ANGLIA RUSKIN UNIVERSITY

FACULTY OF SCIENCE AND ENGINEERING

AN INVASIVE LADYBIRD IN RURAL HABITATS: *HARMONIA AXYRIDIS* (COLEOPTERA: COCCINELLIDAE) IN THE UK

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A thesis in partial fulfilment of the requirements of Anglia Ruskin University for the degree of DOCTOR OF PHILOSOPHY

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ABSTRACT

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This study investigates how the invasive alien harlequin ladybird *Harmonia axyridis* affects the ladybird (Coleoptera: Coccinellidae) community in rural habitats in the UK. A novel approach to species distribution models was used to determine where *H. axyridis* may next spread under various climate change scenarios. Field surveys were carried out in England and Wales to investigate how *H. axyridis* affects native coccinellids in rural habitats, including the rare 5-spot ladybird *Coccinella quinquepunctata*. Molecular techniques were employed to determine if intraguild predation occurred between *H. axyridis* and *C. quinquepunctata*.

A combination of variables had an impact on the establishment and spread of *H. axyridis* with human influence being the most important factor. The future spread of *H. axyridis* is predicted to be affected by climate change, with a shift in global distribution expected north and west. In the UK, this species is predicted to spread further into areas such as Scotland and mid-Wales. Unlike urban habitats, rural woodlands are not dominated by *H. axyridis.* Furthermore, a distinct community of coccinellids is evident in both coniferous and deciduous woodland. *Coccinella quinquepunctata* appears not be negatively affected by *H. axyridis* at this time. It is thought this is due to the inhospitable habitat that *C. quinquepunctata* occupies. However, using molecular techniques, it was not possible to confirm if intraguild predation had occurred at the sites where *C. quinquepunctata* and *H. axyridis* were both observed.

A range of interacting factors are necessary for *H. axyridis* to establish in a region and once established this species will spread to rural habitats yet does not dominate the coccinellid community as it does in urban habitats. Continued monitoring thorough Citizen Science is essential in further understanding this dynamic species and its impacts on native coccinellids.

KEY WORDS: CITIZEN SCIENCE, *COCCINELLA QUINQUEPUNCTATA,* COCCINELLIDAE, *HARMONIA AXYRIDIS*, INVASIVE ALIEN SPECIES, MOLECULAR ECOLOGY, RURAL HABITATS, SPECIES DISTRIBUTION MODELS

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1 General introduction

1.1 Invasive alien species

The natural environment is steadily being changed by increased human activity (IPBES, 2019). This activity has led to an increase in animal and plant movement, locally and globally, often resulting in species being relocated to habitats where they would not naturally occur (Blackburn *et al*., 2014; Lucy *et al*., 2016), particularly through trade (Chapman *et al*., 2017). The number of species being introduced to non-native habitats has increased steadily in the last two centuries (Seebens *et al*., 2017). Additionally, this global rise in the establishment of invasive species appears only to be increasing, likely due to ineffective prevention measures and a continued increase of facilitating factors such as land use change, climate change and pollution (Seebens *et al*., 2017; IPBES, 2019).

A wide range of terminology has evolved with the growing interest in non-native species, which can lead to confusion as to the status of a species (Colautti & MacIsaac, 2004) especially as not every non-native species has a negative effect on other species. Throughout this body of work, the term 'invasive alien species' (IAS) is used to refer to a species that as a result of human activities, has moved beyond its native geographic range to an area where it does not naturally occur, resulting in a negative impact on biodiversity (Blackburn *et al*., 2014). Invasive alien species are considered to be one of the leading drivers for biodiversity loss globally, potentially are a contributing factor to species extinctions and are considered the third largest hazard to European threatened species (Gurevitch & Padilla, 2004; Lucy *et al*., 2016; IUCN, 2018). There are several critical stages of an invasion; movement of a taxa to a location not previously inhabited by said taxa, introduction of that taxa into a suitable habitat, subsequent establishment with a population increase in the invaded habitat followed by dispersal from the primary invasion point (Blackburn *et al*., 2011).

There are more than 12,000 non-native species in Europe with between 10-15% becoming IAS over time (European Commission, 2016). Concerning the United Kingdom (UK) alone, of 591 species considered, 30 were deemed to have a high risk of establishing with subsequent negative impacts on biodiversity while 63 species fell into the medium risk category (Roy *et al*., 2014). In response to this threat to biodiversity, the EU introduced EU Regulation 1143/2014 on Invasive Alien Species which has led to the List of Invasive Alien Species of Union Concern (European Commission, 2019). This list consists of 66 species that are considered particularly damaging economically or ecologically and for which measures need to be taken by Member States to ensure the prevention, early detection and management of these species (European Commission, 2017). The list is updated regularly (most recently in August 2019) to take into account new species that may need to be included. There are many pathways by which a species may arrive in any given region or

country and so Member States are required to abide by restrictions pertaining to these species (keeping, importing, selling, breeding or growing) (Essl *et al*., 2015).

1.1.1 Pathways of introduction

Animal and plant species have been introduced deliberately by people into habitats outside of their native range for centuries. The reasons behind deliberate introductions tended to be for either aesthetic or leisure purposes or more practically as a food source or method of biological control. In the mid-1800s several attempts were made to introduce rabbits into Australia and in 1859 one landowner successfully introduced wild *Oryctolagus cuniculus* (European rabbits) to provide 'sport-shooting' for the wealthy (Fenner, 2010). In Ireland (as well as the UK), in the 19th century, *Rhododendron ponticum* (rhododendron) was introduced as an ornamental plant and has since affected biodiversity in mature oak woodlands (Stephenson *et al*., 2006; Maguire *et al*., 2008). In the 1900s, sugar cane crops in Australia were greatly affected by two beetles which resulted in the introduction of *Bufo marinus* (cane toad) in 1935 as a method of biological control (Australian Government, 2010).

There are also several ways in which a species can be introduced accidentally. Continually increasing global trade routes and commercial travel routes play a large role in accidental introductions (Hulme, 2009; Chapman *et al*., 2017). Hulme *et al.* (2008) describe six pathways for accidental introductions: release, escape, contaminant, stowaway, corridor and unaided. This was subsequently updated to exclude unaided by Saul *et al*. (2017) as the databases used were unable to fully capture the scope of this category. Species can have more than one introduction pathway and those species that do, are more likely to have an ecological impact (Pergl *et al*., 2017; Saul *et al*., 2017). One example of an accidental introduction reported by Bartlett *et al*. (2019) was the spread of the *Eretmoptera murphyi* (a flightless midge) in Antarctica which was following footpaths between research sites. An increase in man-made canals has connected European waterways to the Ponto-Caspian basin. A number of freshwater invertebrates and fish originating from the Ponto-Caspian basin are now considered to be IAS (Bij de Vaate *et al*., 2002). Different taxonomic groups tend to utilise different pathways of introduction, for example, mammals are associated with either release or escape and less often unaided while terrestrial invertebrates tend to follow the contaminant pathway (Pergl *et al*., 2017). Many of the introductions cited above (intentional and accidental) result in negative impacts on biodiversity and ecosystem function (Constable & Birkby, 2016) but also often have a high economic impact.

1.1.2 Economic costs

The indirect effects of IAS on biodiversity and ecosystem services are difficult to quantify and it is particularly difficult to acquire accurate information on the costs incurred by IAS. There are, however, examples for certain taxonomic groups although the figures are a conservative estimate at best (Pimentel *et al*., 2001). Globally, £52 billion annually is spent dealing with just invasive alien insects and this estimate is on the conservative side due to large areas that have not been sampled (Bradshaw *et al*., 2016). The estimated cost of IAS to Europe is €12-20 billion annually (Scalera, 2010; Gallardo *et al*., 2016) while the direct cost of IAS in the UK is estimated to be at least £1.7 billion per year (Kelly *et al*., 2013). The majority of these costs are due to effects on agriculture and horticulture, due to IAS weed control, with construction and infrastructure being second on the list of industries directly affected by IAS (Williams *et al*., 2010). Plant invasions exact the highest cost in the UK, directly and indirectly. One example is *Fallopia japonica* (Japanese knotweed) which not only grows at an impressive speed of up to a metre a month but can take several years to eradicate at great cost. Furthermore, as a result of its ability to grow through concrete and tarmac, *F. japonica* affects the building industry and there are mortgage providers who will not provide mortgages for a property if the neighbouring property has *F. japonica* growing there (Williams *et al*., 2010). The costs cited for the UK are reported to be just 2% of the actual costs of IAS and these costs are predicted to rise as more IAS establish in the UK (Kelly *et al*., 2013).

1.2 Ecological impacts of IAS

Aside from the economic impact of IAS, there are costs to biodiversity and ecosystem function. Even though it seems difficult to assign a cost to the ecological impacts, it has been reported that the economic impacts of IAS correlate with ecological impacts (Vila *et al*., 2010). Gurevitch & Padilla (2004) postulated that only a small percentage of taxa threatened with extinction are as a result of IAS. However, Clavero & Garcia-Berthou (2005) investigated the cause of extinction for several hundred species and from the quarter of cases where a cause of extinction could be gleaned, at least half could be attributed to the effect of IAS. When looking at the drivers of avian extinctions based on IUCN criteria, Clavero *et al.* (2009) found that IAS were a much greater risk factor for extinction than habitat destruction. More recently, Bellard *et al*. (2016) found that IAS were the were the top driver for the loss of extinct amphibians, mammals and reptiles. Furthermore, in comparison to native species, IAS were significantly more likely to be the extinction driver for plants and animals (Blackburn *et al*., 2019). Additionally, those species that were threatened by IAS tended to be endemic to islands, often with small ranges (Clavero *et al*., 2009), however, IAS as extinction drivers is also now becoming more of an issue for mainland populations (Bellard *et al*., 2016). The success of freshwater amphipods as IAS, e.g. *Dikerogammarus villosus* ('killer' shrimp), is often

accompanied by sharp declines in native biodiversity (Bollache *et al*., 2008). At some sites in Germany this invasive amphipod constituted 90% of the total abundance of all benthic macroinvertebrates (Arndt *et al*., 2009). Establishment of invasive alien *Gammarus* spp. in the UK have also had negative effects on aquatic biodiversity and the subsequent discovery of *D. villosus* in 2010 resulted in increased concern for freshwater habitat biodiversity due to the high predatory nature of this species (MacNeil *et al*., 2012). There were also concerns that routine biomonitoring would be affected: when assessing water quality under the purview of the Water Framework Directive, it became increasingly apparent that the presence of IAS was affecting the accuracy of biomonitoring in the UK and other European countries (Arndt *et al*., 2009; MacNeill *et al*., 2012). Invasive alien plants can alter geomorphic characteristics by reducing or increasing erosion, increasing sedimentation, altering dunes spatially and impacting the topography of an area (Fei *et al*., 2014). Due to increased sedimentation as a result of invasive *Spartina* spp. in China, over 100, 000 hectares of mudflats are now salt marshes (An *et al*., 2007; Liao *et al*., 2007). *Pacifastacus leniusculus* (signal crayfish) was introduced in the UK as a food source in the 1960s and is now widespread in the UK (Crawford *et al*., 2006). This IAS has negatively affected riparian biodiversity with the abundance of bivalves and gastropods being severely affected (Mathers *et al*., 2016). Additionally, the presence of *Pacifastacus leniusculus* has a negative effect on riverbank stability, which increases sedimentation in gravels beds, affecting invertebrate diversity which has an additional economic cost due to subsequent necessary dredging (Rice *et al*., 2014; Mathers *et al*., 2016). This rapid invasion has also had a negative effect on *Austropotamobius pallipes* (native or white-clawed crayfish) abundance (Mathers *et al*., 2016) as they are more susceptible to crayfish plague than *P. leniusculus*. Additionally, *A. pallipes* are smaller than *P. leniusculus* and can be outcompeted physically through intraguild predation.

1.2.1 Intraguild predation

Another way that IAS can affect native biodiversity is through intraguild predation (IGP). Intraguild predation occurs when the competition between two predators of the same prey results in one of those predators preying on the other (Polis *et al*., 1989). The two main factors affecting the direction of IGP are body size and trophic specialisation, where the biggest and less specialised species are more likely to act as the predator and the smaller and more specialised species become the prey (Polis *et al*., 1989). In California, investigation of the intraguild relationship among several desert scorpion species revealed that the dominant species preyed on at least two of the other intraguild species, and they reported an increase in the numbers of these two species when the dominant scorpion species was removed (Polis & McCormick, 1987). Longhorn beetle larvae of the species *Monochamus carolinensis* are phytophagous and found on pine trees alongside bark

beetles, specifically *Ips calligraphus*. However, during a laboratory trial, Dodds *et al*. (2001) revealed that *M. carolinensis* larvae preyed on *I. calligraphus* in over 70% of encounters. This form of intraguild predation is thought to occur due to a need for additional nutrients (Dodds *et al*., 2001). *Dikerogammarus villosus* has colonised many central European rivers resulting in a reduction in the abundance of native gammarids (Dick *et al*., 2002). When this predatory IAS reached the Netherlands, MacNeill & Platvoet (2005) carried out laboratory experiments to assess the impact this species may have on their native gammarid, *Gammarus pulex*. The native gammarid was preyed on by *D. villosus* but never vice versa. The invasive gammarid is larger than the native *G. pulex,* which is one explanation for this disparity in predation (MacNeill & Platvoet, 2005). This brief introduction to intraguild predation illustrates that IAS are not necessarily passive or so called "passengers" of change.

1.2.2 Ecosystem function

When introduced to a new habitat, generalist species are more likely to become invasive than specialist species; this can result in native specialist species being outcompeted and thereby leading to functional homogenisation (the increase in similarity of a functional variable over time) (Clavel *et al*., 2011). It can be difficult to ascertain the effect that the presence of an IAS may have on ecosystem function, however in recent years, research started to reveal the effects on the invaded ecosystem and community (Simberloff *et al*., 2013). In China, *Spartina alterniflora* (Cord grass spp.) increased carbon and nitrogen levels in the soil when it invaded an area, which in turn negatively affected native plant growth, thereby altering ecosystem function (Liao *et al*., 2007). In the UK, the microbial community of soil is changed when the habitat is invaded by *Impatiens glandulifera* (Himalayan balsam) which prevents the establishment of native flora thus homogenising the plant community (Pattison *et al*., 2016). Additionally, *I. glandulifera* has been shown to negatively affect terrestrial invertebrate diversity (Seeney *et al*., 2019). In aquatic systems, *D. haemobaphes* negatively affected ecosystem function in aquatic ecosystems by impacting native gammarids that are essential in the breakdown of leaf litter (Constable & Birkby, 2016). The presence of *P. leniusculus* in an ecosystem brought about a significant change in the macroinvertebrate community with a decrease in abundance of *Glossiphonia complanata* (leech), *Hydropsyche* spp. (caddisfly), *Caenis* spp. (mayfly) (Mathers *et al*., 2016). If these native species decrease then a subsequent decrease in fish abundance and an increase in Diptera will occur. Together with the negative effect *P. leniusculus* can have on riverbank stability, a change in ecosystem function would be inevitable (Crawford *et al*., 2006; Mathers *et al*., 2016). Globally, coccinellids have been used as biological control of aphids, however some species become established outside of this role and have subsequent negative effects on native coccinellid species. When this occurs, the native

coccinellid community becomes homogenised, leading to a decrease in ecosystem services provided against crop pests (Roy *et al*., 2012; Grez *et al*., 2013; Grez *et al*., 2016).

From the examples cited above, it is clear that IAS can exact serious effects both environmentally and economically. MacDougall & Turkington (2005) concluded that IAS were only passengers and not directly the cause of biodiversity loss, whereas Clavero *et al.* (2009) argue that IAS are not simply passengers when it comes to changes in biodiversity but have a more defining role as drivers of biodiversity loss. More recently, Roy *et al*. (2012) propose that instead of leading to extinction, the presence of IAS is more likely to change the relative abundance of species. Furthermore, global invertebrate biodiversity loss is a major concern and the presence of IAS is often associated with such loss (Didham *et al*., 2005; Mikanowski, 2017) which is likely to impact ecosystem function negatively.

1.3 Coccinellids

Approximately 6000 species of coccinellid have been described globally (Nedvěd & Kovář, 2012), with 47 species resident in the UK and 27 species in Ireland (Roy *et al.,* 2013). In the UK there are 26 species, within the subfamilies Chilocorinae, Coccinellinae and Epilachninae, which are conspicuous and readily identifiable as coccinellids. The remaining 21 coccinellid species in the UK are small, generally without spots and do not obviously look like a ladybird and are often referred to as inconspicuous (Roy *et al*., 2011). The majority of the conspicuous coccinellid species (21 of 26 species) in the UK and Ireland are predators of a wide variety of prey including aphids, coccids, lacewing larvae, thrips, other coleopteran larvae, lepidopteran larvae and coccinellid larvae (Hodek & Evans, 2012). Non-predaceous coccinellids feed on pollen, fungus and other plant material (Evans, 2009). Coccinellids gravitate towards habitats that will provide them with an adequate source of food (Majerus *et al*., 2016). Coccinellid populations as a result will leave an area with poor food resources and move from one habitat to another as aphids begin to populate a given area (Michaud *et al*., 2016). If the primary food source is scarce, aphidophagous species can survive on alternative food sources, such as pollen, coccids, mites, mildew, honeydew etc. Due to the ephemeral nature of aphid species, there are a number of generalist feeding coccinellid species, including *Harmonia axyridis* (harlequin ladybird), *Adalia bipunctata* (two-spot ladybird) and *Coccinella septempunctata* (seven-spot ladybird) (Roy *et al*., 2011). However, not all native aphidophagous ladybird species are generalist feeders, for example, *Myzia oblongoguttata* (striped ladybird) can survive on other prey but can only successfully reproduce when feeding on aphids

from the genera *Schizolachnus* or *Cinara* (Majerus *et al*., 2016). Additionally, *C. septempunctata* and *A. bipunctata* must have access to high numbers of aphids prior to egg-laying, whereas, *Coccinella quinquepunctata* (5-spot ladybird) and *Propylea quattuordecimpunctata* (14-spot ladybird) can begin egg-laying when aphid numbers are lower (Majerus *et al*., 2016).

1.3.1 Coccinellids and biological control

Coccinellids have been used in the biological control of aphids and coccids for almost 140 years (Iperti, 1999). Classical biological control is the use of a natural enemy to reduce a pest that occurs in large numbers. Generally, both the pest and natural enemy are not native to the geographical area affected (Majerus *et al*., 2016). The first noted instance of classical biological control is that of the introduction of *Rodolia cardinalis* (vedalia ladybird) from Australia into California in the 1880s. This introduction of *R. cardinalis* was an attempt to control the cottony cushion scale (*Icerya purchasi*) on citrus crops (Iperti, 1999). Coccinellid use in biological control became more widespread in the 1970s and 1980s and has had success in pest management, for example, on pecans and soybean (Koch & Galvan, 2008). Both *C. septempunctata* and *H. axyridis* have been used extensively as biological control species in countries where they are not native, however, these species have spread to non-target habitats, resulting in adverse effects on native species (Evans, 2000; Brown *et al.,* 2011a; Roy *et al.*, 2016; Sloggett, 2017). When an invasive coccinellid has become established in any ecosystem it has been reported that numbers of native coccinellids decline (Koch & Galvan, 2008). Harmon *et al.* (2007) highlighted the decline of *A. bipunctata* over a broad geographic range after the invasion of *C. septempunctata* and *H. axyridis* in North America. The dramatic decline of *Coccinella novemnotata* in North America has also been attributed to a combination of pressures exerted by both *C. septempunctata* and *H. axyridis* (Losey *et al*., 2012b; Tumminello *et al*., 2015; Ducatti *et al*., 2017).

1.3.2 *Harmonia axyridis* Harlequin ladybird

Harmonia axyridis has a native range in central and eastern Asia (Roy *et al.*, 2011; Orlova-Bienkowskaja *et al.,* 2015). *Harmonia axyridis* is a large coccinellid and is well defended from predators; larvae have large spines, its chemical defences are stronger than its coccinellid counterparts and it is not as susceptible to parasitic or fungal infections as are other coccinellid species (Koch & Galvan, 2008; Roy *et al.,* 2008; Sloggett *et al*., 2011). *Harmonia axyridis* is a generalist species reportedly able to prey on up to 60 aphid species (Majerus *et al*., 2016). If preferred prey numbers are low, other arthropods are also known to be prey for *H. axyridis,* e.g.

lepidopteran eggs, psyllids and lacewing larvae (Koch & Galvan, 2008). If there is a lack of prey for *H. axyridis* it may turn to intraguild predation and prey upon the eggs and larvae of other coccinellid species (Brown *et al*., 2011a; Roy *et al*., 2011). Intraguild predation has been observed in coccinellid species, especially when *H. axyridis* is present (Pell *et al*., 2008; Lucas, 2012). In laboratory trials with 11 other coccinellid species, *H. axyridis* was the dominant predator in the majority of intraguild interactions with the exception of three coccinellid species (Ware & Majerus, 2008).

Harmonia axyridis was used in biological control in 1916 in North America (Brown *et al*., 2011b), yet, it failed to establish until 1988 (Majerus *et al*., 2016), however once established it spread quickly. This coccinellid is now globally established either as a result of its use in North and South America, Asia and Europe in biological control of pest aphids and coccids or accidental introduction and is now a threat to native coccinellids and other non-target species (Harmon, 2007; Brown *et al.,* 2011a; Honěk *et al*., 2016). Although it is described as semi-arboreal, *H. axyridis* has been recorded in a wide range of habitats in the UK: urban areas and gardens, grassland, arable land and deciduous and coniferous woodland (Brown *et al*., 2011b). The majority of UK records of *H. axyridis* have been in urban areas and Labrie *et al.* (2008) reported *H. axyridis* surviving very cold winters only where people dwell, as this species prefers to over-winter in anthropogenic structures. Additionally, Brown *et al*. (2011b) reported that *H. axyridis* tended to oviposit and feed at sites that have human structures nearby. *Harmonia quadripunctata* is closely related to *H. axyridis* and was initially recorded in the UK in 1937 (Roy *et al*., 2011). *Harmonia quadripunctata* is not considered to be an IAS and took fifty years to spread from East Anglia to Devon whereas *H. axyridis* took two years to spread a similar distance (Brown *et al*., 2008; Brown *et al*., 2011a). The majority of native coccinellids are univoltine, i.e., they have just one brood per year. *Harmonia axyridis* can have up to five broods per year (multivoltine) in its home range. In the Czech Republic, this species is reported to be capable of producing up to three broods per year if sufficient resources are present as well as suitable thermal conditions (Honěk *et al*., 2018a). Furthermore, Brown *et al*. (2008) reported increases in larval numbers later in the calendar year indicating that in the UK, this species is bi-voltine, which would partly explain the rapid spread of *H. axyridis* in the UK.

1.3.3 Effects of *Harmonia axyridis* on native coccinellids in the UK and Europe

In Belgium, there has been a decline of almost a third of *A. bipunctata* since the establishment of *H. axyridis* (Roy *et al*., 2012). Prior to the arrival of *H. axyridis* in the Czech Republic, some coccinellid species were already in decline, however, two species (*Adalia decempunctata* & *Calvia quattuordecimguttata*) declined only after *H. axyridis* had established (Honěk *et al*., 2016). In the UK, there was an initial decline of *A. bipunctata*, *C. septempunctata* and *P. quattuordecimpunctata*

within three years of *H. axyridis* establishment (Brown *et al*., 2011a). Recently, however, from a continuing 11-year study it was evident that *A. bipunctata* continued to decline while numbers of the other two species recovered (Brown & Roy, 2017). The change seen here in less than a decade highlights how important continued data collection is, however, gathering large volumes of data can be costly both financially and in terms of people hours. Increasingly, researchers are recruiting members of the public as Citizen Scientists in an effort to acquire quality data in large numbers and these efforts are proving to be successful.

1.4 Citizen Science

Citizen Science is when members of the public volunteer their personal time in order to collect data. This method of recording data is used to help inform research across a range of areas such as climate change, pollution effects, psychology, health and social care, genetics and ecology (Silvertown, 2009). From this point forward, Citizen Science will be discussed within the remit of ecology. One of the earliest Citizen Science projects is the Audubon Christmas Bird Count, which has been underway since 1900, and contributes to determining national trends and conservation of bird species in North America (National Audubon Society, 2020). In the UK, Citizen Scientists collect data either for specific projects (e.g. Big Garden Birdwatch, UK) or for their own records that are made available to researchers or the general public on platforms such as iRecord (https://www.brc.ac.uk/irecord/). Thus, engaging in Citizen Science results in mutual benefits for both those running the project and those who are volunteering their time. Large quantities of valuable usable data are collected for those running the project while Citizen Scientists benefit in a range of ways from engaging with the environment and playing their part in protecting their local or national habitats, learning about new species or habitats to feeling like they are contributing to research (Silvertown, 2009; Pages *et al*., 2019).

As with any large-scale endeavour, however, there are some disadvantages. For example, the reliability of Citizen Science data has previously been questioned as not every citizen scientist will have the knowledge to know exactly what they are looking for and may mis-identify plants or animals that are the focus of a project. To overcome this, some Citizen Science projects develop excellent field guides that enable volunteers to make accurate recordings (Silvertown, 2009). Additionally, the ever-increasing use of smartphones has facilitated Citizen Science in several ways. Smartphone apps have been developed to guide volunteers in the identification of organisms (Roy *et al*., 2018). In addition to this, being able to take a photograph of an organism and upload it for identification/verification by an expert reduces the pressure on volunteers to be expert on a given

organism. These technological developments, together with improved data modelling mean than much of the data gathered through Citizen Science are reliable, and quality assured, as well as being essential in discovering more about a range of species (Crall *et al*., 2015).

Citizen science has been used successfully on a global level to prevent, detect and manage IAS or determine the effects of IAS on native species (Losey *et al*., 2012a; Roy *et al*., 2015; Brown & Roy, 2017; Adriaens, 2019). In the UK, *Vespa velutina* (Asian hornet) has been recorded 17 times over the last three years in the UK and all of these sightings were from members of the public (gov.uk, 2019). As a result of engagement from the general public and the subsequent eradication of reported individuals, this species has not yet established in the UK. In recent years it has been increasingly recognised that there is a need for continuous monitoring of invertebrate species in order to accurately assess the decline of not just rare invertebrate species but also common species (Roy *et al*., 2012; Mikanowski, 2017). The UK Ladybird Survey is an excellent example of how successful a Citizen Science project can be. The efforts of thousands of recorders mean that researchers have been able to not only map the distribution of *H. axyridis* from the time it was established but also the distributions of the UK's native coccinellids (Roy *et al*., 2011). Additionally, long-term conservation trends have been published as a result of Citizen Science efforts in the form of the Field Guide to the Ladybirds of Great Britain and Ireland (Roy *et al*., 2018b). These efforts in turn have increased knowledge in relation to native coccinellid species in addition to how an IAS effects native species.

1.5 Summary

Invasion processes involve complex mechanisms influenced by a range of environmental and geographical factors. Given the negative effect of plant, invertebrate and vertebrate IAS in Europe and more specifically the UK, it is important to learn more about the mechanisms and interactions of IAS on native flora and fauna. Roy *et al*. (2016) stated the necessity for research to evaluate the negative and positive impacts of IAS at the ecosystem level. Using *H. axyridis* as a model species will provide knowledge on the effect of an IAS on native species at the community level. A particular knowledge gap in relation to *H. axyridis*, relates to the prevalence and effects of this species in the wider countryside (i.e. as opposed to in urban / suburban habitats or agricultural systems). When the potential spread of *H. axyridis* in the UK was mapped by Purse *et al*. (2014), it was predicted that rural areas would be less affected by this IAS and would be a potential refuge for native coccinellids. Considering the rapid spread of *H. axyridis* and its detrimental effect on native coccinellids in north America (Koch & Galvan, 2008; Losey *et al*., 2012b), Europe (Adriaens *et al*.,

2008; Roy *et al*., 2012) and UK urban areas (Brown & Roy, 2017), the overall aim of this research is to determine how *H. axyridis* is affecting the native coccinellid assemblage in specified rural habitats.

This thesis initially looks at the global reach of *H. axyridis* and determines what global, continental (Europe) and national (UK) impact climate change may have on the continued spread and distribution of *H. axyridis*. From this global, European and national view, the thesis moves to the investigation of *H. axyridis* within England in rural habitat to determine how prolific the species is in these areas and how the species sits within the coccinellid community. The thesis continues to narrow in scope by looking at the presence of *H. axyridis* in a very specific and rare habitat and how the species may be affecting a nationally rare coccinellid, *C. quinquepunctata* five-spot ladybird. The final data chapter of this thesis attempted to determine through molecular analysis if *H. axyridis* may be affecting *C. quinquepunctata* in its habitat through intraguild predation. In summary, the thesis attempts to reveal and explain the current place *H. axyridis* holds within the coccinellid community in the UK but also realises the necessity in viewing this IAS on a global scale allows greater interpretation of national and regional data.

2 Determining the spread of *Harmonia axyridis* in the UK and Europe using data from Citizen Science.

2.1 Introduction

2.1.1 Invasive alien species

Invasive alien species (IAS) are a global concern and although the UK is an island nation, it is increasingly at risk, like the rest of Europe, to the establishment of IAS, due to busy trade routes, movement of people and climate change (Great Britain Non-Native Species Secretariat, 2015; Roy *et al*., 2019). The influx of alien species into Europe has not slowed down and is not expected to in the immediate future (Seebens *et al*., 2017). The threat from IAS is taken seriously by the EU, who have developed regulations (EU Regulation 1143/2014) in an attempt to minimise and manage the impact of current IAS and, importantly, to prevent any further establishment of new IAS.

2.1.2 *Harmonia axyridis* – Harlequin ladybird

One species that has established throughout Europe is the coccinellid, *Harmonia axyridis*, harlequin ladybird. This species has been described as the most invasive ladybird on earth (Majerus, 2004) and has spread to all continents except Antarctica. The presence of this coccinellid is reportedly responsible for the decline of several native coccinellids; *Coccinella novemnotata* in North America (Harmon *et al*., 2007; Losey *et al*., 2012b) and overall native coccinellid diversity in Chile (Grez *et al*., 2016). In Europe, *H. axyridis* was initially introduced to 13 European countries and has since spread to other European countries (Brown *et al*., 2011b) with varying impacts to the coccinellid communities. *Adalia decempunctata* and *Calvia quattuordecimguttata* have declined in the Czech Republic (Honěk *et al*., 2016), in Italy, *Adalia bipunctata* has also decreased in abundance (Masetti *et al*., 2018) while in Serbia, *H. axyridis* has increased in abundance to become the dominant coccinellid in urban areas (Markovic *et al*., 2018). In the UK, a number of species initially declined (Brown *et al*., 2011a) but *A. bipunctata* has declined considerably since the establishment of *H. axyridis* (Brown & Roy, 2017). Evidence in the above studies is from long-term studies, many of which are based on observations from members of the public and are large-scale citizen science projects such as the Lost Ladybug Project in the USA, Chinita arlequín in Chile and the UK Ladybird Survey in the United Kingdom.

2.1.3 Citizen Science

Data from citizen science initiatives contributes to the detection and control of IAS as well as learning more about the dynamics of IAS (Thomas *et al*., 2017). Such initiatives result in a large quantity of data being gathered in a short time frame and with an increase in the use of smartphone technology such as smartphone apps, a greater number records can be verified globally than if the data were being collected by a handful of ecologists (Roy *et al*., 2018). Citizen science projects are particularly useful in helping to detect the first records of an IAS or tracking the spread and distribution of a species (Roy *et al*., 2018). One example is *Thaumetopoea processionea* (oak processionary moth) which can be successfully detected by moth recorders (Pocock *et al*., 2017). *Vespa velutina* (Asian hornet) is another species that concerns Europe as a whole and there are initiatives in place where members of the public can submit a suspected sighting (CEH, 2017). As a result, sightings are dealt with promptly and this species is currently not established in the UK (CEH, 2017).

Citizen Science data can be stored and accessed from a range of places both locally (databases for certain species), nationally (national data recording centres) and internationally and is often made available as open data. There is a move by a large number of researchers to share data in order to progress the scientific process (Molloy, 2011). The Global Biodiversity Information Facility (GBIF) is an example of a global database portal for all taxonomic groups. Records from such databases can be used to determine where a species may next spread to, which allows regions and countries to prepare in the hope of prevention and or control of the species (Chapman *et al*., 2019). Information submitted through citizen science projects is essential for learning more about coccinellid interactions (Roy *et al*., 2016). This is evident globally from research carried out in Chile (Grez *et al*., 2016), USA (Losey *et al*., 2012a), Belgium (Adriaens *et al*., 2008) and the UK (Brown & Roy, 2017). In the UK there is excellent data on the spread of *H. axyridis* in urban and anthropogenically disturbed areas, with the most recent predictions of further spread of the species being made by Purse *et al*. (2014) using Bayesian spatial survival models.

2.1.4 Species distribution models

Species distribution models (SDMs) are a useful tool in horizon scanning for IAS and one set of such models being used more frequently are CLIMEX models. These models aid in determining the effect climate change may have on the spread of a species, whether it is invasive or not. Previously, these SDMs used one of two methods when defining the background region for pseudo-absences. One method used the area accessible to the species in relation to its occurrence (Mainali *et al*., 2015), while the other method used the area outside the species range by considering it to be unsuitable

(Thuiller *et al*., 2004). Recently a different approach was taken by Chapman *et al*. (2019) who combined both methods using real data to create an average, which resulted in more accurate projections based on five plant species that are IAS in Europe. This was achieved using current biological knowledge of the limiting factors for the species (Chapman *et al*., 2019). These models relied on large datasets, many of which are only available as a result of the efforts of citizen science data collection. As CLIMEX models are useful in predicting where an IAS may next spread to and considering the continuous spread of *H. axyridis* in certain regions, it would be prudent to investigate what effect climate change may have on the distribution and presence of *H. axyridis* in the UK and wider continent of Europe in the future.

2.1.5 Aim and hypotheses

Using citizen science data, the aim of this study was to use the methods adapted by Chapman *et al*. (2019) to determine where *H. axyridis* has established and where it may spread in the future in relation to climate data. It is expected that climate will affect the range accessible to *H. axyridis* and that an increase in temperature resulting from climate change will facilitate the range expansion of *H. axyridis* in the UK and continental Europe.

2.2 Methods

The species distribution models laid out below follow closely those described in Chapman *et al*. (2019). The methods below have also been used for several risk assessments of IAS carried out by D. Chapman, O. Pescott and B. Beckman (e.g. Pescott *et al*., 2017).

2.2.1 Data for modelling

Harmonia axyridis records (n=199,902) were acquired from four databases: GBIF (Global Biodiversity Information Facility, n = 141,176), BISON (Biodiversity Information Serving Our Nation, n = 7,278), iDigBio (Integrated Digitized Biocollections, n = 402) and UKLS (UK Ladybird Survey, n = 51,046). The native range (latitude and longitude) for *H. axyridis* was taken from Orlova-Bienkowskaja *et al*. (2015). There are areas of Japan that appear as part of the invaded range of *H. axyridis* (Figure 2.1a), however the literature does not explicitly discuss a divide of native and invaded range across Japan (Brown *et al*., 2011b; Orlova-Bienkowskaja *et al*., 2015; Roy *et al*., 2016). During this analysis, the region of Japan indicated as invaded is outside the latitude/longitude

ranges that were used in the modelling. Furthermore, records in areas where *H. axyridis* was not previously confirmed as established were removed, for example small numbers of records from Turkey, Iran, and north west Canada. Records where georeferencing was imprecise (e.g. records referenced to a country or island centroid) or outside the coverage of the predictor layers (e.g. small island) were also removed (Chapman *et al*., 2019). The remaining records were gridded at a 0.25 x 0.25 degrees of longitude/latitude resolution for modelling (Chapman *et al*., 2019), resulting in 4,693 grid cells with occurrences (Figure 2.1a). The density of Insecta records held by GBIF was also compiled on the same grid (Figure 2.1b) as a proxy for recording effort.

Climate data were selected from the 'Bioclim' variables contained within the WorldClim database (Hijmans *et al*., 2005). Based on the biology of *H. axyridis*, the following climate variables and habitat layer were used in the modelling:

- Bio6: Minimum temperature of the coldest month
- Bio10: Mean temperature of the warmest quarter
- Climatic Moisture Index (CMI): ratio of mean annual precipitation to potential evapotranspiration, log+1 transformed.
- Human Influence Index (HII): the Global Human Influence Index Dataset of the Last of the Wild Project (WCS & CIESIN, 2005) was developed from nine global data layers. These layers incorporate human population pressure (population density), human land use and infrastructure (built-up areas, night time lights, land use/land cover) and human access (coastlines, roads, railroads, navigable rivers). The index ranges between 0 and 1 and was ln+1 transformed for the modelling to improve normality.

To estimate the effect of climate change on the potential distribution of *H. axyridis*, equivalent modelled future climate conditions for the 2070s under the four Representative Concentration Pathways (RCP) 2.6, 4.5, 6.0 and 8.5 were also obtained. The RCPs represent scenarios of low to high emissions, respectively. The above variables were obtained as averages of outputs of eight Global Climate Models (BCC-CSM1-1, CCSM4, GISS-E2-R, HadGEM2-AO, IPSL-CM5A-LR, MIROC-ESM, MRI-CGCM3, NorESM1-M), downscaled and calibrated against the WorldClim baseline (see http://www.worldclim.org/cmip5_5m). Future scenarios are presented for Europe only and as RCP8.5 is considered quite extreme and RCP6.0 is quite similar to RCP4.5, only RCP2.6 and 4.5 are presented here.

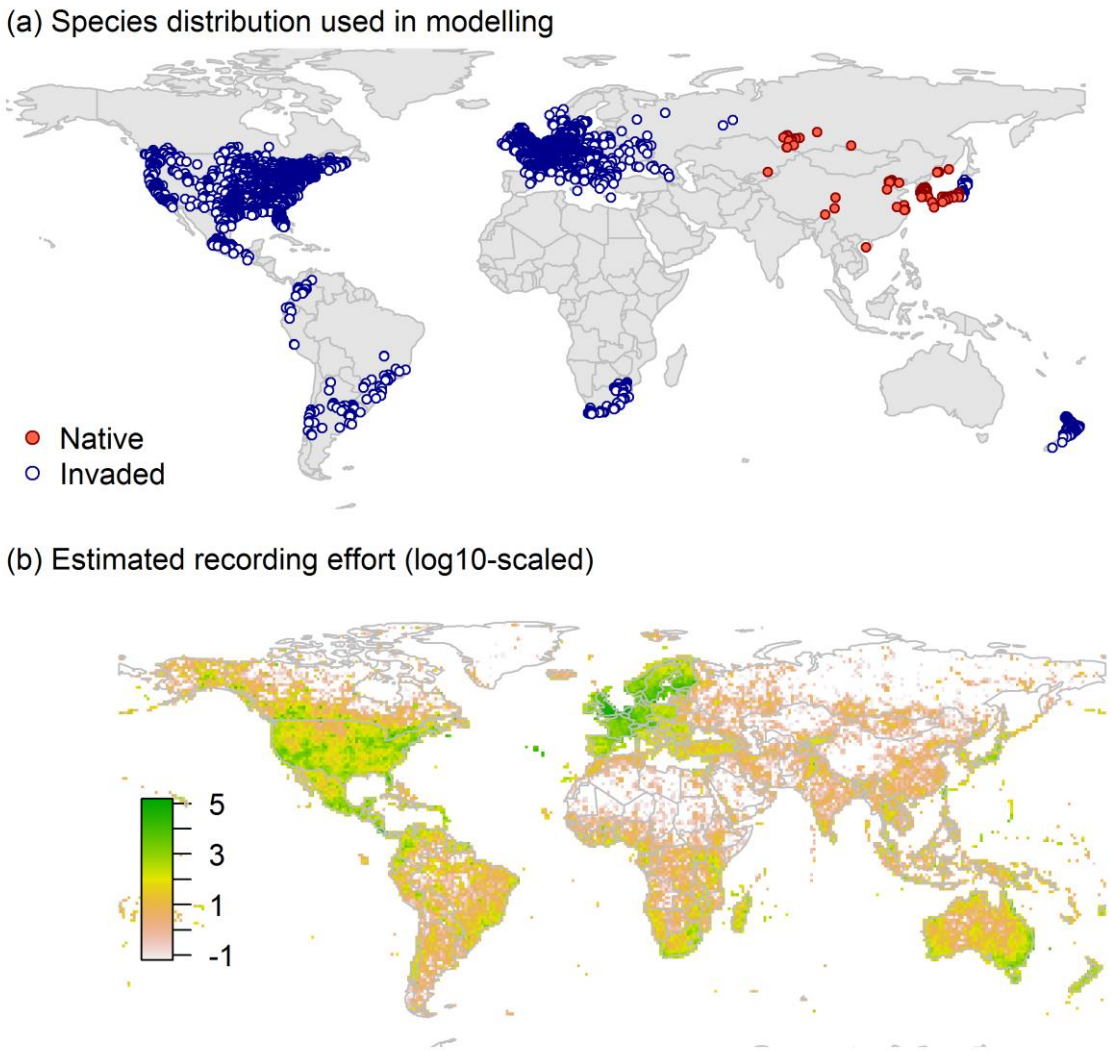


Figure 2.1: (a) Occurrence records obtained for *Harmonia axyridis* and used in the modelling, showing native and invaded distributions; (b) The recording density of Insecta on GBIF, which was used as a proxy for recording effort.

2.2.2 Species distribution model

A presence-only ensemble modelling strategy was employed using the BIOMOD2 R package v3.3-7.1 (Thuiller *et al*., 2009, Thuiller *et al*., 2019). These models contrast the environment at the species' occurrence locations against a random sample of the global background environmental conditions (often termed 'pseudo-absences') in order to characterise and project suitability for occurrence. This approach was developed for distributions that are in equilibrium with the environment, however, IAS distributions are not at equilibrium and subject to dispersal constraints at a global scale. As a result, the inclusion of locations suitable for *H. axyridis* but where it had not been able to disperse to were minimised (Chapman *et al*., 2019). Accordingly, the background sampling region included:

- the area accessible by native *H. axyridis* populations, where the species is likely to have had sufficient time to disperse to all locations. Based on presumed maximum dispersal distances, the accessible region was defined as a 500km buffer around the native range occurrences.
- a 50km buffer around the occurrences of *H. axyridis* in a non-native area, encompassing regions likely to have had high propagule pressure for introduction by humans and/or dispersal of the species
- regions where there was an *a priori* expectation of high unsuitability for the species so that absence was assumed irrespective of dispersal constraints (Figure 2.2). The following rules were applied to define a region expected to be highly unsuitable for *H. axyridis* at the spatial scale of the model:
  - Minimum temperature of the coldest month (Bio6) < -23°C
  - Mean temperature of the warmest quarter (Bio10) < 11°C
  - Climatic moisture index (CMI) < log1p (0.23)

Only 1.3% of occurrence grid cells were located in the unsuitable background region. Within the background region, 10 samples of 5000 randomly sampled grid cells were obtained, weighting the sampling by recording effort (Figure 2.2).

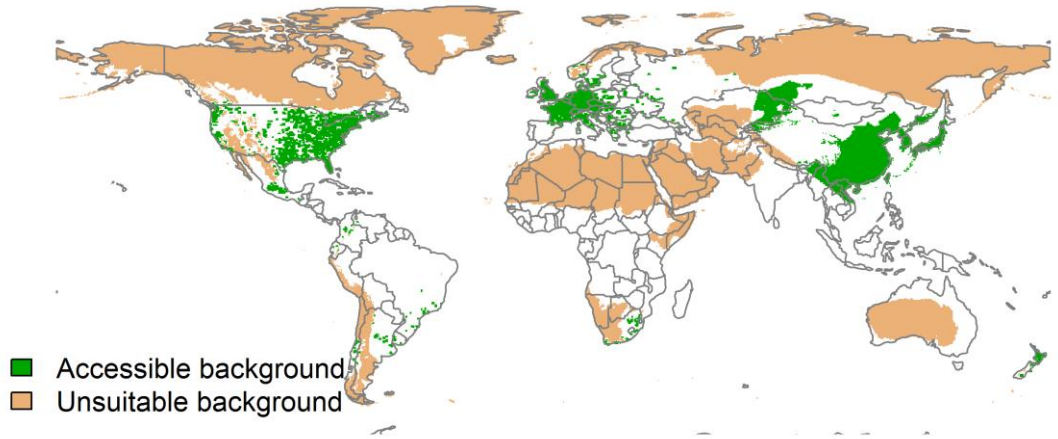


Figure 2.2: The background from which pseudo-absence samples were taken in the modelling of *Harmonia axyridis*. Samples were taken from a 500km buffer around the native range and a 50km buffer around non-native occurrences (together forming the accessible background) and from areas expected to be highly unsuitable for the species (the unsuitable background region). Samples were weighted by a proxy for recording effort (Figure 2.1(b)).

Each dataset (i.e. combination of the presences and the individual background samples) was randomly split into 80% for model training and 20% for model evaluation (Chapman *et al*., 2019). With each training dataset, seven statistical algorithms were fitted with the default BIOMOD2 settings: Generalised Linear Model (GLM), Generalised Boosting Model (GBM), Generalised Additive Model (GAM) with a maximum of four degrees of freedom per smoothing spline, Artificial Neural Network (ANN), Multivariate Adaptive Regression Splines (MARS), Random Forest (RF) and Maxent (Thuiller *et al*., 2016; Chapman *et al*., 2019). The background sample was larger than the number of occurrences so prevalence fitting weights were applied to give equal overall importance to the occurrences and the background. Normalised variable importance was assessed and variable response functions were produced using BIOMOD2's default procedure. Model predictive performance was assessed by three measures; AUC (Area Under the Curve), Cohen's Kappa, and TSS (True Skill Statistic) (detailed descriptions in Appendix A2.2a-A2.2c). AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected absence (Allouche *et al*. 2006). Cohen's Kappa corrects the overall accuracy of model predictions by the accuracy expected to occur by chance (Cohen, 1960). TSS compares the number of correct forecasts, minus those attributable to random guessing, to that of a hypothetical set of perfect forecasts (Allouche *et al*. 2006).

An ensemble model was created and ensemble projections were made for each dataset and then averaged to give an overall suitability, as well as its standard deviation. The projections were then classified into suitable and unsuitable regions using the 'minROCdist' method, which minimizes the distance between the ROC plot and the upper left corner of the plot (point (0,1)). A limiting factor map was subsequently produced following Elith *et al*. (2010) (Figure 2.6). For this, projections were made separately with each individual variable fixed at a near-optimal value. These were chosen as the median values at the occurrence grid cells. Finally, the most strongly limiting factors were identified as those resulting in the highest increase in suitability in each grid cell.

2.2.3 Caveats to the modelling

To remove spatial recording biases, the selection of the background sample was weighted by the density of Insecta records on the GBIF database. While this is preferable to not accounting for recording bias at all, it may not provide the perfect measure of recording bias. There was substantial variation among modelling algorithms in the partial response plots (Figure 2.3). In part this will reflect their different treatment of interactions among variables. Since partial plots are made with other variables held at their median, there may be values of a particular variable at

which this does not provide a realistic combination of variables to predict from. Other variables potentially affecting the distribution of the species, such as land cover, were not included in the model.

2.3 Results

The ensemble model suggested that the Human Influence Index (HII) was the most important factor when determining suitability for *H. axyridis*, accounting for 31.7% of the variation. Mean temperature of the warmest quarter (Bio10), climatic moisture index (CMI) and minimum temperature of the coldest month (Bio6) accounted for 28.3%, 24% and 16% respectively (Figure 2.3; Table 2.1).

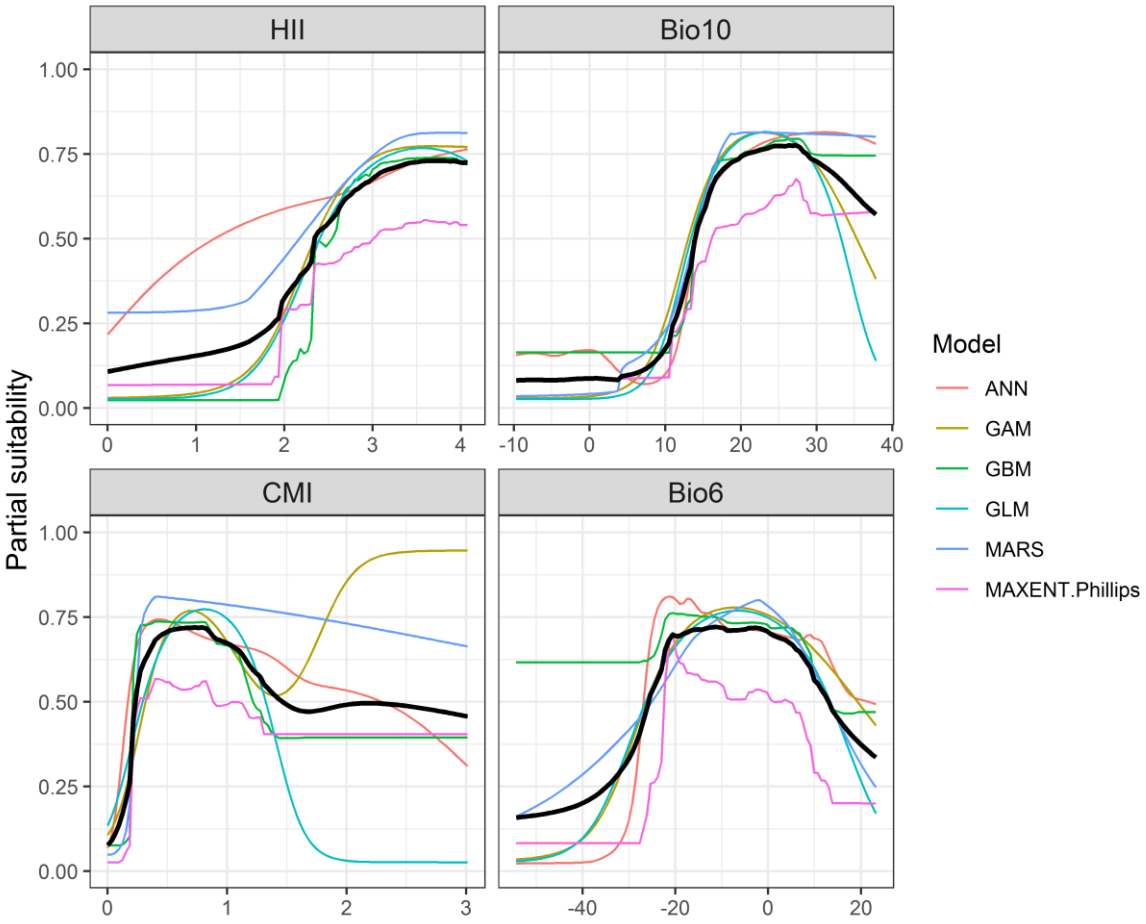


Figure 2.3: Partial response plots from the fitted models. Thin coloured lines show responses from the algorithms in the ensemble, while the thick black line is their ensemble. In each plot, other model variables are held at their median value in the training data. Some of the divergence among algorithms is because of their different treatment of interactions among variables.

Table 2.1: Summary of the cross-validation predictive performance (AUC, Kappa, TSS) and variable importance of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the average from models fitted to 10 different background samples of the data.

					variable importance (%)			
Algorithm	**AUC**	**Kappa**	**TSS**	**Used in the ensemble**	**Human Influence Index (HII)**	**Mean temperature of the warmest quarter (Bio10)**	**Climatic Moisture Index (CMI)**	**Minimum temperature of the coldest month (Bio6)**
GLM	0.804	0.436	0.526	yes	37	27	19	16
GAM	0.805	0.435	0.527	yes	36	26	21	17
ANN	0.813	0.458	0.537	yes	17	34	27	22
GBM	0.814	0.453	0.534	yes	47	26	24	3
MARS	0.806	0.442	0.528	yes	25	32	28	15
RF	0.673	0.401	0.500	no	29	30	24	17
Maxent	0.811	0.448	0.536	yes	28	26	24	22
Ensemble	**0.812**	**0.449**	**0.534**		**32**	**28**	**24**	**16**

2.3.1 Current climate

Given the distribution that was used for the modelling process (Figure 2.1a), it was interesting that the model predicted that certain countries in Africa (Morocco, Algeria, Ethiopia, east Uganda, Kenya, north Tanzania, Angola, Zambia, Zimbabwe), Asia (Turkey, Georgia, Azerbaijan, Iran, Nepal, north Pakistan, India), Oceania (east Australia) and Europe (Portugal, Spain, Greece) would be suitable under current climatic conditions for *H. axyridis* to establish (Figure 2.4a & Figure 2.5). Similar to UK suitability, the east and south of Ireland appear suitable for *H. axyridis*, with the west and north of Ireland being unsuitable, similar to much of Scotland, parts of north-west England and mid-Wales (Figure 2.5).

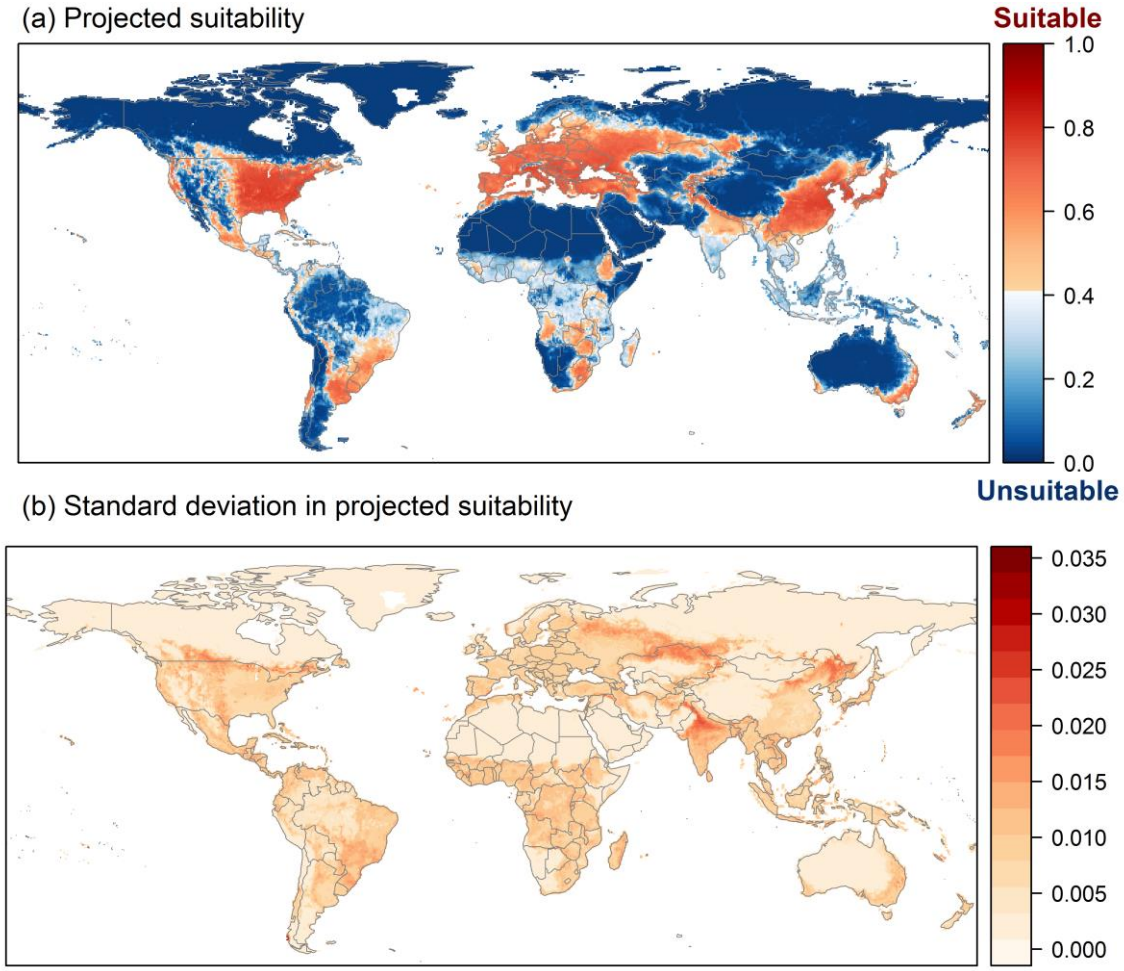


Figure 2.4: (a) Projected global suitability for *Harmonia axyridis* establishment in the current climate. For visualisation, the projection has been aggregated to a 0.5 x 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Values > 0.41 may be suitable for the species. White land areas have climatic conditions outside the range of the training data so were excluded from the projection. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the four datasets.

In northern and western Europe, including the UK, Bio10 (mean temperature of the warmest quarter) was the strongest limiting factor. In the Mediterranean, Bio6 (mean temperature of the coldest month) and CMI (Climate Moisture Index) were the strongest limiting factors (Figure 2.6). Outside of Europe, these two factors together with HII (Human Influence Index) were the predominant limiting factors with very few areas limited by Bio10 (Figure 2.6).

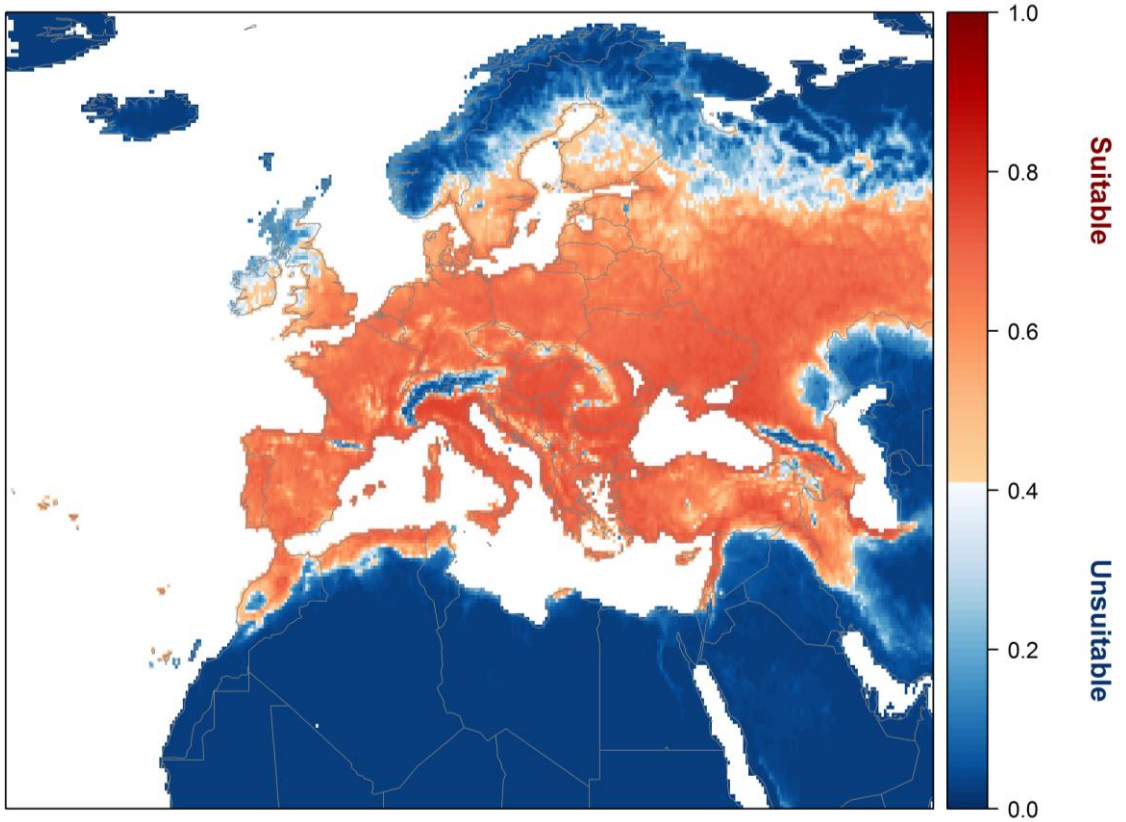


Figure 2.5: Projected current suitability for *Harmonia axyridis* establishment in Europe and the Mediterranean region. The white areas have climatic conditions outside the range of the training data so were excluded from the projection.

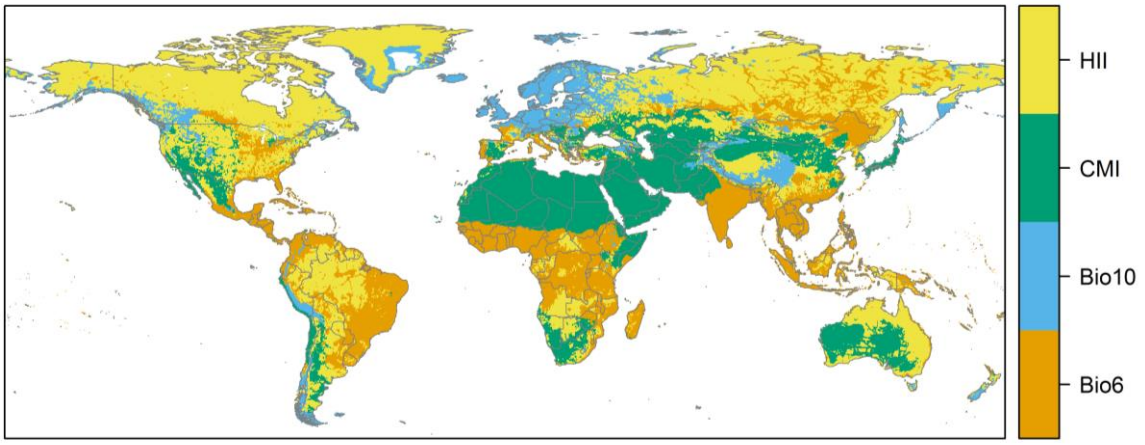
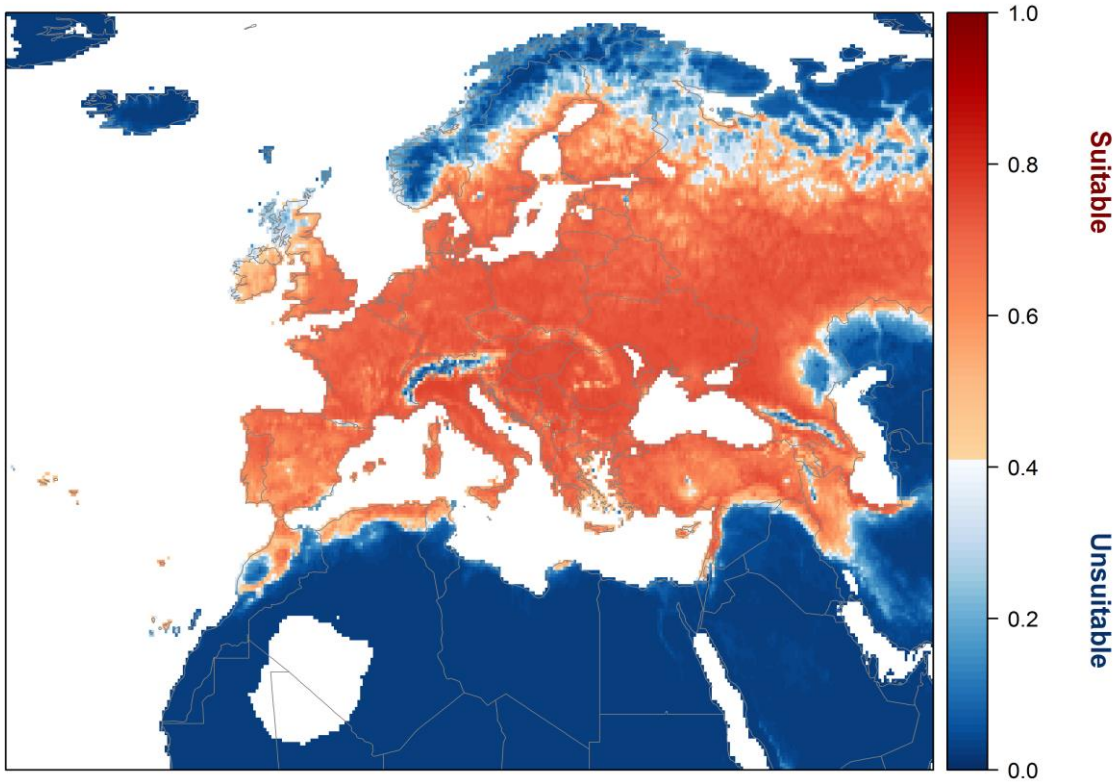


Figure 2.6: The most strongly limiting factors for *Harmonia axyridis* establishment estimated by the model in current climatic conditions; HII = Human Influence Index; CMI = Climatic Moisture Index; Bio10 = mean temperature of the warmest quarter; Bio6 = mean temperature of the coldest month.

2.3.2 Future climate scenarios in Europe

Looking ahead to the 2070s in Europe, under both RCP2.6 and RCP4.5, the parts of Ireland and the UK that are currently unsuitable for the establishment of *H. axyridis* are predicted to become more suitable. Similarly, the same was predicted for areas of Norway, Sweden and Finland that are currently unsuitable. Conversely, those areas in the Mediterranean that are currently suitable are predicted to become less suitable (south-east Spain, central Turkey, west Morocco) (Figure 2.7a & Figure 2.7b).

(7a) RCP2.6



(7b) RCP4.5

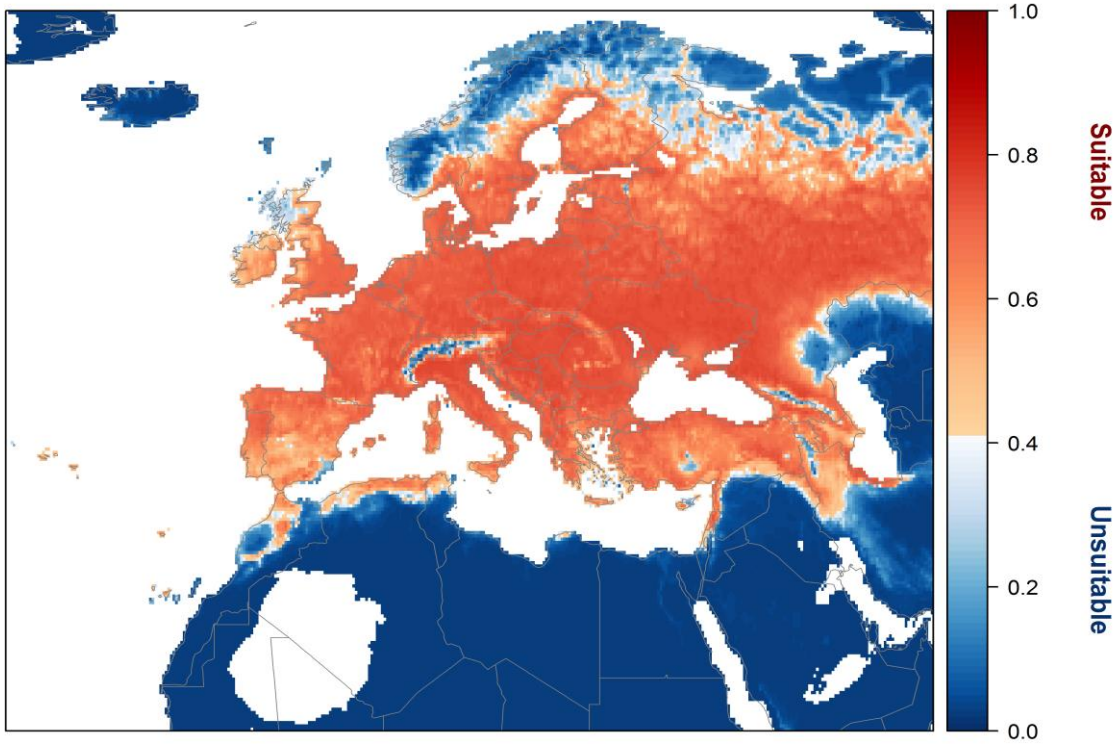


Figure 2.7: Projected suitability for *Harmonia axyridis* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario (a) RCP2.6 and (b) RCP4.5, equivalent to Figure 2.5. The white areas have climatic conditions outside the range of the training data so were excluded from the projection.

Taking a closer look at the UK and Ireland, it is predicted under both climate change scenarios that *H. axyridis* will increase its distribution by spreading further north into Scotland and westward into Wales and Northern Ireland. Under future scenarios, areas of Ireland that are currently predicted as unsuitable become more suitable with an almost all island suitability predicted under RCP4.5 (Figure 2.8).

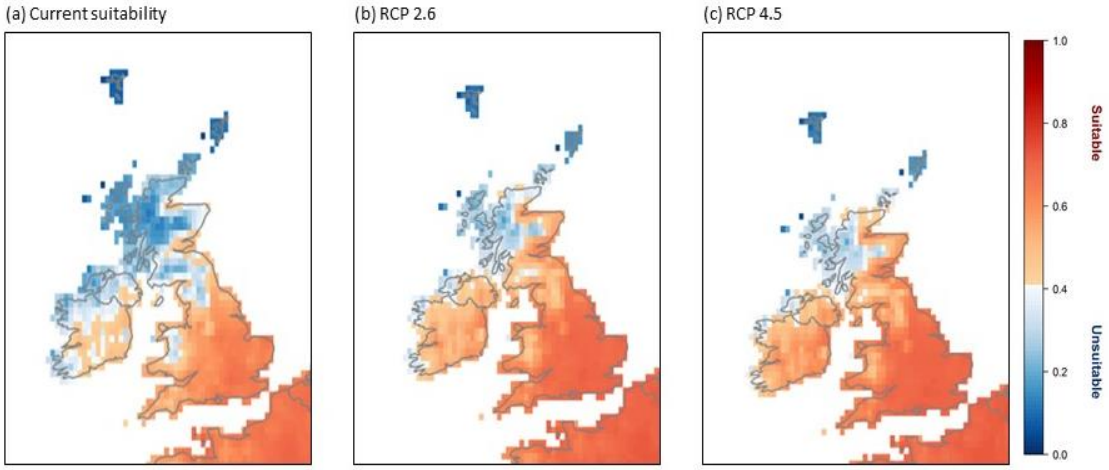


Figure 2.8: Projected suitability for *Harmonia axyridis* establishment in the UK and Ireland (a) current suitability and under climate change scenario (b) RCP2.6 and (c) RCP4.5. The white areas have climatic conditions outside the range of the training data so were excluded from the projection.

Figure 2.9 further emphasises this predicted change in suitability for *H. axyridis* by illustrating the suitability of the Biogeographical regions of Europe both currently and under future scenarios. The Arctic region is currently highly unsuitable and remains relatively unsuitable under RCP2.6 and RCP4.5. The Alpine and Boreal regions have low suitability currently but will increase under both RCP2.6 and RCP4.5 however, these regions are not predicted to be as suitable as the Continental, Pannonian, Black Sea, Anatolian or Mediterranean regions.

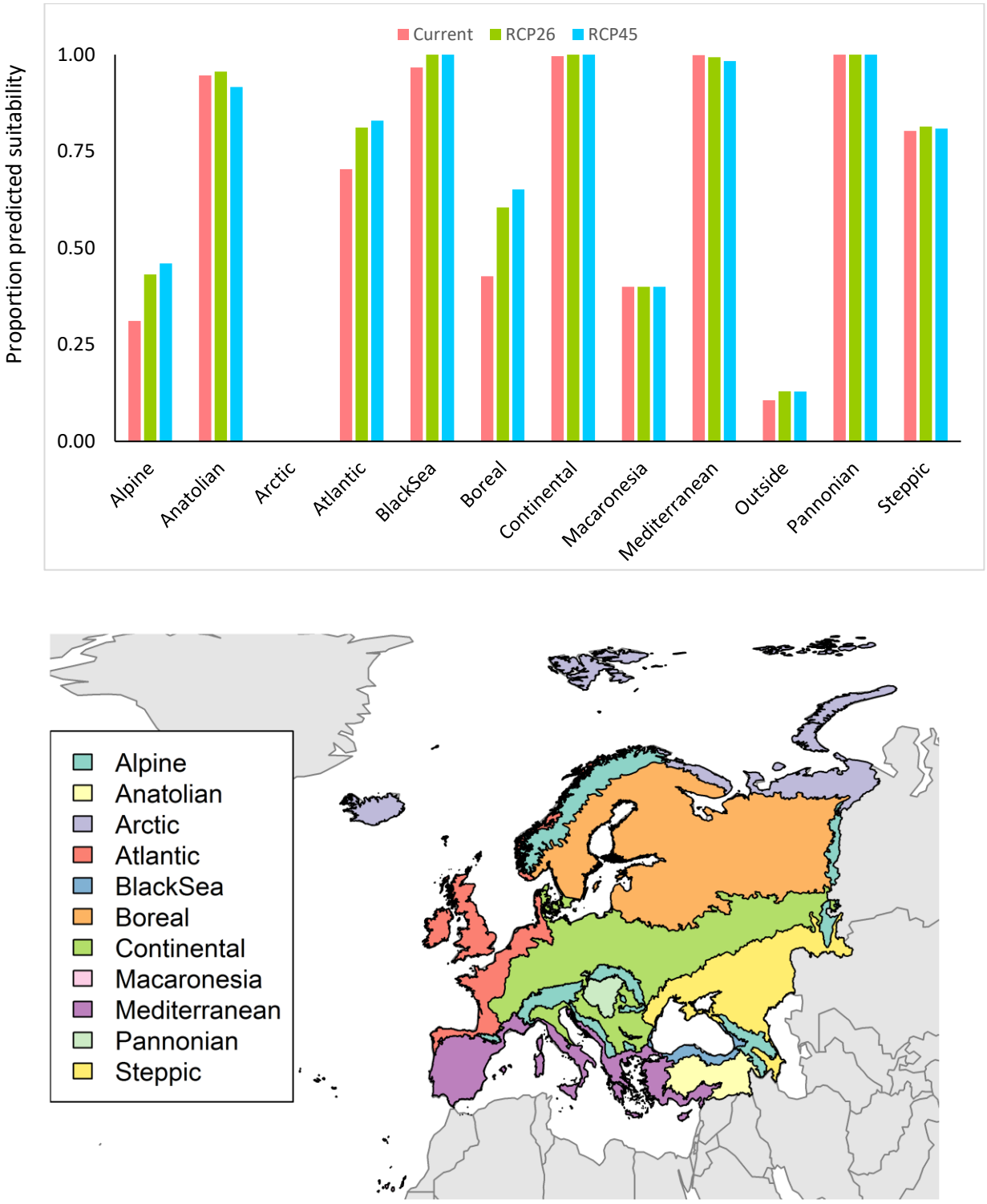


Figure 2.9: Variation in projected suitability for *Harmonia axyridis* establishment among Biogeographical regions of Europe (Bundesamt für Naturschutz (BfN), 2003). The bar plots show the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios, RCP2.6 and RCP 4.5. The location of each region is also shown.

Europe appears to be the continent with countries that have a higher proportion of predicted suitability for the establishment of *H. axyridis* in the current climate as well in the future scenarios. Increases are predicted across the majority of the continent with just a small number of decreased suitability in the very far south of Europe (Figure A2.3a). Predictions for South America and the majority of North American countries are almost the reverse of Europe with most countries predicted to become less suitable for the establishment of *H. axyridis* under future scenarios with the exception of Canada, Chile and Peru. These countries have a low proportion of suitable grid cells currently and the predicted future increases are also low (Figure A2.3b). Africa is similar to the Americas in that many countries have a low proportion of suitable grid cells in the current scenario and the majority are predicted to be less suitable under future scenarios (Figure A2.3c). Asia also has a high number of countries with a low proportion of grid cells that are predicted to be suitable for the establishment of *H. axyridis*. However, under future scenarios, countries in Asia, mainly in the north and west, are predicted to have an increase in suitability while countries in the south and east are predicted to decrease in suitability (Figure A2.3d).

2.4 Discussion

2.4.1 Global

The approaches to modelling adopted here highlight that human influence had the greatest impact on the global spread of *H. axyridis*. Additionally, this approach indicated that it is the interaction of a suite of variables that facilitate the spread of *H. axyridis,* with Bio10 (mean temperature of the warmest quarter), CMI (Climatic Moisture Index) and Bio6 (minimum temperature of the coldest month) all playing a role in various global regions. There is a considerable body of evidence concluding that urbanisation and an increase in anthropogenic areas positively contribute to the spread and establishment of *H. axyridis* (Adriaens *et al*., 2008; Brown *et al*., 2008; Brown *et al*., 2011a; Purse *et al*., 2014; ; Roy *et al*., 2016; Veran *et al*., 2016; Viglášová *et al*., 2017; Brown & Roy, 2017). Globally, it is apparent that the regions suitable in the current climate for *H. axyridis* tend to have high levels of anthropogenic influence. Intensification of agricultural practices (Honěk *et al*., 2016) and increased industrialisation (Poutsma *et al*., 2008) have been attributed to an increase in the occurrence of *H. axyridis* in recent years, which is particularly evident in Europe and North America. When investigating the spread of *H. axyridis* in France, Veran *et al*. (2016) reported that increased land area under agriculture and vines was a factor in the dispersal of the species. Furthermore, Pons *et al*. (2015) reported concerns for Spain's vineyards which amounts to a large percentage of Europe's grape and wine production (Ameixa *et al*., 2019). Africa is a large continent with a range of limiting factors (Figure 2.6; CMI, Bio6 & HII) preventing the establishment and spread of *H. axyridis*. The species has established in South Africa, Lesotho (Brown *et al*., 2011b) and Swaziland (Roy *et al*., 2016) with a potential establishment in Algeria (Lakhal *et al*., 2018). There are individual records of *H. axyridis* from Kenya (Nedvěd *et al*., 2011) and Zanzibar, Tanzania (Nedvěd & Halva, 2016) but as of yet there is no confirmation of establishment of it in these countries. The global model in this study predicted that the inland areas of these countries would be suitable for *H. axyridis* but it is likely that high winter temperatures indicative of the tropical climate would limit its establishment (Nedvěd & Halva, 2016).

2.4.2 Europe

This model indicated that southern Europe would be suitable for the establishment of *H. axyridis,* as did Poutsma *et al*. (2008). Unlike much of the rest of the northern part of the globe (i.e. Canada and Russia) human influence was not considered the strongest limiting factor in Europe, but instead it was Bio10 (mean temperature of the warmest quarter). However, in southern Europe Bio6 (mean temperature of the coldest month) and CMI (climatic moisture index) were the limiting factors for the establishment of *H. axyridis*. The optimum temperature for *H. axyridis* is reported to be 20^{o}C (Barahona-Segovia *et al*., 2016) so it is not surprising that western and northern Europe would have

different limiting factors. If the mean temperature of the warmest quarter increased sharply, say by 10°C, then this may have a negative impact on *H. axyridis* abundance in these regions (Veran *et al*., 2016).

When investigating the spread of *H. axyridis* in France, Veran *et al*. (2016) predicted that *H. axyridis* would have difficulty establishing and spreading in southern France as well as Spain, Portugal and Greece. Occurrences of *H. axyridis* are particularly low in southern Europe to the extent that it does not affect coccinellid diversity (Soares *et al*., 2017), indicating that other factors or the species' interaction with climatic and/or environmental variables are involved in *H. axyridis* not establishing in the Azores, much of the Iberian Peninsula and Greece. When investigating why *H. axyridis* had not established in the Azores, Soares *et al*. (2017) concluded prey availability to be the main cause of this phenomena which is not surprising given that aphids favour a temperate climate. Aphid colonies develop on young plants and numbers can increase rapidly to create very large populations in temperate areas. However, in southern Europe the host plants differ and the climate is drier, leading to less substantial food sources for aphids (Poutsma *et al*., 2008). Furthermore, the coccinellid community in Portugal is generally dominated by smaller coccinellids such as *Scymnus* spp., which are more often predators of coccids than aphids (Magro & Hemptinne, 1999), indicating that aphids may not always be the most abundant food source in these regions.

Another explanation for the non-establishment of *H. axyridis* in southern Europe could be the warm winter conditions in these regions. *Harmonia axyridis* needs to enter diapause in the winter and, with reduced prey, will exhaust fat reserves before the establishment of new aphid colonies in the spring (Poutsma *et al*., 2008). Alaniz *et al*. (2020) recently reported that *H. axyridis* had not established in the Azores, Portugal, due to high winter temperatures which prevent the necessary diapause for this species. Recently, however, a population of *H. axyridis* was recorded in north-eastern Spain with concerns that this species will continue to spread and impact on the economy through damage to vineyards (Pons *et al*., 2015; Ameixa *et al*., 2019). Subsequently, Ameixa *et al*. (2019) reported that high temperatures had a positive effect on *H. axyridis*. However, Ameixa *et al*. (2019) did not state how high these temperatures are or what the upper limit may be for *H. axyridis*. In contrast, this current study predicted that those areas in southern Europe currently suitable will, with climate change, become more unsuitable over time and Veran *et al*. (2016) reported that extreme high (and low) temperatures had a negative effect on *H. axyridis*. Additionally, *H. axyridis* was reported to function optimally at a temperature range of 15°C to 20°C with an upper limit of 27.2°C and has a significantly reduced survival rate at 30°C (Barahona-Segovia *et al*., 2016).

2.4.3 UK and Ireland

Both the UK and Ireland have the same limiting factors to the establishment of *H. axyridis* (Bio10). The UK has been heavily industrialised for two centuries and an extensive road & rail network is evident. *Harmonia axyridis* is not often recorded in Scotland and is thought to be limited due to there being fewer urban areas than England (Purse *et al*., 2014) as well as a less favourable climate (Majerus *et al*., 2016). The future scenarios, however, predict that suitability will increase in Scotland, Northern Ireland and Wales as well as Ireland. It was predicted that *H. axyridis* would arrive in Ireland (Brown *et al*., 2008) and the first records were observed in Cork and Carlow in 2010. However, *H. axyridis* has spread at a considerably slower rate in Ireland in comparison to rates in Europe and the UK (Weyman *et al*., 2019) which could be partly due to less human influence than in the UK. Additionally, temperatures also tend to be lower in Ireland and Scotland in comparison to England while precipitation tends to be higher. These aspects of decreased anthropogenic disturbance and a less hospitable climate are likely to explain the currently slow spread of *H. axyridis* in Ireland and limited distribution in Scotland (Roy & Brown, 2015). If the climate changes as predicted over the next 50 years, however, these Irish and Scottish habitats will be vulnerable to further spread of *H. axyridis*.

2.4.4 Marginal areas

Harmonia axyridis is recently considered established in Turkey (Bukejs & Telnov, 2014) and Iran, whilst occurrences have also been recorded in Israel and Saudi Arabia (Biranvand *et al*., 2019; Mardani-Talaee *et al*., 2019). The Turkish records are either from areas with a considerable amount of anthropogenic disturbance or central areas of higher elevation, meaning cooler winters. The Iranian records are from an area with anthropogenic disturbance in the form a particularly busy port. It is likely that these recent establishments are due to a combination of increased human influence together with *H. axyridis* becoming more tolerant of higher temperatures to the norm. How likely is it that *H. axyridis* will continue to spread and establish in these countries, particularly under climate change scenarios RCP2.6 and RCP4.5? As previously mentioned, *H. axyridis* can tolerate higher temperatures but is likely to struggle during warm winters (Poutsma *et al*., 2008; Barahon-Segovia *et al*., 2016; Alaniz *et al*., 2020). It is predicted that this species will find these areas even less suitable under climate change scenarios RCP2.6 and RCP4.5 unless suitable areas at higher elevation are available during the winter months to facilitate diapause. Barahon-Segovia *et al*. (2016) reported that *H. axyridis* (as an IAS) species performed better at lower temperatures than other coccinellid species (as native species) indicating, higher temperatures may not be of benefit to *H. axyridis*. Additionally, Logan *et al*. (2019) reported that when faced with climate change, *H. axyridis* has low evolutionary potential, leading to the conclusion that this species may not spread

or remain in the Middle East, particularly should temperatures increase in this area. Coleoptera (beetles) have previously been shown to adapt to different thermal scenarios (extreme cold or extreme heat) through phenotypic plasticity. The reproductive organs of both male and female beetles have been shown to adapt to these scenarios, particularly extreme heat, and remain capable of reproducing (Vasudeva *et al*., 2014; Farrow, 2016). Barahona-Segovia *et al*. (2016) also reported *H. axyridis* ability to survive at higher temperatures (30^{o}C), although survival rate was greatly reduced from optimum temperatures (20^{o}C). Furthermore, these studies were carried out under laboratory conditions at constant temperatures which do not represent natural conditions or take into account that individuals can move to avoid adverse conditions.

2.4.5 The importance of Citizen Science data

With an increase in public interest in recording wildlife and the advent of smartphones and improved accuracy through GPS, citizen science data is reliable and generates sizeable datasets that would not previously have been possible to acquire (Roy *et al*., 2018). This study would not have been possible without the efforts of thousands of people, yet there are still gaps in the data. Citizen science relies on people to upload their records to platforms, such as GBIF, and gaps in the data become evident when reviewing the literature on a species. For example, the records from countries the Middle East, particularly Turkey and Iran (Birnavand *et al*., 2019) were not recorded on the databases utilised in searches and so were unintentionally omitted from analysis. From the models here, Turkey is a predicted suitable area where *H. axyridis* could establish so its presence is unsurprising and the area in Iran where a large number were observed is also predicted suitable under the current climate. Under the different climate change scenarios, small areas of Turkey are predicted to become unsuitable for *H. axyridis*, however this species could establish and remain in other parts of this country that have suitable climate (e.g. suitable winter temperatures to facilitate diapause).

Just recently, *H. axyridis* was discovered in Costa Rica, Guatemala, Honduras, Panama and Puerto Rico solely as a result of the efforts of citizen scientists (Hiller & Haelewaters, 2019). As climate changes, *H. axyridis* is likely to be recorded in new countries and regions and an increased network of citizen scientists can help track these movements. Encouragement of data recording by citizen scientists globally is recommended, not just to observe *H. axyridis* but also to gain a more detailed account of newly arrived and/or potential invaders. As humans continue to increase global movement due to trade and travel, the risk of a new invasive alien species will only increase.

2.5 Conclusion

This research has illustrated that human influence is the most important factor globally for facilitating the spread and establishment of *H. axyridis*. However upon closer inspection, it is clear that a single factor alone cannot determine if *H. axyridis* (or any other potential IAS) may establish in a region or not and that it is a combination of factors, climatic, environmental and/or biological, that are necessary to work together to make a region suitable for this prolific coccinellid. Once established, *H. axyridis* facilitated by anthropogenic disturbance and climate change will affect native coccinellid biodiversity, by changing the community dynamics as it behaves both as a passenger and driver of change. The inclusion of records from recently established populations of *H. axyridis* would make the models more robust and give a more comprehensive overview of the future spread of *H. axyridis*.

3 *Harmonia axyridis* in rural woodlands in England

3.1 Introduction

3.1.1 Coccinellids in rural habitat

Research concerning coccinellids often tends to focus on a small number of species (Sloggett, 2005) including *Harmonia axyridis, Coccinella septempunctata, Adalia bipunctata, Hippodamia convergens* or *Coleomegilla maculata*, many of which focus on the species as an IAS. As a result, specialist or less frequently recorded native coccinellid species are often overlooked. Even though the coccinellid species above provide ecosystem services in the form of pest control in agricultural landscapes, so too do other native coccinellid species (Roy *et al*., 2012; Honěk *et al*., 2017). It is likely that these services are strengthened by the presence of less managed areas (wild herb, grassland or woodland) adjacent to crop landscapes (Woltz & Landis, 2014). Additionally, trees and grassland tend to have a more diverse coccinellid community than crops (Honěk, 2012). Many studies investigating coccinellid assemblages or diversity appear to concentrate on urban green spaces (Brown *et al*., 2011a; Viglášová *et al*., 2017), urban woodlands, or crop-only systems such as alfalfa (Grez *et al*., 2008; Grez *et al*., 2014), cereals or canola (Bianchi *et al*., 2007) with only a small number focussing on natural landscape adjacent to cropped fields (Amaral *et al*., 2015). It has been suggested that an ongoing increase of *H. axyridis* numbers may lead to the extinction of coccinellid species locally (Adriaens *et al*., 2010; Comont *et al*., 2014). In the UK, distribution of *H. axyridis* is well known within urban and other anthropogenic habitats, but much less is known on detailed habitat use in the wider countryside (Brown *et al*., 2011a; Brown & Roy, 2017). Considering the aforementioned studies together with the decline of native coccinellids in urban areas as a result of *H. axyridis* in the UK, it is important to understand how native coccinellids are faring in rural areas (Viglášová *et al*., 2017). In terms of the distinction between rural and urban areas, there is no specific definition of rural area as the boundary between urban and rural is often unclear (JNCC, 2010). There are governmental definitions (UK), however, these relate to human population size within an area and are not suitable for the purposes here. In this chapter (and Chapter 4), the term rural is used to describe the habitats surveyed that were based in woodland areas that were not within a town or city. In contrast, urban areas describe wooded areas or tree stands within towns.

3.1.2 Coccinellid communities

In any habitat, a small number of dominant species (between two and four) are expected to comprise around 90% of the coccinellid community. Selyemová *et al*. (2007) reported a diverse coccinellid community in rural coniferous woodland in Slovakia that was dominated by four species, however, *H. axyridis* was not established in the region at the time. When investigating overwintering coccinellids in coniferous woodland, Holecová *et al*. (2018) reported that *H. axyridis*

was not the top dominant coccinellid. It has been suggested that some native coccinellids tend to be more abundant in rural habitats (with less anthropogenic disturbance) as opposed to *H. axyridis*, which prefers habitats altered by human activity (Roy *et al*., 2016). In the UK, just as *H. axyridis* was establishing, Brown *et al.* (2011a) reported that *H. axyridis* was largely absent from coniferous woodland. Furthermore, Purse *et al.* (2014) predicted that areas with less anthropogenic disturbance, particularly coniferous woodland, could be a refuge for native coccinellids.

3.1.3 Vegetation structure

Vegetation structure of a habitat can also influence coccinellid assemblages. Grassland has been shown to be a refuge for native coccinellid species with very few invasive coccinellids recorded in this habitat (Diepenbrock & Finke, 2013). Rural woodland generally consists of a range of tree species and areas of wild herbs/grassland. The heterogeneity of a habitat is associated with increased animal species diversity (Tews *et al*., 2004) as well as population stability in butterflies (Oliver *et al*., 2010). In Michigan (USA) coccinellid species richness was higher when the habitat was more complex and contained a range of vegetation structures from deciduous trees, to grassland and crops (Colunga-Garcia *et al*., 1997). When non-crop vegetation was added to an agricultural habitat, coccinellid activity increased (Woltz & Landis, 2014) and intraguild predation between a native coccinellid and *H. axyridis* decreased (Amaral *et al*., 2015). Additionally, Honěk (2012) reported that trees facilitate greater coccinellid diversity than herbaceous stands which in turn have greater coccinellid diversity than crops.

3.1.4 Prey and competitors associated with coccinellids

Beginning to understand how and why certain habitats are used by particular coccinellid species would be beneficial to understanding the relationship between *H. axyridis* and native specialist coccinellid species (Sloggett & Majerus, 2000). Competition for food resources from *H. axyridis* is one of the reasons why native coccinellids may be negatively affected (Brown *et al*., 2011a). However, Vandereycken *et al*. (2013) found that *H. axyridis* was the dominant predator on corn but not on other crops and so monitoring aphid abundance adds another dimension to studies of coccinellid community dynamics. Lacewings (Neuroptera) and ants (Formicidae) also interact strongly with aphids and so sit in the same guild as coccinellids. Lacewings are aphid predators and have been found in greater abundance than *H. axyridis* on crops (Vandereycken *et al*., 2013). Some ants tend aphids for their honeydew and if coccinellids attempt to prey on these aphids, the ants will protect the aphids by attacking the coccinellids (Lucas, 2012). Investigating these guild relationships is important in determining whether or not *H. axyridis* is negatively affecting these additional taxonomic groups.

3.1.5 Aims & Hypotheses

The aim of the research presented in this chapter was to explore the relationship between the invasive *H. axyridis* and native coccinellid species in rural, non-anthropogenic habitats. With the wealth of literature on *H. axyridis* in urban areas, a comparison between urban and rural habitat was also carried out to illustrate habitat preferences.

Considering the information presented above, the following hypotheses were postulated:

- Given previous findings of low IAS abundance in rural habitat, the proportion of *H. axyridis* is expected to be lower in woodlands than in urban sites and the proportion of native specialist coccinellids is expected to be higher in woodlands than in urban sites.
- Vegetation structure is expected to have an effect on the coccinellid communities, with the trees hosting a greater abundance and diversity of native coccinellids than grassland.
- A relationship between the abundance of coccinellids and their competitors as well as between coccinellids and their prey is expected.

3.2 Method

3.2.1 Field Sites

Field sites were identified based on the presence of enough native tree species and individuals to facilitate robust data collection as well as on proximity to each other. All sites were in Cambridgeshire, Suffolk or Lincolnshire. During the 2016 field season, four deciduous sites (Brampton Wood, Monk's Wood, Raveley Wood and Wistow Wood) and two coniferous sites (two sites at King's Forest) were sampled. Two of the deciduous sites were similar in structure (ancient woodland) and were yielding low numbers of coccinellids. One of these woodlands had fewer individuals of the required tree species and so for the 2017 fieldwork, Wistow Wood was removed from the site list and an additional coniferous site at King's Forest was included. Furthermore, as urban areas have previously been shown to have high numbers of *H. axyridis*, two urban sites (Doddington and Spalding) were added in 2017 to enable meaningful comparison with the rural sites. Thus, during the 2017 field season three deciduous woodlands (Brampton Wood, Monk's Wood and Raveley Wood) and three coniferous woodland (three sites at King's Forest) were surveyed (*Table 3.1*). Surveys took place from May to October inclusive. Brampton and Raveley Woods are managed by the Wildlife Trust for Bedfordshire, Cambridgeshire and Northamptonshire, while Monk's Wood is managed by Natural England. The three sites at King's Forest are managed by the Forestry Commission. The urban site at Doddington is managed by the Church of England Diocese of Ely while the site at Spalding is managed by the South Holland District Council. Maps for the site locations can be found in Appendix 3, (Figures A3.1a, b & c). Written permission from each relevant organisation was acquired prior to any surveys being undertaken. Grid references were recorded using a Garmin GPSmap 60CSx.

Table 3.1: Locations and characteristics for each field site surveyed.

Site	Woodland Type	Grid References	County	2016 Surveys	2017 Surveys
Brampton Wood	Deciduous	TL1787 7018	Cambridgeshire	√	√
Monk's Wood	Deciduous	TL1976 8011	Cambridgeshire	√	√
Raveley Wood	Deciduous	TL2444 8184	Cambridgeshire	√	√
Wistow Wood	Deciduous	TL2963 8214	Cambridgeshire	√	X
King's Forest 01	Coniferous	TL8223 7374	Suffolk	√	√
King's Forest 02	Coniferous	TL8201 7417	Suffolk	√	√
King's Forest 03	Coniferous	TL8088 7153	Suffolk	X	√
Doddington	Urban deciduous	TL4005 9069	Cambridgeshire	X	√
Spalding	Urban deciduous	TF2474 2205	Lincolnshire	X	√

3.2.2 Vegetation layers/structure and survey method

Three vegetation layers were selected for data collection; tree, shrub and herb layer. These layers encompass the key different vegetation types found within a woodland and collectively contain the majority of UK ladybird species (Roy *et al*., 2013). The tree and shrub species selected for surveying are all native to the UK. Additionally, the number of individuals of each tree/shrub species was sufficient to allow regular visits during the sampling season at the respective woodland sites as well as to avoid over-sampling or damage to vegetation. As urban sites were included for comparison purposes, only the tree layer was sampled, predominantly lime (*Tilia x europaea*) and a small number of sycamore (*Acer pseudoplatanus*).

- The herb layer (grassland/grass layer) comprised low vegetation including grasses, wildflowers, thistle, bramble, saplings etc. Vegetation height in the grass margins did not exceed one metre in height.
- The shrub layer (intermediate layer) comprised of species that as mature individuals are considered part of the shrub layer (Hall *et al*., 2004) or are immature individuals of a tree species. Individuals used for surveying were no higher than three metres. The species selected for data collection were hazel (*Corylus avellana*) and hawthorn (*Crataegus monogyna*) in deciduous woodland and immature Scots Pine (*Pinus sylvestris*) and birch (*Betula pendula*) in coniferous woodland.

- The tree layer (mature layer) consisted of trees that were over three metres high with the target species being oak (*Quercus robur*) and field maple (*Acer campestre*) in deciduous woodland and mature Scots pine (*Pinus sylvestris*) and silver birch (*Betula pendula*) in coniferous woodland. The tree and shrub layer are on occasion referred to collectively as woodland and the grass layer referred to as grassland.

Sweep-netting is a common method for surveying insects in grassland (Ausden & Drake, 2006) and was used to sample coccinellids. This method involved the use of a sweep net which comprised of a white canvas bag (46cm in diameter) attached to a metal ring on a large pole. One sweep was carried out for one metre of distance walked with 100 metres of grassland per visit being surveyed at deciduous and coniferous woodland sites only. Sweeping this area took approximately 25 minutes. An estimate of the percentage plant coverage of the grass margin was determined by eye at each sampling point.

Tree beating was used to collect ladybirds from the tree and shrub layers. This method involved using a stick (approximately 1.5 metres in length) to sharply tap tree branches whereby the animals upon the branch fall onto a large white beating tray (110cm x 86cm) so that individuals could be identified (Roy *et al*., 2013). Three individual branches on each tree were sampled by tapping each branch three times in quick succession. Depending on accessibility, each survey was carried out around the full circumference of the tree. Ten trees of each species in both the intermediate and mature layers were surveyed in deciduous and coniferous woodland. At each urban site, 20 mature trees were surveyed. Completion of surveying 10 trees within one gradient took approximately 25 minutes.

All captured coccinellids were identified to species level in the field with the aid of two Field Studies Council (FSC) guides: Guide to ladybirds of the British Isles (Majerus *et al*., 2010) and Guide to the ladybird larvae of the British Isles (Brown *et al*., 2012). Where identification proved difficult or time consuming, detailed photographs of the specimen were taken for later identification and the individual subsequently released. Larvae in the early stages of development, especially first and second instar, are very difficult to identify to species level in the field and so where there was uncertainty the term 'Early Stage Larva' (ESL) was used. Additionally, third instar *Harmonia* spp. larvae are included in the ESL group due to the similarity between *H. axyridis* and *H. quadripunctata* at this life stage. Just over six percent of records were of coccinellid larvae that were not identifiable due to their early life stage (ESL) (Appendix A3.1 & A3.2). These records were removed prior to statistical analysis as the specific species is often unknown and so it would be impossible to draw meaningful conclusions from such analysis.

3.2.3 Prey and competitors associated with coccinellids

The number of aphids (adult and immature) captured during sweeping/tree beating were also recorded. Due to potentially very large numbers being present these numbers were estimated in increments of 5 (for example, 1, 5, 10, 15, 20, 25, etc.). Aphids (Aphidoidea) were identified to superfamily. Lacewing numbers were also noted with two families being identified in the field; Chrysopidae & Hemerobiidae but are grouped for analysis purposes. Ants (Formicidae) that were captured during sweeping/tree beating were also recorded and identified to family level. Aphidoidea, Chrysopidae & Hemerobiidae and Formicidae are referred to as aphids, lacewings and ants respectively from this point forward. Additionally, when referred to as a guild group, aphids, lacewings and ants are referred to as associated insects for clarification in Sections 1.3 and 1.4.

3.2.4 Weather conditions

In order to standardise data collection sampling took place between 10:00 and 16:00 when weather conditions were favourable, i.e. when the temperature was greater than 14°C, conditions were dry and wind speeds were below 5 on the Beaufort scale (Jones *et al*., 2006). Some surveys were carried out when the temperature was below 14^{o}C, however in such instances there was at least 60% sun. Humidity and ambient temperature were recorded using an EasyLog EL-21CFR-2-LCD. Any gaps in the temperature/humidity data were provided by the Met Office.

3.2.5 Data analysis

The majority of the analyses was carried out using R Studio (R Core Team, 2019). The following packages were used for basic analyses and visualisation of data: dplyr (Wickam *et al*., 2019), ggfortify (Horikoshi & Tang, 2016; Tang *et al*., 2016), ggplot2 (Wickham, 2016), ggpubr (Kassambara, 2018). For multivariate analyses three packages were used: lattice (Sarkar, 2008) and vegan (Oksanen *et al*., 2019). The remaining packages used for regression analyses were: fmsb (Nakazawa, 2018), lmtest (Zeileis, 2002), pscl (Zeileis *et al*., 2008), sandwich, (Zeileis, 2004; Zeileis, 2006), lattice and MASS (Venables & Ripley, 2002). As the data were count data, non-parametric tests and generalised linear models (GLM) were applied to the data. Wilcoxon paired tests were used to compare abundances of different coccinellid groups at the same locations, e.g. urban areas. Spearman's correlation was utilised to investigate any association between both *H. axyridis* and native coccinellid abundance and that of aphids, lacewings and ants.

3.2.5.1 Regression models

Generalised linear models (GLM) were utilised to investigate the effects of site location (urban, rural), site type (deciduous, coniferous), vegetation structure (tree, herb or grass layer) and season (summer, autumn) on coccinellid and associated insect abundance. Environmental variables (temperature, humidity) were included in the models. When applying a GLM to count data, the results can often be overdispersed. Overdispersion happens for various reasons with the most common being excess zeros in the data (Beckerman *et al*., 2017). In the case of these data, overdispersion was common and so alternative regression models were applied to the data and a subsequent model selection carried out to determine which was the best fit, if any.

The regression models (poisson, negative binomial (NB), zero-inflated poisson (ZIP) model and zero-inflated negative binomial regression (ZINB) model) were applied to the data. The zero-inflated models treat the zeros differently, either as true or false zeros (Zuur *et al*., 2012). True zeros occur because the habitat is not favoured by the organisms in question, for example, if winters are too harsh. False zeros on the other hand are when an individual was present but not recorded due to survey design or observer error. It is recommended that if a count dataset consists of true and false zeros then zero-inflated regression models should be applied (Zuur *et al*., 2012). Additionally, these models help to explain and clarify the ecology behind the numbers. Zero-inflated models can run using a poisson or negative binomial distribution. A zero-inflated model is essentially two models run at the same time, the count model (models the count data) and the binary model (models the zeros). Both parts of the model are fitted simultaneously and are modelled in terms of the explanatory variables (Zuur *et al*., 2007).

In many cases a zero-inflated negative binomial (ZINB) model was the best fit for the data, however there were cases where the data were overdispersed, but not as a result of the zeros. When a ZINB was not the best fit, a negative binomial regression model was applied to the data. All models were compared to the null model, and reduced models compared with the full model. There are several methods to determine which is the best model to choose (e.g. Akaike Information Criterion, Bayesian Information Criterion) where the model with the lowest value is considered the best (Zuur *et al*., 2009; Beaujean & Morgan, 2016). Justification for the model choices was based on log likelihood, AIC and weighted AIC (see Appendix 3 for full details). Table A3.3 presents which model was the best fit for explaining the effects of the variables on *H. axyridis*, native coccinellid, Aphidoidea, Formicidae and Neuroptera abundance in urban trees, rural woodlands and rural grasslands. Urban data were analysed separately as well as in comparison to rural woodland (tree and shrub layer) data. Data from woodlands were analysed separately to the grassland data due to differences in sampling method. Starting with all variables in the model, a step-wise process was used to determine which variables had an impact on the dependent variable. Any variables

resulting in a p-value of less than 0.2 were removed from the model. The z-statistic is used in these regression models as the variance is known, unlike in Gaussian models where the variance is estimated resulting in a t-statistic (Zuur *et al*., 2009).

Collinearity can be an issue in regression models (Zuur *et al*., 2007). Collinearity is when independent variables can be highly correlated. Temperature and humidity were checked for collinearity with a variance inflation factor (VIF). Neither variables were of concern with a VIF of < 1.2 each, and both were incorporated into the regression models.

3.2.5.2 Diversity Indices

Shannon Diversity was calculated for rural sites only and for native coccinellid species only. Simpson's diversity was not carried out as rare species or those recorded in low numbers are not taken into account by this measure (Magurran, 2004; Morris *et al*., 2014). Differences in diversity across sites types and season were calculated using t-tests while ANOVA was used to assess any differences in diversity within the vegetation structure followed by a post-hoc Tukey if any significances were apparent. Regression models were run to determine if there was a relationship between native coccinellid diversity and the abundance of *H. axyridis*.

3.2.5.3 Ordination

Canonical Correspondence Analysis (CCA) detects patterns of variation in a given community that can be explained by environmental data. The analysis focuses on beta-diversity (how dissimilar sites are) instead of alpha diversity (diversity of a site) (Zuur *et al*., 2007). This method of multivariate analysis generates an ordination diagram where a given species point is at the weighted average or centroid of the sites where it was recorded (ter Braak & Verdonschot, 1995). The qualitative environmental variables (site type and vegetation layer) are illustrated by a point that is the centroid of site points belonging to that group, for example the weighted average of the tree layer where the weight is the total abundance of the tree layer (ter Braak & Verdonschot, 1995). This analysis was used to investigate the relationship that site type (deciduous or coniferous) and vegetation layer (tree, shrub or herb) had on the coccinellid assemblage. The coccinellid data were fourth root transformed to remove any effect of highly abundant species (Chessman, 2003; Pickwell, 2012). Interpretation of the resulting ordination is based on the eigenvalues, statistical significance determined by Monte Carlo permutation test and ecological interpretability (ter Braak & Verdonschot, 1995). In this case, the biplot rule (described below) was applied as the eigenvalues were less than 0.4 and this rule is more informative than the centroid rule when eigenvalues are

low (ter Braak & Verdonschot, 1995). To interpret the relationship between the species and sites, the biplot rule was used. Firstly, the direction of maximum change in the relative abundance of a species (e.g. species X or Y) was determined by drawing a line from Species X to the origin. Subsequently the sites were then projected onto the arrow for Species X, illustrating the share each site (site A or B) has in the total abundance of each species (ter Braak & Verdonschot, 1995; Zuur *et al*., 2007). To interpret how a species relates to an environmental variable, imagine the variable line (e.g. 'Type') is extended in the opposite direction for the same distance, forming an axis of its own. Each species can be projected perpendicular to the axis, indicating the species relationship with that variable (Zuur *et al*., 2007). This analysis was carried out in PAleontological Statistics (PAST) Version 3.23 (Hammer *et al*., 2001). The combination of regression models, the Shannon diversity index and the ordination analysis yielded a detailed representation of coccinellid assemblages.

3.3 Results

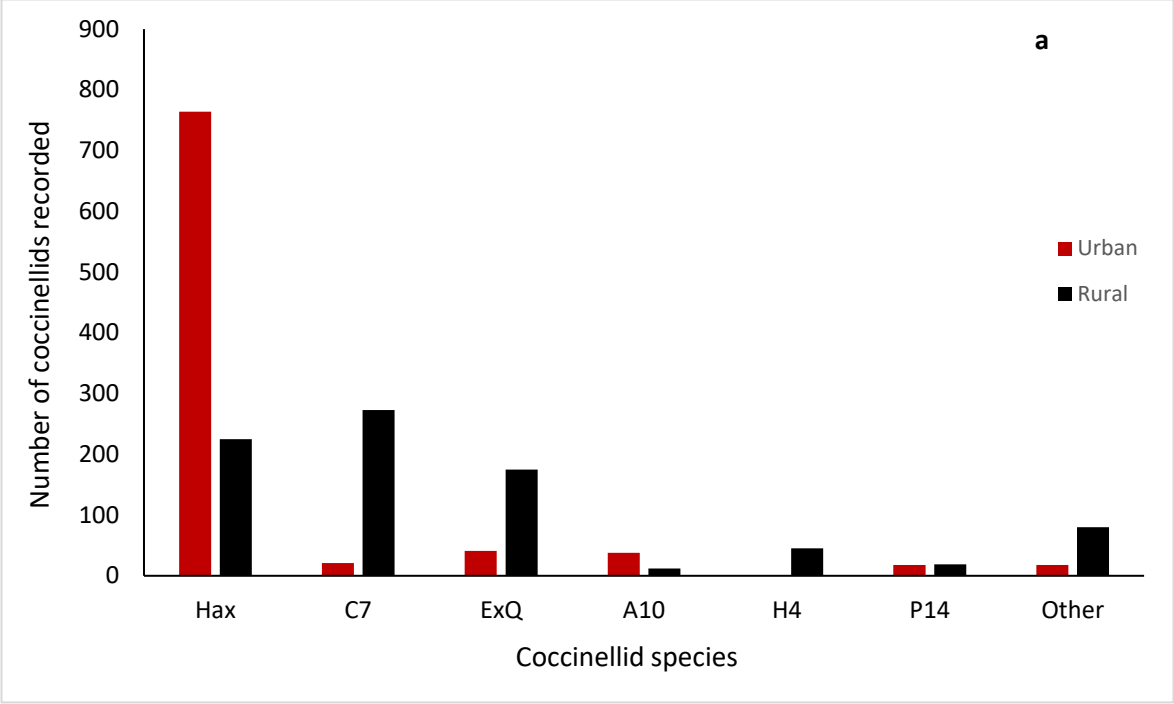
3.3.1 Overview

Eighteen species of coccinellid totalling 2,278 individuals were recorded during the study period across three different vegetation gradients (mature, intermediate, grass) from three distinct site types (deciduous, coniferous, urban). Figure 3.1a & Figure 3.1b illustrates the six most frequently recorded coccinellids on trees and on grassland respectively. Just eight of these species were recorded in grassland in comparison to 16 species on trees in woodland or urban sites (Appendix A3.1 & A3.2). Five species were recorded in coniferous woodland only (*Myzia oblongoguttata, Myrrha octodecimguttata*, *Scymnus suturalis*, *Subcoccinella vigintiquattuorpunctata* and *Tytthaspis sedecimpunctata*), while one species was recorded in deciduous woodland only (*Psyllobora vigintiduopunctata*) and at urban sites only (*Aphidecta obliterata*). In grassland, four coccinellid species were recorded at coniferous sites only (*Exochomus quadripustulatus*, *S. suturalis*, *S. vigintiquattuorpunctata* and *T. sedecimpunctata*). Considering all site types (urban, deciduous, coniferous), the majority of all coccinellids were recorded at rural sites with 20% at deciduous and 38% at coniferous sites while species richness was lower at urban sites (10 spp.) than deciduous (12 spp.) and coniferous (16 spp.) sites.

3.3.2 Comparison of urban and rural habitat

In both urban and rural sites, 1873 individual coccinellids were recorded in trees, with, 50.6% of these records recorded from two urban sites which consisted solely of mature trees. Of the remaining 49.4% of records, *C. septempunctata* was the most recorded coccinellid in woodlands (29.5%) with *H. axyridis* comprising 24.3% of records and *E. quadripustulatus* making up 19.0% of records. The fourth most abundant group was 'Other' which consists of individuals from 12 other coccinellid species, making up 8.6% of the coccinellids recorded in rural areas (Figure 3.1a).

The abundance of *H. axyridis* (median = 35) was significantly higher than that of native coccinellids (median = 5.5) in urban areas (Wilcoxon test: $Z = -3.9$, $p < 0.001$, $r = 0.87$). However, the reverse was shown in rural areas with significantly greater numbers of native coccinellids (median = 7) recorded ($Z = -5.57$, $p < 0.001$, $r = .63$) than *H. axyridis* (median = 1) (Figure 3.2). As revealed by the count model of the ZINB, *H. axyridis* was recorded in significantly greater numbers in urban areas than rural areas ($z = 12.52$, $p < 0.001$) (Figure 3.2). The abundance of *H. axyridis* did not differ between the seasons at urban sites. Native coccinellid abundance was not affected by whether the site was situated in an urban or rural habitat (Figure 3.2).



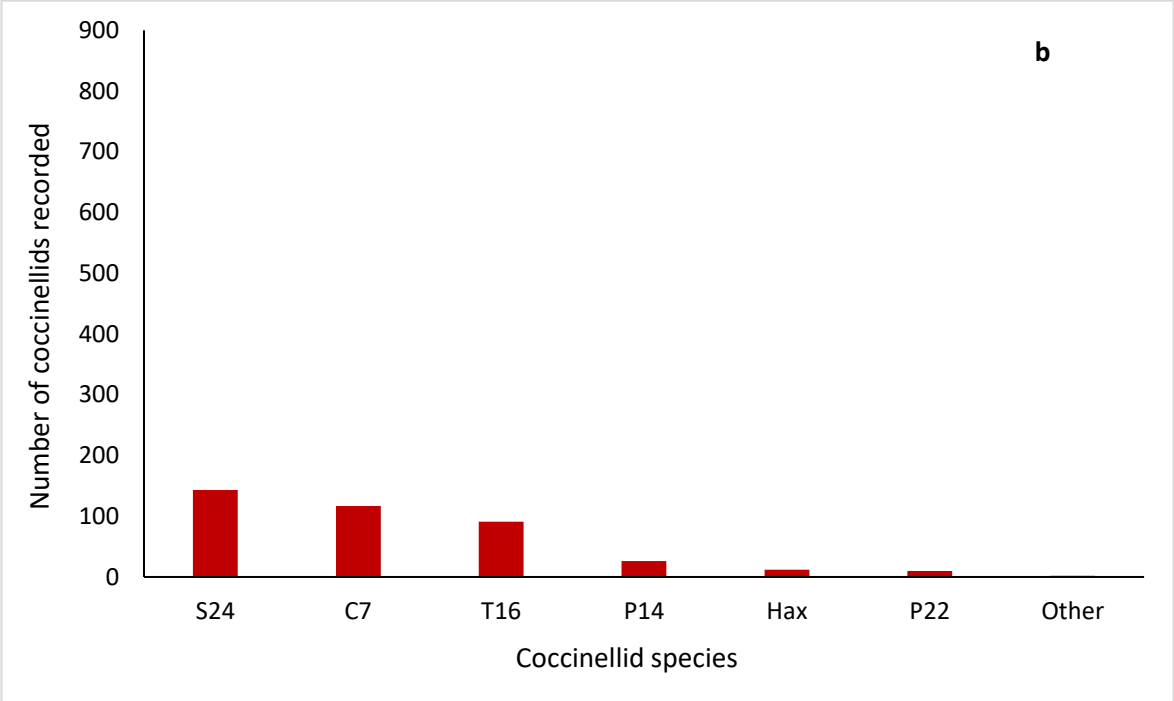


Figure 3.1: Number of coccinellids recorded in woodland (a) and grassland (b) for the six most abundant species; A10 = *Adalia decempunctata*; C7 = *Coccinella septempunctata*; ExQ = *Exochomus quadripustulatus*; Hax = *Harmonia axyridis*; H4 = *H. quadripunctata*; Other = abundance of remaining coccinellid species combined; P14 = *Propylea quattuordecimpunctata*; P22 = *Psyllobora vigintiduopunctata*; S24 = *Subcoccinella vigintiquattuorpunctata*; T16 = *Tytthaspis sedecimpunctata*

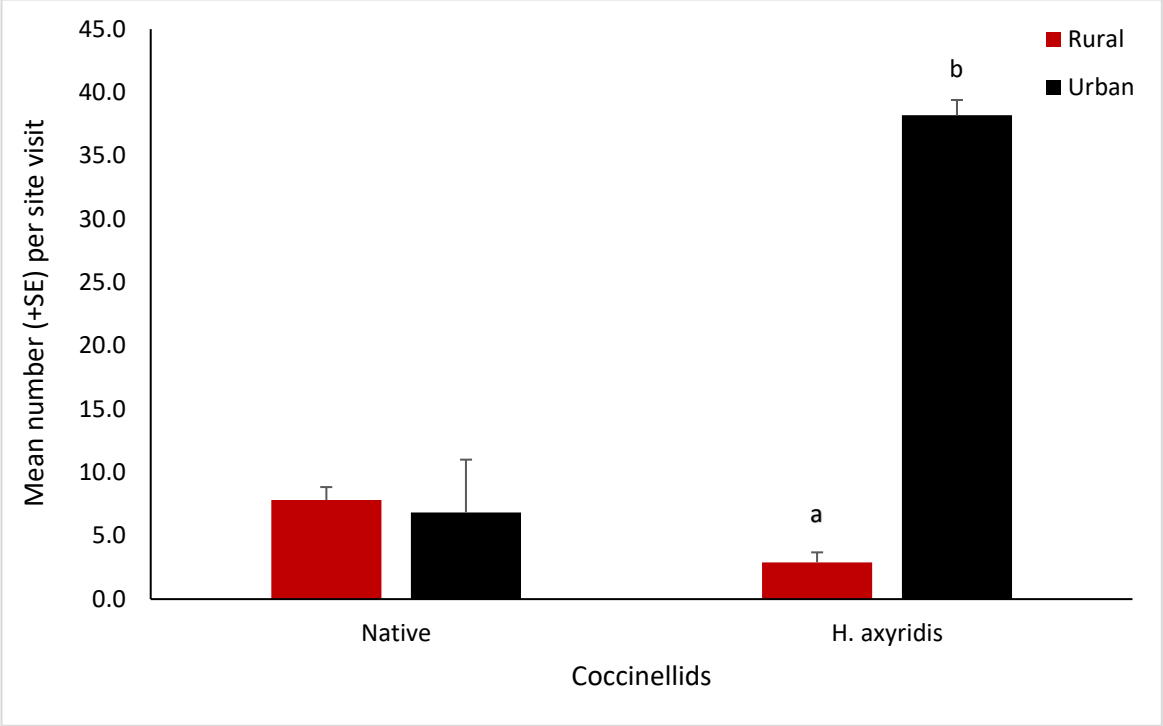


Figure 3.2: Mean number (+SE) per site visit of coccinellids recorded from the woodland at rural sites and the tree layer at urban sites in Cambridgeshire, Suffolk and Lincolnshire. Different letters above bars indicate significant differences as revealed by a regression model -> *H. axyridis*, log likelihood = -249, df = 11, AIC = 519, z = 12.52, $p < 0.001$; Native = all native coccinellids recorded.

3.3.3 Coccinellids in rural woodland

Rural woodland site type (deciduous and coniferous) was analysed separately. Native coccinellid abundance was significantly greater than that of *H. axyridis* in deciduous woodland (median = 2 and 0 respectively, $Z = -5.43$, $p < 0.001$, $r = 0.60$) and coniferous woodland (median = 0 and 1 respectively, $Z = -4.15$, $p < 0.001$, $r = 0.50$) (Figure 3.3). The binary model of the ZINB revealed that the likelihood of recording *H. axyridis* was higher in the summer rather than autumn across rural woodlands combined ($z = -3.011$, $p = 0.003$).

3.3.3.1 Harmonia axyridis

The only variable that affected *H. axyridis* abundance was vegetation layer in both deciduous only and coniferous only woodland, with a greater number recorded in the mature layer ($z = 2.65$, $p = 0.008$ and $z = 2.82$, $p = 0.005$ respectively) (Figure 3.4). The abundance of *H. axyridis* was higher during the summer ($z = 4.78$, $p < 0.001$) in deciduous woodland with no effect of season apparent in coniferous woodland. In addition to the results from the logistic model, the binary model explained in greater detail what the zeros represented in these data, with the likelihood of

recording *H. axyridis* being significantly higher in coniferous woodland in comparison to deciduous woodland (z = 3.67, p = 0.0002).

3.3.3.2 Native coccinellids

In rural woodland, deciduous sites had a significantly lower number of native coccinellids than did coniferous sites (z = -3.16, p = 0.002) (Figure 3.3). In deciduous woodland, vegetation layer had no effect on native coccinellid abundance, however, abundance was significantly higher in the mature layer of coniferous woodland as opposed to the intermediate layer (z = 2.67, p = 0.008) (Figure 3.4). Season did not influence the abundance of native coccinellids in deciduous only or coniferous only woodland.

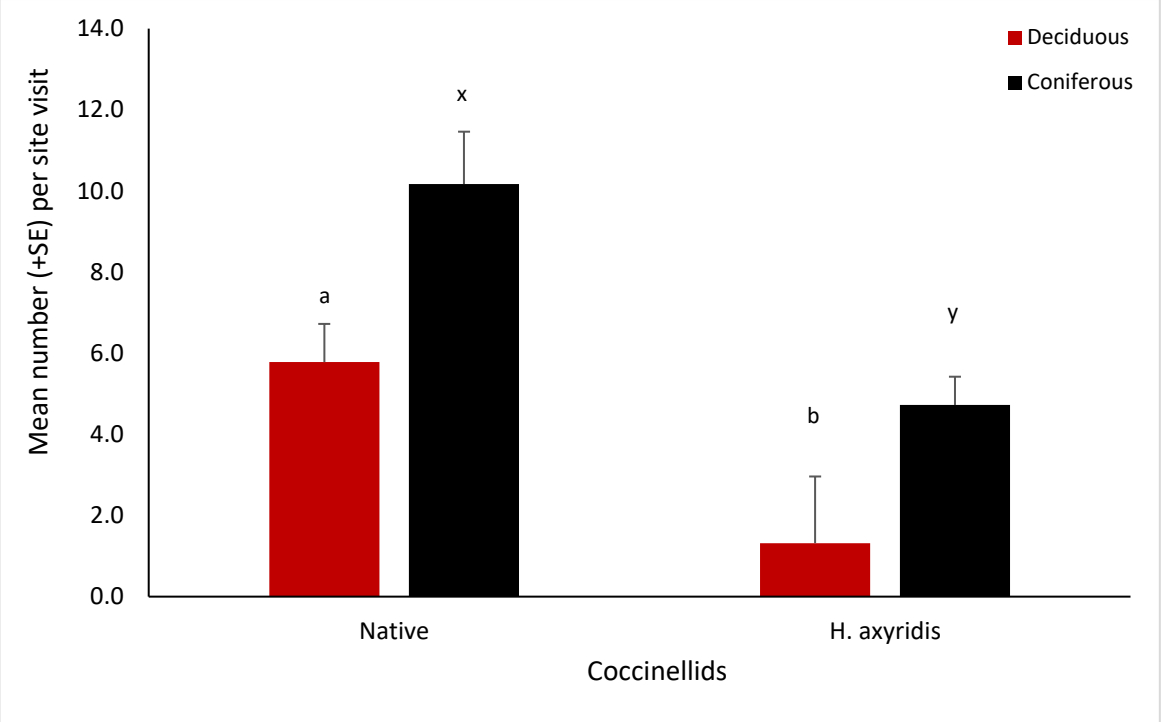


Figure 3.3: Mean number (+SE) per site visit of coccinellids recorded in woodland at deciduous and coniferous sites in Cambridgeshire and Suffolk. Native = all native coccinellids recorded. Consecutive letters indicate where significant differences occur.

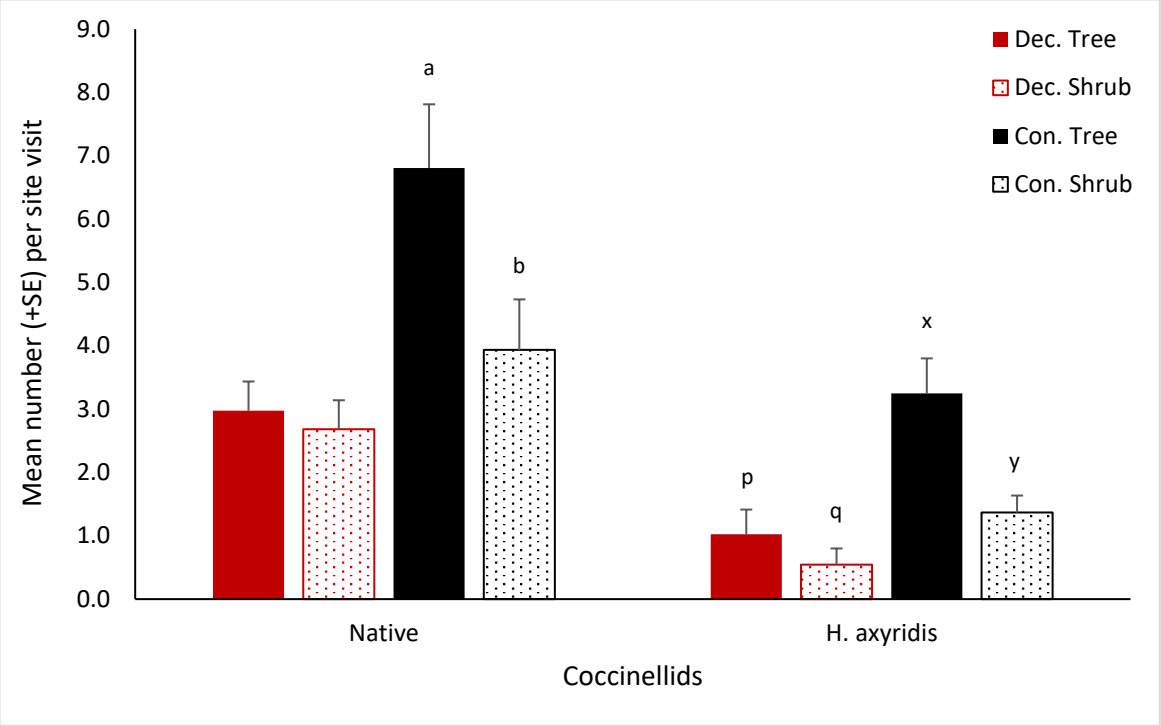


Figure 3.4: Mean number (+SE) per site visit of coccinellids recorded in woodland at deciduous only and coniferous only sites in Cambridgeshire and Suffolk. Native = all native coccinellids recorded; Dec. = Deciduous; Con. = Coniferous; Tree = Tree layer; Shrub = Shrub layer. Consecutive letters indicate where significant differences occur.

3.3.3.3 Coccinellids in grassland

Eight coccinellid species totalling 405 individuals were recorded in the grassland habitat. Two of the species, *S. vigintiquattuorpunctata* and *T. sedecimpunctata* were confined to grassland only (Table A3.2). Very few *H. axyridis* were recorded in the grass gradient (n = 12) and as a result it was not possible to apply any statistical analysis.

Grassland type had an effect on native coccinellid abundance, with significantly fewer recorded in deciduous grassland ($z = -3.09$, $p = 0.002$) as indicated by the reduced negative binomial model (Figure 3.5). In coniferous woodland, native coccinellids were significantly more abundant during summer rather than autumn ($z = 2.47$, $p = 0.001$). The null model was the best fit to investigate coccinellid abundance in grassland at deciduous sites revealing no effect of season on native coccinellid abundance.

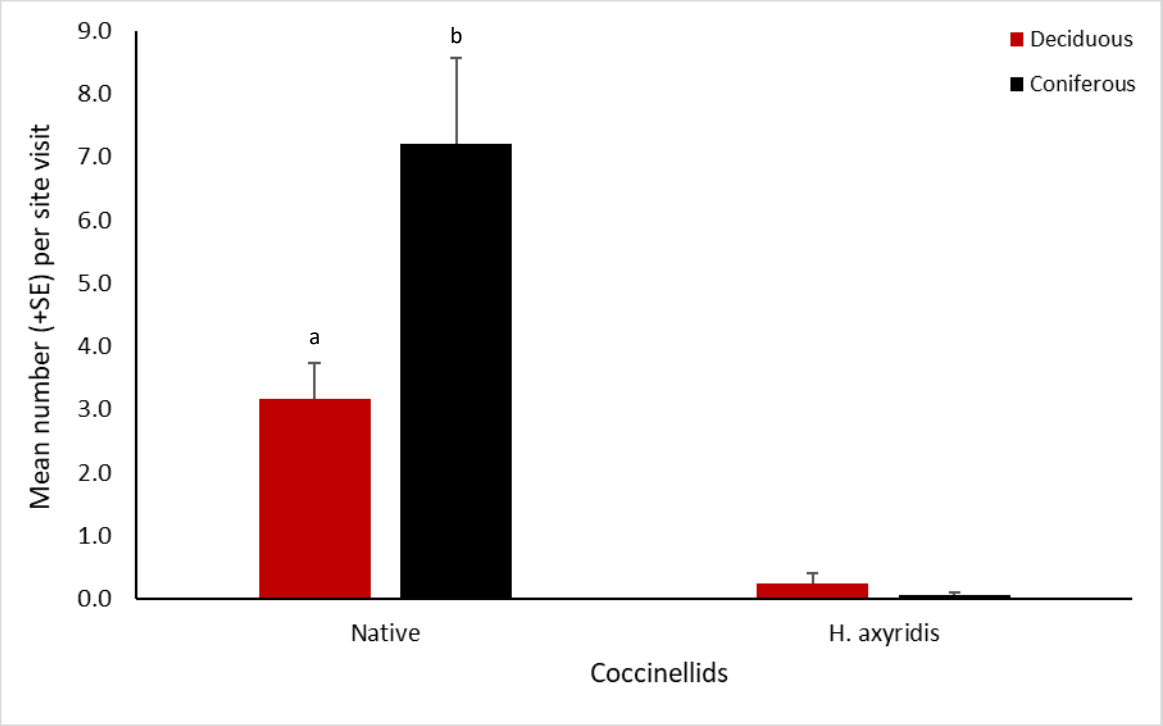


Figure 3.5: Mean number (+SE) per site visit of coccinellids recorded in the grass layer at deciduous and coniferous sites in Cambridgeshire and Suffolk. Native = all native coccinellids recorded. Consecutive letters indicate where significant differences occur.

3.3.4 Native coccinellid community

3.3.4.1 Coccinellid diversity

When considered as an entire habitat, coniferous sites hosted a significantly higher native coccinellid diversity ($t = 5.83$, $p < 0.001$) than deciduous woodlands (Figure 3.6). In deciduous woodland sites, native coccinellid diversity varied significantly (one-way ANOVA: $F = 4.35$, $p = 0.015$) with the tree layer having greater diversity than the grass layer ($p = 0.01$) as revealed by a post-hoc Tukey test. Coniferous sites also exhibited differences between the different vegetation structures ($F = 9.24$, $p < 0.0002$) with a significantly lower diversity in both the shrub layer and grass layer ($p = 0.0005$ & $p = 0.001$ respectively; Figure 3.6) in comparison to tree layer. There was no effect of seasonality on native coccinellid diversity in deciduous woodland, however native coccinellid diversity in coniferous woodlands was higher during the summer ($t = -2.23$, $p = 0.02$). When investigating the relationship between native coccinellid diversity and *H. axyridis* abundance in the entire rural habitat, the count part of the ZINB model revealed that native coccinellid diversity did not affect *H. axyridis* abundance. However, the binary model indicated that the probability of recording *H. axyridis* was significantly lower when native coccinellid diversity was higher ($z = -2.37$, $p = 0.01$). As expected, native coccinellid abundance was higher when native coccinellid diversity was higher ($z = 5.6$, $p < 0.001$).

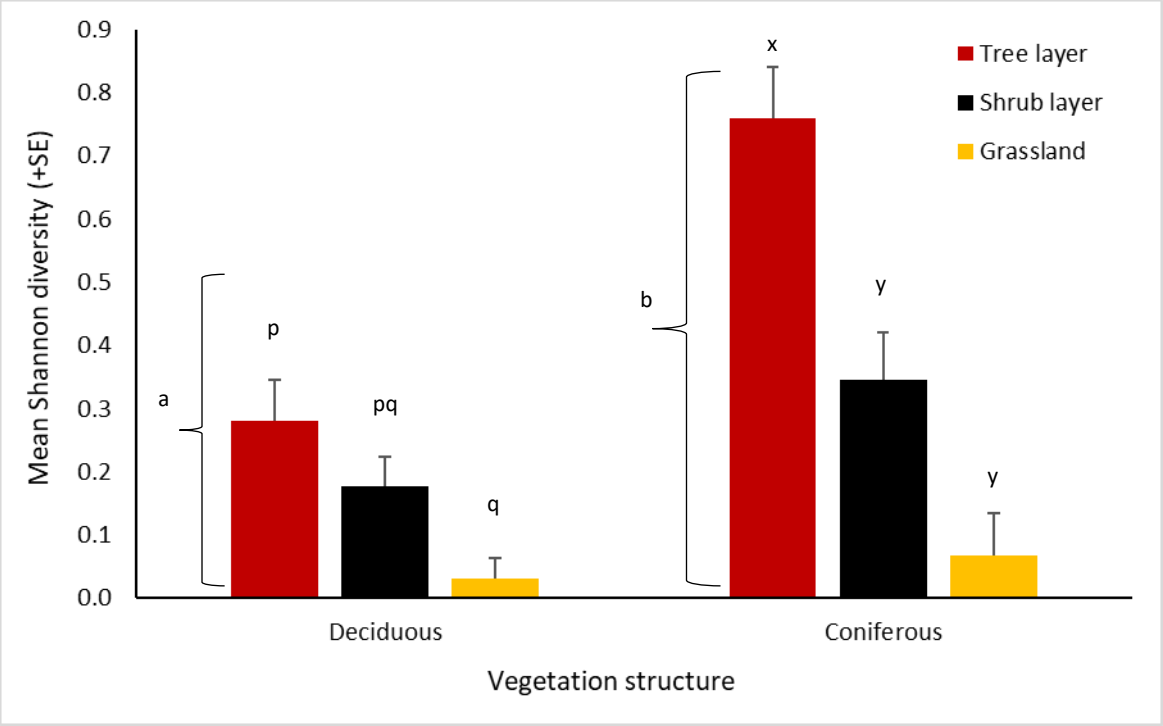


Figure 3.6: Mean Shannon diversity (+SE) of native coccinellid species at deciduous and coniferous sites and at different vegetation layers across all sites in Cambridgeshire and Suffolk. Consecutive letters indicate where significant differences occur. Letters on brackets represent differences between deciduous and coniferous sites collectively.

3.3.4.2 Coccinellid assemblage

The coccinellid assemblage is represented by an ordination diagram which is interpreted below by starting with the environmental variables, Type and Layer. Focusing firstly on the 'Type' axis, there is a clear difference in the coccinellid communities that are associated with coniferous only and deciduous only sites. Some species are positively associated with coniferous (*M. oblongoguttata*, *M. octodecimguttata*, *Scymnus* spp., *H. quadripunctata*) and deciduous (*Halyzia sedecimguttata*, *A. decempunctata*, *P. quattuordecimpunctata*) sites while other species are more generalist and are associated with both sites in varying abundances (*H. axyridis*, *E. quadripustulatus*, *C. septempunctata*) (Figure 3.7).

The 'Layer' axis also reveals that certain species are associated with particular vegetation layers and others are quite generalist in their habitat preferences. Habitat generalist species appear to aggregate along the centre of the 'Layer' axis (*H. axyridis*, *E. quadripustulatus*, *Scymnus* spp., *P. quattuordecimpunctata*) while the herb layer has a distinct coccinellid assemblage (*P. vigintiduopunctata*, *S. vigintiquattuorpunctata*, *T. sedecimpunctata*) (Figure 3.7).

Several species show a preference for the tree layer over the shrub layer in both coniferous (*A. bipunctata, M. oblongoguttata*, *M. octodecimguttata*, *H. quadripunctata*, *Anatis ocellata*) and deciduous sites (*Chilocorus renipustulatus*, *Calvia quattuordecimguttata*, *H. sedecimguttata*, *A. decempunctata*) (Figure 3.7). The herb layer at coniferous sites sits well apart from the other coniferous vegetation layers and as expected is more similar to the deciduous herb layer. At deciduous sites, there is a visible gradient through the vegetation layers being utilised by the coccinellid community, moving left to right from herb layer to shrub layer to tree layer. There was no difference in species diversity between the shrub and tree layer at deciduous sites and from the CCA plot (Figure 3.7) it becomes apparent that coccinellids use both vegetation structures with little variation between them, particularly when comparing the herb layer. For example, *C. septempunctata* (C7) is associated with both the herb and shrub layer, but with a greater abundance associated with the herb layer and a lower abundance associated with the tree layer. The tree and shrub layer at coniferous sites host similar coccinellid assemblages to each other. For example, *E. quadripustulatus* (ExQ) was associated across all coniferous sites for both the tree and shrub layer yet has a greater association with the tree layer. Two coccinellid species (*Tytthaspis sedecimpunctata,* T16 & *Subcoccinella vigintiquattuorpunctata,* S24) dominated the herb layer at coniferous sites that were not associated with any other site type or vegetation layer.

Harmonia axyridis appears as a generalist in the ordination diagram, being situated close to the origin and almost halfway on both variable axes. This species however, was more positively associated with coniferous sites and with the shrub layer (KF01, KF03, KF02 & BW), while *H. axyridis* was negatively associated with the herb layer at both site types. Associations with certain native coccinellid species were evident, however these species were not as abundant as *H. axyridis*. Both *E. quadripustulatus* (ExQ) and *A. bipunctata* (A2) have a similar association with coniferous sites as *H. axyridis*, however *A. bipunctata* is positively associated with the tree layer, unlike *E. quadripustulatus* which seemed to utilise both the tree and shrub layer (Figure 3.7).

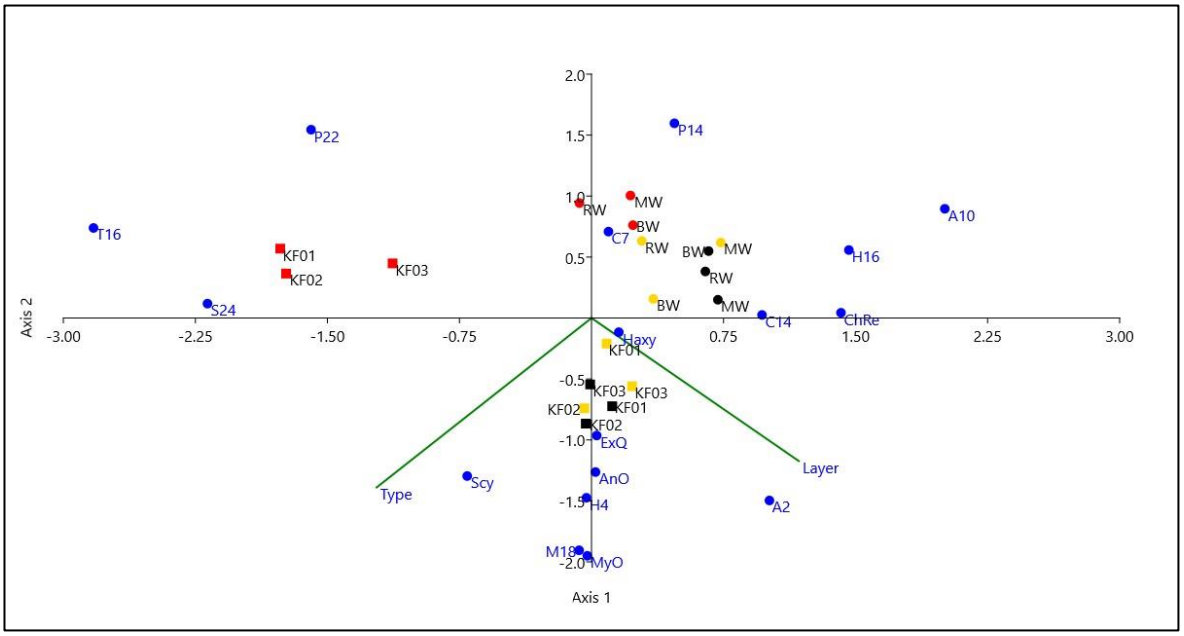


Figure 3.7: Species-conditional CCA triplot based on a canonical correspondence analysis of the coccinellid and environmental data recorded at rural sites in Cambridgeshire and Suffolk. Environmental vectors are amplified by a factor of two. Axis 1 explained 99.3% of the variation in the taxon-environmental structure and axis 2 explained 0.7% of the variation (Eigenvalues were 0.3505 and 0.0024 respectively); Type = coniferous sites (KF01, KF02, KF03 = Kings Forest site 1, 2 and 3) and deciduous sites (BW = Brampton Wood, MW = Monk's Wood, RW = Raveley Wood); Layer = tree, shrub and herb layer; coniferous sites are indicated by filled coloured squares, deciduous sites by filled coloured dots and species by blue circles; coccinellid species = (A2 = *Adalia bipunctata*; A10 = *Adalia decempunctata*; AnO = *Anatis ocellata*; C7 = *Coccinella septempunctata*; C14 = *Calvia quattuordecimguttata*; ChRe = *Chilocorus renipustulatus*; ExQ = *Exochomus quadripustulatus*; H4 = *Harmonia quadripunctata*; H16 = *Halyzia sedecimguttata*; Hax = *Harmonia axyridis*; M18 = *Myrrha octodecimguttata*; MyO = *Myzia oblongoguttata*; P14 = *Propylea quattuordecimpunctata*; P22 = *Psyllobora vigintiduopunctata*; S24 = *Subcoccinella vigintiquattuorpunctata*; Scy = *Scymnus* spp.; T16 = *Tytthaspis sedecimpunctata*).

Table 3.2: Guide to aid interpretation of Figure 3.7 indicating the symbols and colours used to represent site type (deciduous/coniferous) and vegetation structure (tree/shrub/herb layer) where coccinellids were observed.

Site Characteristics	**Symbol & Colour**	**Site Characteristics**	**Symbol & Colour**
DECIDUOUS	Dots	**CONIFEROUS**	Squares
Tree layer	Black	Tree layer	Black
Shrub layer	Gold	Shrub layer	Gold
Herb layer	Red	Herb layer	Red

3.3.5 Coccinellids and associations with other insects

Across all site types (urban, deciduous, coniferous) and vegetation structures (tree layer, shrub layer and grassland) a total of 17,747 aphids (prey) were recorded, along with 10,878 ants (natural enemies) and 925 lacewings (competitors).

3.3.5.1 Prey and competitors associated with coccinellids in urban habitat

A significantly greater number of aphids and lacewings were recorded at urban sites in comparison to rural sites ($z = 3.83$, $p = 0.001$ and $z = 7.99$, $p < 0.001$ respectively), however ant abundance was significantly lower at urban sites ($z = -4.67$, $p < 0.001$) (Figure 3.8). Looking in more detail at urban sites only, season did not affect lacewing, ant or aphid abundance.

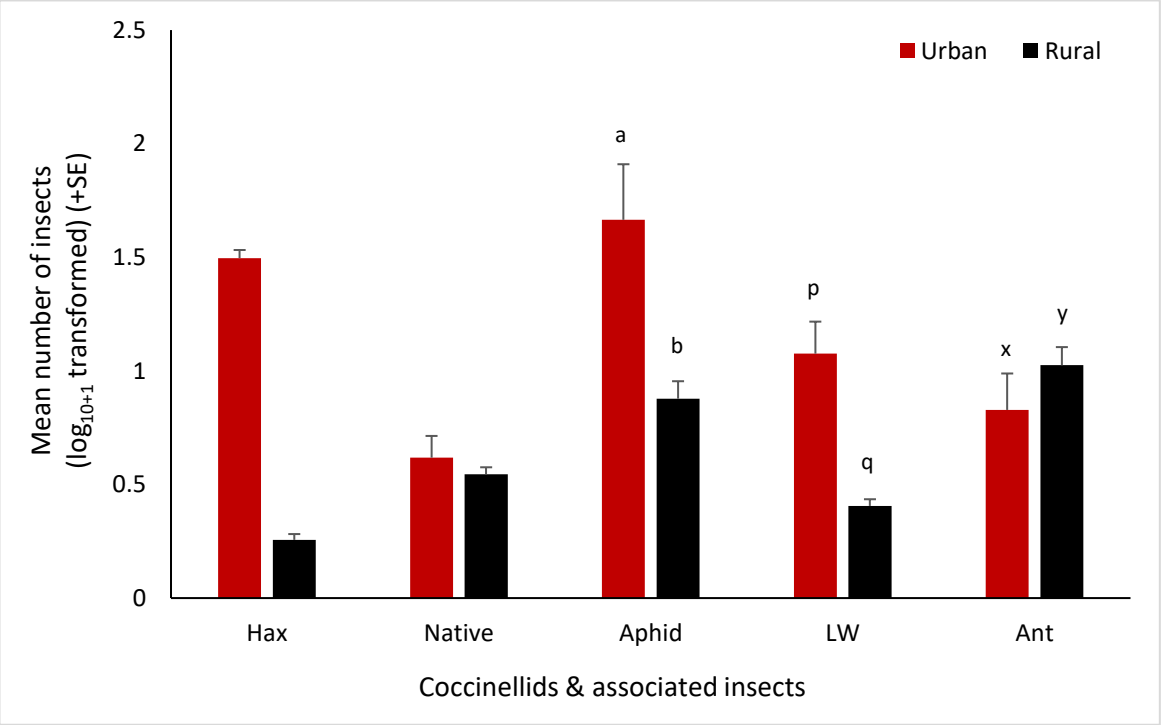


Figure 3.8: Mean number (log_{10+1} transformed) (+SE) per site visit of *H. axyridis* and native coccinellids recorded in relation to records of prey (Aphids), natural enemies (Ants) and competitors (Lacewings) in East Anglia. Hax = *Harmonia axyridis*, Native = native coccinellids recorded, LW = lacewings. Consecutive letters indicate where significant differences occur.

3.3.5.2 Prey and competitors associated with coccinellids rural woodlands

Woodland type had an effect on aphid abundance with significantly fewer observations at deciduous sites ($z = -3.34$, $p = 0.0008$; Figure 3.9). Vegetation type (tree or herb layer) had no effect on aphid abundance at either deciduous or coniferous woodland sites. Season affected aphid abundance, with greater numbers observed in the summer at deciduous ($z = 3.96$, $p < 0.001$) and coniferous woodlands ($z = 4.54$, $p < 0.001$) when investigated separately (Figure 3.10).

Significantly fewer ants were recorded at deciduous woodlands than at coniferous woodlands ($z = -12.59$, $p < 0.001$; Figure 3.9). Vegetation structure had no effect on ant abundance at deciduous only or coniferous only woodland. Season also had no effect on ant abundance at deciduous sites, however it did affect ant numbers at coniferous woodlands, with higher abundance recorded during the summer ($z = 3.19$, $p = 0.001$).

There were significantly more lacewings recorded in deciduous than coniferous woodlands ($z = 4.60$, $p < 0.001$) (Figure 3.9). The null model was the best fit when determining if any of the variables had an effect on lacewing abundance in deciduous or coniferous woodlands, signifying that vegetation structure and season had no effect on lacewing abundance in either woodland type.

Harmonia axyridis abundance was positively associated with aphid abundance at urban sites and coniferous-only woodland while native coccinellid abundance was negatively associated with aphid abundance at deciduous-only woodland. Native coccinellid abundance was positively associated with lacewing abundance at deciduous only woodland while *H. axyridis* was positively associated with lacewing abundance at urban sites (Table 3.3).

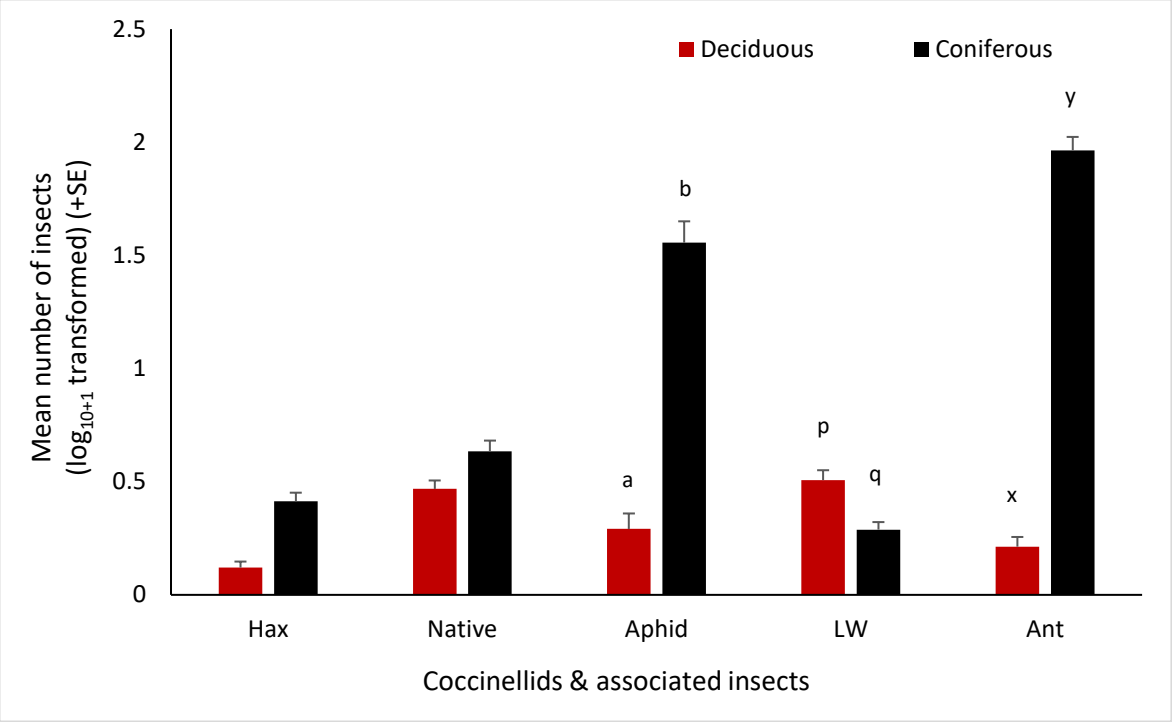


Figure 3.9: Mean number (log_{10+1} transformed) (+SE) per site visit of *H. axyridis* and native coccinellids recorded in relation to records of prey (Aphids), natural enemies (Ants) and competitors (Lacewings) in East Anglia. Hax = *Harmonia axyridis*, Native = native coccinellids recorded, LW = lacewings. Consecutive letters indicate where significant differences occur.

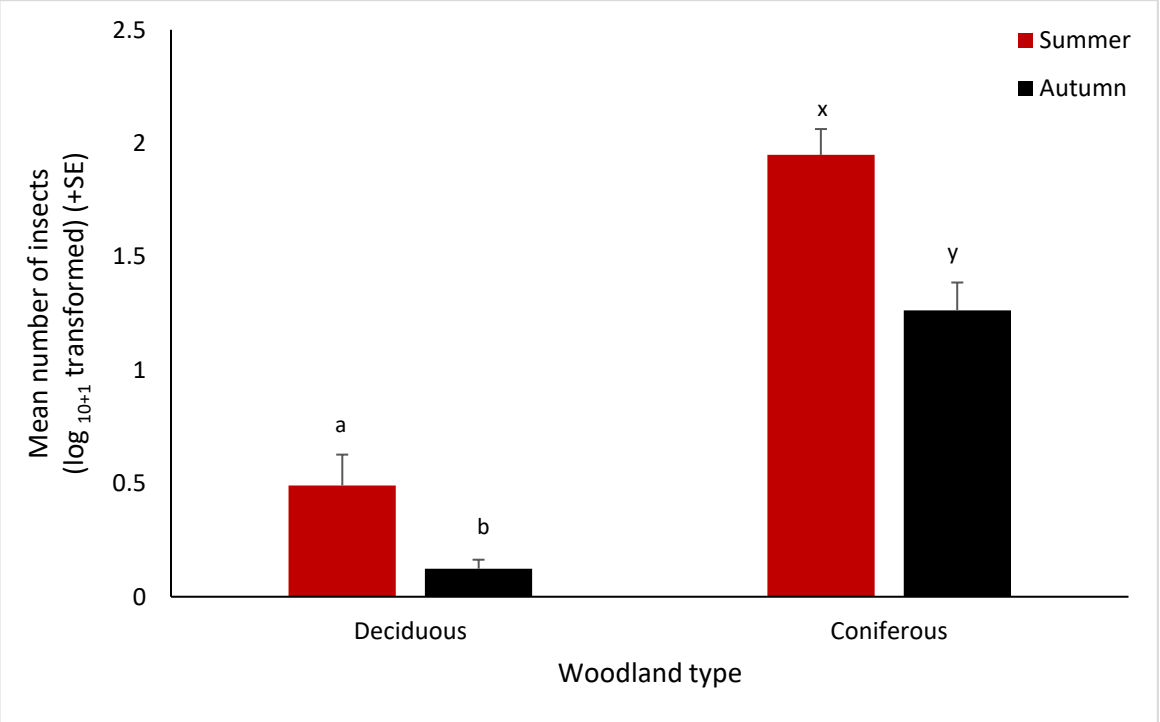


Figure 3.10: Mean number (log_{10+1} transformed) (+SE) per site visit of aphids recorded in deciduous only and coniferous only woodlands. Consecutive letters indicate where significant difference occur.

Table 3.3: Significance of Spearman correlations for coccinellid abundance with associated insects recorded from trees at different site types. Significant r^2 values are shown in bold.

Associated Insects	*Harmonia axyridis*	Native coccinellids
APHIDS		
Urban sites	**0.49**	0.35
Deciduous Woodland	0.086	**-0.35**
Coniferous Woodland	**0.26**	-0.13
Deciduous Grassland	n/a	**-0.48**
Coniferous Grassland	n/a	0.10
FORMICIDAE		
Urban sites	-0.35	-0.35
Deciduous Woodland	0.05	0.022
Coniferous Woodland	-0.18	0.19
Deciduous Grassland	n/a	-0.11
Coniferous Grassland	n/a	**0.40**
NEUROPTERA		
Urban sites	**0.56**	0.44
Deciduous Woodland	0.17	**0.35**
Coniferous Woodland	-0.068	0.13

3.3.5.3 Prey and competitors associated with coccinellids in rural grassland

There was a very low number of lacewings recorded in grassland (n = 5) and so analysis was not applied to these data. Site type had no effect on the abundance of aphids recorded in rural woodlands and season had no effect on aphid abundance in deciduous only or coniferous only woodland. In rural grassland, ant abundance was affected by site type with a significantly lower abundance of ants in deciduous grasslands ($z = -5.1$, $p < 0.001$) as opposed to coniferous grassland. Also when considering both site types together, ant abundance was affected by season in coniferous only grassland with a significantly greater abundance of ants observed during the summer ($z = -3.77$ $p = 0.001$). There was no effect of season on ant abundance in deciduous grassland.

There was a negative association between aphid and native coccinellid abundance in deciduous grassland. Conversely there was a significant positive association between native coccinellid abundance and ant abundance in coniferous only grassland (Table 3.3).

3.4 Discussion

3.4.1 Urban habitat compared with rural habitat

Harmonia axyridis dominated the coccinellid assemblage in urban areas, but not in rural woodland or grassland, in contrast to native coccinellid species. In Italy, Masetti *et al.* (2018) observed that *H. axyridis* was the dominant coccinellid in trees and shrubs within hedgerows, however the habitats surveyed were directly adjacent to intensively farmed crop fields. Less than a decade after its establishment in Belgium, *H. axyridis* also dominated urban areas in preference to non-anthropogenic habitats (Adriaens *et al*., 2008). Within four years of its establishment in the UK, *H. axyridis* had dominated in urban habitats (Brown *et al*., 2008b; Brown *et al*., 2011a). This invasive coccinellid is known to also prefer anthropogenic structures for its overwintering sites (Roy *et al*., 2016). When investigating the ability of *H. axyridis* and native coccinellids to overwinter in cold climates such as Canada, Labrie *et al*. (2008) found that *H. axyridis* only survived when overwintering in buildings, whilst native species were able to survive overwintering outside. This preference for a milder climate would explain the attraction of *H. axyridis* to anthropogenic areas during the cooler months in the UK, however, as this research shows, it is not completely restricted to urban habitats and can also be found in rural areas during active months.

Native species had been more abundant in urban habitats prior to the establishment of *H. axyridis* in the UK (Brown *et al*., 2011a). Native coccinellid abundance in this study did not differ between urban and rural sites. In rural habitat, however, native coccinellids were observed in greater abundance than *H. axyridis*. A similar outcome was observed in Belgium with a greater abundance of native coccinellids observed in semi-natural habitat such as grassland and pioneer vegetation (Adriaens *et al*., 2008) while in central Canada, four native coccinellid species declined from native vegetation when *C. septempunctata* established itself as an invasive coccinellid (Turnock *et al*., 2003). In the Czech Republic, a long-term study also revealed a decline in native coccinellid abundance, however, this decline was apparent prior to the establishment of *H. axyridis* (Honěk *et al*., 2016). Once *H. axyridis* became established, some species abundances remained static, however for two species, *A. bipunctata* and *P. quattuordecimpunctata*, the abundances declined even further (Honěk *et al*., 2016). This could be due to additional pressures such as changes in land use (Honěk *et al*., 2016) that happened to coincide with the establishment of *H. axyridis*. Additionally, Masetti *et al*. (2018) reported a decrease in *A. bipunctata* and two other coccinellid species native to Italy, following the establishment of *H. axyridis*. The coccinellid community of urban habitats tend to be dominated by *H. axyridis*, however, it may be that this species does not have the same dominant effect in rural habitats.

3.4.2 Deciduous and coniferous habitat

In this study, native coccinellids were more abundant than *H. axyridis* in both coniferous and deciduous woodland, indicating that unlike urban habitats, coccinellid assemblages in rural habitats are not dominated by *H. axyridis.* Coniferous woodland hosted a greater number of native coccinellids than deciduous woodland. Brown *et al*. (2011a) proposed that coniferous habitat may provide a refuge for native coccinellids considering the continuing spread of *H. axyridis*. Furthermore, using spatial modelling to determine what habitat type was most likely to be utilised by *H. axyridis*, Purse *et al*. (2014) also proposed that coniferous woodland would act as a barrier to the continuing establishment of *H. axyridis*, resulting in a refuge for native coccinellids. Yet, the abundance of *H. axyridis* in coniferous woodland in this research was higher than at deciduous woodland. The majority of habitat surveyed by Brown *et al*. (2011a) were urban sites with a greater number of lime and sycamore trees available while two of their sites were situated in less anthropogenic areas and yielded either very low abundance or no observations at all of *H. axyridis*. Both Brown *et al*. (2011a) and Purse *et al*. (2014) used data from the beginning of the invasion process to mid-2012 and it is possible, given the penchant that *H. axyridis* has for urban habitats, that it had not yet fully established itself in this rural habitat. *Harmonia axyridis* is highly phenotypically plastic and can overcome habitat and dietary barriers efficiently and successfully (Majerus *et al*., 2006) adapting to climatic extremes and being able to survive on a range of diets where other coccinellids cannot (Sloggett & Majerus, 2000b). Furthermore, when investigating overwintering coccinellid assemblages, Holecová *et al*. (2018) found that even though *H. axyridis* was one of the dominant species on Scots Pine, the majority of the time, either *E. quadripustulatus* or *C. septempunctata* made up a larger proportion of overwintering coccinellids. This report regarding species proportions is similar to that observed here (Appendix Table A3.1).

The differences seen here between coniferous and deciduous woodland, could also be attributed to fragmentation of the deciduous woodlands surveyed, as fragmentation of a habitat is known to reduce diversity of a habitat (Pullin, 2002). Being ancient woodlands, the deciduous sites were heterogenous, with a range of other broadleaved tree species intermingled with the survey species, while coniferous sites were more homogenous with just two tree species which were arranged predominantly in blocks in a plantation style and were relatively young (6-10 years) (Appendix A3.1). Sweaney *et al*. (2015) surmised that pine plantation led to the homogenisation of the habitat and found lower species richness in ground-beetle communities in plantation-only sites in comparison to a combination of plantation and farm sites. However, coniferous sites in this current work, were continuously connected by additional woodland areas, whereas the deciduous sites were stand-alone woodlands, surrounded by crop fields. Rösch *et al*. (2013) investigated the effects of fragmented habitat on leafhoppers and concluded that species richness depended not just on the size of the habitat, but also composition of the surrounding landscape and connectivity of the

habitat. Even though Holecová *et al*. (2018) found no effect of adjacent habitat on overwintering coccinellid assemblages, this was at coniferous sites only. In this current study some other process may be influencing site differences, perhaps connectivity or adjacent land use or a combination of the two.

What constitutes suitable habitat for coccinellids? Comont *et al*. (2012) determined that the ability of a species to feed on a range of prey species was more important in determining the distribution of a species than habitat type. However, prey preference and habitat type are intrinsically linked due to the fidelity of aphid species to specific plants (Dixon *et al*., 1987). Given the homogenous nature of the coniferous sites in comparison to the deciduous woodland with a range of tree and shrub species, resulting in a greater range of prey species, one would expect a greater abundance of *H. axyridis* in deciduous woodland, yet this was not the case. Aside from an adequate food source and suitable sites to facilitate successful reproduction, coccinellids require suitable overwintering sites. Coniferous woodlands experience less extreme temperature variation than deciduous woodlands (Ferrez *et al*., 2011) and greater overwintering success as a result could explain the increased abundance of native coccinellids at these sites. Another explanation is that there are several conifer specialist coccinellids in the UK and fewer deciduous specialists as many recorded at deciduous habitats tend to be generalist in their habitat preferences (Roy *et al*., 2013). Additionally, considering the preference *H. axyridis* has for more sheltered overwintering sites, it is possible that the large area of coniferous plantation in this study provided sufficient shelter to enable this species to overwinter successfully. With a shortage of knowledge on coccinellid assemblages at overwintering sites (Pendleton & Pendleton, 1997-2019; Hodek, 2012; Holecová *et al*., 2018) investigating the overwintering coccinellid assemblages in coniferous woodland would provide further knowledge of coccinellid behaviour and importantly how climate change may influence coccinellid assemblages in the future.

3.4.3 Vegetation structure and the coccinellid community

There was a distinct difference in the coccinellid community in relation to vegetation structure between coniferous and deciduous sites. Within these individual site types, vegetation structure affected both the abundance and distribution of different species. The tree layer in both deciduous and coniferous woodland supported the greatest coccinellid diversity and in both cases differed to the herb layer. At urban sites, Viglášová *et al*. (2017) found that coccinellid diversity also differed across the different vegetation types that were surveyed, with higher species diversity in trees in comparison to nettle stands. In this study, coccinellid diversity differed in the tree and shrub layer at coniferous woodland, but not at deciduous woodland. In field margins, Burgio *et al*. (2006)

reported higher diversity of coccinellids in mature hedgerows in comparison to younger hedgerows. Additionally, older hedgerows have been shown to have greater invertebrate diversity to that of younger hedgerows (Deeming *et al*., 2010). One reason for the lack of a difference at deciduous woodland sites between the tree and shrub layers could be the choice of species for the shrub layer. All three of the deciduous sites were ancient woodland (BCN, 2019), while the coniferous sites were all based at a large Forestry Commission plantation woodland, which is less than a century old (Forestry Commission, 1951). The shrub layer at deciduous sites is likely to have been much older than the shrub layer at coniferous sites (based on a combination of canopy cover, height and guidance from Bennett, 2016). This essentially meant that the shrub layer consisted of immature individuals of the same species surveyed for the tree layer at coniferous sites. Considering this, it is not surprising that no difference was apparent for deciduous sites: all four species surveyed were considerably more established than the shrub layer at coniferous sites. However, the difference between the tree and shrub layer at coniferous sites is understandable as mature trees host a greater diversity of invertebrates, insects and coccinellids (Schowalter, 1989; also see above). Holecová *et al*. (2018) reported a significantly higher number of overwintering coccinellids in mature pine trees as opposed to younger pines. This could be due to larger more mature trees having a greater quantity of less exposed areas thus offering a greater level of protection during the winter months.

As well as greater species diversity, a larger and different coccinellid assemblage was associated with the tree layer than the shrub layer across both site types. Janssen *et al.* (2017) found maturity of tree stands to be the most important factor for the assemblage of saproxylic beetles. The herb layer in this study also hosted a very different coccinellid community to that of the tree and shrub layer, likely due to the very different food sources available in the herb layer (mildew, plant material, different aphids). Similar findings were reported by Viglášová *et al.* (2017) for the coccinellid species observed on nettle stands. The differences reported here relate to both site types with a unique coccinellid assemblage at both coniferous and deciduous sites. Grass specialists dominated the herb layer at coniferous sites (e.g. *T. sedecimpunctata* & *S. vigintiquattuorpunctata*), while generalist coccinellids, such *C. septempunctata* dominated at deciduous sites. Interestingly, Viglášová *et al.* (2017) reported seasonal differences in how *C. septempunctata* used different vegetation structures, with greater numbers in nettles in the summer, and higher abundance on trees later in the year. No such seasonal effect was evident here, however, this species did make use of the different vegetation layers as previously illustrated.

Native coccinellid diversity did not have an effect on the abundance of *H. axyridis*, however when native coccinellid diversity was higher, the probability of recording *H. axyridis* was lower. Viglášová

et al. (2017) reported their lowest native coccinellid species diversity when *H. axyridis* abundance was highest, however their field sites were in urban habitats. It is well discussed that *H. axyridis* is the dominant coccinellid at urban sites, however a different account is being observed in rural woodland and grassland. For example, one would expect *A. bipunctata* to be more positively associated with *H. axyridis*, however, this native species appeared to be almost specialist in terms of habitat, being associated with the tree layer, and not with the shrub or herb layer as expected (Sloggett, 2008). The relationship between *H. axyridis* and *A. bipunctata* is likely to be complex and likely to vary between habitats (urban/rural, tree/grass) and examination of this relationship should take place only when the entire coccinellid community is the focus of a study to elucidate the standing both of these coccinellids within their community as well as their roles in ecosystem function.

3.4.4 Seasonality and coccinellids

In this study, season did not drive the abundance of *H. axyridis* or native species at urban sites. The only instances where season affected coccinellid abundance was at deciduous sites (*H. axyridis* more abundant in the summer) and in coniferous grassland (native species more abundant in the summer). However, seasonality was reported as having an effect on coccinellid abundance in urban areas with Viglášová *et al*. (2017) observing greater numbers in autumn, particularly with common generalist species. Seasonality is not an easy variable to consider based on the range of seasonal differences across large countries and continents as well as climate change resulting in non-seasonal weather patterns happening more frequently (IPCC, 2007). This makes it difficult to compare findings with other studies, however, if studies turn their focus to extreme weather events instead of seasonality on its own, it may be possible to determine the effects on species (Oliver *et al*., 2013).

Environmental variables such as temperature, humidity and sunshine hours all play a role in coccinellid abundance (and that of their prey). Hassan *et al*. (2009) found that relative humidity had a negative impact on aphid abundance while Brown *et al*. (2011a) found that mean maximum temperature correlated with the abundance of native coccinellid larvae. Given that in this study, relative temperature and humidity were only recorded at the time surveying began, it is difficult to arrive at meaningful conclusions. There were instances within the models where higher humidity seemed to negatively affect *H. axyridis* abundance. This, however, would need to be followed up with a more comprehensive dataset with continuous environmental data readings. It is possible to acquire environmental data from meteorological institutions, although these data would be coarse and not reflective of microclimates at survey sites. Microclimate is often underestimated and some

coccinellids are known to prefer areas that are warm and sheltered (*C. septempunctata*) while others will tolerate a cooler microclimate (*P. quattuordecimpunctata*) (Honěk, 2012). Taking into consideration the previously mentioned differences in microclimate of coniferous and deciduous woodland sites (Ferrez *et al*., 2011) and the differences between urban and rural areas in terms of temperature (George *et al*., 2007), drawing conclusions from analyses with these data would not give a complete picture of how environmental variables influence assemblages at specific sites. In future research, continuous temperature and humidity should be recorded at survey sites using automatic weather stations which can remain at sites throughout the study period. Automatic weather stations are capable of recording temperature, humidity, sunshine, rainfall and wind speed. Incorporating this technology in future studies should help reveal the complex and connected way that environmental variables affect the coccinellid community.

3.4.5 Prey and competitors associated with coccinellids

3.4.5.1 Aphids

At urban sites, there was a positive relationship between *H. axyridis* abundance and that of aphids. Honěk *et al*. (2018b) also reported a positive relationship with aphid abundance and that of *H. axyridis* in urban areas. Additionally, Vandereycken *et al*. (2013) reported a positive relationship between aphids and coccinellids in a range of crop habitats. However, when investigating coccinellids in urban areas, Viglášová *et al* (2017) found the relationship between common coccinellid species and aphid abundance to be non-linear with coccinellid abundance increasing with that of aphids, however when aphid abundance became very high, coccinellid abundance decreased.

At rural sites, a negative relationship was observed between native coccinellid and aphid abundance at deciduous sites with the reverse observed with *H. axyridis* and aphid abundance at urban and coniferous sites. The majority of coccinellids recorded at deciduous sites were *C. septempunctata*, which is a species that is known to tolerate areas with lower aphid density (Honěk, 1985). The third and fourth most recorded coccinellids at deciduous sites were *P. quattuordecimpunctata* and *A. decempunctata,* both of which are also tolerant of low aphid abundance (Honěk, 1985). The relationship between coccinellid abundance and that of aphids, however, is not an easy one to tease apart. As mentioned above, positive and non-linear relationships have been observed while, Brown *et al*. (2011a) and Brown & Roy (2017) did not find any correlation between *H. axyridis* or aphidophagous coccinellids and aphid abundance.

Michaud *et al*. (2016) suggested that aphidophagous coccinellids are able to adapt to aphid colonies as they develop. As an aphid colony increases, coccinellid numbers (as well as other aphidophagous predators) will increase in order to feed and oviposit, so their offspring will have sufficient food resources upon emergence. However, if there are too many individual predators, the aphid colony will collapse, leaving many offspring without sufficient resources to fully develop (Michaud *et al*., 2016). As a result, coccinellid predators need to be able to read the signals of when an aphid colony micro-habitat is at carrying capacity which could result in some researchers observing a non-linear or negative relationship between coccinellid and aphid abundance.

In contrast seasonality affected aphid numbers, with greater numbers recorded in the summer. This is supported by Sequeira & Dixon (1997) who found that not only was aphid density seasonal but that this variation did not differ from one year to the other, even in years with low aphid abundance. Burgio *et al*. (2006) also reported higher aphid abundance in summer, along with a higher abundance of coccinellids. This corresponds in part with the results here of a greater number of *H. axyridis* observed in the summer in deciduous woodland, however not at coniferous sites, nor was there any seasonal effect on native coccinellid abundance at either rural site type. However, in a longer-term study of aphids in pecan trees, Dutcher *et al*. (2012) reported that aphid abundances varied widely from one season to another. Additionally, aphid abundance is dependent on the quality of the host plant (Sequeira & Dixon, 1997) and with many studies focussing on lime, sycamore and other urban tree species, it is possible that the lack of some expected relationships could be as a result of host species choice on the part of the researcher.

Each study records aphid abundance in a slightly different way, however consistently within their own study. Furthermore, the aphid data is often considered as an addition to the main question, for example the diversity of coccinellids, or the effect of invasive coccinellids on native counterparts. More work is needed to investigate the relationship between aphids and their predators, approaching it from the aphid perspective. It is often considered that the aphids present at the same time as coccinellids are suitable prey, however this may not be the case. There are examples of *A. bipunctata* feeding on certain aphid species that are in fact inadequately nutritious for them (Sloggett, 2008). Further studies could reveal more details about the aphid preferences of coccinellids and if species such as *A. bipunctata* are declining because of competition for food, IGP or an insufficient supply of appropriate food. Additionally, the coccinellid species most often the focus of research tended to be generalist and ubiquitous species, for example *C. septempunctata, P. quattuordecimpunctata, A. bipunctata* and *H. axyridis*, with specialist coccinellids ignored. However, Sloggett *et al*. (2008) illustrated that specialist coccinellids were more likely to remain in situ feeding on aphids, while generalist species left the area relatively

quickly after arrival. Investigating the relationship between specialist coccinellids and aphids would be useful from the perspective of conservation as well as biological control. There is a large volume of literature on aphid dynamics and their relationship with coccinellids (see Dixon & Dixon, 2000). These relationships are complex and the details are largely beyond the remit of the present study.

3.4.5.2 Ants (Formicidae)

More ants were recorded at coniferous sites than deciduous sites. At coniferous sites, vegetation structure had no effect on ant or aphid abundance while a greater number of coccinellids were noted in the tree layer. Conversely, season affected the ant and aphid abundance at coniferous sites, with a greater number of both taxonomic groups recorded in the summer, while season had no effect on native coccinellid abundance at the same sites. Sloggett & Majerus (2000b) investigated the spatial relationship between coccinellids and ants on pine trees and also observed that ants were more abundant in summer, decreasing to almost zero observations by the end of September. Furthermore, coccinellids tend to co-occur with ants and aphids when aphid numbers are particularly low (Sloggett *et al*., 2000b) which may indicate that aphids were sufficiently abundant for both the ant and coccinellid communities at coniferous sites. The relationship between aphids and ants is a mutual one: ants tend to aphids, feeding on the honeydew produced, while the aphid colony expands and is protected from aphid predators such as coccinellids (Way, 1963). The relationship between ants, aphids and coccinellids is a complex one and without additional investigation at these sites, it is difficult to infer any further conclusions.

3.4.5.3 Lacewings (Neuroptera)

In contrast to ants, lacewing abundance was higher at deciduous woodlands. It is possible that *H. axyridis* abundance at deciduous sites was lower as a result of greater abundance of lacewings. Firstly, some coccinellid species choose their oviposition sites dependent on the presence of other coccinellid species and/or lacewings (Ruzicka, 2001; Evans, 2003). Secondly in laboratory tests, Nedvěd *et al*. (2013) showed that lacewing species outcompeted *H. axyridis* during intraguild predation. At urban areas, both lacewing and *H. axyridis* numbers were higher than at rural areas. Given that lacewings and *H. axyridis* are thought to share the same habitat with little conflict (Nedvěd *et al*., 2013), it is possible that the abundance of prey was more than sufficient for these two taxonomic groups to inhabit the same space.

3.5 Conclusion and future work

It is perhaps not appropriate to attribute the decline of native coccinellids solely to *H. axyridis*, as there have been cases where some native species were in decline prior to its arrival (e.g. *A. bipunctata* & *C. quinquepunctata* in Czech Republic, Honěk *et al.*, 2016). Environmental pressures such as climate change, intensification of agricultural practices (Honěk *et al*., 2016) and increased anthropogenic disturbance (Brown & Roy, 2018) may all have contributed to the initial decline of these species, but these pressures were compounded by the arrival of *H. axyridis*. There are suggestions that this initial decline of native species will reverse and that the invasive and native populations may stabilise and co-exist (Hentley *et al*., 2016). The long-term research by Honěk *et al*. (2016) illustrates just how important long-term population studies are in having baseline data prior to the establishment of an IAS but also in determining how native coccinellid abundance can fluctuate over several years. More long-term studies in a range of habitats are needed to reveal a more complete picture on native coccinellid communities and how they change in the presence of IAS and other drivers of change.

Coccinellid communities are not often the sole focus of studies and information on their structure tends to come as an add-on to other works (Honěk, 2012). More research needs to be initiated to investigate the coccinellid community as a whole and not just focus on individual species. Considering the complex relationship between aphids and generalist coccinellids it is important to further understand the significance of a diverse coccinellid community, how the communities exist in different habitats and their role in ecosystem functioning, especially given the aforementioned evidence that specialists are likely to be more effective as biological control agents than generalist species.

In this study there was a distinct difference in *H. axyridis* abundance between urban and rural sites. However, this IAS was less abundant than native coccinellids as a group within both rural woodland and rural grassland. A distinct native coccinellid assemblage was present at all three vegetation layers. Ancient woodlands as opposed to younger woodlands are a likely refuge for native coccinellids, particularly specialist species. With increasing pressures from multiple drivers, it is important to continue research into the dynamics of complex native coccinellid communities.

4 The ecology of *Coccinella quinquepunctata* in the presence of *Harmonia axyridis*

4.1 Introduction

A large volume of literature exists concerning invasive alien species (IAS) and the effect they have on native flora and fauna. The majority of studies relate to terrestrial systems, however, within this group, the vast majority of research focusses on plants with a small percentage investigating herbivorous species and very few concerning predators or organisms at other trophic levels (Lowry *et al*., 2013). Kenis *et al*. (2009) reviewed primary research on invasion ecology relating to insects and examined a set of 403 papers. The research tended to focus on species that have a negative economic impact such as invasive ants or invasive pollinators, with just six percent of publications concentrating on *Harmonia axyridis* (Kenis *et al*., 2009). In the USA, researchers have also focussed on the negative impacts of *Coccinella septempunctata* and *Propylea quattuordecimpunctata* (Harmon *et al*., 2007; Losey *et al*., 2012b) but not to the same extent as the research on *H. axyridis*.

Harmonia axyridis is popular in biological control due to its effectiveness as a pest controller in agricultural systems, however at the same time, several native coccinellid species have been displaced (Adriaens *et al*., 2008; Brown *et al*., 2011a; Sloggett, 2017). Additionally, it is not yet clear if *H. axyridis* can take over the role that native coccinellids play in biological control should local extinctions occur (Roy *et al*., 2012). As a result, the research on these biological control agents often concerns the effect the IAS may be having on native species, but more so on native species that were once abundant and have noticeably declined since the establishment of an IAS, such as *Coccinella novemnotata* in North America (Losey *et al*., 2012b) and *Adalia bipunctata* in the UK (Brown *et al*., 2011a). Research that investigates the effect of IAS on coccinellid species that are considered specialists or are rare and may be at risk of local/national extinction as a result of pressure from IAS is uncommon. Additionally, few studies have been undertaken to determine how rare/local coccinellid species contribute to their assemblages/habitat or how they may be affected by the presence of IAS such as *H. axyridis* (Sloggett, 2017). *Coccinella quinquepunctata*, is a generalist coccinellid, abundant in Europe yet considered a specialist in the UK that may be at risk from *H. axyridis*.

4.1.1 *Coccinella quinquepunctata* – Five-spot ladybird

Coccinella quinquepunctata (five-spot ladybird) is a small conspicuous ladybird, typically 5mm in length and red with black spots. This species is not found in Ireland, whilst in the UK *C. quinquepunctata* is always recorded in a restricted habitat of unstable river shingle (Roy *et al*., 2011). Due to only a handful of records since 1913, *C. quinquepunctata* was considered extinct in the UK until 1987 (Majerus & Fowles, 1988). As a result of the restricted distribution of *C. quinquepunctata* in the UK, this species falls under the Red Data Book Category 3 (RDB3) Rare. The RDB3 classification is for taxa that are not yet endangered or vulnerable but are at risk due to restrictions in their habitat or geographical area (Hyman, 1992).

Upon the rediscovery of *C. quinquepunctata* in the UK, more information became available regarding vegetation that this species was associated with on the shingle banks. It was also noted that the species was more likely to be observed on low vegetation, not more than 30-45 cm in height (Majerus & Fowles, 1988). In the late 1980s, surveys reported the species to be well established in west Wales on both the River Ystwyth and Rheidol as well as in south east Wales on Afon Tywi, with reports of up to 50 individuals recorded at some sites (Majerus & Fowles, 1988). *Coccinella quinquepunctata* was easily found on thistle or dock growing on river shingle along the Afon Tywi and River Severn in 2002 and 2003 (Bates & Sadler, 2004). In Scotland, there were previous records of *C. quinquepunctata* in the early 1900s (Majerus & Fowles, 1988) and upon the rediscovery in Wales, surveys were subsequently undertaken at previously recorded sites in Scotland. Since then, other sites of suitable habitat have been identified along the River Dee and surveys carried out resulting in further observations of this species in Scotland (Littlewood, 2015).

4.1.2 Specialised habitat

Climatic conditions in the UK are considered suboptimal for some coccinellids, resulting in the UK being the edge of the acceptable range for several coccinellid species (Brown & Roy, 2015). *Coccinella quinquepunctata* only persists in the UK in specialised habitats, in contrast to their mainland European populations (Majerus & Fowles, 1988). This habitat is Exposed Riverine Sediment (ERS) or shingle banks that form along river bends, and several rivers that traverse Wales contain this feature. Exposed riverine sediment is in a constant state of alteration due to the nature of the river systems and water levels rise and fall regularly (O'Callaghan *et al*., 2013). Water levels not only rise in terms of height but water also moves inwards across the shingle to the extent of the terrestrial habitat during high/maximal flow periods. The water level can rise in this way quite quickly (several metres in 30 minutes) and can reduce just as quickly, depending on the underlying geology of the upstream river catchment (Baker *et al*., 2004). As a result, the invertebrate

community in these habitats are well adapted to the unpredictability of these shingle banks (Sadler *et al.*, 2004).

Bates & Sadler (2004) have described *C. quinquepunctata* as having an ERS fidelity grade of 1, like many invertebrates inhabiting ERS. Essentially this means that *C. quinquepunctata* is dependent on unstable river shingle for at least one stage of its life cycle and is not found in other habitat unless it happens to resemble ERS in some way, for example lakes that have wave action resulting in a sediment similar to ERS (Bates & Sadler, 2004; Sadler *et al*., 2004). However, due to anthropogenic disturbances such as gravel extraction, livestock access, channel modification and the establishment of invasive alien species, the quality of habitat is being degraded to the point that specialised invertebrate species are at risk (Hyman, 1992; Hewitt *et al*., 2010). *Impatiens glandulifera* (Himalayan balsam) is an invasive herbaceous plant that is one of the tallest in the UK reaching 2.5 metres in height (Beerling & Perrin, 1993). This IAS outcompetes native plants through its height by blocking light for smaller plant species (Pyšek & Prach, 1995; Tanner *et al*., 2014). Additionally, *I. glandulifera* alters the microbial soil community making it difficult for native plants to take root (Pattison *et al*., 2016). Furthermore, the annual nature of *I. glandulifera* and its root structure, work together to de-stabilise the river bank leaving it more susceptible to erosional transportation away from situ during flooding (Pyšek & Prach, 1995; WISE Network, 2014).

In central Europe, *C. quinquepunctata* is found in more generalist habitat such as trees, wild herbaceous vegetation and cereal fields (Honěk *et al.*, 2014; Majerus *et al*., 2016). This species, however, has been declining in central Europe over the last 40 years (Honěk *et al*., 2016). Aside from the short studies above, few details are known about the one of UK's rarest and most specialist ladybird species. Discovering why *C. quinquepunctata* is so specialist in the UK would be a step towards being able to develop and implement an effective conservation plan for this species in the UK. Given the RDB3 classification of *C. quinquepunctata*, discovering how this species lives alongside the invasive species *H. axyridis*, would prove insightful in an effort to fully understand how *H. axyridis* affects vulnerable native coccinellids in rural habitats (Roy *et al*., 2016).

4.1.3 Effects of invasive coccinellids

Invasive alien species are one of the biggest drivers of biodiversity loss (Sala *et al*., 2000; Roy *et al*., 2014; IPBES, 2019). A number of coccinellid species have established themselves in many countries outside of their native range. In the USA, *H. axyridis* and *C. septempunctata* are well established (Harmon *et al*., 2007) and are considered to have played a role in the decline of *C. novemnotata* in North America (Tumminello *et al*., 2015; Ducatti *et al*., 2017; Ugine *et al*., 2018). *Coccinella*

septempunctata was first established in 1983 and since then, *C. novemnotata* has gone from being the most prevalent coccinellid in the assemblage to not being found at all in 11 states (Harmon *et al*., 2007; Losey *et al*., 2012b). When comparing *C. novemnotata* and *C. septempunctata*, Tumminello *et al*. (2015) found that the native *C. novemnotata* experienced a higher death rate when grouped with *C. septempunctata* rather than a conspecific. Even though *C. novemnotata* was declining prior to the establishment of *H. axyridis* in the late 1980s*, C. novemnotata* was further impacted by resource competition and intraguild predation (Ducatti *et al*., 2017).

In the UK, there was a 41% decline in the proportion of native aphidophagous coccinellids within three years of the first record of *H. axyridis* (Brown *et al*., 2011a). Additionally, three native coccinellid species experienced a decline in numerical abundance in this timeframe and this decline continued for *A. bipunctata* over an 11-year period (Brown & Roy, 2017). The decline of *C. quinquepunctata* in central Europe has some parallels to that of *C. novemnotata* in North America, in that this decline was underway prior to the establishment of *H. axyridis* (Honěk *et al*., 2016). In contrast, *C. quinquepunctata*, although classified as rare and low in abundance, is stable in the UK (Brown & Roy, 2015; Roy *et al*., 2018). With probable negative impacts to its habitat, *C. quinquepunctata* is particularly susceptible to negative impacts from *H. axyridis* through competition for prey and intraguild predation (Roy *et al*., 2016).

Regardless of other pressures affecting native coccinellids, *H. axyridis* is a factor in how coccinellid communities have changed over recent years (Brown & Roy, 2017; Honěk *et al*., 2019a). This is concerning, given that a diverse native coccinellid assemblage delivers invaluable services to their habitat by controlling aphids, coccids and other plant-predators (Sloggett *et al*. 2008; Grez *et al*., 2014). It is possible that *C. quinquepunctata* carries out such a role for the plant community that survives on ERS. If *H. axyridis* were to become numerous or even dominant in this habitat, it is possible that, together with other invasive pressures, this habitat would become irreparably damaged thereby negatively effecting *C. quinquepunctata* and the wider community of specialised invertebrates (Sadler *et al*., 2004). Together with the specialist characteristics of *C. quinquepunctata* and the negative effects IAS can have on native coccinellids, it is important to discover if *H. axyridis* is having a negative impact on the rare *C. quinquepunctata* in the UK.

4.1.4 Aims & Hypotheses

The aim of the research presented in this chapter was to discover more about the ecology of *C. quinquepunctata* and if this nationally rare species may be at risk from *H. axyridis*.

Taking into consideration the research presented above the following hypotheses are postulated

- It was expected that *C. quinquepunctata* would be recorded but in low numbers
- It was expected that *H. axyridis* would co-occur with *C. quinquepunctata* and in higher numbers than other native species.
- Native coccinellids were expected to occur in the same habitat as *C. quinquepunctata* but in lower numbers to that of *H. axyridis*.

4.2 Methods

4.2.1 Field Sites

Field sites were identified based on where *C. quinquepunctata* had previously been recorded as well as their proximity to each other to maximise number of survey locations within the timeframe available. Within these sites, survey locations were randomly selected. Twelve sites were identified from the NBN Atlas (https://nbnatlas.org/) on the Rivers Severn, Towy, Usk and Wye in Wales where surveys were carried out (Table 4.1). In 2017, surveys were carried out in mid-June, mid-August and late-September under the same conditions as those outlined in Chapter 3 (section 3.2.2). All sites were surveyed at least twice but poor weather conditions resulted in just eight of the sites being surveyed for a third time. OS location and elevation from sea level were recorded using Garmin GPSmap 60CSx (Figure 4.2a). Written permission from each respective landowner was acquired prior to any surveying taking part.

Table 4.1: Location of all 12 sites surveyed in 2017.

Location	Codes	Grid Refs.	River
Hay-on-Wye	HW	SO22964 42815	Wye
Glasbury	GL	SO17930 39176	
Llandinam	LL01	SO02206 89053	Severn
	LL02	SO02727 89387	
	LL03	SO02562 89828	
Llandovery	LLGC	SN75424 33439	Towy /
	LLCW	SN74434 32124	Afon Tywi
The Bryn	BR01	SO33073 09419	Usk
	BR02	SO33329 09571	
	BR03	SO34237 08932	
Abergavenny	AB01	SO29276 13866	Usk
	AB02	SO29761 13667	

4.2.2 Survey methods

Coccinella quinquepunctata has adapted to hide quickly if disturbed and instead of sweep netting, a survey method known as 'direct search' was carried out (Ausden & Drake, 2006) to survey the ERS/shingle banks. Direct search is when the researcher(s) moves slowly through the habitat, to observe individuals of the target species. Direct searching of ERS was carried out for one hour (30 minutes on one occasion when two researchers were present). The search was carried out by moving from the water's edge to where the shingle bordered with grassland (became terrestrial in nature) and continued laterally over and back across the shingle (Figure 4.1). Where each survey started was dependent on time of day to ensure that the researcher's shadow did not disturb any individuals prior to observation or impair detection of individuals. The area of shingle bank searched varied due to both changeable water levels and varying vegetation density throughout the season.

The density of the vegetation on the shingle banks was assessed in broad categories based on percentage cover of the area surveyed: low (0-30%), medium (31-60%) or high (> 60%). Plant species that the target species were observed on were recorded to genera or species level. When *C. quinquepunctata* was recorded, the distance the individual was from the water's edge was recorded as was its elevation from the substrate.

Sweep-netting is a common method for surveying insects in grassland (Ausden & Drake, 2006) and was used to survey for coccinellids in such vegetation adjacent to the shingle banks (Figure 4.1). This method involves the use of a sweep net which is a white canvas bag (46 cm diameter) attached to a metal ring on a large pole. One sweep was carried out for one metre of distance walked. The net contents were checked every five metres for coccinellids, which were recorded and the net subsequently emptied. This was carried out 20 times resulting in 100 metres of grassland being surveyed at each site. Sweeping this size area took approximately 20 minutes.

All coccinellids encountered during both methods were recorded and initially identified with the aid of two Field Studies Council (FSC) guides: Guide to ladybirds of the British Isles (Majerus *et al.*, 2010) and Guide to the ladybird larvae of the British Isles (Brown *et al*., 2012). Some coccinellid species were group together to form the category 'Other' as there were too few of each species to apply meaningful analysis to. These species were *P. quattuordecimpunctata*, *T. sedecimpunctata*, *P. vigintiduopunctata* and *S. vigintiquattuorpunctata.*

Figure 4.1: Illustration of a typical site with ERS/shingle bank bordered by grassland/pasture with the start point for direct search highlighted.

4.2.3 Environmental conditions

In order to standardise data collection, surveys took place between 10:00 and 16:00 when weather conditions were favourable. Data collection was carried out when the temperature was greater than 14°C, weather conditions were dry and wind speeds were below 5 on the Beaufort scale (Met Office, 2016). Some surveys were carried out when the temperature was below 14°C, however in these instances there was at least 60% sun. Humidity and ambient temperature were recorded using an EasyLog EL-21CFR-2-LCD. Any gaps in the temperature/humidity data were generously provided by the Met Office.

4.2.4 Data analysis

The analysis was carried out using R Studio (R Core Team, 2019). As the data were count data, non-parametric tests and generalised linear models (GLM) were applied. Wilcoxon paired tests were used to compare abundances of difference coccinellid groups at the same locations, e.g. *C. quinquepunctata* and *H. axyridis* abundance on shingle. The following R packages were used for basic analyses and visualisation of data: dplyr (Wickam *et al*., 2019), ggfortify (Horikoshi & Tang, 2016; Tang *et al*., 2016), ggplot2 (Wickham, 2016), ggpubr (Kassambara, 2018). For multivariate analyses three packages were used: Hotelling (Curran, 2018), lattice (Sarkar, 2008) and vegan (Oksanen *et al*., 2019). The remaining packages used for regression analyses were: fmsb (Nakazawa, 2018), lmtest (Zeileis, 2002), pscl (Zeileis *et al*., 2008), sandwich, (Zeileis, 2004; Zeileis, 2006), lattice and MASS (Venables & Ripley, 2002).

4.2.4.1 Regression analysis

Generalised linear models (GLM) were utilised to investigate the effects of habitat (shingle or grass), season (Visit – June, August, September), coccinellid diversity (Shannon diversity) and vegetation cover (Cover) on *C. quinquepunctata* abundance. Environmental variables (temperature, humidity)

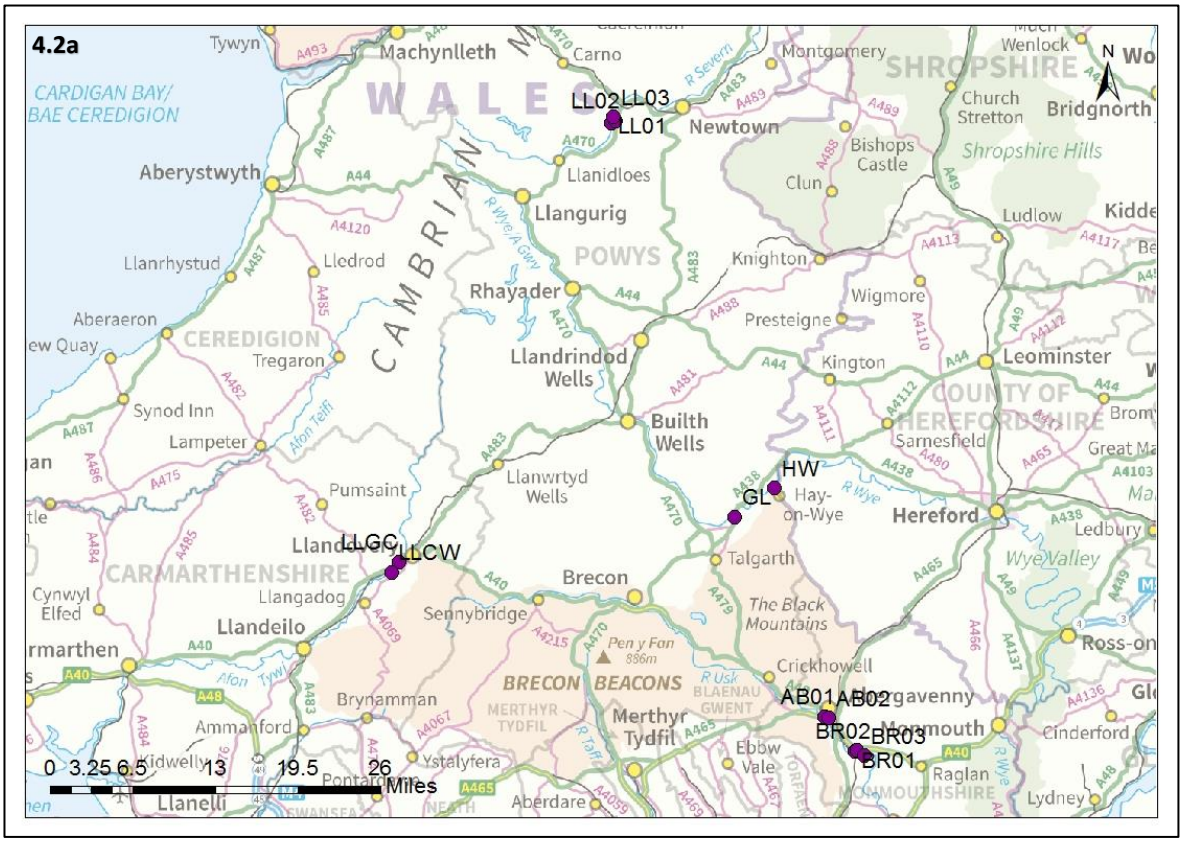
were included in the models. The regression models (poisson, negative binomial (NB), zero-inflated poisson (ZIP) model and zero-inflated negative binomial regression (ZINB) model) were applied to the data. Model selection was carried out using the same methods as those described in Chapter 3 and resulting outputs can be found in Appendix 4 (Table A4.2a – A4.7b inclusive)

4.2.4.2 Diversity Indices

Shannon diversity was calculated for shingle and grass habitat separately and only for native coccinellid species. Simpson's diversity was not carried out as this measure is not sensitive to rare species or those recorded in low numbers (Magurran, 2004; Morris *et al*., 2014) and there are instances in this dataset where there are a number of species recorded in low numbers. Differences in diversity across sites types and season were calculated using t-tests while ANOVA was used to assess any differences in diversity within the vegetation structure followed by a post-hoc Tukey, if any significances were apparent. Regression models were applied to determine if native coccinellid diversity had any effect on the abundance of *C. quinquepunctata* and *H. axyridis*.

4.3 Results

In 2017, nine coccinellid species were recorded at 12 river sites in Wales with 687 individuals being recorded across both the shingle and grass habitat types (Appendix A4.1). *Coccinella quinquepunctata* was present at all sites, while *H. axyridis* was only recorded at seven of the 12 sites surveyed and was only more abundant than *C. quinquepunctata* at one site (Figure 4.2). A significantly greater number of *C. quinquepunctata* were recorded on the shingle habitat in comparison to the grass habitat ($z = 6.72$, $p < 0.0001$), however there was no such difference when comparing *H. axyridis* abundance at both habitat types (Figure 4.3). Coccinellid diversity was higher in the grass habitat than the shingle, however not significantly so.



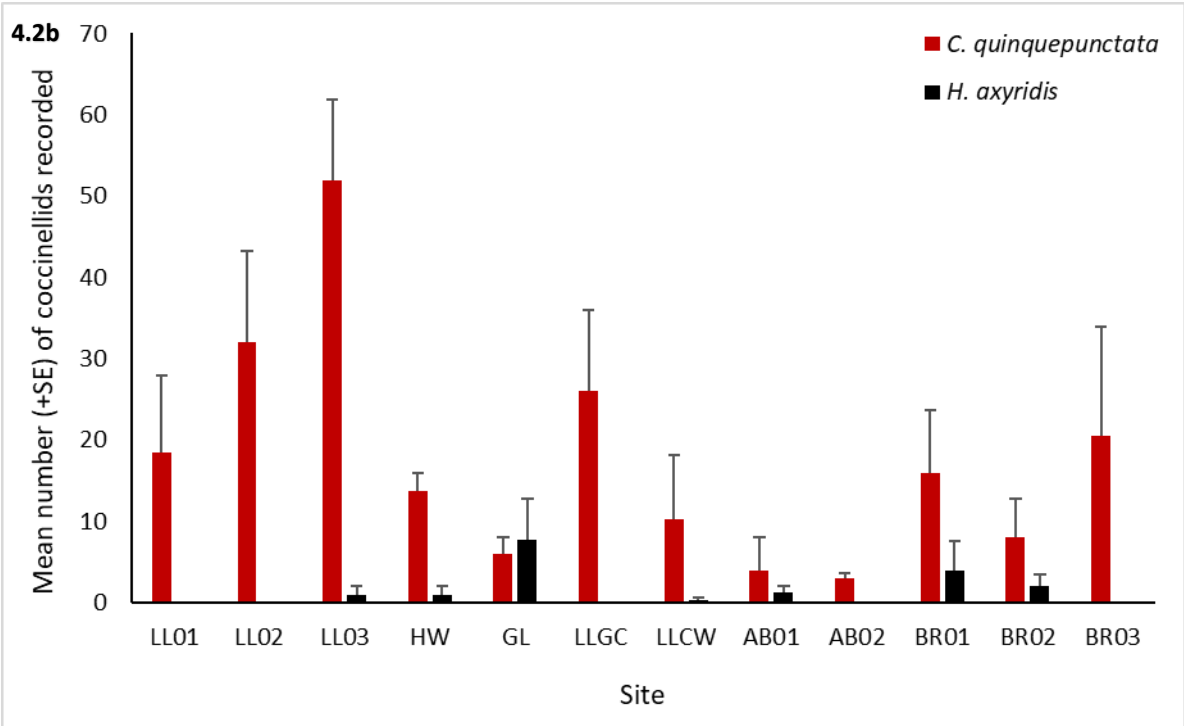


Figure 4.2a & b: Map of survey sites along with mean number (+SE) per site visit of *Coccinella quinquepunctata* and *Harmonia axyridis* recorded at each site from both survey methods combined in 2017. Sites: AB01 & AB02 = Abergavenny site 1 & 2; BR01, BR02 & BR03 = Bryn sites 1,2 & 3; GL = Glasbury; HW = Hay-on-Wye; LL01, LL02 & LL03 = Llandinam sites 1,2 & 3; LLCW = Cwmgwyn Farm; LLGC = Llandovery

4.3.1 Shingle habitat

Six species of coccinellid were observed by direct searching (DS) of the shingle bank habitat. In total, 592 coccinellids were observed by DS with the vast majority (77%) being *C. quinquepunctata* (Figure 4.3). The second most abundant coccinellid on shingle was *A. bipunctata* (10.5%) with *H. axyridis* (7%) being the third most abundant species. The abundance of *C. quinquepunctata* was significantly greater than that of *H. axyridis* on shingle habitat as revealed by the Wilcoxon signed-rank test ($Z = -4.32$, $p < 0.0001$) (Figure 4.3). Significantly more *C. quinquepunctata* were recorded in June ($z = 2.57$, $p = 0.01$) as opposed to in August and September (Figure 4.4). Coccinellid diversity had no effect on *C. quinquepunctata* numbers on shingle, nor did vegetation cover. There was no effect of season or vegetation cover on *H. axyridis* abundance on shingle banks. However, the reduced model revealed that *H. axyridis* abundance was higher when coccinellid diversity was higher ($z = 4.71$, $p < 0.0001$) on shingle.

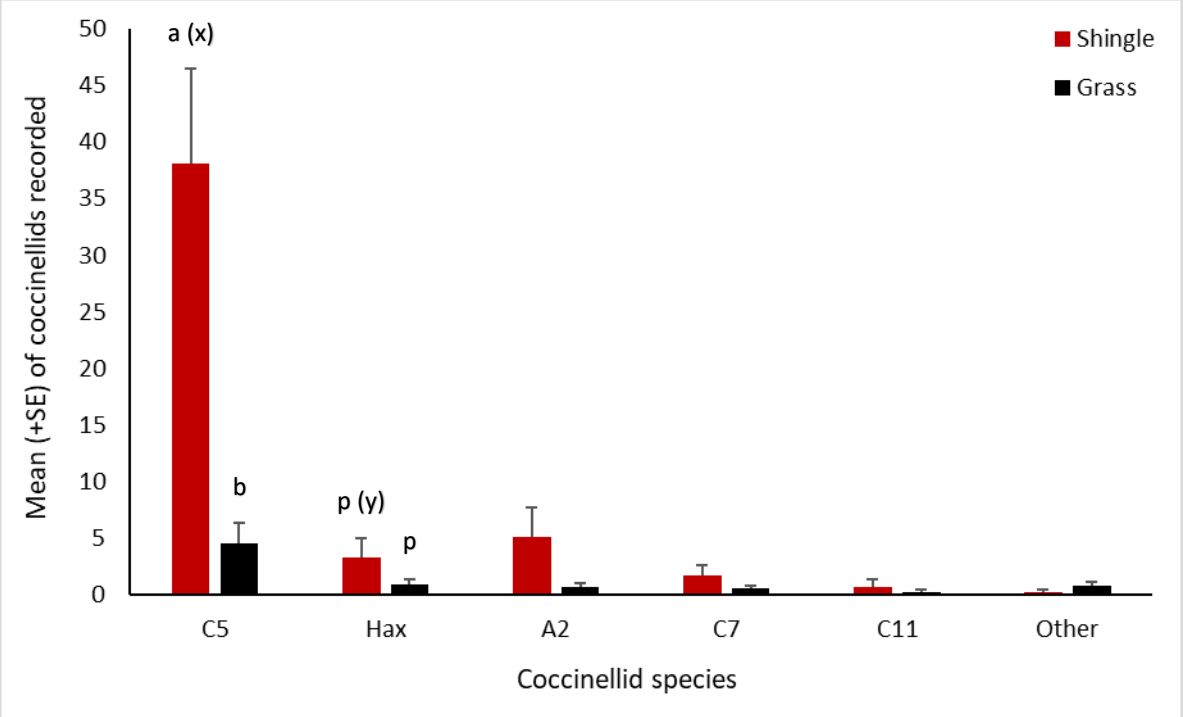


Figure 4.3: Mean number (+SE) per site visit of coccinellids recorded on ERS and grassland in Wales in 2017. C5 = *Coccinella* quinque*punctata*; Hax = *Harmonia axyridis;* A2 = *Adalia bipunctata*; C7 = *Coccinella septempunctata*; C11 = *Coccinella undecimpunctata*; Other = *Propylea quattuordecimpunctata, Psyllobora vigintiduopunctata, Subcoccinella vigintiquattuorpunctata* & *Tytthaspis sedecimpunctata.* Consecutive letters indicate where significant differences occur for each test, i.e. a/b, p/q or x/y are three different tests.

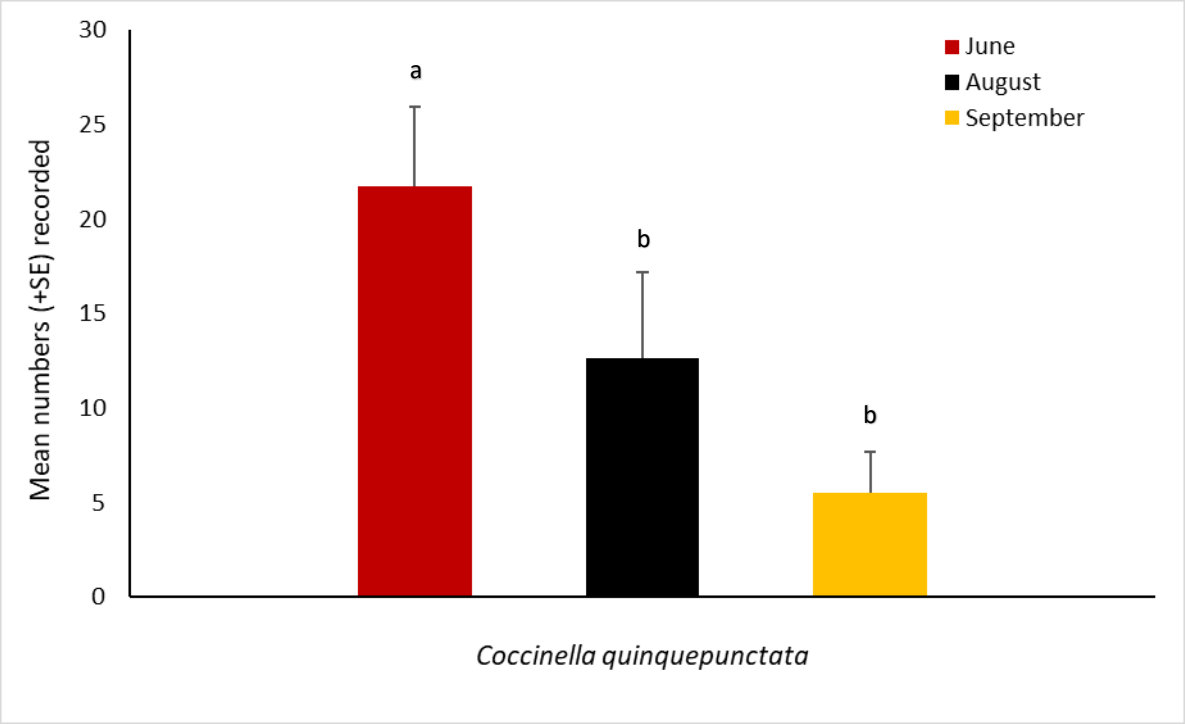


Figure 4.4: Mean number (+SE) per site visit of *Coccinella quinquepunctata* recorded on shingle only at 12 sites in Wales over three visits in June, August and September 2017. Consecutive letters indicate where significant differences occur.

4.3.2 Grassland habitat

Of the 95 coccinellids recorded in grass adjacent to the shingle banks, the majority again were *C. quinquepunctata* (58%). This habitat had a higher species richness than the shingle with nine species of coccinellid recorded. This difference was due to the presence of specialist coccinellids that are only found in grassland habitat (*Psyllobora vigintiduopunctata, Subcoccinella vigintiquattuorpunctata* and *Tytthaspis sedecimpunctata*). There were significantly more *C. quinquepunctata* recorded in grassland than *H. axyridis* (Z = -2.728, p = 0.02) (Figure 4.3). In contrast to the ERS habitat, abundance of *C. quinquepunctata* was higher when coccinellid diversity was higher (z = 2.99, p = 0.002) in the grassland.

4.3.3 Distance and Elevation

Coccinella quinquepunctata was found at a range of distances from the water's edge. Significantly fewer individuals were recorded further from the water's edge at 16-20 metres and 26-30 metres (z = -2.76, p = 0.006 & z = -3.51, p = 0.0004 respectively). The numbers recorded at the other three distances were also lower but not significantly so. (Figure 4.5).

Coccinella quinquepunctata was observed at various heights from the shingle substrate, but was found more frequently at lower elevations with significantly fewer individuals recorded above three-quarters of a metre from the ground (z = -2.85, p = 0.004) (Figure 4.6).

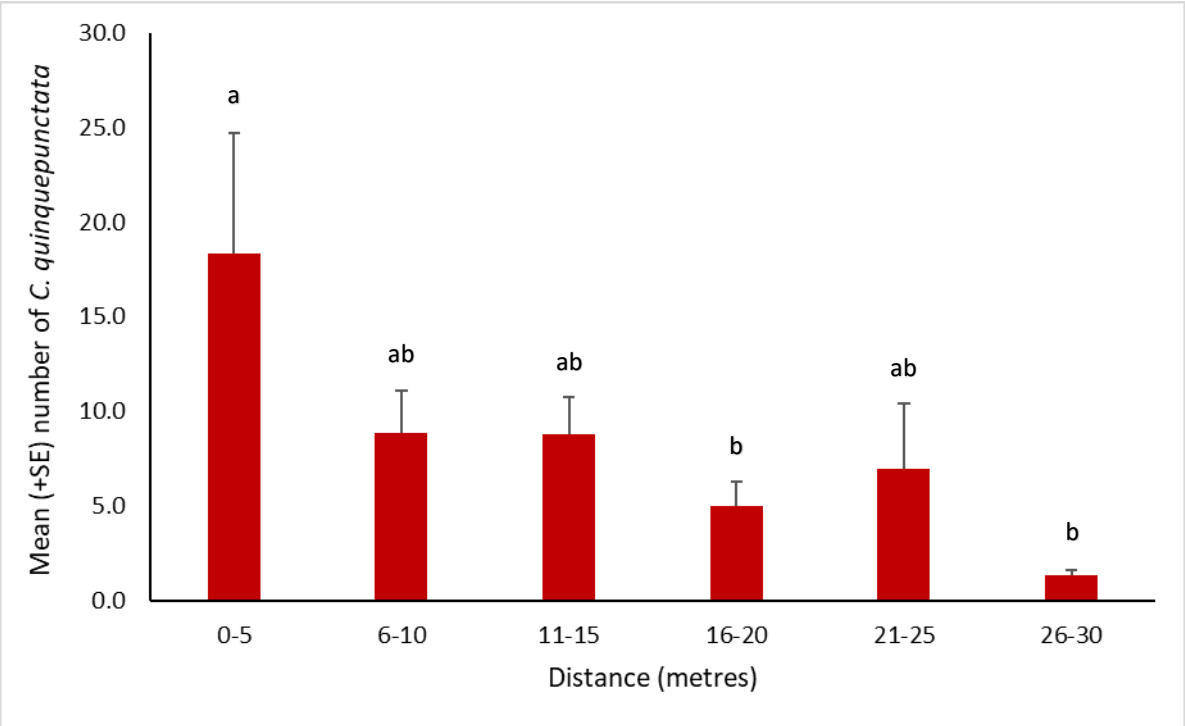


Figure 4.5: Mean number (+SE) per site of *Coccinella quinquepunctata* and the distance (in metres) from the water's edge they were recorded at in 2017. Consecutive letters indicate where significant differences occur.

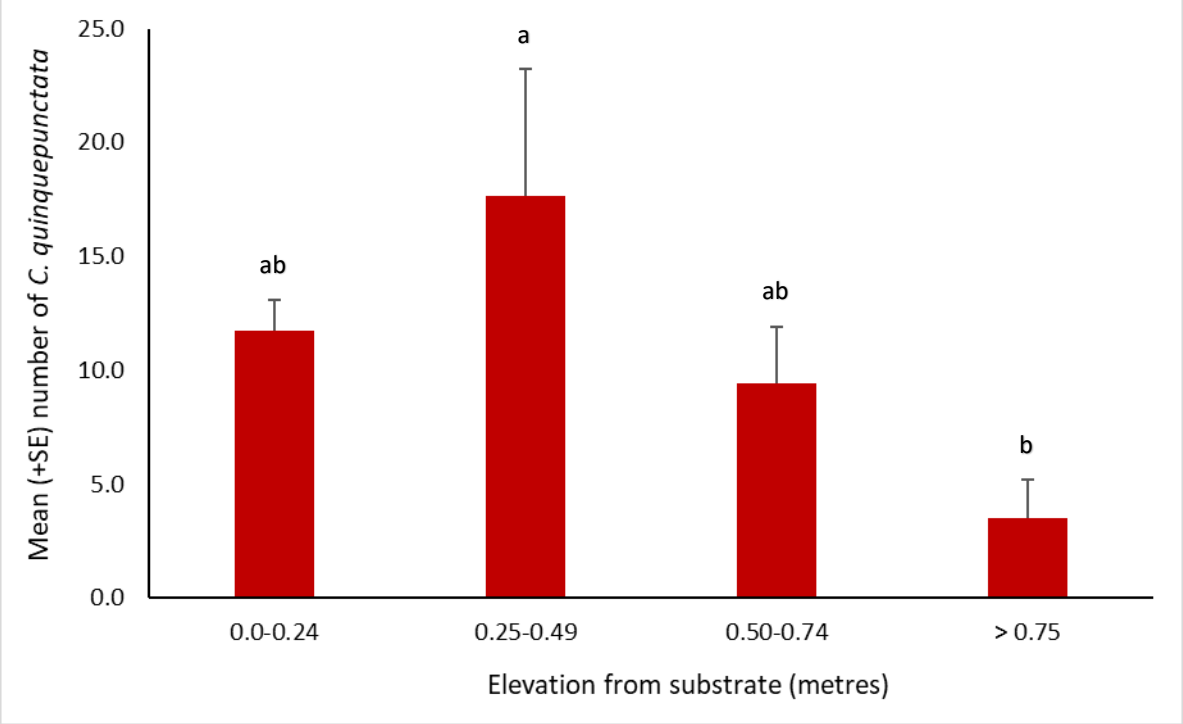


Figure 4.6: Mean number (+SE) per site of *Coccinella quinquepunctata* and the distance (in metres) from the substrate they were recorded at in 2017. Consecutive letters indicate where significant differences occur.

4.4 Discussion

4.4.1 *Coccinella quinquepunctata*, *Harmonia axyridis* and other coccinellids

Coccinella quinquepunctata was recorded in higher numbers than expected during the course of this research and more *C. quinquepunctata* were observed than *H. axyridis* on both the shingle bank and grassland habitat. The habitat and generally rural nature of the habitat is likely to be less suitable for *H. axyridis* and consequently a refuge for *C. quinquepunctata.* The low number of *H. axyridis* was surprising, however this species has a well-documented preference for urban habitats (Adriaens *et al*., 2008, Purse *et al*., 2014, Roy & Brown 2015; Viglášová *et al*., 2017) and in this case, all sites surveyed were in rural areas or on the edge of small rural villages. Urban and anthropogenic habitats are more suitable for *H. axyridis* by providing secure overwintering sites in buildings (Roy *et al*., 2011; Roy *et al*., 2016). Furthermore, the exposed riverine sediment (ERS) is a unique habitat with sparse vegetation stands where aphid numbers are perhaps too low to sustain a predator such as *H. axyridis*. Honěk *et al*. (2018b) reported an increase in *H. axyridis* numbers when aphid numbers increased but also with an increase in the level of urbanisation. Considering the lack of overwintering sites and reduced prey availability, it is interesting that *H. axyridis* was recorded at all. Native coccinellid species were present in both habitats and *A. bipunctata* was present on ERS in greater numbers than *H. axyridis*, albeit not significantly so. The overall low number of other coccinellid species recorded further reiterates that ERS is not a particularly suitable habitat for most coccinellids.

In the grass habitat the low number of *H. axyridis* mirrored that of the overall number of coccinellids. Despite the low number of coccinellids in grassland, there was a greater diversity of native coccinellids in this habitat than on the ERS. Even though this was most likely due to the presence of three grass-specialist coccinellid species, the difference was not significant. The time spent searching grass habitat was just less than half the time spent searching on ERS, which partially accounts for the lower numbers recorded. Additionally, it is not possible to directly compare the two sampling techniques. The number of *C. quinquepunctata*, however, was higher when coccinellid diversity was higher in the grass habitat only. This could be due to a very low number of *H. axyridis* recorded in the grass habitat. However, it is more likely that coccinellid diversity and abundance was higher where the habitat was less managed or disturbed thereby creating a more suitable habitat for coccinellids (Diepenbrock & Finke, 2013; Grez *et al*., 2014; Honěk *et al*., 2014). Further investigation into coccinellid diversity and the heterogeneity/disturbance of the habitat adjacent to ERS would reveal more about the interaction between *C. quinquepunctata* and other coccinellids as well as the native coccinellid community.

Given that *C. quinquepunctata* was considered extinct in the UK just over 30 years ago, it was interesting to find that this species was the dominant coccinellid at both habitat types. Prior research indicated that *H. axyridis* would represent a negative pressure for this species. When carrying out laboratory trials to determine rates of intraguild predation (IGP), *H. axyridis* was the dominant predator when placed with *C. quinquepunctata* (Ware & Majerus, 2008). Thus, it is likely that should IGP occur, it would have a negative effect on *C. quinquepunctata*. However, the results in this study indicate that habitat separation on ERS limit the interactions of these two species thereby limiting the opportunities for IGP. In North America, pressure in the form of IGP and competition for resources from *H. axyridis* exacerbated the situation with *C. novemnotata*, which is found in only a small number of states and those numbers are greatly reduced (Ducatti *et al*., 2017). In Europe, *C. quinquepunctata* had been considered an abundant habitat generalist, however, this decline in abundance was evident prior to presence of *H. axyridis* (Honěk *et al*., 2016). It is thought that changes in land use together with the intensification of agricultural practices has impacted *C. quinquepunctata* in the Czech Republic (Honěk *et al*., 2016). The results here indicate that *H. axyridis* is not currently impacting *C. quinquepunctata* negatively, due to very low abundance of the former, however, a combination of other pressures may negatively impact this nationally rare species.

4.4.2 Distance & Elevation

Less *C. quinquepunctata* were found further from the river's edge. This is surprising given that water levels can rise surprisingly quickly at such locations, which would not necessarily be a problem for adults but would be for eggs and pupae and possibly for larvae as well. However, the vegetation that *C. quinquepunctata* was recorded on the majority of the time was thistle (*Cirsium* spp.) which tends to reach a maximum height of approximately one metre. Moreover, a greater number of individuals were recorded above ground level, between a quarter and a half a metre from the substrate. This plant species is architecturally quite stable and even if the water levels rise quickly (section 4.1.2), individuals that cannot fly or reach drier parts of the shingle are likely to find refuge higher up on the vegetation for the duration of the high flow period. It is possible that *C. quinquepunctata* is closer to water to evade potential IGP or competition for resources from other native coccinellids such as *A. bipunctata* and *C. septempunctata*.

Considering the unstable nature of the habitat in terms of structure but also climatically, *C. quinquepunctata* appears to have adapted well to ERS. Adaptations in ground beetles (Carabidae)

that enable them to move more easily in ERS have been observed (Fowles, 1988). Both adult and larval *C. quinquepunctata* move differently to other coccinellid species and tend to scuttle/run quickly across the shingle while if disturbed on vegetation, individuals will drop to the shingle and disappear very quickly (pers. obs.). This behaviour is most likely an adaptation to life in an unstable and exposed habitat such as ERS (Sadler *et al*., 2004).

4.4.3 Vegetation cover

The density of vegetation cover on the shingle had no effect on the abundance of *C. quinquepunctata*, however there were instances when one site had yielded high numbers but subsequent visits resulted in considerably lower numbers with the only difference being that the vegetation density was considerably higher than at the time of the first visit. During surveys in Scotland, Littlewood (2015) reported finding greater numbers on shingle with less vegetative cover. Given that the majority of plants that *C. quinquepunctata* was recorded on and the ability of this species to disperse at speed, it is possible that this could be a result of an issue with the survey method. When sampling in Wales, it was difficult to move across the shingle during a search without brushing against the vegetation when it was present at high density. Considering the reaction of *C. quinquepunctata* when disturbed, it is not surprising that less individuals could be recorded in densely vegetated areas of the shingle. Alternatively, it may be that ERS that is sparsely vegetated is warmer. Exposed riverine sediment is an exposed habitat and heats up quickly (Bates *et al*., 2009). Given the high temperatures in central Europe where *C. quinquepunctata* has been more frequently observed, it may be that ERS is the only thermally suitable habitat for this species in the UK. Furthermore, *Cirsium* spp. were the dominant taxonomic group on ERS and was the vegetation that *C. quinquepunctata* were most likely to be recorded on. Even though it is a dietary generalist (Hodek & Evans, 2012), little is known about the specific dietary requirements of *C. quinquepunctata.* In Europe it is found in cereal stands with low aphid numbers (Honěk *et al*., 2016) as well as on wild flower or grass stands that are well populated by aphids (Honěk *et al*., 2014). Meanwhile, in the UK, *C. quinquepunctata* is found on *Cirsium* spp. (thistle), *Urtica dioica* (stinging nettle) and *Cytisus* spp. (broom) (Majerus & Fowles, 1988). Some *Cirsium* spp. have several phenologically different aphid species as predators (Völkl, 1989) which, combined with the persistence of *Cirsium* spp. on ERS and the higher temperature of this habitat, could explain why *C. quinquepunctata* has a preference for this unstable habitat in the UK. Research into specific prey preferences and/or requirements of *C. quinquepunctata* would reveal more about why this species is so specialist in terms of its habitat preferences in the UK as well as increase our understanding of this species' ecology.

4.4.4 Additional pressures

The main finding here, that *H. axyridis* does not appear to be impacting *C. quinquepunctata* in Wales, was unexpected. However, there are pressures that negatively impact the ERS which in turn are likely to have a subsequent effect on *C. quinquepunctata*. The reason for the RDB3 (Rare) categorisation of this species is due to the habitat where it is found being at risk. There are several threats to this habitat; invasive plant species, livestock access to shingle banks, gravel extraction and river modification (Fowles, 1988; Bates *et al*., 2007a; Hewitt *et al*., 2010). Although IAS represent a considerable pressure, they are not solely responsible for the decline of native species. There are instances where IAS take advantage of a system that is already vulnerable and when removed from the habitat in question, native species that were in decline do not recover as expected, thereby indicating that IAS are not always the drivers of change (Vitousek *et al*., 1997; Didham *et al*., 2005). Multiple IAS and various anthropogenic activities together culminate into drivers of change (Vitousek *et al*., 1997), and being clear on which factor happens to be the greatest threat will facilitate effective conservation plans for native species (Majerus *et al*., 2016).

More than one invasive plant species was identified on or near the shingle habitat (e.g. Japanese knotweed, *Fallopia japonica*; monkey flower, *Erythranthe guttatus*), however, the species most likely to have the greatest negative and immediate impact is *I. glandulifera* (Himalayan balsam). Seven of the 12 sites surveyed here had established stands of *I. glandulifera* present. This species potentially impacts *C. quinquepunctata* in two ways. Firstly, *I. glandulifera* changes the microbial community of the soil which prevents native plant species from taking root (Pattison *et al*., 2016), thereby homogenising the ERS plant community. During surveys, neither aphids nor any coccinellid species were seen on *I. glandulifera* plants (pers. obs.). This is not surprising, given that Tanner *et al*. (2013) reported a reduction in coccinellid numbers on areas invaded by *I. glandulifera* in comparison to non-invaded areas, which is likely due to this species not having any natural enemies in its invaded range (Tanner *et al*., 2014). Considering the significantly reduced abundance of *C. quinquepunctata* in the grassland adjacent to the shingle, the potential and inevitable lack of native plant species, as a result of *I. glandulifera*, providing a source of prey for *C. quinquepunctata*, could see the species become locally extinct in areas where *I. glandulifera* is not adequately controlled. If this habitat becomes too stable, vegetation succession becomes an issue and the specialised invertebrate community is negatively affected (Sadler *et al*., 2004). On the other hand, *I. glandulifera* de-stabilises the shingle bank as it has shallow roots and the soil around it becomes more fragmented, so when the rivers are in flood, considerably more substrate than usual will be removed. The ERS is in a constant state of flux (Fowles, 1994), however, this increased pressure is

likely to have an adverse effect not just on *C. quinquepunctata* but also the many other invertebrates (many of which are also nationally rare) that inhabit the shingle (Sadler *et al*., 2004). Livestock regularly have access to the shingle bank for water and will also graze on the bank. This is likely to have a negative impact on *C. quinquepunctata* due to the additional disturbance of the ERS, given this species reliance on this habitat type. Bates *et al*. (2007a) determined that trampling by livestock reduced the conservation value of the beetle assemblages on river shingle. However, a small number of sites in this research, that were grazed by sheep during the entire field season, yielded the highest number of observations of *C. quinquepunctata*. These sites also were clear of *I. glandulifera* and Day (2015) reported that grazing can be used to help control *I. glandulifera* successfully. Nevertheless, this IAS can be readily removed by hand and uncontrolled livestock access is more likely to be negative rather than a positive influence for ERS. Additionally, if the beetle and wider invertebrate assemblage of the shingle is compromised, this could have a knock-on negative effect on *C. quinquepunctata*.

One of the sites in this study had gravel extracted from it just prior to the final survey. This process resulted in complete removal of the vegetation and a large layer of the shingle bank. This site was the closest site to an urban area and in addition to the gravel extraction, the vegetation was highly managed throughout the entire survey period. *Coccinella quinquepunctata* was present at the site but in lower numbers than elsewhere. If the vegetation had not been cut back so severely and so frequently, it is possible that a greater number of *C. quinquepunctata* would have been recorded. This degree of disturbance to the shingle habitat and adjacent grassland mainly as a result of gravel extraction is a serious concern for *C. quinquepunctata* and other shingle-dwelling invertebrates (Sadler *et al*., 2004; Bates *et al*., 2007b). This level of disturbance is especially concerning, considering that after river system modification took place, it was reported that all trace of ERS had disappeared from the midlands and south east of England (O'Callaghan *et al*., 2013).

In Europe, *C. quinquepunctata* is in decline, however, in the UK, the species is stable (Roy *et al*., 2018) and the numbers recorded during this research indicate this to be the case. However, several coccinellid species have declined in recent years (Roy *et al*., 2018) and not necessarily solely as a result of the increased presence of *H. axyridis*. Like all insects, coccinellids rely on an external source of heat and so are more sensitive to changes in temperature (Facey *et al*., 2014). It is possible the decline in many UK species as well as the increase in *C. quinquepunctata* numbers (and other native coccinellids e.g. *Hippodamia variegata*, Adonis ladybird) is due to an increase in temperature in the UK over recent decades (Carrington, 2020). Herbivorous insects such as aphids are particularly sensitive to temperature and could decline with a combination of continued increases in temperature as well as due to climatic induced changes to their food source (Clissold

& Simpson, 2015). This would impact *C. quinquepunctata* and other aphidophagous coccinellids negatively and the expectation is that at the very least, the coccinellid community will change and, in certain cases, some coccinellid species numbers may decline (Honěk *et al*., 2017). However, there are numerous pressures on ecological communities and what is clear is that no one particular pressure is solely responsible for biodiversity decline (Harvey, 2015) and different components of each individual community will react in their own way to these pressures (Stewart *et al*., 2015). Furthermore, should temperatures continue to rise, particularly in winter months, overwintering for coccinellids may be interrupted thereby resulting in fewer individuals surviving to the breeding season, resulting in an overall decline of coccinellids (Alaniz et al., 2020).

4.4.5 Further work

In European countries where *C. quinquepunctata* is relatively abundant (at least until recent years), there seem to be drier summers with higher temperatures. Wales and Scotland are quite dissimilar in these terms, but on open ERS, the temperature can get quite high in comparison to nearby grassland (Bates *et al*., 2009). This species may be highly phenotypically plastic and capable of adapting where optimal conditions are not present. This plasticity would further enable the species to adapt its movement for survival in this inhospitable habitat, like other invertebrates inhabiting ERS have done (Sadler *et al*., 2004). In the US, *Coccinella novemnotata* has exhibited morphological changes since the establishment of *C. septempunctata* indicating a degree plasticity (Losey *et al*., 2012b). Evolutionary ecology studies would help in learning more about how *C. quinquepunctata* has adapted to survive in such a marginalised habitat in the UK as well as indicating how well the species may fare in the face of climate change. Additionally, molecular analysis into any genetic variation between the UK populations of *C. quinquepunctata* and those in other European countries (e.g. Czech Republic, Slovakia, Netherlands) would possibly reveal more information concerning this unusual habitat choice for *C. quinquepunctata*.

Continued monitoring of *C. quinquepunctata* (both in Wales and Scotland) is necessary to detect any future changes in the population. In the event of a decline in numbers, the continued monitoring of *H. axyridis* would further inform researchers if the IAS started to have an effect on *C. quinquepunctata* or if a different pressure may be having a negative impact. Monitoring will also help determine a more complete distribution in the UK. Investigation of any differences between urban and rural areas would further increase the small volume of knowledge on this species, as it is likely that *C. quinquepunctata* would be less inclined to inhabit urban areas given the disparity in abundance between it and *H. axyridis*. Given that ERS is an important riparian habitat that is also

terrestrial in nature, additional studies into how the specialised invertebrate community contribute to ecosystem function would help bridge the gap between aquatic and terrestrial ecology in the UK. Finally, considering the specialist habitat preference of *C. quinquepunctata,* and numerous other rare invertebrate species, it would be prudent to designate habitat protection status on ERS in order to control livestock access, prevent gravel abstraction, river channel modification and initiate restoration or enhancement of the habitat where it has been damaged or removed.

4.5 Conclusion

This work adds to the small volume of knowledge on *C. quinquepunctata*. It is evident that *C. quinquepunctata* is doing well in terms of abundance in Wales and is relatively unaffected by *H. axyridis* through IGP or resource competition. The RDB3 Rare categorisation is justified for *C. quinquepunctata* considering the multiple pressures effecting ERS. However, if this unique habitat continues not to be properly protected, then *C. quinquepunctata* is likely to decline to the point of extinction in the UK.

5 Differentiating between coccinellid species using a molecular method

5.1 Introduction

5.1.1 Molecular ecology

The field of molecular biology has advanced significantly assisting in answering evolutionary and ecological questions generally unanswerable by observation alone. Polymerase chain reaction (PCR) is the method used to amplify the DNA regions or loci of interest, resulting in so many copies that the product can then be observed/visualised using electrophoresis. Another form of PCR is quantitative or real-time PCR (qPCR) for which there are two different methods. One method uses a non-specific dye, such as SYBR Green, together with a pair of primers. When SYBR Green binds to the double stranded DNA (dsDNA), it fluoresces. Therefore, the more DNA there is, the more dye can bind to it and the greater the fluorescence (Rowe *et al*., 2017). A melting curve analysis can be done after to differentiate between sequences by length or composition (Rowe *et al*., 2017). The second qPCR method is more specific and uses a fluorescent probe together with a primer pair. The probe attaches to a specific area of the sequence and will only fluoresce if it has successfully attached to the complementary sequence it was designed to match.

Primers are essential when synthesising DNA and PCR cannot operate without them. They tend to be between 15-25 bp long and are specific to a target section of sequence of DNA (Rowe *et al.,* 2017). Primers can be relatively general in the sequence they target, for example, a primer can be designed to detect insects only in an owl pellet to determine if insects make up part of their diet. Primers can also be quite specific, being able to detect just a known sequence of a species, for example, to detect coyote DNA in wolf scat to answer the question do wolves predate and eat coyotes. The use of primers is wide-ranging in molecular analysis and the ecological method termed "DNA barcoding" also requires primers (Rowe *et al.,* 2017).

5.1.2 DNA Barcoding

DNA barcoding is the identification of a specific species or group of organisms by using short sequences from a specific section of the genome, such as COI or 18S (Deagle *et al.,* 2014). The proposed locus choice for animals was COI, however, while this region seemed suitable for many taxa, there were several taxonomic groups where it was not possible to use COI (Deagle *et al*., 2014). For example, when targeting COI in nematodes, the amplification was regularly inconsistent mainly due to very high mutation rates of the nematode mitochondria which in turn led to struggles

in primer design (Creer *et al*., 2010). As a result, there is currently no consensus as to which locus is best to use (Lawson Handley, 2015), however many animal focussed studies continue to use COI.

5.1.3 Environmental DNA

Environmental DNA (eDNA) is any DNA that has been released by an organism into its environment, for example in faeces, shed hair or skin, exuviae, pollen, etc. (Valentini *et al*., 2016). With the refinement of molecular techniques, the use of eDNA was expected to make possible the detection of cryptic or rare species, especially in areas where it has perhaps been perceived they did not inhabit (Lawson Handley, 2015). The process of identifying all the taxa present (or taxa from a specific group of interest) from an environmental sample, such as river water, is known as metabarcoding and utilises regions of approximately 600 bp (Deagle *et al*., 2014). In the last 15 years or so, researchers have investigated increasingly degraded DNA such as that found in faecal samples or gut contents of invertebrates (Symondson, 2002; Hoogendoorn & Heimpl, 2003; Dodd, 2004; Sloggett *et al*., 2009) (see Section 5.14 for details). This was made possible when, instead of targeting longer fragments of 500-800 bp, shorter fragments of between 90-250 bp were targeted, called mini-barcoding, which allowed the amplification of DNA in degraded samples (Chen, 2000). Following from this discovery Hänfling *et al.* (2016) suggested smaller fragments be used when detecting prey during gut content analysis and found that more species could be detected using 12S (~100 bp) as opposed to CytB (~460 bp) due to larger fragments being broken down more quickly during the digestion process (Symondson, 2002). When using species-specific primers, standard PCR can be used to analyse eDNA, however quantitative (qPCR) is becoming increasingly popular as it is more sensitive when DNA concentrations are very low (Freeland, 2016).

5.1.4 Prey detection

Detecting what a predator consumes can be achieved by observing the predation act itself or by microscopically going through gut content (Chen, 2000). However, less invasive and less time-consuming methods have been investigated in the last two decades. Protein electrophoresis identifies different enzymes by producing different bands depending on the enzyme, however it is unable to determine if more than one species has been consumed (Symondson, 2002). Enzyme linked immunosorbent assays (ELISA) have frequently been used in studies investigating prey detection in predator gut. This method utilises monoclonal antibodies and a resulting colour change to determine/identify the prey (Symondson, 2002; Dodd, 2004; Aebi *et al*, 2011). This method, however, is costly and there are some issues with cross-reactivity (Symonds, 2002; Aebi *et*

al., 2011). Gas chromatograph-mass spectrometer (GC-MS) can also be used in prey detection (Gladyshev *et al.,* 2000) however it is expensive and can rarely identify prey to species level (Aebi *et al*., 2011). In the last decade or so, molecular methods have been employed to determine prey species in gut or faecal samples. A series of early studies successfully used DNA-based methods to successfully detect Collembola (springtail) species in Linyphidae (spiders) (Augusti *et al*., 2003), aphids in Chrysopidae (lacewings) (Chen *et al*., 2000), Lepidoptera (butterfly) prey in Coccinellids (ladybird) (Hoogendoorn & Heimpel, 2001) and Arionidae (slug) species in Carabidae (ground beetle) species (Dodd, 2004). These molecular methods are also proving effective in the detection of intraguild predation (IGP) from gut content analysis as species-specific markers can be designed to detect prey.

5.1.5 Intraguild predation

Intraguild predation (IGP) occurs when the competition between two predators of the same prey results in either one of these predators preying on the other (Polis *et al*., 1989). IGP generally occurs if there is a scarcity of usual prey items and can result in a considerable decrease of the intraguild prey species (Holt & Polis, 1997). More often the scenario results in the smaller of the predators becoming prey as documented by Fedriani *et al*. (2000) when they found coyote to prey on both gray foxes and bobcats but not vice versa. IGP has been documented in several species, specifically invertebrates; larval Cerambycidae (longhorn beetle) as predators of Scolytidae larvae (bark beetle) (Dodds *et al*., 2001); *Dikerogammarus villosus* predation on *Gammarus* species (MacNeill & Platvoet, 2005); Carabidae predating Linyphidae species (Davey *et al*., 2013); *Harmonia axyridis* predating on both *Adalia* spp. (Brown *et al*., 2015). Many occurrences of IGP often transpire when an invasive alien species (IAS) is predating on a native species, perhaps in the same family or genus. In addition to this pressure, IAS also affect native species by competing for resources. One such instance of an IAS impacting native species in this manner is *H. axyridis* (Brown *et al*., 2015). The impact this species can have on native generalist coccinellids is severe and there is concern that specialist coccinellids could be at greater risk (Majerus *et al*., 2016).

5.1.6 *Coccinella quinquepunctata* – Five-spot ladybird

Coccinella quinquepunctata (five-spot ladybird) is a medium-sized ladybird that was considered extinct in the UK until 1987 (Majerus & Fowles, 1988). Whilst more generalist in some other parts of its range, in the UK this species is usually recorded in a restricted habitat of unstable river shingle (Roy *et al*., 2011). The river banks that *C. quinquepunctata* is recorded on are in constant flux and

as a result, the invertebrate community are adapted to the unpredictability of such habitats (Sadler *et al*., 2004). When *C. quinquepunctata* was rediscovered, a small number of studies were undertaken to reveal the characteristics resulting in good habitat for this species (Majerus & Fowles, 1988) (See Chapter 4.1 for a more in-depth description of *C. quinquepunctata* in the UK).

The three short studies cited above constitute most of the research done on *C. quinquepunctata*, which shows that relatively little is known about one of the UKs rarest and most specialist ladybird species. Given the RDB3 classification of *C. quinquepunctata*, and how *H. axyridis* can so negatively affect other native coccinellids (see Section 5.1.8), it is essential to discover what effect the presence of *H. axyridis* may be having on this specialist coccinellid (Roy *et al*., 2016).

5.1.7 *Harmonia axyridis* – Harlequin ladybird

Harmonia axyridis (Harlequin ladybird) is a large ladybird native to Asia and often used as a biocontrol method for the control of aphids (Iperti, 1999). Because of its success as biocontrol, it has been used extensively on most continents resulting in population explosions to the point that it is now considered a global IAS (Majerus *et al*., 2006 a/b; Brown *et a*l., 2008/2011; Roy *et al*., 2016/2012/2014; Rondoni *et al*., 2014). *Harmonia axyridis* outcompetes native coccinellids and other insects, such as lacewings, for prey. As previously mentioned, this species is an intraguild predator of other coccinellids and lacewings and is implicated in the decline of some species (*Adalia bipunctata, Coccinella septempunctata, Propylea quattuordecimpunctata*) in the last two decades (Brown *et al*., 2011a). It has been suggested that this decline is in part due to IGP by *H. axyridis* (Brown & Roy, 2018). Thomas *et al*. (2013) successfully developed specific markers for *Adalia* species and revealed that *H. axyridis* was indeed a predator of these species in the UK. Following from this success, Brown *et al*. (2015) investigated IGP by *H. axyridis* in five European countries and also determined that whilst IGP occurred in France, Slovakia and the Czech Republic, *A. bipunctata* was not detected as prey in these countries whereas *A. decempunctata* was. Additionally, Rondoni *et al*. (2015) revealed IGP of *A. bipunctata* by *H. axyridis* in Italy, but at a lower occurrence to that in the UK with *Oenopia conglobata* (a coccinellid species not found in the UK) being detected more frequently that *A. bipunctata*. In China, Yang *et al*. (2017) successfully detected IGP between *H. axyridis* and *C. septempunctata*. From the evidence, it is apparent that *H. axyridis* is a top intraguild predator and it is possible that this species is a serious threat to rare coccinellids such as *C. quinquepunctata*.

5.1.8 Aims & hypotheses

This study aimed to design a molecular marker specific for *C. quinquepunctata,* which could enable the detection of the species as well as being able to differentiate it from other coccinellid species, including *H. axyridis.* With such a molecular marker it would be possible to investigate if *H. axyridis* preys upon *C. quinquepunctata* by analysing the gut content of *H. axyridis* collected in or near *C. quinquepunctata* habitat. It was hypothesised that:

- enough mutations unique to *C. quinquepunctata* would be found to allow the development of species-specific primers.
- With these species-specific primers, it would be possible to determine if intraguild predation occurred between *C. quinquepunctata* and *H. axyridis.*

5.2 Methods

5.2.1 Field Collection

Native coccinellids were collected for molecular analysis during field data collection at Welsh field sites in 2017 (Chapter 4.2). A direct search was carried out on shingle banks to assess *C. quinquepunctata* numbers in their specialised habitat. This survey method of the shingle bank was carried out for one hour (30 minutes if two researchers present). A more in-depth account of the field methods can be found in Chapter 4.2.2. A maximum of three individuals (either adult or 4th instar larvae) were collected at sites where *C. quinquepunctata* was encountered more than three times. When other native coccinellid species were encountered more than twice, up to a maximum of three individuals of that species were also collected and stored in 70% ethanol and placed in a -20^{o}C freezer. In this case, four additional coccinellids species were collected; *Coccinella undecimpunctata*, *C. septempunctata, Adalia bipunctata,* and *Propylea quattuordecimpunctata*. All individuals of *H. axyridis* that were recorded were also collected.

5.2.2 DNA Extraction and determination of DNA concentration

DNA extraction was carried out using a Qiagen DNeasy Blood and Tissue kit and according to the manufacturer's instructions. Each individual coccinellid was placed in an Eppendorf tube (1.5ml) with 180µl of ATL buffer (Qiagen® DNeasy® Blood and Tissue kit) and 20µl of proteinase K (600mAU/ml). The contents were then crushed using a sterile micro pestle and subsequently placed in a vortex for 10 seconds. The samples were incubated overnight at 56 ^{o}C. After incubation, extraction continued as per the manufacturer's instructions. Stock DNA was stored at -20^{o}C. A

working aliquot of each DNA extract was kept at 4°C. To determine the concentration of DNA and confirm that the extraction process was successful, a NanoDrop (ThermoFisher) was used. Any samples with concentrations greater than 250 ng/µl were diluted to ensure that the PCR reactions were not inhibited by a high concentration of DNA.

5.2.3 DNA sequence assessment for species-specific primer design

Sequences for several coccinellid species, including for *C. quinquepunctata*, *H. axyridis* and *C. septempunctata* were acquired from GenBank®. At the time, just one author had provided sequences for *C. quinquepunctata* and so all sequences of this species came from the same study by Magro *et al.* (2010) (Table 5.1). For consistency the sequences for *C. septempunctata* and *H. axyridis* utilised in the alignment process were also taken from this study. Using T-Coffee Multiple Sequence Alignment (EMBI-EBL, 2018), sequences were aligned and any differences between them identified by eye to determine if a specific primer could be developed for *C. quinquepunctata*. Sequences from five regions were aligned: COI, 12s, 16s, 18s and 28s. There were no available sequences for COII for *C. quinquepunctata* and so this region was excluded from alignment. Differences between the sequences for the three coccinellid species *C. quinquepunctata*, *C. septempunctata* and *H. axyridis* were assessed. The rationale behind this choice of species is as follows. Given that *H. axyridis* is an IAS that is a highly successful generalist predator, guaranteeing that specific primers for *C. quinquepunctata* would not amplify *H. axyridis* is essential for successful detection of intraguild predation. As well as being highly abundant, *C. septempunctata* is in the same genus as *C. quinquepunctata*. Therefore, any primers that could differentiate between these two species, would have a high probability of being species specific. Percentage identity was calculated to compare the similarity of the *C. quinquepunctata* and *C. septempunctata* sequences as well as for *C. quinquepunctata* and *H. axyridis*. The percentage identity was calculated using T-Coffee Multiple Sequence Alignment (EMBL-EBI, 2018).

Table 5.1: Three target coccinellid species and their GenBank Accession numbers available for mitochondrial regions (mtDNA – COI, 12S & 16S) and nuclear regions (18S &28S).

GenBank Accession Number					
Species	**COI**	**12S**	**16S**	**18S**	**28S**
C. quinquepunctata	GU073928	FJ621320	GU073841	GU073684	FJ621326
H. axyridis	GU073932	FJ621323	GU073846	GU073689	FJ621330
C. septempunctata	GU073929	FJ621321	GU073842	GU073685	FJ621328

5.2.4 Testing of cross-species markers

Literature searches were carried out to check for species-specific markers that were developed for coccinellid species. Just two papers reported such markers; Thomas *et al*. (2013) and Yang *et al*. (2017). Subsequently, five primer pairs were selected and tested (Table 5.2) for cross-species amplification in *C. quinquepunctata* and other coccinellid species including, *H. axyridis*, *C. septempunctata*, *Adalia bipunctata*, *A. decempunctata*, *C. undecimpunctata*, *Propylea quattuordecimpunctata* and *Exochomus quadripustulatus*.

Table 5.2: Primer characteristics

Primer	**Gene**	**Target species**	**Sequence**	**T_A(°C)**	**Amplicon (bp)**
COI-Abip	COI	*A. bipunctata*	**F**: GACCCAATGGATGAAACC **R**:GGATTAAGAGGAATACCACGAC	63 → 58*	80
COI-Adecem	COI	*A. decempunctata*	**F**:GGATTACTCCAGTTAAGCC **R**:GACTTGCAACATTACACGG	63 →52*	105
ITS-AD2	ITS1	*Adalia spp.*	**F**:CGTAGAGAACGGGATTCGTC **R**:TTATGTTTGTGTTGTCTCACGTC	53	99
SEP	COI	*C. septempunctata*	**F**:AATATGAGCCGGAATAATT **R**:TCCAATTATTAAAGGAACAAG	52-56**	196
HAX	COI	*H. axyridis*	**F**:AATTGTTACAGCTCATGCT **R**:CCCCTATTTCTACGATTG	54-58**	197

* These protocols were both touchdown PCR, see Section 5.2.4 for details; ** These primers were tested at three different temperatures within the ranges above, see Section 5.2.4 for details; COI-Abip, COI-Adecem & ITS-AD2 from Thomas *et al*., 2013; SEP & HAX from Yang *et al*., 2017.

All amplifications were performed in 20µl reactions containing: 10µl MyTaq Mix (Bioline), 4µl ddH_2O, 2µl DNA and 2µl of Primer-F and Primer-R (0.5µM). The PCR protocol for COI-Abip was modified from the protocol reported by Thomas *et al.* (2013) with: 94°C for 3 minutes, followed by 10 cycles of [94°C for 1 minute, 63°C for 1 minute and 72°C for 1 minute] with each cycle's annealing temperature decreasing from 63°C by 0.5°C to 58°C in the last cycle. This was followed by 35 cycles of [94°C for 1 minute, 58°C for 1 minute and 72°C for 1 minute], and a final extension at 72°C for 10 minutes and held at 4°C. COI-Adecem was also run on touch down protocol that was taken from Thomas *et al*. (2013) with: 94°C for 3 minutes, followed by 10 cycles of [94°C for 1 minute, 62°C for 1 minute and 72°C for 1 minute] with each cycle's annealing temperature decreasing from 63°C by

1°C to 52°C in the last cycle. This was followed by 30 cycles of [94°C for 1 minute, 52°C for 1 minute and 72°C for 1 minute], and a final extension at 72°C for 10 minutes and held at 4°C. The PCR programme for ITS1-AD2, also modified from Thomas *et al*. (2013) was: 94°C for 3 minutes, followed by 40 cycles of [94°C for 1 minute, 53°C for 1 minute and 72°C for 1 minute], a final extension at 72°C for 10 minutes and held at 4°C.

The initial trials of the SEP & HAX primers were carried out using the reported conditions by Yang *et al.* (2017) with one exception; the number of cycles to test the HAX primer were increased from 40 to 45 in line with the SEP primers. The first protocol using SEP was: 94°C for 4 minutes, followed by 45 cycles of [94°C for 30 seconds, 56°C for 30 seconds and 72°C for 30 seconds], a final extension at 72°C for 10 minutes and held at 4°C. The first protocol using HAX was the same as for SEP except the annealing temperature was 54°C for the duration. Based on the results found using these protocols, the PCRs of these two primers were repeated with changes made to the annealing temperatures. In order to decrease the specificity, the annealing temperature for two additional PCR trials involving SEP was decreased to 54°C and 52°C respectively. Conversely, to increase the specificity of the HAX primer, the annealing temperature was increased for additional PCR trials to, 56°C and 58°C respectively.

Electrophoresis was used to separate the resulting PCR amplicons. A 2% agarose gel in 0.5% TAE buffer stained with 9µl of GelRed®(Biotium) was used. Depending on the well size, between 1.5µl and 3µl of Coral Red loading dye was added to 5µl and 7µl PCR product respectively. This mix was then loaded into the gel alongside a Hyperline ™ 25bp ladder (Bioline) (Figure 5.2 a, b & c). The gel was run at 70-90V for between 60 and 90 minutes. The agarose gels were visualised under a UV Transilluminator and photographs taken for analysis.

5.3 Results

5.3.1 DNA sequence assessment for species-specific primer design

When sequences were aligned for each of the five regions, using three coccinellid species as a starting point, the sequences exhibited very few differences from each other. The percentage identity calculations (Table 5.3) illustrate how similar both the *C. septempunctata* and *H. axyridis* sequences are to those of *C. quinquepunctata*, with a mean percentage identity of almost 95% for the two closely related species and over 90% for *H. axyridis* and *C. quinquepunctata*.

Table 5.3: Percentage Identity generated using T-Coffee Multiple Sequence Alignment to compare similarity of *C. quinquepunctata* sequences to *C. septempunctata* and *H. axyridis*.

	COI	12S	16S	18S	28S	Mean Percentage Identity
C. quinquepunctata & *C. septempunctata*	87.79	93.62	95.38	99.45	97.90	94.83
C. quinquepunctata & *H. axyridis*	84.69	87.16	88.31	98.78	93.75	90.54

When comparing the three species' sequences at the five different loci, there were a few instances per locus where the sequences all differed and this was only by one base pair (Figure 5.1). It was discussed and decided that at least three bases changes/mutations would be necessary that were unique to *C. quinquepunctata* in a short region (<10bp) to develop a species-specific primer. The lack of differences found between sequences meant that it was not possible to develop a species-specific primer for *C. quinquepunctata*.

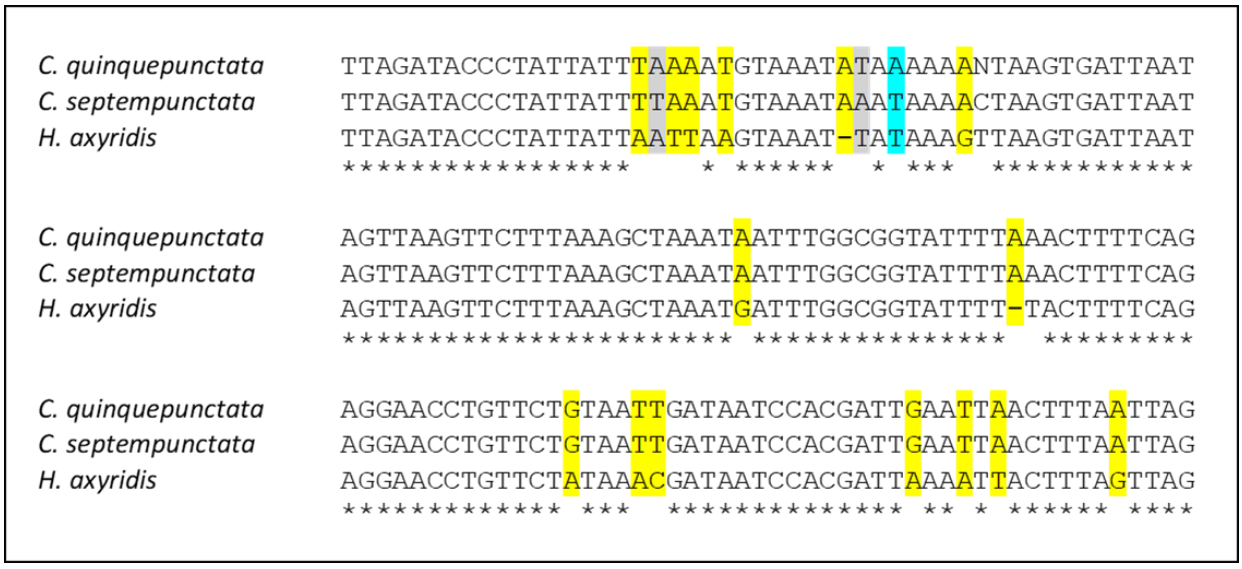


Figure 5.1: Example of aligned sequences illustrating the similarity between the three coccinellid species using a section of the mitochondrial 12S ribosomal RNA gene; blue = differences between *C. quinquepunctata* and other two species; yellow = differences between *C. quinquepunctata* and *H. axyridis* but not *C. septempunctata*; grey = differences between *C. quinquepunctata* and *C. septempunctata* only.

5.3.2 Testing of cross-species markers

All five primer pairs amplified their target species. There was non-specific amplification of large sections of DNA from some primers for *C. quinquepunctata* (COI-Abip, ITS1-AD2, Hax), *C. septempunctata* (ITS1-AD2, Hax) and *H. axyridis* (COI-Abip, ITS1-AD2, Sep). Both COI-Abip and COI-

Adecem were proven to be species-specific primers, amplifying just the target species, *Adalia bipunctata* and *A. decempunctata* respectively, with only a few instances of non-specific amplification, which was of fragments of over 300bp (Figure 5.2a). The ITS-AD2 primer was designed to amplify *Adalia spp*. (Thomas *et al*., 2013), however, in this case *C. septempunctata* was also amplified by this primer (Figure 5.2a).

The primer SEP was expected to be species-specific to *C. septempunctata* (Yang *et al*., 2017), which was the case when the annealing temperature was 56°C, however, when the annealing temperature was reduced to 52°C, *H. axyridis* was also amplified (Figure 5.2b). The HAX primer, reported to be species-specific to *H. axyridis* (Yang *et al*., 2017), amplified several other coccinellid species consistently and amplifying segments of a very similar length to those expected for *H. axyridis*. This non-species-specific amplification included *C. septempunctata* and *C. undecimpunctata,* both of which are the same genus as *C. quinquepunctata.* This was apparent at different annealing temperatures (Figure 5.2c).

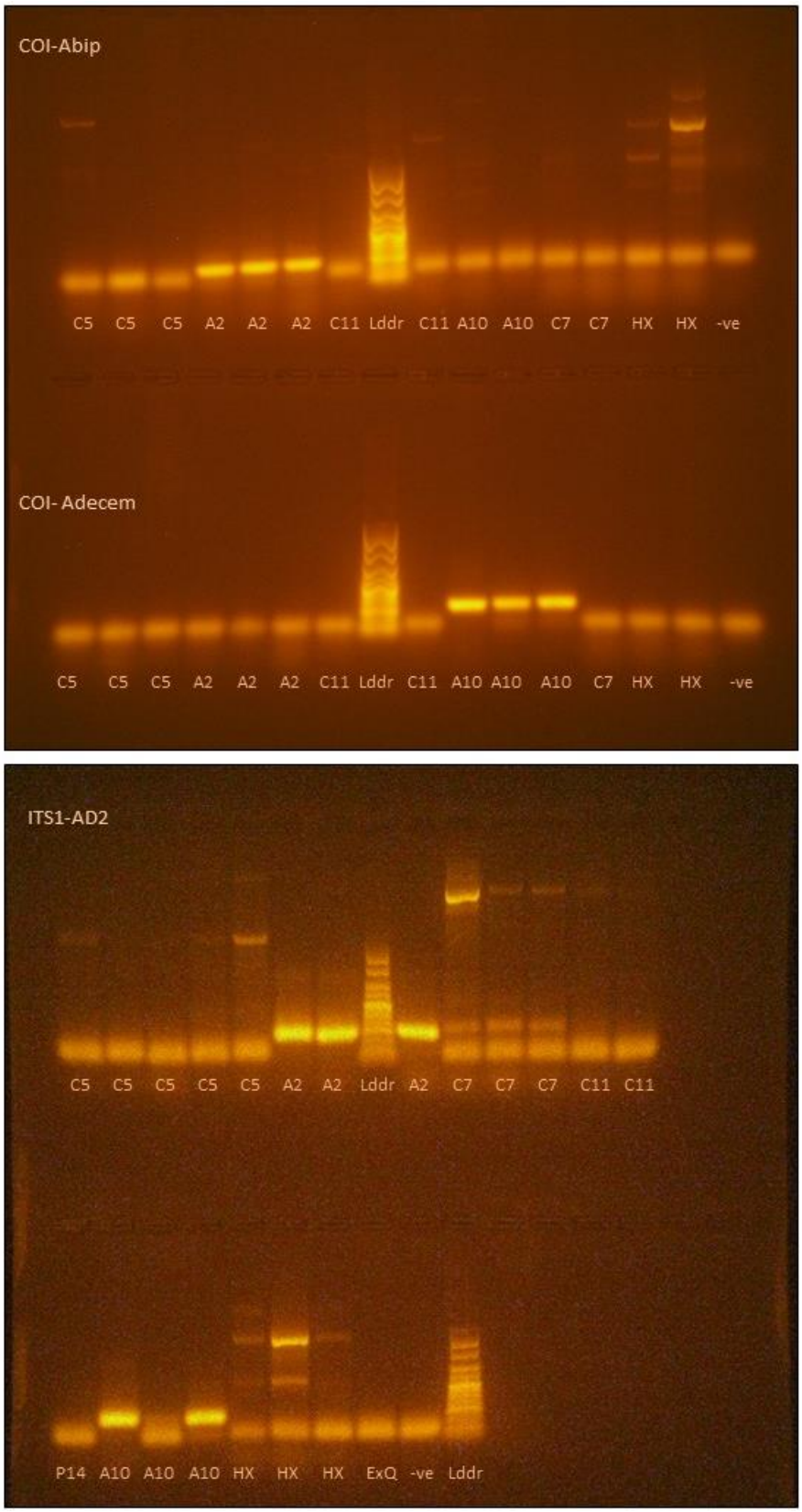


Figure 5.2a: Gel images illustrating the PCR results of the COI-Abip, COI-Adecem and ITS1-AD2 primers. A2 = *A. bipunctata*; A10 = *A. decempunctata*; C5 = *C. quinquepunctata*; C7 = *C. septempunctata*; C11 = *C. undecimpunctata*; ExQ = *E. quadripustulatus*; HX = *H. axyridis*; Lddr = Ladder; P14 = *P. quattuordecimpunctata*; -ve = negative control.

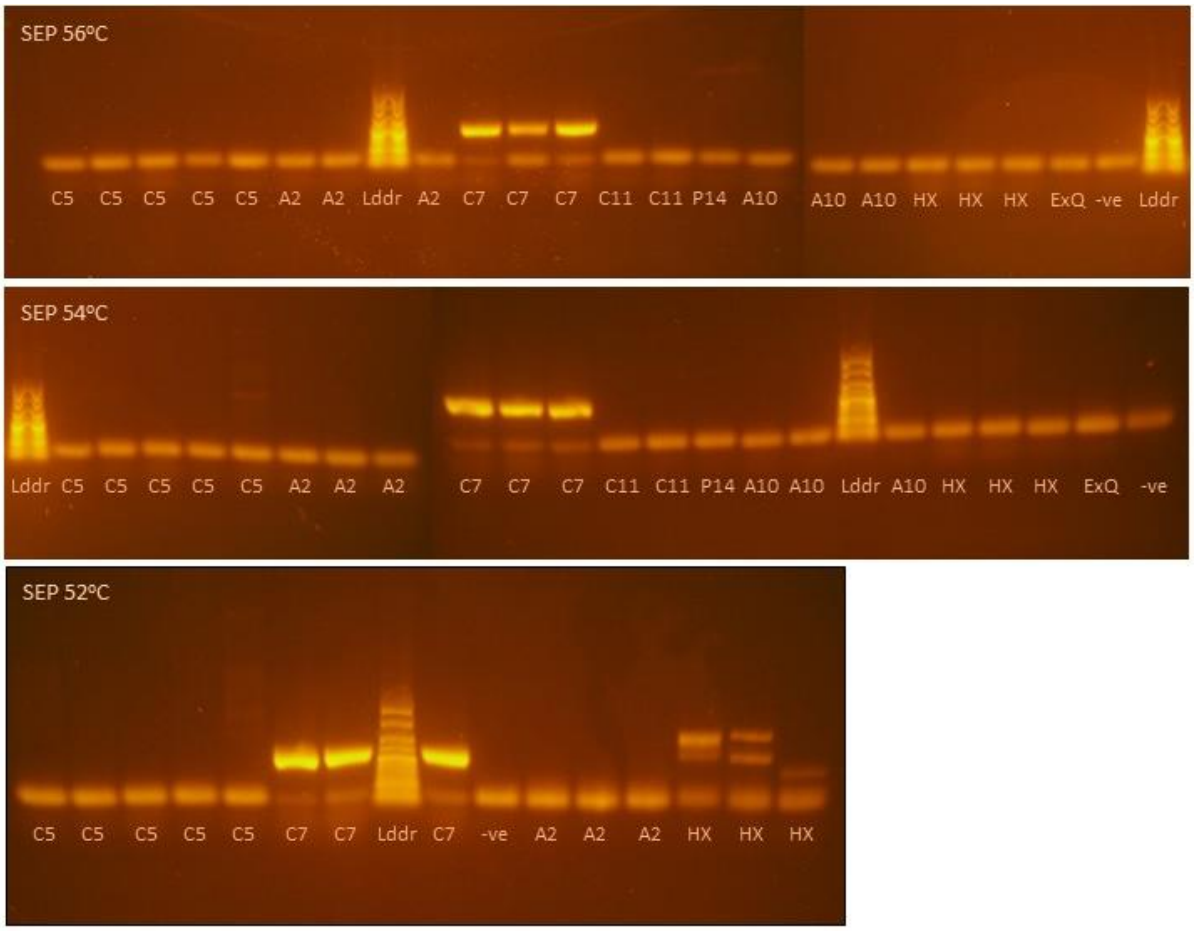


Figure 5.2b: Gel images illustrating the PCR results of the SEP primers at annealing temperatures of 56°C, 54°C and 52°C. A2 = *A. bipunctata*; A10 = *A. decempunctata*; C5 = *C. quinquepunctata;* C7 = *C. septempunctata*; C11 = *C. undecimpunctata*; ExQ = *E. quadripustulatus*; HX = *H. axyridis*; Lddr = Ladder; P14 = *P. quattuordecimpunctata*; -ve = negative control.

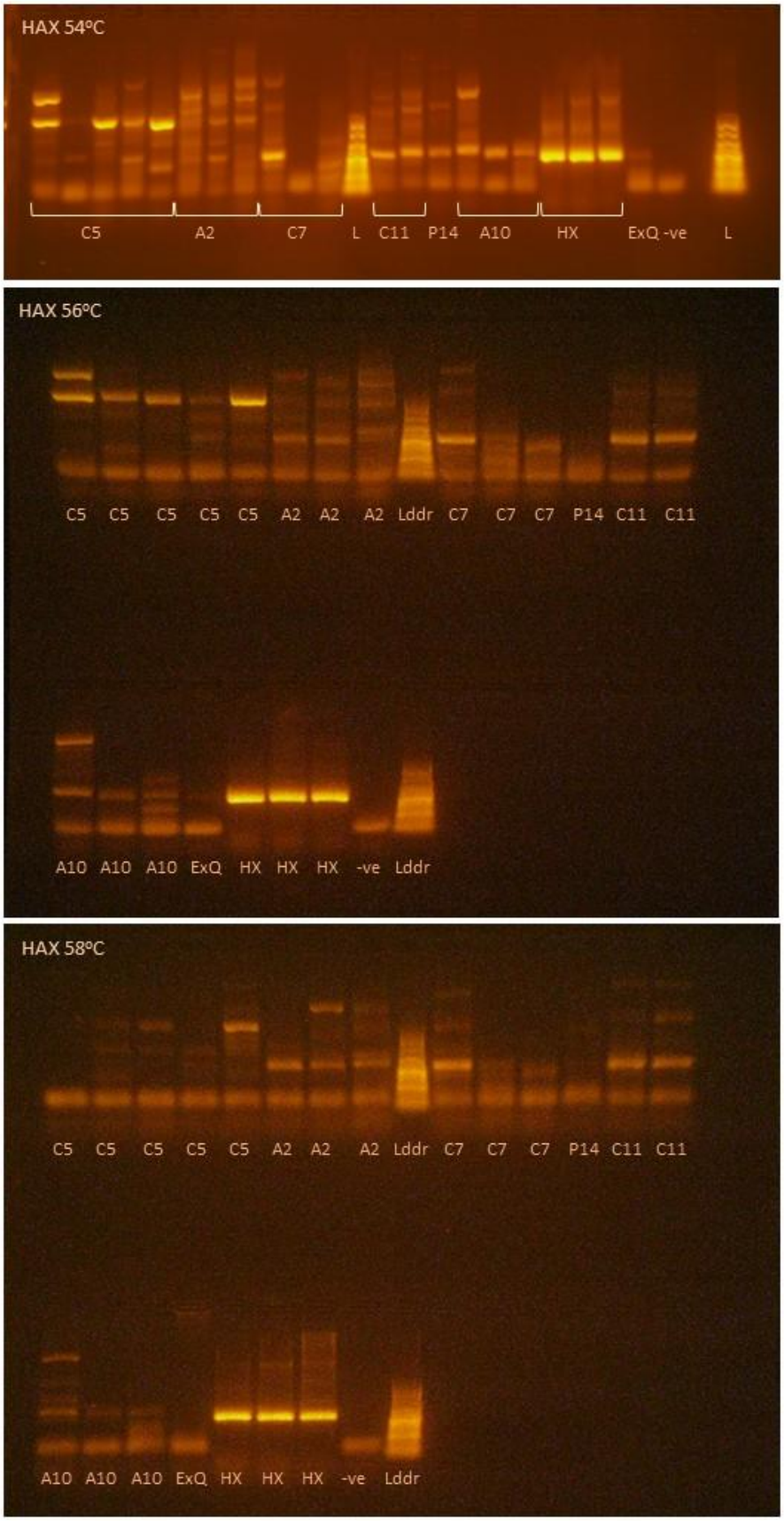


Figure 5.2c: Gel images illustrating the PCR results of the HAX primers at annealing temperatures of 54°C, 56°C and 58°C. A2 = *A. bipunctata*; A10 = *A. decempunctata*; C5 = *C. quinquepunctata;* C7 = *C. septempunctata*; C11 = *C. undecimpunctata*; ExQ = *E. quadripustulatus*; HX = *H. axyridis*; L / Lddr = Ladder; P14 = *P. quattuordecimpunctata*; -ve = negative control.

5.4 Discussion

5.4.1 Primer design

Previous studies investigating intraguild predation (IGP) in coccinellids have focussed on predator interactions between *H. axyridis* and other generalist and typically abundant species such as *C. septempunctata*, *A. bipunctata*, *A. decempunctata*, *Propylea japonica* and *P. quattuordecimpunctata* (Hautier *et al*., 2008; Sloggett *et al*., 2009; Brown, 2010; Thomas *et al*., 2013; Brown *et al*., 2015; Rondoni *et al*., 2015; Yang *et al.,* 2017). This current research aimed to develop a molecular marker to determine if *H. axyridis* preyed upon a rare and specialist coccinellid, *C. quinquepunctata*. The marker needed to be species-specific to differentiate clearly *C. quinquepunctata* from the potential predator (*H. axyridis*) and other possible related prey. Despite previous studies having successfully developed and used markers to detected IGP in coccinellids (Thomas *et al.*, 2013; Brown *et al.,* 2015; Rondoni *et al.,* 2015, Yang *et al.,* 2017), it became apparent early on that in this case, developing a species-specific marker was not straightforward and may not be possible with the time and resources available for this study.

The aforementioned studies succeeded in developing species-specific primers based on unique sequence sections identified when comparing sequences available from GenBank (Thomas *et al*., 2013). This method is not only cost effective but also saves a considerable amount of researcher time. However, King *et al*. (2008) recommended that researchers rely less on sources of sequences such as GenBank and instead carry out their own sequencing. There are several reasons for this, including not enough individuals being sequenced and the possibility of false-negatives when trying to detect prey (King *et al*., 2008). Due to the timescale of this piece of work, this option was not available and so GenBank was used as a source of sequences. There is a plethora of sequences available on GenBank for the more common coccinellids such as *H. axyridis*, *C. septempunctata*, *A. bipunctata* etc. However, there are very few sequences available for the less common or more specialist coccinellids. There was only one study (Magro *et al*., 2010) that had provided sequences for *C. quinquepunctata.* The sequences available were only for the regions, COI, 12S, 16S, 18S and 28S and were obtained from two individuals from a population in Wales. For consistency, the sequences used for *H. axyridis* and *C. septempunctata* were also from Magro *et al*. (2010).

When comparing the sequences available a very high proportion of similarity was found between *C. quinquepunctata* and both *C. septempunctata* and *H. axyridis*. This high level of similarity between the different species was unexpected. Therefore, it was decided that the sequences analysed did not provide a unique enough anchor to develop species-specific primers. If the development of molecular markers that allow the investigation into less common or more specialist coccinellid IGP is to be pursued, it is recommended that the laboratory carry out their own

sequencing to avoid any concerns over reliability of sequences from other sources. In this case, there were concerns in particular about the origin of the sample and if the sequence reflected the mutations of a single individual or were representative of the population. Having sequences available from more individuals (e.g. five individuals per population) would allow detection of which mutations are unique to the species and which differ between individuals or populations. Finally, increasing the loci that are sequenced would increase the likelihood of finding a region with enough unique mutations to develop a reliable species-specific marker. Unfortunately, it was not possible to investigate this within the timeframe of this research.

When attempting to design primers that are to be species-specific, it is widely accepted that mitochondrial loci such as COI or 12S are more appropriate than loci in nuclear DNA, including the ITS1 region (Dodd, 2004; King *et al*., 2008). However, it has also been noticed that COI is not a universal answer. For example, when attempting to design species-specific primers for lumbricid earthworms, the COI region showed too much diversity within this species group and it was only possible to design group-specific primers using 12S for the same species group (Harper *et al*., 2005). Another factor is that, some primers that are designed for the COI region can on occasion coamplify COII which can lead to double banding which could cause confusion when interpreting the results (Symondson, 2002). It could be worth considering a variety of regions from the mitochondrial and the nuclear DNA such as 12S or a combination of 12S and 16S (Freeland, 2016). Due to the deteriorated nature of the DNA, sequences are short and in low concentration. Therefore, a good marker for IGP needs to be able to amplify mitochondrial DNA or repeated sections of nuclear DNA, as well as being species specific (Lawson-Handley, 2015).

5.4.2 Previously designed primers

Primers available from previous studies (Thomas *et al*., 2013; Yang *et al*., 2017) were tested under a variety of PCR conditions to determine if they amplified *C. quinquepunctata*, even if it was known that they also amplified other species, including *H. axyridis*. If the amplicon obtained had been clear and single banded, it may have been possible to design a way to reliably differentiate between the amplicons of different species (e.g. using a melting curve or restriction enzymes). However, this was not the case, since these primers were either too specific or not specific enough.

The COI-Adecem and COI-Abip primers (Thomas *et al*., 2013) were too specific while ITS1-AD2 (Thomas *et al*., 2013) was not so specific, amplifying also *C. septempunctata,* but unfortunately not *C. quinquepunctata* (except for some large non-specific bands). The HAX primers (Yang *et al*., 2017), did not show specificity for *H. axyridis*, producing amplicons for all the tested species with many

bands produced for most of them, which made it impossible to design a way to distinguish the amplicons of *C. quinquepunctata* from those of other species. Due to this finding, it is recommended that this marker is not used to assess IGP, since it would result in an overestimation of *H. axyridis* predation.

The SEP primers were specific at their recommended annealing temperature (Yang *et al*., 2017), but by decreasing the temperature (and therefore the specificity; King *et al.,* 2008) replicons were also obtained for *H. axyridis*, but not for *C. quinquepunctata.* This was surprising since *C. septempunctata* is in the same genus as *C. quinquepunctata* and therefore their DNA sequences are likely to be more similar (as confirmed in section 5.4.1). It appears, however, that in the case of the target sequences of these primers, *H. axyridis* shows a more similar sequence to *C. septempunctata* than *C. quinquepunctata*. Another possibility could be that the samples of *H. axyridis* used contained *C. septempunctata* DNA, maybe due to IGP. This is unlikely from an ecological perspective because *H. axyridis* was in low abundance at the collection sites (personal observation). Additionally, this seems improbable, since all three samples amplified, but only when decreasing the annealing temperature and produced multiple bands. As well as being highly abundant, *C. septempunctata* is in the same genus as *C. quinquepunctata* and both species share habitat (see Chapter 4). Therefore, any primer that could amplify and differentiate between these two species, would have a high probability of being species specific. Unfortunately, in this case, neither ITSI-AD2 (Thomas *et al*., 2013), SEP nor HAX (Yang *et al*., 2017) were suitable for this.

A potential reason for the non-specific amplicons is the formation of chimeric sequences which is a common occurrence in degraded DNA such as that found in gut content (Dodd, 2004; Rowe *et al*., 2017). Chimeric sequences are the formation of a new sequence from two sequences joining together (Edgar, 2016). These sequences have been observed in relatively high numbers of PCRs, up to a third in some cases (Cronn *et al*., 2002; Dodd, 2004). Using low annealing temperatures, may have resulted in the formation of chimeric sequences. Moreover, the nonspecific amplicons could correspond to non-target species, including gut bacteria. For example, Dodd (2004) revealed that some non-target amplicons apparent in ground beetles after PCR were likely to be bacteria, namely *Lactobacillus* spp.

A further difficulty is that there are many cases in the literature with incomplete reported methodologies. For example, Brown *et al*. (2015) did not discuss the molecular process and report the findings from the work carried out. Yang *et al*. (2017) created primers that they used to determine IGP by *H. axyridis* on *C. septempunctata* and *P. japonica*. However, the authors did not indicate which mitochondrial region was targeted by these primers and so it is difficult to elucidate

why these primers were so specific in their study, but not in this current research. Perhaps as in previous IGP studies, the COI region was the target.

5.4.3 PCR as a method

The choice of the PCR method over other prey detection methods in this research is justified. The ELISA method is currently the least expensive method for detecting IGP once the protocol has been developed. However, there are some disadvantages of ELISA, in that it can take significantly longer to perfect the method: as it requires the development of specific monoclonal antibodies for the target species and more importantly, it is only possible to detect one prey species resulting in an incomplete picture of predation (Symondson, 2002; Dodd, 2004; Aebi *et al*., 2011). Using GC/MS has proved successful in some cases (Hautier *et al*., 2008; Sloggett *et al*., 2009) and it has the advantage of being able to detect prey up to 36 hours after it has been consumed (Thomas *et al*., 2013). This is particularly useful for IGP tests on wild predators, however it is quite costly and it is not always possible to detect prey to species level (Sloggett *et al*., 2009; Aebi *et al*., 2011). Using a PCR-approach, molecular markers that are species-specific can often be developed at relatively low cost. Sequences for many species are readily available in databanks such as GenBank and the considerable cost reduction of sequencing opens the possibility of sequencing de novo when needed. The invention and subsequent cost reduction of qPCR, has made the method more reliable, without adding much more to the costs. Therefore, the use of species-specific markers amplified via PCR or qPCR is the most feasible option in terms of time and financial costs (Dodd, 2004; Aebi *et al*., 2011; Lawson Handley, 2015).

This method was unsuccessful in this case due to the time and resources restrictions; however, it was and remains the most viable option. The scope of this research did not allow for potentially years in developing clonal antibodies to detect one species using ELISA as it has been shown that *H. axyridis* can consume a large biomass in a short period of time (Majerus *et al*., 2016). GC/MS may or may not be capable of detecting coccinellids to species level and again, the development of the technique is expected to be long. Sloggett *et al*. (2009) has had some success but to genus level only. The current research had a short amount of time in which to be completed and also hoped to determine quite specifically if *C. quinquepunctata* was the species being consumed and so attempting to achieve this using GC/MS would have been futile. Thomas *et al*., (2013) and Brown *et al*. (2015) had success with species specific primers with *Adalia spp*. Furthermore, the more recent research from Yang *et al*. (2017) claiming to have specific primers for both *H. axyridis* and *C. septempunctata* indicated that this project could be successful in detecting if *H. axyridis* had consumed *C. quinquepunctata*.

5.4.4 Does *H. axyridis* prey on *C. quinquepunctata*?

From Chapter 4 it is evident that *H. axyridis* rarely came into contact with *C. quinquepunctata*. Moreover, given the paucity of prey options on river shingle, the adapted movement of *C. quinquepunctata* adults and larvae on the river shingle along with the potentially aphid rich habitat away from the river shingle, it is very likely that *H. axyridis* does not prey on *C. quinquepunctata,* or that predation is infrequent and limited mainly to eggs. Little is known about *C. quinquepunctata* and there is a lack of detailed information about the time of year that this coccinellid produces eggs. When collecting samples for this research, fourth instar larva were observed in mid-June, indicating that eggs may have been produced approximately 4-5 weeks prior, depending on ambient temperature (Roy *et al*., 2013). It is also possible that this species lays eggs prior to *H. axyridis* and so its larvae are further along in their development before any *H. axyridis* larvae may appear on the shingle habitat. During sample collection, *C. quinquepunctata* were not see near *H. axyridis* larva (personal observation), however, if these species developed in parallel, then *H. axyridis* would likely prey on *C. quinquepunctata* (Ware & Majerus, 2008).

Previous studies on *H. axyridis* intra-guild predation show a range of results. A low rate of IGP on *A. bipunctata* and *Oenopia conglobata* was reported by Rondoni *et al*. (2015). However, they targeted a longer than recommended fragment (237 and 167 respectively) for prey detection in the gut which probably resulted in an underestimation of the predation rate. They did report a greater rate of IGP on a coccinellid native to Italy, *Oenopia conglobata,* than *A. bipunctata* (native to both Italy and the UK). Brown *et al*. (2015) stated that the UK was the only country from five where *H. axyridis* was found to prey on *A. bipunctata*, with *A. decempunctata* being preyed on in France, Slovakia and the Czech Republic. In laboratory feeding trials, however, *H. axyridis* tends to be offered *A. bipunctata* only after a period of starvation and with no other prey option (Brown, 2010; Thomas *et al*., 2013). In addition, Rondoni *et al*. (2015) found that *H. axyridis* had a higher survival rate when preying on aphids or *H. axyridis* eggs as opposed to when preying on *A. bipunctata*. It is possible then that if prey biomass is sufficient then *H. axyridis* will opt not to preyed on *A. bipunctata*, which could also apply to other coccinellid species including *C. quinquepunctata* Furthermore, even when IGP of other species has been confirmed due to the habitat mismatch this may not be the case for *C. quinquepunctata.* Further field investigations would be necessary to confirm that the results in Chapter 4 are not a one-off occurrence. Together with successfully developing a species-specific marker for this rare coccinellid, this would add vital information towards the conservation of a nationally (and potentially internationally) rare beetle.

5.4.5 Conclusion

Using the available sequences found in GenBank, it was not possible to develop species specific primers due to the high similarity of the *C. quinquepunctata* and *H. axyridis* and *C. septempunctata* sequences. Additionally, sequences of only one or two *C. quinquepunctata* were available, which made it impossible to infer if the mutations were due to individual variation or were present in the whole species. Primers were tested that had been developed for other related species in order to see if they amplified also *C. quinquepunctata* and, if so, if we could use the melting curve or a restrictive enzyme protocol to identify the fragments. Unfortunately, in this case, the cross-species amplification was not successful.

Research that has attempted primer design but been unsuccessful, needs to be disseminated to help inform others who may try the same research unknowingly. Moreover, the testing of the HAX primers (developed by Yang *et al*., 2017) showed they were not sufficiently specific. Details about primer specificity also should be disseminated to the wider community to prevent replication that can be costly instead of only publishing when a positive outcome has been achieved in this area. This would help researchers to develop realistic expectations and make informed choices when considering using molecular methods.

Previous studies have shown that it is possible to reliably detect IGP using PCR or qPCR after extensive primer design (Dodd, 2003; Thomas *et al*., 2013; Rondoni *et al*., 2015). Many of these studies tend to focus on abundant species, such as *H. axyridis*, *A. bipunctata*, etc. (Thomas *et al*., 2013, Brown *et al*., 2015; Rondoni *et al*., 2015). More efforts into including less abundant species would benefit future studies of IGP and potentially yield a more in-depth picture of coccinellid interactions. Developing molecular markers for more species, based or tested on individuals of different populations would ensure reliability of markers and therefore build on the strength of molecular methods which in turn would lead to greater success in IGP studies.

6 General Discussion: Invasive alien species and science communication

Invasive alien species (IAS) are one of the biggest direct threats to biodiversity globally (IUCN, 2018; IPBES, 2019), however, there has been some debate around whether or not IAS are passengers of change (MacDougall & Turkington, 2005) or do they drive biodiversity loss (Clavero *et al*., 2009)? It is more likely that IAS facilitate loss of biodiversity as well as negatively affecting ecosystem function (Clavel *et al*., 2011) by altering the abundance of native species with cascading effects across communities and/or assemblages (Roy *et al*., 2012). A large volume of literature has been amassed for over a decade building on the knowledge of *H. axyridis* in its preferred habitat of urban areas (Adriaens *et al*., 2008; Brown *et al*., 2011a; Purse *et al*., 2014; Viglášová *et al*., 2017; Honěk *et al*., 2018b). Taking a snapshot of coccinellid abundance is just a starting point for researchers to begin building a clearer picture of what is really happening with the establishment of a specific IAS. This thesis builds on existing knowledge of IAS, specifically *H. axyridis*, and starts to unravel the complex relationship *H. axyridis* has with native coccinellids in rural habitats in the UK.

6.1 Thesis summary

The overall outcome of this thesis was multifaceted and highlights how complex a process it is to determine the effect of the presence of one species on a community. This thesis revealed how several factors in combination have led to the current establishment and spread of *Harmonia axyridis.* Climate change is predicted to affect the distribution and spread of *H. axyridis*, globally and nationally, with the species establishing in areas previously free of the species. *Harmonia axyridis* appears to have habitat preferences and within these habitat types there are distinct coccinellid communities, not all of which are dominated by *H. axyridis*. This IAS does not appear to affect rare coccinellid species, however, this is more due to habitat preferences of the respective species. Many questions and/or recommendations have been generated and the most important are highlighted below in relation to the relevant data chapters of this thesis.

Chapter 2 illustrates how human activities were the most important factor in determining if *H. axyridis* would establish in a region. Additionally, the future range shift of *H. axyridis* under various climate scenarios was revealed thereby concluding that a combination of factors is necessary for successful establishment of *H. axyridis*. To improve future predictions, increased sharing of records of recently established *H. axyridis* populations as well as use of Citizen Science are necessary. Citizen science has been successful in some regions (Europe and North America) but is less widely used in many other places (Asia, South America and Africa) (Figure 1b).

Chapter 3 of this work reveals that *H. axyridis* does not dominate the coccinellid communities in rural habitat in contrast to its domination of urban coccinellid communities. Previous research illustrates how this community fluctuates over time, regardless of whether an IAS has established in these habitats or not (Honěk *et al*., 2016). In order to know more about how ecosystem function may be affected by the arrival of IAS, it is necessary to know what species are in that community and what role they play within it. Chapter 3 also illustrated how different the coccinellid community is at different habitat types with a unique suite of generalist and specialist species. The next step is to determine how these specific communities support the ecosystem, which can only be achieved through additional studies on a range of species, including specialists (Sloggett, 2005).

Chapter 4 demonstrates how a rare coccinellid was faring following the arrival of *H. axyridis*. The probability of *C. quinquepunctata* being negatively impacted by *H. axyridis* was low in this instance. *Coccinella quinquepunctata* could face pressures, however, from other quarters such as invasive alien plant species, agricultural practices and disruption of its habitat by human activity. This species and in particular it's habitat require continued monitoring to ensure *C. quinquepunctata* is protected against these pressures as well as the potential of *H. axyridis* range expansion due to climate change. Not only is Citizen Science a useful method for monitoring IAS (Hiller & Haelewaters, 2019) but it is also essential for the conservation of specialist species.

Chapter 5 used molecular techniques in an attempt to assess feeding interactions between an IAS and a rare specialist species. In this case, due to the similarity of coccinellid sequences it was not possible to develop the primers required. Even though the expected outcome was not achieved, with greater resources (time and financial) it would be possible to detect if IGP occurred between *H. axyridis* and *C. quinquepunctata* in a natural habitat. Moreover, considering the results of Chapter 4, it was unlikely that *C. quinquepunctata* was prey for *H. axyridis*.

Ultimately, this thesis emphasizes that attempting to determine how an IAS integrates into a new range/habitat is not a straightforward process. The effect of one IAS on biodiversity cannot be viewed without also looking at the interactions between other drivers of change (land use change, climate change and pollution) all of which have increased over the last five decades (IPBES, 2019). As a global community, scientists and the general public need to cooperate to ensure that biodiversity is protected from these drivers of change.

6.2 Published research

6.2.1 Insufficient species

The majority of research around IAS has tended to focus on terrestrial systems and plants (Lowry *et al*., 2013). Plants are of course important, however, other taxonomic groups in terms of IAS can be as economically and ecologically damaging as plants (e.g. Varroa mite *Varroa destructor* and Muntjac deer *Muntiacus reevesi*; Figure 6.1) (Eschen & Williams, 2011). In much ecological research, model species are used to answer a range of questions and to test hypotheses, however, it can be easy to forget that there may be other species worthy of study. Moving to the literature on coccinellids, the species of focus in publications has generally been *H. axyridis*. There are regions where other coccinellids have established as IAS (e.g. *Coccinella septempunctata, Exochomus quadripustulatus, Hippodamia variegata* and *Propylea quattuordecimpunctata* in USA), prior to *H. axyridis*. However, apart from *C. septempunctata* in North America (e.g. Evans, 2004), the other species have received considerably less research attention (Sloggett, 2005; Harmon, 2007). Furthermore, a Web of Science search (09.02.2020) for 'invasive ladybird' and 'invasive coccinellid' revealed the first 20 results to be dominated by work focussed on *H. axyridis* with 12 and 16 results respectively with the other entries concerning a range of other species. There is no denying the effect that *H. axyridis* has on biodiversity, however these studies tend to focus on specific native species also, e.g. *A. bipunctata*. Sloggett (2005) observed that the majority of coccinellid studies focussed on just a handful of generalist species and argued that having a narrow group of model species would likely lead to bias in the scientific process. Coccinellids are renowned for their use in biological control (Harmon, 2007) but just a small number have become IAS and generally, these IAS have been generalist species such as *C. septempunctata* or *H. axyridis*. However, when comparing a generalist and specialist coccinellid species, Sloggett *et al*. (2008) illustrated that the specialist coccinellid remained in situ for considerably longer than the generalist species, suggesting that specialist species would be more suitable in this role. By excluding specialist species from investigation, we have an incomplete picture without the scientific evidence available to advise on best methods to conserve habitats and species.

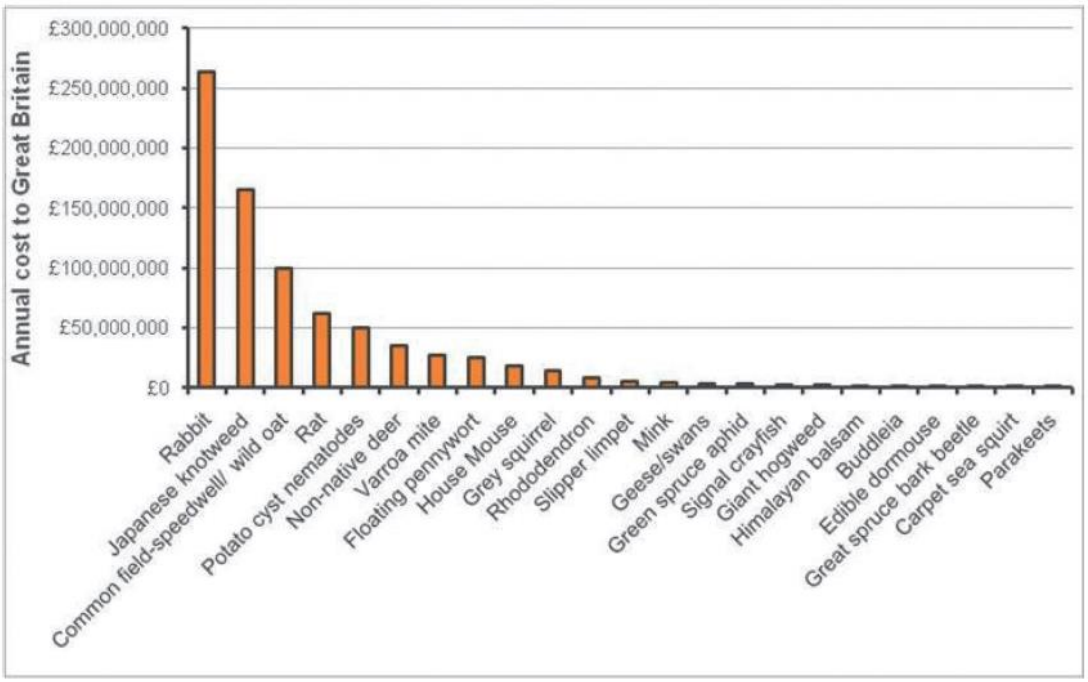


Figure 6.1: From Eschen & Williams (2011) Aliens: The Invasive Species Bulletin 31: 47-51

6.2.2 Habitats

Another aspect of the coccinellid literature is the habitat in which this research takes place tended to be anthropogenic in nature, such as urban areas and agricultural fields (e.g. alfalfa). This is valuable research but there are a range of habitat types with distinct coccinellid communities that have been ignored completely or studied insufficiently. This could be due to the fact that the coccinellid species that have established as IAS have tended to do so because they have been used in biological control and so it is of benefit to landowners and environmental groups to understand the effects of these IAS on native species in these habitats. However, these anthropogenic habitats are not in isolation and neighbouring forest, grassland or riverine habitats should also be included. One particular reason for this paucity of data is likely to be the difficulty in collecting data in such habitats. Being more rural, these habitats cover large areas. There are also fewer people living in these areas, thus limiting participation levels in any Citizen Science projects that may be underway, resulting in fewer records. Monitoring cannot be completed by ecologists alone as there simply are not enough people to survey all of these places. However, engaging people in Citizen Science leads to a huge quantity of data and information that can be verified by experts to ensure high quality of datasets. These data can be analysed and meaningful results determined, not only locally but globally (Gardiner *et al*., 2012; Chapman *et al*., 2019; Alaniz *et al*., 2020). In order to engage more

people in Citizen Science, the current research needs to be framed in a way that is easily understood by the maximum number of people. Both the general public and the editors/reviewers need to be confident that Citizen Science data are reliable and just as usable as data collected by professional scientists (Gardiner *et al*., 2012). Moving beyond peer reviewed journals is essential for this to work, with researchers promoting their findings not just at conferences but at public events such as the British Science Festival or Science Week.

6.3 Bias in published results

One problem with publications is the pressure on researchers to have 'positive' or significant results. This pressure to publish only 'positive' results leads to bias in the publication process with very few 'negative' results being reported (Fanelli, 2010) which skews the reality of working in science. Furthermore, not publishing non-significant results creates a cycle of unnecessary repetition of research, resulting in wasted finances, time and a potential publication. It was not possible to answer the molecular question being asked in Chapter 5 of this research. Although eDNA and barcoding can be utilised to determine what a certain predator may consume, the current reality is that very few species currently have specific primers and of these, very few that are available on GenBank have not been published (Ardura, 2019). Is the lack of species-specific primers a result of researchers not working in this field or is it that researchers have tried and it has not been possible? In molecular ecology it is just as important to publish what does not work, as it is to know what does work. Studies or methods that have not worked are less likely to be published than those that have worked (Fanelli, 2010). Undergraduate students around the UK are taught not to get disheartened about a non-significant result, because it is in itself a result. However, by the time these keen, energised and motivated undergraduates become postgraduates and start on the path to publishing their own work, this approach to science seems to be moot. Publications outlining something that has not worked are invaluable, in particular to PhD researchers and early career investigators.

6.4 Communicating science

As mentioned above, science needs to be communicated more effectively, not just to academics and researchers but also to members of the public. It is no longer enough to simply have your research published in an academic journal as increasingly, funders wish to know how academics will communicate their results to wider audiences. In the UK, the Research Excellence Framework

(REF) evaluates research quality from higher education. All submissions are judged on several criteria including their impact, which is how significant and far-reaching the research has been on society, the economy, the environment or quality of life (REF, 2019). This impact aspect accounts for 25% of the overall assessment. One method of achieving far-reaching impact is for researchers to publish their raw data or their statistical code so others can use this information for future work. Nature Scientific Data is a journal that was developed to facilitate the sharing and re-use of datasets under the guidelines of FAIR Data Principles. These principles aim to ensure that data are Fair, Accessible, Interoperable and Reusable (Wilkinson *et al*., 2016). One example of data published in this way is the UK Ladybird Survey data which was recently made available (Brown *et al*., 2018). An example of the importance of sharing data was when carrying out data checks for Chapter 2 of this work. I excluded the records (n = 2) from Turkey, which I assumed to be incorrect as I had not found any evidence to state that *H. axyridis* had established in this country. Having completed my analysis, I subsequently discovered that *H. axyridis* was now considered established in Turkey (Bukejs & Telnov, 2014) as well as in Iran (Biranvand *et al*., 2019). The records reported in these papers for both countries were not present on GBIF and so could not be included in the analysis. It is important for new species establishments to be published, but these records also need to be shared on biodiversity portals to allow for more complete analysis, particularly on a global scale.

Many researchers do not have any specific training in science communication (Davis *et al*., 2018) and so there is no clear method on how to communicate science apart from publishing papers. In the case of IAS, timely and effective communication is especially important. It is imperative that globally, people know and understand what is occurring in their environment and that IAS can be prevented from establishing or how they can be controlled by the most appropriate methods possible. Effectively communicating IAS research to people who do not all speak one language can be quite an undertaking, however, there are initiatives underway to bring together global communities in tackling the issue of IAS (Lucy *et al*., 2016; Roy *et al*., 2018). The methods being undertaken are communication of science as well as engagement of people as Citizen Scientists.

6.5 Citizen Science

Regularly we hear academics bemoan the fact that people are disconnected from the environment and the current the state of the environment, and this disconnect is partly due to increased urbanisation. Additionally, it has been reported that people are in general spending less time on outdoor activities that would place them in contact with the natural environment (Pergams &

Zaradic, 2008). The results of research need to be made accessible to as many people as possible in order to initiate behavioural change and re-connect people with their environment. People who consider themselves connected with the natural environment are more likely to take action to help protect it (Mackay & Schmitt, 2019). However, much of the information regarding the environment and climate change does not enter mainstream media until the situation is at crisis level. Much of what has been in the media in the last year concerning climate change, has been known by scientists for several decades, indicating another disconnected relationship, this time between research, politicians taking the science seriously and the global populace. The point at where this particular relationship seems to break down is with the media. A recent study highlighted that climate change scientists featured in 49% less media articles than climate change deniers (Petersen *et al*., 2019). As scientists we need to do more to engage with and inform the general public about published research, thereby building a relationship with them. This is increasingly useful as members of the public are all potential Citizen Scientists and without them, large scale studies would not be possible.

The UK Ladybird Survey is an incredible success story in engaging the general public as Citizen Scientists, of which tens of thousands helped record 135,504 coccinellids over 20 years (Roy *et al*., 2011). The beauty of this project is that anyone can partake regardless of level of expertise. Not only were native coccinellids recorded but the Citizen Scientists also helped to map the establishment and subsequent spread of *H. axyridis* (Brown *et al*., 2008). Additionally, these data have been made open access for anyone to make use of (Brown et al., 2018). This is something the science community needs to embrace; include as many people in data collection by communicating effectively what you want to achieve and then share the data once you have answered your questions.

6.6 Future work

As with any thesis, many more questions were generated with several avenues for further research being revealed. These are discussed in greater detail in their respective chapters, however, below is a summary of future work that would be beneficial.

Investigating the climate characteristics of Scotland and Ireland may reveal more information about the distribution and establishment of *H. axyridis* in these areas. Even though the Climate Moisture Index was included in the model, rainfall specifically was not included. Including such factors (e.g. precipitation of wettest quarter, precipitation of driest quarter) in the species distribution model

may lead to a better understanding as to why these geographical regions have not yet seen large populations of *H. axyridis* become established.

There is a wealth of coccinellid research available yet there are still areas where the surface has only just been scratched. One such area is the dynamics of overwintering coccinellid assemblages. Further research into this aspect of coccinellid ecology in a range of habitats would provide further knowledge of coccinellid behaviour and importantly how climate change may influence coccinellid assemblages in the future. Investigating the coccinellid community as a whole instead of focussing on one or two species would likely reveal the complex relationship not just between individual coccinellid species but also between coccinellid species, their prey and natural enemies resulting in a better understanding of the role of coccinellids in ecosystem function.

There is just a small volume of information available regarding *C. quinquepunctata* in the UK and this thesis has highlighted many other potential avenues for further research on this species. Given the differences between this species' range in mainland Europe and the UK, it would be prudent to investigate not just prey preferences or requirements in different locations but also investigate if there may be genetic variation between the UK populations of *C. quinquepunctata* and those in other European countries. Given the differences observed between urban and rural sites for generalist coccinellids, investigating if there is a similar effect with *C. quinquepunctata* would add to the knowledge for this species. Considering the unusual habitat *C. quinquepunctata* is observed at in the UK, exposed riverine sediment (ERS), investigating the invertebrate community in this habitat would reveal more about ecosystem function as such sites as well as provide more justification to designate habitat protection status for ERS.

The outcome of chapter 5 was not expected, however, some important conclusions were drawn. Firstly, developing a species-specific marker for *C. quinquepunctata* would help considerably towards the conservation of a nationally (and potentially internationally) rare beetle leading to confirmation regarding *H. axyridis* not currently having a negative impact on *C. quinquepunctata*. Secondly, molecular research that has been unsuccessful should be disseminated to prevent replication in an area that is time-consuming and potentially expensive. Publishing only 'successful' research is damaging and costly to the entire research community.

Finally, further encouragement and continued support for Citizen Science projects, globally, nationally and regionally would result in richer datasets leading more accurate species distribution models, allow the detection of fluctuations in populations of rare coccinellids or the increased present of IAS and in either of these cases, allow swift action to be taken where necessary.

6.7 Conclusion

Invasive alien species are dynamic and complex and generate seemingly endless questions and hypotheses. The way forward in tackling the issue of IAS is to continue with high quality research but to include more variation in the taxonomic groups and habitats being studied, as has been carried out in this thesis by including rural (Chapter 3) and marginalised (Chapter 4) habitats as well as focussing on *C. quinquepunctata* (Chapters 4 & 5). Applying a combination of approaches such as field surveys (Chapters 3 & 4), molecular ecology (Chapter 5) and use of data models (Chapter 2) adds depth and gives multi-dimensional outputs. Science communication as a whole can improve and the responsibility for this lies with editors and researchers alike, while finding a way to include Citizen Science will only enrich the research process (Pocock *et al.*, 2018).

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Appendix for Chapter 2

A2.1 Calculation of Climatic Moisture Index

Monthly potential evapotranspirations were estimated from the WorldClim monthly temperature data and solar radiation using the simple method of Zomer *et al*. (2008), which is based on the Hargreaves evapotranspiration equation (Hargreaves, 1994).

A2.2a AUC (area under the curve; Fielding & Bell 1997)

Predictions of presence-absence models can be compared with a subset of records set aside for model evaluation (here 20%) by constructing a confusion matrix with the number of true positive, false positive, false negative and true negative cases. For models generating non-dichotomous scores (as here) a threshold can be applied to transform the scores into a dichotomous set of presence-absence predictions. Two measures that can be derived from the confusion matrix are sensitivity (the proportion of observed presences that are predicted as such, quantifying omission errors) and specificity (the proportion of observed absences that are predicted as such, quantifying commission errors). A receiver operating characteristic (ROC) curve can be constructed by using all possible thresholds to classify the scores into confusion matrices, obtaining sensitivity and specificity for each matrix, and plotting sensitivity against the corresponding proportion of false positives (equal to 1 - specificity). The use of all possible thresholds avoids the need for a selection of a single threshold, which is often arbitrary, and allows appreciation of the trade-off between sensitivity and specificity. The area under the ROC curve (AUC) is often used as a single threshold-independent measure for model performance (Manel, Williams & Ormerod 2001).

A2.2b Cohen's Kappa (Cohen, 1960)

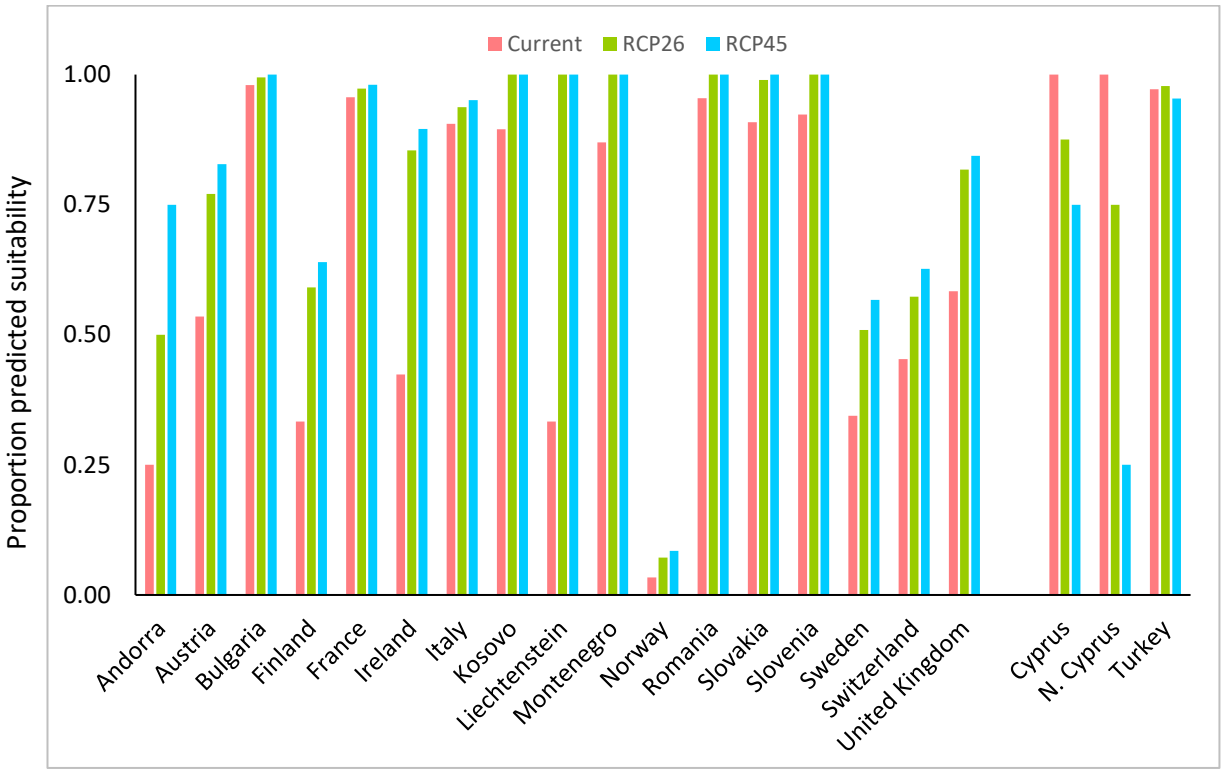
This measure corrects the overall accuracy of model predictions (ratio of the sum of true presences plus true absences to the total number of records) by the accuracy expected to occur by chance. The kappa statistic ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random. Advantages of kappa are its simplicity, the fact that both commission and omission errors are accounted for in one parameter and its relative tolerance to zero values in the confusion matrix (Manel, Williams & Ormerod 2001). However, Kappa has been criticised for being sensitive to prevalence (the proportion of sites in which the species was recorded as present) and may therefore be inappropriate for comparisons of model accuracy between species or regions (McPherson *et al*., 2004; Allouche *et al*. 2006).

A2.2c TSS (the true skill statistic; Allouche *et al*. 2006)

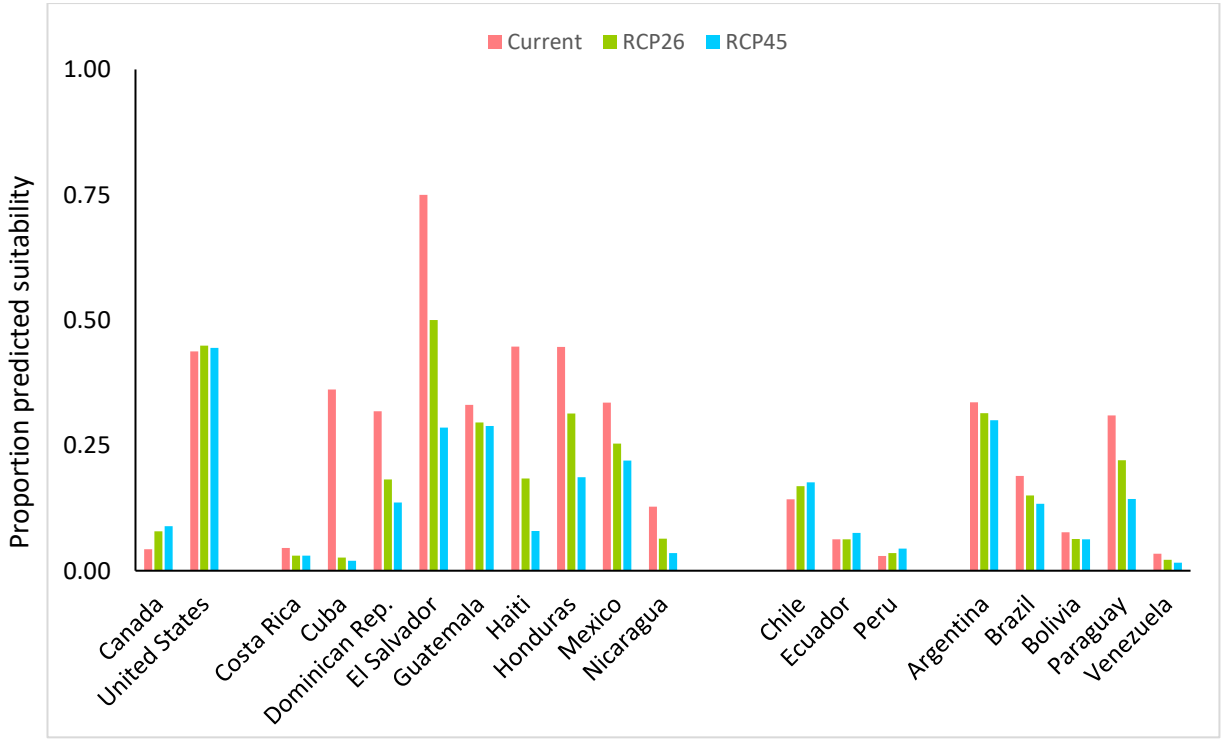
TSS is defined as sensitivity + specificity - 1, and corrects for Kappa's dependency on prevalence. TSS compares the number of correct forecasts, minus those attributable to random guessing, to that of a hypothetical set of perfect forecasts. Like kappa, TSS takes into account both omission and commission errors and success as a result of random guessing, and ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random (Allouche *et al*. 2006).

A2.3 Variation in projected suitability for *Harmonia axyridis* establishment across the continents

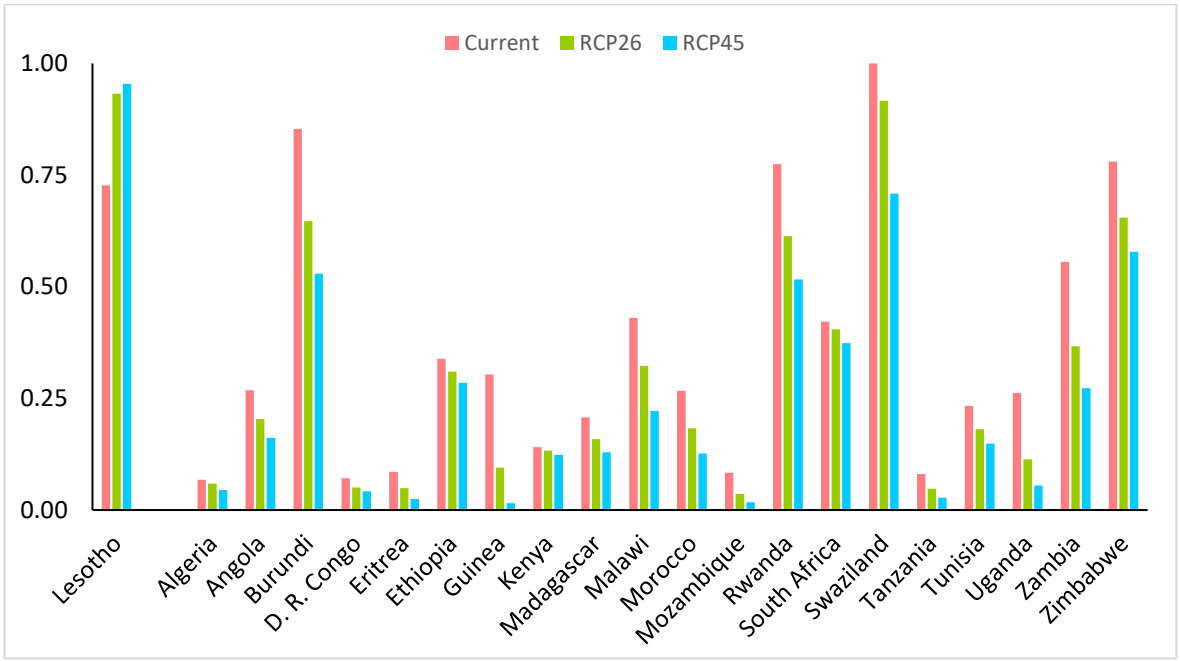
(a) Europe



(b) North and South America



(c) Africa



(d) Asia and Oceania

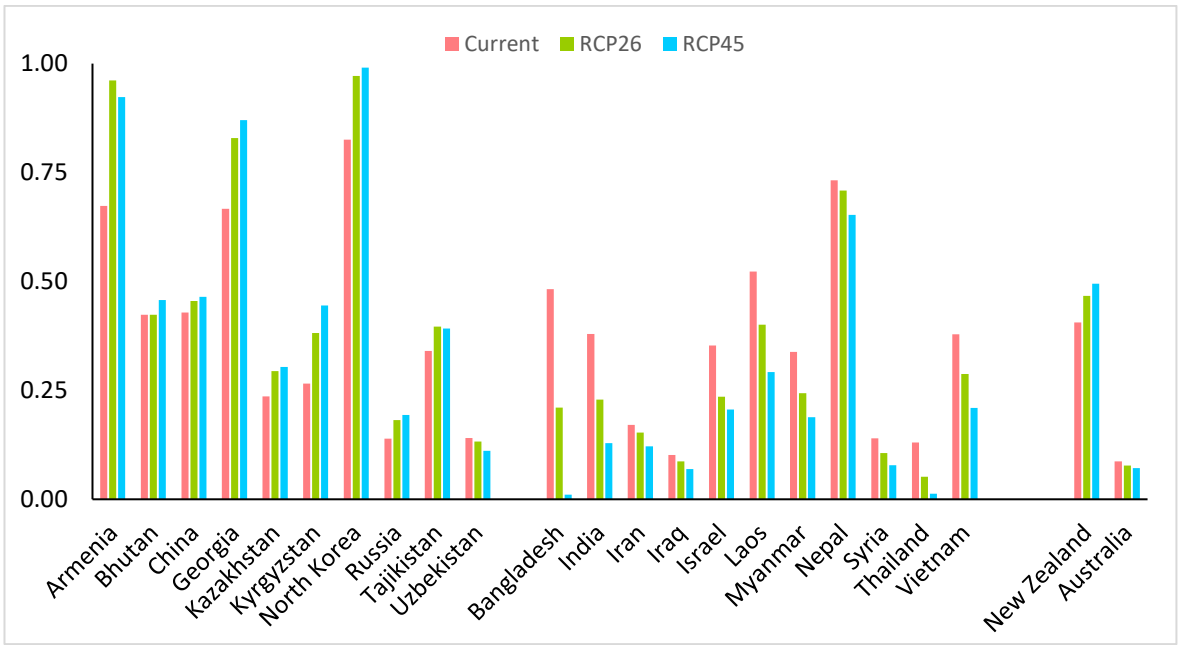


Figure A2.3: Variation in projected suitability for *Harmonia axyridis* establishment across the continents. The bar plots show the proportion of grid cells in each country classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios (RCP26 & RCP45). For each continent (Europe, North America, South America, Africa, Asia, Oceania) countries with increasing suitability are to the left and countries with decreasing suitability are to the right. Where two continents are represented in the same figure (North & South America; Asia & Oceania), there is a clear division between each continent and increasing/decreasing suitability are depicted as described above. Each figure represents selected countries where changes in suitability are predicted in (a) Europe, (b) North and South America, (c) Africa and (d) Asia and Oceania. Countries with a predicted proportion of 1 (e.g. Netherlands, Belgium) or 0 (e.g. Iceland, Chad) under both current and future scenarios were excluded from the figures.

Appendix for Chapter 3

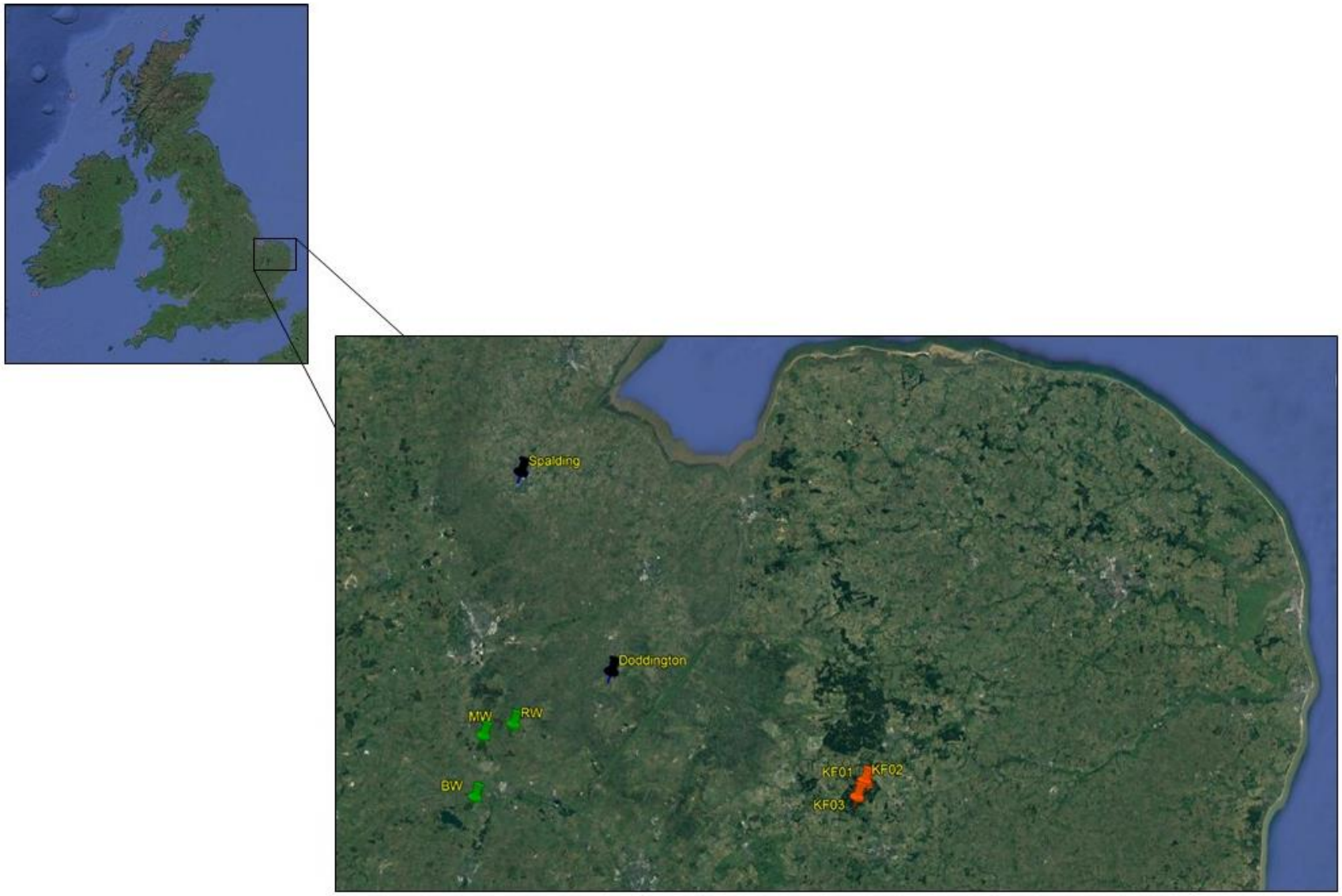


Figure A3.1a: Location of rural and urban field sites surveyed in 2016 and 2017. Dark Blue = Urban sites (Spalding and Doddington). Green = Deciduous sites (BW = Brampton Wood; MW = Monks Wood; RW = Raveley Wood). Orange = Coniferous sites (KF01, KF02, KF03 = Kings Forest 01, 02 and 03).

Figure A3.1b: Location of coniferous sites surveyed in 2016 and 2017; KF01, KF02, KF03 = Kings Forest 01, 02 and 03.

Figure A3.1c: Location of deciduous sites surveyed in 2016 and 2017; BW = Brampton Wood; MW = Monks Wood; RW = Raveley Wood.

Table A3.1: Total number of coccinellids recorded on trees at different site types in Cambridgeshire, Suffolk and Lincolnshire.

Species/Site Type	Deciduous	Coniferous	Urban	TOTAL
Harmonia axyridis	55	170	764	**989**
Coccinella septempunctata	175	98	21	**294**
Exochomus quadripustulatus	6	169	41	**216**
Adalia decempunctata	12	0	38	**50**
Harmonia quadripunctata	2	43	1	**46**
Propylea quattuordecimpunctata	18	1	18	**37**
Calvia quattuordecimguttata	11	4	5	**20**
Anatis ocellata	1	17	0	**18**
Adalia bipunctata	1	4	8	**13**
Halyzia sedecimguttata	7	1	4	**12**
Myzia oblongoguttata	0	11	0	**11**
Myrrha octodecimguttata	0	9	0	**9**
Scymnus suturalis	0	6	0	**6**
Chilocorus renipustulatus	4	1	0	**5**
Subcoccinella vigintiquattuorpunctata	0	2	0	**2**
Aphidecta obliterata	0	0	1	**1**
Psyllobora vigintiduopunctata	1	0	0	**1**
Tytthaspis sedecimpunctata	0	0	0	**0**
Early stage larva (ESL)	20	76	47	**143**
TOTAL	**313**	**612**	**948**	**1873**

Table A3.2: Total number of coccinellids species recorded from the grassland in Cambridgeshire and Suffolk.

Species	**Deciduous**	**Coniferous**	**Total**
Subcoccinella vigintiquattuorpunctata	0	143	**143**
Coccinella septempunctata	105	12	**117**
Tytthaspis sedecimpunctata	0	91	**91**
Propylea quattuordecimpunctata	24	2	**26**
Harmonia axyridis	10	2	**12**
Psyllobora vigintiduopunctata	1	9	**10**
Exochomus quadripustulatus	0	1	**1**
Scymnus suturalis	0	1	**1**
Early Stage Larvae (ESL)	2	2	**4**
TOTAL	**142**	**263**	**405**

Table A3.3 Final model that was best fit when variables applied under the following conditions. See Appendix 3 for details regarding model selection. ZINB = Zero-inflated negative binomial; NB = Negative binomial; N/A = not applicable due to data not collected or not enough data available for analysis.

Location	**Urban & rural trees**	**Rural woodland**	**Deciduous woodland**	**Coniferous woodland**
Dependent variable				
H. axyridis	Full ZINB	Full ZINB	Full ZINB	Reduced NB
Native coccinellids	Null model	Reduced NB	Full ZINB	Reduced NB
Aphidoidea	Reduced ZINB	Full ZINB	Full ZINB	Reduced NB
Formicidae	Full ZINB	Full ZINB	Full ZINB	Reduced NB
Neuroptera	Full ZINB	Reduced NB	Null model	Null model
Location		**Rural grassland**	**Deciduous grassland**	**Coniferous grassland**
Dependent variable				
H. axyridis	N/A	N/A	N/A	N/A
Native coccinellids	N/A	Reduced NB	Null model	Full ZINB
Aphidoidea	N/A	Full ZINB	Full ZINB	Full NB
Formicidae	N/A	Reduced NB	Full ZINB	Full NB
Neuroptera	N/A	N/A	N/A	N/A

Table A3.4a Comparison of regression models *H. axyridis* from both urban and woodland sites.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	1.30	2.70	1.17	-6.67	1.16	-7.38
UR[a]	2.62	2.67	2.20	-18.04	2.27	-18.04
Season[c]	-0.05	0.11	-0.15	-0.96	-0.15	-1.12
Temp	0.04	0.02	0.06	0.11	0.06	0.13
Humidity	-0.02	-0.04	-0.01	0.07	-0.02	0.07
Standard Errors						
Intercept	0.36	1.21	0.36	3.02	1.00	3.50
UR[a]	0.08	0.26	0.08	2329.45	0.18	2584.88
Season[c]	0.70	0.23	0.07	0.56	0.18	0.69
Temp	0.01	0.04	0.01	0.08	0.03	0.10
Humidity	0.004	0.01	0.004	0.03	0.01	0.04
Likelihood						
Log Likelihood	-348	-260	-294		-249	
Model *df*	5	6	10		11	
Fit measures						
AIC	707	531	609		519	
AIC Weight	0.00	0.00	0.00		0.99	

[a] Reference category is urban; [c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.4b Results for final ZINB regression model (full ZINB) for *H. axyridis* from both urban and woodland sites

Variable	ZINB Count			ZINB Logistic		
	Coefficients	SE	z-value	Coefficients	SE	z-value
Intercept	1.16	1.00	1.16	-7.38	3.50	-2.11
UR[a]	2.27	0.18	12.52***	-18.04	2584.88	-0.007
Season[c]	-0.15	0.18	-0.83	-1.12	0.69	-1.62
Temp	0.06	0.03	2.07	0.13	0.10	1.32
Humidity	-0.02	0.01	-1.31	0.07	0.04	1.91

Log likelihood = -249, Model df = 11, AIC = 519; ZINB = Zero Inflated negative binomial; SE = Standard error; [a] Reference category is Urban; [c] Reference category is Summer; *** = $p < 0.0001$; ** = $p < 0.001$; * = $p < 0.01$; AIC: Akaike information criterion.

Table A3.5 Comparison of regression models native coccinellids from both urban and woodland sites.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	1.67	1.58	1.54	-4.35	1.44	-14.59
UR[a]	-0.13	-0.13	-0.07	0.62	-0.09	25.30
Season[c]	-.06	-0.06	-0.002	0.40	0.01	54.05
Temp	.003	0.006	0.004	0.02	0.00	-8.32
Humidity	0.006	0.006	0.009	0.02	0.01	1.34
Standard Errors						
Intercept	0.39	1.02	0.39	3.72	1.01	824.40
UR[a]	0.10	0.24	0.10	0.75	0.23	96.53
Season[c]	0.08	0.20	0.08	0.70	0.19	237.64
Temp	0.01	0.03	0.01	0.11	0.03	60.68
Humidity	0.004	0.01	0.004	0.04	0.01	4.13
Likelihood						
Log Likelihood	-435	-298	-386		-293	
Model *df*	5	6	10		11	
Fit measures						
AIC	880	609	793		608	
AIC Weight	0.00	0.39	0.00		0.60	

[a] Reference category is urban; [c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.6a Comparison of regression models for *H. axyridis* in rural woodland.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	1.38	1.65	-0.35	-9.03	-0.77	-13.03
Type[a]	-1.2	-1.48	-0.25	3.15	-0.40	4.04
Gradient[b]	0.86	0.97	0.75	-0.42	0.80	-0.52
Season[c]	0.16	0.68	-0.26	-2.10	-0.04	-2.62
Temp	0.02	0.00	0.05	0.14	0.06	0.23
Humidity	-0.03	-0.03	0.00	0.09	0.002	0.11
Standard Errors						
Intercept	0.79	1.41	0.88	3.32	1.56	4.85
Type[a]	0.16	0.26	0.19	0.73	0.32	1.10
Gradient[b]	0.15	0.25	0.17	0.53	0.25	0.76
Season[c]	0.14	0.25	0.16	0.69	0.26	0.87
Temp	0.02	0.04	0.02	0.09	0.04	0.13
Humidity	0.01	0.02	0.01	0.03	0.02	0.05
Likelihood						
Log Likelihood	-273	-221	-231		-211	
Model *df*	6	7	12		13	
Fit measures						
AIC	558	456	487		448	
AIC Weight	0.00	0.01	0.00		0.99	

[a] Reference category is deciduous; [b] Reference category is mature; [c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.6b Results for final ZINB regression model (full ZINB) for *H. axyridis* in rural woodland

Variable	ZINB Count			ZINB Logistic		
	Coefficients	SE	z-value	Coefficients	SE	z-value
Intercept	-0.77	1.56	-0.49	-13.03	4.85	**-2.69***
Type[a]	-0.40	0.32	-1.26	4.04	1.10	**3.67****
Gradient[b]	0.80	0.25	**3.24***	-0.52	0.76	-0.68
Season[c]	-0.04	0.26	-0.16	-2.62	0.87	-**3.01***
Temp	0.06	0.04	1.46	0.23	0.13	1.76
Humidity	0.002	0.02	0.10	0.11	0.05	2.30

Log likelihood = -211, Model df = 13, AIC = 448; [a] Reference category is Deciduous; [b] Reference category is Mature; [c] Reference category is Summer; *** = $p \leq 0.0001$; ** = $p \leq 0.001$; * = $p \leq 0.01$; AIC: Akaike information criterion.

Table A3.7a Comparison of regression models for *H. axyridis* species in deciduous woodland.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	3.61	2.32	1.44	11.69	1.55	46.95
Gradient[b]	1.22	1.29	1.19	-0.03	1.26	-10.01
Season[c]	2.59	2.48	2.81	3.50	2.89	20.94
Temp	-0.21	-0.19	-0.32	-2.29	-0.35	-17.25
Humidity	-0.04	-0.03	0.04	0.42	0.04	3.77
Standard Errors						
Intercept	1.83	2.95	1.86	9.78	2.47	74.54
Gradient[b]	0.33	0.52	0.33	1.66	0.48	38.77
Season[c]	0.47	0.62	0.49	2.58	0.60	43.52
Temp	0.06	0.10	0.06	1.02	0.10	32.63
Humidity	0.02	0.03	0.02	0.18	0.03	7.67
Likelihood						
Log Likelihood	-68	-65	-65		-57	
Model *df*	5	6	10		11	
Fit measures						
AIC	184	149	151		137	
AIC Weight	0.00	0.002	0.00		0.99	

[b] Reference category is mature; [c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.7b Results for final ZINB regression model (full ZINB) for *H. axyridis* in deciduous woodland

Variable	ZINB Count			ZINB Logistic		
	Coefficients	SE	z-value	Coefficients	SE	z-value
Intercept	1.55	2.47	0.63	46.95	74.54	0.63
Gradient[b]	1.26	0.48	2.65*	-10.01	38.77	-0.26
Season[c]	2.89	0.60	4.78***	20.94	43.52	0.48
Temp	-0.35	0.10	-3.56**	-17.25	32.63	-0.53
Humidity	0.04	0.03	1.38	3.77	7.67	0.49

ZINB = Zero Inflated negative binomial; Log likelihood = -57, Model df = 11, AIC = 137; [b] Reference category is Mature; [c] Reference category is Summer; *** = $p < 0.0001$; ** = $p < 0.001$; * = $p < 0.01$; AIC: Akaike information criterion.

Table A3.8a Comparison of regression models for *H. axyridis* in coniferous woodlands.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	1.46	1.00	-.74	-12.70	N/A	N/A
Gradient[b]	0.74	0.71	0.75	0.25	N/A	N/A
Season[c]	-0.35	-0.18	-0.48	-1.93	N/A	N/A
Temp	0.05	0.05	0.07	0.12	N/A	N/A
Humidity	-0.03	-0.03	0.001	0.14	N/A	N/A
Standard Errors						
Intercept	0.98	1.53	1.20	6.80	N/A	N/A
Gradient[b]	0.17	0.25	0.19	1.08	N/A	N/A
Season[c]	0.16	0.26	0.18	2.03	N/A	N/A
Temp	0.02	0.04	0.03	0.15	N/A	N/A
Humidity	0.01	0.02	0.01	0.07	N/A	N/A
Likelihood						
Log Likelihood	-155	-138	-142		N/A	
Model *df*	5	6	10		N/A	
Fit measures						
AIC	321	289	303		N/A	
AIC Weight	0.00	0.99	0.00		N/A	

[b] Reference category is mature; [c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.8b Results for final NB regression model (reduced NB model) for *H. axyridis* in coniferous woodland

Variable	Negative binomial		
	Coefficients	SE	z-value
Intercept	2.33	0.96	2.42*
Gradient[b]	0.73	0.26	2.82*
Hum	-0.03	0.02	-1.99

NB = Negative binomial; Log likelihood = 139, Model df = 4, AIC = 287; [b] Reference category is mature; *** = $p < 0.0001$; ** = $p < 0.001$; * = $p < 0.01$; AIC: Akaike information criterion.

Table A3.9a Comparison of regression models for native coccinellid species in rural woodlands.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	1.12	1.45	1.21	-3.10	N/A	N/A
Type[a]	-0.62	-0.64	-0.61	0.21	N/A	N/A
Gradient[b]	0.39	0.32	0.18	-1.23	N/A	N/A
Season[c]	-0.06	-0.08	-0.06	0.14	N/A	N/A
Temp	-0.02	-0.03	-0.01	0.08	N/A	N/A
Humidity	0.01	0.01	0.01	0.001	N/A	N/A
Standard Errors						
Intercept	0.45	0.91	0.44	2.59	N/A	N/A
Type[a]	0.08	0.17	0.09	0.49	N/A	N/A
Gradient[b]	0.08	0.17	0.09	0.52	N/A	N/A
Season[c]	0.09	0.18	0.09	0.50	N/A	N/A
Temp	0.01	0.03	0.01	0.07	N/A	N/A
Humidity	0.004	0.01	0.01	0.03	N/A	N/A
Likelihood						
Log Likelihood	-475	-368	-433		N/A	
Model *df*	6	7	12		N/A	
Fit measures						
AIC	963	750	890		N/A	
AIC Weight	0.00	1.00	0.00		N/A	

[a] Reference category is deciduous; [b] Reference category is mature; [c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.9b Results for final NB regression model (reduced NB model) for native coccinellid species in rural woodland

Variable	Negative binomial		
	Coefficients	SE	z-value
Intercept	1.45	0.15	9.55***
Type[a]	-0.54	0.17	-3.16**
Gradient[b]	0.34	0.17	1.96

Log likelihood = 370, Model df = 4, AIC = 748; NB = Negative binomial; [a] Reference category is deciduous; [b] Reference category is mature; *** = $p < 0.0001$; ** = $p < 0.001$; * = $p < 0.01$; AIC: Akaike information criterion.

Table A3.10a Comparison of regression models native coccinellids in deciduous woodlands.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	1.69	1.88	0.85	-34.93	1.25	-766.52
Gradient[b]	0.02	0.07	-0.006	-0.88	0.10	0.02
Season[c]	-0.23	-0.15	0.17	13.43	0.09	147.15
Temp	-0.08	-0.09	-0.07	0.44	-0.10	8.52
Humidity	0.02	0.02	0.03	0.19	0.03	6.80
Standard Errors						
Intercept	0.62	1.08	0.65	69.38	0.94	639.30
Gradient[b]	0.13	0.22	0.13	1.01	0.20	6.64
Season[c]	0.14	0.23	0.15	68.10	0.22	137.14
Temp	0.02	0.03	0.02	0.26	0.03	7.12
Humidity	0.006	0.01	0.007	0.11	0.01	5.75
Likelihood						
Log Likelihood	-200	-174	-182		-164	
Model *df*	5	6	10		11	
Fit measures						
AIC	409	360	384		349	
AIC Weight	0.00	0.004	0.00		0.99	

[b] Reference category is mature; [c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.10b Results for final ZINB regression model (full ZINB) for native coccinellids in deciduous woodland

Variable	ZINB Count			ZINB Logistic		
	Coefficients	SE	z-value	Coefficients	SE	z-value
Intercept	1.25	0.94	1.33	-766.52	639.30	-1.20
Gradient[b]	0.10	0.20	0.50	0.02	6.64	0.002
Season[c]	0.09	0.22	0.39	147.15	137.14	1.07
Temp	-0.10	0.03	-3.01*	8.52	7.12	1.20
Humidity	0.03	0.01	2.56*	6.80	5.75	1.18

Log likelihood = -164, Model df = 11, AIC = 349; ZINB = Zero Inflated negative binomial; SE = Standard error; [b] Reference category is Mature; [c] Reference category is Summer; *** = $p < 0.0001$; ** = $p < 0.001$; * = $p < 0.01$; AIC: Akaike information criterion.

Table A3.11a Comparison of regression models for native coccinellids in coniferous woodlands.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	0.03	-0.37	0.77	1.78	-0.29	65.48
Gradient[b]	0.64	0.66	0.39	-1.90	0.61	-2.80
Season[c]	0.10	0.10	-0.09	-1.76	-0.02	-24.24
Temp	0.02	0.04	0.02	-0.03	0.06	0.51
Humidity	0.01	0.01	0.008	-0.03	0.007	-1.53
Standard Errors						
Intercept	.65	1.49	0.63	4.56	1.50	365.25
Gradient[b]	0.11	0.24	0.11	0.86	0.23	2.39
Season[c]	0.11	0.25	0.11	0.96	0.24	573.37
Temp	0.02	0.04	0.02	0.12	0.04	0.52
Humidity	0.007	0.02	0.007	0.05	0.02	7.29
Likelihood						
Log Likelihood	-258	-188	-232		-183	
Model *df*	5	6	10		11	
Fit measures						
AIC	526	388	484		389	
AIC Weight	0.00	0.53	0.00		0.46	

[b] Reference category is mature; [c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.11b Results for final NB regression model (reduced NB model) for native coccinellids in coniferous woodland

Variable	Negative binomial		
	Coefficients	SE	z-value
Intercept	1.27	0.18	7.06***
Gradient[b]	.65	0.24	2.67*

Log likelihood = -189, Model df = 3, AIC = 383; NB = Negative binomial; [b] Reference category is mature; *** = $p < 0.0001$; ** = $p < 0.001$; * = $p < 0.01$; AIC: Akaike information criterion.

Table A3.12a Comparison of regression models for native coccinellids from rural grasslands.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	3.78	3.68	2.99	-10.07	N/A	N/A
Type[b]	-0.85	-0.76	-0.66	0.69	N/A	N/A
Season[c]	0.61	0.49	0.52	-0.15	N/A	N/A
Temp	-0.63	-0.08	-0.03	0.26	N/A	N/A
Humidity	-0.01	-0.01	-0.008	0.05	N/A	N/A
Standard Errors						
Intercept	0.44	1.11	0.46	3.98	N/A	N/A
Type[b]	0.11	0.25	0.11	0.67	N/A	N/A
Season[c]	0.10	0.25	0.11	0.69	N/A	N/A
Temp	0.02	0.04	0.02	0.11	N/A	N/A
Humidity	0.004	0.01	0.004	0.04	N/A	N/A
Likelihood						
Log Likelihood	-287	-201	-259		N/A	
Model *df*	5	6	10		N/A	
Fit measures						
AIC	584	414	537		N/A	
AIC Weight	0.00	1.00	0.00		N/A	

[b] Reference category is deciduous; [c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.12b Results for final NB regression model (reduced NB model) for native coccinellids from rural grasslands.

Variable	Negative binomial		
	Coefficients	SE	z-value
Intercept	3.18	0.78	4.09***
Type[a]	-0.77	0.25	-3.09*
Season[b]	0.54	0.25	2.16
Temp	-0.07	0.04	-1.98

Log likelihood = -201, Model df = 5, AIC = 412; NB = Negative binomial; [a] Reference category is deciduous; [b] Reference category is summer; *** = $p < 0.0001$; ** = $p < 0.001$; * = $p < 0.01$; AIC: Akaike information criterion.

Table A3.13 Comparison of regression models for native coccinellids in deciduous grasslands.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	2.87	3.09	2.34	-8.10	3.12	-86.11
Season[c]	-0.15	-0.08	0.02	0.93	0.28	30.78
Temp	-0.06	-0.07	-0.04	0.17	-0.09	0.62
Humidity	-0.006	-0.01	-0.002	0.05	-0.001	0.66
Standard Errors						
Intercept	0.58	1.31	0.61	5.00	1.19	5536.61
Season[c]	0.19	0.36	0.21	0.92	0.38	5536.19
Temp	0.03	0.05	0.03	0.14	0.05	0.82
Humidity	0.006	0.01	0.005	0.05	0.01	0.63
Likelihood						
Log Likelihood	-117	-94	-104		-90	
Model *df*	4	5	8		9	
Fit measures						
AIC	242	197	224		198	
AIC Weight	0.00	0.58	0.00		0.42	

[c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.14a Comparison of regression models for native coccinellids in coniferous grasslands.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	3.49	2.74	2.92	-252.85	1.70	-246.62
Season[c]	0.99	1.02	0.80	-19.85	0.80	-19.78
Temp	-0.05	-0.04	-0.02	8.09	0.01	1.89
Humidity	-0.02	-0.01	-0.02	0.98	-0.006	0.95
Standard Errors						
Intercept	0.72	1.97	0.76	436.40	1.77	415.24
Season[c]	0.14	0.34	0.14	642.44	0.33	657.48
Temp	0.02	0.05	0.02	14.10	0.05	13.45
Humidity	0.008	0.02	0.01	1.93	0.02	1.84
Likelihood						
Log Likelihood	-155	-105	-140		-97	
Model *df*	4	5	8		9	
Fit measures						
AIC	318	219	295		211	
AIC Weight	0.00	0.016	0.00		0.98	

[c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.14b Results for final ZINB regression model (full ZINB) for native coccinellids from grass in coniferous woodland

Variable	ZINB Count			ZINB Logistic		
	Coefficients	SE	z-value	Coefficients	SE	z-value
Intercept	1.70	1.77	0.96	-246.62	415.24	-0.59
Season[c]	0.80	0.33	2.47*	-19.78	657.48	-0.03
Temp	0.01	0.05	0.30	1.89	13.45	0.59
Humidity	-0.006	0.02	-0.31	0.95	1.84	0.52

Log likelihood = -97, Model df = 9, AIC = 211; ZINB = Zero Inflated negative binomial; SE = Standard error; [c] Reference category is Summer; *** = $p < 0.0001$; ** = $p < 0.001$; * = $p < 0.01$; AIC: Akaike information criterion.

Table A3.15a Comparison of regression models for *H. axyridis* and Shannon diversity of native coccinellids in rural habitats.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	-0.49	-0.44	0.84	1.03	0.25	0.09
Shannon	1.16	1.05	0.37	-1.39	0.57	-1.76
Standard Errors						
Intercept	0.10	0.18	0.11	0.20	0.31	0.53
Shannon	0.13	0.31	0.13	0.35	0.29	0.74
Likelihood						
Log Likelihood	-403	-288	-316		-285	
Model *df*	2	3	4		5	
Fit measures						
AIC	809	581	640		579	
AIC Weight	0.00	0.02	0.00		0.98	

[c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.15b Results for final ZINB regression model (full ZINB) for *H. axyridis* and Shannon diversity of native coccinellids in rural habitats.

Variable	ZINB Count			ZINB Logistic		
	Coefficients	SE	z-value	Coefficients	SE	z-value
Intercept	0.25	0.31	0.82	0.09	0.53	0.17
Shannon	0.57	0.29	1.96	-1.76	0.74	-2.37*

Log likelihood = -97, Model df = 9, AIC = 211; ZINB = Zero Inflated negative binomial; SE = Standard error; [c] Reference category is Summer; *** = $p < 0.0001$; ** = $p < 0.001$; * = $p < 0.01$; AIC: Akaike information criterion.

Table A3.16a Comparison of regression models for native coccinellids and Shannon diversity of native coccinellids in rural habitats.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	1.04	0.97	1.36	-0.80	1.18	-1.43
Shannon	0.99	1.15	0.67	-37.99	0.89	-37.99
Standard Errors						
Intercept	0.05	0.09	0.05	0.19	0.10	0.36
Shannon	0.06	0.15	0.07	4378.9	0.16	5987.12
Likelihood						
Log Likelihood	-761	-564	-698		-559	
Model *df*	2	3	4		5	
Fit measures						
AIC	1525	1133	1403		1127	
AIC Weight	0.00	0.00	0.00		1.00	

[c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.16b Results for final ZINB regression model (full ZINB) for native coccinellids and Shannon diversity of native coccinellids in rural habitats.

Variable	ZINB Count			ZINB Logistic		
	Coefficients	SE	z-value	Coefficients	SE	z-value
Intercept	1.18	0.10	11.60**	-1.43	0.36	-4.01**
Shannon	0.89	0.16	5.63**	-37.99	5987.12	0.99

Log likelihood = -97, Model df = 9, AIC = 211; ZINB = Zero Inflated negative binomial; SE = Standard error; [c] Reference category is Summer; *** = $p < 0.0001$; ** = $p < 0.001$; * = $p < 0.01$; AIC: Akaike information criterion.

Table A3.17a Comparison of regression models for aphids from trees at both urban sites and rural woodlands.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	4.89	7.22	5.99	0.17	8.94	1.97
UR [a]	1.69	2.00	1.30	-1.45	1.66	-2.5
Temp	-0.02	-0.11	-0.02	0.002	-0.13	-0.04
Humidity	-0.01	-0.03	-0.02	-0.005	-0.05	-0.03
Season[c]	0.86	1.56	0.57	-0.66	1.46	-0.50
Standard Errors						
Intercept	0.07	1.65	0.09	1.43	2.20	3.13
UR [a]	0.02	0.57	0.02	0.65	0.46	3.98
Temp	0.002	0.06	0.002	0.05	0.06	0.09
Humidity	0.001	0.02	0.001	0.01	0.02	0.04
Season[c]	0.02	0.37	0.02	0.33	0.37	0.57
Likelihood						
Log Likelihood	-18561	-698	-12806		-692	
Model *df*	5	6	10		11	
Fit measures						
AIC	37131	1407	25632		1407	
AIC Weight	0.00	0.39	0.00		0.61	

[a] Reference category is urban; [c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.17b Results for final ZINB regression model (reduced ZINB) aphids from trees at both urban sites and rural woodlands

Variable	ZINB Count			ZINB Logistic		
	Coefficients	SE	z-value	Coefficients	SE	z-value
Intercept	3.83	0.26	14.50	-0.59	0.38	-1.56
UR[a]	1.76	0.46	3.83**	-1.83	1.50	-1.22
Season[c]	1.12	0.33	3.35**	-0.61	0.48	-1.27

Log likelihood = -695, Model df = 7, AIC = 1404; ZINB: Zero-inflated negative binomial; [a]Reference category is Urban; [c]Reference category is Summer; *** = p < 0.0001; ** = p < 0.001; * = p < 0.01; AIC: Akaike information criterion.

Table A3.18a Comparison of regression models for ants from trees at both urban sites and rural woodlands.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	3.95	2.56	3.52	-2.91	1.50	-4.62
UR [a]	-1.42	-1.54	-1.71	-1.20	-1.87	-10.11
Season[c]	0.43	0.62	0.34	-0.17	0.72	-0.11
Temperature	-0.01	0.02	0.002	0.01	0.03	0.02
Humidity	0.003	0.01	0.01	0.04	0.04	0.05
Standard Errors						
Intercept	0.09	1.55	0.10	1.72	1.69	2.84
UR [a]	0.06	0.53	0.06	0.66	0.40	127.61
Season[c]	0.02	0.35	0.02	0.34	0.32	0.48
Temperature	0.003	0.05	0.003	0.05	0.05	0.07
Humidity	0.001	0.02	0.001	0.02	0.02	0.03
Likelihood						
Log Likelihood	-10609	-697	-6078		-687	
Model *df*	5	6	10		11	
Fit measures						
AIC	21228	1406	12177		1397	
AIC Weight	0.00	0.01	0.00		0.99	

[a] Reference category is urban; [c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.18b Results for final ZINB regression model (full ZINB) ants from trees at both urban sites and rural woodlands

Variable	ZINB Count			ZINB Logistic		
	Coefficients	SE	z-value	Coefficients	SE	z-value
Intercept	1.50	1.69	0.89	-4.62	2.84	-1.63
UR[a]	-1.87	0.40	-4.67***	-10.11	127.61	-0.08
Season[c]	0.72	0.32	2.27’	-0.11	0.48	-0.23
Temperature	0.03	0.05	0.55	0.02	0.07	0.27
Humidity	0.04	0.02	1.86	0.05	0.03	1.90

Log likelihood = -687, Model df = 11, AIC = 1397; ZINB: Zero-inflated negative binomial; [a]Reference category is Urban; [c]Reference category is Summer; *** = p < 0.0001; ** = p < 0.001; * = p < 0.01; ‘ = p < 0.05; AIC: Akaike information criterion.

Table A3.19a Comparison of regression models for lacewings from trees at both urban sites and rural woodlands.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	0.84	1.87	1.14	-1.97	1.92	-245.11
UR [a]	2.27	2.28	1.97	-2.02	2.32	52.73
Season[c]	-0.31	0.05	-0.21	0.24	0.13	118.85
Temperature	0.01	-0.05	.01	0.05	-0.06	-16.57
Humidity	0.001	0.001	0.001	-0.001	0.003	5.84
Standard Errors						
Intercept	0.32	0.92	0.32	1.68	0.77	284.74
UR [a]	0.07	0.29	0.07	1.04	0.29	75.30
Season[c]	0.07	0.21	0.08	0.38	0.21	184.02
Temperature	0.01	0.03	0.01	0.06	0.03	30.47
Humidity	0.003	0.01	0.003	0.02	0.01	7.26
Likelihood						
Log Likelihood	-807	-405	-730		-399	
Model *df*	5	6	10		11	
Fit measures						
AIC	1623	822	1479		819	
AIC Weight	0.00	0.17	0.00		0.83	

[a] Reference category is urban; [c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.19b Results for final ZINB regression model (full ZINB) lacewings from trees at both urban sites and rural woodlands

Variable	ZINB Count			ZINB Logistic		
	Coefficients	SE	z-value	Coefficients	SE	z-value
Intercept	1.92	0.77	2.49	-245.11	284.74	-0.86
UR[a]	2032	0.29	7.99***	52.73	75.30	0.70
Season[c]	0.13	0.21	0.63	118.85	184.02	0.65
Temperature	-0.06	0.03	-1.85	-16.57	30.47	-0.54
Humidity	0.003	0.01	0.44	5.84	7.26	0.80

Log likelihood = -399, Model df = 11, AIC = 820; ZINB: Zero-inflated negative binomial; [a]Reference category is Urban; [c]Reference category is Summer; *** = $p < 0.0001$; ** = $p < 0.001$; * = $p < 0.01$; AIC: Akaike information criterion.

Table A3.20a Comparison of regression models for aphids from trees in rural woodlands.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	2.31	-1.22	2.28	-1.65	6.49	-8.61
Type[a]	-1.99	-3.57	-0.99	3.92	-1.66	14.36
Gradient[b]	0.06	0.99	0.13	0.46	0.23	-0.02
Season[c]	1.63	3.03	1.40	-1.31	2.05	-0.83
Temperature	-0.01	-0.01	0.001	0.04	-0.11	-0.05
Humidity	0.03	0.08	0.03	-0.03	-0.009	-0.06
Standard Errors						
Intercept	0.13	1.93	0.13	2.51	2.38	139.79
Type[a]	0.03	0.36	0.03	0.59	0.49	139.75
Gradient[b]	0.02	0.35	0.02	0.46	0.35	0.58
Season[c]	0.02	0.37	0.02	0.49	0.37	0.61
Temperature	0.003	0.06	0.003	0.07	0.06	0.09
Humidity	0.001	0.02	0.001	0.03	0.03	0.04
Likelihood						
Log Likelihood	-9352	-543	-7545		-520	
Model *df*	6	7	12		13	
Fit measures						
AIC	18715	1100	15115		1067	
AIC Weight	0.00	0.00	0.00		0.99	

[a] Reference category is deciduous; [b] Reference category is mature; [c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.20b Results for final ZINB regression model (full ZINB) aphids from trees in rural woodlands

Variable	ZINB Count			ZINB Logistic		
	Coefficients	SE	z-value	Coefficients	SE	z-value
Intercept	6.49	2.38	2.73*	-8.61	139.79	-0.06
Type[a]	-1.66	0.49	-3.34**	14.36	139.75	0.10
Gradient[b]	0.23	0.35	0.65	-0.02	0.58	-0.03
Season[c]	2.05	0.37	5.46***	-0.83	0.61	-1.37
Temp	-0.11	0.06	-1.94	-0.05	0.09	-0.53
Humidity	-0.009	0.03	-0.35	-0.06	0.04	-1.51

Log likelihood = -520, Model df = 13, AIC = 1067; ZINB: Zero-inflated negative binomial; [a]Reference category is Deciduous; [b] Reference category is Mature; [c] Reference category is Summer; *** = $p < 0.0001$; ** = $p < 0.001$; * = $p < 0.01$; AIC: Akaike information criterion.

Table A3.21a Comparison of regression models for aphids from trees in deciduous woodlands.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	-7.72	-17.5	-16.78	-4.04	-23.13	-394.10
Gradient[b]	2.34	1.40	2.67	0.42	1.52	-2.97
Season[c]	4.25	3.17	4.72	0.37	3.22	33.28
Temperature	0.07	0.22	0.27	0.15	0.32	9.16
Humidity	0.06	0.19	0.15	0.01	0.25	2.46
Standard Errors						
Intercept	0.49	4.42	0.70	4.62	6.04	298.20
Gradient[b]	0.10	0.75	0.10	0.58	0.81	4.27
Season[c]	0.18	0.80	0.21	0.75	0.81	24.86
Temperature	0.01	0.12	0.02	0.12	0.14	7.11
Humidity	0.003	0.04	0.01	0.04	0.06	1.83
Likelihood						
Log Likelihood	-2436	-130	-741		-123	
Model *df*	5	6	10		11	
Fit measures						
AIC	4882	273	1502		269	
AIC Weight	0.00	0.12	0.00		0.88	

[b] Reference category is mature; [c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.21b Results for final ZINB regression model (full ZINB) aphids from trees in deciduous woodlands

Variable	ZINB Count			ZINB Logistic		
	Coefficients	SE	z-value	Coefficients	SE	z-value
Intercept	-23.13	6.04	-3.83**	-394.10	298.20	-1.32
Gradient[b]	1.52	0.81	1.88	-2.97	4.27	-0.70
Season[c]	3.22	0.81	3.96***	33.28	24.86	1.34
Temp	0.32	0.14	2.38’	9.16	7.11	1.29
Humidity	0.25	0.06	3.96***	2.46	1.83	1.34

Log likelihood = -123, Model df = 11, AIC = 269; ZINB: Zero-inflated negative binomial; [b] Reference category is Mature; [c] Reference category is Summer; *** = p < 0.0001; ** = p < 0.001; * = p < 0.01; ‘ = p < 0.05; AIC: Akaike information criterion.

Table A3.22a Comparison of regression models for aphids from trees in coniferous woodlands.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	3.56	8.67	3.36	-30.17	7.56	-30.67
Gradient[b]	-0.23	-0.35	-0.15	19.61	-0.20	19.12
Season[c]	1.38	1.57	1.26	-19.37	1.42	-19.37
Temperature	-0.02	-0.14	-0.01	0.27	-0.11	0.29
Humidity	0.01	-0.03	0.02	0.06	-0.02	0.07
Standard Errors						
Intercept	0.13	1.99	0.14	7226.66	2.16	6506.68
Gradient[b]	0.02	0.32	0.02	7226.65	0.31	6506.67
Season[c]	0.03	0.34	0.03	7589.92	0.33	8826.57
Temperature	0.003	0.05	0.004	0.19	0.06	0.22
Humidity	0.001	0.02	0.001	0.07	0.02	0.08
Likelihood						
Log Likelihood	-5880	-378	-5615		-375	
Model *df*	5	6	10		11	
Fit measures						
AIC	11770	768	11250		772	
AIC Weight	0.00	0.89	0.00		0.11	

[b] Reference category is mature; [c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.22b Results for final NB regression model (reduced NB model) for aphids from trees in coniferous woodlands.

Variable	Negative binomial		
	Coefficients	SE	z-value
Intercept	8.13	2.00	4.07***
Season[a]	1.55	0.34	4.54***
Temperature	-0.13	0.05	-2.47*
Humidity	-0.03	0.02	-1.23

Log likelihood = -378, Model df = 5, AIC = 767; NB = Negative binomial; [a] Reference category is summer; *** = $p < 0.0001$; ** = $p < 0.001$; * = $p < 0.01$; AIC: Akaike information criterion.

Table A3.23a Comparison of regression models for ants from trees in rural woodlands.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	3.32	0.55	3.30	-21.2	1.88	-21.97
Type[a]	-4.26	-4.36	-3.21	20.52	-3.54	19.73
Gradient[b]	0.24	1.17	0.23	-1.18	0.36	-1.29
Season[c]	0.57	-0.04	0.56	-0.74	0.33	-0.65
Temperature	-0.01	0.19	-0.01	0.05	0.09	0.10
Humidity	0.02	-0.00	0.02	0.02	0.01	0.03
Standard Errors						
Intercept	0.12	1.37	0.12	2012.6	1.22	1573.56
Type[a]	0.07	0.25	0.08	2012.58	0.28	1573.56
Gradient[b]	0.02	0.25	0.02	0.52	0.22	0.60
Season[c]	0.02	0.25	0.02	0.52	0.23	0.59
Temperature	0.003	0.04	0.003	0.08	0.04	0.09
Humidity	0.001	0.01	0.001	0.03	0.01	0.03
Likelihood						
Log Likelihood	-3068	-562	-2924		-532	
Model *df*	6	7	12		13	
Fit measures						
AIC	6147	1139	5873		1090	
AIC Weight	0.00	0.00	0.00		1.00	

[a] Reference category is deciduous; [b] Reference category is mature; [c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.23b Results for final ZINB regression model (full ZINB) ants from trees in rural woodlands

Variable	ZINB Count			ZINB Logistic		
	Coefficients	SE	z-value	Coefficients	SE	z-value
Intercept	1.88	1.22	1.54	-21.97	1573.56	-0.01
Type[a]	-3.54	0.28	-12.59***	19.73	1573.56	0.01
Gradient[b]	0.36	0.22	1.59	-1.29	0.60	-2.14
Season[c]	0.33	0.23	1.45	-0.65	0.59	-1.09
Temp	0.09	0.04	2.44*	0.10	0.09	1.00
Humidity	0.01	0.01	1.08	0.03	0.03	0.89

Log likelihood = -532, Model df = 13, AIC = 1090; ZINB: Zero-inflated negative binomial; [a]Reference category is Deciduous; [b] Reference category is Mature; [c] Reference category is Summer; *** = $p < 0.0001$; ** = $p < 0.001$; * = $p < 0.01$; AIC: Akaike information criterion.

Table A3.24a Comparison of regression models for ants from trees in deciduous woodlands.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	-7.89	N/A	-6.49	-5.28	-6.19	-11.29
Gradient[b]	2.47	N/A	-0.39	-1.95	-0.36	-3.44
Season[c]	-0.50	N/A	-0.001	-0.26	0.91	1.05
Temperature	0.35	N/A	0.39	0.26	0.41	0.57
Humidity	-0.01	N/A	0.003	0.02	-0.02	0.004
Standard Errors						
Intercept	1.06	N/A	1.17	4.36	2.19	7.40
Gradient[b]	0.28	N/A	0.39	0.83	0.64	1.60
Season[c]	0.17	N/A	0.20	0.71	0.52	1.48
Temperature	0.03	N/A	0.04	0.16	0.07	0.29
Humidity	0.008	N/A	0.008	0.03	0.02	0.07
Likelihood						
Log Likelihood	-201		-127		-100	
Model *df*	5	N/A	10		11	
Fit measures						
AIC	412	N/A	273		223	
AIC Weight	0.00	N/A	0.00		0.99	

[b] Reference category is mature; [c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.24b Results for final ZINB regression model (full ZINB) ants from trees in deciduous woodlands

Variable	ZINB Count			ZINB Logistic		
	Coefficients	SE	z-value	Coefficients	SE	z-value
Intercept	-6.19	2.19	-2.82*	-11.29	7.40	-1.53
Gradient[b]	-0.36	0.64	-0.56	-3.44	1.60	-2.15
Season[c]	0.91	0.52	1.77	1.05	1.48	0.71
Temp	0.41	0.07	5.78***	0.57	0.29	1.97
Humidity	-0.02	0.02	-0.90	0.004	0.07	0.06

Log likelihood = -100, Model df = 11, AIC = 223; ZINB: Zero-inflated negative binomial; [b] Reference category is Mature; [c] Reference category is Summer; *** = $p < 0.0001$; ** = $p < 0.001$; * = $p < 0.01$; AIC: Akaike information criterion.

Table A3.25a Comparison of regression models for ants from trees in coniferous woodlands.

Variable	Poisson	NB
Coefficients		
Intercept	3.36	2.60
Gradient[b]	0.21	0.21
Season[c]	0.60	0.67
Temperature	-0.02	-0.01
Humidity	0.03	0.03
Standard Errors		
Intercept	0.12	1.22
Gradient[b]	0.02	0.20
Season[c]	0.02	0.21
Temperature	0.003	0.03
Humidity	0.001	0.01
Likelihood		
Log Likelihood	-2646	-410
Model *df*	5	6
Fit measures		
AIC	5302	832
AIC Weight	0.00	1.00

[b] Reference category is mature; [c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.25b Results for final NB regression model (reduced NB model) for ants from trees in coniferous woodlands.

Variable	Negative binomial		
	Coefficients	SE	z-value
Intercept	2.52	0.80	3.14**
Season[a]	0.67	0.21	3.19**
Humidity	0.04	0.01	2.73*

Log likelihood = -410, Model df = 4, AIC = 829; NB = Negative binomial; [a] Reference category is summer; *** = $p < 0.0001$; ** = $p < 0.001$; * = $p < 0.01$; AIC: Akaike information criterion.

Table A3.26a Comparison of regression models for lacewings from trees in rural woodlands.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	0.82	0.35	0.98	-1.37	N/A	N/A
Type[a]	0.93	0.90	0.85	-0.25	N/A	N/A
Gradient[b]	0.32	0.28	0.24	0.30	N/A	N/A
Season[c]	0.12	0.20	0.23	0.42	N/A	N/A
Temperature	-0.04	-0.03	-0.04	0.03	N/A	N/A
Humidity	0.003	0.007	0.004	-0.003	N/A	N/A
Standard Errors						
Intercept	0.52	1.07	0.56	2.35	N/A	N/A
Type[a]	0.12	0.21	0.14	0.45	N/A	N/A
Gradient[b]	0.10	0.20	0.11	0.43	N/A	N/A
Season[c]	0.10	0.21	0.11	0.45	N/A	N/A
Temperature	0.02	0.03	0.02	0.07	N/A	N/A
Humidity	0.005	0.01	0.006	0.02	N/A	N/A
Likelihood						
Log Likelihood	-401	-311	-355		N/A	
Model *df*	6	7	12		N/A	
Fit measures						
AIC	813	635	734		N/A	
AIC Weight	0.00	1.00	0.00		N/A	

[a] Reference category is deciduous; [b] Reference category is mature; [c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.26b Results for final NB regression model (reduced NB model) for lacewings from trees in rural woodlands.

Variable	Negative binomial		
	Coefficients	SE	z-value
Intercept	0.20	0.19	1.05
Type[a]	0.95	0.21	4.60***
Gradient[b]	0.29	0.20	1.42

Log likelihood = -312, Model df = 4, AIC = 632; NB = Negative binomial; [a] Reference category is deciduous; [b] Reference category is mature; *** = $p < 0.0001$; ** = $p < 0.001$; * = $p < 0.01$; AIC: Akaike information criterion.

Table A3.27 Comparison of regression models for lacewings from trees in deciduous woodlands.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	2.32	2.04	2.43	-1.58	2.37	70.31
Gradient[b]	0.37	0.36	0.31	-0.24	0.36	-0.01
Season[c]	0.04	0.10	0.10	0.16	-0.005	-16.41
Temperature	-0.07	-0.07	-0.05	0.06	-0.06	0.59
Humidity	0.001	0.01	0.01	-0.01	-0.001	-1.72
Standard Errors						
Intercept	0.57	1.32	0.59	2.82	1.30	137.24
Gradient[b]	0.12	0.26	0.12	0.54	0.26	3.33
Season[c]	0.12	0.27	0.12	0.56	0.27	76.29
Temperature	0.02	0.04	0.02	0.09	0.04	1.69
Humidity	0.01	0.01	0.007	0.03	0.01	2.99
Likelihood						
Log Likelihood	-268	-195	-231		-192	
Model *df*	5	6	10		11	
Fit measures						
AIC	564	401	482		406	
AIC Weight	0.00	0.88	0.00		0.12	

[b] Reference category is mature; [c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.28 Comparison of regression models for lacewings from trees in coniferous woodlands.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	-1.95	-2.08	-3.26	-9.45	-2.42	-16.19
Gradient[b]	0.14	0.13	0.07	-0.28	0.08	-0.24
Season[c]	0.46	0.43	1.00	2.60	0.90	11.04
Temperature	0.03	0.04	0.04	0.06	0.03	-0.03
Humidity	0.02	0.02	0.04	0.09	0.03	0.09
Standard Errors						
Intercept	1.27	1.87	1.61	7.55	1.75	90.35
Gradient[b]	0.20	0.30	0.22	0.82	0.28	1.05
Season[c]	0.21	0.31	0.24	1.60	0.31	90.11
Temperature	0.03	0.05	0.04	0.17	0.04	0.17
Humidity	0.01	0.02	0.01	0.07	0.02	0.07
Likelihood						
Log Likelihood	-127	-114	-114		-111	
Model *df*	5	6	10		11	
Fit measures						
AIC	263	239	247		243	
AIC Weight	0.00	0.87	0.02		0.11	

[b] Reference category is mature; [c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.29a Comparison of regression models for aphids from grassland at rural sites.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	-1.83	-4.35	5.92	2.75	0.72	-4.39
Type [a]	1.20	-0.002	1.54	1.27	1.89	11.68
Season[c]	2.66	2.41	1.56	-0.47	0.96	-2.02
Temperature	-0.07	0.09	-0.21	-0.08	.01	-0.16
Humidity	0.04	0.05	-0.01	-0.02	-0.003	-0.04
Standard Errors						
Intercept	0.60	3.44	0.95	30.5	5.16	67.09
Type [a]	0.10	0.61	0.12	0.53	0.82	66.81
Temperature	0.13	0.63	0.14	0.54	0.70	1.04
Humidity	0.02	0.10	0.03	0.09	0.11	0.19
Season[c]	0.01	0.04	0.01	0.03	0.06	0.06
Likelihood						
Log Likelihood	-853	-155	-381		-149	
Model *df*	5	6	10		11	
Fit measures						
AIC	1715	321	782		319	
AIC Weight	0.00	0.27	0.00		0.73	

[a] Reference category is deciduous; [c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.29b Results for final ZINB regression model (full ZINB) aphids from grassland at rural sites.

Variable	ZINB Count			ZINB Logistic		
	Coefficients	SE	z-value	Coefficients	SE	z-value
Intercept	0.72	5.16	0.14	5.16	67.09	-0.07
Type [a]	1.89	0.82	2.29	0.82	66.81	.18
Season[c]	0.96	0.70	1.37	0.70	1.04	-1.95
Temperature	.01	0.11	.08	0.11	0.19	-0.86
Humidity	-0.003	0.06	-0.06	0.06	0.06	-0.66

Log likelihood = -149, Model df = 11, AIC = 319; ZINB: Zero-inflated negative binomial; [a]Reference category is Deciduous; [c]Reference category is Summer; *** = $p < 0.0001$; ** = $p < 0.001$; * = $p < 0.01$; AIC: Akaike information criterion.

Table A3.30a Comparison of regression models for ants from grassland at rural sites.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	1.65	0.36	1.61	-2.87	-0.61	-16.07
Type [a]	-1.58	-1.60	-1.42	0.56	-1.74	-2.23
Season [c]	1.16	1.17	0.84	-1.13	0.99	-10.11
Temperature	-0.03	-0.01	-0.002	0.13	0.04	0.54
Humidity	0.01	0.02	0.01	-0.01	0.03	0.06
Standard Errors						
Intercept	.56	1.70	0.60	3.33	1.92	15.77
Type [a]	0.11	0.30	0.12	0.60	0.28	1.99
Season [c]	0.10	0.31	0.11	0.64	0.31	66.13
Temperature	0.01	0.05	0.02	0.10	0.05	0.37
Humidity	0.006	0.02	0.01	0.04	0.02	0.13
Likelihood						
Log Likelihood	-349	-196	-292		-191	
Model *df*	5	6	10		11	
Fit measures						
AIC	708	404	605		405	
AIC Weight	0.00	0.62	0.00		0.38	

[a] Reference category is deciduous; [c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.30b Results for final NB regression model (full NB) ants from grassland at rural sites.

Variable	Negative binomial		
	Coefficients	SE	z-value
Intercept	0.36	1.70	0.21
Type [a]	-1.60	0.30	-5.37***
Season [c]	1.17	0.31	3.77**
Temperature	-0.01	0.05	-0.12
Humidity	0.02	0.02	1.33

Log likelihood = -196, Model df = 6, AIC = 404; NB: Negative binomial; [a]Reference category is Deciduous; [c]Reference category is Summer; *** = $p < 0.0001$; ** = $p < 0.001$; * = $p < 0.01$; AIC: Akaike information criterion.

Table A3.31a Comparison of regression models for aphids from grassland at deciduous sites.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	-0.89	-13.52	10.09	6.52	19.37	303.96
Season[c]	3.99	4.02	1.70	-1.80	1.28	-18.66
Temperature	-0.16	0.31	-0.32	-0.12	-0.51	-7.92
Humidity	0.06	0.11	-0.02	-0.03	-0.11	-2.35
Standard Errors						
Intercept	0.81	6.24	1.28	4.87	12.10	424.18
Season[c]	0.23	1.11	0.24	0.86	1.26	41.19
Temperature	0.02	0.17	0.03	0.14	0.30	12.06
Humidity	0.007	0.06	0.01	0.05	0.10	3.33
Likelihood						
Log Likelihood	-584	-75	-221		-69	
Model *df*	4	5	8		9	
Fit measures						
AIC	1177	460	458		155	
AIC Weight	0.00	0.08	0.00		0.92	

[c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.31b Results for final ZINB regression model (full ZINB) aphids from grassland at deciduous sites.

Variable	ZINB Count			ZINB Logistic		
	Coefficients	SE	z-value	Coefficients	SE	z-value
Intercept	19.37	12.10	1.60	303.96	424.18	0.72
Season[c]	1.28	1.26	1.02	-18.66	41.19	-0.45
Temperature	-0.51	0.30	-1.68	-7.92	12.06	-0.66
Humidity	-0.11	0.10	-1.10	-2.35	3.33	-0.71

Log likelihood = -69, Model df = 9, AIC = 155; ZINB: Zero-inflated negative binomial; [c]Reference category is Summer; *** = p < 0.0001; ** = p < 0.001; * = p < 0.01; AIC: Akaike information criterion.

Table A3.32a Comparison of regression models for aphids from grassland at coniferous sites.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	1.39	1.79	2.93	-0.80	N/A	N/A
Season[c]	0.41	0.25	0.59	0.48	N/A	N/A
Temperature	0.07	0.07	0.02	0.02	N/A	N/A
Humidity	-0.03	-0.04	-0.03	0.002	N/A	N/A
Standard Errors						
Intercept	1.23	3.78	1.90	4.26	N/A	N/A
Season[c]	.20	0.66	0.21	0.73	N/A	N/A
Temperature	0.03	0.10	0.04	0.11	N/A	N/A
Humidity	0.01	0.04	0.02	0.05	N/A	N/A
Likelihood						
Log Likelihood	-154	-73	-107			
Model *df*	4	5	8		N/A	
Fit measures						
AIC	317	156	230			
AIC Weight	0.00	1.00	0.00		N/A	

[c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.32b Results for final NB regression model (full NB) aphids from grassland at coniferous sites.

Variable	Negative binomial		
	Coefficients	SE	z-value
Intercept	1.79	3.78	0.47
Season[c]	0.25	0.66	0.38
Temp	0.07	0.10	0.68
Humidity	-0.04	0.04	-0.88

Log likelihood = -73, Model df = 5, AIC = 156; NB: Negative binomial; [c]Reference category is Summer; *** = $p < 0.0001$; ** = $p < 0.001$; * = $p < 0.01$; AIC: Akaike information criterion.

Table A3.33a Comparison of regression models for ants from grassland at deciduous sites.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	-6.03	-4.26	-5.91	-3.41	-6.29	-6.26
Season[c]	-0.50	0.34	-0.08	-0.50	0.58	0.01
Temperature	0.35	0.27	0.34	0.15	0.37	0.24
Humidity	-0.01	-0.02	0.005	0.02	-0.01	0.02
Standard Errors						
Intercept	1.02	2.93	1.11	3.07	2.24	5.09
Season[c]	0.17	0.58	0.20	0.55	0.56	0.89
Temperature	0.03	0.09	0.04	0.10	0.07	0.16
Humidity	0.008	0.03	0.008	0.03	0.02	0.03
Likelihood						
Log Likelihood	-276	-113	-131		-107	
Model *df*	4	5	8		9	
Fit measures						
AIC	561	236	278		232	
AIC Weight	0.00	0.10	0.00		0.90	

[c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.33b Results for final ZINB regression model (full ZINB) ants from grassland at deciduous sites

Variable	ZINB Count			ZINB Logistic		
	Coefficients	SE	z-value	Coefficients	SE	z-value
Intercept	-6.29	2.24	-2.80	-6.26	5.09	-1.23
Season[c]	0.58	0.56	1.04	0.01	0.89	.01
Temperature	0.37	0.07	5.18***	0.24	0.16	1.54
Humidity	-0.01	0.02	-0.31	0.02	0.03	0.65

Log likelihood = -107, Model df = 9, AIC = 232; ZINB: Zero-inflated negative binomial; [c]Reference category is Summer; *** = p < 0.0001; ** = p < 0.001; * = p < 0.01; AIC: Akaike information criterion.

Table A3.34a Comparison of regression models for ants from grassland at coniferous sites.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	3.43	2.59	N/A	N/A	N/A	N/A
Season[c]	0.59	0.67	N/A	N/A	N/A	N/A
Temperature	-0.01	-0.002	N/A	N/A	N/A	N/A
Humidity	0.03	0.04	N/A	N/A	N/A	N/A
Standard Errors						
Intercept	0.12	1.23	N/A	N/A	N/A	N/A
Season[c]	0.02	0.21	N/A	N/A	N/A	N/A
Temperature	0.003	0.03	N/A	N/A	N/A	N/A
Humidity	0.001	0.01	N/A	N/A	N/A	N/A
Likelihood						
Log Likelihood	-2703	-410				
Model *df*	4	5		N/A		N/A
Fit measures						
AIC	5422	842				
AIC Weight	0.00	1.00		N/A		N/A

[c] Reference category is summer; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; AIC: Akaike information criterion.

Table A3.34b Results for final NB regression model (full NB) ants from grassland at coniferous sites.

Variable	Negative binomial		
	Coefficients	SE	z-value
Intercept	2.59	1.23	2.11
Season[c]	0.67	0.21	3.20**
Temperature	-0.002	0.03	-0.08
Humidity	0.04	0.01	2.55*

Log likelihood = -410, Model df = 5, AIC = 842; NB: Negative binomial; [c]Reference category is Summer; *** = $p < 0.0001$; ** = $p < 0.001$; * = $p < 0.01$; AIC: Akaike information criterion.

Appendix for Chapter 4

Table A4.1: Total numbers of coccinellid species that were recorded across all sites on river shingle (by direct search) and grass (by sweep netting)

Species/Site Type	Shingle	Grass	TOTAL
Coccinella quinquepunctata	457	55	**512**
Harmonia axyridis	40	11	**51**
Adalia bipunctata	62	9	**71**
Coccinella septempunctata	21	7	**28**
Coccinella undecimpunctata	9	3	**12**
Propylea quattuordecimpunctata	3	5	**8**
Tytthaspis sedecimpunctata	0	3	**3**
Psyllobora vigintiduopunctata	0	1	**1**
Subcoccinella vigintiquattuorpunctata	0	1	**1**
TOTAL	**592**	**95**	**687**

Table A4.2a Comparison of regression models for *C. quinquepunctata* from both habitats in Wales.

Variable	Poisson	Negative Binomial
Coefficients		
Intercept	-4.18	-3.76
Habitat[a]	2.03	2.14
Visit[b]	0.57	0.69
Visit[c]	-0.78	-0.66
Temperature	0.07	0.07
Humidity	0.05	0.04
Standard Errors		
Intercept	0.93	2.49
Habitat[a]	0.14	0.31
Visit[b]	0.13	0.49
Visit[c]	0.17	0.43
Temperature	0.02	0.07
Humidity	0.008	0.02
Likelihood		
Log Likelihood	-285	-162
Model *df*	6	7
Fit measures		
AIC	583	339
AIC Weight	0.00	1.00

[a] Reference category is shingle; [b] Reference category is June; [c] Reference category is September; df = degrees of freedom; AIC: Akaike information criterion.

Table A4.2b Results for final NB regression model (reduced NB model) for *C. quinquepunctata* from both habitats in Wales.

Variable	Negative binomial		
	Coefficients	SE	z-value
Intercept	-1.76	1.26	-1.39
Habitat[a]	2.12	0.32	6.72***
Visit[b]	1.07	0.36	2.98*
Visit[c]	-0.57	0.42	-1.36
Humidity	0.03	0.02	1.61

Log likelihood = -163, Model df = 6, AIC = 338; [a] Reference category is shingle; [b] Reference category is June; [c] Reference category is September; *** = $p < 0.0001$; ** = $p < 0.001$; * = $p < 0.01$; NB = Negative binomial; AIC: Akaike information criterion.

Table A4.3a Comparison of regression models *H. axyridis* from both habitats in Wales.

			ZIP		ZINB	
Variable	Poisson	NB	Count	Logistic	Count	Logistic
Coefficients						
Intercept	-9.33	-10.0	-14.7	-8.70	-16.07	-538.5
Habitat[a]	1.25	1.13	.72	-0.65	0.20	-25.7
Visit[b]	1.91	1.82	0.96	-1.43	1.02	-39.9
Visit[c]	0.53	0.83	-0.52	-1.70	-0.33	-63.3
Temperature	0.11	0.14	0.29	0.26	0.31	11.38
Humidity	0.07	0.07	0.13	0.08	0.13	4.88
Standard Errors						
Intercept	3.21	5.94	5.02	10.48	7.36	519.1
Habitat[a]	0.34	0.70	0.39	0.81	0.76	25.95
Visit[b]	0.54	1.12	0.76	1.42	1.08	45.11
Visit[c]	0.62	1.00	0.92	1.72	1.16	65.35
Temperature	0.07	0.16	0.11	0.25	0.19	11.32
Humidity	0.03	0.05	0.05	0.09	0.06	4.67
Likelihood						
Log Likelihood	-92	-56	-62		-51	
Model *df*	6	7	12		13	
Fit measures						
AIC	195	127	148		127	
AIC Weight	0.00	0.55	0.00		0.45	

[a] Reference category is shingle; [b] Reference category is June; [c] Reference category is September; NB: Negative binomial; ZIP: Zero-inflated Poisson; ZINB: Zero-inflated negative binomial; df = degrees of freedom; AIC: Akaike information criterion.

Table A4.3b Results for final NB regression model (reduced NB model) for *H. axyridis* from both habitats in Wales.

Variable	Negative binomial		
	Coefficients	SE	z-value
Intercept	-2.46	0.84	-2.92*
Habitat[a]	1.20	0.71	1.70
Visit[b]	2.16	0.85	2.53*
Visit[c]	1.06	0.96	1.10

Log likelihood = -57, Model df = 5, AIC = 125; [a] Reference category is shingle; [b] Reference category is June; [c] Reference category is September; *** = $p < 0.0001$; ** = $p < 0.001$; * = $p \leq 0.01$; NB = Negative binomial; AIC: Akaike information criterion.

Table A4.4a Comparison of regression models for *C. quinquepunctata* from shingle habitat in Wales.

Variable	Poisson	Negative Binomial
Coefficients		
Intercept	-2.14	-1.60
Cover[d]	0.25	-0.04
Cover[e]	-0.22	-0.52
Visit[b]	0.56	0.99
Visit[c]	-0.88	-0.65
Shannon	-0.38	-0.35
Temperature	0.06	0.03
Humidity	0.05	0.05
Standard Errors		
Intercept	0.99	2.82
Cover[d]	0.14	0.52
Cover[e]	0.11	0.39
Visit[b]	0.13	0.51
Visit[c]	0.18	0.47
Shannon	0.15	0.49
Temperature	0.02	0.08
Humidity	0.01	0.03
Likelihood		
Log Likelihood	-206	-112
Model *df*	8	9
Fit measures		
AIC	428	242
AIC Weight	0.00	1.00

[b] Reference category is June; [c] Reference category is September; [d] Reference category is low; [e] Reference category is medium; df = degrees of freedom; AIC: Akaike information criterion.

Table A4.4b Results for final NB regression model (reduced NB model) for *C. quinquepunctata* from shingle habitat in Wales.

Variable	Negative binomial		
	Coefficients	SE	z-value
Intercept	-0.69	1.47	0.64
Visit[b]	1.03	0.39	0.01*
Visit[c]	-0.44	0.45	0.33
Humidity	0.04	0.02	0.03

Log likelihood = -112, Model df = 5, AIC = 235; [a] Reference category is shingle; [b] Reference category is June; [c] Reference category is September; *** = $p < 0.0001$; ** = $p \leq 0.001$; * = $p \leq 0.01$; NB = Negative binomial; AIC: Akaike information criterion.

Table A4.5a Comparison of regression models for *H. axyridis* from shingle habitat in Wales.

Variable	Poisson	Negative Binomial
Coefficients		
Intercept	-4.59	-18.37
Cover[d]	-1.45	-1.37
Cover[e]	0.49	-0.20
Visit[b]	1.59	1.02
Visit[c]	0.39	1.1
Shannon	3.25	4.71
Temperature	-0.04	0.27
Humidity	0.04	0.14
Standard Errors		
Intercept	5.52	8.90
Cover[d]	0.81	1.25
Cover[e]	0.74	1.15
Visit[b]	0.66	1.00
Visit[c]	0.95	1.23
Shannon	0.53	1.03
Temperature	0.13	0.19
Humidity	0.05	0.08
Likelihood		
Log Likelihood	-39	-28
Model *df*	8	9
Fit measures		
AIC	94	73
AIC Weight	0.00	0.99

[b] Reference category is June; [c] Reference category is September; [d] Reference category is low; [e] Reference category is medium; df = degrees of freedom; AIC: Akaike information criterion.

Table A4.5b Results for final NB regression model (reduced NB model) for *H. axyridis* from shingle habitat in Wales.

Variable	Negative binomial		
	Coefficients	SE	z-value
Intercept	-25.54	9.69	-2.64*
Shannon	4.54	0.96	4.71***
Temperature	0.44	0.17	2.51
Humidity	0.20	0.08	2.28

Log likelihood = -29, Model df = 5, AIC = 67; [a] Reference category is shingle; [b] Reference category is June; [c] Reference category is September; *** = $p < 0.0001$; ** = $p \leq 0.001$; * = $p \leq 0.01$; NB = Negative binomial; AIC: Akaike information criterion.

Table A4.6a Comparison of regression models for *C. quinquepunctata* from grass habitat in Wales.

Variable	Poisson	Negative Binomial
Coefficients		
Intercept	-10.87	-3.28
Visit[b]	0.04	0.48
Visit[c]	-1.59	-1.44
Shannon	0.82	1.88
Temperature	0.24	0.12
Humidity	0.09	0.01
Standard Errors		
Intercept	4.33	4.84
Visit[b]	0.56	1.14
Visit[c]	0.70	1.09
Shannon	0.27	0.62
Temperature	0.09	0.16
Humidity	0.04	0.04
Likelihood		
Log Likelihood	-63	-44
Model *df*	6	7
Fit measures		
AIC	138	102
AIC Weight	0.00	1.00

[b] Reference category is June; [c] Reference category is September; df = degrees of freedom; AIC: Akaike information criterion.

Table A4.6b Results for final NB regression model (reduced NB model) for *C. quinquepunctata* from grass habitat in Wales.

Variable	Negative binomial		
	Coefficients	SE	z-value
Intercept	-0.78	0.57	-1.37
Visit[b]	1.28	0.71	1.81
Visit[c]	-1.17	1.03	-1.14
Shannon	1.83	0.61	2.99*

Log likelihood = -44, Model df = 5, AIC = 99; [a] Reference category is shingle; [b] Reference category is June; [c] Reference category is September; *** = $p < 0.0001$; ** = $p \leq 0.001$; * = $p \leq 0.01$; NB = Negative binomial; AIC: Akaike information criterion.

Table A4.6a Comparison of regression models for *C. quinquepunctata* from shingle habitat in Wales.

Variable	Poisson	Negative Binomial
Coefficients		
Intercept	2.46	2.46
Elevation[f]	0.41	0.41
Elevation[g]	-0.22	-0.22
Elevation[h]	-1.21	-1.21
Standard Errors		
Intercept	0.08	0.22
Elevation[f]	0.11	0.32
Elevation[g]	0.13	0.33
Elevation[h]	0.23	0.42
Likelihood		
Log Likelihood	-208	-129
Model *df*	4	5
Fit measures		
AIC	423	268
AIC Weight	0.00	1.00

[f] Reference category is 0.25-0.49 metres; [g] Reference category is 0.50-0.74 metres; [h] Reference category is > 0.75 metres; df = degrees of freedom; AIC: Akaike information criterion.

Table A4.6b Results for final NB regression model (full NB model) for *C. quinquepunctata* from shingle habitat in Wales.

Variable	Negative binomial		
	Coefficients	SE	z-value
Intercept	2.46	0.22	11.15***
Elevation[f]	0.41	0.32	1.29
Elevation[g]	-0.22	0.33	-0.67
Elevation[h]	-1.21	0.42	-2.85*

Log likelihood = -129, Model df = 5, AIC = 268; [f] Reference category is 0.25-0.49 metres; [g] Reference category is 0.50-0.74 metres; [h] Reference category is > 0.75 metres; *** = $p < 0.0001$; ** = $p \leq 0.001$; * = $p \leq 0.01$; NB = Negative binomial; AIC: Akaike information criterion.

Table A4.7a Comparison of regression models for *C. quinquepunctata* and distance from water's edge.

Variable	Poisson	Negative Binomial
Coefficients		
Intercept	2.91	2.91
Distance[i]	-0.73	-0.73
Distance[j]	-0.74	-0.74
Distance[k]	-1.30	-1.30
Distance[l]	-0.96	-0.96
Distance[m]	-2.62	-2.62
Standard Errors		
Intercept	0.07	0.26
Distance[i]	0.12	0.37
Distance[j]	0.13	0.39
Distance[k]	0.19	0.47
Distance[l]	0.20	0.53
Distance[m]	0.50	0.75
Likelihood		
Log Likelihood	-272	-146
Model *df*	6	7
Fit measures		
AIC	555	307
AIC Weight	0.00	1.00

[i] Reference category is 6-10 metres; [j] Reference category is 11-15 metres; [k] Reference category is 16-20 metres; [l] Reference category is 21-25 metres; [m] Reference category is 26-30 metres; df = degrees of freedom; AIC: Akaike information criterion.

Table A4.7b Results for final NB regression model (full NB model) for *C. quinquepunctata* and distance from water's edge.

Variable	Negative binomial		
	Coefficients	SE	z-value
Intercept	2.91	0.26	11.00***
Distance[i]	-0.73	0.37	-1.96
Distance[j]	-0.74	0.39	-1.88
Distance[k]	-1.30	0.47	-2.76**
Distance[l]	-0.96	0.53	-1.81
Distance[m]	-2.62	0.75	-3.51**

Log likelihood = -129, Model df = 5, AIC = 268; [i] Reference category is 6-10 metres; [j] Reference category is 11-15 metres; [k] Reference category is 16-20 metres; [l] Reference category is 21-25 metres; [m] Reference category is 26-30 metres; *** = $p < 0.0001$; ** = $p \leq 0.001$; * = $p \leq 0.01$; NB = Negative binomial; AIC: Akaike information criterion.