Nottingham Trent University

UNTANGLING THE ROLES OF PREY AVAILABILITY, HABITAT QUALITY AND PREDATION AS PREDICTORS OF HEDGEHOG ABUNDANCE.

Katie Alexandra Lee

September 2021.

A thesis submitted in partial fulfilment of the requirements of Nottingham Trent University for the degree of Doctor of Philosophy.

Acknowledgements

This PhD was match-funded by Nottingham Trent University and People's Trust for Endangered Species. Thank you to NTU for facilitating my research, it is an institution I am proud to be associated with and thank you to everyone at PTES who not only supported this project financially but provided continual assistance and a friendly reception at annual meetings.

A huge thank you to Dr Richard Yarnell for giving unfaltering support since day one of the project. From the endless advice and the promptest feedback, your guidance has been second to none. I thank Dr Louise Gentle for all her support and specifically for her expertise in helping shape the genomic work in this thesis. I thank Dr Antonio Uzal for all his support with statistical analysis and for lots of useful feedback that improved this thesis considerably. And to my external supervisors, Dr Phillip Baker and Professor Richard Delahay, who have provided further feedback that I am immensely grateful for.

I wish to thank the team at the University of Sheffield, NERC Biomolecular Facilities, Dr Deborah Dawson, Dr Kathryn Maher and Dr Gavin Wilson. To Debs for your continuous support and for persisting to see my work through to the finish despite the pandemic. To Gav for helping with a vast amount of laboratory work and to Katy for supporting me with bioinformatic analysis. Also, thanks to Ellen and Lorna, for being the best lab pals, our lunch excursions and the daily moral support meant so much.

Thank you to Nida Al-Fulaij, Dr Lucy Bearman-Brown and Dr Ben Williams for assisting with study site selection and to all the landowners who allowed me to survey their farms across the summers of 2018/19. Thank you to Becky Favier, Ben Harris, Li Catzel and Roisin Jones, who were amongst the students who helped me with fieldwork, you were a fantastic team, and I could not have completed the data collection without you all. Thank you to my PhD friends, especially Alex, Aurelie, Beth, Jess and Lauren. From our Nottingham antics, holidaying in Portugal and Teams pub nights, your support really did keep me going.

Lastly, thank you to the people who have definitely heard more than their fair share about hedgehogs and badgers, my family. Thank you to James, Grandma Liz, Cathy, Jon and Isabel, for supporting me along the way. A special thanks to Ashley for enduring the whole write up in lockdown with me, thank you for being the best 'bubble'. And lastly the biggest thank you to my parents Alison and Gavin for their relentless support. Thank you both for accompanying me on fieldwork, helping devise fieldwork hacks and for generally making my life easier when times were tough. The emotional support has been immeasurable, and I will be forever grateful.

Contribution Statement

Field work and data collection (camera trapping and invertebrate sampling) were carried out by myself in 2018 and with the assistance of several Masters students from Nottingham Trent University in 2019.

Benjamin Harris, Li Catzel and Roisin Jones assisted with camera trapping and invertebrate sampling throughout May – August 2019.

For Chapter 4, scats were collected for badgers and hedgehogs across Brackenhurst campus and Hartpury College campus. Scat collection at Brackenhurst was carried out by myself in 2018 and 2019. Sampling of scats at Hartpury College was conducted by myself and Becky Favier (Hartpury University).

Training in DNA metabarcoding was provided at the NERC Biomolecular Analysis Facility by Dr Deborah Dawson, Dr Katy Maher and Dr Gavin Wilson. All laboratory work and bioinformatic analysis was conducted by myself.

This project was match-funded by Nottingham Trent University and The People's Trust for Endangered Species.

Abstract

Badgers (*Meles meles*) are the principal predator of hedgehogs (*Erinaceus europeaus*) and have been implicated in the decline of hedgehog populations in Britain and elsewhere. Sharing an intra-guild predation relationship, badgers may negatively impact hedgehog populations via predation, creating a 'landscape of fear' leading to avoidance behaviours and / or competition for shared food resources. Previous studies have evaluated the apparent negative relationship between badgers and hedgehogs, suggesting that hedgehogs may be excluded from areas of high badger density. Despite this, the mechanism by which badgers exert a negative pressure on hedgehogs remains unclear. This study aimed to identify the mechanisms that facilitate coexistence amongst badgers and hedgehogs by assessing densities of both species, habitat and food availability, and dietary niche overlap across multiple sites, to establish the importance of potential competition and predation amongst these two species of concern.

Camera trapping and invertebrate sampling were conducted between 2018 and 2019 across twenty-three sites in England and Wales. Of these, two sites were surveyed all year round, to assess seasonal variation in invertebrate prey resources and, through scat analysis, dietary niche assessment for both badgers and hedgehogs. Dietary assessment was performed by analysing scat samples using the DNA metabarcoding technique. Density estimates were calculated for both species using the Random Encounter Model, and occupancy analysis at individual camera locations was used to assess the spatiotemporal relationship.

To date, no studies have compared the diet of badgers and hedgehogs at the same location to determine the extent of dietary competition and the frequency of hedgehog consumption within the diet of rural badgers. Both species exploited many of the same dietary Families, however dietary composition of prey within each scat sample was significantly dissimilar between badger and hedgehog across all seasons, indicating niche partitioning between the two guild members. Hedgehog DNA was identified in only 1.3% of badger samples at sites where hedgehogs were present, suggesting that hedgehogs are not a key prey item for badgers, but are consumed opportunistically. Diet selection indices showed that neither species consume invertebrate prey relative to its abundance and instead exhibit dietary preferences, suggesting that they may compete for the most common shared dietary items.

Furthermore, this study is the first to estimate densities of co-occurring badgers and hedgehogs across multiple sites, showing that hedgehog densities are significantly higher in mixed farmland landscapes, compared with arable-dominated landscapes. Although both species can co-exist at the regional scale, occupancy modelling in this study showed spatial

segregation at individual camera trap locations which was driven by species-specific differences in habitat selection. Hedgehogs were found in close proximity to buildings, whereas badgers were found away from buildings, closer to arable habitat. Badger and hedgehog temporal activity showed a high degree of overlap, providing no evidence for temporal separation.

Findings from this novel study have identified dietary and spatial partitioning that are likely important mechanisms facilitating the coexistence of badgers and hedgehogs by reducing competitive and predatory interactions, particularly in mixed farmland habitat. Future studies may look to establish whether habitat selection demonstrated in this study is consistent in different land uses and whether hedgehogs are exhibiting their natural preferences or a landscape of fear response in the presence of badgers.

Table of Contents

List of Figures

List of Tables

Chapter 1 Introduction

1.1 Agriculture and biodiversity

Anthropogenic pressures such as habitat destruction, climate change and urbanisation, have triggered the sixth mass extinction (Chapin et al. 2000), resulting in severe and sustained declines in global biodiversity, beyond the background rate (Butchart et al. 2010). As ecosystem structure and function are directly related to biodiversity, changes in species richness, composition and abundance can affect how species interact with one another and have important effects on ecosystem properties. As such, global changes induced by anthropogenic pressures have increased the vulnerability of ecosystems to disturbance, affecting their stability (Hooper et al. 2005) and potential to provide services of importance to humans (Montoya et al. 2012).

Forecasting suggests that changes in agricultural land-use, measured as more than 50% of an area being converted for agriculture, will have the greatest impact on biodiversity across all major terrestrial biomes between 1990 and 2100 (Sala, 2000). It is estimated that 48.7% of all terrestrial habitat had lost ≥10% of its original species richness by 2005 and for 28% of all terrestrial habitat, overall abundance of all species was reduced by >20% (Newbold et al. 2016). The true rate of the loss of biodiversity is expected to be higher, as other factors such as abiotic conditions and climate change are likely to interplay, exacerbating the effects on biodiversity (Newbold et al. 2016).

A widespread reduction in biodiversity is evident across farmland habitat and has been driven in Britain by the agricultural revolution since the late 17th century when agricultural output increased significantly (Pretty 1991). The second, most current agricultural revolution of the 20th century is characterised by advances to chemical and mechanical technologies (Robinson and Sutherland 2002), which has led to the intensification of farming practices to increase productivity (Benton et al. 2003). Together, the increased application of fertilisers by heavy machinery that causes soil compaction, usage of pesticides that affects invertebrate availability for higher trophic feeders, and

broader habitat simplification that has reduced heterogeneity, have contributed to the sustained decrease of biodiversity across the rural landscape in recent decades (Benton et al. 2003; Emmerson et al. 2016). The effect of these farming practices on biodiversity often varies across spatial scales, and therefore both local and landscape level effects should be considered as they may differ between species (Tscharntke et al. 2005).

Furthermore, the relationship between a species and the environment is strongly influenced by its interactions with other species (Deng et al. 2012) that form part of the same ecological network (Tylianakis et al. 2010). To determine the resilience of these networks to disturbance, whether natural or anthropogenic, the underlying mechanisms of species interactions must be understood. For example, antagonistic interactions such as predator-prey relationships are susceptible to drivers of global environmental change including biochemical cycling, climate change and land use, causing instability that can lead to ecosystem level impacts such as the removal of a species from the system (Tylianakis et al. 2010). The complexity of these networks is further increased when they are dependent on more than one type of interaction (Melián et al. 2009) such as competitive and predatory interactions within an intraguild predation (IGP) relationship.

1.2 Intraguild predation

Incidences of IGP, in which two or more species engage in both competition and predation (Polis et al. 1989), are widespread (Arim and Marquet 2004). All species that share a common resource such as food or sheltering sites, irrespective of the taxa they belong to, are regarded as members of the same guild, such as the eagle owl (*Bubo bubo*), and their intraguild prey stone martens (*Martes foina*) (Virgós et al. 2020). The relationship exhibited by fishers and martens is an example of asymmetrical IGP in which predation between the two species is unidirectional, with fishers predating on martens (Polis et al. 1989). For intra-guild members like these, resource partitioning provides a mechanism for promoting coexistence which can be achieved through either alteration of activity,

foraging, habitat selection or a combination of these (Polis et al. 1989). Where resources are spatially homogenised, antagonistic interactions are more likely and this has been shown to result in incidences of predation as in the example of fishers (*Pekania pennanti*) predating on their intraguild prey American martens (*Martes americana*) due to niche compression (Manlick et al. 2017).

In the simplest three species model of IGP, the intraguild predator gains a direct energetic advantage through consuming the intraguild prey and this act of predation reduces further competition for shared prey (Polis and Holt 1992). However, IGP can occur between several species within an assemblage, such as that exhibited by lynx (*Lynx lynx*) and wolf (*Canis lupus*), who are both intraguild predators of the red fox (*Vulpes vulpes*) though their effect on fox abundance is different and varies depending on the spatiotemporal scale (Wikenros et al. 2017).

Incidences of IGP are common and have been observed across all major animal groups including arthropods (Brown et al. 2015), birds (Sergio and Hiraldo 2008), fish (Bachiller et al. 2015) and mammals (Arim and Marquet 2004). However, invertebrate guilds have been studied more frequently, possibly due to logistical challenges associated with studying mammals and birds (Sergio and Hiraldo 2008). Nonetheless, where field studies exist for mammals, they provide an opportunity to assess the mechanistic factors that promote the coexistence of intraguild species, either through spatial, temporal, or dietary niche partitioning.

Many studies of IGP focus on assessing the spatial relationship between species, establishing the importance of habitat and its management on influencing these interactions (Janssen et al. 2007). Habitat availability is frequently identified as an important factor that allows fine-scale partitioning between intraguild species and has even been shown to be more important than the presence of the intraguild-predator in shaping the spatial relationship between intraguild species. For example, the intraguild prey, kit foxes *(Vulpes*

macrotis), have been observed utilising habitat that their intraguild predator, coyotes *(Canis latrans),* cannot exploit, as opposed to avoiding the coyotes directly (Robinson et al. 2014). Similarly, occupancy modelling showed the probability of an area being occupied by either stone marten or their intraguild predators (badgers (*Meles meles)* and red foxes) was more strongly affected by habitat type and structure, than by interspecific interactions (Cruz et al. 2015).

Conversely, there is evidence of intraguild-predator and intraguild-prey species overlapping spatially, with temporal partitioning providing an alternative mechanism for reducing predator-prey interactions. Like spatial partitioning, the activity of intraguild-prey is not always in direct response to the intraguild-predator. For example, red foxes are temporally partitioned from their intraguild predator the lynx. However, whereas the red foxes mirror the changes in activity of rabbit (*Oryctolagus cuniculus*) prey in response to lunar cycles, the lynx do not alter their activity (Penteriani et al. 2013). The temporal activity of fox and lynx is therefore sufficiently different, which is likely to benefit foxes as the subordinate intraguild-prey species, by reducing competition and lowering the risk of predation. However, in food-limiting conditions, fox activity deviates and they will hunt when lynx are more active, regardless of the increased risk of predation (Penteriani et al. 2013). This highlights the variability of observed responses by interacting species in relation to the availability of resources at the patch level.

Hence, the suitability of an area for a species is partly influenced by the localised availability of potential food which can vary between habitat type. There is evidence that species need not be spatially separated when in prey rich areas, as this can reduce competitive and predatory interactions, facilitating coexistence (Lesmeister et al. 2015). For example, high spatial and temporal overlap was observed amongst a guild of five mammalian carnivores, with species co-occurring more frequently than expected, supported by an abundance of prey (Lesmeister et al. 2015). Alternatively, temporal

variability in food availability may promote spatial partitioning as in the example of red fox and their intraguild prey stone marten. In this example, both species utilise available habitat differentially, with martens exploiting arboreal fruits in summer, spring and autumn, a resource that foxes cannot exploit (Padial et al. 2002). Therefore, the strength of competition between these two species is likely reduced during seasons when fruits are abundant (Prigioni et al. 2008). This highlights how diet often reflects seasonal availability of food, resulting in varying levels of competition throughout, and between, years (Davis et al. 2015). These fluctuations in food resources can indirectly lead to population level effects within the IGP relationship, on either the intraguild predator or prey species.

Fewer studies have assessed the importance of potential dietary niche overlap between intraguild-predator and intraguild-prey, which is likely intrinsically linked to the local environment and available habitat, and directly influences the intensity of competition amongst intraguild-species (Manlick et al. 2017; Tsunoda et al. 2017). Niche overlap theory states that coexistence is less likely to occur when niche overlap is greater (Letten et al. 2017). This has been evidenced in American martens, where high dietary overlap with fishers prevented their recovery (Manlick et al. 2017). Dietary niche partitioning may facilitate coexistence (Tsunoda et al. 2017) and this is more likely where the intra-guild prey has a broader niche than the intraguild-predator (Polis et al. 1989). Importantly, where dietary niche partitioning is prominent, there must be sufficient prey availability to enable coexistence by this mechanism (Balme et al. 2017).

Additionally, prey-switching may be a mechanism employed by intraguild-prey when competition for food with intraguild-predators is high. For example, dietary assessment of the spotted-tailed quoll *(Dasyurus maculatus)* revealed that different prey types were consumed when their intraguild-predator the Tasmanian devil *(Sarcophilus harrisii)* had declined (Andersen et al. 2017). Quolls benefitted from mesopredator release,

consuming larger mammal prey when competition with devils was reduced (Andersen et al. 2017). Therefore, determining whether the utilisation of different food resources by the intraguild prey species is density dependent, requires the assessment of diet, food availability and predator densities together.

Coexistence within an IGP relationship can therefore be facilitated by several mechanisms including dietary, spatial, and temporal partitioning. The strength of these relationships and the intensity of competition and predation frequently reflects the relative densities of intraguild predator and prey species (Polis et al. 1989), together with the availability of habitat and shared prey resources. Alternate states of IGP have been observed reflecting the intensity of IGP interactions which can vary between localities from causing little or no negative effect on intraguild-prey, typically when resource availability is abundant, to significant effects that can exclude intraguild-prey completely. Identifying the factors that limit coexistence within an IGP relationship requires the key components to be assessed simultaneously, so their relative importance can be determined. However, studies usually assess only one aspect of the IGP relationship, without considering other factors that could be at play. In some cases, this results in behavioural observations that cannot be definitively explained. Therefore, studies should aim to provide a holistic approach where possible, to aid a more thorough understanding of the factors that drive IGP relationships.

The European badger and European hedgehog (*Erinaceus europaeus*) share an asymmetrical intraguild predation relationship. This is a complex and extreme case of interspecific competition, which can ultimately lead to the exclusion of the subordinate intraguild prey species (Périquet et al. 2015), in this example, hedgehogs. Ecological theory states that there are three potential states that can exist within this relationship; 1) the intraguild-predator is initially present alone at its carrying capacity preventing invasion by the intraguild prey, 2) the intraguild predator excludes the intraguild prey, or 3) the

intraguild prey is a superior exploiter of resources, supporting coexistence of both species (Polis et al. 1989). Competitive interactions can have a multitude of effects on the populations involved, leading to behavioural, physiological or spatiotemporal shifts and significant demographic change (Périquet et al. 2015).

Badgers and hedgehogs are a valuable mammalian model IGP system to explore mechanisms that facilitate or limit coexistence as there are areas in the United Kingdom (UK) where the two species mutually coexist and areas where each species exist exclusively. Physiological and behavioural responses of hedgehogs to badgers (Ward et al. 1996; Hof et al. 2012) coupled with evidence of predation (Doncaster 1994; Hof et al. 2012), demonstrates the effects of IGP as a result of predator-prey interactions between these two species, implicating badgers in the recent population decline of hedgehogs (Young et al. 2006). Despite this, there is evidence to suggest that these two species can coexist with one another at the landscape level and this has been shown in geographically distinct areas in the UK and Netherlands (Poel et al. 2015; Williams et al. 2018). Establishing what factors facilitate the coexistence of these two species is important for promoting their persistence in the rural landscape.

1.3 Population history of hedgehogs and badgers

During the Last Glacial Maximum (23000-16000 BP), the European hedgehog was almost exclusively restricted to the Iberian and Italian Peninsulas that offered glacial refugia (Sommer 2007), whereafter its broad distribution during the early Holocene (9600-8600 BC) across western Europe to northern Russia reflected temperate climates (Seddon et al. 2001). Similarly the postglacial expansion of the European badger, led to its widespread distribution from the British Isles to the west bank of the Volga River in Europe (Kinoshita et al. 2020). During the early Holocene badgers inhabited the Ural Mountains in Russia but have since suffered contractions in their distribution within the last 2500 years, likely due

to changes in availability of resources associated to warmer climates (Kinoshita et al. 2020).

Across the UK, badgers and hedgehogs have co-occurred throughout the rural landscape, though their distribution has altered spatiotemporally, reflecting the more recent anthropogenic pressures that have affected the population abundance of both species within the last century. Badgers were relatively uncommon in the 1920-30's following a period of persecution and control by gamekeepers (Cresswell et al. 1990). A period of steady recovery, including reintroduction efforts to areas such as East Anglia where badgers were scarce, led to an estimate of 250,000 badgers in Britain in the 1980's (Wilson et al. 1997), though proportionately more badgers were distributed in southwest England (24.9 %) than in southeast England (21.9 %), Wales (14%) or Scotland (9.9%) (Cresswell et al. 1989). Within the last two decades further increases in badger abundance have been reported, with an estimated 424,000 badgers in England and 61,000 badgers in Wales between 2012-2014 (Judge et al. 2017). Regional differences in badger abundance appear to be associated to habitat type, with over 55% of the estimated 485,000 badgers present in England and Wales, occupying lowland pastoral habitat, predominantly found in the southwest of England (Judge et al. 2017).

Conversely, UK hedgehog population abundance has been declining rapidly, from an estimated abundance of around 30 million in the 1950's to less than 1.5 million in the mid 1990's (Harris et al. 1995), coinciding with a marked reduction in semi-natural agricultural habitat (Moorhouse et al. 2014) . A comparison of UK hedgehog distribution between the period of 1960 – 1975 and 2000 – 2015, showed a 5.0 – 7.4 % decline in the number of occupied grid cells, suggesting a more patchy distribution across the UK (Hof and Bright 2016).

1.4 Factors influencing hedgehog populations

Hedgehogs are declining in the UK (Roos et al. 2012; Wembridge and Wilson 2018) and multiple factors have been suggested to explain the decline. Hedgehog road casualties are estimated to be between 167,000 – 335,000 per year (Wembridge et al. 2016), leading to direct reductions in hedgehog populations by mortality and reduced gene flow through fragmentation and isolation between areas (Moore et al. 2020). Between 2002 – 2018 road casualties fell by a third to a half (Wembridge and Wilson 2018), though urban and suburban areas remain hotspots for mortality (Wright et al. 2020), suggesting additional factors are important for declines in rural hedgehogs. Agricultural intensification is highlighted as a major cause of habitat loss and loss of heterogeneity, across the rural landscape (Wembridge 2011), coupled with poorer availability of invertebrate food resources (Yarnell and Pettett 2020). Another factor proposed is the impact of badgers on hedgehogs, as a negative relationship between the abundance of both species has been shown (Young et al. 2006; Trewby et al. 2014) warranting further investigation. An understanding of how these factors interplay and impact hedgehog populations is needed to better target conservation action for hedgehogs.

The hedgehog is listed as a species of "principal importance for the purpose of conserving biodiversity" under section 41 (England) of the NERC Act (2006), recognising the need for immediate conservation action due to population losses of up to 50% in some regions of the United Kingdom between 1990 and 2001 (Wembridge 2011). Severe declines are estimated for rural hedgehogs, with road casualties recorded between 2002 and 2017 decreasing between a third and a half across Great Britain (Wilson and Wembridge 2018). This is reflected in estimates of hedgehog densities which have been recorded 7.5 times higher in urban (32.3 km^{-2}) contrasted with rural (4.3 km^{-2}) sites (Schaus et al. 2020). The rural landscape covers 70% of the UK (DEFRA 2012) and throughout this hedgehog occupancy is estimated to be as low as 22% (Williams et al.

2018), demonstrating the patchy distribution of hedgehogs across largely agricultural habitats.

Hedgehogs show an affinity to mown grassland, here termed amenity grassland habitat (Pettett et al. 2017), and also to suburban village habitat, which has been associated with good food availability and habitat for shelter (Hubert et al. 2011). Conversely, hedgehogs have been shown to avoid arable habitat (Williams et al. 2018) and, as agricultural intensification has increased field sizes, coupled with the loss of hedgerows (Tscharntke et al. 2005), this suggests that large expanses of the agricultural landscape provide unfavourable habitat for hedgehogs.

As a dietary generalist, hedgehogs naturally consume a broad range of prey items, with invertebrate prey being most dominant and supplemented by food items such as vertebrates including small mammals and birds, and plant species, namely grasses (Wroot 1984; Yalden 1976). Anthropogenic food resources such as pet food are also prominent in the diet of suburban hedgehogs (Pettett 2016) and likely to be one of the significant attractions of villages and garden habitats (Hubert et al. 2011; Pettett et al. 2017), particularly due to the decreases in invertebrate biodiversity across agricultural habitat (Hooper et al. 2005). Agricultural intensification has been suggested as a cause of hedgehog declines in the rural landscape, the effects of which may be compounded by climatic changes, that can reduce foraging opportunities and increase energetic demands on hedgehogs (Pettett et al. 2017; Geiser 2020).

In addition, the increase in badger numbers from estimates of 250,000 across Britain in the 1980's (Wilson et al. 1997) to 485,000 by 2014 (Judge et al. 2017) may have negatively impacted on hedgehogs via IGP interactions. Competition for shared prey resources may be increased in food limiting conditions and as a result, the risk of predation of hedgehogs by badgers may also increase. Evidence suggests that hedgehogs avoid areas with higher badger sett densities (Williams et al. 2018) and that they may be excluded from

areas of high badger density (Young et al. 2006). Despite this, hedgehogs are absent from many areas of potentially suitable habitat that are unoccupied by badgers (Williams et al. 2018), thus highlighting the multifaceted nature of their decline.

1.5 Badger ecology and population trends

Badgers are omnivores that live in social clans of between 2 – 25 individuals that occupy exclusive territories and share underground burrows, termed setts (Da Silva et al. 1993). Over 50% of rural badger setts are located in deciduous woodland, though hedgerows are used when woodland is scarce (Thornton 1988). Setts are important, providing refugia for resting and reproduction (Roper 1992), and are typically found in areas with sandy soils and sloping topography which is beneficial for sett excavation and drainage (Neal 1972).

Badgers forage throughout the surrounding rural landscape, utilising pasture habitat that is typically abundant in earthworms, the badger's main prey (Kruuk et al. 1979). They also forage in arable habitat, particularly when there is a seasonal abundance of cereal crops (Thornton 1988). Badgers are broad generalists that commonly consume invertebrates such as earthworms and beetles, vertebrates including rabbits and rodents and plant species such as fruits and cereal crops (Kruuk and Parish 1981; 1985). Their diet typically reflects the local availability of these different prey types (Roper 1994). Moreover, the quality of foraging habitat can affect badger home range size, with higher densities supported in prey rich areas (Kruuk 1978).

Badgers have a long history of persecution as both a pest species and for sport through the act of badger baiting (Macdonald et al. 2015). Protective legislation was first introduced in the late 20th century, at a time when badger numbers had declined to a point where they were regarded as being scarce throughout the UK (Cresswell et al. 1989). The Wildlife and Countryside Act (1981), and later the Protection of Badgers Act (1992), initiated a period of recovery in Great Britain that saw sett density stabilise in Wales and increase by 103% in England (see Sainsbury et al. 2019). The latest population estimate for

England and Wales is 485,000 individuals (Judge et al. 2017), almost double that of the estimate in the 1980's, which predicted 250,000 individuals (Cresswell et al. 1989). However, this figure conceals some wide geographic variation, with little or no population increase in some areas such as Wales (Judge et al. 2014).

Changes in badger abundance have been identified as possible drivers of population change in other species. For instance, reduced badger abundance through culling to control bovine Tuberculosis resulted in an increase in fox abundance through competitive release (Trewby et al. 2008). Similarly, hedgehog abundance in amenity grassland habitat was shown to double over a five year period, post badger culling (Trewby et al. 2014). However, the mechanism behind the latter observation was not investigated and could have been caused by a reduction in predation, competitive release, or a combination of these factors.

Evidently, legislative changes are a powerful tool for conserving and protecting wildlife and, in relation to badgers, have helped increase their national abundance (Judge et al. 2017; Wilson et al. 1997). Nevertheless, conservation efforts that are targeted towards a specific species are likely to have wider ecological consequences as other species will also be impacted (Trewby et al. 2008). Badgers and hedgehogs have co-occurred for many decades though this may be an artefact of widespread badger persecution within a heavily managed rural landscape prior to legislation being brought in. Therefore, if increased badger densities can have a negative impact on hedgehogs, it is important to establish under what conditions both species can coexist, and which factors promote the persistence of both species within the rural landscape.

1.6 Intraguild predation amongst badgers and hedgehogs

1.6.1 Habitat preferences

1.6.1.1 Urban

Sparse to moderately urbanised areas are recognised as attractive habitat for hedgehogs (MacGregor-Fors 2011), as often these areas provide good quality suitable amenity grassland habitat, selected by hedgehogs (Young et al. 2006). Hedgehogs can be considered urban adaptors, responding positively to development and degrees of urbanisation, which is likely due to their generalist foraging nature and behavioural flexibility (Rodewald and Gehrt 2014) that allows them to exploit low predator density areas that are plentiful in food and nesting sites. Hedgehogs, are similar to other urban adapted species such as foxes, in being able to exploit anthropogenic artificial food resources which may support higher densities than would be found in more natural environments (Murray et al. 2016). Hubert et al. (2011) showed that the difference between average hedgehog densities in rural (4.4 ± 1.3 individuals km^{-2}) and urban environments (36.5 ± 15.2 individuals km^{-2}) differed by an order of magnitude, in accord with more recent findings of Schaus et al. (2020). Interestingly, higher urban densities were not caused by higher reproductive rates, suggesting that other factors such as increased overwintering survival and reduced mortality may be important (Hubert et al. 2011). This study concluded that the population effects identified were best explained by a combination of greater food availability and availability of refugia and sheltering in urban areas, irrespective of badger activity (Hubert et al. 2011).

Evidence from one study indicates that there may be a sex bias in hedgehog utilisation of specific urban habitat features (Dowding et al. 2010). For example, female hedgehogs were shown to avoid gardens of detached houses more frequently than males, and it was suggested that this may have been because these gardens were more suitable for badgers (Dowding et al. 2010). Occupying niches that the predator species does not utilise

provides refugia and a means of spatial avoidance. Therefore, hedgehogs appear to show a preference for built up areas, opting to stay close to buildings (Pettett et al. 2017), which also increases the likelihood of survival (Doncaster, 1994). Hof et al. (2012), found that hedgehogs were observed in suburban village habitat on 25% more occasions when badgers were present in surrounding habitat, suggesting that predation risk was causing hedgehogs to be displaced away from rural areas.

1.6.1.2 Rural

Rural, rather than urban, hedgehog ecology has received more attention, and therefore the severe declines in hedgehog abundance reported in rural hedgehogs may not be representative of the whole picture (Wembridge 2011; Wilson and Wembridge 2018). Nonetheless, hedgehog occupancy is just 22% across rural England and Wales, and in 77% of the areas where badgers are absent, hedgehogs were also absent (Williams et al. 2018). Therefore, in some areas of the rural landscape, hedgehogs are unlikely to be limited by badgers alone, as other factors such as agricultural intensification have drastically altered the rural landscape for badgers and hedgehogs alike.

Studies have shown that in rural landscapes hedgehogs favour amenity grassland habitat, and seemingly avoid arable and woodland habitat in relation to its availability in the presence and absence of badgers (Pettett et al. 2017). Radiotracking has shown that hedgehog's use of arable habitat was 50% less in sites occupied by badgers (Pettett et al. 2017). The presence of badger activity was also negatively correlated with hedgehog use of suitable habitat such as pasture, an effect which was further exacerbated by hedgehogs being isolated from other suitable habitat (Micol et al. 1994). This suggests that the agricultural landscape is becoming an increasingly hostile environment for hedgehogs due to lack of suitable habitat (Yarnell and Pettett 2020). Therefore, it has been suggested that preserving and restoring hedgerows and field margins would be beneficial, as this would increase habitat heterogeneity and connectivity across the rural landscape, whilst

potentially reducing predator-prey encounters (Hof and Bright 2010; Yarnell and Pettett 2020).

There is evidence to suggest that the behaviour exhibited by hedgehogs, namely their use of spatial refugia (Young et al. 2006), supports the 'landscape of fear' hypothesis (Hof et al. 2012), in which the prey species alters their behaviour in direct response to the level of predation risk (Laundré et al. 2001). For example, hedgehog home ranges have been shown to be smaller in sites occupied by badgers (Pettett et al. 2017), and their use of edge features is greatly increased with badger presence (Hof et al. 2012), suggesting that hedgehogs avoid badgers spatially, presumably due to the perceived risk of predation.

1.6.1.3 Spatial

The relationship between interacting species can differ at various spatial scales, and this was demonstrated by Pettett et al. (2018), revealing that fox abundance was negatively associated with hedgehog numbers at the 10 km² scale but positively associated at the county level. Importantly, these correlations do not imply causation as habitat selection and food availability could also be influencing these trends. Nonetheless, Pettett et al. (2018) found that badger abundance was negatively associated with hedgehog abundance at both scales, analogous to findings of Williams et al. (2018).

At the finer scale, there is strong evidence for habitat selection by hedgehogs, as they are infrequent users of arable, favouring amenity and other grassland habitats. Other habitat features such as buildings, hedgerows and gardens are also positively selected by hedgehogs (Hof and Bright 2010; Dowding et al. 2010b). In order to establish whether these are normal habitat preferences or caused by the presence of badgers, other variables, namely prey availability, must be considered simultaneously. The likelihood is that both factors may play a role in hedgehog habitat selection and therefore an assessment of their relative importance is needed. Hof et al. (2012) assessed the use of edge features by hedgehogs in relation to invertebrate food availability and the presence of badgers. Food

availability did not differ significantly with distance from the edge habitat, although hedgehogs were found disproportionately nearer to edge features when badgers were present, suggesting that factors other than food availability influence habitat selection by hedgehogs.

1.6.2 Prey preferences

Dietary assessment is required to determine how a species utilises locally available prey resources, to establish their dietary niche. Assessment of the diet of hedgehogs in village environments revealed a high frequency of occurrence of artificial food types. Pettett (2015), utilised molecular DNA metabarcoding to identify prey species consumed, and found that faecal samples contained Carabid beetles, Lumbricidae and Lepidoptera, in 100%, 95% and 89% of samples, respectively. High levels of DNA belonging to Bovidae (93%) and Suidae (89%), were also found in the diet, and confirmed as originating from pet food (Pettett 2015). The ubiquity of these food types suggests that artificial food may be an important attractant to urban areas for hedgehogs.

Many common invertebrate prey that hedgehogs consume are likely shared with badgers, particularly those frequently consumed such as earthworms which have been shown to be important in the diet of badgers (Shepherdson et al.1990; Zabala and Zuberogoitia 2003b; Cleary et al. 2011). However, badgers have a wide diet which also includes fruits, vegetables, crops and berries (Shepherdson et al. 1990). Roper (1994) described badgers as dietary specialists at the individual and population level, as badgers in northwest Europe reportedly most commonly consumed earthworms, whereas fruits were most readily consumed in southern Europe. Therefore, this demonstrates that the importance of each prey type is likely to vary in relation to its local and seasonal availability.

The theory of IGP suggests that increased dietary breadth of the intraguild-prey species can promote coexistence with the intraguild predator as the species can adapt and utilise different prey in relation to its availability (Michalko and Pekár 2017). The stable state of coexistence is also most likely to exist in areas with moderate levels of food productivity (Holt and Polis 1997). Whereas, intraguild-prey that are dietary specialists may be more easily outcompeted if food became limiting as they would be unable to adapt through mechanisms such as prey switching (van Zoeren et al. 2018). Therefore, for competition to be minimised, hedgehogs must be able to exploit resources that are either not favoured or are less available to badgers, possibly using niches that may be relatively poorer in terms of prey availability that are avoided or underutilised by badger, in order to coexist at the local level. To date, no studies have directly compared hedgehog and badger diet from the same location, and so it has not been possible to ascertain to what degree hedgehogs and badgers are competing for food at the local site level.

1.6.3 Predation of hedgehogs by badgers

Hedgehogs have been shown to associate badger odour with a risk of predation (Ward et al. 1996), and this has been attributed to their preference for low predator risk suburban habitat. Moreover, hedgehogs daily energy expenditure has been shown to be 30% lower than when in the presence badgers (Pettett et al. 2017). This suggests that hedgehogs alter their activity levels in response to predation risk, restricting their movement in the presence of badgers (Pettett et al. 2017) and avoiding areas tainted with badger odour (Ward et al. 1997). However, the response of hedgehogs to badger presence is not ubiquitous, as they have been shown to forage within close proximity to one another, sharing gardens (Tysnes 2016) and even food resources (PTES 2021), suggesting the relationship is complex and likely locally and temporally context dependent.

In conditions where food resources are limited, species may broaden their dietary niches, to allow them to better exploit the food available (Michalko and Pekár 2017). For badgers this could include predating on hedgehogs under food limiting conditions but not at other times, as suggested by Hof and Bright (2010) to be the cause of their observed high predation rates, particularly in areas with low earthworm availability. Across two large arable farms, Hof and Bright (2010) recorded a hedgehog mortality rate of 20%, of which 18% was caused by badger predation. When extrapolated over a full season, an unsustainable mortality rate of 52% was predicted. Moreover, 88% of hedgehogs predated by badgers were male, a sex bias most likely caused by larger home ranges of male hedgehogs during the mating season which potentially increases chances of encountering foraging badgers. Similarly, the translocation of hedgehogs into badger dense areas resulted in predation, and the dispersal of hedgehogs away from these introduction sites, which returned the abundance of hedgehogs to its initial number just a month after the translocation event took place (Doncaster, 1994).

For predation to negatively affect a hedgehog population and drive its decline, the predation rate must exceed the birth rate and immigration rate combined. Intraguild predators can sustain high predation rates on intraguild prey as the latter decline in number, as shared prey becomes more plentiful, sustaining higher intraguild predator densities (Polis et al. 1989). Alternatively, predation may impact individuals but because of density dependent factors this may in fact help increase the survival of the remaining individuals by reducing encounter rates between predator and prey (Janssen et al. 2007). Therefore, it is difficult to establish whether predation itself has the potential to limit and reduce hedgehog abundance and, depending on the local hedgehog population size, whether this could occur at an unsustainable level, ultimately leading to localised extinctions of hedgehogs.

1.6.4 Numerical relationship between badgers and hedgehogs

Micol et al. (1994) was the first to suggest a 'landscape of fear' hypothesis for explaining the relationship exhibited between hedgehogs and badgers and predicted that the former would be mostly absent from rural areas where badger sett density was ≥ 2.27 km^{-2} (Micol et al.1994). Furthermore, there is evidence to suggest that hedgehogs can be excluded from amenity grassland habitat in areas with >10 setts km^{-2} (Young et al. 2006). More recent estimates predict that hedgehogs would be excluded from an area that had a sett density of 5.21 setts km^{-2} or 3.29 main setts km^{-2} (Williams et al. 2018). However, these studies are mostly correlative, large scale and limited to the use of indirect indices of abundance which may be imperfect.

Whilst hedgehog numbers are decreasing in both rural and urban areas (Wilson and Wembridge 2018), there are large differences in the densities of hedgehogs in these two broad landscapes. Recent estimates of rural hedgehog densities ranged from 1.2 – 6.8 km^{-2}, whereas urban densities were much higher, ranging from 13.9 – 25.9 km^{-2} (Schaus et al. 2020). Long term monitoring suggests that the rate of decline could be four times greater in rural areas as hedgehog abundance decreased by up to 13.7% (between 2000 – 2014) and 3.1% (between 2003 – 2014) in rural and urban areas, respectively (Wembridge and Langton 2015), suggesting that different processes may be driving declines in these environments.

At the local scale, hedgehog abundance can vary within and between habitat types, with evidence of fine-scale variation related to earthworm availability and badger sett density across pasture habitat (Micol et al. 1994). The availability of earthworms was shown to increase incrementally with the age of mown grassland between 4 and 40 years old and this was positively associated with hedgehog abundance (Doncaster, 1994). However, in a study by Parrott et al. (2014), hedgehogs were only detected on 2% of pasture fields compared to 26% of amenity grassland habitat. The almost absence of

hedgehogs in pasture fields is consistent with Young et al. (2006), who again found them concentrated on amenity grassland that is rarely found in the rural landscape and more associated with urban landscapes where hedgehog densities are known to be higher (Schaus et al. 2020). Trewby et al. (2014) provided experimental evidence showing that hedgehog abundance doubled from 0.9 ha^{-1} to 2.4 ha^{-} in their preferred amenity grassland habitat, over a five-year period in areas with badger culling. Hedgehog abundance did not change in control areas (0.3–0.3 ha^{-1}) where there was no badger culling, suggesting that hedgehogs benefited from mesopredator release in in areas where badger density was reduced (Trewby et al. 2014). However, these studies did not account for variable detection probabilities of hedgehogs in each habitat.

1.7 Summary and aims

The available evidence suggests that badgers exert a negative pressure on hedgehogs, through both competition and predation (Doncaster et al. 2001; Hof and Bright 2010). Past studies have concluded that hedgehogs avoid badgers (Ward et al. 1996; Hof et al.2012; Williams et al. 2018), but there is no evidence for how other factors such as habitat preference and food availability may influence the resulting pattern of occupancy. Therefore, it is necessary to try to disentangle these complex interacting factors in order to explain hedgehog abundance and distribution in relation to badgers and other factors.

Integrating habitat, prey, and predator presence into a broader, more holistic study, is essential to better understand the recent decline of the hedgehog and how badgers may influence hedgehog abundance and distribution. By including multiple rural study sites, hedgehog spatial use at the landscape and local level can be determined. Habitat use should be assessed in conjunction with a predictor of food resources, to better assess the importance of each component that may influence competition and predation amongst badgers and hedgehogs. Therefore, the main aim of this study is to explore which factors better explain the ecological mechanisms of coexistence within this IGP relationship.

The objectives of this study are to i) investigate whether hedgehogs and badgers coexist at the landscape and local scale across the rural landscape; ii) identify the best predictors of hedgehog abundance, including habitat, prey availability and predator abundance, and iii) provide an overview of the circumstances under which badgers and hedgehogs appear to co-exist, or otherwise, across the rural landscape.

To meet these objectives, the following research questions are posed:

- What is the numerical relationship between badger and hedgehog density at the local scale?

It is anticipated that the relationship between hedgehog and badger density will be negative due to predation risk and competition for food.

- Does habitat use by hedgehogs alter in the presence of badgers within their home ranges at the patch scale (i.e., is there spatial segregation from badgers)?

Based on past research it is expected that hedgehog and badgers will show spatial separation due to hedgehogs avoiding predation from badgers.

- Do hedgehogs exhibit temporal segregation from badgers?

IGP theory depicts that niche separation can be achieved through temporal segregation and as this has been shown to facilitate coexistence of other intraguild species it may also be important for co-occurring hedgehog and badger.

- To what extent does the diet of badgers and hedgehogs overlap and does this vary seasonally?

As badgers and hedgehogs are both dietary generalists that consume invertebrate prey it is expected that their diets will be overlapping and that competition for food between these two species may vary in response to seasonal availability of different prey types.

- How does hedgehog diet and habitat use reflect local food availability?

Under IGP theory, as badgers and hedgehogs share prey species, hedgehogs are expected to occupy areas of relatively low food availability in relation to badgers that may outcompete them. This would further explain their predicted spatial separation. Lastly, dietary assessment is expected to reveal predation of hedgehogs by badgers, which may exacerbate the requirement for spatial segregation.

- Is hedgehog abundance best predicted by habitat availability, competition for prey or predation risk?

These predictions will be tested and then evaluated in the thesis discussion.

1.8 Thesis structure

Chapter 1: Provides background information so that the subsequent chapters can be put in context.

Chapter 2: Provides a general methodology, including site descriptions.

Chapter 3: Investigates the availability of invertebrate prey across different rural habitats.

Chapter 4: Identifies the diet of badger and hedgehog, assessing the level of dietary overlap and identification of predation events. Seasonal variation in the diet is also discussed.

Chapter 5: Assesses whether badger and hedgehog diet reflect local food availability across two sites and four seasons.

Chapter 6: Models camera trapping data from 23 sites to investigate spatial and temporal patterns of hedgehog and badger across rural habitats.

Chapter 7: Evaluates and synthesises the findings of this thesis to draw conclusions as to the importance of intraguild predation on hedgehog populations. The best predictors of hedgehog abundance are discussed alongside how the research findings could inform the ongoing conservation of hedgehogs.

2.1 Study Site Designation

In total, 23 rural sites ranging from 0.5 – 1.2 km² were surveyed for badger and hedgehogs between April and September in 2018 or 2019 (Figure 2.1). The total survey effort (23 sites) was determined by logistical constraints related to the scope of the PhD and therefore sites were selected (Table 2.1) to provide a representation of rural areas across England and Wales where there had been recent evidence of badger and/or hedgehog activity (Williams et al. 2018). Survey efficacy was maximised to incorporate localised areas fulfilling three species mixes: badger only, hedgehog only and areas where both species co-occur at the local 1 km² scale. The People's Trust for Endangered Species (PTES) provided support for raising awareness of the study to landowners, with several offering access to their farms. Three agricultural Campuses: Nottingham Trent University, Hartpury College and Riseholme College, were included, and one National Trust site, Clumber Park. Remaining sites were either arable-dominated, pasture-dominated or mixed farms, which constitutes the vast proportion of the rural landscape in England and Wales. Badgers and hedgehogs co-occurred at 11 of the 23 sites (Table 2.1). This occurred at 7 out of 12 mixed farms, 4 out of 6 pasture farms and none of the 5 arable farms. Therefore, co-occurrence was more likely at pasture farms, followed by mixed farms and less likely at arable farms.

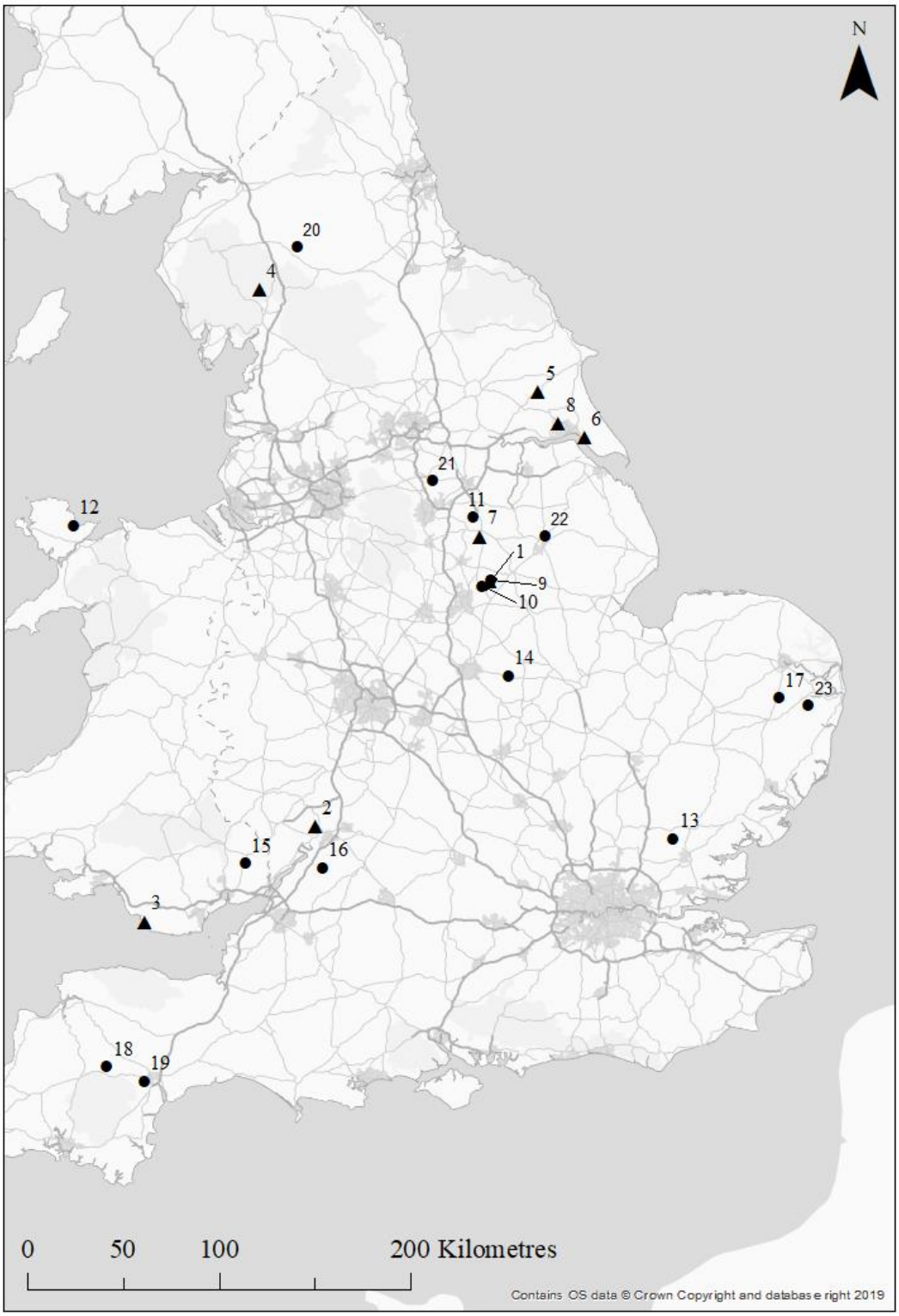


Figure 2.1 Location of 23 sites where camera and invertebrate surveys were conducted to investigate badger and hedgehog intraguild predation between April 2018 and September 2019. Sites surveyed in 2018 (n = 8) are depicted by triangles and those surveyed in 2019 (n = 15) by circles. Numbering depicts the order sites were visited.

Table 2.1 Summary of site characteristics for 23 rural sites surveyed between April-September 2018-2019. Site numbers correlate with the sites illustrated in Figure 2.1.

Site No.	Site Name and County	Grid Ref.	Survey dates	No. of survey nights	Site area (km^2)	Badger (ü = present)	Hedgehog (ü= present)	Land-use Description
1	Brackenhurst A - Nottinghamshire	SK 69533 52275	05/04/2018 - 24/04/2018	20	0.53	Ü	ü	Mixed farm (college)
2	Hartpury- Gloucestershire	SO 78752 22977	30/04/2018 -10/05/2018	10	0.70	Ü	ü	Mixed farm (college)
3	Slade - Glamorgan	SS 89139 73102	17/05/2018 - 28/05/2018	11	0.60	Ü	ü	Organic Pasture/Livestock farm
4	Kendal - Cumberland	NY 49572 03867	18/06/2018 - 28/06/2018	10	0.40	Ü	ü	Pasture/Livestock farm
5	Driffield - East Yorkshire	SE 95322 50697	30/06/2018 - 10/07/2018	10	0.84	Ü	ü	Mixed farm
6	Keyingham - East Yorkshire	TA 22393 23519	14/07/2018 - 24/07/2018	10	0.60	Ü		Mixed farm
7	Clumber - Nottinghamshire	SK 62587 75134	03/08/2018 - 17/08/2018	15	0.65	Ü		Arable (National Trust site)
8	Thorn - East Yorkshire	TA 19519 27495	20/08/2018 - 30/08/2018	10	0.72	Ü		Arable farm
9	Brackenhurst B - Nottinghamshire	SK 69533 52275	15/04/2019 - 26/04/2019	10	0.77	Ü	ü	Mixed farm
10	Epperstone - Nottinghamshire	SK 66100 49191	07/05/2019 - 17/05/2019	10	0.78	Ü		Arable farm
11	Hodsock - Nottinghamshire	SK 61271 85391	14/05/2019 - 24/05/2019	10	0.67	Ü	ü	Mixed farm
12	Anglesey	SH 51785 80988	04/06/2019 -14/06/2019	10	0.69		ü	Mixed farm
13	Dunmow - Essex	TL 65600 17534	04/06/2019 -14/06/2019	11	1.00	Ü		Arable farm
14	Loddington - Leicestershire	SK 79094 02431	17/06/2019 - 27/06/2019	10	1.00	Ü		Mixed farm
15	Usk - Monmouthshire	SO 41668 04553	19/06/2019 -29/06/2019	10	0.50	Ü	ü	Pasture/Livestock farm
16	Woodchester - Gloucestershire	SO 81385 01635	02/07/2019 -12/07/2019	10	0.61	Ü		Dense woodland, Pasture
17	Long Stratton - Norfolk	TM 21040 90882	11/07/2019 - 23/07/2019	12	0.79	Ü	ü	Mixed farm
18	Spreyton - Devon	SX 69828 97398	16/07/2019 - 26/07/2019	10	0.63	Ü	ü	Organic Pasture/Livestock farm
19	Ide - Devon	SX 89042 90300	17/07/2019 - 17/07/2019	10	0.58	Ü		Mixed organic farm
20	Knock - Cumberland	NY 68769 27063	05/08/2019 - 15/08/2019	10	1.00	Ü		Pasture/Livestock farm
21	Barnsley - South Yorkshire	SE 39426 05206	09/08/2019 - 19/08/2019	10	1.10		ü	Mixed farm
22	Riseholme - Lincolnshire	SK 96962 78065	12/09/2019 - 22/09/2019	10	0.96	Ü	ü	Mixed farm (college)
23	Suffolk	TM 36890 87186	16/09/2019 - 26/09/2019	10	0.53			Arable farm

2.2 Landowner Permissions

Landowners were identified and contacted initially by telephone or email to discuss participation in the study and to gain permission for access. The methods and rationale for the study were explained prior to the start of survey work.

2.3 Land Cover

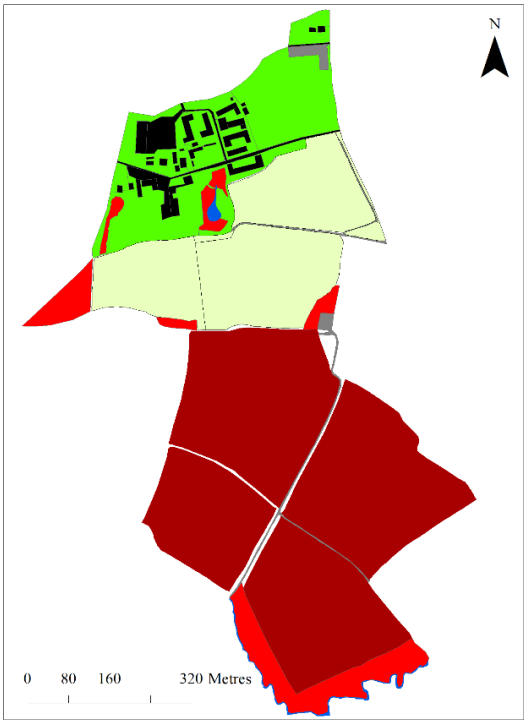
Site boundaries were designated primarily to ensure the inclusion of multiple broad habitats at the local scale (Table 2.2).

Table 2.2 Broad habitat definitions adapted from the JNCC Phase 1 Habitat Survey Handbook (JNCC 2010) used to categorise and map the habitats across 23 study sites across the UK.

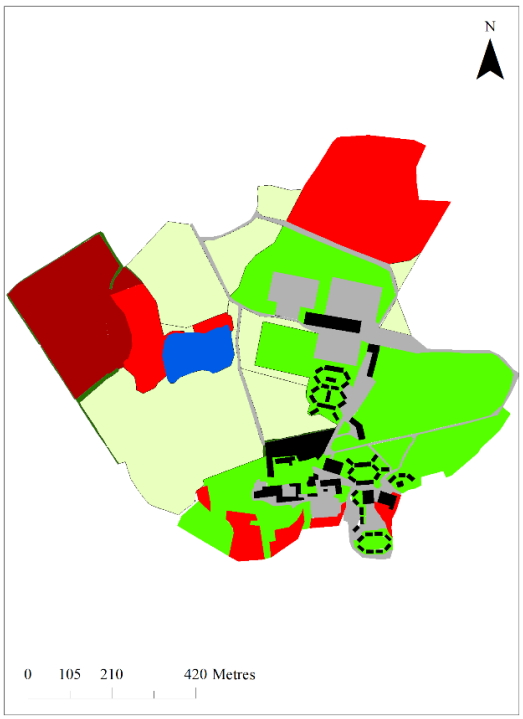
Habitat Type	Definition
Arable	Arable cropland, horticultural land (for example nurseries, vegetable plots, flower beds), freshly ploughed land and recently reseeded grassland, such as rye grass and rye clover leys, often managed for silage
Amenity	Intensively managed and regularly mown grasslands, typical of lawns, playing fields, golf course fairways and many urban 'savannah' parks
Building	Includes agricultural, industrial, and domestic buildings.
Grassland	Acid grassland (unimproved and semi-improved) Neutral grassland (unimproved and semi-improved) Calcareous grassland (unimproved and semi-improved) Improved and poor semi-improved grassland. Marsh/marshy grassland.
Hedges	Includes native species-rich, intact, defunct and hedges with trees.
Open water	Standing water (all types, including coastal lagoons). Running water (all types)
Woodland	Semi-natural broadleaved woodland Semi-natural coniferous woodland Semi-natural mixed woodland Plantation woodland (broadleaved, coniferous, and mixed) Dense/continuous scrub and recently felled woodland.
Urban	Built up areas with infrastructure such as hardstanding, roads and buildings.

2.4 Site Mapping

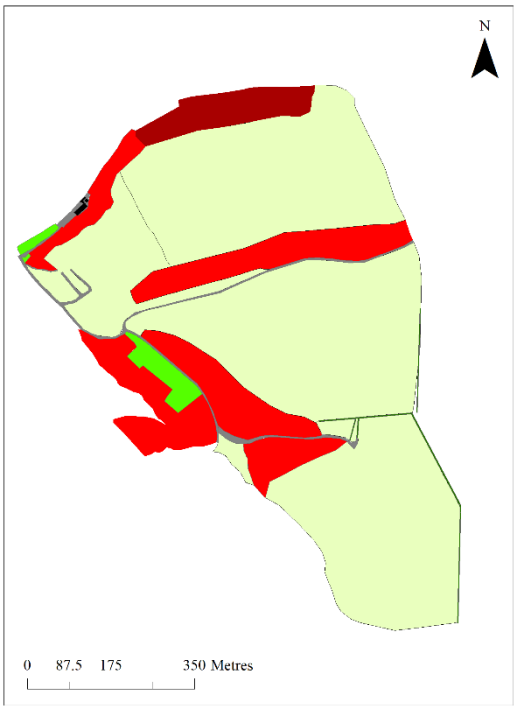
The boundary of each study site was mapped using ArcMap GIS software (ESRI (Environmental Systems Research Institute.) 2018). Habitat mapping was conducted using aerial orthophotographs that were downloaded from the EDINA Digimap service providing detailed 25cm resolution of habitat features (Figure 2.2)



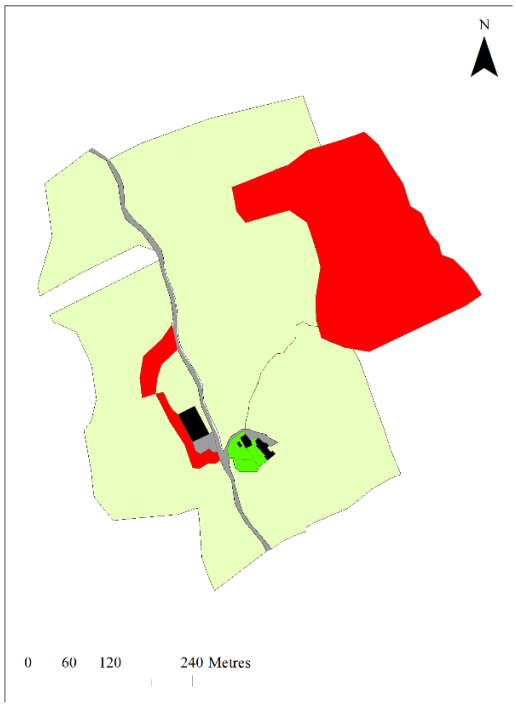
Site 1) Brackenhurst A



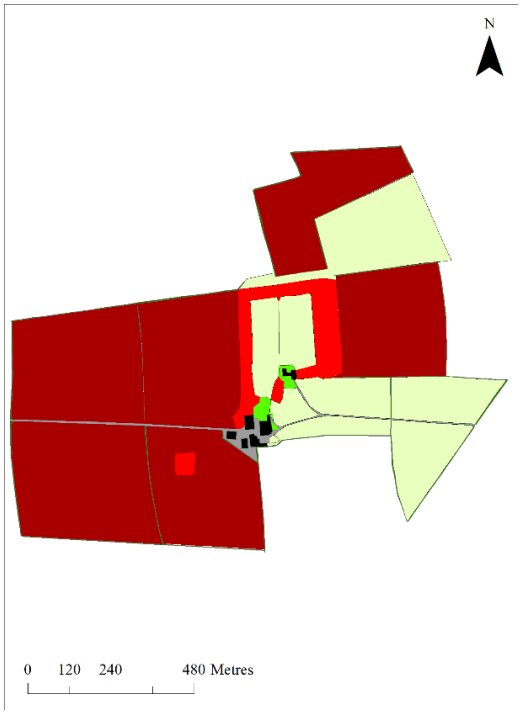
Site 2) Hartpury



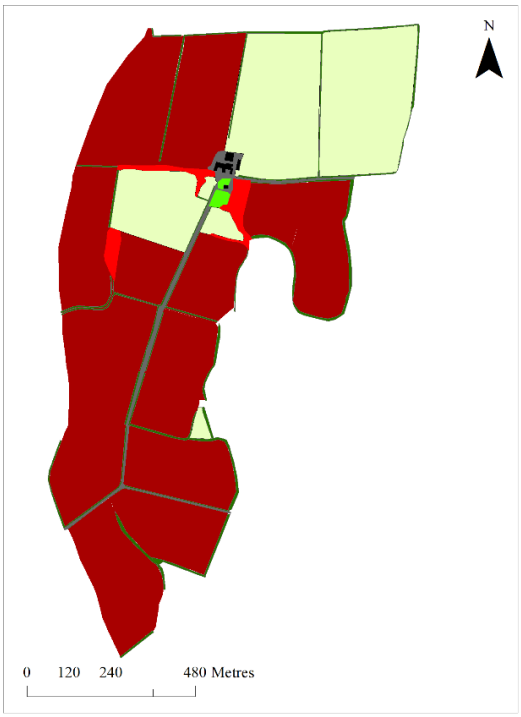
Site 3) Slade



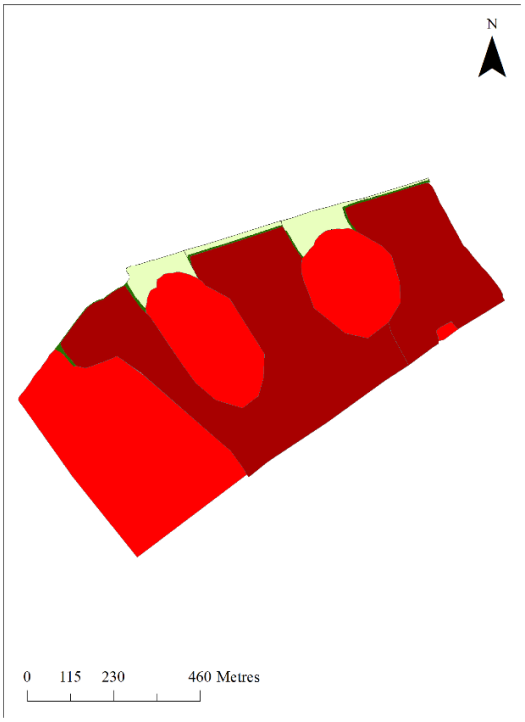
Site 4) Kendal



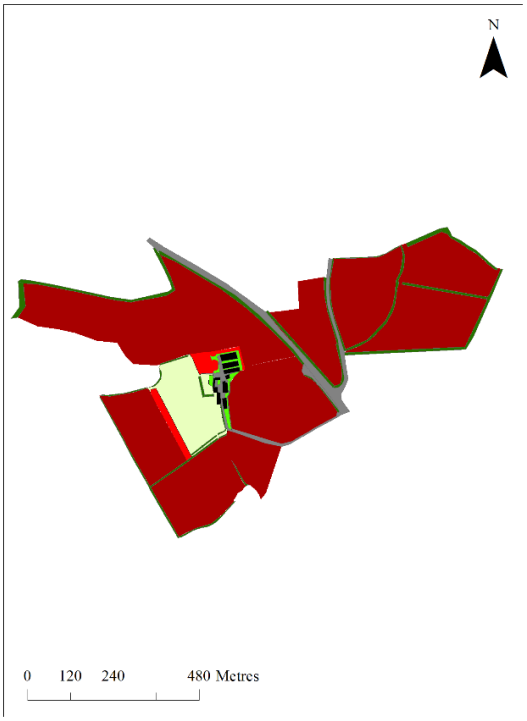
Site 5) Driffield



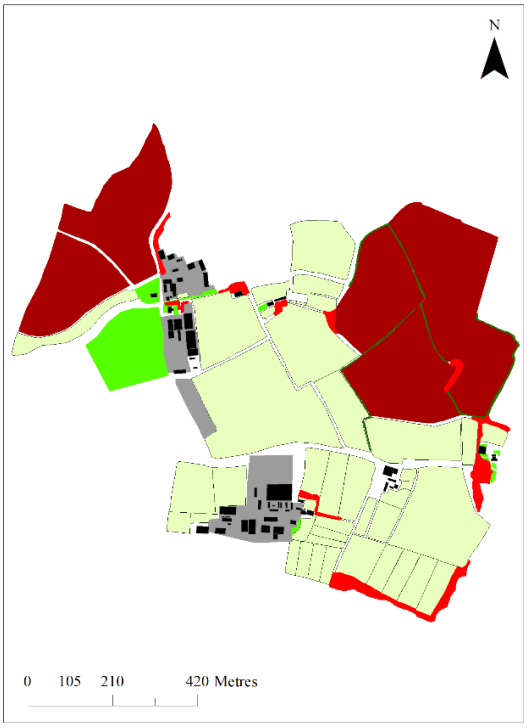
Site 6) Keyingham



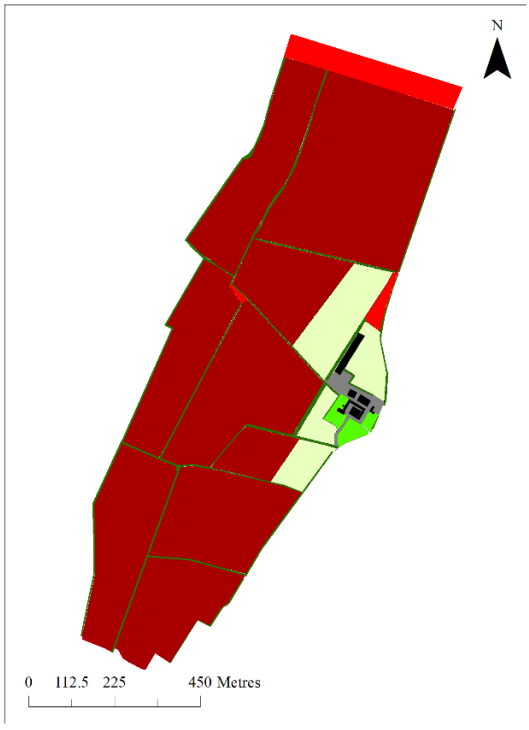
Site 7) Clumber



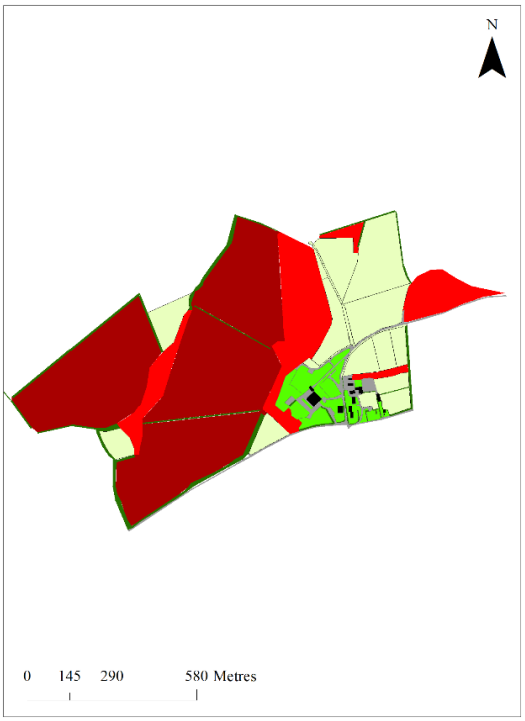
Site 8) Thorn



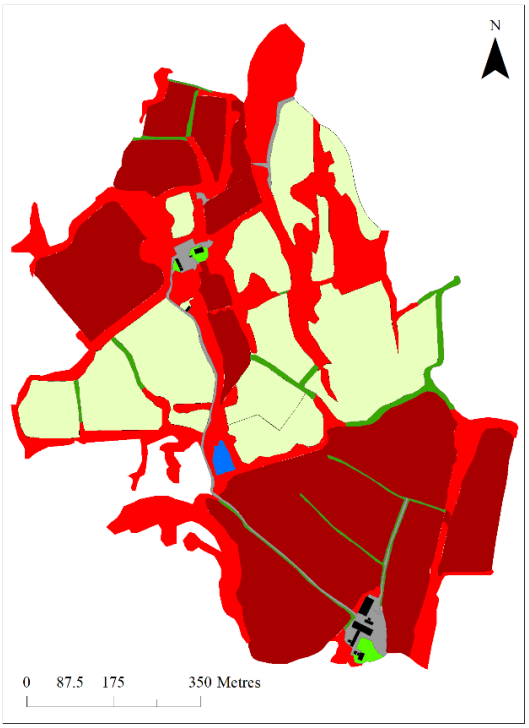
Site 9) Brackenhurst B



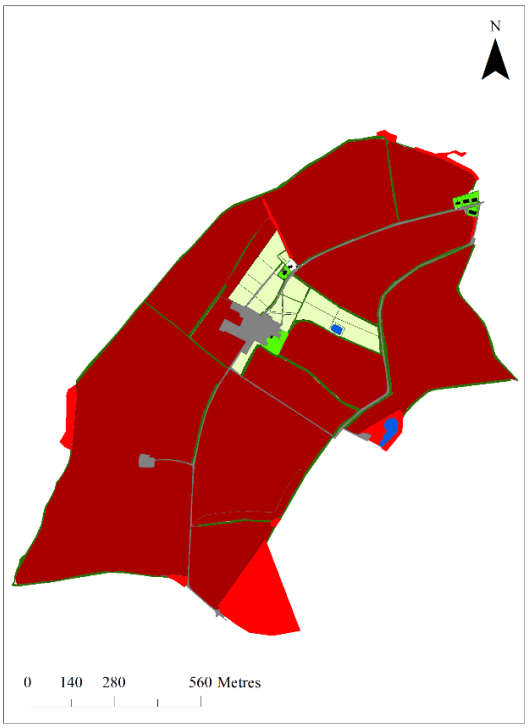
Site 10) Epperstone



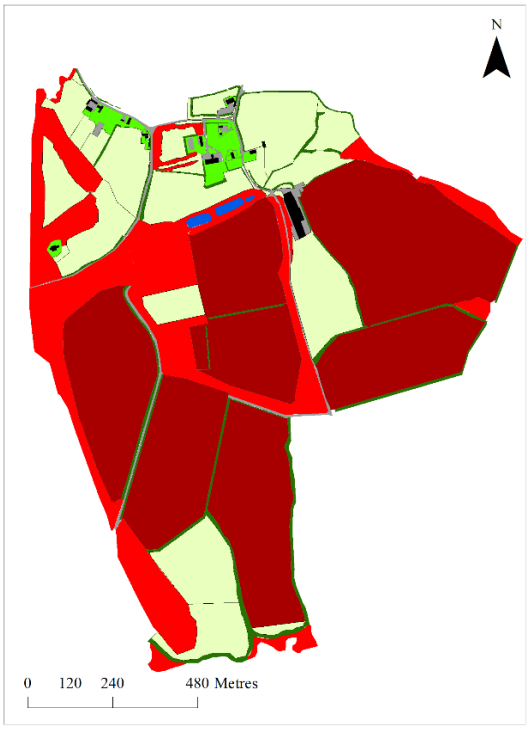
Site 11) Hodsock



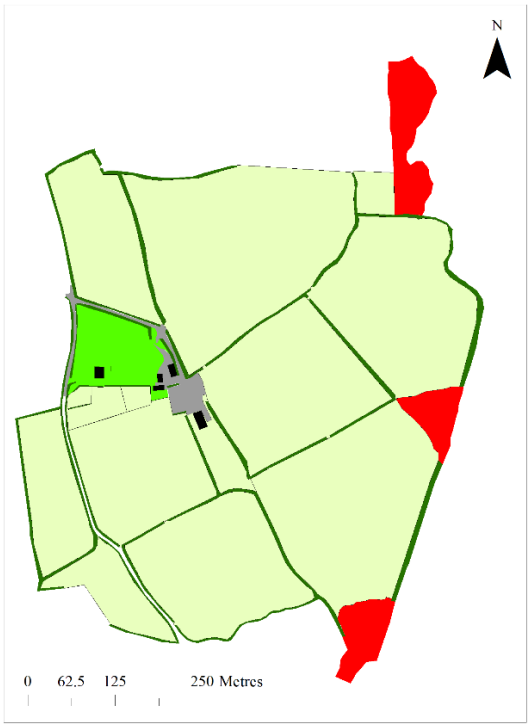
Site 12) Anglesey



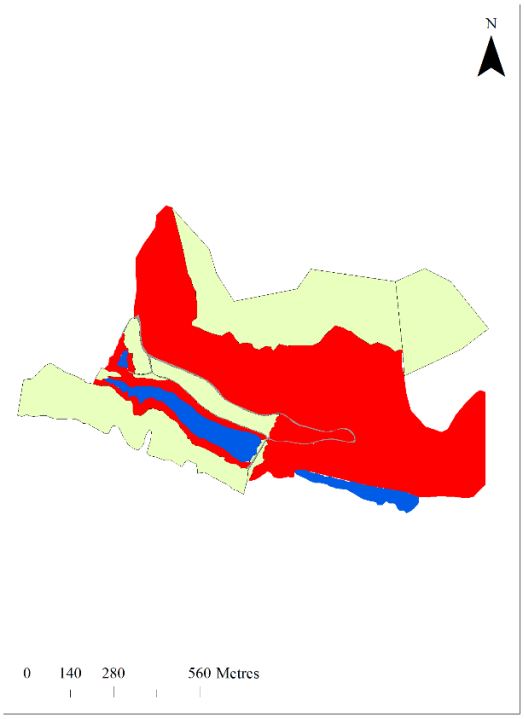
Site 13) Dunmow



Site 14) Loddington

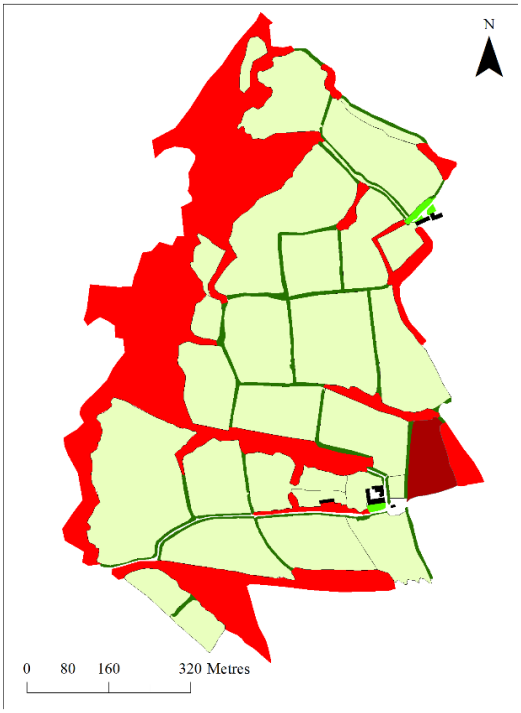


Site 15) Usk

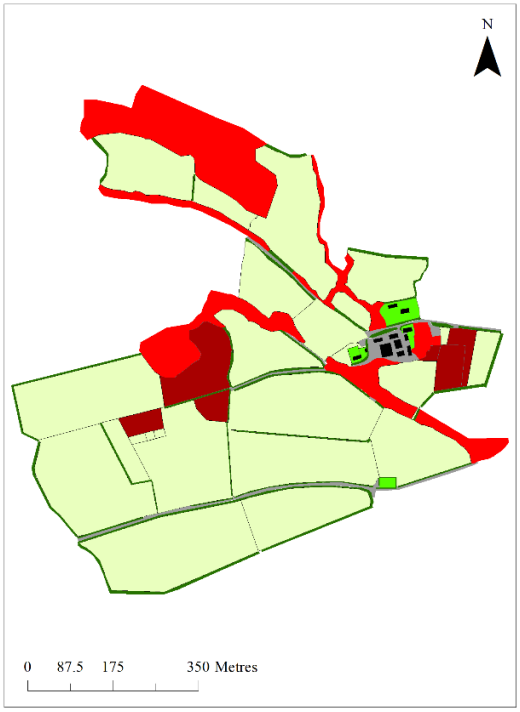


Site 16) Woodchester

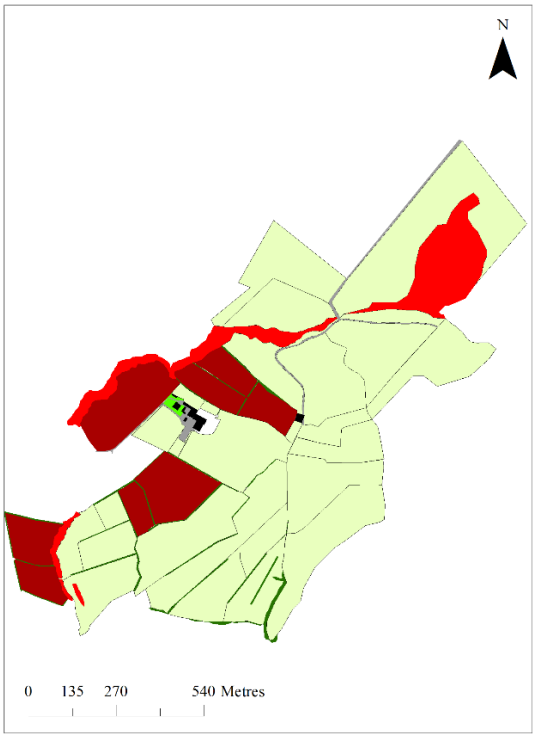
Site 17) Long Stratton



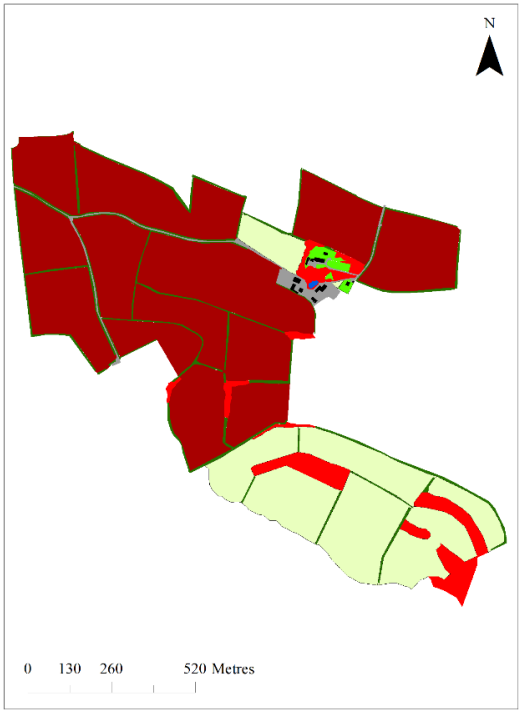
Site 18) Spreyton



Site 19) Ide

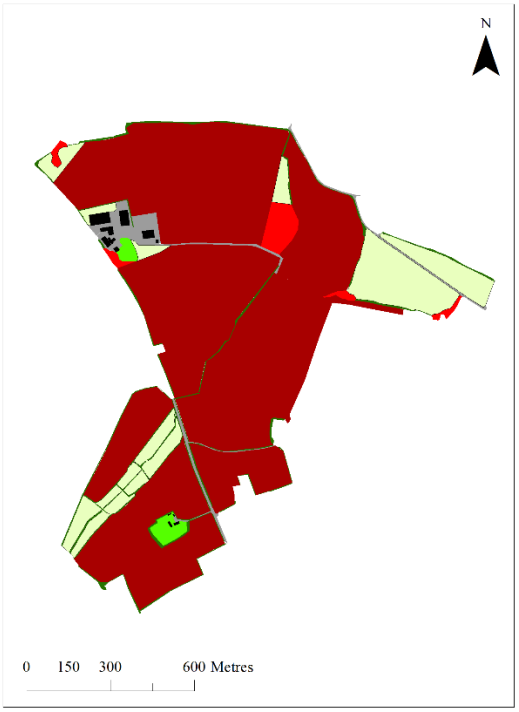


Site 20) Knock



Site 21) Barnsley

Site 22) Riseholme



Site 23) Suffolk

Figure 2.2 Habitat mapping for sites surveyed between April 2018 and September 2019. Habitats are coded as follows: brown = arable, lime green = amenity grassland, black = buildings, light green = grassland, dark green = hedges, blue = open water, red = woodland and grey = urban.

2.5 Weather

The mean temperature in the UK was 9.5°C and 9.4°C in 2018 and 2019, respectively, 0.6°C higher than the 1981-2010 average (Table 2.3) (Met Office 2020). In 2018, total rainfall was 1064 mm, 8% less than the average rainfall between 1981-2010, whereas 2019 was generally wetter, with 1240 mm of rainfall, 7% more than the 1981-2010 average.

Table 2.3 Seasonal temperatures and rainfall for the UK between 2018 and 2019 (Met Office 2020).

2018	Winter	Spring	Summer	Autumn
Rainfall (average mm)	317	239	176	332
Temperature (average °C)	3.6	8.1	15.8	9.8
2019	Winter	Spring	Summer	Autumn
Rainfall (average mm)	256	249	337	357
Temperature (average °C)	5.2	8.4	15.1	9.1

2.6 General Methods

2.6.1 Invertebrate community sampling

As invertebrates are known to constitute a major component of both hedgehog and badger diet, an assessment of the macroinvertebrate community was used as a proxy for food availability. Surveying was carried out at all sites, except for Thorn in 2018 which was omitted due to particularly hot and dry conditions that made sampling challenging. A standardised sampling effort was applied across each site for assessment of the macroinvertebrate community which included three earthworm cores and nine singular pitfall traps, placed at randomly generated locations within each habitat according to the types described in Table 2.2. Pitfall traps provided a standardised comparative method for comparing macroinvertebrate communities (Boetzl et al. 2018), however sampling effort that includes number of pitfalls and number of trapping nights, was limited at each site due to the logistical constraints of the project. Therefore singular pitfall traps were included to allow an increase in the number of spatial replicates that correlates with higher species richness and catch abundance (Boetzl et al. 2018). As earthworms are soil fauna, cores were included as an additional measure for this taxa, providing an efficient method of assessing abundance and density with limited disturbance (J. Smith et al. 2008).

2.6.1.1 Pitfall trapping

Plastic tapered cups with the dimensions 9.5 cm height, 8 cm width at the top and 6 cm at the base, were dug into the ground. The rim of the plastic cup was flush with the ground substrate to ensure a consistent catch rate between cup, habitat and location. Each cup was filled halfway with propylene glycol (Special Ingredients Ltd), an odourless preservative that is not harmful to livestock or other wildlife. The preservative also prevented predatory action between the macroinvertebrates that were caught in the traps (Schmidt et al. 2006). A wooden stick was placed within each cup to provide a ramp for non-target species such as small mammals and amphibian species to escape. To avoid the traps flooding and to

minimise disturbance, each trap was covered by a 15 cm by 15cm plastic half pipe, with the sides removed to leave a support in each corner. Traps were left unattended for ten nights before being collected and stored until identification took place. Individual pitfall traps were sorted through and organisms >5mm were identified to Order level, and abundance was recorded. Organisms from individual pitfall traps were dried in an oven at 60°C for 72 hours. Organisms from each Order were dried separately, and their dry biomass recorded. Two measurements were recorded, initially at 48hrs and again at 72hrs, to ensure that a constant biomass had been reached.

2.6.1.2 Earthworm coring

Earthworm coring was performed following the protocol described by Valckx et al. (2011), whereby each 25cm³ square soil core was thoroughly sorted by hand in the field. Earthworm abundance and wet biomass (g) were recorded before returning organisms to the environment.

2.6.1.3 Seasonal invertebrate surveys

The invertebrate sampling protocols were repeated at Brackenhurst Campus and Hartpury College for five seasons commencing in summer 2018 and ending in summer 2019. This provided an assessment of the local availability of invertebrates as potential prey items throughout the year that could be compared against dietary preferences (Chapter 5). On each sampling occasion, new sampling locations were randomly generated for each site using the sampling tool in ArcMap (ESRI (Environmental Systems Research Institute.) 2018).

2.6.2 Scat analysis overview

Two working farms set in rural University estates in the UK were selected to quantify the diet of badgers and hedgehogs: Hartpury College in Gloucestershire (0.70 km², 51° 54'N, -2° 18'E) which is pasture-dominated and Nottingham Trent University's Brackenhurst

Campus in Nottinghamshire (0.55 km², 53° 3'N, 0° 57'E) which is arable-dominated. Although next generation deoxyribonucleic acid (DNA) sequencing costs have rapidly reduced, the cost per sequencing run remains substantial (Hert et al. 2008) and therefore funding constraints of the present study, limited molecular dietary analysis of co-occurring badgers and hedgehogs to two study sites. These sites were selected as the presence of badger and hedgehog was known at both sites prior to commencing sampling, with density estimates as given in Chapter 6.

Scat (faecal) samples were collected throughout the period May 2018 – August 2019, representing four and five sampling seasons for hedgehog and badger, respectively. Seasons were as follows; Summer 2018 = June - August 2018, Autumn 2018 = September - November 2018, Winter 2018 = December 2018 – February 2019, Spring 2019 = March – May 2019 and Summer 2019 = June – August 2019. Sampling for hedgehog scats was omitted during the winter season, as this reflects their hibernation period when activity levels are low.

Walkover site surveys were carried out to search for hedgehog scats systematically, with a minimum of two surveys per month, and additional sporadic sampling. Hedgehog scats were identified by their distinctive features, typically being about 3-5 cm in length and shiny black in appearance. Remnants of insect prey were usually clearly visible too (Olsen 2014). Badger scats were sampled by bimonthly surveys of active latrines found near badger setts. The appearance of badger scats varied and depended on what had been consumed. Often, scats were slimy and black, indicative of an abundance of earthworms, and deposited in distinctive shallow pits (latrines), up to 10 cm deep (Olsen 2014).

Samples were taken from each individual scat, using 15 ml Kartel stool vials with integrated spoons that were filled with a ratio of 1:10, stool to absolute ethanol. To ensure that each sample was representative of the scat, three replicates were taken where possible, each from a unique sampling location on the scat. To minimise contamination from the

surrounding substrate, the external surface was avoided where possible. This was especially important for sampling badger scats that are typically found in shallow latrines (Buesching et al. 2016) where the substrate is often exposed soil, as the microbial community in soil would likely dominate genetic sequencing and therefore reduce the data on actual prey species (McInnes et al. 2017). Sample freshness was important for retaining prey DNA within the scats that is otherwise lost over time through DNA degradation (McInnes et al. 2017). Multiple studies have shown scats < 5 days old provide the highest concentration and quality of DNA (Deagle et al. 2005; Oehm et al. 2011). Therefore, only fresh scats were sampled then frozen at -18°C until further processing took place.

A total of 215 scats were collected, of which 80 badger and 64 hedgehog scats (144 in total) were selected for subsequent analysis as follows: eight samples were included per species, per season, from each of the two sites. A sufficient number of scats (> 59) for detecting principal prey items within the diet (Trites and Joy 2005; Foster et al. 2010) were analysed for both species, though the sample size was limited by the number of scats that could be analysed using the available sequencing resources, whilst maintaining adequate sequencing read depth per sample (Forin-Wiart et al. 2018). To ensure the diets of badgers and hedgehogs were assessed year-round, an equal number of scats were taken per season for each species. Seasonal variation in the diet was assessed at the species level across both sites combined due to the limited sample size. Samples were selected to represent unique sampling dates, ensuring independence of samples. Records of sample freshness were also consulted to preferentially analyse fresher scats that would be more suitable for molecular assessment (Reed et al. 1997). All scats were analysed at the UK Natural Environment Research Council (NERC) Biomolecular Analysis Facility (NBAF) at the University of Sheffield. The full molecular workflow is shown in Figure 2.3.

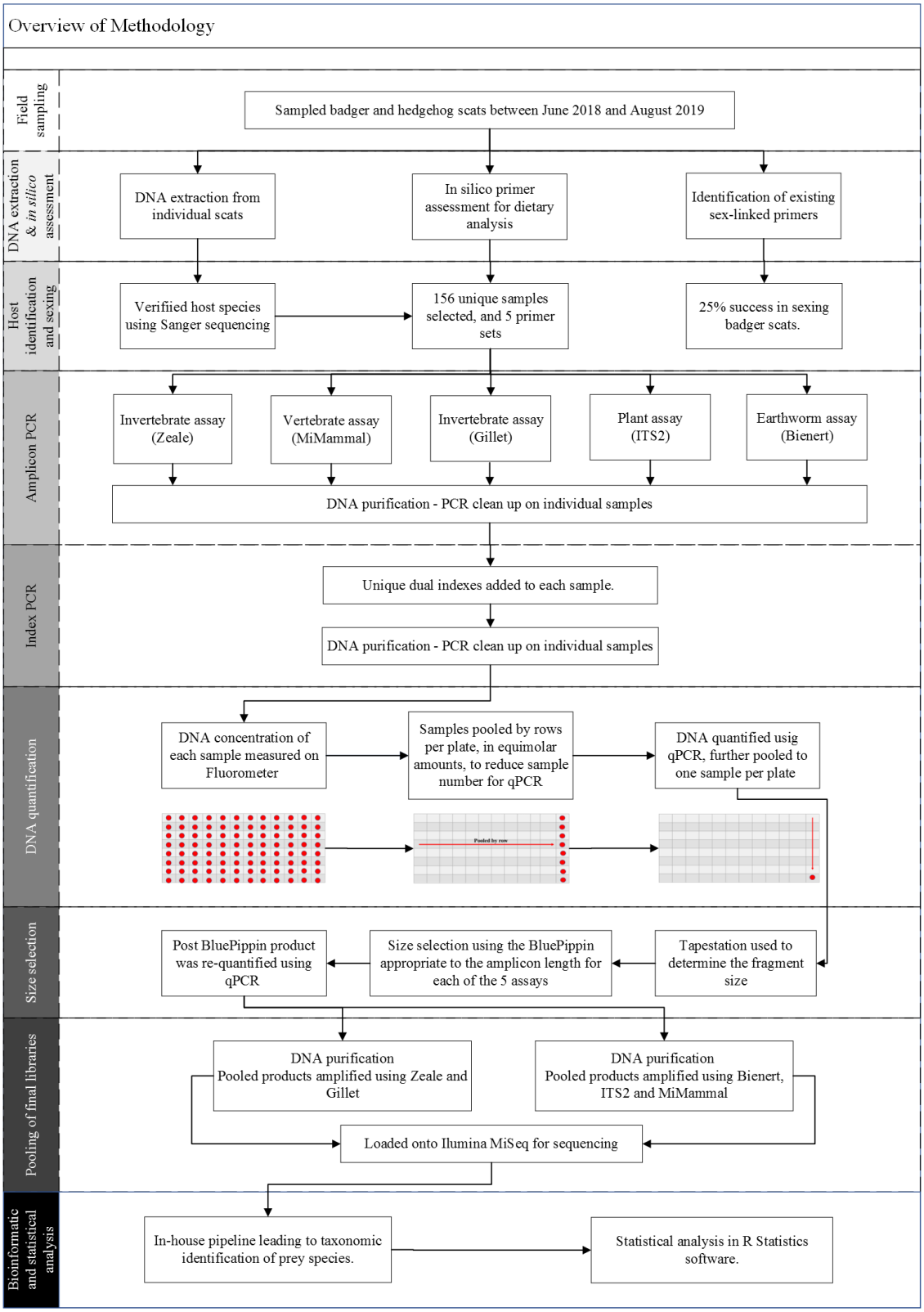


Figure 2.3 Description of the molecular protocol implemented for the metabarcoding of 144 badger and hedgehog faecal samples, amplified using five primer sets and sequenced on the Illumina Miseq.

2.6.3 Confirming species' identity and sexing host species

To verify the host species of the scat samples, DNA was extracted from badger and hedgehog tissue samples using an ammonium acetate method (section 2.6.3.1). Existing extracted genomic DNA was available for dog (*Canis lupus*), mallard (*Anas platyrhynchos)*, house sparrow (*Passer domesticus*) and fox (*Vulpes vulpes)*, that were also included to assess primer amplification success. Including controls from a diverse range of species with varying numbers of mismatches between the species and the primer sequence allowed the effect of these primer mismatches and the capabilities of the primers to be tested. Increasing numbers of mismatches between the primer sequence and species-specific DNA, especially at the 3-prime end of the primer, decreases the likelihood of successful amplification and thus species identification. All samples were amplified using the 'MiMammal' primer set (Ushio et al. 2017), generic to the DNA of many mammals, and polymerase chain reaction (PCR) methods (section 2.6.3.2). Amplicons were then Sanger sequenced (Sanger et al. 1977), and analysed using an ABI 3730 DNA analyser (section 2.6.3.3) and BioEdit software (Hall 1997). The sequences were then compared to reference sequences in the NCBI GenBank nr sequence database using the BLASTN tool and MEGA (Kumar et al. 2016) software (section 2.6.3.4), to identify which species produced the sample and select those samples for subsequent analysis that were from the target species.

2.6.3.1 DNA extraction from tissue samples

Genomic DNA was extracted from hedgehog and badger tissue using an ammonium acetate method (Nicholls et al. 2000; Richardson et al. 2001). Eight ear tissue samples, taken from known sex roadkill badgers, were provided by Roger Cottis of Scottish Badgers. One hedgehog ear tissue sample was provided from a deceased male that had been housed in captivity by Holderness Hedgehog Hospital (a registered charity 1178929). A 1 cm^3 sample of tissue (taken from the ear of either species), was cut into small pieces

on a sterile glass tile using sterilised tweezers and a sharp scalpel. All equipment was bleached (10%) thoroughly between samples. Each sample was added to a 1.5 ml Eppendorf tube containing 250µl Digsol buffer and 10µl Proteinase K (10mg/ml). Samples were vortexed before placing in a rotating oven at 55°C for 2 hours to allow complete digestion. To precipitate the proteins, 300µl of 4M ammonium acetate was added to each sample, and these were vortexed several times over a 15-minute period at room temperature. All samples were then centrifuged for 10 minutes at 13000 rpm. The supernatant was discarded, then the remaining pellet was washed with 500µl of absolute ethanol. The ethanol was decanted off and the ethanol wash was repeated, but with 70% ethanol on the second occasion. After removal of any remaining ethanol, samples were air-dried by placing them upside down on a clean paper towel. Once dry, 200µl of Low TE (10mM Tris-HCl, pH 8.0, 0.1nM EDTA) was added per sample and, after dislodging the DNA pellet, placed in a water bath at 57°C for 30 minutes. Once the pellet was fully dissolved, samples were removed and stored at -20°C.

2.6.3.2 Polymerase Chain Reaction (PCR) and DNA quantification

Polymerase Chain Reaction (PCR) of the scat DNA was carried out on a 96-well PCR plate, using 10µl reaction volume per sample (5µl Qiagen PCR Mastermix (hereby referred to as mastermix throughout), 1µl Forward MiMammal primer (0.5 µM), 1µl Reverse MiMammal primer (0.5 µM), 2µl ddH20 and 1µl DNA template) and performed on a DNA Engine Tetrad thermocycler (which was used for all subsequent PCR). PCR conditions were as follows; 95°C for 15 min, 35 cycles: 98°C for 20 s; 65°C for 15 s and 72°C for 15 s, with a final extension at 72°C for 5 min. PCR products were visualised on 1% agarose gel with Tris-Borate-EDTA (TBE), stained with ethidium bromide. The Quick-load 100 bp DNA ladder (New England BioLabs) was used on each gel to provide a scale from which the approximate size of the PCR products could be assessed. Gels were run for approximately 40 minutes at 90 watts.

To quantify the DNA concentration of PCR products, each sample was analysed using a FlouroSTAR fluorometer. A 2µl sample of each individual PCR product was loaded onto a clean BMG LABTECH black microplate. Quantiflour mix was added to each well (200µl), which was made up as follows; 1 ml 20X TBE and 19 ml ddH20, added to 50µl of Quantiflour dsDNA dye. A set of calf thymus DNA standards were prepared at following concentrations: 0, 3.125, 6.25, 12.5, 50, 100 ng/µl. The quality of DNA extracted from the tissue samples were assessed on 1% agarose gel and concentration estimated based on comparison to the calf thymus DNA standards.

2.6.3.3 Sanger sequencing

To prepare samples for Sanger sequencing, 2µl of EXO-sap was added to the PCR products in each plate well, before incubating at room temperature in the dark for 15 minutes. Sequence PCR was then carried out on all samples separately for the forward and reverse reactions (adding 8µl of master mix to 2µl of EXO-sapped PCR product). PCR conditions were as follows; 96^{o}C 1 min, 39 cycles of 94^{o}C 10 secs, 50^{o}C 5 secs and 60^{o}C for 4 minutes. Following this, 66.5µl of mastermix containing 95% ethanol, 125mM EDTA and 3M sodium acetate was added to each sample and these were incubated in the dark for 15 minutes.

Each plate was spun down in a plate centrifuge at 24,000 RPM for 30 mins. Plates were tapped lightly to remove liquid without dislodging DNA pellets and spun at 120 RPM for 30 secs. Samples were then washed with 70% ethanol, decanted, and then air-dried. Next, in a fume hood, 10µl of formamide was pipetted into each well, before being covered and denatured for 3 mins at 95°C, then loaded onto the ABI1370 sequencer. As a time and cost saving measure, only the reverse primer was sequenced for badger scat samples, and the forward for hedgehog scat samples. The reverse was used for badger scat sequencing as there were fewer base pair mismatches and therefore more likely to amplify badger DNA successfully.

2.6.3.4 Analysis of Sanger sequences using BioEdit and MEGA

Once the samples had been sequenced, the files were analysed using BioEdit (Hall 1997), to remove any sequencing noise, and aligned using MEGA (Tamura et al. 2007), to construct a clean sequence for the entire target fragment. Using the Basic Local Alignment Search Tool (BLAST), each sequence was compared against the National Centre for Biotechnology Information (NCBI) nr nucleotide database, to find sequences with high similarity. The results of the BLAST search were then analysed to find the most fitting sequence, with full sequence overlap and a low e-value. The results of Sanger sequencing using the MiMammal primer set allowed identification of the most probable host of each scat sample (Appendix A), ensuring samples for subsequent analysis were from the target species only.

2.6.3.5 In silico primer selection for identification of badger and hedgehog scats

Primer fit was assessed using *in-silico* analysis. Reference sequences for the complete 12S rRNA gene region, which the MiMammal primers amplify, were obtained for hedgehog and badger from Genbank (Sayers et al. 2020), a repository for DNA sequences, in 'fasta' format. These were imported into the alignment software MEGA, to identify the position of the forward and reverse primer in reference sequences. For imperfect matches between the primer and reference sequence, the number of mismatching bases were counted. A reference matrix, developed in R using the package 'stringdist', was consulted to examine mismatches of the four control species (dog, mallard, house sparrow and fox), to test the breath of taxa that were amplifiable using the MiMammal primer set. Hedgehog had three primer base mismatches on both the forward and reverse primers, whereas badger had only one base mismatching, located on the reverse primer. The four controls had between one and three primer base mismatches on the forward and/or reverse primers (Appendix B). The primer sets successfully amplified for both badger and hedgehog, and the other

species. Therefore, these primers were suitable for ensuring the host species was identified and for identifying other vertebrate species in the diet.

2.6.3.6 Sexing of badger scat samples

Two sex-typing primer sets RG4 (Y-linked, SRY) (Griffiths and Tiwari 1993), and Mel592 (X-linked) (Annavi et al. 2011), exist for sexing badger samples. Amplification of the Y-linked SRY gene identifies males (XY), with proven success on known-sex genomic badger DNA (Kinoshita et al. 2017; Tashima et al. 2011). The pitfall of using only the SRY primer set, is that absence of detection could be due to PCR failure or poor-quality samples, as opposed to definitive evidence of a female (XX). The primer set Mel592 amplifies a microsatellite which is X-linked (Annavi et al. 2011). A heterozygous indicates the individual is female (XX), but samples that are homozygous cannot be identified as either male or female because males (XY) have only one copy of this X-linked locus, which would be confused with homozygous females. Nevertheless, when used in tandem, the two sex markers complement one another to increase confidence in the assignment of female identity in samples. Currently, no sex-typing markers have been successfully developed for the sexing of hedgehog samples. The existing RG4 primer set was tested, however it was unsuccessful in sexing genomic hedgehog DNA, therefore sexing of hedgehogs was omitted from in this study.

2.6.3.7 PCR using sex-linked primers

Both primer sets were used to amplify genomic badger DNA from the eight badger tissue samples, as four were known females and four were known males, to validate the sexing of scats in this study. A 10µl PCR reaction was used per sample (5µl Qiagen PCR mastermix, 1µl Forward primer (0.5 µM), 1µl Reverse primer (0.5 µM), 2µl ddH20 and 1µl DNA template), and a touchdown PCR profile was used as follows; 95°C for 15 mins, 34 cycles of 94° for 30 secs, 61°C for 90 secs (reducing by 1°C for the first 5 cycles), 72°C for 1 min and, finally, 60°C for 30 min. Due to low DNA concentrations of scat samples,

amplification was optimised further by air-drying 2µl DNA before performing amplicon PCR on a 2µl PCR reaction volume (1µl Qmix, and 1µl PrimerMix) following Kenta et al. (2008).

2.6.3.8 Genotyping and Analysis

Genomic DNA was diluted to 1:26000 prior to loading on the ABI 3730. For scat samples, 8µl of water was added to the 2µl of undiluted PCR product, to allow easy removal of 1µl of DNA template to a new plate for sex determination. In a fume hood, 9µl of formamide (with Applied Biosystems ROX500 added as the internal standard) was added to each well containing 1µl DNA template. Plates were denatured for 3 mins at 95°C and then PCR products loaded onto the ABI13730 sequencer. Due to high DNA degradation, each DNA sample was genotyped three times, to increase the reliability of the genetic profiles produced (following Frantz et al. 2003; Frantz et al. 2006). GeneMapper (Chatterji and Pachter 2006) was used to assign allele sizes and these were manually assessed to assign sex to each sample (Appendix C).

2.6.4 Molecular dietary assessment – DNA metabarcoding preparation

Initially, primer selection was tested *in silico*, to ensure key prey items were detectable in this study (section 2.6.2.5). DNA was then extracted (section 2.6.3.2) from the selected scat samples, and amplified using five primer sets, targeting earthworms, insects (two sets used to account for the large number of species), mammals, and plants to provide thorough coverage of badger and hedgehog diet, plus the MiMammal primer set to identify the defecating species (see section 2.6.4.1). Next, DNA was amplified in primer set-specific single-plexes, to prevent primer associated bias in amplification and non-target amplification (section 2.6.3.3). To prepare samples for sequencing on a Miseq, samples were labelled with unique-tag dual indexes, that identify individuals in a pool (section 2.6.3.4). Samples were pooled in several stages, to ensure equal representation of each

amplicon, before being sequenced on an Illumina Miseq. Finally, raw sequence data was analysed to identify prey species to Family level (section 2.6.3.5).

2.6.4.1 In silico primer assessment

Following a thorough search of the existing literature, an extensive list of potential prey items was constructed for both badger and hedgehog. Full mtDNA reference sequences for potential prey items were imported from the NCBI nucleotide database into MEGA for sequence alignment (as per section 2.6.3.4**)**. *In silico* primer assessment was performed for all primer sets used, allowing primer fit and primer biases to be identified against potential prey items. The primer sets selected for *in-vitro* primer testing were chosen due to their relatively short amplicon lengths, and breadth of coverage of potential prey items (Table 2.4). Primer sets were modified with unique identifying tags and the Illumina 'small RNA sequencing primer' and 'read 2 sequencing primer' adaptors to allow sequencing on the Ilumina MiSeq.

Table 2.4 Primer sets included for diet assessment of hedgehog and badger scat samples.

Primer set	Target taxa	Forward primer sequence	Reverse primer sequence	Amplicon length (bp)	Reference
Bienert	Earthworm	ATTCGGTTGGGGCGACC	CTGTTATCCCTAAGGTAGCTT	70	Bienert et al. 2012
MiMammal	Vertebrate	GGGTTGGTAAATTTCGTGCCAGC	CATAGTGGGGTATCTAATCCCAGTTTG	171	Ushio et al. 2017
Gillet	Invertebrate	ATTCHACDAAYCAYAARGAYATYGG	ACTATAAAARAAATYTDAYAAADGCRTG	133	Gillet et al. 2015
Zeale	Invertebrate	AGATATTGGAACWTTATATTTTATTTTTGG	WACTAATCAATTWCCAAATCCTCC	157	Zeale et al. 2011
ITS2	Plant	TGTGAATTGCARRATYCMG	CCCGHYTGAYYTGRGGTCDC	187–387	Moorhouse-Gann et al. 2018

2.6.4.2 DNA extraction from scats

DNA was extracted from ~300 ng of faecal sample using a QIAamp DNA Stool Mini Kit (QIAGEN) following the manufacturer's instructions, with some minor modifications. The eluted DNA volume was reduced to 50 µl to increase the DNA concentration, and this was put back through the spin column once more to further optimise DNA recovery. Mock extractions without samples were performed systematically to monitor levels of contamination.

2.6.3.3 PCR techniques

Once DNA was extracted from the scat samples, PCRs were undertaken to amplify DNA for each of the five primer sets. PCRs were carried-out in volumes of 20 µl, comprising 10 µl of QIAGEN Multiplex PCR Master Mix, 2 µl of each primer (1µl forward primer reduced to a concentration of 5 µM, 1 µl reverse primer reduced to a concentration of 5 µM), 4 µl of ultra-pure water, and 2 µl of DNA from each scat sample of varying concentrations. Each plate was spun down before loading amplicons onto the PCR machine, ensuring all reagents were mixed at the bottom of each well pre-loading. Single-use PCR lids were used to cover plates throughout library preparation, to reduce contamination and evaporation of the PCR product. The PCR thermal cycling conditions varied for each primer set and were performed on a TETRAD2 Peltier Thermal Cycler. A touchdown PCR program was used for amplification of the Zeale primer set (Zeale et al. 2011) under the following conditions: 15 min at 95ºC followed by 16 cycles of 30 s at 94 ºC, 30 s at 72 ºC followed in turn by 24 cycles of 30 s at 94 ºC, 30 s a 53 ºC and 20 s at 72 ºC followed by a final incubation of 10 min at 72 ºC. For the Gillet primer set (Esnaola et al. 2018) the conditions were as follows; 15 min at 95 ºC, followed by 40 cycles of 30 s at 94 ºC, 45 s at 45 ºC, 30 s at 72 ºC and 10 min at 72 ºC. For the Bienert primer set (Bienert et al. 2012) the conditions were as follows; 95 ºC 15 min, 50 cycles of 95 ºC 30 s, 58 ºC 30 s and 72 ºC 60 s. For the ITS2 primer set (Moorhouse-Gann et al. 2018) the

conditions were as follows; 95 °C 15 min, 40 cycles of 95 °C for 30 s, 56 °C for 30 s and 72 °C for 1 min and finally 72 °C for 10 min. For the MiMammal primer set, the same conditions were used as stated in section 2.6.3.2.

Amplicon PCR products were cleaned to remove excess reagent, primer dimers and non-specific amplicons (D'aquila et al. 1991), using AMPure XP paramagnetic beads in a 1:1 ratio of beads to product. The beads were mixed by pipetting the PCR mixture up and down ten times before placing the plate on a magnetic stand. The beads bound to the DNA and sunk to the bottom of the well, and the supernatant (containing any contaminates) was discarded, ensuring the bead was not disturbed. The beads were then washed twice with 70% ethanol before being allowed to air-dry at room temperature for a maximum of 10 minutes. The purified amplicon was then eluted in 20 µl of LowTE, prior to a second PCR step being performed that added the identifying indexes.

2.6.4.4 Dual-indexing PCR

To enable the simultaneous sequencing of all species in this mixed samples, each purified amplicon was labelled uniquely with dual-indexed illumina adaptor tags, by combining different forward and reverse indexes (1µl of each at 0.2µM) from a Nextera XT Index Kit (FC-131-1002). The same dual index was used for each sample in each PCR for each of the five primer sets (see Appendix D for plate organisation), as the primer sequence itself allowed demultiplexing in post-sequencing analysis. Thermal cycling conditions for the index PCR were as follows; 15 min at 95 ºC, followed by 8 cycles of 30 s at 95 ºC, 30 s at 55 ºC, 30 s at 72 ºC and 5 min at 72 ºC. This was repeated for all 5 PCR assays. The post-index PCR product was cleaned using AMPure XP beads (Beckman Coulter, High Wycombe, UK). Initially, beads were added in a ratio of 0.5 beads to 1 PCR product. After placing on the magnetic stand, the supernatant was removed to a new plate. Beads were added at 0.9x concentration and after being placed on the magnetic rack, the supernatant was discarded, leaving the DNA bound to the pellet. An 80% ethanol wash was performed

twice and then left to dry at room temperature. Samples were removed from the magnetic rack and eluted in 15 µl of Low TE (10mM Tris-HCl, pH 8.0, 0.1nM EDTA). Samples were placed back onto the magnetic rack to separate the magnetic beads from the samples.

The concentration of DNA in each sample was measured in ng/µl on the FluroSTAR fluorimeter. Samples were combined to eight pools per plate before quantifying DNA in each pool using quantitative PCR (qPCR), a highly accurate method for analysing the amount of indexed PCR product (Mardis and McCombie 2017). Unlabelled DNA is not detected during qPCR, so this qPCR provides a quality assurance step, ensuring each pool showed the anticipated amount of indexed DNA present. After obtaining the initial qPCR concentrations, further pooling in equimolar amounts was carried out, resulting in one pooled 40nM sample per plate.

The resulting pooled sample that remained per plate, was run on the Agilent 4200 TapeStation (Agilent 2018) to check the size of the indexed-amplicon; inclusive of Illumina adaptors and dual-indexes. The Tapestation revealed that amplicon PCR was often longer than expected, indicating substantial primer dimers in the samples. Therefore, samples were run on a BluePippin (Wang et al. 2015), to limit the DNA for sequencing to that of the required fragment size expected for each of the primer sets.

The BluePippin was calibrated, and a new cassette containing an external marker was inserted before each use. Fresh electrophoresis buffer (40 µl) was added to each elution module before sealing with adhesive tape strips. Sample wells were topped up with buffer to ensure they were at maximum capacity (70 µl) prior to running the continuity test, ensuring the current in each sample column fell within the expected values. The BluePippin internal standard (10 µl) was added to each sample (30 µl), and was mixed by pipetting, vortexing and being spun down on the centrifuge. Samples were then washed with Tween 20, which increases DNA yield by up to 30% (Sage Science Inc. 2016). Clean-up was performed on the post-BluePippin samples to remove the electrophoresis buffer,

and the remaining DNA was suspended in Low TE (10mM Tris-HCl, pH 8.0, 0.1nM EDTA). Post BluePippin products were re-quantified using qPCR to ascertain how much DNA had been recovered, prior to diluting to 4nM.

Primer sets were were pooled at 4nM in 15 µl and these were further diluted to 20 pM before loading on to an Illumina MiSeq Platform using v2 chemistry with 300 cycles and a 2 x 150 bp paired end read length, to provide sequences for further analysis.

2.6.4.5 Bioinformatics analysis

The Illumina sequencing yielded raw sequence data, where two sequences (essentially the forward and reverse DNA strands) were produced per primer set, for each sample, which were uploaded to the High Performance Computing (HPC) server Iceberg, supported by the University of Sheffield, and quality-filtered using strict criteria; a minimum average Phred score of 30 (Shi et al. 2016), a measurement for assessing sequence quality, over a 4 bp sliding window, and minimum sequence length of 60 bp. Illumina adaptors and primer sequences were then trimmed from sequence data using the software 'Mothur', and only sequences >60bp were retained for analysis. The smallest amplicon expected was 70bp for the primer set amplifying earthworms (Bienert et al. 2012). Trimmed paired sequences were aligned using 'FLASH' software (Magoč and Salzberg 2011), allowing 1 mismatch in 10 bp. Both identical and chimeric sequences were removed, to condense the data to unique, high-quality sequences only. These were then clustered using 'Usearch v9.2'(Edgar 2013), into molecular Operational Taxonomic Units (mOTUs), where each cluster showed 97% similarity (Edgar 2013; Johnson et al. 2019) between sequences.

Taxonomic assignment to Family level was obtained by comparing the mOTUs against the NCBI nucleotide reference database, using the BLAST function, ensuring 95% similarity between the mOTU and reference sequence. Following taxonomic assignment, the sequence matrix depicting prey items in each sample was processed to remove contamination using microDecon in R Statistics (McKnight et al. 2019). This program

compares the proportions of contaminant taxa in blank samples to systematically remove contaminant reads. Assessment of the post-microDecon matrix, demonstrated that likely contamination had been removed.

2.6.5 Camera trapping

674 camera trap locations were surveyed across the 23 sites, with an average of 29 camera traps per study site, stratified by four key habitat categories: Arable, Amenity + Urban + Buildings, Grassland, and Woodland (Table 2.5).

Table 2.5 Camera deployment information for 23 independent study sites surveyed between 2018 and 2019. Zeros indicate habitat present, but no cameras placed, dashes indicate absence of habitat within the site boundary. Camera trapping surveys were conducted for the initial 10 nights of the survey period depicted in Table 2.1.

Site number	Site name	Number of cameras deployed	Habitat (camera facing)			
			Arable	Amenity, Urban and Buildings	Grassland	Woodland
1	Brackenhurst A	40	23	4	11	2
2	Hartpury	38	0	13	15	10
3	Slade	40	0	2	32	6
4	Kendal	29	0	2	23	4
5	Driffield	29	8	3	15	3
6	Keyingham	29	18	2	8	1
7	Clumber	20	8	-	3	9
8	Thorn	27	17	2	5	3
9	Brackenhurst B	30	13	4	13	0
10	Epperstone	30	19	3	6	2
11	Hodsock	25	8	4	8	5
12	Anglesey	30	5	1	19	5
13	Dunmow	27	24	-	0	3
14	Loddington	30	14	1	12	3
15	Usk	31	1	2	24	4
16	Woodchester	32	-	-	6	26
17	Long Stratton	29	20	1	3	5
18	Spreyton	25	0	0	20	5
19	Ide	24	2	1	14	7
20	Knock	27	5	2	15	5
21	Barnsley	26	12	1	10	3
22	Riseholme	30	6	4	13	7
23	Suffolk	26	16	1	6	3

The ‘Sampling Tool’ in ArcGIS 10.6.1 (ESRI (Environmental Systems Research Institute.) 2018) was used to generate random camera positions within each habitat, producing co-ordinates that could be located in the field (see Appendix F for site maps including camera trap locations). Cameras that failed during the survey period were removed from the study to avoid overestimation of sampling effort.

Camera trapping surveys were conducted between June 2018 and September 2019, surveying each site for 10 consecutive nights. Camera deployment took 1-2 days and was supported by MSc students from Nottingham Trent University. A handheld ‘Garmin GPS, 60’ device was used to locate, within 3-5 metres (95% accuracy), the randomly generated GPS position for each camera location. However, it was not possible to place the cameras precisely at the GPS locations, as the random point generation did not account for habitat features such as impermeable fences, dense cereal crop or livestock that would damage cameras and/or prevent the cameras working reliably. In these cases, the nearest available location for deployment of a camera was used. The random deployment of cameras was therefore fulfilled, despite most cameras being placed near linear features.

Bushnell 119837 Essential E3 Trophy Cam HD (Bushnell Corp., Overland Park, KS, USA) cameras were used consistently throughout the study to reduce any detection bias that may have been introduced by using a camera with different technical specifications. Suitable features to attach a camera included trees, hedgerows, telegraph poles, fence posts and occasionally staked wooden posts. A good, clear field of view in front of the proposed camera location was sought to provide a reasonable chance of detecting the target species (Rowcliffe et al. 2008). In addition, camera detection zones, specified by its radius and angle, were calculated at individual camera locations (Cusack et al. 2015). Camera settings were as follows; mode = ‘Video’, LED = ‘High’, video size = ‘640x480’, video length = ‘15 secs’, interval = ‘5 mins’, sensor level = ‘automatic’, night mode, time stamp = ‘On’, field scan = ‘Off’ and sound = ‘Off’. Each camera was fitted

with a 16GB micro-SD card and placed in the 'ON' mode before positioning in the field. Cameras were placed approximately 30 cm from the ground, with a slight downwards tilt which was achieved by securing a small block of wood/stick with a diameter of approximately 2cm, between the camera and the object it was being fastened to. All camera data were copied from the SD card and the corresponding camera location was recorded.

2.6.6 Data analysis and software packages used

All statistical analysis was carried out using 'R Studio' software version 1.2.5 (R Studio Team 2020) and individual packages are referred to in each chapter. To compare site-specific data including habitat and invertebrate covariates (Chapters 5 and 6), Z Scores were calculated. Bioinformatic analysis (Chapter 4) was performed on the University of Sheffield's High-Performance Computing resource, Iceberg. An in-house pipeline was followed that utilised Python (Python Software Foundation, NH, USA) and Perl scripts (Cranor 1994), leading to taxonomic assignment.

2.7 Ethical Approval

The full methodologies implemented were granted ethical approval by Nottingham Trent University's School Ethics Committee on 16/02/2018 under the project code ARE721.

3.1 Introduction

Habitat and any associated management, have considerable effects on the abundance and diversity of invertebrate assemblages inhabiting it (Chapin et al. 2000). In intra-guild predation (IGP) relationships, these basal resources can underpin ecological interactions at higher trophic levels, causing bottom-up effects on intraguild species (Sergio et al. 2003; Sergio et al. 2005; Dicks et al. 2019). A reduction in basal prey abundance and / or diversity can influence the level of competition and rate of predation amongst intra-guild predator and intra-guild prey species (Polis et al. 1989; Navarrete et al. 2000; Takimoto et al. 2012). Prey availability is often closely linked to habitat availability and its quality (Marshall et al. 2006; McHugh et al. 2019), which varies at the local scale (Rosalino et al. 2005; Andersen et al. 2017). Furthermore, as seasonal fluctuations in food availability are typical at certain latitudes and can affect the abundance and range of prey items available at a given time, there is potential for prey-switching, either increasing the level of competition between intraguild-competitors, or leading to more frequent predation events (Périquet et al. 2015). Assessing the availability of invertebrate assemblages as food resources is therefore a necessity when investigating the interactions amongst higher trophic species (McHugh et al. 2019).

Food availability can indirectly shift the balance of competition and predation, with the potential to cause population level effects (Sergio and Hiraldo 2008). Despite this, these data are often missing from studies investigating the spatial and temporal relationship between IGP competitors, resulting in inferences that rely on habitat type only, and the presence of the intra-guild competitor as explanatory variables (Cruz et al. 2015). For example, a comparison of red foxes, badgers, and stone martens demonstrated that the probability of an area being occupied by a species was more strongly affected by habitat type and structure than by interspecific interactions (Cruz et al. 2015). However, the patterns of habitat selection exhibited by each of these species may also have been

influenced by the availability of food but, without its assessment, this cannot be ascertained.

The main aim of this study was to evaluate the availability of prey for two IG competitors as a potential driver of competition. In particular, this study assesses the availability of invertebrate prey for badgers and hedgehogs across different rural habitats in Britain. Whilst badger and hedgehog diets are not composed exclusively of invertebrate prey, it does represent the most common food group for both species (Wroot 1984; Shepherdson et al. 1990) and therefore was assessed as a proxy for food availability in this study. Due to the high frequency in which some invertebrate prey are consumed, this is likely to lead to competition between badgers and hedgehogs. In contrast, vertebrate species such as small mammals are consumed at lower frequencies by both species (Kruuk and Parish 1981; Yalden 1976), likely resulting in weaker competition for these prey types. Notably, not all prey items are shared between badgers and hedgehogs such that the degree of dietary niche overlap (Chapter 4) will also affect the magnitude of competition for prey between these two species

In this study, habitat type was predicted to be a key factor affecting the composition of invertebrate communities that are basal prey resources for badgers and hedgehogs. Prey availability can influence the stability of the IGP relationship as it is an important driver of both competitive and predatory interactions (Chapter 5). Variability between month, season and site were also investigated to assess their importance in explaining fluctuations in prey availability and hence the potential pattern of competition over the course of the year.

This study used pitfall traps and earthworm cores to analyse the invertebrate community across 22 rural sites in England and Wales, to: 1) compare the abundance of invertebrates from pitfall trap captures between different habitats, seasons and sites, 2) compare the biomass of invertebrates from pitfall trap captures between different habitats,

seasons and sites, 3) compare the abundance of earthworms as measured by earthworm cores between different habitats, seasons and sites, and 4) compare the biomass of earthworms as measured by earthworm cores between different habitats, seasons and sites. This assessment of invertebrate communities provides an understanding of the availability of potential invertebrate prey resources for badgers and hedgehogs, with prey preferences considered in Chapter 5 and prey resource utilisation assessed in Chapter 6.

3.2 Methods

Surveying was conducted across the four distinct habitats described in Chapter 2: arable, amenity grassland, other grassland and woodland. Pitfall trapping was selected to measure the availability of ground dwelling invertebrates that are potentially consumed by badgers and hedgehogs; soil cores were used to estimate earthworm availability, a key prey type of both species. By assessing abundance and biomass together, a more thorough assessment of the availability of prey can be attained, potentially highlighting the relative quantitative importance of different prey items.

3.2.1 Surveying invertebrate communities

Pitfall trapping and earthworm cores were sampled as described in Chapter 2, section 2.6.2. Pitfall trap data were excluded if the traps had been disturbed by cattle or wildlife during the 10-day survey period to minimize errors from inconsistent survey effort. Similarly, data from Thorn (Site 8) were excluded due to particularly dry soil conditions which inhibited the deployment of pitfall traps. In total, 493 pitfall traps and 212 earthworm cores taken from 22 study sites were included for analysis.

Three earthworm cores were taken in each of the following habitats: amenity grassland, arable, grassland and woodland, present within the site boundaries of 22 study sites (see Chapter 2 for habitat mapping). From each core, measures of earthworm abundance and biomass were collected in the field before returning organisms back into

the environment. The number of adult and juvenile worm abundance per core was recorded, as was fresh biomass (g).

Nine unbaited pitfall traps containing the preservative propylene glycol, were deployed in each habitat present at each of the 22 sites for a ten-night trapping period. Pitfall trap captures were sealed and stored in a cool place until processing took place, typically within six weeks of surveying. Invertebrates that measured > 5 mm in length were identified to the Order level using taxonomic identification guides (Tilling 1987; Barnard 2011) and abundance was recorded. Order level identification was carried out as this accounts for differences in functional traits of prey such as mobility, that may influence the likelihood of being potential prey to either badger or hedgehog (Kennedy et al. 2019). Following identification, organisms were dried at 60°C for 72 hours to obtain measures of dry biomass (g) (Chapter 2.6.1.1).

Pitfall captures were pooled across each of the broad habitat types and the diversity of invertebrate communities was measured using the Reciprocal Simpson Diversity Index (D) that assesses richness and evenness of the invertebrate structure (Lande 1996):

$$D = \frac{N(N-1)}{\sum n\ (n-1)}$$

where N = total number of organisms of all species (Order level) found within each habitat and n = number of individuals of a particular species (Order level) found in each habitat.

The principal means of testing for differences in abundance, biomass, and diversity of invertebrates between habitat types and months (Table 3.1) was through a series of Generalised Linear Mixed Models (GLMMs), where both interactions and main effects were tested and site was included as a random factor in all models (Ostfeld et al. 2018; Hothorn et al. 2008). Interaction terms were included in each analysis but were excluded where they were not significant via a stepwise backwards elimination. Data processing and formatting were performed in Microsoft Excel (Microsoft Corporation 2018) and all

figures and statistical analysis were conducted using R Studio version 1.2.5 (R Studio Team 2020).

3.2.2 Model Selection

The best fitting models for each of the four invertebrate measures; pitfall abundance, pitfall biomass, earthworm abundance and earthworm biomass are reported below.

3.2.2.1 Pitfall abundance

A negative binomial GLMM was used to investigate the factors affecting the abundance of invertebrates captured in pitfall traps.

$$Abundance_i \sim Negative\ binomial(\mu_{i,}\sigma^2)$$

$$E(Abundance_i) = \mu_i \ and \ var(abundance_i) = \sigma^2$$

$$\mu_i = \mu_i = \beta_1 + \beta_2 \ X \ habitat_i \ \mathrm{X} \ month_i + \mathrm{Site_j} + \mathrm{e_{ij}}$$

$$\mathrm{Site_j} \sim \mathrm{N}(0, \mathrm{s^2_{Site}})$$

$$\mathrm{e_{ij}} \sim \mathrm{N}(0, \mathrm{s^2})$$

Where $\mathrm{Abundance_i}$ is the abundance of organisms *i* from Site *j* assuming a negative binomial distribution with mean $\mu_{i,}$ and variance σ^2. $Habitat_i \ month_i$ are categorical covariates indicating the habitat and month in which the pitfall sample was taken from. A significant interaction between habitat and month was identified and included in the model. The random intercept $\mathrm{Site_j}$ is included in the model to introduce a correlation structure between observations for different abundances from different sites, with variance $\mathrm{s^2}_{Site}$ distributed normally and equal to 0. e_{ij} is the residual variance in the model, again with the assumption of normality and equal to 0.

3.2.2.2 Pitfall biomass

A Gamma GLMM was fitted to the data to examine the effects of invertebrate biomass of pitfall trap captures sampled from UK rural landscapes.

$$Biomass_i \sim Gamma(\mu_i, \sigma^2)$$
$$E(Biomass_i) = \mu_i \; and \; var(biomass_i) = \sigma^2$$
$$\mu_i = \mu_i = \beta_1 + \beta_2 \; X \; habitat_i + month_i + \text{Site}_j + e_{ij}$$
$$\text{Site}_j \sim N(0, s^2_{\text{Site}})$$
$$e_{ij} \sim N(0, s^2)$$

Where Biomass_i is the biomass (g) of organisms *i* from Site *j* assuming a gamma distribution with mean μ_i, and variance σ^2. $Habitat_i$ is a categorical covariate indicating the habitat in which the pitfall sample was taken from. The random intercept Site_j, is included in the model to introduce a correlation structure between observations for different abundances from different sites, with variance s^2_{Site} distributed normally and equal to 0. e_{ij} is the residual variance in the model, again with the assumption of normality and equal to 0.

3.2.2.3 Earthworm abundance

A Negative Binomial GLMM was fitted to the data to examine the effects of earthworm abundance of earthworm cores sampled from UK rural landscapes

$$Abundance_i \sim Negative \; binomial(\mu_i, \sigma^2)$$
$$E(Abundance_i) = \mu_i \; and \; var(abundance_i) = \sigma^2$$
$$\mu_i = \mu_i = \beta_1 + \beta_2 \; X \; habitat_i + month_i + \text{Site}_j + e_{ij}$$
$$\text{Site}_j \sim N(0, s^2_{\text{Site}})$$
$$e_{ij} \sim N(0, s^2)$$

Where earthworm abundance_i is the abundance of earthworms *i* from Site *j* assuming a negative binomial distribution with mean μ_i, and variance σ^2. $Habitat_i$ is a categorical

covariate indicating the habitat in which the pitfall sample was taken from. The random intercept $Site_{j,}$ is included in the model to introduce a correlation structure between observations for different abundances from different sites, with variance s^2_{Site} distributed normally and equal to 0. e_{ij} is the residual variance in the model, again with the assumption of normality and equal to 0.

3.2.2.4 Earthworm biomass

A Gamma GLMM was fitted to the data to examine the effects of earthworm biomass of earthworm cores sampled from UK rural landscapes.

$$Biomass_i \sim Gamma(\mu_{i,}\sigma^2)$$

$$E(Biomass_i) = \mu_i \; and \; var(biomass_i) = \sigma^2$$

$$\mu_i = \mu_i = \beta_1 + \beta_2 \; X \; habitat_i + month_i + Site_j + e_{ij}$$

$$Site_j \sim N(0, s^2_{Site})$$

$$e_{ij} \sim N(0, s^2)$$

Where $Biomass_i$ is the biomass (g) of earthworms *i* from Site *j* assuming a gamma distribution with mean $\mu_{i,}$ and variance σ^2. $Habitat_i$ is a categorical covariate indicating the habitat in which the pitfall sample was taken from. The random intercept $Site_{j,}$ is included in the model to introduce a correlation structure between observations for different abundances from different sites, with variance s^2_{Site} distributed normally and equal to 0. e_{ij} is the residual variance in the model, again with the assumption of normality and equal to 0.

3.2.2.5 Order level abundance

A series of Negative binomial GLMM's were used to investigate the factors affecting the abundance of invertebrates belonging to Coleoptera, Isopoda and Pulmonata captured in pitfall traps.

$$Abundance_i \sim Negative\ binomial(\mu_{i,}\sigma^2)$$

$$E(Abundance_i) = \mu_i\ and\ var(abundance_i) = \sigma^2$$

$$\mu_i = \mu_i = \beta_1 + \beta_2\ X\ habitat_i + month_i + \text{Site}_j + e_{ij}$$

$$\text{Site}_j \sim N(0, s^2_{\text{Site}})$$

$$e_{ij} \sim N(0, s^2)$$

Where Abundance_i is the abundance of organisms *i* (belonging to either Coleoptera, Isopoda or Pulmonata) from Site *j* assuming a Poisson distribution with mean $\mu_{i,}$ and variance σ^2. $Habitat_i\ month_i$ are categorical covariates indicating the habitat and month in which the pitfall sample was taken from. The random intercept $\text{Site}_{j,}$ is included in the model to introduce a correlation structure between observations for different abundances from different sites, with variance s^2_{Site} distributed normally and equal to 0. e_{ij} is the residual variance in the model, again with the assumption of normality and equal to 0.

Table 3.1 List of variables included in Generalised Linear Mixed Models of invertebrate assemblages.

Variable	Distribution and (link function)	Data type	Variable type	Description
Abundance	Negative binomial(log)	Count	Response	Number of organisms > 5mm in a pitfall capture (n = 493)
Biomass	Gamma (log)	Continuous	Response	Dry biomass (g) of pitfall capture
Earthworm abundance	Negative binomial(log)	Count	Response	Number of adult and juvenile earthworms per core
Earthworm biomass	Gamma (log)	Continuous	Response	Fresh biomass (g) of adult and juvenile earthworms per core
Habitat_	N/A	Categorical	Predictor	Habitat 1 = Amenity, 2 = Arable, 3 = Grassland and 4 = Woodland
Month_	N/A	Categorical	Predictor	Month of survey: 1 = April, 2 = May, 3 = June, 4 = July, 5 = August and 6 = September
Site	N/A	Categorical	Random factor	Sites 1 – 23 (omitting Thorn - site 8) *see Chapter 2 for site description*.

3.3 Results

A total of 24 orders were identified across all pitfall captures, (Table 3.2). Coleoptera was the most abundant order within the arable, grassland and woodland habitats whereas Isopoda was the most abundant order within the amenity grassland habitat. The mean number of organisms per pitfall capture was greatest in woodland habitat (24.8), followed by grassland (21.9), arable (19.8) and amenity (19.4). Species richness, as assessed at the Order level, followed the same trend, with greatest richness (23 orders) identified within woodland, followed by grassland, arable and amenity where 21, 19 and 18 orders were identified, respectively.

Table 3.2 Taxonomic rank showing total abundance of invertebrates captured by pitfall trapping (>5 mm) for individual taxa ranked from most to least abundant. Data were merged from 22 rural sites in Britain with an average of 7 pitfall traps per broad habitat type. Total number of pitfall traps per habitat was as follows;Amenity grassland = 86, Arable = 103, Grassland = 145 and Woodland = 159. A 10-night trapping period using pitfall traps containing propylene glycol was conducted across all sites to obtain captures.

Rank	Total abundance							
	Amenity (n=86)		Arable (n=103)		Grassland (n=145)		Woodland (n=159)	
	Taxon	Tot.	Taxon	Tot.	Taxon	Tot.	Taxon	Tot.
1	Isopoda	485	Coleoptera	939	Coleoptera	1091	Coleoptera	1047
2	Coleoptera	432	Isopoda	317	Isopoda	714	Isopoda	1018
3	Pulmonata	188	Araneae	186	Araneae	389	Pulmonata	346
4	Araneae	185	Diptera	184	Hemiptera	323	Dermaptera	269
5	Hemiptera	101	Dermaptera	97	Pulmonata	246	Hemiptera	266
6	Polydesmidae	67	Pulmonata	94	Collembola	109	Collembola	240
7	Collembola	57	Polydesmidae	54	Diptera	58	Polydesmidae	159
8	Dermaptera	43	Opiliones	43	Opiliones	55	Araneae	157
9	Julidae	25	Collembola	38	Dermaptera	37	Julidae	101
10	Geophilomorpha	23	Hemiptera	33	Oligochaeta	35	Opiliones	99
11	Diptera	15	Hymenoptera	21	Hymenoptera	25	Diptera	49
12	Opiliones	15	Julidae	11	Geophilomorpha	24	Oligochaeta	48
13	Oligochaeta	13	Oligochaeta	8	Neuroptera	15	Geophilomorpha	46
14	Lepidoptera	12	Geophilomorpha	6	Polydesmidae	13	Hymenoptera	20
15	Ephemeroptera	4	Neuroptera	5	Lepidoptera	11	Glomerida	15
16	Hymenoptera	1	Lepidoptera	3	Julidae	10	Amphipoda	13
17	Lithobiidae	1	Lithobiidae	2	Ephemeroptera	5	Lepidoptera	12
18	Stylommatophora	1	Ephemeroptera	1	Glomerida	4	Ephemeroptera	11
19	Amphipoda	0	Glomerida	1	Orthoptera	2	Stylommatophora	9
20	Glomerida	0	Stylommatophora	0	Lithobiidae	1	Lithobiidae	6
21	Glossiphoniidae	0	Amphipoda	0	Stylommatophora	1	Orthoptera	4
22	Neuroptera	0	Glossiphoniidae	0	Amphipoda	0	Glossiphoniidae	1
23	Odonata	0	Odonata	0	Glossiphoniidae	0	Odonata	1
24	Orthoptera	0	Orthoptera	0	Odonata	0	Neuroptera	0
All Taxa		1668		2043		3168		3937

The taxonomic group Coleoptera was identified in the highest proportion of individual pitfall traps (n = 493) across all four habitats, indicative of its commonness (Figures 3.1 – 3.4).

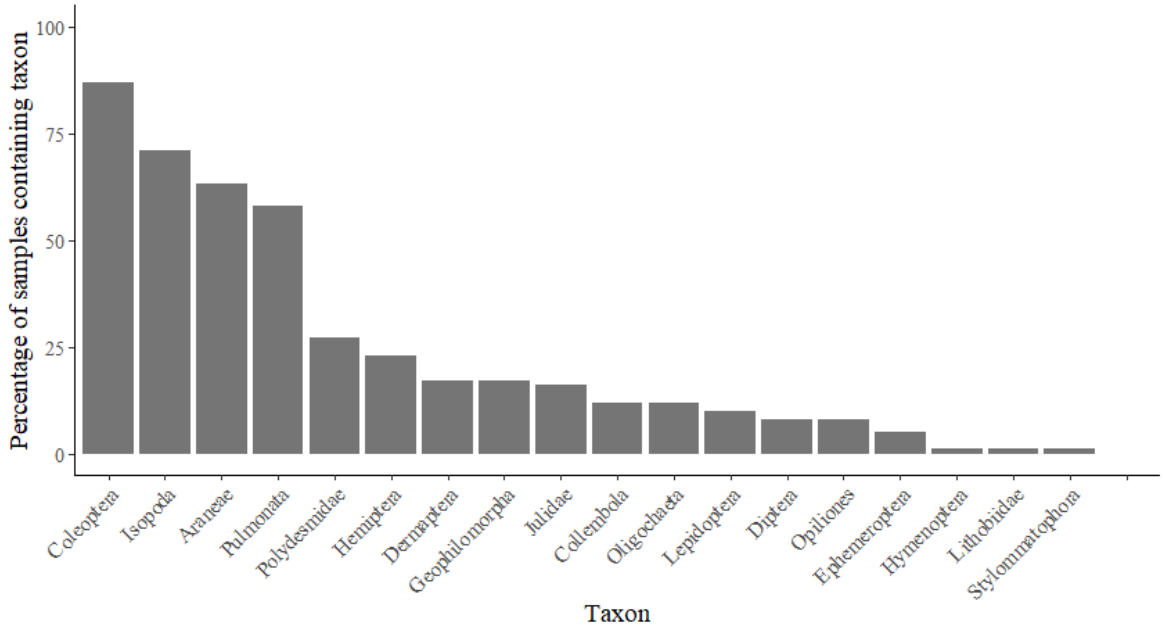


Figure 3.1 Proportion of pitfall captures (n = 86) sampled from amenity grassland habitat across 22 sites containing organisms belonging to different taxonomic Orders.

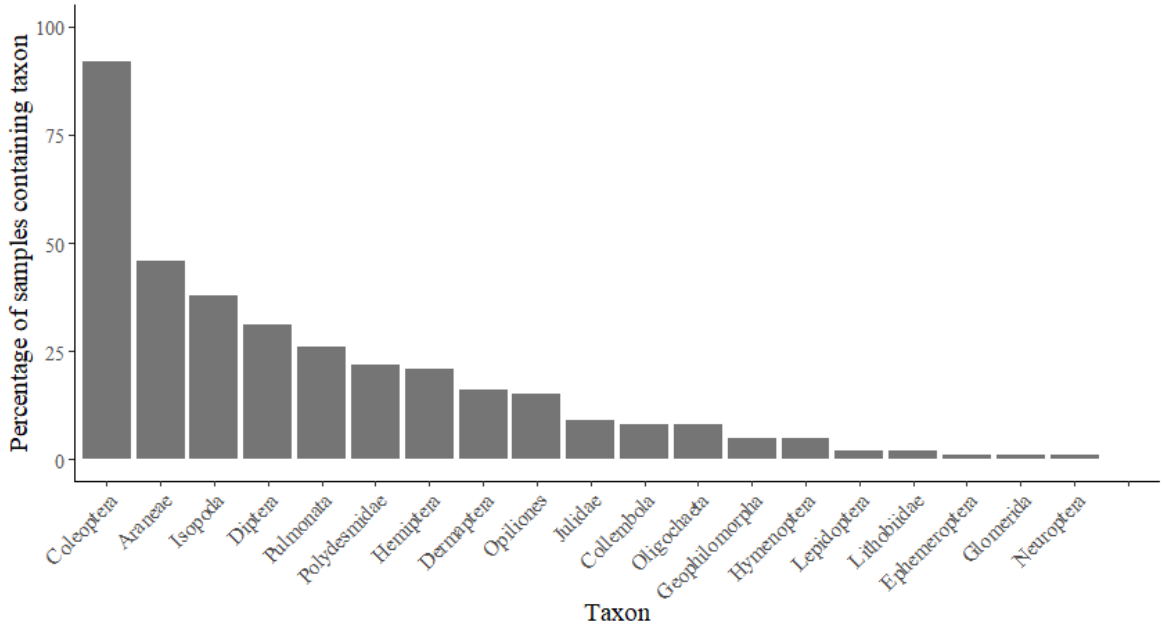


Figure 3.2 Proportion of pitfall captures (n = 103) sampled from arable habitat across 22 sites containing organisms belonging to different taxonomic Orders.

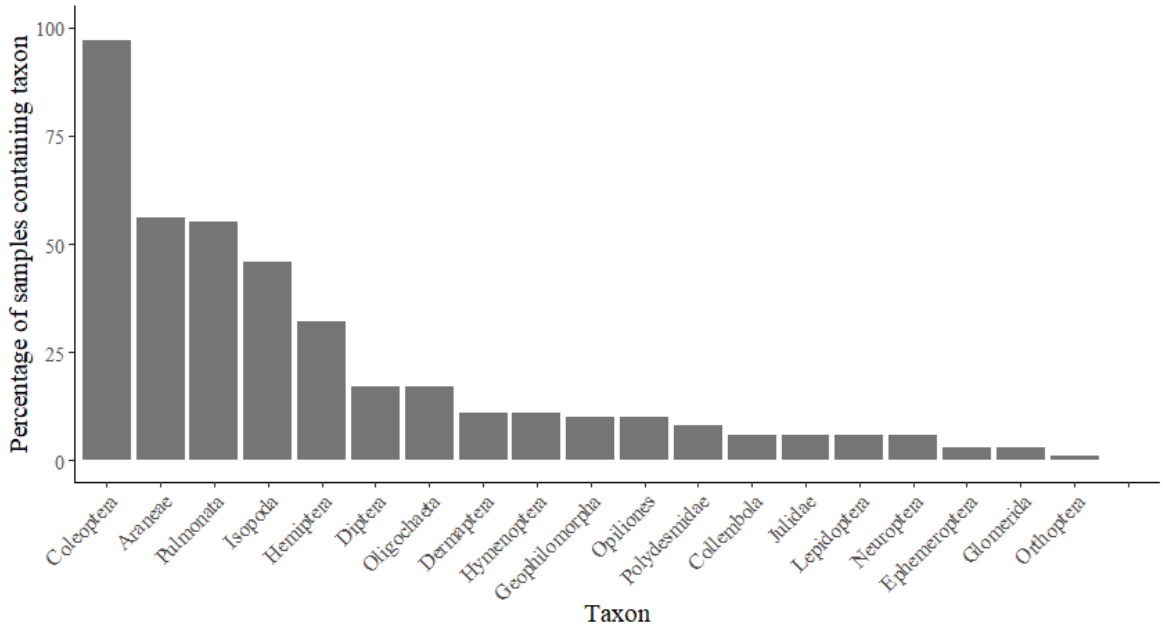


Figure 3.3 Proportion of pitfall captures (n = 145) sampled from grassland habitat across 22 sites containing organisms belonging to different taxonomic Orders.

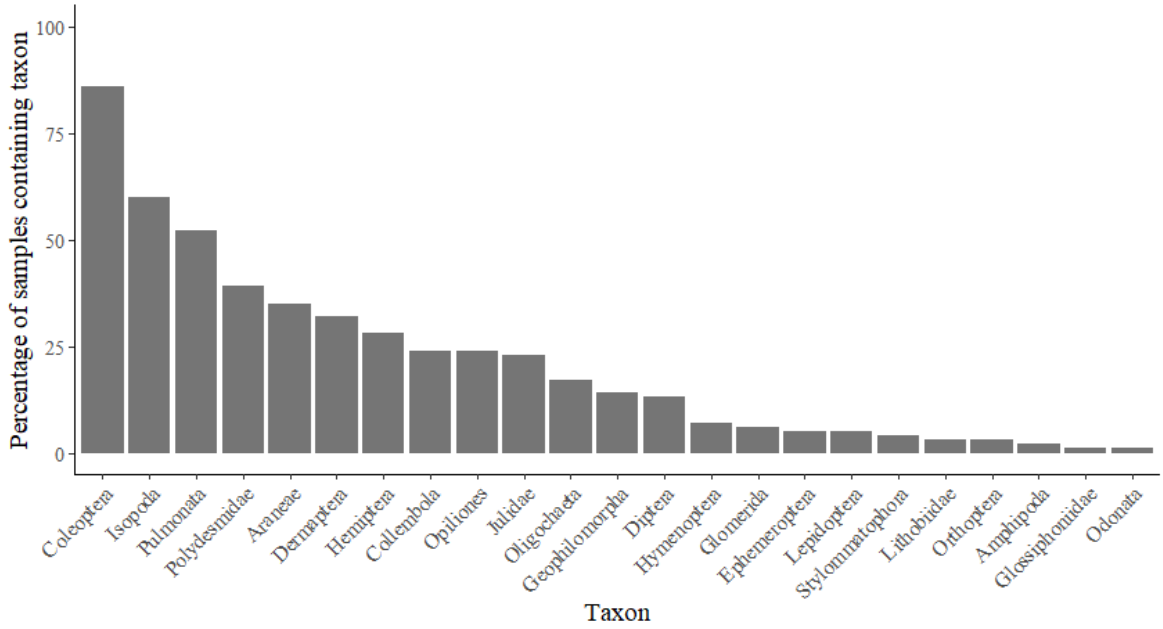


Figure 3.4 Proportion of pitfall captures (n = 159) sampled from woodland habitat across 22 sites containing organisms belonging to different taxonomic Orders.

Comparison of diversity between habitat type showed woodland to be the most diverse habitat and arable to be the least diverse habitat for invertebrate community composition (Table 3.3).

Table 3.3 Comparison of invertebrate diversity of organisms captured by pitfall trapping and identified to the level of Order, using Simpson's Reciprocal Index, whereby higher values indicate greater diversity amongst sample group.

Habitat	Simpson's Reciprocal Index	Diversity Rank
Amenity	5.42	2
Arable	3.88	4
Grassland	4.92	3
Woodland	6.12	1

3.3.1 Pitfall invertebrate abundance

A negative binomial GLMM was used to investigate the factors affecting the abundance of invertebrates captured in pitfall traps. The marginal and conditional R^2 values for the

GLMM were 0.16 and 0.33, respectively. There was a significant interaction between habitat and month (Table 3.4) showing significant variability in the abundance across habitats throughout the sampling period, which explains 16% of the variance within the dataset. There were significantly more invertebrates in grassland habitat in May, June, July and August, and significantly fewer invertebrates in woodland habitat in May and June (Table 3.4). Site, as the random factor, accounted for 17% of the variance within the dataset, demonstrating that invertebrate abundance varied both between habitats and on a local scale.

Table 3.4 Summary of Negative Binomial Generalised Linear Mixed Model (GLMM) for the effect of month and habitat on invertebrate abundance of pitfall trap captures sampled from the UK rural landscape. Site was included as a random factor with standard deviation of 0.30. Nobs = 484. Bold P values indicate statistical significance at 0.05 level. SE = Standard Error.

Model Parameter	Estimate	SE	P-value
(Intercept)	2.96	0.24	**<0.001**
Habitat_arable:Month_august	0.31	0.28	0.281
Habitat_grassland:Month_august	1.15	0.34	**0.001**
Habitat_arable:Month_july	0.09	0.28	0.735
Habitat_grassland:Month_july	1.14	0.35	**0.001**
Habitat_wood:Month_july	-0.48	0.28	0.087
Habitat_arable:Month_june	-0.02	0.27	0.954
Habitat_grassland:Month_june	0.60	0.33	**0.070**
Habitat_wood:Month_june	-0.82	0.25	**0.001**
Habitat_arable:Month_may	-0.28	0.31	0.375
Habitat_grassland:Month_may	1.03	0.34	**0.002**
Habitat_wood:Month_may	-0.84	0.26	**0.001**
Habitat_arable:Month_september	0.41	0.33	0.213
Habitat_grassland:Month_september	0.28	0.41	0.501
Habitat_wood:Month_september	-0.32	0.33	0.342

3.3.2 Pitfall invertebrate biomass

A Gamma GLMM was fitted to the data to examine the effects of invertebrate biomass of pitfall trap captures sampled from UK rural landscapes. The marginal and conditional R^2 values for the GLMM were 0.15 and 0.25 respectively. There was significant variability in the biomass across habitats throughout the sampling period, which explained 15% of the variance within the dataset (Table 3.5). Invertebrate biomass was significantly higher in July than in any of the other months surveyed and there was significantly lower biomass in May. Between habitats there was significantly higher invertebrate biomass in woodland, grassland and arable habitat compared with amenity grassland habitat. Site, as the random factor, accounted for 10% of the variance within the dataset, demonstrating that invertebrate biomass varied both between habitat and on a local scale.

Table 3.5 Summary of Gamma Generalised Linear Mixed Model (GLMM) for the effect of month and habitat on invertebrate biomass of pitfall trap captures sampled from the UK rural landscape. Site was included as a random factor with standard deviation of 0.35. Nobs = 484. Bold P values indicate statistical significance at 0.05 level. SE =Standard Error.

Model Parameter	Estimate	SE	P
(Intercept)	-0.97	0.29	**0.001**
Habitat_arable	0.35	0.14	**0.015**
Habitat_grassland	0.46	0.14	**0.001**
Habitat_woodland	0.78	0.13	**<0.001**
Month_may	-0.78	0.34	**0.023**
Month_june	-0.63	0.33	0.055
Month_july	-0.67	0.32	**0.040**
Month_august	-0.21	0.37	0.574
Month_september	0.31	0.39	0.436

3.3.3 Earthworm abundance

A Negative Binomial GLMM was fitted to the data to examine the effects of earthworm abundance of earthworm cores sampled from UK rural landscapes. The marginal and conditional R^2 values for the GLMM were 0.37 and 0.63 respectively (Table 3.6). There was significant variability in the abundance of earthworms across habitat and month throughout the sampling period, which explains 37% of the variance within the dataset. Earthworm abundance was significantly lower in arable and woodland habitat, also during June, July and September. Site, as the random factor, accounted for 26% of the variance within the dataset, demonstrating that earthworm abundance varied both between habitat and on a local scale.

Table 3.6 Summary of Negative Binomial Generalised Linear Mixed Model (GLMM) for the effect of month and habitat on earthworm abundance of earthworm cores sampled from the UK rural landscape. Site was included as a random factor with standard deviation of 0.55. Nobs = 212. Bold P values indicate statistical significance at 0.05 level. SE = Standard Error.

Model Parameter	Estimate	SE	P
(Intercept)	2.63	0.42	**<0.001**
Habitat_arable	-1.37	0.18	**<0.001**
Habitat_grassland	-0.28	0.14	**0.050**
Habitat_woodland	-0.30	0.14	**0.029**
Month_may	-0.49	0.50	0.329
Month_june	-0.97	0.48	**0.042**
Month_july	-1.21	0.49	**0.014**
Month_august	-0.59	0.53	0.273
Month_september	-1.44	0.60	**0.017**

3.3.4 Earthworm biomass

A Gamma GLMM was fitted to the data to examine the effects of earthworm biomass of earthworm cores sampled from UK rural landscapes. The marginal and conditional R^2

values for the GLMM were 0.36 and 0.59 respectively (Table 3.7). There was significant variability in the biomass across habitats and months throughout the sampling period, which explains 36% of the variance within the dataset. Earthworm biomass was significantly lower in arable habitat and in July and September. Site, as the random factor, accounted for 23% of the variance within the dataset, demonstrating that earthworm biomass varied both between habitat and on a local scale.

Table 3.7 Summary of Gamma Generalised Linear Mixed Model (GLMM) for the effect of month and habitat on earthworm biomass of earthworm cores sampled from the UK rural landscape. Site was included as a random factor with standard deviation of 0.69. Nobs = 212. Bold P values indicate statistical significance at 0.05 level. SE =Standard Error.

Model Parameter	Estimate	SE	P
(Intercept)	2.00	0.61	**0.001**
Habitat_arable	-1.76	0.27	**<0.001**
Habitat_grassland	-0.33	0.26	0.193
Habitat_woodland	-0.22	0.25	0.376
Month_may	-0.68	0.72	0.342
Month_june	-1.01	0.68	0.136
Month_july	-1.54	0.70	**0.027**
Month_august	-0.91	0.76	0.230
Month_september	-1.84	0.83	**0.027**

3.3.5 Order level abundance

Overall, invertebrate abundance showed more variance than invertebrate biomass. Therefore, a series of GLMMs were run to investigate whether the abundance of specific Orders varied by habitat and whether this was influenced by seasonal differences between the months of April – September. Coleoptera, Isopoda and Pulmonata were assessed due their dominance in the landscape across habitats, relative to other Orders identified.

3.3.5.1 Coleoptera

The abundance of Coleoptera was uneven between habitats and months surveyed and was least abundant in amenity grassland habitat (Figure 3.5). Seasonally, the lowest abundance of Coleoptera was in grassland habitat in April with a mean abundance of 1.6 individuals and the highest abundance was in woodland habitat in August with a mean abundance of 14.3 individuals per pitfall capture.

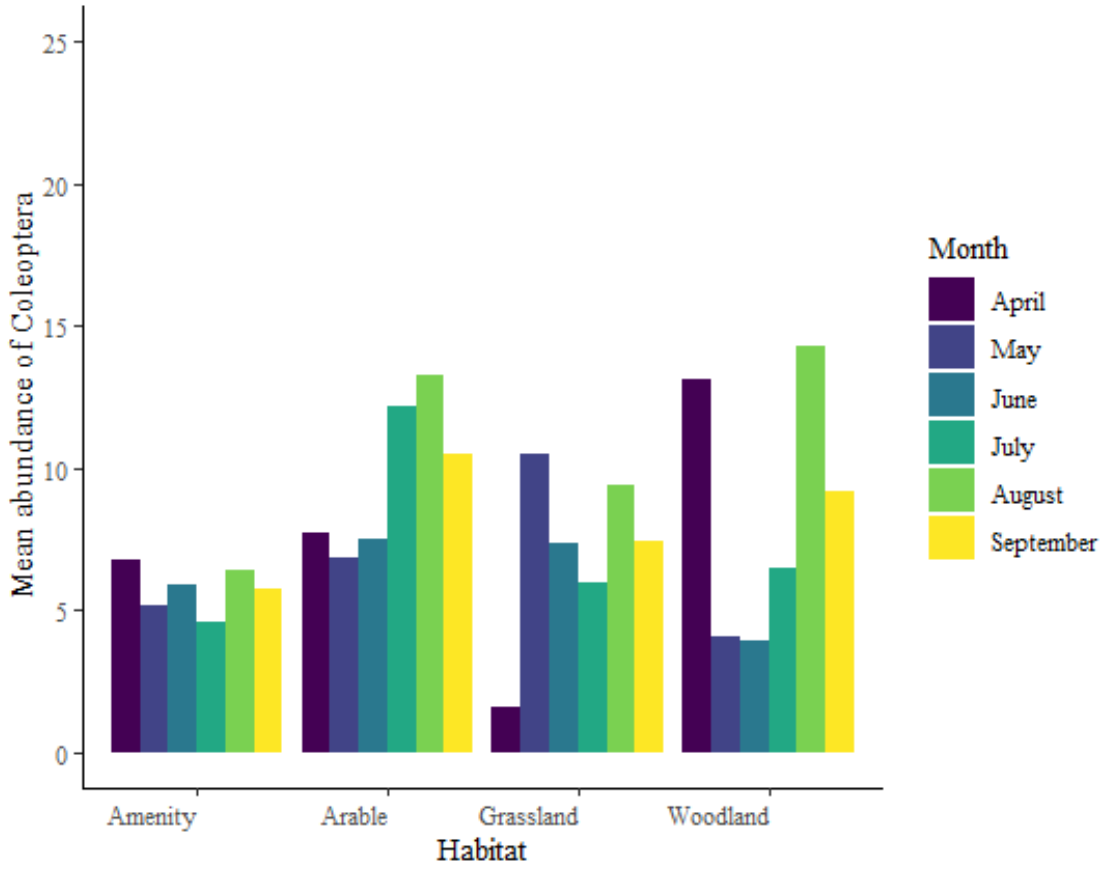


Figure 3.5 Mean abundance of Coleoptera in pitfall trap captures across four broad habitats and six different months. Averages taken from 493 unique pitfall traps sampling in the UK between April 2018 and September 2019.

A Negative binomial GLMM was used to investigate the factors affecting the abundance of Coleoptera captured in pitfall traps. The marginal and conditional R^2 values for the GLMM were 0.11 and 0.24, respectively. There was a significant variability in the abundance of Coleoptera across habitats throughout the sampling period, which explains 11% of the

variance within the dataset. There were significantly more Coleoptera in arable, grassland and woodland habitat than in amenity grassland habitat (Table 3.8). Site, as the random factor, accounted for 13% of the variance within the dataset, demonstrating that abundance of Coleoptera varied more greatly at the local site than between habitats.

Table 3.8 Summary of Negative Binomial Generalised Linear Mixed Model (GLMM) for the effect of month and habitat on the abundance Coleoptera of pitfall trap captures sampled from the UK rural landscape. Site was included as a random factor with standard deviation of 0.44. Nobs = 484. Bold P values indicate statistical significance at 0.05 level. SE = Standard Error.

Model Parameter	Estimate	SE	P-value
(Intercept)	1.38	0.35	**0.00**
Habitat_arable	0.80	0.17	**0.00**
Habitat_grassland	0.71	0.16	**0.00**
Habitat_woodland	0.38	0.16	**0.01**
Month_may	-0.16	0.42	0.70
Month_june	-0.41	0.41	0.32
Month_july	-0.16	0.40	0.69
Month_august	0.47	0.45	0.30
Month_september	0.00	0.48	1.00

3.3.5.2 Isopoda

The abundance of Isopoda differed between the four broad habitats, with the lowest overall average abundance recorded in arable habitat and the highest in woodland (Figure 3.6). The abundance of Isopoda was highest in woodland habitat in May with a mean abundance of 22.0 individuals and lowest in arable habitat in May with a mean abundance of 0 individuals per pitfall capture.

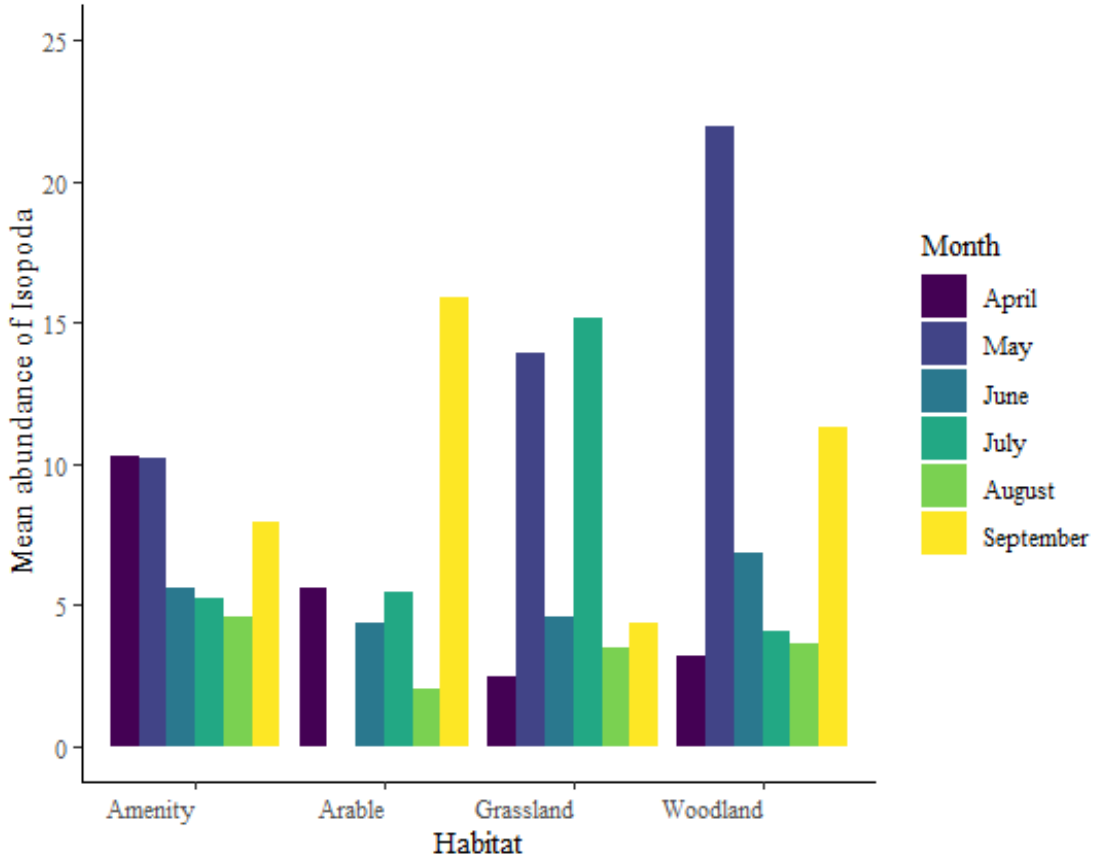


Figure 3.6 Abundance of Isopoda in pitfall trap captures across four broad habitats and six different months. Averages taken from 493 unique pitfall traps sampling in the UK between April 2018 and September 2019.

A Negative binomial GLMM was used to investigate the factors affecting the abundance of Isopoda captured in pitfall traps. The marginal and conditional R^2 values for the GLMM were 0.17 and 0.28, respectively. There was a significant variability in the abundance of Isopoda across habitats throughout the sampling period, which explains 17% of the variance within the dataset. There were significantly less Isopoda in arable and grassland habitats (Table 3.9). Site, as the random factor, accounted for 11% of the variance within the dataset, demonstrating that abundance of Isopoda varied between habitats with some variation at the local scale.

Table 3.9 Summary of Negative Binomial Generalised Linear Mixed Model (GLMM) for the effect of month and habitat on the abundance Isopoda of pitfall trap captures sampled from the UK rural landscape. Site was included as a random factor with standard deviation of 0.69. Nobs = 484. Bold P values indicate statistical significance at 0.05 level. SE = Standard Error.

Model Parameter	Estimate	SE	P-value
(Intercept)	1.58	0.56	**0.00**
Habitat_arable	-1.02	0.31	**0.00**
Habitat_grassland	-0.64	0.30	**0.03**
Habitat_woodland	-0.49	0.29	0.09
Month_may	1.02	0.68	0.13
Month_june	-0.70	0.66	0.29
Month_july	0.36	0.65	0.58
Month_august	-1.05	0.75	0.16
Month_september	1.08	0.78	0.17

3.3.5.3 Pulmonata

The abundance of Pulmonata was greatest in woodland habitat, and lowest in arable habitat (Figure 3.7). The highest abundance of Pulmonata was in woodland habitat in April with a mean abundance of 9.0 individuals per pitfall capture. In amenity and grassland habitat, abundance of Pulmonata was greatest in August with a mean abundance of 6.4 and 9.4 individuals. In arable habitat Pulmonata was most abundant in May with a mean abundance of 5.1 individuals per pitfall capture.

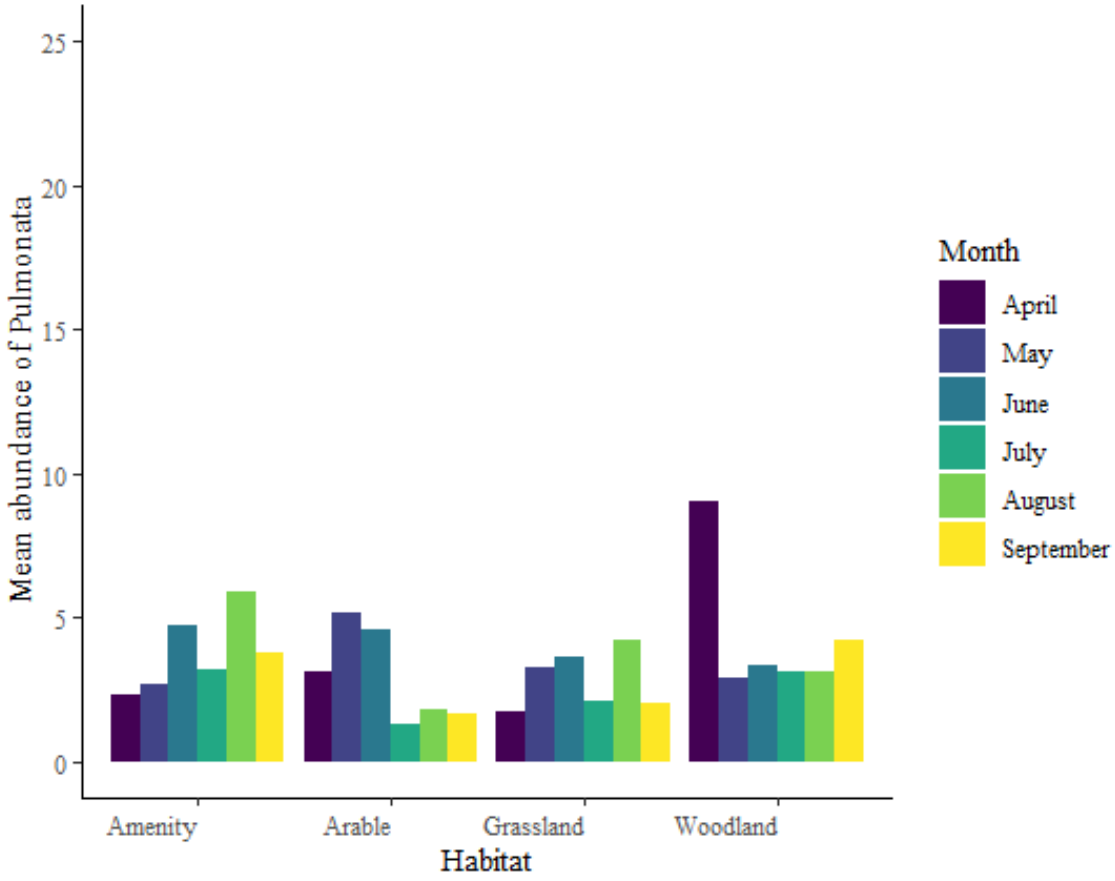


Figure 3.7 Abundance of Pulmonata in pitfall trap captures across four broad habitats and six different months. Averages taken from 493 unique pitfall traps sampling in the UK between April 2018 and September 2019.

A Negative binomial GLMM was used to investigate the factors affecting the abundance of Pulmonata captured in pitfall traps. The marginal and conditional R^2 values for the GLMM were 0.24 and 0.77, respectively. There was a significant variability in the abundance of Pulmonata across habitats throughout the sampling period, which explains 24% of the variance within the dataset. There were significantly less Pulmonata in arable habitat (Table 3.10) though no significant differences in abundance were observed between months. Site, as the random factor, accounted for 53% of the variance within the dataset, demonstrating that abundance of Pulmonata varied both between habitats and on a local scale.

Table 3.10 Summary of Negative binomial Generalised Linear Mixed Model (GLMM) for the effect of month and habitat on the abundance Pulmonata of pitfall trap captures sampled from the UK rural landscape. Site was included as a random factor with standard deviation of 1.04. Nobs = 484. Bold P values indicate statistical significance at 0.05 level. SE = Standard Error.

Model Parameter	Estimate	SE	P-value
(Intercept)	0.69	0.74	0.35
Habitat_arable	-0.45	0.13	**0.00**
Habitat_grassland	-0.15	0.10	0.14
Habitat_woodland	0.09	0.10	0.35
Month_may	0.04	0.91	0.97
Month_june	0.00	0.88	1.00
Month_july	-1.21	0.87	0.16
Month_august	-0.76	0.97	0.43
Month_september	-1.49	1.11	0.18

3.4 Discussion

Changes to farming practices can have significant effects on biodiversity across anthropogenic landscapes (Robinson and Sutherland 2002; Tscharntke et al. 2005; Angus et al. 2009). Modern farming, characterised by the application of chemicals, use of heavy machinery and intensified management practices, has introduced and exacerbated many of the negative pressures that affect ground dwelling invertebrate communities (Bruce 2016; Dicks et al. 2019). This study investigated the differences in invertebrate communities across multiple habitats and sites inhabited by hedgehogs and badgers, to assess the availability of prey and how this varied at the local scale, between habitats and seasons. The results highlight significant differences in abundance and biomass between habitat types, showing that higher abundances also correlated with overall greater diversity. Moreover, abundance of specific invertebrate Orders varied both seasonally and between sites. Coleoptera and Araneae were most abundant in grassland habitat, whereas Isopoda

and Pulmonata were most abundant in woodland habitat. This study revealed significant variation in the abundance and biomass of invertebrates between habitat type that was partly explained by seasonal differences, though Site had a more profound effect, explaining comparatively high proportions of the variance. This study demonstrates the highly variable nature of invertebrate assemblages at both the habitat and local scale, which will likely influence interactions amongst higher trophic levels.

3.4.1 Richness and diversity of pitfall captures

Modelling showed that the overall abundance of invertebrates varied significantly between all habitats. Woodland habitat was associated with the highest abundance of invertebrates, followed by grassland, arable and finally amenity grassland habitat. In this study, amenity habitat refers to mown grassland which is typically short, and its lower diversity and abundance of invertebrates is attributed to its regular maintenance (Fischer et al. 2013). Similarly, lower diversity and abundance of invertebrates is also attributed to intensively managed grassland habitat, which is subject to grazing and compaction (Vickery et al. 2001), and arable habitat, which is increasingly intensively managed (Poschlod et al. 2005). These results align with existing literature that depicts relatively poor biodiversity across cultivated land (Poschlod et al. 2005; V. Smith et al. 2008) and demonstrate how management practices can have detrimental effects on the richness and diversity of invertebrate communities across different habitats.

3.4.2 Biomass of pitfall captures

The strength of correlation between abundance and biomass inevitably varies between different invertebrate groups and can depend on the sampling technique used to assess community structure (Saint-Germain et al. 2007). For example, the positive correlation between the abundance and biomass of ground beetles sampled by pitfall trapping has been shown to be strong, though weaker correlation is expected for less active species which

tend to be smaller (Saint-Germain et al. 2007). In this study, biomass was included to allow the functional importance of different groups of taxa to be assessed, as biomass may be more informative in terms of competitive interactions between badger and hedgehog (Saint-Germain et al. 2007). Results showed that invertebrate biomass was not significantly different between arable, amenity and grassland habitat, but greater in woodland habitat. Woodland habitat is typically patchily distributed across the rural landscape and yet it can be an important habitat for supporting higher abundances of generalist species at higher trophic levels, particularly those which are generalists within the broader agricultural environment (Fuentes-Montemayor et al. 2020).

3.4.3 Availability of earthworms

Amenity grassland habitat supported a significantly higher abundance of earthworms than woodland, grassland and arable habitats. Earthworm biomass correlated with abundance, suggesting the availability of earthworms was greatest in amenity grassland followed by woodland, grassland, and lastly arable habitat. The lowest earthworm abundance in arable habitat was an expected result, as earthworm populations have suffered markedly with modern farming practices, namely increased frequency of tillage (Chan 2001), and with the use of heavy modern machinery which has led to the removal of some species of earthworm from local environments (Decaëns and Jiménez 2002). Lower earthworm availability in grassland habitat may reflect its use as grassland for livestock, which can cause significant soil compaction (Vickery et al. 2001). Whereas, earthworm availability in woodland habitat is more strongly affected by soil structure which often develops in complexity as the woodland matures (Ashwood et al. 2019). Earthworms are also affected by the soil's microclimate and are sensitive to changes in temperature and moisture (Eggleton et al. 2009). These factors may contribute to the lower earthworm availability observed in woodland habitat compared with amenity grassland habitat in this study.

Earthworm biomass was affected significantly by month, though abundance was not, and this may be reflective of the species of earthworm active in the upper layer of the soil. For example, during cold and dry periods, earthworms migrate deeper and therefore less may be available for capture in earthworm cores which are taken near the ground's surface (Fraser et al. 2012). These results are consistent with other studies demonstrating that earthworm captures decreased between April – June, reflective of the drier surface soils and earthworm migration (Peach et al. 2004; Blouin et al. 2013).

3.4.4 Site level differences

The results of this study showed that the abundance and biomass of invertebrates was partly explained by habitat and season, demonstrating broad trends that are likely common across rural landscapes. However, Site accounted for between 27% to 53% of variation in abundance indices. This result highlights that invertebrate assemblages are highly variable at the local scale, typically reflecting local land use and its management at small spatial scales. Typically, studies have focused on a specific management practice, or habitat type, to quantify the impacts on invertebrate diversity (Hof and Bright 2010; Yarnell and Pettett 2020; Atkinson et al. 2005; Frazão et al. 2017). However, the results here indicate that broad relationships are not applicable when considering local differences in abundance and biomass of invertebrates.

Management practices at the local level, which includes the application of pesticides that often has unintended consequences due to their broad specificity, can result in negative effects on non-target organisms. Invertebrate assemblages have suffered reductions at the landscape level as a result of the widespread use of pesticides to meet the increasing demand for higher agricultural productivity (Mancini et al. 2020; Wilson and Tisdell 2001) that has resulted in increasing pesticide resistance due to its overuse (Wilson and Tisdell 2001). Whereas, organic farming has shown that a combination of reducing

pesticide application and providing semi-natural habitat that increases habitat heterogeneity, can have a positive effect on invertebrate abundance and richness (Gonthier et al. 2014). This demonstrates the importance of land management decisions and the potential for both positive and negative effects on invertebrate biodiversity.

In addition, some management practices differentially affect certain groups of invertebrate taxa. For example, hedgerow cutting regimes and meadow cutting techniques can unequally affect ground dwelling and flying insects (Amy et al. 2015; Humbert et al. 2010), demonstrating that site specifics can cause variation in invertebrate assemblages. The results of this study suggest that variation in invertebrate diversity and abundance are better predicted by site specifics than by generalised patterns between broad habitats. This further highlights the need for invertebrate sampling at individual sites to clarify the role of prey in intra-guild studies as patterns of invertebrate availability reported in other studies may not apply to specific sites.

3.4.5 Effects of invertebrate assemblages on predators

The general patterns of decline that have been observed in invertebrates as a reflection of farming intensification have been shown to cause lower densities of farmland bird species (Benton et al. 2003) and this is likely to be the same for other taxa that utilise rural landscapes too. Within the IGP relationship shared by badger and hedgehog, the local availability of invertebrate food may influence both competitive and predatory interactions. Studies of bird distributions showed that densities were higher in mixed farmland followed by pastoral farmland, with comparatively lower densities in arable landscapes due to lower invertebrate and plant food resources (Atkinson, Fuller, and Vickery 2002). Seasonal variation can further exacerbate the differences in food availability between pastoral, arable and mixed farming landscapes. For example song thrush (*Turdus philomelos*) declines across arable dominated landscapes in Britain, have been attributed to lower

invertebrate abundance due to fast drying arable soils in summer and a lack of alternative grassland and woodland habitat (Peach et al. 2004). Like many farmland bird species, badgers and hedgehogs forage on invertebrates, which suggests it is possible that these mammals may also benefit from the resources supported by mixed farmland environments. Therefore, badger and hedgehog may be expected to occupy areas of mixed farmland more frequently and perhaps together, in comparison with other habitat mixes.

3.4.6 Habitat selection

Often, habitat selection has been shown to drive the spatial relationships between intraguild-competitors, resulting in spatial overlap or partitioning. The habitat in which a species forages will influence the encounter rate of potential prey (Crowder and Cooper 1982; Glaspie and Seitz 2018). IGP theory depicts that the intraguild-predator species will occupy optimal habitat, including areas that are abundant in prey, potentially forcing intraguild-prey into resource-poor areas (Zabalo 2012). In this respect, badgers might be expected to utilise woodland habitat which is supported by the highest abundance and diversity of invertebrate prey, perhaps excluding hedgehog from habitat they have been shown to rest in (Hof and Bright 2010).

For the intraguild-prey species, dietary breadth and prey switching may provide an alternative means for niche partitioning. For example the dietary breadth of tigers (*Panthera tigris*), leopards *(P. pardus*) and dholes (*Cuon alpinus*), doubled in food-limiting circumstances, with more abundant but less energetically profitable prey being more readily consumed (Steinmetz et al. 2020). Similarly, the hog badger (*Arctonyx collaris*) consumed different prey in relation to its seasonal availability, although they showed preferences too, choosing fruits over more abundant earthworms (Zhou et al. 2015). Again, hedgehogs might be expected to exploit resources that badgers do not utilise in order to reduce potential competition for shared resources. Dietary partitioning has been observed

to promote the coexistence of intraguild-competitors in other species (Kamler et al. 2007) and this is investigated amongst badger and hedgehog in Chapter 4.

Notably, many of the invertebrate and also plant prey that badger and hedgehog compete for, such as Poaceae, Coleoptera and Lepidoptera, are also common prey types for many farmland bird species. Therefore, badger and hedgehog are also competing for these prey types with many other species (Vickery et al. 2002). This food network highlights how IGP relationships are influenced by broader ecological networks which may have important effects on overall resource availability within the rural landscape.

3.4.7 Limitations

Identification of potential invertebrate prey for badgers and hedgehog in the present study was limited to Order level, providing an assessment of abundance and biomass of broad taxonomic groups across each study site. Species level identification would provide a more detailed assessment, though this was unlikely to be informative for assessing patterns in resource use between the 23 sites in this present study.

Furthermore, whilst pitfall trapping is routinely used as the primary method for assessing ground dwelling invertebrates (Brown and Matthews 2016), the capture rate is likely to be affected by habitat characteristics such as vegetation type and differences in the activity between taxa, with species more active on the substrate's surface being more readily captured (Melbourne 1999) . Moreover local weather conditions are likely to influence the capture rate of both pitfall traps and earthworm cores due to the sensitivity of invertebrates to climatic conditions (Saska et al. 2013).

3.4.8 Conclusions and further research

Invertebrates respond to environmental change and this study describes the differences in invertebrate assemblages between broad rural habitats and how the availability of different

prey groups varies between seasons. It also highlights site-specific differences in the abundance and biomass of invertebrates. This indicates that localised management practices may strongly influence the availability of invertebrate prey and provides important information on the availability of resources for species which utilise these different broad habitats, potentially providing explanatory power for their habitat preferences which may be driven by prey preferences and availability. Further investigation would be necessary to understand the nature of these site-specific differences, to determine what factors enhance diversity of invertebrate assemblages within the same broad habitat types. Understanding how to improve habitat quality for invertebrates would likely be beneficial to the wider ecosystem, which is dependent on them. The information gained in this study is utilised in Chapter 6 to assess badger and hedgehog spatial distributions and habitat preferences, considering local food availability of invertebrate prey. In Chapter 4, the diet of both generalists is discussed and compared in relation to the seasonal availability of invertebrate prey in Chapter 5.

4.1 Introduction

Predation and competition can lead to rapid demographic change by reducing population densities and altering community structure (Mack et al. 2000; Hiltunen and Laakso 2013). Within Intra-Guild Predation (IGP), guild members are subject to both competitive and predator-prey, antagonistic interactions (Polis and Holt 1992). These interactions can lead to multiple states, including coexistence of intraguild-predator and intraguild-prey, alternative stable states such as the intraguild predator existing alone, or exclusion of the intraguild-prey. For example, predation is typically a 'top down' pressure that can limit prey populations and result in an increase in the abundance of shared prey resources for the intraguild predator (Holt et al. 1999). However, another common limit on population size is often due to 'bottom up' processes related to the availability of food resources and the strength of competition for them (White 2008). Understanding what conditions (competition, predation or their interaction) become limiting factors to intraguild-prey populations is difficult to establish (Hiltunen and Laakso 2013), though dietary niche assessment provides an opportunity to identify the level of niche overlap and potentially quantify the rate of predation between intraguild-predator and intraguild-prey species.

Anthropogenic causes such as habitat alteration, loss of habitat heterogeneity, or climate change, can exacerbate 'bottom up' pressures that influence the IGP relationship (North and Ovaskainen 2007; Elmhagen et al. 2010). Reductions in food resources are associated with primary production, levels of biodiversity and the number of available niches for organisms. This is often accompanied by cascading effects for species at higher trophic levels (Carson 1962; Fey et al. 2008; Tucker and Rogers 2014). The resilience of a species engaged in IGP to respond to environmental change is likely influenced by their dietary niche. For example, for an intraguild-prey species to persist and coexist with the intraguild-predator species, IGP theory states it must be the superior exploiter of resources (Holt and Polis 1997). Therefore, intraguild-prey species that are dietary generalists may

benefit from being able to adjust their diet to fluctuating availability of food (Holt et al. 1999). Whereas, if a reduction in one food resource cannot be compensated for by switching to an alternative prey item, then competition intensity will increase (Chase et al. 2002), which may lead to a population reduction in the predator species. Being a generalist may support dietary partitioning within IGP and may be particularly important for intraguild-prey that are known to utilise prey-poor areas in the presence of intraguild-predators (Thompson et al. 2007; Steinmetz et al. 2013). Hence, the availability of suitable habitat for the intraguild-prey species will indirectly affect the distribution of a species (Cross et al. 2019).

The theory of IGP states that the level of predation between intraguild-competitors, such as badgers predating on hedgehogs, is typically assumed to be density-dependent and can increase through either high, unstable levels of intraguild-prey, or low availability of shared food (Polis et al. 1989). Not only does the act of predation result in an immediate energetic gain, but it reduces competition further (Polis et al. 1989). Predation between badgers and hedgehogs is asymmetrical, with badgers preying on hedgehogs (Neal 1986; Doncaster 1992; Trewby et al. 2014). To understand the dynamic balance of predation and competition (i.e. under what circumstances does IGP increase) the degree of dietary niche overlap between the predator and prey species must first be established (Kartzinel et al. 2015). Understanding the level of niche overlap provides evidence for how changes in shared prey resources might affect the species concerned.

According to Niche Overlap Theory the level of overlap is fundamentally important with a maximum threshold below which species can coexist (Pianka 1974). Often, it is niche partitioning itself, or behavioural adaptation, that allows species to coexist when sharing resources (Salinas-Ramos et al. 2020). Within IGP, intraguild-prey may utilise prey poorer areas to avoid riskier high predator density areas, which may lead to an increased dietary breadth and individual specialism, as animals opportunistically utilise

what is available, and this may be outside their typical dietary preferences (Michalko and Pekár 2014). Similarly, if the intraguild-predator and intraguild-prey compete for a common food type that becomes depleted, the intraguild-prey may broaden their niche to attain enough food (Michalko and Pekár 2014). The complexity of predator-prey relationships and the prevalence of multi-species effects (Reddy et al. 2019), highlights the potential benefits of wide dietary breadth for allowing niche partitioning within the IGP relationship.

Existing studies have documented the generalist foraging nature of both badgers (Roper 1994; Roper 2010) and hedgehogs (Reeve 1994; Hof and Bright 2010). Invertebrates constitute an important part of both diets, though the items consumed, and their relative proportions will vary by site and season. For example, the reported frequency of earthworms within the diet of hedgehogs has previously ranged greatly from 34% (Yalden 1976) to 95% of samples analysed (Wroot 1984), although this may in part be associated with the limitations of using morphological analyses, as soft-bodied organisms can be difficult to identify (Wroot 1984), potentially leading to over and under representation of different prey types (Berry et al. 2017). Although traditional methods are inexpensive and have been used widely, morphological analysis is time consuming (de Sousa et al. 2019) and provides poorer taxonomic resolution than newer molecular methods (Berry et al. 2015). However, existing morphological studies have been shown to identify important prey types (Granquist et al. 2018), showing that hedgehogs commonly consume beetles (Coleoptera), moths (Lepidoptera) and earwigs (Dermaptera), amongst other invertebrates and less frequently vertebrate prey, including mammals, birds and eggs (Yalden 1976; Wroot 1984).

The breadth of badger diet is also well evidenced and although many studies have highlighted the ubiquity and importance of earthworms (Shepherdson et al. 1990; Zabala and Zuberogoitia 2003; Cleary et al. 2011), other food types such as wheat may be

consumed in similar volumes when seasonally available (Shepherdson et al. 1990). Roper (1994) reported that in two studies more than 50% of the diet by volume consisted of fruits, with earthworms representing no more than 50% of diet by volume across eleven studies. This demonstrates the wide variation in the diets of badgers and hedgehogs, and despite both species consuming many similar food types (Hof et al.2012), the extent of possible competition where the two species co-occur has not been investigated. Therefore, to establish the level of dietary niche overlap and to understand if this results in competition for food resources, a comparison of diets from badgers and hedgehogs at sites where they coexist is needed.

In addition to the potential for competition, the importance of predation of hedgehogs by badgers requires investigation. Several studies have provided evidence for badger predation on hedgehogs which increases with badger density (Doncaster 1992; 1994; Hof and Bright 2010). A mortality rate of 20% was recorded in a 75 day trapping period of 44 wild hedgehogs, of which 89% of deaths were caused by badger predation and when extrapolated over a whole year, this resulted in an unsustainable mortality rate of 52% (Hof and Bright 2010). This demonstrates the potential for predation to cause population level effects, though the extent to which this occurs across the UK badger and hedgehog system is unknown.

Increasingly, the value and versatility of molecular methods for investigating ecological networks is being realised and these methods have been used successfully on elusive species such as giant otters (*Pteronura brasiliensis*), (Quéméré et al. 2021), in logistically challenging environments (Casper et al. 2007), and as an alternative to more traditional surveying (Valentini et al. 2016). Molecular methods are non-invasive and are replacing morphological analyses of food remains due to the sensitivity of these techniques (Pompanon et al. 2012). Progression from deoxyribonucleic acid (DNA) barcoding that identifies specific species, to next-generation sequencing, such as DNA metabarcoding,

where a whole community can be identified simultaneously, has been revolutionary (Yu et al. 2012). These techniques can be applied to studies of environmental DNA (eDNA) derived from faeces, mucous and gametes that have been left behind in the environment by the host species (Rees et al. 2014). Although typically more degraded than genomic DNA (Marshall and Stepien 2017), eDNA can be analysed successfully using next-generation sequencing as it targets only short regions of a gene which are often still well preserved (Ruppert et al. 2019). Therefore, the diet of rare, elusive, and protected species that are difficult to observe continuously in the field, can be assessed through faecal eDNA sampling.

This study used DNA metabarcoding to analyse the faeces ("scats") of badgers and hedgehogs, collected from two rural sites in the UK where the species co-occur, to: 1) compare the diet of the two species, 2) assess the level of dietary niche overlap between them, 3) identify seasonal variation in their diets, and 4) identify occurrences of badger predation on hedgehogs. Ultimately, this novel, comparative study will assess the level of competition for food resources between hedgehogs and badgers and the importance of badgers as predators of hedgehogs in a rural environment.

4.2 Methods

4.2.1 Study sites and sample collection

Two working mixed farms, set in University rural estates in the UK, were selected for the study: Hartpury College in Gloucestershire (0.8 km²,51° 54'N, -2° 18'E), and Nottingham Trent University's Brackenhurst Campus in Nottinghamshire (0.55 km², 53° 3'N, 0° 57'E). The presence of badgers and hedgehogs at both sites was known prior to sampling, with density estimates given in Chapter 6.

Faecal samples were collected throughout, May 2018 – August 2019, representing four and five sampling seasons for hedgehog and badger, respectively. Seasons were as

follows; Summer 2018 = June - August 2018, Autumn 2018 = September - November 2018, Winter 2019 = December 2018 – February 2019, Spring 2019 = March – May 2019 and Summer 2019 = June – August 2019. Sampling for hedgehog scats was omitted during the winter season, as this reflects their hibernation period when activity levels are low (Reeve 1994; South et al. 2020).

Walkover site surveys were carried out to search for hedgehog scats systematically, following linear features such as hedgerows and field margins, with a minimum of two surveys per month, and additional sporadic sampling. Hedgehog scats were identified by their distinctive features, typically cylindrical and about 3-5 cm in length, often tapered at one end and shiny black in appearance. Remnants of insect prey were also usually clearly visible (Olsen 2014). Badger scats were sampled during walkover surveys and additionally by bimonthly surveys of latrines located at active setts. The appearance of badger scats varied from solid sausage-shaped, to sloppy with a jam-like consistency depending on what had been consumed. Often scats were slimy and black, indicative of an abundance of earthworms (Skoog 1970) and deposited in distinctive shallow pits, up to 10 cm deep (Olsen 2014). Although scats were collected in the field based on their size, shape and smell etc., “species” was confirmed by subsequent genetic analyses.

A total of 215 scats were collected, of which a subset of 144 were selected for analysis to attain suitable sequencing read depth. In total, 80 badger and 64 hedgehog scats were selected for subsequent analysis as follows: eight samples were included per species, per season, from the two UK sites, Brackenhurst in Nottinghamshire and Hartpury in Gloucestershire, with sample records being consulted to preferentially analyse fresher scats that would be more suitable for molecular assessment (Reed et al. 1997). All scats were analysed at the UK Natural Environment Research Council (NERC) Biomolecular Analysis Facility (NBAF) at the University of Sheffield.

4.2.2 Overview of molecular methodology

Genomic DNA was extracted from the 144 scat samples, and the originator species was verified using Sanger sequencing with MiMammal primers (Ushio et al. 2017), to be sure that samples taken forward originated from the target hosts. Subsequently, two existing sex-markers RG4 (Y-linked, SRY) (Griffiths and Tiwari 1993), and Mel592 (X-linked) (Annavi et al. 2011) were tested to investigate whether the originator of each scat sample could be accurately sexed, with a view of investigating dietary niche partitioning between the sexes (Jones et al. 2015).

To investigate diet, five primer sets were selected to provide broad coverage of badger and hedgehog diets, and *in silico* assessment ascertained that these primers were likely to amplify the broad range of potential prey items. Scat samples were assayed four times to identify contents, using combined primer sets based on short (invertebrate) and variable (plant, vertebrate, and earthworm specific) DNA amplicon length. Samples were pooled in equimolar amounts, then sequenced using an Illumina MiSeq (Illumina, San Diego, California, USA) next generation sequencing platform. Finally, raw sequencing data was analysed using an in-house bioinformatic pipeline, and further statistical analysis was conducted using R Studio version 1.2.5 (R Studio Team 2020). Low level contamination was systematically removed from the dataset using the program microDecon (McKnight et al. 2019).

Family level comparisons of different food groups were made to ascertain dietary overlap between badger and hedgehog, and whether this was exacerbated between the sites surveyed or temporally, as prey availability varied. Analysis of data at the Family level increases the proportion of data that can be included in analysis, as not all sequences can be identified to species level. Also, species level may be too sensitive for ecological interpretation (Cristescu 2014). It is unlikely that predators, in this case badgers or hedgehogs, distinguish between similar species of prey and, perhaps instead, consume

them relative to their encounter frequency (Clare et al. 2016). Subsequent data analysis was conducted in R 4.0.5 (R Core Team 2019) and is described in Chapter 6. DNA from the originator species was omitted for downstream analysis, therefore hedgehog DNA was not analysed for hedgehog scats and badger DNA was not analysed for badger scats. Hedgehog DNA identified in badger scats was included as this may represent potential predation events.

4.2.3 Data analysis

Dietary composition was analysed at the Family level and two analytical methods were used for quantitative interpretation of the metabarcoding datasets: frequency of occurrence (FOO), and relative read abundance (RRA) (Deagle et al. 2019). FOO of each Family is expressed as the percentage of samples (individual scats) that contain a given food item, inferring that a food group found in a high proportion of samples is likely to be important (Deagle et al. 2019). Food items present in >10% of samples were considered common dietary items in this study. A threshold value, typically around 1%, appropriate to the study question, can also be applied to remove non-dietary food items that may exist due to contamination or secondary ingestion (Deagle et al. 2019). Shorter amplicon lengths increase the possibility of sequencing items representing secondary predation, but are often required in dietary studies to simultaneously increase the probability of identifying rare prey items (Uiterwaal and DeLong 2020). RRA is a proxy for relative abundance that compares the number of DNA sequences amplified per dietary item within each sample and can be used to assess the volume of each dietary item that is consumed (Deagle et al. 2019). However, there are many avenues for introducing biases that can lead to unequal representation of prey items during sequencing. For example, some primers have a better binding affinity to a particular species' DNA (Piñol et al.2019). Therefore, caution must be taken when interpreting RRA results.

Sequence data were translated into both a binary format (presence/absence) from FOO data, and proportion of reads per sample from RRA data. Initially, species richness was assessed using FOO data to construct prey accumulation curves, in order to assess whether the sample size was sufficient to quantify the diet of each species.

Dietary niche breadth was assessed using Levin's diversity index (Levins 1968). This was calculated using the frequency of occurrence of each dietary Family (n = 94), with the 'invsimpson' function in the R package 'Vegan' (Oksanen et al. 2016), using the following formula:

$$\hat{B} = \frac{Y^2}{\sum N_j^2}$$

where $\hat{B}$ = Levins' measure of niche breadth, N_j = number of individuals scats containing each food type, Y= $\sum N_j$ = total number of scats sampled. Values range from 1 to a maximum value of the number of Families in the sample, with higher values indicating greater dietary breadth. In addition, dietary richness rarefaction curves were generated using 'specacc' in the R package 'Vegan' using the 'Chao' method (Chao et al. 2014), to establish the level of undetected mOTUs. This allows the sampling effort to be assessed, to determine whether sampling was representative of the community being measured.

Dietary niche overlap between badgers and hedgehogs was calculated using Pianka's Index, which describes overlap on a scale of 0 to 1, with 1 indicating high overlap and 0 indicating no overlap (Pianka 1973), using the following formula:

$$\hat{O}_{jk} = \frac{\sum_j^n \hat{p}_{ij}\,\hat{p}_{ik}}{\sqrt{\sum_i^n \hat{p}_{ij}^2\ \sum_i^n \hat{p}_{ik}^2}}$$

where O_{jk} = Pianka's measure of niche overlap between species j and k, p_{ij} = proportion of resource *i* of the total resources used by species *j*, p_{ik} = proportion of resource *i* of the total resources used by species *k*, n = total number of resource states (94 unique Families).

Pianka's Index was calculated from FOO data for badger and hedgehog, relative to null models of randomised occurrence data, in the R package 'EcoSimR' (Gotelli and Ellison 2015). Niche overlap was also calculated using RRA, and an ecological network was constructed using the R package 'Barpartite' to visualise the results. Where RRA was used, the ratio of sequence reads for each prey Family present in negative control samples was used to deduce the proportion of reads that were likely due to contamination (McKnight et al. 2019). Contamination values were removed from each sample before calculating the RRA of food Families present within each sample.

Dietary composition was analysed, providing an assessment of the dissimilarity between the food items present in individual scats of each species (Arrizabalaga-Escudero et al. 2018; Kartzinel et al. 2015). Friedman's test was carried out using the R package 'Rstatix' (Kassambara 2021) to find significant seasonal differences in the FOO of common food types for each species. Badger and hedgehog dietary niches were visualised using non-metric multidimensional scaling (NMDS) of FOO data, a technique that uses rank orders to collapse information from multiple dimensions that can be analysed and interpreted (Clarke and Gorley 2015). NMDS was carried out using the R package 'Vegan', specifically 'metaMDS'(Kruskal 1964). Permutational multivariate analysis of variance (PERMANOVA) was undertaken using the R package 'Vegan' and function 'adonis' which calculated Jaccard distance matrices based on 999 permutations, to compare food Family composition in the diets between species season and site (Anderson 2005).

4.3 Results

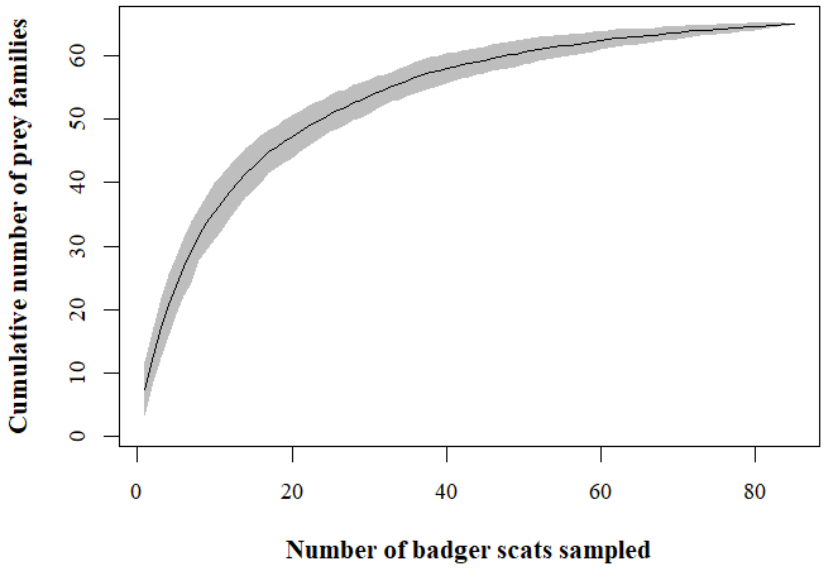
A total of 144 faecal samples were analysed, of which 80 were from badgers and 64 from hedgehogs based on Sanger sequencing using the MiMammal primer set (Ushio et al. 2017). Sexing of the hedgehog faecal samples was not possible using the RG4 vertebrate sex-linked primer set (Griffiths and Tiwari 1993) and, despite correctly sexing known male

and female badgers from genomic DNA extracted from badger tissue, only 35.7% of scat samples tested (n = 16) provided reliable indicators of badger sex. This was likely the result of using highly degraded DNA from faecal samples and, therefore, neither badger nor hedgehog sex was included as a covariate in analyses.

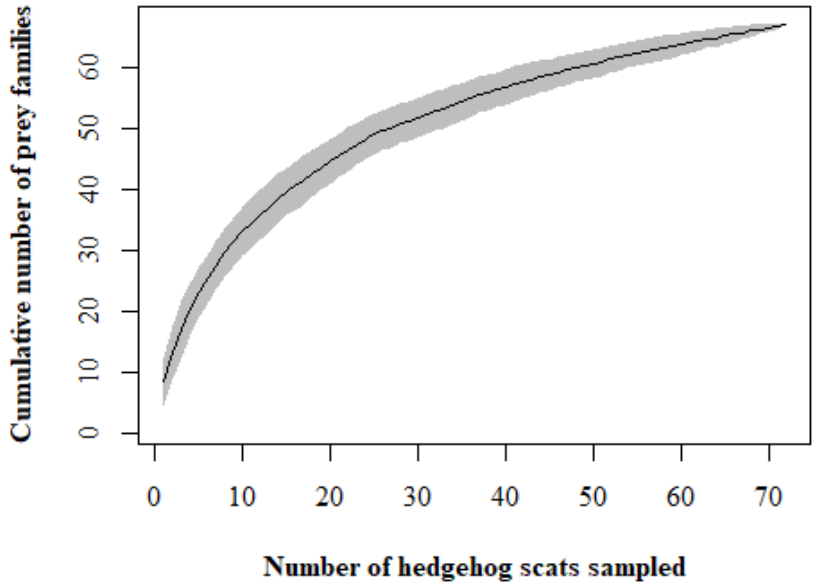
Following successful DNA extraction, amplification and sequencing, 1,180,396 high quality sequences were clustered into taxonomic units to allow identification of prey items to Family level.

4.3.1 Species richness

Prey accumulation curves demonstrate that the total sampling effort implemented in this study was sufficient to allow a high proportion of both badger and hedgehog dietary items to be described (Figure 4.1). For both species, richness begins to plateau at approximately 55 – 60 unique scats, suggesting that few further Families present in the diet remained unidentified. As scat samples per season and site were lower than 55 – 60 unique scats, season and site comparisons should be treated with caution as species richness may be underestimated.



a)



b)

Figure 4.1 Accumulation curves for prey Families in badger (a) and hedgehog (b) scat samples, with 95% confidence intervals shown by the shaded area. Data were randomly resampled 100 times, and only prey Families that represented >1% of sequence reads per sample were included to minimise the risk of including contaminant DNA.

In terms of broad dietary categories, hedgehog scats contained invertebrate DNA in 100% of scats, followed by plant 96.9%, bird 14.0%, other mammal 15.6%, amphibian 1.6%, and fungi 1.6%. By comparison, badger scats had similar broad dietary composition and rankings, with invertebrates being found in 98.8% of badger scats, followed by plant 72.5 %, other mammal 43.8%, bird 18.8%, amphibian 1.3%, and fungi 1.3%.

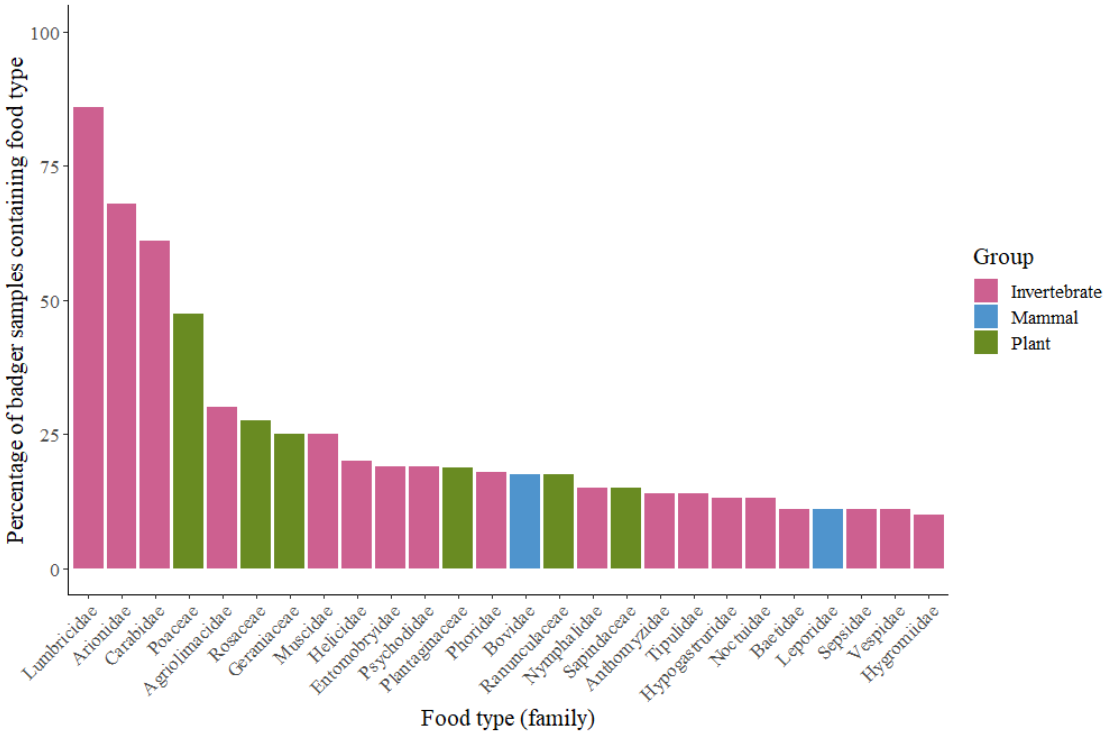
Several different dietary items were found in both species, suggesting generalist dietary preferences. A larger number of prey Families (75) were detected in badger samples than in hedgehog samples (63) (Appendix E), suggesting that badgers have a broader dietary niche, though slightly more scat samples were analysed for badgers too. The most frequently detected prey Families in hedgehog samples were beetles (Carabidae in 89.0% of samples), slugs and snails (Arionidae in 71.0%) and earthworms (Lumbricidae

in 68.0%) (Table 4.2), whereas in badger samples the most common prey detected were earthworms (86.0%) followed by slugs and snails (68.0%) and beetles (61.0%). Despite the large number of Families found in the scats of badgers and hedgehogs, several were found exclusively in either hedgehog (10 unique Families) or badger (15 unique Families) samples (Appendix E), suggesting some dietary niche partitioning. In addition, hedgehog (Erinaceidae) DNA was only detected in one badger scat (1.3%) (see section 4.3.6). Badgers consumed other mammalian prey, including Muridae (5.0%) and Leporidae (11.0%), more frequently than hedgehogs in this study.

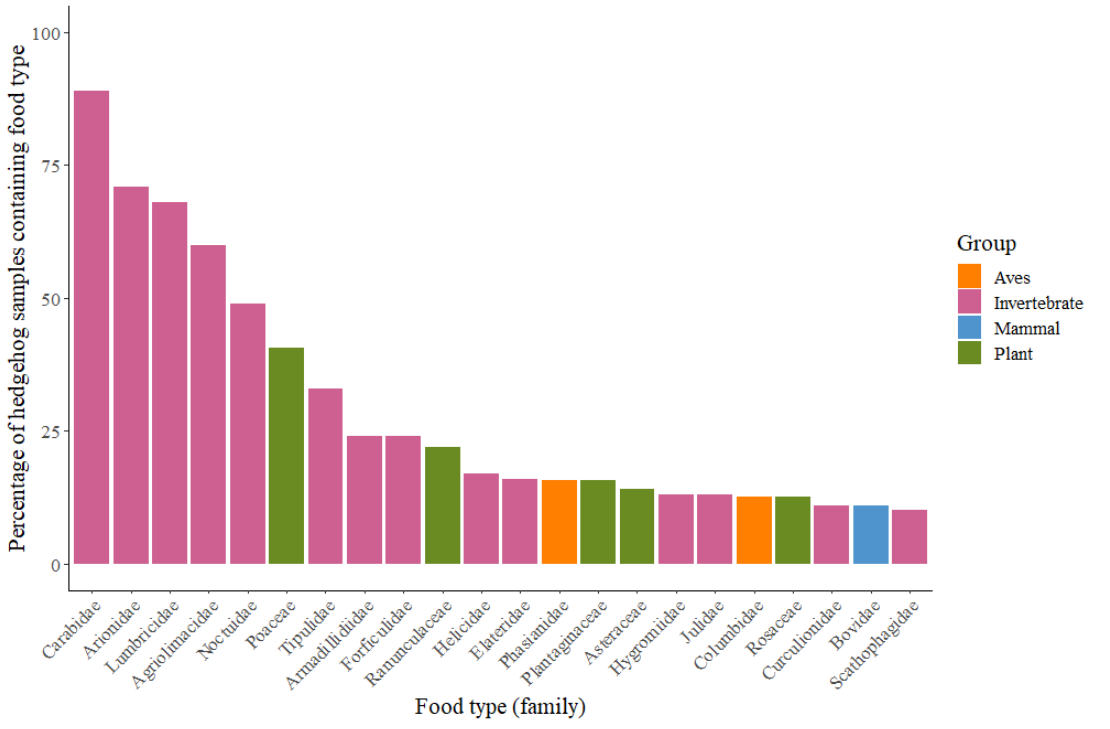
Prey items were unevenly represented by RRA in both hedgehog and badger diet (Table 4.1). Beetles (Carabidae) represented 45.2% of RRA in hedgehog samples, followed by earthworms (Lumbricidae) 16.7%, moths (Noctuidae) 8.9% and slugs (Arionidae) 8.4%. Together, these four Families accounted for 79.2% of RRA in hedgehog scats, demonstrating their abundance in sequence data. In contrast, slugs (Arionidae) were proportionally greatest in badger samples 27.0%, followed by earthworms (Lumbricidae) 26.9%, beetles (Carabidae) 12.6% and grasses (Poaceae) 4.5%, accounting for 71.0% of total read abundance of prey items in badger samples. Therefore, it appears that both species consume similar dietary Families, but in differing quantities.

Table 4.1 The presence of dietary plant and animal Families detected in >10% of hedgehog or badger scat samples from two rural farms in England, between summer 2018 and summer 2019, using DNA metabarcoding. Information is presented in descending order of frequency of prey types from hedgehog scats. RRA indicates the proportion of sequence reads identified to each food group from data pooled across all scats from each species. A complete dietary list is given in Appendix E.

Prey type	Prey Family	Percentage of hedgehog scats containing Family DNA (n=64)	Percentage RRA hedgehog scats (n=64)	Percentage of badger scats containing Family DNA (n=80)	Percentage RRA badger scats (n=80)
Invertebrate	Carabidae	89.0	45.2	61.0	12.6
Invertebrate	Arionidae	71.0	8.4	68.0	27.0
Invertebrate	Lumbricidae	68.0	16.7	86.0	26.9
Invertebrate	Agriolimacidae	60.0	2.1	30.0	1.5
Invertebrate	Noctuidae	49.0	8.9	13.0	1.5
Plant	Poaceae	40.6	0.6	47.5	4.5
Invertebrate	Tipulidae	33.0	0.7	14.0	<0.01
Invertebrate	Armadillidiidae	24.0	0.9	0.0	0.0
Invertebrate	Forficulidae	24.0	<0.1	0.0	0.0
Plant	Ranunculaceae	21.9	0.1	17.5	1.0
Invertebrate	Helicidae	17.0	<0.1	20.0	0.4
Invertebrate	Elateridae	16.0	<0.1	0.0	0.0
Aves	Phasianidae	15.6	<0.1	1.3	0.1
Plant	Plantaginaceae	15.6	0.2	18.8	2.4
Plant	Asteraceae	14.1	0.1	3.8	<0.1
Invertebrate	Hygromiidae	13.0	0.5	10.0	<0.1
Invertebrate	Julidae	13.0	<0.1	1.0	<0.1
Aves	Columbidae	12.5	0.4	1.3	<0.1
Plant	Rosaceae	12.5	0.7	27.5	0.2
Invertebrate	Curculionidae	11.0	0.2	0.0	0.0
Mammal	Bovidae	10.9	0.2	17.5	0.4
Invertebrate	Scathophagidae	10.0	0.1	1.0	<0.1
Plant	Geraniaceae	6.3	<0.1	25.0	1.8
Invertebrate	Entomobryidae	6.0	<0.1	19.0	0.1
Invertebrate	Muscidae	6.0	0.7	25.0	<0.1
Invertebrate	Phoridae	6.0	1.8	18.0	0.1
Plant	Sapindaceae	3.1	<0.1	15.0	0.1
Invertebrate	Psychodidae	3.0	0.9	19.0	2.1
Invertebrate	Sepsidae	2.0	0.1	11.0	2.3
Invertebrate	Anthomyzidae	0.0	4.2	14.0	4.5
Invertebrate	Baetidae	0.0	0.0	11.0	0.1
Invertebrate	Hypogastruridae	0.0	0.0	13.0	0.3
Invertebrate	Nymphalidae	0.0	0.0	15.0	<0.1
Invertebrate	Vespidae	0.0	0.0	11.0	2.4
Mammal	Leporidae	0.0	0.0	11.0	1.1



a)



b)

Figure 4.2 Frequency of occurrence (%) of animal and plant Families detected in badger (a) and hedgehog (b) scat samples. Only Families represented in >10 % of samples are shown.

4.3.2 Diet composition

Diet composition was assessed across species, site and season using FOO data, revealing differences in the diet composition between badgers and hedgehogs (Table 4.2). There was no significant intraspecific variation in diet composition due to site for hedgehogs (P=0.22) or badgers (P=0.32), (Table 4.2). Therefore, as site had no significant effect, samples were pooled for each species across the two sites, resulting in 16 samples per species per season. Season had a significant effect on badger diet composition (P<0.001) and marginal differences were identified for hedgehog diet (P=0.08). Also, there was a near significant result for the interaction between species and season on dietary composition (P=0.08), suggesting seasonal differences in the diet were different for each species.

Table 4.2 Results of the permutational multivariate analysis of variance with Jaccard distance matrices based on 999 permutations, to compare the diet composition (Family level) between (a) badgers, (b) hedgehogs and (c) badgers and hedgehogs. For (c) data from the two sites were merged as there was no significant difference for Site in either single-species analysis.

Variables	F	df	R^2	P
Badger				
Season	2.53	4	0.12	**<0.001**
Site	1.17	1	0.01	0.32
Season*Site	1.53	4	0.07	**0.07**
Hedgehog				
Season	1.59	3	0.07	**0.08**
Site	1.43	1	0.02	0.22
Season*Site	1.06	4	0.05	0.38
Badger & Hedgehog				
Species	90.59	1	0.38	**<0.001**
Season	1.48	1	0.01	0.17
Species*Season	2.04	1	0.01	**0.08**

4.3.3 Prey diversity

At both sites, badger samples show greater prey diversity than hedgehogs (Table 4.3). However, between sites, badger prey diversity was highest at Hartpury, whereas the

reverse was true for hedgehogs (Table 4.3). Levin's index ranged from 17.73 for the diversity of hedgehog's diet at Hartpury to 28.07 for badgers at Hartpury, demonstrating high levels of diversity amongst different sample groups (Table 4.3). There was more inter-site variation in the diversity of prey taken by hedgehogs than that taken by badgers. Moreover, there was a greater difference in diversity indices for badger and hedgehog diet at Hartpury (difference of 10.34) than at Brackenhurst (difference of 2.92).

Table 4.3 The diversity of dietary Families as measured by Levin's Index found in hedgehog and badger scat samples. Higher index values are indicative of greater diversity within samples.

Group	Number of samples	Levin's Diversity Index
Badger (all)	80	28.36
Badger (Brackenhurst)	40	26.21
Badger (Hartpury)	40	28.07
Hedgehog (all)	64	21.49
Hedgehog (Brackenhurst)	32	23.29
Hedgehog (Hartpury)	32	17.73

4.3.4 Dietary Niche Overlap

The level of dietary niche overlap exhibited between badger and hedgehog samples was high (Table 4.4). Also, pairwise comparisons of sites (Brackenhurst and Hartpury) showed high niche overlap between the two different populations of hedgehogs and badgers, suggesting high similarity in diet, regardless of location.

Table 4.4 Pianka's Niche Overlap Index, calculated from the presence–absence matrix (FOO) including 144 scats from two sites, Brackenhurst and Hartpury.

Pair-wise comparison	Pianka's Index (FOO)
Hedgehog diet between sites	0.93
Badger diet between sites	0.92
Hedgehog and badger diet (sites pooled)	0.73

Relative Read Abundance

The level of dietary niche overlap measured using Pianka's index was substantial from both RRA and FOO analysis, with values of 0.60 and 0.73 respectively. This shows slightly more overlap when considering the presence, rather than relative proportion, of diet items within scats. Network analysis revealed the Family found in greatest abundance in hedgehog samples was Carabidae, accounting for 45.2% of total sequence reads. For badgers, the most abundant Families were Lumbricidae and Arionidae, accounting for 26.9% and 27.0% of total read abundance, respectively, illustrating differential selection of diet Families (Figure 4.3).

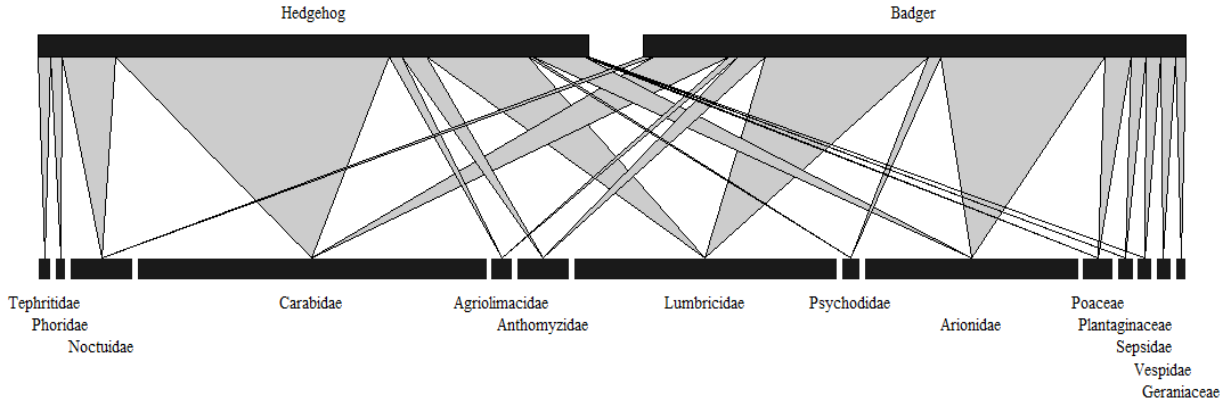


Figure 4.3 Network analysis between badger and hedgehog prey, constructed using the Bipartite package in R Statistics software. Each shaded segment represents a predator-prey interaction, where width is relative to the proportion of reads that were obtained from sequencing. Only taxa that constituted >10% of overall reads from prey items of either species are shown in the figure.

4.3.5 Seasonality

To investigate seasonal differences in the diet of badger and hedgehog, samples from across the two sites were pooled for each species as no significant intraspecific differences in diet composition were found between sites (Table 4.2). DNA from invertebrate and plant Families was consistently present in badger and hedgehog samples irrespective of season (Table 4.4). Bird DNA was identified in badger and hedgehog scats in all seasons, though lower relative frequencies were observed in Autumn and Spring. Amphibian and

fungal DNA was rare in both badger and hedgehog samples relative to the other taxa, each being recorded only once per species, although in different seasons. Mammal DNA occurred more often in badger scats than hedgehog scats in all seasons, except for the summer of 2019.

Table 4.4 Proportion of badger and hedgehog scat samples (n = number of scat samples) containing broad prey types across five seasons in 2018 and 2019. Winter data were not recorded for hedgehogs because this is the hibernation season.

Food type	Species	Summer 2018 (n=16)	Autumn 2018 (n=16)	Winter 2018/19 (n=16)	Spring 2019 (n=16)	Summer 2019 (n=16)
Invertebrate	Hedgehog	100.0	100.0	NA	100.0	100.0
	Badger	100.0	100.0	93.8	100.0	100.0
Plant	Hedgehog	100.0	87.5	NA	100.0	100.0
	Badger	87.5	68.8	68.8	62.5	75.0
Mammal	Hedgehog	6.3	18.8	NA	12.5	25.0
	Badger	62.5	56.3	43.8	43.3	12.5
Bird	Hedgehog	18.8	6.3	NA	12.5	18.8
	Badger	25.0	18.8	6.3	18.8	25.0
Amphibian	Hedgehog	0.0	0.0	NA	0.0	6.3
	Badger	6.3	0.0	0.0	0.0	0.0
Fungi	Hedgehog	6.3	0.0	NA	0.0	0.0
	Badger	0.0	0.0	0.0	6.3	0.0

The proportion of scats containing the five most common diet Families varied seasonally (Table 4.5). A Friedman test was carried out to compare the FOO of the most five most common Families (Table 4.5) for the different seasons for each species. For hedgehogs no significant difference between seasons was found $\chi^2(3) = 6.8$, $p > 0.05$. For badgers, there was found to be a significant difference between the seasons, $\chi^2(4) = 10.5$, $p < 0.05$. Dunn-Bonferroni post hoc tests were carried out and there was a significantly higher proportion of badger scat samples containing the five most common dietary Families in Summer 2018 than Autumn 2018 ($p < 0.05$) and in Summer 2019 compared

with Autumn 2018 ($p < 0.05$) after Bonferroni adjustments. There were no significant differences between any other seasons for badgers' diet.

Table 4.5 Proportion of badger and hedgehog scat samples containing the five common food types across five seasons sampled in 2018 and 2019.

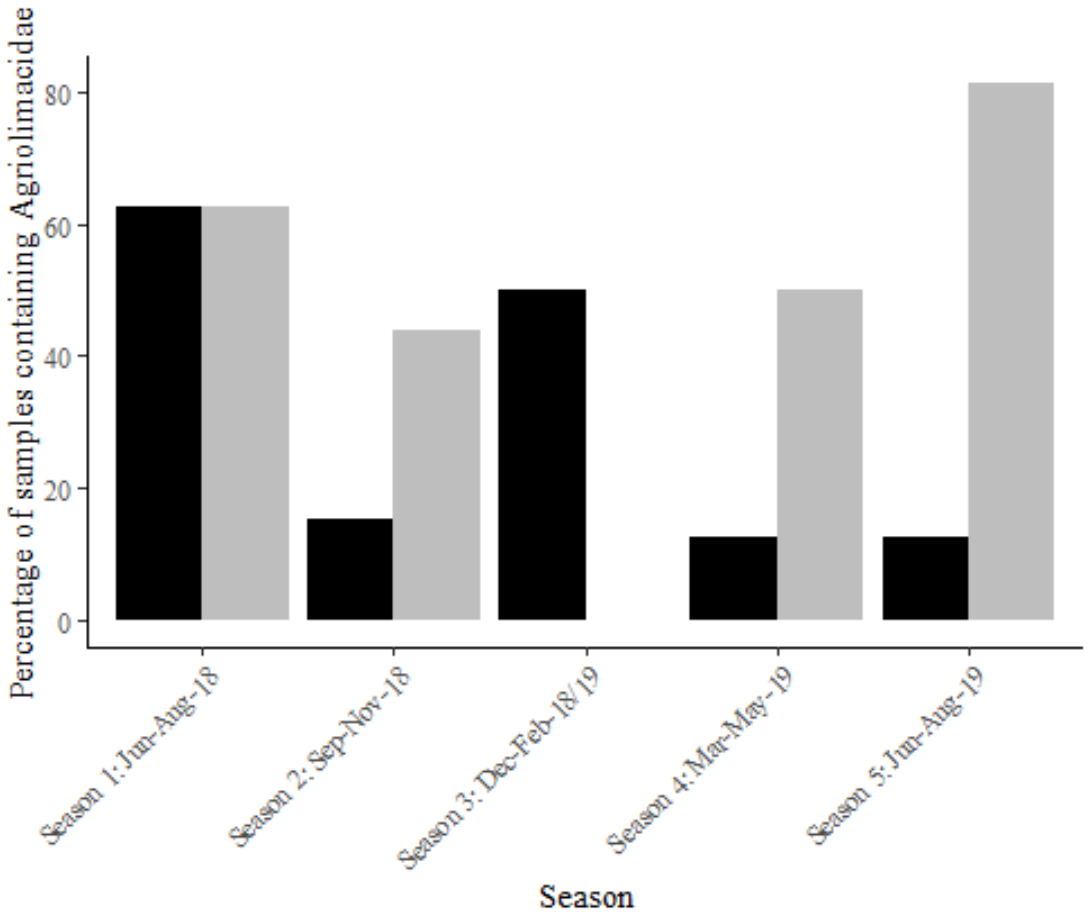
Food type	Species	Summer 2018	Autumn 2018	Winter 2018/19	Spring 2019	Summer 2019
Agriolimicidae	Hedgehog	62.5	43.8	NA	50.0	81.3
	Badger	62.5	15.4	50.0	12.5	12.5
Arionidae	Hedgehog	87.5	62.5	NA	56.3	75.0
	Badger	100.0	38.5	64.3	31.3	87.5
Carabidae	Hedgehog	93.8	81.3	NA	81.3	93.8
	Badger	87.5	15.4	50.0	56.3	68.8
Lumbricidae	Hedgehog	68.8	43.8	NA	93.8	62.5
	Badger	87.5	76.9	92.9	87.5	75.0
Poaceae	Hedgehog	7.8	4.7	NA	17.2	10.9
	Badger	10.0	6.3	8.8	8.8	7.5

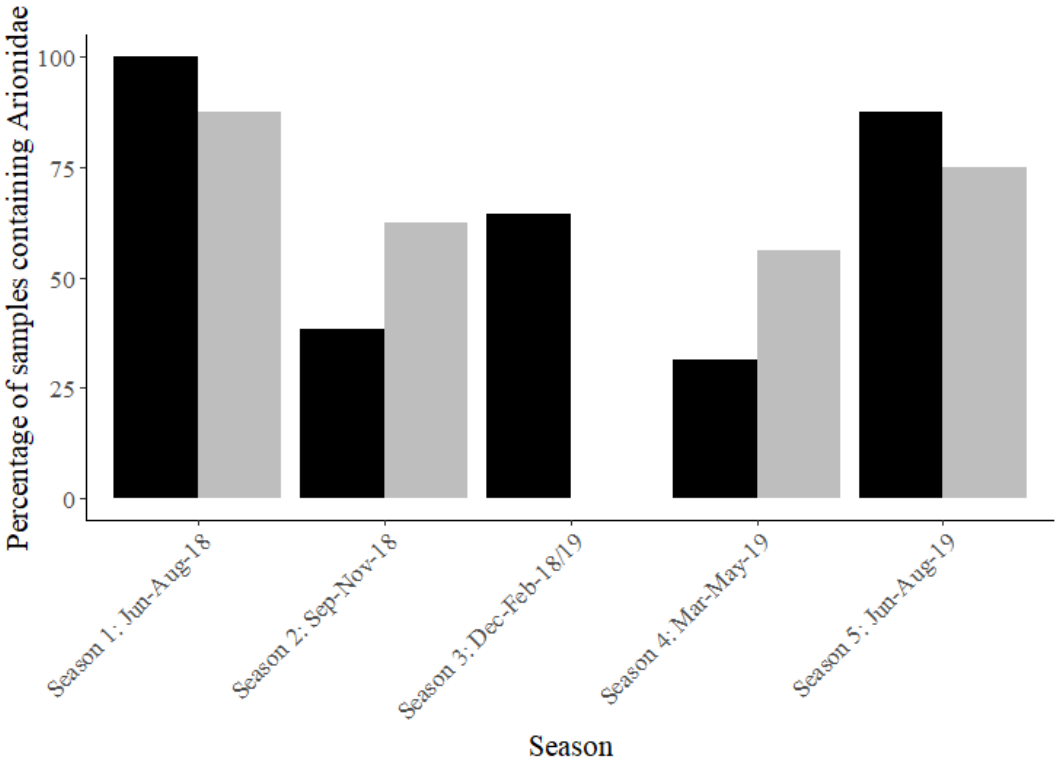
Carabidae were consistently found in a greater proportion of hedgehog samples than badger samples irrespective of season. Lumbricidae were found in higher proportions in badger samples, with the exception of spring 2019 (March – May), when its occurrence in hedgehog (93.8%) samples was greater than badgers (75.0%).

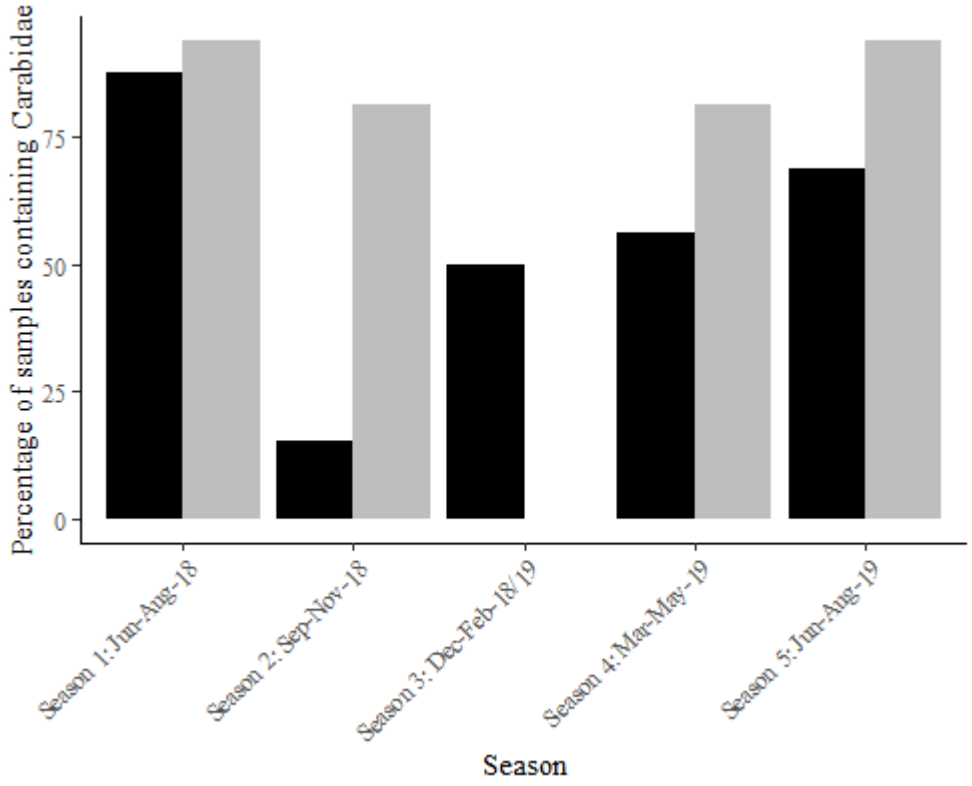
Arionidae were found in 12.5% more badger samples than hedgehog samples in both Summer 2018 and Summer 2019, demonstrating a consistent pattern between years. In Summer 2018, 62.5% of both badger and hedgehog samples contained Agriomicidae, whereas in summer 2019 these increased in hedgehog diet (81.25) and decreased in badger samples (12.5 %).

To visualise patterns in dietary composition amongst badgers and hedgehogs, NMDS plots were produced considering all dietary items (Figure 4.5). Samples were placed in the 2D ordination space, whereby distance along each axis relates to the level of

dissimilarity between the dietary composition of each sample. The higher proportions of badger samples containing Lumbricidae were important in driving the dissimilarity between the diet of both species in all seasons (Figure 4.5). Moreover, Arionidae was a driver of dissimilarity, in the hedgehog diet in Summer 2019, Autumn 2018 and Spring 2019. Between season, the level of dissimilarity changes, as does the overall composition of the diet. Niche dissimilarity was lowest in Autumn 2018, with fewer Families driving the dissociation between the two groups.




Percentage of samples containing Arionidae
100
75
50
25
0
Season 1: Jun-Aug-18
Season 2: Sep-Nov-18
Season 3: Dec-Feb-18/19
Season 4: Mar-May-19
Season 5: Jun-Aug-19
Season


Percentage of samples containing Carabidae
75
50
25
0
Season 1: Jun-Aug-18
Season 2: Sep-Nov-18
Season 3: Dec-Feb-18/19
Season 4: Mar-May-19
Season 5: Jun-Aug-19
Season

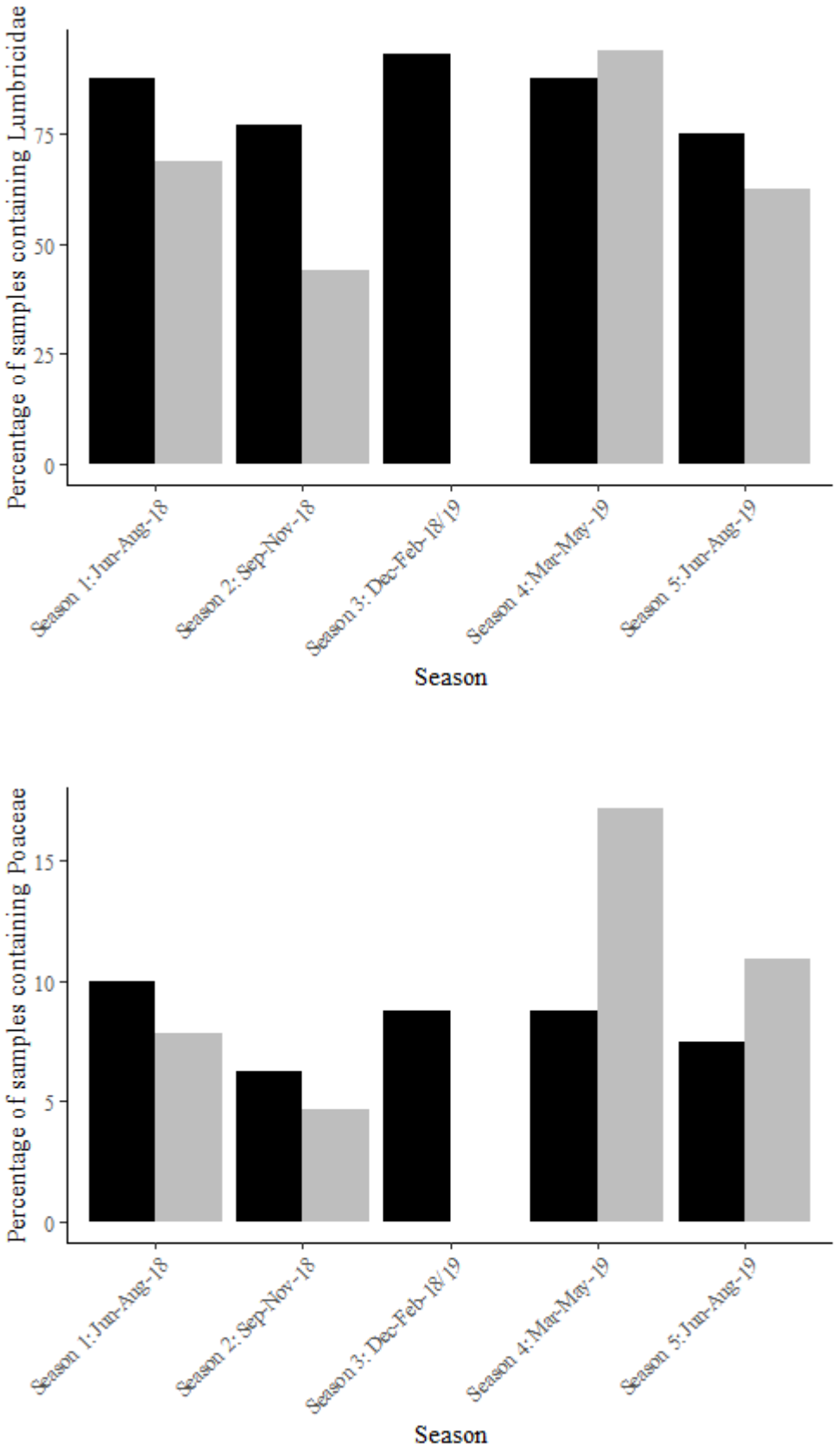
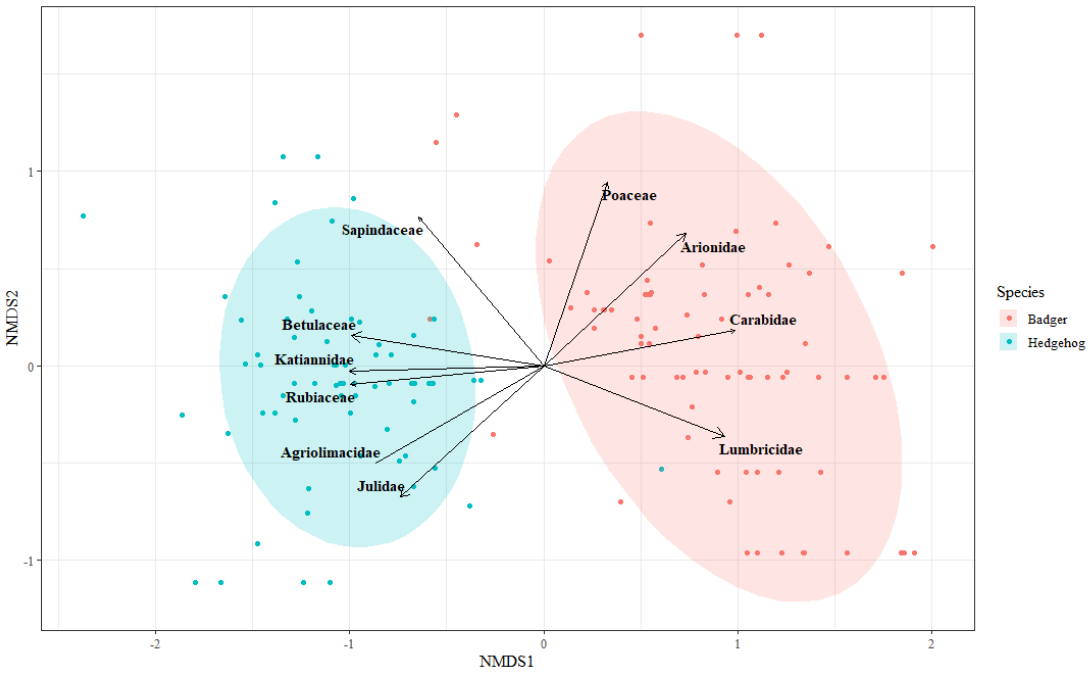
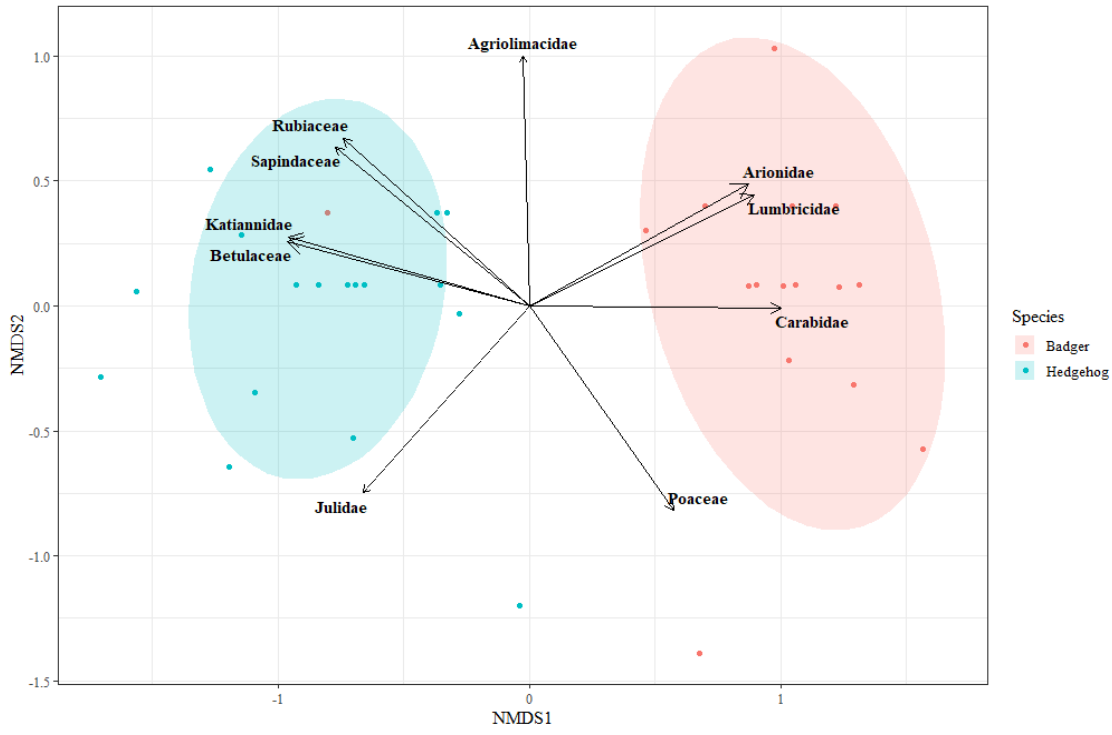


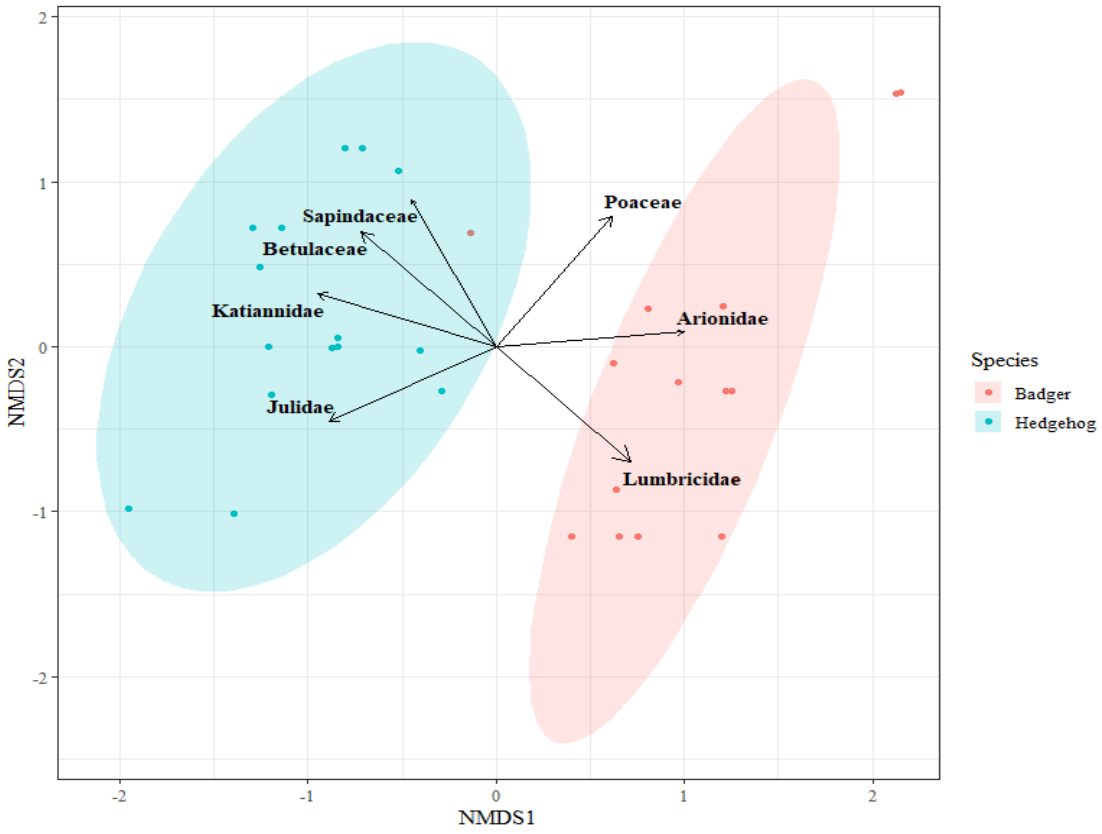
Figure 4.4 Frequency of occurrence of key food Families present in the scats of hedgehogs (grey bars) and badgers (black bars). Hedgehog scats were not collected in Season 3 as they are inactive during winter.



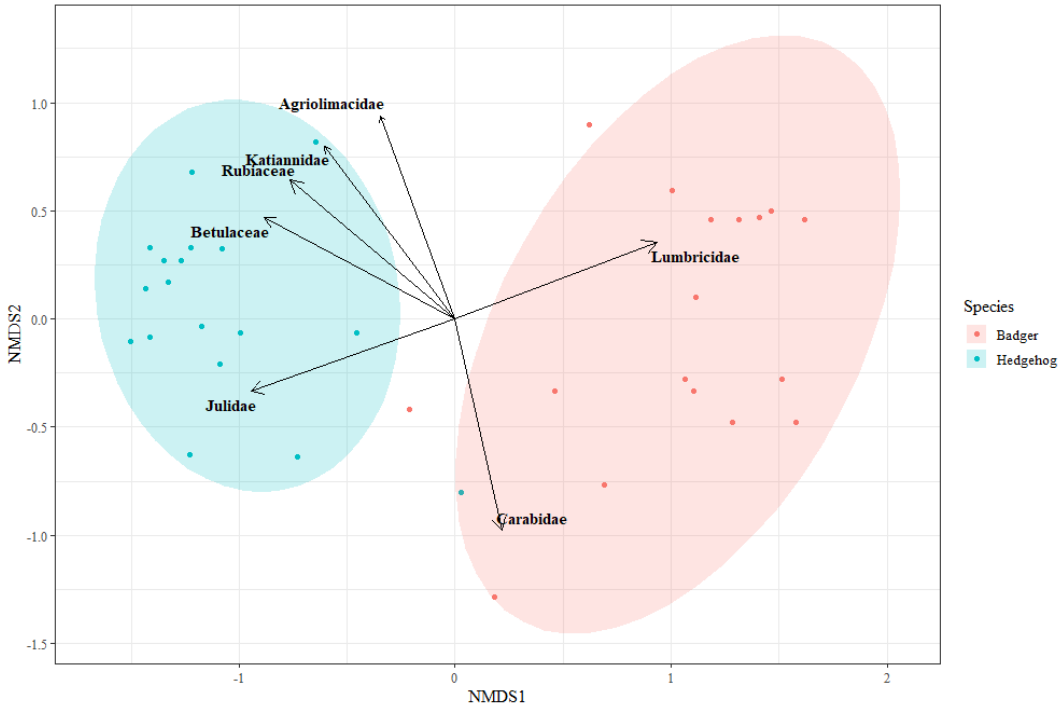
A) All scats from badgers and hedgehogs pooled, excluding scats sampled during Winter 2018/19.



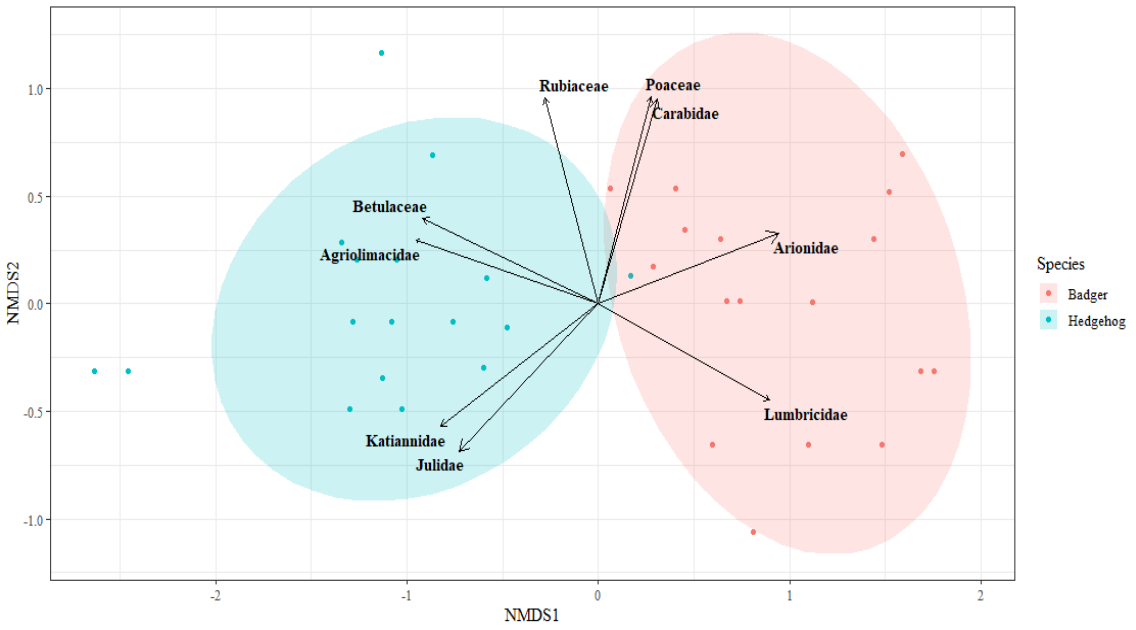
B) Summer 2018



C) Autumn 2018



D) Spring 2019



E) Summer 2019

Figure 4.5 Non-metric multidimensional scaling (NMDS) plots based on a Bray-Curtis distance matrix, to show the difference in Family composition of different food types of badger (red) and hedgehog (blue) scat samples, for Families identified in >5% samples. The unconstrained ordination plots show the compositional differences between both species' diets. Badger and hedgehog diet is shown across all sampling periods (Plot A), in Summer 2018 (Plot B), in Autumn 2018 (Plot C), in Spring 2019 (Plot D), and in Summer 2019 (Plot E). Arrows depict the prey Families that are significantly driving the disassociation between dietary composition of badgers and hedgehogs. Ellipse (shaded) show 95% confidence intervals.

4.3.6 Badger predation of hedgehogs

Hedgehog DNA was present in 1.3% (n=1) of badger samples that were analysed. The sample containing hedgehog DNA was collected between April – September 2018 at the Brackenhurst site.

4.4 Discussion

This study presents the first comparison of badger and hedgehog diet, using faecal samples from the same localities, in an attempt to understand the potential for competition for food between these intra-guild species. Although the diets of both species have been described in specific locations using traditional morphological methods (Yalden 1976; Shepherdson et al.1990; Hof et al.2012), a comparison of their diets at the same time and place, which is a pre-requisite for understanding how they may compete for food, has not yet been undertaken. High-throughput sequencing of scats in this study allowed precise identification of a diverse range of invertebrate, plant and vertebrate Families consumed by badgers and hedgehogs. The results are consistent with existing literature (Cleary et al. 2009; Shepherdson et al. 1990; Yalden 1976), which shows many shared food items, leading to assumptions of competition between the species (Wembridge 2011). However, the present study also shows that the composition of the diet of hedgehogs and badgers was sufficiently different to be consistent with dietary niche partitioning between the species within local areas. In addition, several Families were found to be unique to either hedgehog or badger diets, suggesting some specificity and significant differences in diet composition between the species. Hedgehog DNA was only extracted from one badger scat out of 80, suggesting infrequent predation. This comparative DNA metabarcoding approach has provided a novel, in-depth assessment of potential competitive interactions between these two species and, through identification of niche partitioning, and limited direct predation, increases our understanding of the conditions of their coexistence.

4.4.1 Species Richness

Hedgehog and badger diets, as measured across two rural sites over five consecutive seasons, showed that both species have broad generalist diets, comprising largely of invertebrates and supplemented by vertebrates and likely incidental ingestion of plants,

similar to previous studies (Reeve 1994; Neal and Cheeseman 1996). Overall, species richness of taxa (to Family level) consumed by badgers was 12.5% and 58.3% higher than that of hedgehogs at Brackenhurst and Hartpury respectively. Both species showed high diversity of taxa in their diet (as measured by the Levin's Index) with the diversity of food items taken by badgers being only slightly greater than that of hedgehogs, supporting their generalist dietary niche (Hof et al. 2019). The diversity of food items taken by hedgehogs was 31.4% higher at Brackenhurst than Hartpury, whereas the diversity of badger diet was 7.1% greater at Hartpury in comparison with badger diet at Brackenhurst. Levin's index ranged from 26.21 – 28.36 for badgers, much greater than that observed in other taxa, such as the genus *Martes*, where diversity only ranges between 2.07 and 3.96 (Zhou et al. 2011). Collectively, hedgehog and badger diets contained 94 Families (see Appendix E) of which 35 were common food types (>10% faecal samples) to either one or both species. Hedgehog diet contained 29 common food types of which 25 were also present in badger diet, suggesting these species have access to many of the same food resources. Many of the food items found in only one of the two species' diet were relatively rare, whereas the more common prey occurred in the diets of both.

The most common food types identified in hedgehog and badger diets in this study are broadly consistent with the findings of previous studies. For hedgehogs, Carabidae (ground beetles) was the most common Family, identified in 89% of samples taken from the two sites. This is greater than the 60% of stomach samples containing Carabidae identified by Yalden (1976), but agrees broadly with the work of Pettett (2015) which identified Carabidae in 100% of hedgehog fecal samples (n = 57). Pettett's (2015) study used similar methods to those used in the present study, possibly accounting for the difference with Yalden's (1976), however differences in food availability between sampling different sites and times may also account for the variation shown. What all three studies highlight, is a high frequency of occurrence of Carabidae within hedgehog diets.

Similarly for hedgehog, Lumbricidae (earthworms) were found in 69% of samples, twice as common as the 34% of samples Yalden (1976) reported, though in accord with Pettett (2015). Interestingly, the present study identified slugs and snails in a much higher percentage of hedgehog samples (Arionidae in 71% and Agriolimacidae in 60%) than recorded by Pettett (2015) in which only 4% of samples contained either Arionidae or Agriolimacidae.

For badger scats, Lumbricidae was the most common prey taxa identified, present in 87% of samples, followed by Arionidae (land slugs), found in 67% of samples. The ubiquity of Lumbricidae in the badgers diet is well-reported (eg. Shepherdson et al. 1990; Zabala and Zuberogoitia, 2003a; Cleary et al. 2011), emphasising earthworms as a key prey taxon for badgers. However, slugs have not previously been shown to be a key prey type of badgers, with Cleary et al. (2011) identifying slugs in just 0.7% of gut and faecal samples (n=281) from badgers in Ireland. Moreover, slugs accounted for 28.5% of RRA in this study, which is considerably greater than the bulk volume of slugs found in badger stomach (0.14%) and faecal samples (0.01%) through morphological analysis by Cleary (2011). This suggests that slugs may previously have been underrepresented in the diet of badgers in comparison to earthworms, and this is likely to be because of identification issues caused by the differential digestibility of prey (Strauss 1979). After digestion, there may be no visually distinguishable remains of slugs, unlike earthworms that can be identified by the presence of undigested chaetae (Wroot 1984; Battisti et al. 2019).

The main prey types that were consumed frequently by both badgers and hedgehogs are also abundant and widespread in rural habitats (Chapter 3; Hof et al.2012), and therefore are likely the most readily available prey too. Interestingly, vertebrate prey were taken more frequently by badgers than hedgehogs, which may reflect the ability of the former to handle larger prey. The ability of badgers to predate other mammals may reduce competition for food with hedgehogs further, although this was not identified as a

main driver of dissimilarity in diet. The thirteen Families of taxa found exclusively in badger samples included several arthropods and mammals. Leporidae (rabbits and hares) DNA was found in 11.0% of badger scats which may indicate a higher level of consumption compared to the lower frequencies of mammals (0.36%, n = 281) reported in the diet of badgers before (Cleary et al. 2011). DNA from several other mammal and bird species was found in low numbers of samples in the present study (Appendix E), including Felidae (cats) and Canidae (fox and dogs) in 1.3% and 5.0% of samples respectively, and is likely to represent environmental contamination or incidental consumption of cat or dog DNA whilst badgers foraged.

The presence of Bovidae (cows and sheep) DNA in hedgehog (10.9%) and badger (17.5%) scats suggests either environmental contamination, from soil (Buesching et al. 2016), incidental consumption of other animals faeces (Mychek-Londer et al. 2020), or perhaps food provision by humans (e.g. pet food). Suidae (pigs) DNA was also found, and their absence in the vicinity of either study site most likely indicates the consumption of pet food. Nevertheless, possible supplementary feeding in the present study was relatively low in comparison with hedgehog scats analysed from rural villages, in which sources of pet food (Bovidae and Suidae) were identified in 93% and 89% of hedgehog scats respectively (Pettett 2015).

FOO data showed Muridae (rodents), Soricidae (shrews) and Talpidae (moles/shrews) DNA was present in low numbers of badger samples and therefore are unlikely to represent very important prey items. Similarly, Erinaceidae (hedgehog DNA) was found in only 1.3% of badger samples, indicating one potential predation or consumption event. These prey items are likely consumed opportunistically and may represent either scavenging or predation events.

The hedgehog's diet is mainly comprised of invertebrate prey (Wroot 1984; Yalden 1976), with ten Families of prey taxa found only in hedgehog scats, eight of which were

arthropods, one belonged to Annelida and the last was a mollusc. There are, however, distinct morphological differences between some of these Families such as Lumacidae, a Family of soft-bodied large slugs, and Forficulidae, a Family that includes earwigs. Hedgehog samples contained relatively low proportions of mammal prey species in comparison with badgers.

Determining why a species consumes one prey and not another could require extensive dietary assessment, taking into account factors such as size, locomotion, palatability, and digestibility of prey. However, one potentially important factor could be the encounter rate of different prey, attributed to the habitats used by badgers and hedgehogs, and this is investigated in Chapter 5. Poaceae (grasses) were the most common plant Family identified in scats, being found in 40.6% and 47.5% of hedgehog and badger samples, respectively. RRA analysis also suggests that badgers consumed greater quantities of this food type. Unlike other studies that have demonstrated the presence of fruits, vegetables, crops and berries in relatively high proportions in badger diet (Shepherdson et al. 1990), the present study did not find them to contribute significantly to the diet, irrespective of season. This could, however, reflect the local availability of food resources at the particular sites surveyed. Alternatively, grasses may have been incidentally consumed by either species, either whilst foraging in grassland habitat or through secondary predation of other prey.

4.4.2 Dietary Overlap

Dietary niche overlap between badgers and hedgehogs, measured using Pianka's index, was 0.73 across the five seasons surveyed, demonstrating that both species have, at some point, consumed many of the same plant and animal Families. This finding has not been demonstrated previously. Plateauing of prey accumulation curves demonstrates that the sampling effort employed in the present study was sufficient to capture most of the

Families present in badger and hedgehog diets. Therefore, the high level of overlap observed is likely to reflect the availability of similar resources within the home ranges of both species. Badgers and hedgehogs both currently occupy similar habitats in the UK rural landscape, both for example utilising grassland for foraging (Parrott et al. 2014). At the local scale, there is much variation in food availability between different habitats and land uses (see chapter 3; Poschlod et al. 2005) and in Chapter 5 this is investigated in relation to the diet of both species.

Despite hedgehogs and badgers consuming many of the same food types, their frequency of occurrence in their respective diets differed, suggesting dietary niche partitioning between the species. Each Family represented different proportions of the diet in each sample, with differences between badger and hedgehog diets accounting for 38% of the variance in dietary composition. Hence, hedgehogs consumed beetles more frequently than badgers, and badgers consumed earthworms more frequently than hedgehogs. RRA also suggested that slugs were proportionately more important to badgers and were a major driver of the dissimilarity between badger and hedgehog diet.

Previous studies have defined badgers and hedgehogs as broad generalists (Hof and Bright 2010; Roper 1994) that share invertebrate prey (Trewby et al. 2014). The results from the current study suggest that niche partitioning exists between the two species, which is evident across all seasons, and this likely acts to reduce the intensity of competition for prey resources.

Seasonally, the dissimilarity between species varied too, which is likely to reflect seasonal variability in food availability (Saska et al. 2013). NMDS plots indicated that the composition of plant and animal Families identified in badger and hedgehog scats was dissimilar in all seasons, suggesting dietary niche partitioning between the species throughout the year. December to February was omitted from analysis as this reflects the period when hedgehogs are largely inactive due to hibernation (Morris 1973). The

magnitude of dietary divergence was greatest between the species from September to November, when fewer Families of food items drove the dissimilarity, suggesting a narrower diet during this period when hedgehogs are putting on fat for hibernation (South et al. 2020) and badgers for torpor (Woodroffe 1995). This fits well with Byrne et al's (2012) review that documents the breadth of Irish badger diets being greatest in the summer and spring. Although specific compositional data varies between studies that have been undertaken across Europe, there is uniformity in finding strong seasonal variation in badger diet (Byrne et al. 2012). Hedgehogs also exhibit seasonal dietary differences (Wroot 1984) as identified in the present study which also indicated that despite this variation their diet remained dissimilar to that of badgers in all seasons, with beetles, worms and slugs being significant drivers of those differences (figure 4.6).

The pattern of resource use for badgers and hedgehogs, as assessed by the FOO of plant and animal Families in scats, was relatively consistent for Carabidae, Lumbricidae and Arionidae in June – August of 2018 and 2019. This suggests that there may be regular seasonal fluctuations in the availability of some food items available to both species. However, Agriomicidae was found in fewer badger samples in Summer 2019 than in the previous year, demonstrating the ability of these generalists to alter their diet in relation to resource availability, which may also affect the overall level of competition between badgers and hedgehogs. However, these patterns should be interpreted cautiously due to the relatively low sample number that was assessed for each season and species.

4.4.3 Relative Read Abundance

Although the breadth of hedgehog and badger diets may reduce opportunities for competition, the intensity of competition for their common prey groups may still remain high (Holbrook and Schmitt 1989). The RRA data provides a measure of how often the DNA of a particular Family was found in individual hedgehog or badger scats, giving an

indication of the volume consumed of each food type. For example, previous work analysing 490 badger scats found that despite identifying 9 food types, only fruits and invertebrates accounted for 89% of the biomass of the diet (Rosalino et al. 2005). In the case of RRA, we assume that sequence reads reflect the amounts of different food items consumed, which is notably caveated as shorter DNA fragments are preferentially sequenced (De Barba et al. 2014; Deagle et al. 2019) though evidence is growing that shows it does indeed reflect biomass (Smith et al. 2018; Young et al. 2020; Browett et al. 2021).

RRA findings were in agreement with those from the FOO analysis, with both approaches highlighting the importance in the diet of hedgehogs of the Carabidae, Arionidae and Lumbricidae. A comparison of RRA showed that, proportionally, beetles were more important for hedgehogs (45.2%) than badgers (12.6%). However, whilst FOO showed Arionidae was detected in only 18% fewer hedgehog samples than Carabidae, it was represented by 37% fewer reads. This demonstrates how FOO and RRA can be used to complement one another, showing the importance of specific food groups that are both frequently detected in the diet and likely consumed in higher proportions than others.

4.4.4 Evidence for Predation

Badgers are considered to be the main predator of hedgehogs in the UK, with recorded rates of predation of 18% following a 75 day tracking period of 44 hedgehogs (Hof and Bright 2010). The avoidance of badgers by hedgehogs was shown by Doncaster (1992), who compared two sites and showed that hedgehog mortality and dispersal was greater in the area with higher badger density, despite offering similar availability of suitable habitat for hedgehogs. Furthermore, Trewby et al. (2014) showed that a decrease in badger density was followed by an increase in hedgehog density, and speculated that a reduction in predation was possibly the cause. However, the impact of predation on hedgehog

population sizes is unknown and would require birth rate, other sources of mortality and population sizes to be accounted for. It also requires an assessment of whether badger predation is likely to be additive to other forms of mortality, or compensatory, i.e., whether badgers predate on animals that would otherwise die from other causes.

In the present study dietary assessment allowed potential predation events to be identified and quantified for the two rural sites surveyed. To enable the confident identification of IGP events, hedgehog and badger samples were processed separately in the laboratory. No badger DNA was found in any hedgehog samples, nor negative controls. Similarly, no hedgehog DNA was found in negative controls for badgers. However, hedgehog DNA was present in 1.3% (n=1) of badger samples that were analysed. Although there is high confidence that this represents consumption of hedgehog tissue by a badger, it is not possible to ascertain whether this represents predation, scavenging, incidental consumption of faeces, or environmental contamination.

Much higher rates of predation than that suggested by the present study have been reported previously. For example, 7 out of 24 hedgehogs (Doncaster (1994) and 8 out of 44 hedgehogs (Hof and Bright (2010). However, these reflect local environmental conditions as Doncaster (1994) released hedgehogs into unfamiliar territory, and these animals may have been more vulnerable to predation. In addition, Hof and Bright (2010) assessed hedgehogs across two large arable farms, whereas the present study assessed badgers and hedgehogs occupying mixed farmland habitat. However, it is also possible that predation may have been underestimated in the current study, as the likelihood of detecting predation events will depend on how long hedgehog DNA persists in badger scats after consumption and the frequency that samples were collected.

Badgers are known to be opportunistic exploiters of food resources (Delahay et al. 2001). The infrequent detection of hedgehogs in the present study may simply suggest that they are an opportunistic prey item. Indeed, hedgehog DNA was only present in one

badger scat collected from the Brackenhurst site in June – November 2018. During this period, the mean UK summer temperature was the highest on record since 1884 (Kendon et al. 2019; Petch et al. 2020), perhaps presenting harsher than usual foraging conditions for badgers and hedgehogs, making some food types such as earthworms inaccessible due to very dry soils, which may have caused badgers to switch to other food types including hedgehogs. The results of the present study suggest that incidences of hedgehog predation by badgers may be rare at these two study sites and highlight that hedgehogs do not make a major contribution to badger diet. However, a larger sample size would be required to ascertain the broader significance of badger predation on hedgehogs and their contribution to badger diet more generally.

4.4.5 Limitations

Identification of food items in the present study was limited to Family level in order to maximise the amount of sequence data that could be accurately taxonomically assigned and included in the analysis. Ideally, dietary items would be identified to species level, providing a more detailed assessment of the food types consumed by badgers and hedgehogs. It is possible that badgers and hedgehogs may differentially select species within the same Family, and this may reveal further niche partitioning than identified in this study.

Furthermore, it is not possible to infer whether the presence of DNA in the diet represents scavenging, predation, incidental predation, or environmental contamination when using molecular methods such as DNA metabarcoding in this study. Similarly, it was not possible to make the distinction between pheasant ‘meat’ and pheasant ‘eggs’ using these molecular methods. Whereas classical morphological scat analysis would indicate the presence of eggshell which might allow discrimination.

4.4.6 Further Research

There is evidence that sex differentiation in diet exists between male and female hedgehogs in New Zealand (Jones et al. 2015) and that juvenile and adult hedgehogs take different prey (Dickman 1988). Similarly, individual badgers within the same social group can specialise on certain prey items (Robertson et al. 2014). Sex biases in the diet may identify further niche partitioning, and individual food preferences within each species. Thus, the identification of sex-linked primers, specific to hedgehogs, would be useful to analyse non-invasive DNA samples, whether it be for dietary or genetic studies. Findings from sexing badger scats do, however, demonstrate the limitations of using degraded DNA samples (Fernando et al. 2003), as successful sexing of badger scats was just 37.5%, much lower than that achieved using DNA from genomic tissue samples.

4.4.7 Conclusions

The present study has shown that, in localities where badgers and hedgehogs co-occurred, they consumed many similar plant and animal Families, although the relative contributions of these to the diet of each species was sufficiently different to reduce competition. Dissimilarity in diet composition was evident across all seasons, revealing that niche partitioning exists at a site scale. However, it is not clear if this pattern is due to the species foraging on different prey and frequencies, or encountering prey and frequencies based on differential space use at each site. Therefore, the dissimilarity in diet may be driven by hedgehogs either selecting different foraging areas or being forced into them by badgers, which is discussed in Chapter 6.

Both RRA and FOO analysis highlighted the commonness of beetles and slugs in the diet of hedgehogs, and of earthworms in the diet of badgers. Despite this, badgers and hedgehogs have the ability to take a wide range of prey and this likely helps promote the coexistence of the two species. Moreover, it is important to incorporate a measure of local

food availability at the sites where scats are sampled, to establish whether these prey choices reflect availability (see Chapter 5). Food availability is likely to vary between habitats and should be measured to assess whether hedgehogs are using optimal habitats for foraging, or whether badger presence, or other factors, can explain hedgehog habitat use (see Chapter 6). Furthermore, this study looked at two sites, with a range of habitats including rural and mixed farming with some urban infrastructure. Niche partitioning was observed at these sites, although whether these patterns remain in other habitats and sites requires further investigation. Similarly, how competition between these species may vary in different habitats or in response to lower food availability remains to be seen. Future studies should attempt to measure diet changes in hedgehogs and badgers in response to changes in density or, in areas with and without badgers, to better untangle the influence of badgers on hedgehog diet.

5.1 Introduction

For a species to occupy and persist within the local environment, it must be able to efficiently exploit available food resources (Emlen, 1966). The conceptualisation of Optimal Foraging Theory (hereafter OFT) described the relationship between maximum net energy intake (calories) per unit of time spent foraging (Emlen, 1966; MacArthur and Pianka, 1966), such that individuals who make optimal decisions and maximise their net energy intake will survive long enough to pass on their genes to the next generation. Therefore, in conditions where food is limited and uncertain, animals spend more time foraging, often over larger patches, as their motivation to forage and build fat reserves increases (Anselme et al. 2017). Dietary niche breadth also alters in response to variability in the availability of food. It is predicted that a more productive environment decreases niche breadth (MacArthur and Pianka, 1966) as the predator can be more selective, increasing the degree of dietary specialism. For example, the great tit (*Parus major*) selected profitable prey when it was abundant, ignoring less profitable prey, irrespective of the rate at which it encountered it (Krebs et al. 1977).

Dietary selection, defined as the consumption of food in relation to food availability (Gillis et al. 2020), is an important mechanism for studying ecological networks, namely predation and competition (Amundsen et al. 1996). Not all fauna and flora within the local environment represent potential food species, some may be inaccessible due to the size and feeding posture of a species, whilst other food items may be recognised as being unpalatable due to toxicity or physical defence mechanisms such as thorns (Westoby, 1974). Similarly, species do not utlise all available foraging habitat which can affect the encounter rate and abundance of different prey types (Gillis et al. 2020). A criticism of OFT is it assumes all prey are encountered at the same frequency and does not differentiate between stationary (plant) and mobile (animal) prey (Sih and Christensen 2001). Mobility can effect the ability of a predator to capture potential prey

and therefore can affect the likelihood of prey acquisiton between different predator species (Sih and Christensen, 2001).

Several indices have been developed to study the electivity of prey (Lechowicz, 1982), assessing whether particular prey items are consumed at random, in proportion to their avaialiblity, or selected for, either postively or negatively (Jacobs, 1974). Molecular methods are increasingly being utilised for dietary assessment from stomach or faecal samples, to demonstrate the frequency of dietary items in relation to their availability in the landscape (Hayward et al. 2017). For example, Kowalczyk et al. (2011) demonstrated that the most frequently consumed tree species (*Carpinus/Corylus)* by European bison *(Bison bonasus)* was also highly represented in the forest understory, indicating minimal negative impact on tree diversity. This highlights that dietary patterns can be observed, allowing inferences to be made about the relative importance of particular food types for species that could not be ascertained from diet assessment alone.

Badger and hedgehog are dietary generalists, both omnivores that consume a broad range of prey (Morris, 2006; Neal and Cheeseman, 1996). However, the consumption of different prey likely varies in response to local availability (Serbent et al. 2011) and accessibility (Westoby, 1974). The dynamic balance of competition and predation within the IGP relationship shared by badger and hedgehog can be further understood by assessing dietary preferences in relation to prey availability. Wroot (1984) assessed the selection of invertebrate prey by hedgehog from the environment, though no comparative study exists for comparing prey selection of both badgers and hedgehogs from areas where they co-occur. The results from Chapter 4 provide the first comparative dietary study, identifying the level of dietary niche overlap between badgers and hedgehogs from the same localities, which showed similar and wide niche breadth between the two species, though badger diet was more diverse overall. Both species exhibited a broad dietary niche which may indicate poor food resource availability for these two generalists across the two

study sites. Composition analysis revealed dissimilarity between the two species, occupying the same local environment. This indicates that they utilise available resources differently to one another. Further dietary selection assessment could potentially provide a better understanding of whether each species under or over utilises each prey species in relation to its availability, and whether the dietary dissimilarity shown in chapter 4 is due to preferential feeding habits of badgers and hedgehogs.

This study aimed to assess the patterns of resource use of badger and hedgehog on invertebrate prey. The specific research questions were to: (1) assess the effect of seasonality and site on prey availability, (2) investigate whether badger and hedgehog utilise invertebrate prey in relation to its availability, (3) identify any potential prey preferences of either species, (4) compare selectivity of prey between badger and hedgehog and (5) assess whether there are seasonal prey preferences driven by resource availability. Under IGP theory, badger would be expected to be the dominant forager for certain prey types, potentially excluding hedgehog from these food resources. This study will deepen our understanding of how co-occurring badger and hedgehog utilise locally available resources and its implications for competition between these two IGP competitors.

5.2 Methodology

5.2.1 Study sites

To investigate patterns of resource use by badger and hedgehog, diet and prey availability were studied across two sites, Brackenhurst Campus and Hartpury College Estate (see Chapter 2) across 4 seasons, giving 8 comparisons between diet and invertebrate biomass as assessed by pitfall traps. The diet was analysed molecularly, utilising DNA metabarcoding of faecal samples (Chapter 4) that were collected throughout April 2018 – August 2019. The four seasons used for comparison were as follows, Summer 2018 (June – August 2018), Autumn 2018 (September – November 2018), Spring 2019 (March – May

2019) and Summer 2019 (June – August 2019). The period between December – February was omitted from electivity analysis to reflect the inactivity of hedgehogs during this period. Complementary invertebrate sampling (Chapter 2) was conducted during each quarterly period to give a measure of seasonal invertebrate food availability.

5.2.2 Seasonal invertebrate food availability

Ideally, a measure of food availability would include all possible food types and, in this study, would include vertebrate, invertebrate and plant species. Suitable survey methods for estimating the availability of these diverse taxa are not comparable with one another and therefore this assessment of food availability considers exclusively invertebrate prey using pitfall trapping. Dietary assessment (Chapter 4) demonstrated that this prey group is proportionally greater than other food types and therefore the most likely prey that badger and hedgehog compete over (Kruuk and Parish 1985; Wroot 1984; Roper 1994).

Pitfall traps were used to assess the availability of ground dwelling invertebrates as this method has been shown to capture higher species richness (94%) than other survey methods such as visual searching (41%) or sweep netting (25%) (Hancock and Legg 2012). Despite this, there is criticism for its use in quantitative studies due to the bias towards species more active on the soil's surface, essentially providing an index of activity rather than abundance (Sabu and Shiju 2010). Nonetheless, invertebrates that are more active are also more likely to be encountered by their predators (O'Donnell 2000), and therefore activity also reflects what food is available. Here, the measure of abundance and biomass calculated from this method is coarse and is used in this study to assess the resource availability of key ground dwelling invertebrate prey types shared by badgers and hedgehogs.

Seasonal variation of invertebrate prey was assessed quarterly at two study sites (Brackenhurst and Hartpury – see Chapter 4). Pitfall traps were sampled as described in

Chapter 2, section 2.6.1.1. In total 247 pitfall traps were set across both campuses and in proportion to habitat availability. Pitfall captures, submerged in the preservative propylene glycol, were removed from the field and dried to obtain measures of dry biomass (g), (Chapter 2.6.1.2). Pitfall trap captures were sealed and stored in a cool place until processing took place, typically within 6 weeks of surveying. Macro-invertebrates that measured > 5 mm in length were identified to the Order level and abundance per trap was recorded. Order level identification was carried out as this accounts for differences in functional traits of prey such as mobility, that may influence the likelihood of being potential prey to either badger or hedgehog (Kennedy et al. 2019). Following identification, dry biomass was calculated for each Order within each individual pitfall trap, giving a second measure of the invertebrate communities present in the environment.

To assess prey availability, potential variation in invertebrate communities between habitat and season should be accounted for. Therefore, seasonal differences in abundance of invertebrates (>5mm) and biomass of invertebrates between habitat types and months (Table 5.1) was investigated through a series of Generalised Linear Mixed Models (GLMMs), where both interactions and main effects were tested and site was included as a random factor in all models (Ostfeld et al. 2018; Hothorn et al. 2008). Data processing and formatting were performed in Microsoft Excel (Microsoft Corporation 2018) and all figures and statistical analysis was conducted using R Studio version 1.2.5 (R Studio Team 2020).

Table 5.1 List of variables included in Generalised Linear Mixed Models of invertebrate assemblages.

Variable	Distribution and (link function)	Type	Variable	Description
Abundance	Negative Binomial	Count	Response	Number of organisms > 5mm in a pitfall capture
Biomass	Gamma (log)	Continuous	Response	Dry biomass (g) of pitfall capture
Habitat	N/A	Categorical	Predictor	Habitat 1 = Amenity, 2 = Arable, 3 = Grassland and 4 = Woodland
Season	N/A	Categorical	Predictor	Season: 1 = Summer 2018, 2 = Autumn 2018, 3 = Winter 2019, 4 = Spring 2019, 5 = Summer 2019
Site	N/A	Categorical	Random factor	Sites 1 and 2, Brackenhurst and Hartpury respectively (*see Chapter 2 for site description).*

5.2.3 Diet of hedgehog and badger

In total 144 faecal samples, of which 80 were sampled from badger scats and 64 from hedgehog scats, were analysed using two primer sets which identified invertebrate taxa in the diet (see Molecular methodology Chapter 2). Of the 80 badger samples, 16 were not included in the assessment of diet selection, reflecting the period between December 2018 to February 2019 that had been omitted for hedgehog, due to their hibernation activity in this season. After sequencing, taxonomic identification and removal of potential contamination, data was converted into a binary format, allowing the presence/absence of each prey group (Order level) within each individual sample to be shown. For each species Frequency of occurrence (FOO) of each dietary item was calculated, that is the number of scat samples that contain each prey type expressed as a percentage (% FOO) (Deagle et al. 2019). FOO was further rescaled to give the Percent of occurrence (POO), so that the sum of all food groups was 100% (Deagle et al. 2019).

To assess diet selection, POO was calculated again, using only invertebrate prey, therefore excluding other prey types. This allowed invertebrate prey availability within the

environment and invertebrate prey in the diet to be compared using Ivlev's electivity index (section 5.2.5).

5.2.4 Availability of invertebrate prey

Invertebrate data measuring the biomass of organisms collected from pitfall trapping was collated from the five sampling occasions throughout April 2018 – August 2019. Survey effort was consistent in each broad habitat (Arable, Amenity, Grassland and Woodland) across study sites (see Chapter 2). Biomass data were collated from both sites and POO was calculated to show the proportion of each invertebrate prey group (Order level), present in the environment.

5.2.5 Electivity Index

The selection of invertebrate prey, measured by POO (McLachlan-Troup et al. 2010), by badger and hedgehog was calculated using Ivlev's electivity index, D (Jacobs, 1974).

$$D = (r - p)/(r + p - 2pr)$$

Where *r* is the proportion of samples containing a given prey group and *p* is the proportion of a given prey group available in the environment as measured by pitfall traps. *D* ranges from -1 to +1, indicating extreme negative and positive selection respectively, with values around zero indicating similar selection of prey to the relative proportion of that prey type in the environment. As the foraging activity of each species within different habitat was unknown, Ivlev's index was calculated at the site level, with a unique value calculated per season per site.

5.3 Results

In total, 247 pitfall traps were successfully recovered from seasonal field sampling at Brackenhurst and Hartpury, resulting in, on average, 25 pitfall traps per season, per site.

5.3.1 Seasonal invertebrate community variation

A Gamma GLMM was fitted to the data to investigate seasonal differences in invertebrate biomass of pitfall trap captures sampled from two UK rural sites (Brackenhurst and Hartpury). The marginal and conditional R^2 values for the GLMM were 0.37 and 0.42, respectively. There was a significant interaction between habitat and season (Table 5.3) with significant variability in the biomass across habitats throughout the sampling period, which explains 37% of the variance within the dataset. Site, as the random factor, accounted for 5% of the variance within the dataset, demonstrating that invertebrate biomass varied most greatly at the habitat level between the seasons surveyed. Invertebrate biomass was significantly higher in woodland habitat during Autumn 2018 than in woodland across other seasons. Biomass was also significantly greater in Grassland and Arable habitat during Winter 2018/19 than in these habitats across other seasons. In Spring 2019, invertebrate biomass was significantly higher in Grassland and Woodland habitat than comparison with other seasons.

Table 5.2 Summary of Gamma Generalised Linear Mixed Model (GLMM) for the effect of season and habitat on invertebrate biomass of pitfall trap captures sampled from two rural sites in the UK. Site was included as a random factor with standard deviation of 0.23. Nobs = 247. Bold P values indicate statistical significance at 0.05 level. SE =Standard Error.

Model Parameter	Estimate	SE	P-value
(Intercept)	-0.59	0.31	0.06
Arable:Autumn2018	0.85	0.57	0.14
Grassland:Autumn2018	0.98	0.53	0.06
Woodland:Autumn2018	1.77	0.55	**< 0.001**
Arable:Winter2018/19	1.55	0.58	**0.01**
Grassland:Winter2018/19	1.67	0.56	**< 0.001**
Woodland:Winter2018/19	-0.33	0.56	0.56
Arable:Spring2019	0.34	0.60	0.57
Grassland:Spring2019	1.40	0.59	**0.02**
Woodland:Spring2019	1.49	0.56	**0.01**
Arable:Summer2019	0.10	0.50	0.84
Grassland:Summer2019	0.26	0.56	0.65
Woodland:Summer2019	0.78	0.51	0.13

5.3.2 Diet composition of prey groups

A total of 64 hedgehog scat and 64 badger scats sampled evenly across four seasons and two sites, provided complementary dietary data. A total of 97 prey types were identified in the diet of badger and hedgehog together, of which POO showed that invertebrate prey were proportionally the greatest dietary component for both species (Figure 5.1).

Broad prey groups were found in similar proportions of scat samples of badger and hedgehog, though invertebrate prey were taken more frequently by hedgehog than badger. Invertebrate prey typically constituted 73.8% of prey items in hedgehog scats, 10% greater than in badger diet (63%). The second most common prey group was plant species, representing 18.9% of prey items found in hedgehog scats and 25.5 % of prey items within

badger scats. Mammal prey species made up 8.6% of prey items within badger scats, in comparison to a lower proportion of 4.9% of prey items in hedgehog scats. Therefore, mammal and plant prey were more frequently identified in badger scats than hedgehog scats, with other broad groups being identified in low numbers of badger and hedgehog samples. Bird DNA represented 1.7% of prey items and fungi and amphibian DNA represented <1% of the food items identified in both badger and hedgehog scats.

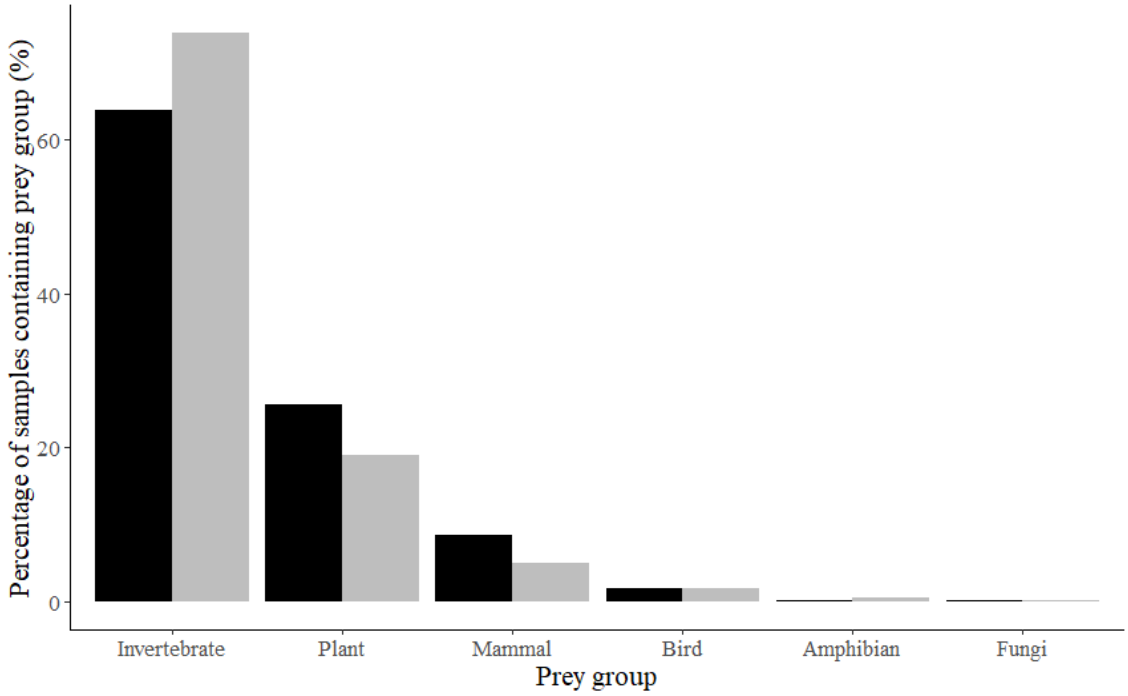


Figure 5.1 Diet composition shown as the POO of major food groups for the European badger (black bars) and hedgehog (grey bars) in rural England.

5.3.3 Diet composition of invertebrate prey

Percentage of occurrence (POO) analysis showed that the most abundant invertebrate prey type consumed by hedgehog, according to its relative frequency in all samples pooled across seasons was Coleoptera (beetles) followed by Stylommatophora (slugs and snails), and Haplotaxida (earthworms). This was consistent across the two study sites, constituting 27.7 % of hedgehog diet at Brackenhurst, and 32.0 % of hedgehog diet at Hartpury (Figure

5.2). For badger, Stylommatophora (land snails and slugs) was proportionately greatest in the diet of badgers at Hartpury representing 30.5 %, followed by Haplotaxida (28.4%) and Coleoptera (27.4%). Whereas at Brackenhurst the Order Haplotaxida, that includes earthworms, was proportionately greatest in the diet of badgers (31.9 %), followed by Stylommatophora (25.9%) and Coleoptera (21.6%).

Haplotaxida (earthworms) consistently constituted a greater proportion of badger diet (28.4 – 31.9 %) in comparison to hedgehog diet (14.8 - 18.2 %). The top five ranking invertebrate prey in hedgehog diet were Coleoptera, Stylommatophora, Haplotaxida, Lepidoptera and Isopoda (Table 5.4). Except for Isopoda, the remaining four prey types were also ranked within the five most common invertebrate prey in badger diet.

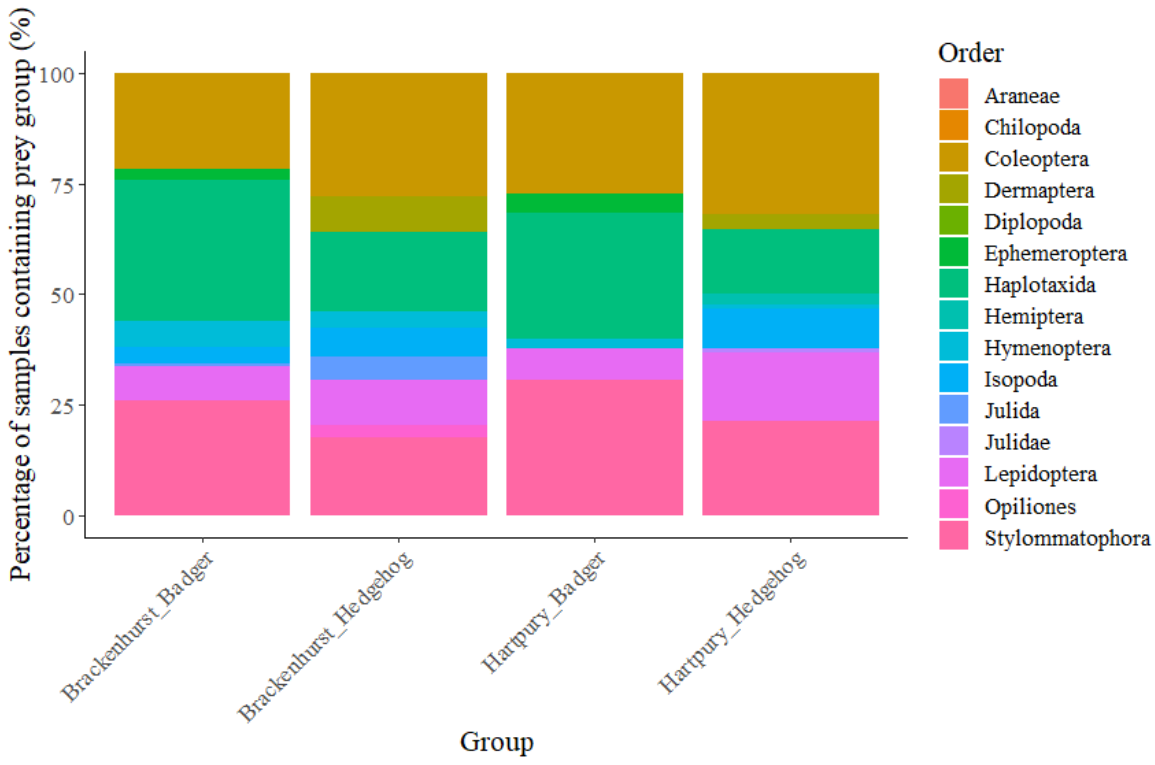


Figure 5.2 Proportion of different invertebrate prey (grouped by Order) consumed by European badger and hedgehog at two sites in England (Brackenhurst ad Hartpury).

Despite being available in the environment, Araneae (spiders) and Chilopoda (centipedes), Hemiptera (true bugs) and Trichoptera (caddisflies) were not present in the

diet of badger or hedgehog. Similarly, badger did not consume Psocoptera (booklice), Dermaptera (earwig) or Opiliones (harvestmen).

Throughout the four seasons that badger and hedgehog diet was simultaneously assessed, both species showed seasonal variation in the prey that they selected for at each site. Ivlev's electivity index showed that hedgehog and badger most strongly selected for Haplotaxida (earthworms), Diptera (flies), Ephemeroptera (mayflies) and Lepidoptera (butterflies and moths) relative to their availability (Table 5.3).

Table 5.3 Selection of invertebrate prey by hedgehog and badger across Brackenhurst and Hartpury estates. Hedgehog and badger diet is shown as POO and available prey is shown as proportion of biomass from pitfall trap captures. Invertebrate prey Orders that represented <1% of available prey (Collembola, Diptera, Ephemeroptera, Opiliones, Psocoptera, Trichoptera) are represented by category Other.

Invertebrate Prey (Order)	Proportion of hedgehog diet (%)	SE	Proportion of badger diet (%)	SE	Proportion available (%)	Selectivity index D	
						Hedge-hog	Bad-ger
Araneae	0.00	-	0.00	-	3.91	-1.00	-1.00
Chilopoda	0.00	-	0.00	-	2.63	-1.00	-1.00
Coleoptera	21.91	1.26	15.32	1.88	23.04	-0.03	-0.20
Dermaptera	4.32	1.25	0.00	-	2.08	0.35	-1.00
Haplotaxida	15.29	1.35	21.37	2.61	3.68	0.61	0.71
Hemiptera	0.00	-	0.00	-	4.54	-1.00	-1.00
Hymenoptera	2.02	0.64	3.56	1.55	6.50	-0.53	-0.29
Isopoda	1.73	0.49	1.88	0.97	12.38	-0.75	-0.74
Julida	2.50	1.06	0.33	0.31	2.72	-0.04	-0.78
Lepidoptera	12.49	1.75	7.56	1.59	1.37	0.80	0.69
Stylommato-phora	18.81	1.25	19.77	2.23	35.51	-0.31	-0.28
Other	20.93	4.54	30.21	3.56	1.65	0.85	0.90

Amongst the most common prey within the diet, both species selected against Stylommatophora in relation to the availability of this prey type. Badger selected against Coleoptera (Index = -0.20), whereas hedgehog utilised Coleoptera proportionate to its availability (Index = -0.03).

5.3.4 Seasonal and Site patterns of prey use

5.3.4.1 Summer 2018

The utilisation of Coleoptera was similar between badger and hedgehog in Summer 2018, differing between the two sites. At Brackenhurst, Coleoptera was selected for by both species, whereas Coleoptera was selected against at Hartpury. This may reflect the increased availability of Coleoptera at Hartpury in which 35.0 % of available prey biomass was represented by Coleoptera, as opposed to 10.1% at Brackenhurst. Both populations of hedgehog strongly selected for Dermaptera during this period, though availability was <1% across both sites. Haplotaxida was positively selected for by both species across the two sites, though the strength of selection was greater at Brackenhurst.

5.3.4.2 Autumn 2018

During the period September – November 2018, the percentage availability of Stylommatophora was twice as great at Brackenhurst than at Hartpury. This was reflected in the diet of badger and hedgehog at Brackenhurst, as Stylommatophora occurred in higher percentages of samples than any other Order of invertebrate prey. Both species positively selected for Lepidoptera, though selection was stronger for both badger and hedgehog at the Brackenhurst site (Table 5.6). Coleoptera was underutilised relative to its availability at Hartpury College, whereas Coleoptera was again positively selected for by both species at Brackenhurst. Collembola was selected for by badger at both sites and was absent from hedgehog diet.

5.3.4.3 Spring 2019

Coleoptera was underutilised by both species relative to its availability from March – May 2019 at Hartpury College, whereas Coleoptera was positively selected for by both species at Brackenhurst (Table 5.7). Collembola was selected for by badger at both sites whereas it was selected against by hedgehog. Hymenoptera was consumed in similar frequencies by

hedgehog at both sites. However, the availability measured by pitfall traps was lower at Hartpury College, which resulted in hedgehog underutilising Hymenoptera at Brackenhurst and positively selecting for Hymenoptera at Hartpury. Hymenoptera was only selected for by hedgehog at Hartpury as was Opiliones by hedgehog at Brackenhurst. Hedgehog diet contained proportionately more Stylommatophora than badger diet. Despite this, selectivity was consistent within each site, showing that Stylommatophora was selected for at Brackenhurst and selected against at Hartpury by both species.

5.3.4.4 Summer 2019

A similar pattern in resource use was observed for Coleoptera in the Summers of 2018 and 2019, with hedgehog and badger utilising Coleoptera similarly across both sites (Table 5.8). However, selection in 2019 was positive at Hartpury and negative at Brackenhurst, a reversal on the previous year for both species. The selection of Dermaptera by hedgehog was comparable to the previous year, with strong selection at both sites. Similarly, Haplotaxida and Stylommatophora were again positively selected for by both species and at both sites during this period.

5.3.5 Seasonal selection of invertebrate prey Orders

The utilisation of individual Orders of invertebrate prey varied between seasons. Selectivity indices showed that the utilisation of Coleoptera varied between seasons, though the pattern of resource use was similar for badger and hedgehog at each site (Figure 5.3). From June – August 2018, badger and hedgehog positively selected for Coleoptera at Brackenhurst, whereas both species selected against Coleoptera at Hartpury, relative to its availability. This suggests that the selection of Coleoptera varies at the local scale, in relation to its availability. Between September and November 2018, badgers at both sites strongly selected against Coleoptera, with much lower proportions of Coleoptera in the diet than any other season.

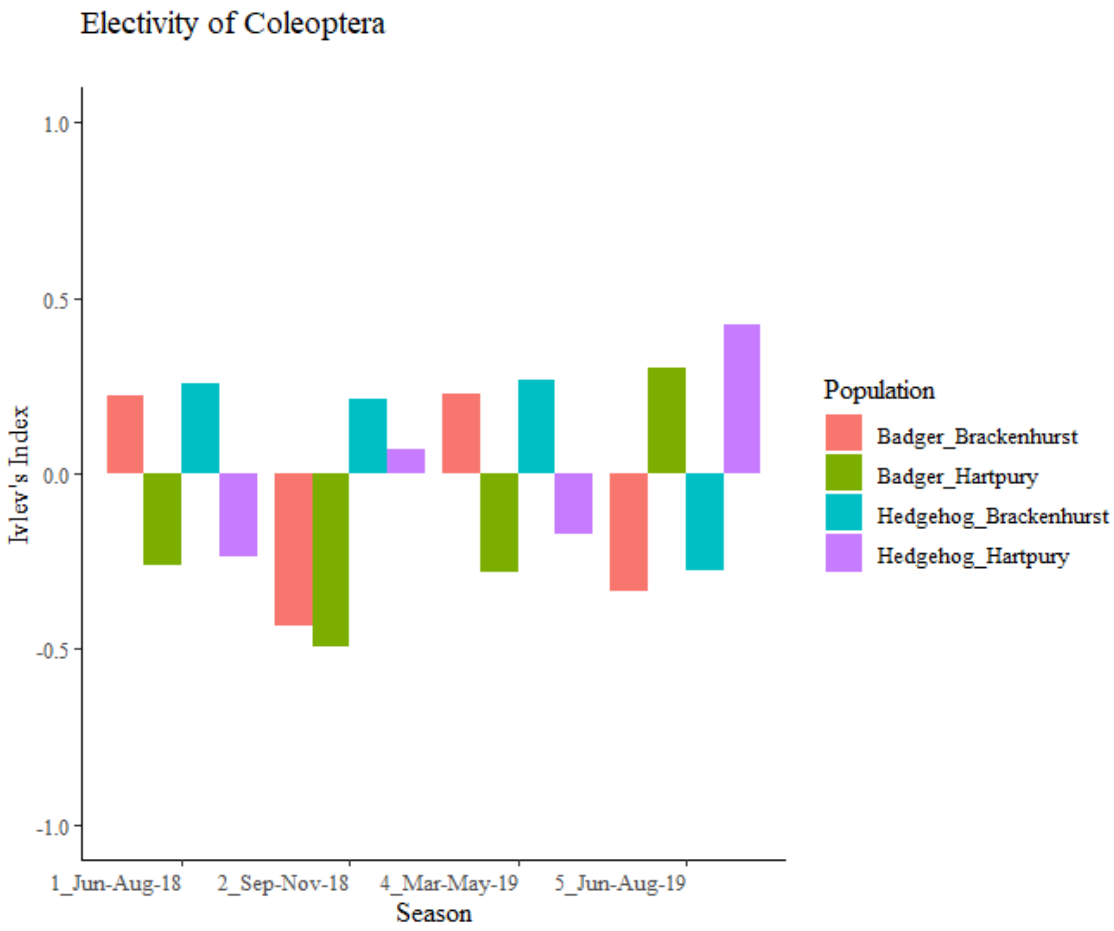


Figure 5.3 Selection of Coleoptera as shown by Ivlev's electivity index, demonstrating prey selection by badger and hedgehog populations between June 2018 and August 2019. The prey index was calculated for FOO of dietary data obtained by molecular analysis of faecal samples and availability of prey was measured as biomass of organisms caught by pitfall trapping.

In contrast, the Order Lepidoptera, was almost continuously positivity selected for, across all seasons for badger and hedgehog (Figure 5.4). The strength of selection may be overstated due to poor capture rates of Lepidoptera in pitfall traps. At each site, selection of Lepidoptera was slightly greater by hedgehog than by badger, except for March – May 2019 when selection was strongest (+1) and consistent across all groups.

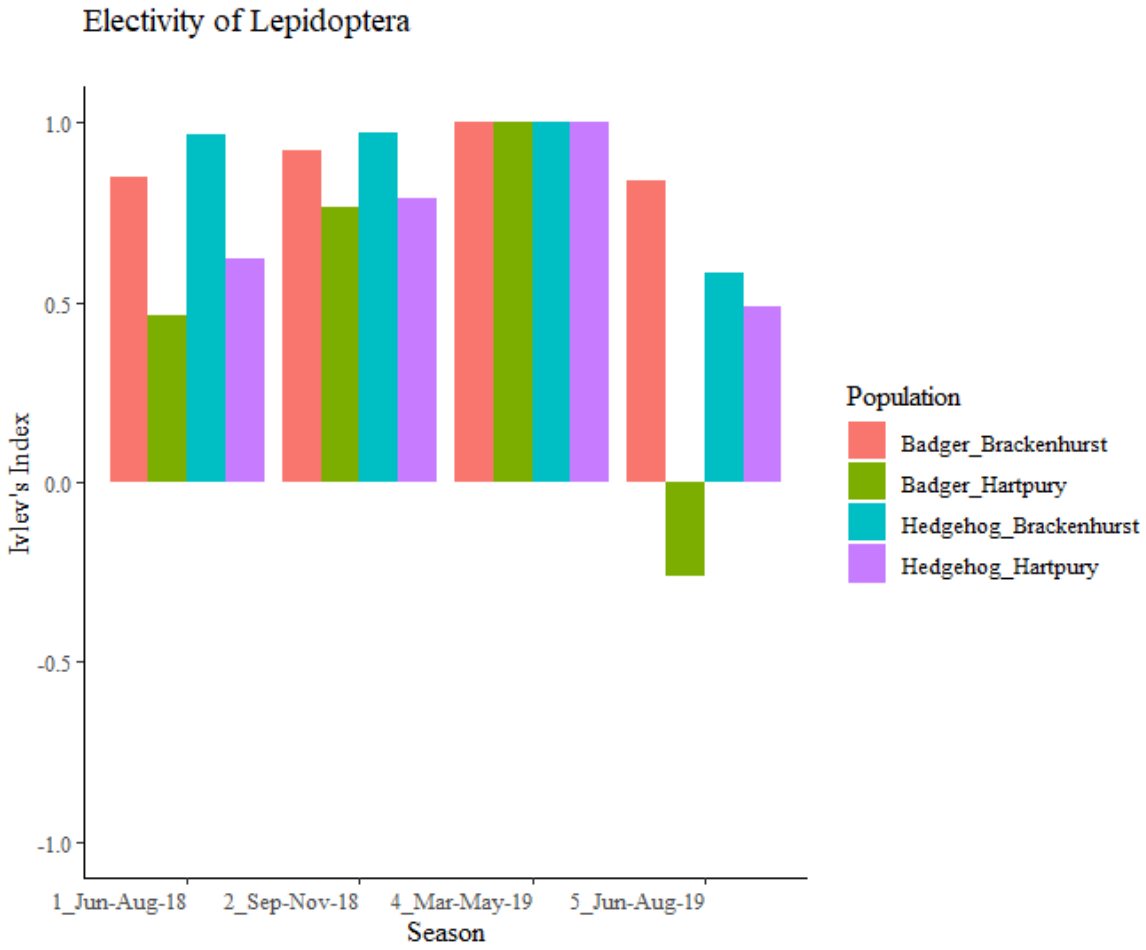


Figure 5.4 Selection of Lepidoptera as shown by Ivlev's electivity index, demonstrating prey selection by badger and hedgehog populations between June 2018 and August 2019. The prey index was calculated for FOO of dietary data obtained by molecular analysis of faecal samples and availability of prey was measured as biomass of organisms caught by pitfall trapping.

There were seasonal patterns in the selection of Stylommatophora, with both species positively selecting for this prey type between June and August 2019 (Figure 5.5). However, during this period in 2018, positive selection was only identified in badger and hedgehog at Hartpury. Between September to November 2018, neither species selected for Stylommatophora, potentially demonstrating underutilisation of this prey resource. Pitfall trap captures were highly variable for this prey type, and this may be reflected in the selection indices for Stylommatophora.

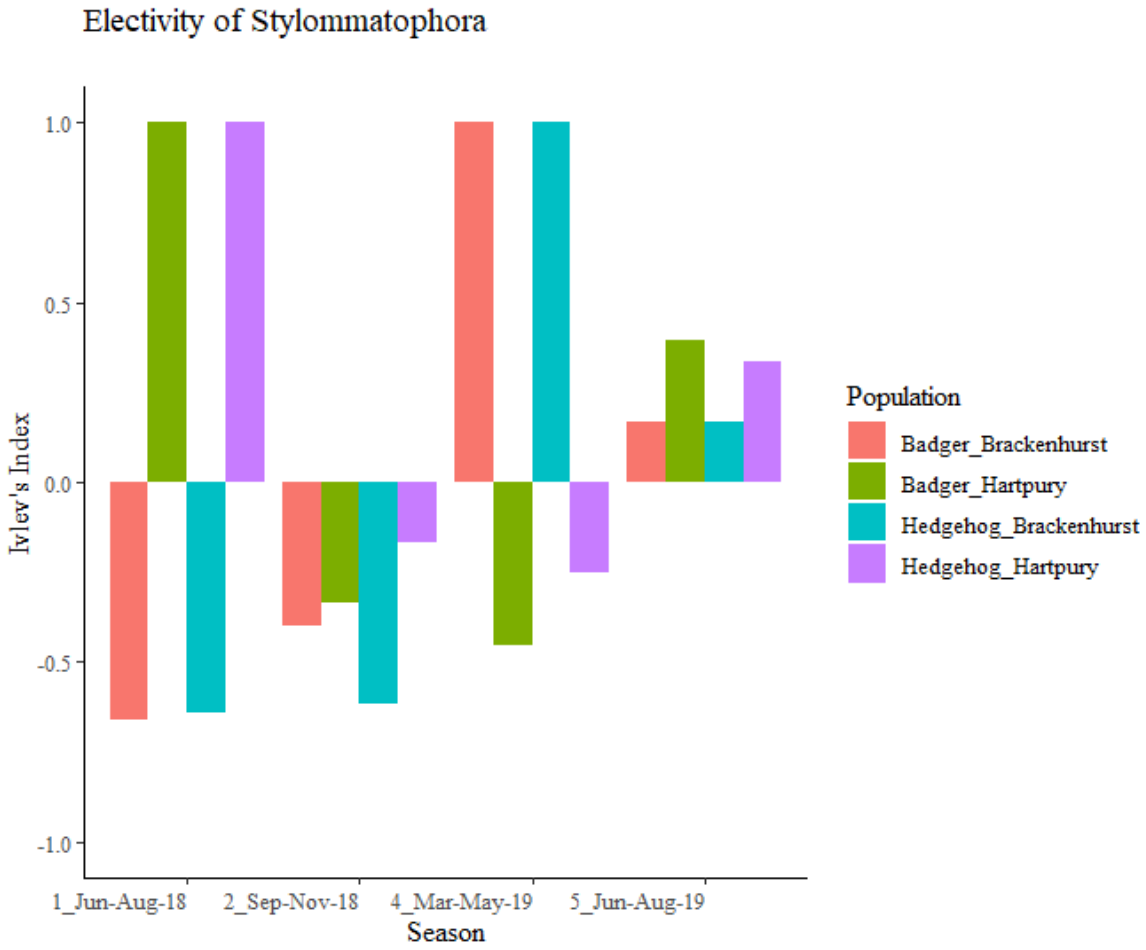


Figure 5.5 Selection of Stylommatophora as shown by Ivlev's electivity index, demonstrating prey selection by badger and hedgehog populations between June 2018 and August 2019. The prey index was calculated for FOO dietary data obtained by molecular analysis of faecal samples and availability of prey was measured as biomass of organisms caught by pitfall trapping.

Haplotaxida was positively selected for across both sites and all four seasons. During the period June – August in both 2018 and 2019, hedgehog more strongly selected for Haplotaxida than badger (Figure 5.6). Conversely, between September and November 2018, badger showed stronger selection of Haplotaxida across both sites. In the period March – May 2019, selection for Haplotaxida was similar between each species, with selection being greater at the Hartpury site. Low capture rates of Haplotaxida in pitfall traps likely influence the strength of selection observed here.

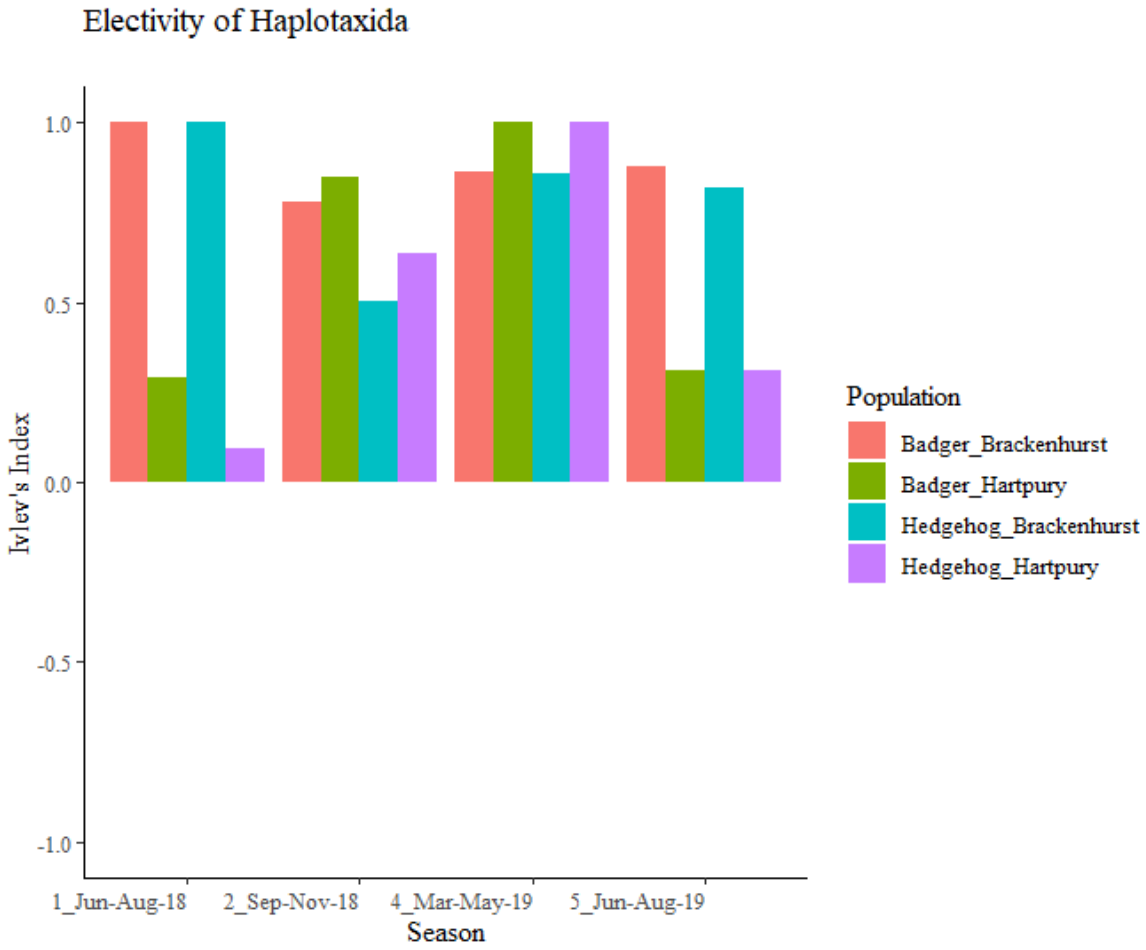


Figure 5.6 Selection of Haplotaxida as shown by Ivlev's electivity index, demonstrating prey selection by badger and hedgehog populations between June 2018 and August 2019. The prey index was calculated for FOO of dietary data obtained by molecular analysis of faecal samples and availability of prey was measured by was biomass or organisms caught by pitfall trapping.

The selection of Hymenoptera (Figure 5.7) varied between season and site. There was no consistent pattern for either species. Between March and May 2019, hedgehog at Hartpury showed a strong selection for Hymenoptera, whilst all other groups avoided this prey type. In June – August 2019, both badger and hedgehog selected for Hymenoptera, whereas both species underutilised Hymenoptera at Hartpury.

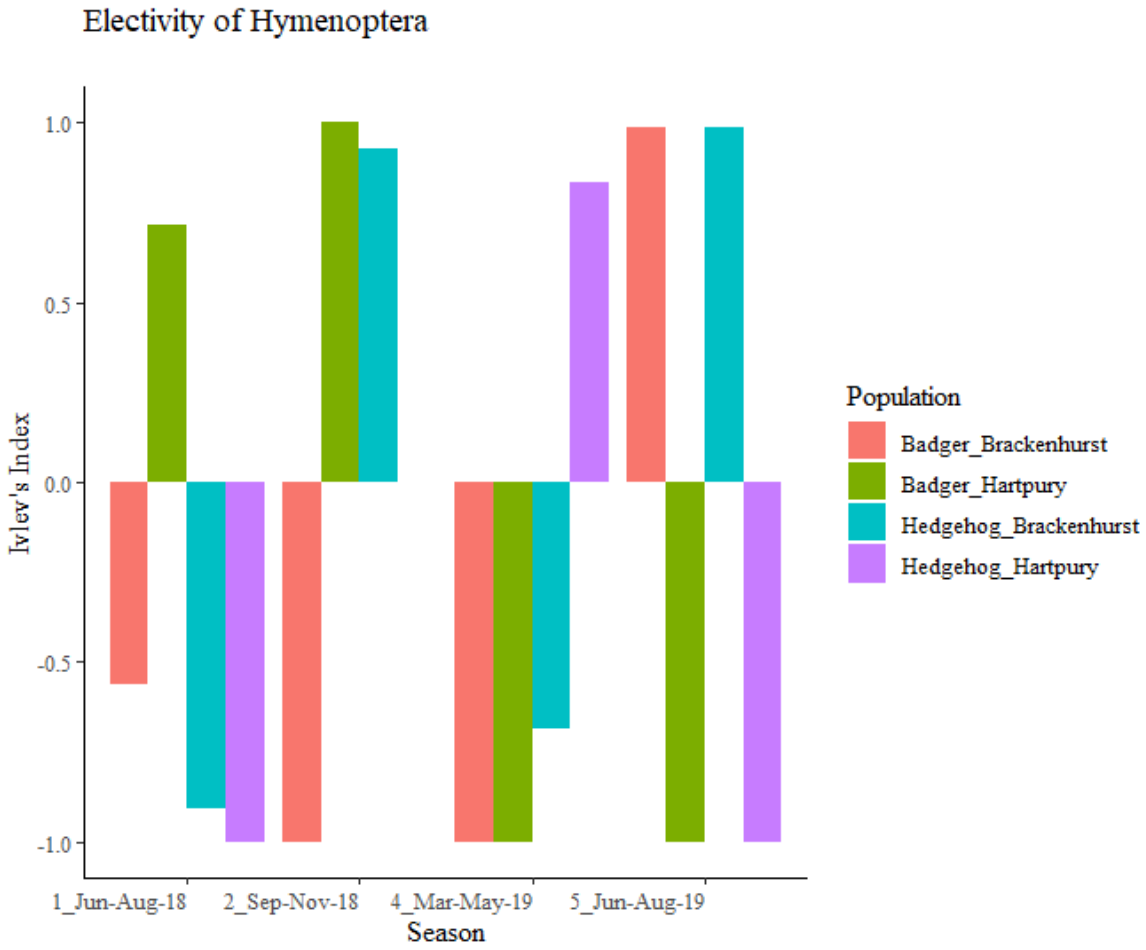


Figure 5.7 Selection of Hymenoptera as shown by Ivlev's electivity index, demonstrating prey selection by badger and hedgehog populations between June 2018 and August 2019. The prey index was calculated for FOO of dietary data obtained by molecular analysis of faecal samples and availability of prey was measured as biomass of organisms caught by pitfall trapping.

Selection of Isopoda was observed at Brackenhurst, by both badger and hedgehog between the months of June – August 2018 and March – May 2019 (Figure 5.8). Hedgehog at Brackenhurst also selected for Isopoda between September and November. However, neither species selected for Isopoda at Hartpury during any season.

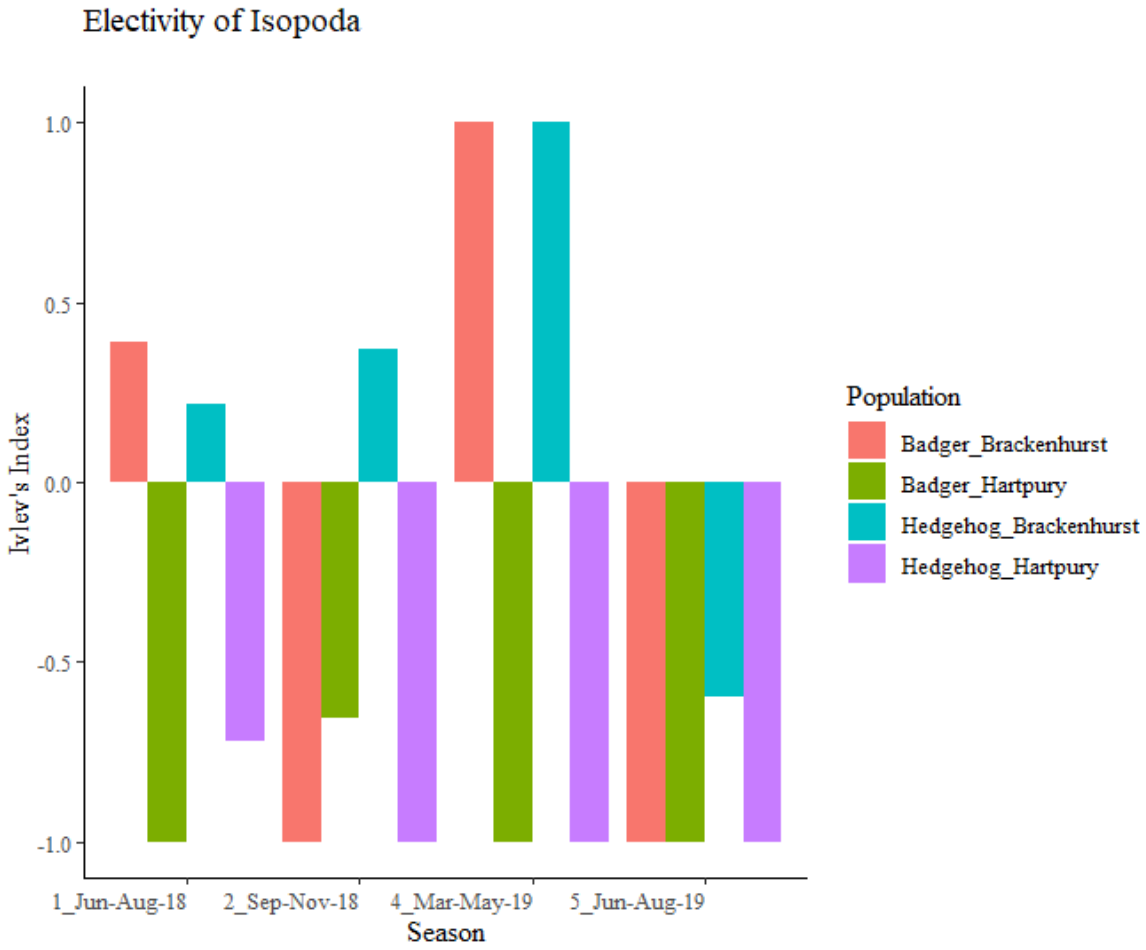


Figure 5.8 Selection of Isopoda as shown by Ivlev's electivity index, demonstrating prey selection by badger and hedgehog populations between June 2018 and August 2019. The prey index was calculated for FOO of dietary data obtained by molecular analysis of faecal samples and availability of prey was measured as biomass of organisms caught by pitfall trapping.

The selection of Dermaptera was specific to hedgehog, and evident in the period June – August in both 2018 and 2019 (Figure 5.9). At Brackenhurst, hedgehog selected for Dermaptera in all seasons apart from September to November. Dermaptera was not present in the diet of badger despite being available in the environment.

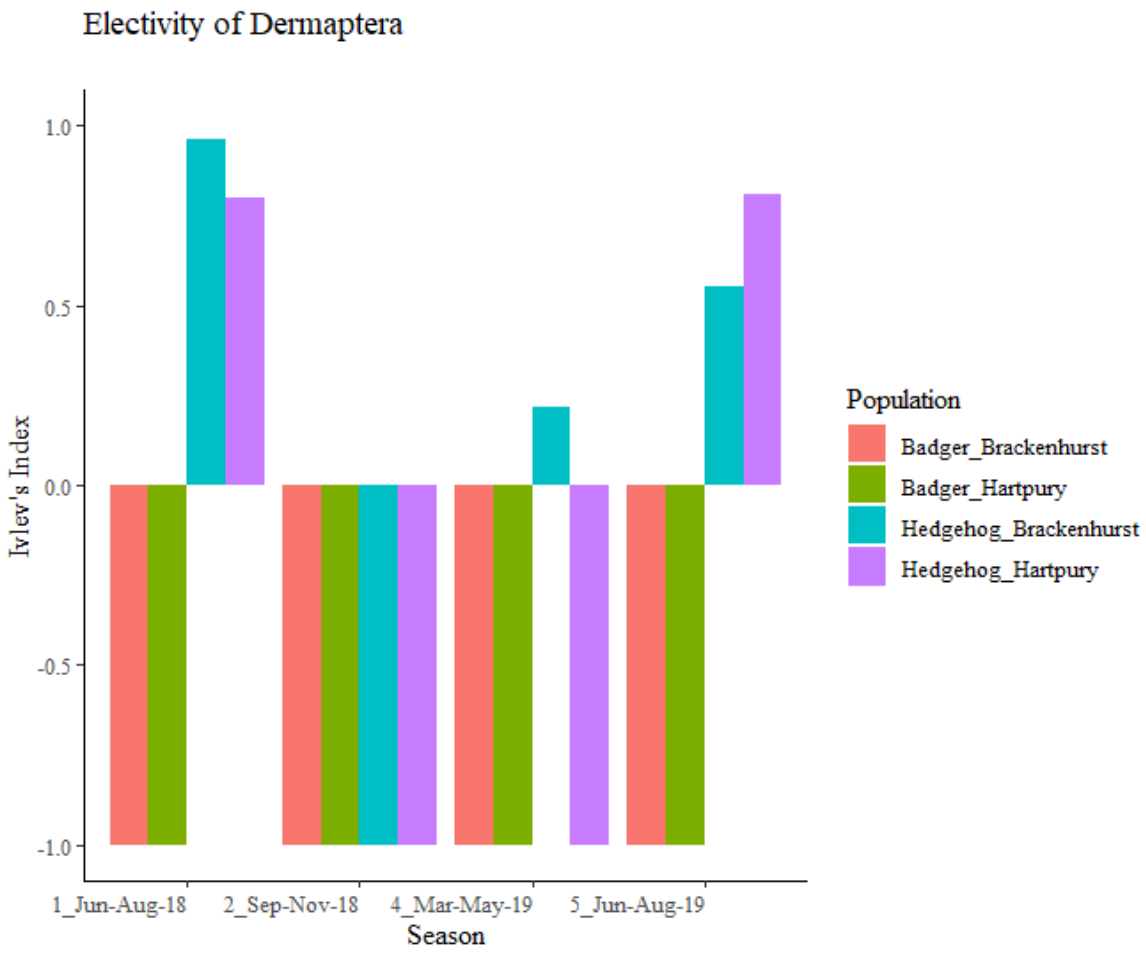


Figure 5.9 Selection of Dermaptera as shown by Ivlev's electivity index, demonstrating prey selection by badger and hedgehog populations between June 2018 and August 2019. The prey index was calculated for FOO of dietary data obtained by molecular analysis of faecal samples and availability of prey was measured as biomass of organisms caught by pitfall trapping.

Other Orders that were exclusively utilised by hedgehog were often consumed during specific seasons. Psocoptera (booklice) constituted 2.7% of hedgehog diet at Hartpury from June -September 2018. This rose to 9.9% between the period September- November. This prey type was not captured by pitfall trapping, indicating its low abundance within the environment. The availability of the Order Opiliones (harvestmen), was greatest at Brackenhurst between March and May 2019, representing 2.6% of available prey. Hedgehogs at Brackenhurst positively selected for Opiliones during this period and through until August 2019. Opiliones were not however identified in the diet of hedgehog at Hartpury throughout the period surveyed.

5.4 Discussion

Competition for shared prey resources constitutes a key component of the Intra-Guild Predation (IGP) relationship between badgers and hedgehogs. Invertebrate prey dominates the diet of both species (Chapter 4), (Cleary et al. 2009; Wroot, 1984), though the degree of competition for shared prey items is dependent on local prey availability. IGP theory states that the level of competition indirectly affects the rate of predation between intraguild-predator and intraguild-prey (Holt and Polis 1997). Until now, there has been no comparative dietary assessment of badger and hedgehog with reference to local food availability. The diets of both species are extremely broad and often includes species from distinct groups of taxa, posing a significant challenge when attempting to quantify the relative availability of potential food items within the environment. In this study, pitfall trapping provides an index of the availability of ground dwelling invertebrate prey (Chapter 3), which dominates the diet of badger and hedgehog (Chapter 4) and therefore represents the prey types most likely to be competed over. However, this study exposed the nuances in prey biomass that likely exist between different prey Orders captured by pitfall trapping. Therefore, these results should be interpreted with appropriate caution, although for the most common prey items consumed by both species, namely ground dwelling invertebrates (Chapter 4), pitfall trapping does provide a reliable method of assessment. The results of this study show that neither species demonstrates strong selection of preferential prey, but instead tend to select what is available, reflective of their generalist statuses. Differences in prey selection showed that hedgehog take proportionally more beetles than badgers, whereas badgers consume proportionally more slugs and earthworms which may facilitate niche partitioning (Cloyed and Eason 2017) between co-occurring badger and hedgehog. Moreover, seasonal variation of available prey appears to affect prey selection, thus driving the strength of competition between seasons too.

Dietary assessment (Chapter 4) of hedgehog showed that Coleoptera (beetles) was in the highest proportion of samples, followed by Stylommatophora (slugs) and Haplotaxida (earthworms). Hedgehogs utilised Coleoptera proportionate to its availability (Index = -0.03), whereas badger selected against this prey type (Index = -0.20). This perhaps reflects the hedgehog's smaller size, making it better suited for finding and consuming this prey type, unlike badgers, where foraging for Coleoptera is likely to be energetically inefficient in comparison. Seasonally, Coleoptera consistently represented a substantial proportion of prey biomass within the environment, though this varied between each site. In Autumn 2018, badgers at both sites showed a substantial reduction in the consumption of Coleoptera that did not correspond to a decrease in availability, suggesting that badgers may be utilising other prey during this period. As badgers are known to consume proportionately more plant and mammal prey than hedgehog (Chapter 4), and previous studies have highlighted the consumption of cereal crops during Autumn (Kruuk and Parish 1981), it is probable that badgers may have utilised other prey types that were seasonally available during this period. In the other three seasons assessed, the strength of selection appeared to be driven by the site-specific availability of Coleoptera, which was broadly similar between badger and hedgehog within each site. Again, this highlights a common prey type for both species which is likely competed over, with the strength of competition being affected by the local availability of prey.

Both species selected for Haplotaxida (earthworms), highlighting the importance of this prey group for both species and the potential for competition over this prey resource. Selection for Haplotaxida was consistently high throughout all seasons, further reinforcing the understanding of Haplotaxida as a key prey type. Relatively low proportions of available prey biomass were constituted by Haplotaxida, and this may highlight a sampling bias between different prey groups. However, as hedgehogs are known to forage mainly on

surface dwelling invertebrates, pitfall trapping is likely to provide a realistic estimate of available prey for which badger and hedgehog may compete for (Doncaster 1992).

Arable habitat (Chapter 3) supports a high abundance of Coleoptera, though hedgehogs are known to widely avoid arable habitat (Pettett et al. 2017; Williams et al. 2018). Despite this, hedgehog were shown to select Coleoptera relative to its site-wide availability, indicating that there was accessibility to this prey type across the wider site. The utilisation of different habitats, which likely affects the availability and encounter rate of certain prey, is discussed in Chapter 6 and considers whether hedgehogs avoid arable land in response to badgers, due to the difficulty they face in navigating this habitat, or to avoid badgers.

In contrast, there were other Orders that accounted for relatively small proportions of available prey biomass that were still strongly selected for. Lepidoptera, for example, exceeded no more than 6.7% of available prey in any season. Although pitfall traps have been used similarly in other studies to assess broad invertebrate communities (Bhandari et al. 2018), the results here indicate that capture rate likely fluctuates between Orders. Caterpillars (Lepidoptera), for example, may be underrepresented in pitfall trapping, and this demonstrates a limitation of this method that must be recognised when comparing across different Orders.

In this analysis, both species demonstrated strong selection of Lepidoptera, highlighting a preference for this prey type. Despite the strong selection exhibited towards Lepidoptera, neither badger nor hedgehog diet contained substantial proportions of this prey group. Therefore, it is unlikely that badger and hedgehog are frequently in competition for this prey type. More realistically, it highlights an opportunistic prey resource, that both species will select for, should they encounter this rarer prey type. This has been shown in badgers previously, with predation rates on bumble bees ranging from 1 – 6.5 % (Cleary et al. 2009; Roberts et al. 2020) in reflection of seasonal availability of this

prey type. This also supports the level of Hymenoptera observed in the diet of badgers in this study, which was on average 3.6% of badger diet. Whereas, other studies have shown higher frequencies of badger predation on wasp nests in Summer, reflecting their seasonal importance (Goszczynski et al. 2000).

Several of the prey groups available in the environment were not utilised by one or both species. Badger did not consume Dermaptera, nor Opiliones or Psocoptera. Araneae Chilopoda, Hemiptera and Trichoptera were not identifed in the diet of either species, despite representing between 0.90 and 4.5 % of available prey in pitfall traps. Several factors could potentially explain the non-consumption of these prey groups, such as the ability of each predator species to detect the prey and capture these prey types (Deudero and Morales-Nin, 2001). Moreover, the encounter rate of different prey may influence the likelihood of a prey type being consumed by either species (Sih and Christensen 2001).

Another aspect that is infrequently considered is the macronutrient content of different prey. It has been suggetsed that badger densities and group size may potentially be limited by the availability of macronutrients, in additon to the abundance of prey (Balestrieri et al. 2019). Therefore, it is possible for there to be an abundance of prey yet the predator to still be nutrient deficent. Both species strongly selected for Lepidoptera, perhaps indicating its nutritional value. Indeed high levels of protein (Ramos-Elorduy et al. 2011) are needed for 40-45 % of badger nutritional requriements (Balestrieri et al. 2019). Perhaps the integration of agri-environment schemes that promote the recovery of insects (Kleijn et al. 2003; McHugh et al. 2019) may also provide unintentional benefits to the wider ecosystem, providing an abundance of these preferred prey types for badger and hedgehog.

Competition for prey items between badger and hedgehog is more likely for the common prey types and therefore this study assessed the selection of prey items that consitutied >1% of available prey biomass. However, for both species, the cateory ‘Other’

represented a sizable proportion of the diet. This shows that many other prey items that are relatively less available in the envronment, are important collectively within the diet of both species. This supports the findings of Chapter 4 which demonstrated the broad dietary niche of both species. High dietary breadth, as exhibited by both species across the two sites surveyed (Chapter 4), may be indiciative of poor resource availability (MacArthur and Pianka 1966), whereby species broaden their niche to compensate for limited resource availability in order to meet their energetic demands.

5.4.1 Limitations

Whilst invertebrate prey dominates the diet composition of both badger and hedgehog, it does not describe their foraging patterns completely. Here, a measure of ground dwelling invertebrate biomass served as an index for available prey via pitfall traps, although plant, mammal, bird and amphibian species were not considered. As invertebrate prey dominate the diet of both badger and hedgehog, these are the prey they most likely compete for regularly. The measure of invertebrate biomass was obtained from pitfall trapping, which was chosen as it presented the broadest assessment of ground dwelling invertebrate prey that could be used exclusively as a measure of prey availability. For some taxa, namely Haplotaxida (earthworms) that only emerge on the soils surface to forage and mate (Butt et al. 2003), this may underrepresent the measure of available prey, particularly for badger that can dig and capture worms from beneath the soil's surface. Therefore, although the method of assessing available prey was imperfect, it provided a useful estimate for the broad array of invertebrate prey consumed by these competitors.

Patterns of prey selection revealed the complete avoidance of some Orders, namely Chilopoda and Araneae, despite their notable availability. There are several unmeasured factors that may have accounted for this, such as the palatability or motility of prey. These interactions would be logistically difficult to observe, particularly without disturbing the

natural foraging behaviour of badger and hedgehog. Potentially, food trials (Peterson and Renaud 1989) could be used to better understand these apparent prey preferences exhibited by badger and hedgehog.

Another limitation of the study was the number of faecal samples that could be analysed molecularly, which in turn constrained the number of study sites. The results of this study were obtained from sampling two mixed farms, and therefore the assessment of several additional sites would be beneficial to assess whether the results observed in this study are consistent across other geographical locations. For example, areas with different land use may support different prey that would affect the level of niche segregation and competition between badger and hedgehog. Moreover, given the evidence for sex biases and individual level specialism in the diet of many species (Catry et al. 2014; Jacquier et al. 2020; Terraube et al. 2014), including the badger (Robertson et al. 2014, 2015), it would perhaps have been revealing to include host identification of individual scats. Technical constraints currently prevent hedgehog faecal samples from being sexed as there are no known sexing primers. Moreover, existing genotyping of badger faecal samples (Frantz et al. 2003), and that performed as part of this wider study, document variable success rates when analysing poor quality samples such as degraded faecal samples (see Chapter 4).

5.4.2 Further Research

This study allowed dietary patterns of badger and hedgehog to be analysed across the two sites studied. However, accessibility and utilisation of the available foraging habitat is an important driver of diet selection indices, which alters considerably with spatial scale (Gillis et al. 2020). Therefore, by using home range analysis to investigate the proportion of available habitat utilised by both species, a more accurate assessment of prey availability could be calculated (Gillis et al. 2020). Moreover, a measure of the other prey

groups would be beneficial. For example, incorporating a seasonal assessment of the plant species available would reveal whether either species exhibit prey switching, in relation to the seasonal availability of cultivated crops. Farmland habitat has been associated with higher levels of individual specialism in badgers, driven by limited resources that also was associated with larger territory size (Robertson et al. 2015).

In addition, the two sites in this study included mixed farms where both species co-occur. Prey availability is likely to differ more greatly in arable dominated sites and prey selectivity of one species may alter in the absence of the other. Inclusion of a broader range of sites would be interesting, to ascertain whether the dietary selection shown in this study is consistent amongst sites with differing land use and species composition.

5.4.3 Conclusions

The results of this study highlight that both badgers and hedgehogs do not consume invertebrate prey randomly, relative to its availability, but rather the dietary patterns of these species show that they positively select certain invertebrate prey. The most common prey types Coleoptera and Haplotaxida were positively selected for by badgers and hedgehogs, indicating that there is competitive pressure for these prey resources. However, hedgehogs utilised prey that badgers selected against. This could reflect palatability, encounter rate at a finer level, and the ability of these species to capture different mobile prey types (Sih and Christensen 2001). Differential selection of prey may facilitate niche partitioning, highlighting an important mechanism whereby hedgehogs exploit resources that badgers do not. This is in line with IGP theory that hedgehogs, the intra-guild prey, must be a superior exploiter of resources to enable coexistence with the intra-guild predator, badgers (Polis et al. 1989). Understanding the utilisation of available foraging habitat by badgers and hedgehogs (Chapter 6) will enhance the understanding of the accessibility of available prey further.

Chapter 6 The spatio-temporal and abundance relationships between badgers and hedgehogs across the rural landscape

6.1 Introduction

The relationship between badgers (*Meles meles*) and hedgehogs (*Erinaceuas europaeus*) is complex due to their intra-guild predation relationship (Doncaster 1992), whereby they compete for food resources within the same habitats and badgers predate hedgehogs, thereby acting as the principle driver of interactions between these two species (Polis et al. 1989). Studying intra-guild predation relationships amongst wild animals is difficult due to the wide range of variables that need to be quantified to understand the interplay between predation and competition. Typically, research on badgers and hedgehogs has focused on one dimension of the relationship, either spatial, density or temporal, whilst omitting the effects of prey availability and dietary niche partitioning and involving too few study sites to incorporate context-specific habitat preferences.

Spatial avoidance strategies represent a key mechanism for facilitating the coexistence of some species, relying on the utilisation of the same resources but in different locations, or switching to different resources which occur in different locations, namely habitat and food (Darmon et al. 2012). Often, multiple study sites are required to investigate these spatial patterns and therefore by collecting data uniformly, larger datasets can be produced, providing greater explanatory power (Scotson et al. 2017). At the regional scale, both badgers and hedgehogs are widely distributed across the United Kingdom, suggesting their frequent co-occurrence (Judge et al. 2017; Wembridge and Wilson 2018). However, at a finer scale they exhibit different habitat associations, with hedgehogs showing an affinity for amenity grassland and suburban areas that is not commonly shared by badgers (Doncaster 1992; Young et al. 2006). Such relationships have been attributed to badgers creating a 'landscape of fear' for hedgehogs, precluding them from suitable habitat where badgers are present. Indeed, hedgehog occupancy at the 1 km^2 scale has been shown to be negatively related to the presence of badgers in rural England and Wales (Yarnell et al. 2014; Williams et al. 2018). This solidifies the growing

consensus that badgers exert a negative pressure on hedgehogs (Young et al. 2006; Hof et al.2012; Williams et al. 2018; Hof et al. 2019b), possibly "excluding" them from suitable habitat as they seek refuge from badger predation and competition. Therefore, at a finer habitat scale, hedgehogs may be spatially excluded from suitable habitat by the competitive and predatory action of badgers leading to reduced densities or occupancy rates which could then result in population declines.

Theory also suggests that the intraguild-prey species may occupy lower quality habitats to minimize the risk of interactions with the intraguild-predator. This has been observed in coyotes (*Canis latrans*) and their intraguild-prey the kit fox (*Vulpes macrotis*), (Robinson et al. 2014). This highlights that habitat use can also impact the availability and quality of food resources, and typically results in subordinate species avoiding optimal foraging habitat in the presence of a predator (Morris 2009). Accordingly, in the case of badgers and hedgehogs, the latter would be hypothesised to be found in suboptimal habitat, in terms of prey availability.

The dynamic nature of resource availability means that there is some variation in the subordinate species' response to predation risk. For example, bobcats (*Lynx rufus*) avoid coyotes when basal prey are abundant but are forced to use areas of higher coyote presence when prey resources in suboptimal habitats are diminished (Wilson et al 2010). This demonstrates that the response to predation risk in bobcats varies with fluctuating food availability. As stated in Chapter 4, there is a maximum level of dietary overlap that allows species to coexist, and alternative prey items increase the likelihood of establishing this balance. When food is limiting, intra-guild predators may switch their prey, consuming their occasional prey more readily. Therefore, the rate of intraguild predation may rise under these circumstances. Without incorporating co-occurrence data and a measure of food availability, these interactions would go undetected, and the mechanisms for coexistence would not be identified.

Temporal segregation is another predator avoidance strategy that has been shown to promote the coexistence of some species. In the example of coyotes avoiding wolves *(Canis lupus)* (Arjo and Pletscher 1999), temporal segregation was only evident during the winter when home ranges were highly overlapping, showing the fluidity of these behavioural shifts in response to the threat posed by intraguild predators. By altering their daily activity between seasons, coyotes effectively occupied the same prey-rich area as wolves, benefitting from scavenging on wolf kills whilst avoiding predator encounters. This demonstrates that when shared prey are sufficiently abundant to support both intraguild predator and prey species, temporal segregation may provide another mechanism for promoting the coexistence of these competitors. Though the nocturnal habits of badgers and hedgehogs are well known (Dowding et al. 2010; Garnett et al. 2002), there has been no direct comparison of temporal patterns of hedgehog and badger activity to date. Evidence showing that hedgehogs physiologically respond to predator olfactory cues (Ward et al. 1997) suggests that they perceive badgers as a threat which may in turn initiate a shift in activity, though this remains unstudied.

There is evidence that badgers predate hedgehogs (Micol et al., 1994), which was demonstrated by the detection of hedgehog DNA within badger scats in the wider study (Chapter 4), though this may represent scavenging behaviour on an already dead hedgehog. Furthermore, decreases in badger abundance can result in increases in hedgehog density in their preferred habitat, amenity grassland (Trewby et al. 2014). Indeed, other studies have highlighted the negative correlation between increasing badger activity and hedgehog abundance (Hof et al.2012; Hubert et al. 2011; Williams et al. 2018; Yarnell et al. 2014). However, these studies have relied on using badger sett density, or main sett density, as a proxy for badger abundance, which may be erroneous leading to over or underestimation of badger activity within an area. Despite this, scientific consensus is that badgers exert a negative population response on hedgehog (Doncaster et al. 2001; Young et al. 2006).

However, the mechanism of this negative population response (competition for food or direct predation) has not been identified. Furthermore, habitat selection by each species is different and use of amenity grassland by hedgehogs may either be because it provides optimal habitat for them, or because it reflects their landscape of fear response caused by the presence of badgers in the surrounding habitat (Doncaster 1992). However, hedgehog densities are considerably higher in urban areas (Schaus et al. 2020), which suggests that urban areas may offer the best habitat for them, irrespective of badger presence.

The aim of this study was to test whether the presence of badgers, habitat preference, or an index of prey availability, best predicted hedgehog presence, density and temporal activity across rural England and Wales. Based on intraguild predation theory (Polis et al. 1989) and past literature, it is hypothesised that badger density and presence will negatively influence hedgehog presence and density, and that hedgehogs will avoid areas of high prey availability shared by badgers. Finally, it is hypothesised that badgers and hedgehogs will exhibit spatial or temporal partitioning and, should they be spatially separated, there will be no change in the temporal niche of hedgehogs in order to avoid badgers.

6.2 Methods

The study took place across all 23 study sites described in Chapter 2. Camera trapping was used to estimate focal species density and occupancy (Chapter 6), whilst invertebrate sampling was used as an index of prey availability for both species (Chapter 3). Methodologies were consistent between sites and years, allowing data to be pooled for analysis.

6.2.1 Data analysis

6.2.1.1 Density estimation

The density of badgers and hedgehogs at each site was estimated using the Random Encounter Model (REM) methodology (Rowcliffe et al. 2008). REM allows density estimates to be obtained from camera trapping data for species such as hedgehogs that do not have any unique identification features (Schaus et al. 2020). The REM formula calculates density (D) as a function of trapping rate (the number of detections per unit time, y/t), speed of movement (v), radial distance to the animal (r) and camera detection zone (θ), ($2a$, where a is the angle of detection, in radians):

$$\boldsymbol{D = \frac{y}{t}\frac{\pi}{(vr(2+\theta))}}$$

Total trapping effort was calculated by multiplying the number of trapping hours per survey night, defined as the time between the first and last detection of either target species at each site, by the number of survey nights. Number of detections were recorded by counting the number of individuals within any one 15-second video. A 5-minute delay between camera triggers was considered sufficient to assume that video recordings represented unique detection events. A minimum of 10 independent detections per species was required to calculate reliable density estimates using site-specific parameters (Rowcliffe et al. 2008).

The position of the animal as the first point of detection was used to calculate distance from the camera and angle of detection. Landmark features such as trees, shrubs and hedgerows were important for positioning the location of the animal and describing the path travelled by the animal over the course of the video. Where possible, the movement of the animal was tracked and measured using a measuring tape and the overall distance travelled (m) was recorded. For each species, the speed of movement (m/s) was calculated as the average, taken from all detections across each site. For sites where badgers or

hedgehogs were detected but the number of detections was low, mean parameters for species daily ranges were calculated across all sites.

The 95% confidence intervals around the density estimates were calculated by resampling camera locations using replacement bootstrapping analysis based on 1000 iterations, as per Rowcliffe et al. (2008). Standard errors were also calculated for the independent estimation of parameters V, r, and θ. A linear regression was used to establish whether hedgehog density was associated with badger density. The scatterplot of standardised predicted values versus standardised residuals indicated that the data met the assumptions of homogeneity of variance and linearity, and the residuals were approximately normally distributed.

Inferential analyses were performed to assess whether land-use affected densities of badger and hedgehog. Assumptions of normality were not met for hedgehog density, therefore the non-parametric Kruskal–Wallis test (Kruskal and Wallis 1952) was performed to assess hedgehog density between different land-uses. Pairwise comparisons were assessed using Bonferroni adjusted Wilcoxon rank sums tests. A one-way ANOVA (Chambers et al. 1992) was performed to assess the relationship between badger density and land-use as the assumptions of normality were met.

6.2.1.2 Temporal activity analysis

Diel activity patterns were assessed by fitting kernel density functions to the patterns of timing of observations of badgers and hedgehogs (Ridout and Linkie 2009). Analyses were conducted in R Statistics software using the packages 'Activity' (Rowcliffe 2016) and 'Overlap' (Meredith and Ridout 2016). The 'overlap' package produced a non-parametric estimator of the coefficient of overlap between badgers and hedgehogs, 'dhat 1' ($\Delta 1$), ranging from 0 (no overlap) to 1 (complete overlap). To minimise biases due to small sample sizes, a minimum of ten detections per species at each site was required to fit kernel density estimates and calculate the coefficient of overlap (Lashley et al. 2018). High

confidence in activity estimation requires 100 detections per species (Rowcliffe et al. 2014; Dykes et al. 2018), therefore preventing within site analyses in this study due to relatively low detection levels. The degree of overlap was visualised with the function 'adjust', using the value 0.8, which produced smooth density estimates. Bootstrapping using 10,000 iterations was carried out and 95% confidence intervals were generated. The Wald Test was used to compare activity level estimates by assessing whether the difference between two activity estimates was significantly different from zero (Ridout and Linkie 2009).

Nightly temporal activity patterns for each species were first estimated from the pooled time stamps of all videos recorded across all sites. To assess whether the presence of badgers at a site resulted in a change in hedgehog temporal activity, temporal data from sites where both species co-occurred were compared to the temporal activity of the same species at sites where badgers or hedgehogs were found exclusively. Comparison of badger and hedgehog activity at individual sites was conducted for 6 of the 11 sites where both species co-occurred; low detection levels (<10 timed observations per species per site (Lashley et al. 2018) at the remaining 5 sites prevented activity overlap from being quantified.

6.2.1.3 Occupancy Modelling

Occupancy modelling estimates the probability of a species being present whilst accounting for imperfect detection. Repeated surveys allow the detection probability to be estimated, either as a constant or using detection covariates. Single-species single-season occupancy models (MacKenzie et al. 2017) were used to estimate badger and hedgehog presence/absence in relation to habitat and food covariates. Multi-species occupancy models, using the best predictors identified for the occupancy of each species, were used to test which variables influence species co-occurrence. Each camera survey night was treated as a repeat survey. For occupancy analysis, a site was defined as a camera trap location, which allowed potential spatial segregation at the habitat scale to be assessed. Cameras

were spaced a minimum of 40 metres apart, which is less than one home range, meaning individuals of both species could have visited >1 camera location per night, potentially violating assumptions of independence. However, occupancy was calculated as a measure of relative activity at a site rather than as a measure of true occupancy (MacKenzie et al. 2017). Differences in the abundance of the target species may affect the detection of the target species, therefore to account for the perceived change in detection, a relative abundance score was used as a covariate for detection occupancy (MacKenzie et al. 2017). Measures of relative species abundance were calculated as the number of photos taken at each camera station divided by the number of trapping days, averaged for each study site. No other detection covariates were included as there was no evidence to suggest detection would vary with any other covariate. Camera locations were pooled across 2018 and 2019 prior to analysis to provide occupancy estimates for both badgers and hedgehogs.

Data was collected from 674 cameras, though data was omitted from the 27 cameras placed at Thorn site, due to missing invertebrate data, and from 7 cameras that had extremely high detection rates due to random location generation placing cameras near badger setts that caused issues with over-dispersion. A further 2 camera sites with high pitfall capture biomass (g) were identified and removed as outliers, resulting in 638 camera sites that were included in subsequent occupancy models. All occupancy analyses were conducted in R Statistics software using the package 'Unmarked' (Fiske et al. 2013).

To assess the occurrence of each species in relation to habitat availability (amenity, arable, building, grassland and woodland: Table 6.1), nearest Euclidean distances (m) from each camera location to each habitat type were included as covariates in occupancy models. Distances were calculated in ArcMap 10.6.1 (ESRI (Environmental Systems Research Institute.) 2018) using the "Near" tool. Distance to amenity grassland and distance to the nearest building were co-linear, therefore only distance to buildings was included in the occupancy models. Covariates that were continuous data were standardised

using z-scores (Table 6.1). Habitat diversity was quantified as the number of habitat types present within a 10m circular buffer of each camera location (ranging from 1 – 4).

Table 6.1 Summary of the covariates used in the single-season single-species occupancy models and the data format for each.

Variable name	Description	Variable type
Dist_to_arable	Distance from camera location to nearest habitat feature – Arable	Z-scores
Dist_to_amenity	Distance from camera location to nearest habitat feature – Amenity grassland	Z-scores
Dist_to_building	Distance from camera location to nearest habitat feature – Buildings	Z-scores
Dist_to_grassland	Distance from camera location to nearest habitat feature – Grassland	Z-scores
Dist_to_woodland	Distance from camera location to nearest habitat feature – Woodland	Z-scores
Badger_relative	Relative abundance of badger at each camera location.	Binary
Hedgehog_relative	Relative abundance of hedgehog at each camera location.	Binary
Habitats	Number of habitats within 10m buffer of camera location	Count
Earthworm_abundance	Camera location specific estimate of earthworm abundance	Z-scores
Earthworm_biomass	Camera location specific estimate of earthworm biomass	Z-scores
Pitfall_abundance	Camera location specific estimate of pitfall abundance	Z-scores
Pitfall_biomass	Camera location specific estimate of pitfall biomass	Z-scores
Beetle_abundance	Camera location specific estimate of beetle abundance	Z-scores
Beetle_biomass	Camera location specific estimate of beetle abundance	Z-scores

To test whether the availability of food (see Chapter 3) was associated with the occurrence of either species, the abundance and biomass of both earthworms and pitfall trap captures within each habitat was measured and used as an index of prey availability. Average values from invertebrate sampling sites were calculated for each habitat type, specific to each unique study site (n = 22 sites). Unique values were calculated for each camera location (638) by extracting the proportion of each broad habitat within a 10-metre buffer and weighting the average food availability measure by these proportions. This resulted in six unique covariates for the index of food availability per camera location; earthworm abundance, earthworm wet biomass, pitfall capture abundance, pitfall capture dry biomass, beetle abundance and beetle biomass. Data specific to earthworms and beetles were included in the analysis as these contributed large components of both species diet (Chapter 4). The relative abundance of species was added as a variable influencing detection probability (p), to account for variations in abundance between study sites.

For each species, a global model was used to test each combination of the following variables using the dredge function from the *'MuMIn'* package (Bartoń 2019): 1) distance to arable; 2) distance to building; 3) distance to grassland: 4) distance to woodland; 5) earthworm biomass; 6) pitfall biomass 7) beetle biomass and 8) number of habitats within 10m. Models produced a value for detection probability, naive occupancy, and estimated occupancy (considering the detection probability). Models were ranked using ΔAIC scores (Akaike 1974) and only those with a value < 2 were selected for subsequent analysis (Burnham and Anderson 2002). Models that did not converge were omitted. The goodness of fit for the highest-ranking model was assessed using a bootstrap method (1000 replications) (MacKenzie and Bailey 2004). Hedgehog occupancy models were not over-dispersed $\hat{c} = 1.04$, whereas badger models were, resulting in a variation inflation factor (VIF) of $\hat{c} = 2.58$ for badger occupancy models. Standard errors were

inflated by a factor of $\sqrt{\hat{C}}$ = 1.61 for badger models and ranked by quasi-AIC (ΔQAIC) values. Models with ΔQAIC values >2 were excluded.

Multispecies models were used to evaluate interactions between hedgehogs and badgers. The models were built with the best single-species occupancy parameters yielded from the single species occupancy modelling which included pitfall biomass, distance to buildings, arable, and woodland habitat, with parameters for interaction.

6.3 Results

Of the 23 study sites, hedgehogs were detected at 13 sites (Table 6.2), of which badgers co-occurred at 11 of these sites. Badgers were found at 20 sites, of which 9 had no hedgehogs. All 11 sites where both species were found were either livestock farms with predominantly pasture habitat or classified as mixed farms, that had both arable and recently grazed pasture, except for Norfolk where grassland was present but was not grazed by livestock. One study site (Suffolk) failed to detect either species; this was also the largest arable-dominated site included in the study.

Of the 638 unique camera locations included in the occupancy analysis across 23 study sites, 218 were surveyed in 2018 and 420 in 2019. A Pearson's Chi-Squared test was carried out to assess whether the presence of each species and land-use were related. There was significant evidence of an association for hedgehog, (χ^2 (2) = 10.59 p = 0.01) but not for badger (χ^2 (2) = 0.96, p = 0.62). Hedgehogs were detected at 0.0% of the 5 sites classified as arable, 66.7% of the 6 pasture sites, and 75.0% of the 12 mixed farm sites (Figure 6.1). Therefore, hedgehogs were more likely (Chi-squared result) found at pasture and mixed farming sites than arable. Badgers were present at 80.0% of the 5 sites classified as arable, 100.0% of the 6 pasture sites, and 83.3% of the 12 mixed farm sites (Figure 6.1).

Table 6.2 Camera trap derived density estimates for badgers and hedgehogs across 23 sites in England and Wales between April 2018 and August 2019. Site number, number of camera detections and the estimated Day Range used to calculate random encounter model (REM) density estimates are also given. Activity represents the proportion of time animals spent being active and is one of the parameters required to obtain REM estimates. Standard deviations and 95% confidence intervals are given for density estimates.

Site	Land-use	No. of detections	Day Range (speed*activity)	Density per km²	SD	95% CI	No. of detections	Day Range (speed*activity)	Density per km²	SD	95% CI
		Hedgehog					Badger				
1	Mixed farm (college)	11	0.46	4.73	2.18	1.96 - 9.78	54	1.23	4.49	1.42	2.21 - 7.31
2	Mixed farm (college)	44	0.66	12.05	3.19	6.50 -19.42	17	1.03	3.04	0.81	1.51 - 4.87
3	Organic Pasture/Livestock farm	16	0.43	4.05	1.94	2.00 - 9.54	26	1.74	4.16	1.55	1.03 - 7.39
4	Pasture/Livestock farm	2	1.84	0.96	1.41	0.50 – 3.97	42	0.74	15.21	4.26	8.36 - 24.18
5	Mixed farm	9	1.00	3.81	0.48	3.03 - 4.92	36	0.75	7.89	2.52	3.72 - 14.49
6	Mixed farm	0		0			63	0.46	34.1	7.66	20.73 - 52.08
7	Arable (National Trust site)	0		0			22	0.96	4.07	1.50	2.14 - 7.54
8	Arable farm	0		0			3	0.97	1.39	NA	0.71 - NA
9	Mixed farm	14	1.52	1.71	0.78	0.68 - 3.60	33	0.50	15.25	8.31	3.93 - 32.37
10	Arable farm	0		0			8	2.23	1.60	0.60	0.90 - 3.03
11	Mixed farm	31	0.78	7.08	2.61	3.16 - 13.35	1	1.10	1.50	0	0.34 – 0.34
12	Mixed farm	12	0.67	3.91	0.23	3.54 - 4.62	0		0		
13	Arable farm	0		0			57	1.03	7.43	1.79	4.92 - 11.67
14	Mixed farm	0		0			22	0.86	11.07	10.81	2.62 - 43.62
15	Pasture/Livestock farm	10	0.56	2.88	0.92	0.98 - 3.50	1	1.10	0.55	0	0.55 – 0.55
16	Dense woodland, Pasture	0		0			35	1.15	4.64	1.05	2.97 - 7.31
17	Mixed farm	10	0.35	7.61	2.78	4.02 - 14.34	1	1.10	0.59	0	0.59 – 0.59
18	Organic Pasture/Livestock farm	1	0.65	0.30	0	0.27 – 0.27	18	1.33	3.76	1.29	2.02 - 6.61
19	Mixed organic farm	0		0			14	1.95	1.64	0.43	0.98 - 2.62
20	Pasture/Livestock farm	0		0			23	0.51	5.53	3.52	9.76 - 22.76
21	Mixed farm	16	0.40	8.37	4.74	9.95 - 27.73	0		0		
22	Mixed farm (college)	6	0.37	2.05	0.40	1.63 - 3.18	7	1.25	3.3	1.00	1.60 - 5.11
23	Arable farm	0		0			0		0		

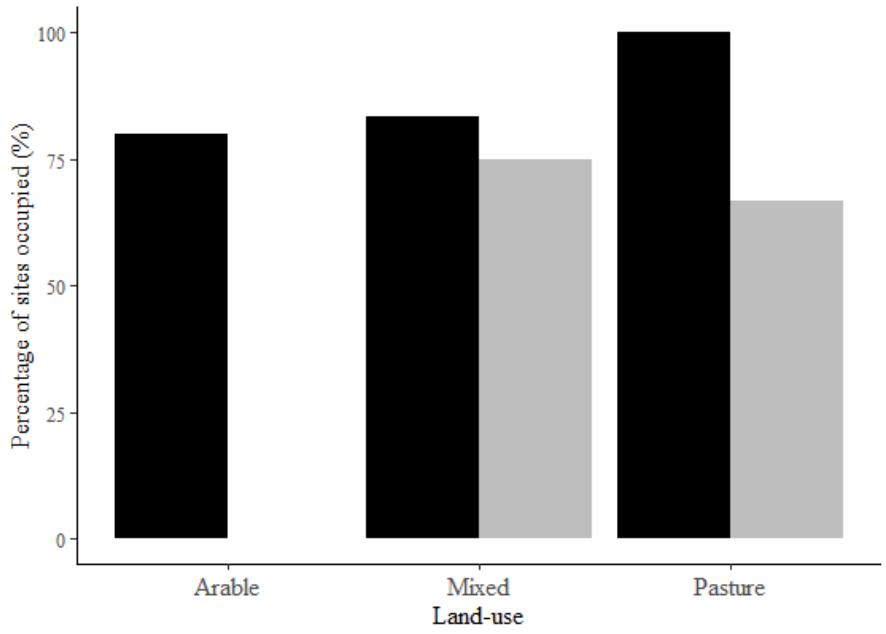


Figure 6.1 The percentages of sites (n = 23) where badger and hedgehog were detected using camera traps, showing an association between land-use and detection of hedgehogs. Black bars = badgers, grey bars = hedgehogs.

Badgers were also recorded at more individual camera trap locations than hedgehogs (n = 158 (24.6%) and n = 64 (10.0%), respectively). At the 11 sites where both species were detected, both species were recorded at just 11 individual camera trap locations (1.7%), suggesting spatial separation within sites at a local level.

6.3.1 Numerical relationship between badgers and hedgehogs

Using site-specific parameters, density estimates were calculated for hedgehog and badger for 11 and 16 sites, respectively (Table 6.2). Hedgehogs and badgers were detected at a further 2 and 4 sites, respectively but, due to low detections rates, average parameters were used to calculate density estimates at these sites. Hedgehog densities ranged from 1.7 to 12.1 km^{-2}. In comparison, badger densities ranged from 1.6 to 15.3 km^{-2}. Hedgehogs were not detected at

10 sites and badgers were not detected at 3 sites. The density of a species that was not detected at a site was assumed to be zero and included in this analysis.

Due to the variability in hedgehog densities, both linear and non-linear approaches were used to investigate the relationship between hedgehog and badger density. Linear regression showed a non-significant negative relationship (Figure 6.2) between the density of both species ($R^2 = 0.09$, $F(1,14) = 2.05$, $p = 0.17$), with the plot indicating a non-linear relationship. As the distribution of hedgehog density was skewed, a non-linear Gamma GLM was carried out, however the model failed to converge and was likely due to the small sample size of sites where both species co-occurred (Montez-Rath et al. 2006).

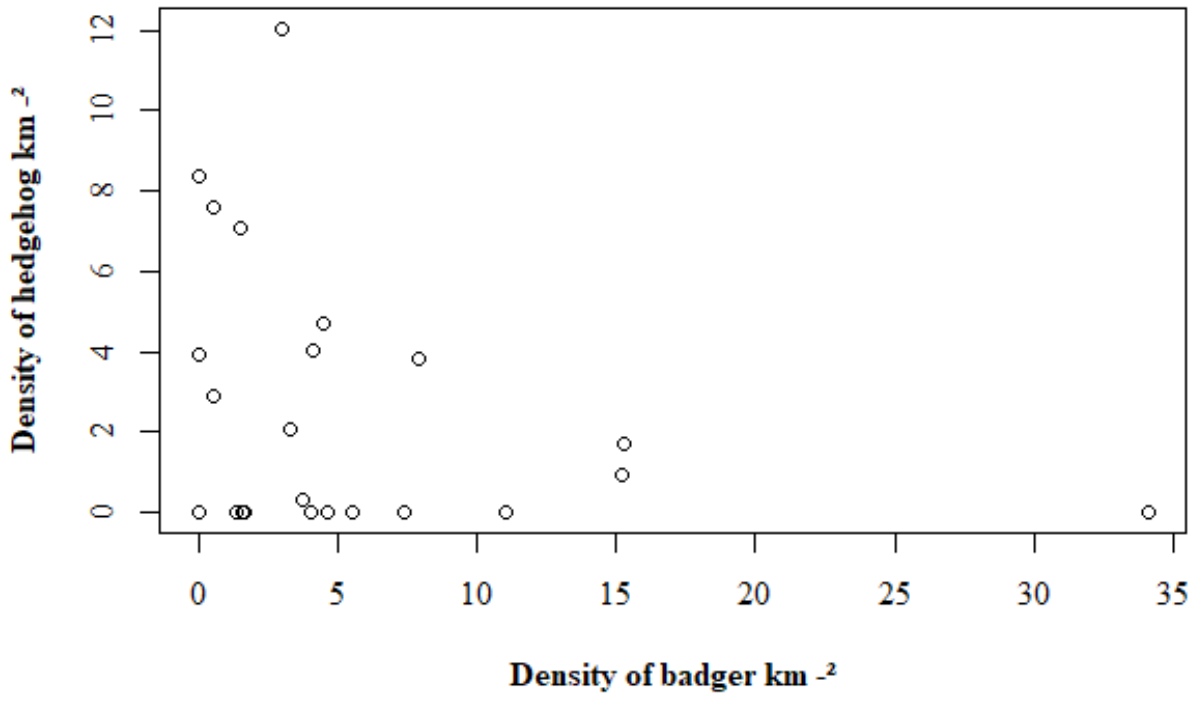


Figure 6.2 Linear regression showing the relationship between badger and hedgehog density (km^{-2}) across 23 rural sites in England and Wales.

6.3.1.1 Land-use

A Kruskal-Wallis test was carried out to compare hedgehog densities between arable, pasture and mixed farming landscapes. The results indicated that the density of hedgehogs differed between the three types of land-use, H(2) = 7.87, p = 0.02. Wilcoxon signed rank pairwise tests were carried out for the three pairs of groups. Hedgehog densities were significantly higher (p = 0.02, adjusted using Bonferroni correction) in mixed farmland landscapes in comparison with arable-dominated landscapes (Figure 6.3). The median density of hedgehogs in mixed farmland was 3.86 km^{-2} compared to 0.63 km^{-2} and 0.00 km^{-2} in pasture and arable dominated landscapes, respectively.

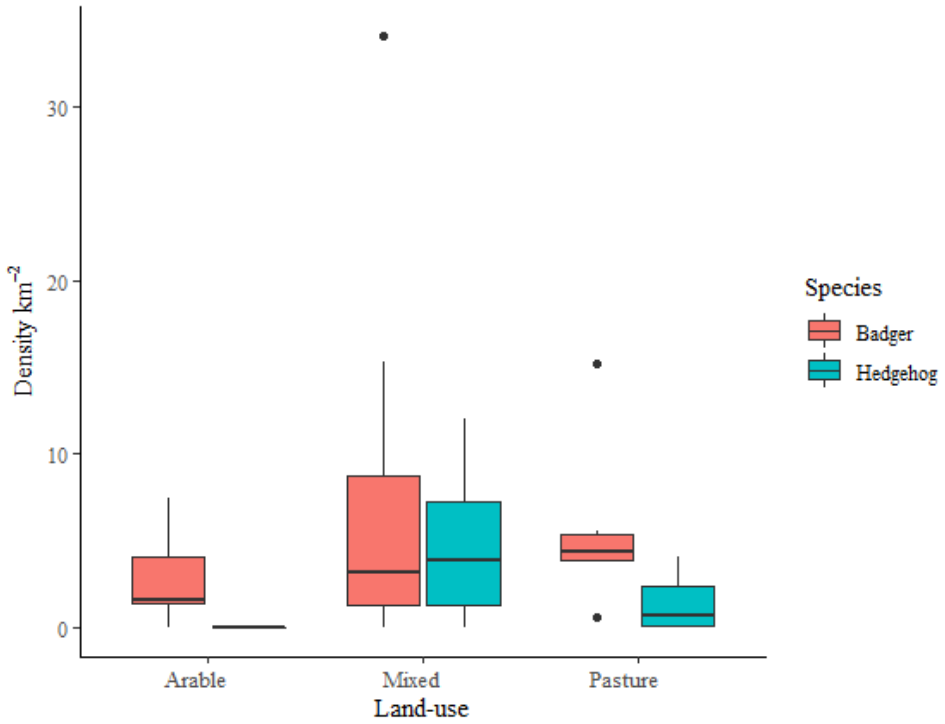


Figure 6.3 Boxplots (median, 25% and 75% quartiles, and 95% confidence interval) of the density of hedgehogs (blue) and badgers (pink) in Arable (n = 5), Mixed (n = 12) and Pasture (n = 6) dominated landscapes.

A one-way ANOVA was conducted to compare badger densities between arable, pasture and mixed farming landscapes. Normality checks and Levene's test were carried out and the assumptions met. There was no significant difference in mean badger density, $F_{2,20} = 0.47$, $p = 0.63$, between the three different types of land-use.

6.3.2 Factors predicting hedgehog occupancy

Naïve hedgehog occupancy across the 638 camera locations was 10.0 %. Of these 64 detections, 42 (66%) were located less than 200 metres from buildings. The number of habitats present within the 10-metre buffer of each camera location ranged from 1- 4 (1.5 ± 0.6). The mean (± sd) distance (m) from locations to each habitat was: arable = 121 ± 157, amenity = 282 ± 247, building = 254 ± 183, grassland = 83 ± 130 and woodland = 96 ± 125. Grassland was the most commonly occurring habitat, present within the 10m buffer of 46% of cameras, followed by followed by arable (40%), woodland (35%), buildings (17%) and amenity grassland (12%).

The best fitting models for hedgehog occupancy (Table 6.3) all included distance to the nearest building (Dist_to_building) and relative pitfall biomass (Pitfall_biomass). Badger presence as a covariate for explaining hedgehog occupancy was not in the top models with $\Delta AIC < 2$.

Detection of hedgehogs was influenced by their relative abundance (Hedgehog_relative), ($\beta = 32.14$, 95% CI= 23.19 – 40.22). In the highest ranked model, hedgehog occupancy was significantly negatively associated with distance to the nearest building (Dist_to_building), ($\beta = -0.47$, 95% CI= -0.77 – 0.14) and pitfall capture biomass (Pitfall_biomass), ($\beta = -2.69$, 95% CI= -5.35 – -0.26). The combined AIC weight of the six best fitting models with an AIC < 2 was 0.19, with all models including distance to nearest building (Figure 6.4) and pitfall biomass (Figure 6.5).

Table 6.3 Summary of single-species occupancy models ΔAIC<2 (N = 638 locations) used to evaluate detection (p) and occupancy (ψ) for hedgehogs. Variable names relate to those depicted in Table 6.1. K = number of parameters; QLL = Quasi Log Likelihood; wi = model weight, cum.wi = cumulative model weight. Final retained model is in bold.

Model	K	AIC	ΔAIC	QLL	w_i	Cum. w_i
p (Hedgehog_relative), *ψ* (Dist_to_building + Pitfall_biomass)	5	941.21	0.00	-465.56	0.05	0.05
p (Hedgehog_relative), *ψ* (Dist_to_building + Dist_to_arable + Pitfall_biomass)	6	941.79	0.58	-464.83	0.04	0.09
p (Hedgehog_relative), *ψ* (Dist_to_building + Pitfall_biomass + Earthworm_biomass)	6	941.92	0.71	-464.90	0.04	0.13
p (Hedgehog_relative), *ψ* (Dist_to_building + Dist_to_grassland + Pitfall_biomass)	6	942.53	1.32	-465.20	0.03	0.16
p (Hedgehog_relative), *ψ* (Dist_to_building + Pitfall_biomass + Beetle_biomass)	6	943.16	1.95	-465.51	0.02	0.18
p (Hedgehog_relative), *ψ* (Dist_to_building + Dist_to_woodland + Pitfall_biomass)	6	943.19	1.98	-465.53	0.01	0.19

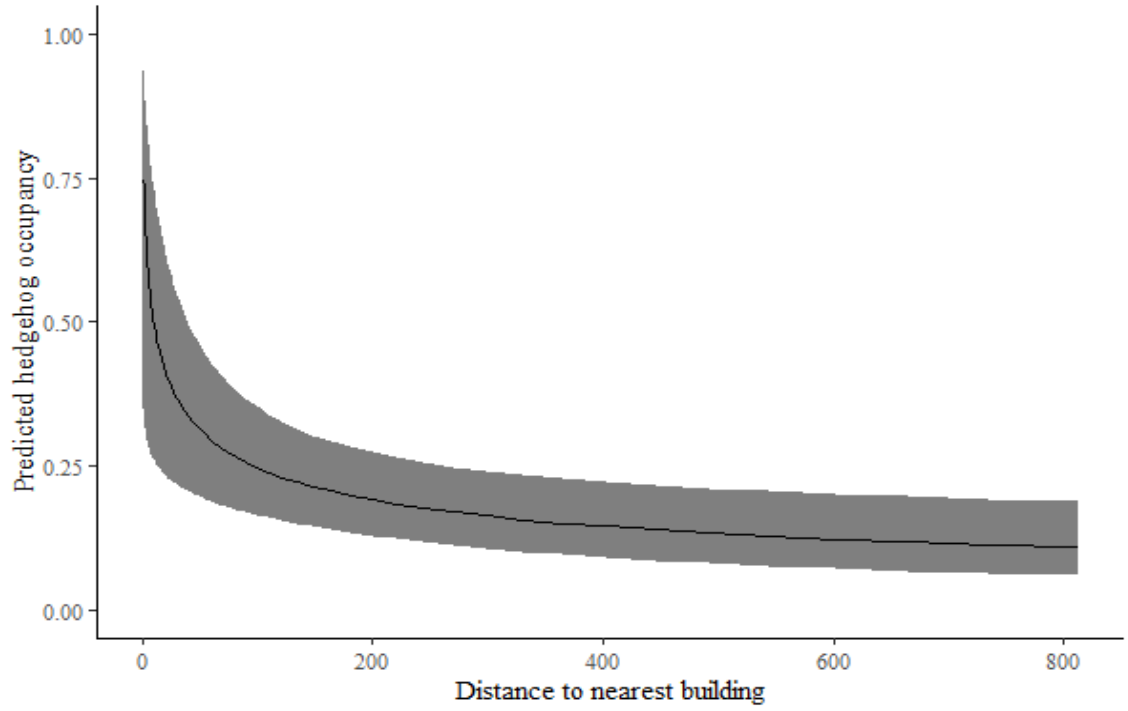


Figure 6.4 Relationship between distance to nearest building (m) and hedgehog occupancy across 638 camera sites in England and Wales in 2018 – 19, based on an occupancy model with distance to nearest building added as a covariate and constant detection.

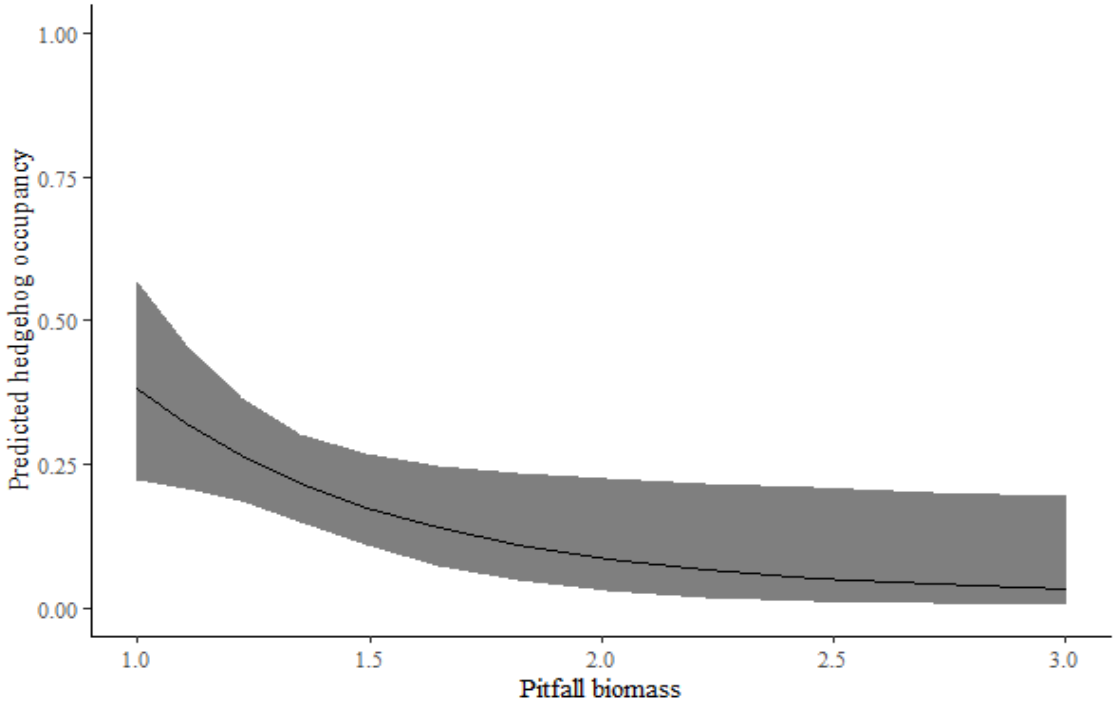


Figure 6.5 Relationship between pitfall capture biomass (g) and hedgehog occupancy across 638 camera sites in England and Wales in 2018 – 19, based on an occupancy model with pitfall biomass as a covariate and constant detection.

6.3.3 Factors predicting badger occupancy

Badgers were found at 158 (24.8 %) of the 638 locations. Of these 158 detections, 80% were located less than 200 metres from arable habitat, whilst only 30 % were located within 200 metres of buildings. There were 196 camera locations in arable habitat, of which 32.1 % were occupied by badgers. Badgers were only detected at 2 of the 41 camera locations located in amenity grassland habitat (4.8 %).

The best fitting models for badger occupancy (Table 6.4) included distance to nearest building (Dist_to_building), distance to arable (Dist_to_arable) and distance to woodland (Dist_to_woodland). Hedgehog presence as a covariate for explaining badger occupancy was not in the top models with $\Delta AIC<2$.

Detection probability was positively influenced by relative badger abundance (Badger_relative) (β= 17.32, 95% CI=13.46 – 21.17). In the highest ranked model, badger occupancy was significantly positively associated with distance to the nearest building (β= 0.43, 95% CI= -0.17 – -0.70), and negatively associated with distance to arable (β= -0.20, 95% CI= -0.32 – -0.07) and distance to woodland (β= -0.17, 95% CI= -0.29 – -0.04). The combined QAIC weight of the eight best fitting models with an AIC < 2 was 0.46, with all models including distance to buildings (Figure 6.6), woodland (Figure 6.7) and arable habitat (Figure 6.8).

Table 6.4 Summary of single-species occupancy models ΔAIC<2 (N = 638 locations) used to evaluate detection (p) and occupancy (ψ) for badgers. Variable names relate to those depicted in Table 6.1. K = number of parameters; QLL = Quasi Log Likelihood; wi = model weight, cum.wi = cumulative model weight. Final retained model is in bold.

Model	K	QAIC	ΔQAIC	QLL	w_i	Cum. w_i
p (Badger_relative), *ψ* (Dist_to_arable + Dist_to_building + Dist_to_woodland)	6	832.33	0.00	-1055.4	0.09	0.09
p (Badger_relative), *ψ* (Dist_to_arable + Dist_to_building + Dist_to_woodland + Earthworm_biomass)	7	833.66	1.33	-1054.49	0.09	0.18
p (Badger_relative), *ψ* (Dist_to_arable + Dist_to_building + Dist_to_woodland + Pitfall_biomass)	7	833.77	1.44	-1054.62	0.08	0.26
p (Badger_relative), *ψ* (Dist_to_arable + Dist_to_building + Dist_to_woodland + Coleoptera_biomass)	7	834.23	1.90	-1055.22	0.04	0.34
p (Badger_relative), *ψ* (Dist_to_arable + Dist_to_building + Dist_to_woodland + Dist_to_grassland)	7	834.26	1.93	-1055.26	0.04	0.38
p (Badger_relative), *ψ* (Dist_to_arable + Dist_to_building + Dist_to_woodland + Dist_to_grassland + Habitats)	8	834.33	2.00	-1055.36	0.04	0.46

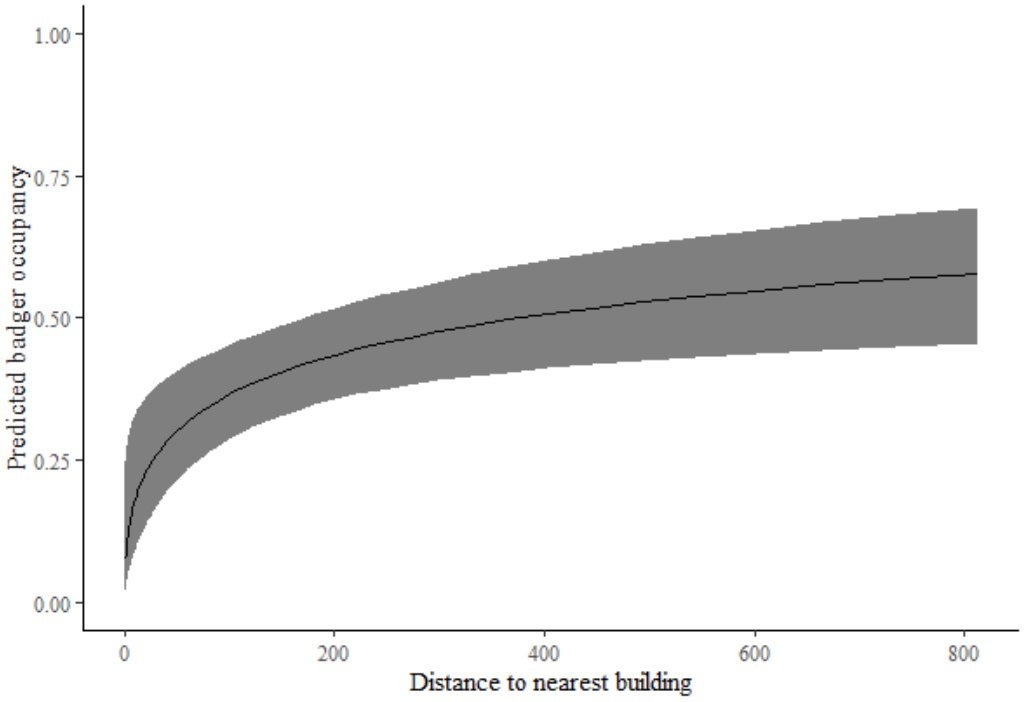


Figure 6.6 Relationship between distance to nearest building (m) and badger occupancy across 638 camera sites in England and Wales in 2018 – 19, based on an occupancy model with distance to nearest building added as a covariate and constant detection.

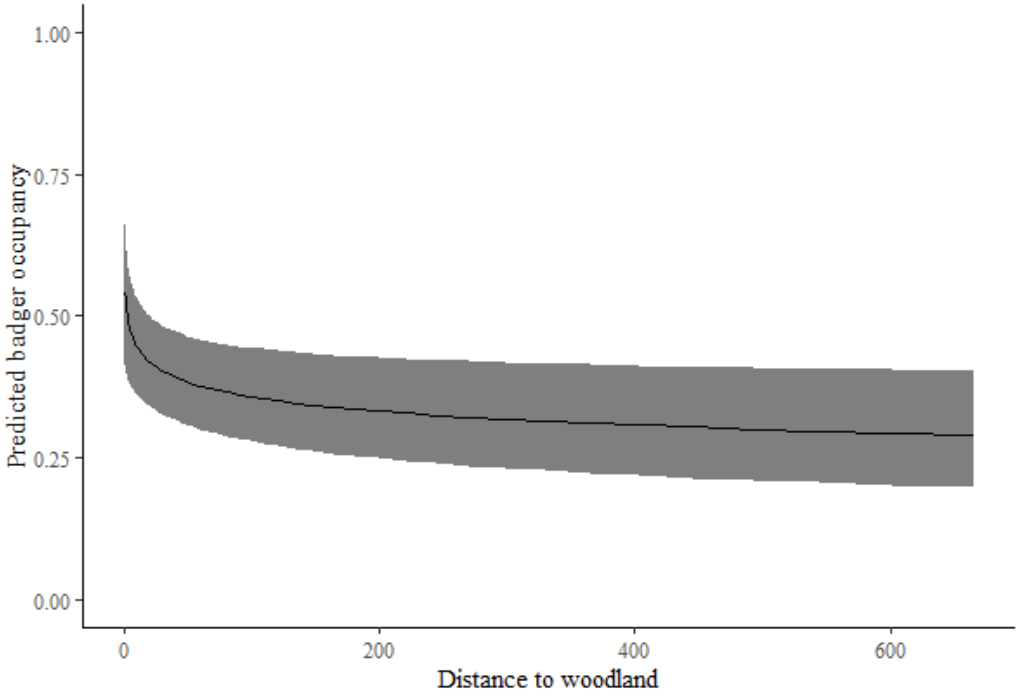


Figure 6.7 Relationship between distance to woodland habitat (m) and badger occupancy across 638 camera sites in England and Wales in 2018 – 19, based on an occupancy model with distance to woodland added as a covariate and constant detection.

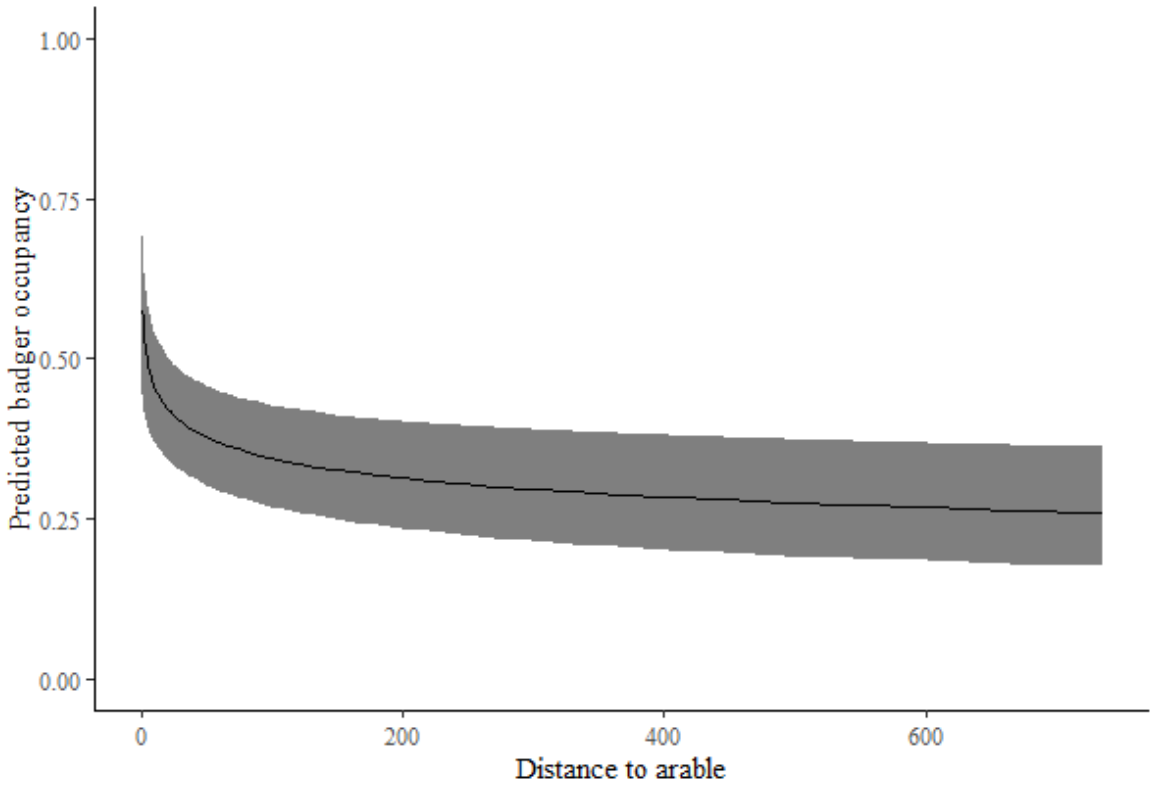


Figure 6.8 Relationship between distance to arable habitat (m) and badger occupancy across 638 camera sites in England and Wales in 2018 – 19, based on an occupancy model with distance to arable added as a covariate and constant detection.

6.3.4 Multi-species occupancy

The environmental predictors of hedgehog and badger occupancy identified above, were incorporated into multi-species occupancy models to test which variables influence species co-occurrence (Table 6.5). The best ranking model showed no species interaction between the occupancy of badgers and hedgehogs (Table 6.5). The majority of cameras did not detect both species during the sampling period, demonstrated by their fine scale separation (see Appendix F) which limited any inference and statistical power associated with this test.

Table 6.5 Multi-species occupancy models used to evaluate species interactions between badgers and hedgehogs. Covariates from single-season-single species occupancy models for hedgehog (Table 6.3) and badger (Table 6.4) were incorporated.

Multi-species model	K	AIC	ΔAICc	QLL	wi	Cum. wi
No Interaction	11	3064.22	0.00	-1520.9	0.45	0.45
Constant	13	3065.98	1.75	-1519.7	0.19	0.64
Dist_to_woodland	12	3066.2	1.98	-1520.85	0.17	0.81
Pitfall_biomass	13	3068.09	3.87	-1520.75	0.07	0.88
Dist_to_building	13	3068.14	3.92	-1520.78	0.06	0.94
Dist_to_arable	13	3068.28	4.05	-1520.85	0.06	1.00

6.3.5 Temporal analysis

A total of 181 hedgehog detections and 482 badger detections were recorded across all sites. Analysis of activity levels showed that both species were principally nocturnal, with peaks in activity between 21.00-03.00hrs and showing a high coefficient of overlap (0.93 ± 0.05 95% CI; Figure 6.9).

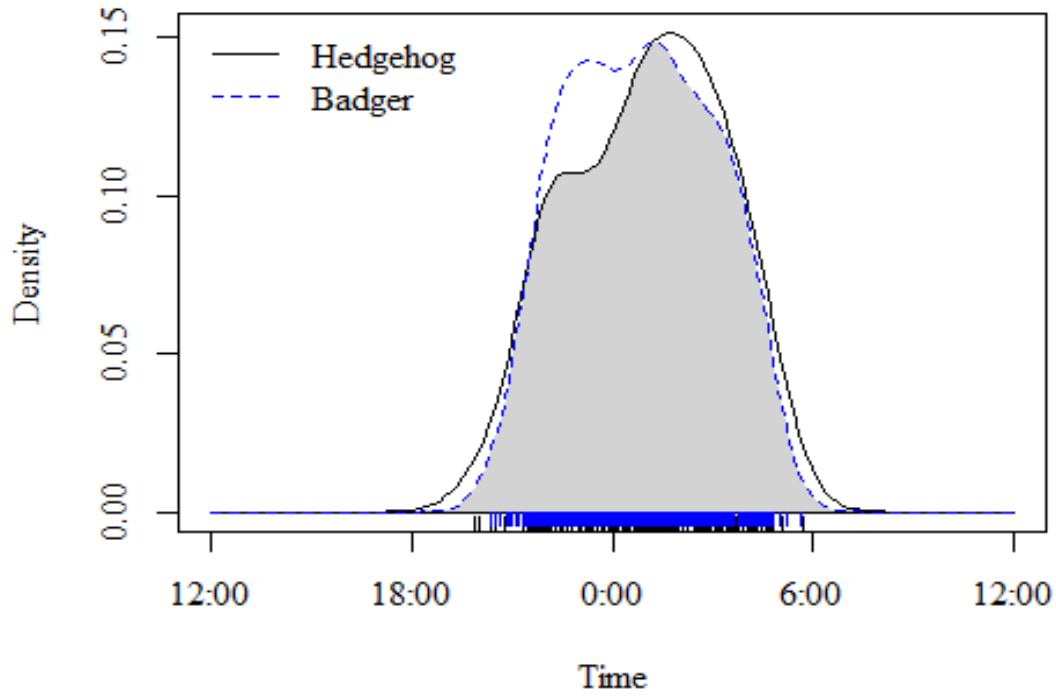


Figure 6.9 Overlap plot comparing diel activity pattern density curves of badger and hedgehog as obtained from camera trap detections (181 hedgehog and 482 badger detections) across 23 sites in England and Wales. The grey shaded area indicates activity overlap and individual detection times at shown along the X axis as rings.

Comparison of badger and hedgehog activity where both species were present, and a sufficient number of detections were recorded (n = 6), showed substantial overlap at all sites (Appendix G). However, as the number of detections per species per site was low i.e. < 100 and > 10, patterns of activity should be treated with caution, as recommended by Rowcliffe et al. (2014) and Lashley et al. (2018).

Of the 181 hedgehog detections, 153 (84.5%) were at camera locations on sites where badgers were also detected and 28 (15.5%) were at camera locations on sites where badgers were not recorded. Consequently, there was no difference in hedgehog activity at camera traps with and without badgers (Wald statistics: $\chi 2 = 0.09$, $p = 0.76$; Figure 6.10), with a high

degree of activity overlap shown (0.87 ± 0.10 95% CI). Therefore, there is no evidence of temporal partitioning between badgers and hedgehogs.

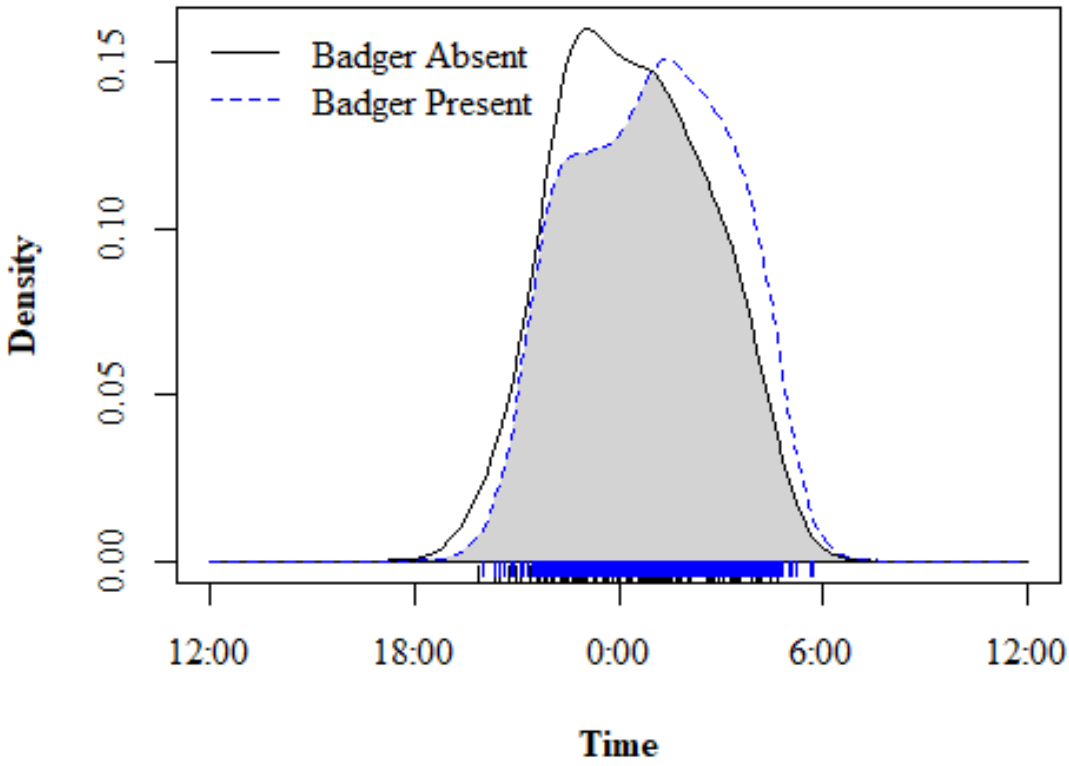


Figure 6.10 Overlap plot comparing diel activity pattern density curves of hedgehog detections at sites where badgers were present (n = 153) and sites where badgers were absent (n =28). The grey shaded area indicates activity overlap and individual detection times at shown along the X axis by rings.

6.4 Discussion

Badgers have been implicated in the decline of hedgehogs in the UK and elsewhere which could represent a negative effect due to direct predation and / or as a consequence of predator avoidance in areas where they would be competing for access to shared resources (Micol et al. 1994; Young et al. 2006; Trewby et al. 2014; Hof et al.2012; Williams et al. 2018). However, the exact relationship between these two species remains open to conjecture, due to the omission of potential explanatory variables such as food availability and habitat

preference at multiple sites that would allow the importance of competition to be assessed, in addition to predation risk. This study found no significant relationship between hedgehog and badger densities. Hedgehogs may be unlikely to persist in areas where badger density exceeds a threshold value, however this will be site specific, based on the availability of habitat, and scale dependent (Yarnell and Pettett 2020). Further, the results of this study showed that hedgehog density was significantly greater in mixed farmland landscapes compared to arable dominated landscapes (Wembridge and Langton 2015). Occupancy modelling supported this finding, demonstrating that hedgehog presence was best predicted by models containing habitat and indices of food availability covariates, with no evidence of significant species interactions. In accord with IGP theory, hedgehogs were associated with prey-poor habitats (Pitfall_biomass), close to buildings, whereas badger occupancy models showed that they were more likely to be present at greater distances from buildings, closer to arable and woodland habitat. Such spatial segregation may negate the necessity for temporal avoidance, consistent with the high overlap in activity observed. These patterns of occupancy at sites where badgers and hedgehogs co-occur could suggest that badgers are excluding hedgehogs (Young et al. 2006), as shown by the association between hedgehog and prey-poor areas (Pitfall_biomass).

Despite this, the models suggest that hedgehog and badger occupancy is best predicted by species-specific habitat preferences. Hedgehog habitat utilisation was consistent at the two sites where badgers were absent, with hedgehog remaining close to buildings. This suggests that these areas support resources that are important to hedgehogs. Future studies should investigate habitat use by hedgehog at sites occupied and unoccupied by badgers to confirm or refute the landscape of fear hypothesis, as the sample size for badger-absent sites was limited to two. An alternative argument is that badgers might be restricting their own

activity by avoiding buildings due to fear of humans (Sévêque et al. 2020), creating pockets of habitat that is suitable for hedgehogs.

Badgers were detected more frequently than hedgehogs and at more geographical locations throughout this study, which is similar to occupancy trends at the 1km scale in rural areas (Williams et al. 2018b). All three scenarios (hedgehogs only, badgers only and both species present) were identified, although sites where hedgehogs were absent were more common than sites where badgers were absent, limiting the ability to measure hedgehog habitat selection at sites without badgers. Badger abundance is estimated to have increased by 88% since the 1980s across parts of England, though abundance in Wales has remained unchanged (Judge et al. 2017), leading to hypotheses that badgers may be partly responsible for concomitant hedgehog declines (Hof et al. 2019). Previous comparisons of indices of hedgehog abundance and badger sett density predicted that hedgehogs would be largely absent from rural areas with ≥ 2.27 badger setts 10 km^{-2} (Micol et al. 1994) and may be excluded from amenity grassland habitat with >10 setts km^{-2} (Young et al. 2006). More recently, sett densities of >5 km^{-2} were shown to exclude hedgehogs from sites at the 1 km^2 scale (Williams et al. 2018). To date, all studies of badger and hedgehog relationships have used badger sett density as a proxy for badger abundance (Micol et al.1994; Young et al. 2006; Williams et al. 2018). However, this approach is likely to be less reliable than using directly comparable estimates of badger and hedgehog density, because of the wide geographic variability in mean social group size (estimated between 2.67 to 7.92 mean number of badgers per social group (Judge et al. 2017) across different land types. The present study provides the first assessment of the numerical relationship between badgers and hedgehogs using locally derived density estimates for both species. In this study, no hedgehogs were found on sites with badgers when badger density exceeded 15.25 km^{-2}, consistent with the argument that areas with high badger activity may exclude hedgehogs.

Therefore, depending on badger group sizes, these estimates are in line with the predictions of Williams et al. (2018), and suggest that although badger presence alone may not be influential on excluding hedgehogs, badger density could be.

A weak negative correlation was observed between badger and hedgehog densities, that explained only 9 % of the variation exhibited. This implies that other factors may be more important in determining the density of hedgehogs across the rural landscape, than badger density alone. This finding is in agreement with Williams et al (2018), who showed that hedgehogs were absent from many 1 km² sites in England and Wales when badgers were absent. Habitat availability is likely an important factor, as the results of this study showed hedgehog densities were higher in mixed farmland sites than in arable. Hedgehogs' association to buildings has been demonstrated at the site level (Williams et al. 2018) and the results of this study show this is consistent at the finer local scale, affecting occupancy too.

Covariates representing both habitat composition and prey availability were included in occupancy models to examine their relative importance in explaining hedgehog and badger presence. Modelling identified that hedgehog occupancy was positively influenced by proximity to buildings, and therefore proximity to amenity grassland habitat, which was correlated with distance to buildings in this study. This is consistent with previous work showing hedgehog preference for amenity grassland and proximity to buildings (Trewby et al. 2014; Young et al. 2006; Pettett et al. 2017; Williams et al. 2018). Hedgehogs have been shown to be associated with buildings, with higher densities found in urban compared to rural areas (Hubert et al. 2011; Williams et al. 2018; Schaus et al. 2020) indicating a preference for urban infrastructure even in rural environments. Their positive association with buildings may reflect the climatic benefits associated with urbanised habitat, providing shelter and refugia (Hubert et al. 2011). Higher hedgehog density in urban areas (Schaus et al. 2020) may be linked with greater food resources in this environment in the form of anthropogenic

resources (Pettett et al. 2017), but studies on the abundance of natural prey in rural and urban habitats are needed to inform whether natural food is also more abundant. Currently, it is often suggested that hedgehogs are in towns to escape predation, but if food is also more numerous, this may provide an alternative hypothesis.

Whilst other studies have shown similar trends regarding the affinity between hedgehog and 'green infrastructure' associated with the built environment (Williams et al. 2018; Yarnell et al. 2014; Schaus et al. 2020; Hof et al. 2012), the question remained as to whether this is driven by a landscape of fear, with hedgehogs seeking refuge in habitat infrequently utilised by badgers, or because it offers the best quality habitat in terms of shelter and food availability. If hedgehogs were primarily choosing their foraging habitat based on the avoidance of badgers, we might expect them to be found in relatively lower quality habitat in terms of prey availability, compared with badgers. For invertebrates captured by pitfall traps, this was found to be the case, as models indicated that hedgehog occupancy was negatively influenced by higher pitfall biomass. Moreover, beetles (Coleoptera) were the most abundant order captured in pitfall traps (Chapter 3) and were identified in the highest proportion of hedgehog scats, in comparison to any other prey type. Arable habitat which often dominates land-use in rural landscapes (Angus et al. 2009), supported the highest abundance of beetles but, despite this, hedgehog occupancy was negatively associated with distance to arable habitat, indicating avoidance which is not fully understood. Furthermore, no hedgehogs were found in arable-dominated study sites, suggesting that this land use type is unsuitable for hedgehogs in England and Wales.

Interestingly, the relative presence of badgers at a location was not a significant predictor of hedgehog occupancy in any of the models, and multi-species models did not identify any species interactions affecting species co-occurrence. This shows that badgers and hedgehogs rarely co-occur in the same space, though it was not possible to determine whether

this is due to species-specific habitat preferences, or some form of landscape of fear. The results are consistent with the idea that hedgehogs are not compatible with the modern arable agricultural landscape, as hedgehogs were detected at most mixed farming and pasture sites, raising questions as to the role badgers play in reported hedgehog population decline in the UK.

Badger occupancy was associated with shorter distances to arable and woodland habitat and, in contrast to hedgehogs, large distances to buildings. Badgers were detected more often in arable habitat (35.2 %) and grassland habitat (27.6 %) than in amenity grassland (1.9 %), possibly reflecting foraging preferences or human activity levels which may be indicative of badger-human conflict (Huck et al. 2006). Badgers are generally more common in rural than urban areas (Harris 1984), but increasing high urban badger sett density areas (Davison et al. 2009) can be comparable with rural densities (Davison et al. 2008). Therefore, perhaps low-level human disturbance in the rural landscape results in avoidance by badgers, demonstrated by their evasion of amenity grassland and built-up habitats associated with proximity to humans.

In addition to the study showing that badgers and hedgehogs were spatially separated at the local habitat scale, this is the first study to compare potential temporal partitioning of both species. As hedgehogs have shown short-term physiological responses to badger odour (Ward et al. 1996; Ward et al. 1997) and alteration of their movement in the presence of badgers (Hof et al. 2012), it is conceivable that they could utilise temporal avoidance to reduce encounter rates with badgers. However, as expected, daily nocturnal activity was highly overlapping, revealing no significant difference in activity between species. At sites with badgers, hedgehog and badger activity mostly overlapped, and when comparing across sites, there was no difference.

The absence of temporal avoidance may suggest that spatial partitioning is sufficient for reducing competition for both species, and predatory pressure on hedgehogs. Therefore, this study has demonstrated a clear difference in habitat use, meaning that at the small spatial scale, these two species are spatially segregated, as previously indicated (Hubert et al. 2011; Pettett et al. 2017). Hedgehogs appear to occupy sites with proportionately low arable habitat and show a preference for proximity to buildings. Until now, studies have not considered that hedgehogs and badgers may be exhibiting different preferences, providing an alternative hypothesis to the landscape of fear caused by badgers. To differentiate these further, work is required assessing hedgehog habitat preferences in badger-free sites or, alternatively, assessing hedgehogs' use of urban sites with badgers. Potentially, more caution should be taken when inferring 'landscapes of fear' driven by predation, as other factors such as habitat selection and prey availability need considering simultaneously. Additionally, the accessibility of the rural landscape likely differs between each species, as badger activity may be somewhat constricted by the location of their setts. If suitable habitat for setts were located away from towns, this again would create pockets of space for hedgehogs. This is especially important for understanding the population dynamics for declining species such as the hedgehog.

6.4.1 Limitations

By design, this study aimed to investigate three species mixes of badger and hedgehog (both species, badger only and hedgehog only) equally, though this was logistically challenging at the very fine local scale due to the scarcity of areas where hedgehogs were abundant and badgers absent. Also, at the camera level, there were very few locations where both species were detected. A more balanced study design could be achieved by the addition of more hedgehog only sites and this would increase confidence in the findings. Additionally, most

study sites had relatively low densities of hedgehogs and badgers ranging between 1.7 - 12.1 km^{-2} and 1.6- 15.3 km^{-2} respectively. For example, mean urban hedgehog densities of 32.3 km^{-2} have been recorded (Schaus et al. 2020) and badger densities of up to 25.3 km^{-2} in south-west England (Rogers et al. 1997). Additional sites including those of higher densities of each species may strengthen the relatively weak negative correlation shown between densities of badgers and hedgehogs in this study.

There were also limitations in the measures of prey availability, as it was not possible to quantify if the invertebrates that were caught were available for each species. Moreover, there are other food resources that were not quantified, such as higher vertebrates and plant species which are often consumed by badgers (Kruuk and Parish 1981; Shepherdson et al. 1990). A complete assessment of potential food items would more accurately reflect local food availability and is recommended for future studies of this kind, as opposed to extrapolating the invertebrate indices by habitat, as in this study. The feasibility of this is hampered by the labour-intensive nature of these studies and the necessity to survey many sites to ensure a representative study.

6.4.2 Further research

This study highlights the importance of incorporating measures of prey and habitat availability when studying interactions amongst IG predator and prey species. Understanding the mechanisms that facilitate their coexistence provides a better understanding of how these species are interacting with one another and the intensity of these interactions. Generally, quantitative data for IGP is sparse, particularly in mammals, and although field studies of this nature are challenging, more quantitative evidence is needed to establish under what conditions coexistence occurs.

This study showed that both species can co-occur at the landscape scale on pasture and mixed farming landscapes, but hedgehogs cannot occur in arable landscapes. Furthermore, locally within sites where the two species co-exist, they are separated almost exclusively, with hedgehogs being found near buildings and badgers being found away from buildings. This fine scale spatial segregation exhibited by badgers and hedgehogs may be driven by habitat selection, although it was not possible to rule out whether a landscape of fear exists and, to do so, a more balanced study of each species mix would be required. Nevertheless, the spatial niche segregation identified may also be important for reducing competitive and predatory interactions between the two species. To further understand the dynamic balance between predation and competition between badgers and hedgehogs, it would be useful to quantify the effects of food availability on predation rates. This was beyond the scope of the present study and would require long term monitoring of hedgehog and badger abundance, alongside food availability.

6.4.3 Conclusions

Many studies have documented a negative association between badgers and hedgehogs in the UK and badgers have been implicated in hedgehog declines. This study sought to investigate this association further by including information on habitat associations and prey availability and showed that patterns of hedgehog and badger occupancy were strongly related to the availability of different habitats. Data on activity levels of the two species suggest that spatial segregation may provide the mechanism for reducing competitive and predatory risk, negating the need for temporal avoidance. Further, these results confirm low hedgehog occupancy in the rural landscape of the UK, likely due to a combination of factors, rather than an increase in badgers *per se*. Certainly, both species can co-occur in mixed farming and pasture landscapes, though arable landscapes appear unsuitable for hedgehogs. More broadly,

this study highlights the need for including habitat and other resources such as food into studies investigating intra-guild predation in mammals, to ensure that the roles of predation and competition are not overemphasised.

Chapter 7 General discussion – The relationship between hedgehogs and badgers across the rural landscape

7.1 Overview

This thesis set out to investigate what mechanistic factors facilitate the coexistence of the European hedgehog *(Erinaceus europaeus*) and their intra-guild predator, the European badger (*Meles meles*) which has been implicated in their decline. Using the framework of Intra-Guild Predation (IGP), these two species exhibit predation and compete for shared prey resources (Polis et al. 1989), namely invertebrate prey, that has been shown to vary in its availability between habitat types and locations (Hof and Bright 2010). In this study, the numerical relationship between badgers and hedgehogs was negative but weak, suggesting that both species can coexist where badger densities are relatively low. Whilst previous studies have demonstrated that high badger density can lead to the exclusion of hedgehogs from an area (Doncaster 1992; 1994; Young et al. 2006), there are also many sites at the 1 km scale without badgers where hedgehogs are still absent such as upland habitat that is rarely inhabited by hedgehogs (Williams et al. 2018).

In this study, hedgehog density was highly variable amongst sites, which suggests that site-specific characteristics other than badger density may be influential. These factors may be related to major land use types at the 1 km scale, with the presence of hedgehogs being more likely in areas dominated by pasture or mixed farmland compared to arable. Several sites in this study supported both species, although they were representative of areas with relatively low densities of both species. Where both species can co-occur, habitat and food availability are likely to be important for facilitating their coexistence (Lesmeister et al. 2015). Moreover, in the present study the spatial distribution of hedgehogs and badgers revealed that despite both species being present at the site scale, there was clear segregation at the finer habitat scale. The main predictor of hedgehog presence and density was proximity to buildings, which is potentially important for reducing predator-prey interactions, as badgers avoided buildings (Poel et al. 2015).

There is some evidence that suggests hedgehogs may exhibit a 'landscape of fear' response in the presence of badgers (Doncaster 1992; Hof et al. 2012). The results of the present study show fine scale spatial segregation that is strongly associated with habitat type, which is consistent with this hypothesis. However, it was not possible to ascertain whether these patterns reflected species-specific habitat preferences or avoidance of badgers by hedgehogs. Nonetheless, in the present study there were too few co-occurrences of badgers and hedgehogs to be certain, necessitating further investigation. If there was a 'landscape of fear' effect, then hedgehogs might be expected to shift their temporal activity patterns (de Satgé et al. 2017), but this was not observed in the present study. However, this may not be necessary if the two species are sufficiently spatially segregated within the local environment.

Additionally, dietary niche overlap between badgers and hedgehogs was assessed to determine the potential for competition for food. Despite high overlap in dietary breadth, with the two species consuming many of the same food items, the composition of individual hedgehog and badger scats was distinctly dissimilar throughout all seasons assessed, demonstrating that they utilised available prey differently and suggesting dietary niche partitioning. This might result from individual prey preferences of badgers and hedgehogs, or alternatively, may reflect differences in fine scale habitat selection that in turn influence the encounter rates for different prey types (Sih and Christensen 2001). The generalist nature of both species may provide opportunities for niche partitioning through prey switching (Kang and Wedekin 2013) and may therefore facilitate coexistence within the IGP relationship. This is likely to depend on the densities of both species (Anderson and Semlitsch 2014), which is highly variable at the local scale. In the present study both species were at relatively low densities of <10 km^{-2} at the majority of sites, so the potential for one to compete and deplete the food resources for the other was marginal. Interestingly however, similar to hedgehogs, other generalist invertebrate feeders such as farmland birds have also declined (Chamberlain

et al. 2000; Benton et al. 2002). Therefore, declining invertebrate food resources in rural Great Britain may have increased potential competition and predation between hedgehogs and badgers. A study including a large number of sites with varying abundances of invertebrate prey would be required to investigate whether the level of competition between badgers and hedgehogs varied with available resources.

The present study attempted to assess the importance of hedgehogs in the diet of badgers. Hedgehog DNA was only detected in a single badger scat out of eighty assessed, showing that the frequency of occurrence of hedgehogs in the diet of badgers was low across the two study sites where both species were known to be present. However, these findings are likely influenced by low densities of both species and spatial separation, reducing the likelihood of badgers encountering and predating hedgehogs.

The present study focused only on rural environments, and so other habitat types and associated badger and hedgehog densities were not represented. Therefore, it would be useful to include more study sites in other landscapes (e.g., suburban environments) where the two species co-occur. The patterns shown in this study may apply to the rural agricultural countryside and not urban areas, where the relationships may differ due to differing food availability and interspecific densities. Furthermore, the present study assessed a limited number of study sites and so looking at more sites would help determine whether the observations made are representative.

In conclusion, this study identified both dietary and spatial partitioning as mechanisms that likely facilitate the coexistence of badgers and hedgehogs in rural landscapes. Impacts of competition are limited due to both species being generalists and exhibiting some dietary differentiation, predation rates of badgers on hedgehogs appear low potentially due to low densities and fine scale spatial separation which appears to be driven

by species specific habitat preferences with hedgehogs being found closer to buildings than badgers. These clear patterns can be explained by either a 'landscape of fear' whereby hedgehogs avoid badgers, or differential habitat selection as both species exhibit their habitat preferences. However, surveying across a wider range of habitats and densities would be required to determine this. Results of the present study suggest that these species are more likely to co-occur in mixed farmland or pasture dominated farmland (Figure 6.1). This chapter presents the evidence from the complete study that supports the above conclusions. The findings of this work are discussed in relation to IGP theory and previous research, to demonstrate how this work has progressed the understanding of hedgehog-badger relationships, by discussing mechanisms that likely facilitate the coexistence of these two species. These findings may inform future management efforts towards promoting the persistence of hedgehogs within the rural landscape and highlight opportunities for further research that enhance our understanding of hedgehog declines.

7.2 Numerical relationship between badger and hedgehog densities at the local scale

This study sought to investigate the negative relationship between badgers and hedgehogs described in other studies, which includes the prediction that hedgehogs will be excluded from areas of high badger density (Young et al. 2006; Williams et al. 2018). In the present study, the relationship between badger and hedgehog densities, assessed across twenty-three rural sites, was found to be negative, albeit weak, suggesting a non-linear relationship, with hedgehogs present at several sites where badger densities were below 15.25 km^{-2}. Hedgehog densities were highly variable amongst sites and likely depend on the local context, as significantly higher hedgehog densities were associated with mixed farmland sites in comparison to arable sites, demonstrating that other local factors are likely to be at play in determining rural hedgehog abundance.

Previous studies have typically calculated hedgehog densities by capture, mark, recapture methods and have relied on using variations in sett density as a proxy for badger density (Young et al. 2006; Williams et al. 2018). More recently, the reliability of the Random Encounter Model (Rowcliffe et al. 2008) for estimating hedgehog densities has been demonstrated (Schaus et al. 2020), and this was implemented within the present study to provide estimates of the numbers of badgers and hedgehogs at each study site.

The weak negative relationship between badger and hedgehog numbers identified here may reflect the observation that several study sites supported both species and others supported low densities of only one species. The inclusion of sites supporting extremely high densities of either species may have altered the relationship observed here, perhaps reflecting the strongly negative relationship described by Young et al. (2006) more closely. However, the relatively low hedgehog densities observed in rural locations in the present study are consistent with earlier reports that hedgehog densities are frequently higher in urban areas (Hubert et al. 2011; Schaus et al. 2020). Hedgehog occupancy at the 1 km scale has been predicted to be as low as 22 %, demonstrating that hedgehogs do not occupy large proportions of the rural landscape, even in the absence of badgers (Williams et al. 2018). In the present study, finer scale analysis at camera sites showed that hedgehog occupancy was just 10 %, whereas badgers occupied 30 % of sites, showing how localised their distribution can be. This suggests that other factors related to the local environment have the potential to have an influence on hedgehog densities than badgers. Specifically, local site level conditions likely influence whether the two species can co-occur. Where both species were identified together at the 1 km scale, this tended to be in mixed farmland landscapes, as opposed to arable dominated areas where only badgers were found, although still at low densities.

7.3 Frequency of badger predation on hedgehogs

Dietary assessment allowed potential incidences of hedgehog predation by badgers to be quantified over a period of a year and a half, across two populations. The observed frequency of potential predation events was low, with only one badger scat (from Brackenhurst) out of a total of 80 scats containing hedgehog DNA. This could indicate a predation or scavenging event (Sheppard and Harwood 2005) and suggests that in these two co-occurring populations, badgers consume hedgehogs infrequently. In the present study, densities of both species were low, and perhaps at higher densities the encounter rate between the two species would be greater potentially resulting in more predation. However, for predation rates to be calculated and extrapolated over the year, a greater sampling effort would be needed and longer-term monitoring would be necessary to estimate whether predation is likely to influence hedgehogs at the population level. Higher predation rates have been observed in studies of other hedgehog populations (Doncaster 1994; Bearman-Brown et al. 2020) which demonstrates that the frequency of predation varies at the local scale and is likely dependent on the density of both species.

7.4 'Landscape of fear' hypothesis

At the site scale, badgers and hedgehogs were found coexisting, especially where land use was classified as mixed farming. This landscape is likely to be associated with a heterogenous habitat structure, which has been shown to facilitate coexistence amongst other intraguild-competitors (Janssen et al. 2007). However, comparison of hedgehog and badger activity within each site, at the finer patch level, revealed spatial segregation between badgers and hedgehogs across multiple sites where both species co-occurred. Very few camera positions were visited by both species throughout the survey period, demonstrating that hedgehogs and badgers rarely coexist at the patch level (Appendix F), and this may be important for reducing the risk of predation and helping to limit competitive interactions too

(Krauze-Gryz et al. 2012). Moreover, there were no instances of both species being detected on the same camera at the same time, although footage of this does exist elsewhere (Wildlife Online n.d.).

Previous studies have stated that hedgehogs likely exhibit a 'landscape of fear' response, remaining closer to linear features when foraging in the presence of badgers (Hof et al. 2012). Also, the abundance of invertebrate prey was shown to be significantly lower in sites occupied by only badgers (Hof et al. 2012), which is consistent with the possibility that hedgehogs may have been removed from some rural areas by competitive exclusion. To investigate these possibilities, hedgehog behaviour in the presence and absence of badgers must be compared.

In the present study, hedgehogs were present at two of the twenty-three sites where badgers were not identified. Across these badger-free sites, hedgehog activity was again predominately clustered around buildings (Appendix F), although hedgehogs did use woodland and arable habitat that was infrequently visited by hedgehogs on sites where both species co-occurred. Generalisations as to whether hedgehog space-use is affected by the presence of badgers cannot be ascertained from these limited study sites. However, the patterns of habitat use by hedgehogs appear consistent across the twenty-three sites suggesting that the patterns observed in this study may reflect general habitat preferences of rural hedgehogs co-occurring with badgers.

In the present study, habitat, and correlates of invertebrate food availability, were the best predictors of hedgehog presence, as opposed to the presence of their potential predator, badgers. Hedgehog presence was strongly associated with proximity to buildings that commonly co-occurred with amenity grassland habitat which has been shown to be preferred by hedgehogs (Micol et al. 1994; Parrott et al. 2014). Moreover, amenity areas supported a

relatively high abundance of earthworms, though lower pitfall trap biomass. This suggests that hedgehogs utilise habitat rich in earthworms, though this is possibly a poorer habitat in relation to the availability of beetles, their most common prey item. Inversely, badgers were found to be negatively influenced by proximity to buildings and grassland habitat, suggesting that they did not frequently utilise the same habitats as hedgehogs. Therefore, this spatial segregation may potentially reflect the natural habitat preferences of both species, as opposed to hedgehogs displaying a 'landscape of fear' response. Nevertheless, due to the low number of co-occurrences of badgers and hedgehogs in the present study, more sites would be needed to confirm this.

Another consideration that may provide an alternative explanation for the habitat choices by badgers and hedgehogs is the availability of suitable nesting/denning habitat. Badgers are known to select woodland, scrub and hedgerows for sett location, supporting higher sett densities than other open habitat including arable, pasture and amenity grassland habitat (Feore and Montgomery 1999). Whereas, hedgehogs have been shown to nest in close proximity to hedgerows, roads and woodland features (Bearman-Brown et al. 2020), with urban environments suggested as providing good day nesting sites (Pettett et al. 2017). In the present study, distance to setts or hedgehog nests was not measured though their location in the landscape may partly describe the local occupancy of each species. Therefore, the avoidance of amenity grassland habitat by badgers may be due to the proximity to woodland habitat where setts are commonly found (Feore and Montgomery 1999). Similarly, the lack of hedgerow and connected woodland within hedgehog home ranges may partly explain their affinity with buildings and amenity grassland habitat. Finer scale habitat features associated with these habitats, including gardens, may provide hedgehogs with refuge from predation, suitable nesting habitat and an abundance of food, whether it be from natural sources and/or supplementary feeding (Pettett et al. 2017; Schaus et al. 2020).

Under IGP theory, hedgehogs might be expected to alter their temporal activity patterns in the presence of badgers (Palmer et al. 2017). The present study is the first to quantify the degree of temporal overlap between badgers and hedgehogs at the same locations, and reveals high temporal overlap between their activity patterns. Hedgehog activity was similar amongst sites occupied by badgers and those where badgers were absent. This suggests that hedgehogs do not alter their activity patterns in response to badger presence, at least at low densities where perhaps the risk of predation is lower. Short-term physiological and behavioural responses in hedgehogs have been observed in response to badger odour (Ward et al. 1996; Ward et al. 1997), though the present study demonstrates that at a site level hedgehogs do not necessarily temporally avoid badgers. Rather hedgehogs may utilise other methods of niche partitioning that likely facilitate their coexistence such as spatial partitioning as demonstrated here (Appendix F and Chapter 6).

7.5 Competition for food

Dietary niche partitioning is another mechanism that can facilitate the coexistence of intra-guild competitors (Tsunoda et al. 2017). In the present study, dietary analysis of co-occurring badgers and hedgehogs was used to assess the level of dietary niche overlap and the potential for competition for shared food items. The novelty of this work is not only in the methodological approach, which provides a highly sensitive technique for identifying prey items (Sousa et al. 2019), but also in the assessment of the seasonal consumption of prey by both species in the same locations. The results identified the broad dietary breadth of badgers and hedgehogs, and considerable overlap between the two species. This demonstrates their generalist nature, which may be important for weakening the potential for dietary competition (Kang and Wedekin 2013). For hedgehogs, as the subordinate intraguild-prey species, this broad diet may also provide opportunities for prey switching (Andersen et al. 2017), to further reduce competition for shared resources. However, the results of the present study

revealed that hedgehog and badger diet composition was significantly dissimilar throughout the year, indicating that they select prey differentially, perhaps as a result of foraging in different areas within a site or due to variations in prey handing, capture or palatability (Westoby 1974). Therefore coexistence within the IGP relationship may be more likely when both intraguild-predator and prey are generalist feeders (Kang and Wedekin 2013).

Several prey items were common to both species' diets including beetles, earthworms and slugs, and are therefore likely to be important prey items for both (Deagle et al. 2019). Given the low densities of badgers and hedgehogs across the two sites, the possible impact of either species on these common prey types is likely to be limited, again reducing the chance of competition at these sites. This highlights the potential importance of intra-guild predator densities on the strength of competitive interactions within the IGP relationship (Anderson and Semlitsch 2014).

By assessing the diet of both species in areas where they co-occur, it was possible to also compare resource use. Prey availability is logistically challenging to measure, particularly when assessing resource availability of two generalist and opportunistic feeders, as this requires all possible dietary items to be assessed (Rutz and Bijlsma 2006). For badgers and hedgehogs, this would require many groups of potential prey to be assessed simultaneously using a range of methods, that would each introduce biases and ultimately may not represent availability. Invertebrate prey is frequently the principal food type for both species (Roper 1994; Yalden 1976; Wroot 1984; Kruuk and Parish 1981; Cleary et al. 2009), as shown by the dietary assessment component of this study. Therefore, selection of invertebrate prey is likely to be a meaningful indication of the strength of competition between the two species.

Hedgehogs consume invertebrate prey in disproportionately greater amounts than any other food type (Yalden 1976; Wroot 1984) and this was evidenced in the present study, emphasising the importance of invertebrate prey availability in habitat occupied by hedgehogs. Diet selection indices showed that neither badgers nor hedgehogs consumed invertebrate prey relative to availability. Broadly, the abundance and biomass of invertebrate prey varied seasonally, at the site level, and also between habitats. This demonstrates that environmental characteristics at the local scale are important drivers of prey availability (Sperry and Weatherhead 2009). Therefore, dietary selection will likely vary between populations, and this may also affect the strength of competition between badgers and hedgehogs. In the present study both species showed strong positive selection of Haplotaxida (earthworms), although methodological limitations may have underrepresented this prey type. Nevertheless, both species consumed earthworms at high frequencies, demonstrating the importance of this prey item irrespective of season and site.

There was, however, no uniform pattern in prey selection in the present study, which varied between species, season, and site, across different Orders of invertebrate prey. This suggests that prey selection is complex and therefore difficult to disentangle. Dissimilarity in the diet may be steered by the utilisation of different habitats observed in the study, which in turn dictates the accessibility of different prey, including their encounter rates (Padial et al. 2002). For example, hedgehogs infrequently utilised arable habitat, abundant in beetles, though this may be compensated for by the overall availability of beetles and additional prey types in other habitats. Further investigation of prey preferences would require a more focused assessment that could possibly be achieved through feeding experiments as opposed to field observations. Nonetheless, the present work has shown that prey availability is a crucial aspect of the IGP relationship, and that dietary partitioning is likely an important mechanism that promotes coexistence amongst hedgehogs and badgers.

7.6 Limitations

The twenty-three rural sites selected for the present study were chosen to represent areas occupied by both species at the finer habitat scale, and areas where each species is found separately. By selecting sites that were thought to support both species, those supporting higher densities of either species were likely excluded from the study. Badger-free areas were far scarcer than hedgehog-free areas, reflecting the low persistence of hedgehogs within the rural landscape (Williams et al. 2018). The present study found relatively low levels of activity of both species across several sites which arguably constrained analysis of the numerical relationship between badgers and hedgehogs, which would have benefited from additional sites, particularly those with high densities of either species. Logistically, the number of sites that could be included in the study had to be balanced against the resources available, resulting in a compromise between sample size and the survey effort required at each site.

Deciphering a suitable measure of food availability is challenging and, in this study, invertebrate prey was determined to be a suitable proxy for food availability, as it was not possible to combine multiple survey methods assessing the availability of different broad taxonomic groups. However, the trapping rate produced by pitfall trapping, differed between taxa (Hancock and Legg 2012) and may have caused some, including earthworms, to be underrepresented. Consequently, diet selection of some prey types should be interpreted cautiously. Diet selection by both species was considered by assessing the diet of co-occurring badgers and hedgehogs from two sites in conjunction with food availability, showing that both species can have strong prey preferences. However, this assumed that both species had equal access to the resources available across the whole site, and the spatial distributions of each species (Appendix F) suggest that this is not the case. Badgers and

hedgehogs are therefore likely to encounter prey and food types at different rates, depending on the habitats they utilise.

7.7 Conservation of hedgehogs

The results of the present study show that hedgehogs can coexist with their intraguild-predator, though this may be dependent on a multitude of factors and the scale at which coexistence is considered. Fine-scale spatial segregation appears to facilitate co-occurrence within the rural landscape, with seemingly infrequent use of the same locations which will also limit costly encounters. The results show that hedgehogs avoided arable habitat, despite it being relatively abundant in one of their principal prey items, Coleoptera. Hedgehogs have been shown to utilise grassy field margins, maintaining their proximity to hedgerows in the presence of badgers (Hof et al. 2012). These features increase heterogeneity within arable habitat, improving connectivity, providing refuge and reasonably therefore, increasing the suitability of arable habitat for hedgehogs (Yarnell and Pettett 2020). This emphasizes the need for the maintenance and restoration of valuable habitats such as grassy field margins and hedgerows for the benefit of hedgehogs amongst other species (Benton et al. 2002).

Moreover, another factor that may influence the likelihood of hedgehog presence within the rural landscape is the availability of prey, which can also influence the strength of competitive and predatory interactions. Differences in dietary preferences may reflect palatability, encounter rate at a finer spatial scale and the ability of these species to capture different mobile prey types (Westoby 1974), requiring further investigation. Nonetheless, these dietary dissimilarities may be important for reducing competition and promoting coexistence between badgers and hedgehogs across mixed farmland habitat. Conservation efforts should therefore employ a bottom-up approach, managing land and creating habitat that best supports the prey utilised by hedgehogs (Yarnell and Pettett 2020). Furthermore,

promoting the recovery of invertebrate biodiversity is likely to benefit a wide range of other species including farmland birds, which also frequently rely on invertebrate prey (Benton et al. 2002).

7.8 Further research

The results of the present study have progressed our understanding of the mechanisms that likely facilitate coexistence amongst badgers and hedgehogs across rural landscapes in England and Wales. However, this has invoked further research questions that would be of interest in the future. Habitat was the best predictor of hedgehog occupancy, and this revealed that hedgehogs avoided arable, woodland and grassland habitats, and were found more frequently near to buildings. This, coupled with diet analysis, suggests that they utilise areas with high earthworm abundance and therefore these observed patterns in habitat utilisation may reflect their preferences in the absence of badgers. However, it would be beneficial to include further study sites where there are no badgers, to reaffirm whether hedgehogs do or do not utilise other habitats in the absence of their principal predator, and also areas with higher hedgehog density such as urban areas both with and without badgers. This would help ascertain whether hedgehogs do behave in accordance with the 'landscape of fear' hypothesis at the habitat scale. Similarly, dietary analysis revealed partitioning between badgers and hedgehogs, although diet was studied at locations where both species co-occur. It would again be useful to broaden the assessment of diet to include more study sites, both with alternative land-uses and alternative species mixes to establish if both species dietary preferences are consistent under these different conditions.

7.9 Conclusions

Badgers have been implicated in the rapid decline of the European hedgehog (Micol et al. 1994; Doncaster 1992; Young et al. 2006; Wembridge, Wilson 2018) and an understanding of the relationship between hedgehogs and their principle predator and potential competitor will help inform future conservation efforts. More specifically, understanding the circumstances that allow both native species to co-occur may be advantageous.

Badgers and hedgehogs engage in complex intra-guild interactions involving both competition and predation (Holt and Polis 1997), making the drivers of their relationship difficult to disentangle. Previous studies have shown that areas supporting high badger densities likely exclude hedgehogs (Young et al. 2006; Williams et al. 2018), reflected in the low occupancy (Williams et al. 2018) and relatively low densities of rural hedgehogs compared with urban areas (Schaus et al. 2020). Historically, the association between hedgehogs and buildings has been viewed as being the result of them avoiding badgers, but few have considered that hedgehogs may prefer urban areas regardless of the presence or absence of badgers (Hubert et al. 2011; Pettett et al. 2017). The present study of IGP between badgers and hedgehogs represents the first of its kind, assessing the potential for temporal partitioning and comparing the densities of both species across a number of sites. Potential competition and predation were also assessed by comparing the diet of the two species, both seasonally and at the same site.

The results of this study describe for the first time, the characteristics of badger and hedgehog coexistence. The holistic nature of this study has revealed fine scale spatial partitioning and dietary niche partitioning which likely supports the coexistence of badgers and hedgehogs in mixed farmland and pasture habitat within the rural landscape. Alternative states of the IGP relationship, such as the exclusion of hedgehogs by badgers, may result in

environments with higher badger densities or lower prey availability, that make it increasingly difficult for hedgehogs to survive using strategies such as spatial or dietary partitioning, potentially leading to their eventual exclusion. Co-occurrence was not identified at any arable sites, suggesting either that hedgehogs actively avoid arable habitat or that badgers exclude them from this habitat.

In high density areas, competition and demand for resources will be higher and potentially reduces the likelihood of coexistence relative to areas of moderate to low densities (Holt and Polis 1997; Polis et al. 1989). In conditions where both species can co-occur, hedgehogs and badgers are partitioned spatially at the local scale and show some partitioning in diet, as both species consume a wide array of food types. Therefore, low densities of both badgers and hedgehogs, together with spatial and dietary segregation, suggests that these species are unlikely to be in strong competition with one another and, as such, there may be no need for temporal partitioning or perhaps temporal partitioning is more costly. Whether the driver of the spatial segregation exhibited is a 'landscape of fear' or hedgehog preference for habitats features near buildings, requires further study. To better support the coexistence of badgers and hedgehogs, more high-quality habitat such as hedgerows and grassy margins are needed to increase connectivity across the rural landscape, provide cover and nesting habitat for hedgehogs and support an abundance of shared invertebrate prey, which would likely benefit other wildlife too.

References

Agilent, 2018. *Agilent 2100 Bioanalyzer System Agilent 4200 TapeStation System Applications for DNA, RNA, and Protein Analysis Application Compendium*.

Akaike, H., 1974. A New Look at the Statistical Model Identification. *IEEE Transactions on Automatic Control*, 19(6), pp.716–723. 10.1109/TAC.1974.1100705.

Amy, S.R., Heard, M.S., Hartley, S.E., George, C.T., Pywell, R.F. and Staley, J.T., 2015. Hedgerow rejuvenation management affects invertebrate communities through changes to habitat structure. *Basic and Applied Ecology*, 16(5), pp.443–451. 10.1016/j.baae.2015.04.002.

Andersen, G.E., Johnson, C.N., Barmuta, L.A. and Jones, M.E., 2017. Dietary partitioning of Australia's two marsupial hypercarnivores, the Tasmanian devil and the spotted-tailed quoll, across their shared distributional range. *PLOS ONE*, 12(11). 10.1371/journal.pone.0188529.

Anderson, M., 2005. PERMANOVA, Permutational multivariate analysis of variance. A computer program. *Journal of Optoelectronics and Advanced Materials*. 10.1002/9781118445112.stat07841.

Anderson, T.L. and Semlitsch, R.D., 2014. High intraguild predator density induces thinning effects on and increases temporal overlap with prey populations. *Population Ecology*, 56(2), pp.265–273. 10.1007/s10144-013-0419-9.

Angus, A., Burgess, P.J., Morris, J. and Lingard, J., 2009. Agriculture and land use: Demand for and supply of agricultural commodities, characteristics of the farming and food industries, and implications for land use in the UK. *Land Use Policy*, 26(1), pp.230–242. 10.1016/j.landusepol.2009.09.020.

Annavi, G., Dawson, D.A., Horsburgh, G.J., Greig, C., Dugdale, H.L., Newman, C., Macdonald, D.W. and Burke, T., 2011. Characterisation of twenty-one European badger (Meles meles) microsatellite loci facilitates the discrimination of second-order relatives. *Conservation Genetics Resources*, 3(3), pp.515–518. 10.1007/s12686-011-9392-9.

Arim, M. and Marquet, P.A., 2004. Intraguild predation: A widespread interaction related to species biology. *Ecology Letters*, 7(7), pp.557–564. 10.1111/j.1461-0248.2004.00613.x.

Arjo, W.M. and Pletscher, D.H., 1999. Behavioral responses of coyotes to wolf recolonization in northwestern Montana. *Canadian Journal of Zoology*, 77(12), pp.1919–1927. 10.1139/z99-177.

Arrizabalaga-Escudero, A., Clare, E.L., Salsamendi, E., Alberdi, A., Garin, I., Aihartza, J. and Goiti, U., 2018. Assessing niche partitioning of co-occurring sibling bat species by DNA metabarcoding. *Molecular Ecology*, 27(5), pp.1273–1283. 10.1111/mec.14508.

Ashwood, F., Watts, K., Park, K., Fuentes-Montemayor, E., Benham, S. and Vanguelova, E.I., 2019. Woodland restoration on agricultural land: long-term impacts on soil quality. *Restoration Ecology*, 27(6), pp.1381–1392. 10.1111/REC.13003.

Atkinson, P.W., Fuller, R.J., Vickery, J.A., Conway, G.J., Tallowin, J.R.B., Smith, R.E.N., Haysom, K.A., Ings, T.C., Asteraki, E.J. and Brown, V.K., 2005. Influence of agricultural management, sward structure and food resources on grassland field use by birds in lowland England. *Journal of Applied Ecology*, 42(5), pp.932–942. 10.1111/j.1365-2664.2005.01070.x.

Atkinson, P.W., Fuller, R.J. and Vickery, J.A., 2002. Large-scale patterns of summer and winter bird distribution in relation to farmland type in England and Wales. *Ecography*, 25(4), pp.466–480. 10.1034/j.1600-0587.2002.250409.x.

Bachiller, E., Cotano, U., Ibaibarriaga, L., Santos, M. and Irigoien, X., 2015. Intraguild predation between small pelagic fish in the Bay of Biscay: impact on anchovy (Engraulis encrasicolus L.) egg mortality. *Marine Biology*, 162(6), pp.1351–1369. 10.1007/s00227-015-2674-0.

Balme, G.A., Pitman, R.T., Robinson, H.S., Miller, J.R.B., Funston, P.J. and Hunter, L.T.B., 2017. Leopard distribution and abundance is unaffected by interference competition with lions. *Behavioral Ecology*, 28(5), pp.1348–1358. 10.1093/beheco/arx098.

De Barba, M., Miquel, C., Boyer, F., Mercier, C., Rioux, D., Coissac, E. and Taberlet, P., 2014. DNA metabarcoding multiplexing and validation of data accuracy for diet assessment: Application to omnivorous diet. *Molecular Ecology Resources*, 14(2), pp.306–323. 10.1111/1755-0998.12188.

Barnard, P.C., 2011. *The Royal Entomological Society Book of British Insects*. 10.1002/9781444344981.

Bartoń, K., 2019. MuMIn: Multi-Model Inference [software], Version 1.43.6.

Battisti, A., Giuliano, D. and Balestrieri, A., 2019. Detection of earthworm chaetae in mammal faeces: Methodological implications. *Folia Zoologica*, 68(1), pp.43–47. 10.25225/fozo.075.2019.

Bearman-Brown, L.E., Baker, P.J., Scott, D., Uzal, A., Evans, L. and Yarnell, R.W., 2020. Over-Winter Survival and Nest Site Selection of the West-European Hedgehog (Erinaceus europaeus) in Arable Dominated Landscapes. *Animals*, 10(9), p.1449. 10.3390/ani10091449.

Benton, T.G., Vickery, J.A., Wilson, J.D. and Wilson, J.D., 2003. Farmland biodiversity: is habitat heterogeneity the key? *Trends in Ecology & Evolution*, 18(4), pp.182–188. 10.1016/S0169-5347(03)00011-9.

Benton, T.G., Bryant, D.M., Cole, L. and Crick, H.Q.P., 2002. Linking agricultural practice to insect and bird populations: A historical study over three decades. *Journal of Applied Ecology*, 39(4), pp.673–687. 10.1046/j.1365-2664.2002.00745.x.

Berry, O., Bulman, C., Bunce, M., Coghlan, M., Murray, D.C. and Ward, R.D., 2015. Comparison of morphological and DNA metabarcoding analyses of diets in exploited marine fishes. *Marine Ecology Progress Series*, 540, pp.167–181. 10.3354/MEPS11524.

Berry, T.E., Osterrieder, S.K., Murray, D.C., Coghlan, M.L., Richardson, A.J., Grealy, A.K., Stat, M., Bejder, L. and Bunce, M., 2017. DNA metabarcoding for diet analysis and biodiversity: A case study using the endangered Australian sea lion (Neophoca cinerea). *Ecology and Evolution*, 7(14), pp.5435–5453. 10.1002/ece3.3123.

Bhandari, K.B., West, C.P., Longing, S.D., Brown, C.P. and Green, P.E., 2018. Comparison of Arthropod Communities among Different Forage Types on the Texas High Plains using Pitfall Traps. *Crop, Forage & Turfgrass Management*, 4(1), pp.1–7. 10.2134/cftm2018.01.0005.

Bienert, F., De Danieli, S., Miquel, C., Coissac, E., Poillot, C., Brun, J.J. and Taberlet, P., 2012. Tracking earthworm communities from soil DNA. *Molecular Ecology*, 21(8), pp.2017–2030. 10.1111/j.1365-294X.2011.05407.x.

Blouin, M., Hodson, M.E., Delgado, E.A., Baker, G., Brussaard, L., Butt, K.R., Dai, J., Dendooven, L., Peres, G., Tondoh, J.E., Cluzeau, D. and Brun, J.J., 2013. A review of earthworm impact on soil function and ecosystem services. *European Journal of Soil Science*, 64(2), pp.161–182. 10.1111/ejss.12025.

Boetzl, F.A., Ries, E., Schneider, G. and Krauss, J., 2018. It's a matter of design-how pitfall trap design affects trap samples and possible predictions. *PeerJ*, 2018(6), p.e5078. 10.7717/PEERJ.5078/SUPP-2.

Browett, S.S., Curran, T.G., O'Meara, D.B., Harrington, A.P., Sales, N.G., Antwis, R.E., O'Neill, D. and McDevitt, A.D., 2021. Primer biases in the molecular assessment of diet in multiple insectivorous mammals. *Mammalian Biology*, 1, p.3. 10.1007/s42991-021-00115-4.

Brown, G.R. and Matthews, I.M., 2016. A review of extensive variation in the design of pitfall traps and a proposal for a standard pitfall trap design for monitoring ground-active arthropod biodiversity. *Ecology and Evolution*, 6(12), pp.3953–3964. 10.1002/ECE3.2176.

Brown, P.M.J., Ingels, B., Wheatley, A., Rhule, E.L., de Clercq, P., van Leeuwen, T. and Thomas, A., 2015. Intraguild predation by Harmonia axyridis (Coleoptera: Coccinellidae) on native insects in Europe: Molecular detection from field samples. *Entomological Science*, 18(1), pp.130–133. 10.1111/ens.12092.

Bruce, T.J.A., 2016. The CROPROTECT project and wider opportunities to improve farm productivity through web-based knowledge exchange. *Food and Energy Security*, 5(2), pp.89–96. 10.1002/fes3.80.

Buesching, C.D., Newman, C., Service, K., Macdonald, D.W. and Riordan, P., 2016. Latrine marking patterns of badgers (Meles meles) with respect to population density and range size. *Ecosphere*. 10.1002/ecs2.1328.

Burnham, K.P. and Anderson, D.R., 2002. Model selection and multi-model inference: a practical information. *Model Selection and Multimodel Inference*. 10.1007/B97636.

Butt, K.R., Nuutinen, V. and Sirén, T., 2003. Resource distribution and surface activity of adult Lumbricus terrestris L. in an experimental system. In: *Pedobiologia*. Elsevier GmbH, pp. 548–553. 10.1078/0031-4056-00227.

Byrne, A.W., Paddy Sleeman, D., O'Keeffe, J. and Davenport, J., 2012. The ecology of the European badger (Meles meles) in Ireland: A review. *Biology and Environment*, 112(1), pp.105–132. 10.3318/BIOE.2012.02.

Carson, R., 1962. *Silent Spring*. Boston: Houghton Mifflin.

Casper, R.M., Jarman, S.N., Gales, N.J. and Hindell, M.A., 2007. Combining DNA and morphological analyses of faecal samples improves insight into trophic interactions: A case study using a generalist predator. *Marine Biology*, 152(4), pp.815–825. 10.1007/s00227-007-0732-y.

Chamberlain, D.E., Fuller, R.J., Bunce, R.G.H., Duckworth, J.C. and Shrubb, M., 2000. Changes in the abundance of farmland birds in relation to the timing of agricultural intensification in England and Wales. *Journal of Applied Ecology*, 37(5), pp.771–788. 10.1046/j.1365-2664.2000.00548.x.

Chambers, J.M., Freeny, A. and Heiberger, R.M., 1992. *Analysis of variance; designed experiments. Chapter 5 of Statistical Models in S eds* ,. Pacific Grove, California: Wadsworth & Brooks/Cole.

Chan, K.Y., 2001. An overview of some tillage impacts on earthworm population abundance and diversity - Implications for functioning in soils. *Soil and Tillage Research*, 57(4), pp.179–191. 10.1016/S0167-1987(00)00173-2.

Chao, A., Gotelli, N.J., Hsieh, T.C., Sander, E.L., Ma, K.H., Colwell, R.K. and Ellison, A.M., 2014. Rarefaction and extrapolation with Hill numbers: A framework for sampling and estimation in species diversity studies. *Ecological Monographs*. 10.1890/13-0133.1.

Chapin, F.S., Zavaleta, E.S., Eviner, V.T., Naylor, R.L., Vitousek, P.M., Reynolds, H.L., Hooper, D.U., Lavorel, S., Sala, O.E., Hobbie, S.E., Mack, M C, Díaz, S, Mack, Michelle C and Díaz, Sandra, 2000. Consequences of changing biodiversity. *Nature*, 405(6783), pp.234–42. 10.1038/35012241.

Chase, J.M., Abrams, P.A., Grover, J.P., Diehl, S., Chesson, P., Holt, R.D., Richards, S.A., Nisbet, R.M. and Case, T.J., 2002. The interaction between predation and competition: a review and synthesis. *Ecology Letters*, 5(2), pp.302–315. 10.1046/j.1461-0248.2002.00315.x.

Chatterji, S. and Pachter, L., 2006. Reference based annotation with GeneMapper. *Genome Biology*, 7(4). 10.1186/gb-2006-7-4-r29.

Clare, E.L., Chain, F.J.J., Littlefair, J.E. and Cristescu, M.E., 2016. The effects of parameter choice on defining molecular operational taxonomic units and resulting ecological analyses of metabarcoding data1. *Genome*. 10.1139/gen-2015-0184.

Clarke, K.R. and Gorley, R.N., 2015. *PRIMER v7: User Manual/Tutorial Plymouth Routines In Multivariate Ecological Research*.

Cleary, G.P., Corner, L.A.L., O'Keeffe, J., Marples, N.M., O'Keeffe, J. and Marples, N.M., 2011. Diet of the European badger (Meles meles) in the Republic of Ireland: A comparison of results from an analysis of stomach contents and rectal faeces. *Mammalian Biology*, 76(4), pp.470–475.

Cleary, G.P., Corner, L.A.L.L., O'Keeffe, J., Marples, N.M., O'Keeffe, J., Marples, N.M., O'Keeffe, J. and Marples, N.M., 2009. The diet of the badger Meles meles in the Republic of Ireland. *Mammalian Biology*, 74(6), pp.438–447.

Cloyed, C.S. and Eason, P.K., 2017. Niche partitioning and the role of intraspecific niche variation in structuring a guild of generalist anurans. *Royal Society Open Science*, 4(3), p.170060. 10.1098/rsos.170060.

Cranor, L.F., 1994. Programming Perl. *Crossroads*. 10.1145/197149.197157.

Cresswell, P., Harris, S., Bunce, R.G.H. and Jefferies, D.J., 1989. The badger (Meles meles) in Britain: present status and future population changes. *Biological Journal of the Linnean Society*, 38(1), pp.91–101. 10.1111/j.1095-8312.1989.tb01566.x.

Cresswell, P., Harris, S. and Jefferies, D.J., 1990. The history, distribution, status and habitat requirements of the badger in Britain. *Nature Conservancy Council, Peterborough*, pp.1–84. 10.2/JQUERY.MIN.JS.

Cristescu, M.E., 2014. From barcoding single individuals to metabarcoding biological communities: Towards an integrative approach to the study of global biodiversity. *Trends in Ecology and Evolution*, 29(10), pp.566–571. 10.1016/j.tree.2014.08.001.

Cross, S.L., Craig, M.D., Tomlinson, S. and Bateman, P.W., 2019. I don't like crickets, I love them: invertebrates are an important prey source for varanid lizards. *Journal of Zoology*, p.jzo.12750. 10.1111/jzo.12750.

Crowder, L.B. and Cooper, W.E., 1982. Habitat stuctural complexity and the interaction between bluegills and their prey (Lepomis macrochirus). *Ecology*, 63(6), pp.1802–1813. 10.2307/1940122.

Cruz, J., Sarmento, P. and White, P.C.L., 2015. Influence of exotic forest plantations on occupancy and co-occurrence patterns in a Mediterranean carnivore guild. *Journal of Mammalogy*, 96(4), pp.854–865. 10.1093/jmammal/gyv109.

Cusack, J.J., Swanson, A., Coulson, T., Packer, C., Carbone, C., Dickman, A.J., Kosmala, M., Lintott, C. and Rowcliffe, J.M., 2015. Applying a random encounter model to estimate lion density from camera traps in Serengeti National Park, Tanzania. *Journal of Wildlife Management*, 79(6), pp.1014–1021. 10.1002/jwmg.902.

D'aquila, R.T., Bechtel, L.J., Videler, J.A., Eron, J.J., Gorczyca, P. and Kaplan, J.C., 1991. Maximizing sensitivity and specificity of PCR by preamplification heating. *Nucleic Acids Research*, 19(13), p.3749. 10.1093/nar/19.13.3749.

Darmon, G., Calenge, C., Loison, A., Jullien, J.M., Maillard, D. and Lopez, J.F., 2012. Spatial distribution and habitat selection in coexisting species of mountain ungulates. *Ecography*, 35(1), pp.44–53. 10.1111/j.1600-0587.2011.06664.x.

Davis, N.E., Forsyth, D.M., Triggs, B., Pascoe, C., Benshemesh, J., Robley, A., Lawrence, J., Ritchie, E.G., Nimmo, D.G. and Lumsden, L.F., 2015. Interspecific and Geographic Variation in the Diets of Sympatric Carnivores: Dingoes/Wild Dogs and Red Foxes in South-Eastern Australia Crowther, M. S., ed. *PLOS ONE*, 10(3), p.e0120975. 10.1371/journal.pone.0120975.

Davison, J., Huck, M., Delahay, R.J. and Roper, T.J., 2009. Restricted ranging behaviour in a high-density population of urban badgers. *Journal of Zoology*, 277(1), pp.45–53. 10.1111/j.1469-7998.2008.00509.x.

Davison, J., Huck, M., Delahay, R.J. and Roper, T.J., 2008. Urban badger setts: characteristics, patterns of use and management implications. *Journal of Zoology*, 275(2), pp.190–200. 10.1111/j.1469-7998.2008.00424.x.

Deagle, B.E., Thomas, A.C., McInnes, J.C., Clarke, L.J., Vesterinen, E.J., Clare, E.L., Kartzinel, T.R. and Eveson, J.P., 2019. Counting with DNA in metabarcoding studies: How should we convert sequence reads to dietary data? *Molecular Ecology*, 28(2), pp.391–406. 10.1111/mec.14734.

Decaëns, T. and Jiménez, J.J., 2002. Earthworm communities under an agricultural intensification gradient in Colombia. *Plant and Soil*, 240, pp.133–143.

DEFRA, 2012. Agriculture in the United Kingdom. *Agriculture in the United Kingdom*, pp.1–116.

Delahay, R.J., Cheeseman, C.L. and Clifton-Hadley, R.S., 2001. Wildlife disease reservoirs: The epidemiology of Mycobacterium bovis infection in the Eeuropean badger (Meles meles) and other British mammals. In: *Tuberculosis*. pp. 43–49. 10.1054/tube.2000.0266.

Deng, Y., Jiang, Y.H., Yang, Y., He, Z., Luo, F. and Zhou, J., 2012. Molecular ecological network analyses. *BMC Bioinformatics*, 13(1), pp.1–20. 10.1186/1471-2105-13-113.

Dickman, C.R., 1988. Age-related dietary change in the European hedgehog, Erinaceus europaeus. *Journal of Zoology*, 215(1), pp.1–14. 10.1111/j.1469-7998.1988.tb04881.x.

Dicks, L. V., Rose, D.C., Ang, F., Aston, S., et al., 2019. What agricultural practices are most likely to deliver "sustainable intensification" in the UK? *Food and Energy Security*, 8(1). 10.1002/fes3.148.

Doncaster, C.P., 1994. Factors regulating local variations in abundance: field tests on hedgehogs, Erinaceus europaeus. *Oikos*, 69, pp.182–192. 10.2307/3546136.

Doncaster, C.P., Rondinini, C., Johnson, P.C.D., Carlo, R. and Paul C. D., J., 2001. Field test for environmental correlates of dispersal in hedgehogs Erinaceus europaeus. *Journal of Animal Ecology*, 70(1), pp.33–46. 10.1111/j.1365-2656.2001.00471.x.

Doncaster, C.P., 1992. Testing the role of intraguild predation in regulating hedgehog populations. *Proceedings. Biological sciences / The Royal Society*, 249(1324), pp.113–7. 10.1098/rspb.1992.0092.

Dowding, C. V., Harris, S., Poulton, S. and Baker, P.J., 2010. Nocturnal ranging behaviour of urban hedgehogs, Erinaceus europaeus, in relation to risk and reward. *Animal Behaviour*, 80(1), pp.13–21. 10.1016/j.anbehav.2010.04.007.

Dykes, J.L., Strickland, B.K., Demarais, S., Reynolds, D.B. and Lashley, M.A., 2018. Soil nutrients indirectly influence intraspecific plant selection in white-tailed deer. *Basic and Applied Ecology*, 32, pp.103–109. 10.1016/j.baae.2018.08.001.

Edgar, C.R.C., 2013. USEARCH. *Journal of the National Cancer Institute*. 10.1093/jnci/djs546.

Edgar, R.C., 2013. UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nature Methods*, 10(10), pp.996–998. 10.1038/nmeth.2604.

Eggleton, P., Inward, K., Smith, J., Jones, D.T. and Sherlock, E., 2009. A six year study of earthworm (Lumbricidae) populations in pasture woodland in southern England shows their responses to soil temperature and soil moisture. *Soil Biology and Biochemistry*. 10.1016/j.soilbio.2009.06.007.

Elmhagen, B., Ludwig, G., Rushton, S.P., Helle, P. and Lindén, H., 2010. Top predators, mesopredators and their prey: interference ecosystems along bioclimatic productivity gradients. *Journal of Animal Ecology*. 10.1111/j.1365-2656.2010.01678.x.

Emmerson, M., Morales, M.B., Oñate, J.J., Batáry, P., Berendse, F., Liira, J., Aavik, T., Guerrero, I., Bommarco, R., Eggers, S., Pärt, T., Tscharntke, T., Weisser, W., Clement, L. and Bengtsson, J., 2016. How Agricultural Intensification Affects Biodiversity and Ecosystem Services. *Advances in Ecological Research*, 55, pp.43–97. 10.1016/BS.AECR.2016.08.005.

Esnaola, A., Arrizabalaga-Escudero, A., González-Esteban, J., Elosegi, A. and Aihartza, J., 2018. Determining diet from faeces: Selection of metabarcoding primers for the insectivore Pyrenean desman (Galemys pyrenaicus). *PLoS ONE*, 13(12). 10.1371/journal.pone.0208986.

ESRI (Environmental Systems Research Institute.), 2018. ArcMap [software], Version 10.6.1. *Redlands, California*.

Feore, S. and Montgomery, W.I., 1999. Habitat effects on the spatial ecology of the European badger (Meles meles). *Journal of Zoology*, 247(4), pp.537–549. 10.1111/j.1469-7998.1999.tb01015.x.

Fernando, P., Vidya, T.N.C., Rajapakse, C., Dangolla, A. and Melnick, D.J., 2003. Reliable Noninvasive Genotyping: Fantasy or Reality? *Journal of Heredity*, 94(2), pp.115–123. 10.1093/JHERED/ESG022.

Fey, K., Banks, P.B. and Korpimäki, E., 2008. Voles on small islands: Effects of food limitation and alien predation. *Oecologia*, 157(3), pp.419–428. 10.1007/S00442-008-1084-0.

Fischer, L.K., von der Lippe, M. and Kowarik, I., 2013. Urban land use types contribute to grassland conservation: The example of Berlin. *Urban Forestry and Urban Greening*. 10.1016/j.ufug.2013.03.009.

Fiske, A.I., Chandler, R., Miller, D., Royle, A., Kery, M. and Chandler, M.R., 2013. Package 'unmarked' Models for data from unmarked animals [software], Version 1.1.1. *CRAN Repository*.

Forin-Wiart, M.A., Poulle, M.L., Piry, S., Cosson, J.F., Larose, C. and Galan, M., 2018. Evaluating metabarcoding to analyse diet composition of species foraging in anthropogenic landscapes using Ion Torrent and Illumina sequencing. *Scientific Reports 2018 8:1*, 8(1), pp.1–12. 10.1038/s41598-018-34430-7.

Foster, R.J., Harmsen, B.J. and Doncaster, C.P., 2010. Sample-size effects on diet analysis from scats of jaguars and pumas. *Mammalia*, 74, pp.317–321. 10.1515/MAMM.2010.006.

Frantz, A.C., Fack, F., Muller, C.P. and Roper, T.J., 2006. Faecal DNA typing as a tool for investigating territorial behaviour of badgers (Meles meles). *European Journal of Wildlife Research*, 52(2), pp.138–141. 10.1007/s10344-005-0016-0.

Frantz, A.C., Pope, L.C., Carpenter, P.J., Roper, T.J., Wilson, G.J., Delahay, R.J. and Burke, T., 2003. Reliable microsatellite genotyping of the Eurasian badger (Meles meles) using faecal DNA. *Molecular Ecology*, 12(6), pp.1649–1661. 10.1046/j.1365-294X.2003.01848.x.

Fraser, P.M., Schon, N.L., Piercy, J.E., MacKay, A.D. and Minor, M.A., 2012. Influence of summer irrigation on soil invertebrate populations in a long-term sheep irrigation trial at Winchmore (Canterbury). *New Zealand Journal of Agricultural Research*, 55(2), pp.165–180. 10.1080/00288233.2012.662902.

Frazão, J., de Goede, R.G.M., Brussaard, L., Faber, J.H., Groot, J.C.J. and Pulleman, M.M., 2017. Earthworm communities in arable fields and restored field margins, as related to management practices and surrounding landscape diversity. *Agriculture, Ecosystems and Environment*, 248, pp.1–8. 10.1016/j.agee.2017.07.014.

Fuentes-Montemayor, E., Ferryman, M., Watts, K., Macgregor, N.A., Hambly, N., Brennan, S., Coxon, R., Langridge, H. and Park, K.J., 2020. Small mammal responses to long-term large-scale woodland creation: the influence of local and landscape-level attributes. *Ecological Applications*. 10.1002/eap.2028.

Garnett, B.T., Delahay, R.J. and Roper, T.J., 2002. Use of cattle farm resources by badgers (Meles meles) and risk of bovine tuberculosis (Mycobacterium bovis) transmission to cattle. *Proceedings of the Royal Society B: Biological Sciences*, 269(1499), pp.1487–1491. 10.1098/rspb.2002.2072.

Geiser, F., 2020. Seasonal Expression of Avian and Mammalian Daily Torpor and Hibernation: Not a Simple Summer-Winter Affair. *Frontiers in Physiology*, 11, p.436. 10.3389/fphys.2020.00436.

Glaspie, C.N. and Seitz, R.D., 2018. Habitat complexity and benthic predator-prey interactions in Chesapeake Bay. *PLOS ONE*, 13(10). 10.1371/journal.pone.0205162.

Gonthier, D.J., Ennis, K.K., Farinas, S., Hsieh, H.Y., Iverson, A.L., Batáry, P., Rudolphi, J., Tscharntke, T., Cardinale, B.J. and Perfecto, I., 2014. Biodiversity conservation in agriculture requires a multi-scale approach. *Proceedings of the Royal Society B: Biological Sciences*, 281(1791). 10.1098/rspb.2014.1358.

Goszczynski, J., Jedrzejewska, B. and Jedrzejewski, W., 2000. Diet composition of badgers (Meles meles) in a pristine forest and rural habitats of Poland compared to other European populations. *Journal of Zoology*, 250(4), pp.495–505. 10.1111/j.1469-7998.2000.tb00792.x.

Gotelli, N. and Ellison, A., 2015. R Package 'EcoSimR' - Null Model Analysis for Ecological Data [software] Version 1.00.

Granquist, S.M., Esparza-Salas, R., Hauksson, E., Karlsson, O. and Angerbjörn, A., 2018. Fish consumption of harbour seals (Phoca vitulina) in north western Iceland assessed by DNA metabarcoding and morphological analysis. *Polar Biology*, 41(11), pp.2199–2210. 10.1007/S00300-018-2354-X.

Griffiths, R. and Tiwari, B., 1993. Primers for the differential amplification of the sex-determining region Y gene in a range of mammal species. *Molecular Ecology*, 2(6), pp.405–406. 10.1111/j.1365-294X.1993.tb00034.x.

Hall, T.A., 1997. BioEdit Sequence Aligment Editor. *BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT*. 10.1002/cphc.201600586.

Hancock, M.H. and Legg, C.J., 2012. Pitfall trapping bias and arthropod body mass. *Insect Conservation and Diversity*, 5(4), pp.312–318. 10.1111/j.1752-4598.2011.00162.x.

Harris, S., Morris, P., Wray, S. and Yalden, D., 1995. *A review of British mammals: population estimates and conservation status of British mammals other than cetaceans*.

Harris, S., 1984. Ecology of Urban badgers Meles meles: Distribution in Britain and habitat selection, persecution, food and damage in the city of Bristol. *Biological Conservation*, 28(4), pp.349–375. 10.1016/0006-3207(84)90041-7.

Hayward, M.W., Porter, L., Lanszki, J., Kamler, J.F., Beck, J.M., Kerley, G.I.H., Macdonald, D.W., Montgomery, R.A., Parker, D.M., Scott, D.M., O'Brien, J. and Yarnell, R.W., 2017. Factors affecting the prey preferences of jackals (Canidae). *Mammalian Biology*, 85(1), pp.70–82. 10.1016/j.mambio.2017.02.005.

Hert, D.G., Fredlake, C.P. and Barron, A.E., 2008. Advantages and limitations of next-generation sequencing technologies: A Comparison of electrophoresis and non-electrophoresis methods. *Electrophoresis*, 29(23), pp.4618–4626. 10.1002/elps.200800456.

Hiltunen, T. and Laakso, J., 2013. The relative importance of competition and predation in environment characterized by resource pulses - an experimental test with a microbial community. *BMC Ecology*, 13, p.29. 10.1186/1472-6785-13-29.

Hof, A.R., Allen, A.M. and Bright, P.W., 2019. Investigating the role of the eurasian badger (Meles meles) in the nationwide distribution of the western european hedgehog (Erinaceus europaeus) in England. *Animals*, 9(10), p.759. 10.3390/ani9100759.

Hof, A.R. and Bright, P.W., 2016. Quantifying the long-term decline of the West European hedgehog in England by subsampling citizen-science datasets. *European Journal of Wildlife Research*, 62(4), pp.407–413. 10.1007/S10344-016-1013-1.

Hof, A.R. and Bright, Paul W., 2010. The impact of grassy field margins on macro-invertebrate abundance in adjacent arable fields. *Agriculture, Ecosystems and Environment*, 139(1–2), pp.280–283. 10.1016/j.agee.2010.08.014.

Hof, A.R. and Bright, P. W., 2010. The value of agri-environment schemes for macro-invertebrate feeders: Hedgehogs on arable farms in Britain. *Animal Conservation*, 13(5), pp.467–473. 10.1111/j.1469-1795.2010.00359.x.

Hof, A.R., Snellenberg, J. and Bright, P.W., 2012. Food or fear? Predation risk mediates edge refuging in an insectivorous mammal. *Animal Behaviour*, 83(4), pp.1099–1106. 10.1016/J.ANBEHAV.2012.01.042.

Holbrook, S.J. and Schmitt, R.J., 1989. Resource overlap, prey dynamics and the strength of competition. *Ecology*, 70(6), pp.1943–1953. 10.2307/1938124.

Holt, R.D., Lawton, J.H., Polis, G.A. and Martinez, N.D., 1999. Trophic rank and the species-area relationship. *Ecology*, 80(5), pp.1495–1504. 10.1890/0012-9658(1999)080[1495:TRATSA]2.0.CO;2.

Holt, R.D. and Polis, G.A., 1997. A theoretical framework for intraguild predation. *American Naturalist*, pp.745–764.

Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setälä, H., Symstad, A.J., Vandermeer, J. and Wardle, D.A., 2005. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecological Monographs*, 75(1), pp.3–35. 10.1890/04-0922.

Hothorn, T., Bretz, F. and Westfall, P., 2008. Simultaneous inference in general parametric models. *Biometrical Journal*, 50(3), pp.346–363. 10.1002/bimj.200810425.

Hubert, P., Julliard, R., Biagianti, S. and Poulle, M.L., 2011. Ecological factors driving the higher hedgehog (Erinaceus europeaus) density in an urban area compared to the adjacent rural area. *Landscape and Urban Planning*, 103(1), pp.34–43. 10.1016/j.landurbplan.2011.05.010.

Huck, M., Davison, J. and Roper, T.J., 2006. Predicting European badger Meles meles sett distribution in urban environments. *Wildlife Biology*, 14(2), pp.188–198. 10.2981/0909.

Humbert, J.Y., Ghazoul, J., Sauter, G.J. and Walter, T., 2010. Impact of different meadow mowing techniques on field invertebrates. *Journal of Applied Entomology*, 134(7), pp.592–599. 10.1111/j.1439-0418.2009.01503.x.

Janssen, A., Sabelis, M.W., Magalhães, S., Montserrat, M. and Van Der Hammen, T., 2007. Habitat Structure Affects Intraguild Predation. *Ecology*, 88(11), pp.2713–2719.

Johnson, J.S., Spakowicz, D.J., Hong, B.Y., Petersen, L.M., Demkowicz, P., Chen, L., Leopold, S.R., Hanson, B.M., Agresta, H.O., Gerstein, M., Sodergren, E. and Weinstock, G.M., 2019. Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. *Nature Communications*, 10(1), pp.1–11. 10.1038/s41467-019-13036-1.

Jones, C., Moss, K., Sanders, M. and Jones, C., 2015. New Zealand Ecological Society Diet of hedgehogs (Erinaceus europaeus) in the upper Waitaki Basin , New Zealand : Implications for conservation. *New Zealand Journal of Ecology*, 29(1), pp.29–35.

Judge, J., Wilson, G.J., Macarthur, R., McDonald, R.A. and Delahay, R.J., 2017. Abundance of badgers (Meles meles) in England and Wales. *Scientific Reports*, 7(1), p.276. 10.1038/s41598-017-00378-3.

Judge, J., Wilson, G.J., Macarthur, R., Delahay, R.J. and McDonald, R.A., 2014. Density and abundance of badger social groups in England and Wales in 2011–2013. *Scientific Reports*, 4. 10.1038/SREP03809.

Kamler, J.F., Ballard, W.B., Wallace, M.C., Gilliland, R.L. and Gipson, P.S., 2007. Dietary overlap of swift foxes and coyotes in Northwestern Texas. *American Midland Naturalist*. 10.1674/0003-0031(2007)158[139:DOOSFA]2.0.CO;2.

Kang, Y. and Wedekin, L., 2013. Dynamics of a intraguild predation model with generalist or specialist predator. *Journal of Mathematical Biology*, 67(5), pp.1227–1259. 10.1007/s00285-012-0584-z.

Kartzinel, T.R., Chen, P.A., Coverdale, T.C., Erickson, D.L., John Kress, W., Kuzmina, M.L., Rubenstein, D.I., Wang, W. and Pringle, R.M., 2015. DNA metabarcoding illuminates dietary niche partitioning by African large herbivores. *Proceedings of the National Academy of Sciences of the United States of America*, 112(26), pp.8019–8024. 10.5061/dryad.2fg10.

Kassambara, A., 2021. R package rstatix: Pipe-Friendly Framework for Basic Statistical Tests [software] Version 0.7.0.

Kendon, M., McCarthy, M., Jevrejeva, S., Matthews, A. and Legg, T., 2019. State of the UK climate 2018. *International Journal of Climatology*, 39(S1), pp.1–55. 10.1002/joc.6213.

Kennedy, S., Lim, J.Y., Clavel, J., Krehenwinkel, H. and Gillespie, R.G., 2019. Spider webs, stable isotopes and molecular gut content analysis: Multiple lines of evidence support trophic niche differentiation in a community of Hawaiian spiders. *Functional Ecology*, 33(9), pp.1722–1733. 10.1111/1365-2435.13361.

Kenta, T., Gratten, J., Haigh, N.S., Hinten, G.N., Slate, J., Butlin, R.K. and Burke, T., 2008. Multiplex SNP-SCALE: A cost-effective medium-throughput single nucleotide polymorphism genotyping method. *Molecular Ecology Resources*, 8(6), pp.1230–1238. 10.1111/j.1755-0998.2008.02190.x.

Kinoshita, E., Kosintsev, P.A., Abramov, A. V., Solovyev, V.A., Saveljev, A.P., Nishita, Y. and Masuda, R., 2020. Holocene changes in the distributions of Asian and European badgers (Carnivora: Mustelidae: Meles) inferred from ancient DNA analysis. *Biological Journal of the Linnean Society*, 129(3), pp.594–602. 10.1093/BIOLINNEAN/BLAA007.

Kinoshita, E., Kosintsev, P.A., Raichev, E.G., Haukisalmi, V.K., Kryukov, A.P., Wiig, Ø., Abramov, A. V., Kaneko, Y. and Masuda, R., 2017. Molecular phylogeny of Eurasian badgers (Meles) around the distribution boundaries, revealed by analyses of mitochondrial DNA and Y-chromosomal genes. *Biochemical Systematics and Ecology*, 71, pp.121–130. 10.1016/j.bse.2017.01.011.

Klymus, K.E., Marshall, N.T. and Stepien, C.A., 2017. Environmental DNA (eDNA) metabarcoding assays to detect invasive invertebrate species in the Great Lakes. *PLOS ONE*, 12(5), p.e0177643. 10.1371/journal.pone.0177643.

Krauze-Gryz, D., Gryz, J.B., Goszczyński, J., Chylarecki, P. and Żmihorski, M., 2012. The good, the bad, and the ugly: space use and intraguild interactions among three opportunistic predators—cat (*Felis catus*), dog (*Canis lupus familiaris*), and red fox (*Vulpes vulpes*)—under human pressure. *Canadian Journal of Zoology*, 90(12), pp.1402–1413. 10.1139/cjz-2012-0072.

Kruskal, J.B., 1964. Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. *Psychometrika*. 10.1007/BF02289565.

Kruskal, W.H. and Wallis, W.A., 1952. Use of Ranks in One-Criterion Variance Analysis. *Journal of the American Statistical Association*, 47(260), p.583. 10.2307/2280779.

Kruuk, H., 1978. Foraging and Spatial Organisation of the European Badger, Meles meles. *Sociobiology*, 4(1), pp.75–89.

Kruuk, H., Parish, T., Brown, C.A.J. and Carrera, J., 1979. The Use of Pasture by the European Badger (Meles meles). *Source: Journal of Applied Ecology*, 16(2), pp.453–459.

Kruuk, H. and Parish, T., 1981. Feeding Specialization of the European Badger Meles meles in Scotland. *The Journal of Animal Ecology*, 50(3), p.773. 10.2307/4136.

Kruuk, H. and Parish, T., 1985. Food, Food Availability and Weight of Badgers (Meles meles) in Relation to Agricultural Changes. *The Journal of Applied Ecology*, 22(3), p.705. 10.2307/2403223.

Kumar, S., Stecher, G. and Tamura, K., 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular biology and evolution*. 10.1093/molbev/msw054.

Lande, R., 1996. Statistics and Partitioning of Species Diversity, and Similarity among Multiple Communities. *Oikos*, 76(1), p.5. 10.2307/3545743.

Lashley, M.A., Cove, M. V., Chitwood, M.C., Penido, G., Gardner, B., DePerno, C.S. and Moorman, C.E., 2018. Estimating wildlife activity curves: comparison of methods and sample size. *Scientific Reports*, 8(1), pp.1–11. 10.1038/s41598-018-22638-6.

Laundré, J.W., Hernández, L. and Altendorf, K.B., 2001. Wolves, elk, and bison: Reestablishing the 'landscape of fear' in Yellowstone National Park, U.S.A. *Canadian Journal of Zoology*, 79(8), pp.1401–1409. 10.1139/z01-094.

Lesmeister, D.B., Nielsen, C.K., Schauber, E.M. and Hellgren, E.C., 2015. Spatial and temporal structure of a mesocarnivore guild in midwestern north America. *Wildlife Monographs*, 191(1), pp.1–61. 10.1002/wmon.1015.

Letten, A.D., Ke, P.J. and Fukami, T., 2017. Linking modern coexistence theory and contemporary niche theory. *Ecological Monographs*, 87(2), pp.161–177. 10.1002/ecm.1242.

Levins, R., 1968. Chapter one. On theories and Models. In: *Evolution in Changing Environments*. Princeton University Press, 1968.

MacArthur, R.H. and Pianka, E.R., 1966. On Optimal Use of a Patchy Environment. *The American Naturalist*, 100(916), pp.603–609. 10.1086/282454.

Macdonald, D.W., Newman, C. and Beusching, C.D., 2015. *Wildlife Conservation on Farmland: Conflict in the countryside*. Oxford University Press.

MacGregor-Fors, I., 2011. Misconceptions or misunderstandings? On the standardization of basic terms and definitions in urban ecology. *Landscape and Urban Planning*, 100(4), pp.347–349. 10.1016/J.LANDURBPLAN.2011.01.013.

Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M. and Bazzaz, F.A., 2000. Biotic invasions: Causes, epidemiology, global consequences, and control. *Ecological Applications*. 10.1890/1051-0761(2000)010[0689:BICEGC]2.0.CO;2.

MacKenzie, D.I., Nichols, J.D., Royle, J.A., Pollock, K.H., Bailey, L.L. and Hines, J.E., 2017. *Occupancy Estimation and Modeling: Inferring Patterns and Dynamics of Species Occurrence: Second Edition*. Elsevier. 10.1016/C2012-0-01164-7.

MacKenzie, D.I. and Bailey, L.L., 2004. Assessing the fit of site-occupancy models. *Journal of Agricultural, Biological, and Environmental Statistics*. 10.1198/108571104X3361.

Magoč, T. and Salzberg, S.L., 2011. FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, 27(21), pp.2957–2963. 10.1093/bioinformatics/btr507.

Mancini, F., Woodcock, B.A., Redhead, J., Spurgeon, D.J., Jarvis, S.G., Pywell, R.F., Shore, R.F., Johnson, A.C. and Isaac, N.J.B., 2020. Detecting landscape scale consequences of insecticide use on invertebrate communities. *Advances in Ecological Research*, 63, pp.93–126. 10.1016/bs.aecr.2020.07.001.

Manlick, P.J., Woodford, J.E., Zuckerberg, B. and Pauli, J.N., 2017. Niche compression intensifies competition between reintroduced American martens (Martes americana) and fishers (Pekania pennanti). *Journal of Mammalogy*, 98(3), pp.690–702. 10.1093/jmammal/gyx030.

Mardis, E. and McCombie, W.R., 2017. Library quantification using sybr green-quantitative polymerase chain reaction (qPCR). *Cold Spring Harbor Protocols*, 2017(6), pp.485–488. 10.1101/pdb.prot094714.

Marshall, E.J.P., West, T.M. and Kleijn, D., 2006. Impacts of an agri-environment field margin prescription on the flora and fauna of arable farmland in different landscapes. *Agriculture, Ecosystems and Environment*, 113(1–4), pp.36–44. 10.1016/j.agee.2005.08.036.

McHugh, N.M., Bown, B.L., Hemsley, J.A. and Holland, J.M., 2019. Relationships between agri-environment scheme habitat characteristics and insectivorous bats on arable farmland. *Basic and Applied Ecology*, 40, pp.55–66. 10.1016/j.baae.2019.09.002.

McKnight, D.T., Huerlimann, R., Bower, D.S., Schwarzkopf, L., Alford, R.A. and Zenger, K.R., 2019. MicroDecon: A highly accurate read-subtraction tool for the post-sequencing removal of contamination in metabarcoding studies. *Environmental DNA*, 1(1), pp.14–25. 10.1002/edn3.11.

McLachlan-Troup, T.A., Dickman, C.R. and Grant, T.R., 2010. Diet and dietary selectivity of the platypus in relation to season, sex and macroinvertebrate assemblages. *Journal of Zoology*, 280(3), pp.237–246. 10.1111/j.1469-7998.2009.00645.x.

Melbourne, B.A., 1999. Bias in the effect of habitat structure on pitfall traps: An experimental evaluation. *Australian Journal of Ecology*, 24(3), pp.228–239. 10.1046/J.1442-9993.1999.00967.X.

Melián, C.J., Bascompte, J., Jordano, P. and Křivan, V., 2009. Diversity in a complex ecological network with two interaction types. *Oikos*, 118(1), pp.122–130. 10.1111/j.1600-0706.2008.16751.x.

Meredith, M. and Ridout, M., 2016. Package ‘ overlap ’. Estimates of Coefficient of Overlapping for Animal Activity Patterns. *CRAN Repository*.

Met Office, 2020. *Climate summaries - Met Office* [online]. *Metoffice.Gov.Uk*. Available at: Metoffice.Gov.Uk [Accessed 29 February 2020].

Michalko, R. and Pekár, S., 2014. Is different degree of individual specialization in three spider species caused by distinct selection pressures? *Basic and Applied Ecology*, 15(6), pp.496–506. 10.1016/j.baae.2014.08.003.

Michalko, R. and Pekár, S., 2017. The Behavioral Type of a Top Predator Drives the Short-Term Dynamic of Intraguild Predation. *The American naturalist*, 189(3), pp.242–253. 10.1086/690501.

Micol, T., Doncaster, C.P. and Mackinlay, L.A., 1994. Correlates of Local Variation in the Abundance of Hedgehogs Erinaceus europaeus. *The Journal of Animal Ecology*, 63(4), p.851. 10.2307/5262.

Microsoft Corporation, 2018. Microsoft Excel.

Montez-Rath, M., Christiansen, C.L., Ettner, S.L., Loveland, S. and Rosen, A.K., 2006. Performance of statistical models to predict mental health and substance abuse cost. *BMC Medical Research Methodology*, 6(1), pp.1–11. 10.1186/1471-2288-6-53/FIGURES/4.

Montoya, D., Rogers, L. and Memmott, J., 2012. Emerging perspectives in the restoration of biodiversity-based ecosystem services. *Trends in Ecology and Evolution*, 27(12), pp.666–672. 10.1016/j.tree.2012.07.004.

Moore, L.J., Petrovan, S.O., Baker, P.J., Bates, A.J., Hicks, H.L., Perkins, S.E. and Yarnell, R.W., 2020. Impacts and Potential Mitigation of Road Mortality for Hedgehogs in Europe. *Animals*, 10(9), p.1523. 10.3390/ANI10091523.

Moorhouse-Gann, R.J., Dunn, J.C., De Vere, N., Goder, M., Cole, N., Hipperson, H. and Symondson, W.O.C., 2018. New universal ITS2 primers for high-resolution herbivory analyses using DNA metabarcoding in both tropical and temperate zones. *Scientific Reports*, 8(1), p.8542. 10.1038/s41598-018-26648-2.

Moorhouse, T.P., Palmer, S.C.F., Travis, J.M.J. and Macdonald, D.W., 2014. Hugging the hedges: Might agri-environment manipulations affect landscape permeability for hedgehogs? *Biological Conservation*, 176, pp.109–116. 10.1016/j.biocon.2014.05.015.

Morris, D.W., 2009. Apparent predation risk: Tests of habitat selection theory reveal unexpected effects of competition. *Evolutionary Ecology Research*, 11(2), pp.209–225.

Morris, P., 1973. Winter nests of the hedgehog (Erinaceus europaeus L.). *Oecologia*, 11(4), pp.299–313. 10.1007/BF00345702.

Morris, P.A., 2006. *The new Hedgehog book.* Whittet Books, Stansted.

Murray, M.H., Becker, D.J., Hall, R.J. and Hernandez, S.M., 2016. Wildlife health and supplemental feeding: A review and management recommendations. 10.1016/j.biocon.2016.10.034.

Mychek-Londer, J.G., Chaganti, S.R. and Heath, D.D., 2020. Metabarcoding of native and invasive species in stomach contents of Great Lakes fishes. *PLOS ONE*, 15(8). 10.1371/journal.pone.0236077.

Navarrete, S.A., Menge, B.A. and Daley, B.A., 2000. Species interactions in intertidal food webs: Prey or predation regulation of intermediate predators? *Ecology*, 81(8), pp.2264–2277. 10.1890/0012-9658(2000)081[2264:SIIIFW]2.0.CO;2.

Neal, E.G., 1972. The National Badger Survey. *Mammal Review*, 2(2), pp.55–64. 10.1111/j.1365-2907.1972.tb00157.x.

Neal, E.G., 1986. *The natural history of badgers*. Croom Helm.

Neal, E.G. and Cheeseman, C., 1996. *Badgers*. London: T & AD Poyser.

NERC, 2006. *Section 41: Species of Principal Importance in England*.

Nicholls, J.A., Double, M.C., Rowell, D.M. and Magrath, R.D., 2000. The evolution of cooperative and pair breeding in thornbills Acanthiza (Pardalotidae). *Journal of Avian Biology*, 31(2), pp.165–176. 10.1034/j.1600-048X.2000.310208.x.

North, A. and Ovaskainen, O., 2007. Interactions between dispersal, competition, and landscape heterogeneity. *Oikos*, 116(7), pp.1106–1119. 10.1111/j.0030-1299.2007.15366.x.

O'Donnell, C.F.J., 2000. Influence of season, habitat, temperature, and invertebrate availability on nocturnal activity of the new zealand long-tailed bat (chalinolobus tuberculatus). *New Zealand Journal of Zoology*, 27(3), pp.207–221. 10.1080/03014223.2000.9518228.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O 'hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Wagner, H. and Oksanen, M.J., 2016. Package 'vegan' Title Community Ecology Package [software], Version 2.3-5.

Olsen, L.-H., 2014. *Tracks and Signs of the Animals and Birds of Britain and Europe*. 10.1515/9781400847921.

Ostfeld, R.S., Levi, T., Keesing, F., Oggenfuss, K. and Canham, C.D., 2018. Tick-borne disease risk in a forest food web. *Ecology*, 99(7), pp.1562–1573. 10.1002/ecy.2386.

Padial, J.M.M., Avila, E., Sanchez, J.M., Âvila, E., Sánchez, J.M., Avila, E. and Sanchez, J.M., 2002. Feeding habits and overlap among red fox (Vulpes vulpes) and stone marten (Martes foina) in two Mediterranean mountain habitats. *Mammalian Biology*, 67(3), pp.137–146. 10.1078/1616-5047-00021.

Palmer, M.S., Fieberg, J., Swanson, A., Kosmala, M. and Packer, C., 2017. A 'dynamic' landscape of fear: prey responses to spatiotemporal variations in predation risk across the lunar cycle. *Ecology Letters*, 20(11), pp.1364–1373. 10.1111/ele.12832.

Parrott, D., Etherington, T.R. and Dendy, J., 2014. A geographically extensive survey of hedgehogs (Erinaceus europaeus) in England. *European Journal of Wildlife Research*, 60(2), pp.399–403. 10.1007/s10344-014-0795-2.

Peach, W.J., Denny, M., Cotton, P.A., Hill, I.F., Gruar, D., Barritt, D., Impey, A. and Mallord, J., 2004. Habitat selection by song thrushes in stable and declining farmland populations. *Journal of Applied Ecology*, 41(2), pp.275–293. 10.1111/j.0021-8901.2004.00892.x.

Penteriani, V., Kuparinen, A., del Mar Delgado, M., Palomares, F., López-Bao, J.V., Fedriani, J.M., Calzada, J., Moreno, S., Villafuerte, R., Campioni, L. and Lourenço, R., 2013. Responses of a top and a meso predator and their prey to moon phases. *Oecologia*, 173(3), pp.753–766. 10.1007/s00442-013-2651-6.

Périquet, S., Valeix, M., Claypole, J., Drouet-Hoguet, N., Salnicki, J., Mudimba, S., Revilla, E. and Fritz, H., 2015. Spotted hyaenas switch their foraging strategy as a response to changes in intraguild interactions with lions. *Journal of Zoology*, 297(4), pp.245–254. 10.1111/jzo.12275.

Périquet, S., Fritz, H. and Revilla, E., 2015. The Lion King and the Hyaena Queen: Large carnivore interactions and coexistence. *Biological Reviews*, 90(4), pp.1197–1214. 10.1111/brv.12152.

Petch, J.C., Short, C.J., Best, M.J., McCarthy, M., Lewis, H.W., Vosper, S.B. and Weeks, M., 2020. Sensitivity of the 2018 UK summer heatwave to local sea temperatures and soil moisture. *Atmospheric Science Letters*, 21(3). 10.1002/asl.948.

Peterson, C.H. and Renaud, P.E., 1989. Analysis of feeding preference experiments. *Oecologia*, 80(1), pp.82–86. 10.1007/BF00789935.

Pettett, C. E, Johnson, P.J., Moorhouse, T.P., Hambly, C., Speakman, J.R. and MacDonald, D.W., 2017. Daily energy expenditure in the face of predation: Hedgehog energetics in rural landscapes. *Journal of Experimental Biology*, 220(3), pp.460–468. 10.1242/jeb.150359.

Pettett, C. E, Moorhouse, T.P., Johnson, P.J. and Macdonald, D.W., 2017. Factors affecting hedgehog (Erinaceus europaeus) attraction to rural villages in arable landscapes. *European Journal of Wildlife Research*, 63(3), pp.1–12. 10.1007/s10344-017-1113-6.

Pettett, C.E., 2016. *Factors Affecting Hedgehog Distribution and Habitat Selection in Rural Landscapes*. University of Oxford.

Pettett, C.E., Johnson, P.J., Moorhouse, T.P. and Macdonald, D.W., 2018. National predictors of hedgehog Erinaceus europaeus distribution and decline in Britain. *Mammal Review*, 48(1), pp.1–6. 10.1111/mam.12107.

Pianka, E.R., 1974. Niche Overlap and Diffuse Competition (desert lizards/resource partitioning/community structure/species diversity). *Proceedings of the National Academy of Sciences of the United States of America*, 71(5), pp.2141–2145.

Pianka, E.R., 1973. The Structure of Lizard Communities. *Annual Review of Ecology and Systematics*. 10.1146/annurev.es.04.110173.000413.

Poel, J.L. van de, Dekker, J. and Langevelde, F. van, 2015. Dutch hedgehogs *Erinaceus europaeus* are nowadays mainly found in urban areas, possibly due to the negative Effects of badgers *Meles meles*. *Wildlife Biology*, 21(1), pp.51–55. 10.2981/wlb.00072.

Polis, G.A. and Holt, R.D., 1992. Intraguild predation: The dynamics of complex trophic interactions. *Trends in Ecology and Evolution*, 7(5), pp.151–154. 10.1016/0169-5347(92)90208-S.

Polis, G.A., Myers, C.A. and Holt, R.D., 1989. The Ecology and Evolution of Intraguild Predation: Potential Competitors That Eat Each Other. *Annual Review of Ecology and Systematics*, 20(1), pp.297–330. 10.1146/annurev.es.20.110189.001501.

Pompanon, F., Deagle, B.E., Symondson, W.O.C., Brown, D.S., Jarman, S.N. and Taberlet, P., 2012. Who is eating what: Diet assessment using next generation sequencing. *Molecular Ecology*, 21(8), pp.1931–1950. 10.1111/j.1365-294X.2011.05403.x.

Poschlod, P., Bakker, J.P. and Kahmen, S., 2005. Changing land use and its impact on biodiversity. *Basic and Applied Ecology*, 6(2), pp.93–98. 10.1016/j.baae.2004.12.001.

Pretty, J.N., 1991. Farmers' extension practice and technology adaptation: Agricultural revolution in 17-19th century Britain. *Agriculture and Human Values*, 8(1–2), pp.132–148. 10.1007/BF01579666.

Prigioni, C., Balestrieri, A., Remonti, L. and Cavada, L., 2008. Differential use of food and habitat by sympatric carnivores in the eastern Italian Alps. *Italian Journal of Zoology*, 75(2), pp.173–184. 10.1080/11250000701885521.

PTES, 2021. *Hedgehog predators - Hedgehog Street* [online]. Available at: https://www.hedgehogstreet.org/predators/ [Accessed 23 May 2021].

Quéméré, E., Aucourd, M., Troispoux, V., Brosse, S., Murienne, J., Covain, R., Le Bail, P., Olivier, J., Duplaix, N., Tysklind, N. and Galan, M., 2021. Unravelling the dietary diversity of Neotropical top predators using scat DNA metabarcoding: a case study on the elusive Giant Otter. *Molecular Ecology Resources*, p.195. 10.1002/edn3.195.

R Core Team, 2019. R: A language and environment for statistical computing [software] Version 4.0.5. *R Foundation for Statistical Computing*.

R Studio Team, 2020. RStudio: Integrated Development for R. [software].

Reddy, C.S., Yosef, R., Calvi, G. and Fornasari, L., 2019. Inter-specific competition influences apex predator-prey populations. *Wildlife Research*, 46(7), pp.628–638. 10.1071/WR19011.

Reed, J.Z., Tollit, D.J., Thompson, P.M. and Amos, W., 1997. Molecular scatology: the use of molecular genetic analysis to assign species, sex and individual identity to seal faeces. *Molecular Ecology*, 6(3), pp.225–234. 10.1046/j.1365-294X.1997.00175.x.

Rees, H.C., Maddison, B.C., Middleditch, D.J., Patmore, J.R.M. and Gough, K.C., 2014. The detection of aquatic animal species using environmental DNA - a review of eDNA as a survey tool in ecology. *Journal of Applied Ecology*, 51(5), pp.1450–1459. 10.1111/1365-2664.12306.

Reeve, N., 1994. *Hedgehogs*. London: T and AD Poyser Natural History.

Richardson, D.S., Jury, F.L., Blaakmeer, K., Komdeur, J. and Burke, T., 2001. Parentage assignment and extra-group paternity in a cooperative breeder: The Seychelles warbler (Acrocephalus sechellensis). *Molecular Ecology*, 10(9), pp.2263–2273. 10.1046/j.0962-1083.2001.01355.x.

Ridout, M.S. and Linkie, M., 2009. Estimating overlap of daily activity patterns from camera trap data. *Journal of Agricultural, Biological, and Environmental Statistics*, 14(3), pp.322–337. 10.1198/jabes.2009.08038.

Roberts, B.R., Cox, R. and Osborne, J.L., 2020. Quantifying the relative predation pressure on bumblebee nests by the European badger (Meles meles) using artificial nests. *Ecology and Evolution*, 10(3), pp.1613–1622. 10.1002/ece3.6017.

Robertson, A., McDonald, R.A., Delahay, R.J., Kelly, S.D. and Bearhop, S., 2014. Individual foraging specialisation in a social mammal: the European badger (Meles meles). *Oecologia*. 10.1007/s00442-014-3019-2.

Robinson, Q.H., Bustos, D. and Roemer, G.W., 2014. The application of occupancy modeling to evaluate intraguild predation in a model carnivore system. *Ecology*, 95(11), pp.3112–3123. 10.1890/13-1546.1.

Robinson, R.A. and Sutherland, W.J., 2002. Post-war changes in arable farming and biodiversity in Great Britain. *Journal of Applied Ecology*, 39(1), pp.157–176. 10.1046/j.1365-2664.2002.00695.x.

Rodewald, A.D. and Gehrt, S.D., 2014. Wildlife population dynamics in urban landscapes. *Urban Wildlife Conservation: Theory and Practice*, pp.117–147. 10.1007/978-1-4899-7500-3_8.

Rogers, L.M., Cheeseman, C.L., Mallinson, P.J. and Clifton-Hadley, R., 1997. The demography of a high-density badger (Meles meles) population in the west of England. *Journal of Zoology*, 242(4), pp.705–728. 10.1111/j.1469-7998.1997.tb05821.x.

Roos, S., Johnston, A. and Noble, D., 2012. UK Hedgehog datasets and their potential for long-term monitoring. *British Trust for Ornothology Research Report*, (598), pp.9–59.

Roper, T.J., 2010. *Badger*. London, UK.: Harper Collins.

Roper, T.J., 1992. Badger Meles meles setts–architecture, internal environment and function. *Mammal Review*, 22(1), pp.43–53. 10.1111/j.1365-2907.1992.tb00118.x.

Roper, T.J., 1994. The European badger Meles meles: food specialist or generalist? *Journal of Zoology*, 234(3), pp.437–452. 10.1111/j.1469-7998.1994.tb04858.x.

Rosalino, L.M., Loureiro, F., Macdonald, D.W. and Santon-Reis, M., 2005. Dietary shifts of the badger (Meles meles) in Mediterranean woodlands: An opportunistic forager with seasonal specialisms. *Mammalian Biology*, 70(1), pp.12–23. 10.1078/1616-5047-00172.

Rowcliffe, J.M., Field, J., Turvey, S.T. and Carbone, C., 2008. Estimating animal density using camera traps without the need for individual recognition. *Journal of Applied Ecology*, 45(4), pp.1228–1236. 10.1111/j.1365-2664.2008.01473.x.

Rowcliffe, J.M., Kays, R., Kranstauber, B., Carbone, C. and Jansen, P.A., 2014. Quantifying levels of animal activity using camera trap data. *Methods in Ecology and Evolution*, 5(11), pp.1170–1179. 10.1111/2041-210X.12278.

Rowcliffe, M., 2016. R Package 'activity': Animal Activity Statistics [software], Version 1.1.

Ruppert, K.M., Kline, R.J. and Rahman, M.S., 2019. Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. *Global Ecology and Conservation*, 17. 10.1016/j.gecco.2019.e00547.

Rutz, C. and Bijlsma, R.G., 2006. Food-limitation in a generalist predator. *Proceedings of the Royal Society B: Biological Sciences*, 273(1597), pp.2069–2076. 10.1098/RSPB.2006.3507.

Sabu, T.K. and Shiju, R.T., 2010. Efficacy of pitfall trapping, winkler and berlese extraction methods for measuring ground-dwelling arthropods in moist-deciduous forests in the western ghats. *Journal of Insect Science*, 10(98), pp.1–17. 10.1673/031.010.9801.

Sage Science Inc., 2016. *Blue Pippin DNA Size Selection System Operations Manual*. Beverly, MA.

Sainsbury, K.A., Shore, R.F., Schofield, H., Croose, E., Campbell, R.D. and Mcdonald, R.A., 2019. Recent history, current status, conservation and management of native mammalian carnivore species in Great Britain. *Mammal Review*, 49(2), pp.171–188. 10.1111/mam.12150.

Saint-Germain, M., Buddle, C.M., Larrivée, M., Mercado, A., Motchula, T., Reichert, E., Sackett, T.E., Sylvain, Z. and Webb, A., 2007. Should biomass be considered more frequently as a currency in terrestrial arthropod community analyses? *Journal of Applied Ecology*, 44, pp.330–339. 10.1111/j.1365-2664.2006.01269.x.

Salinas-Ramos, V.B., Ancillotto, L., Bosso, L., Sánchez-Cordero, V. and Russo, D., 2020. Interspecific competition in bats: state of knowledge and research challenges. *Mammal Review*, 50(1), pp.68–81. 10.1111/mam.12180.

Sanger, F., Nicklen, S. and Coulson, A.R., 1977. DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences of the United States of America*, 74(12), pp.5463–5467. 10.1073/pnas.74.12.5463.

Saska, P., van der Werf, W., Hemerik, L., Luff, M.L., Hatten, T.D. and Honek, A., 2013. Temperature effects on pitfall catches of epigeal arthropods: A model and method for bias correction Pocock, M., ed. *Journal of Applied Ecology*, 50(1), pp.181–189. 10.1111/1365-2664.12023.

de Satgé, J., Teichman, K. and Cristescu, B., 2017. Competition and coexistence in a small carnivore guild. *Oecologia*, (4), pp.873–884. 10.1007/s00442-017-3916-2.

Sayers, E.W., Cavanaugh, M., Clark, K., Ostell, J., Pruitt, K.D. and Karsch-Mizrachi, I., 2020. GenBank. *Nucleic Acids Research*. 10.1093/nar/gkz956.

Schaus, J., Uzal, A., Gentle, L.K., Baker, P.J., Bearman-Brown, L., Bullion, S., Gazzard, A., Lockwood, H., North, A., Reader, T., Scott, D.M., Sutherland, C.S. and Yarnell, R.W., 2020. Application of the Random Encounter Model in citizen science projects to monitor animal densities. *Remote Sensing in Ecology and Conservation*, 6(4), pp.514–528. 10.1002/rse2.153.

Scotson, L., Johnston, L.R., Iannarilli, F., Wearn, O.R., Mohd-Azlan, J., Wong, W. ming, Gray, T.N.E., Dinata, Y., Suzuki, A., Willard, C.E., Frechette, J., Loken, B., Steinmetz, R., Moßbrucker, A.M., Clements, G.R. and Fieberg, J., 2017. Best practices and software for the management and sharing of camera trap data for small and large scales studies. *Remote Sensing in Ecology and Conservation*, pp.1–15. 10.1002/rse2.54.

Seddon, J.M., Santucci, F., Reeve, N.J. and Hewitt, G.M., 2001. DNA footprints of European hedgehogs, Erinaceus europaeus and E. concolor: Pleistocene refugia, postglacial expansion and colonization routes. *Molecular Ecology*, 10(9), pp.2187–2198. 10.1046/j.0962-1083.2001.01357.x.

Sergio, F., Blas, J., Forero, M., Fernández, N., Donázar, J.A. and Hiraldo, F., 2005. Preservation of wide-ranging top predators by site-protection: Black and red kites in Doñana National Park. *Biological Conservation*, 125(1), pp.11–21.

Sergio, F. and Hiraldo, F., 2008. Intraguild predation in raptor assemblages: A review. *Ibis*, 150(1), pp.132–145. 10.1111/j.1474-919X.2008.00786.x.

Sergio, F., Marchesi, L. and Pedrini, P., 2003. Spatial refugia and the coexistence of a diurnal raptor with its intraguild owl predator. *Journal of Animal Ecology*, 72(2), pp.232–245. 10.1046/j.1365-2656.2003.00693.x.

Sévêque, A., Gentle, L.K., López-Bao, J. V., Yarnell, R.W. and Uzal, A., 2020. Human disturbance has contrasting effects on niche partitioning within carnivore communities. *Biological Reviews*, 95(6), pp.1689–1705. 10.1111/brv.12635.

Shepherdson, D.J., Roper, T.J. and Lüps, P., 1990. Diet, food availability and foraging behaviour of badgers (Meies meles L.) in southern England. *Z. Säugetierkunde*, 55(2), pp.81–93.

Sheppard, S.K. and Harwood, J.D., 2005. Advances in molecular ecology: Tracking trophic links through predator-prey food-webs. *Functional Ecology*, 19(5), pp.751–762. 10.1111/j.1365-2435.2005.01041.x.

Shi, H., Li, W. and Xu, X., 2016. Learning the Comparing and Converting Method of Sequence Phred Quality Score. In: Atlantis Press, pp. 260–263. 10.2991/meici-16.2016.55.

Sih, A. and Christensen, B., 2001. Optimal diet theory: when does it work, and when and why does it fail? *Animal Behaviour*, 61(2), pp.379–390. 10.1006/anbe.2000.1592.

Da Silva, J., Woodroffe, R. and Macdonald, D.W., 1993. Habitat, food availability and group territoriality in the European badger, Meles meles. *Oecologia*, 95, pp.558–564.

Skoog, P., 1970. The food of the Swedish badger, Meles meles L. *Yiltrevy*, 7, pp.1–120.

Smith, J., Potts, S. and Eggleton, P., 2008. Evaluating the efficiency of sampling methods in assessing soil macrofauna communities in arable systems. *European Journal of Soil Biology*, 44(3), pp.271–276. 10.1016/J.EJSOBI.2008.02.002.

Smith, J.A., Thomas, A.C., Levi, T., Wang, Y. and Wilmers, C.C., 2018. Human activity reduces niche partitioning among three widespread mesocarnivores. *Oikos*, 127(6), pp.890–901. 10.1111/oik.04592.

Smith, V., Bohan, D.A., Clark, S.J., Haughton, A.J., Bell, J.R. and Heard, M.S., 2008. Weed and invertebrate community compositions in arable farmland. *Arthropod-Plant Interactions*, 2(1), pp.21–30. 10.1007/s11829-007-9027-y.

Sommer, R.S., 2007. When east met west: The sub-fossil footprints of the west European hedgehog and the northern white-breasted hedgehog during the Late Quaternary in Europe. *Journal of Zoology*, 273(1), pp.82–89. 10.1111/j.1469-7998.2007.00302.x.

de Sousa, L.L., Silva, S.M. and Xavier, R., 2019. DNA metabarcoding in diet studies: Unveiling ecological aspects in aquatic and terrestrial ecosystems. *Environmental DNA*, 1(3), pp.199–214. 10.1002/edn3.27.

Sousa, L.L., Silva, S.M. and Xavier, R., 2019. DNA metabarcoding in diet studies: Unveiling ecological aspects in aquatic and terrestrial ecosystems. *Environmental DNA*, 1(3), pp.199–214. 10.1002/edn3.27.

South, K.E., Haynes, K. and Jackson, A.C., 2020. Hibernation Patterns of the European Hedgehog, Erinaceus europaeus, at a Cornish Rescue Centre. *Animals*, 10(8), p.1418. 10.3390/ANI10081418.

Sperry, J.H. and Weatherhead, P.J., 2009. Does prey availability determine seasonal patterns of habitat selection in Texas ratsnakes. *Journal of Herpetology*, 43(1), pp.55–64. 10.1670/08-058R1.1.

Steinmetz, R., Seuaturien, N., Intanajitjuy, P., Inrueang, P. and Prempree, K., 2020. The effects of prey depletion on dietary niches of sympatric apex predators in Southeast Asia. *Integrative Zoology*, 16(1), pp.19–32. 10.1111/1749-4877.12461.

Steinmetz, R., Seuaturien, N. and Chutipong, W., 2013. Tigers, leopards, and dholes in a half-empty forest: Assessing species interactions in a guild of threatened carnivores. *Biological Conservation*, 163, pp.68–78. 10.1016/j.biocon.2012.12.016.

Strauss, R.E., 1979. Reliability Estimates for Ivlev's Electivity Index, the Forage Ratio, and a Proposed Linear Index of Food Selection. *Transactions of the American Fisheries Society*, 108(4), pp.344–352. 10.1577/1548-8659(1979)108<344:refiei>2.0.co;2.

Takimoto, G., Post, D.M., Spiller, D.A. and Holt, R.D., 2012. Effects of productivity, disturbance, and ecosystem size on food-chain length: Insights from a metacommunity model of intraguild predation. *Ecological Research*, 27(3), pp.481–493. 10.1007/s11284-012-0929-5.

Tamura, K., Dudley, J., Nei, M. and Kumar, S., 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*. 10.1093/molbev/msm092.

Tashima, S., Kaneko, Y., Anezaki, T., Baba, M., Yachimori, S., Abramov, A. V., Saveljev, A.P. and Masuda, R., 2011. Phylogeographic Sympatry and Isolation of the Eurasian Badgers (Meles , Mustelidae, Carnivora): Implications for an Alternative Analysis using Maternally as Well as Paternally Inherited Genes. *Zoological Science*, 28(4), pp.293–303. 10.2108/zsj.28.293.

Thompson, C.M. and Gese, E.M., 2007. Food webs and intraguild predation: Community interactions of a native mesocarnivore. *Ecology*, 88(2), pp.334–346. 10.1890/0012-9658(2007)88[334:FWAIPC]2.0.CO;2.

Thornton, P.S., 1988. Density and distribution of Badgers in south-west England–a predictive model. *Mammal Review*, 18(1), pp.11–23. 10.1111/j.1365-2907.1988.tb00066.x.

Tilling, S.M., 1987. *A key to the major groups of British terrestrial invertebrates.* Field Studies Council.

Trewby, I.D., Wilson, G.J., Delahay, R.J., Walker, N., Young, R., Davison, J., Cheeseman, C., Robertson, P.A., Gorman, M.L. and McDonald, R.A., 2008. Experimental evidence of competitive release in sympatric carnivores. *Biology Letters*, 4(2), pp.170–172. 10.1098/rsbl.2007.0516.

Trewby, I.D., Young, R., McDonald, R.A., Wilson, G.J., Davison, J., Walker, N., Robertson, A., Doncaster, C.P. and Delahay, R.J., 2014. Impacts of removing badgers on localised counts of hedgehogs. *PLoS ONE*, 9(4). 10.1371/journal.pone.0095477.

Trites, A.W. and Joy, R., 2005. Dietary analysis from fecal samples: How many scats are enough? *Journal of Mammalogy*, 86(4), pp.704–712. 10.1644/1545-1542(2005)086[0704:DAFFSH]2.0.CO;2.

Tscharntke, T., Klein, A.M., Kruess, A., Steffan-Dewenter, I. and Thies, C., 2005. Landscape perspectives on agricultural intensification and biodiversity - Ecosystem service management. *Ecology Letters*, 8(8), pp.857–874. 10.1111/j.1461-0248.2005.00782.x.

Tsunoda, H., Raichev, E.G., Newman, C., Masuda, R., Georgiev, D.M., Kaneko, Y. and Hayward, M., 2017. Food niche segregation between sympatric golden jackals and red foxes in central Bulgaria. *Journal of Zoology*, 303(1), pp.64–71. 10.1111/jzo.12464.

Tucker, M.A. and Rogers, T.L., 2014. Examining predator–prey body size, trophic level and body mass across marine and terrestrial mammals. *Proceedings of the Royal Society B: Biological Sciences*, 281(1797). 10.1098/RSPB.2014.2103.

Tylianakis, J.M., Laliberté, E., Nielsen, A. and Bascompte, J., 2010. Conservation of species interaction networks. *Biological Conservation*, 143(10), pp.2270–2279. 10.1016/J.BIOCON.2009.12.004.

Tysnes, T.M., 2016. *Factors Affecting Hedgehog (Erinaceus europaeus) - Presence in Residential Areas in Southern Norway Assessed by a Questionnaire Survey*. Norwegian University of Life Sciences,.

Uiterwaal, S.F. and DeLong, J.P., 2020. Using patterns in prey DNA digestion rates to quantify predator diets. *Molecular Ecology Resources*, 20(6), pp.1723–1732. 10.1111/1755-0998.13231.

Ushio, M., Fukuda, H., Inoue, T., Makoto, K., Kishida, O., Sato, K., Murata, K., Nikaido, M., Sado, T., Sato, Y., Takeshita, M., Iwasaki, W., Yamanaka, H., Kondoh, M. and Miya, M., 2017. Environmental DNA enables detection of terrestrial mammals from forest pond water. *Molecular Ecology Resources*, 17(6), pp.e63–e75. 10.1111/1755-0998.12690.

Valentini, A., Taberlet, P., Miaud, C., Civade, R., et al., 2016. Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Molecular Ecology*, 25(4), pp.929–942. 10.1111/mec.13428.

Vickery, J., Carter, N. and Fuller, R.J., 2002. The potential value of managed cereal field margins as foraging habitats for farmland birds in the UK. *Agriculture, Ecosystems and Environment*, 89(1–2), pp.41–52. 10.1016/S0167-8809(01)00317-6.

Vickery, J.A., Tallowin, J.R., Feber, R.E., Asteraki, E.J., Atkinson, P.W., Fuller, R.J. and Brown, V.K., 2001. The management of lowland neutral grasslands in britain: Effects of agricultural practices on birds and their food resources. *Journal of Applied Ecology*, 38(3), pp.647–664. 10.1046/j.1365-2664.2001.00626.x.

Virgós, E., Baniandrés, N., Burgos, T. and Recio, M.R., 2020. Intraguild predation by the eagle owl determines the space use of a mesopredator carnivore. *Diversity*, 12(9), p.359. 10.3390/D12090359.

Wang, M., Beck, C.R., English, A.C., Meng, Q., Buhay, C., Han, Y., Doddapaneni, H. V., Yu, F., Boerwinkle, E., Lupski, J.R., Muzny, D.M. and Gibbs, R.A., 2015. PacBio-LITS: A large-insert targeted sequencing method for characterization of human disease-associated chromosomal structural variations. *BMC Genomics*, 16(1), pp.1–12. 10.1186/s12864-015-1370-2.

Ward, J.F., MacDonald, D.W., Doncaster, C.P. and Mauget, C., 1996. Physiological response of the European hedgehog to predator and nonpredator odour. *Physiology and Behavior*, 60(6), pp.1469–1472. 10.1016/S0031-9384(96)00245-4.

Ward, J.F., MacDonald, D.W. and Doncaster, C.P., 1997. Responses of foraging hedgehogs to badger odour. *Animal Behaviour*, 53(4), pp.709–720. 10.1006/anbe.1996.0307.

Wembridge, D., Newman, M.R., Bright, P.W. and Morris, P.A., 2016. Hedgehog road casualties in Great Britain. *Mammal communications*, (2), pp.8–14.

Wembridge, D., 2011. *The state of Britain's hedgehogs 2011*.

Wembridge, D. and Langton, S., 2015. *The State of Britain's Hedgehogs 2015*.

Wembridge, D. and Wilson, E., 2018. *The State of Britain's Hedgehogs 2018*.

Westoby, M., 1974. An analysis of diet selection by large generalist herbivores. *American Naturalist*, 108(961), pp.290–304. 10.1086/282908.

White, T.C.R., 2008. The role of food, weather and climate in limiting the abundance of animals. *Biological Reviews*, 83(3), pp.227–248. 10.1111/J.1469-185X.2008.00041.X.

Wikenros, C., Aronsson, M., Liberg, O., Jarnemo, A., Hansson, J., Wallgren, M., Sand, H. and Bergström, R., 2017. Fear or food – abundance of red fox in relation to occurrence of lynx and wolf. *Scientific Reports*, 7(1). 10.1038/s41598-017-08927-6.

Wildlife Online, *How significant are foxes and badgers as predators of hedgehogs? | Wildlife Online* [online]. Available at: https://www.wildlifeonline.me.uk/questions/answer/how-significant-are-foxes-and-badgers-as-predators-of-hedgehogs [Accessed 7 June 2021].

Williams, B.M., Mann, N., Neumann, J.L., Yarnell, R.W. and Baker, P.J., 2018. A prickly problem: developing a volunteer-friendly tool for monitoring populations of a terrestrial urban mammal, the West European hedgehog (Erinaceus europaeus). *Urban Ecosystems*. 10.1007/s11252-018-0795-1.

Williams, B.M., Baker, P.J., Thomas, E., Wilson, G., Judge, J. and Yarnell, R.W., 2018. Reduced occupancy of hedgehogs (Erinaceus europaeus) in rural England and Wales: The influence of habitat and an asymmetric intra-guild predator. *Scientific Reports*, 8(1). 10.1038/s41598-018-30130-4.

Wilson, C. and Tisdell, C., 2001. Why farmers continue to use pesticides despite environmental, health and sustainability costs. *Ecological Economics*, 39(3), pp.449–462. 10.1016/S0921-8009(01)00238-5.

Wilson, G., Harris, S. and McLaren, G., 1997. *Changes in the British badger population, 1988 to 1997*. London: Peoples Trust for Endangered Species.

Woodroffe, R., 1995. Body condition affects implantation date in the European badger, Meles meles. *Journal of Zoology*, 236(2), pp.183–188. 10.1111/J.1469-7998.1995.TB04486.X.

Wright, P.G.R., Coomber, F.G., Bellamy, C.C., Perkins, S.E. and Mathews, F., 2020. Predicting hedgehog mortality risks on British roads using habitat suitability modelling. *Biodiversity and Conservation*, 2020(1). 10.7717/peerj.8154.

Wroot, A.J., 1984. *Feeding ecology of the European hedgehog, Erinaceus europaeus*. Royal Holloway, University of London.

Yalden, D.W., 1976. The food of the hedgehog in England. *Acta Theriologica*, 21, pp.401–424. 10.4098/AT.arch.76-39.

Yarnell, R.W., Pacheco, M., Williams, B.M., Neumann, J.L., Rymer, D.J. and Baker, P.J., 2014. Using occupancy analysis to validate the use of footprint tunnels as a method for monitoring the hedgehog Erinaceus europaeus. *Mammal Review*, 44(3–4), pp.234–238. 10.1111/mam.12026.

Yarnell, R.W. and Pettett, C.E., 2020. Beneficial Land Management for Hedgehogs (Erinaceus europaeus) in the United Kingdom. *Animals*, 10(9), p.1566. 10.3390/ani10091566.

Young, M.J., Dutoit, L., Robertson, F., Van Heezik, Y., Seddon, P.J. and Robertson, B.C., 2020. Species in the faeces: DNA metabarcoding as a method to determine the diet of the endangered yellow-eyed penguin. *Wildlife Research*, 47(6), pp.509–522. 10.1071/WR19246.

Young, R.P., Davison, J., Trewby, I.D., Wilson, G.J., Delahay, R.J. and Doncaster, C.P., 2006. Abundance of hedgehogs (Erinaceus europaeus) in relation to the density and distribution of badgers (Meles meles). *Journal of Zoology*, 269(3), pp.349–356. 10.1111/j.1469-7998.2006.00078.x.

Yu, D.W., Ji, Y., Emerson, B.C., Wang, X., Ye, C., Yang, C. and Ding, Z., 2012. Biodiversity soup: Metabarcoding of arthropods for rapid biodiversity assessment and biomonitoring. *Methods in Ecology and Evolution*, 3(4), pp.613–623. 10.1111/j.2041-210X.2012.00198.x.

Zabala, J. and Zuberogoitia, I., 2003. Badger, Meles meles (Mustelidae, Carnivora), diet assessed through scat-analysis: A comparison and critique of different methods. *Folia Zoologica*, 52(1), pp.23–30.

Zabalo, J., 2012. Permanence in an Intraguild Predation Model with Prey Switching. *Bulletin of Mathematical Biology*, 74(9), pp.1957–1984. 10.1007/s11538-012-9740-2.

Zeale, M.R.K., Butlin, R.K., Barker, G.L.A., Lees, D.C. and Jones, G., 2011. Taxon-specific PCR for DNA barcoding arthropod prey in bat faeces. *Molecular Ecology Resources*, 11(2), pp.236–244. 10.1111/j.1755-0998.2010.02920.x.

Zhou, Y.-B., Newman, C., Xu, W.-T., Buesching, C.D., Zalewski, A., Kaneko, Y., Macdonald, D.W. and Xie, Z.-Q., 2011. Biogeographical variation in the diet of Holarctic martens (genus Martes, Mammalia: Carnivora: Mustelidae): adaptive foraging in generalists. *Journal of Biogeography*, 38(1), pp.137–147. 10.1111/j.1365-2699.2010.02396.x.

Zhou, Y., Chen, W., Kaneko, Y., Newman, C., Liao, Z., Zhu, X., Buesching, C.D., Xie, Z. and Macdonald, D.W., 2015. Seasonal dietary shifts and food resource exploitation by the hog badger (Arctonyx collaris) in a Chinese subtropical forest. *European Journal of Wildlife Research*, 61(1), pp.125–133. 10.1007/s10344-014-0881-5.

van Zoeren, J., Guédot, C. and Steffan, S.A., 2018. Conserving carnivorous arthropods: an example from early-season cranberry (Ericaceae) flooding. *The Canadian Entomologist*, 150(02), pp.265–273. 10.4039/tce.2017.66.

Appendices

Appendix A: Identification of the originator species of scat samples for hedgehog and badger following Sanger sequencing using DNA amplified with MiMammal primers (a). Sequences were compared against the NCBI database using the BLASTn function. Sample numbers correspond to the position of samples within 96-well plates for hedgehog (b) and badger (c) samples. Samples from each species were processed on separate plates to minimize laboratory contamination.

Sample	Species	Illumina Tag	Site	BLASTn taxonomic assignment match (%)
1	Hedgehog	Fi5_1Ri7_1	Brackenhurst	88.00%
25	Hedgehog	Fi5_2Ri7_1	Brackenhurst	96.00%
49	Hedgehog	Fi5_3Ri7_1	Brackenhurst	98.00%
73	Hedgehog	Fi5_4Ri7_1	Brackenhurst	88.00%
97	Hedgehog	Fi5_5Ri7_1	Brackenhurst	83.00%
121	Hedgehog	Fi5_6Ri7_1	Brackenhurst	83.00%
145	Hedgehog	Fi5_7Ri7_1	Brackenhurst	97.52%
169	Hedgehog	Fi5_8Ri7_1	Brackenhurst	98.00%
2	Hedgehog	Fi5_1Ri7_2	Brackenhurst	91.95%
26	Hedgehog	Fi5_2Ri7_2	Brackenhurst	89.47%
50	Negative	Fi5_3Ri7_2		
74	Hedgehog	Fi5_4Ri7_2	Brackenhurst	98.00%
98	Hedgehog	Fi5_5Ri7_2	Brackenhurst	96.89%
122	Hedgehog	Fi5_6Ri7_2	Brackenhurst	94.23%
146	Hedgehog	Fi5_7Ri7_2	Brackenhurst	96.89%
170	Hedgehog	Fi5_8Ri7_2	Brackenhurst	97.52%
3	Hedgehog	Fi5_1Ri7_3	Brackenhurst	84.46%
27	Hedgehog	Fi5_2Ri7_3	Brackenhurst	90.56%
51	Hedgehog	Fi5_3Ri7_3	Brackenhurst	97.52%
75	Hedgehog	Fi5_4Ri7_3	Brackenhurst	83.77%
99	Hedgehog	Fi5_5Ri7_3	Brackenhurst	97.52%
123	Negative	Fi5_6Ri7_3		
147	Hedgehog	Fi5_7Ri7_3	Brackenhurst	97.52%
171	Hedgehog	Fi5_8Ri7_3	Brackenhurst	97.52%
4	Hedgehog	Fi5_1Ri7_4	Brackenhurst	98.83%
28	Hedgehog	Fi5_3Ri7_4	Brackenhurst	95.65%
52	Hedgehog	Fi5_3Ri7_4	Brackenhurst	95.65%
76	Hedgehog	Fi5_4Ri7_4	Brackenhurst	97.52%
100	Hedgehog	Fi5_5Ri7_4	Brackenhurst	97.52%
124	Hedgehog	Fi5_6Ri7_4	Brackenhurst	97.52%
148	Hedgehog	Fi5_7Ri7_4	Brackenhurst	98.70%
172	Hedgehog	Fi5_8Ri7_4	Brackenhurst	79.02%
5	Negative	Fi5_1Ri7_5		
29	Hedgehog	Fi5_2Ri7_5	Brackenhurst	98.14%

Sample	Species	Illumina Tag	Site	BLASTn taxonomic assignment match (%)
53	Hedgehog	Fi5_3Ri7_5	Brackenhurst	98.42%
77	Hedgehog	Fi5_4Ri7_5	Hartpury	98.92%
101	Hedgehog	Fi5_5Ri7_5	Hartpury	91.16%
125	Hedgehog	Fi5_6Ri7_5	Hartpury	96.27%
149	Hedgehog	Fi5_7Ri7_5	Hartpury	96.89%
173	Hedgehog	Fi5_8Ri7_5	Hartpury	98.66%
6	Hedgehog	Fi5_1Ri7_6	Hartpury	97.52%
30	Hedgehog	Fi5_2Ri7_6	Hartpury	98.83%
54	Hedgehog	Fi5_3Ri7_6	Hartpury	98.00%
78	Negative	Fi5_4Ri7_6		
102	Hedgehog	Fi5_5Ri7_6	Hartpury	98.51%
126	Hedgehog	Fi5_6Ri7_6	Hartpury	96.12%
150	Hedgehog	Fi5_7Ri7_6	Hartpury	98.77%
174	Hedgehog	Fi5_8Ri7_6	Hartpury	98.79%
7	Hedgehog	Fi5_1Ri7_7	Hartpury	97.44%
31	Hedgehog	Fi5_2Ri7_7	Hartpury	97.52%
55	Hedgehog	Fi5_3Ri7_7	Hartpury	96.51%
79	Hedgehog	Fi5_4Ri7_7	Hartpury	96.89%
103	Hedgehog	Fi5_5Ri7_7	Hartpury	94.44%
127	Hedgehog	Fi5_6Ri7_7	Hartpury	97.52%
151	Negative	Fi5_7Ri7_7		
175	Hedgehog	Fi5_8Ri7_7	Hartpury	96.89%
8	Hedgehog	Fi5_1Ri7_8	Hartpury	98.66%
32	Hedgehog	Fi5_2Ri7_8	Hartpury	97.44%
56	Hedgehog	Fi5_3Ri7_8	Hartpury	96.89%
80	Hedgehog	Fi5_4Ri7_8	Hartpury	98.66%
104	Hedgehog	Fi5_5Ri7_8	Hartpury	97.56%
128	Hedgehog	Fi5_6Ri7_8	Hartpury	97.52%
152	Hedgehog	Fi5_7Ri7_8	Hartpury	97.52%
176	Hedgehog	Fi5_8Ri7_8	Hartpury	97.52%
9	Hedgehog	Fi5_1Ri7_9	Hartpury	98.00%
33	Negative	Fi5_2Ri7_9		
57	Hedgehog	Fi5_3Ri7_9	Hartpury	81.82%
81	Hedgehog	Fi5_4Ri7_9	Hartpury	97.52%
105	Hedgehog	Fi5_5Ri7_9	Hartpury	99.89%
129	Hedgehog	Fi5_6Ri7_9	Hartpury	97.52%
153	Samples not applicable to this present study			
177	Samples not applicable to this present study			
10	Samples not applicable to this present study			
34	Samples not applicable to this present study			
58	Samples not applicable to this present study			
82	Samples not applicable to this present study			
106	Negative	Fi5_5Ri7_10		

Sample	Species	Illumina Tag	Site	BLASTn taxonomic assignment match (%)
130	Samples not applicable to this present study			
154	Samples not applicable to this present study			
178	Samples not applicable to this present study			
11	Samples not applicable to this present study			
35	Hedgehog	Fi5_2Ri7_11	Hartpury	98.32%
59	Hedgehog	Fi5_3Ri7_11	Hartpury	98.83%
83	Hedgehog	Fi5_4Ri7_11	Hartpury	98.83%
107	Hedgehog	Fi5_5Ri7_11	Hartpury	97.59%
131	Hedgehog	Fi5_6Ri7_11	Brackenhurst	98.18%
155	Hedgehog	Fi5_7Ri7_11	Brackenhurst	99.44%
179	Negative	Fi5_8Ri7_11		
12	Hedgehog	Fi5_1Ri7_12	Replicates	
36	Hedgehog	Fi5_2Ri7_12	Replicates	
60	Hedgehog	Fi5_3Ri7_12	Replicates	
84	Hedgehog	Fi5_4Ri7_12	Replicates	
108	Hedgehog	Fi5_5Ri7_12	Replicates	
132	Hedgehog	Fi5_6Ri7_12	Replicates	
156	Hedgehog	Fi5_7Ri7_12	Replicates	
180	Samples not applicable to this present study			
13	Badger	Fi5_1Ri7_13	Brackenhurst	85.62%
37	Badger	Fi5_2Ri7_13	Brackenhurst	98.91%
61	Badger	Fi5_3Ri7_13	Brackenhurst	98.36%
85	Badger	Fi5_4Ri7_13	Brackenhurst	97.81%
109	Badger	Fi5_5Ri7_13	Brackenhurst	100.00%
133	Badger	Fi5_6Ri7_13	Brackenhurst	100.00%
157	Badger	Fi5_7Ri7_13	Brackenhurst	100.00%
181	Badger	Fi5_8Ri7_13	Brackenhurst	100.00%
14	Badger	Fi5_1Ri7_14	Brackenhurst	100.00%
38	Badger	Fi5_2Ri7_14	Brackenhurst	100.00%
62	Negative	Fi5_3Ri7_14		
86	Badger	Fi5_4Ri7_14	Brackenhurst	100.00%
110	Badger	Fi5_5Ri7_14	Brackenhurst	87.65%
134	Badger	Fi5_6Ri7_14	Brackenhurst	99.37%
158	Badger	Fi5_7Ri7_14	Brackenhurst	86.42%
182	Badger	Fi5_8Ri7_14	Brackenhurst	96.86%
15	Badger	Fi5_1Ri7_15	Brackenhurst	91.51%
39	Badger	Fi5_2Ri7_15	Brackenhurst	100.00%
63	Badger	Fi5_3Ri7_15	Brackenhurst	100.00%
87	Badger	Fi5_4Ri7_15	Brackenhurst	100.00%
111	Badger	Fi5_5Ri7_15	Brackenhurst	100.00%
135	Negative	Fi5_6Ri7_15		
159	Badger	Fi5_7Ri7_15	Brackenhurst	100.00%
183	Badger	Fi5_8Ri7_15	Brackenhurst	100.00%

Sample	Species	Illumina Tag	Site	BLASTn taxonomic assignment match (%)
16	Badger	Fi5_1Ri7_16	Brackenhurst	100.00%
40	Badger	Fi5_3Ri7_16	Brackenhurst	98.88%
64	Badger	Fi5_3Ri7_16	Brackenhurst	100.00%
88	Badger	Fi5_4Ri7_16	Brackenhurst	87.29%
112	Badger	Fi5_5Ri7_16	Brackenhurst	100.00%
136	Badger	Fi5_6Ri7_16	Brackenhurst	99.35%
160	Badger	Fi5_7Ri7_16	Brackenhurst	100.00%
184	Badger	Fi5_8Ri7_16	Brackenhurst	96.86%
17	Negative	Fi5_1Ri7_17		
41	Badger	Fi5_2Ri7_17	Brackenhurst	85.89%
65	Badger	Fi5_3Ri7_17	Brackenhurst	100.00%
89	Badger	Fi5_4Ri7_17	Brackenhurst	100.00%
113	Badger	Fi5_5Ri7_17	Brackenhurst	98.15%
137	Badger	Fi5_6Ri7_17	Brackenhurst	85.22%
161	Badger	Fi5_7Ri7_17	Brackenhurst	81.97%
185	Badger	Fi5_8Ri7_17	Brackenhurst	100.00%
18	Badger	Fi5_1Ri7_18	Brackenhurst	98.15%
42	Badger	Fi5_2Ri7_18	Brackenhurst	100.00%
66	Badger	Fi5_3Ri7_18	Brackenhurst	100.00%
90	Negative	Fi5_4Ri7_18		
114	Badger	Fi5_5Ri7_18	Hartpury	100.00%
138	Badger	Fi5_6Ri7_18	Hartpury	100.00%
162	Badger	Fi5_7Ri7_18	Hartpury	100.00%
186	Badger	Fi5_8Ri7_18	Hartpury	99.37%
19	Badger	Fi5_1Ri7_19	Hartpury	100.00%
43	Badger	Fi5_2Ri7_19	Hartpury	99.37%
67	Badger	Fi5_3Ri7_19	Hartpury	96.86%
91	Badger	Fi5_4Ri7_19	Hartpury	100.00%
115	Badger	Fi5_5Ri7_19	Hartpury	100.00%
139	Badger	Fi5_6Ri7_19	Hartpury	100.00%
163	Negative	Fi5_7Ri7_19		
187	Badger	Fi5_8Ri7_19	Hartpury	100.00%
20	Badger	Fi5_1Ri7_20	Hartpury	100.00%
44	Badger	Fi5_2Ri7_20	Hartpury	100.00%
68	Badger	Fi5_3Ri7_20	Hartpury	100.00%
92	Badger	Fi5_4Ri7_20	Hartpury	100.00%
116	Badger	Fi5_5Ri7_20	Hartpury	100.00%
140	Badger	Fi5_6Ri7_20	Hartpury	99.87%
164	Badger	Fi5_7Ri7_20	Hartpury	98.18%
188	Badger	Fi5_8Ri7_20	Hartpury	100.00%
21	Badger	Fi5_1Ri7_21	Hartpury	100.00%
45	Negative	Fi5_2Ri7_21		
69	Badger	Fi5_3Ri7_21	Hartpury	100.00%

Sample	Species	Illumina Tag	Site	BLASTn taxonomic assignment match (%)
93	Badger	Fi5_4Ri7_21	Hartpury	96.23%
117	Badger	Fi5_5Ri7_21	Hartpury	100.00%
141	Badger	Fi5_6Ri7_21	Hartpury	100.00%
165	Badger	Fi5_7Ri7_21	Hartpury	97.42%
189	Badger	Fi5_8Ri7_21	Hartpury	100.00%
22	Badger	Fi5_1Ri7_22	Hartpury	100.00%
46	Badger	Fi5_2Ri7_22	Hartpury	100.00%
70	Badger	Fi5_3Ri7_22	Hartpury	100.00%
94	Badger	Fi5_4Ri7_22	Hartpury	99.84%
118	Negative	Fi5_5Ri7_22		
142	Badger	Fi5_6Ri7_22	Hartpury	100.00%
166	Badger	Fi5_7Ri7_22	Hartpury	100.00%
190	Badger	Fi5_8Ri7_22	Hartpury	99.37%
23	Badger	Fi5_1Ri7_23	Hartpury	100.00%
47	Badger	Fi5_2Ri7_23	Hartpury	98.88%
71	Badger	Fi5_3Ri7_23	Hartpury	99.15%
95	Badger	Fi5_4Ri7_23	Hartpury	99.40%
119	Badger	Fi5_5Ri7_23	Hartpury	92.74%
143	Badger	Fi5_6Ri7_23	Hartpury	91.98%
167	Badger	Fi5_7Ri7_23	Hartpury	97.53%
191	Negative	Fi5_8Ri7_23		
24	Badger	Fi5_1Ri7_24	Replicates	
48	Badger	Fi5_2Ri7_24	Replicates	
72	Badger	Fi5_3Ri7_24	Replicates	
96	Badger	Fi5_4Ri7_24	Replicates	
120	Badger	Fi5_5Ri7_24	Replicates	
144	Badger	Fi5_6Ri7_24	Replicates	
168	Badger	Fi5_7Ri7_24	Replicates	
192	Badger	Fi5_8Ri7_24	Replicates	

b)

1	2	3	4	5	6	7	8	9	10	11	12
25	26	27	28	29	30	31	32	33	34	35	36
49	50	51	52	53	54	55	56	57	58	59	60
73	74	75	76	77	78	79	80	81	82	83	84
97	98	99	100	101	102	103	104	105	106	107	108
121	122	123	124	125	126	127	128	129	130	131	132
145	146	147	148	149	150	151	152	153	154	155	156
169	170	171	172	173	174	175	176	177	178	179	180

c)

13	14	15	16	17	18	19	20	21	22	23	24
37	38	39	40	41	42	43	44	45	46	47	48
61	62	63	64	65	66	67	68	69	70	71	72
85	86	87	88	89	90	91	92	93	94	95	96
109	110	111	112	113	114	115	116	117	118	119	120
133	134	135	136	137	138	139	140	141	142	143	144
157	158	159	160	161	162	163	164	165	166	167	168
181	182	183	184	185	186	187	188	189	190	191	192

Appendix B: Number of primer mismatches between several mammal species full mtDNA and MiMammal primers.

Common name	Latin Name	Complete mtDNA used	MiMammal Forward primer	MiMammal Reverse primer
Badger	*Meles meles*	Y	1	0
Dog	*Canis lupus familiaris*	Y	0	0
Hedgehog	*Erinaceus europaeus*	Y	3	3
House sparrow	*Passer domesticus*	Y	3	0
Mallard	*Anas platyrhynchos*	Y	3	0
Red fox	*Vulpes vulpes*	Y	0	0

Appendix C: Identification of badger sex from DNA obtained from facecal samples (n=16) using RG4 (Y-linked, SRY) (Griffiths and Tiwari 1993), and Mel592 (X-linked) (Annavi et al. 2011) primer sets. Sex was successfully assigned to 37.5% of badger samples analysed.

Sample	Mel592		SRY		Sex
Name	Allele 1	Allele 2	Allele 1	Allele 2	confirmed
40	238	238	123	123	Male
48	238	240			Female
41	238	238	124	124	Male
49					-
42	248	248	125	125	Male
50					-
43	248	248			-
51			124	124	-
44	232	232	124	124	Male
52	238	240			Female
45	238	238			-
53			125	125	-
46			124	124	-
56	240	240			-
47					-
57	240	240			-

Appendix D: Plate organisation (96-well) with dual indexes for hedgehog (a) and badger (b) DNA scat samples including negatives (shaded blue) and replicates (shaded green) in preparation of sequencing on the Illumina MiSeq Platform.

a)

Ri7_1Fi5_1	Ri7_1Fi5_2	Ri7_1Fi5_3	Ri7_1Fi5_4	Ri7_1Fi5_5	Ri7_1Fi5_6	Ri7_1Fi5_7	Ri7_1Fi5_8	Ri7_1Fi5_9	Ri7_1Fi5_10	Ri7_1Fi5_11	Ri7_1Fi5_12
Ri7_2Fi5_1	Ri7_2Fi5_2	Ri7_2Fi5_3	Ri7_3Fi5_4	Ri7_2Fi5_5	Ri7_2Fi5_6	Ri7_2Fi5_7	Ri7_2Fi5_8	Ri7_2Fi5_9	Ri7_2Fi5_10	Ri7_2Fi5_11	Ri7_2Fi5_12
Ri7_3Fi5_1	Ri7_3Fi5_2	Ri7_3Fi5_3	Ri7_3Fi5_4	Ri7_3Fi5_5	Ri7_3Fi5_6	Ri7_3Fi5_7	Ri7_3Fi5_8	Ri7_3Fi5_9	Ri7_3Fi5_10	Ri7_3Fi5_11	Ri7_3Fi5_12
Ri7_4Fi5_1	Ri7_4Fi5_2	Ri7_4Fi5_3	Ri7_4Fi5_4	Ri7_4Fi5_5	Ri7_4Fi5_6	Ri7_4Fi5_7	Ri7_4Fi5_8	Ri7_4Fi5_9	Ri7_4Fi5_10	Ri7_4Fi5_11	Ri7_4Fi5_12
Ri7_5Fi5_1	Ri7_5Fi5_2	Ri7_5Fi5_3	Ri7_5Fi5_4	Ri7_5Fi5_5	Ri7_5Fi5_6	Ri7_5Fi5_7	Ri7_5Fi5_8	Ri7_5Fi5_9	Ri7_5Fi5_10	Ri7_5Fi5_11	Ri7_5Fi5_12
Ri7_6Fi5_1	Ri7_6Fi5_2	Ri7_6Fi5_3	Ri7_6Fi5_4	Ri7_6Fi5_5	Ri7_6Fi5_6	Ri7_6Fi5_7	Ri7_6Fi5_8	Ri7_6Fi5_9	Ri7_6Fi5_10	Ri7_6Fi5_11	Ri7_6Fi5_12
Ri7_7Fi5_1	Ri7_7Fi5_2	Ri7_7Fi5_3	Ri7_7Fi5_4	Ri7_7Fi5_5	Ri7_7Fi5_6	Ri7_7Fi5_7	Ri7_7Fi5_8	Ri7_7Fi5_9	Ri7_7Fi5_10	Ri7_7Fi5_11	Ri7_7Fi5_12
Ri7_8Fi5_1	Ri7_8Fi5_2	Ri7_8Fi5_3	Ri7_8Fi5_4	Ri7_8Fi5_5	Ri7_8Fi5_6	Ri7_8Fi5_7	Ri7_8Fi5_8	Ri7_8Fi5_9	Ri7_8Fi5_10	Ri7_8Fi5_11	Ri7_8Fi5_12

b)

Ri7_1Fi5_13	Ri7_1Fi5_14	Ri7_1Fi5_15	Ri7_1Fi5_16	Ri7_1Fi5_17	Ri7_1Fi5_18	Ri7_1Fi5_19	Ri7_1Fi5_20	Ri7_1Fi5_21	Ri7_1Fi5_22	Ri7_1Fi5_23	Ri7_1Fi5_24
Ri7_2Fi5_13	Ri7_2Fi5_14	Ri7_2Fi5_15	Ri7_3Fi5_16	Ri7_2Fi5_17	Ri7_2Fi5_18	Ri7_2Fi5_19	Ri7_2Fi5_20	Ri7_2Fi5_21	Ri7_2Fi5_22	Ri7_2Fi5_23	Ri7_2Fi5_24
Ri7_3Fi5_13	Ri7_3Fi5_14	Ri7_3Fi5_15	Ri7_3Fi5_16	Ri7_3Fi5_17	Ri7_3Fi5_18	Ri7_3Fi5_19	Ri7_3Fi5_20	Ri7_3Fi5_21	Ri7_3Fi5_22	Ri7_3Fi5_23	Ri7_3Fi5_24
Ri7_4Fi5_13	Ri7_4Fi5_14	Ri7_4Fi5_15	Ri7_4Fi5_16	Ri7_4Fi5_17	Ri7_4Fi5_18	Ri7_4Fi5_19	Ri7_4Fi5_20	Ri7_4Fi5_21	Ri7_4Fi5_22	Ri7_4Fi5_23	Ri7_4Fi5_24
Ri7_5Fi5_13	Ri7_5Fi5_14	Ri7_5Fi5_15	Ri7_5Fi5_16	Ri7_5Fi5_17	Ri7_5Fi5_18	Ri7_5Fi5_19	Ri7_5Fi5_20	Ri7_5Fi5_21	Ri7_5Fi5_22	Ri7_5Fi5_23	Ri7_5Fi5_24
Ri7_6Fi5_13	Ri7_6Fi5_14	Ri7_6Fi5_15	Ri7_6Fi5_16	Ri7_6Fi5_17	Ri7_6Fi5_18	Ri7_6Fi5_19	Ri7_6Fi5_20	Ri7_6Fi5_21	Ri7_6Fi5_22	Ri7_6Fi5_23	Ri7_6Fi5_24
Ri7_7Fi5_13	Ri7_7Fi5_14	Ri7_7Fi5_15	Ri7_7Fi5_16	Ri7_7Fi5_17	Ri7_7Fi5_18	Ri7_7Fi5_19	Ri7_7Fi5_20	Ri7_7Fi5_21	Ri7_7Fi5_22	Ri7_7Fi5_23	Ri7_7Fi5_24
Ri7_8Fi5_13	Ri7_8Fi5_14	Ri7_8Fi5_15	Ri7_8Fi5_16	Ri7_8Fi5_17	Ri7_8Fi5_18	Ri7_8Fi5_19	Ri7_8Fi5_20	Ri7_8Fi5_21	Ri7_8Fi5_22	Ri7_8Fi5_23	Ri7_8Fi5_24

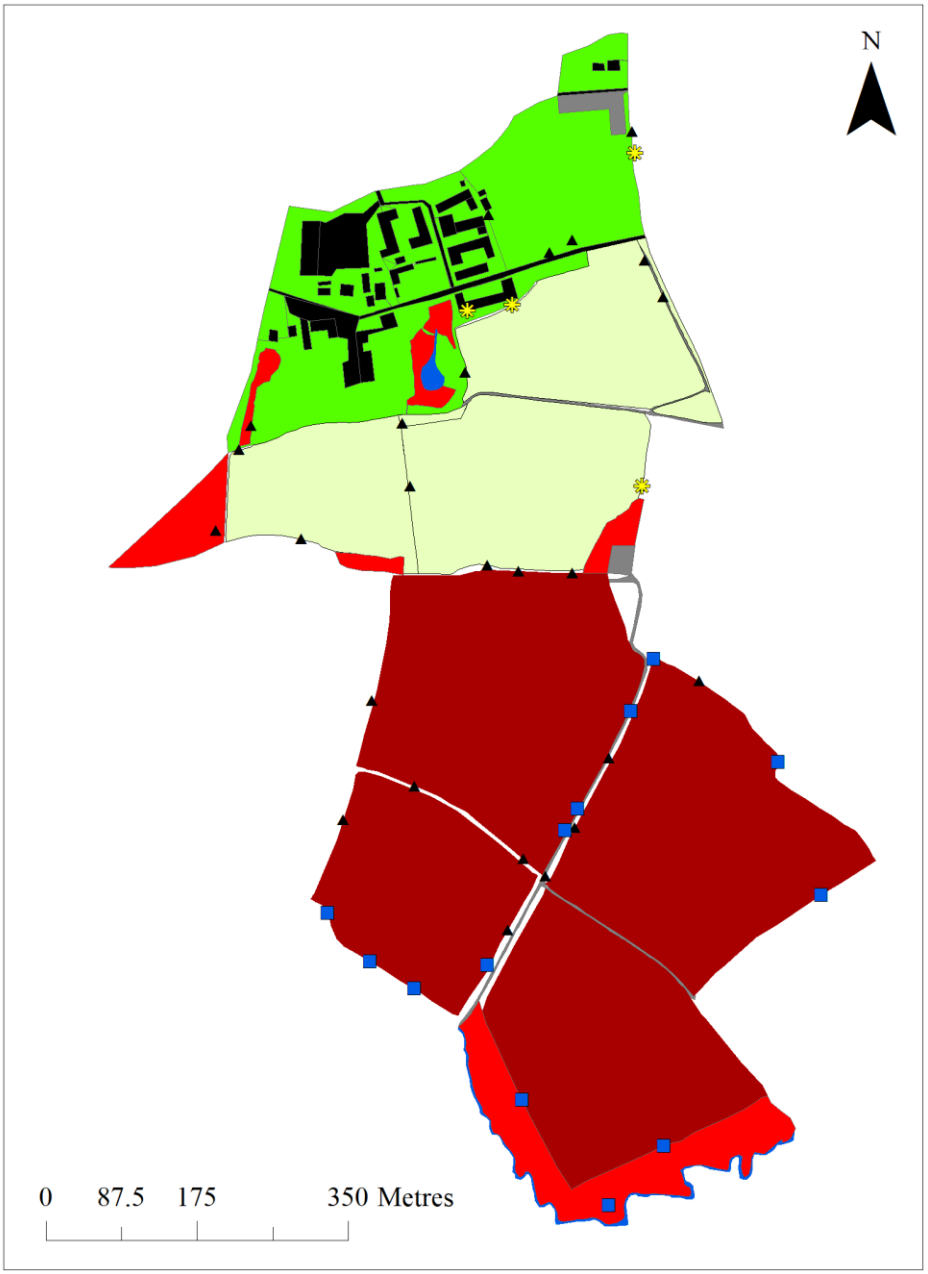
Appendix E: The presence (P) and absence (A) of 94 food types (Families) detected across hedgehog and badger scat samples from two rural farms in England, between summer 2018 and summer 2019, using DNA metabarcoding.

Prey Family	Prey type	Hedgehog P/A	Badger P/A	Percentage of hedgehog samples (n=64)	Percentage of badger samples (n=80)
Bufonidae	Amphibian	P	A	1.6	0.0
Ranidae	Amphibian	P	A	1.6	0.0
Salamandridae	Amphibian	A	P	0.0	1.3
Phasianidae	Aves	P	P	15.6	1.3
Columbidae	Aves	P	P	12.5	1.3
Anatidae	Aves	P	P	1.6	3.8
Corvidae	Aves	A	P	0.0	3.8
Gruidae	Aves	A	P	0.0	5.0
Hyaloriaceae	Fungi	A	P	0.0	1.3
Cladosporiaceae	Fungi	P	A	1.6	0.0
Carabidae	Invertebrate	P	P	89.0	61.0
Arionidae	Invertebrate	P	P	71.0	68.0
Lumbricidae	Invertebrate	P	P	68.0	86.0
Agriolimacidae	Invertebrate	P	P	60.0	30.0
Noctuidae	Invertebrate	P	P	49.0	13.0
Tipulidae	Invertebrate	P	P	33.0	14.0
Armadillidiidae	Invertebrate	P	A	24.0	0.0
Forficulidae	Invertebrate	P	A	24.0	0.0
Helicidae	Invertebrate	P	P	17.0	20.0
Elateridae	Invertebrate	P	A	16.0	0.0
Hygromiidae	Invertebrate	P	P	13.0	10.0
Julidae	Invertebrate	P	P	13.0	1.0
Curculionidae	Invertebrate	P	A	11.0	0.0
Scathophagidae	Invertebrate	P	P	10.0	1.0
Limacidae	Invertebrate	P	A	8.0	0.0
Entomobryidae	Invertebrate	P	P	6.0	19.0
Formicidae	Invertebrate	P	A	6.0	0.0
Muscidae	Invertebrate	P	P	6.0	25.0
Phalangiidae	Invertebrate	P	A	6.0	0.0
Phoridae	Invertebrate	P	P	6.0	18.0
Porcellionidae	Invertebrate	P	P	6.0	4.0
Staphylinidae	Invertebrate	P	P	6.0	6.0
Tephritidae	Invertebrate	P	A	6.0	0.0
Caeciliusidae	Invertebrate	P	A	5.0	0.0

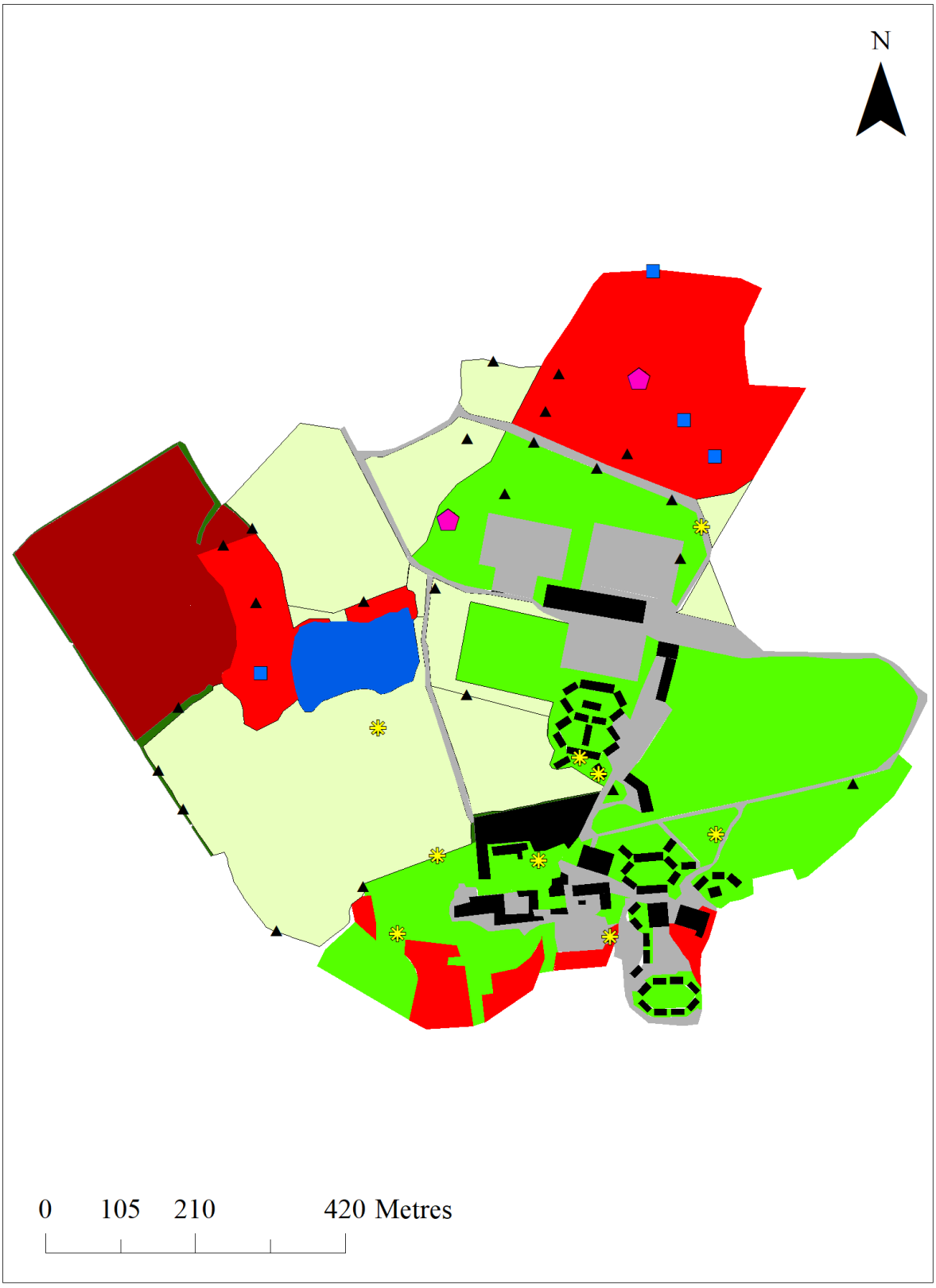
Prey Family	Prey type	Hedgehog P/A	Badger P/A	Percentage of hedgehog samples (n=64)	Percentage of badger samples (n=80)
Braconidae	Invertebrate	P	P	3.0	3.0
Hepialidae	Invertebrate	P	A	3.0	0.0
Katiannidae	Invertebrate	P	P	3.0	3.0
Parasitidae	Invertebrate	P	P	3.0	9.0
Psychodidae	Invertebrate	P	P	3.0	19.0
Drosophilidae	Invertebrate	P	P	2.0	5.0
Dryomyzidae	Invertebrate	P	P	2.0	4.0
Euzetidae	Invertebrate	P	P	2.0	1.0
Sepsidae	Invertebrate	P	P	2.0	11.0
Sphaeroceridae	Invertebrate	P	P	2.0	5.0
Syrphidae	Invertebrate	P	P	2.0	4.0
Trichoniscidae	Invertebrate	P	P	2.0	3.0
Anthomyzidae	Invertebrate	A	P	0.0	14.0
Baetidae	Invertebrate	A	P	0.0	11.0
Hypogastruridae	Invertebrate	A	P	0.0	13.0
Limoniidae	Invertebrate	A	P	0.0	8.0
Lonchopteridae	Invertebrate	A	P	0.0	4.0
Nymphalidae	Invertebrate	A	P	0.0	15.0
Ripiphoridae	Invertebrate	A	P	0.0	5.0
Sciaridae	Invertebrate	A	P	0.0	9.0
Succineidae	Invertebrate	A	P	0.0	5.0
Vespidae	Invertebrate	A	P	0.0	11.0
Bovidae	Mammal	P	P	10.9	17.5
Suidae	Mammal	P	A	6.3	0.0
Felidae	Mammal	P	P	1.6	1.3
Canidae	Mammal	A	P	0.0	5.0
Cricetidae	Mammal	A	P	0.0	2.5
Leporidae	Mammal	A	P	0.0	11.0
Muridae	Mammal	A	P	0.0	5.0
Soricidae	Mammal	A	P	0.0	1.3
Talpidae	Mammal	A	P	0.0	1.3
Erinaceidae	Mammal	N/A	P	N/A	1.3
Poaceae	Plant	P	P	40.6	47.5
Ranunculaceae	Plant	P	P	21.9	17.5
Plantaginaceae	Plant	P	P	15.6	18.8
Asteraceae	Plant	P	P	14.1	3.8
Rosaceae	Plant	P	P	12.5	27.5
Fagaceae	Plant	P	P	9.4	8.8

Prey Family	Prey type	Hedgehog P/A	Badger P/A	Percentage of hedgehog samples (n=64)	Percentage of badger samples (n=80)
Brassicaceae	Plant	P	P	7.8	8.8
Geraniaceae	Plant	P	P	6.3	25.0
Aquifoliaceae	Plant	P	A	4.7	0.0
Fabaceae	Plant	P	P	4.7	6.3
Apiaceae	Plant	P	A	3.1	0.0
Boraginaceae	Plant	P	P	3.1	1.3
Sapindaceae	Plant	P	P	3.1	15.0
Adoxaceae	Plant	P	A	1.6	0.0
Berberidaceae	Plant	P	A	1.6	0.0
Cupressaceae	Plant	P	P	1.6	5.0
Lamiaceae	Plant	P	A	1.6	0.0
Polygonaceae	Plant	P	P	1.6	1.3
Araliaceae	Plant	A	P	0.0	1.3
Betulaceae	Plant	A	P	0.0	2.5
Caryophyllaceae	Plant	A	P	0.0	3.8
Convolvulaceae	Plant	A	P	0.0	2.5
Euphorbiaceae	Plant	A	P	0.0	7.5
Onagraceae	Plant	A	P	0.0	1.3
Pinaceae	Plant	A	P	0.0	5.0
Primulaceae	Plant	A	P	0.0	2.5
Rubiaceae	Plant	A	P	0.0	6.3
Salicaceae	Plant	A	P	0.0	1.3

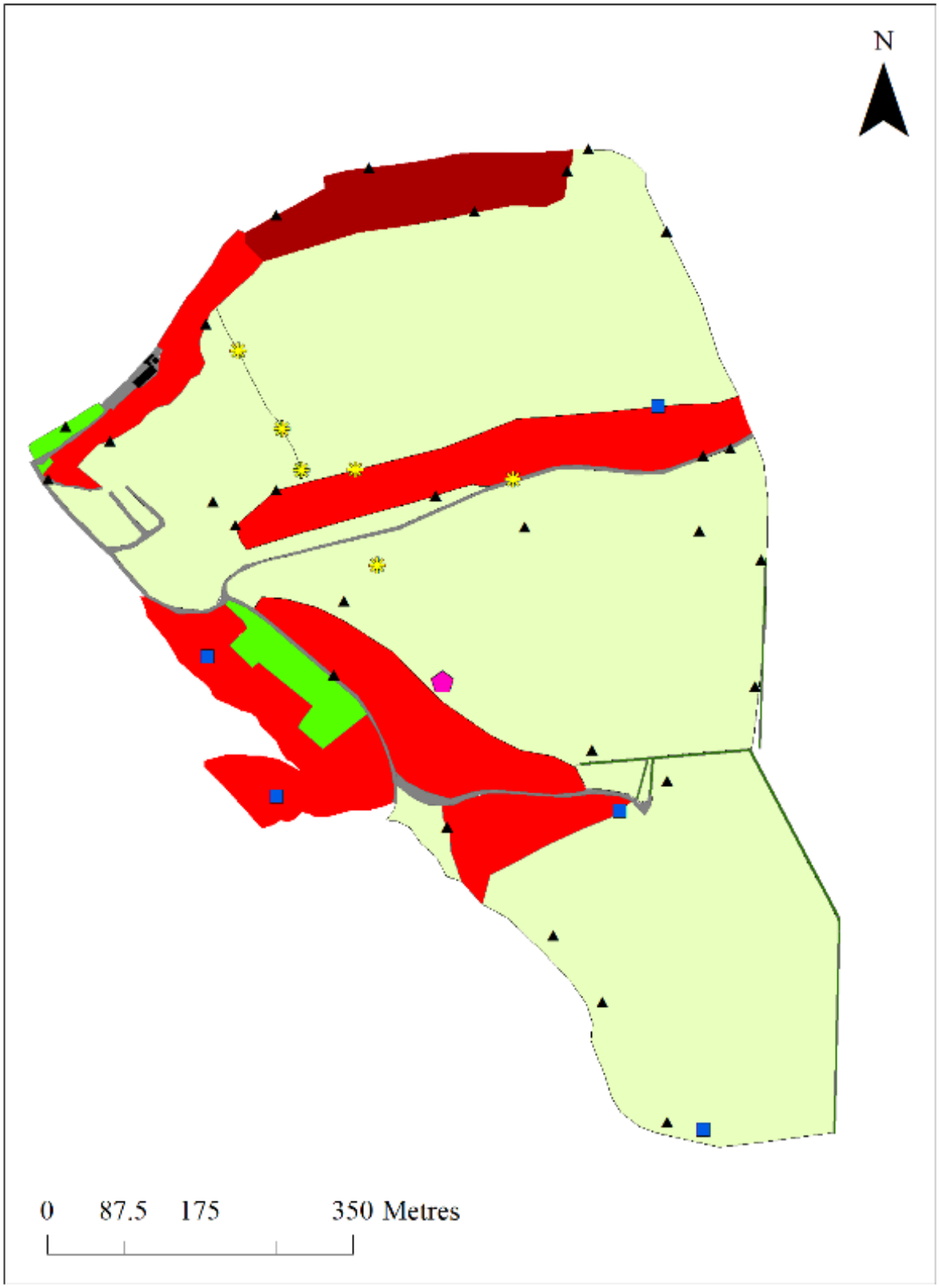
Appendix F: The location of trial cameras across 23 sites surveyed between April 2018 and September 2019 were generated and mapped using ArcMap GIS software (ESRI (Environmental Systems Research Institute 2018). Habitats are coded as follows: brown = arable, lime green = amenity grassland, black = buildings, light green = grassland, dark green = hedges, blue = open water, red = woodland and grey = urban. Detection data during 10-night surveys are coded as follows: black triangle = camera location, yellow asterisk = hedgehog detected, blue square = badger detected and pink pentagon = hedgehog and badger detected (although at separate times).



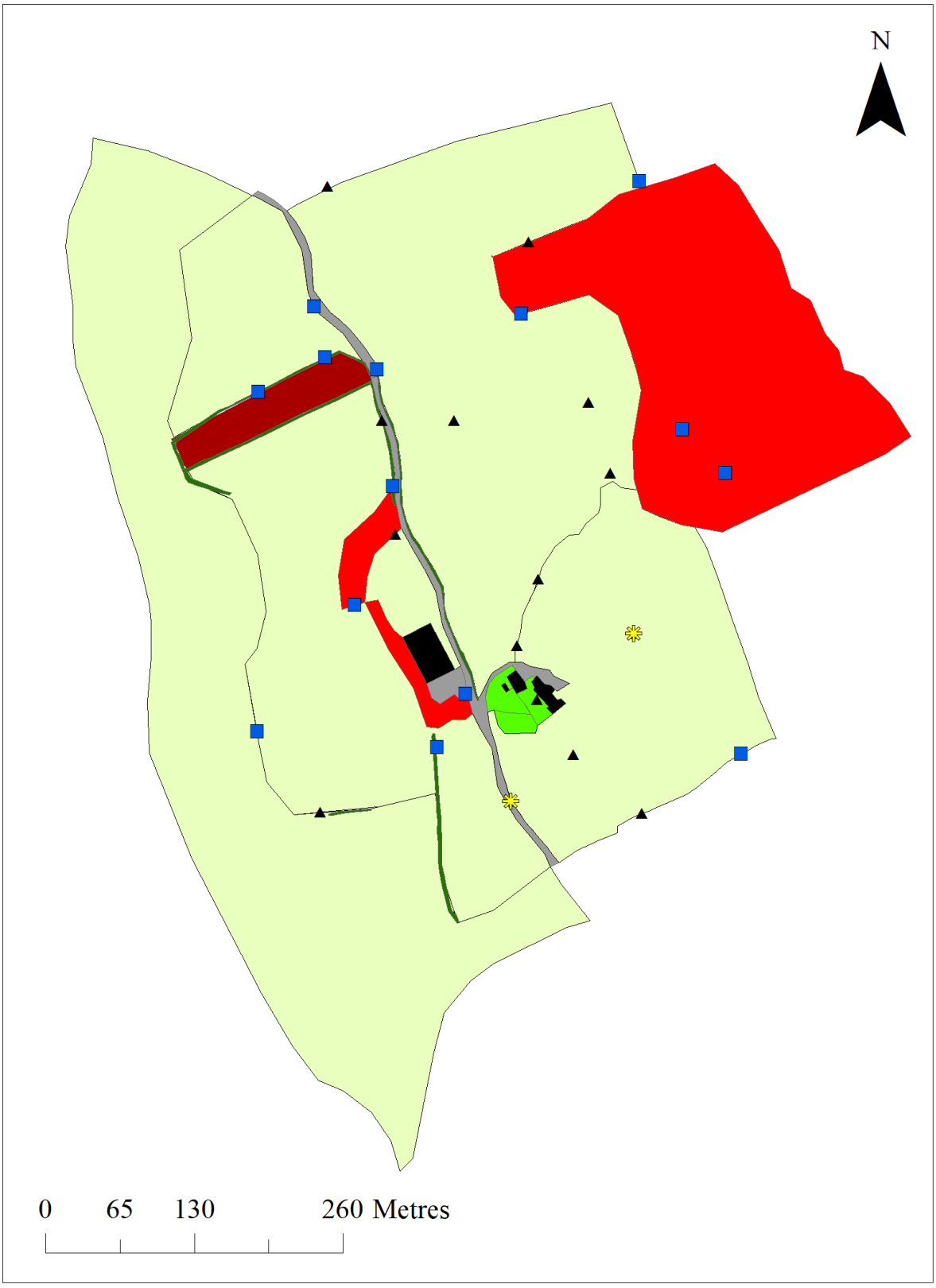
Site 1) Brackenhurst A



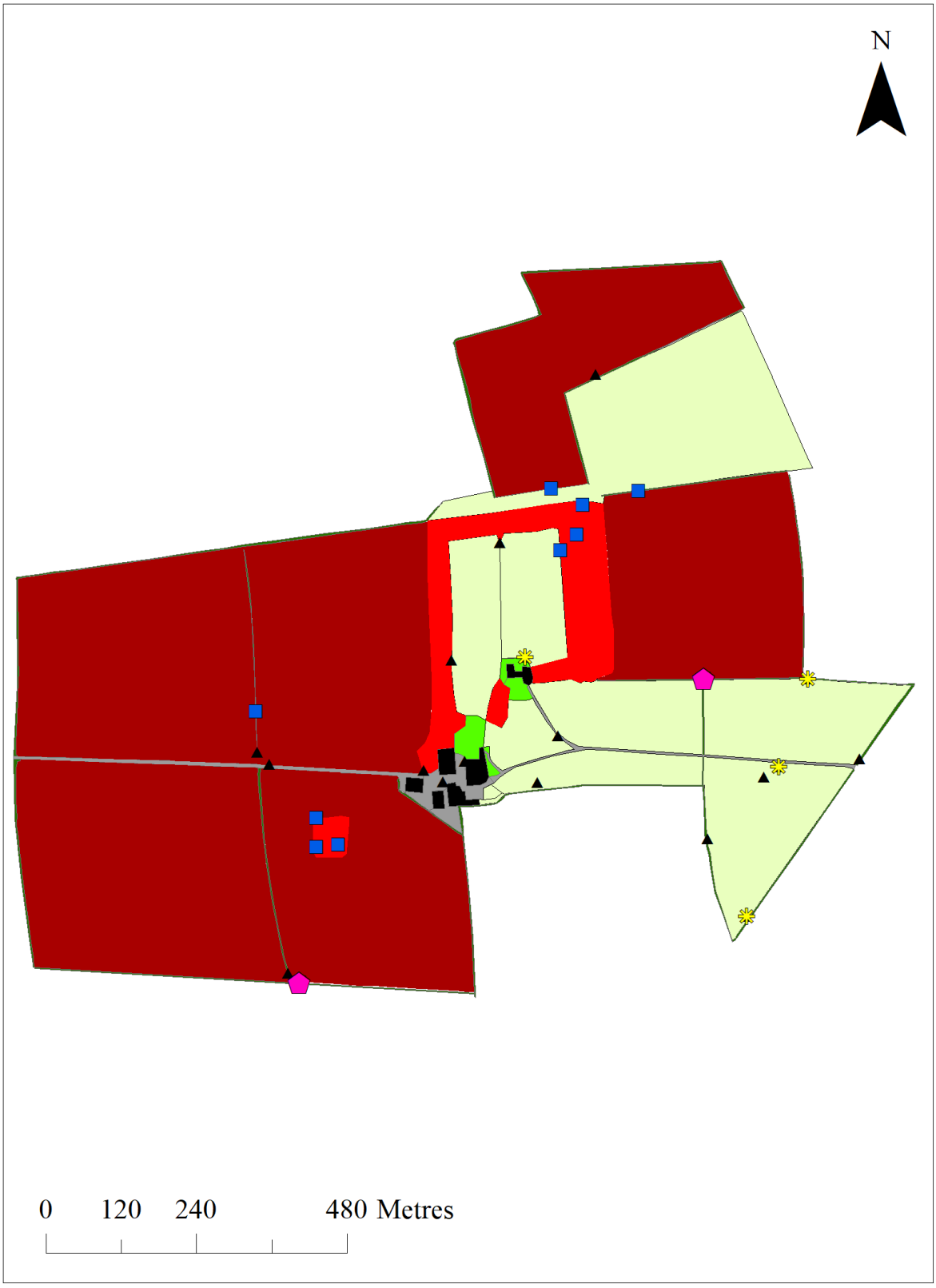
Site 2) Hartpury



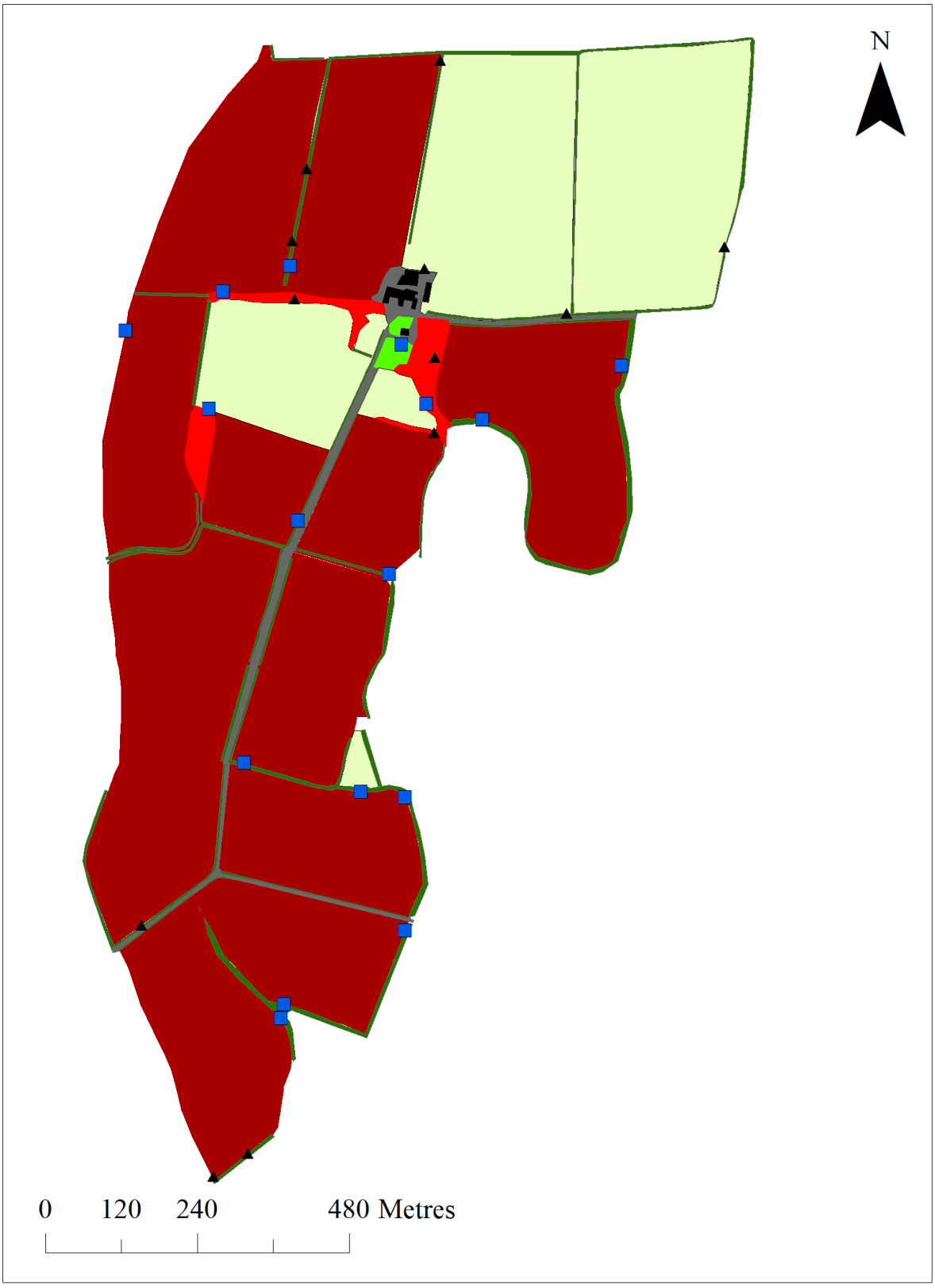
Site 3) Slade



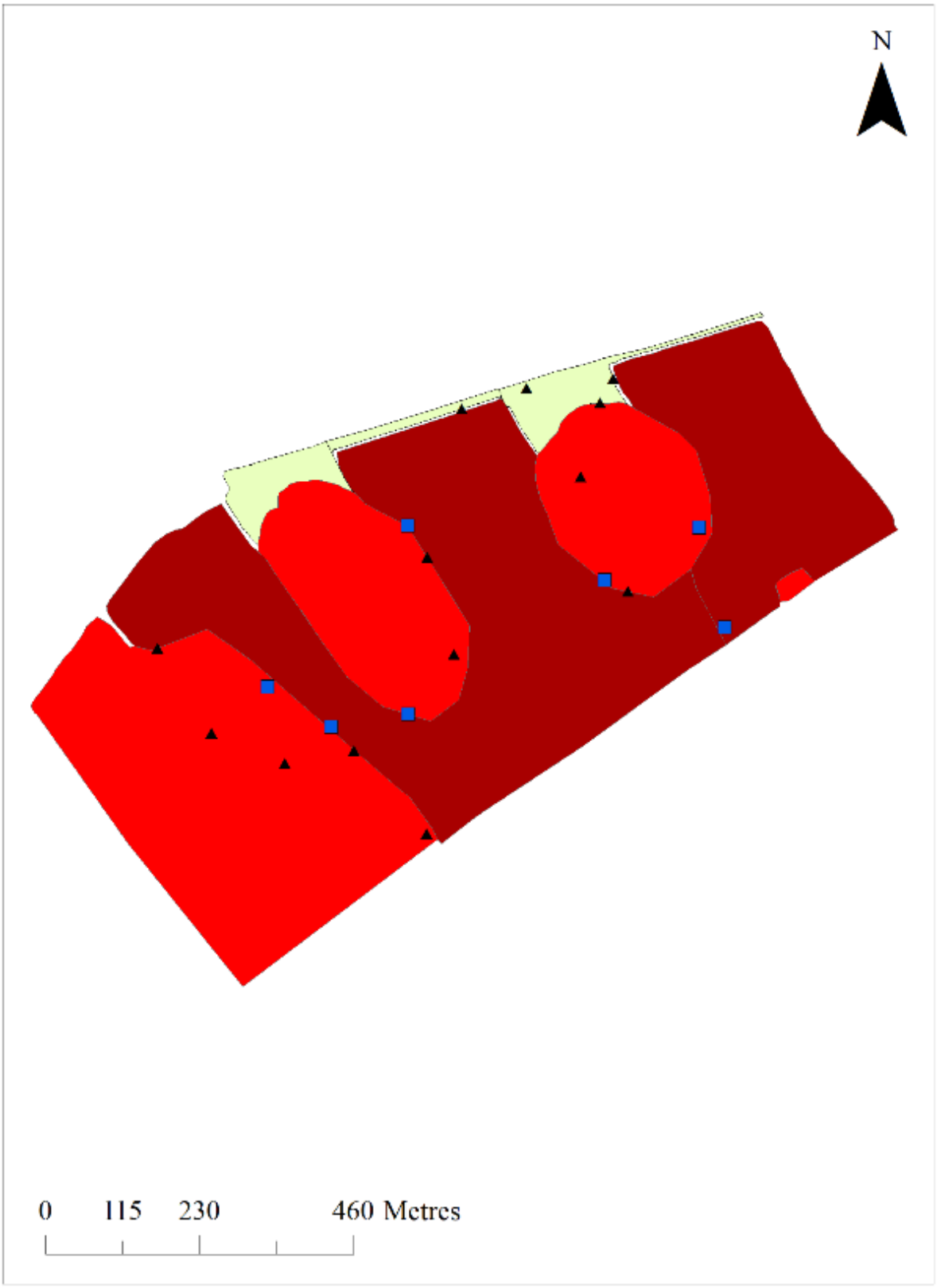
Site 4) Kendal



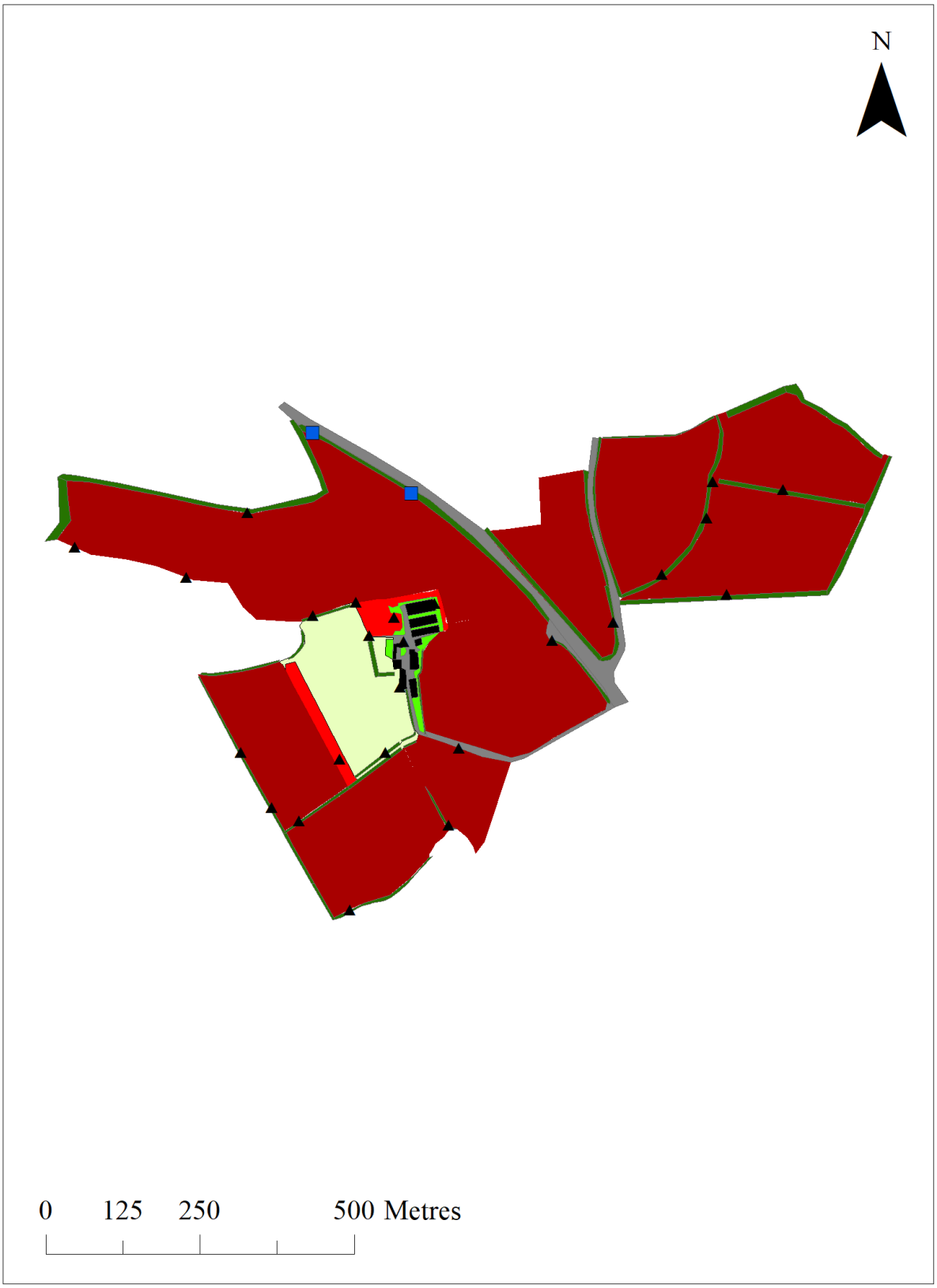
Site 5) Driffield



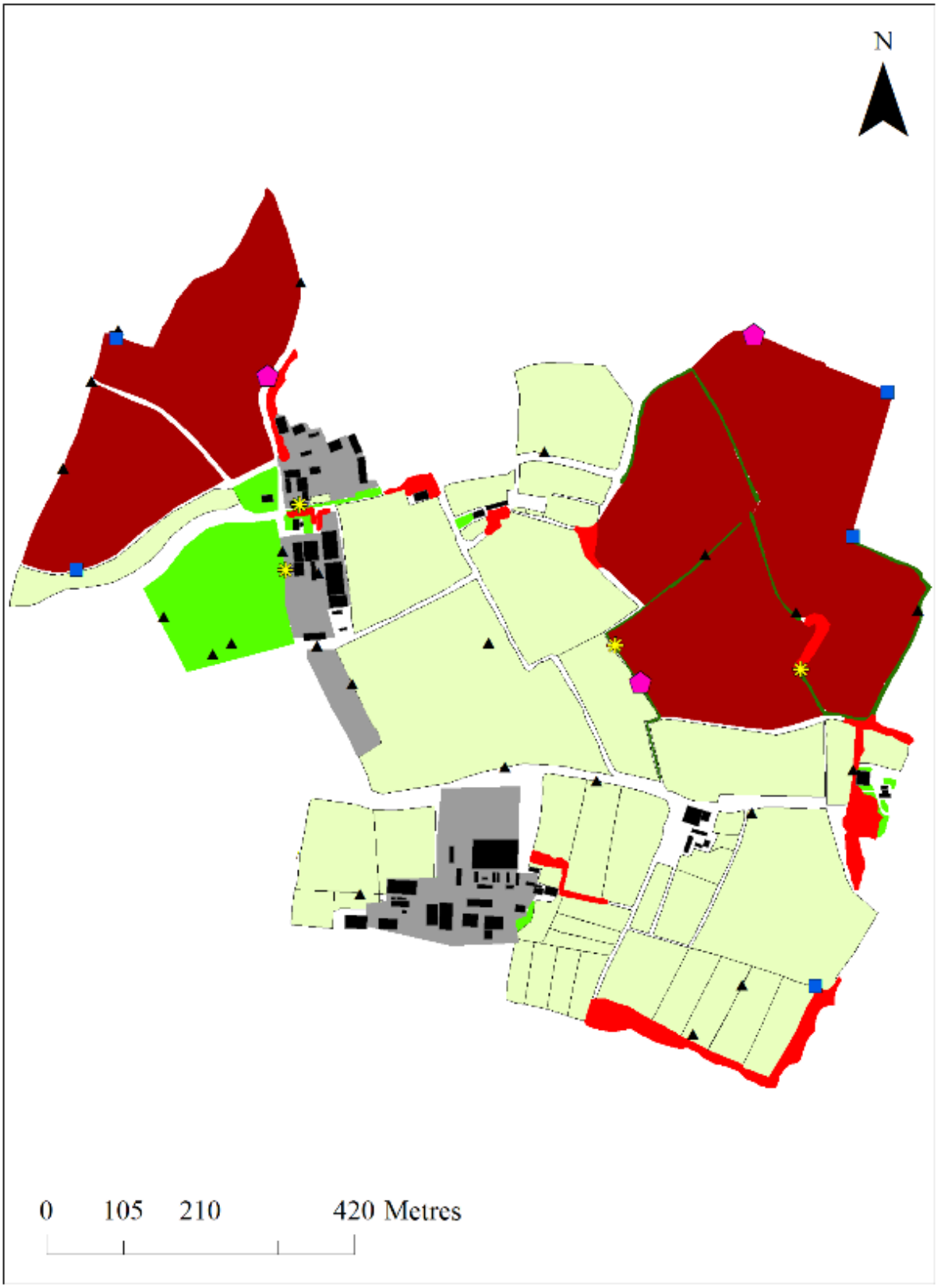
Site 6) Keyingham



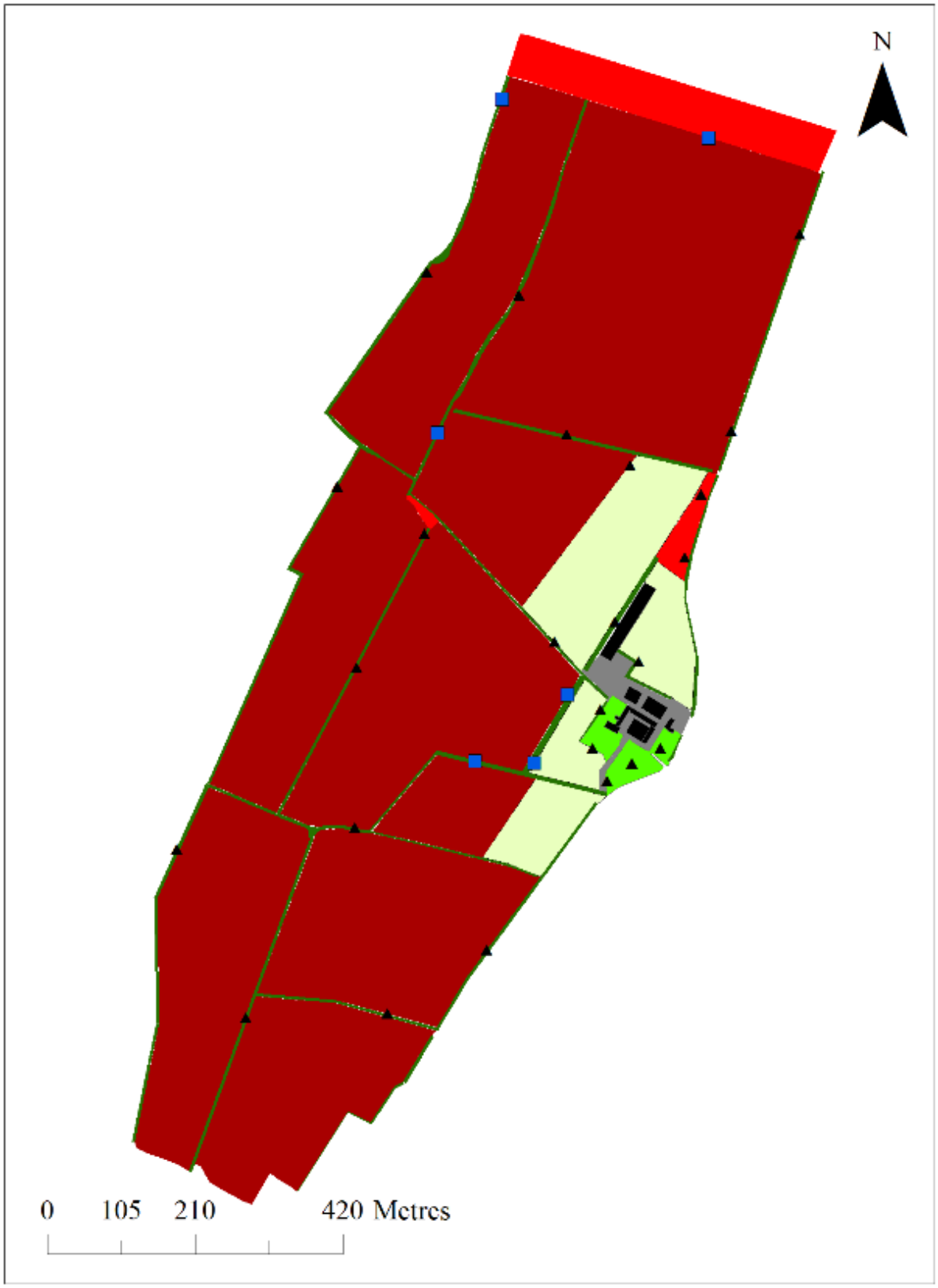
Site 7) Clumber



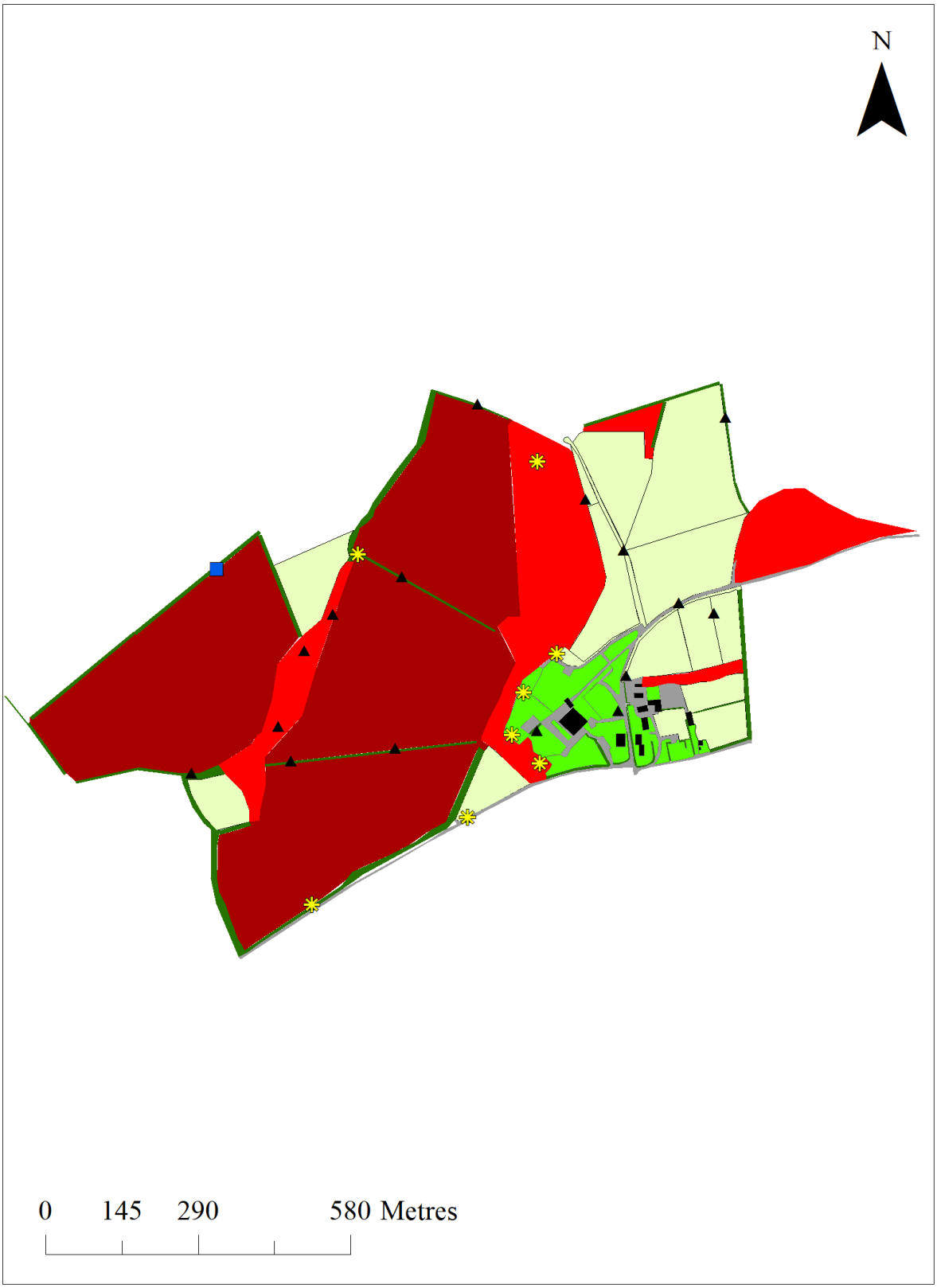
Site 8) Thorn



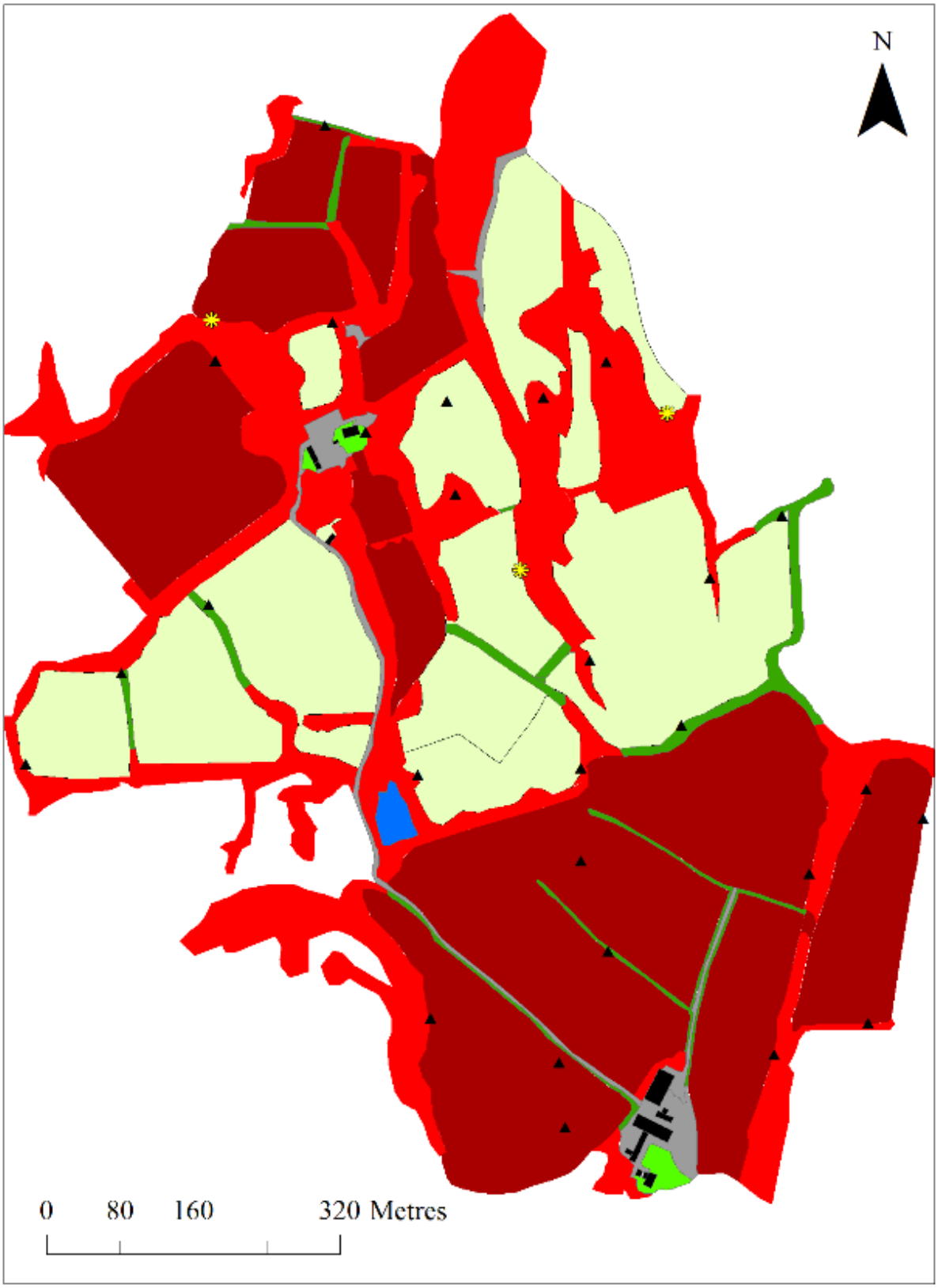
Site 9) Brackenhurst B



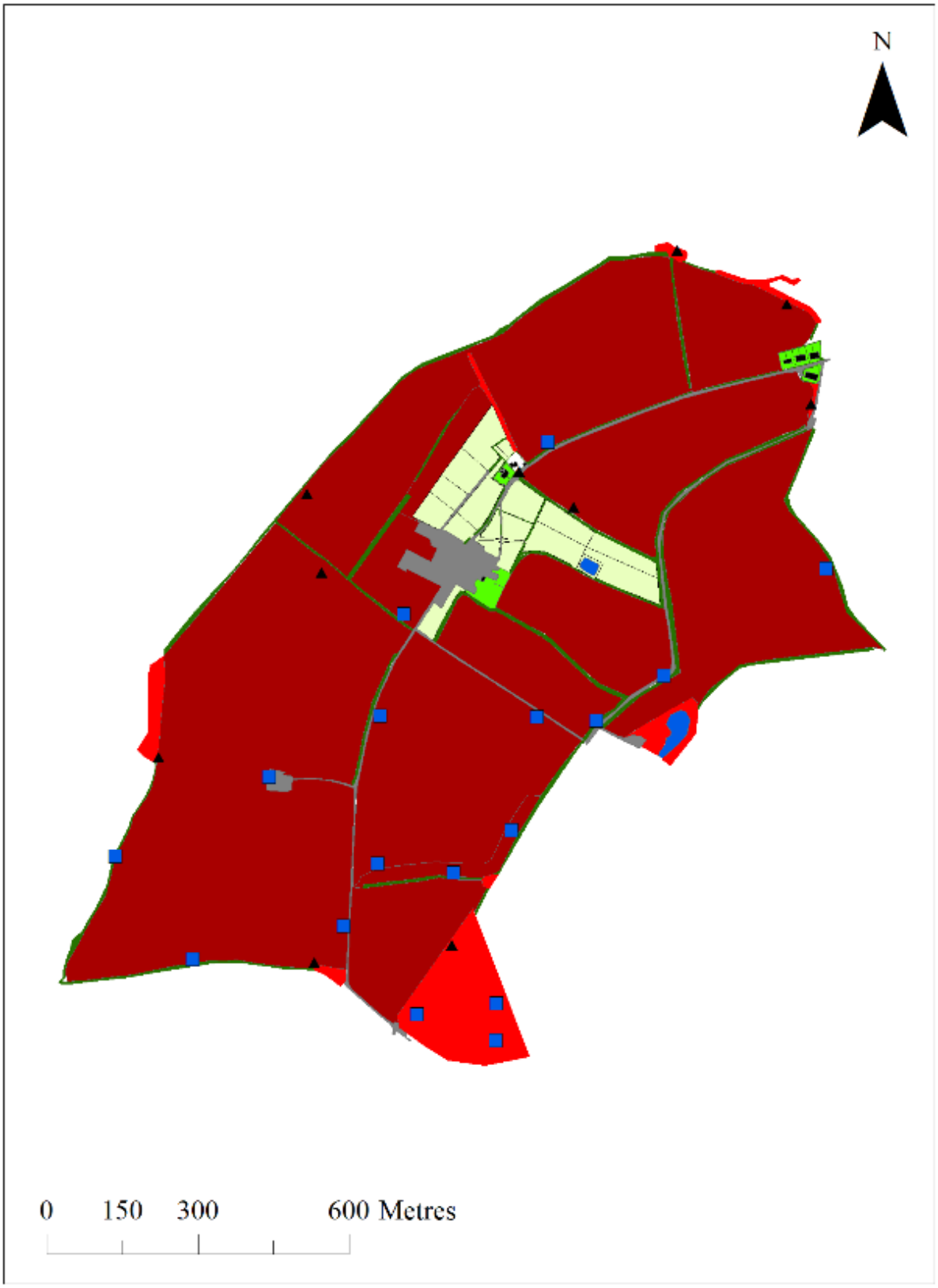
Site 10) Epperstone



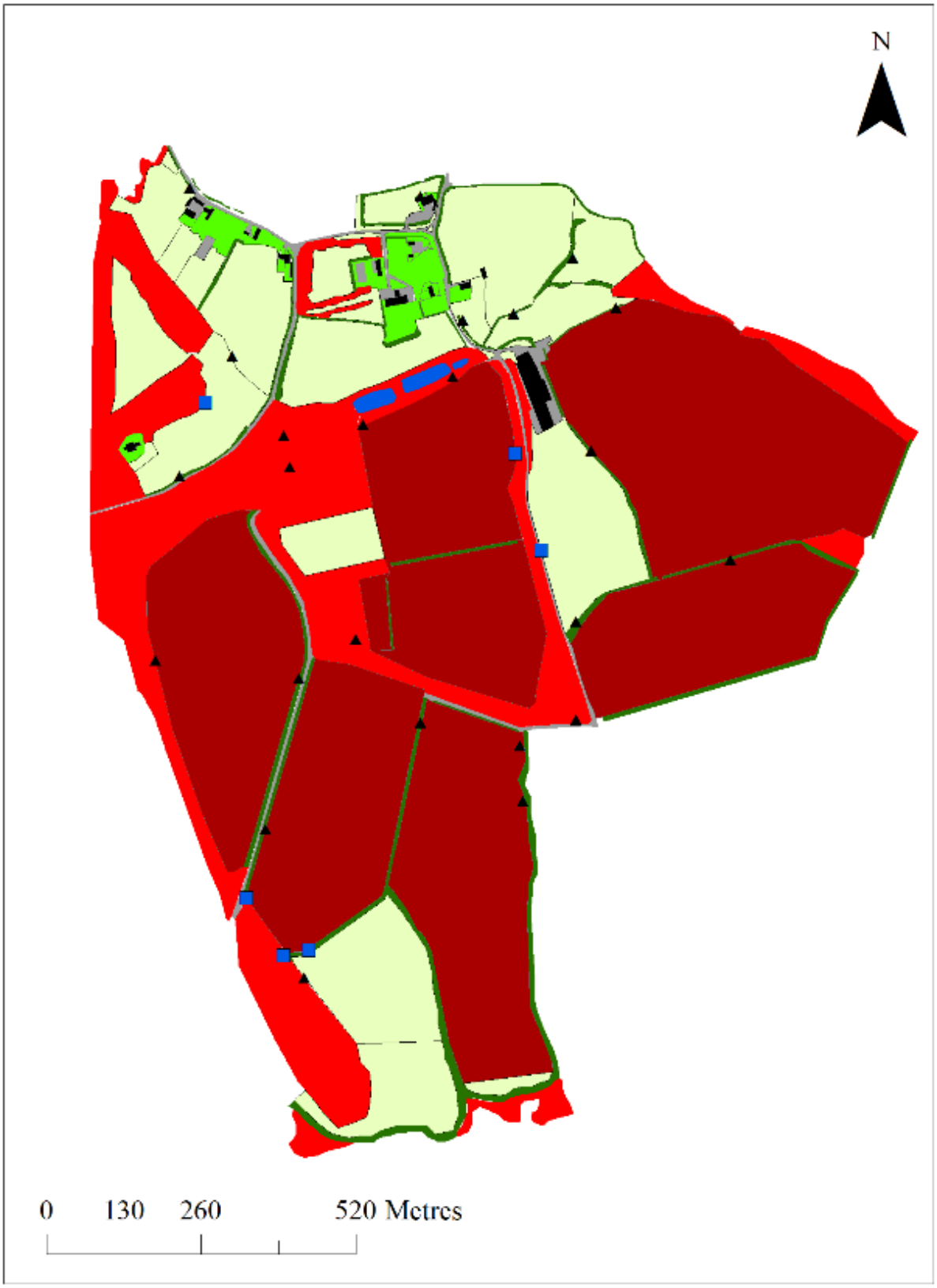
Site 11) Hodsock



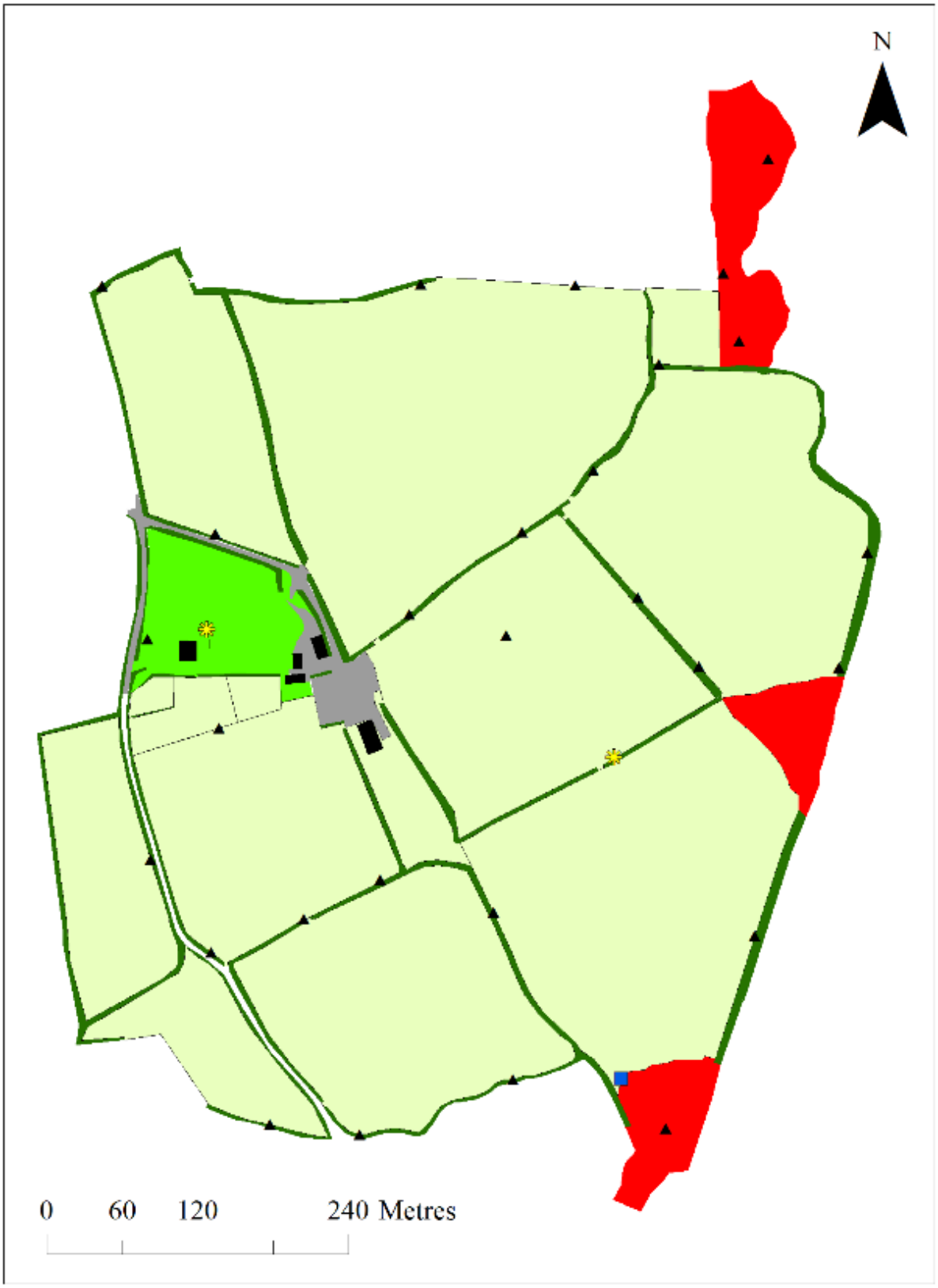
Site 12) Anglesey



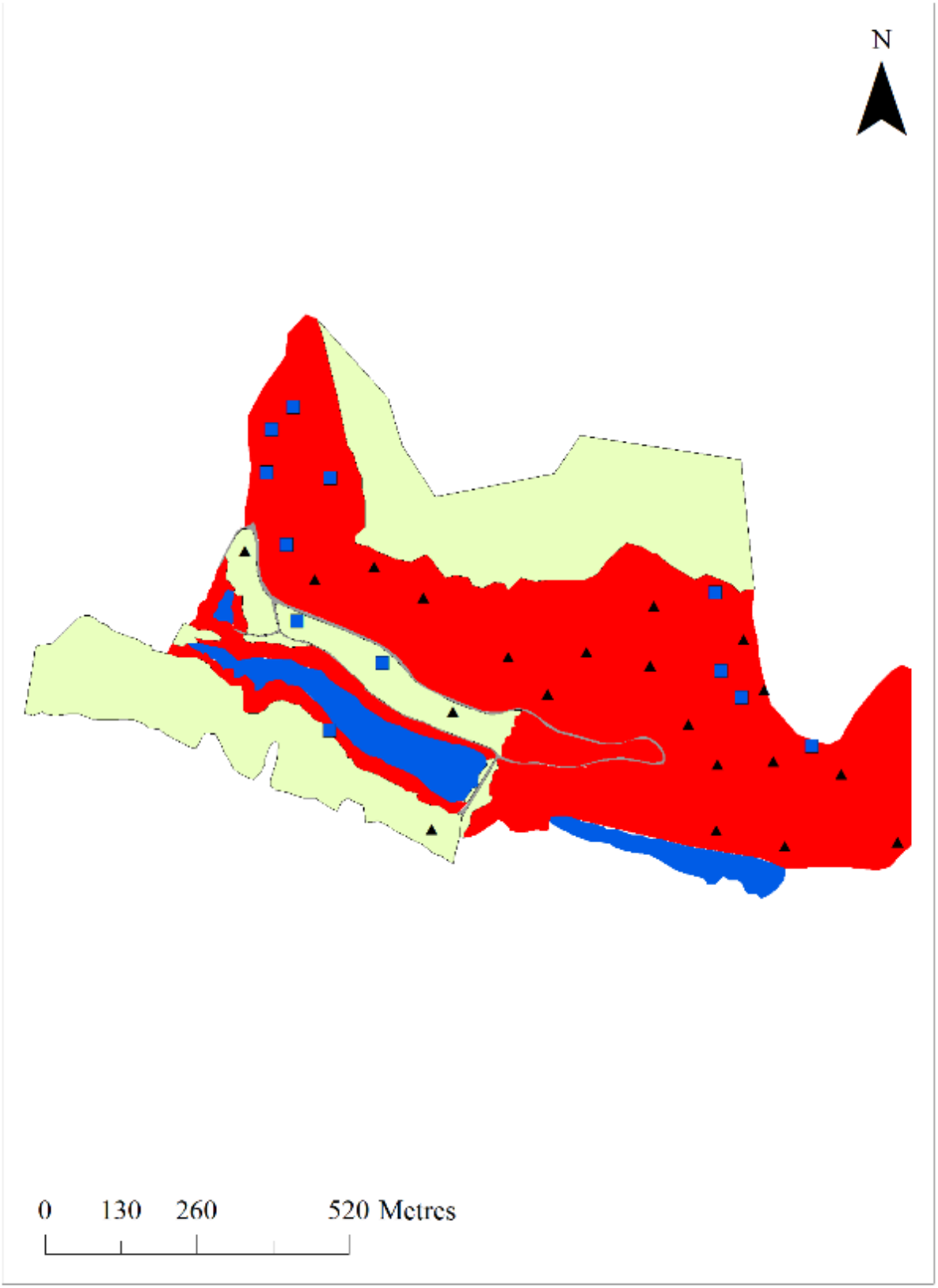
Site 13) Dunmow



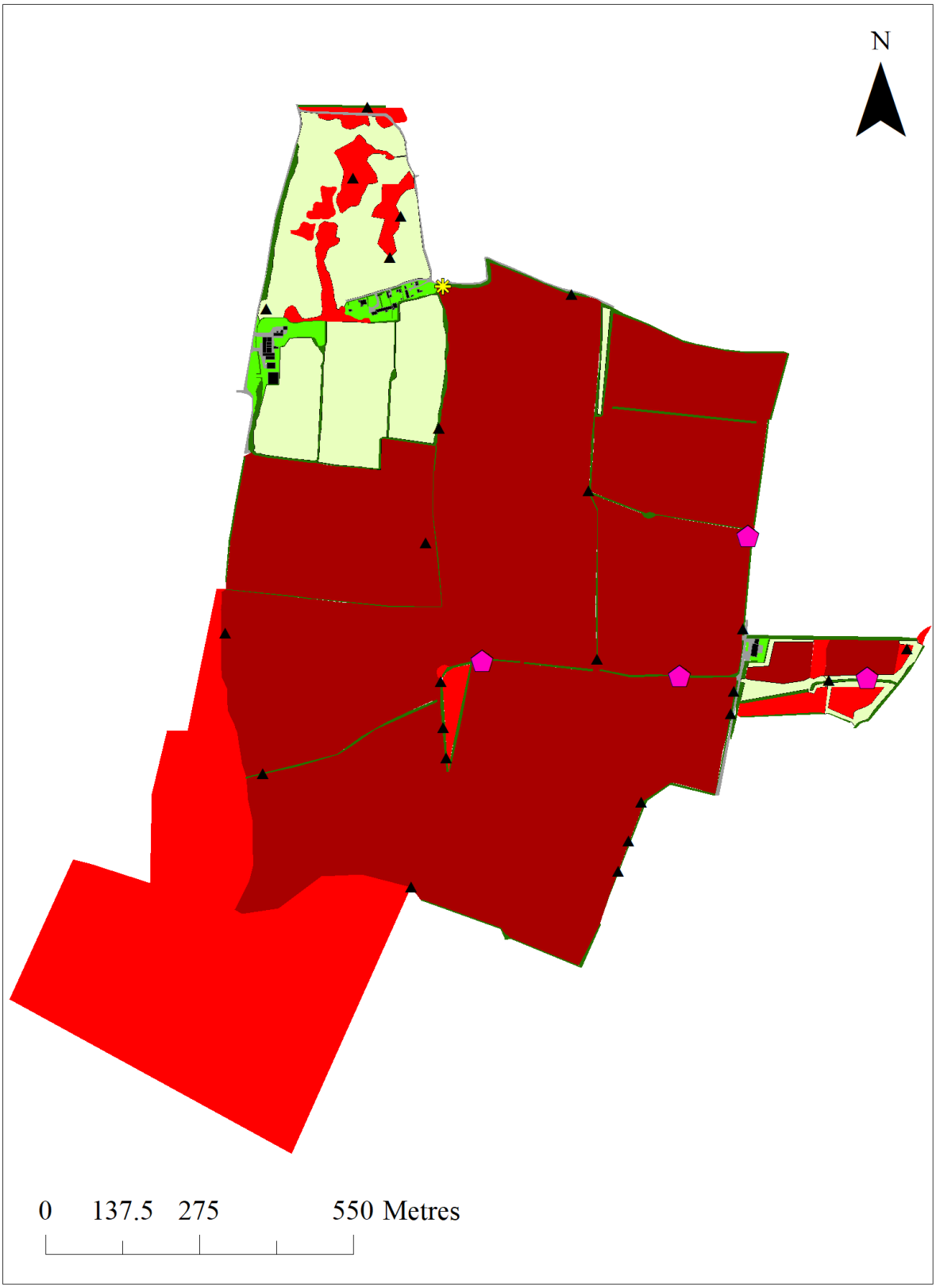
Site 14) Loddington



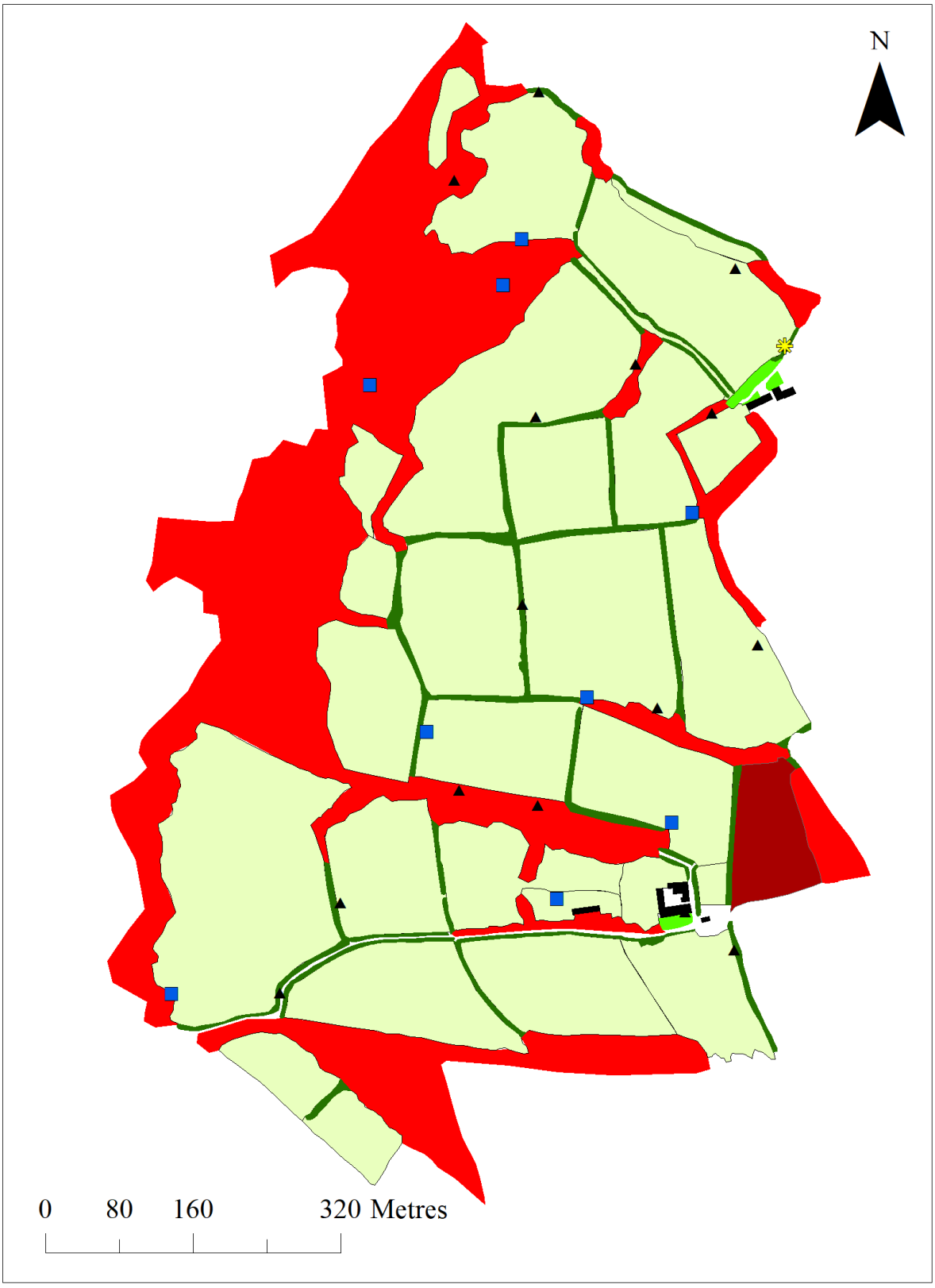
Site 15) Usk



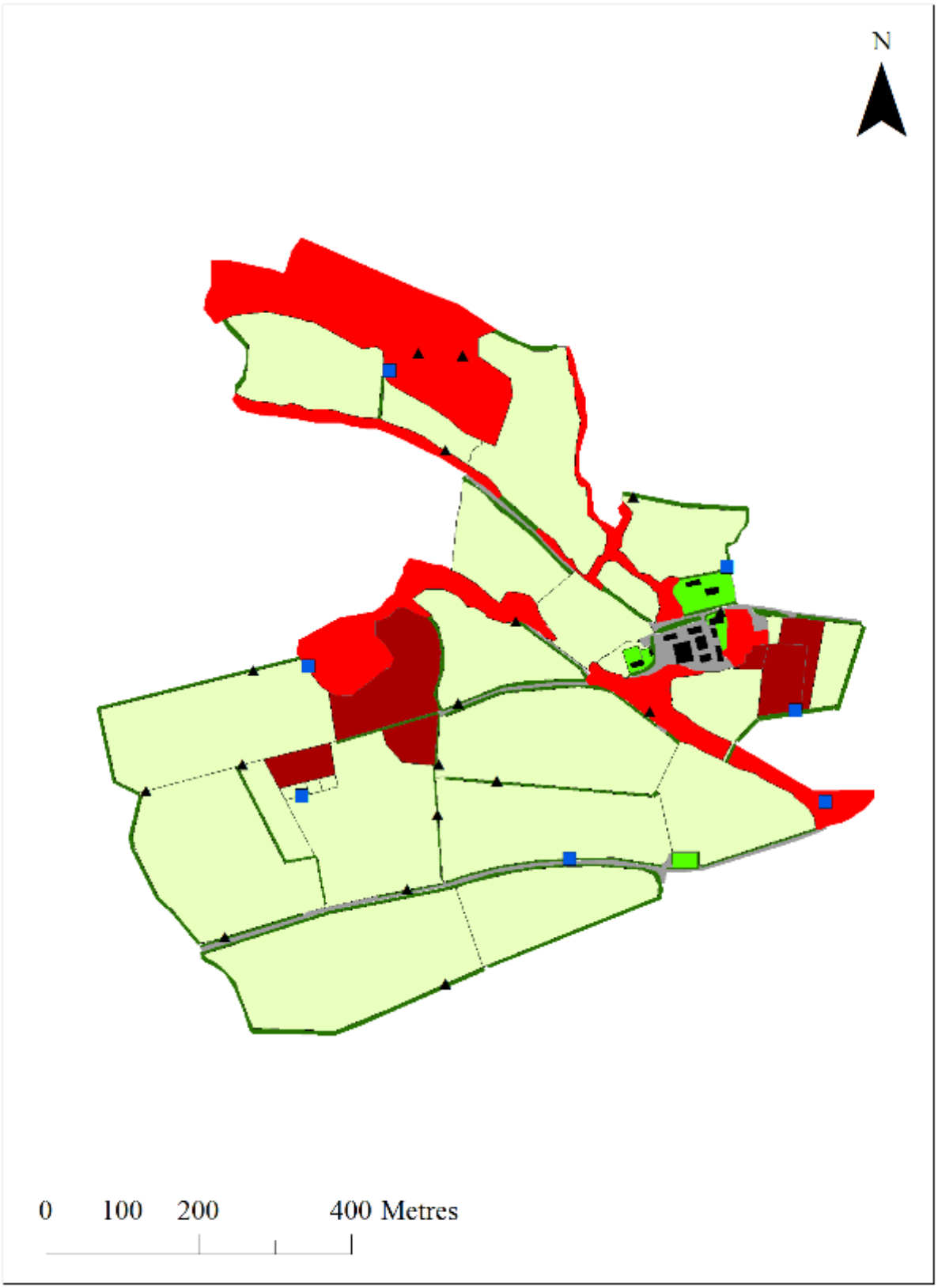
Site 16) Woodchester



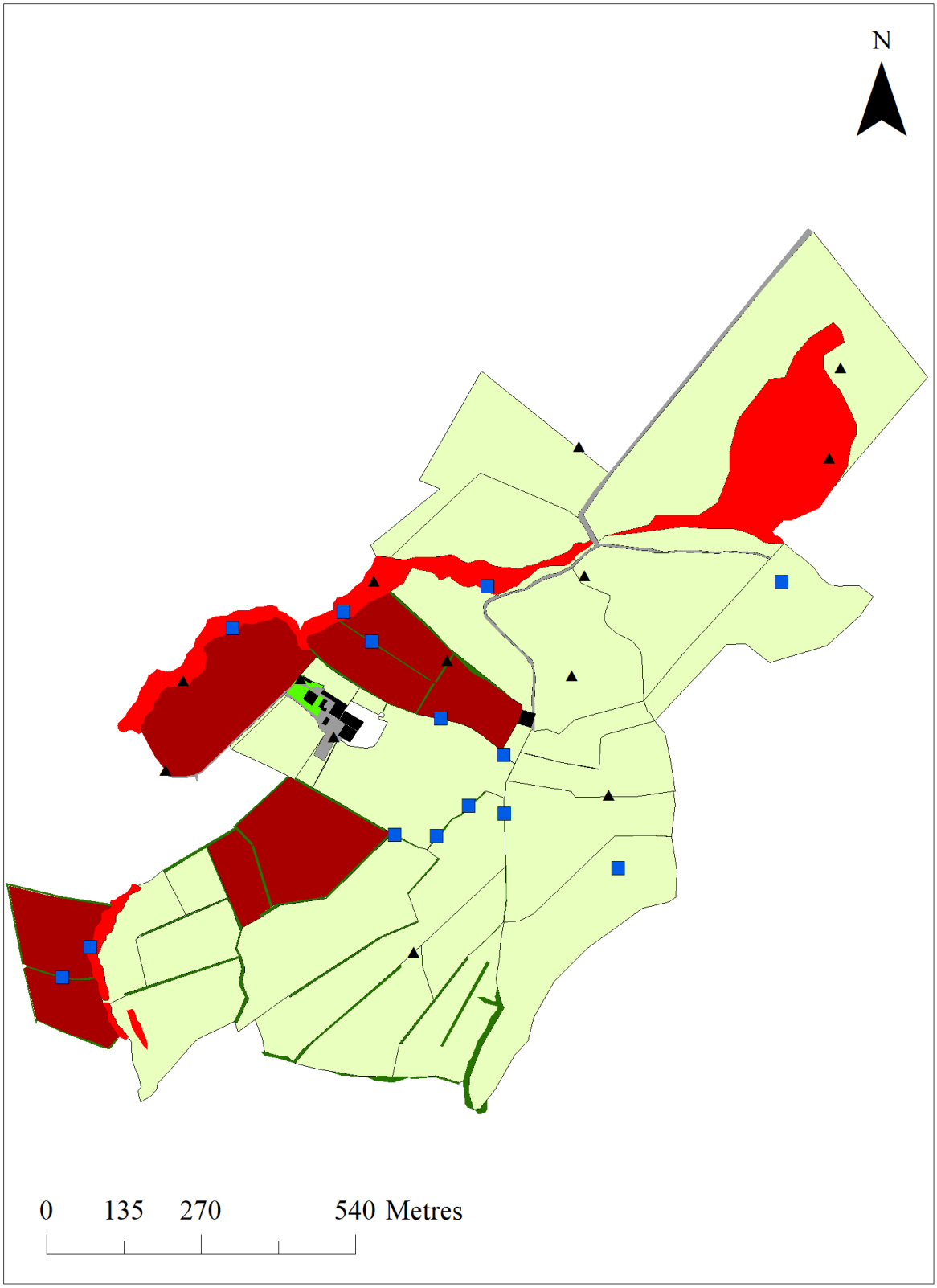
Site 17) Long Stratton



Site 18) Spreyton



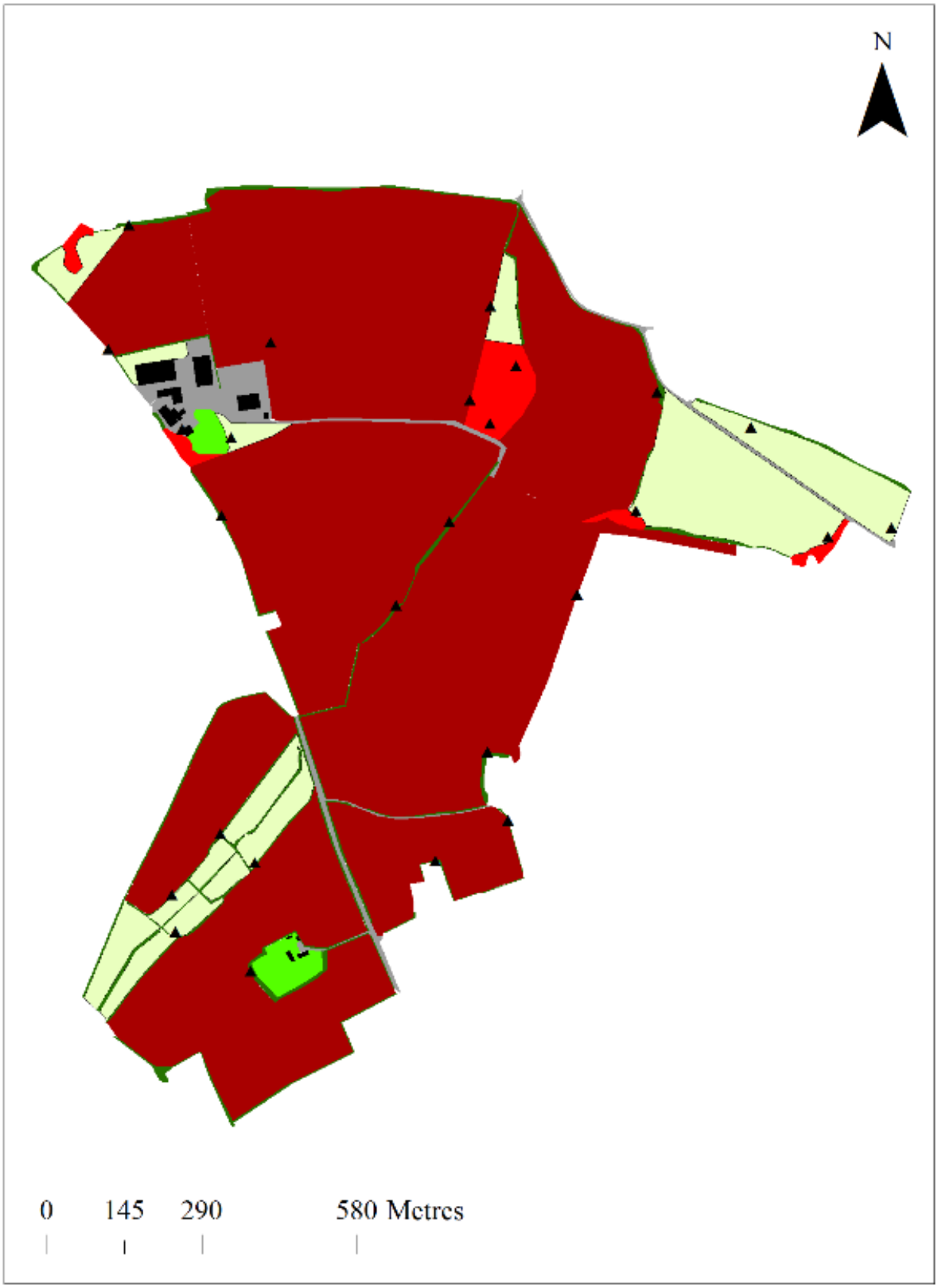
Site 19) Ide



Site 20) Knock

Site 21) Barnsley

Site 22) Riseholme

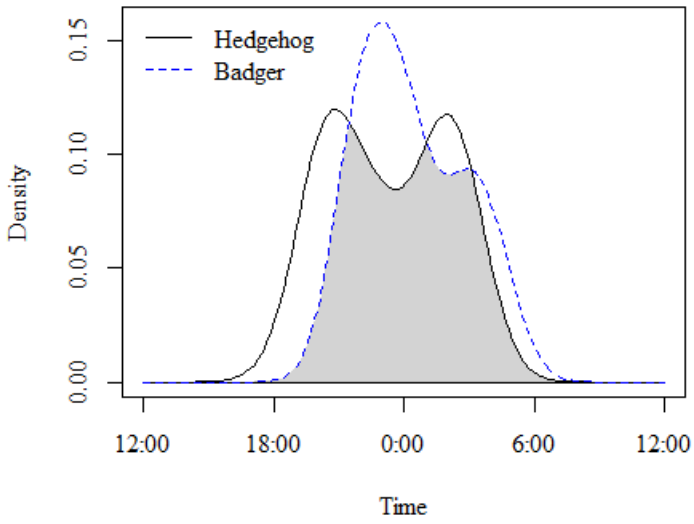


Site 23) Suffolk

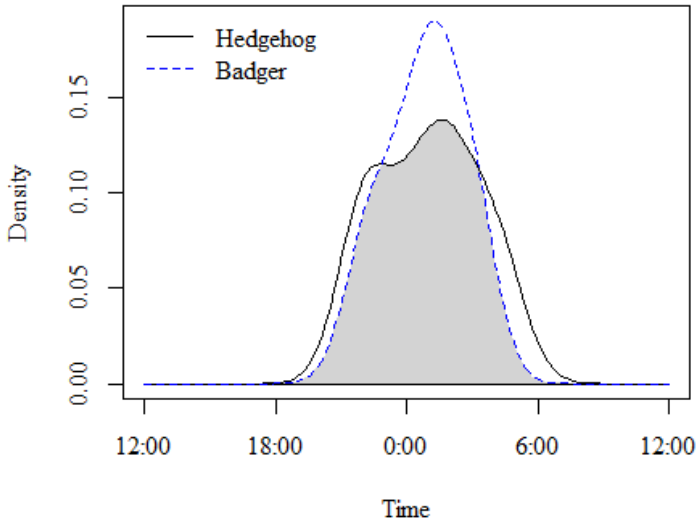
Appendix G: Comparison of activity levels of badgers and hedgehogs at six sites where they co-occurred. Overlap plot comparing diel activity pattern density curves of badger and hedgehog at the following sites; A) Brackenhurst A, B) Hartpury, C) Slade, D) Driffield, E) Brackenhurst B and F) Riseholme, as obtained from camera trap detections of hedgehog, and badger. The grey shaded area indicates activity overlap and individual detection times at shown along the X axis as rings.

Site	No. badger detections	No. hedgehog detections	Coefficient of overlap	Bootstrap coefficient of overlap	95% confidence intervals	Wald statistic (χ^2)	P-value
Bracken-hurst 2018	54	11	0.74	0.74	0.54-0.94	1.10	0.31
Hartpury	17	43	0.84	0.80	0.67 1.0	2.17	0.14
Slade	26	16	0.75	0.74	0.58-0.92	4.06	0.05
Driffield	36	9	0.86	0.78	0.55-0.93	0.37	0.55
Bracken-hurst 2019	33	14	0.71	0.70	0.50-0.92	3.14	0.08
Rise-holme	7	6	0.59	0.50	0.27-0.90	0.01	0.97

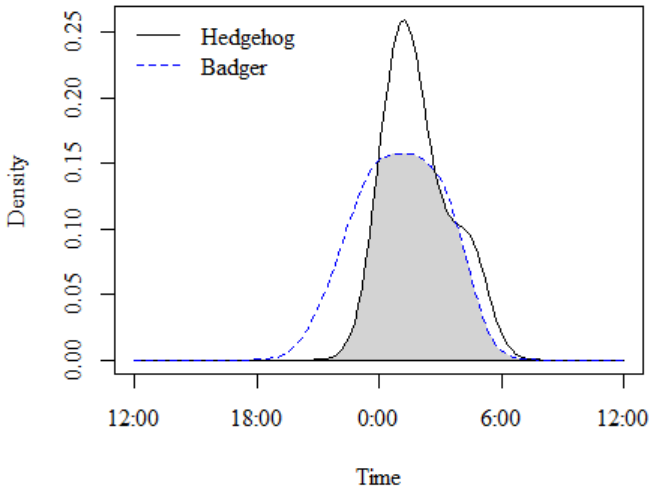
The difference in badger and hedgehog activity levels bordered on statistical significance (P=0.05) for Slade, where hedgehog activity was particularly concentrated between 24:00 and 01:00 hours (C).



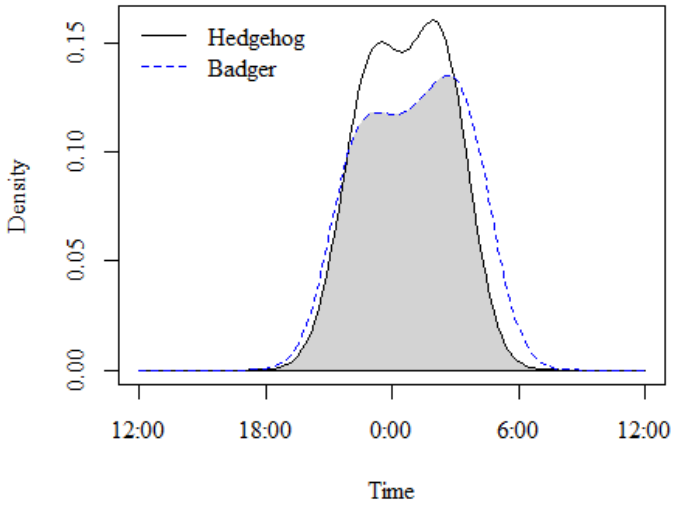
A) Brackenhurst A



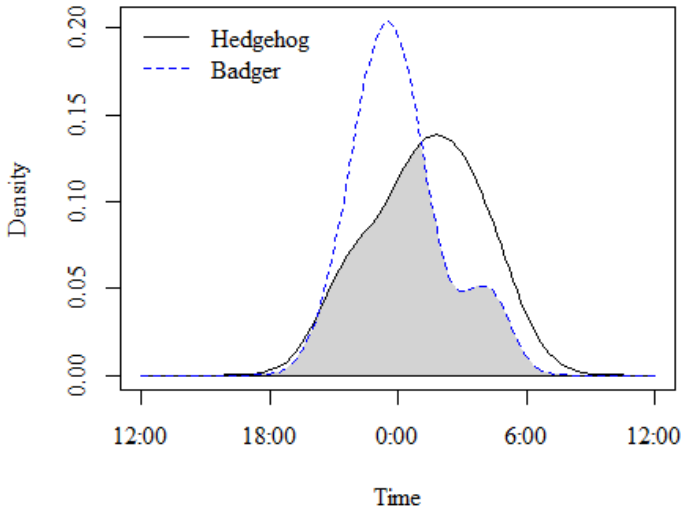
B) Hartpury



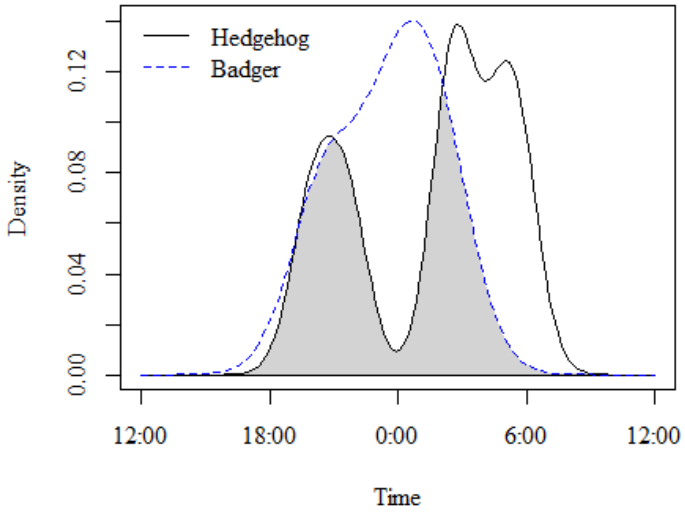
C) Slade



D) Driffield



E) Brackenhurst B


Hedgehog
Badger
Density
0.00
0.04
0.08
0.12
12:00
18:00
0:00
6:00
12:00
Time

F) Riseholme