Modelling the relationship between native amphibian species and the non-native marsh frog

Aidan Mackay

Durrell Institute of Conservation and Ecology (DICE)

School of Anthropology and conservation

University of Kent, UK

DICE

University of Kent

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Author's Declaration

All the chapters in this thesis were written my A. J. T. Mackay. R. A. Griffiths had the original idea for the thesis while the author developed the design and analysis with advice from both supervisors. Editorial feedback was provided by R. A. Griffiths and Z. G. Davies. All field work was conducted by A. J. T. Mackay with assistance from volunteers. The University of Kent, School of Anthropology and Conservation Research Ethics Committee approved all research within this thesis.

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Abstract

Due to increasing globalization the rate of non-native introductions has rapidly increased. This is likely to continue as climate change leads to increases in range of some species. In some cases, the effect of non-native species on biodiversity has been very severe. However, due to the complex nature of ecosystems it is sometimes difficult to determine if a non-native species is having a negative impact. This can be the case if interactions with other species are involved or native species are declining due to other threats such as habitat loss. This thesis investigates whether the marsh frog (*Pelophylax ridibundus*), a non-native species introduced to the UK in 1935, is affecting the distribution and abundance of the common frog *(Rana temporaria*) in Kent, south east England.

Species distribution modelling predicted marsh frogs to be present in areas where common frog presence was low. Many of these areas were in coastal regions with lots of watercourses that have higher salinity levels. These conditions are more suited to marsh frogs than common frogs, thereby explaining the predicted distributions. However, an area in the centre of Kent with high pond density was also predicted as less suitable for common frogs. This prediction fitted the hypothesis that in areas of high pond density common frog numbers have been reduced by the combined presence of marsh frogs, and great crested newts (*Triturus cristatus*). To test this hypothesis, a local level study compared the presence of common frogs in this high pond density area in Kent with ponds in Sussex where marsh frogs were absent. To make the comparison more meaningful, propensity modelling was used to match the ponds to be compared in the two areas by other characteristics as far as possible. Occupancy modelling was used to determine probability of detection for great crested newts and marsh frogs from survey data collected in ponds in Kent. This showed that accounting for variation in detectability did not increase the predicted occupancy of the survey ponds for these species. The presence of common frogs was found to be much higher in the ponds in Sussex. Logistic regression showed that common frogs were positively associated with shaded ponds, which marsh frogs tended to avoid. This suggested predation and/or competition by

marsh frogs on common frogs was unlikely because of these different habitat preferences. Therefore, a higher proportion of great crested newts in the survey ponds in Kent may be the main cause of the lower number of common frogs in that area.

Results from an eDNA metabarcoding analysis of water samples taken in 2014 from ponds in central Kent were obtained. The data provided the presence/absences of common frogs, great crested newts, and marsh frogs. Logistic regression showed that common frogs were not negatively associated with great crested newt presence. However, there was a much higher proportion of ponds with great crested newts compared to ponds with common frogs. In contrast, the proportion of ponds occupied by marsh frogs was very low. This supported the hypothesis that marsh frogs are unlikely to be the cause of lower common frog presence in the area. Common frog spawn surveys were conducted in 2017 on a subset of the same ponds analysed by the DNA metabarcoding in 2014. These showed a change in pond occupancy between 2014 and 2017. This could be due to natural changes in occupancy or metabarcoding could be detecting non-breeding common frog ponds and missing some breeding ponds.

Both landscape and local level studies have indicated that common frog presence is lower in an area of high pond density in Kent. This is unlikely to be caused by the presence of marsh frogs because of a difference in pond preference between common frogs and marsh frogs reducing the risk of predation or competition. There was also a relatively low presence of marsh frogs in areas that were showing lower proportions of common frogs compared to great crested newts. The high proportion of ponds occupied by great crested newts is more likely to be the reason for lower common frog presence. Therefore, more active measures to control the spread of marsh frogs is not required when considering conservation measures to protect common frogs.

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Chapter 1. General Introduction

The rate of non-native species introductions have increased as a result of human activity (Seebens et al. 2018; Wilson et al. 2009; Ricciardi 2007; Tatem and Hay 2007). Climate change may also result in the alterations in species ranges (Hulme 2017; Ihlow et al. 2016; Araújo, Thuiller and Pearson 2006). Therefore, the spread of non-native species seems likely to continue (Tatem and Hay 2007). Non-native species can cause severe damage to the ecosystem they invade as well as causing economic damage (Shine 2010; Shine et al. 2006). The impacts of non-native species are diverse. They can range from relatively direct impacts such as predation and the spreading of disease, or have more complicated origins involving trophic cascades (Penk et al. 2017; Kraus 2015; Roemer, Donlan and Courchamp 2002). Therefore, assessing the effects of non-native species can be challenging.

The loss of biodiversity is a concern for many taxa including amphibians (Stuart et al. 2004). There are multiple causes of amphibian declines and the increasing presence of non-native amphibians is not a trivial one (Measey et al. 2016; Kraus 2015; Bielby et al. 2008; Beebee and Griffiths 2005). Species such as the American bullfrog (*Lithobates catesbeianus*) and African clawed frogs (*Xenopus laevis*) have had major impacts on native species all over the world (Laufer et al. 2018; Barbosa, Both and Araujo 2017; Ihlow et al. 2016).

The UK has had its fair share of problems with non-native species. However, amphibian invaders have been less successful. There have been introductions of some of the notorious amphibian invasive species into the UK including the American bullfrog and African clawed frog. So far, their impacts have not been severe. The UK climate has had its advantages in this respect and limited the spread of these species (Kernan 2015; Measey and Tinsley 1998). There is one non-native amphibian which has been by far the most successful introduction to date, the marsh frog (*Pelophylax ridibundus*) (Zeisset

and Beebee 2003). However, the impact of this species is far from clear. The marsh frog's impact (or not) on the common frog (*Rana temporaria*) is the subject of this research.

1.1. An introduction to the marsh frog (*Pelophylax ridibundus*)

1.1.1. Distribution and taxonomy

The marsh frog is distributed widely through western, central and eastern Europe and has a native area extending from eastern France to Kazakhstan (Kuzmin et al. 2009) (Figure 1.1). Marsh frogs belong to Ranidae, sometimes known as 'true fogs' or 'leaping frogs'. Characteristically the members of this family have long powerful hind legs for swimming and leaping (Greenhalgh and Ovenden 2007). The Ranidae can be separated into the 'brown frogs' that tend to be more terrestrial and the 'green frogs' or 'water frogs' that are in general more aquatic and noisier (Greenhalgh and Ovenden 2007). Marsh frogs are classified as a green frog; it has a loud communal call and the aquatic behaviour is typical of this group. This is in contrast to the common frog, a member of the brown frogs which displays the typical more terrestrial behaviour of this group e.g. leaving ponds soon after spawning and having a quieter call (Greenhalgh and Ovenden 2007).

Figure 1.1. Distribution of marsh frogs showing the extant (resident) population (yellow) and introduced populations (purple). Reproduced from IUCN Red List (Kuzmin et al. 2009).

1.1.2. Interactions between marsh frogs and common frogs

In the UK marsh frogs emerge from hibernation around late April early May but if the spring has been very cold this can be delayed until late May (Beebee and Griffiths 2000). Mating and then spawning ensues in May to early June usually over about two weeks or longer if interrupted by bad weather (Beebee and Griffiths 2000). The males tend to congregate during breeding usually in areas that have lots of floating vegetation that can be used as rafts for mating on (Beebee and Griffiths 2000). The congregation of male marsh frogs during mating is not as pronounced as for common frogs because marsh frog males are very territorial and aggressive (Menzies 1962).

Several other features that aided marsh frogs successful introduction into the UK are also related to breeding. Marsh frogs lay many eggs (Cayuela, Besnard and Joly 2013); a trait that has increased the invasive potential of many species including amphibians (Measey et al. 2016; Van Bocxlaer et al. 2010; Urban et al. 2008; Shine et al. 2006; Kats and Ferrer 2003). A study in Russia found that female absolute fecundity ranges from 1,199 to 13,252 (Ivanova and Zhigalski 2011). The estimates of the numbers of eggs laid

by a single female each year in the UK vary for both marsh frogs and common frogs. Estimates for the common frog have been in the range of 1000 to 2000 (Inns 2009; Beebee and Griffiths 2000). Examination of one marsh frog female just prior to spawning found 2,900 eggs (Smith 1973). Another feature that could benefit an invading species is survival of their young. Rather than the eggs being laid in a large clump that is visible from above like common frogs, marsh frogs lay small clumps of eggs that are deposited about 5 to 10 cm below the surface and attached to dense aquatic vegetation (Wycherley and Anstis 2001; Kyriakopoulou-Sklavounou and Kattoulas 1990). Tadpole behaviour also gives marsh frogs an advantage over common frogs. Rather than congregating like other anuran tadpoles marsh frog tadpoles tend to prefer deeper water and dense vegetation and are rarely caught in open water like common frogs (Beebee and Griffiths 2000; Innocenzi 1995).

The differences in behaviour and habitat preference that common frogs exhibit compared with marsh frogs may prove to be a significant factor in determining if marsh frogs are affecting the distribution of common frogs (Cayuela, Besnard and Joly 2013). Both species have a relatively broad habitat range; however, marsh frogs have a preference for ditches, sewers and larger open ponds (Roth, Buehler and Amrhein 2016; Beebee and Griffiths 2000; Morand and Joly 1995). Common frogs can be found in both ditches or ponds and larger lakes but can also inhabit much smaller, shallower waterbodies compared to the marsh frog (Inns 2009; Beebee and Griffiths 2000). Compared with common frogs, marsh frogs have a much higher tolerance of brackish water (Viertel 1999; Innocenzi 1995; Beebee 1980; Menzies 1962). This tolerance has enabled marsh frogs to successfully colonise tidal marshes like Romney Marshes in Kent (Firth 1984). The differing preference in habitat has led to the belief that they are occupying a vacant niche and that the disappearances of the common frogs from these areas is due to a change in habitat favouring marsh frogs rather than common frogs (Beebee and Griffiths 2000; Beebee 1980). For example, an increase of intensive agricultural farming in an

area is likely to affect the more terrestrial common frog compared to marsh frogs that reside by the water all year round (Beebee 1980).

Several studies looking at the diet of marsh frogs, both in England and across Europe, indicate that marsh frogs are a generalist and their diet is strongly influenced by availability of prey (Cicek and Mermer 2007; Merry 2004). The majority of their prey tends to be invertebrates but amphibians, reptiles, fish, mammals and plant material have all been found in diet studies (Mollov, Boyadzhiev and Donev 2010; Cicek and Mermer 2007; Yilmaz and Kutrup 2006; Merry 2004; Ruchin and Ryzhov 2002). Common frogs share a similar diet consisting of mainly invertebrates (Beebee and Griffiths 2000). Despite this, competition for food between the two species is unlikely because common frog adults leave ponds to forage on land before marsh frogs have emerged from hibernation (Merry 2004). More prey is caught on land than in water (Cicek and Mermer 2007; Merry 2004). Marsh frogs will both sit and wait and actively forage to catch their prey (Merry 2004). Variability in the type of prey was seen in different populations, localities and at different times of year (Mollov, Boyadzhiev and Donev 2010; Cicek and Mermer 2007; Yilmaz and Kutrup 2006; Ruchin and Ryzhov 2002). Cannibalism was also found in many studies (Mollov, Boyadzhiev and Donev 2010; Cicek and Mermer 2007; Yilmaz and Kutrup 2006; Ruchin and Ryzhov 2002; Smith 1973) and this may indicate that the marsh frog is an opportunistic feeder (Mollov, Boyadzhiev and Donev 2010). In the most comprehensive assessment of diet under natural conditions to be carried out in England there was a high mortality of common frog larvae prior to marsh frogs emerging from hibernation so predation of marsh frogs on common frogs could not be tested (Merry 2004).

1.1.3. Introduction of marsh frogs into the UK

There have been numerous reports of introductions of green frogs into the UK taking place in the 19th and 20th century (Arnold 1995; Menzies 1962; Smith 1939) the first possibly being in 1837 at Morton and Hockering in Norfolk (Arnold 1995). However, the

differences in the species were not fully understood and introductions of any of the green frogs into the UK were referred to as edible frogs sometimes noted as being 'the subspecies of *ridibunda*', *'Rana esculenta* the Hungarian variety' or 'the Italian form *Rana esculenta lessonae*' (Arnold 1995; Boulenger 1884). Due to this it is possible that the first introduction of marsh frogs in the UK occurred in Childworth and Shere, Surrey in 1884 when several types of green frog were introduced (Arnold 1995). Many of the introductions of green frogs did not survive (Frazer 1983; Smith 1939). The reasons for this are unclear - anecdotal reports suggest enough of the frogs appear to have been released; Smith (1939) refers to 200 marsh frogs being sold to a firm in Barking Creek, [East London] and 1500 specimens from France and Belgium being released in the Fens near Stoke Ferry [Norfolk]. The Stoke Ferry area was very likely to have had suitable habitat but as these are anecdotal reports it is difficult to draw any conclusions. There are several introduced populations of green frogs present in southern England often with a mix of pool frogs (*Pelophylax lessonae*), edible frogs (*Pelophylax esculentus*) and marsh frogs. There is also a population of the Iberian marsh frog (*Pelophylax perezi*) in Somerset (Reptile and Amphibian Group for Somerset n.d.).

The most well-known introduction of marsh frogs which is likely to have been the progenitor of the population in southern Kent occurred in the winter of 1934-35 when Edward Percy Smith introduced 12 marsh frogs into his garden at Stone-in-Oxney, near Romney and Walland Marshes, East Kent (Smith 1939). The 12 frogs had originally been brought over from the neighbourhood of Debreczen in Hungary by Professor A.V. Hill (Mandeville and Spurway 1949). Since then, the species has become abundant in the low-lying wetlands of the south eastern counties (Zeisset and Beebee 2003). However, there are also records of sightings in the Midlands (Nottinghamshire Biological and Geological Records Centre 2012; Worcestershire Biological Records Centre 2012), as well as a water frog population in Yorkshire which may contain marsh frogs (Inns 2009). After their introduction marsh frogs spread rapidly along watercourses but their

dependence on water has prevented natural colonisation of suitable habitat that is geographically separated (Zeisset and Beebee 2003). Accidental or deliberate translocations are likely to be the cause of the occurrence of new populations in these areas (Zeisset and Beebee 2003). The dispersal of marsh frogs in Kent and Sussex proceeded in a distinctive pattern along rivers with smaller populations radiating from these.

1.2. Detecting impacts of invasive species

It is notoriously difficult to identify the effects of a non-native species (Pilliod, Griffiths and Kuzmin 2012; Shine 2010; Shine et al. 2006; Parker et al. 1999). Even in research into the spread of the cane toad (*Bufo marinus*) in Australia, which is one of the most studied invasions, it has been difficult to quantify the effects on native species (Shine 2010; Shine et al. 2006). Identifying effects of a non-native species is hindered by several sources of ambiguity. Evidence can be derived from experiments designed to test the effect of the non-native on a particular species; or research investigating correlation between abundance and distribution of one species with the non-native (Pilliod, Griffiths and Kuzmin 2012). With experiments, there are trade-offs with replication and scale. Pure field experiments are often un-replicated (or pseudo-replicated) so may be realistic but confounded. At the other extreme, more contrived mesocosm or laboratory experiments can be well-designed with appropriate replication but may be more divorced from the real world.

Experiments have shown that marsh frogs will eat common frog tadpoles (Innocenzi 1995). However, studies on the diet of marsh frogs have not shown significant levels of predation on other amphibians (Mollov, Boyadzhiev and Donev 2010; Cicek and Mermer 2007; Yilmaz and Kutrup 2006; Merry 2004; Ruchin and Ryzhov 2002). Further experimental studies along these lines may not adequately answer the question of why there appears to be fewer common frogs in areas where there are marsh frogs. Without

answering this question doubts may still remain about the impact of marsh frogs on common frogs. To help answer this more general question this research will start at a landscape level to identify whether the distribution of common frogs appears altered in relation to the distribution of marsh frogs. Species distribution models (SDMs) associate species presence records with environmental variables to predict species distributions (Peterson et al. 2011; Pearson 2007). This technique has been used to make inferences about interactions between species (Buckland et al. 2014; Anderson, Peterson and Gómez-Laverde 2002). A more focussed local level study will then be used to identify if the findings of the landscape level study are reproduced at a much smaller scale.

To achieve the aim of determining whether the presence of marsh frogs is likely to have caused lower presence of common frogs, four main objectives were set: 1) Use species distribution modelling to predict the distributions of common frogs, marsh frogs, great crested newts, and grass snakes to locate areas in Kent and Sussex where these species may be interacting; 2) Identify which variables are associated with the presence of these species to help determine whether species interactions or habitat preferences are responsible for the predicted distributions; 3) Compare common frog pond occupancy at a local level to find out if it is lower in areas where marsh frogs are present while controlling for differences in habitat; 4) Identify what pond variables may be associated with the presence of common frogs, marsh frogs, and great crested newts and whether these variables reveal anything about the likelihood that marsh frogs are preying on common frogs.

1.3. Thesis structure

- Chapter 2 uses SDMs to compare the predicted distributions of marsh frogs and common frogs in order to determine areas of possible overlap and interaction.

- Chapters 3 and 4 aim to validate the findings in Chapter 2. A local level study was used to identify if any reduced presence of common frogs found by the

landscape study are reflected at a pond level by the presence of marsh frogs. To take account of variation in detectability, occupancy modelling was used to calculate the probability of detection and pond occupancy.

- Chapter 5 compares the results of the local level study to species presence/absence data identified using eDNA metabarcoding by Harper, University of Hull (Harper et al. 2018a; Harper et al. 2018b; Harper et al. 2018c). Common frog spawn surveys and eDNA techniques were then used on a subset of these ponds to identify if occupancy has changed between 2014 and 2017 and/or whether the eDNA survey detects the same ponds as the spawn surveys.

- Chapter 6 is a general discussion of the main points raised in the other chapters and makes recommendations regarding conservation.

Chapter 2. Impacts of potential competitors and predators on the distribution of common frogs

2.1. Abstract

The introduction of non-native species can lead to a loss in biodiversity. However, as there may be multiple factors driving population trends it is often difficult to determine the impacts of a non-native species. In such situations assessing the distributions of the native and non-native species can indicate where impacts may be occurring to help target a more focussed study. The non-native marsh frog (*Pelophylax ridibundus*) may have been exacerbating declines in a native common frog (*Rana temporaria*) in south east England. Species distribution models (SDMs) were used to determine the overlap in distribution between marsh frogs, common frogs and a native predator of the common frog, the great crested newt (*Triturus cristatus*). The SDMs revealed common frogs predicted presence was lower in areas predicted suitable for marsh frogs. The strong positive association with linear water features was the main predictor of marsh frog distribution. The lower probability of common frogs in areas with lots of linear water features may be due to these areas having less suitable habitat for common frogs, rather than the presence of marsh frogs. An area in central Kent with high pond density was predicted as less suitable for common frogs and more suitable for both marsh frogs and great crested newts. Great crested newts and marsh frogs were positively associated with pond density and could be the reason for fewer common frogs in this area.

2.2. Introduction

The global decline in amphibians is now well recognised by the conservation community (Bielby et al. 2008; Beebee and Griffiths 2005; Stuart et al. 2004). The impact of non-native amphibian species is an important factor in this decline (Measey et al. 2016). However, determining if a non-native is the cause of a native species' decline can be

difficult, particularly if any impacts are due to indirect effects (Pilliod, Griffiths and Kuzmin 2012; Preston, Henderson and Johnson 2012; Shine 2010; Shine et al. 2006; Parker et al. 1999). Even if there is a strong correlation between a decline of a native and an increase in a non-native species, the non-native may not be the cause of the decline. Changes in habitat that are favourable to the non-native and detrimental to the native species could be the reason for the negative relationship (Didham et al. 2005; Byers 2002). The non-native species may be exacerbating the decline or having no impact. Establishing whether the non-native species is an important factor in the decline is essential if conservation efforts are to be effective.

In some areas of lowland Kent and Sussex the native common frog (*Rana temporaria*) has declined (Brady 2009; Firth 1984; Beebee 1980; Menzies 1962). A non-native species, the marsh frog (*Pelophylax ridibundus*), was introduced into Kent in 1935 and Sussex in 1973 (Beebee 1980; Smith 1939) (Figure 2.1). It has been suggested that marsh frogs may be one of the causes of the decline in common frogs (Brady 2009; Firth 1984; Menzies 1962). There is also evidence that in Switzerland, another part of the marsh frog's introduced range, it has affected the density and distribution of native amphibians (Roth, Buehler and Amrhein 2016). However, other factors such as changes in land use, may be causing the common frog decline in Kent and Sussex, possibly simultaneously with the spread of marsh frogs (Beebee 1980).
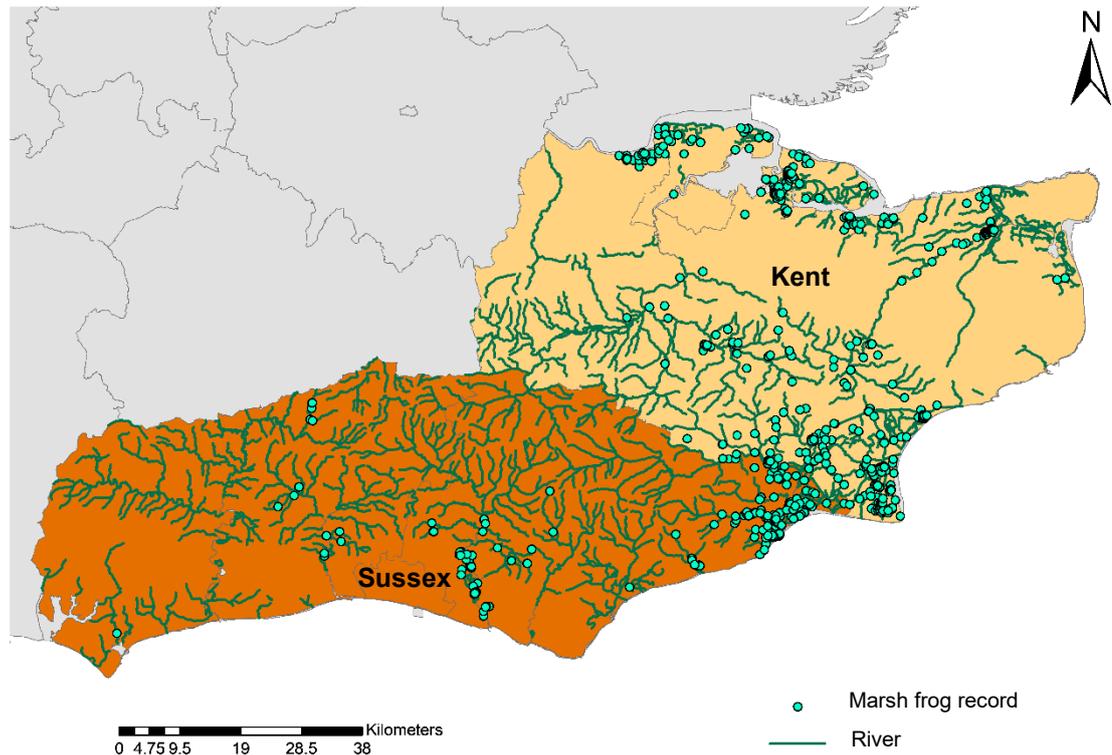
Figure 2.1. The recorded presence of marsh frogs in Sussex and Kent using all validated records until 2014 held by Kent Reptile and Amphibian Group (KRAG) and the Sussex Biodiversity Records Centre (SxBRC).

It is possible that predation of common frog tadpoles and froglets by marsh frogs could be reducing common frog numbers (Menzies 1962). However, more complex competitive and predatory interactions may be involved. The great crested newt (*Triturus cristatus*), another predator of the common frog that is associated positively with high pond density (Oldham et al. 2000), could be driving or exacerbating the situation (Brady 2009). Marsh frogs inhabit ponds as well as marsh dykes and ditches, and rarely stray far from water (Kovar et al. 2009; Beebee and Griffiths 2000). Areas of high pond density are likely to be suitable habitat for both these species. Great crested newts are negatively affected by predatory fish (Hartel et al. 2007; Van Buskirk 2005). Therefore, it has been suggested that common frogs may have been using ponds occupied by fish to avoid predation by great crested newts (Brady 2009). Marsh frogs tend to be unaffected by the presence of fish (Hartel et al. 2007; Van Buskirk 2003), so they could be reducing the

number of ponds where common frogs can breed successfully (Brady 2009). Another possible explanation may lie with grass snakes (*Natrix helvetica*), which have large home ranges (Reading and Jofre 2009; Madsen 1984) and feed on both frog species and have become very abundant in marsh frog inhabited areas (Gregory and Isaac 2004). It is possible that grass snakes are moving from marsh frog sites to ponds occupied by common frogs, thereby impacting upon their populations.

Due to the possible complex interactions that could be involved if marsh frogs are affecting common frogs, a broader scale approach examining species distributions was undertaken in this study. Species' distribution models (SDMs) can be used to predict presence by associating species records with environmental conditions that may affect their occurrence (Pearson 2007). SDMs have been used to predict the extent of non-native invasions, estimate the overlap of non-native and endemic species, and highlight where competitive exclusion may be occurring between species (Buckland et al. 2014; Urban et al. 2008; Anderson, Peterson and Gómez-Laverde 2002). SDMs can thus indicate where a non-native species has potentially displaced a native species.

The aim of this study was to determine whether the distribution patterns of common frogs, marsh frogs, great crested newts, and grass snakes were consistent with the hypothesis that marsh frogs were affecting the distribution of common frogs in Kent and Sussex. To achieve this SDMs were used to: (i) identify if there are areas where impacts between the species could be occurring; and (ii) whether the predicted distributions and the environmental variables most associated with them support a mechanism of impact on common frogs such as predation by marsh frogs, great crested newts or grass snakes. The environmental variables driving the distribution of all species were considered, to avoid falsely attributing the absence of common frogs to the presence of marsh frogs.

2.3. Methods

2.3.1. Study area

The study area comprises the counties of Kent (Lat 51° 11'N, Long 0° 44,E) and Sussex (Lat 50°56'N, Long 0°2'E) in south east England (Figure 2.1). These counties are characterised by landscapes consisting of chalk hills, lowland arable land, wetland marshes, and patches of woodland. The 25 m resolution raster Land Cover Map 2007 (LCM2007), which identifies the most widespread habitat in 25 m x 25 m pixels (Morten et al. 2011), shows the east of Kent and west of Sussex dominated by arable and horticultural land; while in the west of Kent and central and east Sussex improved grassland is the predominating land cover (Figure 2.2a). There are also extensive urban and suburban areas across Kent and Sussex, providing potentially suitable habitat for all four species in the form of urban and suburban parks and gardens (Beebee and Griffiths 2000). Access to surface water such as ponds and watercourses is important for amphibians. Central Kent has a large area where pond density is very high (Figure 2.2b). In Sussex there are smaller areas of higher pond density. The southernmost part of Kent has many watercourses (Figure 2.2c). In some parts of north Kent and the south coast of Sussex there are also smaller areas with a high density of watercourses.
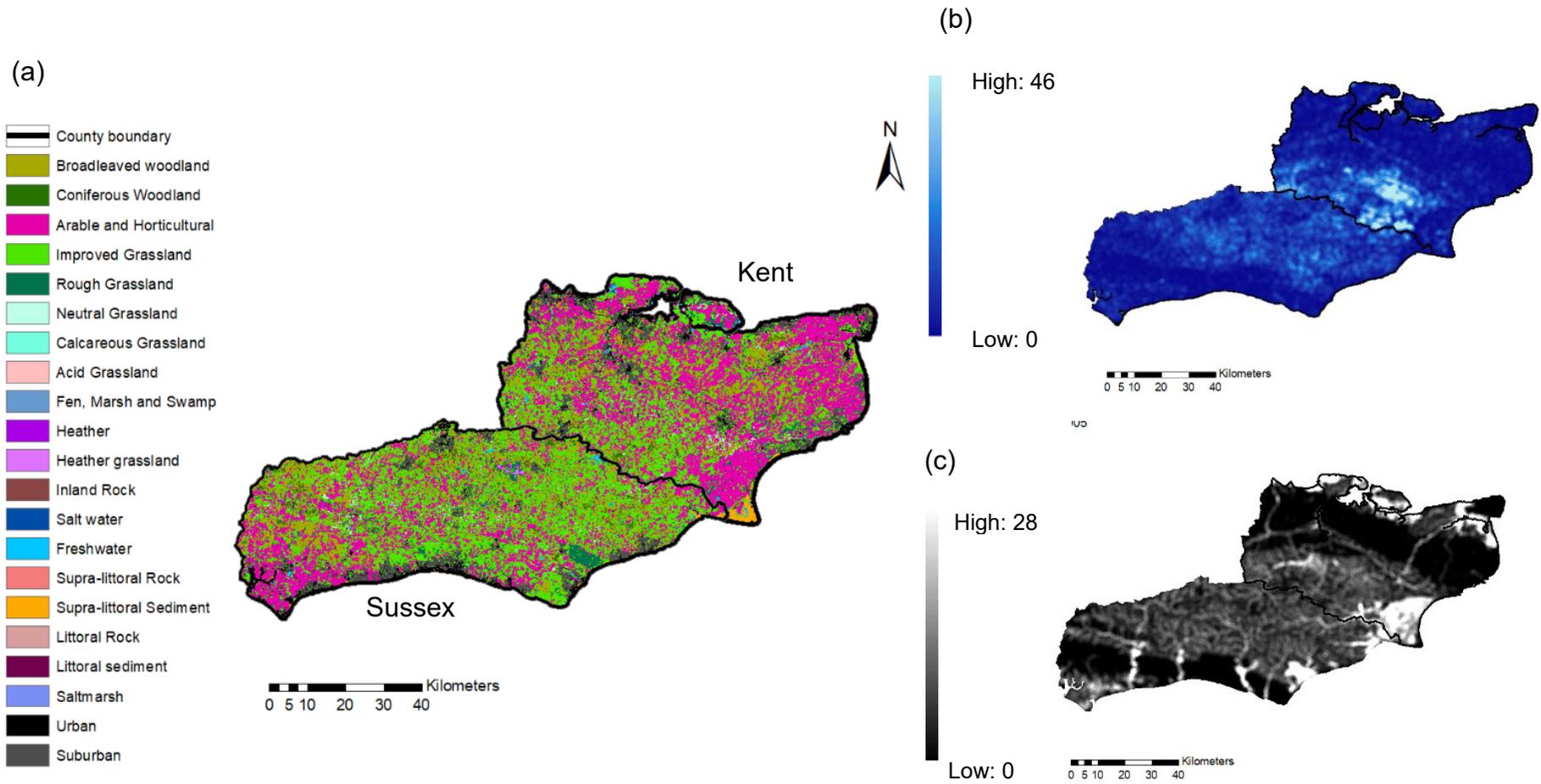
Figure 2.2. Land cover maps of Kent and Sussex. (a) Map showing habitats in Kent and Sussex using the 25 m x 25 m raster Land Cover Map 2007 (Morton et al. 2011). Each 25 m x 25 m raster pixel represents the habitat identified as dominant in that location. The habitat categories are based on the UK Biodiversity Action Plan Broad Habitats (Morton et al. 2011). (b) Map showing pond density per (km²) in Kent and Sussex. Lighter blue areas indicate higher pond density. (c) Map showing linear water (length per km²). Lighter areas indicate greater amounts of linear water features.

2.3.2. Species data collation

Species presence records were obtained from the Kent Reptile and Amphibian Group (KRAG) and the Sussex Biodiversity Records Centre (SxBRC). The KRAG records used were either 'validated' or 'within expected range' based on the KRAG validation system. 'Validated' records are defined as being supported by compelling evidence, such as a clear photograph or the record was from a trusted recorder (Brady 2010). A record that is 'within expected range' is defined as being submitted from an unknown recorder, but which is within the expected range for that species (Brady 2010). SxBRC records are validated by the Sussex county recorder using five different levels: 'correct', 'considered correct', 'plausible', 'unable to verify', and 'not accepted/incorrect' (Foreman 2018). The levels 'correct' and 'considered correct' are similar to the KRAG 'validated' and 'within expected range' categories. Records from only these categories were used in the SDMs for Sussex and Kent. The KRAG and SxBRC species records are from a variety of sources including records collected by the public, records from environmental consultants, and other surveys to assess biodiversity in areas such as nature reserves. Therefore, the recording techniques and survey effort for the collection of species records varies.

The habitat where species have been recorded in the past may have changed through time, particularly in the case of older observations. To increase the likelihood that the type of habitat present when a species was recorded matches the habitat variable used in the SDM, only species records from 2000 to 2014 were used. Only species presence records reported to a precision of 100 m x 100 m or lower, equivalent to a six figure British National Grid reference, were included in the analyses. This resolution was chosen to maximise the number of species records that could be used, without being too coarse for comparison with the 25 m x 25 m land cover map (see below). Duplicated records were removed. Using these criteria, the number of grid cells with species present were as follows:

Kent: 1253 grid cells with common frog, 218 grid cells with marsh frog, 685 grid cells with great crested newt, and 985 grid cells with grass snake. Sussex: 1088 grid cells with common frog, 84 grid cells with marsh frog, 554 grid cells with great crested newt, and 730 grid cells with grass snake.

2.3.3. Environmental variable selection

For each species, environmental variables that were considered likely to affect the distribution, based on previous research and expert knowledge, were selected for use in the SDMs (Table 2.1). These included a subset of the WorldClim bioclimatic variables (http://www.worldclim.org/bioclim) (Table 2.1) taken from records between 1950 and 2000 (Hijmans et al. 2005). The WorldClim variables have a spatial resolution of 30 arc seconds which equates to approximately 0.86 km² resolution at the equator (reducing with increasing distance from the equator) (Hijmans et al. 2005). Each WorldClim variable was created using temperature or precipitation records from weather stations to model an interpolated surface for each variable (Hijmans et al. 2000). The relatively small spatial extent of Kent and Sussex means that the bioclimatic variables may be approaching their limit of accuracy given the spatial scale. However, the higher elevation of the North Downs and South Downs in Kent and Sussex means there are temperature and precipitation gradients within the counties which could affect amphibian distribution. Therefore, inclusion of bioclimatic variables may result in more accurate distribution models.

Explanatory variables identifying the distance of each pixel from a habitat category were created using the Land Cover Map 2007 (LCM2007) (Table 2.1).The LCM2007 is a 25 m x 25 m raster product derived from satellite imagery and digital cartography, and delineates habitats according to the UK Biodiversity Action Plan Broad Habitats (Morton et al. 2011). A minimum mappable unit of 0.5 hectares is achieved in the LCM2007; segments of habitat smaller than this are 'dissolved', into surrounding segments so the

dominant habitat is represented (Morton et al. 2011). Other habitats smaller than 0.5 hectares may be present but not shown.

The LCM2007 has six different classes of grassland habitat ('Improved grassland', 'Rough grassland', 'Neutral grassland', 'Calcareous grassland', and 'Acid grassland') that were merged to form two aggregate classes: 'Distance from improved grassland' and 'Distance from semi-natural grassland'. This was done to differentiate, as far as possible, between highly managed grassland, such as sports fields, and grassland with more natural characteristics that may provide some habitat for amphibians. The habitat classes 'urban' and 'suburban' from the LCM2007 correspond to the UK Biodiversity Action Plan Broad Habitat class 'Built-up areas and gardens' (Morten et al. 2011; Jackson 2000). This category covers: urban and rural settlements (including domestic gardens and allotments), farm buildings, caravan parks, industrial estates, retail parks, waste and derelict ground, urban parkland, and urban transport infrastructure (Jackson 2000). The LCM2007 classes 'urban' and 'suburban' were combined to create the variable 'Distance from urban/suburban areas' (Table 2.1).

Table 2.1. Environmental variables used in Kent and Sussex species distribution models for common frogs, marsh frogs, great crested newts, and grass snakes.

Variable Name	Description	Reason for inclusion
Linear water features	Combined length of linear water features per km². Derived from Ordnance Survey MasterMap Water Network (OS MasterMap Water Network 2015).	Marsh frogs are a highly aquatic species and often associated with linear water features such as drainage ditches (Inns 2009; Beebee and Griffiths 2000).
Distance from broadleaved woodland	A measurement of the distance in metres of each raster pixel from the closest pixel classified as broadleaved woodland. Derived from the LCM2007 (Morton et al 2011).	Common frog presence has been associated with woodland (Boissinot et al. 2015; Van Buskirk 2005).
Distance from arable land	A measurement of the distance in metres of each raster pixel from the closest pixel classified as arable or horticultural land. Derived from the LCM2007 (Morton et al. 2011).	Areas of arable land are likely to be detrimental to amphibians due to issues such as agricultural runoff and overgrowth of ditches (Beebee and Griffiths 2000). Arable land has also been found to be negatively associated with grass snake presence (Ward 2017).
Distance	A measurement of the distance	Improved grassland could have a

from improved grassland	in metres of each raster pixel from the closest pixel classified as improved grassland. Derived from the LCM2007 (Morton et al. 2011).	negative effect on amphibians due to eutrophication of ponds from fertilisers (Beebee 2013).
Distance from semi-natural grassland	A measurement of the distance in metres of each raster pixel from the closest pixel classified as semi-natural grassland. Derived from the LCM2007 (Morton et al 2011).	Semi-natural grassland can often provide habitat for amphibians and reptiles. It was found to be positively associated with great crested newt presence in Kent and grass snakes in Jersey (Ward 2017; Bormpoudakis et al. 2015).
Distance from urban/ suburban areas	A measurement of the distance in metres of each raster pixel from the closest pixel classified as urban or suburban areas. Derived from the LCM2007 (Morton et al 2011).	Amphibians, particularly common frogs, can often occupy urban and suburban ponds leading to quite high populations (Beebee 1979).
Pond density	Number of ponds within a 1 km radius divided by pi. Derived from a UK wide pond dataset provided by ARC Trust.	Presence of great crested newts has been linked to higher pond densities (Bormpoudakis et al. 2015; Oldham et al. 2000).
Distance from major road	A measurement of the distance in metres of each raster pixel from the closest pixel classified as a motorway, primary road, or A-road. Derived from Ordnance Survey MasterMap Highways Network (OS MasterMap Highways Network 2015).	Busy roads have been found to affect common frogs and great crested newts negatively (Hartel et al. 2010).
Distance from minor road	A measurement of the distance in metres of each raster pixel from the closest pixel classified as a B-road or minor road. Derived from Ordnance Survey MasterMap Highways Network (OS MasterMap Highways Network 2015).	Roads with less traffic have been found to have a positive effect on some amphibian species (Hartel et al. 2010).
Temperature seasonality	The standard deviation of temperature x 100 at a resolution of 30 arc seconds. Recorded between 1950 and 2000. Obtained from the World Clim data set http://www.worldclim.org/bioclim (Hijmans et al. 2005).	Temperature seasonality has been found to be negatively associated with great crested newt presence in Kent (Bormpoudakis et al. 2015).
Maximum temperature warmest month	Maximum temperature warmest month recorded between 1950 and 2000. Obtained from the WorldClim data set http://www.worldclim.org/bioclim (Hijmans et al. 2005).	Marsh frogs emerge from hibernation later than UK native amphibians (Beebee and Griffiths 2000) and therefore are likely to be more associated with warmer areas.
Minimum temperature coldest month	Minimum Temperature coldest month recorded between 1950 and 2000. Obtained from the WorldClim data set http://www.worldclim.org/bioclim	Warmer winters can result in hibernating animals depleting energy reserves without being able to feed. Previous research has found great crested newts to be negatively associated with warmer winters

	(Hijmans et al. 2005).	(Bormpoudakis et al. 2015; Griffiths, Sewell and McCrea 2010).
Precipitation coldest quarter	Precipitation coldest quarter recorded between 1950 and 2000. Obtained from the WorldClim data set http://www.worldclim.org/bioclim (Hijmans et al. 2005).	High winter rainfall, leading to waterlogged soils, could mean hibernating amphibians can only utilise gaseous exchange across their skin. This could prevent pumping of air into their lungs thus leading to respiratory problems (Griffiths, Sewell and McCrea 2010).

Highly correlated variables in SDMs can lead to very complex models, making it difficult to interpret them ecologically (Peterson et al. 2011). Therefore, Spearman's rank tests were used to measure inter-correlation between all the variables. There were no correlations between variables where Spearman's rho was > 0.70 (Supplementary information Table S2.1, S2.2) so all the variables were retained (Proosdij et al. 2016; Ihlow et al. 2016; Dormann et al. 2013).

2.3.4. Model development

Maxent modelling was chosen because it can be used with just species presence records, where no information exists on species absence. Also, studies comparing the predictive performance of different models have shown Maxent to be one of the most accurate modelling methods (Wachtel et al. 2018; Schank et al. 2017; Elith et al. 2006; Phillips, Anderson and Schapire 2006). Maxent models were run using Maxent version 3.3k (Phillips, Dudik and Schapire 2011).

The accuracy of an SDM can be measured by identifying the fraction of species presence records predicted correctly (the sensitivity) and the fraction of absence records predicted correctly (the specificity) (Pearson 2007). The Receiver Operating Characteristic (ROC) is a plot of sensitivity against 1 minus the specificity over a range of thresholds (Pearson 2007). The proportion of the area under the ROC curve (the AUC) provides a single-value measure of the fraction of absence and presence records correctly predicted by the model (Fielding and Bell 1997; Swets 1988). AUC is used to assess the SDMs in this study because it measures accuracy over the full range of possible thresholds (Pearson

2007; Fielding and Bell 1997). However, as Maxent does not use species absence data, specificity is calculated based on the predicted area composed of the background environmental data for the entire study area including the places with presence records (Pearson 2007). The value of each variable at a random selection of points is used as the background environmental data. In this study approximately 10,000 background points were used for each model run so a good representation of the study area was achieved (Elith et al. 2011).

Maxent splits the species data into two parts. Data used to train the model and data used to test the model's predictive ability using AUC. The AUC value provides a measure of how well the model classifies a sample of species presences compared to a random prediction (Pearson 2007). An AUC score of 0.5 means that the model has been able to rank random presence sites over random background sites no better than chance (Phillips et al. 2009). The AUC values for SDMs using different species or different regions are not comparable because the area covered by a species' predicted distribution relative to the study region would vary (Peterson et al. 2011; Franklin et al. 2009; Phillips, Anderson and Schapire 2006). Therefore, AUC will only be used to assess performance in models using the same species in the same region.

The AUC calculated from model testing is referred to in this study as AUCtest. Different methods can be used to split the data. In this study two methods are tested: (1) the subsample method (SS) and (2) the cross-validation method (CV). The SS method splits the species data into 80% training data and 20% test data. This level of data splitting ensured there was enough training and testing data for even the SDM with the lowest number of presence records (Proosdij et al. 2016). The training and testing of the model were repeated 10 times, with a different set of test data used for each run. The mean AUCtest from the 10 runs was used as the measure of predictive performance. The CV method divided the species data into five equal parts, with one of these being used to test the model (20% again, to be comparable with the SS method) and the others to train

the model. Each part can only be used as test data once (Peterson et al. 2011), meaning that a total of five repeat tests could be conducted. The CV testing method provided more consistent results and was therefore used in the model tuning analysis (Supplementary information S2).

While AUCtest is a measure of discriminatory power of the model, it does not account for overfitting and can therefore support overly complicated models (Radosavljevic and Anderson 2014). Overfitting is where a model fits to the training data too closely making it less able to predict independent test data (Boria et al. 2014). To provide a measure of overfitting, the AUC difference (AUCdiff) was calculated by subtracting AUCtest from the AUC calculated by Maxent during model training (AUCtrain) (Boria et al. 2014). Therefore, a low AUCdiff means the model can predict test data well and is not fitting too closely to the species presence data that was used to develop the model.

Research has shown it is also important to identify what model settings are likely to produce the best performing models for the data, environmental variables, and study area being analysed (Morales, Fernandez and Baca-Gonzalez 2017; Radosavljevic and Anderson 2014; Anderson and Gonzalez Jr. 2011). Adjustments can be made to alter how closely models are fitted to the data by a regularisation mechanism (Phillips and Dudík 2008). Regularisation reduces overfitting by lowering or setting some coefficients to zero, only keeping those coefficients that improve the ability of the model to distinguish between presence and background locations (Merow, Smith and Silander 2013). In this study a model tuning analysis was conducted for common frogs, great crested newts, and grass snakes. Models were run with regularisation levels of 0.5, 1, 2, 3, 4, 5, and 6 to identify the most appropriate setting (Supplementary Information S2). Marsh frogs are unlikely to be occupying all suitable habitats in Kent and Sussex because their unfacilitated range expansion has been limited to contiguous suitable habitat (Zeisset and Griffiths 2003). Due to this the default regularisation level of 1 was used which has the effect of fitting the predicted distribution closer to the records of species presences.

2.3.5. Mitigating sampling bias

Record centres often hold data from opportunistic sightings which can be subject to sampling bias (Fourcade et al. 2014; Kadmon, Farber and Danin 2004; Dennis and Thomas 2000). Reducing the clumping of species records by spatially filtering them can improve model performance (Boria et al. 2014; Radosavljevic and Anderson 2014; Kramer-Schadt et al. 2013). The data for each species was filtered by implementing a minimum distance between species records using the R package SpThin (Aiello-Lammens et al. 2014). The minimum distances used were 250 m for great crested newts and marsh frogs, 500 m for common frogs and 1 km for grass snakes. These distances were chosen based on evidence of dispersal distances for each species (Safner et al. 2011; Kovar et al. 2009). For instance, marsh frogs rarely move far from their pond (Kovar et al. 2009; Holenweg 2001; Wycherley and Joslin 1996), although there is evidence that they can disperse greater distances along watercourses (Menzies 1962). As such 250 m between records was used to reduce spatial autocorrelation.

A second method of bias reduction that uses a bias file was compared with spatial filtering. Bias files account for the clustering of records from uneven sampling by using background data with the same bias as the presence data (Kramer-Schadt et al. 2013; Phillips et al. 2009). It has the benefit of not reducing the amount of species data, unlike the use of spatial filters, which is beneficial if sample sizes are low (Kramer-Schadt et al. 2013). The bias files were generated using the function kde2d from the R package MASS (Venables and Ripley 2002). The comparison of bias reduction techniques was conducted with the analysis of model testing methods and regularisation levels (Supplementary information S2). The SDMs using either spatial filtering or a bias file indicated they may be predicting a higher probability of marsh frog presence than is likely given the limited dispersal of marsh frogs. For this reason, the marsh frog SDMs used in the results were run without spatial filtering or a bias file for both Kent and Sussex. Most of the common frog and grass snake records are clustered in urban and suburban areas.

The use of spatial filtering or bias files did not change the position of 'Distance from urban/suburban areas' as the most important variable in the SDMs. Indeed, the dominance of 'Distance from urban/suburban areas' made it difficult to assess what other variables may be associated with common frog and grass snake presence. Sampling bias may be the cause of this dominance. Nevertheless, common frogs are common in urban garden ponds (Beebee and Griffiths 2000). Grass snakes have also become established in urban parks and gardens; probably attracted by the food source provided by common frogs and other amphibians (Beebee and Griffiths 2000). It is possible, therefore, that large numbers of records in urban and suburban areas may be a reasonable representation of common frog distribution. This could also be the case for grass snakes, although their more cryptic nature may reduce their likelihood of being detected in rural areas. Consequently, the urban and suburban areas were not used as an environmental variable in the SDMs predicting the distribution of common frogs and grass snakes. Initially, all the other variables were used in the urban and suburban removed models. However, the distribution maps showed that the variables 'Distance from major road' and 'Distance from minor road' were acting as proxies for 'Distance from urban/suburban areas', so the models were re-run without these variables.

2.3.6. Identifying overlapping distributions

The final model settings used for each species were determined from the results of the model tuning analysis (Supplementary information S2). Distribution maps of the common frogs, marsh frogs, great crested newts, and grass snakes were created in ArcGIS 10.5 using the logistic output from Maxent. Binary presence/absence was determined for each species by implementing a threshold at the 10th percentile training presence (10%TP) (Radosavljevic and Anderson 2014; Pearson et al. 2007). This threshold allows for 10 percent omission of presence records before a species is classed as absent. The area of predicted sympatry between common frogs and great crested newts using the 10%TP was identified. The proportion that the area of predicted sympatry constituted out of the

total area predicted as having common frogs present was calculated. Chi-squared tests were used to identify if the proportion of sympatric area was significantly different in Sussex compared to Kent. This process was repeated using the predicted area of sympatry between common frogs and marsh frogs to indicate whether common frogs were predicted to be absent from areas of high pond density that were otherwise predicted suitable for marsh frogs, great crested newts, or both.

The marsh frog population in Sussex is much smaller and more recently established than in Kent, so there may be areas which are suitable for the species that are yet to be colonised. Consequently, the distribution of common frogs may not yet have been affected by presence of marsh frogs. Comparing the common frog distribution in Kent and Sussex may show that common frogs are predicted to inhabit areas in Sussex that were predicted as unsuitable in Kent. This would suggest marsh frogs were having an influence on the distribution of common frogs in Kent. The predicted distributions for common frogs and great crested newts were also compared to see if the patterns differed.

2.3.7. Comparing species distributions in Kent and Sussex

The differences in habitat and landscape between two areas can affect species distribution patterns. Although Kent and Sussex have a similar habitat and landscape, the differences that do exist may affect the predicted species distributions. To help determine what might be driving any differences in distribution, the variables having most influence in the Kent and Sussex SDMs were identified using percent contribution, permutation of importance, and jackknife test on variable importance. The percentage contribution measures how influential a variable is by adding (or subtracting) the log likelihood of the presence samples (penalised by regularisation) to the contribution of the corresponding variable after each iteration of the model training process (Phillips, Dudik and Schapire 2011). The permutation of importance assesses the contribution of each variable by measuring the decrease in AUCtrain when the values of that variable are

randomly distributed between the species presence and background pixels (Phillips, Dudik and Schapire 2011). The greater the drop in AUCtrain, the more the model relies on that variable (Phillips 2010). The jackknife test identifies which variable has the highest AUCtest when used in isolation, and which variable decreases the AUCtest the most when removed from the model. Response curves were used to identify how each variable affects the species predicted probability when run in a Maxent model using only that variable.

2.4. Results

2.4.1. Common frog

The AUCtest for the best performing common frog SDM for Kent was 0.68. Therefore, this model has been able to rank presence sites over random background sites. However, the model is not doing this very well given an AUCtest score of 0.5 means it would be ranking sites no better than random. The four variables that contributed most to the model were 'Linear water features', 'Distance from broadleaved woodland', 'Pond density', and 'Distance from arable land' (Table 2.2). The response curves showed 'Linear water features' and 'Pond density' were negatively associated with common frog presence (Figure 2.3a, c). The probability of presence of common frogs increased with the 'Distance from arable land' until approximately 700 m, after which point there is a lot of variation (Figure 2.3d). 'Distance from broadleaved woodland' had the most useful information for the model by itself as indicated by jackknife tests on AUCtest (Figure 2.5a). The proximity of broadleaved woodland (up to ~250 m) had a positive effect on common frog probability of presence (Figure 2.3b).

Table 2.2. Variable contributions for the common frog SDM in Kent. The percent contribution is a measure of the increase or decrease in the average log likelihood of presence records for the study area after each iteration of the model training. Permutation importance provides a measure of the drop in AUC calculated during the model training if the contribution of a variable is randomly distributed between the species presence and background pixels.

Variable	Percent contribution	Permutation of importance
Linear water features	21.9	28.3
Distance from broadleaved woodland	17.4	18.1
Pond density	14.4	7.7
Distance from arable land	13	5.4
Precipitation coldest quarter	8.7	1.1
Distance from semi-natural grassland	8.2	8.5
Minimum temperature coldest month	6.7	10.9
Maximum temperature warmest month	5	7.7
Distance from improved grassland	3.2	11.2
Temperature seasonality	1.4	1.2
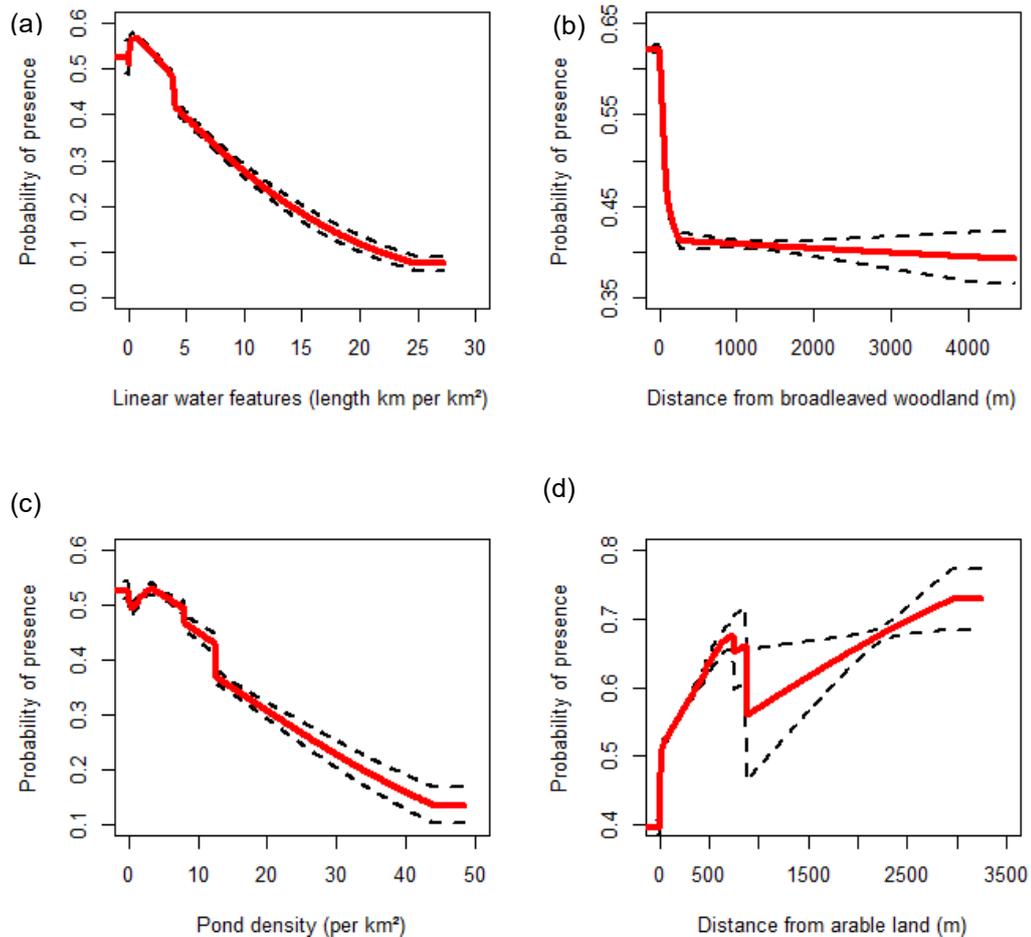
Figure 2.3. Response curves for the four variables that had the highest percentage contribution in the common frog SDM for Kent: (a) 'Linear water features' (b) 'Distance from broadleaved woodland' (c) 'Pond density' (d) 'Distance from arable land'. The red line is the mean response of the common frog probability of presence to the variable, calculated from five replicate runs. The black dashed line indicates the mean +/- one standard deviation.

The common frog SDM for Sussex had an AUCtest of 0.61 indicating that the model prediction is relatively poor. As in the Kent SDM, the percentage contribution of 'Distance from broadleaved woodland' and 'Pond density' indicated they were important to the model (Table 2.3). In the Sussex model the common frog probability of presence initially increases in response to the variable 'Pond density' (Figure 2.4c). This is the opposite to the response in the Kent model. However, the density of ponds is much higher in Kent than in Sussex as can be seen from the x axis of the 'Pond density' response curves (Figures 2.3c and 2.4c). Therefore, in Sussex there is less information for the model to use to determine common frog response to higher pond density leading to much more

variation (Figure 2.4c). 'Linear water features' in Sussex had similar permutation of importance as the Kent SDM but this was initially associated with an increase in common frog probability of presence. 'Minimum temperature coldest month' was a reasonably high contributor in Sussex. The response curve indicating common frogs prefer areas with higher temperatures in winter (Figure 2.4d).

Table 2.3. Variable contributions for the common frog SDM in Sussex.

Variable	Percentage contribution	Permutation of importance
Distance from broadleaved woodland	16.5	10.0
Distance from semi-natural grassland	14.4	5.0
Pond density	13.7	16.4
Minimum temperature coldest month	13.4	24.7
Linear water features	9.5	20.2
Maximum temperature warmest month	9.1	6.4
Temperature seasonality	7.5	9.9
Precipitation coldest quarter	7.4	0
Distance from arable land	7.2	5.2
Distance from improved grassland	1.3	2.2
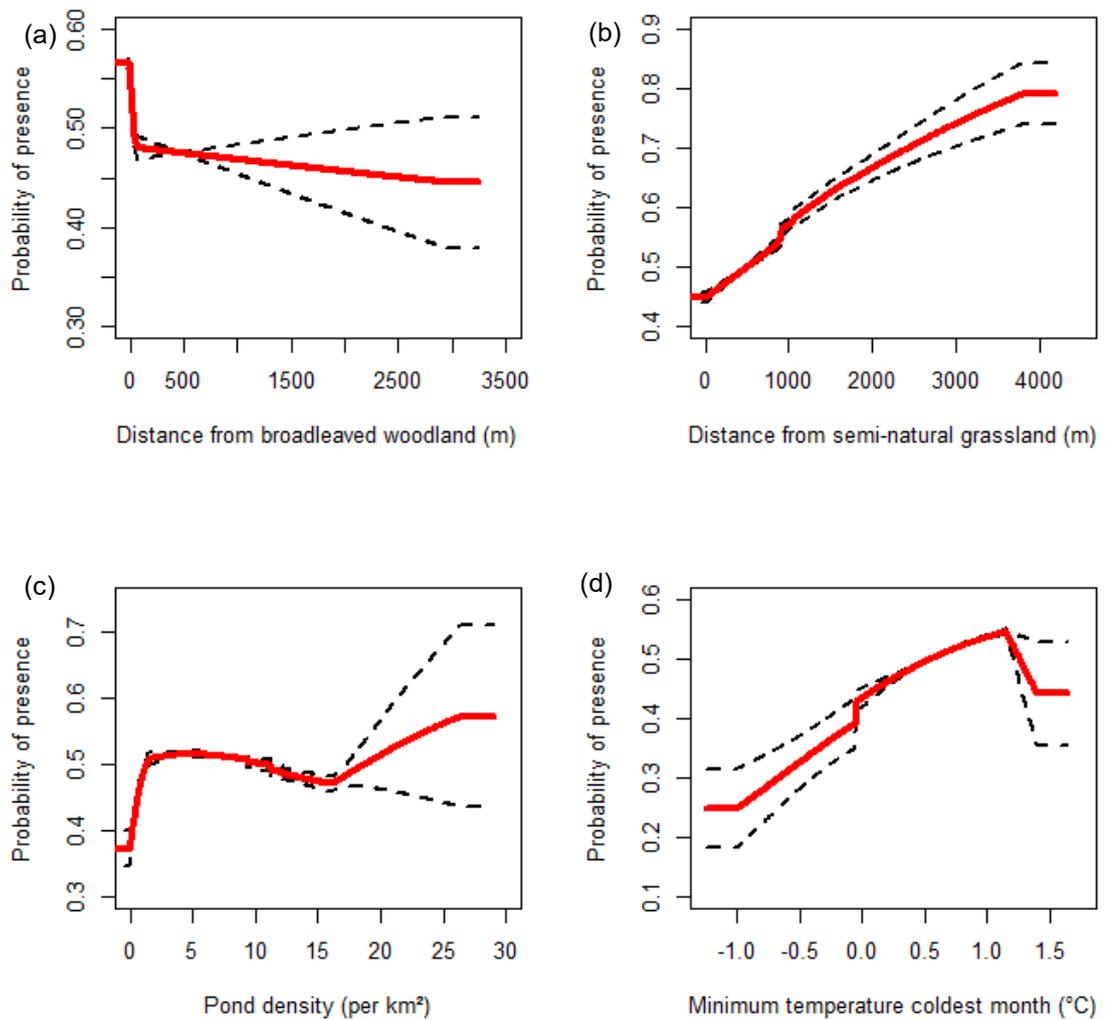
Figure 2.4. Response curves for the four variables that had the highest percentage contribution in the common frog SDM in Sussex; (a) 'Distance from broadleaved woodland' (b) 'Distance from semi-natural grassland' (c) 'Pond density' (d) 'Minimum temperature coldest month'. The red line is the mean response of the common frog probability of presence to the variable, calculated from five replicate runs. The black dashed line indicates the mean +/- one standard deviation.

For the Sussex SDM the variables 'Temperature seasonality' closely followed by 'Minimum temperature warmest month' contained the most information (Figure 2.5b). However, for both the Kent and Sussex models there was very little information that was not contained in the other variables (Figure 2.5).
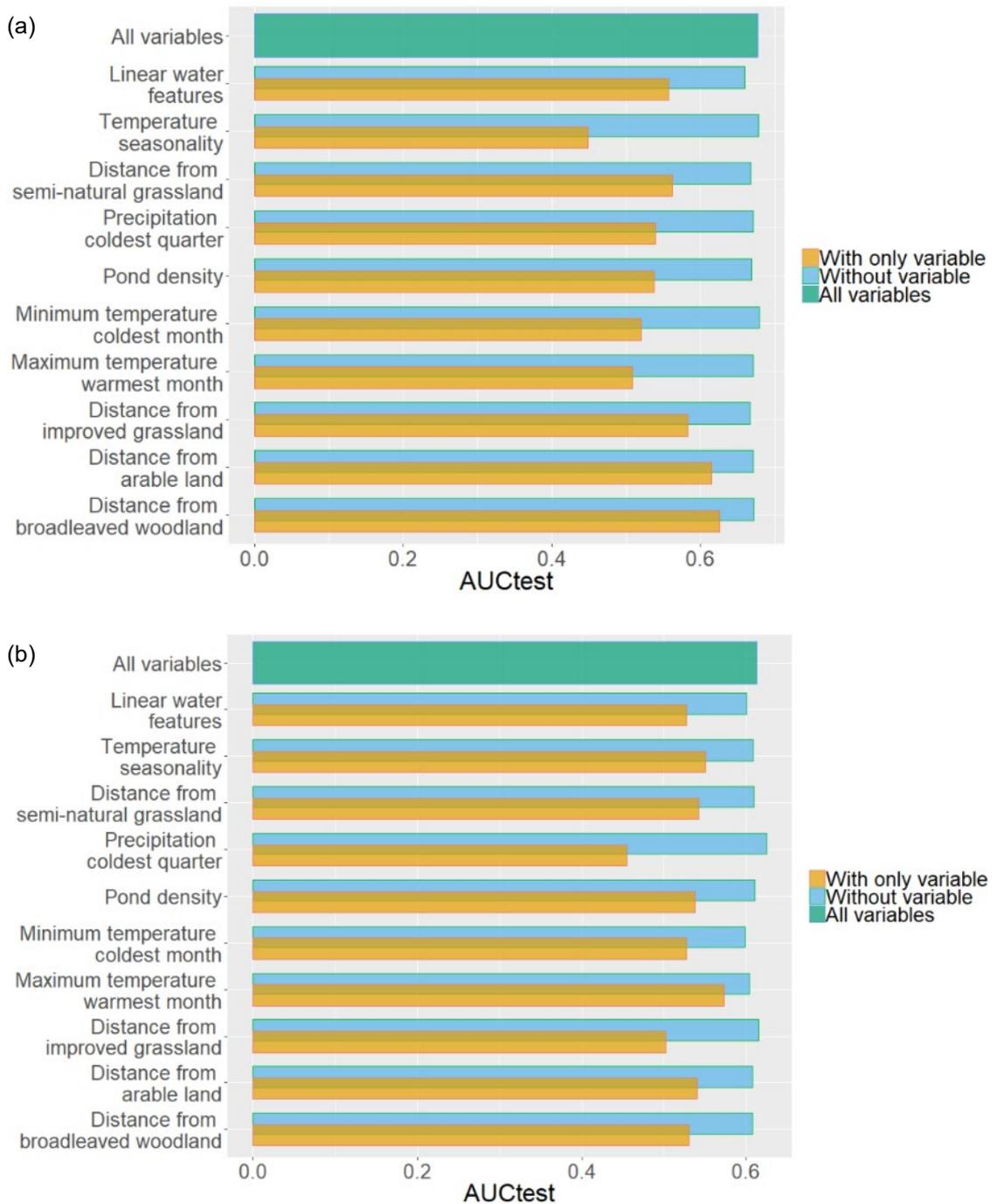
Figure 2.5. Jackknife tests of variable importance using AUCtest for the common frog SDMs in: (a) Kent and (b) Sussex. The orange bars indicate level of gain in AUCtest when a variable is used in isolation. The light blue bars indicate the amount of AUCtest lost when a variable is removed from the model. The AUCtest using all the variables in the model is shown by the green bar.

2.4.2. Marsh frog

The AUCtest scores of the marsh frog SDMs for Kent and Sussex were 0.91 and 0.92

respectively indicating the models performed well. 'Linear water features' was positively

associated with probability of presence in Kent (Figure 2.6a) and was also the most

influential variable (Table 2.4). In Kent marsh frog probability of presence tended to increase with Pond density (Figure 2.6b). In the Sussex SDM, 'Minimum temperature coldest month' and 'Linear water features' were the highest contributors to the model (Table 2.5). The response curves show that in the Sussex SDM 'Linear water features' was initially positively associated with presence of marsh frogs but at higher levels of linear water the probability of marsh frog presence declined (Figure 2.6c). Probability of presence of marsh frogs increased in response to 'Minimum temperature coldest month' after 0°C (Figure 2.6d). The jackknife tests indicated that 'Linear water features' provided the most information to the models when used without the other variables for both Kent and Sussex SDMs (Figures 2.7a, b).

Table 2.4. Variable contributions for the marsh frog SDM in Kent.

Variable	Percentage contribution	Permutation of importance
Linear water features	45.9	38.9
Distance from arable land	9.0	6.3
Minimum temperature coldest month	7.4	8.3
Maximum temperature warmest month	7.0	5.4
Distance from urban/suburban areas	6.7	7.3
Pond density	6.6	14.4
Distance from broadleaved woodland	6.1	5.5
Precipitation coldest quarter	5.1	5.8
Distance from minor road	1.9	2.6
Temperature seasonality	1.3	0.7
Distance from semi-natural grassland	1.1	2.3
Distance from improved grassland	1.1	1.4
Distance from major road	0.7	1.1

Table 2.5. Variable contributions for the marsh frog SDM in Sussex.

Variable	Percentage contribution	Permutation of importance
Minimum temperature coldest month	47.1	56.7
Linear water features	31.2	10.7
Precipitation coldest quarter	10.0	21.0
Distance from broadleaved woodland	2.7	0.9
Temperature seasonality	2.6	3.7
Distance from major road	1.6	0.3
Maximum temperature warmest month	1.2	3.0
Distance from semi-natural grassland	1.2	0.2
Distance from arable land	0.8	1.5
Pond density	0.7	0.1
Distance from improved grassland	0.6	1.6
Distance from urban/suburban areas	0.2	0.1
Distance from minor road	0.1	0.2
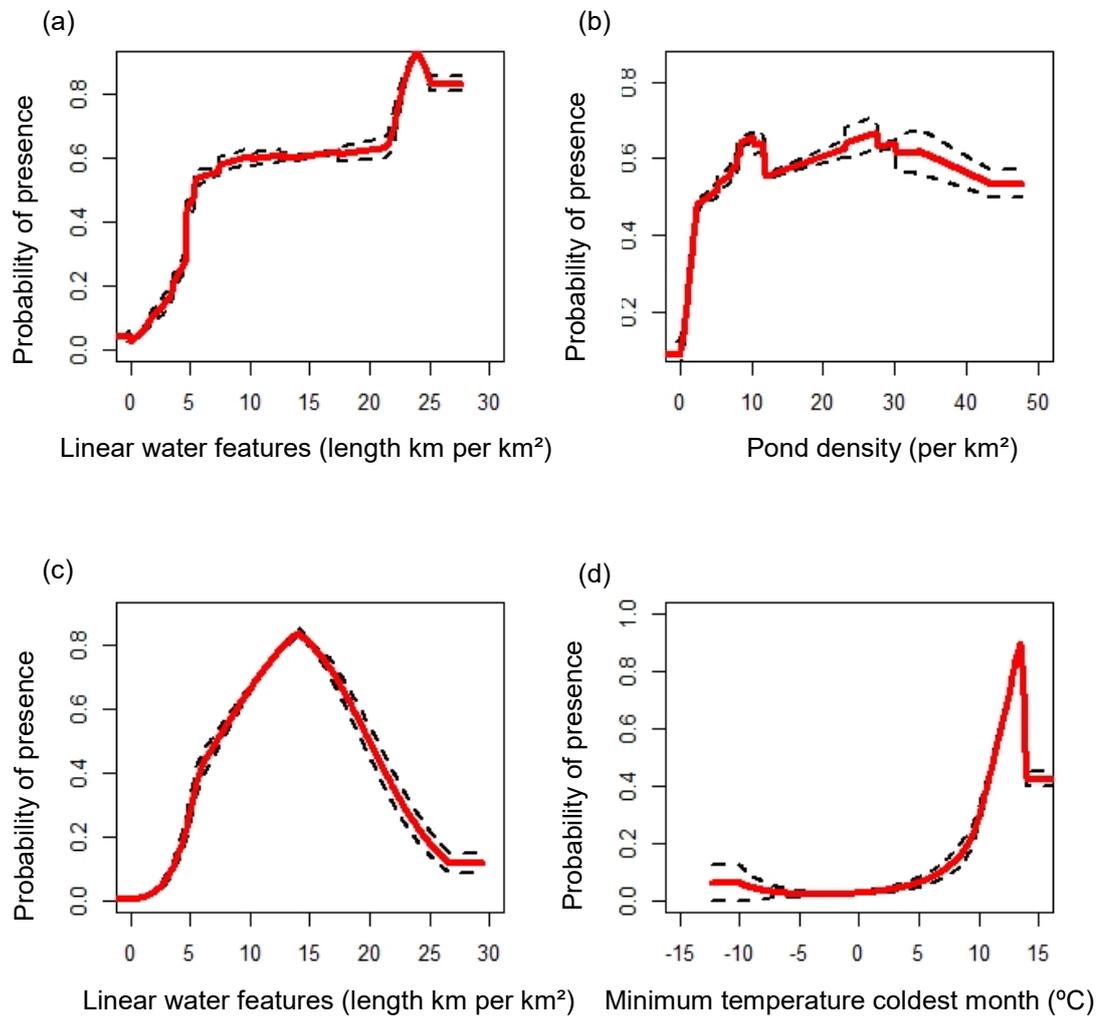
Figure 2.6. Response curves from the marsh frog SDMs in Kent and Sussex; (a) 'Linear water features' in the Kent SDM (b) 'Pond density' in Kent (c) 'Linear water features' in the Sussex SDM. (d) 'Minimum temperature coldest month' in Sussex. The red line is the mean response of the marsh frog probability of presence to the variable, calculated from five replicate runs. The black dashed line indicates the mean +/- one standard deviation.

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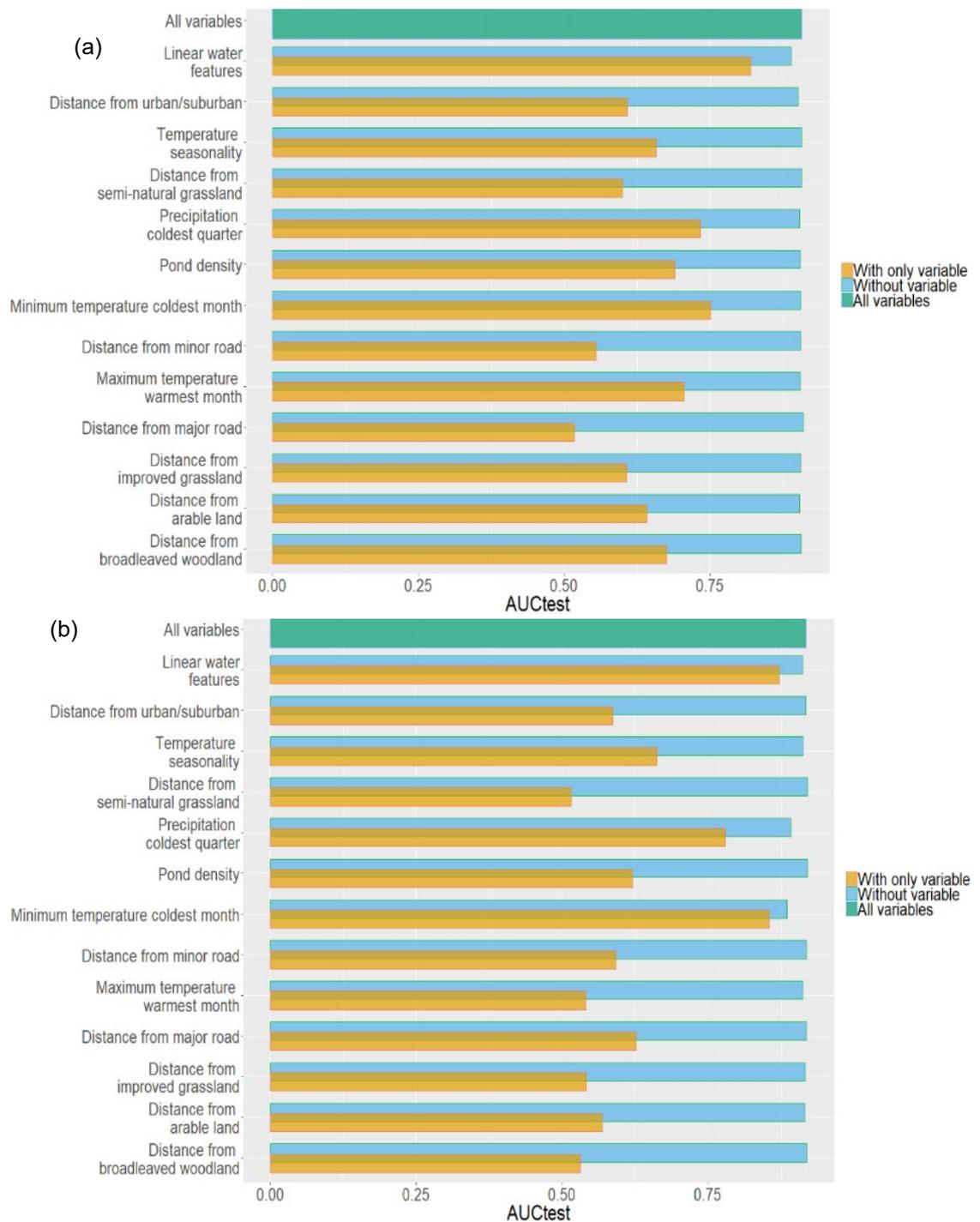
Figure 2.7. Jackknife tests of variable importance using AUCtest for the marsh frog SDMs in: (a) Kent and (b) Sussex. The orange bars indicate level of gain in AUCtest when a variable is used in isolation. The light blue bars indicate the amount of AUCtest lost when a variable is removed from the model. The AUCtest using all the variables in the model is shown by the green bar.

2.4.3. Great crested newt

'Pond density' was the most important variable to the great crested newt SDMs of Kent and Sussex and was positively associated with great crested newt probability of presence (Table 2.6 and 2.7; Figure 2.8a, b). The AUCtest score for the Kent model was

0.80 and for the Sussex model was 0.71 indicating the models predicted the great crested newt distributions well. Jackknife tests on the Kent great crested newt SDM showed only 'Pond density' and, to a lesser extent, 'Distance from urban/suburban areas' were providing information to the model not supplied by other variables (Figure 2.9). 'Linear water features' were much more important to the Kent SDM compared to Sussex (Table 2.6 and 2.7). The response curves showed that probability of presence of great crested newts decreased with 'Distance from urban/suburban areas' in both Kent and Sussex (Figure 2.8c and 2.8d). This may be an indication that sampling bias is still a problem because great crested newts are not usually associated with urban areas (Bormpoudakis et al. 2015; Hartel et al. 2010).
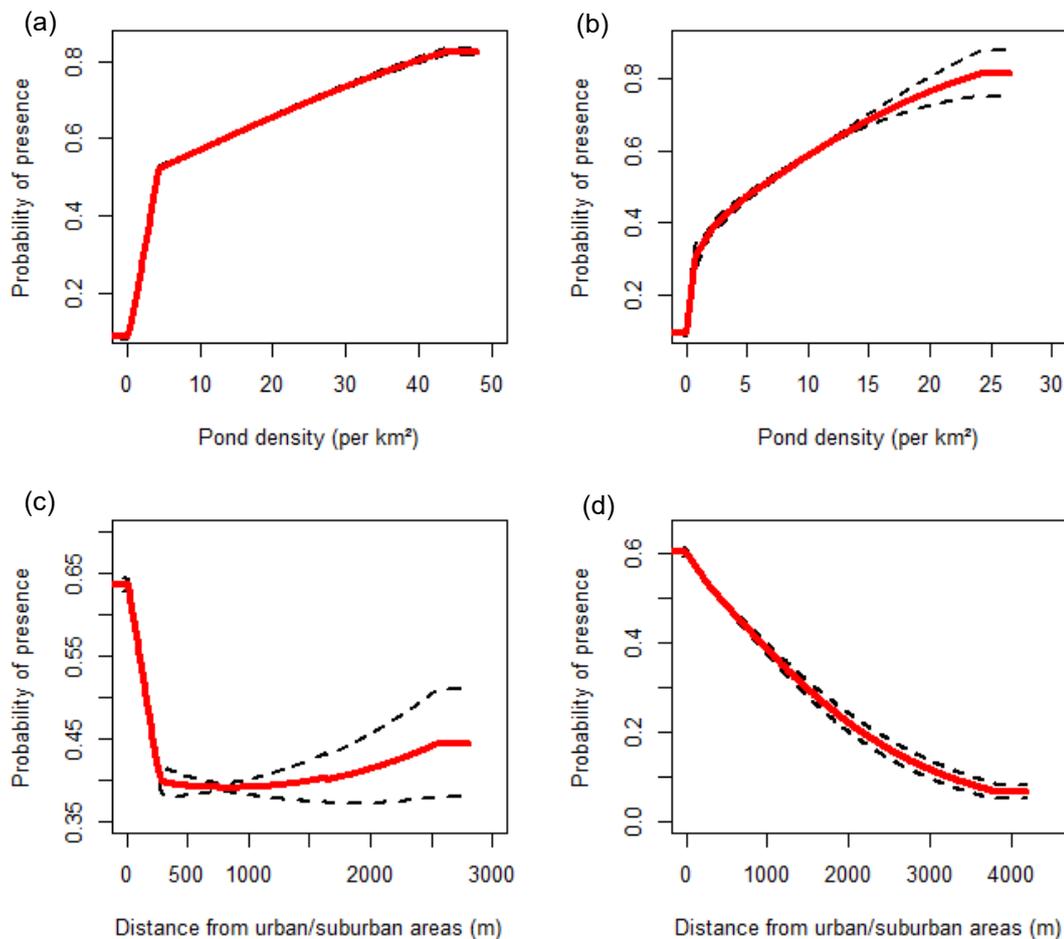
Figure 2.8. Response curves from the great crested newt SDMs in Kent and Sussex: (a) Kent Pond density; (b) Sussex Pond density; (c) Kent 'Distance from urban/suburban areas' (d) Sussex 'Distance from urban/suburban areas'. The red line is the mean response of the great crested newt probability of presence to the variable, calculated from five replicate runs. The black dashed line indicates the mean +/- one standard deviation.

Table 2.6. Variable contributions for the great crested newts SDM in Kent.

Variable	Percentage contribution	Permutation importance	of
Pond density	42.6	40.1	
Linear water features	17.6	11.8	
Distance from urban/suburban areas	16.1	19.1	
Distance from arable land	8.4	3.8	
Maximum temperature warmest month	3.2	1.5	
Distance from major road	3.2	6.3	
Minimum temperature coldest month	2.8	5.4	
Temperature seasonality	1.9	1.2	
Precipitation coldest quarter	1.7	4.4	
Distance from broadleaved woodland	1	2.7	
Distance from minor road	0.8	1.8	
Distance from semi-natural grassland	0.5	1.1	
Distance from improved grassland	0.2	0.5	

Table 2.7. Variable contributions for the great crested newts SDM in Sussex.

Variable	Percentage contribution	Permutation of importance
Pond density	43.1	23.2
Distance from urban/suburban areas	21.3	24.9
Precipitation coldest quarter	10.1	10.8
Distance from major road	5.1	10.5
Temperature seasonality	4.5	0.6
Distance from minor road	4.2	10.1
Maximum temperature warmest month	2.8	0.1
Minimum temperature coldest month	2.2	2.9
Distance from improved grassland	1.9	9
Linear water features	1.6	2.8
Distance from semi-natural grassland	1.2	0.9
Distance from arable land	1	3
Distance from broadleaved woodland	0.8	1.1

2.4.4. Grass snake

The Kent grass snake SDM had an AUCtest of 0.67 again indicating that this is a relatively poor performing model. The four most influential variables were 'Distance from arable land', 'Minimum temperature coldest month', 'Distance from broadleaved woodland', and 'Distance from semi-natural grassland' (Table 2.8). The probability of presence increased with 'Distance from arable land' and decreased with 'Distance from broadleaved woodland' and 'Distance from semi-natural grassland' (Figure 2.10). There is a rapid increase in the probability of presence of grass snakes at a temperature of 1.4°C which peaks at just above 1.6°C for 'Minimum temperature coldest month' (Figure 2.10b).

'Linear water features' was the variable that contributed the most to the Sussex grass snake SDM. The response to 'Linear water features' was positive and similar to the response in Kent. However, the response to 'Distance from broadleaved woodland' differed but only after approximately 500 m.

Table 2.8. Variable contributions for the grass snake SDM in Kent.

Variable	Percentage contribution	Permutation of importance
Distance from arable land	36.7	22.4
Minimum temperature coldest month	16	7.5
Distance from broadleaved woodland	15.5	24.7
Distance from semi-natural grassland	9.3	8.7
Linear water features	7.1	11.8
Precipitation coldest quarter	5.1	0
Pond density	4.1	4.6
Temperature seasonality	3.2	7.5
Distance from improved grassland	1.6	9.3
Maximum temperature warmest month	1.4	3.5
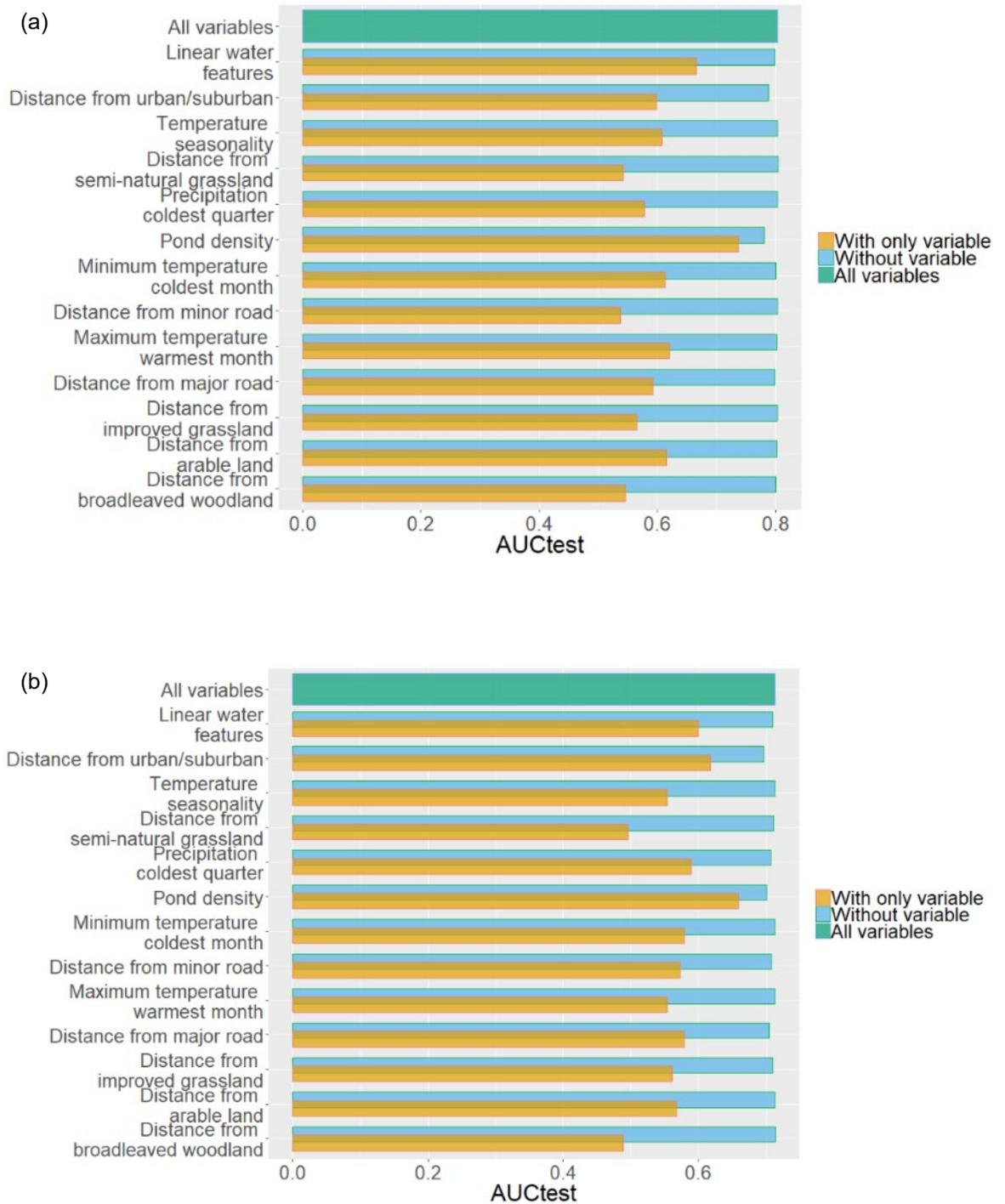
Figure 2.9. Jackknife tests of variable importance using AUCtest for the great crested newt SDMs in: (a) Kent and (b) Sussex. The light blue bars indicate the amount of AUCtest lost when a variable is removed from the model. The AUCtest using all the variables in the model is shown by the green bar.

Table 2.9. Variable contributions for the grass snake SDM in Sussex.

Variable	Percentage contribution	Permutation importance	of
Linear water features	30.6	24.8	
Distance from broadleaved woodland	15.8	5.4	
Distance from improved grassland	10.6	15	
Distance from arable land	9.5	18.8	
Maximum temperature warmest month	9.3	7.3	
Distance from semi-natural grassland	7.5	8	
Minimum temperature coldest month	7.1	5.3	
Pond density	6.2	7.1	
Precipitation coldest quarter	2	0	
Temperature seasonality	1.5	8.2	
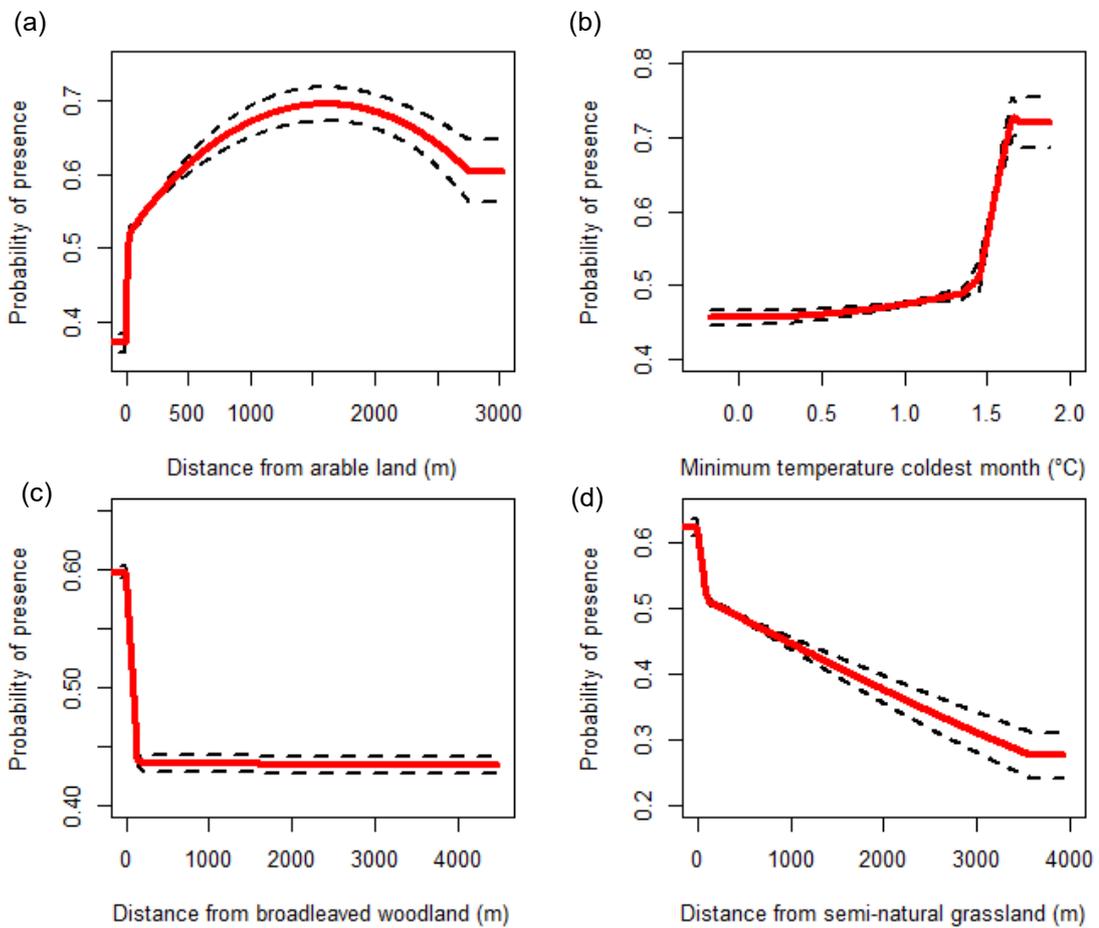
Figure 2.10. Response curves for the four variables that had the highest percentage contribution in the grass snake SDMs in Kent: (a) 'Distance from arable land'; (b) 'Minimum temperature coldest month'; (c) 'Distance from broadleaved woodland'; (d) 'Distance from semi-natural grassland'. The red line is the mean response of grass snake probability of presence to the variable, calculated from five replicate runs. The black dashed line indicates the mean +/- one standard deviation.

2.4.5. Results of comparing species distributions in Kent and Sussex

The distribution maps showed that in Kent, the distributions of common frogs and marsh frogs appear negatively associated. The areas with a higher predicted probability of presence of marsh frogs had a lower predicted probability of presence of common frogs (Figure 2.11a, b), particularly in coastal regions. The areas where common frog and marsh frog distributions overlap using the 10%TP threshold was relatively small and tended to be away from the areas with highest pond density (Figure 2.11d).

A much higher proportion of the common frog predicted distribution overlaps the marsh frog distribution in Sussex compared to Kent (Table 2.10). The results of a chi-square test of independence, using number of 25 m² cells predicted to be occupied by common frogs using the 10% TP, showed this to be significant, X^2 (df = 1, N = 7,436,486) = 223,480, p < 0.00. Unlike in Kent, the predicted distributions of common frogs and marsh frogs in Sussex did not appear to be negatively associated (Figure 2.12a and 2.12c).

The proportion of the predicted common frog distribution which overlapped the great crested newt distribution was also significantly higher in Sussex compared to Kent, X^2 (df = 1, N = 7,436,486) = 600,250, p < 0.00 (Table 2.10). Again, the areas where the common frog and great crested newt distribution overlapped were situated away from the larger areas of high pond density in Kent (Figure 2.11). In areas of higher pond density common frog and great crested newt predicted presence were still largely overlapping in Sussex (Figure 2.12b). Sussex also has a lower maximum pond density and many of the areas of higher pond density are smaller than in Kent (Figure 2.11d, 2.11e, 2.12d, 2.12e).

Table 2.10. The size of the predicted distribution of common frogs, marsh frogs, and great crested newts from the Kent and Sussex SDMs. A threshold at the 10th percentile training presence (10%TP) was used to determine presence or absence of each species. The proportion of the common frog distribution that overlaps with the marsh frog and great crested newt distributions in Kent and Sussex is given in the last two columns.

County	Area of common frog predicted distribution (km²)	Area of marsh frog predicted distribution (km²)	Area of great crested newt predicted distribution (km²)	Proportion of common frog distribution overlapping marsh frog distribution	Proportion of common frog distribution overlapping great crested newt distribution
Kent	2129	969	1596	0.072	0.39
Sussex	2519	663	2244	0.19	0.67
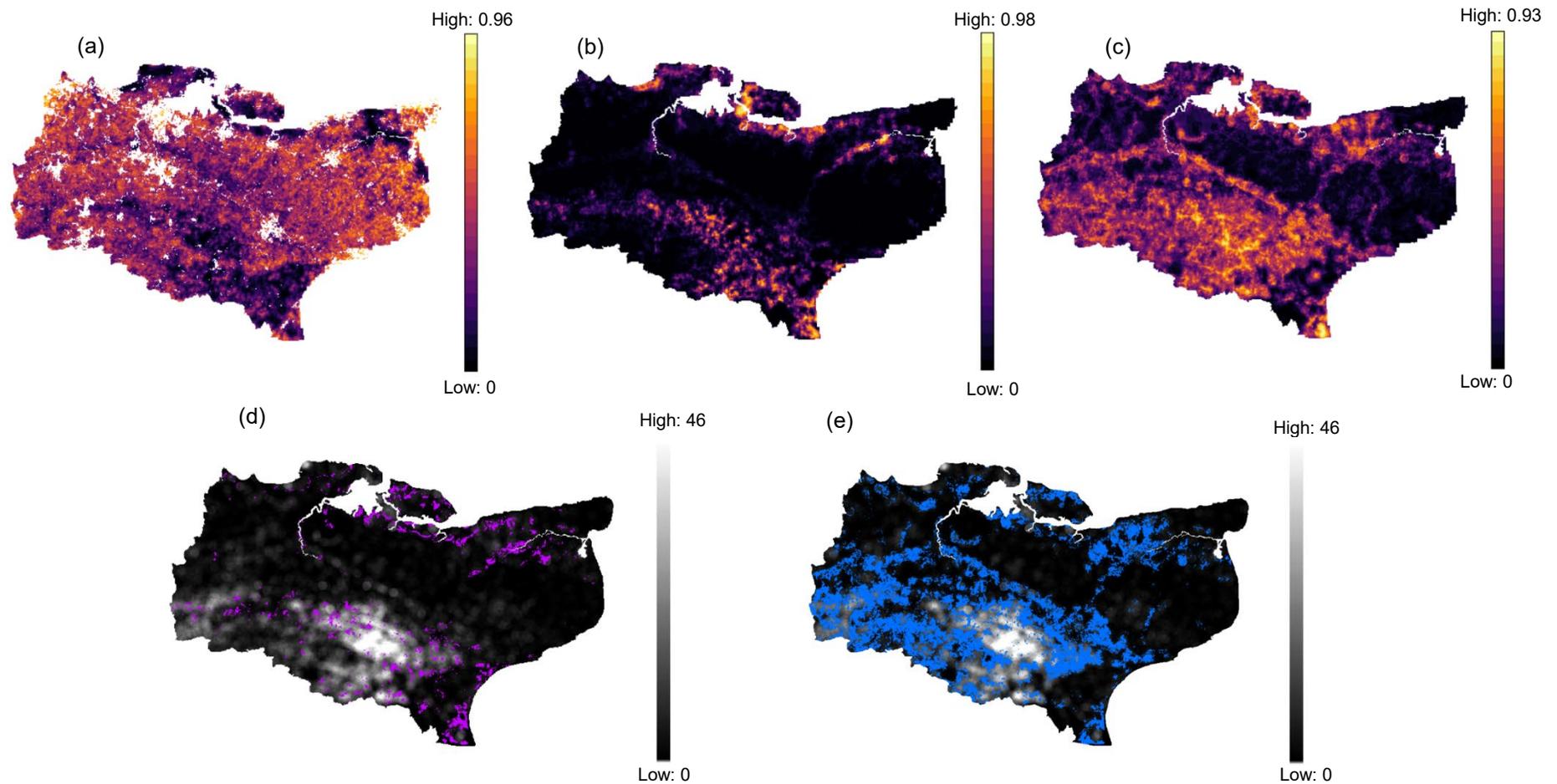
Figure 2.11. Species distribution maps and pond density maps for Kent: (a) common frog predicted distribution; (b) marsh frog predicted distribution; (c) great crested newt predicted distribution; (d) pond density map showing areas where common frog and marsh frog predicted distributions overlap (purple); (e) pond density map of Kent showing areas where common frog and great crested newt predicted distributions overlap (blue). Lighter greyscale shading indicates higher pond density. Species distributions were determined using a 10% training presence threshold. The SDMs were generated using Maxent's logistic output. Warmer colours indicated a higher probability of presence.
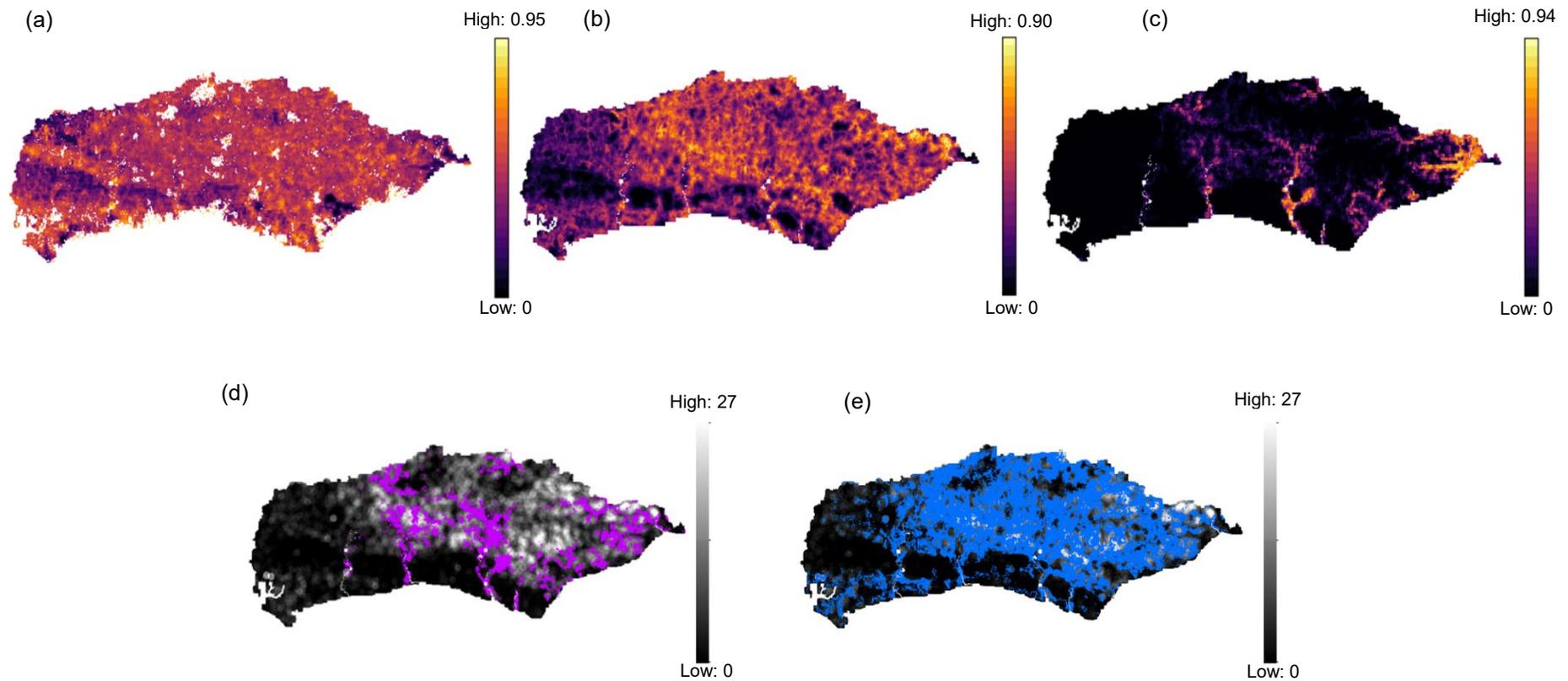
Figure 2.12. Species distribution maps and pond density maps for Sussex: (a) common frog predicted distribution; (b) great crested newt predicted distribution (c) marsh frog predicted distribution; (d) pond density map showing areas where common frog and marsh frog predicted distributions overlap (purple); (e) pond density map of Sussex showing areas where common frog and great crested newt predicted distributions overlap (blue). Lighter greyscale shading indicates higher pond density. The presence/absence species distributions were determined for each species using a 10% training presence threshold. The SDMs were generated using Maxent's logistic output. Warmer colours indicated a higher probability of presence.

2.5. Discussion

The pattern in predicted distributions of common frogs and marsh frogs may be reflecting these species different responses to habitat rather than a negative interaction between them. The areas predicted by the SDMs as suitable for marsh frogs and unsuitable for common frogs are related to the positive and negative associations of these species respectively with 'Linear water features'. The distribution of marsh frogs was largely predicted by 'Linear water features' (Table 2.4 and 2.5). This reflects previous research that has shown marsh frogs are highly aquatic and spread along watercourses, not moving far from water very often (Kovar et al. 2009; Holenweg 2001; Beebee and Griffiths 2000; Wycherley and Joslin 1996). The areas with most 'Linear water features' are predominantly coastal marsh dyke habitats. Marsh frogs are abundant in some of these areas, and may be better adapted to them than common frogs because they are more tolerant of higher levels of salinity (Beebee and Griffiths 2000; Innocenzi 1995; Beebee 1980). Livestock grazing in marshland areas may also reduce its suitability as habitat for the native amphibians, which spend more of the time foraging on land than marsh frogs (Beebee 1980).

Common frogs are negatively associated with areas of high pond density in Kent (Figure 2.3c and 2.10a). A study in Luxembourg also found the same trend with still and running water variables after using principle component analysis (Wood 2010). The SDMs of both Kent and Sussex are consistent with other studies in finding great crested newts are positively associated with pond density (Bormpoudakis et al. 2015; Oldham et al. 2000). A significantly higher proportion of the common frog distribution overlaps the great crested newt distribution in Sussex compared to Kent, and Kent has larger areas with high pond density than Sussex. This evidence provides support for the hypothesis that a higher abundance of great crested newts is linked to lower numbers of common frogs in areas characterised by high pond density.

The Sussex common frog response curve shows a different response to pond density compared to Kent (Figure 2.4c). This could be due to Sussex having lower pond densities than Kent, and the areas of higher pond density in Sussex being much smaller in area of extent (Figure 2.12d). Smaller areas of high pond density may not be able to sustain a great crested newt population large enough to impact common frog numbers. At very high pond densities great crested newts could occupy ponds less suitable for their needs, such as those with higher fish presence (Brady 2009). As a result, more predation on common frogs may be occurring.

Marsh frogs are also positively associated with high pond density in Kent (Figure 2.6b). Therefore, marsh frogs may still be one of the causes of the reduced probability of common frog being present in high pond density areas. The lack of marsh frogs in many of the higher pond density areas in Sussex could be the reason that these areas are more suitable for common frogs. However, it could be that any impact of marsh frogs on common frogs is more likely to be caused in combination with predation by great crested newts. Further investigations into the pond preferences of each species at a local level may provide additional insights. In particular, identifying whether common frogs are using ponds occupied by fish in high pond density areas would be worth exploring.

'Distance from broadleaved woodland' was negatively associated with common frog presence in Kent and Sussex. Other studies have also found a positive association between woodland habitat and common frog presence (Boissinot et al. 2015; Van Buskirk 2005). This could be because common frog spawn may be less subject to predation by waterfowl in woodland where it is less easily found. Woodland areas may also aid movement between ponds (Van Buskirk 2005). However, woodland is relatively sparse in the area of high pond density in central Kent. This may be another reason for the reduced predicted probability of common frog presence in this region. It is worth noting that the Sussex and Kent common frog SDMs have quite a low AUCtest values.

This may be due to common frogs being habitat generalists with no strong distribution predictors at the spatial scale examined by the models (Franklin et al. 2009).

In the grass snake SDM in Kent, the response curves for 'Distance from broadleaved woodland', 'Linear water', and 'Distance from arable land' are similar to those produced by the common frog SDM for the county (Figures 2.3 and 2.10). The grass snake distribution could reflect the distribution of one of its frequent prey items the common frog. Distance to grass snake prey can be a good predictor of grass snake distribution (Ward 2017) and may be the reason for the positive association with 'Distance from broadleaved woodland'. Large populations of grass snakes have been found around marsh frog populations (Gregory and Isaac 2004), which would explain the positive association with 'Linear water features'. However, there is no clear evidence to support or reject the hypothesis that grass snake numbers are inflated in areas dominated by marsh frogs, with individuals subsequently dispersing into common frog areas.

The results of the predicted species distributions in this study are correlative and not sufficient to prove or disprove that marsh frogs or great crested newts are affecting common frogs. Field studies are required to confirm whether a predicted lower probability of presence of common frogs in high pond density areas in Kent is a real phenomenon. It will also be necessary to test whether the presence of great crested newts and/or marsh frogs are significantly negatively associated with the presence of common frogs in ponds. However, this type of correlative study can be used to focus research on the hypotheses that are most likely to prove correct (Anderson, Peterson and Gómez-Laverde 2002). With the often limited resources available for conservation research this is particularly useful.

2.6. Supplementary information

Table S2.1. Spearman's rho correlation coefficients between explanatory variables in Kent

Variable Name	Linear water features	Distance from major road	Distance from minor road	Pond density	Distance from urban/ suburban areas	Distance from arable land	Distance from improved grassland	Distance from semi-natural grassland	Distance from broadleaved woodland	Temperature seasonality	Maximum temperature warmest month	Minimum temperature coldest month	Precipitation coldest quarter
Linear water features	1	0.07	0.26	0.51	0.16	-0.05	-0.08	-0.09	0.24	-0.21	0.35	0.48	0.05
Distance from major road	0.07	1	-0.02	0	0.46	-0.11	-0.01	0.01	0.02	-0.02	-0.17	0.03	0.06
Distance from minor road	0.26	-0.02	1	0.05	0.2	0.03	0.09	0.02	0.06	-0.04	0.11	0.11	-0.01
Pond density	0.51	0	0.05	1	0.09	0.07	-0.29	-0.16	-0.12	-0.03	0.33	0	0.42
Distance from urban/ suburban areas	0.16	0.46	0.2	0.09	1	-0.21	-0.02	-0.06	0.02	-0.07	-0.07	0.01	0.24
Distance from arable land	-0.05	-0.11	0.03	0.07	-0.21	1	-0.34	-0.01	-0.26	0.11	0.11	-0.15	0.07
Distance from improved grassland	-0.08	-0.01	0.09	-0.29	-0.02	-0.34	1	0.14	0.15	-0.06	-0.14	0.14	-0.16
Distance from semi-natural grassland	-0.09	0.01	0.02	-0.16	-0.06	-0.01	0.14	1	0.08	0.04	0.08	-0.02	-0.09
Distance from broadleaved woodland	0.24	0.02	0.06	-0.12	0.02	-0.26	0.15	0.08	1	-0.13	0.03	0.37	-0.25
Temperature seasonality	-0.21	-0.02	-0.04	-0.03	-0.07	0.11	-0.06	0.04	-0.13	1	0.37	-0.7	0.15
Maximum temperature warmest month	0.35	-0.17	0.11	0.33	-0.07	0.11	-0.14	0.08	0.03	0.37	1	-0.18	0.08
Minimum temperature coldest month	0.48	0.03	0.11	0	0.01	-0.15	0.14	-0.02	0.37	-0.7	-0.18	1	-0.42
Precipitation coldest quarter	0.05	0.06	-0.01	0.42	0.24	0.07	-0.16	-0.09	-0.25	0.15	0.08	-0.42	1

Table S2.2. Spearman's rho correlation coefficients between explanatory variables in Sussex

Variable Name	Linear water features	Distance from major road	Distance from minor road	Pond density	Distance from urban/ suburban areas	Distance from arable land	Distance from improved grassland	Distance from semi- natural grassland	Distance from broadleaved woodland	Temperature seasonality	Maximum temperature warmest month	Minimum temperature coldest month	Precipitation coldest quarter
Linear water features	1	0.02	0.03	0.46	0.01	-0.13	-0.12	-0.09	0.09	-0.17	0.38	0.51	-0.22
Distance from major road	0.02	1	0	-0.05	0.43	-0.01	-0.05	-0.09	-0.09	0.11	-0.08	-0.1	0.11
Distance from minor road	0.03	0	1	-0.13	0.21	-0.01	0.05	0.04	0.02	-0.01	-0.03	-0.01	-0.03
Pond density	0.46	-0.05	-0.13	1	0.03	-0.05	-0.26	-0.1	-0.15	-0.2	0.04	0.37	-0.33
Distance from urban/ suburban areas	0.01	0.43	0.21	0.03	1	-0.1	-0.14	-0.1	-0.1	0.06	-0.16	-0.03	0.03
Distance from arable land	-0.13	-0.01	-0.01	-0.05	-0.1	1	-0.21	0.04	-0.2	-0.06	-0.2	-0.03	-0.03
Distance from improved grassland	-0.12	-0.05	0.05	-0.26	-0.14	-0.21	1	0.09	-0.04	0.15	0.05	-0.1	0.2
Distance from semi-natural grassland	-0.09	-0.09	0.04	-0.1	-0.1	0.04	0.09	1	0.11	-0.1	-0.02	0.07	-0.08
Distance from broadleaved woodland	0.09	-0.09	0.02	-0.15	-0.1	-0.2	-0.04	0.11	1	-0.24	0.22	0.28	-0.02
Temperature seasonality	-0.17	0.11	-0.01	-0.2	0.06	-0.06	0.15	-0.1	-0.24	1	0.05	-0.51	0.47
Maximum temperature warmest month	0.38	-0.08	-0.03	0.04	-0.16	-0.2	0.05	-0.02	0.22	0.05	1	0.12	-0.13
Minimum temperature coldest month	0.51	-0.1	-0.01	0.37	-0.03	-0.03	-0.1	0.07	0.28	-0.51	0.12	1	-0.09
Precipitation coldest quarter	-0.22	0.11	-0.03	-0.33	0.03	-0.03	0.2	-0.08	-0.02	0.47	-0.13	-0.09	1

2.6.1. Model tuning methods

The AUCdiff and AUCtest results for common frogs, marsh frogs, great crested newts, and grass snakes using the subsample (SS) and cross-validation (CV) methods were plotted over a range of regularisation values and using either a bias file or spatial filtering (Figures S2.1-13). If the results were close the lower regularisation value was selected to avoid underfitting (Radosavljevic and Anderson 2014).

2.6.2. Model tuning results

The general trend over the different regularisation values was similar for the SS and CV methods. For most of the models the SS results, over different regularisation levels, were more erratic compared to CV methods for both AUCdiff and AUCtest scores. In the CV method, each species record occurs in at least one testing set. This ensures that the model can predict all environments where there are species records. The simple splitting into test and training data by the SS method can mean that the training data is missing key atypical records (Peterson et al. 2011). The slightly more erratic results from the SS method may be due to this.

Table S2.3. Model specifications for the models selected to be in the main analysis

Species	No. records	County	Regularisation	Bias method	Testing method	AUC diff	AUC test
Common frog	549	Kent	2	Bias file	CV	0.02	0.67
Common frog	547	Sussex	2	Bias file	CV	0.04	0.61
Great crested newt	435	Kent	2	Spatial thinning (250 m)	CV	0.02	0.80
Great crested newt	402	Sussex	4	Spatial thinning (250 m)	CV	0.03	0.71
Marsh frog	154	Kent	1	None	CV	0.02	0.88
Marsh frog	72	Sussex	1	None	CV	0.03	0.87
Grass snake	759	Kent	4	Bias file	CV	0.03	0.65
Grass snake	604	Sussex	2	Bias file	CV	0.03	0.66

Common frog results

For the Kent SDMs the bias file models produced lower AUCdiff results, but the spatially filtered models generally had higher AUCtest values (Figure S2.1 and S2.2). The spatial filtering may not have reduced the spatial autocorrelation in the training data. This can lead to higher AUCtest results and would decrease the ability of the models to predict the test data causing the higher AUCdiff values (Radosavljevic and Anderson 2014; Hijmans 2012). Therefore, the bias file model was used with the CV method for the analysis. A regularisation level of 2 was used which achieved a low AUCdiff of 0.022 and high AUCtest of 0.67 (Figure S2.1 and S2.2; Table S2.3).

The Sussex SDM models with data spatially thinned by 500 m using a regularisation level of 2 produced the lowest AUCdiff and highest AUCtest (Figures S2.3 and S2.4). The model was heavily influenced by 'Temperature seasonality' (percent contribution = 38.9 and permutation contribution = 38.7) which is clearly visible in the distribution map (Figure S2.5). Common frogs are found in a wide variety of habitats (Beebee and Griffiths 2000), this may be the cause of the relatively low AUCtest because it is harder for a

model to discriminate between suitable and unsuitable habitat (Proosdij et al. 2016; Franklin et al. 2009). However, under this scenario just having one dominant environmental predictor such as 'Temperature seasonality' is less likely. The variation in 'Temperature seasonality' is also low across Sussex so there is not a strong environmental pressure to cause this sort of distribution. For these reasons, the bias file model using a regularisation value of 2 with the CV method was chosen for the main analysis. This model had an AUCdiff of 0.037 and an AUCtest of 0.61 (Table S2.3).
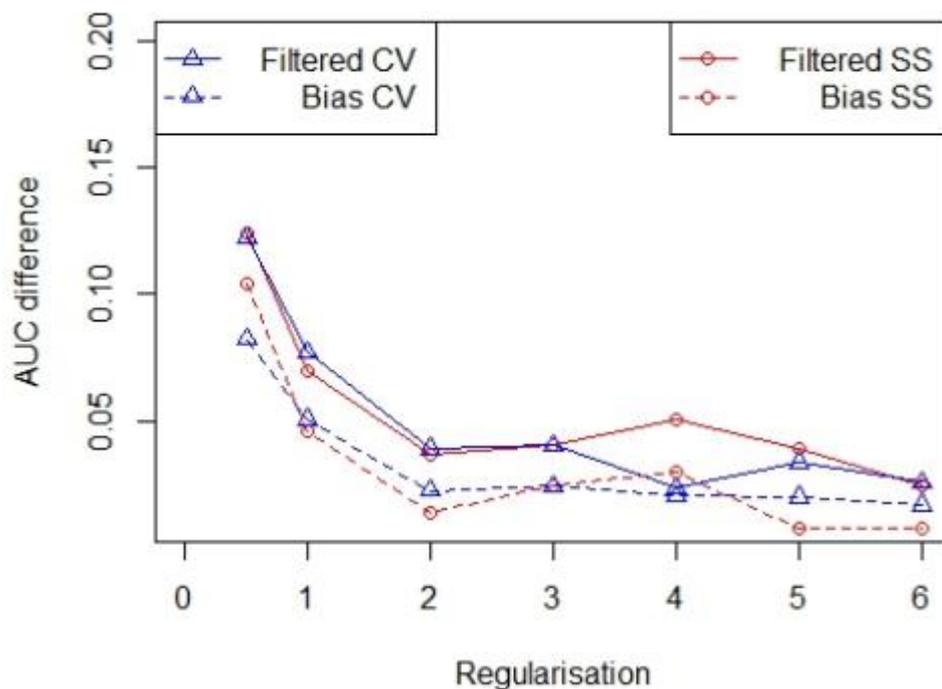
Figure S2.1. Results of the AUCdiff for the Kent common frog SDM. Comparing models over regularisation levels 0.5 to 6 using a bias file (Bias) or spatial filtering of data at 500 m (Filtered). Each model was run using the subsample testing method (SS) or the cross-validation testing method (CV).
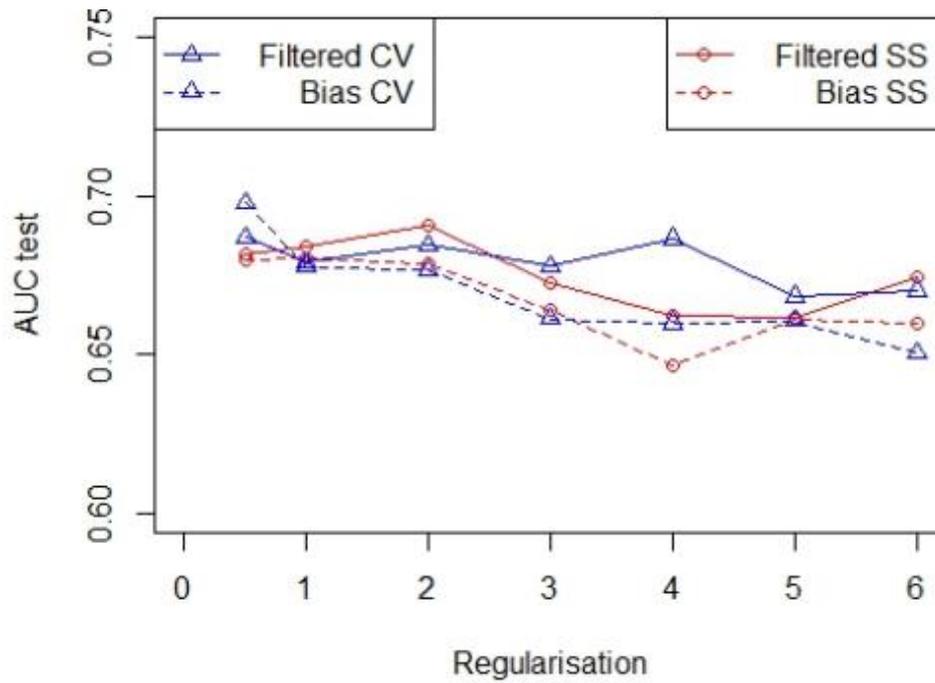
Figure S2.2. Results of the AUCtest for the Kent common frog SDM comparing models over regularisation levels 0.5 to 6 using a bias file (Bias) or spatial filtering of data at 500 m (Filtered). Each model was run using the subsample testing method (SS) or the cross-validation testing method (CV).
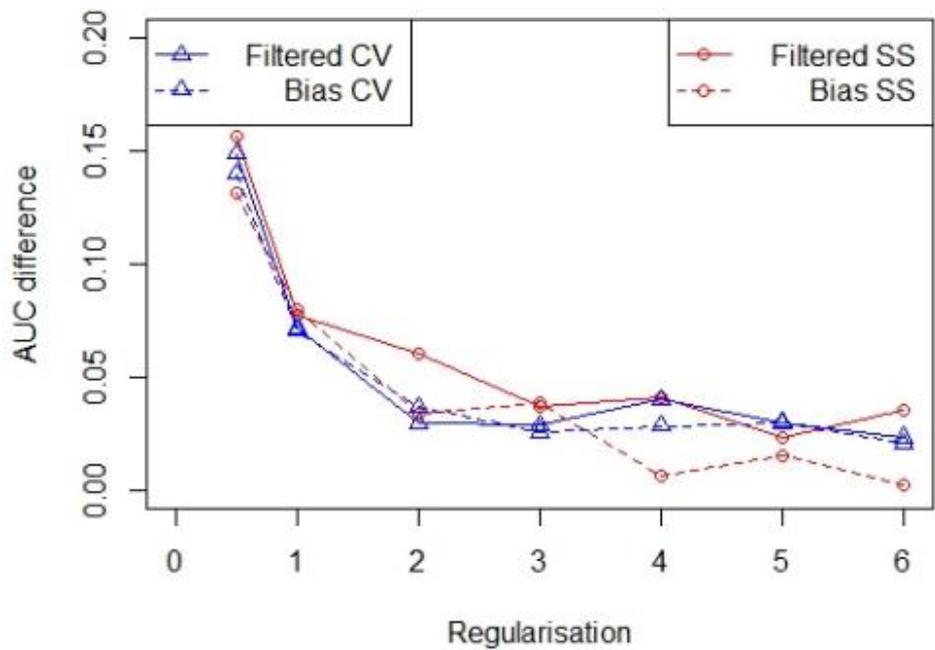
Figure S2.3. Results of the AUCdiff for the Sussex common frog SDM comparing models over regularisation levels 0.5 to 6 using a bias file (Bias) or spatial filtering of data at 500 m (Filtered). Each model was run using the subsample testing method (SS) or the cross-validation testing method (CV).
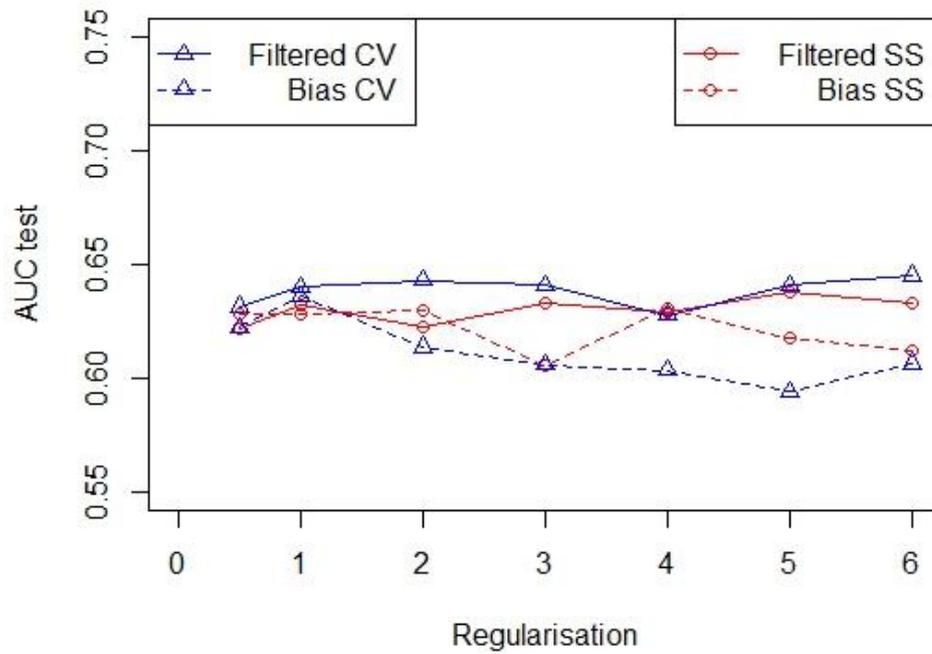
Figure S2.4. Results of the AUCtest for the Sussex common frog SDM comparing models over regularisation levels 0.5 to 6 using a bias file (Bias) or spatial filtering of data at 500 m (Filtered). Each model was run using the subsample testing method (SS) or the cross-validation testing method (CV).
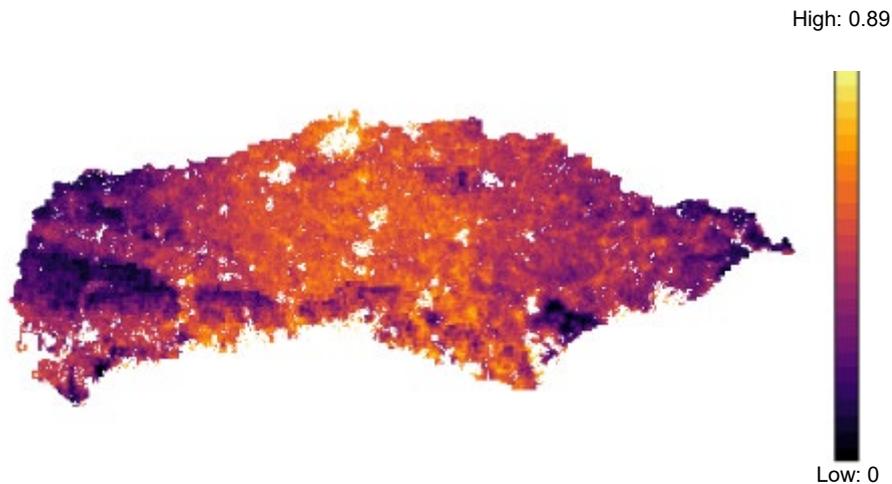
Figure S2.5. Common frog species distribution map of Sussex using spatial thinning at 500 m and a regularisation level of 2 using Maxent's logistic output. Warmer colours indicated a higher probability of presence. There is a high suitability for common frogs in the centre of Sussex which reflects the over reliance on the variable 'Temperature seasonality' and so this model was not used in the main analysis.

Great crested newt results

In the Kent models a regularisation value of 2, spatial filtering the data, and using the CV validation method provided a reasonably high AUCtest score of 0.80 (Figure S2.6; Table S2.3). There was not much loss of generality in prediction, indicated by the relatively low AUCdiff of 0.021 (Figure S2.7, Table S2.3).

The AUCdiff and AUCtest for the Sussex SDMs were all fairly similar. The spatially thinned model at a regularisation level of 4 using the CV method was the model used in the main analysis. This model used the variable 'Distance from urban/suburban areas' less than others with similar AUCtest and AUCdiff results (Figures S2.8 and S2.9). Great crested newts are not known to be positively associated with urban areas, so a high use of this variable is likely to be due to survey bias. This model produced an AUCdiff of 0.026 and AUCtest of 0.71 (Table S2.3).
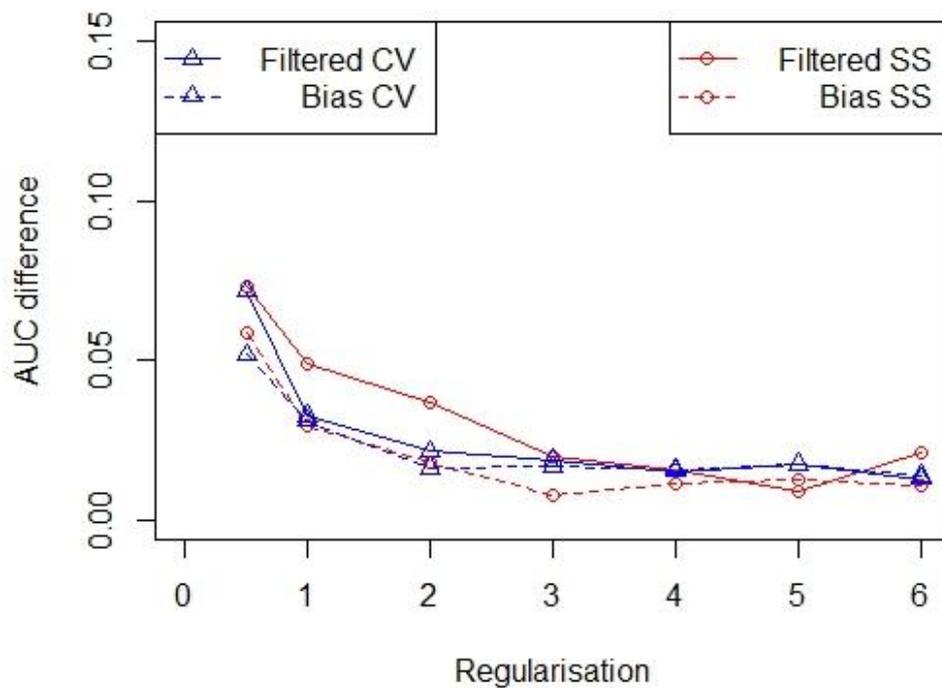
Figure S2.6. Results of the AUCdiff for the great crested newt SDM in Kent comparing models over regularisation levels 0.5 to 6 using a bias file (Bias) or spatial filtering of data at 250 m (Filtered). Each model was run using the subsample testing method (SS) or the cross-validation testing method (CV).
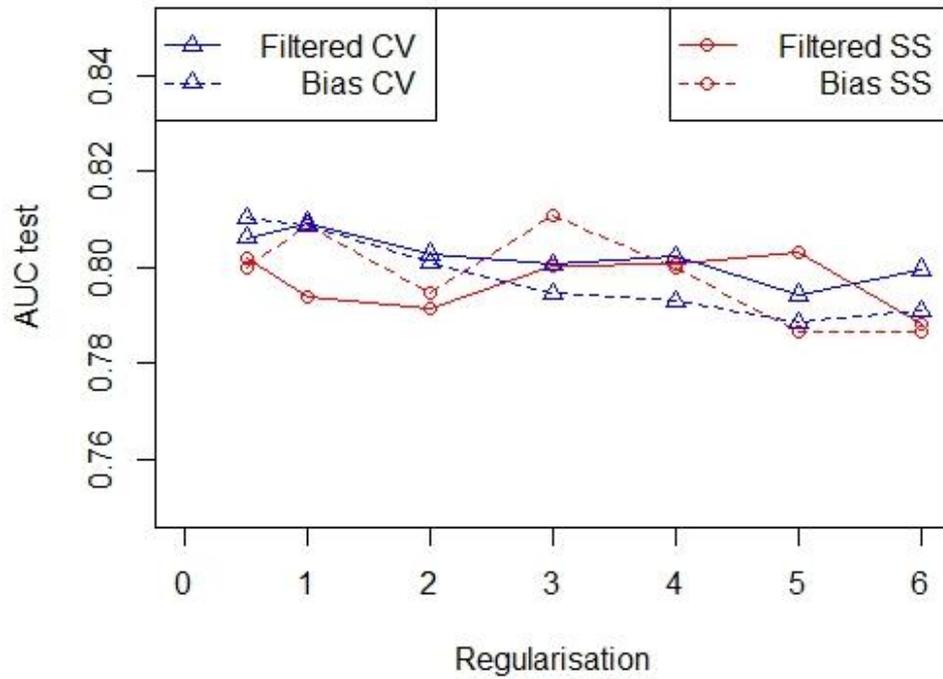
Figure S2.7. Results of the AUCtest for the great crested newt SDM in Kent comparing models over regularisation levels 0.5 to 6 using a bias file (Bias) or spatial filtering of data at 250 m (Filtered). Each model was run using the subsample testing method (SS) or the cross-validation testing method (CV).
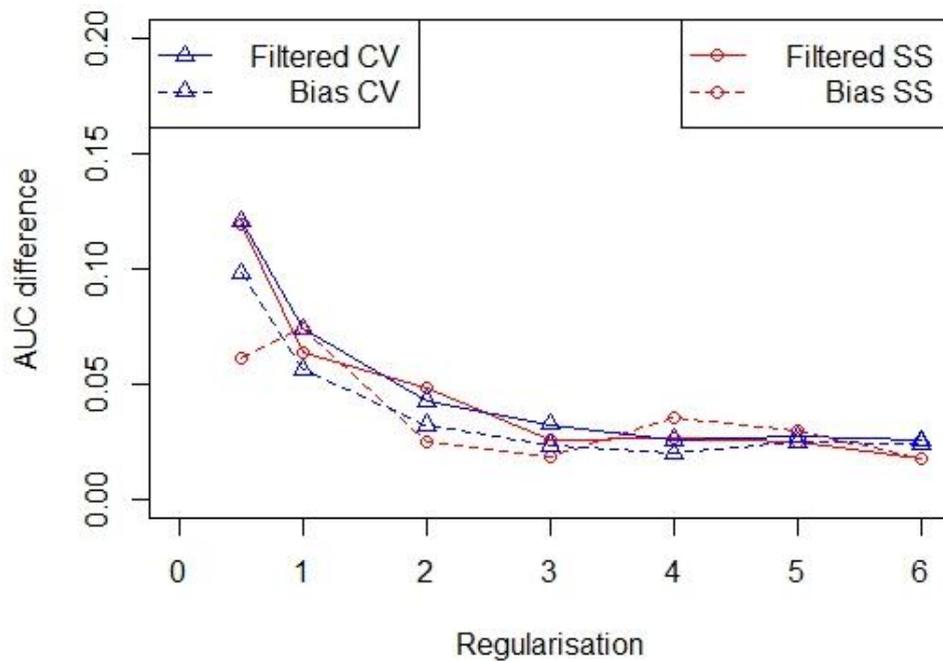
Figure S2.8. Results of the AUCdiff for the great crested newt SDM in Sussex comparing models over regularisation levels 0.5 to 6 using a bias file (Bias) or spatial filtering of data at 250 m (Filtered). Each model was run using the subsample testing method (SS) or the cross-validation testing method (CV).
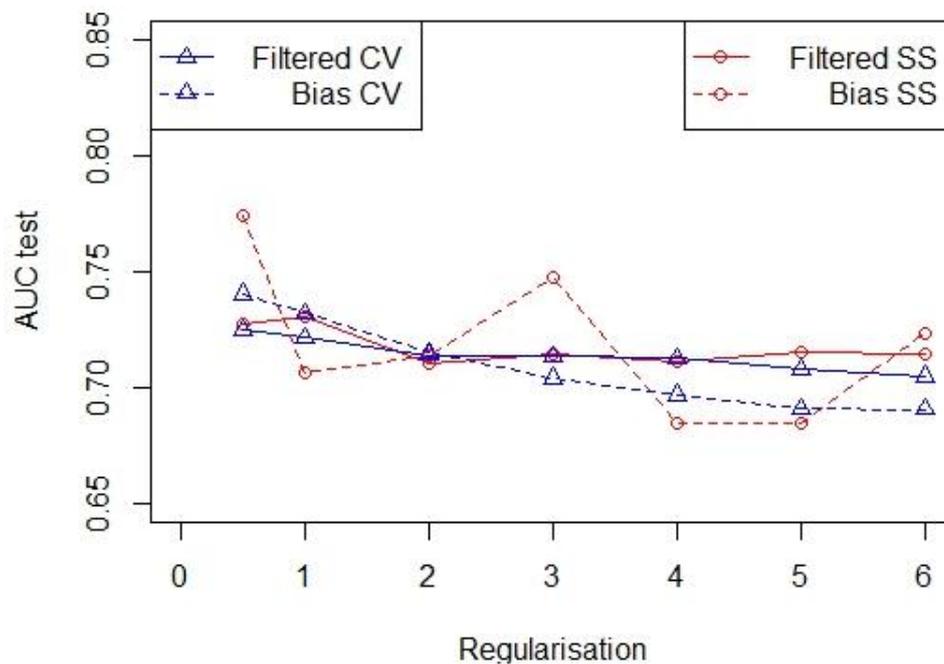
Figure S2.9. Results of the AUCtest for the great crested newt SDM in Kent comparing models over regularisation levels 0.5 to 6 using a bias file (Bias) or spatial filtering of data at 250 m (Filtered). Each model was run using the subsample testing method (SS) or the cross-validation testing method (CV).

Grass snake results

There was a lot of variation in both AUCdiff and AUCtest in the filtered SS models for Kent (Figures S2.10 and S2.11). The AUCdiff was the lowest in the SS bias file model but the CV bias file models were also quite close. The AUCtest results for the bias models were higher than for the spatially filtered models using the CV method. A regularisation value of 4 using the CV method was chosen for use in the main analysis, resulting in an AUCtest of 0.65 and an AUCdiff of 0.033 (Table S2.3).

In the Sussex grass snake SDMs the models using bias files tended to perform better for both AUCdiff and AUCtest (Figures S2.12and S2.13). A regularisation level of 2 using the CV method and a bias file was used for the main analysis.
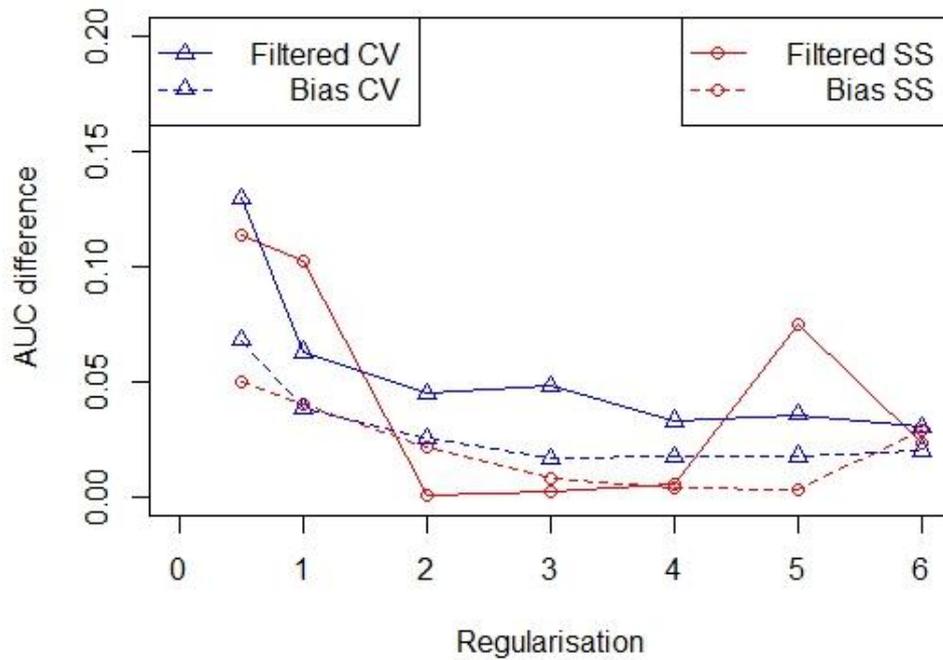
Figure S2.10. Results of the AUCdiff for the grass snake SDMs in Kent comparing models over regularisation levels 0.5 to 6 using a bias file (Bias) or spatial filtering of data at 250 m (Filtered). Each model was run using the subsample testing method (SS) or the cross-validation testing method (CV).
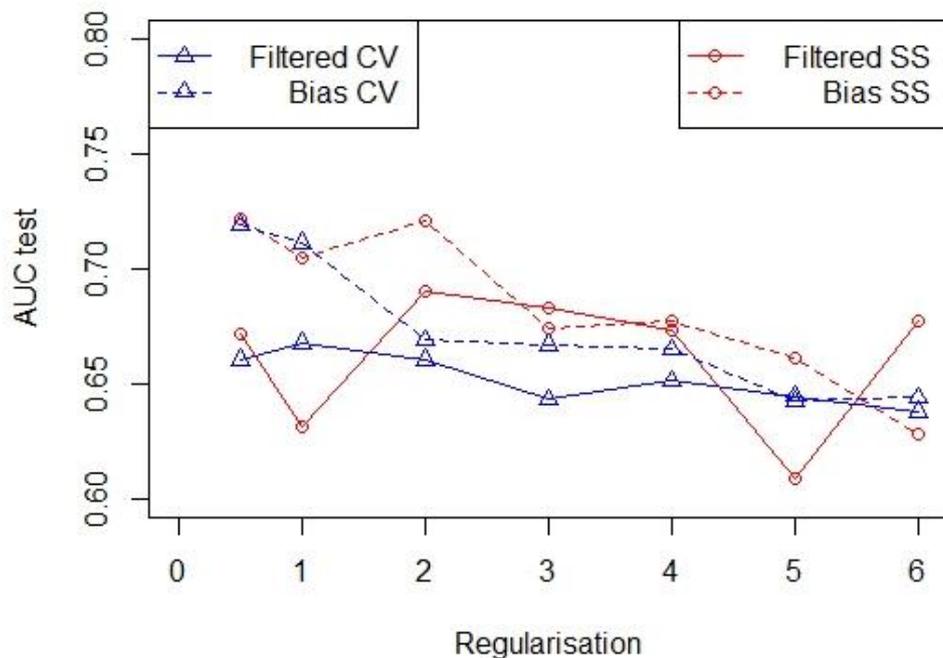
Figure S2.11. Results of the AUCtest for the grass snake SDMs in Kent comparing models over regularisation levels 0.5 to 6 using a bias file (Bias) or spatial filtering of data at 250 m (Filtered). Each model was run using the subsample testing method (SS) or the cross-validation testing method (CV).
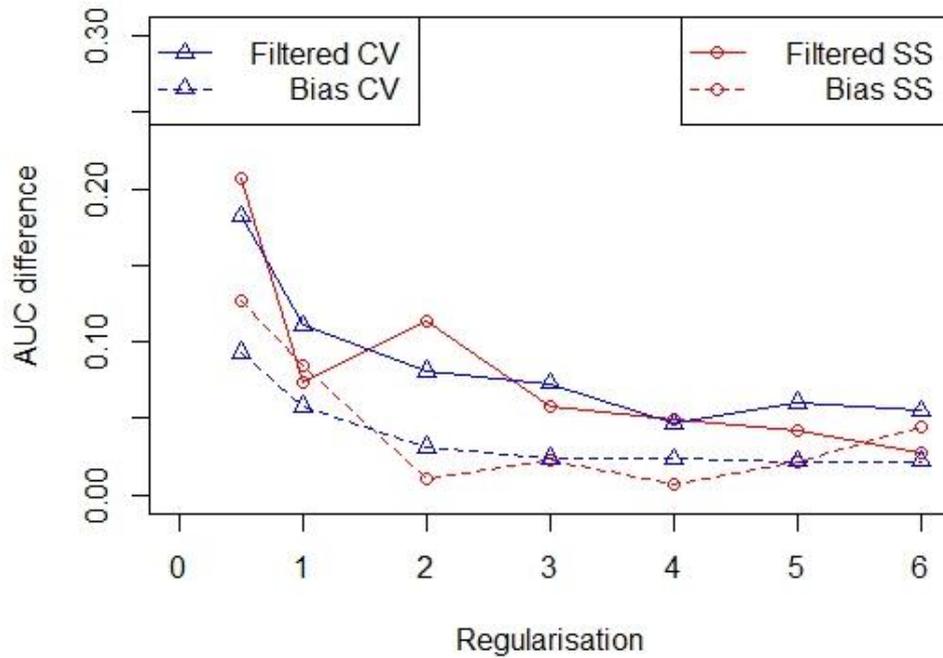
Figure S2.12. Results of the AUCdiff for the grass snake SDMs in Sussex comparing models over regularisation levels 0.5 to 6 using a bias file (Bias) or spatial filtering of data at 250 m (Filtered). Each model was run using the subsample testing method (SS) or the cross-validation testing method (CV).
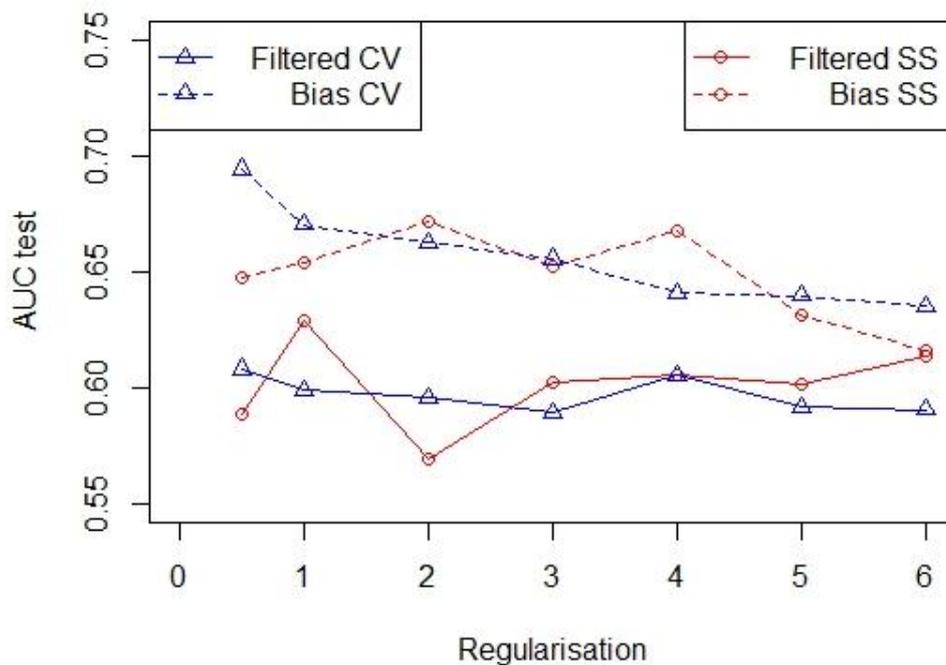
Figure S2.13 Results of the AUCtest for the grass snake SDMs in Sussex comparing models over regularisation levels 0.5 to 6 using a bias file (Bias) or spatial filtering of data at 250 m (Filtered). Each model was run using the subsample testing method (SS) or the cross-validation testing method (CV).

Chapter 3. Now you see them now you don't: Determining probability of detection and occupancy for two amphibian species

3.1. Abstract

When using presence/absence data it is important to account for variation in detectability of the species involved. Failing to do so may lead to erroneous conclusions. If there is a low sample size, non-detection of a species when it is present, will have a greater impact on any inferences from the data. Common frogs (*Rana temporaria*) may be affected by the presence of marsh frogs (*Pelophylax ridibundus*) and great crested newts (*Triturus cristatus*). Prior to an assessment of whether common frog presence was higher in the absence of marsh frogs, occupancy modelling was used to determine the probability of detection and occupancy for great crested newts and marsh frogs in the study site. The turbidity of the water was the most important variable associated with great crested newt detection. The detection of marsh frogs was associated with water temperature. However, the predicted site occupancy of neither species increased when compared to the results of logistic regression which just uses naïve measures of occupancy. This suggests that species occupancy was not being underestimated when using models that do not account for detectability. Therefore, logistic regression could be used, and an extra variable incorporated into a model without risking model over-parameterisation. Adding extra variables to a model can affect which variables are most associated with a species by the logistic regression. This may alter an inference about the likelihood of marsh frogs sharing the same ponds as common frogs.

3.2. Introduction

If marsh frogs (*Pelophylax ridibundus*) are having an impact on common frogs (*Rana temporaria*) this may be caused by direct interactions between the two species within

ponds. Marsh frogs are large frogs and can reach a snout ventral length of 13 cm or more (Inns 2009; Frazer 1983), so there is the potential for predation on common frogs at all its life stages (Measey et al. 2015; Innocenzi 1995; Smith 1973; Ahrenfeldt 1953). Equally, marsh frogs could be out-competing common frogs for resources such as food or space (Merry 2004). It is also possible that other species may be involved in increasing this type of direct impact. For example, it has been hypothesised that in places with high numbers of great crested newts (*Triturus cristatus*), common frogs may occupy ponds with fish because fish ponds are less likely to have great crested newts. This could result in the predation of common frogs by marsh frogs because marsh frogs are less affected by fish (Brady 2009; Hartel et al. 2007; Van Buskirk 2003). However, it is also possible that great crested newts are the only cause of reduced common frog presence in areas with large great crested newt populations.

Attributing the decline of a native species to a cause such as the presence of a non-native species can be difficult if the impact has already taken place (Shine 2010; Merry 2004). However, this can be explored by identifying significant associations with environmental variables that affect the presence of each species (Cayuela, Besnard and Joly 2013); or by identifying differences in presence or abundance of the native species in areas with and without the non-native species (Buckland et al. 2014). In the next chapter**,** these techniques will be used to assess whether marsh frogs are having an impact on common frogs. However, such analyses require an assessment of the effect of imperfect detection in surveys used to determine presence (or abundance) of the species involved. If detectability is not accounted for this can lead to underestimates of occupancy (Kéry and Royle 2016; Guillera-Arroita, Ridout and Morgan 2010).

Occupancy modelling provides a means to determine levels of confidence in species detection in presence-absence studies (MacKenzie and Bailey 2004; MacKenzie et al. 2002). Variables that may affect the probability of detection (ρ) or the probability of occupancy (ψ) of a species can be incorporated into occupancy models. The objectives

of this chapter are to: (1) determine what degree of confidence can be given to the results of surveys to detect the presence of marsh frogs and great crested newts in Kent; and (2) identify whether occupancy modelling indicates that naïve counts of occupied sites underestimates true occupancy for the two species. The variables found to be associated with occupancy and detection provided information on what may be causing variation in detection and occupancy between surveys and ponds. The results of the occupancy modelling are compared with logistic regression models that use naïve estimates of occupancy, to assess whether these models underestimate species occupancy. If naïve estimates of occupancy are reliable, logistic regression has the advantage that one extra covariate of occupancy can be used (to replace the covariate of detection). Several environmental variables have been associated with the presence of great crested newts (Oldham et al. 2000); this may be also the case for marsh frogs. Therefore, even one extra variable may produce a better fitting model.

Common frog spawn is very visible when laid. If surveys are conducted when spawning occurs, occupancy models achieve a detection rate of $p=1.0$, but this reduces in later surveys when spawn has hatched or is predated (Sewell, Beebee and Griffiths 2010). Therefore, timing surveying to coincide with the spawning period (late February to March) provided the highest chance of detecting common frogs. The use of occupancy modelling was considered unnecessary because frog spawn if present is unlikely to be missed. Occupancy modelling also assumes that the population is closed meaning a site will not change from absent to present over the survey period (MacKenzie et al. 2002). This assumption is more easily broken when conducting spawn surveys because there can be several weeks difference in spawning times between ponds. Spawning times may also be associated with migration distance to a pond (Loman 2016). If common frogs have not reached the pond before a survey visit occurs this would falsely lower detection probabilities. For these reasons, in this chapter, occupancy models have only been developed for marsh frogs and great crested newts. The next chapter will investigate the

variables associated with common frogs by logistic regression to identify if they influence the likelihood that marsh frogs could be impacting on common frogs. The aim of this chapter is to determine if accounting for the probability of detection affects the presence/absences of marsh frogs and great crested newts in the surveys in Kent.

3.3. Methods

3.3.1. Study Area

The study area was in southern and central Kent in the UK. The landscape consists of low-lying clay valleys with some outcrops of sandstone, limestone or chalk. Due to the clay the area is predominantly pastoral and has many ponds. To provide the best opportunity of assessing any impacts of marsh frogs on common frogs, ponds within the dispersal distances of known marsh frog and common frog sites were identified. A dispersal distance of 1.5 km has been shown to be a good approximation for common frogs (Safner et al. 2011; Kovar et al. 2009). Estimates of marsh frog dispersal distances vary but there is consensus that marsh frogs do not move far from water frequently (Kovar et al. 2009; Holenweg 2001). For example, in Surrey, most sites colonised by frogs of the genus *Pelophylax* found in the UK (including marsh frogs) were within 100 m of a watercourse (Wycherley and Joslin 1996). For this reason, the survey ponds were selected to be within 100 m of a watercourse and within 500 m from a marsh frog record. Species presence records were supplied by the Kent Reptile and Amphibian Group (KRAG) and the Kent and Medway Biological Records Centre (KMBRC). This selection process resulted in a set of ponds that could potentially be occupied by both common frogs and marsh frogs.

3.3.2. Pond Surveys

From the ponds identified using common and marsh frog dispersal distances, a subset of 45 ponds were surveyed for marsh frogs and great crested newts between March and early June 2014. The surveys were conducted in the evening. A 1,000,000 candle-power

torch was used to search the area extending to approximately 2 m from the water's edge. All the accessible banks of each pond were searched in this way. The numbers of great crested newts and marsh frogs were recorded (including all aquatic life stages of both species). Each pond was surveyed three times with a minimum of 7 days and maximum of 25 days between surveys. Variables that may affect the detectability (Table 3.1) or occupancy (Table 3.2) of either great crested newts or marsh frogs were recorded during the surveys. Habitat and bioclimatic variables were also used as covariates of occupancy (Table 3.2).

Table 3.1. Environmental variables that may affect the detectability of marsh frogs and great crested newts in amphibian surveys.

Variable Name	Description	Reason for inclusion
Date	The number of days from 1st January 2014 to the date the survey was conducted.	Both great crested newts and marsh frogs hibernate during the winter (Inns 2009; Beebee and Griffiths 2000). Therefore, amphibian surveys conducted earlier in the season may reduce the likelihood of detection of both species.
Water temperature	The water temperature 5 cm below the water surface recorded during a survey.	As ectotherms, marsh frogs and great crested newts are likely to be sensitive to temperature. Sewell et al. (2010) found that detectability of great crested newts increased with temperature. Marsh frogs are often seen basking on the banks of ponds and ditches (Inns 2009; Beebee and Griffiths 2000; Merry 2004) so detection may also be affected by water or air temperature.
Air temperature	The mean air temperature calculated from the minimum and maximum air temperatures 1 m above ground level, recorded during a survey visit.	As above.
Torchable area	An estimate of the percentage of a pond's perimeter that could be searched by torch. This also took account of torching that was prevented by lack of access or obscuring of water surface by vegetation growth.	Probability of detection may be reduced if there are areas of a pond that cannot be surveyed.
Time from sunset	A measure of the amount of time (minutes) from sunset the survey was started.	Great crested newts can be more active in open areas of a pond at night (Beebee and Griffiths 2000). Torching may also be more effective when it is darker.
Turbidity	Turbidity of the water scored from 1 = able to see the bottom of the	The depth the torch beam can penetrate the water reduces with higher levels of

	pond clearly, 2 = moderately clear, to 3 = turbid (Sewell, Beebee and Griffiths 2010).	turbidity. Great crested newts also tend to spend more time in deeper areas of ponds (Beebee and Griffiths 2000) so detection of great crested newts and marsh frogs may be reduced.

Table 3.2. Environmental variables that may affect the occupancy of great crested newts ([1]) and marsh frogs ([2]) in amphibian surveys.

Variable Name	Description	Reason for inclusion
Conductivity[2]	The mean conductivity (µS) of the pond from three surveys measured 5 cm below surface of the pond using a conductivity meter.	Marsh frogs may be more tolerant to saline conditions than UK native amphibians including great crested newts (Innocenzi 1995; Morand and Joly 1995; Beebee 1980).
Shade[1,2]	The mean of three surveys of a pond where the percentage of a pond's perimeter shaded to at least 1 m from the shore was estimated at each survey visit.	Excessive shading of a pond by trees can lead to eutrophication because of leaves accumulating in the pond making the pond less suitable for great crested newts (ARG UK 2010; Oldham et al. 2000). Marsh frogs are often found basking on the banks of ponds and ditches (Merry 2004; Beebee and Griffiths 2000; Smith 1973). This may mean shaded ponds are less suitable for marsh frogs.
Macrophytes[1,2]	The mean surface area covered by macrophytes at each of three surveys calculated from the estimates of the percentage of the pond's surface covered.	Macrophytes are used as an egg substrate for great crested newts and provide a food source for their prey (ARG UK 2010; Oldham et al. 2000). Marsh frogs lay their spawn in weeds and bask on floating vegetation (Frazer 1983; Smith 1973) so may also be positively associated with the presence of macrophytes.
Fish[1]	A subjective assessment of the presence and abundance of fish in the pond, scored from; 1 = fish population is dense; 2 = small numbers of goldfish, crucian carp, or stickleback; 3 = presence of fish is possible given pond conditions; to 4 = no records of fish or any fish seen in torch surveys.	In certain conditions great crested newts may be negatively affected by the presence of predatory fish (ARG UK 2010; Hartel et al. 2007; Oldham et al. 2000).
Fowl[1]	A subjective assessment of the likely impact of water fowl on the presence of amphibians, considering: the number of water	Water fowl may affect the presence of great crested newts through predation, removing aquatic vegetation and polluting the water (ARG UK 2010;

[1] Used as an occupancy covariate in the great crested newt models.
[2] Used as an occupancy covariate in marsh frog models.

	fowl seen; the likelihood of predation of amphibians; lack of pond or bank vegetation due to water fowl grazing; and water pollution due to waterfowl. The pond was scored from: 1 = major; 2 = minor; to 3 = absent.	Oldham et al. 2000).
Pond area[1,2]	The approximate area of the pond (m²) measured by pacing out the length and width of ponds.	There is evidence that pond area could affect great crested newt occupancy and that a pond area of 500 to 750 m² is the optimum size (ARG UK 2010; Oldham et al. 2000). Pond area may also affect marsh frog presence as marsh frogs tend to be associated with larger water bodies (Beebee and Griffiths 2000; Frazer 1983).
Pond permanence[1,2]	The permanence of the pond determined by the frequency a pond dries from: never dries = 1; dries every 1 or 2 years in 10 = 2; dries 3 to 8 years in 10 = 3; and dries 8 or 9 years in 10 = 4.	Marsh frogs rarely leave ponds so are more likely to be found in ponds that seldom dry out (Kovar et al.2009; Holenweg 2001). Great crested newts need ponds where metamorphosis can be completed before drying, but pond drying does prevent the build-up of predators (ARG UK 2010; Indermaur et al. 2010; Oldham et al. 2000).
Pond density[1,2]	Number of ponds within a 1 km radius divided by pi. Derived from a UK wide pond dataset provided by ARC Trust[1].	The presence of great crested newts has been linked to higher pond densities (Bormpoudakis et al. 2015; ARG UK 2010; Oldham et al. 2000). The marsh frog's aquatic nature may also mean that there is a positive association with high pond density.
Mean water temperature[1,2]	The mean water temperature 5 cm below the water surface recorded over the three surveys.	Colder water temperature may affect great crested newt or marsh frog occupancy because warmer temperatures may lead to a longer breeding season and more time for larval development.
HSI[1]	The Habitat Suitability Index for great crested newts is a numerical index indicating the suitability of a pond for great crested newts scored from 0 to 1 developed by Oldham et al. (2000) and updated in 2010 (ARG UK 2010).	The HSI is made up of 10 indices (including some of the variables in this table). These are: 'Pond area', 'Pond permanence', 'Shade', 'Fowl', 'Macrophyte', 'Fish', 'Pond density', 'Geographic location', 'Water quality', and 'Terrestrial habitat'.
Linear water features[2]	Combined length of linear water features per km². Derived from Ordnance Survey MasterMap Water Network (OS MasterMap Water Network 2015).	Marsh frogs are a highly aquatic species and often associated with linear water features such as drainage ditches (Inns 2009; Beebee and Griffiths 2000).
Elevation[2]	Recorded using a hand-held GPS unit.	Elevation was included as an occupancy covariate in the marsh frog models because it may be correlated to 'Linear water features', 'Water temperature', and 'Air temperature'. In models that can only have one

		covariate it may be a better predictor than any one of these variables singly.
Distance from broadleaved woodland[1]	Distance from broadleaved woodland (m). Derived from the LCM2015 (Rowland et al. 2017).	Woodland areas can provide undisturbed habitat for great crested newts and some studies have found a positive association between woodland and great crested newts although presences can differ with species range (Van Buskirk 2005; Jehle 2000; Latham et al. 1996).
Distance from arable land[1,2]	Distance from arable or horticultural land (m). Derived from the LCM2015 (Rowland et al. 2017).	Areas of arable land are likely to be detrimental to amphibians due to issues such as agricultural runoff and overgrowth of ditches (Beebee and Griffiths 2000).
Distance from semi-natural grassland[1]	Distance from semi-natural grassland (m). Derived from the LCM2015 (Rowland et al. 2017)).	Semi-natural grassland can often provide habitat for amphibians and reptiles. It was found to be positively associated with great crested newt presence in Kent (Bormpoudakis et al. 2015).
Temperature seasonality[1]	The standard deviation of temperature x 100 at a resolution of 30 arc seconds. Recorded between 1950 and 2000. Obtained from the World Clim data set http://www.worldclim.org/bioclim (Hijmans et al. 2005).	Temperature seasonality has been found to be negatively associated with great crested newt presence in Kent (Bormpoudakis et al. 2015).
Maximum temperature warmest month[2]	Maximum temperature warmest month recorded between 1950 and 2000. Obtained from the WorldClim data set http://www.worldclim.org/bioclim (Hijmans et al. 2005).	Marsh frogs emerge from hibernation later than UK native amphibians (Beebee and Griffiths 2000) and therefore are likely to be more associated with warmer areas.
Mean temperature coldest quarter[1]	Mean temperature coldest quarter recorded between 1950 and 2000. Obtained from the WorldClim data set http://www.worldclim.org/bioclim (Hijmans et al. 2005).	Warmer winters can result in hibernating animals depleting energy reserves without being able to feed. Previous research has found great crested newts to be negatively associated with the warmer winters (Bormpoudakis et al. 2015; Griffiths, Sewell and McCrea 2010).
Precipitation coldest quarter[1,2]	Precipitation coldest quarter recorded between 1950 and 2000. Obtained from the WorldClim data set http://www.worldclim.org/bioclim (Hijmans et al. 2005).	High winter rainfall, leading to waterlogged soils, could mean hibernating amphibians can only utilise gaseous exchange across their skin. This could prevent pumping of air into their lungs thus leading to respiratory problems (Griffiths, Sewell and McCrea 2010).

3.3.3. Data analysis

Single Season Occupancy Model

A separate single season occupancy model was developed for marsh frogs and great crested newts using the R package 'unmarked' (Fiske and Chandler 2011). Single season occupancy models assume: a) the population is closed so a site does not change its occupancy state during the survey period; b) a species is never falsely reported as present (false positive); c) and detection of a species at one site is independent of all others (MacKenzie et al. 2002).

Marsh frogs hibernate in ponds over winter and have a low migration rate between ponds (Kovar et al. 2009; Holenweg 2001; Beebee and Griffiths 2000). Therefore, it is reasonable to assume that the population was closed over the survey period of the study. Great crested newts usually migrate to ponds for breeding in March or April (Beebee and Griffiths 2000). The length of stay in the ponds can vary from just a few days to the rest of the year (Beebee and Griffiths 2000). However, although some great crested newts may have left the pond before the end of the survey period, it was unlikely that all would have done, and the assumption of a closed population is not violated (Sewell, Beebee and Griffiths 2010). The surveys were conducted by the researcher and one of five volunteers trained in amphibian survey procedures. Volunteers were also given training in how to identify marsh frogs by the researcher. On a few occasions surveys were undertaken by the researcher alone. All pond metrics were recorded by the researcher during surveys. Both marsh frogs and great crested newts are easily distinguished from other native amphibians so false positives were unlikely.

Spatial autocorrelation (the presence of a species at one site means the nearby sites are more likely to have the species) can lead to a lack of independence between surveys sites. The join count permutation test from the R package spdep (Bivand and Piras 2015) was used to identify spatial autocorrelation. The join count permutation test works by counting the numbers of neighbours within a specified distance that are the same state

or a different binary state. For example, for each of the survey ponds the presence or absence of marsh frogs is recorded then the number of neighbouring ponds with or without marsh frogs is counted. These calculations are used to determine whether there are a significantly higher number of neighbours that are the same state than is predicted when the state of the ponds is repeatedly randomly simulated. The number of simulations used was 999. Research has shown that most great crested newts and marsh frogs will not disperse further than 250 m (Kovar et al. 2009; Holenweg 2001; Wycherley and Joslin 1996), so a maximum distance of 250 m was used to indicate what was considered a neighbouring pond. The number of neighbours with the same state was not significantly higher than predicted by the simulation for either marsh frogs or great crested newts. This suggests that spatial autocorrelation within the data is not an issue and independence between sites can be assumed.

The data from the occupancy and detection environmental variables were assessed for skewness in the data or outliers that may affect the models. The variables 'Pond area', and 'Distance from broadleaved woodland' were square root transformed. The continuous variables were standardised, so they had a mean of zero and a standard deviation of one (Fiske and Chandler 2011).

Model Selection

The Akaike Information Criterion for small sample sizes (AICc) was used to rank models (Mazerolle 2006; Burnham and Anderson 2002). Those models within two delta AICc of the highest ranked model were considered to have substantial support and were assessed for model fit using the MacKenzie-Bailey test (M&B test) with 1000 simulations (MacKenzie and Bailey 2004; Burnham and Anderson 2002). The M&B test was implemented using the mb.gof.test with the R package AICcmodav (Mazerolle 2015). The overdispersion factor (ĉ) was calculated for each model using the M&B test. If ĉ >1 for all the models ranked by AICc (except the null model which assumes a constant occupancy and detection $\rho(\cdot),\psi(\cdot)$), overdispersion (the variation in the observed data is

higher than expected by the model) was an issue so QAICc was used instead of AICc (Anderson and Burnham 2002). The function modavgPred of AICcmodav (Mazerolle 2015) was used to increase confidence intervals where $\hat{c} > 1$ for the predicted probability of detection. This inflating of confidence intervals shows the increased uncertainty in the prediction due to overdispersion (Kéry and Royle 2016).

Model selection was undertaken on the detection covariates first and the best performing model (lowest (Q)AICc) was used when selecting models for the occupancy part (Kéry and Royle 2016). Only variables that had an *a priori* hypothesis concerning the species detection or occupancy were included in the model selection process to avoid models that have no causal basis for inclusion but may be correlated with presence of the species (Mazerolle 2006; Anderson and Burnham 2002; Burnham and Anderson 2002; Anderson et al. 2001). Due to the low number of sites a maximum of two variables per model was used (Sewell, Beebee and Griffiths 2010; Burnham and Anderson 2002). To reduce the number of covariates in a model, the ordinal variables 'Fish' and 'Pond permanence' were reduced from four categories to two. For example, this meant for 'Fish' the categories 1 (fish population is dense) and 2 (small numbers of goldfish, crucian carp, or stickleback) were combined into a single category; similarly, categories 3 (presence of fish is possible given pond conditions) and 4 (no records of fish or any fish seen in torch surveys) were combined. This created an ordinal variable with two categories which corresponded to 1 ('Fish high') and 2 ('Fish low'). The best performing occupancy models for great crested newts and marsh frogs were identified based on (Q)AICc and the M&B test. Logistic regression models using naïve occupancy data were then run using the occupancy covariate from the highest ranked models for each species. These were compared to the occupancy models to identify if using the detection covariates increased the predicted occupancy for the species (Kéry and Royle 2016).

3.4. Results

3.4.1. Great crested newt

Out of the 40 ponds surveyed great crested newts were detected at least once in 22 of the ponds. The great crested newt model selection process for detectability identified three models with a delta AICc <2.0 (Table 3.3). These models were treated as having considerable support (Burnham and Anderson 2002). The evidence ratio between the two top models (the model with 'Turbidity' as a variable and the null model) was 2.9 indicating the top model ('Turbidity') was 2.9 times more likely to be the best model than the null model out of the candidate models (Mazerolle 2006). A 'Turbidity' score of 1 (able to see the bottom of the pond clearly) was positively associated with detection of great crested newts in a pond (estimate = 1.67, p = 0.002) and a 'Turbidity' score of 3 (turbid) was negatively associated with detection (estimate = -2.63, p = 0.004). No other variables were significantly associated with detection of great crested newts. Therefore, 'Turbidity' was used as the detection variable in all the models in the model selection process to select the most important variable for probability of occupancy (ψ) of great crested newts.

Table 3.3 Model selection on the detection part of the great crested newt model using constant occupancy ψ(·). The parameter count (K) includes intercept.

Model	K	AICc	Delta AICc	AICc Weight	Cumulative weight	Log likelihood
ρ(Turbidity) ψ(·)	5	103.72	0	0.31	0.31	-45.98
ρ(·) ψ(·)	3	104.14	0.42	0.25	0.57	-48.74
ρ(Water temperature) ψ(·)	4	105.13	1.41	0.15	0.72	-48
ρ(Time from sunset) ψ(·)	4	105.98	2.26	0.1	0.82	-48.42
ρ(Torchable area) ψ(·)	4	106.06	2.34	0.1	0.92	-48.46
ρ(Date) ψ(·)	4	106.46	2.74	0.08	1	-48.66

When selecting for probability of occupancy the model with ρ(Turbidity) and ψ(Fish) was the highest ranked model based on AICc (Table 3.4). This model produced a mean predicted occupancy of 0.62 (95% CI 0.29 – 0.83) and a mean detection probability of 0.65 (95% CI 0.46 – 0.82) (Table 3.5). For this model a pond with a 'Fish' value of 2 (a low fish presence) increased the log odds of great crested newts being present by 2.76 (estimate = 2.76, p = 0.007). The log odds of detecting great crested newts in a pond increased with a pond 'Turbidity' value of 1 (estimate = 1.66, p = 0.002) and decreased with a 'Turbidity' value of 3 (estimate = -2.69, p = 0.002). A pond 'Turbidity' value of 2 did not significantly affect great crested newt detection. The logistic regression modelling of the presence/absence of great crested newts using 'Fish' as a covariate produced a mean predicted occupancy of 0.55 (95% CI 0.36 – 0.73).

Table 3.4. The ten highest ranked models after model selection on the occupancy part of the great crested newt model using 'Turbidity' as the detection covariate. The parameter count (K) includes intercept.

Model	K	AICc	Delta AICc	AICc Weight	Cumulative weight	Log likelihood
ρ(Turbidity) ψ(Fish)	5	116.41	0	0.55	0.55	-52.32
ρ(Turbidity) ψ(HSI)	5	117.51	1.11	0.31	0.86	-52.87
ρ(Turbidity) ψ(Precipitation coldest quarter)	5	121.09	4.68	0.05	0.91	-54.66
ρ(Turbidity) ψ(Mean water temperature)	5	122.43	6.02	0.03	0.94	-55.33
ρ(Turbidity) ψ (Mean temperature coldest quarter)	5	123.31	6.9	0.02	0.96	-55.77
ρ(Turbidity) ψ(Temperature seasonality)	5	124.45	8.04	0.01	0.97	-56.34
ρ(Turbidity) ψ(shade)	5	124.74	8.34	0.01	0.98	-56.49
ρ(Turbidity) ψ(·)	4	125.01	8.6	0.01	0.98	-57.93
ρ(Turbidity) ψ(Distance from semi-natural grassland)	5	126.67	10.26	0	0.99	-57.45
ρ(·) ψ(·)	2	127.14	10.73	0	0.99	-61.41

Only one other model had a delta AICc <2.0, the model with ρ(Turbidity) and ψ(HSI) (Table 3.4). 'HSI' was positively associated with occupancy of great crested newts, so for every one unit increase in 'HSI' the log odds of great crested newts being present increased by 1.23 (estimate = 1.23, p = 0.004). Detection of great crested newts was significantly positively associated with a pond 'Turbidity' value of 1 (estimate = 1.67, p = 0.002) and negatively associated with a 'Turbidity' value of 3 (estimate = -1.90, p = 0.04). The evidence ratio between these models was 1.74. The results of the M&B goodness

of fit test indicated that the observed detection histories of the two highest ranked models were not significantly different from the expected detection histories as predicted by the models. However, the overdispersion parameter ($\hat{c}$) for the top model (ρ(Turbidity) and ψ(Fish)) was greater than 1 ($\hat{c}$ = 1.58). This indicated there was more variation in the observed data than expected by the model (MacKenzie et al. 2006). A comparison of the detection histories from the M&B test for the model using ρ(Turbidity) and ψ(HSI) showed a better fit and indicated very little overdispersion ($\hat{c}$ = 1.11). The predicted occupancy for this model was 0.56 (95% CI 0.36 – 0.76) (Table 3.5) which is not much higher than the naïve occupancy of 0.55. Comparing the logistic regression (which uses the naïve occupancy) with 'HSI' as a covariate and the occupancy model ρ(Turbidity) and ψ(HSI) showed they had a similar relationship with HSI (Figure 3.1). Only the upper 95% confidence intervals for the occupancy model were slightly wider at lower occupancy values compared to the logistic regression (Figure 3.1). 'HSI' in the logistic regression also had a similar positive affect on great crested newt presence (estimate = 1.35, *p* = 0.002). Although occupancy models show that turbidity affects detection, ignoring this in logistic regressions results in occupancies that do not differ from those provided by occupancy models.
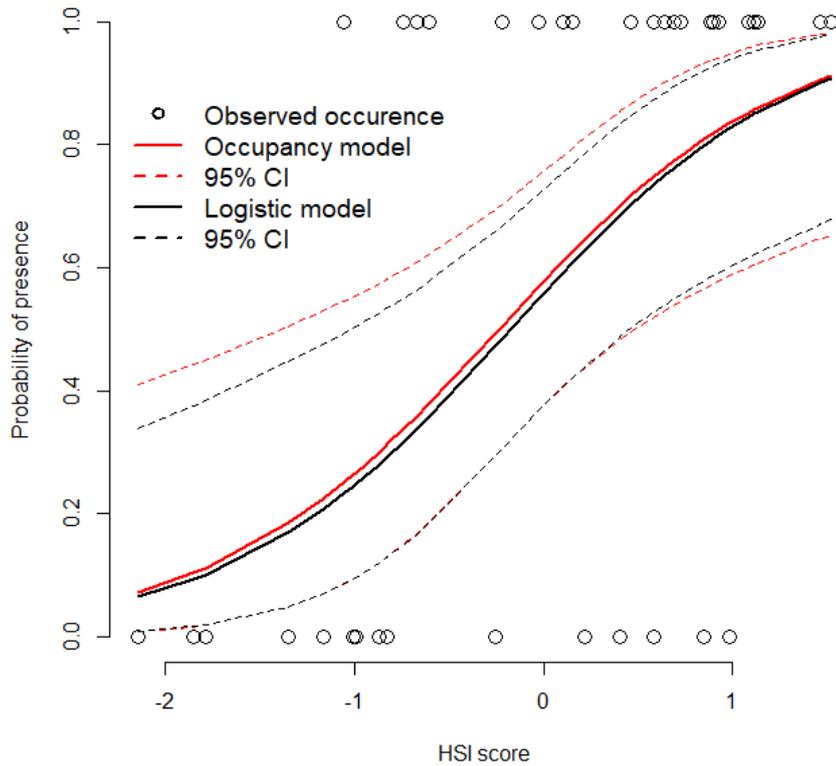
Figure 3.1. The observed great crested newt occurrence state (presence or absence) of each pond (open circles) with plots of the estimated likelihood of presence against the scaled HSI score from: an occupancy model using 'Turbidity' as the detection covariate and 'HSI' as the occupancy covariate (solid red line); and a logistic regression model (solid black line). Dashed lines are 95% confidence intervals for the occupancy model (red) and logistic regression model (black). HSI score has been scaled to a mean of zero and a standard deviation of one.

Table 3.5. Estimated probability of detection (ρ) and occupancy (ψ) of great crested newts for the four highest ranked occupancy models using AICc. Confidence intervals for the probability of occupancy have been inflated by the overdispersion factor (ĉ).

Model	ĉ	Estimate of ρ	95% CI of ρ	Estimate of ψ	95% CI of ψ
ρ(Turbidity) ψ(Fish)	1.58	0.65	0.46 – 0.82	0.62	0.29 – 0.83
ρ(Turbidity) ψ(HSI)	1.11	0.71	0.49 – 0.88	0.56	0.36 – 0.76
ρ(Turbidity) ψ(Precipitation coldest quarter)	1.55	0.64	0. 47 – 0.79	0.65	0.29 – 0.84
ρ(Turbidity) ψ(Water temperature)	1.45	0.64	0.47 – 0.79	0.65	0.29 – 0.84

The three highest ranked models in the detection model selection process were ranked closely. Therefore, model selection for the occupancy models was also run with constant detection ρ(·) and detection with 'Water temperature' as the detection covariate. When using either constant detection or 'Water temperature' the top ranked model used 'HSI' as the occupancy covariate and 'Fish' in the next highest ranked model based on AICc (Table S3.1 and S3.2). The evidence ratios indicated that the top models were very close when ranking models using constant detection or 'Water temperature' as the detection covariate. The M&B goodness of fit test showed better fit for both constant detectability and when using 'Water temperature' as the detection covariate compared to the model ρ(Turbidity) and ψ(Fish). There was also little or no overdispersion in the models. The predicted occupancy for the model ρ(Water temperature) and ψ(HSI) was 0.55 (95% CI 0.35 – 0.72). The model ρ(Water temperature) and ψ(Fish) had a predicted occupancy of 0.56 (95% CI 0.36 – 0.74). The better fit for the models using either ρ(Water temperature) or ρ(·) compared to using ρ(Turbidity) indicates that using 'Turbidity' as a detection covariate may be producing less reliable predictions of the probability of occupancy and detection.

3.4.2. Marsh frog

In terms of detectability, 'Water temperature' produced the highest AICc ranking (Table 3.6). 'Water temperature' was positively associated with the detection of marsh frogs (estimate = 1.02, p = 0.01). There were no other models within two delta AICc. The evidence ratio between the top two models was 11.9 indicating the model with 'Water temperature' as the detection variable had considerably more support. Therefore, 'Water temperature' was used as the detection covariate for model selection on the occupancy part of the model. Model fit using the M&B tests for ρ(Water temperature) and ψ(·), indicated that the observed (i.e. naïve) detection histories were not significantly different from those predicted by the model (p = 0.089) but the p value was not far off significance and there was evidence of overdispersion (ĉ = 1.79).

Table 3.6. Model selection on the detection part of the marsh frog model using constant occupancy $\psi(\cdot)$. The parameter count (K) includes intercept.

Model	K	AICc	Delta AICc	AICc Weight	Cumulative weight	Log likelihood
ρ(Water temperature)	3	123.58	0	0.81	0.81	-58.46
$\rho(\cdot)$ $\psi(\cdot)$	2	128.53	4.95	0.07	0.88	-62.1
ρ(Torchable area) $\psi(\cdot)$	3	129.43	5.85	0.04	0.92	-61.38
ρ(Time from sunset) $\psi(\cdot)$	3	130.53	6.96	0.03	0.95	-61.93
ρ(Date) $\psi(\cdot)$	3	130.69	7.11	0.02	0.97	-62.01
ρ(Air temperature) $\psi(\cdot)$	3	130.87	7.29	0.02	0.99	-62.1
ρ(Turbidity) $\psi(\cdot)$	4	132.57	8.99	0.01	1	-61.71

Table 3.7. The ten highest ranked models after selection on the occupancy part of the marsh frog model using 'Water temperature' as the detection covariate. The parameter count (K) includes intercept.

Model	K	QAICc	Delta_QAICc	QAICcWt	Cumulative weight	Quasi-Log likelihood
ρ(Water temperature) ψ(Macrophytes)	5	72.06	0	0.33	0.33	-30.15
ρ(Water temperature) ψ(Shade)	5	72.51	0.45	0.26	0.59	-30.37
ρ(Water temperature) ψ(Conductivity)	5	74.84	2.78	0.08	0.68	-31.54
ρ(Water temperature) ψ(Maximum temperature warmest month)	5	75.01	2.95	0.08	0.75	-31.62
ρ(Water temperature) ψ(Linear water features)	5	76.21	4.15	0.04	0.79	-32.22
ρ(Water temperature) ψ(Elevation)	5	76.33	4.27	0.04	0.83	-32.28
ρ(Water temperature) ψ(Pond density)	5	76.9	4.84	0.03	0.86	-32.57
ρ(Water temperature) ψ(Precipitation coldest quarter)	5	76.95	4.89	0.03	0.89	-32.59
ρ(Water temperature) ψ(Mean water temperature)	5	77.1	5.04	0.03	0.92	-32.67
ρ(Water temperature) ψ(Distance from arable land)	3	77.24	5.18	0.02	0.94	-35.29

The top ranked model after model selection to determine the variable most important for probability of occupancy (ψ) was ρ(Water temperature) and ψ(Macrophytes) (Table 3.7). In this model 'Macrophytes' was significantly positively associated with the presence of marsh frogs (estimate = 1.37, p = 0.006). This model predicted a marsh frog probability of occupancy of 0.53 (95% CI 0.25 – 0.77) and a probability of detection of 0.65 (95% CI 0.46 – 0.81) (Table 3.8). The detection of marsh frogs was significantly positively associated with 'Water temperature' (estimate = 0.89, p = 0.02). The logistic regression

using naïve observations of occupancy and 'Macrophytes' as a covariate produced a mean occupancy probability of 0.50 (95% CI 0.31 – 0.73) and was similarly positively associated with marsh frog presence (estimate = 1.31, p = 0.003). The occupancy model and the logistic model had a similar relationship to 'Macrophytes', but the occupancy model had wider confidence intervals (Figure 3.2). The next highest ranked model ρ(Water temperature) and ψ(Shade) produced a probability of occupancy of 0.52 (95% CI 0.26 – 0.79). 'Shade' was negatively associated with the presence of marsh frogs (estimate = -1.26, p = 0.004). Again, 'Water temperature' was positively associated with the detection of marsh frogs (estimate = 0.80, p = 0.03). There were no other models within two delta AICc of the top model. The M&B tests showed that the difference between observed and expected detection histories was not significant for either model, although the model ρ(Water temperature) and ψ(Shade) was close to being significant (p = 0.06) suggesting a better fit for the model using 'Macrophytes' as the occupancy covariate. The predicted occupancy produced by using 'Water temperature' as a detection covariate is therefore only marginally higher if variation in detection is not considered. Consequently, logistic regression models are considered more appropriate because including an extra covariate is likely to produce a better fitting model and may affect which variable are the best predictors of marsh frog presence.

Table 3.8. Estimated probability of detection (ρ) and occupancy (ψ) of marsh frogs for the four highest ranked models using AICc. Confidence intervals for the probability of occupancy have been inflated by the overdispersion factor (ĉ).

Model	ĉ	Estimate of ρ	95% CI of ρ	Estimate of ψ	95% CI of ψ
ρ(Water temperature) ψ(Macrophytes)	1.76	0.65	0.46 – 0.80	0.53	0.25 – 0.77
ρ(Water temperature) ψ(Shade)	2.01	0.66	0.47 – 0.82	0.52	0.26 – 0.79
ρ(Water temperature) ψ(Conductivity)	1.94	0.59	0.43 – 0.75	0.60	0.21 – 0.86
ρ(Water temperature) ψ(Maximum temperature warmest month)	1.87	0.64	0.45 – 0.81	0.54	0.26 – 0.81
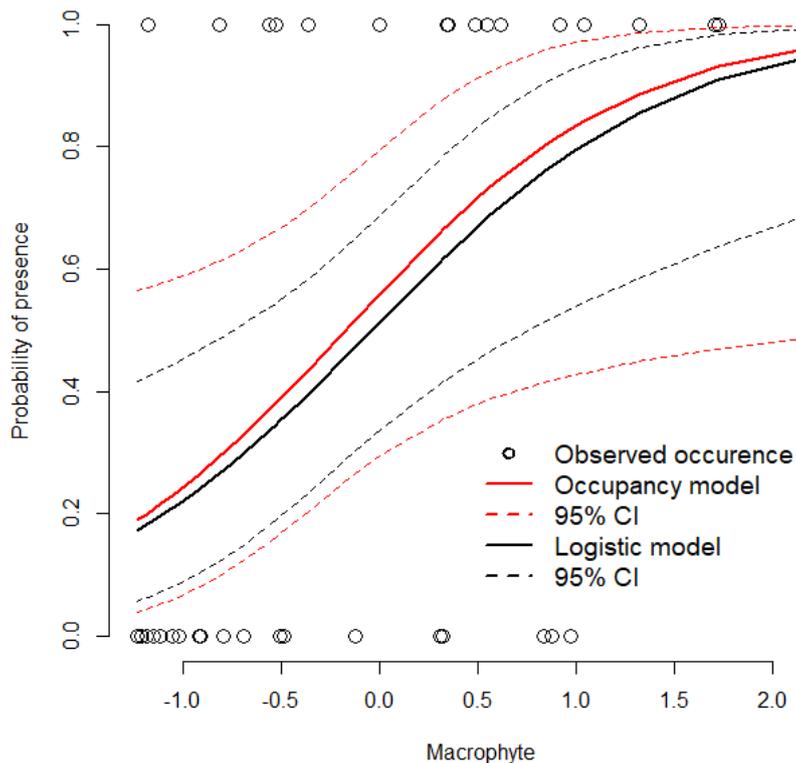
Figure 3.2. The observed marsh frog occurrence state (presence or absence) of each pond (open circles) with plots of the estimated likelihood of presence against the scaled 'Macrophytes' score from: an occupancy model using 'Water temperature' as the detection covariate and 'Macrophytes' as the occupancy covariate (solid red line); and a logistic regression model (solid black line). Dashed lines are 95% confidence intervals for the occupancy model (red) and logistic regression model (black). 'Macrophytes' has been scaled to a mean of zero and a standard deviation of one.

3.5. Discussion

3.5.1. Great crested newt models

The estimated occupancy of great crested newts from the highest ranked model ρ(Turbidity) and ψ(Fish) was higher and had broader confidence intervals than the logistic regression model which does not account for variation in detectability (Figure 3.1). The lower estimated occupancy could be due to accounting for variations in detectability due to 'Turbidity'. However, when 'HSI' is used as the occupancy covariate the predicted occupancy is very similar to the estimates produced by the logistic regression (Figure 3.1). The model ψ(Fish) and ρ(·) also produces an estimate of occupancy similar to the observed occupancy. This suggests the combination of ψ(Fish) and ρ(Turbidity) is the cause of the lower occupancy and higher predicted detection. The low p-value and high ĉ calculated from the M&B test indicates that it is not a good fitting model despite its higher AICc value. Fish at high densities can be the cause of high turbidity in ponds as well as being detrimental to the presence of great crested newts (Chan 2011; Hartel et al. 2007; Oldham et al. 2000). However, turbidity in ponds can also be caused by other factors that are unfavourable to great crested newts, such as high numbers of waterfowl or eutrophic ponds (Oldham et al. 2000). The reduced probability of detection in the ψ(Fish) and ρ(Turbidity) model may be the result of the true absence of great crested newts in ponds with no observed detections and high 'Turbidity', but with this absence being due to a factor other than the presence of fish. Plotting the variable 'HSI' against the mean 'Turbidity' score of each pond shows low 'HSI' scores are associated with high 'Turbidity' scores. There are almost as many ponds with a high 'Turbidity' level and low 'Fish' score (Figure 3.3a, b).

(a)   (b)
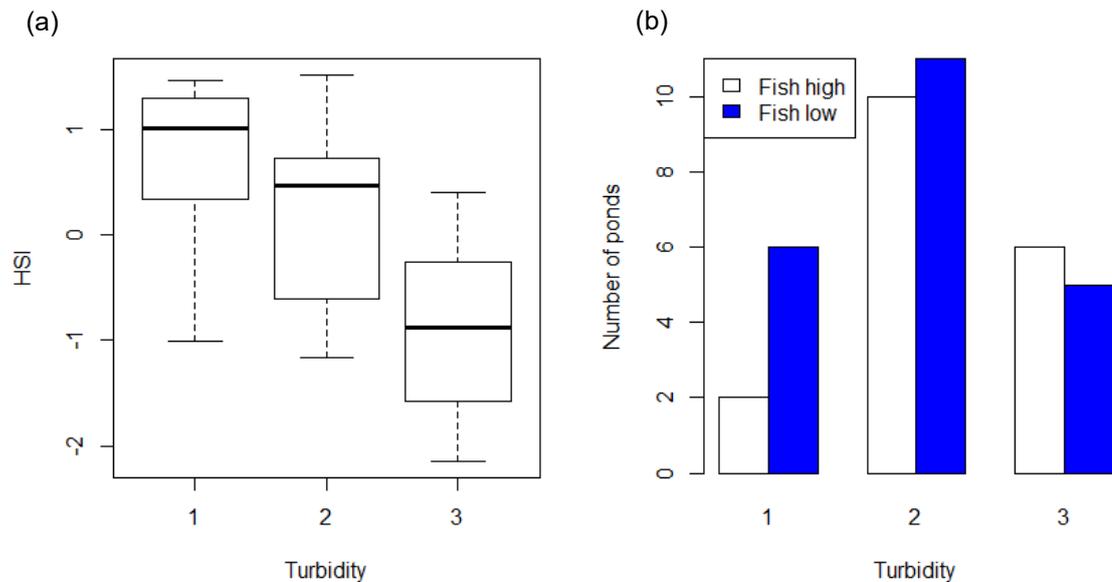
Figure 3.3. The variable 'Turbidity' plotted against: (a) the variable 'HSI' and (b) the variable 'Fish'. 'Turbidity' is an ordinal variable with three categories: 1 (able to see the bottom of the pond clearly), 2 (moderately clear), and 3 (turbid). The variable 'Fish' was turned from a four-category ordinal variable to a two-category variable. The categories 1(fish population is dense) and 2 (small numbers of goldfish, crucian carp, or stickleback) were combined into a single category 'Fish high'. The categories 3 (presence of fish is possible given pond conditions) and 4 (no records of fish or any fish seen in torch surveys) were combined to form 'Fish low'. 'HSI' has been centred and scaled. 'HSI' decrease as ponds increase in turbidity, which suggests turbid ponds may be less suitable for great crested newts. Ponds with high 'Turbidity' and 'Fish low', and high 'Turbidity' and 'Fish high' are similar in number. If very turbid ponds are less suitable for great crested newts, models with 'Turbidity' as a detection covariate and 'Fish' as an occupancy covariate may be attributing some great crested newt absences to non-detections and therefore, increasing the predicted occupancy.

The variable 'HSI' is a conglomeration of several environmental variables that have evidence to support their influence on great crested newts (ARG UK 2010; Oldham et al. 2000). It is very likely that there are several variables that affect the presence of great crested newts so the HSI score provides this broad spectrum without having a complex model. Sewell et al. (2010) also found HSI score to be important in predicting occupancy for great crested newts. Based on this evidence the model ψ(HSI) and ρ(Turbidity) is likely to be the best predictor of great crested newt occupancy from this analysis. The predicted occupancy of this model is very similar to the results from the logistic regression using 'HSI' as a covariate (Figure 3.1). Therefore, there is reasonable justification in this case for ignoring probability of detection and considering non-detections as absences.

A previous study on presence of great crested newts in Kent found evening water temperature as having the greatest effect on great crested newt detectability (Sewell, Beebee and Griffiths 2010). The results in my study showed 'Water temperature' to be ranked third by AICc but ranked lower than the model using constant detectability (Table 3.3). However, mean water temperatures for the three survey events in this study were 13.7°C, 14.2°C, and 16.2°C. Even the first surveys in this study were closer to temperatures recorded later in the season (April to end of May) by Sewell et al. (2010). The higher water temperature in this study may be the reason why 'Water temperature' was found to be much less of a limiting factor on detectability. Bottle trapping as well as torching was used in surveys by Sewell et al. (2010) so this could also have increased the importance of water temperature.

3.5.2. Marsh frog models

The results show that 'Water temperature' is by far the strongest predictor associated with probability of detection for marsh frogs. Marsh frog behaviour suggests there is a link between temperature and detectability; for example, marsh frogs emerge later from hibernation than UK native amphibians (Beebee and Griffiths 2000). The variable 'Air temperature' was not a strong predictor. However, during the surveys, which took place after sunset, it was observed that marsh frogs tend to be found just submerged at the pond edge, so air temperature would be less important.

The model selection to find variables that are important in predicting marsh frog occupancy indicated 'Macrophytes' and 'Shade' are important predictors. 'Macrophytes' and 'Shade' were negatively correlated (Spearman's rho = 0.62); a reasonable explanation for this is that the more shaded a pond is, the less light there is to stimulate pond vegetation to grow. It is possible therefore, that one variable may be a proxy for the other. However, both 'Macrophytes' and 'Shade' have biologically meaningful reasons to be associated with marsh frog presence. The marsh frog behaviour of basking in the sun particularly on south facing banks is indicative of a preference for unshaded ponds (Inns

2009; Merry 2004; Beebee and Griffiths 2000). Marsh frogs tend to lay their eggs among plants (Kyriakopoulou-Sklavounou and Kattoulas 1990). Macrophytes are also likely to provide cover from predators and experiments have shown that marsh frog tadpoles tend to stay around aquatic vegetation (Innocenzi 1995).

The results of the M&B goodness of fit tests for the top two marsh frog occupancy models ρ(Water temperature) and ψ(Macrophyte), and ρ(Water temperature) and ψ(Shade) showed that there was not a significant lack of fit for either of the above models. However, the p values were low suggesting there was overdispersion in both models. Overdispersion and a lack of fit can be caused by several factors such as important covariates being left out of the model, interactions unmodeled, or a lack of independence in the data (Kéry and Royle 2016; Anderson and Burnham 2002). The low sample size meant using more complex models was not possible. Therefore, some of the lack of fit could be due to missing covariates. A higher sample size would allow more covariates to be used which is likely to increase model fit.

The lack of fit of the occupancy model did not lead to differences in the estimated occupancy of the survey ponds compared to the estimates from the logistic regression using 'Macrophytes' as a covariate (Figure 3.2). Therefore, logistic regression could be used instead. This would allow an increase in the number of variables or interaction terms providing more information about predictors of marsh frog presence.

Including covariates of detection for great crested newts or marsh frogs only increased the predicted occupancy for either species a marginal amount. This indicates the three repeat surveys for each pond have achieved high detection rates and false absences are unlikely. Therefore, the environmental variables found to be associated with great crested newts and marsh frogs can be used without consideration of false absences affecting the results. Using logistic regression rather than occupancy modelling will allow an extra covariate to be used in the model to predict presence of the species. Including more variables in the model is likely to increase model fit. It may also affect which

variables are most associated with the presence of a species. This could alter the conclusion concerning whether marsh frogs and common frogs are likely to be found in the same ponds.

3.6. Supplementary information

Table S3.1. Model selection on the occupancy part of the great crested newt model using constant detection ρ(·). The parameter count (K) includes intercept.

Model	K	AICc	Delta AICc	AICc Weight	Cumulative Weight	Log likelihood
ρ(·) ψ(HSI)	3	116.86	0	0.73	0.73	-55.1
ρ(·) ψ(Fish)	3	119.28	2.42	0.22	0.95	-56.31
ρ(·) ψ(Mean water temperature)	3	124.45	7.59	0.02	0.97	-58.89
ρ(·) ψ(Precipitation coldest quarter)	3	126.57	9.71	0.01	0.98	-59.95
ρ(·) ψ(·)	2	127.14	10.28	0	0.98	-61.41
ρ(·) ψ(Temperature seasonality)	3	127.18	10.32	0	0.98	-60.26
ρ(·) ψ(Mean temperature coldest quarter)	3	127.44	10.58	0	0.99	-60.39
ρ(·) ψ(Shade)	3	128.16	11.3	0	0.99	-60.75
ρ(·) ψ(Pond density)	3	128.81	11.95	0	0.99	-61.07
ρ(·) ψ(Distance from semi-natural grassland)	3	129.18	12.32	0	0.99	-61.25
ρ(·) ψ(Macrophyte)	3	129.36	12.5	0	1	-61.35
ρ(·) ψ(Pond permanence)	3	129.38	12.52	0	1	-61.36
ρ(·) ψ(Distance from broadleaved woodland)	3	129.47	12.61	0	1	-61.4
ρ(·) ψ(Distance from arable land)	3	129.47	12.61	0	1	-61.4
ρ(·) ψ(Fowl)	4	130.59	13.73	0	1	-60.72

Table S3.2. Model selection on the occupancy part of the great crested newt model using 'Water temperature' as the detection covariate. The parameter count (K) includes intercept.

Model	K	AICc	Delta AICc	AICc Weight	Cumulative Weight	Log likelihood
ρ(Water temperature) ψ(HSI)	4	117.54	0	0.72	0.72	-54.2
ρ(Water temperature) ψ(Fish)	4	119.68	2.13	0.25	0.96	-55.27
ρ(Water temperature) ψ(Precipitation coldest quarter)	4	127.03	9.49	0.01	0.97	-58.95
ρ(·) ψ(·)	2	127.14	9.6	0.01	0.97	-61.41
ρ(Water temperature) ψ(·)	3	127.62	10.07	0	0.98	-60.47
ρ(Water temperature) ψ(Temperature seasonality)	4	127.79	10.24	0	0.98	-59.32
ρ(Water temperature) ψ(Mean temperature coldest quarter)	4	127.97	10.43	0	0.99	-59.41
ρ(Water temperature) ψ(Shade)	4	128.67	11.13	0	0.99	-59.76
ρ(Water temperature) ψ(Pond density)	4	129.4	11.86	0	0.99	-60.13
ρ(Water temperature) ψ(Distance from semi-natural grassland)	4	129.76	12.21	0	0.99	-60.31
ρ(Water temperature) ψ(Macrophyte)	4	129.98	12.44	0	1	-60.42
ρ(Water temperature) ψ(Pond permanence)	4	130.01	12.46	0	1	-60.43
ρ(Water temperature) ψ(Distance from broadleaved woodland)	4	130.08	12.53	0	1	-60.47
ρ(Water temperature) ψ(Distance from arable land)	4	130.08	12.54	0	1	-60.47
ρ(Water temperature) ψ(Fowl)	5	131.38	13.83	0	1	-59.81

Chapter 4. Detecting the effects of marsh frogs on common frogs at a local level

4.1. Abstract

Non-native species can cause changes in the ecosystems they invade, inflicting indirect impacts on native species. Landscape scale studies can indicate where impacts may be occurring because of the presence of a non-native. However, to determine whether suspected changes in native distributions are a result of the non-native, rather than another cause, more localised fine-scale studies are required. A landscape study has suggested that in high pond density areas of Kent, marsh frogs (*Pelophylax ridibundus*), in combination with great crested newts (*Triturus cristatus*), may be reducing the presence of common frogs (*Rana temporaria*). To test whether common frog presence is lower in areas with marsh frogs present, pond surveys were conducted to determine common frog and great crested newt occupancy in an area with, and an area without, marsh frogs. Propensity modelling was used to limit the difference between areas to provide a clearer comparison with fewer confounding variables. Logistic regression was used to identify the environmental variables associated with each species. The results showed there were fewer common frogs in areas where marsh frogs were present. However, common frogs were found to be positively associated with pond shading in areas where marsh frogs were absent. Marsh frogs were negatively associated with pond shading suggesting that common frogs and marsh frogs were likely to use different ponds in areas where their ranges overlap. Therefore, negative impacts on common frogs by marsh frogs was not likely to reduce common frog population numbers. However, there was much higher great crested newt occupancy in the areas with marsh frogs, suggesting predation by great crested newts may be the cause of a lower presence of common frogs in areas with marsh frogs.

4.2. Introduction

Landscape scale studies can be used to assess the actual and potential distributions of non-natives (Ihlow et al. 2016; Buckland et al. 2014) and to make predictions about species interactions such as competition (Anderson, Peterson and Gómez-Laverde 2002). However, landscape scale studies may lack sufficient accuracy to forecast species distributions at a local level (Bormpoudakis et al. 2015; Moreira et al. 2015). The presence of a non-native species can lead to changes in community structures often due to complex interactions between several species (Klop-Toker et al. 2018; Shulse, Semlitsch and Trauth 2013; Roemer, Donlan and Courchamp 2002). Therefore, it is often necessary to investigate what is happening at a much more local level where changes in native species presence or abundance can be assessed more easily.

There are still challenges associated with identifying if a non-native species is having an impact at a local level. An assessment of the status of a native species before and after an invasion can provide evidence that the non-native is the cause of the decline although this is rarely achievable in practice (Doody et al. 2009). In some instances, the assessment of a non-native species impact must be conducted long after its introduction and when declines in native species have already taken place (Willson 2017). In these situations, there are usually several competing hypotheses that may explain the observed reductions in a native population, such as habitat loss and fragmentation.

An alternative strategy is to compare two areas with and without the invasive species, while attempting to control for any other variables that may confound a comparison between the two (Buckland et al. 2014). Patterns in the presence and abundance of native and non-native species can be used to indicate whether there is a potential for predation or competition between species based on the type of habitat they use (Klop-Toker et al. 2018; Roth, Buehler and Amrhein 2016; Cayuela, Besnard and Joly 2013). The aims of this chapter are to: 1) identify if fewer ponds are occupied by common frogs (*Rana temporaria*) in areas where marsh frogs (*Pelophylax ridibundus*) are present; and

2) determine if any differences in common frog occupancy between areas with and without marsh frogs are due to the presence of marsh frogs, differences in habitat use, or the presence of another predator of common frogs the great crested newt (*Triturus cristatus*). To achieve these aims the interactions between the abundance of common frogs was compared between two areas, one with and one without marsh frogs. To make a comparison more meaningful, propensity modelling was used to limit the difference between areas as far as possible. Variables associated with the presence of common frogs, marsh frogs, and great crested newts were identified to investigate whether habitat preferences affect the likelihood of interactions between these species.

4.3. Methods

4.3.1. Study system

The study area was located in the Wealden area of Kent and Sussex in south east England. Survey ponds were classified into two groups 'ponds within a marsh frog area' and 'ponds in areas without marsh frogs'. The former group contained 49 ponds in southern and central Kent (also used in the occupancy modelling study, Chapter 3) hereafter known as the Kent survey ponds. These ponds had been selected to be within the dispersal distance of a common frog and marsh frog record. A distance of 1.5 km was used as the dispersal distance for common frogs (Safner et al. 2011; Kovar et al. 2009) and 500 m for marsh frogs (Kovar et al. 2009; Holenweg 2001) (Chapter 3, methods section). The 'ponds in areas without marsh frogs' were all selected from the neighbouring county of Sussex and hereafter known as the Sussex survey ponds. The exact distribution of marsh frogs in Sussex is not known. However, the Sussex survey ponds were selected to be at least 5 km from a known marsh frog record to increase the likelihood that the species inhabiting the ponds were not affected by the presence of marsh frogs. Records from 1973 to 2014, held by the Sussex Biodiversity Records

Centre, were used for this purpose. Ponds were identified in Kent and Sussex from a UK pond map provided by ARC trust.

To reduce the likelihood of differences between the ponds in the two survey areas propensity modelling was used to match the Kent survey ponds with ponds in Sussex. Propensity modelling is a technique borrowed from medical research. It is used in situations where the effect of a treatment on subjects needs to be assessed but a properly structured randomised trial is not possible. In randomised experiments comparisons between treatment and control subjects is possible because the subjects in each group represent unbiased samples from a wider population. Direct comparisons may be misleading in non-randomised experiments because the treated subjects may differ systematically from the control subjects (Rosenbaum and Rubin 1983). Propensity modelling works by ensuring the distribution of baseline covariates are as close as possible between control subjects and treatment subjects by calculating a propensity score for each subject (Austin 2011). The propensity score is defined as the probability of a subject receiving the treatment given the covariates (Ho et al. 2011). It is estimated using logistic regression to determine the probability of a subject getting the treatment given the covariates used in the propensity modelling (Ho et al. 2007).

This technique was used to select the survey ponds in Sussex by considering presence of marsh frogs as the 'treatment'. Therefore, ponds in locations where marsh frogs are present are the 'subjects' given the 'treatment' (treatment ponds) and ponds in marsh frog absent areas are the control 'subjects' (control ponds). The effect that was measured was the presence or absence of common frogs, and whether this was affected by the 'treatment' i.e. the presence of marsh frogs in the area. The treatment ponds were the 49 Kent survey ponds already selected and the control ponds were chosen from ponds in Sussex, 5 km from a known marsh frog record. The R package MatchIt (Ho et al. 2011) was used to calculate the propensity score and match the scores for the Kent survey ponds with all ponds in Sussex that were 5 km away from a marsh frog record. Nearest

neighbour matching was used, where the most similar score to the propensity score is used rather than an exact match (Ho et al. 2007).

Environmental variables that may affect the presence of common frogs, according to previous research, were used to match the two survey areas (Table 4.1). For each of the 49 Kent survey ponds eight similar ponds in Sussex were identified (a total of 392 ponds). This provided a greater number of ponds that could be surveyed in Sussex. From the 392 ponds a subset of 52 were surveyed to approximately match the number of Kent survey ponds (Figure 4.1). If permission to survey the pond was denied by the landowner, the nearest accessible pond with values similar to those of the original pond was surveyed. To identify if spatial autocorrelation could lead to a lack of independence between the Sussex survey sites the joint count permutation test was used from the R package spdep (Bivand and Piras 2015) (Chapter 3, methods section).

Table 4.1. Environmental variables that may influence the presence of common frogs. The variables were used in the propensity modelling to find ponds in Sussex similar to survey ponds in Kent to enable a comparison of common frog presence between the two areas. These variables were also used in the logistic regression to determine those which were associated with common frog presence.

Variable Name	Description	Reason for inclusion
Pond density	Number of ponds within a 1 km radius divided by pi. Derived from a UK wide pond dataset provided by ARC Trust.	There is evidence that high pond density is associated with presence of great crested newts (Chapter 3) (Bormpoudakis et al. 2015; ARG UK 2010; Oldham et al. 2000). Great crested newts are known to prey on common frogs (Covaciu-Marcov et al. 2010) and may be affecting common frog abundance. Common frogs need ponds to breed and may initially increase with pond density before higher great crested newt presence starts to have an impact. Therefore, pond density may not have a linear relationship with common frog presence, so the squared version of the variable was also included in the logistic regression.
Linear water features	Combined length of linear water features within a 1 km radius divided by pi. Derived from Ordnance Survey MasterMap Water Network (OS MasterMap Water Network 2015).	In some areas lots of linear water features are linked to livestock grazing which may negatively affect the presence of common frogs (Beebee 1980).
Distance from broadleaved woodland	Distance from broadleaved woodland (m). Derived from the LCM2015 (Rowland et al. 2017)	Common frog presence has been associated with woodland (Boissinot et al. 2015; Van Buskirk 2005). The species distribution modelling results in Chapter 2 also found a positive association.
Distance from arable land	Distance from arable or horticultural land (m). Derived from the LCM2015 (Rowland et al. 2017).	Areas of arable land are likely to be detrimental to amphibians due to issues such as agricultural runoff and overgrowth of ditches (Beebee and Griffiths 2000).
Distance from urban/ suburban areas	Distance from urban and suburban areas (m). Derived from the LCM2015 (Rowland et al. 2017).	Common frogs can often occupy urban and suburban ponds leading to quite high populations (Beebee and Griffiths 2000; Beebee 1979). An interaction with 'Pond density' was included in the logistic regression because great crested newts are less common in urban/suburban areas (Beebee and Griffiths 2000; Inns 2009), so higher densities of ponds near urban areas may not be as detrimental to common frog presence.
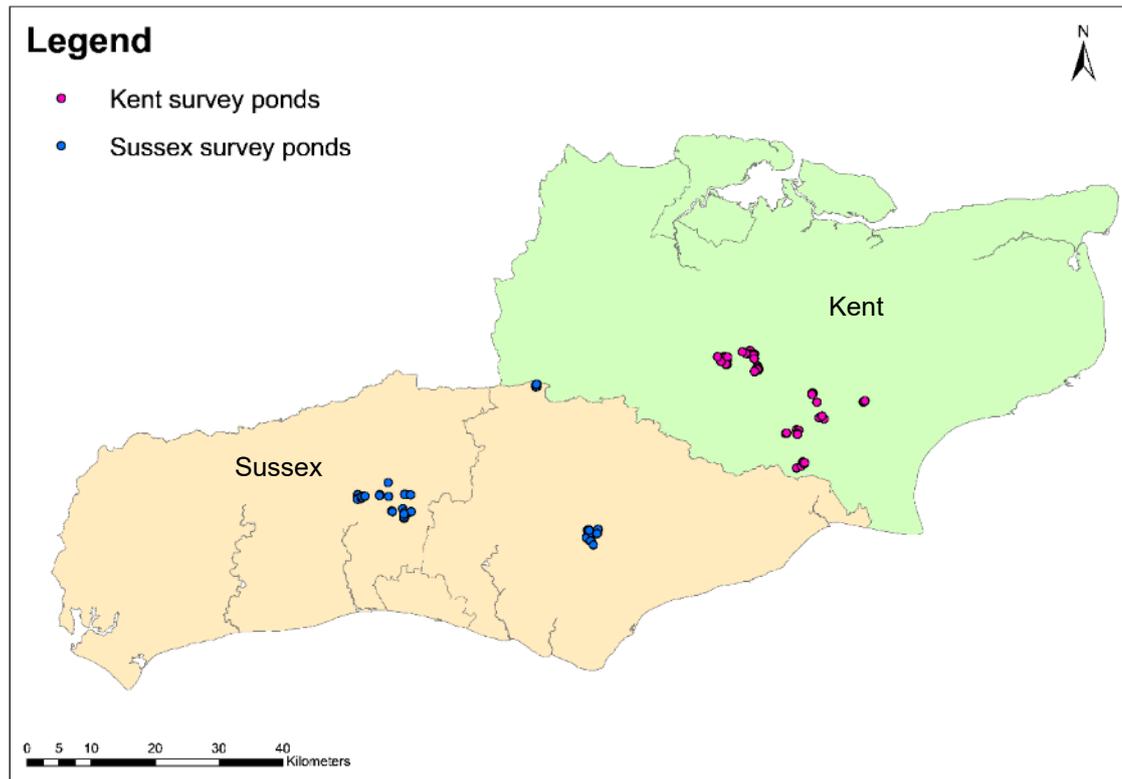
Figure 4.1. Locations of the survey ponds in Kent and Sussex. The 49 survey ponds in Kent were selected to be a maximum of 1500 m from a common frog record and a maximum of 500 m from a marsh frog record. The 52 survey ponds in Sussex were chosen to match, as far as possible, the habitat surrounding the 49 survey ponds in Kent by using propensity modelling. The Sussex ponds were also selected to be at least 5 km from a marsh frog record.

4.3.2. Amphibian survey methods

For both the Kent and Sussex survey areas, the first spawn surveys were carried out in late February or early March 2015, with one or two further surveys in mid to late March. All accessible banks of the ponds were walked around, and individual common frogs spawn clumps were counted in daylight. Spawn mats were measured using the protocol from Griffiths, Raper and Brady (1996). If it was not possible to access all banks, binoculars were used to look for spawn. To establish the presence of great crested newts in the Sussex survey ponds three surveys were conducted between March and early June to coincide with great crested newt breeding season. Due to the withdrawal of permission only 45 of the 52 Sussex survey ponds were surveyed. Surveys were conducted after sunset and the entire margin of the pond was searched using a 1,000,000 candle power torch. Great crested newts at all life stages were recorded.

Distinguishing female smooth and female palmate newts while torching was very difficult because of their similar appearance, so counts of these species were combined under the category 'small newts'. The same variables used in Chapter 3 to model occupancy for great crested newts and marsh frogs were recorded during the spawn surveys or added afterwards using ArGIS10.5. These were: 'Conductivity', 'Shade', 'Macrophytes', 'Fish', 'Fowl', 'Pond area', 'Pond permanence', 'Mean water temperature', 'Linear water features', 'Elevation', 'Distance from broadleaved woodland', 'Distance from arable land', 'Distance from semi-natural grassland', 'HSI', and 'Temperature seasonality' (Chapter 3, Table 4.2).

The matching of variables taken during ponds surveys (e.g. 'Conductivity') was obviously not possible before selection of the Sussex survey ponds. Therefore, these could differ between the Kent and Sussex survey sites. If there is a difference between the number of ponds with common frog spawn present in the survey ponds in Sussex compared to Kent this could be due to disparities between the environmental conditions of the two areas rather than the presence of marsh frogs. For this reason, hypothesis tests were used to identify if there were significant differences between the environmental variables measured in Kent and Sussex. This also provided a measure of how successful the propensity modelling had been.

Several of the environmental variable samples for the Kent and Sussex survey ponds were not normally distributed or had different distributions or variances. Therefore, using the Student's t-test or the Mann-Whitney U-test to test for significant differences in means or medians between samples may be unreliable. For this reason, bootstrapping of the t-statistic was carried out using the function boot.t.test from the R package tpepler/nonpar (Pepler 2017; Efron and Tibshirani 1993). The values from the Kent and Sussex surveys were combined for a variable. From this, random sampling with replacement was undertaken to produce two groups matching the number of survey ponds in Kent and Sussex. The test statistic was calculated, and the process repeated 10,000 times. Each

of the 10,000 replicate t-scores was compared to the observed t-score (the t-score from the original data). The probability of getting a value more extreme than the observed t-score gives the p-value.

4.3.3. Logistic regression analysis

In Kent only eight of the 49 ponds were found to have common frogs. This was too few ponds to reliably identify variables which were significant in predicting common frog presence. For this reason, the analysis was limited to identifying variables affecting presence of common frogs in Sussex and presence of marsh frogs and great crested newts in Kent. The presence/absence of great crested newts was not used as an explanatory variable in the Sussex logistic regression because only five ponds in Sussex were found to contain them.

4.3.4. Analysis

The environmental variable values from the survey ponds in Sussex and Kent were checked for outliers and skews in the distributions. For the logistic regression of the Sussex data the variables 'Pond area', and 'Distance from urban/suburban areas' were square root transformed to reduce effects of outliers. The same variables for the Kent data also needed to be square root transformed.

Spearman's rank correlation coefficients were calculated to identify collinearity between the continuous predictor variables. Where a Spearman's rho ≥ 0.70 was identified the variable that was least likely to be biologically meaningful was not used in the logistic regressions. The continuous variables were centred around zero by subtracting the sample mean and scaled by dividing by the covariate sample standard deviation. Using the survey data from Kent 'Elevation' and 'Maximum temperature warmest month' were found to be correlated (Spearman's rho = - 0.8). Therefore, 'Elevation' was not used in the marsh frog logistic regression because higher temperatures were more likely to directly affect marsh frogs.

4.3.5. Model selection process

A set of biologically meaningful candidate models were specified based on knowledge of common frogs spawning habits, possible interactions with predators, and previous studies (Table 4.2). This reduced the likelihood of identifying spurious associations with variables (Mazerolle 2006; Burnham and Anderson 2002).

Table 4.2. Variables used in the models to predict the common frog spawn presence in Sussex survey ponds.

Variable	Reason for inclusion
Shade	To identify whether the link between common frogs and woodland found in the species distribution models in Chapter 2 is also due to shading of a pond. For example, tree roots around shaded ponds may be providing areas where predatory fish cannot access the frog spawn. Very shaded ponds my dry more frequently because they are close to succession, frequent drying can reduce the presence of predators (Oldham et al. 2000). The squared version of 'Shade' was also included in the models because it may only be high levels of pond shading that affect common frog presence.
Pond area	Common frogs often spawn in urban and suburban garden ponds which tend to be smaller. There may also be a non-linear association with 'Pond area'. Great crested newt presence is less likely in smaller ponds (Oldham et al. 2000) and their impact on common frogs in larger ponds may also be less severe because of the greater possibility of separation. This could mean the impact of great crested newts on common frogs could be more severe in medium sized ponds.
Fish	Fish can prey on common frogs. However, the presence of fish may not affect common frog occupancy (Hartel et al. 2007; Van Buskirk 2005), or have a positive affect as common frogs may use these ponds to avoid predation by great crested newts (Brady 2009).
Pond drying	Ponds that dry out more frequently may reduce the numbers common frog predators such as fish (Oldham et al. 2000).

The same survey data was used in marsh frog and great crested newt logistic regressions as in the occupancy modelling in Chapter 3. However, it was possible to use more variables per model to try and achieve a better fitting model with logistic regression because an extra degree of freedom was available as variation in detection between ponds was not being accounted for (Babyak 2004). The variables that had a low AICc ranking in the occupancy modelling were not used in the marsh frog and great crested newt logistic regressions in this chapter. The variable 'HSI' is based on the great crested newt Habitat Suitability Index (Oldham et al. 2000) and is calculated using several

metrics used as variables on their own e.g. 'Pond density' (Chapter 3, Table 4.2) due to this 'HSI' was not combined with variables used in the calculation of the HSI.

The models were ranked based on the AIC for small sample sizes (AICc) (Mazerolle 2006; Burnham and Anderson 2002). Ranking of the models was carried out using the R package AICcmodavg (Mazerolle 2015). Model averaging was used to determine the strength and precision of each variable (Mazerolle 2006). Due to the low sample size and number of predictor variables, rather than using the global model to calculate the overdispersion factor ($\hat{c}$), candidate models were divided into subsets each with their own 'sub-global' model (Tables, S4.1, S4.2, S4.3) (Burnham and Anderson 2002). If all the sub-global models had a $\hat{c}$ higher than 1 the lowest value of $\hat{c}$ was used as the estimate in the model averaging process and QAICc used to rank the models (Mazerolle 2015; Burnham and Anderson 2002).

4.4. Results

4.4.1. Species occupancy in areas with and without marsh frogs

The results of the spawn surveys found significantly fewer ponds with common frogs present in the Kent survey ponds (Fisher's Exact Test p = 0.005; Table 4.3). The odds ratio of 3.7 indicates that the odds of finding common frog spawn in the Sussex survey ponds is approximately 3.6 times that of finding them in the Kent survey ponds. However, there also were significantly more great crested newts found in the Kent survey ponds than in the Sussex ponds (Fisher's Exact Test p < 0.001). The number of ponds with small newts and common toads were not significantly different between areas (Table 4.3). Two of the Kent survey ponds had both common frog spawn and great crested newts; and two of the ponds had both marsh frogs and common frog spawn. In both cases there were only a few marsh frogs or great crested newts.

Table 4.3. Results of the local level survey showing fractions of amphibian species found in survey ponds in Kent and Sussex. The odds ratios, 95% confidence intervals for the odds ratio, and p values are shown from the Fisher's Exact Test which tests whether proportions of the species are significantly different between Kent and Sussex survey areas. Smooth newts (*Lissotriton vulgaris*) and palmate newts (*Lissotriton helveticus*) were grouped together as small newts.

	Area with marsh frogs present (Kent)	Area with marsh frogs absent (Sussex)	Odds ratio	95% Confidence intervals for the odds ratio	p values
Fraction of ponds with common frog spawn present	8/49	22/52	3.7	1.36 – 11.02	0.005
Fraction of ponds with great crested newts present	22/40	5/44	9.2	2.83 – 36.43	<0.001
Fraction of ponds with small newts present	28/40	31/44	1.0	0.36 – 2.89	1
Fraction of ponds with common toads present	8/40	13/45	1.77	0.587 – 5.65	0.319
Fraction of ponds with marsh frogs present	20/40	0/45	NA	NA	NA

Despite the propensity modelling that attempted to match the Kent and Sussex pond samples, there were differences in environmental variables that may also explain the disparities in common frog occupancy between the Kent and Sussex survey ponds. The results of the bootstrapped Student's t-test on the data from Sussex and Kent showed that there were significant differences between the two areas for 'Pond density' and 'Elevation' (Table 4.4). The mean for 'Pond density' in the Kent survey ponds was higher than in Sussex. 'Elevation' was higher in the Sussex survey ponds.

Table 4.4. Student's t-test results using bootstrapped t-distributions of the environmental variables from the Kent and Sussex survey ponds. Ten thousand replicates were used for the bootstrap.

Variable	Variable mean for the Kent survey ponds (n = 49)	Variable mean for the Sussex survey ponds (n = 52)	Observed t-statistic for Kent Sussex survey ponds	p value for Student's t-test using bootstrapped t-distribution
Pond density	23 ponds/km²	14 ponds/km²	9.43	<0.01
Distance from urban areas	185 m	137 m	1.46	0.15
Distance from broadleaved woodland	249 m	203 m	1.35	0.18
Pond area	372 m²	335 m²	0.53	0.60
Linear water features	4.4 water/km²	4.4 water/km²	0.077	0.94
Shade	40%	53%	-1.81	0.08
Distance from arable land	218 m	316 m	-2.23	0.03
Elevation	36 m	50 m	-3.44	<0.01

4.4.2. Common frog logistic regression Sussex

The highest ranked model for the common frog logistic regression was Model 6 which contained the variables 'Shade', 'Pond density' and 'Pond density²' (Table 4.5). Dividing the QAICc weight of the top model and the next best model to get the evidence ratio indicates that Model 6 is 2.5 times more likely to be the best model compared to Model 11 (Mazerolle 2006). However, Model 6, Model 11, and Model 18 have a Delta QAICc < 2 indicating that the three top models have substantial support (Burnham and Anderson 2002). The Hosmer and Lemeshow goodness of fit tests indicated that there was no evidence that these models were a poor fit. In Model 6 'Shade' and 'Pond density²' were both significantly positively associated with common frog presence (Table 4.6). 'Pond density²' was also positively associated with common frog presence in Model 11 (Table 4.7). The covariates in the three highest ranked models ('Shade', 'Pond density', and

'Distance from urban/suburban areas') are most likely to be important in predicting common frog presence.

Table 4.5. Top ten models rank by QAICc of the logistic regression of common frog spawn presence using survey data from Sussex in areas without marsh frogs. A total of 52 ponds were used in the analysis. An extra parameter is included for the estimation of over dispersion in the parameter count (K).

Model specification	Model no.	K	QAICc	Delta QAICc	QAICc Weight	Quasi log-likelihood
Shade + Pond density + Pond density²	6	5	59.5	0	0.24	-24.1
Pond density + Pond density²	11	4	60.53	1.03	0.15	-25.84
Shade + Distance from urban/suburban areas	18	4	61.49	1.99	0.09	-26.32
Pond area + Distance from urban/suburban areas	16	4	61.62	2.12	0.08	-26.39
Shade	1	3	61.63	2.13	0.08	-27.57
Shade + Pond density	5	4	62.34	2.84	0.06	-26.75
Shade + Distance from urban/suburban areas + Pond area	17	5	63.1	3.6	0.04	-25.9
Shade + Pond area	3	4	63.39	3.89	0.03	-27.27
Shade + Shade²	2	4	63.66	4.16	0.03	-27.41
Linear water features + Shade	9	4	63.69	4.19	0.03	-27.42

Table 4.6. Variable regression coefficients, standard error and p values from Model 6 which was the top ranked model using QAICc in the Sussex common frog logistic regression.

Variable	Regression coefficient estimate	Standard Error	*p* value
Shade	0.67	0.34	0.050
Pond density	-0.34	0.35	0.34
Pond density²	0.74	0.36	0.037

Table 4.7. Variable regression coefficients, standard error and p values from Model 11 which was the model ranked second using QAICc in the Sussex common frog logistic regression.

Variable	Regression coefficient estimate	Standard Error	*p* value
Pond density	-0.29	0.34	0.39
Pond density²	0.79	0.34	0.019

The model averaging showed that 'Shade' and 'Distance from urban/suburban areas' had the most influence and were both positively associated with common frog presence (Table 4.8). The model averaged estimate for 'Pond density' was -0.38 indicating a negative relationship with the presence of common frogs. However, there were broad confidence intervals that stretched across zero for 'Pond density' so the negative association of 'Pond density' is less certain. Three of the four highest ranked models containing 'Pond density' had either a polynomial or interaction term. These were excluded from the model averaging because polynomials or interactions containing the main effect terms have a different interpretation (Mazerolle 2015). The estimates for both 'Pond density' and its polynomial 'Pond density²' show a positive association with presence of common frogs in Model 6 and Model 11 (Table 4.9). Plotting Model 11 (Common frog presence ~ Pond density + Pond density²) shows the predicted presence of common frogs is higher at low and high 'Pond density' but dips at medium levels (Figure 4.2). The model ranking suggests that 'Pond area', 'Distance from broadleaved woodland', and 'Linear water features' are not likely to be good predictors of common frog presence. This is also apparent in the lower model average estimates and the wider confidence intervals (Table 4.8).

The logistic regression is indicating that common frog presence is more likely in more shaded ponds and ponds that are further from urban and suburban areas. There may also be a higher likelihood of common frog presence in ponds in high and low pond density areas.

Table 4.8. Results of model averaging over the candidate models for all the explanatory variables used in the Sussex common frog logistic regression. An over dispersion factor (ĉ) of 1.20 calculated from the eight sub-global models.

Variable	Model-averaged estimate	Unconditional Standard Error	95% Confidence intervals
Shade	0.62	0.38	-0.14, 1.37
Pond density	-0.38	0.34	-1.04, 0.28
Distance from urban/suburban	0.65	0.38	-0.10, 1.40
Pond area	0.35	0.34	-0.32, 1.02
Linear water features	-0.07	0.33	-0.72, 0.58
Distance from broadleaved woodland	-0.28	0.34	-0.94, 0.38

Table 4.9. Estimates of the covariates of the three highest ranked models in common frog logistic regression.

Model	Shade	Pond Density	Pond density^2	Distance from urban areas	Pond Area
Model 6	0.67	-0.34	0.74	NA	NA
Model 11	NA	-0.29	0.79	NA	NA
Model 18	0.44	NA	NA	0.57	NA
Model 16	NA	NA	NA	0.57	0.40
Model 1	0.64	NA	NA	NA	NA
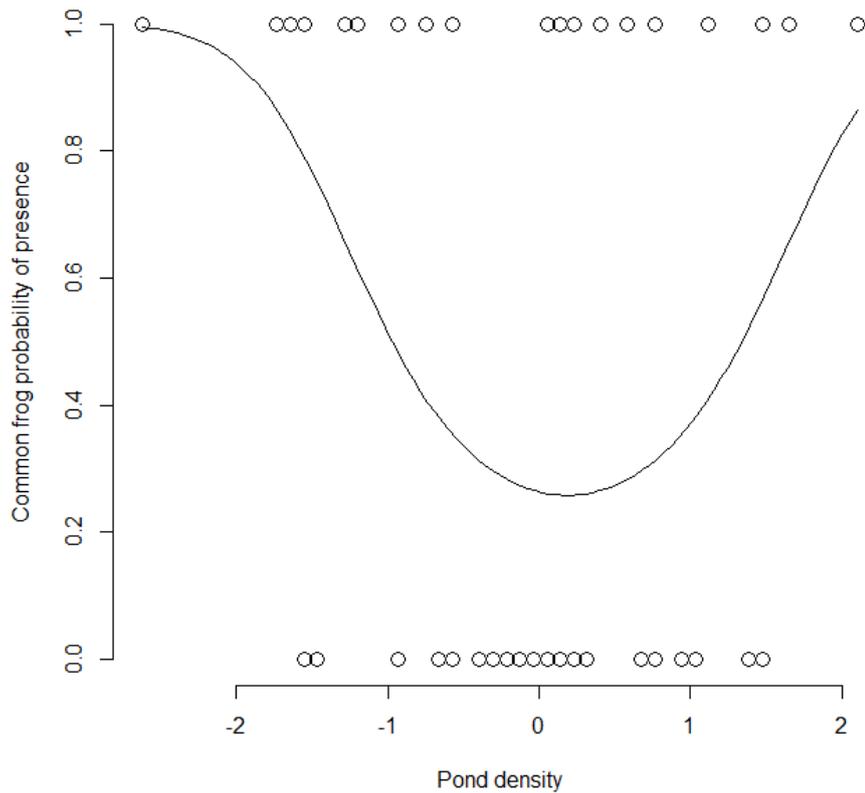
Figure 4.2. The observed common frog occurrence state (presence or absence) of each pond (open circles). The predicted curve of the logistic regression model Common frog presence ~ Pond density + Pond density^2 (Model 11). The x-axis is the centred and scaled version of 'Pond density'.

4.4.3. Marsh frog logistic regression Kent

The top model ranked by AICc was Model 6 which contained 'Linear water features' and 'Shade' (Table 4.10). In Model 6 'Shade' had a negative relationship with marsh frog presence (estimate = -1.36, p = 0.0028) by contrast 'Linear water features' had a positive relationship (estimate = 0.86, p = 0.038). This was also the case for the model averaged estimate of 'Shade' which was -1.30 and the model averaged estimate of 'Linear water features' which was 0.94 (Table 4.11). By plotting this model, the strong influence of both these variables can be seen (Figure 4.3).

Table 4.10. Top ten models rank by AICc of the marsh frog logistic regression models. A total of 40 ponds were used in the analysis. The parameter count (K) includes the intercept.

Model	Model no.	K	AICc	Delta AICc	AICc Weight	log-likelihood
Shade + Linear water features	6	3	45.83	0	0.25	0.25
Shade + Maximum temperature warmest month	8	3	46.26	0.43	0.2	0.44
Macrophyte + Linear water features	16	3	46.42	0.58	0.18	0.63
Shade + Conductivity	9	3	47.43	1.59	0.11	0.74
Shade	3	2	48.47	2.64	0.07	0.81
Macrophyte + Maximum temperature warmest month	15	3	48.78	2.94	0.06	0.86
Shade + Pond density	7	3	49.61	3.78	0.04	0.9
Macrophyte	5	2	49.89	4.05	0.03	0.93
Macrophyte + Conductivity	17	3	50.41	4.57	0.03	0.96
Shade + Distance from broadleaved woodland	10	3	50.81	4.98	0.02	0.98

Table 4.11. Results of model averaging over the candidate models for all the explanatory variables used in the Kent marsh frog logistic regression.

Variable	Model-averaged estimate	Unconditional Standard Error	95% Confidence intervals
Shade	-1.30	0.46	-2.20, -0.40
Linear water features	0.94	0.47	0.03, 1.86
Maximum temperature warmest month	0.81	0.41	0, 1.61
Macrophytes	1.37	0.57	0.26, 2.49
Conductivity	0.74	0.47	-0.18, 1.65
Pond density	-0.45	0.42	-1.26, 0.37
Distance from broadleaved woodland	0.04	0.40	-0.74, 0.83
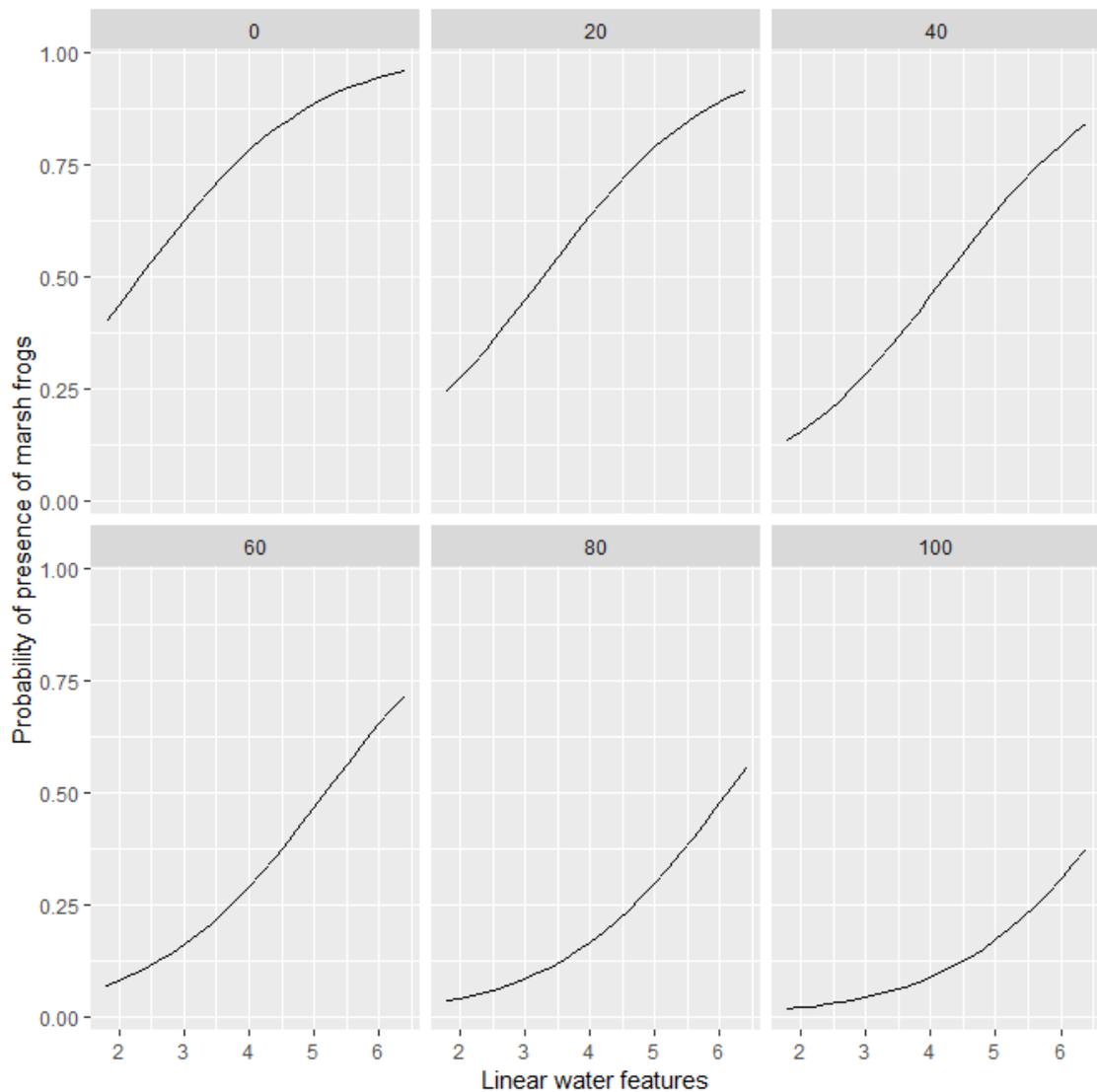
Figure 4.3. Plots of the logistic regression model of marsh frog probability of presence against linear water features length/km² at pond shading levels of 0%, 20%, 40%, 60%, 80%, and 100%. Variables were not scaled and centred so they are more easily interpreted.

As well as 'Linear water features' the model averaging results show that marsh frog presence increases with 'Macrophytes', 'Maximum temperature warmest month', and 'Conductivity'. These four variables were all in models with a delta AICc < 2, so are likely to be important predictors of marsh frog presence. In Model 8 'Maximum temperature warmest month' is positively associated with marsh frog presence and in Model 16 'Macrophytes' also has a positive effect on marsh frog presence (estimate = 1.55, p = 0.007). However, in Model 9 'Conductivity' does not have a significant affect and the model averaging shows that the 95% confidence intervals for 'Conductivity' span across

zero (Table 4.11). This indicates that the positive relationship between marsh frog presence and 'Conductivity' is less certain. Therefore, the results indicate that marsh frogs are less likely to be found in more shaded ponds and are strongly associated with ponds in areas with lots of watercourse in areas with higher summer temperatures.

4.4.4. Great crested newt logistic regression

The results of the great crested newt logistic regression were similar to those in the occupancy modelling in Chapter 3 with both 'HSI' and 'Fish' in high ranking models based on AICc (Table 4.10). Model 5 ('HSI' + 'Water temperature') and Model 4 ('HSI' and 'Precipitation coldest quarter') were the only two with a delta AICc < 2 suggesting these models have the strongest support. The regression coefficient estimates indicated that the likelihood of great crested newt presence increased with 'HSI' in both Model 5 (estimate = 1.61, p = 0.002) and Model 4 (estimate = 1.83, p = 0.002). This positive effect was also indicated by the model averaging for 'HSI' (Table 4.11). 'Water temperature' was negatively associated with great crested newt presence in Model 5 (estimate = -1.25, p = 0.023). This negative effect was also found in the model averaging (Table 4.11). Although 'Fish' was not ranked in the top model using AICc, 'Fish' factor level 2 (ponds where fish are unlikely to be impacting on amphibians) was positively associated with great crested newt presence in Model 2 (estimate = 2.1, p = 0.003) and in the model averaging (Table 4.11). Interestingly, there was a positive association with 'Precipitation coldest quarter' in Model 4 (estimate = 1.3, p = 0.030) which was also found in the model averaging, but this is opposite to what was predicted. A negative relationship was thought likely because higher winter rainfall has been linked to lower great crested newt survival in ponds in Kent (Griffiths, Sewell and McCrea 2010).

The top performing variables based on the AICc rankings and model averaging suggest that great crested newts are associated with ponds that have high HSI scores, few fish, and lower water temperatures, which are in areas with more winter rainfall (Table 4.11). These habitat preferences would not prevent great crested newts from occupying the

same ponds in the same areas as common frogs. Therefore, predation of common frogs

by great crested newts could be occurring in Kent.

Table 4.10. Top ten models rank by AICc of the great crested newt logistic regression models. A total of 40 ponds were used in the analysis. The parameter count (K) includes the intercept.

Model	Model no.	K	AICc	Delta AICc	AICc Weight	log-likelihood
HSI + Water temperature	5	3	41.81	0	0.52	0.52
HSI + Precipitation coldest quarter	4	3	42.3	0.49	0.41	0.93
HSI	1	2	46.74	4.94	0.04	0.97
Fish	2	2	49.18	7.37	0.01	0.99
Fish + Water temperature	3	3	49.91	8.11	0.01	1
Precipitation coldest quarter + Water temperature	7	3	54.42	12.61	0	1
Mean temperature coldest quarter + Water temperature	9	3	55.78	13.98	0	1
Precipitation coldest quarter	6	2	56.47	14.66	0	1
Temperature seasonality	13	2	57.09	15.29	0	1
Precipitation coldest quarter + Water temperature	8	2	57.35	15.55	0	1

Table 4.11. Results of model averaging over the candidate models for all the explanatory variables used in the Kent great crested newt logistic regression. The variable 'Fish' is an ordinal variable with two levels (1 = Fish impacting on amphibians is likely and 2 = Fish are unlikely to be impacting on amphibians).

Variable	Model-averaged estimate	Unconditional Standard Error	95% Confidence intervals
HSI	1.69	0.56	0.59, 2.79
Fish (2)	2.07	0.76	0.69, 3.55
Water temperature	-1.23	0.55	-2.32, -0.15
Precipitation coldest quarter	1.29	0.60	0.12, 2.47
Mean temperature coldest quarter	0.34	0.34	-0.32, 1.01
Temperature seasonality	-0.44	0.36	-1.15, 0.28
Shade	0.34	0.34	-0.32, 1.01
Pond density	0.1	0.35	-0.59, 0.78
Distance from broadleaved woodland	0.15	0.35	-0.53, 0.83
Distance from semi-natural grassland	0.01	0.40	-0.77, 0.79

4.5. Discussion

Common frog presence in the Kent survey ponds that fell within the range of marsh frogs was significantly lower compared to the Sussex survey ponds. Indeed, common frog presence in the Kent ponds was so low that it was impossible to construct meaningful models of their site preferences there. However, this result does not necessarily support the hypothesis that marsh frogs are the cause of a reduced common frog population in Kent. The results of the logistic regressions provided evidence that other factors are at work. In Kent marsh frogs tended to avoid shady ponds. In contrast in Sussex shady ponds tended to be preferred by common frogs. This suggests that common frogs and marsh frogs are less likely to be occupying the same ponds because of different habitat preferences. Therefore, the lower common frog occupancy seen in the Kent survey ponds is less likely to be due to competition or predation by marsh frogs.

The positive association with 'Maximum temperature warmest month' and 'Linear water features' found in the marsh frog logistic regression indicates that marsh frogs favoured

ponds in areas with higher summer temperatures and large areas of linear water features. These results are consistent with the behaviour and preferences of marsh frogs. Marsh frogs tend to bask in sunny spots on the banks of ponds or on floating vegetation (Inns 2009; Firth 1984; Frazer 1983). More shaded ponds are likely to have fewer areas for basking. Marsh frogs rarely stray far from water, and watercourses are also a likely means of spread (Ivanova and Zhigalski 2011; Beebee and Griffiths 2000; Wycherley and Joslin 1996).

The positive link between common frog presence and pond shading is not an intuitive one because frog spawn is often found in shallower unshaded parts of ponds (Inns 2009; Beebee and Griffiths 2000; Savage 1961). Hypotheses can be developed to try and explain the positive association. Where fish are presence spawn is often laid in shallower areas inaccessible to predators (Beebee and Griffiths 2000), it is possible that shaded ponds are more likely to have tree roots growing on the edge of ponds providing safe havens from fish for common frog spawn. Waterfowl that eat frog spawn might not be able to spot spawn when flying over shaded ponds. The positive association with shade may also be linked to the proximity of woodland. Several studies have found a positive association with woodland and common frogs (Boissinot et al. 2015; Vági et al. 2013; Hartel et al. 2010; Van Buskirk 2005; Beebee 1985). There was no strong positive association with 'Distance from broadleaved woodland' in the common frog logistic regression models. However, the LCM2015 has a minimum mappable unit of > 0.5 hectares (Rowland et al. 2017). In areas where broadleaf woodland is not the dominant habitat class patches of woodland smaller than this may be dissolved into the surrounding landscape. Ten of the survey ponds with common frog spawn present were in small patches of woodland which were not represented in the LCM2015 'Broadleaved woodland' layer. Therefore, it is possible that the proximity of small patches of woodland could increase the likelihood of common frogs being present.

The higher occupancy of great crested newts found in the survey ponds in Kent compared to Sussex was consistent with the results of Chapter 2 and other research in suggesting that great crested newts are positively associated with high pond densities (Bormpoudakis et al. 2015; Oldham et al. 2000). 'Pond density' was not found to be associated with great crested newt presence in the logistic regression. However, this may be due to the survey ponds all being in or near a high pond density area. Therefore, a lower pond density in the sample of survey ponds would not affect great crested newt presence. The results of the great crested newt logistic regression do not identify any habitat features that would prevent coexistence with common frogs, unlike the marsh frog result. Consequently, given that great crested newts are known predators of common frogs (Covaciu-Marcov et al. 2010), and the high numbers of great crested newts found in the Kent survey ponds, they may be at least one of the drivers of low common frog occupancy in the Kent ponds.

'Pond density' was found to be a good predictor of common frog presence (Table 4.5). However, rather than confirming the reasons for its inclusion which were that common frog presence would initially increase with pond density then reduce as great crested newt presence became more abundant, the opposite model fit was produced. At lower pond density common frogs were more likely to be present, this dipped at medium values then increased again at higher levels of pond density (Figure 4.2). It is possible that ponds in medium pond density areas are more likely to have characteristics that are less suitable for common frogs, such as deeper ponds that tend to have fish and other predators. There is some evidence to suggest that there are more medium sized ponds in medium and high pond density areas (Brady 2009). Great crested newts are less likely to be found in smaller ponds (Oldham et al. 2000) so at medium pond densities great crested newts could be occupying the medium and larger ponds to the detriment of common frogs. However, the predicted lower probability of common frog presence at

medium pond densities could be a spurious association and an example of the dangers in specifying too complex a model with a low sample size.

The evidence from this study suggests that great crested newts are reducing the presence of common frogs in the study area in Kent, rather than marsh frogs. Common frogs tended to spawn in ponds that were shaded, and marsh frogs were predominantly found in unshaded ponds. Therefore, predation by marsh frogs is unlikely to be reducing common frog presence. The sample sizes were relatively low in this study, so it is possible these results are not representative of the population the ponds were drawn from. However, the results do fit with the behaviour known about the species. Therefore, it would be informative to identify if the relationships found in this study are reflected in a larger sample size.

4.6. Supplementary Information

Table S4.1. Sub-global models created for the Sussex common frog logistic regression (n = 52). The variance inflation factor is calculated by dividing the residual deviance of the model by the degrees of freedom.

Subglobal model	degrees of freedom	Residual Deviance	Over dispersion factor (ĉ)
Shade + Pond area + Shade : Pond area	48	62.96	1.31
Shade + Pond density + Pond density²	48	57.84	1.20
Pond area + Pond area² + Pond density	48	67.08	1.40
Distance from broadleaved woodland + Pond density + Pond area	48	67.08	1.40
Linear water features + Pond density + Shade	48	63.20	1.32
Pond density + Pond permanence	48	67.28	1.40
Shade + Fish	48	61.32	1.28
Pond density + Distance from urban/suburban areas + Pond density : Distance from urban/suburban areas	48	62.16	1.32

Table S4.2. Sub-global models created for the Kent marsh frog logistic regression (n = 40). The variance inflation factor is calculated by dividing the residual deviance of the model by the degrees of freedom.

Sub-global model	degrees of freedom	Residual Deviance	Over dispersion factor (ĉ)
Pond permanence	36	52.63	1.46
Maximum temperature warmest month + Macrophytes	37	42.11	1.14
Broadleaved woodland + Pond density	37	56.15	1.52
Shade + Linear water features + Conductivity	36	36.00	1.00

Table S4.3. Sub-global models created for the Kent great crested newt logistic regression (n = 40). The variance inflation factor is calculated by dividing the residual deviance of the model by the degrees of freedom.

Sub-global model	degrees of freedom	Residual Deviance	Over dispersion factor (ĉ)
Fish + Mean temperature coldest quarter	37	40.10	1.08
HSI + Precipitation Coldest quarter + Water temperature	36	27.58	0.77
Distance from broadleaved woodland + Shade + Pond density	36	52.98	1.47
Temperature seasonality + Distance from semi-natural grassland	37	52.12	1.41

Chapter 5. The use of eDNA to determine the relationship between common frogs, great crested newts and marsh frogs

5.1. Abstract

eDNA techniques can increase the number of sites surveyed by removing the need for repeated site visits. This study benefitted from this by using a large data set of the presence/absence of common frogs (*Rana temporaria*), marsh frogs (*Pelophylax ridibundus*), and great crested newts (*Triturus cristatus*) determined by eDNA metabarcoding from previous research. These data were used to investigate common frog pond occupancy in an area of Kent with marsh frogs and great crested newts. Logistic regression was used to identify the variables associated with common frog presence. Only a weak negative association between common frogs and great crested newts was found. However, a high proportion of the ponds had great crested newts while relatively few had common frogs, supporting the hypothesis that common frog populations were lower in areas with large numbers of great crested newts. Common frog presence was negatively associated with permanent ponds. Only one pond was identified by the eDNA metabarcoding to have marsh frogs suggesting that predation by marsh frogs was not contributing to lower a common frog population. Common frog spawn surveys were conducted on a subset of the ponds from the eDNA metabarcoding The spawn surveys found that there was no link between the detection of common frogs by the eDNA metabarcoding in 2014 and the common frog spawn survey results in 2017.

5.2. Introduction

Recording the absence of a species accurately can often be challenging in ecological research. With rare or cryptic species, it can be difficult to determine true absence

(Barata, Griffiths and Ridout 2017; MacKenzie et al. 2006; Gu and Swihart 2004; MacKenzie et al. 2002). If the potential for false absence is not accounted for this can lead to erroneous conclusions (Guillera-Arroita and Lahoz-Monfort 2012; Guillera-Arroita, Ridout and Morgan 2010). Occupancy modelling solves this problem but requires repeated surveys, which may limit the achievable research objectives (Barata, Griffiths and Ridout 2017). Amphibians in most cases breed in water bodies which provides a convenient focal point for detection. However, even in the UK where the road network is extensive and there are very few landscape features that make access difficult, resurveying of ponds to enable adequate detection of amphibians can be time-consuming. Two to nine surveys using standard survey methods are required to reach a 95% confidence level for detection of the widespread UK native amphibians (Sewell, Beebee and Griffiths 2010).

The development of eDNA techniques to detect species from water samples in many cases has reduced the need to repeat surveys to account for detection (Biggs et al. 2015; Jerde et al. 2011; Ficetola et al. 2008). DNA is shed by organisms in skin cells, faecal matter, and other waist products (Buxton, Groombridge and Griffiths 2017; Lydolph et al. 2005; Waits and Paetkau 2005). If the environmental conditions are favourable this DNA can remain in water for over three weeks (Dejean et al. 2012) and can be detected from water samples with high accuracy using PCR techniques such as quantitative PCR (qPCR) or DNA metabarcoding (Valentini et al. 2016; Goldberg et al. 2013; Ficetola et al. 2008). This means only one visit may be required to a site to determine a species' presence, so many more sites can be surveyed. For example, in the case of UK amphibians this has been utilised to estimate occurrence of the great crested newt (*Triturus cristatus*) in three counties in England (Bormpoudakis et al. 2015).

Another benefit of eDNA techniques is that once water samples have been taken it is possible to use the same samples to detect other species or even a broad spectrum of species which had not been planned for in the original survey (Harper et al. 2018a;

Deiner et al. 2017; Taberlet et al. 2012). The present study has benefitted from this utility. eDNA metabarcoding had been used to detect vertebrate species in water samples taken as part of the Natural England's Evidence Enhancement Programme (Harper et al. 2018a). The metabarcoding results for common frog (*Rana temporaria*), great crested newts, and marsh frogs (*Pelophylax ridibundus*) from water samples taken from ponds in Kent were made available for this study. The water samples were taken from ponds surveyed in the Ashford area of Kent that corresponded to areas of high pond density. This area is similar to the locations of the survey ponds used in Chapters 3 and 4 where common frogs were only found in low numbers, but marsh frogs and great crested newts were more common. It was possible to relate the results of the metabarcoding to environmental variables that affect the presence of common frogs in this area. Additionally, these data can be used to identify if great crested newts or marsh frogs are negatively associated with common frog presence in ponds.

Common frog presence in ponds established by eDNA techniques do not distinguish between ponds where common frogs breed (breeding ponds) and where they are present only (non-breeding ponds). Great crested newts prey on common frog spawn and tadpoles (Covaciu-Marcov et al. 2010) but not on adult frogs. Therefore, it may only be spawning ponds that are negatively associated with great crested newts. The accuracy of eDNA techniques can also vary depending on the timing of surveys (Buxton et al. 2017; Dejean et al. 2012). The water samples used in the metabarcoding were taken in May and June 2014. These months are when the concentration of great crested newt eDNA within ponds peaks as breeding is coming to an end (Buxton et al. 2017). Although common frogs breed earlier in the year, it is possible frog eDNA may still be released in May-June if tadpoles are present.

This chapter aims to determine: 1) if the presence of common frogs in a high pond density area of Kent is affected by great crested newts or marsh frogs based on eDNA metabarcoding presence/absence data; 2) what environmental variables are associated

with common frog presence using this data; and 3) whether common frog breeding ponds alone have different associations with environmental variables compared to the eDNA metabarcoding data. To test these issues, logistic regression was used to identify significant associations with environmental variables and the presence of common frogs from eDNA metabarcoding data recorded in 2014. A subset of the ponds surveyed using eDNA metabarcoding were resurveyed for common frog spawn in 2017 to identify breeding ponds.

5.3. Methods

5.3.1. Comparing eDNA detection with conventional visual encounter surveys

Water samples from 508 ponds collected during the spring of 2014 for Natural England's Evidence Enhancement Programme were subjected to DNA metabarcoding (Harper et al. 2018a; Harper, et al. 2018b). The collection of the water samples was conducted following a standardized protocol (Biggs et al. 2015). The presence/absence status of common frog, great crested newts, and marsh frogs for the 508 ponds was determined using eDNA metabarcoding by Harper (2018a,b) at the University of Hull. The DNA metabarcoding workflow is fully described in Harper et al. (2018b). A subset of these data containing 123 ponds in the Low Weald in central Kent were made available for this study. From the 123 ponds, the metabarcoding identified 24 ponds that contained common frogs, 40 that contained great crested newts, and one that contained marsh frogs.

To test if the metabarcoding results were likely to be detecting both breeding and non-breeding common frog ponds, spawn surveys were conducted on 44 ponds in Kent that had been tested by the metabarcoding. Common frogs had been identified as being present in 11 of these ponds in 2014 by the eDNA metabarcoding. The chi-squared test was used to measure whether detection of common frogs in 2014 was linked to presence of common frog spawn in 2017. The McNemar's test was used to identify if there were more changes from common frog presences in 2014 to absences in 2017 or the reverse.

The spawn surveys were conducted between 13th March and 27th March 2017. For each spawn survey the banks of the pond were walked around in daylight and the presence of spawn recorded. If some banks were inaccessible binoculars were used to search these areas for spawn. Two spawn surveys were conducted on each pond between one and two weeks apart to increase the chances of encountering frog spawn which can last approximately ten days to two weeks (Beebee and Griffiths 2000).

5.3.2. Determining predictors of common frog occupancy

The great crested newt habitat suitability Index metrics (ARG UK 2010; Oldham et al. 2000) were recorded in May 2014 for each of the 123 ponds subjected to the DNA metabarcoding. This information was combined with the environmental variables used to predict presence of common frog and great crested newts in Chapters 2,3 and 4. These were: 'Pond density', 'Linear water features', 'Distance from broadleaved woodland', Distance from urban/suburban areas', 'Distance from arable land', and 'Precipitation coldest quarter'.

A variable was included that was a measure of the distance of the survey ponds to the nearest marsh frog record ('Distance from marsh frogs'). Marsh frog presences recorded between 2005 and 2014 to 100 m x 100 m precision from the Kent Reptile and Amphibian Group (KRAG) were used. The records were verified as correct by KRAG using photographs or were from a trusted recorder (Brady 2010). Marsh frog records recorded by the author during amphibian surveys were also included. This variable was created to see if the proximity of a marsh frogs could negatively affect common frog presence. Grass snakes (*Natrix helvetica*) prey on both marsh frogs and common frogs. Large grass snake populations can coincide with the presence of marsh frogs (Gregory and Isaac 2004). It is possible that the presence of marsh frogs in an area is increasing the numbers of grass snakes to a level higher than would normally be present. Grass snakes may then be preying on common frogs in the vicinity and reducing their population size. This variable may link proximity of marsh frogs to a reduced likelihood of common frog

presence in a pond. However, there are many other parameters that may affect the size of grass snake populations so it cannot be assumed that the presence of marsh frogs means a higher population of grass snakes.

Logistic regression was used to identify variables associated with the presence of common frogs. Separate analyses were conducted on the results from the metabarcoding on 123 ponds and the results of the spawn surveys of 44 ponds in 2017. For both analyses a set of candidate models were specified. Each model was specified to test a hypothesis or combination of hypotheses relating to common frog presence (Mazerolle 2006) (Table 5.1).

Table 5.1. Variables used in the logistic regressions of common frog presence data. Each variable was used in the model to test a hypothesis (third column). Data used in the logistic regression was obtained in 2014 and analysed with eDNA metabarcoding (Harper et al. 2018a). Spawn survey data from 2017 was also used in a separate modelling process.

Variables	Description of variable	Hypothesis to test
Great crested newt	Presence or absence of great crested newts in 2014 determined using eDNA metabarcoding.	Whether common frogs were negatively associated with great crested newts.
Great crested newt : Pond area	Presence or absence of great crested newts with an interaction term with 'Pond area' included.	Whether any negative effects of great crested newts on common frogs reduced with increasing 'Pond area'. In large ponds common frog tadpoles may have less chance of being eaten by great crested newts because of the spatial separation particularly if the great crested newt population is small.
Shade	Percentage of pond shaded to at least 1 m from the shore.	Whether the positive association of common frog presence and 'Shade' identified in Chapter 4 was replicated in Kent.
Distance from broadleaved woodland	Distance from broadleaved woodland (m). Derived from the LCM2015 (Rowland et al. 2017).	Whether research showing woodland to be linked to common frog presence (Boissinot et al. 2015; Van Buskirk 2005) would be reproduced in this model.
Pond Density	Number of ponds within a 1 km radius divided by pi. Derived from a UK wide pond dataset provided by ARC	If 'Pond density' was negatively associated with common frog presence.

	Trust.	
Distance from marsh frogs	Distance from the nearest marsh frog species record using records from 2005 to 2014.	If 'Distance from marsh frogs' affected common frog presence. Presence of marsh frogs may inflate the grass snake population and reduce common frog presence in the area due to increased predation.
Pond permanence	The permanence of the pond determined by the frequency a pond dries from: dries 8 or 9 years in 10 = 1; dries 3 to 8 years in 10 = 2; dries every 1 or 2 years in 10 = 3; and never dries = 4. The frequency a pond dried was assessed using local knowledge and surveyor judgement. Surveyor judgement was based on water level at the time of the survey taking seasonality into account (Harper et al. 2018a; ARG-UK, 2010; Oldham et al. 2000).	If common frogs were negatively associated with permanent ponds that may have increased numbers of predators.
Distance from urban/suburban areas	Distance from urban and suburban areas (m). Derived from the LCM2015 (Rowland et al. 2017).	Whether the distance from urban and suburban areas affects common frog presence because of their ability to utilise urban and suburban garden ponds (Beebee and Griffiths 2000; Beebee 1979).
Linear water features	Combined length of linear water features per km². Derived from Ordnance Survey MasterMap Water Network (OS MasterMap Water Network 2015).	Whether density of watercourses is a negative predictor of common frog presence because of a link between intensive grazing and areas with watercourses (Beebee 1980).

The continuous variables specified in the models were plotted using histograms and Cleveland plots to identify extreme values that may affect the models produced (Zuur, Ieno and Smith 2007). The continuous variables were put into the same scale by subtracting the variable's mean and dividing by the standard deviation. Spearman's rank correlation coefficients were calculated to identify collinearity between variables. There were no Spearman's rho greater the 0.7 so collinearity was not considered a problem (Proosdij et al. 2016; Dormann et al. 2013).

5.4. Results

5.4.1. Assessing change in common frog occupancy between 2014 and 2017

The 2017 common frog spawn survey found 13 ponds with common frog spawn. In nine

of the 13 ponds no common frog DNA was identified in the metabarcoding of the water

samples taken in 2014 (Table 5.2). Common frogs were identified as absent from 24

ponds in both 2014 metabarcoding and the 2017 spawn surveys. Only four ponds were

found to be positive for common frogs in both 2014 and 2017 spawn surveys. The chi-

squared test indicated that there was no relationship between the results of the 2014

metabarcoding and 2017 spawn surveys (X-squared = 0.04 p = 0.85). There was also a

non-significant result for the McNemar's test (McNemar's chi-squared = 0.063, p = 0.80)

meaning that ponds were no more likely to change from present to absent than absent

to present between the two survey periods.

Table 5.2. Contingency table indicating the agreement between the results of metabarcoding of 44 ponds surveyed in 2014 and the results of common frog spawn surveys on the same ponds in 2017.

		Spawn surveys on 44 ponds in 2017	
		Common frog spawn present	Common frog spawn absent
DNA metabarcoding on 44 ponds surveyed in 2014	Common frog present	4	7
	Common frog absent	9	24

5.4.2. Logistic regression on common frog presence identified by eDNA metabarcoding in 2014

In the logistic regression identifying which variables were most associated with common

frog presence in the 2014 survey data, the ordinal variable 'Pond permanence' was in

the three highest ranked models (Table 5.3). In the highest ranked model (Model 19) the

regression coefficient for 'Pond Permanence' factor level 4 (ponds that never dried) was

negatively associated with the presence of common frogs (estimate = -2.02, p = 0.007).

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Model averaging uses averages of the regression coefficients for each variable from all the models assessed in the model selection. It weights the regression coefficients using the AICc weights from their respective models. This can reduce bias and increase precision because it uses information from all the models rather than just relying on the top ranked model (Mazerolle 2006; Burnham and Anderson 2002). Confidence intervals from the model averaging can then be used to assess the magnitude of the effect for each variable; narrow confidence intervals indicating a precise estimate (Mazerolle 2006).

The relatively high regression coefficient in the model averaging showed there was a strong negative effect of ponds that never dried on common frog presence (Table 5.4). The 95% confidence intervals did not cross zero indicating there is little doubt about the direction of the effect. The wide confidence intervals suggest the degree of negative relationship is less certain. In Model 19 'Pond density' was negatively associated with common frog presence (estimate = -0.67, p = 0.024). This was also reflected in the model averaging of 'Pond density' (Table 5.4). However, in both cases the regression coefficient was small suggesting the negative association of common frog presence with 'Pond density' was not a strong effect. The model averaging indicated the negative effect was consistent across the models in which 'Pond density' was included. All the other variables used in the models had results indicating their effect on the presence of common frogs may be marginal. The 95% confidence intervals from the model averaging for each of the other variables crossed zero. There was little evidence of any other strong effects on common frog presence in the model.

Table 5.3. Top ten models ranked by AICc of the logistic regression of common frog presence using eDNA metabarcoding results from survey ponds in Kent. A total of 123 ponds were used in the analysis.

Model specification	Model no.	K	AICc	Delta AICc	AICc Weight	Cumulative weight	Log-likelihood
Pond density + Distance from broadleaved woodland + Pond permanence	19	6	116.57	0	0.26	0.26	-51.92
Pond permanence	11	4	117.43	0.87	0.17	0.43	-54.55
Pond density + Pond permanence + Distance from urban/suburban areas	20	6	117.91	1.34	0.13	0.56	-52.59
Pond permanence + Pond area	14	5	119.09	2.53	0.07	0.64	-54.29
Pond density	17	2	119.51	2.95	0.06	0.7	-57.71
Great crested newt + Pond permanence	12	5	119.52	2.96	0.06	0.76	-54.51
Distance from urban/suburban areas + Pond density + Distance from broadleaved woodland	2	5	119.66	3.1	0.06	0.81	-54.57
Distance from urban/suburban areas	5	2	120.48	3.92	0.04	0.85	-58.19
Pond density + Distance from broadleaved woodland	18	3	120.51	3.95	0.04	0.89	-57.16
Distance from marsh frog + Pond density	24	3	121.22	4.65	0.03	0.91	-57.51

Table 5.4. Results of model averaging over the candidate models for all the explanatory variables used in the logistic regression of common frog presence from metabarcoding of eDNA. 'Pond permanence' is an ordinal with four factors: dries 8 or 9 years in 10 = 1; dries 3 to 8 years in 10 = 2; dries every 1 or 2 years in 10 = 3; and never dries = 4. The factor level 1 is represented as the intercept. The variables highlighted in bold have the most support in the model because the confidence intervals indicate there is little doubt in the direction of their effect (confidence intervals do not cross zero).

Variable	Model-averaged estimate	Unconditional Standard Error	95% Confidence intervals
Great crested newt	-0.09	0.36	-0.8, 0.62
Pond area	-0.22	0.31	-0.83, 0.31
Pond permanence (2)	-0.19	0.66	-1.49, 1.11
Pond permanence (3)	-0.48	0.86	-2.16, 1.21
Pond permanence (4)	**-1.88**	**0.76**	**-3.38, -0.38**
Distance from urban/suburban	0.36	0.34	-0.30, 1.01
Pond density	**-0.60**	**0.29**	**-1.18, -0.02**
Distance from broadleaved woodland	0.36	0.31	-0.24, 0.97
Distance from marsh frogs	0.07	0.26	-0.43, 0.57
Linear water features	0.12	0.27	-0.41, 0.64

5.4.3. Logistic regression on common frog presence identified by spawn surveys in 2017

The variables 'Great crested newt' and 'Distance from broadleaved woodland' are in the top two models as ranked by QAICc (Table 5.5). In the highest ranked model (Model 9) 'Distance from broadleaved woodland' is significantly negatively associated with spawn presence (estimate = -0.93, p = 0.039) indicating that the likelihood of spawn presence decreases with distance from woodland. However, the regression coefficient is not very high suggesting a weak negative relationship. The model averaging also shows a negative association with 'Distance from broadleaved woodland' but the 95% confidence intervals cross zero and are quite wide suggesting there is a lack of precision (Table 5.6). The model averaging shows that 'Great crested newt' is negatively associated with presence of common frog spawn but has very wide confidence intervals that span across zero, again suggesting a lack of precision (Table 5.6). Also, 'Great crested newt' is not significant in the top ranked model (estimate = -2.15, p = 0.070). The model averaging

showed all the other variables had 95% confidence intervals which crossed zero and

QAICc weights were also low. This suggests there are no variables with very strong

support.

Table 5.5. Top ten models rank by QAICc of the logistic regression of common frog spawn presence in 2017 using survey data from Kent. A total of 44 ponds were used in the analysis. An extra parameter is included for the estimation of over dispersion in the parameter count (K). (c-hat estimate = 1.17).

Model specification	Model no.	K	QAICc	Delta QAICc	QAICc Weight	Quasi log-likelihood
Great crested newt + Distance from broadleaved woodland	9	4	47.14	0	0.24	0.24
Distance from broadleaved woodland	8	3	48.69	1.55	0.11	0.35
Shade + Great crested newt	15	4	49.05	1.91	0.09	0.45
Great crested newt	4	3	49.3	2.16	0.08	0.53
Great crested newt + Distance from urban/suburban areas	12	6	49.43	2.29	0.08	0.6
Shade	14	3	50.13	2.99	0.05	0.66
Pond density	16	3	50.65	3.5	0.04	0.7
Pond permanence	11	5	50.68	3.53	0.04	0.74
Pond density + Distance from broadleaved woodland	17	4	51.1	3.96	0.03	0.77
Great crested newt + Distance from urban/suburban areas	6	4	51.47	4.33	0.03	0.8

Table 5.6. Results of model averaging over the candidate models for all the explanatory variables used in the common frog spawn logistic regression (c-hat estimate = 1.17). 'Pond permanence' is an ordinal with four factors: dries 8 or 9 years in 10 = 1; dries 3 to 8 years in 10 = 2; dries every 1 or 2 years in 10 = 3; and never dries = 4. The factor level 1 is represented as the intercept.

Variable	Model-averaged estimate	Unconditional Standard error	95% Confidence intervals
Great crested newt	-2.04	1.28	-4.55, 0.47
Pond area	0.20	0.44	-0.67, 1.06
Pond permanence (2)	1.93	1.37	-0.76, 4.62
Pond permanence (3)	2.93	1.73	-0.47, 6.31
Pond permanence (4)	0.38	1.37	-2.3, 3.06
Distance from urban/suburban	0.12	0.40	-0.68, 0.91
Pond density	-0.30	0.44	-1.16, 0.56
Distance from broadleaved woodland	-0.84	0.49	-1.79, 0.11
Distance from marsh frogs	-0.18	0.39	-0.94, 0.58
Linear water features	-0.08	0.41	-0.88, 0.72
Shade	0.61	0.41	-0.19, 1.42

The results of the logistic regression on the common frog spawn survey data indicate that none of the variables are reliable predictors of common frog spawn presence. There may be a weak positive relationship with proximity to woodland and common frog presence. Absence of common frogs may be weakly linked to presence of great crested newts. The 'Pond permanence' factor level 3 (indicating a pond drying every 1 or 2 years in 10) is positively associated with common frog presence but again with wide confidence intervals. There is no evidence that proximity of marsh frogs sites has an effect.

5.5. Discussion

The logistic regression on the metabarcoding results suggests the presence of common frogs is not associated with the presence of great crested newts (Table 5.4). Predation by great crested newts may still be a reason for lower common frog populations. If great crested newt numbers are uniformly high in this area common frogs may have to occupy ponds with great crested newts because they have a presence in most of the ponds

suitable for amphibians. The number of ponds with common frog DNA detected was relatively low (24/123) compared to the number of ponds with great crested newts (40/123). This reflects the results of the species distribution modelling which predicted lower probability of presence for common frogs and higher presence for great crested newts in this area (Chapter 2).

It was not possible to assess whether eDNA techniques picked up both common frog breeding and non-breeding ponds. Common frogs will use ponds they do not breed in. During surveys for common frog spawn carried out in Sussex in 2015 (Chapter 4), adult common frogs were discovered in April in several ponds where no spawn was found earlier in the year. If non-breeding ponds were being detected it would provide another reason for the absence of a negative association between great crested newt presence and common frog presence in the metabarcoding logistic regression.

The difference in breeding times between common frogs and great crested newts could explain why the metabarcoding may be detecting adult common frogs rather than spawning ponds. Peak detection of eDNA can vary depending on breeding times (Buxton et al. 2017; Thomsen et al. 2012). The eDNA detection rate for great crested newts peaks in May (Buxton et al. 2017), which is when the water samples for the metabarcoding analysis were taken. Common frogs usually breed in early spring (Beebee and Griffiths 2000), so detection of common frogs may be lower by May. Degradation of DNA within ponds varies depending on the pond conditions but can be just a few days and is unlikely to be beyond 22 days (Buxton, Groombridge and Griffiths 2017; Barnes et al. 2014; Thomsen et al. 2012). Abundance has an effect on the detectability of eDNA (Biggs et al. 2015). It is possible that the metabarcoding results were only detecting ponds with larger common frog breeding populations. In these ponds there are likely to be higher numbers of tadpoles which survived long enough for their DNA to be present in the ponds when sampling took place. This would also explain the non-significant chi-squared

results suggesting that presence/absence of common frogs in 2014 metabarcoding results were not linked to presence/absence of common frog spawn in 2017.

Neither the metabarcoding or spawn logistic regressions found a negative association with 'Distance from a marsh frog record'. Only one pond with marsh frogs was detected in the metabarcoding results despite the survey ponds being within marsh frog inhabited areas. This suggests it is unlikely that predation by marsh frogs is reducing common frog presence in this survey sample.

The results of the McNemar's test indicated that there was no significant difference in the proportion of ponds changing from common frog positive to common frog negative compared to the reverse. The difference in common frog presence between 2014 and 2017 may just indicate changes in occupancy of spawning ponds. Common frogs do show a certain amount of breeding pond fidelity although spawn may not be found in the same ponds every year (Loman 2016; Beebee and Griffiths 2000; Savage 1961). It is possible false positives could have been caused by the transfer of common frog DNA between ponds by waterfowl or other terrestrial species that visit different ponds (Harper et al. 2018b; Klymus et al. 2017). There was variation between the metabarcoding and qPCR results from the same water samples when detecting great crested newts (Harper, Lawson Handley et al. 2018). The spawn surveys do not produce false positives, but false negatives are possible although less likely given the visibility of frog spawn (Sewell, Beebee and Griffiths 2010).

The logistic regression on the metabarcoding data showed that there was a strong negative association with common frog presence and ponds that did not dry out (Table 5.4). Common frogs can be present in a wide variety of waterbody types (Beebee and Griffiths 2000). In permanent ponds predators of amphibians, such as predatory fish, can build up making them less favourable habitat (Oldham et al. 2000). Modelling of the full metabarcoding data set of 508 ponds over Kent, Cheshire, and Lincolnshire indicated that great crested newts were positively associated with pond depth (Harper et al.

2018a). 'Pond depth' is likely to be correlated to 'Pond permanence'. The negative association with deeper ponds could reflect the common frogs ability to adapt to pond drying (Laurila and Kujasalo 1999), compared to the great crested newt that needs a longer hydroperiod.

Chapter 6. General discussion

Non-native species can exert a variety of different effects on the ecosystems they invade (Kraus 2015; Bucciarelli et al. 2014). Effects can range from the negative to positive depending on the native species being studied (Shine 2010; Copp et al. 2009; Crossland, Alford and Shine 2009; Gregory and Isaac 2004). Many other threats such as habitat loss and fragmentation will continue to affect species all over the planet so there will be a continual debate about resource allocation in conservation (Wilson et al. 2006). In this context it will be vitally important to determine the likely impact of invading species on native species so money can be allocated to where it is most needed. This research has looked at whether a non-native species the marsh frog (*Pelophylax ridibundus*) is affecting the distribution of a native species (*Rana temporaria*) in the UK. In doing so, it provides insights into how the impact of a non-native can be assessed in situations where it is difficult to separate other detrimental forces from the impacts of the non-native species.

The methodology behind this thesis has been to use species distribution models (SDMs) at both regional and local level to examine the distribution and potential interactions between the species. SDMs can be a useful tool at a landscape scale for many purposes such as: identifying suitable habitat for endangered species; predicting invasive species range expansions; and predicting species loss under climate change (Ihlow et al. 2016; Struebig et al. 2015; Araújo, Thuiller and Pearson 2006). However, care must be taken to reduce bias towards more easily sampled areas that can often be found in presence only species records (Kramer-Schadt et al. 2013; Syfert, Smith and Coomes 2013; Phillips et al. 2009). The presence only nature of the data also means that when inferences are made, they should allow for bias due to variability in detection probabilities of species (Guillera-Arroita et al. 2015; Lahoz-Monfort, Guillera-Arroita and Wintle 2014). These limitations do not prevent SDMs from being able to predict where a non-native

may be present and where impacts may occur on native species (Klop-Toker et al. 2018; Ihlow et al. 2016; Sen et al. 2016; Buckland et al. 2014).

The SDMs in Chapter 2 showed that predicted presence for common frogs in Kent was lower in areas where marsh frogs had a higher likelihood of presence. It was then necessary to determine what may be causing these divergent distributions. The aquatic nature of marsh frogs fitted the predicted distribution which was strongly associated with the presence of linear water features and pond density. In contrast, the most influential variables in the common frog SDM were the negative effects of linear water features and pond density. This explained the patterns seen in the distribution models but not the reasons behind them.

Many of the areas with the greatest density of linear water features in Kent and Sussex are coastal or are next to tidal areas of rivers. The surface water in such areas can have higher salinity levels (Firth 1984; Beebee 1980). Common frogs are less tolerant of high salinity levels than marsh frogs (Beebee and Griffiths 2000; Innocenzi 1995). This may be one of the reasons why common frogs were negatively associated with linear water features. Beebee (1980) also suggested that increased livestock grazing may be another of the causes of low common frog presence in a marsh dyke habitat in Sussex where marsh frogs had established themselves. Marsh frogs tend to stay by water and are less reliant on terrestrial habitat than common frogs so are likely to be less affected by grazing (Kovar et al. 2009; Holenweg 2001; Beebee 1980). This may be another reason for the negative association with common frogs and linear water features. However, further investigation into whether there is a positive correlation between areas with many watercourses and intensively grazed grassland would be needed to provide stronger evidence for this hypothesis.

In high pond density areas great crested newts (*Triturus cristatus*) may be reducing the presence of common frogs through predation. This might be exacerbated by the existence of marsh frogs in these areas (Brady 2009). The SDMs in Chapter 2 indicated

that great crested newts had a higher predicted probability in high pond density areas of Kent. Kent has a much higher pond density than Sussex. This could be causing higher densities of great crested newts resulting in fewer common frogs compared to places with lower levels of pond density. The SDMs showed that a greater proportion of the common frog distribution overlapped with the great crested newt distribution in Sussex. This was consistent with the hypothesis that greater densities of great crested newts were the cause of lower common frog presence, although other factors may also be involved.

The SDMs identified an area indicating a lower likelihood of common frogs and higher likelihood of great crested newts and marsh frogs. The area in question was in central and southern Kent and had a higher pond density level compared to other areas in Kent. The local level study (Chapter 3 and 4) was used to identify whether common frog presence was lower in this area and if this was likely to be due to the presence of great crested newts and marsh frogs.

The local level study found common frog presence was lower in areas with marsh frogs present (Kent survey ponds) compared to areas where marsh frogs were absent (Sussex survey ponds). Great crested newts occurred more frequently in ponds in Kent. However, the logistic regression models identified a negative association with marsh frog presence and pond shading in the survey ponds in Kent. This was in direct contrast to the positive association of common frog presence with pond shading found by the logistic regression on survey ponds in Sussex. These results indicate marsh frogs and common frogs may have differing habitat preferences which could reduce the likelihood that marsh frogs were negatively affecting common frog presence. No such difference was found between common frog and great crested newt pond preferences. Predation by great crested newts therefore, was more likely to explain the low presence of common frogs in the survey ponds in Kent.

Pond shading has been linked to reduced abundance in some amphibian species because it can lead to slower larval growth rates (Schiesari, Peacor and Werner 2006; Halverson et al. 2003; Skelly, Freidenburg and Kiesecker 2002). Common frogs tend to spawn in the warmer shallower areas of a pond and faster larval growth rates have been observed in warmer water (Oromi et al. 2015; Inns 2009; Beebee and Griffiths 2000). Therefore, the positive association with common frogs and pond shading in Chapter 4 could be due to other factors such as a correlation between pond shading and small patches of woodland. Woodland and hedgerows can provide benefits for terrestrial amphibians such as food, shelter, or as corridors for dispersal (Boissinot, Besnard and Lourdais 2019; Boissinot et al. 2015; Vos et al. 2007; Denoel and Lehmann 2006; Van Buskirk 2005; Marnell 1998). Boissinot et al. (2015) found a positive association with common frog presence and patches of woodland in a farming landscape in western and central France which had a network of hedgerows connected to small areas of woodland. This type of habitat is similar to the landscape of the survey areas for this study. It is feasible that the positive association with pond shading is reflecting benefits to common frog populations of woodland rather than pond shading.

The negative association of marsh frog to pond shading is consistent with marsh frog behaviour of basking on sunny banks or floating vegetation (Inns 2009; Innocenzi 1996; Firth 1984; Frazer 1983). The tendency of marsh frogs to stay near water means that woodland patches may not be as important for foraging compared to other species of amphibian (Beebee 1980). Boissinot, Besnard and Lourdais (2019) found a negative association with marsh frogs and percentage of woodland cover. Potentially the negative association of marsh frogs with woodland from this study could again be due to a correlation between pond shading and small patches of woodland.

No correlation was found between the variable 'Distance from broadleaved woodland' and 'Shade' in any of the logistic regressions in Chapter 4. However, the LCM2015 used to derive 'Distance from broadleaved woodland' has a minimum mappable unit of 0.5

hectares. Parcels of habitat less than this may be dissolved into the surrounding habitat (Rowland et al. 2017). Therefore, a finer scale layer quantifying the percentage cover of woodland around survey ponds may be needed to determine if there is a correlation between pond shading and small patches of woodland.

The local scale study in Chapter 4 identified variables associated with the presence of common frogs in the Sussex survey ponds. However, there were too few of the survey ponds in Kent with common frog spawn present to carry out a similar analysis. Chapter 5 provided this analysis using common frog presence/absence data, assessed using eDNA metabarcoding carried out by Harper from the University of Hull (Harper et al. 2018a; Harper et al. 2018b). The results of a logistic regression analysis on this data showed that common frogs were negatively associated with permanent ponds but not with the presence of great crested newts. However, the eDNA metabarcoding data showed a much higher proportion of ponds occupied by great crested newts (40 ponds out of 123) compared to common frogs (24 ponds out of 123). These results were consistent with the results from the local level analysis on ponds in Kent (Chapter 4) which also showed much higher presence of great crested newts (22 ponds out of 40) compared to common frogs (8 ponds out of 49). This supports the hypothesis that high numbers of great crested newts in an area could be reducing the presence of common frogs.

Despite the presence of marsh frogs in the area, only one pond was found to have marsh frogs from the eDNA metabarcoding data of ponds in Kent. In the local level analysis (Chapter 4) the survey ponds were selected to be 500 m from a marsh frog record and 100 m from a watercourse. This may have increased the number of ponds with marsh frogs present in the analysis in Chapter 4. The results of the eDNA metabarcoding justify this approach, given that if it was not taken a very low number of ponds with marsh frogs may have been identified. The results of the eDNA metabarcoding indicate that the proportion of ponds occupied with marsh frogs or common frogs is relatively low

compared to great crested newts when ponds are selected randomly. Therefore, the higher proportion of common frogs found in Sussex by the local level study is more likely to be due to a lower population of great crested newts in the area rather than the absence of marsh frogs.

The results of this study indicate that marsh frogs are not affecting the distribution of common frogs in Kent. However, marsh frogs are a generalist predator and tend to consume the prey that is most abundant (Merry 2004). There have been examples of marsh frogs eating common frog tadpoles under experimental conditions (Innocenzi 1995). Therefore, predation on common frogs could be occurring in the few ponds where common frogs are breeding, and marsh frogs are present. Roth et al. (2016) found that the population size of three native toads species, yellow bellied toads (*Bombina variegata*), common midwife toads (*Alytes obstetricans*), and natterjack toads (*Epidalea calamita*) were negatively related to expanding water frog populations (including marsh frogs). But as Roth et al. (2016) notes, "…water frogs may only marginally affect the total population size of an endangered toad species if they do not share the same habitat niche and, thus, if they rarely occur at the same sites". The evidence provided here indicates this may be the situation existing between common frogs and marsh frogs in Kent and Sussex, with common frogs and marsh frogs rarely occurring in the same ponds due to their differing habitat preferences. The implications for conservation practice are therefore neutral and there is no need for a more active policy regarding eradication of the species when considering their impacts on common frogs.

There are other potential risks to native amphibian species from marsh frogs. Marsh frogs can breed with other members of the genus *Pelophylax.* In areas where the pool frog is a native species the marsh frog can contaminate the gene pool through hybridisation (Luquet et al. 2011; Holsbeek et al. 2008). Pool frogs (*Pelophylax lessonae*) have been recently identified as native to the UK although the last population is thought to have died out (Beebee et al. 2005). However, a reintroduction programme has been

established in Norfolk (Baker and Foster 2015). Therefore, potentially if marsh frogs spread across the UK this could be a cause for concern (Innocenzi 1995). However, apart from their rapid dispersal through the marsh dyke habitat of Kent, marsh frog spread has been slow since their introduction in 1935 and limited to contiguous suitable habitat (Zeisset and Beebee 2003). Therefore, marsh frogs are unlikely to spread naturally to East Anglia unless moved there.

Pool frogs and edible frogs (*Pelophylax esculentus*) which are the same genus as marsh frogs can be carriers of amphibian disease like ranavirus and the chytrid fungus (*Batrachochytrium dendrobatidis*) (Stoehr et al. 2013; Duffus and Cunningham 2010; Ariel et al. 2009). There is evidence that they may be more tolerant to the effects of these diseases which may lead to increased persistence in an area (Beebee 2012; Woodhams, Bigler and Marschang 2012). However, the limited distribution of marsh frogs will also reduce its potential impact as a disease carrier. Therefore, introductions of marsh frogs to other parts of the UK should be avoided to maintain this lower disease risk.

6.1. Conclusion

The effects of non-native species can be severe and lead to changes in the distribution of native species (Willson 2017; Buckland et al. 2014). However, the distribution of a species can be influenced by many different factors. Identifying the cause of a distribution pattern can be difficult particularly if there are complex interactions involved. This research has used a combination of species distribution modelling and local level comparative studies to provide evidence indicating that marsh frogs are unlikely to be causing a change in the distribution of common frogs.

The distribution modelling enabled the teasing apart of the variables driving common frog, marsh frog, and great crested newt distributions in Kent and Sussex. The SDMs predicted that common frogs and marsh frogs have a largely non-overlapping distribution in Kent. The main cause of this was likely to be the negative association of common frogs

with linear water and pond density compared to the positive response of marsh frogs to these variables. The positive association of great crested newts with pond density suggested that this species could also be affecting the abundance of common frogs in an area of Kent with high pond density.

The local level study showed that the differing habitat preferences of common frogs and marsh frogs reduced the likelihood of interactions between them. In high pond density areas of Kent, the proportion of ponds occupied by marsh frogs was found to be low. Therefore, the opportunity for marsh frogs to prey on common frogs is also less likely. In ponds where marsh frogs and common frogs are both present some predation may occur, but this is unlikely to be enough to affect the distribution of common frogs. The high presence of great crested newts, a known predator of common frogs, could account for a lower common frog presence in the areas of high pond density in Kent. Survey ponds in Sussex had a lower proportion of great crested newts and a higher proportion of common frogs which is consistent with this hypothesis, although other factors could influence the abundance of these species.

Tools to aid the assessment of invasive species impacts continue to be developed and have often focussed on assessing the invasive potential of non-natives (Dick et al. 2017; Penk et al. 2017; Measey et al. 2016). The objective is to be able to detect the non-native species that are most likely to cause impacts, so funding can be appropriately targeted. This study has also demonstrated a method that could be used to avoid resources being spent on unnecessary non-native species control measures. The advent of eDNA techniques has made this type of study less costly by providing a means of increasing the sample size with less effort. In situations where the impact of a non-native species is difficult to determine this technique is a viable option. Indeed, if eDNA can result in early detection of invasives that have hitherto only been detectable using traditional methods that can only be used after populations are well-established, it will mean that appropriate interventions can be applied earlier and more effectively.

References

Ahrenfeldt, R. H. (1953). Attacks by *Rana esculenta* on *Rana temporaria* and *Bufo bufo* in captivity. *British Journal of Herpetology*, 1, 159-160.

Aiello-Lammens, M. E., Boria, R. A., Radosavljevic, A., Vilela, B. and and Anderson, R. P. (2014) *Functions for spatial thinning of species occurrence records for use in ecological models*. [spThin]. 0.1.0. CRAN.

Anderson, D. R., Burnham, K. P., Gould, W. R. and Cherry, S. (2001). Concerns about finding effects that are actually spurious. *Wildlife Society Bulletin*, 29, 311-316.

Anderson, D. R. and Burnham, K. P. (2002). Avoiding pitfalls when using information-theoretic methods. *The Journal of Wildlife Management*, 66, 912-918.

Anderson, R. P., Peterson, A. T. and Gómez-Laverde, M. (2002). Using niche-based GIS modeling to test geographic predictions of competitive exclusion and competitive release in South American pocket mice. *Oikos*, 98, 3-16.

Anderson, R. P. and Gonzalez Jr., I. (2011). Species-specific tuning increases robustness to sampling bias in models of species distributions: an implementation with Maxent. *Ecological Modelling*, 222, 2796-2811.

Araújo, M. B., Thuiller, W. and Pearson, R. G. (2006). Climate warming and the decline of amphibians and reptiles in Europe. *Journal of Biogeography*, 33, 1712-1728.

ARG UK (2010). *Great Crested Newt Habitat Suitability Index - ARG Advice Note 5*. Amphibian and Reptile Groups of the UK [Online]. Last updated: May 2010. Available from: http://www.arguk.org/advice-and-guidance/view-category [Accessed 10 January 2014].

Ariel, E., Kielgast, J., Svart, H. E., Larsen, K., Tapiovaara, H., Jensen, B. B. and Holopainen, R. (2009). Ranavirus in wild edible frogs *Pelophylax kl. esculentus* in Denmark. *Diseases of Aquatic Organisms*, 85, 7-14.

Arnold, H. R. (1995). *Atlas of Amphibians and Reptiles in Britain*. London: HMSO.

Austin, P. C. (2011). An introduction to propensity score methods for reducing the effects of confounding in observational studies. *Multivariate Behavioral Research*, 46, 399-424.

Babyak, M. A. (2004). What you see may not be what you get: a brief, nontechnical introduction to overfitting in regression-type models. *Psychosomatic Medicine* [Online], 66, 14 September 2018-411-421. Available from: http://www.cs.vu.nl/~eliens/sg/local/theory/overfitting.pdf [Accessed 14 September 2018].

Barata, I. M., Griffiths, R. A. and Ridout, M. S. (2017). The power of monitoring: optimizing survey designs to detect occupancy changes in a rare amphibian population. *Scientific Reports*, 7, 16491.

Barbosa, F. G., Both, C. and Araujo, M. B. (2017). Invasive American bullfrogs and African clawed frogs in South America: high suitability of occurrence in biodiversity hotspots. *Zoological Studies*, 56, 28.

Barnes, M. A., Turner, C. R., Jerde, C. L., Renshaw, M. A., Chadderton, W. L. and Lodge, D. M. (2014). Environmental conditions influence eDNA persistence in aquatic systems. *Environmental Science & Technology*, 48, 1819-1827.

Beebee, T. J. C. (2013). *Amphibians and Reptiles*. Naturalists' Handbooks. Vol. 31. Exeter: Pelagic.

Beebee, T. J. C. (2012). Impact of Ranavirus on garden amphibian populations. *Herpetological Bulletin*, 1-3.

Beebee, T. J. C. (1985). Discriminant analysis of amphibian habitat determinants in South-East England. *Amphibia-Reptilia*, 6, 35-43.

Beebee, T. J. C. (1980). Habitats of the British amphibians (3): river valley marshes. *Biological Conservation*, 18, 281-287.

Beebee, T. J. C. (1979). Habitats of the British amphibians (2): suburban parks and gardens. *Biological Conservation* [Online], 15, 241-257.

Beebee, T. J. C. and Griffiths, R. (2000). *Amphibians and Reptiles: A Natural History of the British Herpetofauna*. The New Naturalist. London: HarperCollins.

Beebee, T. J. C. and Griffiths, R. A. (2005). The amphibian decline crisis: a watershed for conservation biology?. *Biological Conservation*, 125, 271-285.

Bielby, J., Cooper, N., Cunningham, A. A., Garner, T. W. J. and Purvis, A. (2008). Predicting susceptibility to future declines in the world's frogs. *Conservation Letters*, 1, 82-90.

Biggs, J., Ewald, N., Valentini, A., Gaboriaud, C., Dejean, T., Griffiths, R. A., Foster, J., Wilkinson, J. W., Arnell, A., Brotherton, P., Williams, P. and Dunn, F. (2015). Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (*Triturus cristatus*). *Biological Conservation* [Online], 183, 19-28.

Bivand, R. and Piras, G. (2015). Comparing implementations of estimation methods for spatial econometrics. *Journal of Statistical Software*, 63, 1-36.

Boissinot, A., Besnard, A. and Lourdais, O. (2019). Amphibian diversity in farmlands: combined influences of breeding-site and landscape attributes in western France. *Agriculture Ecosystems & Environment*, 269, 51-61.

Boissinot, A., Grillet, P., Besnard, A. and Lourdais, O. (2015). Small woods positively influence the occurrence and abundance of the common frog (*Rana temporaria*) in a traditional farming landscape. *Amphibia-Reptilia*, 36, 417-424.

Boria, R. A., Olson, L. E., Goodman, S. M. and Anderson, R. P. (2014). Spatial filtering to reduce sampling bias can improve the performance of ecological niche models. *Ecological Modelling*, 275, 73-77.

Bormpoudakis, D., Foster, J., Gent, T., Griffiths, R. A., Russell, L., Starnes, T., Tzanopoulos, J. and and Wilkinson, J. (2015). *Developing Models to Estimate the Occurrence in the English Countryside of Great Crested Newts, a Protected Species Under the Habitats Directive [Defra Project Code WC1108]* [Online]. Available from: http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=19272&FromSearch=Y&Publisher=1&SearchText=WC1108&SortString=ProjectCode&SortOrder=Asc&Paging=10 [Accessed 18 January 2017].

Boulenger, G. A. (1884). On the origin of the edible frog in England. *The Zoologist*, 8, 265-269.

Brady, L. (2010). *Record Validation*. Lee Brady's Recording Blog [Online]. Last updated: 5 August 2010. Available from:

http://calumma.typepad.com/lee_bradys_recording_blog/2010/08/index.html [Accessed 9 August 2016].

Brady, L. (2009). Further Thoughts on Common Frog Pond Occupancy. *Lee Brady's Recording Blog Reptile and Amphibian Conservation* [Online]. Available from: http://calumma.typepad.com/lee_bradys_recording_blog/2009/10/further-thoughts-on-common-frog-pond-occupancy.html [Accessed 29 August 2011].

Bucciarelli, G. M., Blaustein, A. R., Garcia, T. S. and Kats, L. B. (2014). Invasion complexities: the diverse impacts of non-native species on amphibians. *Copeia*, 611-632.

Buckland, S., Cole, N. C., Aguirre-Gutierrez, J., Gallagher, L. E., Henshaw, S. M., Besnard, A., Tucker, R. M., Bachraz, V., Ruhomaun, K. and Harris, S. (2014). Ecological effects of the invasive giant Madagascar day gecko on endemic Mauritian geckos: applications of binomial-mixture and species distribution models. *Plos One*, 9, e88798.

Burnham, K. P. and Anderson, D. R. eds. (2002). *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. 2 edn. New York: Springer-Verlag.

Buxton, A. S., Groombridge, J. J. and Griffiths, R. A. (2017). Is the detection of aquatic environmental DNA influenced by substrate type?. *Plos One*, 12, e0183371.

Buxton, A. S., Groombridge, J. J., Zakaria, N. B. and Griffiths, R. A. (2017). Seasonal variation in environmental DNA in relation to population size and environmental factors. *Scientific Reports*, 7, 46294.

Buxton, A. S., Groombridge, J. J. and Griffiths, R. A. (2018). Comparison of two citizen scientist methods for collecting pond water samples for environmental DNA studies. *Citizen Science: Theory and Practice* [Online], 3, 31 August 2018-1-9. Available from: http://doi.org/10.5334/cstp.151 [Accessed 31 August 2018].

Byers, J. E. (2002). Impact of non-indigenous species on natives enhanced by anthropogenic alteration of selection regimes. *Oikos*, 97, 449-458.

Cayuela, H., Besnard, A. and Joly, P. (2013). Multi-event models reveal the absence of interaction between an invasive frog and a native endangered amphibian. *Biological Invasions*, 15, 2001-2012.

Chan, K. (2011). *Can great crested newts (Triturus cristatus) coexist with fish?*. MSc edn. Department of Geography, University College London.

Cicek, K. and Mermer, A. (2007). Food composition of the marsh frog, *Rana ridibunda* Pallas, 1771, in Thrace. *Turkish Journal of Zoology*, 31, 83-90.

Copp, G. H., Britton, J. R., Cucherousset, J., Garcia-Berthou, E., Kirk, R., Peeler, E. and Stakenas, S. (2009). Voracious invader or benign feline? A review of the environmental biology of European catfish *Silurus glanis* in its native and introduced ranges. *Fish and Fisheries*, 10, 252-282.

Covaciu-Marcov, S. D., Cicort-Lucaciu, A. S., Mitrea, I., Sas, I., Caus, A. V. and Cupsa, D. (2010). Feeding of three syntopic newt species (*Triturus cristatus*, *Mesotriton alpestris* and *Lissotriton vulgaris*) from Western Romania. *North-Western Journal of Zoology*, 6, 95-108.

Crossland, M. R., Alford, R. A. and Shine, R. (2009). Impact of the invasive cane toad (*Bufo marinus*) on an Australian frog (*Opisthodon ornatus*) depends on minor variation in reproductive timing. *Oecologia*, 158, 625-632.

Deiner, K., Bik, H. M., Machler, E., Seymour, M., Lacoursiere-Roussel, A., Altermatt, F., Creer, S., Bista, I., Lodge, D. M., de Vere, N., Pfrender, M. E. and Bernatchez, L. (2017). Environmental DNA metabarcoding: transforming how we survey animal and plant communities. *Molecular Ecology*, 26, 5872-5895.

Dejean, T., Valentini, A., Miquel, C., Taberlet, P., Bellemain, E. and Miaud, C. (2012). Improved detection of an alien invasive species through environmental DNA barcoding: the example of the American bullfrog *Lithobates catesbeianus*. *Journal of Applied Ecology*, 49, 953-959.

Dennis, R. and Thomas, C. (2000). Bias in butterfly distribution maps: the influence of hot spots and recorder's home range. *Journal of Insect Conservation* [Online], 4, 73-77. Available from: https://doi-org.chain.kent.ac.uk/10.1023/A:1009690919835 [Accessed 19 April 2018].

Denoel, M. and Lehmann, A. (2006). Multi-scale effect of landscape processes and habitat quality on newt abundance: implications for Conservation. *Biological Conservation*, 130, 495-504.

Dick, J. T. A., Laverty, C., Lennon, J. J., Barrios-O'Neill, D., Mensink, P. J., Britton, J. R., Medoc, V., Boets, P., Alexander, M. E., Taylor, N. G., Dunn, A. M., Hatcher, M. J., Rosewarne, P. J., Crookes, S., MacIsaac, H. J., Xu, M., Ricciardi, A., Wasserman, R. J., Ellender, B. R., Weyl, O. L. F., Lucy, F. E., Banks, P. B., Dodd, J. A., MacNeil, C., Penk, M. R., Aldridge, D. C. and Caffrey, J. M. (2017). Invader Relative Impact Potential: a new metric to understand and predict the ecological impacts of existing, emerging and future invasive alien species. *Journal of Applied Ecology*, 54, 1259-1267.

Didham, R. K., Tylianakis, J. M., Hutchison, M. A., Ewers, R. M. and Gemmell, N. J. (2005). Are invasive species the drivers of ecological change?. *Trends in Ecology & Evolution* [Online], 20, 470-474. Available from: https://www.sciencedirect.com/science/article/pii/S0169534705002272 [Accessed 5 May 2018].

Doody, J. S., Green, B., Rhind, D., Castellano, C. M., Sims, R. and Robinson, T. (2009). Population-level declines in Australian predators caused by an invasive species. *Animal Conservation*, 12, 46-53.

Dormann, C. F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carre, G., Garcia Marquez, J. R., Gruber, B., Lafourcade, B., Leitao, P. J., Muenkemueller, T., McClean, C., Osborne, P. E., Reineking, B., Schroeder, B., Skidmore, A. K., Zurell, D. and Lautenbach, S. (2013). Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. *Ecography*, 36, 27-46.

Duffus, A. L. J. and Cunningham, A. A. (2010). Major disease threats to European amphibians. *Herpetological Journal*, 20, 117-127.

Efron, B. and Tibshirani, R. (1993). *An Introduction to the Bootstrap; Monographs on Statistics and Applied Probability*. Monographs on Statistics and Applied Probability. London: Chapman and Hall.

Elith, J., Graham, C. H., Anderson, R. P., Dudik, M., Ferrier, S., Guisan, A., Hijmans, R. J., Huettmann, F., Leathwick, J. R., Lehmann, A., Li, J., Lohmann, L. G., Loiselle, B. A., Manion, G., Moritz, C., Nakamura, M., Nakazawa, Y., Overton, J. M., Peterson, A. T., Phillips, S. J., Richardson, K., Scachetti-Pereira, R., Schapire, R. E., Soberon, J., Williams, S., Wisz, M. S. and Zimmermann, N. E. (2006). Novel methods improve prediction of species' distributions from occurrence data. *Ecography*, 29, 129-151.

Elith, J., Phillips, S. J., Hastie, T., Dudik, M., Chee, Y. E. and Yates, C. J. (2011). A statistical explanation of MaxEnt for ecologists. *Diversity and Distributions*, 17, 43-57.

Ficetola, G. F., Miaud, C., Pompanon, F. and Taberlet, P. (2008). Species detection using environmental DNA from water samples. *Biology Letters*, 4, 423-425.

Fielding, A. and Bell, J. (1997). A review of methods for the assessment of prediction errors in conservation presence/absence models. *Environmental Conservation*, 24, 38-49.

Firth, F. M. (1984). *The Natural History of Romney Marsh*. Rainham: Meresborough Books.

Fiske, I. and Chandler, R. (2011). An R package for fitting hierarchical models of wildlife occurrence and abundance. *Journal of Statistical Software*, 43, 1-23.

Foreman, B., to Mackay, A., Email from the Biodiversity Data Lead of the Sussex Biodiversity Record Centre. [Personal email: 11 May 2018].

Fourcade, Y., Engler, J. O., Rödder, D. and and Secondi, J. (2014). Mapping species distributions with MAXENT using a geographically biased sample of presence data: a performance assessment of methods for correcting sampling bias. *PLoS ONE* [Online], 9, 16 September 2014-e97122. Available from: http://doi.org/10.1371/journal.pone.0097122.

Franklin, J., Wejnert, K. E., Hathaway, S. A., Rochester, C. J. and Fisher, R. N. (2009). Effect of species rarity on the accuracy of species distribution models for reptiles and amphibians in southern California. *Diversity and Distributions*, 15, 167-177.

Frazer, D. (1983). *Reptiles and Amphibians in Britain.* London: Collins.

Goldberg, C. S., Sepulveda, A., Ray, A., Baumgardt, J. and Waits, L. P. (2013). Environmental DNA as a new method for early detection of New Zealand mudsnails (*Potamopyrgus antipodarum*). *Freshwater Science*, 32, 792-800.

Greenhalgh, M. and Ovenden, D. (2007). *Freshwater Life: Britain and Northern Europe.*

Gregory, P. T. and Isaac, L. A. (2004). Food habits of the grass snake in southeastern England: is *Natrix natrix* a generalist predator?. *Journal of Herpetology*, 38, 88-95.

Griffiths, R. A., Sewell, D. and McCrea, R. S. (2010). Dynamics of a declining amphibian metapopulation: survival, dispersal and the impact of climate. *Biological Conservation*, 143, 485-491.

Gu, W. and Swihart, R. K. (2004). *Absent or undetected? Effects of non-detection of species occurrence on wildlife–habitat models*. [Online]. Vol. 116., pp. 195-203.

Guillera-Arroita, G. and Lahoz-Monfort, J. J. (2012). Designing studies to detect differences in species occupancy: power analysis under imperfect detection. *Methods in Ecology and Evolution*, 3, 860-869.

Guillera-Arroita, G., Lahoz-Monfort, J. J., Elith, J., Gordon, A., Kujala, H., Lentini, P. E., McCarthy, M. A., Tingley, R. and Wintle, B. A. (2015). Is my species distribution model fit for purpose? Matching data and models to applications. *Global Ecology and Biogeography*, 24, 276-292.

Guillera-Arroita, G., Ridout, M. S. and Morgan, B. J. T. (2010). Design of occupancy studies with imperfect detection. *Methods in Ecology and Evolution*, 1, 131-139.

Halverson, M. A., Massoni, S., Dirksen, J. W., Næsset, E., Gobakken, T. and Ohlson, M. (2003). Forest mediated light regime linked to amphibian distribution and performance. *Oecologia*, 134(3), 360-- 364.

Harper, L. R., Lawson Handley, L., Hahn, C., Boonham, N., Rees, H. C., Gough, K. C., Lewis, E., Adams, I. P., Brotherton, P., Phillips, S. and Hänfling, B. (2018a). *Ground-Truthing Environmental DNA Metabarcoding for Ecological Hypothesis Testing at the Pondscape*. [Preprint]. bioRxiv, https://doi.org/10.1101/278309.

Harper, L. R., Lawson Handley, L., Hahn, C., Boonham, N., Rees, H. C., Gough, K. C., Lewis, E., Adams, I. P., Brotherton, P., Phillips, S. and Hänfling, B. (2018b). Needle in a haystack? A comparison of eDNA metabarcoding and targeted qPCR for detection of the great crested newt (*Triturus cristatus*). *Ecology and Evolution*, 8, 6330-6341.

Harper, L. R., Buxton, A. S., Rees, H. C., Bruce, K., Brys, R., Halfmaerten, D., Read, D. S., Watson, H. V., Sayer, C. D., Jones, E. P., Priestley, V., Mächler, E., Múrria, C., Garcés-Pastor, S., Medupin, C., Burgess, K., Benson, G., Boonham, N., Griffiths, R. A., Lawson Handley, L. and Hänfling, B. (2018c). Prospects and challenges of environmental DNA (eDNA) monitoring in freshwater ponds. *Hydrobiologia* [Online], 15 September 2018. Available from: https://doi.org/10.1007/s10750-018-3750-5 [Accessed 15 September 2018].

Hartel, T., Schweiger, O., Öllerer, K., Cogalniceanu, D. and Arntzen, J. W. (2010). Amphibian distribution in a traditionally managed rural landscape of Eastern Europe: probing the effect of landscape composition. *Amphibia-Reptilia*, 143, 1118-1124.

Hartel, T., Nemes, S., Cogalniceanu, D., Ollerer, K., Schweiger, O., Moga, C. and Demeter, L. (2007). The effect of fish and aquatic habitat complexity on amphibians. *Hydrobiologia*, 583, 173-182.

Hijmans, R. J. (2012). Cross-validation of species distribution models: removing spatial sorting bias and calibration with a null model. *Ecology*, 93, 679-688.

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G. and Andy, J. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, 25, 1965-1978.

Ho, D. E., Imai, K., King, G. and and Stuart, E. A. (2011). *MatchIt: Nonparametric processing for parametric causal inference*. *Journal of Statistical Software* [Online], 42, 1-28. Available from: http://www.jstatsoft.org/.

Ho, D. E., Imai, K., King, G. and Stuart, E. A. (2007). Matching as nonparametric preprocessing for reducing model dependence in parametric causal inference. *Political Analysis* [Online], 15, 199-236 [Accessed 2018/09/14].

Holenweg, P. A. K. (2001). Dispersal rates and distances in adult water frogs, *Rana lessonae*, *R. ridibunda*, and their hybridogenetic associate *R. esculenta*. *Herpetologica*, 57, 449-460.

Holsbeek, G., Mergeay, J., Hotz, H., Ploetner, J., Volckaert, F. A. M. and De Meester, L. (2008). A cryptic invasion within an invasion and widespread introgression in the European water frog complex: consequences of uncontrolled commercial trade and weak international legislation. *Molecular Ecology*, 17, 5023-5035.

Hulme, P. E. (2017). Climate change and biological invasions: evidence, expectations, and response options. *Biological Reviews*, 92, 1297-1313.

Ihlow, F., Courant, J., Secondi, J., Herrel, A., Rebelo, R., Measey, G. J., Lillo, F., De Villiers, F. A., Vogt, S., De Busschere, C., Backeljau, T. and Rodder, D. (2016). Impacts of climate change on the global invasion potential of the African clawed frog *Xenopus laevis*. *Plos One*, 11, e0154869.

Indermaur, L., Schaub, M., Jokela, J., Tockner, K. and Schmidt, B. R. (2010). Differential response to abiotic conditions and predation risk rather than competition avoidance determine breeding site selection by anurans. *Ecography*, 33, 887-895.

Innocenzi, P. (1995). *The Ecology and Conservation of the Marsh Frog*. University of Kent. M.Sc.

Inns, H. (2009). *Britain's Reptiles and Amphibians: A Guide to the Reptiles and Amphibians of Great Britain, Ireland and the Channel Islands*. Old Basing, Hampshire: WILDGuides Ltd.

Ivanova, N. L. and Zhigalski, O. A. (2011). Demographic features of populations of the marsh frog (*Rana ridibunda* Pall.) introduced into water bodies of the Middle Urals. *Russian Journal of Ecology*, 42, 400-406.

Jackson, D.L. (2000). *Guidance on the interpretation of the Biodiversity Broad Habitat Classification (terrestrial and freshwater types): Definitions and the relationship with other classifications*, JNCC Report 307, 73 pages, ISSN 0963 8091 (available online at: http://www.jncc.gov.uk/page-2433).

Jehle, R. (2000). The terrestrial summer habitat of radio-tracked great crested newts (*Triturus cristatus*) and marbled newts (*T-marmoratus*). *Herpetological Journal*, 10, 137-142.

Jerde, C. L., Mahon, A. R., Chadderton, W. L. and Lodge, D. M. (2011). "Sight-unseen" detection of rare aquatic species using environmental DNA. *Conservation Letters*, 4, 150-157.

Kadmon, R., Farber, O. and Danin, A. (2004). Effect of roadside bias on the accuracy of predictive maps produced by bioclimatic models. *Ecological Applications*, 14, 401-413.

Kats, L. B. and Ferrer, R. P. (2003). Alien predators and amphibian declines: review of two decades of science and the transition to conservation. *Diversity and Distributions*, 9, 99-110.

Kernan, M. (2015). Climate change and the impact of invasive species on aquatic ecosystems. *Aquatic Ecosystem Health & Management*, 18, 321-333.

Kéry, M., author. and Royle, J. A., author. (2016). *Applied hierarchical modeling in ecology : analysis of distribution, abundance and species richness in R and BUGS. Volume 1, Prelude and static models*.

Klop-Toker, K., Jose, V., Michelle, S., Simon, C., John, C. and Michael, M. (2018). Community level impacts of invasive mosquitofish may exacerbate the impact to a threatened amphibian. *Austral Ecology*, 43, 213-224.

Klymus, K. E., Richter, C. A., Thompson, N. and Hinck, J. E. (2017). Metabarcoding of environmental DNA samples to explore the use of uranium mine containment ponds as a water source for wildlife. *Diversity* [Online], 9, 12 September 2018-54.

Kovar, R., Brabec, M., Vita, R. and Bocek, R. (2009). Spring migration distances of some Central European amphibian species. *Amphibia-Reptilia*, 30, 367-378.

Kramer-Schadt, S., Niedballa, J., Pilgrim, J. D., Schröder, B., Lindenborn, J., Reinfelder, V., Stillfried, M., Heckmann, I., Scharf, A. K., Augeri, D. M., Cheyne, S. M., Hearn, A. J., Ross, J., Macdonald, D. W., Mathai, J., Eaton, J., Marshall, A. J., Semiadi, G., Rustam, R., Bernard, H., Alfred, R., Samejima, H., Duckworth, J. W., Breitenmoser-Wuersten, C., Belant, J. L., Hofer, H. and Wilting, A. (2013). The importance of correcting for sampling bias in MaxEnt species distribution models. *Diversity and Distributions*, 19, 1366-1379.

Kraus, F. (2015). Impacts from invasive reptiles and amphibians. *Annual Review of Ecology, Evolution, and Systematics*, 46, 75-97.

Kuzmin, S. L., Ishchenko, V., Dujsebayeva, T., Tuniyev, B., Papenfuss, T., Beebee, T., Ugurtas, I. H., Sparreboom, M., Rastegar-Pouyani, N., Disi, A. M. M., Anderson, S., Denoël, M. and Andreone, F. (2009). *Pelophylax Ridibundus*. IUCN 2013. IUCN Red List of Threatened Species. Version 2013.1. [Online]. Available from: www.iucnredlist.org [Accessed 27 August 2013].

Kyriakopoulou-Sklavounou, P. and Kattoulas, M. E. (1990). Contribution to the reproductive biology of *Rana ridibunda* Pallas (Anura, Ranidae). *Amphibia-Reptilia*, 11, 23-30.

Lahoz-Monfort, J. J., Guillera-Arroita, G. and Wintle, B. A. (2014). Imperfect detection impacts the performance of species distribution models. *Global Ecology and Biogeography*, 23, 504-515.

Latham, D. M., Oldham, R. S., Stevenson, M. J., Duff, R., Franklin, P. and Head, S. M. (1996). Woodland management and the conservation of the great crested newt (*Triturus cristatus*). *Aspects of Applied Biology*, 44, 451-459.

Laufer, G., Gobel, N., Borteiro, C., Soutullo, A., Martinez-Debat, C. and de Sa, R. O. (2018). Current status of American bullfrog, *Lithobates catesbeianus*, invasion in Uruguay and exploration of chytrid infection. *Biological Invasions*, 20, 285-291.

Laurila, A. and Kujasalo, J. (1999). Habitat duration, predation risk and phenotypic plasticity in common frog (*Rana temporaria*) tadpoles. *Journal of Animal Ecology*, 68, 1123-1132.

Loman, J. (2016). Breeding phenology in *Rana temporaria*. Local variation is due to pond temperature and population size. *Ecology and Evolution*, 6, 6202-6209.

Luquet, E., Vorburger, C., Hervant, F., Joly, P., Kaufmann, B., Schmeller, D. S., Lena, J. P., Grolet, O., Konecny, L. and Plenet, S. (2011). Invasiveness of an introduced species: the role of hybridization and ecological constraints. *Biological Invasions*, 13, 1901-1915.

Lydolph, M., Jacobsen, J., Arctander, P., Gilbert, M., Gilichinsky, D., Hansen, A., Willerslev, E. and Lange, L. (2005). Beringian paleoecology inferred from permafrost-preserved fungal DNA. *Applied and Environmental Microbiology*, 71, 1012-1017.

Macey, J. R., Strasburg, J. L., Brisson, J. A., Vredenburg, V. T., Jennings, M. and Larson, A. (2001). *Molecular Phylogenetics of Western North American Frogs of the Rana boylii Species Group*. [Online]. Vol. 19., pp. 131-143.

MacKenzie, D. I., Nichols, J. D., Lachman, G. B., Droege, S., Royle, J. A. and Langtimm, C. A. (2002). Estimating site occupancy rates when detection probabilities are less than one. *Ecology*, 83, 2248-2255.

MacKenzie, D. I., Nichols, J. D., Royle, J. A., Pollock, K. H., Bailey, L. L. and Hines, J. E. eds. (2006). *Occupancy Estimation and Modeling Inferring Patterns and Dynamics of Species*. Burlington: Elsevier.

MacKenzie, D. I. and Bailey, L. L. (2004). Assessing the Fit of Site-Occupancy Models. *Journal of Agricultural, Biological, and Environmental Statistics*, 9, 300-318.

Madsen, T. (1984). Movements, home range size and habitat use of radio-tracked grass snakes (*Natrix natrix*) in southern Sweden. *Copeia*, 1984, 707-713.

Mandeville, L. C. and Spurway, H. (1949). The development of hybrids between *Rana esculenta* Linn. and *Rana ridibunda* Pallas. *British Journal of Herpetology*, 1, 39.

Marnell, F. (1998). Discriminant analysis of the terrestrial and aquatic habitat determinants of the smooth newt (*Triturus vulgaris*) and the common frog (*Rana temporaria*) in Ireland. *Journal of Zoology*, 244(1), 1-6.

Mazerolle, M. J. (2015) *Model Selection and Multimodel Inference Based on (Q)AIC(C)*. 2.0-3. CRAN.

Mazerolle, M. J. (2006). Improving data analysis in herpetology: using Akaike's Information Criterion (AIC) to assess the strength of biological hypotheses. *Amphibia-Reptilia*, 27, 169-180.

Measey, G. J., Vimercati, G., de Villiers, F. A., Mokhatla, M. M., Davies, S. J., Edwards, S. and Altwegg, R. (2015). Frog eat frog: exploring variables influencing anurophagy. *Peerj*, 3, e1204.

Measey, G. J. and Tinsley, R. C. (1998). Feral *Xenopus laevis* in South Wales. *Herpetological Journal*, 8, 23-27.

Measey, Vimercati, G., de Villiers, F. A., Mokhatla, M., Davies, S. J., Thorp, C. J., Rebelo, A. D. and Kumschick, S. (2016). A global assessment of alien amphibian impacts in a formal framework. *Diversity and Distributions*, 22, 970-981.

Menzies, J. I. (1962). The marsh frog (*Rana esculenta ridibunda Pallas*) in England. *British Journal of Herpetology*, 3, 43-54.

Merow, C., Smith, M. J. and Silander, J. A. (2013). A practical guide to MaxEnt for modeling species' distributions: what it does, and why inputs and settings matter. *Ecography*, 36, 1058-1069.

Merry, K. (2004). *The Diet of the Marsh Frog (Rana Ridibunda) at the London Wetlands Centre*. University College London. M.Sc.

Mollov, I., Boyadzhiev, P. and Donev, A. (2010). Trophic role of the marsh frog *Pelophylax ridibundus* (Pallas, 1771) (Amphibia, Anura) in the Aquatic Ecosystems. *Bulgarian Journal of Agricultural Science*, 16, 298-306.

Morales, N. S., Fernandez, I. C. and Baca-Gonzalez, V. (2017). MaxEnt's parameter configuration and small samples: are we paying attention to recommendations? A systematic review. *Peerj*, 5, 3093.

Morand, A. and Joly, P. (1995). Habitat variability and space utilization by the amphibian communities of the French upper-Rhone floodplain. *Hydrobiologia*, 300, 249-257.

Moreira, F. D., Ascensao, F., Capinha, C., Rodrigues, D., Segurado, P., Santos-Reis, M. and Rebelo, R. (2015). Modelling the risk of invasion by the red-swamp crayfish (*Procambarus clarkii*): incorporating local variables to better inform management decisions. *Biological Invasions*, 17, 273-285.

Morton, D., Rowland, C., Wood, C., Meek, L., Marston, C., Smith, G., Wadsworth, R. and Simpson, I. G. (2011). *Final Report for LCM2007 – the New UK Land Cover Map*. Countryside Survey. Lancaster: Centre for Ecology and Hydrology. Report number: 11/07.

Nottinghamshire Biological and Geological Records Centre (2012). *Records of Amphibians and Reptiles for Nottinghamshire*. NBN Gateway [Online]. Last updated: 24/04/2012. Available from: https://staging-data.nbn.org.uk/Taxa/NHMSYS0020194860 [Accessed 11/09/2013].

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

Oromi, N., Camarasa, S., Sanuy, I. and Sanuy, D. (2015). Variation of growth rate and survival in embryos and larvae of *Rana temporaria* populations from the Pyrenees. *Acta Herpetologica*, 10(2).

OS MasterMap Highways Network (2015). *OS MasterMap Highways Network [GML3 geospatial data]*. 1:2500. EDINA Digimap Ordnance Survey Service.

OS MasterMap Water Network (2015). *OS MasterMap Water Network [GML3 geospatial data]*. 1:2500. EDINA Digimap Ordnance Survey Service.

Parker, I. M., Simberloff, D., Lonsdale, W. M., Goodell, K., Wonham, M., Kareiva, P. M., Williamson, M. H., Von Holle, B., Moyle, P. B., Byers, J. E. and Goldwasser, L. (1999). Impact: toward a framework for understanding the ecological effects of invaders. *Biological Invasions*, 1, 3-19.

Pearson, R. G. (2007). Species' distribution modelling for conservation educators and practitioners. Synthesis. *American Museum of Natural History* [Online], 5 June 2012. Available from: http://academic.uprm.edu/~jchinea/UIP-MAPR/refs/modelos/pearson2008.pdf.

Pearson, R. G., Raxworthy, C. J., Nakamura, M. and Peterson, A. T. (2007). Predicting species distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. *Journal of Biogeography*, 34, 102-117.

Penk, M., Saul, W., Dick, J. T. A., Donohue, I., Alexander, M. E., Linzmaier, S. and Jeschke, J. M. (2017). A trophic interaction framework for identifying the invasive capacity of novel organisms. *Methods in Ecology and Evolution*, 8, 1786-1794.

Pepler, T. (2017) *Tpepler/Nonpar: Collection of Methods for Non-Parametric Analysis*. GitHub.

Peterson, A. T., Soberón, J., Pearson, R. G., Anderson, R. P., Martínez-Meyer, E., Nakamura, M. and Araújo, M. B. (2011). *Ecological Niches and Geographic Distributions (MPB-49)*. Princeton University Press.

Phillips, S. J. (2010). *Species' Distribution Modeling for Conservation Educators and Practitioners*. Exercise. American Museum of Natural History, Lessons in Conservation [Online]. Available from: http://ncep.amnh.org/index.php?globalnav=resources&sectionnav=modules&sectionsubnav=module_files&module_id=361 [Accessed 12 December 2013].

Phillips, S. J., Anderson, R. P. and Schapire, R. E. (2006). Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, 190, 231-259.

Phillips, S. J., Dudik, M. and Schapire, R. E. (2011) *Maxent sSoftware for modeling species niches and distributions*. 3.3.3k.

Phillips, S. J. and Dudík, M. (2008). Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. *Ecography*, 31, 161-175.

Phillips, S. J., Dudik, M., Elith, J., Graham, C. H., Lehmann, A., Leathwick, J. and Ferrier, S. (2009). Sample selection bias and presence-only distribution models: implications for background and pseudo-absence data. *Ecological Applications*, 19, 181-197.

Pilliod, D. S., Griffiths, R. A. and Kuzmin, S. L. (2012). Ecological impacts of non-native species. In: *Amphibian Biology*. Surrey Beatty and Sons, pp. 3343-3382.

Preston, D. L., Henderson, J. S. and Johnson, P. T. J. (2012). Community ecology of invasions: direct and indirect effects of multiple invasive species on aquatic communities. *Ecology*, 93, 1254-1261.

Proosdij, A. S. J., Sosef, M. S., Wieringa, J. J. and Niels, R. (2016). Minimum required number of specimen records to develop accurate species distribution models. *Ecography*, 39, 542-552.

Radosavljevic, A. and Anderson, R. P. (2014). Making better Maxent models of species distributions: complexity, overfitting and evaluation. *Journal of Biogeography*, 41, 629-643.

Reading, C. J. and Jofre, G. M. (2009). Habitat selection and range size of grass snakes *Natrix natrix* in an agricultural landscape in southern England. *Amphibia-Reptilia*, 30, 379-388.

Reptile and Amphibian Group for Somerset (n.d.). *Green Frogs Or Water Frogs*. Reptile and Amphibian Group for Somerset [Online]. Available from: http://www.somersetarg.org.uk/green_frog.php [Accessed 17 September 2018].

Ricciardi, A. (2007). Are modern biological invasions an unprecedented form of global change?. *Conservation Biology*, 21, 329-336.

Roemer, G. W., Donlan, C. J. and Courchamp, F. (2002). Golden eagles, feral pigs, and insular carnivores: How exotic species turn native predators into prey. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 791-796.

Rosenbaum, P. R. and Rubin, D. B. (1983). The central role of the propensity score in observational studies for causal effects. *Biometrika*, 70, 41-55.

Roth, T., Buehler, C. and Amrhein, V. (2016). Estimating effects of species interactions on populations of endangered species. *American Naturalist*, 187, 457-467.

Rowland, C. S., Morton, R. D., Carrasco, L., McShane, G., O'Neil, A. W. and Wood, C. M. (2017) *Land Cover Map 2015 (25m Raster, GB)*. NERC Environmental Information Data Centre.

Ruchin, A. B. and Ryzhov, M. K. (2002). On the diet of the marsh frog (*Rana ridibunda*) in the Sura and Moksha watershed, Mordovia. *Advances in Amphibian Research in the Former Soviet Union*, 7, 197-205.

Safner, T., Miaud, C., Gaggiotti, O., Decout, S., Rioux, D., Zundel, S. and Manel, S. (2011). Combining demography and genetic analysis to assess the population structure of an amphibian in a human-dominated landscape. *Conservation Genetics*, 12, 161-173.

Savage, M. R. (1961). *The Ecology and Life History of the Common Frog*. London: Pitman.

Schank, C. J., Cove, M. V., Kelly, M. J., Eduardo, M., O'Farrill Georgina, Reyna-Hurtado Rafael, Ninon, M., Jordan, C. A., González-Maya, J. F., Lizcano, D. J., Ricardo, M., Dobbins, M. T., Victor, M., Sáenz-Bolaños Carolina, Jimenez, E. C., Nereyda, E., Cruz Díaz, J. C., Joel, S., Spínola Manuel, Andrew, C., Jessica, F., Nielsen, C. K., Francisco, B., Pozo, M. G., Marina, R., de la, T. J., Brenes-Mora Esteban, Godínez-Gómez Oscar, Wood, M. A.,

Jessica, G. and Miller, J. A. (2017). Using a novel model approach to assess the distribution and conservation status of the endangered Baird's tapir. *Diversity and Distributions*, 23, 1459-1471.

Schiesari, L., Peacor, S. D. and Werner, E. E. (2006). The growth-mortality tradeoff: evidence from anuran larvae and consequences for species distributions. *Oecologia*, 149(2), 194-202.

Seebens, H., Blackburn, T. M., Dyer, E. E., Genovesi, P., Hulme, P. E., Jeschke, J. M., Pagad, S., Pyšek, P., van Kleunen, M., Winter, M., Ansong, M., Arianoutsou, M., Bacher, S., Blasius, B., Brockerhoff, E. G., Brundu, G., Capinha, C., Causton, C. E., Celesti-Grapow, L., Dawson, W., Dullinger, S., Economo, E. P., Fuentes, N., Guénard, B., Jäger, H., Kartesz, J., Kenis, M., Kühn, I., Lenzner, B., Liebhold, A. M., Mosena, A., Moser, D., Nentwig, W., Nishino, M., Pearman, D., Pergl, J., Rabitsch, W., Rojas-Sandoval, J., Roques, A., Rorke, S., Rossinelli, S., Roy, H. E., Scalera, R., Schindler, S., Štajerová, K., Tokarska-Guzik, B., Walker, K., Ward, D. F., Yamanaka, T. and Essl, F. (2018). Global rise in emerging alien species results from increased accessibility of new source pools. *Proceedings of the National Academy of Sciences of the United States of America*, 115, E2264.

Sen, S., Shivaprakash, K. N., Aravind, N. A., Ravikanth, G. and Dayanandan, S. (2016). Ecological niche modeling for conservation planning of an endemic snail in the verge of becoming a pest in cardamom plantations in the Western Ghats biodiversity hotspot. *Ecology and Evolution*, 6, 6510-6523.

Sewell, D., Beebee, T. J. C. and Griffiths, R. A. (2010). Optimising biodiversity assessments by volunteers: the application of occupancy modelling to large-scale amphibian surveys. *Biological Conservation*, 143, 2102-2110.

Shine, R. (2010). The ecological impact of invasive cane toads (*Bufo marinus*) in Australia. *The Quarterly Review of Biology*, 85, 253-291.

Shine, R., Brown, G. P., Phillips, B. L., Webb, J. K. and Hagman, M. (2006). The Biology, Impact and Control of Cane Toads: An Overview of the University of Sydney's Research Program. In: Molloy, K. and Henderson, W. eds. *Science of Cane Toad Invasion and Control. Proceedings of the Invasive Animals CRC/CSIRO/Qld NRM&W Cane Toad Workshop.* Brisbane: Invasive Animals Cooperative Research Centre, pp. 18-32.

Shulse, C. D., Semlitsch, R. D. and Trauth, K. M. (2013). Mosquitofish dominate amphibian and invertebrate community development in experimental wetlands. *Journal of Applied Ecology*, 50, 1244-1256.

Skelly, D. K., Freidenburg, L. K. and Kiesecker, J. M. (2002). Forest canopy and the performance of larval amphibians. *Ecology*, 83(4), 983-992.

Smith, E. P. (1939). On the introduction and distribution of *Rana esculenta* in east Kent. *Journal of Animal Ecology*, 8, 168-170.

Smith, M. (1973). *The British Amphibians and Reptiles.* 5th edn. London: Collins.

Stoehr, A. C., Hoffmann, A., Papp, T., Robert, N., Pruvost, N. B. M., Reyer, H. and Marschang, R. E. (2013). Long-term study of an infection with ranaviruses in a group of edible frogs (*Pelophylax kl. esculentus*) and partial characterization of two viruses based on four genomic regions. *Veterinary Journal*, 197, 238-244.

Struebig, M. J., Manuela, F., Gaveau, D. L., Erik, M., Wich, S. A., Catherine, G., Rachel, S., Andreas, W. and Kramer-Schadt Stephanie (2015). Anticipated climate and land-cover

changes reveal refuge areas for Borneo's orang-utans. *Global Change Biology*, 21, 2891-2904.

Stuart, S., Chanson, J., Cox, N., Young, B., Rodrigues, A., Fischman, D. and Waller, R. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science*, 306, 1783-1786.

Swets, J. A. (1988). Measuring the Accuracy of Diagnostic Systems. *Science*, 240, 1285-1293.

Syfert, M. M., Smith, M. J. and Coomes, D. A. (2013). The effects of sampling bias and model complexity on the predictive performance of MaxEnt species distribution models. *Plos One*, 8, e55158.

Taberlet, P., Coissac, E., Hajibabaei, M. and Rieseberg, L. H. (2012). Environmental DNA. *Molecular Ecology*, 21, 1789-1793.

Tatem, A. J. and Hay, S. I. (2007). Climatic similarity and biological exchange in the worldwide airline transportation network. *Proceedings of the Royal Society B: Biological Sciences* [Online], 274, 1489-1496.

Thomsen, P. F., Kielgast, J., Iversen, L. L., Wiuf, C., Rasmussen, M., Gilbert, M. T. P., Orlando, L. and Willerslev, E. (2012). Monitoring endangered freshwater biodiversity using environmental DNA. *Molecular Ecology*, 21, 2565-2573.

Unglaub, B., Steinfartz, S., Kühne, D., Haas, A. and Schmidt, B. R. (2018). *The relationships between habitat suitability, population size and body condition in a pond-breeding amphibian*. [Online]. Vol. 27., pp. 20-29.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M. and Rozen, S. G. (2012) *Primer3--New Capabilities and Interfaces*. 4.1.0. Nucleic Acids Research.

Urban, M. C., Phillips, B. L., Skelly, D. K. and Shine, R. (2008). A toad more traveled: the heterogeneous invasion dynamics of cane toads in Australia. *American Naturalist*, 171, E134-E148.

Vági, B., Kovács, T., Bănncilă, R., Hartel, T. and Anthony, B. P. (2013). A landscape-level study on the breeding site characteristics of ten amphibian species in Central Europe. *Amphibia-Reptilia*, 34, 63-73.

Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P. F., Bellemain, E., Besnard, A., Coissac, E., Boyer, F., Gaboriaud, C., Jean, P., Poulet, N., Roset, N., Copp, G. H., Geniez, P., Pont, D., Argillier, C., Baudoin, J., Peroux, T., Crivelli, A. J., Olivier, A., Acqueberge, M., Le Brun, M., Møller, P. R., Willerslev, E. and Dejean, T. (2016). Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Molecular Ecology*, 25, 929-942.

Van Bocxlaer, I., Loader, S. P., Roelants, K., Biju, S. D., Menegon, M. and Bossuyt, F. (2010). Gradual adaptation toward a range-expansion phenotype initiated the global radiation of toads. *Science*, 327, 679.

Van Buskirk, J. (2005). Local and landscape influence on amphibian occurrence and abundance. *Ecology*, 86, 1936-1947.

Van Buskirk, J. (2003). Habitat partitioning in European and North American pond-breeding frogs and toads. *Diversity and Distributions*, 9, 399-410.

Venables, W. N. and Ripley, B. D. (2002) *Modern Applied Statistics with S*. [MASS]. 7.3-50. New York: Springer.

Viertel, B. (1999). Salt tolerance of *Rana temporaria*: spawning site selection and survival during embryonic development (Amphibia, Anura). *Amphibia-Reptilia*, 20, 161-171.

Vos, C. C., Goedhart, P. W., Lammertsma, D. R. and Spitzen-Van der Sluijs, A. M. (2007). Matrix permeability of agricultural landscapes: an analysis of movements of the common frog (Rana temporaria). *Herpetological Journal*, 17, 174-182.

Wachtel, I., Zidon, R., Garti, S. and Shelach-Lavi, G. (2018). *Predictive modeling for archaeological site locations: Comparing logistic regression and maximal entropy in north Israel and north-east China*. [Online]. Vol. 92., pp. 28-36.

Waits, L. and Paetkau, D. (2005). Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. *Journal of Wildlife Management*, 69, 1419-1433.

Ward, J. W. (2017). *Status and Conservation of the Grass Snake in Jersey*. Canterbury: University of Kent. PhD Thesis.

Willson, J. D. (2017). Indirect effects of invasive Burmese pythons on ecosystems in southern Florida. *Journal of Applied Ecology*, 54, 1251-1258.

Wilson, Dormontt, E. E., Prentis, P. J., Lowe, A. J. and Richardson, D. M. (2009). Something in the way you move: dispersal pathways affect invasion success. *Trends in Ecology & Evolution* [Online], 24, 136-144.

Wilson, McBride, M. F., Bode, M. and Possingham, H. P. (2006). Prioritizing global conservation efforts. *Nature*, 440, 337-340.

Wood, L. R. (2010). *Diversity and distribution of amphibians in Luxembourg*. PhD Thesis edn. Canterbury: University of Kent.

Woodhams, D. C., Bigler, L. and Marschang, R. (2012). Tolerance of fungal infection in European water frogs exposed to *Batrachochytrium dendrobatidis* after experimental reduction of innate immune defenses. *Bmc Veterinary Research*, 8, 197.

Worcestershire Biological Records Centre (2012). *WBRC Species Data for Worcestershire Collated by Date*. NBN Gateway [Online]. Last updated: 04/04/2012. Available from: https://staging-data.nbn.org.uk/Taxa/NHMSYS0020194860 [Accessed 11/09/2012].

Wycherley, J. T. M. and Anstis, R. (2001). *Amphibians and Reptiles of Surrey*. Surrey: Surrey Wildlife Trust.

Wycherley, J. T. M. and Joslin, P. A. (1996). *Green Frogs in Surrey Studies on Distribution, Means of Dissemination, and Reproductive Activity*.

Yilmaz, Z. C. and Kutrup, B. (2006). Seasonal changes in the diet of *Rana ridibunda* Pallas, 1771 (Anura: Ranidae) from the Gorele River, Giresun, Turkey. In: Vences, M., et al. ed. *Proceedings of the 13th Congress of the Societas Europaea Herpetologica*., pp. 201-204.

Zakaria, N. B. (2017). *Long-Term Population Ecology of the Great Crested Newt in Kent*. PhD Thesis edn. Canterbury: University of Kent.

Zeisset, I. and Beebee, T. J. C. (2003). Population genetics of a successful invader: the marsh frog *Rana ridibunda* in Britain. *Molecular Ecology*, 12, 639-646.

Zuur, A. F., Ieno, E. N. and Smith, G. M. (2007). *Analysing Ecological Data*. Statistics for Biology and Health. New York: Springer.