach. Woodland was one of the main factors in the second component of the PCA and was significant in predicting presence of toads in ponds. Furthermore, woodland coverage can be easily calculated using maps, satellite images or free GIS software and so can be calculated reliably and swiftly.

Though habitat loss and degradation is recognised as the most important factor contributing to amphibians worldwide (Bishop et al. 2012; Gallant et al. 2007; Lemckert et al. 2012) it cannot be considered in isolation. The potential for interaction between habitat change and other causes of decline is substantial, particularly for disease where effects of habitat fragmentation on body condition of toads such as those reported by Janin et al. (2011b) could be expected to translate into increased vulnerability to pathogens in degraded habitats. Similarly, where presence of barriers or low density of ponds inhibits movement of toads this may have consequences for isolated populations unable to respond to climate change with a shift in range. A complete response to the recent declines in common toad populations (Bonardi et al. 2011; Carrier and Beebee 2003) should focus on habitat as the likely main driver but also include the potential exacerbating effects of each potential cause.

Conclusions and recommendations

Chapter 2 demonstrated that uncultivated field margins are used by amphibians and reptiles. Future work should consider the importance of the finer scale vegetation structure, age and proximity of these field margins to habitats expected to positively influence common toad probability of occurrence (woodland, water) before support for the creation and management of these features in our landscape is withdrawn when moving to a new agri-environment scheme (Countryside Stewardship, beginning in 2015). The results of Chapter 2 highlight the opportunity that agri-environment scheme management of farmland offers for supporting species conservation. In this project, it was only feasible to investigate the effect of a small range of management options (field margins, hedgerows, ditches) for a small range of species (common toad, common lizard, grass snake). Future work should seek to determine the value of different agri-environment scheme options for the entire range of widespread amphibian and reptile species that we might reasonably expect to use farmland. In a similar way to Janin et al. (2011b), we could go further and investigate the effect of the habitat types represented by each management option on the body condition and fitness of the animals using them.

Chapter 3 showed that the probability of common toad occurrence in farmland was positively affected by proximity to woodland and water, and with greater distance from urban areas. One of the strengths of the findings of the chapter is the simplicity of the habitats used in developing the RSF, which allows them to be applied over a range of scales. Woodland, water and urban areas are universal and coverage of these habitats can be easily extracted from maps, with data often already available for use in GIS systems in high resolution. The findings can therefore be used widely, as demonstrated by the significant results in Chapter 5 on the ability of coverage of woodland and available habitat to predict pond occupancy by common toad, where the RSF was applied to the area around ponds at a national scale. Future work should however consider repeating this work in a range of different landscape types e.g. dominated by pasture or woodland to investigate whether our findings still hold true.

The RSF was used to identify barriers to toad movement in Chapter 4. These barriers seem to be more isolating at the landscape scale than Euclidean distance. As the barriers were defined as areas with 0% relative probability of toad occurrence we can now make recommendations for reducing the size and strength of barriers in the landscape by improving the probability of toad occurrence i.e. increasing the density of woodland/scrub and wet habitats and therefore reducing the average distance to these areas. This recommendation could be achieved through agri-environment scheme management, through ditch management to hold water, pond creation, woodland creation and management (e.g. through the new Countryside Stewardship woodland capital grants) and hedgerow creation and management. Future work following on from Chapter 4 should investigate whether there is a threshold distance beyond which Euclidean distance becomes important in isolating breeding populations. This information would allow us to identify and perform rescues of isolated populations, either through translocation of individuals/spawn or by management of the landscape between neighbouring populations to reduce the size and strength of barriers.

Chapter 5 showed that both the aquatic and terrestrial environments are important in determining toad pond selection. Some of the findings go against conventional wisdom; in particular, I was surprised to find no relationship with presence of fish which is often considered a major influence on common toad pond use (Beebee 1981). Moving forward with this study I will go on to include further data collected by NARRS since this analysis with a view towards developing a tool for assessing the suitability of ponds for breeding toads. Future work could also consider developing a similar suitability index for use in the terrestrial environment that should draw on the findings of the RSF to include distance to urban, woody vegetation and wet habitats but might also consider finer scale vegetation structure.

In conclusion, this thesis has demonstrated the importance of the terrestrial habitat for the common toad at a range of scales from local, landscape to national and has identified key habitats that influence the species probability of occurrence, ability to move around the landscape and selection of breeding ponds. Crucially, the findings have led to recommendations which have the potential to be achieved through government funded Countryside Stewardship schemes, supporting the value of these schemes for species conservation.

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Appendix I: The effect of artificial cover material on the likelihood of detecting three widespread herptile species in UK lowland arable farmland

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Running head: Refuge material herptile detection

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Appendix I

Abstract

Limited literature exists on the relative effectiveness of different refuge materials. This study presents the significance of three refuge materials and reveals their role in increasing the probability of detecting different herptiles.

Keywords; Survey methods, Refuge, *Natrix*, *Bufo*, *Zootoca vivipara,*

1. Introduction

There is growing concern over the decline of herptiles across the world with habitat loss, invasive species and climate change being the main driver of this decline (Bishop et al. 2012; Edgar et al. 2010; Ryan et al. 2002; Walther et al. 2002). Britain in particular has a paucity of data on the current status of herptile populations which is highlighted in the recent state of nature report (RSPB, 2013) thereby calling for urgent research. There are six terrestrial reptile species and seven amphibian species native to Britain each facing environmental and anthropogenic threats particularly in areas covered by agricultural land e.g. natural habitat destruction (Berry et al. 2005; Driscoll 2004) Beebee & Griffiths, 2005) and chemical exposure (Mann et al. 2009). Homogenous landscapes lack habitat diversity; areas for predator avoidance; connectivity for dispersal; reduced prey avail …… CONNECTING PARA

Since 1984 the Common Agriculture policy (CAP) established financial incentives to encourage farmers to carry out environmentally beneficial actions (Ovenden et al. 1998). In England the ministry of agriculture, fisheries and food (MAFF) created Agri-environment schemes (AES) to promote habitat protection and the conservation of species that inhabit agricultural land (Antle *et al*., 1998; Baldock and Bennet, 2002; Commisssion of the European Communities, 2002). Over six million hectares of British land is covered by AES agreements which accounts for over 65% of the agricultural land (Natural England 2009). The landowners of these properties have all agreed to use less intensive farming methods. At a cost of £400 million a year the benefits of AES for farmland flora and fauna have been well debated with some presenting evidence of great benefits for British biodiversity (Carey et al. 2002; Hanley et al. 1999) and others questioning the true effectiveness of AES for many taxa (Kleijn et al. 2006; Kleijn and Sutherland 2003). As research into this field continues very few projects have investigated the role of AES types on supporting reptile and amphibian populations. Firbank *et al*., (1992) report that undisturbed landscapes with a range of vegetation types and heights tend to attract reptiles as the vegetation provides an important refuge (Norbury, 2000). For amphibians, connectivity across disturbed landscapes has been proven to benefit amphibian populations. Mazerolle and Desrochers (2005) found that when presented with a choice 72% of northern green frogs avoided disturbed landscapes therefore AES should provide connectivity and preferred habitat for herptile populations on farmland. The removal of vegetation such as tussock grasses can reduce the number of safe areas for reptile thermoregulation and reptiles have to travel further to find prey in landscapes devoid of vegetation, which increases predation risks (Bentley et al. 2000; Macarthur and Pianka 1966). Implementing AES regulations reduces the risk of habitat destruction and therefore has likely benefits for herptile populations.

Temperate climates such as those found in Britain offer limited thermal conditions for reptiles, and they therefore have to sacrifice predator avoidance against thermoregulatory requirements (Lelievre et al. 2010). One way reptiles successfully thermoregulate is to utilise natural refuges/shelters such as logs and stones of differing thermal properties (Cox et al. 2009; Engelstoft and Ovaska 2000). Utilising this biological factor with the use of artificial refuge materials (also known as artificial cover boards) during reptile surveys has increased detection rates in recent studies (Scheffers et al. 2009). Many studies have recognised the advantages of artificial refuge materials, and as they attract reptiles and amphibians that are very difficult to survey; they are a non-intrusive method; require nominal funding and limited labour from researchers (Engelstoft and Ovaska 2000; Lelievre et al. 2010) however there is ambiguity regarding the best artificial refuge materials to use when surveying herptile populations.

However, the efficiency of different artificial refuges in increasing detection probability of herptile species is largely unknown. This study trialled three types of artificial refuge commonly used in reptile surveys (and to a lesser extent, amphibian surveys): roofing felt, carpet and corrugated tin. The studies were carried out in two AES options: set-aside areas and field margins on farms with Entry Level Stewardship agreements. We comment on the effect of refuge type, AES option and environmental conditions on the probability of detecting three of the commoner herptile species in UK lowland farmland: grass snake (*Natrix natrix*), common lizard (*Zootoca vivipara*) and common toad (*Bufo bufo*).

2. Methods

2. 1. Study Area

The study was conducted from May-October 2011 across two neighbouring farms under Entry Level Stewardship (ELS), on lowland farmland in the Oxfordshire area (Fig. 1). ELS is an Agri-environment scheme (AES) funded by the EU Common Agriculture Policy and endeavours to conserve existing wildlife (Natural England, 2009). The two farms covered a combined total surface area of 6,676,452 m^2. This study concentrates specifically on two AES land-use options: Set-aside land and field margins. Both land-use types were chosen to assess their impacts on herptile populations, which to date has received little attention (Atauri and de Lucio 2001). Two areas of set-aside land and 51 100m lengths of field margins were identified as potential herptile habitat (Fig. 1). An initial pilot study carried out in May/June 2011 across the study area using 120 artificial refuges (Carpet, Felt, Tin) and visual searches identified the presence of common toad (*Bufo bufo)* grass snake *(Natrix natrix)* and common lizard *(Zootoca vivipara)*.

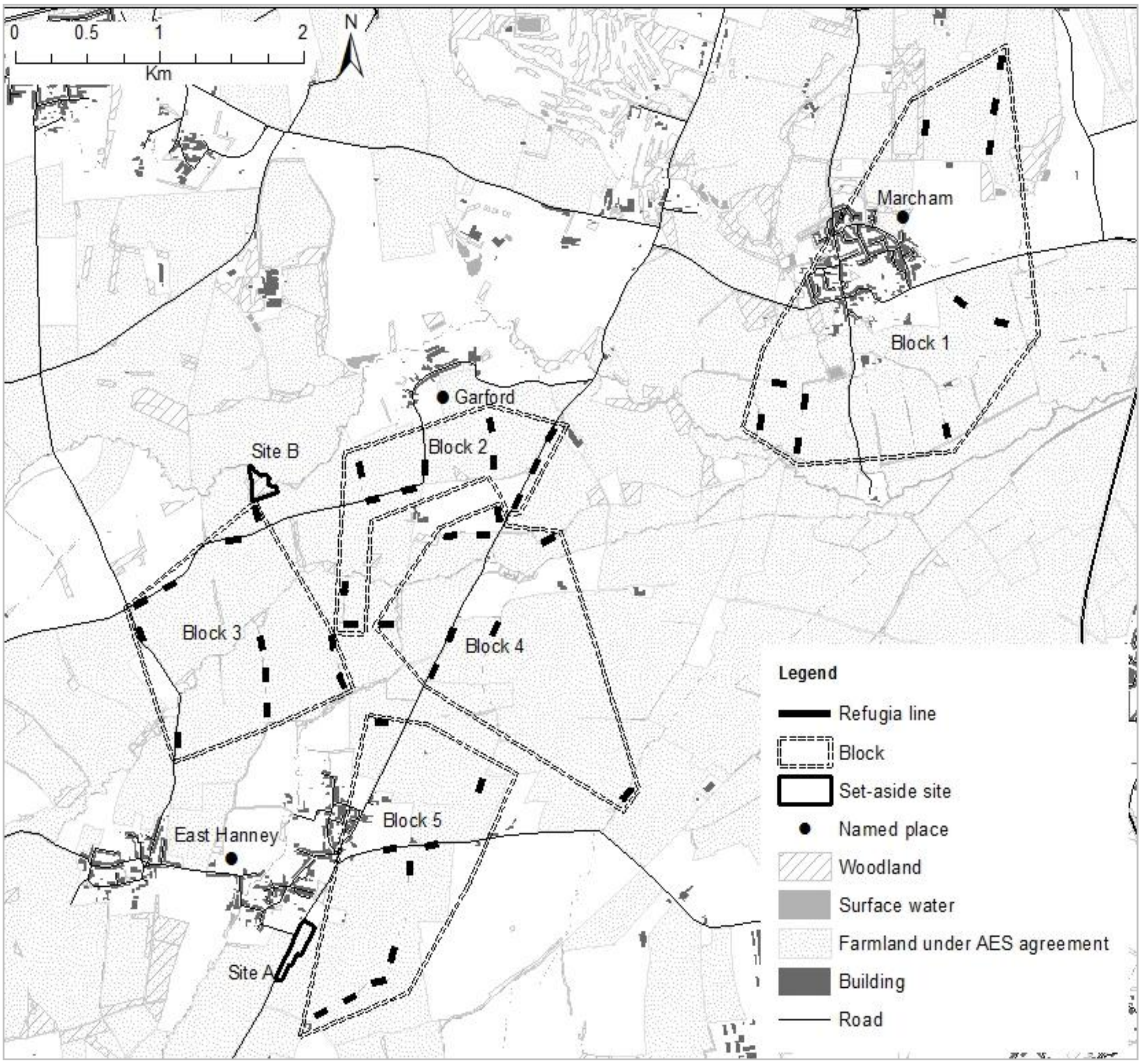

Fig. 1. Location of agri-environment scheme (AES) study sites.

2.2. Set-aside sites

The set-aside sites were three miles apart. Site A (29,848 m^2) was located in East Hanney, Oxfordshire (Fig. 1) was a large open meadow surrounded by a shallow wet ditch and bordered by hedgerow and scattered trees. Site B was (23,265m^2) located in Garford, Oxfordshire (Fig. 1) predominantly covered in deciduous woodland but has large open spaces covered in grassland.

2.3. Field margins

The 51 field margins were located in arable fields (Fig. 1), within 1m of the bordering hedge/ditch or hedge and ditch combination. Each 100m stretch was always a minimum of 100m from the nearest neighbouring stretch. Crops varied between barley, wheat, beans and oil seed rape. The field margins were split into five blocks (Fig. 1) to which could each be surveyed in a single session and to allow analysis for effects of farm owner (Blocks 1 and 5 owned by Farm A, Blocks 2, 3, and 4 owned by Farm B).

2.4. Survey Method

Herptiles were surveyed using the standard survey methodology used by the National Amphibian and Reptile Scheme (NARRS) which incorporated visual searches and refuge surveys (Edgar et al. 2010). The visual searches were conducted during the initial months of the study (May-June) as this period is considered the most effective sampling period just after the reptile brumation phase (Edgar et al. 2010). While searching any sloughed skins, eggs or basking individuals were recorded. For the refuge surveys each set-aside site had 20 sets of refuges (set out in early May 2010) and 255 sets in field margins (late

May 2010). Refuges were placed in the most desirable locations for reptile and amphibian use, choosing open areas and south-facing slopes (Edgar et al. 2010). Each of the 255 sets of refuge materials consisted of a carpet tile and bitumen roofing felt on field margins. In addition to the felt and carpet a corrugated tin sheet was added to the 20 sets on each set-aside land, making a total 295 sets of refugia. Each measured approximately 50cm x 50cm and each set was placed within 30cm of one another. The location of each set was recorded by GPS and marked with a bamboo cane as a visual aid. Once placed out in the field the refugia were left for two weeks to allow the tiles to settle and for herptiles to begin to find and use them.

After a two week period the refuges were checked for reptiles according to the methods of Edgar *et al*., (2010) as each refuge was lifted and the presence or absence of reptile species was recorded. The sites were checked from June-October 2011 and refuge sets were checked on average once a week to minimise disturbance. The refuges were only checked at certain times of day according to the expected basking periods of the herptiles. Morning checks were implemented between 7-11am and evening checks between 3-7pm as advised by Baker and Gleed-Owen (2007). The order of checking each refuge set was random and the same set was not checked more than twice a week to minimise disturbance. Weather conditions were the ruling factor as to when the refugia were checked (air temperature above 10˚C, no rainfall and low wind speed) (Edgar et al. 2010). These conditions increase the probability of the detection of (otherwise cryptic) herptiles (Edgar et al. 2010). The presence of reptiles or amphibians species either on top or under the refugia was recorded.

In addition, weather data from the Wytham Weather station, Oxfordshire (ECN, 1992) was analysed.

2.5. Statistical Analysis

Data was analysed using R statistical software version 2.13.1 (R Development Core Team 2011) to construct generalised linear mixed models (binomial family to test each fixed effect (

Table 1) for each taxon detected (using refuge identity as a random factor). Analysis required use of the lme4 package (Bates *et al*., 2011) and the model code was: lmer (taxon count) ~ (fixed effects) + (1|ID). Fixed effects were divided into two models, (Table 1), namely (a) a weather model and (b) a habitat model. Backward stepwise deletions were then used to remove non-significant variables from the model, so that only significant weather and habitat variables remained to form a final model for each taxon detected (Grass snake, common toad and common lizard).

Appendix I

Table 1: Summary of all variables used in the generalised linear mixed models. The Dependant variable is the abundance of each taxon detected under artificial refuges, The Fixed effects are split according to those initially used in the (a) weather model and (b) Habitat model, keeping refuge ID as the random effect.

Dependent variable	Taxon abundance
Fixed effects	
(a) Weather variables (all continuous)	Dry air temperature (˚C) Wind speed (m/s) Rainfall (mm)
(b) Habitat variables (all categorical)	Month (June/July/August/September/October) AES Type (Set-aside/field margin) Time of survey (AM vs PM) Refuge type (Carpet, Felt, Tin)
Random effect	Refuge ID

3. Results

3.1. Taxon Counts

Between June-October 2011 the 295 artificial refuges yielded a total of 214 observations. *N. natrix*, *B. bufo*. and *Z. vivipara* were found to occupy the refuge materials, the maximum number of observations were recorded during September (68) and the lowest in October (6).

GLMM analysis gave a significant effect on the survey month on the probability of detecting a grass snake (GLMM, Month, $F_{3,295} = 28.14$, $P < 0.001$) and common toad (GLMM, Month, $F_{3,295} = 23.51$, $P < 0.001$). During August there was a significantly higher number of observations (27) of grass snake whereas very low recordings in October (1). More than half of the total observations of the common toad were recorded during September whereas during June and October zero amphibians were detected.

3.2. Refuge material preference

GLMM analysis confirmed that refuge type had a significant effect on the probability of detecting particular reptile species, and was further extended to the preference of amphibians. Felt refuges were significantly more likely to increase the detection rates of grass snakes (GLMM, Refuge, $F_{2,295} = 19.31$, $P < 0.001$; Fig. 2). Felt provided 63% of the grass snake observations whilst carpet and tin only 28% and 9% respectively. GLMM analysis suggested tin may have an impact on the detection of grass snakes due to the large effect size however the high standard error reflects the lower number of tin refuges in set aside land. Significance also indicates that felt refuges are best used to increase the chance of detecting the common lizard (GLMM, Refuge, $F_{2,295} = 6.24$, $P < 0.05$; Fig. 2). Approximately 57% of observations were under felt refuges whilst tin and carpet yielded (16%) and (27%) respectively.

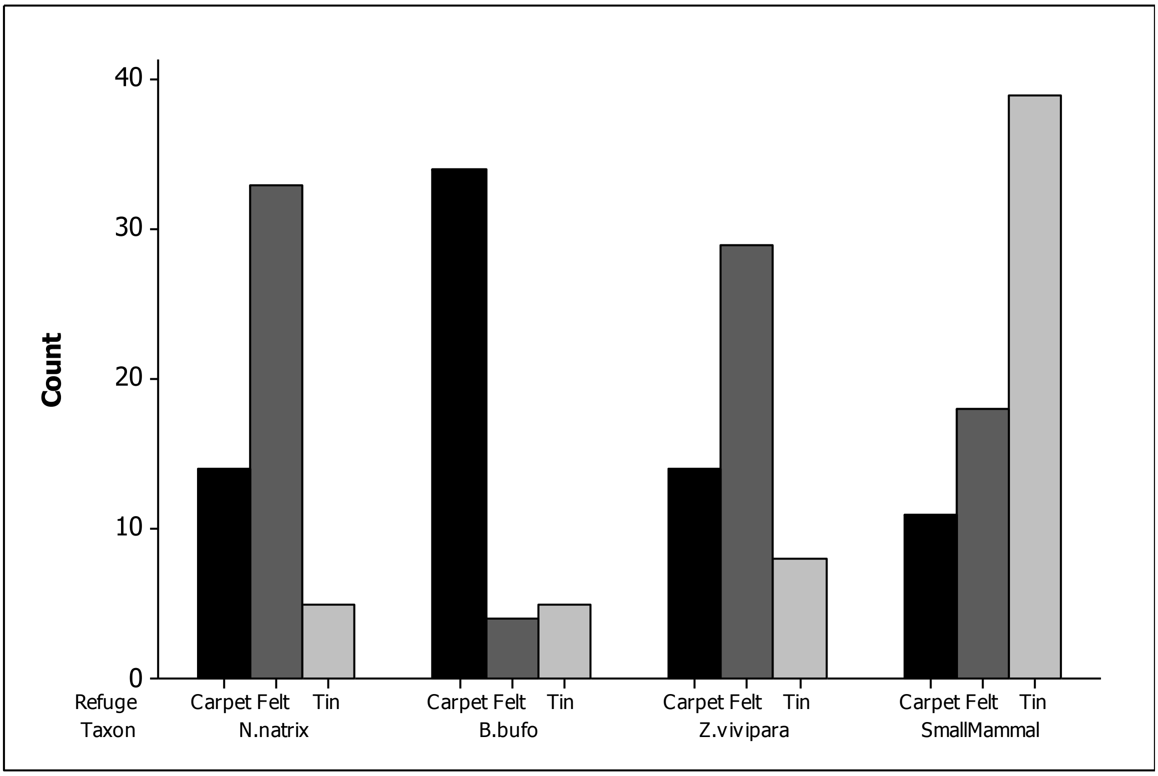

Figure 2. Total counts of taxon (grass snake *N.natrix*, common toad *B.bufo*, common lizard Z.vivipara, and small mammal) detected under each artificial refuge material (Carpet, Felt and Tin) throughout the study period (June-October) showing refuge type

Carpet refuges had a significant effect on the chance of detecting the common toad. (GLMM, Refuge, $F_{2,295} = 26.22$, $P < 0.001$) Approximately 80% of observations for the common toad were recorded under carpet (Fig. 2) however only 10% were detected under tin. In addition to herptiles, small mammals were observed under the artificial refuge materials. Tin refuges significantly increased small mammal detection rates (GLMM, Refuge, $F_{2,295} = 48.02$, $P < 0.001$; Fig. 2).

Over half of small mammal observations were recorded under tin (57%) and only 16% of observations were recorded under carpet.

3.3. Agri-environment Scheme Preference

Agri-environment Scheme (AES) type had a significant effect on the detection of grass snakes (GLMM, AES, $F_{1, 295} = 4.83$, $P < 0.05$) and common toads (GLMM, AES, $F_{1, 295} = 4.83$, $P < 0.05$). Field margins supported a significantly higher number of observations (90%) of grass snakes however caution is needed as the analysis produced a high standard error which may be due to a lower number of refuge materials placed on set-aside land. In contrast for the detection of the common toad set-aside land significantly increased the number of observations (65%).

Survey time

Evening surveys (5-8pm) significantly increased the chance of detecting grass snakes (GLMM, AM vs. PM, $F_{1, 295} = 28.71$, $P < 0.001$, Fig. 3) and common lizards (GLMM, AM vs. PM, $F_{1, 295} = 7.50$, $P < 0.05$, Fig. 3) when compared with morning surveys. Approximately 79% of grass snake and 69% lizard observations were recorded during the evening surveys. For both small mammals and the common toad the survey time did not have a significant effect on the number of observations ($P > 0.05$).

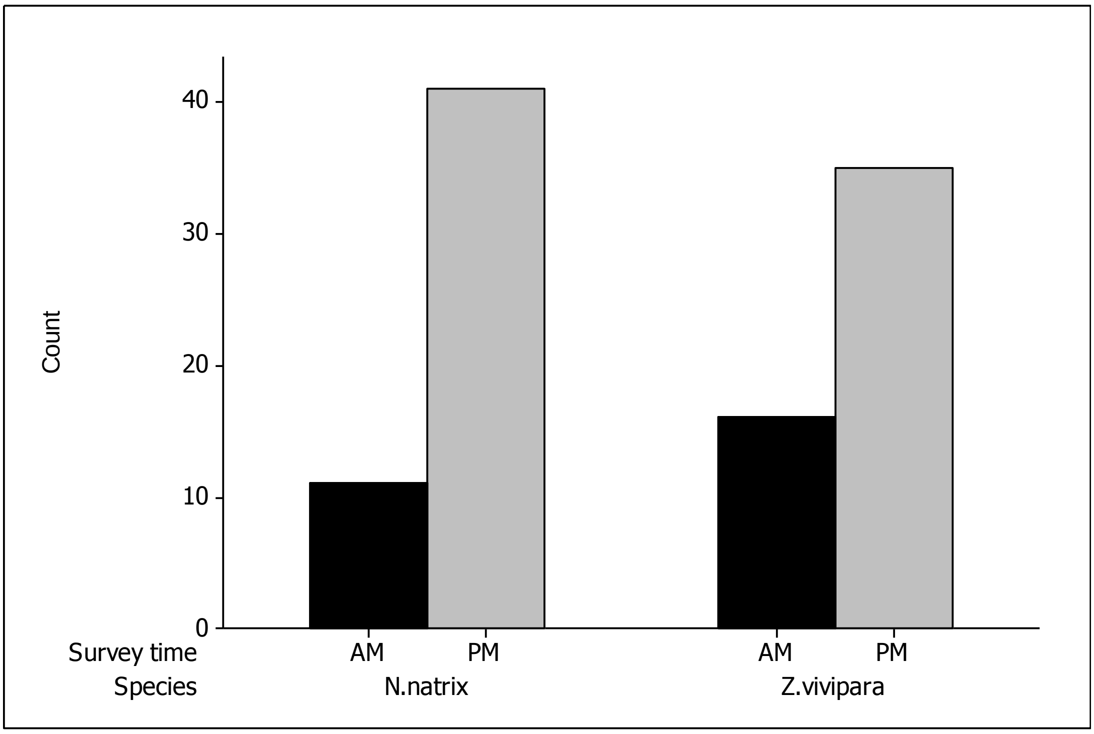

Fig. 3. Catch per unit effort for grass snake, *Natrix natrix* and the common lizard *Zootoca vivipara* during morning (AM) and evening (PM) surveys (per 1000 visits).

3.5. Weather data

Weather data was collected by the Wytham weather station; from June-October 2011. Since air temperature, rainfall, and wind speed had a significant effect on the presence of some taxon these variables were analysed further. The data revealed the highest average air temperature was recorded in August (18.32˚C; Figure 6) and the lowest was June (15.17˚C; Figure 6). June also yielded the lowest number of sightings (Table 3). Temperature had a significant effect on the probability of detecting grass snakes (GLMM, Dry Temperature, $F_{1,295}$ = 5.86, P <0.05, Table 4).

The highest mean wind speed was recorded during September (3.35 m/s). Analysis suggested wind speed had a significant effect on the probability of detecting the common toad (GLMM, Wind speed, $F_{1, 295}$ = 11.93, P <0.001) with the highest number of toads were recorded during September. The summer during 2011 was relatively dry for Oxfordshire as mean rainfall did not exceed 0.2mm. The highest (0.15mm) was recorded during July. Rainfall only had a significant effect on the probability of detecting grass snakes (GLMM, Rain, $F_{1, 295} = 13.10$, $P < 0.001$, Table 4).

4. Discussion

Overview of results

To summarise the significant findings; Felt refuges increased the chance of detecting the common lizard, *Zootoca vivipara* and grass snake *Natrix natrix*; carpet refuges increased the probability of detecting the common toad, *Bufo bufo*. With regards to the agri-environment schemes (AES), set aside land supported a higher number of toads than field margins whereas a significantly higher number of grass snakes were found on field margins compared to set aside. Evening surveys increased the likelihood of detecting grass snakes and the common lizard.

This study confirmed the differential attractiveness of three refuge materials for sampling British herptiles. Although the numbers of sightings were low the presence of felt increased the probability of detecting common lizards and grass snakes; carpet refuges attracted more toads than any other taxa. Artificial refuge materials have been used in previous studies to survey snake (Engelstoft and Ovaska 2000; Kjoss and Litvaitis 2001) Fellers and Drost, 1994) and lizard (Ryan et al. 2002) populations and Houze and Chandler (2002) provide encouraging evidence that artificial refuges attract the same species as natural refuges (logs and branches) albeit at a lower abundance.

Although 295 refuges represent a good sample size, only two (Grass snake and common lizard) out of the 6 native reptile species were detected. This is comparable to a study carried out by Scheffers *et al*. (2009), where 265 paired refuges were used and resulted in the detection of 58% of possible herptofauna in Missouri, USA. The fact that some species were not detected does not suggest their absence from the study area. Different reptile and amphibian species target different habitats which may not exist in the study area e.g. smooth snake *Corinella austriaca* and sand lizard *Lacerta agilis* normally inhabit heathlands and the current study was beyond their native range (Edgar et al. 2010). Extending this study over a longer temporal scale of several years is needed to infer the absence of species, as shown by Reading (1997) who during a three year study on reptile populations in Wareham, Dorset successfully detected all six native reptile species. It is also suggested that future research needs to use alternative sampling regimes to target a range of herptile species (e.g. drift fencing and transects) (Todd et al. 2007). Ryan *et al*. (2002) suggest that there is no single method of surveying that will detect the presence of every reptile species in a study area, this probably being owed to their cryptic nature and specific habitat preferences. In addition to the reptiles the common toad, *Bufo bufo* and a high number of small mammals were recorded during the study. These results suggest an alternative, non-intrusive survey method for sampling these species and may have been overlooked as a method for surveying such taxa during previous studies (Barnet and Dutton, 1995). The large sampling effort employed during this study reinforces the comments of previous authors (e.g. Reading 1997; Scheffers et al. 2009), that surveying reptiles is a notoriously difficult task and one that requires considerable spatial and temporal sampling effort.

4.1. Reptile Survey and weather variables

The temporal frame of surveying reptiles is restricted, as they are only active between March-October (see Table 1). This study confirmed a significant difference in the probability of detecting grass snakes and toads during different months of the study period. Surveying using artificial refuge materials may be highly influenced by environmental factors such as air temperature and rainfall (Fellers and Drost, 1994). Joppa *et al.* (2010) found air temperature to play a significant role on the probability of detecting reptiles as no reptiles were detected below 26 ˚C. In this study (not including October survey data due to lack of weather data) the results suggest that the coldest month (June) yielded the lowest detection which may be attributed to decreased activity in cold weather conditions (Table 3). The highest number of taxon detections were made between 16-20˚C which contrasts with Joppa *et al*. (2010) who detected a maximum number of reptiles between 20-25˚C, however local climatic conditions and community composition may have influenced this (Grant et al. 1992).

August provided peak detection rates for grass snakes which corresponds with the highest mean air temperature (18.32˚C) recorded during the study. However during the first and last month of surveying there was a low number of grass snake observations (see Table 3) which may correspond with the reptile brumation 9reptile hibernation) period (late October/November). This is supported by Kéry (2002) who found a marked decline in reptile sightings at the beginning and end of surveying. Snake detection rates increased between July and August which may reflect their increased activity due to physiological factors such as ecdysis (shedding of skin; Street, 1979) mating and digestion (Isaac and Gregory 2004). These processes increase their energy requirements and they therefore spend longer periods basking, possibly increasing their visibility in open areas during warmer periods. The highest recordings of the common toad in September (Table 3) may be explained by research by Bentley and Yorio (1979) who found that evaporation rates from the skin of common toads increased as air velocity increased. Therefore toads may seek shelter under the artificial refuges (mainly carpet) in moist environments to prevent water loss.

4.2. Agri-Environment Schemes (AES)

This study detected a significantly higher number of common toads on set-aside land compared to field margins. Berger *et al*. (2003) suggest that set aside land is important for adult amphibians (upon leaving their breeding ponds) and juveniles (following metamorphosis) as a safe habitat before the start of brumation (October). Set-aside land often also contains taller and denser vegetation cover than the surrounding landscape which in turn provides more shade and wetter habitats to prevent dehydration, which is a foremost consideration for amphibians (Seebacher and Alford 2002). Greater cover by terrestrial plants has been shown to provide ideal refuge for other amphibians such as the common frog, (*Rana temporaria)* (Marnell 1998; Mazerolle and Desrochers 2005) and this could be true for *B. bufo*.

Lower detection rates of amphibians under artificial refuges on field margins may also be explained due to their close proximity to agricultural fields which are more exposed to operating machinery and agrochemicals (known to harm amphibians; Mann et al. 2009). Berger *et al*. (2003) report a profound decline in amphibian numbers within arable fields compared to set aside land following crop removal in autumn. Cultivation is reported to cause up to 90% mortality in amphibian populations, (Durr et al. 1999) therefore reinforcing the benefits of set-aside areas for amphibians. Further evidence to support the importance of set aside land was shown by Berger *et al*. (2003) who reported that a set aside area of only 32 hectares in 800 hectares of farmland increased amphibian populations by three to four times more than farms with no set aside areas.

In contrast to the common toad a significantly higher number of the grass snakes which were detected on field margins, rather than set aside land. Basking sites are essential for thermoregulating reptiles therefore field margins could offer increased insolation (exposure to sun) compared to set aside sites as Grass snakes tend to avoid over shaded habitat (Wilser *et al*., 2008). Another study by Reading and Jofre (2009) on agricultural land in southern England suggests the preference of grass snakes for habitat boundaries which provide a similar habitat as field margins (Reading and Jofre 2009). In this study grass snakes may have favoured field margins due to their close location to crop fields which potentially support an abundance of prey species (e.g. small mammals). In addition, tall cereal crops (like that grown in Oxfordshire) provide protection from avian predators whilst foraging (Wilser *et al*., 2008).

It has been well documented that Australian reptile populations' benefit from the presence of native vegetation within their habitat ranges (Brown et al. 2008; Michael et al. 2010) and these results are suggestive that the conservation of natural habitat in intensively farmed landscapes is important for reptile conservation. Such AES offer structural habitat diversity (trees, hedgerows and vegetation type) compared to the surrounding homogenous landscape (Bennett et al. 2006) and research by Atauri and Lucio (2001) suggest a positive correlation between the variety of land use types and the species richness of reptiles and amphibians in a Spanish landscape (Atauri and de Lucio 2001). Despite the significance between AES types for the common toad and grass snake it is important to note that all herptiles species detected were all found to occupy each type of AES which may support their role as connecting features, and may allow for the migration of herptiles in British lowland farmland (Berger et al. 2003). This study has established that reptiles do indeed rely on AES schemes as potential habitat; future studies should compare reptile populations on farmland which is not subscribed to Entry-level stewardship schemes with that of AES land to determine the true benefits of AES for reptiles and amphibians.

4.3. Refuge preference

Roofing felt

Felt refuges significantly increased the detection rates of the two reptile species (Grass snake and common lizard). This supports a survey implemented by Riddell (1996) who found a high abundance of lizards under roofing felt which she attributed to its heat absorption and retention properties. Compared to metal refuges, felt heats up slower but retain heat for longer so the dependence of reptiles on warmer refuges to sustain an optimum body temperature throughout the day may explain this preference for the refuge that is warmer than carpet but does not reach extreme highs as a metal refuge such as tin (Riddell, 1996).

Carpet

The significant use of carpet refuges by the common toad can be attributed to the moisture retention of this refuge type. The common toad like many amphibians is nocturnal therefore seeks refuge during the day from heat and predators (Zug and Zug, 1979). Denton and Beebee (1993) showed increased common toad mortality when placed into an environment with no refuge/shelter sites. Toads are unable to control for the loss of water through their skin and therefore are prone to dehydration (Seebacher and Alford 2002). Sheltering in moist, cool environments (Zug and Zug, 1979) is one way to overcome this issue. Seebacher and Alford (2002) showed that adult cane toads lost 25% of their water content in one day without shelter therefore emphasising the preference for moist microhabitats.

Tin

The lack of reptiles and amphibians detected under corrugated tin refuges may have been a reflection of the small sample size however statistical analysis produced a large effect size (Table 3). This indicates that tin plays a positive role on detecting grass snakes therefore future studies should increase the sample size to reveal true significance. However previous studies (e.g. Parmelee and Fitch 1995) suggest that reptiles avoid metal refuges due to the high temperatures underneath the metal sheets. Future research should investigate the effects of different refuge materials (particularly tin) and even the size of refuges.

Conclusions

Overall, this study has succeeded to address the initial aims of this study and provide the following points; (1.) The preference of refuge type indicates a range of different refuge materials should be used in combination to maximise surveys (2.) Weather variables such as air temperature, wind speed and rainfall have an effect on the likelihood of detecting reptiles (3.) The existence of reptiles and amphibians on two AES types (set-aside and field margins). The outcomes of this study provide supporting evidence of the positive impacts that protected habitats (free from anthropogenic threats) provide for reptiles (e.g. Wasiolka and Blaum, 2011; Auttum and Eason, 2006). This reinforces the importance of landscape connectivity and heterogeneous environments which have already been shown to attract amphibians and reptiles (Atauri and de Lucio 2001; Laurila 1998; Mazerolle and Desrochers 2005). Amphibians and reptiles are definitely a less charismatic group of organisms and therefore lack public appeal however; more and more reports on the widespread reptile population decline across agricultural land (Brown et al. 2008; Brown et al.

2011; Driscoll 2004) means that measures to raise awareness and promote conservation are a priority.

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Appendix II: Genetic structure of the common toad (*Bufo bufo*) population in Oxfordshire

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Abstract

This study aims to give insight into the genetic structure of the common toad, *Bufo bufo*, population in Oxfordshire. Using current genetic tools, microsatellites, we described the population status regarding genetic diversity and isolation. Spatial analysis, of the eight breeding patches, using BAPS5 and STRUCTURE failed to find sub-structuring of the population. This suggests extensive gene flow or the absence of differentiation between patches. In contrast, analysis of F_{ST} values indicated differentiation between all but three pairs of breeding patches. Our AMOVA analysis of genetic versus *a priori* geographic structure concluded that grouping following the F_{ST} values explained the most variation and was considered the best estimate of population structure in our study site. To conclude, this study did not show a clear pattern of isolation or loss of genetic diversity of the *Bufo bufo* population in Oxfordshire. Only slight structuring in the population was observed, highlighting either great migration or little differentiation between patches. At a rather local scale no conservation issue was observed. The on-going conservation actions seem to have been fruitful for the population in our study site and should be extended and monitored to a larger scale to confirm the benefit.

Key words.—Bufo bufo; Landscape genetics; Microsatellites; Structure.

Introduction

Successful conservation plans rely on accurate knowledge of the landscape, behavior and genetic structure of the population. Studies have shown that human activities had impacted up to a half of the land surface (Vitousek 1997) leading to a decrease in habitat availability and parceling of species ecosystems (Laurance 2008). Such changes in landscape features affect the connectivity between multiple patches resulting in isolation, and demographic changes (Berry *et al.* 2005; Janin *et al.* 2009; Philcox *et al.* 1999; Saeki and Macdonald 2004). To infer accurate functioning, delimitation and connectivity of populations (Jehle *et al.* 2005; Waples and Gaggiotti 2006), landscape genetic approaches combining genetic and spatial analyses, are appropriate tools (Epps *et al.* 2007; Manel *et al.* 2003; Spear *et al.* 2005). Isolation due to landscape changes impacts population genetics directly, causing reduction of gene flow and potential for evolution (Andersen *et al.* 2004). Small populations are more prone to the effects of genetic drift and inbreeding depression, resulting in compromised immune competence, exposure to deleterious recessive alleles and increased infant mortality. This phenomenon is the principal cause of erosion over time through the extinction vortex phenomenon (Crnokrak and Roff 1999; Frankham 2005). Nowadays, population genetic studies in conservation are mostly based on neutral marker analysis (e.g. microsatellites) as they are known to evolve rapidly and give a good image of the genetic changes affecting populations on a short term scale (Jehle and Arntzen 2002) and allows calculation of genetic distances between pairs of breeding patches (e.g. Allentoft *et al.* 2009; Vos *et al.* 2001; Wilkinson *et al.* 2007). A good understanding of the current population functioning and structure can be achieved thanks to such tools (Coulon *et al.* 2006). This would be very valuable for prediction of population demographic evolution and prioritization of conservation actions (Greenwald *et al.* 2009).

Many amphibian species are endangered worldwide (Houlahan *et al.* 2000) with one third of them now recorded as “Threatened” by the IUCN (IUCN 2012). Decline in amphibian populations is caused by various factors such as disease, habitat fragmentation and destruction through human driven land use changes (e.g. transportation, agricultural conversion) (Biek *et al.* 2002; Houlahan *et al.* 2000). In 2007, the common toad (*Bufo bufo*) was added to section 41 (England) of the UK Biodiversity Action Plan as a priority species (available from jncc.defra.gov.uk [accessed 22 December 2014]) due to concern based on recent, severe and largely unexplained population declines (Carrier and Beebee 2003). This is in recognition of the need for conservation action in order to avoid future decline. The common toad is widely dispersed in Europe (UK, France), northern Eurasia and West Asia (Turkey, Syria, Lebanon) (Agasyan *et al.* 2009) and are highly philopatric (Reading *et al.* 1991), returning to the breeding pond in which they were spawned or to a neighboring pond within a 1.6 km range (Sinsch 1988). Because of this breeding behavior and low dispersal capacity (Sinsch 1987), the common toad is particularly vulnerable to habitat fragmentation (Becker *et al.* 2010). This is especially the case in the UK where more than 60% of the land cover is anthropogenically altered (Natural England 2008) and there is approximately 370 000 kilometers of road (Underhill and Angold 2000). Although locally rare where it has undergone declines (Beebee

2012), it remains widespread in Europe which makes it an ideal study organism for use as a conservation model. Indeed, *Bufo bufo* genetic variability and geographic characteristics have already been studied to some extent (Hitchings and Beebee 1998; Luscher *et al.* 2001; Safner *et al.* 2011; Scribner *et al.* 1994, 1997; Scribner and Chesser 2001; Seppa and Laurila 1999).

In the present study, we aim to give insight into the genetic structure of the population of common toad in Oxfordshire using several ponds sampled in this fragmented area. We used genetic approaches to infer the genetic diversity structure and isolation level of this population (Elzanowski *et al.* 2009; Hels and Buchwald 2001).

MATERIALS AND METHODS

Study area and sample collection

A landscape of 22km by 22km, located in Oxfordshire (United Kingdom) was defined as study site (Figure 1). This area was a combination of agricultural land and urban areas. We focused our study on eight common toad breeding ponds, separated by distances between 3.8 and 21.5km. Sampling was conducted during June and July 2012 and 259 tadpoles, collected from the eight ponds (26 to 48 individuals per pond), were used for DNA extraction based on tail tissues following the DNeasy blood & tissues extraction kit® (QIAGEN) protocol.

Microsatellites genotyping

Polymerase chain reaction (PCR) was performed to amplify seven microsatellite markers (Brede et al. 2001) in two separate multiplex and one singleplex reaction (multiplex Bbufµ15, 14, 65 and 47, multiplex Bbufµ54 and 62 and singleplex Bbufµ11) following instructions from the Type-it microsatellite PCR kit and Taq PCR kit® (QIAGEN) in a 15µl final volume. PCR cycles are provided in Table 1. PCR products were genotyped in an ABI 3730 XL DNA Analyzer (Applied Biosystems); data were edited and analyzed using PeakScanner^{TM} v1.0 (Applied Biosystems) software. In order to reduce scoring error, three independent scores of the microsatellite genotypes were performed.

Data analysis

Number of alleles, observed and unbiased expected heterozygosity H_e (Nei 1978), genetic diversity, allelic richness and inbreeding coefficient (F_{IS}) for each breeding site and each locus were measured using GENEPOP 4.2 (Raymond and Rousset 1995; Rousset 2008) and FSTAT 2.9.3 (Goudet 1995) software. The program FreeNA (Chapuis and Estoup 2007) was used to perform checking for genotyping errors such as allelic dropout, stuttering and null alleles and to infer their possible impact on the population analysis.

We tested each breeding site at each locus for departure from Hardy-Weinberg equilibrium with exact tests and linkage disequilibrium with probability tests across pairs of loci with GENEPOP. Significance levels were adjusted using the sequential Bonferroni correction (Rice 1989), when necessary, in R statistical software (R Development Core Team 2008).

Finally calculation and significance of genetic distance F_{ST} between pairs of breeding patches were estimated using FSTAT 2.9.3.

Population structure

Clustering of the patches was determined using STRUCTURE software (Pritchard et al. 2000) and BAPS5 (Corander et al. 2009) (Bayesian Analysis of Population Structure). Multiple mixture models were tested for K groups of individuals equal one to eight with and without spatial information. AMOVAs were performed to determine significance of genetic differentiation among clusters analyses based on previously defined clustering. We checked for clustering depending on F_{ST} values, using the results obtained with BAPS5 and STRUCUTRE, and using roads as a priori delimitations (Figure 2). AMOVA analyses were performed using Arlequin 3.5.1.2 (Excoffier and Lischer 2010), significance of variation between and within groups and population were calculated based on 10 000 permutations.

Results

Genetic variability

All seven microsatellite loci were successfully amplified and polymorphic. The number of alleles detected varied from 9 (Bbufµ14), to 31 (Bbufµ65), indicating new alleles in all markers compared to Brede et al. (2001). Expected heterozygosity ranged from 0.57 to 0.89 while observed varied from 0.55 to 0.83. The number of alleles, their size ranges, as well as expected and observed heterozygosity were mostly higher than in Brede et al. (2001). The allelic richness values per breeding patch varied from 7.9 for Cumnor to 9.5 for Bagley Wood (Table 2). According to FreeNa, null alleles were present at a very low frequency but, after checking of the corrected values, null alleles did not affect genetic distance calculations.

Only F_{ST} values between the pairs Ducklington/Standlake, Bagley Wood/Fawler and Cothill/Garford were significantly different from zero indicating genetic differentiation between most of the breeding patches (Table 3).

Population structure

Results obtained using BAPS5 and STRUCTURE were unable to detect genetic structure in the study area, suggesting absence of population sub-structuring and extensive gene flow between the breeding patches (Figure 3). Additional clustering using location information followed the same line, the eight breeding patches forming a single population. Of the plausible clustering tested we kept the four most supported, with significant variation values (Table 4). The grouping following the $\mathrm{F_{ST}}$ values for microsatellites gave the highest “among groups” variation value of 2.22%, while the models following a priori road distribution explained very little of the “among groups” variation, 0.74% and 1.44% (P values ranging from 0.02 to 0). Looking at the “among populations within group” variation, the simulation taking $\mathrm{F_{ST}}$ values into account gave the best and lowest value with 0.89% of variation explained. Therefore, grouping following the $\mathrm{F_{ST}}$ values explained the most variation and was considered the best estimate of population structure in our study site.

Discussion

Population structure

Values for genetic variability (heterozygosity and allelic richness) were similar to those found in France (Safner et al. 2011) and Spain (Martinez-Solano and Gonzalez 2008). The H_e and allelic richness values were high for all sampling, around 9 for allelic richness and 0.7 for H_e. F_{ST} estimates brought us to the conclusion that most patches were genetically different. However, BAPS5 and STRUCTURE failed to identify a specific genetic structuration in our population. AMOVA analyses most supported the model based on F_{ST} , showing association of six populations in pairs: Standlake and Ducklington, Garford and Cothill and Bagley Wood and Fawler. The two first pairs can intuitively be seen on the map; they are close to each other and have a high number of water bodies and woodland areas in-between facilitating communication and exchange of individuals. The third pair cannot be explained based on the geography; a plausible explanation could be human intervention, such as artificial translocation of individuals from one pond to the other. Bagley Wood in particular is a likely candidate to have been altered in some way as the sampled pond was located within a school ground. Such grouping between pairs of breeding patches suggests a limited but present migration of individuals between ponds within a local scale of less than 25km.

Presence of non-sampled patches in-between sampling sites or the possible human induced changes (translocation) could explain the observed results.

Conservation strategy

The link between amphibian declines (Cooke and Scorgie 1983) and human driven landscape change has been discussed extensively (Allentoft and O'Brien 2010). However, in the current study the effect of habitat change is not so clear. The decrease in genetic diversity of the Oxfordshire common toad population does not yet seem to be dramatic. Nevertheless, as documented by Piha et al. (2007), local populations can persist for several years in degraded landscapes before going extinct. Divers actions taken by the Amphibian and Reptile Conservation Trust and ToadWatch UK, are apparently fruitful to conserve genetic diversity of toad populations at local scale. In addition, status of the amphibian populations at a larger scale might be different and so conservation action should be considered at the scale most appropriate for the species. For common toad this appears to be beyond the local landscape scale. To minimize extinction risk and loss of genetic diversity, we recommend maintenance of existing patches and improvement of the connectivity between breeding patches by translocation and by the creation of corridors or stepping-stones patches between suitable habitats. Ecological impact assessments of future landscapes change needs to be undertaken and further landscape modeling studies would help decision making for effective conservation action (Beebee 2013).

Conclusion

This study did not show clear pattern of isolation or loss of genetic diversity of the *Bufo bufo* population in Oxfordshire. Only partial structuring in the population was observed, highlighting either great migration or little differentiation between patches. A habitat suitability landscape index would facilitate accurate evaluation of habitats for conservation purposes. It would allow us to determine whether current conservation interventions (e.g. Environmental Stewardship www.defra.gov.uk) are likely to be beneficial, and good value; and if not, to find better management strategies and influence decision making in the future, such as for the forthcoming EU Common Agricultural Policy reforms to improve mitigation and compensation of the impact of human landscape domination.

In May 2013 the UK's “State of Nature” report revealed a decline in 60% of the UK's species (www.rspb.org.uk). Conservation strategies aiming to benefit toad conservation are likely to have benefits on more than just this species. By improving availability, quality and connectivity of natural and semi-natural habitats in what is a human dominated landscape we can improve the chances of sustaining viable populations in a whole suite of species. So called common species are likely not to remain common in their habitat range if conservation actions to halt their decline are not supported.

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Tables

Table 1

Polymerase Chain Reaction conditions for microsatellites amplification.

PCR Name	Gene amplified	Reaction cycles
Multiplex4	Bbufµ15, Bbufµ14, Bbufµ47, Bbufµ65	95°C for 5min denaturation, 94°C 30sec, 55°C 30sec and 70°C 30sec x25 cycles, 70°C for 30min final elongation step.
Multiplex6	Bbufµ54, Bbufµ62	95°C for 5min denaturation, 94°C 30sec, 57°C 30sec and 70°C 30sec x23 cycles, 70°C for 45min final elongation step.
Singleplex11	Bbufµ11	94°C for 3min denaturation, 94°C 30sec, 51°C 1min and 72°C 1min x25 cycles, 72°C for 30min final elongation step.

Table 2

Summary of genetic variation within each of the eight *B.bufo* breeding patches.

Breeding patch	Code	N	Genetic diversity			
			Allelic richness	H_o	H_e	F_{is}
Bagley Wood	BW	26	9.5159	0.6630	0.7046	0.0600 (NS)
Combe	Cb	48	8.9407	0.7050	0.7572	0.0700 (NS)
Cothill	Hi	27	8.5599	0.7715	0.7344	-0.0520 (NS)
Cumnor	Cu	30	7.9241	0.6968	0.7456	0.0670 (NS)
Ducklington	RC	26	8.4656	0.7521	0.7431	-0.0120 (NS)
Fawler	Fa	45	9.2487	0.7054	0.7511	0.0610 (NS)
Garford	Ga	26	9.2804	0.7541	0.7846	0.0400 (NS)
Standlake	St	31	8.0786	0.6640	0.7309	0.0930 (NS)

N refers to the sample size, *Ho* to the observed heterozygosity, *He* to the Nei's unbiased expected

Table 3

Pairwise F_{ST} values between pairs of breeding patches.

Breeding patches	Bagley Wood	Combe	Cothill	Cumnor	Ducklington	Fawler	Garford	Standlake
Bagley Wood	-							
Combe	**0.0280**	-						
Cothill	**0.0389**	**0.0305**	-					
Cumnor	**0.0639**	**0.0298**	**0.0576**	-				
Ducklington	**0.0605**	**0.0182**	**0.0410**	**0.0305**	-			
Fawler	0.0137	**0.0122**	**0.0343**	**0.0453**	**0.0206**	-		
Garford	**0.0364**	**0.0146**	0.0078	**0.0416**	**0.0264**	**0.0229**	-	
Standlake	**0.0373**	**0.0181**	**0.0387**	**0.0442**	0.0033	**0.0107**	**0.0237**	-

Significant values are indicated in bold.

Table 4

Analysis of molecular variance.

	Among groups				Among pop within groups				Within populations			
	df	Variance	*P*-val	%Var.	df	Variance	*P*-val	%Var.	df	Variance	*P*-val	%Var.
1 cluster	x	x	x	x	7	0.0780	<0.0001	2.9026	510	2.6091	<0.0001	97.0974
BW/Fa; Hi/Ga; RC/St; Cb; Cu (*Fst* values)	4	0.0597	<0.0001	2.2158	3	0.0238	0.0040	0.8856	510	2.6091	<0.0001	96.8986
Ga/Hi/BW; Cu/St/RC; Fa/Cb	2	0.0199	0.0184	0.7397	5	0.0627	<0.0001	2.3300	510	2.6091	<0.0001	96.9304
Ga; Hi/BW; Cu; St/RC; Fa/Cb	4	0.0378	0.0062	1.4024	3	0.0450	<0.0001	1.6703	510	2.6091	<0.0001	96.9274
Ga/Hi/BW; Cu; St/RC; Fa/Cb	3	0.0389	0.0011	1.4451	4	0.0458	<0.0001	1.7013	510	2.6091	<0.0001	96.8536

Table 5

Primer sequences, fluorescent dyes used for multiplex PCR and GenBank code for the seven microsatellite loci selected.

Locus name	Primer sequences (5'-3')	GeneBank code
Bbufµ54	(6FAM)-CATTGCGCTGCTGTCAGATTACAC TTAGGGATTGCCGTCCAGTTGTC	AY037820
Bbufµ62	(PET)-GCACATTCCTGTGTCCGTGTATAG ATTCCGAAAACGAAAAGAAAAGAG	AY037821
Bbufµ15	(6FAM)-TCAATATAGGAGTCCCAGAATGTC AATCCCCTAGCGTACACAAGATAC	AY037812
Bbufµ14	(NED)-CGTGCATGCAAGTGTACCTAACC ATGGAGAGTGAAGGGGAAAGAGTG	AY037811
Bbufµ65	(PET)-GGATCTAAGCGCTGTGAGAGTGA CGGTCCGTGTTACCACTGATGC	AY037823
Bbufµ47	(VIC)-GGATCAAGCCCTCAGACAACTC CACAGCAGCAGAAATTTTGACCAG	AY037818
Bbufµ11	GTCACATGGATAATAAATGAGACC TCTAATATTGATGACCAGACAACC	AY037809

Table 6

Summary of the seven microsatellites loci characteristics.

For this study, compared to the referenced characteristics (Brede *et al*. 2001)

Locus name	Size (ref)	Size	Number of alleles (ref)	Number of alleles	Repeat pattern	H_o (ref)	H_o	H_e (ref)	H_e
Bbufµ54	151-167	151-201	7	20	$(CA)_{17}$	0.53	0.81	0.76	0.81
Bbufµ62	195-227	181-207	7	11	$(GT)_{18}$	0.63	0.65	0.7	0.67
Bbufµ15	140-152	151-185	8	10	$(CA)_{16}$	0.53	0.62	0.69	0.71
Bbufµ14	172-182	181-201	2	9	$(TG)3G(GT)_{12}$	0.38	0.55	0.47	0.57
Bbufµ65	133-175	141-205	17	31	$(GT)_{29}$	0.7	0.83	0.93	0.89
Bbufµ47	132-146	134-162	5	10	$(CA)_{18}$	0.32	0.57	0.76	0.58
Bbufµ11	90-122	98-138	10	21	$(CA)_{19}$	0.33	0.79	0.79	0.79

Figure legends

Figure 1

Map of study area in Oxfordshire, UK.

In light grey lines ─ minor roads and local streets, in dark grey lines, black lines ─ single carriageway A roads, bold lines ━ major (dual carriageway) A roads. Dots ○ tadpole collection ponds and squares □ named places. Contains Ordnance Survey data © Crown Copyright and database right 2013.

Figure 2

Patches clustering according to roads distribution in the sampling site.

Top left corner clustering: North of the A40: Fawler and Combe; South of the A420: Garford, Cothill and Bagley Wood; between A40 and A420: Ducklington, Standlake and Cumnor.

Top right corner clustering: North of the A40: Fawler and Combe; South of the A420 left of the A338: Garford; South of the A420 right of the A338: Cothill and Bagley Wood; between A40 and A420 aside A415: Ducklington, Standlake and between A40 and A420 far A415: Cumnor.

Bottom left corner clustering: North of the A40: Fawler and Combe; South of the A420 left of the A338: Garford; South of the A420 right of the A338: Cothill and Bagley Wood; between A40 and A420: Ducklington, Standlake and Cumnor.

Bottom right corner clustering: North of the A40: Fawler and Combe; South of the A420: Garford, Cothill and Bagley Wood; between A40 and A420 aside A415: Ducklington, Standlake and between A40 and A420 far A415: Cumnor.

Figure 3

Individual genetic clustering. Partition graph for STRUCTURE output when K=8 (number of assumed groups). Each vertical line represents an individual and the colors represent its association to genetic clusters.

Figures

Figure 1

Figure 2

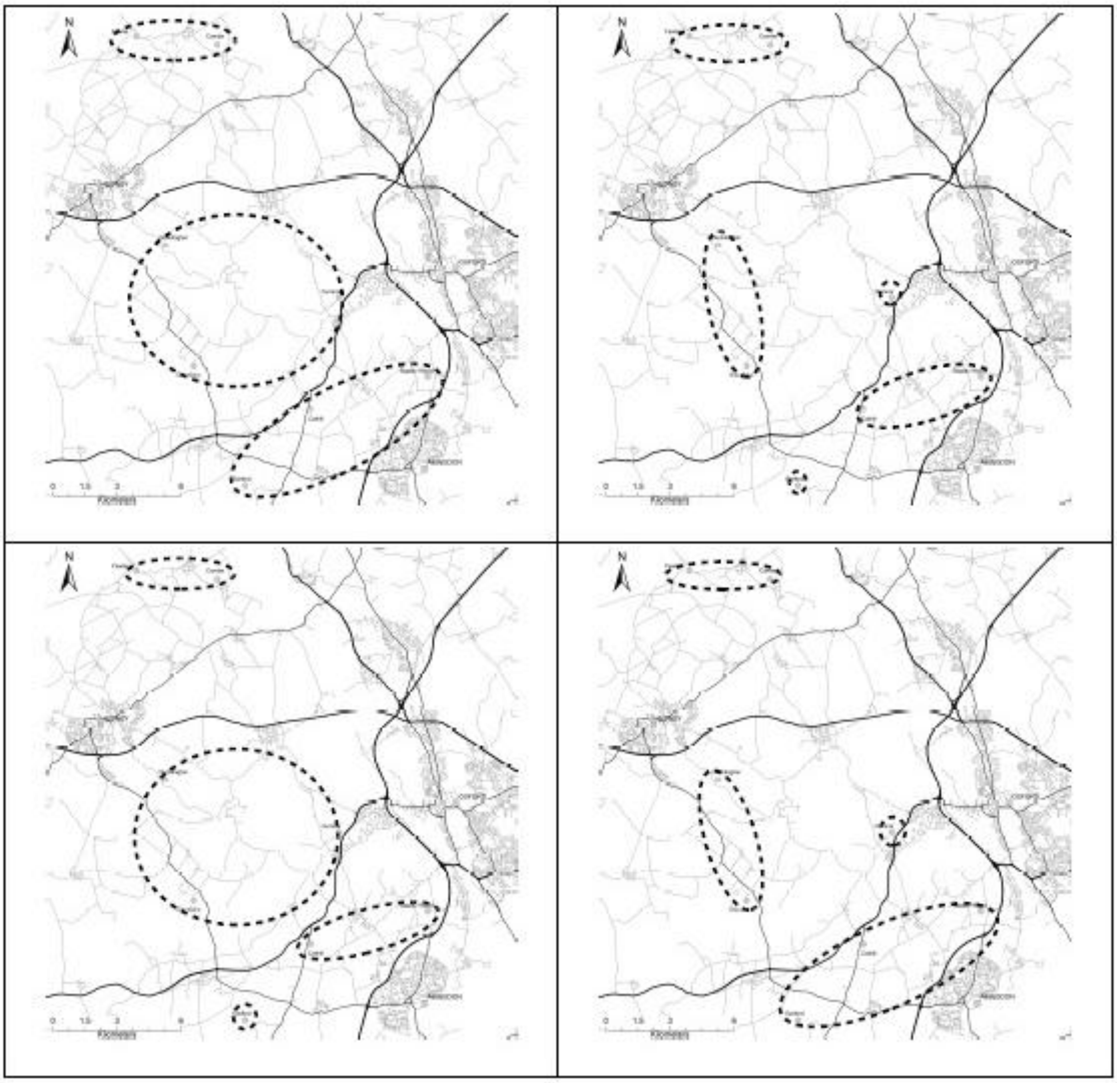
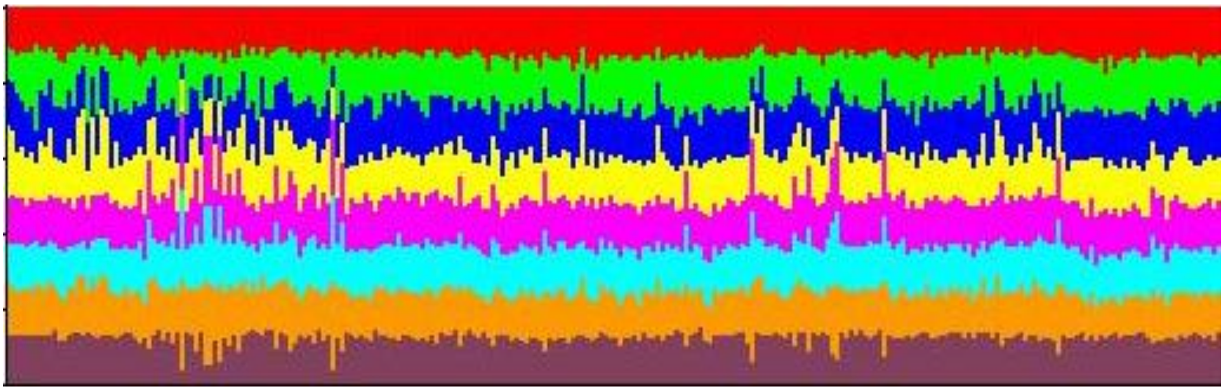


Figure 3



Appendix III: R code

Chapter 2

Carpet temperature trials

```
> carptemp2 <-
+ matrix (c(16.1,16.8,15.7,15.7,15.7,18.4,18.8,16,
+ 15.8,16.6,15.6,15.8,19.6,19.3,19.2,18.9,
+ 15.2,15.1,15.2,20.3,15.2,14.7,14.8,15.2,
+ 21.1,21.2,21.4,21.4,21.4,21.4,21.2,20.9,
+ 10.8,11,11.2,11,10.9,10.8,11.3,11.2),
+ nrow=5,
+ byrow=TRUE,
+ dimnames=list(1:5,
+ c("A","B","C","D","E","F","G","H")))
> friedman.test(carptemp2)

        Friedman rank sum test
data:  carptemp2
Friedman chi-squared = 2.578, df = 7, p-value = 0.9211
```

Chapter 5

Logistic regression of terrestrial variables

```
# add dataframe

NARRS_PCA <- read.csv("NARRS_PCA.csv")
attach(NARRS_PCA)
summary(NARRS_PCA)
names(NARRS_PCA)
dim(NARRS_PCA)

# check significance of each of the habitat variables

wood.mod <- glm(Toads ~ wood500*wood1*wood2*wood3)
summary(wood.mod)

# no significant interaction
# check without interaction

wood.mod2   <-   glm(Toads   ~   wood500+wood1+wood2+wood3,
binomial)
summary(wood.mod2)

# only wood500 significant
```

```
wat.mod <- glm(Toads ~ wat500+wat1+wat2+wat3, binomial)
summary(wat.mod)

# water never significant

RSF.mod <- glm(Toads ~ RSF500+RSF1+RSF2+RSF3, binomial)
summary(RSF.mod)

# RSF signficant at 500,1,2 and 500*3

urb.mod <- glm(Toads ~ urb500+urb1+urb2+urb3, binomial)
summary(urb.mod)

# urb never significant
# select RSF500 and wood500 for PCA to reduce variables going into PCA
```

Principal component analysis and logistic regression of scores

```
# create data frames for completing the PCA
# ponds data frame uses columns 2-12 (only RSF500 and wood500 for terr habitat)

toads <-NARRS_PCA[,1]

ponds <-NARRS_PCA[,2:12]

# this does the pca, cor=TRUE is making sure we are using the correlation matrix
# scale is automatic

toad.pca2 <-princomp(ponds, cor=TRUE)

names(toad.pca2)
summary(toad.pca2)
loadings(toad.pca2)
ponds.cor <- cor(ponds)
eigen(ponds.cor)
# create scree plot to decide how many components to use

plot(toad.pca2, type= "lines")

# the "elbow" is at 5 components this time
# this extracts scores
# all scores
# install vegan first!!

?vegan
scores<-scores(toad.pca2)
```

```
# first 5 axes

scores2<-scores(toad.pca2,choices=c(1,2,3,4,5))
scores2


# logistic regression, binomial family using scores as
predictor

scores.mod <- glm(Toads~scores2,binomial)
scores.mod
summary(scores.mod)

# scores as predictors

score1<-scores(toad.pca2,choices=c(1))
score2<-scores(toad.pca2,choices=c(2))

# logistic regression
# mod1 Toads ~ score1

mod1 <- glm(Toads ~ score1, binomial)
summary(mod1)

# mod2 Toads ~ score2

mod2 <- glm(Toads ~ score2, binomial)
summary(mod2)

mod12<- glm(Toads ~ score1 + score2, binomial)
summary(mod12)

mod1x2 <- glm(Toads ~ score1 * score2, binomial)
summary(mod1x2)

anova(mod1x2, mod12, test="Chi")
anova(mod12, mod1, test="Chi")
anova(mod12, mod2, test="Chi")
anova(mod1, mod2, test="Chi")

# score1+score2 not significantly worse than score1*score 2
# score1 is significantly worse than score1+score2
# score2 is significantly worse than score1+score2

# summary of scores.mod tells us which components are best
component to predict Toads
# Components 1 and 2 significant
```

```
# loadings tell us which variables are important for each
of the components
```

Logistic regression and coefficient plots

```
# Logistic regression and coefficient plots
Frogs.mod <- glm(Toads ~ Frogs, binomial)
summary(Frogs.mod)

# Graph it! par sets up the graphical parameters
par(mfrow=c(3,2))

seq produces a sequence of numbers (from 0, to 4, in 0.01
intervals)
# which will be the x-values (xv)
xv1 <-seq(0,1, 0.01)
# yv are the y-values.
# predict asks R to use the Qual.mod we created, with Newts
at all xv values
# and predicts the y values
yv1 <- predict(Frogs.mod,list(Frogs=xv1), type="response")
# creates the plot with Newts and Toads on the x and y axes
plot(Frogs,Toads, xlab= "Presence/absence of common frog",
ylab= "Toad presence")
# draws the line with x and y values as defined above
lines(xv1,yv1)

Quality.mod <- glm(Toads ~ Quality, binomial)

seq produces a sequence of numbers (from 1, to 4, in 0.01
intervals)
# which will be the x-values (xv)
xv2 <-seq(1,4, 0.01)
# yv are the y-values.
# predict asks R to use the Qual.mod we created, with Newts
at all xv values
# and predicts the y values
yv2 <- predict(Quality.mod,list(Quality=xv2),
type="response")
# creates the plot with Newts and Toads on the x and y axes
plot(Quality,Toads, xlab= "Water quality", ylab= "Toad
presence", xlim=c(1,4))
# draws the line with x and y values as defined above
lines(xv2,yv2)

RSF500.mod <- glm(Toads ~ RSF500, binomial)
# seq produces a sequence of numbers (from 0, to 100, in
0.01 intervals)
# which will be the x-values (xv)
```

```
xv3 <-seq(0,100, 0.01)
# yv are the y-values.
# predict asks R to use the Qual.mod we created, with Newts
at all xv values
# and predicts the y values
yv3        <-        predict(RSF500.mod,list(RSF500=xv3),
type="response")
# creates the plot with Newts and Toads on the x and y axes
plot(RSF500,Toads, xlab= "Percentage available habitat in
500m buffer", ylab= "Toad presence")
# draws the line with x and y values as defined above
lines(xv3,yv3)

Palm.mod <- glm(Toads ~ Palm, binomial)
seq produces a sequence of numbers (from 0, to 4, in 0.01
intervals)
# which will be the x-values (xv)
xv4 <-seq(0,1, 0.01)
# yv are the y-values.
# predict asks R to use the Qual.mod we created, with Newts
at all xv values
# and predicts the y values
yv4 <- predict(Palm.mod,list(Palm=xv4), type="response")
# creates the plot with Newts and Toads on the x and y axes
plot(Palm,Toads, xlab= "Presence/absence of palmate newt",
ylab= "Toad presence")
# draws the line with x and y values as defined above
lines(xv4,yv4)

wood500.mod <- glm(Toads ~ wood500, binomial)
# seq produces a sequence of numbers (from 0, to 100, in
0.01 intervals)
# which will be the x-values (xv)
xv5 <-seq(0,100, 0.01)
# yv are the y-values.
# predict asks R to use the Qual.mod we created, with Newts
at all xv values
# and predicts the y values
yv5       <-       predict(wood500.mod,list(wood500=xv5),
type="response")
# creates the plot with Newts and Toads on the x and y axes
plot(wood500,Toads,  xlab=  "Percentage  woodland  in  500m
buffer", ylab= "Toad presence", xlim=c(0,100))
# draws the line with x and y values as defined above
lines(xv5,yv5)
?ylim

Smooth.mod <- glm(Toads ~ Smooth, binomial)
```

```
# seq produces a sequence of numbers (from 0, to 4, in 0.01
intervals)
# which will be the x-values (xv)
xv6 <-seq(0,1, 0.01)
# yv are the y-values.
# predict asks R to use the Qual.mod we created, with Newts
at all xv values
# and predicts the y values
yv6 <- predict(Smooth.mod,list(Smooth=xv6),
type="response")
# creates the plot with Newts and Toads on the x and y axes
plot(Smooth,Toads, xlab= "Presence/absence of smooth newt",
ylab= "Toad presence")
# draws the line with x and y values as defined above
lines(xv6,yv6)
```