Pollinator monitoring: comparing standardised and novel survey methods

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Submitted in accordance with the requirements for the degree of Doctor of Philosophy

The University of Leeds

School of Biology

September, 2019

The candidate confirms that the work submitted is his own and that appropriate credit has been given where reference has been made to the work of others.

Acknowledgements

First and foremost, I would like to thank my supervisory team: Bill Kunin, Claire Carvell, and Chris Hassall, for their expertise, their dedication, and their patience during the course of this PhD project. You have all been an inspiration to me and I'm looking forward to working with you in future. Chris and Bill were also foolish enough to give me two postdoctoral positions, even after working with me for 3.5 years, so thank you both for the vote of confidence. I would also like to thank Will Hoppitt, who served as my internal assessor during my first three years as a PhD student, and who provided invaluable statistical advise during the early stages of my project.

There are numerous members of staff at the University of Leeds and beyond whom I would also like to thank for helping me throughout this project. Stephen Freeman (Centre for Ecology and Hydrology, Wallingford) who helped me piece together the models for Chapter 2. Richard Gunton, Yoni Gavish, and Charlie Marsh, who provided statistical support at various points during my first two years. Rory O'Connor and Catherine Jones, who both provided an eternally patient and friendly sounding-board for all of my PhD-related queries during my first and second years, and for reminding me that a social life is necessary for maintaining my sanity. Amanda Bretman, for the pub quizzes, the karaoke, and for persuading me that a week in Millport with over 200 undergraduates was just what my third year was missing. Rupert Quinnell, for helping me realise that neither teaching nor statistics are inherently scary. Steve Compton, Roger Key, and Peter Kirby, for helping to foster my growing knowledge of insect taxonomy. And Elizabeth Duncan, for keeping me sane since 2017, and for teaching me everything I did not know about honey bees, genetics, and international flight rules.

A special thank you goes to Sarah L'Amie and Dote Stone in the teaching laboratories, for teaching me to always respect technical staff (an invaluable lesson), as well as for providing a sounding board for my many and varied problems, and for having an endless supply of patience when I inevitably forgot to return equipment.

I also need to thank all the students whom I have worked with, who have helped me through the course of this project, and who have become good friends in the process. These include (in no

particular order) Jude Lane, Cat Shannon, Rob Williams, Solène Guenat, Johan Fruh, Talor Whitham, Steph Bradbeer, Katie Cook, Louise Anderson, James Cant, Harry Tan, Becky Robertson, Chris Pollock, and Hafed Khalil. Two special sets of thanks go to the Friday night dinner and wine crew: Liz Morgan, Sonja Wild, Marianne Mugabo, Dave Ebs, and Phoebe Luckham – I love you all to pieces, thank you for all the wine and whiskey; and to the Brain Trust: Myrna Barjau Pérez Milicua, Dan Warren, and Sofia Biffi, for endless coffee, board games, and pizza.

And to my friends outside of the University of Leeds (an odd concept, I know), thank you for keeping me grounded and for reminding me that life really does exist outside of academia. To my oldest friends: Martin, Jamie, and Helen – thank you for the Dungeons and Dragons campaigns (we can finally restart that damn game!), the late nights on Elder Scrolls Online, and putting up with endless bee facts on long walks. To my friends from my undergraduate degree: Ben Taylor, Laura Michie, Chantel Ingarfield, Shanelle Kohler, Claire Pile, Tammy Lansley, Darrel and Sam Shand, Mark Sollis, and Helen Makaruk; you started me out on this journey and have continued to walk it with me every step of the way - you're all so special, and I wouldn't have made it here without you.

Last, but not least, I would like to thank my family: my mum, Vicki, my dad, Stephen, and my sister, Imogen. You have stuck by me, listened to me ramble on about this project (even when you didn't have the faintest clue what I was talking about), and have supported me through every step of this project. Also, I would like to thank my aunt Lizbeth for *everything*, and for keeping me updated on the presence of her *Andrena cineraria* colony, and my grandparents, on both sides of the family, for keeping the faith even when I completely forgot to call, write, or make contact in any way for vast periods of time. I love you all, and I have a lot of time to catch up on!

Acknowledgements by data chapter

Chapter 2.

Data collection was carried out by Anna Cabriolu and Sarah L'Amie in 2015 (eleven site visits), and by an unknown undergraduate student in 2014 (six site visits). Thomas Dally collected the final two site visits-worth of data in 2015. The study was originally conceived by Bill Kunin, and data analysis was carried out by TD, with advise from Rory O'Connor, Stephen Freeman (CEH, Wallingford) and Will Hoppitt. The parallelised generalised linear mixed-effects models were run using the ARC3 HPC cluster at the University of Leeds. TD wrote the manuscript, and Bill Kunin, Claire Carvell, and Chris Hassall provided edits and suggestions.

Chapter 3

The idea for this chapter was conceived by Bill Kunin and Thomas Dally, building upon research carried out during TD's MRes final project. The field work was carried out by TD and Vaggelis Mizerakis, an undergraduate student from the University of the Aegean, Lesvos, with help from Jelle Devalez, a PhD student from the University of the Aegean, Lesvos. TD was kindly hosted at the University of the Aegean by Professor Theodora Petanidou. Specimen identification was carried out by TD and Jelle Devalez. Data analysis was carried out by TD, who also wrote the manuscript. Bill Kunin, Claire Carvell, and Chris Hassall provided edits and suggestions.

Chapter 4

The idea for this chapter was conceived by Chris Hassall and Thomas Dally. Data collection was carried out by TD, along with Dan Nicholson and Helen Kortjohn. Data analysis was carried out by TD with advise from Chris Hassall, David Chesmore (University of York), and Hafed Khalil (University of York). Osama Eljadi provided Linux-based support, helped build the openSMILE feature extraction tool, and provided code for its use. TD wrote the manuscript, and Bill Kunin, Claire Carvell, and Chris Hassall provided edits and suggestions.

Chapter 5

The idea for this chapter was conceived by Thomas Dally, and the study was designed by TD in collaboration with Bill Kunin, Claire Carvell, and Chris Hassall. The design for the acoustic pan traps was conceived by TD, with help from David Chesmore, Bill Kunin, Chris Hassall, and Claire Carvell. Data were collected by TD, with help from Talor Whitham, and were analysed by TD. TD wrote the manuscript, and Claire Carvell and Chris Hassall provided edits and suggestions.

Chapter 6

Thomas Dally wrote the general discussion and Claire Carvell provided feedback.

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List of abbreviations

PoMS	UK national Pollinator Monitoring Scheme
BWARS	Bees, Wasps, and Ants Recording Society
HRS	Hoverfly Recording Society
GLMM	Generalised linear mixed-effects models
AIC	Akaike's information criterion
ΔAIC	The change in Akaike's information criteria from one model to the next
SE	Standard error
95% CI	95 per cent confidence intervals
MRR	Mark-release-recapture
φ_i	Probability of survival from time period *i* to *i*+1
p_i	Probability of capture at time period *i*
$\widehat{N}$	Estimated size of the superpopulation
$\widehat{N}_i$	Estimated size of the population at time period *i*
AIC_C	Corrected Akaike's information criterion
GLM	Generalised linear models
SVM	Support vector machine
FFO	Focal floral resource observation
SIC	Specialist Insect Classifier

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For my grandpa, Warwick Thomas Dally.

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"… I don't think it was for reading. It was for having written …"

Terry Pratchett, Collegiate Casting-Out of Devilish Devices.

Chapter 1

General introduction: Insect pollinator decline, and the need for standardised, systematic monitoring.

1.1 Thesis outline

Growing evidence of insect pollinator declines and the threat that these pose to ecosystem services has led to international push towards greater conservation and monitoring measures (IPBES, 2016). In order to effectively monitor changes in insect pollinator population trends we need a shift towards long-term, large-scale, systematic monitoring, using standardised sampling protocols. This in turn requires a greater understanding of the biases inherent within all survey methods, so that we can design effective sampling protocols for future monitoring. This thesis will focus on the methods that we use to monitor insect pollinators, where I will concentrate on two main areas of research: 1) the standardisation of current survey methods, together with an exploration of their sampling biases; and 2) the design and exploration of novel technology as a route to future monitoring.

This chapter aims to put these research aims into context. Firstly, I will explore the diversity of insect pollinator taxa, and give an overview of their economic and ecological importance as ecosystem service providers. I will then introduce the growing body of evidence surrounding insect pollinator decline, with a focus on wild as opposed to domestic pollinators, before moving onto the need for systematic monitoring of pollinator population trends in response to this. I will introduce the range of survey methods currently used to sample insect pollinator populations and give an overview of their biases in relation to monitoring. Finally, I will provide a brief summary of the thesis and introduce the main content of each chapter.

1.2 Introduction

1.2.1 The diversity and importance of insect pollinators

The relationship between flowering plants and their insect pollinators is one of the most important on Earth (Ollerton, Winfree, & Tarrant, 2011; Potts et al., 2016; Ollerton, 2017). An estimated 87 per cent of Angiosperms, approximately 308,000 species rely upon animals for pollen transfer, enabling reproduction and maintain genetic diversity within their populations (Ollerton, Winfree, & Tarrant, 2011; Ollerton, 2017). And, of these animals, over 99 per cent are insects (Ollerton, 2017). Insect pollinators are myriad in their diversity, representing approximately 347,487 species belonging to fourteen Orders, of which the Hymenoptera, Diptera, Lepidoptera, and Coleoptera are the most taxonomically diverse (Ollerton, 2017). But the most well-known and well-studied insect pollinator taxon are the bees (Hymenoptera: Apoidea). Bees are also one of the most individually efficient pollinator taxa (Ollerton, 2017), being almost completely reliant on nectar and pollen in both their adult and larval stages. This has led to a wide range of morphological adaptations aimed at enabling individuals to maximise pollen collection, i.e., the scopae (pollen brushes) found in many solitary bee species, together with high densities of branched body hair, for increased surface area; these adaptations also make them excellent movers of pollen between plants. There are approximately 20,000 species of bees worldwide, the majority of which are solitary in nature (Ollerton, 2017), although the social species, as epitomised by the Western Honeybee (*Apis mellifera*), are probably the best known (Smith & Saunders, 2016).

Other members of the Hymenoptera are also highly effective pollinators. Solitary wasps, for example, are the sole pollinators of fig trees (*Ficus* spp.) (Machado et al., 2005), and are common pollinators of sexually-deceptive orchids (Gaskett, 2011). Social wasp species are also common floral visitors, and have been noted as highly efficient pollinators in certain systems (Thomson, 2019).

One of the most well-known non-Hymenopteran pollinator taxa are the hoverflies (Diptera: Syrphidae) (Rotheray & Gilbert, 2011; Jauker et al., 2012), which subsist on pollen and nectar in their adult stage, while the larvae are often either saprophytic or carnivorous . This dual provision of ecosystem services makes hoverflies popular with farmers and gardeners due to the larvae of

some species providing an effective defence against aphids and other plant pests, while the adults provide pollination services to the resulting crop (e.g. Wotton et al., 2019). However, the non-syrphid Diptera are also effective pollinators (Ssymank et al., 2008; Orford, Vaughan, & Memmott, 2015; Rader et al., 2016). The roles of the Coleoptera and Lepidoptera as pollinators are less well researched, but no less important (Kevan & Baker, 1983; Listabarth, 2001; Macgregor et al., 2015).

Other insect taxa also take part in providing pollination services to a greater or lesser degree, including the Thysanoptera, Hemiptera, and Dermaptera (Ollerton, 2017). Within the UK, it has been estimated that there are approximately 6000 species of insects capable of providing pollination services (Falk, S., 2018, personal communication).

1.2.2 The economic and ecological importance of insect pollinators

The importance of insect pollinators is often quantified in terms of their value as ecosystem service providers, specifically their contributions to global agriculture and food production. In 2009, for instance, Gallai et al. (2009) estimated that global pollination services were worth €153 billion. A more recent estimate by the Food and Agriculture Organisation placed between five and eight per cent of global agricultural production by volume, worth an estimated $235-577 billion, as directly attributable to animal-mediated pollination; while around 75 per cent of our most important food crops, accounting for ca. 35 per cent of global agricultural production, are at least partially dependant on pollinators to increase yield (Klein et al., 2007; IPBES, 2016). The majority of the world's staple crops may be wind-pollinated (anemophilous), i.e. wheat, maize, barley, oats, and rice, but there is also evidence to suggest that insect pollinators are common visitors to many species presumed to be entirely anemophilous, and that their visits may enhance crop yield (Saunders, 2018).

Aside from crop production, domesticated pollinator species like the Western honeybee (*Apis mellifera*) provide additional sources of income to the people and communities that keep them (Potts et al., 2016). Honey is a valuable commercial product (García, 2018), and the income gained from hiring out honeybee hives for agricultural pollination services can be considerable. A classic example of this is the hire and transportation of over two million honeybee hives from

across the United States for the purposes of almond crop pollination in California (Lee, Sumner, & Champetier, 2019); beekeeping is also an important poverty-alleviation tool in rural and developing communities (Potts et al., 2016). All of which is in addition to the economic value provided by animal-mediated pollination in terms of employment within the agricultural sector (Potts et al., 2016).

Insect pollinators also contribute to human wellbeing in a more qualitative sense. Current research shows that human wellbeing, including our mental health, can be positively influenced by contact with nature and green spaces (Millennium Ecosystem Assessment, 2005). Since nearly 90 per cent of all flowering plant species rely on animal-mediated pollination for reproduction, maintaining diverse insect pollinator communities, especially in urban centres, is only likely to enhance these benefits.

1.2.3 The extent of our knowledge concerning insect pollinator decline

The importance of insect pollinators, both from an ecological and an economic standpoint, has been highlighted by a growing body of evidence concerning population declines within many insect pollinator taxa (Biesmeijer et al., 2006; Potts et al., 2010; Carvalheiro et al., 2013; Goulson et al., 2015; Powney et al., 2019). This issue is prominent within the international research community, and has captured the public consciousness, having been addressed by the United Nations under both the Convention on Biological Diversity (CBD)[1] and the Food and Agriculture Organisation (FAO) (FAO, 2008), as well as by a number of individual national governments (see DEFRA, 2014, 2015).

The drivers of insect pollinator decline have been well-reviewed by several authors (Potts et al., 2010, 2016; Gonzalez-Varo et al., 2013; Goulson et al., 2015) and primarily consist of habitat loss, resulting in a loss of forage and habitat connectivity at a landscape scale (Carvell et al., 2006; Cranmer, McCollin, & Ollerton, 2012; Goulson et al., 2015), agricultural intensification and the increased use of pesticides (Ollerton et al., 2014; Senapathi et al., 2015), pathogens (Szabo et al.,

[1] https://www.cbd.int/decision/cop/?id=7179

2012; Goulson et al., 2015), climate change, and the resulting potential for phenological mismatches between plants and their insect pollinators (Potts et al., 2010; Potts et al., 2016), and the introduction of non-native pollinator species (Potts et al., 2010; Giannini et al., 2015).

Evidence for insect pollinator declines has primarily been observed across Europe and the United States, with limited research available in other regions (Jamieson et al., 2019). The seminal study by Biesmeijer et al. (2006) gave evidence for declines in the species richness of bee and hoverfly assemblages within the UK, The Netherlands, and Belgium (Carvalheiro et al., 2013), between 1950 and 1980; although these declines may have begun to slow and/or recover as of 1990 (Carvalheiro et al., 2013; but see Van Dooren, 2016). Within the UK, these trends were further investigated by Ollerton et al. (2014), who indicate that we have lost thirteen bee species, and a further thirteen species of flower-visiting wasp, since the mid-1800s; and by Powney et al. (2019), who show that a third of our native wild bee and hoverfly species have declined since 1980, with these declines being found primarily among more specialist species (Biesmeijer et al., 2006). While, in the United States, wild bee abundance was modelled as having decreased across 23 per cent of the country, primarily corresponding to those areas with the highest concentrations of agricultural land (Koh et al., 2016). Bumblebee species (*Bombus* spp.), in particular, have experienced decreases in range size across the United States (Colla & Packer, 2008; Grixti et al., 2008; Cameron et al., 2011); a pattern that has been mirrored here in the UK (Goulson, Lye, & Darvill, 2008).

If declines to insect pollinator populations are allowed to continue or become more severe than is currently predicted, this could put the pollination services that they provide to agriculture at risk (Klein et al., 2007; Potts et al., 2010; IPBES, 2016). Declines are also expected to affect the ecosystem services that they provide to natural and semi-natural habitats. Diverse insect pollinator communities are key to providing pollination services to native flowering plant communities (Potts et al., 2010; Frund et al., 2013; Garibaldi et al., 2013), and Biesmeijer et al. (2006) have linked declines in wild bee and hoverfly species richness in both the UK and The Netherlands to declines in the distribution of flowering plant species, especially those that rely on animal-mediated pollination for reproduction. Christmann (2019) discuss how reducing the pollination

services provided to wild flowering plant populations can lead to their being less able to cope with anthropogenic pressures like climate change due to a lack of genetic diversity.

Several studies have linked specialism in terms of forage or preferred nesting habitat to increased vulnerability to the drivers of insect pollinator decline (Biesmeijer et al., 2006; Carvalheiro et al., 2013; Powney et al., 2019); with generalist species, in particular those that utilise mass-flowering agricultural crops, giving rare instances of increasing population trends (see Powney et al., 2019). This, in turn, has been linked with increasing biotic homogenisation within bee and hoverfly communities since 1950 (Biesmeijer et al., 2006; Carvalheiro et al., 2013). Kleijn et al. (2015) found that 80 per cent of pollinator visits to crops within agricultural landscapes were carried out by two per cent of the bee species present, and suggest that these "super generalists" (Giannini et al., 2015) are unlikely to suffer from population declines due to the commonly agreed-upon drivers of decline. If this is the case, then we need to shift our reasoning for conserving insect pollinator species away from concerns regarding the economic value that they provide to agriculture (i.e. IPBES, 2016), and toward a more holistic approach involving the importance of diverse pollinator communities to the stability of natural and semi-natural habitats (Christmann, 2019), qualitative benefits relating to human health and urban and non-urban greenspaces (Millennium Ecosystem Assessment, 2005; Christmann, 2019), and their intrinsic value as living creatures (Kleijn et al., 2015; Christmann, 2019).

The problems associated with valuing a species based upon its economic worth to human society aside, there are substantial problems associated with the evidence supporting insect pollinator declines (see Ghazoul, 2005). Primarily, that we lack data concerning the abundance of individual insect pollinator populations (Ghazoul, 2005). The vast majority of studies exploring pollinator decline do so in terms of species occurrence, species richness, and range sizes (Biesmeijer et al., 2006; Grixti et al., 2008; Cameron et al., 2011; Carvalheiro et al., 2013; Powney et al., 2019), but none of these measures, although valid, allow scientists to make any judgements regarding how much an individual species has declined by over a given period of time within a given area. The main reason for this relative lack of abundance data is a global absence of centralised, systematic insect pollinator monitoring schemes (but see Carvell et al., 2016). Monitoring schemes do exist

in several countries: the Great Sunflower Project in the United States[2], and the Wild Pollinator Count in Australia[3], for example, but these are not aimed at the collection of species-level data, which are critical to the investigation of population trends. The UK's national Pollinator Monitoring Scheme[4] (PoMS) is, to my knowledge, the only example of a nationwide, systematic pollinator monitoring project collecting species-level data in the world.

Schemes like this are rare due to our relative lack of knowledge concerning the survey methods we use to monitor insect pollinator populations. We are still in the process of understanding the sampling bias inherent to different survey methods, which in turn makes it difficult to standardise the protocols that govern their use (Westphal et al., 2008). This process of standardisation is key because much of the historical data that informs studies like Biesmeijer et al. (2006), Carvalheiro et al. (2013), and Powney et al. (2019) were collected by members of specialist recording schemes like the Bees, Wasps, and Ants Recording Society (BWARS) and the Hoverfly Recording Society (HRS). These are valuable data, but the volunteers who collected them may have done so using multiple survey techniques without a standardised protocol. This makes it difficult to compare findings between individuals and across years, and as well as introducing sampling bias into their data, which is why the findings of these studies are limited to trends in species occurrence or range expansion or contraction, rather than changes in abundance over time (Powney et al., 2019). In order for monitoring schemes like PoMS to be able to analyse and compare changing trends in insect pollinator populations in the long-term, and at a nationwide scale, species records need to be collected using reliable, repeatable methods that provide objective data regarding pollinator diversity (Nielsen et al., 2011; Lebuhn et al., 2013; Popic, Davila, & Wardle, 2013). These methods need to be appropriate to the aims and design of the monitoring scheme in question, as well as to the diverse range of habitats and taxa being studied (Saunders & Luck, 2013).

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[2] See: https://www.greatsunflower.org/
[3] See: https://wildpollinatorcount.com/
[4] See: https://ukpoms.org.uk/home

1.2.4 Insect pollinator survey methods

There are many methods used to survey and monitor insect pollinator populations. These can be separated into two broad categories: active methods, where surveyors are involved in the capture of data or samples, and passive methods, where a range of traps are employed to collect data without the involvement of the surveyor (Potts, Evan, & Boone, 2005). In recent years, there have been many studies that have explored the sampling biases of different insect pollinator survey methods, as well as their performance in relation to other methods. However, there is still no consensus as to which method or combination of methods constitutes the most effective approach to pollinator monitoring.

1.2.4.1 Pan trapping vs. transect surveys

The two most commonly used survey methods for insect pollinator communities are pan trapping and transect surveys (Potts, Evan, & Boone, 2005; Westphal et al., 2008; Nielsen et al., 2011; Popic, Davila, & Wardle, 2013); they are also the two most commonly compared survey methods, in terms of their performance. Pan traps, also called Moericke traps (Cane, Minckley, & Kervin, 2000), are a passive sampling method that uses brightly-coloured bowls, filled with water, as surrogate flowers to attract foraging pollinator species, which then drown in the water (Potts, Evan, & Boone, 2005; Westphal et al., 2008). This method is often referred to as being standardised due to its lack of collector bias relative to active sampling methods (Westphal et al., 2008; Popic, Davila, & Wardle, 2013). Collector bias can be defined as the effect whereby the more sampling experience a surveyor has, the more insects and insect species they are likely to capture (Potts, Evan, & Boone, 2005), However, a wide range of different pan trapping protocols are used by researchers and conservation practitioners, which may result in wildly different findings between studies (Gonzalez et al., 2020).

Pan trapping has been extensively researched in relation to its sources of sampling bias, primarily by directly comparing its results to those of other sampling methods, namely transect surveys (Cane, Minckley, & Kervin, 2000; Roulston, Smith, & Brewster, 2007; Westphal et al., 2008; Wilson, Griswold, & Messinger, 2008; Grundel et al., 2011; Nielsen et al., 2011; Popic, Davila, & Wardle, 2013). Its primary source of sampling bias is the combination of bowl colours used

(Potts, Evan, & Boone, 2005). Different insect groups have evolved specific colour preferences in relation to their preferred source of forage (Kirk, 1984), and research suggests that oligolectic bee species are captured more often in pan traps whose colours are similar to those of their preferred forage (Leong & Thorp, 1999). While Saunders & Luck (2013) indicate that colour preferences are context-driven, and may change depending on habitat or background floral colour (see Toler, Evans, & Tepedino, 2005). There is also research that suggests that pan traps may catch fewer insects in florally-rich habitats, due to competition between flowers and the bowls for insects (Roulston, Smith, & Brewster, 2007; Wilson, Griswold, & Messinger, 2008; Baum & Wallen, 2011). Pan trapping is often considered to be more cost-effective than more active survey methods, since it requires less in-person time within the field (Westphal et al., 2008), however, time is still required to sort through samples and maintain the equipment, so this view is contested by some authors (see Popic, Davila, & Wardle, 2013). This method also collects no behavioural data, providing only measures of species richness and relative abundance, in contrast to active methods like transect surveys and focal floral resource observations (FFOs) (Popic, Davila, & Wardle, 2013).

Transect surveys, on the other hand, are an active sampling method that involve walking along pre-set routes at a slow pace and recording the number of insects observed. If the aim of the study is record species-level data, then it is also common for individuals to be captured using nets. As with all active methods, transect surveys are open to collector bias, and may have additional biases relating to the size and flight speed of individual taxa, for example Potts, Evan, & Boone (2005) list transect surveys as being less likely to sample smaller, faster-flying insect pollinator taxa. Although, unlike passive methods such as pan trapping, transect surveys do allow observations to be made regarding insect pollinator behaviour (Popic, Davila, & Wardle, 2013).

This method is quite labour-intensive, requiring extensive periods of time to be spent in the field, together with taxonomic skills and high-levels of concentration. However, little equipment is needed beyond a net, and since any samples taken are not water-logged, they can be easier to identify (Potts, Evan, & Boone, 2005; Popic, Davila, & Wardle, 2013).

Direct comparisons between pan trapping and transect surveys, in terms of their performance, are common within the literature (Cane, Minckley, & Kervin, 2000; Roulston, Smith, & Brewster, 2007; Westphal et al., 2008; Wilson, Griswold, & Messinger, 2008; Grundel et al., 2011; Nielsen et al., 2011; Popic, Davila, & Wardle, 2013). Of these, fewer studies seem to support the sole use of pan trapping over net sampling; aside from Westphal et al. (2008), who found that pan traps better represented bee species richness than either variable or standardised transect surveys. Based upon their results and the lack of collector bias inherent to the method, Westphal et al. (2008) recommend pan trapping as the most efficient sampling method for insect pollinator surveys; although they also acknowledge that transect surveys still constitute an effective method of pollinator sampling, providing that participants undergo standardised training in order to mitigate collector bias. Popic, Davila, & Wardle (2013), however, recommend the sole use of transect surveys as the most effective method of assessing diversity within pollinator assemblages, since they sample only active floral visitors and therefore more accurately represent the taxa that are providing pollination services. However, the predominant view is that, since both methods have approximately opposing biases, they are likely to be complementary in regard to completing a full inventory of the species present within a site (see Grundel et al., 2011; Nielsen et al., 2011).

Most of the studies that have compared the performance of pan trapping and transect surveys, with the exception of Popic, Davila, & Wardle (2013), did so solely in relation to their ability to sample bee communities (Cane, Minckley, & Kervin, 2000; Roulston, Smith, & Brewster, 2007; Westphal et al., 2008; Grundel et al., 2011; Nielsen et al., 2011). However, net sampling has also been shown to be an effective method of sampling butterflies and flies, including hoverflies (Bates et al., 2011; Popic, Davila, & Wardle, 2013); whereas pan traps have been shown to be effective at sampling flies, beetles, and thrips (Kirk, 1984; Campbell & Hanula, 2007; Popic, Davila, & Wardle, 2013).

1.2.4.2 Malaise trapping

Malaise traps consist of an open-sided tent-like structure (Matthews & Matthews, 1971; Potts, Evan, & Boone, 2005), and function by intercepting flying insects using a central fabric wall. Insects are funnelled up towards the upper-front corner of the tent, where they are captured within

a plastic bottle that is sometimes filled with water, in a manner similar to pan trapping (Potts, Evan, & Boone, 2005). Possibly the greatest source of bias concerning this method is the placement of the trap (Matthews & Matthews, 1971; Potts, Evan, & Boone, 2005); since they work by intercepting foragers they typically placed along flight corridors like hedgerows, but if they are poorly located then this may hamper their sampling ability. Malaise traps have been compared with pan trapping, in terms of their performance, with Bartholomew (2005) showing that pan trapping and Malaise trapping catch approximately similar species, even though pan traps caught a higher overall abundance. Campbell & Hanula (2007) found that pan traps performed better than Malaise traps in terms of both species richness and abundance, while showing that the addition of coloured panels to Malaise traps also increases the abundance of insects captured. The advantage of these traps lies in the fact that surveyors can leave them unattended for long periods of time with little reduction in efficacy (Bartholomew, 2005); however, regular trips must be made to empty the traps, or a preservative but be added to the plastic bottle in which the insects are captured (Bartholomew, 2005; Potts, Evan, & Boone, 2005).

1.2.4.3 Vane trapping

Vane traps are becoming more common within the literature concerning insect pollinator survey methods. Vane traps consist of a plain plastic bottle, filled with water, to which two brightly-coloured, vertical “vanes” are attached above (Stephen & Rao, 2005). Multiple colours could be used, although, so far, only yellow and blue have been tested (Stephen & Rao, 2005; Kimoto et al., 2012; Hall & Reboud, 2019). Vane traps function in a similar way to pan traps, using bright colours to attract foraging insects. On their own, they have been shown capable of catching a diverse selection of bee species in a range of habitats (Stephen & Rao, 2005; Kimoto et al., 2012), with blue vane traps being the most effective (Joshi et al., 2015; Hall, 2018). While, in comparison to other methods, namely pan trapping, blue vane traps have been shown to catch significantly more insects in terms of abundance, than pan trapping (Joshi et al., 2015). In fact, Kimoto et al. (2012) suggest that, due to their effectiveness in initial studies, fewer vane traps may be needed to carry out a monitoring survey than other passive methods, like pan traps.

1.2.4.4 Focal floral resource observations

Focal floral observations (FFOs), sometimes referred to as direct observations or phytometry (Woodcock et al., 2014), involve the recording insect pollinators as they visit focal floral patches (Potts, Evan, & Boone, 2005). This method, in a similar fashion to transect surveys, allows for the observation of foraging behaviour but also requires advanced taxonomic skills depending upon the level of taxonomic resolution required by the survey (Potts, Evan, & Boone, 2005); like transect surveys, this method is also open to collector bias (Westphal et al., 2008). FFOs perform poorly when compared to the results of survey methods like pan trapping and transect surveys (Westphal et al., 2008; Nielsen et al., 2011), but may provide a useful measure pollinator activity and their resulting pollination service for some insect pollinator taxa (Westphal et al., 2008).

FFOs also provide an ideal method for use by citizen scientists (Roy et al., 2016) since the method requires no specialist equipment and works at varying levels of taxonomic resolution. It is also non-destructive, which can be an important consideration when involving citizen scientists in large-scale, long-term monitoring schemes (see Knapton, 2017; Barkham, 2017), especially when charismatic insect pollinator species like bees are a focal taxon. This is exemplified by the choices of the UK's PoMS, Australia's Wild Pollinator Count, and the Great Sunflower Project in the US to use FFO-based methods as part of their survey designs.

1.2.4.5 Trap nesting

Trap nests are a much more specialist survey tool than those listed above, as they are only useful for monitoring the diversity of cavity-nesting bee species, such as the Megachilidae here in the UK (Potts, Evan, & Boone, 2005; Westphal et al., 2008; Nielsen et al., 2011), although this method may also provide data on other cavity-nesting insect species, and their parasites, as well. These sampling ability for these traps is reliant upon their design, specifically the type of materials used to construct the nesting tubes, as well as the size of the tubes themselves, and only collects data on a subset of the overall insect pollinator community (Potts, Evan, & Boone, 2005; Westphal et al., 2008). However, they have been shown to be an effective survey tool when used in conjunction with other methods (Westphal et al., 2008).

1.3 The future of insect pollinator monitoring

In addition, and in response to the scale of insect pollinator decline, survey methods utilising novel technology are being developed and tested (August et al., 2015). Advances in molecular techniques, in particular the use of metabarcoding and environmental DNA (eDNA), have opened up a route to non-destructively monitoring floral visitors by using the DNA traces left on flowers (Thomsen & Sigsgaard, 2019). Video footage may also provide a novel method of monitoring insect pollinator activity and diversity at flowers, in a similar fashion to FFOs, but allowing for footage to be rewound, replayed, and paused, which would enable small, cryptic, or fast-flying taxa to be better detected (Steen, Lene, & Orvedal, 2011; Gilpin, Denham, & Ayre, 2017; Steen, 2017). While the results of several recent studies suggest that acoustics may provide a novel way of non-destructively classifying insect pollinator taxa (Gradišek et al., 2016; Kawakita & Ichikawa, 2019), as well as to passively monitor pollinator activity and pollination services (Heise et al., 2017; Miller-Struttmann et al., 2017; Galen et al., 2019). These methods are still in their infancy, and require testing in relation to more common, traditional survey techniques in order to gauge their importance moving forward in terms of future insect pollinator monitoring schemes.

1.4 Thesis aims

This thesis aims to explore the methods we use to survey insect pollinator populations, together with their biases, and their potential for development as part of a future national monitoring scheme. I will begin by focusing on one of the most commonly-used survey methods for assessing insect pollinator diversity: pan trapping. Pan trapping is a passive survey technique and is thus considered to be standardised due to its lack of collector bias. However, different protocols are employed by users of pan trapping worldwide, making comparisons between studies problematic; in addition, there is little research available to suggest which set of protocols maximises sampling ability. **Chapter 2** will compare different pan trapping protocols in terms of their ability to sample bee and hoverfly populations, and make recommendations for a standardised protocol that can be used by future studies and monitoring schemes. **Chapter 3** will build upon the theme of **Chapter 2**, focusing upon the sampling biases inherent to different survey methods. All survey methods have their own biases that affect the composition of the samples that they collect. Studies of insect

pollinator-related survey methods, so far, have tried to quantify these biases by directly comparing methods to one-another, with pan trapping and transect surveys being the most often compared. But these studies lack any independent knowledge concerning the relative abundance of the insect populations present, and are thus of limited use. I will use a mark-release-recapture experiment in a closed island ecosystem to provide an independent source of relative abundance data, against which the rank abundance of samples collected via pan trapping and transect surveys can be compared, in an attempt to accurately quantify their respective sampling biases.

I then move on to explore the development and performance of a novel method for surveying and monitoring insect pollinator communities: acoustics. Taxonomic skills are in decline worldwide, which is restricting our ability to generate reliable species-level identifications for data records, especially those collected by citizen scientists, and thus our ability to accurately monitor insect pollinator population trends. **Chapter 4** will focus upon the novel use of bioacoustics to identify flower-visiting insect taxa at varying levels of taxonomic resolution, using the sound generated by their wing beats during foraging flights. **Chapter 5** will build upon **Chapter 4**, focusing on the application of passive acoustic monitoring to survey for flower-visiting insects at a landscape scale. Using commercially available automated detection software to extract the number of instances of insect flight sound from soundscape recordings, which can then be compared to the number of insects sampled by traditional sampling methods, in order to test the performance of this novel survey method. I also explore the performance of the automated detection software in terms of both false positive and false negative error rates.

Chapter 6 will then synthesise the findings from **Chapters 2-5**, and put them in the context of the need for future systematic, standardised monitoring in response to insect pollinator decline, while suggesting avenues for future research.

Chapter 2

Standardising pan trapping protocols for future insect pollinator monitoring projects

"The goal remains to design efficient and repeatable sampling methods that effectively represent the diversity of species and how their interactions vary over space and time." Popic, Davila, & Wardle (2013).

2.1 Introduction

Widespread international concern over the extent of insect pollinator declines has led ecologists to reconsider the ways in which we monitor pollinator populations (Westphal et al., 2008; Lebuhn et al., 2013; Popic, Davila, & Wardle, 2013). Many of our current assessments of pollinator status or population trends rely upon records of species occurrence to infer changes in species richness or distribution over broad spatial and temporal scales (see Biesmeijer et al., 2006; Carvalheiro et al., 2013; Ollerton et al., 2014; Powney et al., 2019), but lack the abundance data that would allow us to quantify changes to pollinator populations geographically over time (Ghazoul, 2005). In turn, our ability to collect systematic data on the abundance of different pollinator groups is hampered by our relative lack of knowledge concerning the methods used to survey these organisms. A common consensus is lacking regarding the biases of core survey methods and how best to standardise the use of these methods as part of future monitoring programs (Westphal et al., 2008).

Pan trapping is one of the most commonly used methods for monitoring pollinating insects, in particular bees and hoverflies. It is a passive technique using brightly coloured bowls, partially filled with water and a surfactant, into which the insects land and then drown. Unlike active sampling methods such as hand-netting and direct observation, pan trapping requires little effort or expertise from the field surveyor (although subsequent specimen identification requires both) and suffers from minimal collector bias (Westphal et al., 2008). However, pan traps may miss

gathering functionally important information at the plant-pollinator interface, including behavioural observations that may inform future habitat management or conservation decisions, particularly for rare or specialist species (Popic, Davila, & Wardle, 2013). In addition, pan trap samples seem to differ in composition from those collected by transect surveys, with higher fractions of many solitary bees but lower fractions of honeybees and bumblebees, suggesting potential taxonomic biases between survey methods (Roulston, Smith, & Brewster, 2007; Grundel et al., 2011; Wilson et al., 2016; Portman, Bruninga-Socolar, & Cariveau, 2020).

A recent study criticised the widespread use of pan trapping within pollinator monitoring studies based upon concerns regarding the method's ability to accurately estimate population abundances, highlighting knowledge gaps concerning the percentage of insect populations that are attracted to the bowls and the area over which they sample (Portman, Bruninga-Socolar, & Cariveau, 2020). But the common consensus is that pan traps are a robust and efficient tool for monitoring pollinator populations (Westphal et al. 2008; Nielsen et al. 2011; but see Popic, Davila & Wardle 2013). A report to the Food and Agriculture Organisation of the United Nations (LeBuhn et al., 2016), following on from earlier research by the same authors (Lebuhn et al. 2013), recommended pan trapping as the sole basis for a large-scale pollinator monitoring protocol (but see Tepedino et al. 2015). And the UK Pollinator Monitoring Scheme[5] (PoMS) uses them in combination with transect surveys as part of a standardised insect pollinator monitoring protocol (Carvell et al., 2016).

But, while pan traps represent a valuable monitoring tool, the protocols governing their use are far from standardised. Past studies use a range of different bowl sizes and colour combinations and leave pan traps active over varying periods of time with little in the way of experimental evidence to support their choices (Saunders & Luck, 2013). Gonzalez et al. (2020) reviewed ninety-three studies, published between 2014 and 2018, that involving using pan traps to sample bee diversity. They found that bowl size varied between 96.1 and 2000 ml in volume, 7.25-34 cm in diameter, and 3-13.5 cm in depth. But, despite this high degree of variation, only three studies

[5] https://ukpoms.org.uk/home

to date have explored the effects of bowl size on pan trap samples (Droege, 2002; Wilson et al., 2016; Gonzalez et al., 2020), and with inconsistent results (Droege, 2002; Wilson et al., 2016) Similarly, once reviewed, these same 93 studies left pan traps active for between four hours and ten days (Supplementary Table 1.1). Yet, only a single study has explored the effects on trap duration of sample size (Carboni & Lebuhn, 2003), limiting their comparisons to eight- and twenty-four-hour time periods and leaving longer trap duration periods unexplored, despite several recent monitoring studies using 48-hour trap duration periods as part of their experimental design (see Westphal et al. 2008; Nielsen et al. 2011). There has also been little discussion of whether pan traps might have time- or size-specific patterns of accumulation. Gonzalez et al. (2020) observed that bee abundance was unaffected by bowl diameter, despite a significant increase in bycatch in wider bowls, but a personal observation in Wilson et al. (2016) notes that fewer bees were caught in pan traps that were already full of captured insects (though this effect had yet to be quantified). It is possible that pan traps left out for longer periods might accumulate fewer insects per hour due to a build-up of dead individuals decreasing the attractive nature of the bowls. Equally, smaller bowls might reach their carrying capacity more quickly, potentially necessitating different trap durations for different bowl sizes.

The effects of bowl colour on sample size and diversity have been explored in more detail. The now-standard combination of yellow, blue, and white bowls has been acknowledged as capturing a broad, complementary sample of bee and hoverfly taxa (Bowie et al., 1999; Laubertie, Wratten, & Sedcole, 2006; Campbell & Hanula, 2007; Moreira et al., 2016), although other colour combinations have been tested. While UV-fluorescent paint is often used to further increase their appeal to foraging pollinators (Droege, 2001, 2002; Westphal et al., 2008). However, there is still some debate over which colours are most effective at sampling specific pollinator taxa, and whether this varies based upon the habitat in which the traps are deployed (Saunders & Luck, 2013; Moreira et al., 2016), background floral colour (Toler, Evans & Tepedino 2005; Saunders & Luck 2013), or the preferred forage plants of individual pollinator species (Leong & Thorp, 1999).

In addition to the physical aspects of the method, the ways in which pan traps interact with their surrounding environment also require further investigation. Some research suggests that pan traps become less attractive to their target insects in areas where floral abundance is higher (Cane, Minckley, & Kervin, 2000; Roulston, Smith, & Brewster, 2007; Wilson, Griswold, & Messinger, 2008; Baum & Wallen, 2011; O'Connor et al., 2019b; Westerberg et al., 2021), an effect that has been attributed to increased competition between pan traps and flowers, where the latter may be more attractive to foraging pollinators due to evolved sensory cues (Cane, Minckley & Kervin 2000, but see Toler, Evans & Tepedino 2005). Furthermore, during seasonal periods of increased flowering, pollinators may not need to travel as far in search of forage, potentially reducing their chances of coming into contact with individual pan traps (Baum & Wallen, 2011). However, the evidence supporting these hypotheses is based predominately upon broad, visual assessments of site-level floral abundance, with few quantitative measures of floral abundance surrounding each pan trapping station (but see O'Connor et al., 2019b; Templ et al., 2019; Westerberg et al., 2021). Furthermore, little attention has been paid to the differing levels of floral rewards associated with different plant species, which may have a direct effect on whether pollinators are foraging in an area where pan traps have been placed (but see O'Connor et al., 2019b). Additional research is also needed regarding the effects of weather variables, such as ambient temperature and wind speed, upon the ability of pan traps to sample pollinator populations (Leong & Thorp, 1999; Saunders & Luck, 2013).

2.1.1 Aims

In this study I will investigate the effects of different physical and environmental factors on the abundance and species richness of insect pollinator taxa (focusing specifically upon bees and hoverflies) sampled using pan traps within a standardised experimental design. I address five key questions and provide recommendations for future best-practice based upon my results.

1. Do bowl size and trap duration affect the abundance or richness of bees and hoverflies sampled by pan trapping?
2. Do different bowl colours attract different bee or hoverfly taxa?

3. Do bowl size and trap duration affect the sampled abundance of bee and hoverflies relative to the sampled abundance of invertebrate bycatch?
4. Does the rate of capture of bees and hoverflies in pan traps change with trap duration, and is this change affected by bowl size?
5. How do local biotic and abiotic factors, in particular local floral abundance, affect the abundance or richness of bees and hoverflies sampled by pan trapping?

2.2 Materials & Methods

2.2.1 Study sites

This study was conducted between 2014 and 2015, in four sites surrounding Leeds, West Yorkshire, in the north of England: Meanwood Grove (Leeds, SE280381), St. George's Field (University of Leeds West campus, SE292348), Spen Farm (Tadcaster, SE432408), and Bramham Park (Wetherby, SE49412). These four sites differed in terms of land-use and the diversity of floral resources present. Meanwood Grove is an area of semi-natural grassland where the flora was defined by a high relative abundance of Common Bird's-foot-trefoil (*Lotus corniculatus*) and Red Clover (*Trifolium pratense*). St. George's Field is an area of mown amenity grassland within the grounds of the University of Leeds campus. Some areas are routinely left unmown in order to promote biodiversity, but the overall floral species richness present was low and dominated by hardy species like *Bellis perennis*. The surveys at Spen Farm were carried out in two distinct habitats: an agroforestry plot and a fallow field. The agroforestry plot comprised an abandoned experimental site that has not been cultivated since approximately 2005 (Keesman et al., 2011). The vegetation consisted of rows of Poplar clones (*Populus* spp.) planted ten meters apart (between rows), with a ground cover consisting of grasses combined with species like Spear Thistle (*Cirsium vulgare*) and Common Ragwort (*Senecio jacobaea*). The fallow field had been left uncultivated for an unknown period of time prior to these surveys; it contained a high floral species richness defined by a high relative abundance of Borage (*Borago officinalis*). The Bramham Park site consisted of a managed wildflower meadow with a high floral richness, comprising a number of common grassland species (Rose & O'Reilly, 2006).

Nineteen surveys were carried out during this period: six between July and September 2014, and thirteen between June and October 2015. Surveys were planned so that high and low floral diversity sites would receive an approximately equal number of surveys, although the four individual sites hosted an unequal number of surveys overall. The designations of high or low, in terms of floral diversity, were subjective and made on site after a visual scan of the diversity of flowering plant species present. Meanwood Grove (designated a low resource site) was sampled once, and St. George's Field (designated a low resource site) was sampled twice. At Spen Farm, the agroforestry site (designated a low resource site) was sampled eight times, and the fallow field site (designated a high resource site) was sampled five times. Bramham Park (designated a high resource site) was sampled three times. For a list of sampling dates for each survey, see Supplementary Table 1.2.

2.2.2 Pan trapping protocol

Surveys took place on fine days with an ambient temperature of 15˚C or higher and no sustained rainfall. Maximum day-time temperature (˚C), rainfall (mm), and average wind speed (mph) were recorded each day using data from the UK Met Office MIDAS station at Bramham (SE 448416), accessed via the Centre for Environmental Data Analysis (CEDA) archive (Met Office, 2020).

During each survey, four bowl sizes: 28ml (1 fluid oz.), 57ml (2 fluid oz.), 156ml (5.5 fluid oz.), and 284ml (10 fluid oz.), were tested using three trap duration periods: 7 hours, 24 hours, and 48 hours, and four bowl colours: UV-fluorescent blue, white, yellow, and pink (Sparvar Leuchtfarbe). The combination of UV-fluorescent blue, white, and yellow painted bowls has been noted as highly attractive to insect pollinators (Westphal et al., 2008), and pink was chosen as another naturally prevalent colour of insect-pollinated flowers. The spectral characteristics of each colour bowl were investigated using a spectrophotometer (Shimadzu UV-3101 PC UV-VIS-NIR Scanning Spectrometer).

Each pan trapping station consisted of four bowls of one size (one of each colour) attached to a wooden stand and raised so that the bowls were level with the surrounding flowering vegetation. There were six heights to which the wooden stand could be raised, starting at approximately 10cm above ground level and increasing in 10cm increments to 60cm above ground level. Each of the

three trap duration periods involved four pan trapping stations, one for each differently sized set of coloured bowls (Fig. 2.1).

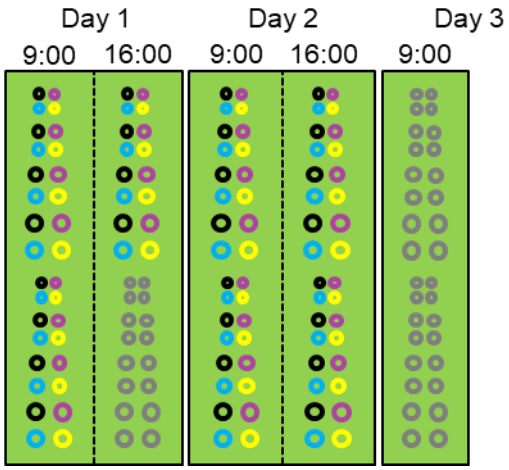

Figure 2.1 A visual representation of the experimental set-up over each three-day sampling period. Each set of four coloured circles represents an active trapping station (one set for each bowl size), and each grey set of four circles represents a trapping station that has been collected.

Each pan trapping survey lasted for forty-eight hours, spread across three calendar days (Fig. 2.1). On the morning of day one the pan trapping stations lasting seven hours and forty-eight hours were set out along a 100m linear transect. In one site: Meanwood Grove, the pan trapping stations were instead set out across areas that were representative of the types of vegetation present, due to the area available for sampling. At least ten meters was left between neighbouring pan trapping stations to limit competition between them (Droege et al., 2010), and the placement of specific pan trapping stations (as designated by bowl size) was randomised. The four bowl sizes contained set volumes of water: 15 ml (28ml), 20 ml (57ml), 85 ml (156ml), and 180 ml (284ml), plus one drop of un-scented detergent (Ecover) as a surfactant. This left an unequal amount of space between the water and the rim of the bowl between different bowl sizes: approximately 1.5cm (28ml), 1.7cm (57ml), 2.2cm (156ml), and 2.4cm (284ml). After seven hours, the 7-hour pan trapping stations were collected, and the contents preserved separately by colour in 70% ethanol for later identification. On the morning of day two, the 24-hour pan trapping stations were placed in the same locations as those previously occupied by the 7-hour pan trapping stations. On the morning of day three, both the 24- and 48-hour pan trapping stations were collected, and the specimens preserved.

Bees (Hymenoptera: Apoidea) and hoverflies (Diptera: Syrphidae), were separated from the other invertebrate specimens (hereafter termed as bycatch), pinned, and identified to species following the keys in Stubbs & Falk (2002) and Falk (2015). Some species were aggregated together due to recognised difficulties in achieving reliable species-level identifications, these were: 1) *Bombus terrestris* and *B. lucorum*; and 2) *Lasioglossum calceatum* and *L. albipes*.

The average height of the vegetation (cm) surrounding each trapping station was recorded, using five haphazardly selected points within a one-meter radius on the first day of sampling during each survey. Floral abundance within a two-meter radius was also assessed on the first day of sampling, following the methodology presented in Baude et al. (2016) and O'Connor et al. (2019b). The number of flowers of each species was counted and multiplied by the estimated volume of nectar sugar produced per flower per 24 hours present in Baude, Kunin, & Memmott (2015), thus accounting for the differing levels of floral reward produced by different plant species. For plants with multiple florets clustered into spikes, racemes, or capitula, such as members of the Asteraceae, each flower "head" was counted as a single floral unit (Carvell et al., 2007). Three representative floral units were then taken from each site and the number of individual florets counted and averaged to provide a mean number of flowers per floral unit. This value could then be standardised into a volume of daily nectar sugar produced per floral unit per day. For further information concerning how each floral species was classified in terms of what constituted a floral unit, together with the average number of florets per floral unit, and the estimated volume of nectar sugar produced per floret per day and per floral unit per day, see Supplementary Table 1.3. For a breakdown of the relative abundance of floral species within a two-meter radius of each pan trapping station per survey visit, see Supplementary Table 1.4.

2.2.3 Data analysis

All data analyses were performed using R, version 3.2.3 (R Core Team, 2016), and version 4.0.5 (R Core Team, 2021).

Bee and hoverfly species were combined into five overlapping taxonomic groups for analysis: bumblebees, solitary bees (any non-*Bombus*/non-*Apis* species), total bees, hoverflies, and total pollinators (total bees + hoverflies). Data on honeybees (*Apis mellifera*. L.) were included in the

"total bees" category, but since the number of honeybees sampled was small (N = 31) they were not treated as their own category for further analysis. Some individuals could not be identified to species-level and were therefore not included in any species richness analyses but were included in the abundance analyses for their pollinator group.

Generalized linear mixed-effects models (GLMMs) were created using the package glmmADMB (Fournier et al., 2012; Skaug et al., 2016). A mixed-effects model structure allowed me to account for naturally occurring variation in bee and hoverfly abundance between survey visits, both between and within sites, by using survey number (1-19) as a categorical random effect within each model (1|survey number). Given that each site received an unequal number of visits, a nested random term (1|site/survey number) would have been inappropriate. A Poisson error structure and a log link were used to analyse count data. Where data were overdispersed, a negative binomial error structure was used. One set of models, focusing on proportional data, used a binomial error structure and this is specified below in section 1.2.3.2. Histograms of both abundance and species richness data from several pollinator groups showed high proportions of zeros, so the *zeroInflation* argument was included in each model to correct for the presence of any false zeros (Zuur et al., 2009). Model selection between zero-inflated and non-zero-inflated models was carried out using the Akaike Information Criterion (AIC).

Model selection was performed via model averaging using the R package MuMIn (Barton, 2016) and the approach laid out by (Grueber et al., 2011); variables were not standardised prior to analysis. Each global model was dredged using the pdredge function, and all models within 2ΔAICC of the best model were averaged. The zero method or "full" coefficients are listed in the results (Grueber et al., 2011; Nakagawa & Freckleton, 2011), and explanatory variables were considered significant where the 95 percent confidence intervals surrounding the parameter estimate did not include zero (Nakagawa & Cuthill, 2007; see Martins, Gonzalez, & Lechowicz, 2015). Results were visualised by plotting the raw data using the geom_bar and geom_smooth functions in R package ggplot2 (Wickham, 2009), alongside the viridis package (Garnier et al., 2021)

2.2.3.1 Effects of bowl size, trap duration, and bowl colour on the abundance and species richness of bees and hoverflies sampled by pan trapping

Bowl size was broken down into two variables: bowl surface area and bowl depth (Table 2.1), as preliminary data exploration indicated that these dimensions may explain more variation in sampled pollinator diversity than bowl volume alone. An interaction between bowl colour and the proportion of similarly coloured flowers within a two-meter radius was also included in these models. Poisson GLMMs were used to assess the effects of bowl surface area, bowl depth, trap duration period, a bowl surface area x bowl depth x trap duration interaction term, and bowl colour on pollinator abundance and species richness.

Global model example 1:

$$Total\ pollinator\ abundance \sim bowl\ surface\ area * bowl\ depth * trap\ duration + bowl\ colour + environmental\ variables + (1|survey\ number)$$

Table 2.1 The dimensions of each size of bowl used in this experiment: total bowl volume (ml), bowl surface area (cm2) and bowl depth (cm).

Bowl volume (ml)	Bowl surface area (cm^2)	Bowl depth (cm)
28	15.90	3.30
57	30.19	3.10
156	44.18	6.00
284	109.36	5.50

2.2.3.2 Effects of bowl size and trap duration on the proportion of bees and hoverflies relative to other invertebrates (bycatch) sampled by pan trapping

The presence of bycatch in a pan trap may be affecting the number of pollinators attracted to the bowl. Binomial GLMMs were used to assess the effects of bowl surface area, bowl depth, trap duration period, and a bowl surface area x bowl depth x trap duration interaction term, on the abundance of pollinators relative to the abundance of bycatch. This variable was created by combining the number of pollinators of a given category (e.g., total bees) and the corresponding counts for non-members of this category (e.g., all non-bees, including hoverflies) using the *cbind*

command in R. I was unable to analyse species richness data in this manner since I was unable to identify the bycatch to species.

Global model example 2:

$$Pollinator\ proportion = cbind(pollinator\ abundance, non - pollinator\ abundance)$$

$$Pollinator\ proportion \sim\ bowl\ surface\ area * bowl\ depth * trap\ duration + bowl\ colour + environmental\ variables + (1|survey\ number)$$

2.2.3.3 Effects of bowl size and trap duration upon the rate of bee and hoverfly capture per hour by pan trapping

Since bees and hoverflies are diurnal, pan traps are only actively sampling for these groups for a portion of the time when bowls are left out for 24- or 48-hour periods. The number of daylight hours each pan trapping station was "active" for was calculated using Leeds-specific data for civil dawn and dusk. A natural log transformation was then applied to these data before they were incorporated into a set of Poisson GLMMs as an offset term (Zuur et al., 2009); allowing for the rate of bee or hoverfly capture to be explored across my three different trap duration periods. These GLMMs were used to assess the effects of bowl surface area, bowl depth, trap duration period, and a bowl surface area x bowl depth x trap duration interaction term on pollinator abundance and species richness. The offset was constrained to be present in every model run during the model averaging process.

Global model example 3:

$$Total\ pollinator\ abundance \sim\ bowl\ surface\ area * bowl\ depth * trap\ duration + bowl\ colour + environmental\ variables + offset(\log(no.\ of\ daylight\ hours)) + (1|survey\ number)$$

2.2.3.4 Effects of local environmental variation on the abundance and species richness of pollinators sampled by pan trapping

The following environmental variables were included as additional fixed effects in all of the models described above: maximum daytime temperature (˚C), daily rainfall (mm), average wind speed (mph), and the average vegetation height surrounding each trapping station (cm). The year

and month in which sampling took place were included to account for temporal variation in sampling effort.

Both wind speed and temperature showed evidence of non-linear patterns when plotted against bee and hoverfly abundance and richness. Maximum daytime temperature ranged between 15.3 and 30.5°C during this experiment, with a median value of 20.1°C across all survey visits. Bee and hoverfly samples peaked between approximately 17 and 22°C, dropping sharply after maximum temperatures rose past 23°C (see Supplementary Fig. 1.1 & 1.2). Wind speed ranged between 3.5 and 19.7mph during this experiment, with a median value of 9mph across all survey visits. Bee and hoverfly samples showed much less sharply delineated trends in relation to average wind speed than maximum day-time temperature (see Supplementary Fig. 1.3 & 1.4), with a broad peak between approximately 7 and 12mph in most groups. In the solitary bee group, this peak extended from the 3.5mph and started dropping after the average wind speed increased past 10mph. While in the total pollinator, total bee, and hoverfly groups, an additional peak in samples between 3.5 and 6mph was observed. In all groups, samples dopped sharply once the average wind speed rose to more than 15mph. To account for these patterns, second-order polynomial terms were fitted to both the maximum daytime temperature and average wind speed variables, using the *poly* function in R, to account for these patterns.

Finally, each site included two proxies of floral abundance. Firstly, the volume of nectar sugar produced per 24 hours (µl) within a two-meter radius of each individual pan trapping station was averaged across all pan trapping stations per survey. This mean was used as a proxy for floral rewards present at the scale of the survey site. Secondly, for each survey, this mean was subtracted from the values for daily nectar sugar production within a two-meter radius of each individual pan trapping station (pan trapping station-scale nectar sugar production – site-scale mean nectar sugar production), providing a positive measure of local daily nectar sugar production within two meters of each pan trapping station relative to the average daily nectar sugar production across the site. This derived variable was used instead of the raw value for nectar sugar production within two meters of each pan trapping station because it reduced the high level of collinearity present between the raw values and their mean within each model. I applied a natural log transformation

to both nectar variables to correct for a strong right-hand skew in the data. For a breakdown of the total volume of nectar sugar produced per day within a two-meter radius of each pan trapping station per survey visit, along with the mean value averaged across all pan trapping stations per survey, see Supplementary Table 1.5.

Global model example 4:

$$\begin{aligned} Total\ pollinator\ abundance \sim\ & bowl\ surface\ area * bowl\ depth * trap\ duration \\ & + bowl\ colour + poly(maximum\ temperature, degree = 2) \\ & + poly(average\ wind\ speed, degree = 2) \\ & + \log(mean\ nectar\ sugar\ per\ survey\ site + 1) \\ & + \mathrm{I}(\log(\text{pan trapping station} - \text{scale nectar sugar production}) \\ & - \log(mean\ nectar\ sugar\ per\ survey\ site + 1)) + daily\ rainfall \\ & + average\ vegetation\ height + Year + Month + (1|survey\ number) \end{aligned}$$

2.3 Results

Nineteen pan trapping surveys were carried out during this experiment, sampling 591 individual pollinators, comprising 36 species of bee and 24 species of hoverfly (Table 2.2). Bumblebees (*Bombus* spp.) were the most abundant pollinator genus in the pan trap samples, representing 48% of all bees and 33% of all pollinators. The Halictidae (*Halictus*, *Lasioglossum*, and *Sphecodes* spp.) accounted for 82% of all solitary bees and 37% of all bees. Hoverflies constituted 31% of all pollinators sampled. By-catch, consisting of 6351 Diptera, 37 Lepidoptera, 687 wasps (both social and solitary), 323 Coleoptera, 368 Hemiptera, and 619 other invertebrates, represented over 90% of all invertebrates sampled by the pan traps.

For a full breakdown of the relative abundance of bee and hoverfly species sampled by site, together with the relative abundance of different insect orders present within the bycatch sampled by site, see Supplementary Tables 1.6 & 1.7.

Table 2.2 Bee and hoverfly species sampled by pan trapping during this experiment.

Pollinator categories					
Solitary bees		Honeybees		Hoverflies	
Species	N	Species	N	Species	N
Andrena (unknown sp.)	1	*Apis mellifera*	31	Unknown Syrphid spp.	3
Andrena bicolor	6			*Cheilosia albitarsis*	2
A. bimaculata	1	Bumblebees		*Dasysyrphus albostriatus*	2
A. chrysosceles	1	Species	N	*Episyrphus balteatus*	45
A. falsifica	1	*Bombus* (unknown spp.)	3	*Eristalis* (unknown sp.)	1
A. haemorrhoa	1	*Bombus hortorum*	10	*Eristalis arbustorum*	11
A. minitula	2	*B. hypnorum*	1	*E. pertinax*	5
A. semilaevis	4	*B. lapidarius*	69	*E. tenax*	14
A. subopaca	2	*B. pascuorum*	17	*Eupeodes corollae*	1
Halictus (unknown spp.)	3	*B. pratorum*	7	*Helophilus* (unknown spp.)	2
Halictus rubicundus	3	*B. rupestris*	8	*Helophilus hybridus*	1
H. tumulorum	43	*B. soroeensis*	3	*H. pendulus*	43
Heriades truncorum	1	*B sylvestris*	2	*H. trivittatus*	1
Hylaeus communis	1	*B. terrestris/lucorum*	60	*Melanostoma mellinum*	6
H. hyalinatus	1	*B. vestalis*	17	*M. scalare*	1
Lasioglossum (unknown sp.)	1			*Merodon equestris*	1
Lasioglussum calceatum/albipes	40			*Neoascia podagrica*	8
L. fulvicorne	3			*Parasyrphus nigritarsis*	1
L. leucopus	33			*Platycheirus albimanus*	1
L. morio	2			*P. manicatus*	1
L. smeathmanellum	19			*P. nielseni*	1
L. villosulum	7			*Rhingia campestris*	2
Megachile willughbiella	1			*Sphaerophoria reuppellii*	1
Osmia aurulenta	1			*Syritta pipiens*	7
O. leaiana	2			*Syrphus ribesii*	15
Sphecodes ephippius	1			*S. vitripennis*	3
Stelis punctulatissima	1			*Xylota segnis*	2
Total	182		197		181

2.3.1 Effects of bowl size and trap duration on the abundance and species richness of bees and hoverflies sampled by pan trapping

Increasing bowl surface area had a weak but significant positive effect upon sampled abundance within the total pollinator (0.073, 95% CI [0.018, 0.127]), bumblebee (0.007 [0.002, 0.012]), and hoverfly groups (0.122 [0.022, 0.221]), and upon bumblebee species richness (0.005 [0.001, 0.009]) (Fig. 2.2A & B). In each of these groups, the bowls with the largest surface area (284ml: 109.36 cm2) sampled the most individuals and/or the most species.

Bowl depth had no significant effect upon sampled abundance or species richness within any group, but a weak significant two-way interaction between bowl surface area and bowl depth upon sampled abundance within the total pollinator (-0.012 [-0.022, -0.001]) and hoverfly groups (-0.020 [-0.039, -0.001]), indicates that as bowl depth increased the positive effect of an increasing surface area decreased.

Pan traps left out for 24 hours sampled significantly greater bumblebee richness (0.883 [0.309, 1.457]) than those left out for 7 hours (Fig. 2.2D). Pan traps left out for 48 hours sampled significantly greater total pollinator abundance (0.531 [0.279, 0.783]) and richness (0.459 [0.226, 0.692]), total bee abundance (0.600 [0.318, 0.882]) and richness (0.598 [0.333, 0.862]), and bumblebee abundance (1.479 [0.143, 2.816]) and richness (1.226 [0.701, 1.752]), than pan traps left out for 7 hours (Fig. 2.2C & D).

The model averaging process doesn't allow for a post-hoc analysis of all possible pairwise comparisons between different levels of categorical explanatory variables. Therefore, in terms of pan trapping duration I can only present the results of pairwise comparisons between the 7- and 24-hour pan traps, and the 7- and 48-hour pan traps (see Supplementary Table 1.8 for all available pairwise comparisons).

2.3.2 Effects of bowl colour on the abundance and species richness of bees and hoverflies sampled by pan trapping

Spectrographic analysis of the different bowl colours showed peaks in reflectance at ca. 470nm for the blue bowls, 440nm and 620nm for the pink bowls, and 520nm for the yellow bowls, while

the white bowls reflected across the whole visual spectrum (Fig. 2.3). The yellow bowls were the only colour to show some limited UV reflectance, between 275 and 320nm.

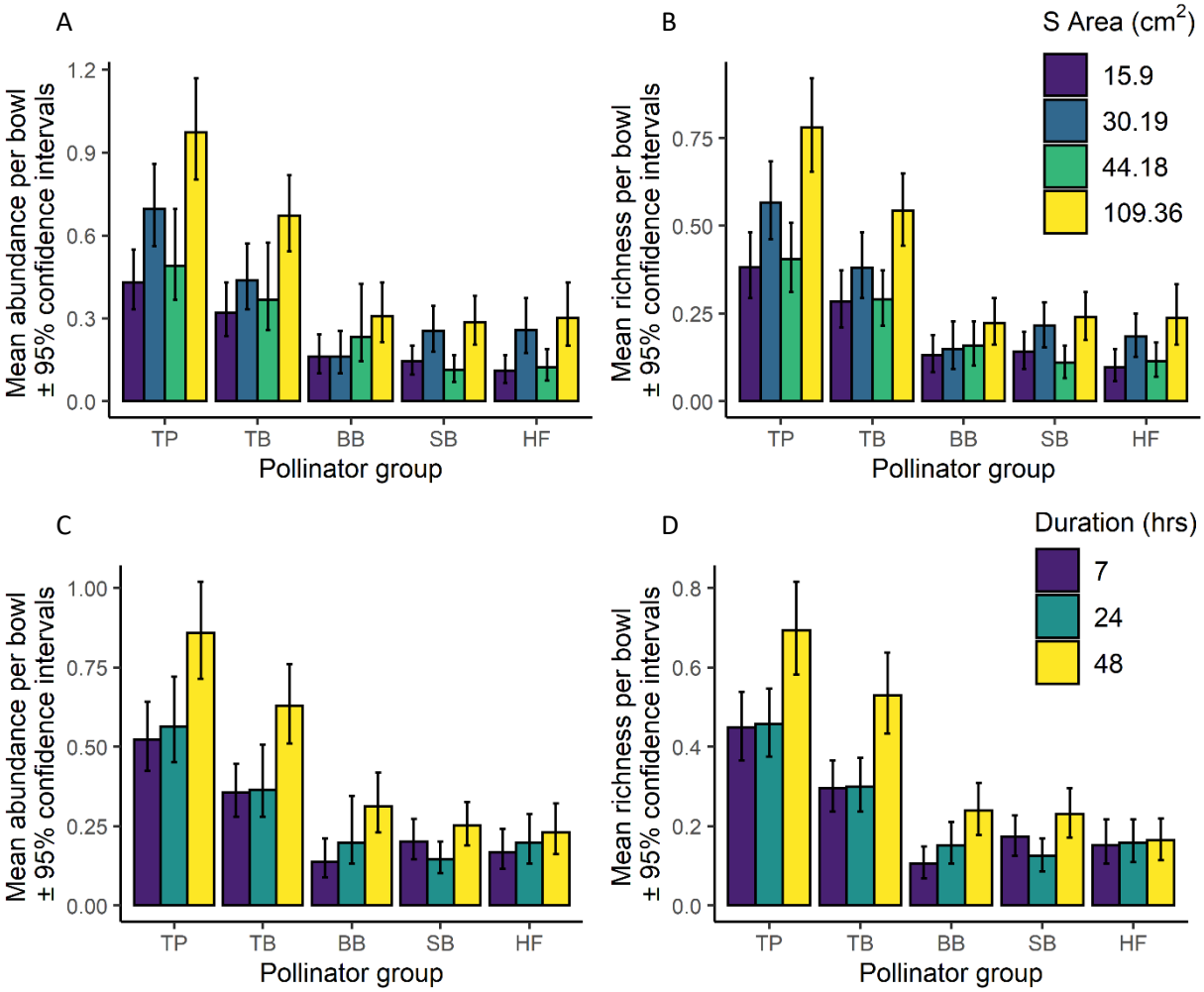

Figure 2.2 A) Mean abundance of each pollinator group sampled per bowl (TP: total pollinators, TB: total bees, BB: bumblebees, SB: solitary bees, and HF: hoverflies) ± 95% confidence intervals, plotted against bowl surface area (cm^2); B) Mean species richness of each pollinator group sampled per bowl ± 95% confidence intervals, plotted against bowl surface area (cm^2); C) Mean abundance of each pollinator group sampled per bowl ± 95% confidence intervals, plotted against trap duration (hours); D) Mean species richness of each pollinator group sampled per bowl ± 95% confidence intervals, plotted against trap duration.

The yellow bowls sampled significantly greater total pollinator abundance and species richness, while the pink bowls sampled the lowest (Table 2.3; Fig. 2.4A & B). The blue bowls sampled a significantly greater abundance and richness of both total bees and bumblebees, whereas solitary bee abundance and richness was significantly higher in both the yellow and white bowls (Table 2.3; Fig. 2.4A & B). Hoverfly abundance and richness was significantly greater in the yellow bowls (Table 2.3; Fig. 2.4A & B).

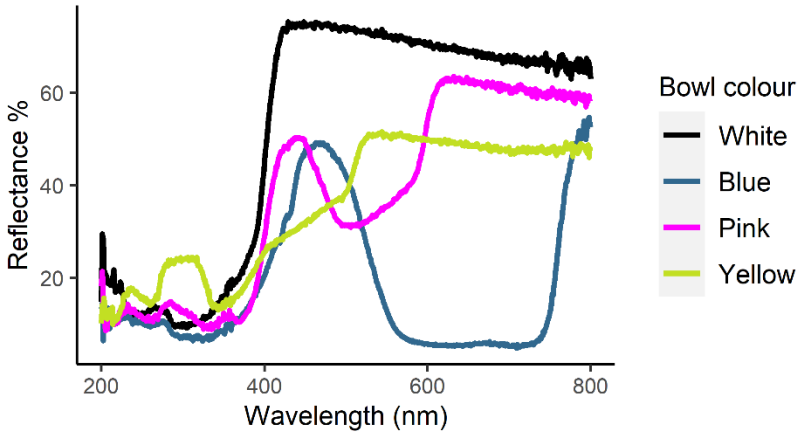

Figure 2.3 The percentage reflectance values for the UV-fluorescent yellow, blue, pink, and white (black line) pan traps.

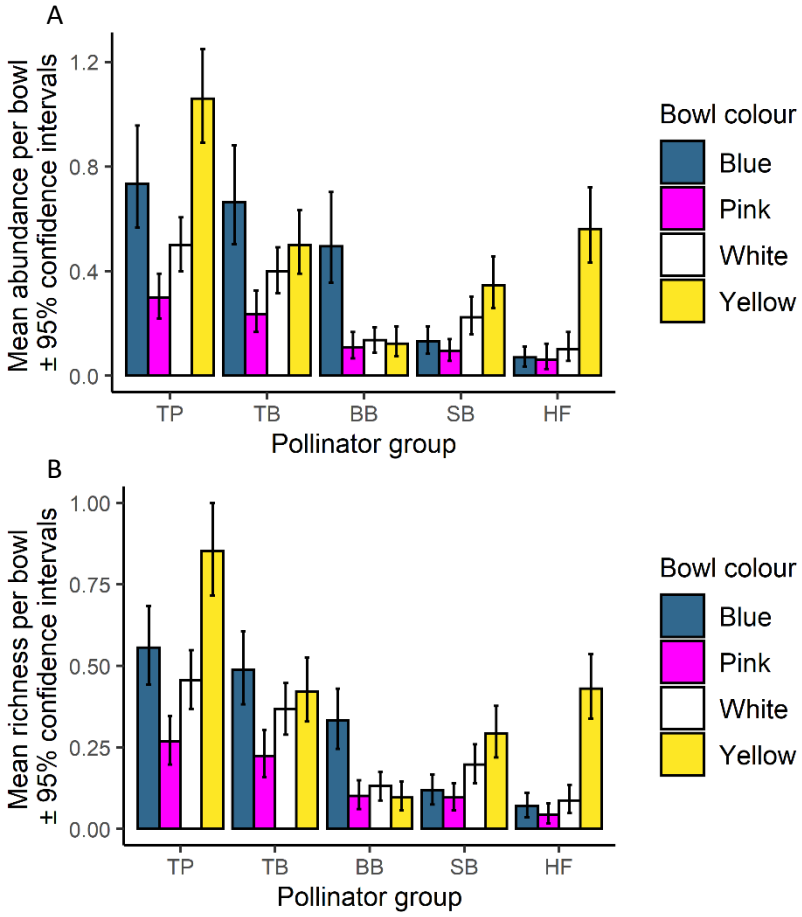

Figure 2.4 A) Mean abundance of each pollinator group sampled per bowl ± 95% confidence intervals, plotted against bowl colour; B) Mean species richness of each pollinator group sampled per bowl ± 95% confidence intervals, plotted against bowl colour.

As previously mentioned, the model averaging process doesn't allow for a post-hoc exploration of all possible pairwise comparisons between different levels of categorical explanatory variables. Therefore the pairwise comparisons shown in Table 2.3, combined with the trends shown in Fig.

2.4A & B, are my only methods of appraising the differences in samples between differently coloured bowls.

Table 2.3 Model averaged parameter estimates (ΔAIC_C <2) and their respective 95% confidence intervals for the effects of bowl colour on the sampled abundance and species richness of each pollinator group. Bold text refers to significant parameter estimates, where the respective 95 percent confidence intervals do not include zero.

			Parameter estimates ± 95% CI	
Group	Parameter		Abundance	Richness
Total pollinators	Intercept		-4.964 [-6.830, -3.097]	-3.862 [-5.720, -2.005]
	Bowl colour†	Pink	**-0.862 [-1.186, -0.539]**	**-0.733 [-1.039, -0.428]**
		White	**-0.322 [-0.608, -0.037]**	-0.200 [-0.459, 0.060]
		Yellow	**0.458 [0.206, 0.711]**	**0.424 [0.200, 0.648]**
Total bees	Intercept		-4.842 [-6.697, -2.986]	-4.087 [-5.648, -2.527]
	Bowl colour	Pink	**-0.981 [-1.339, -0.622]**	**-0.778 [-1.110, -0.446]**
		White	**-0.421 [-0.736, -0.106]**	-0.279 [-0.563, 0.005]
		Yellow	-0.171 [-0.473, 0.130]	-0.145 [-0.419, 0.128]
Bumblebees	Intercept		-6.498 [-8.538, -4.458]	-5.353 [-7.317, -3.389]
	Bowl colour	Pink	**-1.438 [-1.915, -0.961]**	**-1.195 [-1.662, -0.728]**
		White	**-1.185 [-1.634, -0.736]**	**-0.930 [-1.353, -0.506]**
		Yellow	**-1.283 [-1.748, -0.819]**	**-1.240 [-1.715, -0.765]**
Solitary bees	Intercept		-4.051 [-6.794, -1.308]	-3.506 [-5.946, -1.065]
	Bowl colour	Pink	-0.310 [-0.861, 0.241]	-0.205 [-0.768, 0.359]
		White	**0.531 [0.079, 0.982]**	**0.511 [0.033, 0.989]**
		Yellow	**0.968 [0.547, 1.389]**	**0.909 [0.461, 1.356]**
Hoverflies	Intercept		-6.782 [-10.156, -3.407]	-6.809 [-10.522, -3.097]
	Bowl colour	Pink	-0.165 [-0.918, 0.588]	-0.470 [-1.261, 0.321]
		White	0.389 [-0.288, 1.067]	0.223 [-0.435, 0.881]
		Yellow	**2.103 [1.537, 2.669]**	**1.812 [1.283, 2.342]**

† blue bowls are the reference category

2.3.3 Effects of bowl size and trap duration on the proportion of bees and hoverflies relative to other invertebrates (bycatch) sampled by pan trapping

The mean abundance of bycatch sampled in the 28ml (1 fluid oz.), 57ml (2 fluid oz.), 156ml (5.5 fluid oz.), and 284ml (10 fluid oz.) pan traps was: 3.45 ± SE 0.26, 7.65 ± 0.55, 7.98 ± 0.66, and 19.30 ± 1.49, respectively. The abundance of bycatch sampled increased significantly as bowl surface area increased (0.124 [0.095, 0.152]) and as bowl depth increased (0.577 [0.402, 0.753]). However, a weak but significant negative interaction between bowl surface area and bowl depth

indicates that fewer invertebrates were sampled in wider bowls when their depth increased (-0.021 [-0.026, -0.015]).

The proportion of bumblebees in the pan trap samples decreased significantly as bowl surface area increased (-0.133 [-0.228, -0.039]) (Fig. 2.5A), while the proportion of hoverflies decreased significantly as bowl depth increased (-0.278 [-0.444, -0.112]) (Fig. 2.5B). There was also a weak positive interaction between surface area and depth in the bumblebee group (0.023 [0.006, 0.041]), indicating that a significantly greater proportion of bumblebees were sampled in bowls with larger surface areas as their depth increased.

The mean abundance of bycatch sampled in the 7, 24, and 48-hour pan traps was: 5.52 ± 0.41, 9.50 ± 0.81, and 14.00 ± 1.08, respectively. In terms of pan trap duration, the abundance of bycatch sampled in the 24-hour pan traps (0.422 [0.177, 0.667]) and 48-hour pan traps (0.782 [0.462, 1.102]), was significantly higher when compared to pan traps left out for 7 hours.

The proportion of total pollinators (-0.368 [-0.642, -0.095]) and solitary bees (-1.125 [-1.599, -0.651]) was significantly lower in the 24-hour pan traps than in the 7-hour pan traps (Fig. 2.5C). Meanwhile, the proportion of bumblebees was significantly greater in the 48-hour pan traps than in the 7-hour pan traps (2.459 [0.799, 4.119]), while the proportion of solitary bees was significantly lower in the 48-hour pan traps (-0.787 [-1.215, -0.360]) (Fig. 2.5C). See Supplementary Table 1.9 for all available pairwise comparisons between the three trap duration periods.

There was also a significant negative interaction between depth and trap duration in the bumblebee group, with smaller proportions of bumblebees being sampled in deeper bowls after 48-hours than after 7-hours (-0.492 [-0.838, -0.146]).

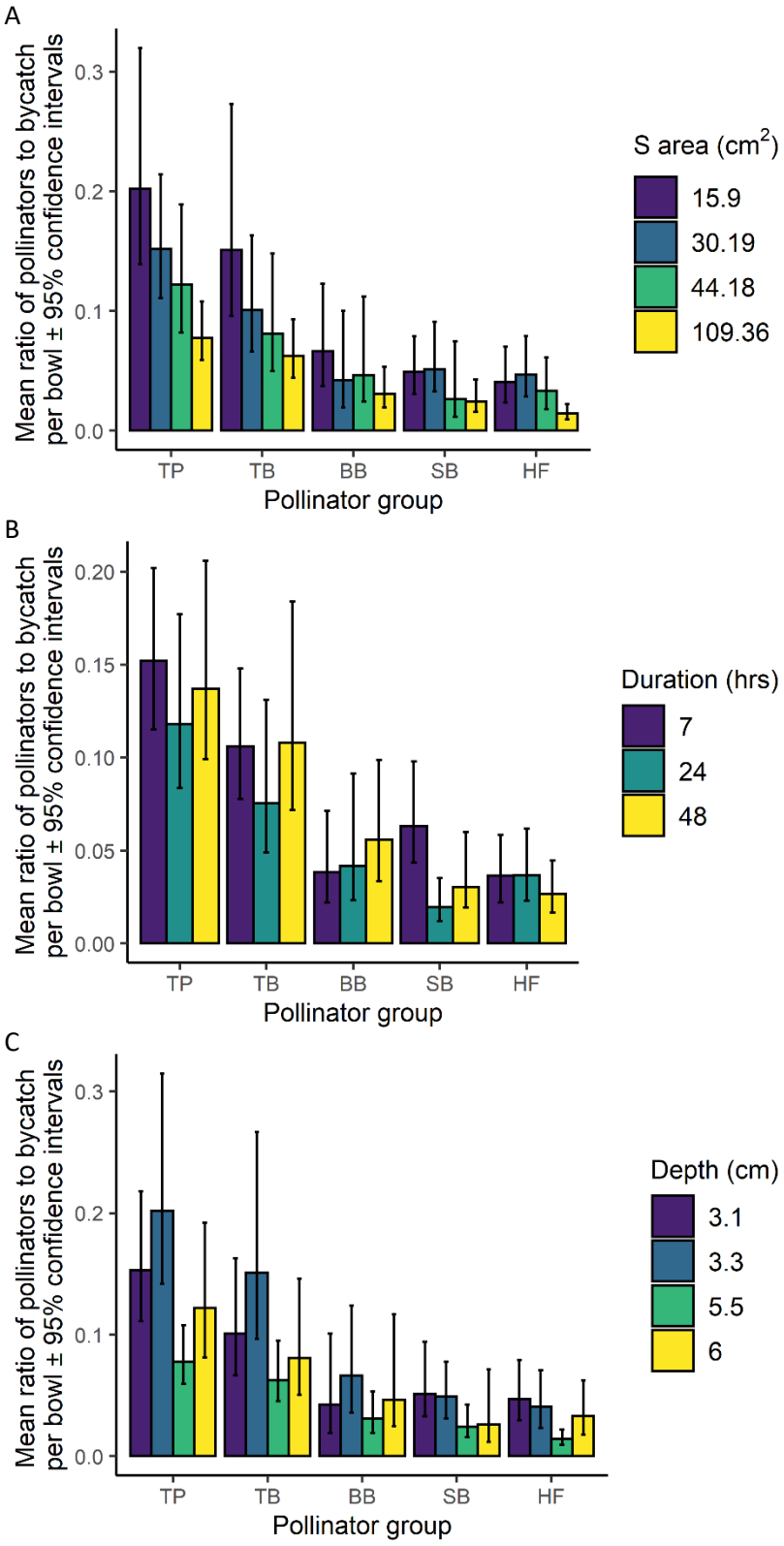

Figure 2.5 A) Mean proportion of each pollinator group sampled per bowl (TP: total pollinators, TB: total bees, BB: bumblebees, SB: solitary bees, and HF: hoverflies) ± 95% confidence intervals, plotted against bowl surface area (cm^2); B) Mean proportion of each pollinator group sampled per bowl (TP: total pollinators, TB: total bees, BB: bumblebees, SB: solitary bees, and HF: hoverflies) ± 95% confidence intervals, plotted against bowl depth (cm); C) Mean proportion of each pollinator group sampled per bowl (TP: total pollinators, TB: total bees, BB: bumblebees, SB: solitary bees, and HF: hoverflies) ± 95% confidence intervals, plotted against trap duration (hours).

2.3.4 Effect of bowl size and trap duration upon the rate of bee and hoverfly capture per hour by pan trapping

Pan traps left out for 24 hours sampled significantly lower total pollinator abundance (-0.733 [-1.007, -0.459]) and richness (-0.780 [-1.035, -0.525]), total bee abundance (-0.821 [-1.132, -0.510]) and richness (-0.808 [-1.107, -0.508]), and hoverfly abundance (-0.743 [-1.463, -0.022]), per daylight hour than those left out for 7 hours (Fig.2.6A & B).

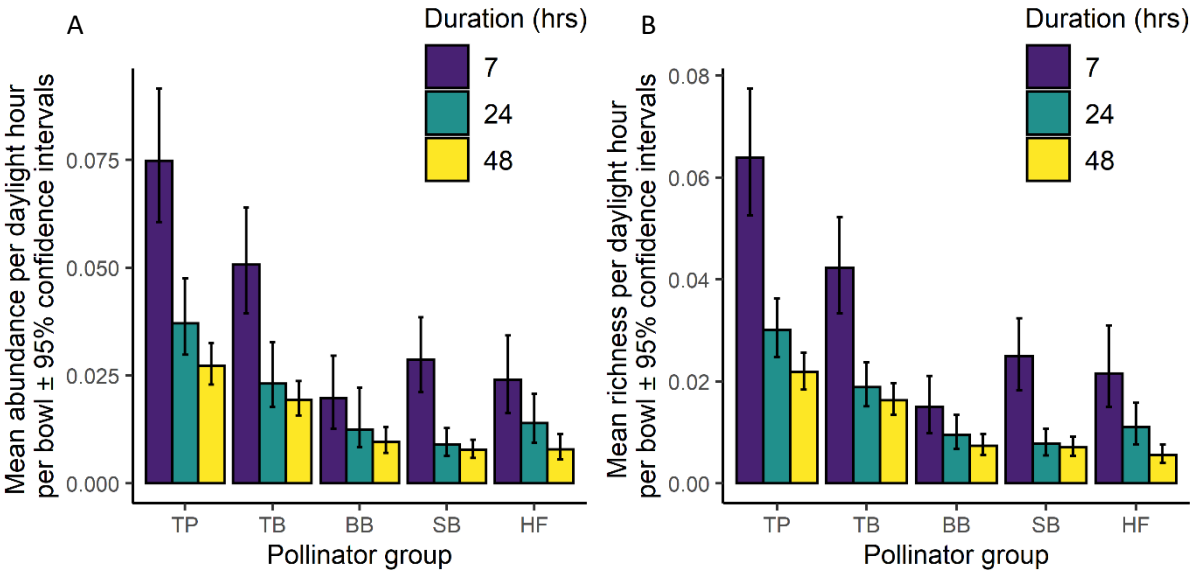

Figure 2.6 A) Mean abundance of each pollinator group sampled per daylight hour per bowl (TP: total pollinators, TB: total bees, BB: bumblebees, SB: solitary bees, and HF: hoverflies) ± 95% confidence intervals, plotted against trap duration (hours); B) Mean species richness of each pollinator group sampled per daylight hour per bowl ± 95% confidence intervals, plotted against trap duration (hours).

Pan traps left out for 48 hours sampled significantly lower total pollinator abundance (-0.963 [-1.218, -0.708]) and richness (-1.037 [-1.270, -0.804]), total bee abundance (-0.921 [-1.206, -0.637]) and richness (-0.928 [-1.193, -0.662]), solitary bee abundance (-3.151 [-4.732, -1.570]) and richness (-2.640 [-4.866, -0.414]), and hoverfly abundance (-1.600 [-2.509, -0.691]) and richness (-1.651 [-2.654, -0.648]), per daylight hour than those left out for 7 hours (Fig. 2.6A & B). See Supplementary Table 1.10 for all available pairwise comparisons between the three trap duration periods.

Two weak significant two-way interaction terms, between trap duration and bowl surface area and trap duration and bowl depth, indicate that leaving a pan trap active for 48 hours reduces the positive effect of increasing bowl surface area (-0.017 [-0.032, -0.002]), while reducing the

negative effect of bowl depth (0.619 [0.135, 1.103]), in terms of solitary bee abundance sampled per daylight hour.

2.3.5 Effects of local environmental variation on the abundance and species richness of pollinators sampled by pan trapping

As the nectar sugar production per floret per day at the scale of the site increased, there were significant increases in total pollinator abundance (0.164 [0.101, 0.227]) and richness (0.138 [0.077, 0.198]), total bee abundance (0.230 [0.143, 0.318]) and richness (0.160 [0.075, 0.244]), bumblebee abundance (0.156 [0.017, 0.295]), and solitary bee abundance (0.241 [0.126, 0.356]) and richness (0.177 [0.070, 0.285]) (Fig. 2.7C & D). Similarly, as the proxy for nectar sugar production per floret per day at the scale of the pan trapping station (pan trap-scale nectar sugar production – site-scale nectar sugar production) increased, there were significant increases in total pollinator abundance (0.075 [0.024, 0.126]) and richness (0.071 [0.023, 0.119]), total bee abundance (0.096 [0.026, 0.167]) and richness (0.099 [0.032, 0.165]), solitary bee abundance (0.121 [0.018, 0.225]) and richness (0.123 [0.020, 0.226]), and hoverfly abundance (0.075 [0.002, 0.148]) (Fig. 2.7A & B). In each group, the effect size for site-level daily nectar sugar production was consistently larger than those for the proxy for daily nectar sugar production at the scale of the pan trapping station.

The effects of maximum day-time temperature and average wind speed were tested for with linear and second-order polynomial terms; the coefficients presented below represent orthogonal polynomial terms. Regarding temperature: in the bumblebee group the linear term was significant, showing a significant decrease in abundance (-18.755 [-28.430, -9.081]) and richness (-16.982 [-26.500, -7.465]) as temperature increased (Fig. 2.8A & B); in the hoverfly group the second-order term was significant, showing a significant decrease in abundance (-12.599 [-24.855, -0.342]) as temperature increased (Fig. 2.8A). Regarding wind speed: the linear term was significant in both the bumblebee and solitary bee groups, showing a significant increase in bumblebee abundance (15.074 [5.686, 24.462]) and richness (15.611 [5.848, 25.374]), and a significant decrease in solitary bee abundance (-11.379 [-19.148, -3.609]) as wind speed increased (Fig. 2.8C & D).

Average vegetation height had a weak significant negative effect upon solitary bee abundance (-0.010 [-0.019, -0.002]) and richness (-0.010 [-0.019, -0.001]) (Fig. 2.8E & F). However, this was not borne out in the raw data, which appears to show both solitary bee abundance and species richness increasing as the average vegetation height increased. Daily rainfall, meanwhile, had no significant effects upon sampled abundance or species richness within any pollinator group.

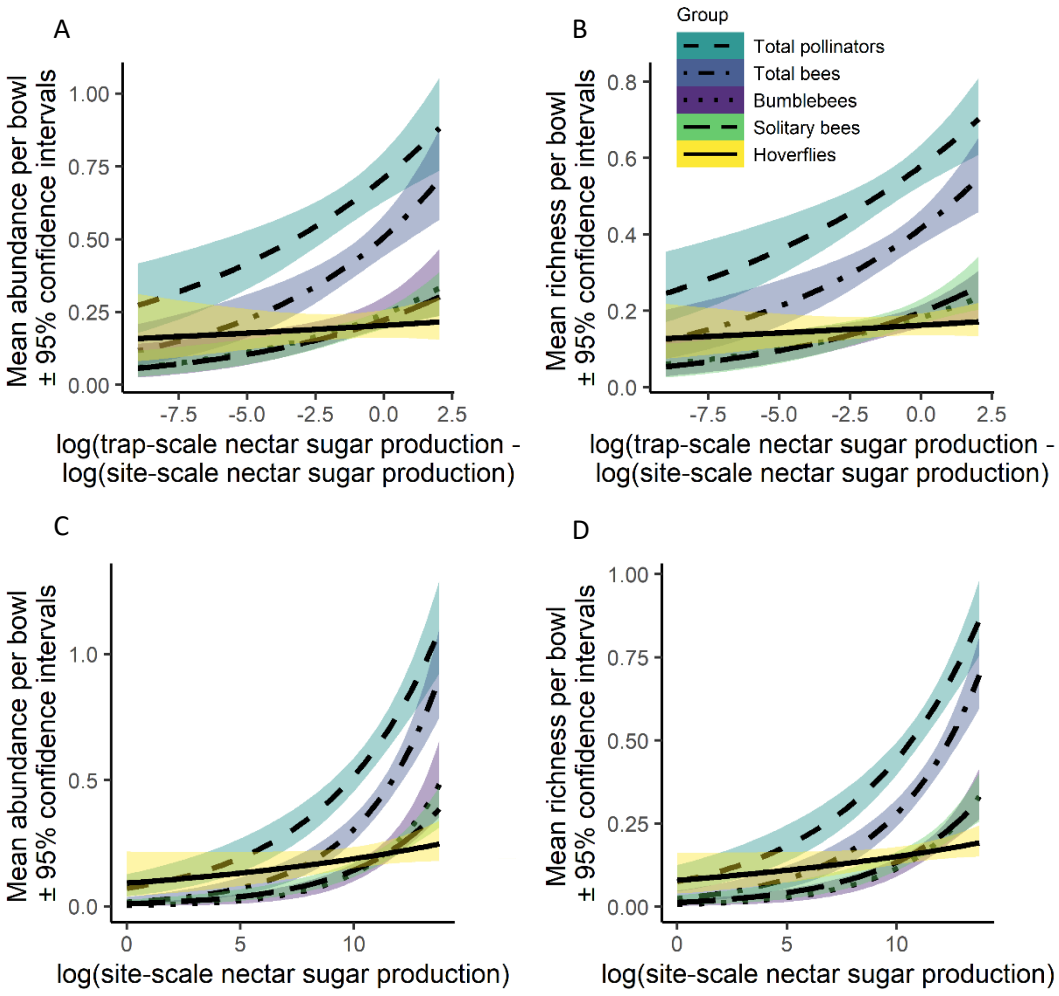

Figure 2.7 A) Mean abundance of each pollinator group sampled per bowl ± 95% confidence intervals, plotted against the proxy for nectar sugar at the scale of the pan trapping station (log(nectar sugar volume (µl) within 2m of each pan trapping station per survey) – log(mean nectar sugar volume (µl) within 2m of each pan trapping station per survey)); B) Mean species richness of each pollinator group sampled per bowl ± 95% confidence intervals, plotted against the proxy for nectar sugar at the scale of the trapping station (log(nectar sugar volume (µl) within 2m of each pan trapping station per survey) – log(mean nectar sugar volume (µl) within 2m of each pan trapping station per survey)); C) Mean abundance of each pollinator group sampled per bowl ± 95% confidence intervals, plotted against the proxy for nectar sugar at the scale of the survey site (log(mean nectar sugar volume (µl) within 2m of each pan trapping station per survey)); D) Mean species richness of each pollinator group sampled per bowl ± 95% confidence intervals, plotted against the proxy for nectar sugar at the scale of the survey site (log(mean nectar sugar volume (µl) within 2m of each pan trapping station per survey)).

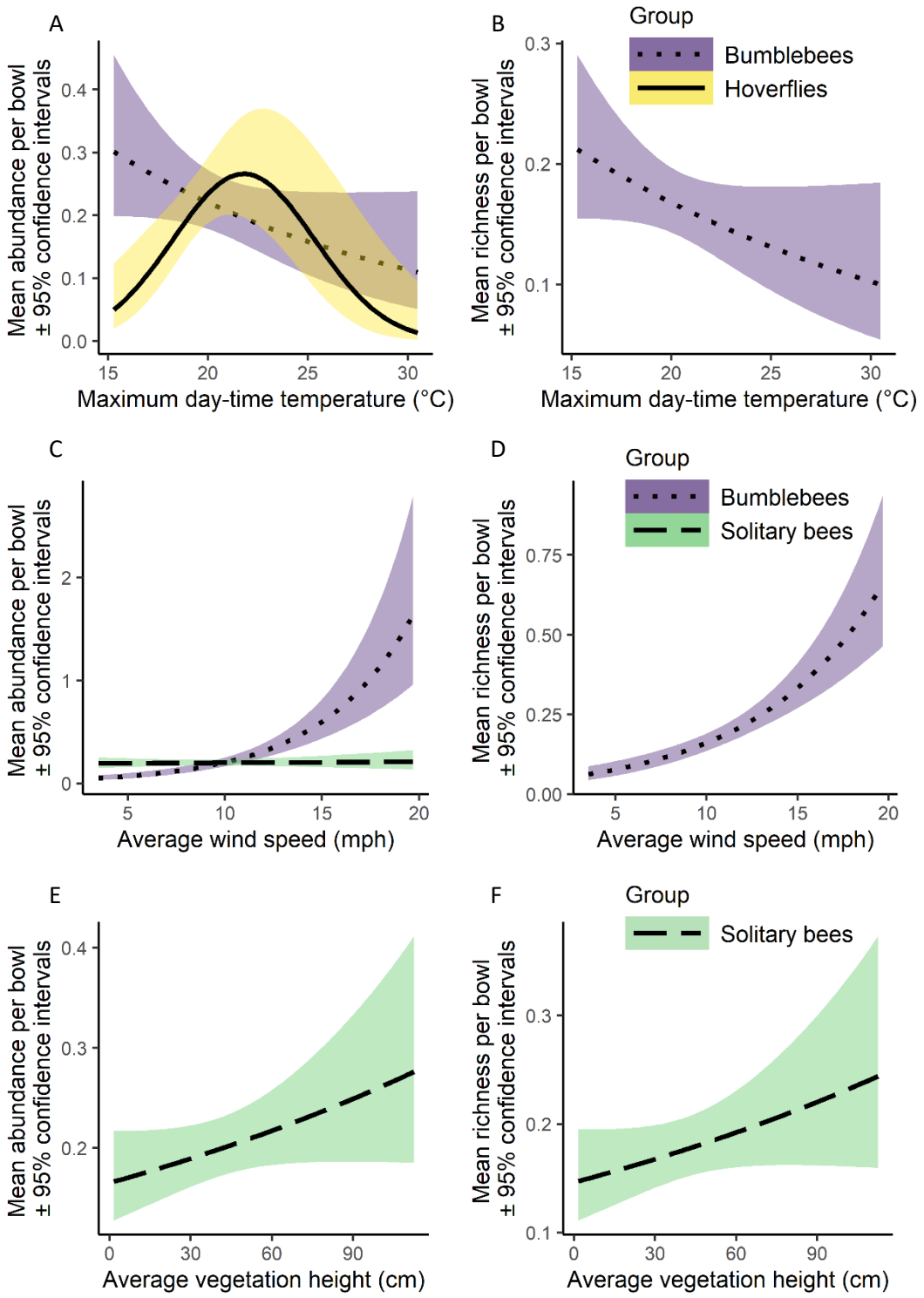

Figure 2.8 A) Mean abundance of bumblebees and hoverflies sampled per bowl ± 95% confidence intervals, plotted against maximum day-time temperature (°C); B) Mean species richness of bumblebees sampled per bowl ± 95% confidence intervals, plotted against maximum day-time temperature (°C); C) Mean abundance of bumblebees and solitary bees sampled per bowl ± 95% confidence intervals, plotted against average wind speed (mph); D) Mean species richness of bumblebees sampled per bowl ± 95% confidence intervals, plotted against average wind speed (mph); E) Mean abundance of solitary bees sampled per bowl ± 95% confidence intervals, plotted against average vegetation height (cm); F) Mean species richness of solitary bees sampled per bowl ± 95% confidence intervals, plotted against average vegetation height (cm).

2.4 Discussion

Pan traps are a commonly used tool for monitoring insect pollinator populations, due both to their ease of use and their relative lack of collector bias. However, while their sampling ability has been compared extensively to that of other methods (Cane, Minckley, & Kervin, 2000; Roulston, Smith, & Brewster, 2007; Westphal et al., 2008; Wilson, Griswold, & Messinger, 2008; Grundel et al., 2011; Popic, Davila, & Wardle, 2013; Prendergast et al., 2020), the protocols governing the use of pan traps have not been as extensively explored. Here, I show that bowl dimensions, trap duration, and the volume of nectar sugar produced per day around the trapping stations all strongly influence the diversity and abundance of bees and hoverflies sampled by pan traps. I also contribute further evidence to the debate surrounding the existence of taxon-specific bowl colour preferences and indications as to how local weather variability may affect pan trap function. I follow up by making specific recommendations for a standardised pan trapping protocol to be deployed in future studies exploring aspects of insect pollinator diversity, occupancy, and population trends.

2.4.1 Effect of bowl size on the abundance and species richness of bees and hoverflies sampled by pan trapping

In terms of the physical characteristics of the pan traps, bowl size had significant effects on both sampled pollinator abundance and species richness. The bowls with the largest surface area (284 ml: 109.36 cm^2) sampled the most sampled the most pollinators overall, as well as greater numbers of bumblebees and hoverflies, presumably because bowls with larger surface areas are more visible to foraging insects. Larger floral displays are easier to detect by aerial foragers from further away (Ohashi & Yahara, 2001; Spaethe, Tautz, & Chittka, 2001), and have been associated with higher pollinator visitation rates (Ohashi & Yahara, 2001); although, Spaethe, Tautz & Chittka (2001) note that this effect may be dependent on the contrast between the floral colour (or that of the pan trap) and that of the background vegetation. Larger floral displays may also be indicative of greater floral rewards (Blarer, Keasar, & Shmida, 2002) which may also affect pollinator visitation rates to pan traps with larger surface areas. My results broadly agree with those of Wilson et al. (2016), who found that larger bowls sampled more bees, but contrast with

those of Droege (2002) who found no differences in sampled bee abundance due to bowl size. Curiously, out of the seven bowl sizes used by Droege (2002), four are approximately similar in volume to those used here, but their results did not mirror the trends in pollinator abundance found here. The use of volume as the primary measure of size (as opposed to diameter, surface area, or depth) in studies like those by Wilson et al. (2016) and Droege (2002) makes direct comparisons between past results and our own difficult. However, Gonzalez et al. (2020), who explored the effect of bowl diameter on sample size, also found no effect of bowl size on bee abundance while using bowls of a similar diameter to those used in this Chapter.

Furthermore, while bowl depth had no effect upon pollinator abundance or richness, the ratio between bowl surface area and depth may be the cause of the apparent reduced sampling ability of the 156ml bowls (44.18 cm^2 x 6 cm) in comparison to the 57ml bowls (30.19 cm^2 x 3.10 cm) in most pollinator groups (Fig. 2.2A & B), despite an increase in surface area. This pattern likely led to the negative interaction term between bowl surface area and bowl depth in the total pollinator and hoverfly groups - since the 57ml bowls have a higher surface area-to-depth ratio than the 156ml bowls (9.74 vs. 7.36), their UV-fluorescent interiors may be more visible to aerial foragers, making the smaller bowls more attractive.

One aspect of bowl size that I did not account for in this analysis was the amount of space left between the water and the rim of the bowls, which was not constant between bowl sizes. This distance was approximately similar between and 28ml and 57ml bowls (1.5cm and 1.7cm), and between the 156ml and 284ml bowls (2.2cm and 2.4cm). One might assume that a smaller gap might facilitate more insect samples, because the water in the bowls would be more immediately accessible to passing foragers, but a smaller gap may also enhance an insects' chance of escaping capture by providing easier access to the rim of the bowl. This distance was greatest in the two deeper bowls (156ml & 284ml), which did sample the most bycatch, indicating that these bowls may generally be more difficult to escape from. But both the 15ml and 156ml bowls seem to be sampling similar numbers of bees and hoverflies per bowl, despite the gap between the water and the rim of the bowl being 7mm larger in the latter. The bowl size which sampled the most bees and hoverflies (and bycatch) per bowl also had the deepest gap between the water and the lip of

the bowl. Since these bowls also had a larger ratio of surface area to depth and were thus likely more attractive to foraging bees and hoverflies, it may be that a larger gap combined with more attractive bowl dimensions may facilitate more samples in the bowls. This warrants further investigation but, in future, I would suggest standardising the volume of water per bowl so that the gap between the water and the rim of the bowl is the same, regardless of bowl size, to reduce potential bias.

When I looked at the proportion of pollinator abundance relative to the abundance of bycatch, I observed different trends in relation to bowl size. For instance, bumblebee abundance sampled per bowl increased with bowl surface area, but when the abundance of non-bumblebees was accounted for, I saw this effect reverse - as bowl surface area increased the proportion of bumblebees sampled per bowl decreased. Since research indicates that bumblebees prefer larger floral displays (Ohashi & Yahara, 2002; Mitchell et al., 2004), this leaves several potential hypotheses to explain this result: 1) since bycatch abundance increased with bowl surface area, this is simply a function of other invertebrates being caught more often than bumblebees; 2) cues associated with increasing bycatch abundance are deterring bumblebees from the pan traps; or 3) a combination of the above. Bumblebees may regard bycatch as visual evidence of competing conspecifics/heterospecifics which, at high densities, may lead to avoidance behaviour (Baude et al., 2011). Bumblebees also actively avoid flowers that have been visited by other bumblebees (Goulson, Hawson, & Stout, 1998; Stout & Goulson, 2001) and hoverflies (Reader et al., 2005), based on olfactory cues left by previous foragers. Since the widest bowls were the most attractive generally, a greater number of insects are likely to have landed on the bowl rims or interiors, potentially leaving scent-marks that may deter foraging bumblebees. Approaching foragers may also react to alarm pheromones released by bumblebees already caught in the pan traps (Dukas, 2001; Llandres, Gonzálvez, & Rodríguez-Gironés, 2013), or to chemical cues linked to the breakdown of dead insect bodies within the bowls (Sun & Zhou, 2013), either of which may deter individuals from landing. Increasing build-up of bycatch may also make pan traps less visually attractive by blocking the attractive colours of the bowls.

The proportion of hoverflies sampled per bowl decreased as bowl depth increased. As the deepest bowls sampled fewer hoverflies overall, I might assume that this effect was simply compounded by the presence of other invertebrates, since the deeper bowls caught significantly more bycatch. However, the second deepest bowls (284 ml: 5.5 cm) also had the greatest surface area (109.36 cm^2), and otherwise sampled the greatest abundance of hoverflies. Despite this, the proportion of hoverflies was also lower in these bowls (Fig. 2.4A), suggesting that this negative effect was not limited to bowls with dimensions that hoverflies already found unattractive. Since the abundance of bycatch was also higher in the 284 ml bowls, this indicates that it is the presence of a greater relative abundance of bycatch that is deterring hoverflies from the deeper bowls, rather than bowl dimensions. There is no evidence to suggest that hoverflies actively avoid flowers occupied by other insects (Reader et al., 2005) or that they reject flowers previously visited by insects based upon scent marks, which merits further research into this potential behaviour within this group. Again, the increasing build-up of bycatch may also have been blocking the colour of the pan traps, making them less attractive to foraging hoverflies.

Overall, these results indicate that pan traps may have size-specific carrying capacities (see Wilson et al. 2016), the specifics of which, along with any associated avoidance behaviours exhibited by bee and hoverfly taxa, warrant further investigation in future observational studies. In terms of recommendations for future monitoring studies, if their aim is to sample a broad range of insect taxa, including bees and hoverflies, then I would recommend using the larger 284 ml (10 fl. oz.) bowls, as they sampled the greatest abundance and species richness of bees, hoverflies, and bycatch within this study. However, if the aim was to sample bees, or bees and hoverflies specifically, then the smaller 57ml (2 fl. oz.) bowls sampled a greater proportional abundance of both taxa in relation to abundance the bycatch. Regardless, I would recommend future studies use pan traps with greater surface area-to-depth ratios, as this seems to increase the attractiveness of the bowls of foraging insects. I also suggest that any future studies looking at the effects of bowl size on insect pollinator diversity should consider investigating other measures of size than volume when planning their experimental design.

2.4.2 Effect of trap duration on the abundance and species richness of bees and hoverflies sampled by pan trapping

Trap duration had stronger effects upon pollinator abundance and richness than either measure of bowl size. The effects of trap durations beyond 24 hours have not been reviewed within the current literature, but shorter trapping periods have been briefly explored and my results largely confirm those of Carboni & Lebuhn (2003), who found no significant differences between pan traps left active for eight hours and those left active for 24 hours in terms of sampled bee abundance. Presumably, pan traps set out in the morning for 7 hours sample the same period of peak pollinator activity as those set out for 24 hours, with the latter gaining little extra sampling power from the additional hours of daylight prior to the following morning. The longest trap duration tested in this study (48 hours) was more successful than the seven and 24-hour pan traps, both in terms of the abundance and richness of total pollinators, total bees, and bumblebees.

However, when pollinator abundance and richness were observed in the context of the rate of capture over time then the per-hour sampled abundance and richness in every group except for the bumblebees decreased as trap duration increased. Since our pan traps were catching, on average, less than one individual pollinator per bowl, we can assume that this is not due to an exhaustion of local bee and hoverfly communities, supporting the findings of (Gezon et al., 2015). The results concerning species richness may relate to the foraging strategies of different groups. Since most bees are central place foragers, limited in their movement by their need to return to their nesting site or colony - especially the smaller solitary bee species (Zurbuchen et al., 2010) - I might expect the number of new species sampled by stationary pan trapping stations to decrease with time. But, since the bumblebee group did not show this trend, and hoverflies (which did) display entirely different forgaing strategies (being highly dispersive, with no nest to return to or larvae to provision (Rotheray & Gilbert, 2011)), this may not be the case.

Alternatively, decreases in the per-hour abundance and richness of different bee and hoverfly groups with increasing trap duration may be explained with reference to the build-up of bycatch over time. The raw abundance of bycatch increased with trap duration, while the proportion of solitary bees significantly decreased with trap duration. The proportion of total pollinators also

decreased in the 24-hour bowls when compared to the 7-hour bowls, but the proportion of total pollinators in the 7-hour and 48-hour bowls was not significantly different. This suggests that the proportion of bees and hoverflies together remain somewhat consistent within the bowls each day, but that the bowls left active for 24-hours may be sampling additional crepuscular insect species later in the evening or earlier the following morning that are increasing the proportion of bycatch present relative to the diurnal bee and hoverfly samples.

This indicates that solitary bees are avoiding pan traps left out for longer due to an increasing abundance of bycatch; avoidance behaviour that may be based around visual and/or olfactory cues (Yokoi, Goulson, & Fujisaki, 2007; Yokoi & Fujisaki, 2009, 2011), potentially representing evidence for a temporal carrying capacity within pan traps (see Wilson et al. 2016). This requires further investigation via observational studies to determine the validity of this effect within different insect pollinator taxa.

Overall I suggest that, while pan traps left out for 48 hours may sample greater abundance and species richness in terms of bees and hoverflies, they also capture fewer individuals and species per hour over time. I therefore suggest that future monitoring protocols use shorter, seven-hour trap duration periods, in line with recent practises present in Carvell et al. (2016) and LeBuhn et al. (2016). A seven hour trap duration also requires only one day spent in the field, as opposed to the two-three days required to carry out a 24- or a 48-hour sampling protocol, increasing the number of total sampling visits that can take place over a survey season. Longer trap durations also attracted larger numbers of non-target taxa, so the use of shorter trap durations may also help to reduce the impact of pan trapping on the wider invertebrate community.

In discussion of bycatch more broadly: many previously understudied insect taxa, e.g., non-syrphid flies, are increasingly being recognised as providing valuable pollination services (Orford, Vaughan, & Memmott, 2015; Rader et al., 2016). If data concerning these and other taxa are already being gathered by pan trapping studies and in large quantities, I suggest that it be reported to provide a more inclusive view of pollinator communities in general as a counterpoint to the often bee-centric view that is generally represented in the current literature (Westphal et al., 2008; Nielsen et al., 2011; Popic, Davila, & Wardle, 2013; O'Connor et al., 2019b).

2.4.3 Effect of bowl colour on the abundance and species richness of bees and hoverflies sampled by pan trapping

Bowl colour had a clear selective effect on the abundance and species richness of pollinator taxa sampled by the pan traps. Yellow bowls sampled the most pollinators overall, and my results support its use in research where only one bowl colour may be required for experimental reasons (Wilson et al., 2016; Gonzalez et al., 2020). But my primary finding was that different insect pollinator taxa show distinct colour preferences: hoverflies for yellow bowls, bumblebees for blue bowls, and solitary bees for both yellow and white bowls, while the pink bowls performed poorly in all groups. The broad appeal of the yellow bowls to both solitary bees and hoverflies may be a result of yellow being the only bowl colour that reflected in the ultraviolet portion of the spectrum, which both bees and hoverflies can detect, and that bees use to locate nectar guides offered by flowers to guide foragers to their nectaries (Horth, Campbell, & Bray, 2014; Orbán & Plowright, 2014). Mulligan & Kevan (1973) also observed higher insect visitation rates in flowers which reflected strongly in the ultraviolet portion of the spectrum.

However, when these results are compared to those of other authors, it is clear that no definite pattern exists with regard to pan trap colour preferences among insect pollinator taxa. For instance, while some hoverfly species have displayed an innate preference for the colour yellow (Ilse, 1949; Lunau, 1988, 2014), and both Bowie et al. (1999) and Laubertie, Wratten & Sedcole (2006) sampled more hoverflies in yellow pan traps, hoverflies can be trained to preferentially visit other colours under laboratory conditions (see Ilse 1949). This presumably reflects a natural response to changes in the seasonal availability of attractive floral rewards. Bees, meanwhile, have displayed diverse preferences for yellow (Abrahamczyk, Steudel, & Kessler, 2010; Gollan, Ashcroft, & Batley, 2011), blue (Joshi et al., 2015; Moreira et al., 2016), blue and white (Campbell & Hanula, 2007), blue and yellow (Droege, 2002), and yellow and white pan traps (Vrdoljak & Samways, 2011; Heneberg & Bogusch, 2014).

Both Toler, Evans & Tepedino (2005) and Saunders & Luck (2013) suggest that the wide disparities in bowl colour preference displayed by different insect pollinator taxa indicates that these inclinations are context-specific rather than fixed or purely innate. Thus, pollinator

assemblages might develop their own colour preferences based upon local plant rewards or phenology, preferences that may vary within and between years (Joshi et al., 2015). The use of only one bowl colour within a study would clearly introduce its own experimental bias by selecting for only a subset of the pollinator community present (Moreira et al., 2016). I therefore recommend maintaining the now-standard combination of UV-fluorescent white, blue, and yellow bowls for future pan trapping studies. My results indicate that they are complementary in their ability to sample broad pollinator communities, and they are more likely to satisfy a wider range of community-specific preferences than any single colour. These results do not justify the addition of UV-fluorescent pink to this combination.

2.4.4 Effect of local environmental variation on the abundance and species richness of pollinators sampled by pan trapping

In addition to the physical characteristics of the pan traps themselves, their local environment can also impact upon their sampling ability. Notably, we found that pan traps located in areas with increased estimated nectar sugar production per day, both at the scale of the site and (to a lesser degree) of the pan trapping station, sampled more individuals and species within most pollinator groups. These results contrast with the widely held hypothesis that pan traps sample fewer insects in areas with higher floral abundance (Cane, Minckley, & Kervin, 2000; Roulston, Smith, & Brewster, 2007; Wilson, Griswold, & Messinger, 2008; Baum & Wallen, 2011; Popic, Davila, & Wardle, 2013; Westerberg et al., 2021), and indicate that pan traps may not be competing for pollinator visits with surrounding flowers as initially proposed by Cane, Minckley & Kervin (2000).

However, most of these previous studies either did not quantify floral resource availability surrounding their pan traps or used proxies without providing data concerning floral abundance, which makes it challenging to compare their results to those presented here. Cane, Minckley & Kervin (2000), for example, counted the number of Creosote bushes along their pan trapping transect (294) but provided no information concerning the number of Creosote blooms, and Roulston, Smith, & Brewster (2007) made no attempt to quantify the floral resources present around their pan traps despite alluding to an effect of floral abundance upon pan trapping efficacy.

Wilson, Griswold, & Messinger (2008) quantified the "average floral richness" across their sites and linked this to bee species richness sampled via pan trapping; showing that, as average floral richness rose to approximately eight species in Mid-May, the number of bee species sampled dropped to the lowest level across the study. While Baum & Wallen (2011) used targeted herbicide application to artificially manipulate floral abundance in two mixed-grass prairie pastures, reducing floral species richness by between 63 and 100% over four months, and reducing floral abundance by over 90% per month. No further information regarding floral availability was provided by the authors, who found the bee species richness was greater in pan trapping samples taken from those sites treated with herbicides when compared to untreated pastures. Finally, Westerberg et al. (2021) quantified floral frequency at a local scale around each pan trapping station ($25m^2$) and at a wider, landscape scale (2-6 ha.), using photographs of individual $1m^2$ vegetation plots. Floral frequency ranged between 9 and 100% across sites, and increasing floral frequency was linked to smaller sample sizes from pan traps. Although, solitary bees showed no negative bias with regard to floral frequency, and social bees were one of the few insect groups sampled to show some limited positive relationships with floral frequency, but only at a local scale.

O'Connor et al. (2019b) provides possibly the closest point of comparison for this study, as the authors measured both floral abundance surrounding their pan trapping stations and converted this into a measure of nectar sugar production per day. They also provided a measure of floral density (per m^2) surrounding their pan traps. O'Connor et al. (2019b) found that, while greater nectar sugar availability had no effect upon bee or hoverfly abundance sampled per pan trap, increasing floral density did have a negative effect upon solitary bee and bumblebee abundance sampled per pan trap in apple and field bean crop monocultures, respectively. The floral abundance surrounding many of their pan traps was comparable to that surrounding many of ours (O'Connor et al., 2019a), which makes this difference in findings puzzling.

One explanation for these contrasting findings may be the spatial heterogeneity present within our respective sampling sites. Rathcke (1983) proposed that co-flowering plant communities which share a common set of pollinator species would facilitate one another in terms of attracting

pollinator visits at lower floral densities. This facilitation increases until the pollinator visitation rate reaches saturation, at which point the co-flowering species move from facilitation to competing for pollinator visits. Rathcke (1983) referred to this as the density-visitation relationship and, if applied to pan trapping, we may expect increasing nectar sugar availability at lower floral densities to facilitate increased pollinator visits to the bowls. O'Connor et al. (2019b) put forward a similar argument concerning their findings, citing that the high levels of homogeneous floral density in the crop monocultures that formed part of their study system may have increased competition between pan traps and the surrounding flowers. In comparison, the floral density present within many of the sites sampled within this Chapter is likely to have been far more heterogeneous, potentially facilitating bee and hoverfly visitation to the pan traps. Templ et al. (2019) also found that the abundance of wild bee species commonly found in pan trap samples correlated with lower levels of floral density (where the average number of floral species per meter square was less than ten, and the average number of flowers was less than one hundred).

Authors of past studies may, likewise, have been sampling from habitats with greater floral densities than those represented in our study sites, initiating competition between pan traps and the surrounding flowering plants for pollinator visitation. If true, this hypothesis may support the common practise of pairing pan trapping with active survey methods like hand-netting, the results of which are positively associated with increased floral abundance and diversity (Popic, Davila, & Wardle, 2013; O'Connor et al., 2019b). Overall, I recommend that future pan trapping studies assess the level of floral rewards present at different scales surrounding their pan traps, and provide measures of both abundance and floral density, to control for and further investigate any effects that these variables might have on pan trap samples. I would also advise doing so at different spatial scales, to account for the level of floral diversity present across the landscape within which a survey is being completed.

My use of the volume of nectar sugar produced per floret per day (µl) in this analysis was an attempt to incorporate the level of nutritional reward offered by different flowering plant species into a measure of floral abundance, using the dataset of 270 plant species present in Baude, Kunin, & Memmott (2015). Nectar provides an immediate, short-term source of energy (in the form of

sugars) that is used by insect species, including bees and hoverflies, to maintain flight whilst foraging (Kevan & Baker, 1983; Rotheray & Gilbert, 2011; Vaudo et al., 2015). Bees can also learn to associate particular plants or patches of plants with high nectar rewards (Cnaani, Thomson, & Papaj, 2006; Vaudo et al., 2015; Nery, Moreno, & Arenas, 2020), and both the volume of nectar available and the sugar concentration have been positively associated with bee visitation rates to flowers (Potts et al., 2004; Cnaani, Thomson, & Papaj, 2006). The use of nectar sugar production, however, disregard those plant species that do not produce nectar, which would bias this approach in habitats where these are common and/or abundant. In addition to nectar, pollen is also an important source of nutrition (proteins and lipids) in bee and hoverfly species (Rotheray & Gilbert, 2011; Vaudo et al., 2015), associated with larval development in bees (Vaudo et al., 2015), reproduction and reproductive development in both taxa (Heinrich, 1979; Rotheray & Gilbert, 2011; Vaudo et al., 2015), and migration in hoverflies (Rotheray & Gilbert, 2011). There are also hoverfly species that primarily feed on pollen rather than nectar, e.g., *Episyrphus balteatus* and *Syrphus ribesii*, although this is also affected by both sex and age (Rotheray & Gilbert, 2011), and the use of nectar sugar production as a proxy for floral abundance may also have biased our analysis against these species.

In addition to the effects of nectar sugar availability, I also collected data on average vegetation height. Across all survey visits, the average vegetation height within a one-meter radius of the pan trapping stations ranged from 1.5 to 112.5 cm, with a median value of 35cm (Supplementary Fig. 1.5). Both solitary bee abundance and species richness appeared to peak between 30 and 35cm, dropping after vegetation reached an average 60-70cm in height (Supplementary Fig. 1.6A & B). The results of the model averaging reported a negative effect of increasing average vegetation height within a one-meter radius of the pan trapping stations on solitary bee abundance and species richness. This, however, was not borne out in the raw data, which appears to show the number of solitary bees and solitary bee species increasing with taller vegetation.

Possibly the first reference to pan trap elevation was in Cane, Minckley, & Kervin (2000), who found that pan traps placed on the ground in a US desert scrub habitat did not sample any specialist bee fauna commonly associated with the locally prevalent Creosote bush (*Larrea tridenatata*). It

has since become common practise to elevate pan traps to the level of the surrounding flowering vegetation (Westphal et al., 2008; Nielsen et al., 2011; O'Connor et al., 2019b), usually citing the results of either Cane, Minckley, & Kervin (2000) or Tuell & Isaacs (2009). The latter tested the effects of elevating pan traps in relation to a highbush blueberry (*Vaccinium corymbosum* L.) crop on sample size, finding that pan traps elevated to approximately one third the height of the crop (0.46-0.6m) sampled the most bees. But very few studies report the height to which pan traps have been raised or that of the surrounding vegetation. Two exceptions are Geroff, Gibbs, & McCravy (2014) and Harris, Braman, & Pennisi (2017). Geroff, Gibbs, & McCravy (2014) tested two different pan trap elevations: bowls set at ground level and bowls suspended one meter from the ground, and found that, while the elevated bowls sampled significantly more native bees and native bee species than those at ground level, both sets of pan trapping samples differed significantly in terms of species composition. Indicating that placing bowls at a range of elevations within a site could help to sample a more complete species inventory. Harris, Braman, & Pennisi (2017), meanwhile, tested two similar elevations: bowls set at ground level and bowls suspended 91.5cm from the ground. Both sets of bowls were successful in terms of sampling native insect pollinator taxa, but locally abundant Halictid bee species were predominately found in pan traps placed upon the ground.

Gumbert & Kunze (1999) found evidence of stratification in bee foraging activity in a tropical aquatic plant community, with smaller bees tending to visit flowers less than 30cm from the ground. In a more recent study, Klecka, Hadrava, & Kolouškova (2018) experimentally manipulated the height of inflorescences in two grassland species: *Centaurea scabiosa* and *Inula salicina*, to between five and 105cm from the ground in short and tall flowering vegetation (averaging 7.2 and 50.1cm, respectively). They found that smaller solitary bee species tended to prefer foraging from *C. scabiosa* flowers at an intermediate height (40-60cm) in both short and tall vegetation, and from *I. salicina* flowers near ground level in short vegetation, and at an intermediate height (ca. 40cm) in tall vegetation. It may be that the solitary bee species commonly found in our pan traps are less likely to be found in habitats characterised by taller vegetation, or that they are present but preferentially forage on plants closer to ground-level. Regardless, my

results suggest that, when pan trapping in sites containing a range of different floral strata, an effort should be made to place individual pan traps or transects across areas that are representative of these strata, otherwise surveys may miss functionally important taxa (Geroff, Gibbs, & McCravy, 2014).

In terms of ambient temperature, almost all sampled pollinators were caught between 17 and 22°C, with a peak occurring between 20 and 21°C (Supplementary Figures 1.2 & 1.3), with significantly fewer bumblebees and hoverflies being sampled as temperatures rose. These results may be an artefact of our sampling methodology: we limited our sampling to days where ambient temperatures were above 15°C, with a median maximum day-time temperature 20.1°C, so I would expect to see some clustering of data points around this value, with little spread towards warmer or cooler temperatures. However, as poikilotherms, insects are vulnerable to overheating when the ambient temperatures increase above a certain point (although many taxa have evolved behavioural and/or physiological methods of combatting heat stress). Heinrich (1979) notes that large *Bombus* species will stop flying in ambient temperatures above 35°C and may die if their internal thoracic temperature reaches 45°C (although smaller workers can forage at higher temperatures). However, given that bumblebee abundance and richness across our surveys started to drop as the maximum temperature rose past 23°C, it's unlikely that this was primarily due to heat stress. Arce et al. (2017) also found that bumblebee foraging activity decreased at warmer temperatures, although they do not list the range of values experienced during their study, so I cannot compare them to my data. Our data do appear to agree with those shown in Corbet et al. (1993), where bumblebee foraging activity peaked between 20 and 25°C. Rotheray and Gilbert (2011), meanwhile, note that flight activity in many UK hoverfly species tends to occur between 15 and 25°C, decreasing above 21°C, which is certainly borne out by my data.

Pan trapping protocols usually indicate a lower ambient temperature boundary of 10-15°C below which pan trapping surveys are inadvisable (see Carvell et al. 2016; LeBuhn et al. 2016), presumably due to lower expected samples. But these data imply that, within the UK, there may also be an upper threshold of between 25°C and 30°C, beyond which certain pollinator groups may be sampled less often. Heinrich (1979) and Rotheray and Gilbert (2011) also note that body

size influences the temperatures at which foraging activity can be maintained within bumblebee and hoverfly species, implying that future research into the effects of ambient temperature on pan trapping samples should take pollinator size into account.

Wind speed should also be considered when planning pan trapping surveys. My results indicate that more solitary bees were sampled when wind speeds were lower, although the raw data suggest this effect may be minimal. Most solitary bees sampled during our surveys consisted of smaller Halictid species which, due to their size, may be less likely to forage under higher wind speeds than larger bee taxa, although there is little published evidence to support this (see Vicens & Bosch 2000). Bumblebees, conversely, were sampled more often as wind speed increased. There is little evidence to suggest that bumblebees preferentially forage under windier conditions (Peat & Goulson, 2005), although they do not appear to avoid them (Tuell & Isaacs, 2010; Crall et al., 2017). Greater wind speeds (within the range of those measured during this study) also do not appear to alter individual bumblebees' foraging patterns or patch use (Comba, 1999). While Arce et al. (2017) found that bumblebee foraging activity actually increased during periods of higher wind speeds.

It is possible that the presence of strong winds may make it more difficult for bumblebees to escape a pan trap once captured. Alternatively, Chang, Crall & Combes (2016) indicate that wind speeds equivalent to 7.8 mph can leave bumblebees unable to alter their flight speed prior to landing, causing them to experience "higher peak decelerations (and thus impact forces) upon landing". This may mean that bumblebees landing on the rim or the dry inner surface of a pan trap during higher winds exhibit less control and are more likely to fall into the water. Based upon these results, I suggest that pan traps are of limited use in environments where higher wind speeds are the norm, and that days with wind speeds in excess of 10 mph (ca. 16 km/hr) should be avoided where possible, particularly where smaller, solitary bee taxa are of primary interest to the survey/surveyor.

2.5 Conclusions

Overall, I recommend that the bowls used for pan trapping surveys are limited to those with wider surface areas and a relatively shallow depth, to increase visibility to aerial foragers. Pan trapping surveys should be carried out within a single day, as the rate of capture in terms of bee and hoverfly abundance and species richness decreased significantly after this length of time. Shorter trap duration periods also minimise both the number of person-hours required to conduct the sampling and the amount of non-target taxa sampled. The standard combination of UV-fluorescent blue, white, and yellow bowls should be maintained by practitioners, in order to sample the broadest assemblage of pollinators and pollinator species regardless of habitat. Greater volumes of nectar sugar produced per day were positively associated with larger pan trapping samples, both at the scale of the pan trapping station and at the scale of the survey site, in contrast to the results of several previous studies. To further investigate these results, future studies should quantify both the floral density and levels of floral rewards at different spatial scales to test for the presence of a density-visitation relationship between local floral resources and pan trap samples. Finally, both ambient temperature and wind speed should be considered when planning pan trapping surveys. Increasing temperatures appear to reduce the number of bees sampled by the bowls, while increasing wind speeds appear to bias pan trap samples in terms of both solitary bee and bumblebee abundance. In future, greater investment in direct observational studies may provide us with a better understanding of how insects behave around pan traps. Video recordings could also be used to verify personal observations without unduly affecting insect pollinator behaviour (Steen, Lene, & Orvedal, 2011; Gilpin, Denham, & Ayre, 2017; Steen, 2017).

Chapter 3

Evaluation of insect pollinator survey methods in a closed system

"Sampling bias means that the samples of a stochastic variable that are collected to determine its distribution are selected incorrectly and do not represent the true distribution because of non-random reasons." (Panzeri, Magri, & Carraro, 2008)

3.1 Introduction

Insect pollinators are a highly diverse community that display a broad range of life-history and behavioural traits (Wardhaugh, 2015; Ollerton, 2017). They are also under threat from population declines on a global scale (Biesmeijer et al., 2006; Cameron et al., 2011; Ollerton et al., 2014; Koh et al., 2016; Powney et al., 2019), leading to renewed calls for action concerning the need to monitor their changing population trends (Dicks et al., 2013). The urgent need to diagnose these declines and their effect upon the diversity of insect pollinator communities are both reflected in the wide range of constantly evolving survey techniques available to monitor insect pollinators (Potts, Kevan, & Boone, 2005; Westphal et al., 2008; August et al., 2015).

Regardless of whether the aim of a survey is to detect the presence of a species, estimate the abundance of a particular population, or estimate the relative abundance of species within a community, the choice of method, or combination of methods used, can have significant impacts upon the success of the survey and the conclusions that can be drawn from the data (Disney, 1982; Tyre et al., 2003; Portman, Bruninga-Socolar, & Cariveau, 2020). Survey methods each have their own inherent sources of sampling bias, and if a particular method is biased against a species or group of species, due to their size or foraging behaviour for example, then this may result in Type II errors or underestimates of these species in the resulting samples (Tyre et al., 2003). In the context of a large-scale, long-term monitoring scheme, such as the UK Pollinator Monitoring

Scheme[6] (PoMS), this could have knock-on effects on the reliability of data for the purposes of modelling population trends and informing conservation policy (Tyre et al., 2003). It is vital, therefore, to design standardised survey protocols that minimise or control for sampling bias, to ensure that the resulting data are as representative as possible of the populations or communities being monitored (Henderson & Southwood, 2016).

One of the first steps towards designing a standardised survey protocol is to quantify the biases associated with a particular survey method or selection of methods. In terms of insect pollinator monitoring, previous studies have often attempted to do this through direct comparisons of the abundance and/or species richness of insects sampled by different survey methods (Westphal et al., 2008; Nielsen et al., 2011; Popic, Davila, & Wardle, 2013; O'Connor et al., 2019b). Among these, the most commonly compared survey methods are pan trapping and transect surveys (Cane, Minckley, & Kervin, 2000; Roulston, Smith, & Brewster, 2007; Westphal et al., 2008; Grundel et al., 2011; Nielsen et al., 2011; Popic, Davila, & Wardle, 2013; Berglund & Milberg, 2019; O'Connor et al., 2019b). As I mentioned in **Chapter 2**, pan trapping is a **passive** survey method, involving the placement of brightly coloured bowls filled with water into which insects are attracted and then drown. By comparison, transect surveys are an **active** survey method, where a surveyor walks a predetermined route and counts the number of insects they observe, occasionally also capturing them with a net for identification at a later date. Transect surveys are labour-intensive and are open to collector bias, in that the relative "success" of the survey (however that may be measured) is directly reliant upon surveyor experience and training (Montgomery et al., 2021). Pan trapping, as a passive survey method, is not open to collector bias, and requires less effort to carry out in the field. However, samples acquired during pan trapping often require more time to sort and identify, as some identifying features can be obscured on wet specimens (Popic, Davila, & Wardle, 2013). In addition, while a surveyor walking a transect tends only to collect data on species of interest, pan traps attract and capture a diverse range of insects (Disney, 1982; Vrdoljak & Samways, 2011; Popic, Davila, & Wardle, 2013; Moreira et al., 2016; Harris, Braman,

[6] https://ukpoms.org.uk/home

& Pennisi, 2017), some of which may not necessarily be of interest to the survey in question and may be discarded as bycatch.

In **Chapter 2**, I discuss the non-standard way in which pan trapping has and is being deployed by researchers to survey insect pollinators. I also investigate how changing aspects of pan trapping survey protocols (i.e., bowl size, shape, colour, and trapping duration) may affect the sampled abundance and species richness of bees and hoverflies. In contrast, several standardised transect sampling protocols have existed for some time now, notably the Pollard transect (Pollard, 1977), and are being used by the UK Butterfly Monitoring Scheme and by the Bumblebee Conservation Trust as part of their Bee Walks, while PoMS makes use of both pan trapping and transect surveys within a single standardised protocol (Carvell et al., 2016).

However, past research notes that, when deployed in parallel, the relative abundance of species sampled by pan trapping and by transect surveys is often different. Several studies indicate that samples collected via pan trapping tend to contain a greater proportion of smaller bee species, particularly those belonging to the Halictidae, and a smaller proportion of larger, social species, such as bumblebees or the western honeybee (*Apis mellifera*), than samples collected via transect surveys (Roulston, Smith, & Brewster, 2007; Westphal et al., 2008; Wilson, Griswold, & Messinger, 2008; Grundel et al., 2011; Wood, Holland, & Goulson, 2015; Rhoades et al., 2017). Meanwhile, Potts, Evan, & Boone (2005) indicate that samples collected via transect surveys tend to be biased towards larger, slower-moving species, since they are likely to be easier to spot. In a rare study focusing on taxa other than bees, Popic, Davila, & Wardle (2013) observed that pan trapping sampled fewer species from several key flower-visiting insect taxa, such as wasps and the Coleoptera, than transect surveys. Indeed, since transect surveys sample at the plant-pollinator interface, they should be more likely to capture data on key flower visitors than passive methods like pan trapping (Popic, Davila, & Wardle, 2013). This does mean, however, that transect surveys are highly dependent on the availability of local floral resources, sampling greater diversity where transects fall in a flower-rich portion of the landscape (Popic, Davila, & Wardle, 2013; O'Connor et al., 2019b). It has also been argued that pan trapping may be biased in the opposite direction, sampling fewer pollinators in florally rich habitats due to competition between the bowls and the

surrounding blooms (Cane, Minckley, & Kervin, 2000; Roulston, Smith, & Brewster, 2007; Baum & Wallen, 2011; O'Connor et al., 2019b; Westerberg et al., 2021), although I present contradictory evidence to this in **Chapter 2**. Moreover, while surveyors along a transect are spatially limited in terms of their sampling, collecting snapshots of insect diversity over time, the area over which a single pan trapping station might sample has not been quantified (Saunders et al., 2021).

As a consequence of past evidence concerning sampling bias, opinion is split regarding whether either one of these two survey methods should be preferred when monitoring insect pollinator diversity (e.g., Westphal et al., 2008; Popic, Davila, & Wardle, 2013), or whether they should routinely be used in tandem, so as to balance out their individual sources of sampling bias (Roulston, Smith, & Brewster, 2007; Wilson, Griswold, & Messinger, 2008; Grundel et al., 2011; Carvell et al., 2016; O'Connor et al., 2019b; Prendergast et al., 2020). However, lacking any independent sources of information regarding the relative abundance of species within a given community, there is no way to prove which survey method samples a more accurate representation of that community. Attempting to do so would be akin to asking two people to draw a picture of the same object before comparing the two pictures to see which provides a better likeness, without using the object itself as a reference.

There are examples of researchers using "gold-standard" methodologies to try and quantify the true relative abundance of a population, so as to assess the sampling bias associated with the methods commonly used to survey that population. Both Lindenmayer et al. (1995) and Craig & Roberts (2001) used the presence of radio-tagged individuals to assess the efficacy of visual searches for estimating bird and mammal abundance. Ecoacoustic studies of both marine and terrestrial soundscape data frequently combine manual counts of animal vocalisations with counts made by automated detection tools, in order to quantify Type I and Type II detection errors (Abrahams & Denny, 2018; Ross et al., 2018; Gibb et al., 2019; Apol, Valentine, & Proppe, 2020). And Gilpin, Denham, & Ayre (2017) used in-person observations of honeybee visitations to focal plants to ground truth counts made through an analysis of static video recordings, in order to assess the efficacy of recorded video footage as a monitoring tool for pollination biologists.

However, while an observer watching a pan trapping station would be able to quantify the number and diversity of individuals that approached the bowls and later compare this to the number and diversity of individuals that were physically sampled, thus helping to quantify the proportion of insects that visited the bowls but were able to escape, or the identity of the taxa that were predominately attracted to the bowls - this kind of ground truthing wouldn't provide any data regarding how the relative abundance of species sampled by pan trapping relates to the relative abundance of species in the surrounding environment.

Mark-release-recapture (MRR) surveys **are** able to provide reliable estimates of the size of a population of a given species within a given area. At its most basic, the method involves capturing and marking a subsample of a population, and then allowing the marked individuals to mix back into the greater population. If a second sample is then captured at a later date, then the proportion of marked individuals within this sample should be the same as the proportion of marked individuals within the total population (Henderson & Southwood, 2016). There are multiple variations on the MRR methodology, depending on whether the focal populations are assumed to be **open** or **closed**; that is, whether a population should be assumed to be open to gains and/or losses through births and deaths or immigration and emigration, or not. MRR experiments have been used to successfully estimate insect population size (Budrys, Budrienė, & Pakalniskis, 2004; Franzén, Larsson, & Nilsson, 2009; Peso & Richards, 2011; Yamamoto et al., 2014), but these estimates have never before been used to ground truth the samples of other survey methods, in order to quantify potential sources of sampling bias. A MRR experiment should enable a researcher to reliably estimate the relative abundance of different insect pollinator species within a community. These data could then be compared against samples taken from this community using different survey methods, like pan trapping and standardised transect surveys, and the rank abundance of different species within these samples should illuminate which survey method (if either) more accurately represents the community as a whole (Avolio et al., 2019), thus providing evidence for any notable sampling biases associated with either method. Robust population estimates do rely on marking a large proportion of the individuals present, which may be problematic when the focus is on highly abundant and mobile insect taxa (Borchers, Buckland, &

Zucchini, 2002; Henderson & Southwood, 2016). However, these issues could be mitigated by sampling from within a closed population, like an island ecosystem (where immigration and emigration can be assumed to be minimal), in combination with an open population analysis that would allow for gains and losses to the population over time via births and deaths (Henderson & Southwood, 2016).

3.1.1 Aims

In this chapter I will use an open population mark-release-recapture experiment within a semi-closed island ecosystem to estimate the relative abundance of bee and hoverfly species within the local insect pollinator community. I will then use these population estimates to ground truth samples made by pan trapping and transect surveys, to test which survey method (if either) best represents the bee and hoverfly community present. Specifically, I will address the following question:

1. Does the rank abundance of bee and hoverfly species sampled by either pan trapping or transect surveys match the relative abundance of those species in a closed island community, as estimated via an open population mark-release-recapture analysis?

In addition, following on from a result within **Chapter 2**, I continue to explore the relationship between local floral abundance and the abundance and species richness of bees and hoverflies sampled by pan trapping and transect surveys.

3.2 Materials & methods

3.2.1 Data collection

Data collection was carried out in April 2016, on the uninhabited islet of Prassológos, approximately 1.1km off the coast of north-east Lesvos, Greece (39°16’16.75”N, 26°24’40.97”E) (Fig. 3.1). Prassológos measures approximately 1ha in area. The islet’s vegetation is dominated by Mediterranean phryganic scrubland species and is regularly grazed by a small herd of resident domestic sheep. Prassológos was chosen because its relatively small size means that it can be surveyed by a single individual within one day, while its distance from the mainland should discourage either immigration or emigration within the local insect fauna. All pollinator sampling

was carried out in appropriate weather: temperature ≥ 13°C, wind speed ≤ 3 on the Beaufort scale, and no sustained rainfall (Pollard, 1977; Pellet, 2008).

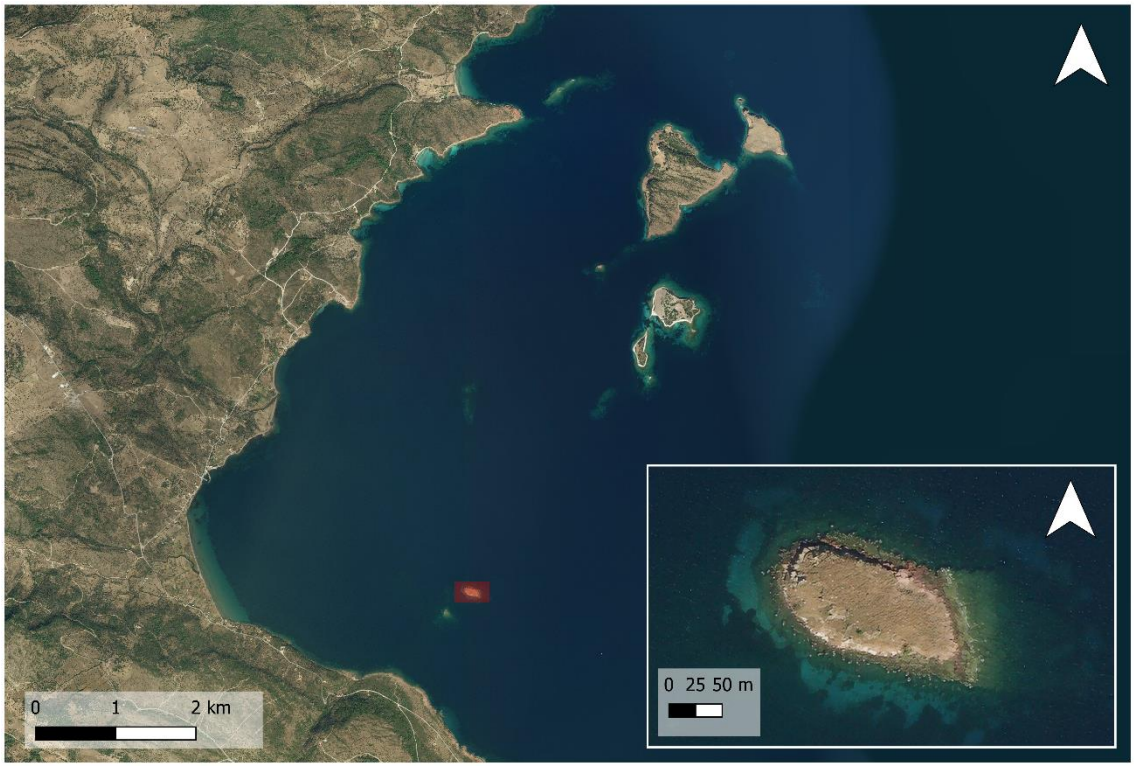

Figure 3.1 A map of Prassológos, Lesvos, Greece, the position of which is highlighted on the main map in red. Created using QGIS 3.18.2 (QGIS.org, 2021)**. Basemap: Microsoft® Bing™ Maps satellite imagery (obtained through the QuickMapServices QGIS plugin), map data © Microsoft 2021. Microsoft product screen shot(s) reprinted with permission from Microsoft Corporation.**

3.2.1.1 Mark-Release-Recapture experiment

A mark-release-recapture (MRR) survey was carried out by two surveyors over seven contiguous days between the 13th and 19th of April 2016. Each day was divided into two sampling periods, the first between 09:00 and 13:00, and the second between 14:00 and 18:00. During each sampling period, each surveyor carried out a variable transect survey. The islet was divided by a central longitudinal rocky ridge. Each surveyor started at an opposing end of the islet and, working their way clockwise around this ridge, walked a slow zigzag pattern around the islet, aiming to cover as much ground as possible. Thus, the whole island was surveyed twice per day by both surveyors, to account for any individual variation in collector experience between surveyors (thus reducing any collector bias) and to maximise potential insect encounters.

During each variable transect walk, bee (Hymenoptera: Apoidea) and hoverfly (Diptera: Syrphidae) species were captured using butterfly nets and partially anaesthetised (if necessary) using short bursts of compressed carbon dioxide (CO_2) gas released into a plastic tube adapted from a home aquarium system (see Supplementary Fig. 2.1). Insects were exposed to CO_2 for approximately 1 minute, until their movement became sluggish, at which point they were marked. Each individual was given a small dot on its wing using a coloured permanent marker pen. A different colour was used for each of the seven days during the experiment, and the position of the mark on the wing denoted the relevant sampling period: AM captures were marked at the tip of the wing, and PM captures at the base of the wing (see Fig. 3.2). Darker colours were used so as to avoid any later confusion over marks after exposure to sunlight. Previous experience, relating to unpublished research by myself, indicates that exposure to permanent marker pen ink has no noticeable effect on insect survival or behaviour. Insects were handled gently during the marking process and, afterwards, were allowed time to recover until they were able to fly of their own accord. Insects were released from the point of capture.

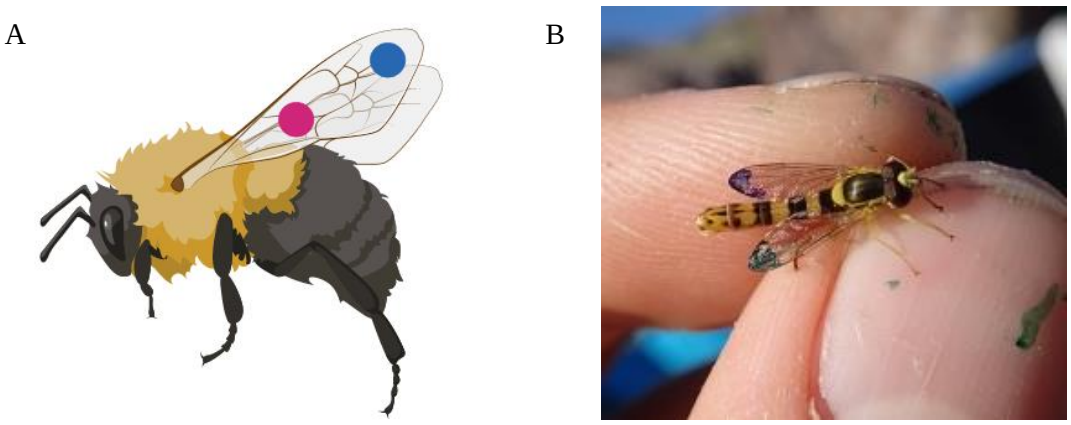

Figure 3.2 An example of the marking system used during the MRR surveys. A) The stylised bumblebee displays two marks from two separate days, with the blue mark representing a mark given during sampling period *i* in the morning, and the magenta mark representing a mark from sampling period *i*+1 during the afternoon. B) A male *Sphaerophoria scripta*, displaying marks from two separate sampling periods, both in the morning.

There is conflicting evidence regarding the effects of short-term exposure (>2 minutes) to CO_2 on insect behaviour and survival. Some authors suggest that the effects are minimal within some insect taxa, providing adequate time was given for the insect to recover (Colinet & Renault, 2012),

while others note that even short periods of CO_2 exposure (<1 minute) can have harmful sub-lethal physiological and behavioural effects in species like *Apis mellifera* (Ribbands, 1950; Nicolas, 1989). In this experiment, only the larger, stinging insects (bumblebees, honeybees, and larger solitary bee species) regularly required anaesthesia in order to mark them. The smaller solitary bee and hoverfly species that formed the majority of individuals encountered on Prassológos could be reliably marked without any exposure to CO_2.

Each sampling period during a given day was considered distinct, i.e., any insect marked on the morning of one day could be re-captured and re-marked on the afternoon of that same day. This maximised the chances of generating accurate population estimates for as many insect species as possible over a short period of time. I judged that the hour-long period in between the morning and afternoon sampling periods gave all marked insects adequate time to mix back into the general population.

Species identification in the field was carried out with help from Dr Jelle Devalez from the University of the Aegean, Lesvos.

3.2.1.2 Pan trapping

Pan trapping was carried out during a four-day period between the 20th and 23rd of April, immediately after the MRR experiment. By doing so I aimed to ensure that the relative abundances of bee and hoverfly species sampled by the pan traps were as representative of the final estimates of population size (provided by the MRR surveys) as possible.

Ten pan trapping stations were set up in fixed locations. Each station was set out between 08:00 and 09:00 each morning and consisted of three 284 ml bowls attached to a wooden stake at approximately the same height as the surrounding flowering vegetation. Three differently coloured bowls were used within each station: UV-fluorescent yellow, blue, and white. Research suggests that these colours are complementary in their ability to sample bee and hoverfly species (Vrdoljak & Samways, 2011; Heneberg & Bogusch, 2014; Moreira et al., 2016), and that UV-fluorescent paint increases the attraction of pan traps to foraging insects (Westphal et al., 2008). Each pan trapping station was emptied between 17:00 and 18:00 in the order in which they were

set out. Within each trapping station, the contents of each differently coloured bowl were strained through a separate square of muslin before being placed together inside a 100ml falcon tube with approximately 50ml of 70% ethanol to act as a preservative.

3.2.1.3 Standardised transect surveys

Ten standardised transects were marked out across the islet. Each transect was 10m long and fixed in a single location – close to one of the fixed pan trapping stations, but more than 10m away in order to prevent competition between the two methods for insects (Droege et al., 2010). Each transect was walked once per day, between 09:00 and 19:00, with one transect completed per hour during this period. The sampling order of the transect surveys was the same each day, starting with transect one. Each transect was surveyed in a similar fashion to a Pollard transect (Pollard, 1977): where the surveyor sampled any insects, either in-flight or visiting flowers, present within 1m of either side of the central transect line, up to 1m in front of themselves, and up to 2m from the ground, using a butterfly net. Approximately one minute was spent in each meter-long section of each transect, to account for any variation in individual's walking speeds, which was timed with a stopwatch. If an insect was caught, the stopwatch was paused and then restarted once the capture was complete. Insects caught during a transect walk were placed directly into vials of 70% ethanol to kill and preserve them.

Four days of standardised transect surveys were originally planned, to coincide with the dates of the pan trapping survey. Unfortunately, shortly after placing the pan traps on the 20th of April, we experienced unexpected high wind speeds in excess of 20mph on and around Prassológos. These continued until late in the afternoon on the 21st and inhibited our ability to catch insects with nets. We also observed very few insects flying during this weather. Standardised transect surveys were, therefore, only carried out between the 22nd and 23rd of April.

3.2.1.4 Floral abundance counts

Additional data were collected regarding the floral abundance within a 2m radius of each of the pan trapping stations and along each of the standardised transects (within 1m of either side of the central line). In a similar fashion to **Chapter 2**, each flower was counted as a single floral unit. Where a plant exhibited multiple florets per flower head, i.e., the capitula evolved by members of

the Asteraceae, each flower head was counted as a single floral unit (Carvell et al., 2007). Three representative flower heads were then taken from each plant species and the number of individual florets counted and averaged to provide a mean number of florets per floral unit. This value was then multiplied across the total number of floral units for a given species found around each pan trapping station or along each standardised transect. For further information concerning how each floral species was classified in terms of what constituted a floral unit, together with the average number of florets per floral unit, see Supplementary Table 2.1. For a breakdown of the relative abundance of floral species within a two-meter radius of each pan trapping station and along each transect, see Supplementary Tables 2.2 and 2.3.

I did not follow the method from Chapter 2 and transform these measures of floral abundance into volumes of nectar sugar produced per 24-hours, as most of the plant species present on the island could not be readily identified to species. In addition, being a Mediterranean phryganic habitat, it may be that the plant species present on Prassológos were not represented in the list of plant species from Baude, Kunin, & Memmott (2015) from which the volumes of nectar sugar produced per day per floret used in **Chapter 2** were taken.

3.2.2 Data analysis

All data analyses were carried out using R, version 3.5.3 (R Core Team, 2019).

3.2.2.1 Mark-release-recapture analysis

The MRR data analyses assumed an open population, in that each bee and hoverfly population present were open to losses, via death or permanent emigration, and gains, via births or immigration, over the course of the experiment (Henderson & Southwood, 2016). I used the superpopulation variant of the Jolly-Seber open-population model to generate population estimates for each bee and hoverfly species captured during this experiment, the background to which is laid out below.

3.2.2.2 The Jolly-Seber model

The Jolly-Seber model (Jolly, 1965; Seber, 1965) is a stochastic open-population model that allows for the estimation of parameters relating to survival (φ_i), capture (p_i), and population size

(N_i), from individual capture histories, based upon maximum likelihood (Pollock & Alpizar-Jara, 2005). Each individual captured during a Jolly-Seber experiment has a unique capture history, e.g., 00101011010000, where a 1 indicates a sampling period in which the individual was captured and marked, and a 0 indicates a sampling period in which it was not captured.

The Jolly-Seber model uses a multinomial maximum likelihood function that is divided into three components (Schwarz & Arnason, 1996; Pollock & Alpizar-Jara, 2005):

$$L = L_1 \times L_2 \times L_3$$

L_1 is a binomial function that estimates the population size for unmarked individuals at sampling period *i*; L_2 is a binomial function that models the probability of losing an individual upon capture; and L_3 is a multinomial function that models the probability of capture (p_i) and survival (φ_i) for marked individuals, often referred to as the Cormack-Jolly-Seber likelihood function (see Cormack, 1964). The product of these three components provides the overall likelihood (L) (Pollock & Alpizar-Jara, 2005). This likelihood function can then be used to estimate the population size for a given species using the following set of equations, adapted from Nichols (2005).

If the total number of individuals caught during sampling period *i* (n_i) is equal to the number of unmarked individuals caught during that period (u_i) plus the number of marked individuals caught during that period (m_i), then:

$$\widehat{N}_i = \frac{n_i}{\hat{p}_i}$$

Where $\widehat{N}_i$ equals the estimated total number of individuals in the population present during sampling at period *i*, and $\hat{p}_i$ is the estimated probability of capture during sampling period *i*. We can then replace $\hat{p}_i$ with its own estimator:

$$\hat{p}_i = \frac{m_i}{\widehat{M}_i}$$

Where $\widehat{M}_i$ equals the estimated number of marked individuals present in the population immediately prior to sampling period *i*.

This provides the true estimate for the total population size present during sampling at period *i*:

$$\widehat{N}_i = \frac{n_i \widehat{M}_i}{m_i}$$

3.2.2.3 The superpopulation approach

A more recent variant of the Jolly-Seber model was introduced by Schwarz & Arnason (1996) (see also Crosbie & Manly, 1985). It posits the idea of a superpopulation (*N*), representing all of the individuals (seen and unseen) that will enter the sampling area during the course of the experiment (Schwarz & Arnason, 1996; Nichols, 2005), where the probability of an individual entering the population from this superpopulation between sampling periods *i* and *i*+1 can be referred to as b_i. In doing so, this approach models new entrants to the population (either by birth or immigration, the model does not differentiate between these two causes) more effectively than the original Jolly-Seber model (Schwarz & Arnason, 2018), providing potentially more reliable population estimates.

In this experiment, the estimated superpopulation ($\widehat{N}$) would represent all of the insects of a given bee or hoverfly species that entered Prassológos over the course of the MRR experiment, while individual population estimates ($\widehat{N}_i$) would represent the number of insects of a given bee or hoverfly species that were active during sampling period *i*. In this case, "active" would denote individuals that were flying, foraging, mating, or involved in any other behaviour that would bring an individual insect into contact with one of the surveyors.

The multinomial likelihood function used by the superpopulation approach is similar to that used by the original Jolly-Seber model, but it replaces the previous component L_1 (originally a binomial function that estimated the population size for unmarked individuals) with a new component describing the probability of initial capture for unmarked individuals using a binomial distribution (Schwarz & Arnason, 1996). Then, as before, the full likelihood (*L*) is the product of all three likelihood components:

$$L = L_1 \times L_2 \times L_3$$

Subsequently, the estimated population size during each sampling period (N_i) can be calculated iteratively, as in Schwarz & Arnason (2018):

$$N_1 = \hat{B}_0$$

$$N_2 = N_1\varphi_1 + \hat{B}_1$$

$$N_3 = N_2\varphi_2 + \hat{B}_2$$

$$\vdots$$

Where $\hat{B}_i$ is the estimated number of entrants to the population directly prior to N_i, and φ_i is the estimated probability of survival from sampling period *i* to period *i*+1.

3.2.2.4 Model assumptions

The following set of assumptions relating to data collection (i.e. how unmarked individuals are caught, marked, and then released) are vital for the accurate estimation of population size by the superpopulation variation on the Jolly-Seber model (Nichols, 2005; Pollock & Alpizar-Jara, 2005; Henderson & Southwood, 2016):

1. All marks on pollinators are read correctly.
2. All marked pollinators retain their marks for the entirety of the experiment.
3. The probability of survival (φ_i) between sampling occasion *i* and *i* +1 is equal for all pollinators, whether marked or unmarked.
4. The probability of capture (p_i) at sampling occasion *i* is equal for all living pollinators within the population, whether marked or unmarked.
5. Sampling is conducted instantaneously.
6. All emigration from the sampled population is permanent.
7. The size of the study area remains constant (if the study area were to change with time, this might affect size of the population present).

Of these, assumptions 3 & 4 are considered the most important, as violations of these assumptions can lead to over- or underestimates of population size ($\widehat{N}_i$) (Jolly, 1965; Seber, 1965; Pollock & Alpizar-Jara, 2005; Henderson & Southwood, 2016).

3.2.2.5 Estimating population size

Population sizes were estimated using the R package RMark (Laake, 2013): an interface between R and the free MRR analysis software MARK (White & Burnham, 1999).

I fitted eight models for each bee or hoverfly species that presented with recapture events, using the *mark.wrapper* function in RMark (see Table 3.1). Each of these models was based on variation in a combination of four parameters: the probabilities of survival (φ_i), capture (p_i), and entry to the population (b_i), along with the size of the superpopulation (N). In each model, φ_i, p_i, and b_i, were either constrained to be constant over time ($\sim \cdot$) or allowed to vary with time ($\sim t$). The estimated size of the superpopulation ($\widehat{N}$) was constrained to remain constant with time due to the relatively short length of the MRR experiment. Model selection was carried out using corrected Akaike's Information Criterion (AIC_C) scores[7], together with their associated ΔAIC_C scores and Akaike weights, where the model with the lowest AIC_C score, and therefore the highest Akaike weight, was assumed to fit the data best.

Table 3.1 The eight models fitted for each species that presented with recapture events.

Model	Explanation
$\varphi_i \sim \cdot, p_i \sim \cdot, b_i \sim \cdot, N \sim \cdot$	All parameters remain constant with time
$\varphi_i \sim t, p_i \sim \cdot, b_i \sim \cdot, N \sim \cdot$	Probability of survival is allowed to vary with time
$\varphi_i \sim \cdot, p_i \sim t, b_i \sim \cdot, N \sim \cdot$	Probability of capture is allowed to vary with time
$\varphi_i \sim \cdot, p_i \sim \cdot, b_i \sim t, N \sim \cdot$	Probability of entering the population is allowed to vary with time
$\varphi_i \sim t, p_i \sim t, b_i \sim \cdot, N \sim \cdot$	Probabilities of survival and capture are allowed to vary with time
$\varphi_i \sim t, p_i \sim \cdot, b_i \sim t, N \sim \cdot$	Probabilities of survival and entry are allowed to vary with time
$\varphi_i \sim \cdot, p_i \sim t, b_i \sim t, N \sim \cdot$	Probabilities of capture and entry are allowed to vary with time
$\varphi_i \sim t, p_i \sim t, b_i \sim t, N \sim \cdot$	All parameters are allowed to vary with time

3.2.2.6 Goodness of fit

As briefly noted earlier, violations of the assumptions of the superpopulation approach, particularly those regarding equal probability of survival and equal probability of capture, may result in over- or underestimates of population size. The ability of our data to meet these two

[7] Computed to account for smaller sample sizes.

primary assumptions can be assessed using the *release.gof* function in RMark. This function calculates two tests: TEST 2 and TEST 3. TEST 2 investigates heterogeneity in the probability of capture (p_i) by assessing whether the likelihood of catching an insect is dependent on when it was first caught (Cooch & White, 2019), while TEST 3 tests for heterogeneity in the probability of survival between sampling period i and i+1 (φ_i) (Cooch & White, 2019). The results from both tests are calculated using contingency tables and yield a chi-square value, degrees of freedom, and a p value; if these p values are non-significant (i.e. $p > 0.05$) then the associated probability of survival or capture can be assumed to be homogeneous. The sum of the two chi-square values divided by the summed degrees of freedom also provides a measure for overdispersion within the dataset (Cooch & White, 2019).

3.2.2.7 Comparing the rank abundance of bee and hoverfly species sampled by pan trapping and standardised transect surveys to the relative abundance estimates made via the MRR experiment

The population estimates for each bee and hoverfly species recorded during the final sampling period ($\widehat{N}_{14}$) were used as expected counts within a Pearson's chi-square test, to test whether there were significant differences between the estimated population size of each species and the relative abundance of each species as sampled by either pan trapping or the standardised transect surveys. Considering that we only completed two of the planned four days of transect surveys, I used both the total abundance of each species sampled by the pan traps and the abundance sampled only over the two-day period during which standardised transect surveys were possible as observed counts within the Pearson's chi-square.

Rank abundance curves were created to visualise the estimated rank abundance of each bee and hoverfly species during the final sampling period ($\widehat{N}_{14}$), as well as the rank abundance of the bee and hoverfly species sampled by pan trapping and by standardised transect surveys. The rank abundance curves were generated using the *geom_point*, *geom_line*, and *geom-text* functions in the ggplot2 package (Wickham, 2009).

Ternary plots, generated using the *ggtern* function in package ggtern (Hamilton & Ferry, 2018), were also used to visualise the proportional composition of the raw samples generated by the

variable transects surveyed as part of the MRR experiment, by pan trapping, and by standardised transect surveys.

3.2.2.8 Correlating bee and hoverfly abundance and species richness sampled by pan trapping and standardised transect surveys with local floral abundance

To test for a relationship between total bee and hoverfly abundance and species richness, as sampled by pan trapping and by standardised transect surveys, and local floral abundance as measured in section 3.2.1.4, both Pearson's (parametric) and Spearman's rank (nonparametric) correlations were applied. Data normality was assessed using the *shapiro.test* function.

3.3 Results

3.3.1 Data collection

Over the course of the MRR surveys we marked 479 insects, representing seven bee species and eight hoverfly species (Table 3.2). Only six species presented with recapture events during the experiment: *Bombus terrestris*, *Halictus phryganicus*, *Episyrphus balteatus*, *Eupeodes corollae*, *Sphaerophoria scripta*, and *Xylocopa violacea*. Of these six species, only four were also sampled by both pan trapping and the standardised transect surveys: *B. terrestris*, *H. phryganicus*, *E. corollae*, and *S. scripta* (see Table 3.6). Therefore, these were the only species for which population estimates were generated and analysed.

3.3.2 Mark-release-recapture analysis

3.3.2.1 Goodness of fit

The population estimation model that provided the best fit for each species is described in Table 3.3. In all cases the Akaike weight for the top model was more than 0.99 (see Supplementary Table 2.4), negating the need for any model averaging.

Goodness-of-fit tests indicate that there was no heterogeneity present in terms of either the probability of capture or the probability of survival for *B. terrestris* (TEST 2: $\chi^2_{(3)} = 2.35$, $p = 0.50$; TEST 3: $\chi^2_{(6)} = 3.90$, $p = 0.69$) or for *S. scripta* (TEST 2: $\chi^2_{(13)} = 10.98$, $p = <0.61$; TEST 3:

$\chi^2_{(17)}$ = 6.92, p = <0.99). No overdispersion was present in the data for either species, although a degree of underdispersion was present in both.

Table 3.2 The abundance of the different insect pollinator species caught during the MRR surveys between 13/04/2016 and 19/04/2016 , together with the number of recapture events for each species (the number of individuals captured once, twice, and three times).

				No. individuals recaptured		
Order	Family	Species	No. marked	1	2	3
Hymenoptera	Andrenidae	*Andrena hesperia*	1	0	0	0
	Apidae	*Apis mellifera*	6	0	0	0
		Bombus terrestris	38	3	2	3
		Xylocopa violacea	13	1	0	0
	Halictidae	*Halictus phryganicus*	32	1	0	0
		Lasioglossum malachurum	1	0	0	0
		Lasioglossum nitidulum	2	0	0	0
Diptera	Syrphidae	*Episyrphus balteatus*	22	1	0	0
		Eristalinus aeneus	5	0	0	0
		Eristalis tenax	29	0	0	0
		Eupeodes corollae	125	19	4	0
		Melanostoma mellinum	12	0	0	0
		Paragus quadrifasciatus	3	0	0	0
		Sphaerophoria scripta	185	42	7	1
		Scaeva pyrastri	5	0	0	0

Table 3.3 The best population estimation model for each pollinator species, as chosen via AIC_C values.

Order	Family	Species	Best model
Hymenoptera	Apidae	*Bombus terrestris*	$\varphi_i \sim \cdot, p_i \sim \cdot, b_i \sim \cdot, N \sim \cdot$
	Halictidae	*Halictus phryganicus*	$\varphi_i \sim \cdot, p_i \sim \cdot, b_i \sim \cdot, N \sim \cdot$ (constrained)
Diptera	Syrphidae	*Eupeodes corollae*	$\varphi_i \sim \cdot, p_i \sim t, b_i \sim \cdot, N \sim \cdot$
		Sphaerophoria scripta	$\varphi_i \sim \cdot, p_i \sim t, b_i \sim \cdot, N \sim \cdot$

Results for TEST 2 and TEST 3 were not estimable for *E. corollae* or *H. phryganicus*. This is likely due to relatively sparse recapture data for both species, leading to the contingency tables for these two tests presenting with observed frequencies of 0 in one or more cells or expected

frequencies of less than 2. I therefore cannot comment on the validity of the assumptions concerning equal probability or survival or capture within these two species, nor can I quantify over- or underdispersion within the data for either species. As a result, I present the parameter estimates for both *E. corollae* and *S. scripta* "as is", but with the joint caveats that I cannot necessarily assume that the probabilities of survival or capture are equal for all individuals from these species between periods *i* and *i*+1 (which may result in over- or underestimates of population size), and that over- or underdispersion may be present within the data.

3.3.2.2 Estimating population size

Population estimates indicate that there were 15 (95% CI [8, 26]) *B. terrestris* individuals active on Prassológos during the final sampling period (Table 3.4; for all population estimates see Supplementary Table 2.5), and that 75 (95% CI [55, 124]) individuals were present on the islet over the course of the MRR experiment (Table 3.5). This may be indicative of a small local colony on Prassológos, or of a subset of workers from a colony or series of colonies on mainland Lesvos repeatedly visiting the islet as a reliable source of forage.

Population estimates for *S. scripta* indicate that 69 (95% CI [44, 111]) individuals were active on Prassológos during the final sampling period (Table 3.4; for all population estimates see Supplementary Table 2.6), and that 659 (95% CI [489, 925]) individuals were present on the islet over the course of the MRR experiment (Table 3.5). These are large population estimates for an islet not much more than a hectare in area. The top model for this species allowed for p_i to vary with time, although this parameter remains relatively low for most of the experiment, only rising above 0.20 during one sampling period (see Fig. 3.3A). These results may be indicative of a large local population of *S. scripta* on the islet, or that regular migration into and out of the population was taking place during the experimental period (or possibly both).

Population estimates for *E. corollae* indicate that 158 (95% CI [70, 358]) individuals were active on Prassológos during the final sampling period (Table 3.4; for all population estimates see Supplementary Table 2.7), and that 526 (95% CI [362, 805]) individuals were present on the islet during the MRR experiment (Table 3.5). As with *S. scripta*, the top model for this species also allowed for p_i to vary with time, showing a decrease over the course of the experiment (Fig. 3.3B).

Again, this may suggest either a large local population exists, or that regular temporary migration in and out of the population was occurring. However, since neither of the goodness-of-fit tests were estimable for *E. corollae*, it is also possible that the model overestimated both *N* and $\widehat{N}_{14}$ due to a violation of the assumptions concerning either p_i or φ_i.

Population estimates for *H. phryganicus* indicate that 429 (95% CI [79, 2315]) individuals were active on Prassológos during the final sampling period (Table 3.4; for all population estimates see Supplementary Tables 2.8 & 2.9), and that 500 (95% CI [118, 2580]) individuals were present on the islet over the course of the MRR experiment (Table 3.5). The population estimates for this species were evidently affected by the low recapture rate, and the large confidence intervals indicate that estimates for both *N* and $\widehat{N}_{14}$ are not reliable. Since neither goodness-of-fit test was estimable, this model is almost certainly overestimating both *N* and $\widehat{N}_{14}$, again due to a violation of the assumptions concerning either p_i or φ_i.

Table 3.4 The estimated population size present on the final sampling period of the experiment ($\widehat{N}_{14}$), together with 95% confidence intervals.

Order	Family	Species	$\widehat{N}_{14}$	95% CI
Hymenoptera	Apidae	*Bombus terrestris*	15.22	[8.87, 26.11]
	Halictidae	*Halictus phryganicus*	428.91	[79.48, 2314.66]
Diptera	Syrphidae	*Eupeodes corollae*	158.30	[69.98, 358.08]
		Sphaerophoria scripta	69.45	[43.61, 110.60]

Table 3.5 The estimated size of the superpopulation present during the course of the experiment ($\widehat{N}$), together with 95% confidence intervals.

Order	Family	Species	$\widehat{N}$	95% CI
Hymenoptera	Apidae	*Bombus terrestris*	77.08	[55.68, 124.36]
	Halictidae	*Halictus phryganicus*	499.90	[117.92, 2579.98]
Diptera	Syrphidae	*Eupeodes corollae*	525.99	[361.50, 804.88]
		Sphaerophoria scripta	659.23	[488.87, 925.10]

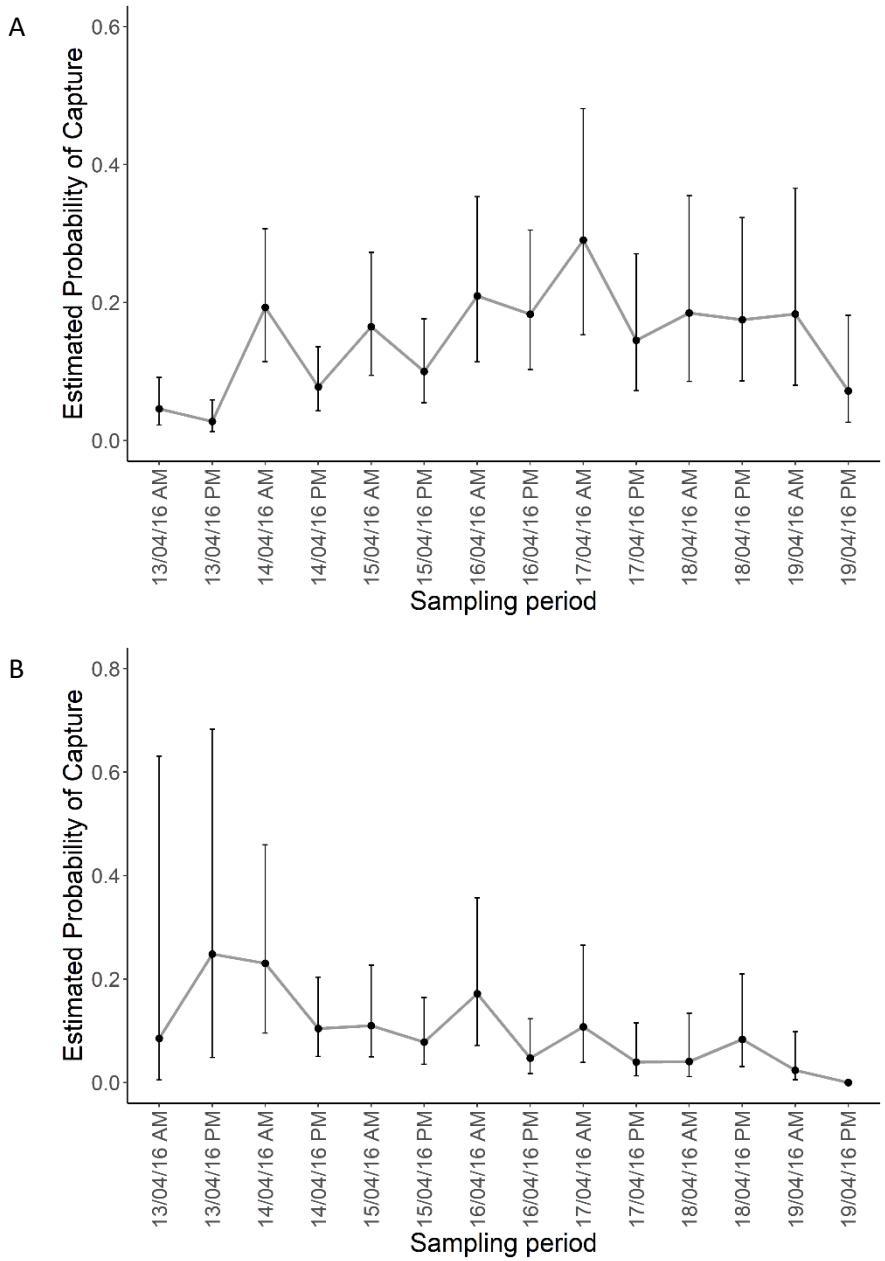

Figure 3.3 The estimated probability of capture during each sampling period ($\widehat{p}_i$) ± 95% confidence intervals for A) *Sphaerophoria scripta,* and B) *Eupeodes corollae*.

3.3.2.3 Comparing the rank abundance of bee and hoverfly species sampled by pan trapping and standardised transect surveys to the relative abundance estimates generated by the MRR experiment

Together both pan trapping and standardised transect surveys sampled fourteen species of bee and hoverfly, seven of which were also sampled during the MRR experiment (Table 3.6). Of these two methods, pan trapping sampled the most species (n = 11 species) and the greatest overall abundance of bees and hoverflies. However, if we limit the pan trap samples to those collected over the two days where both pan trapping and standardised transects were carried out in tandem,

then both survey methods sampled the same species richness (n = 5 species), although pan trapping still sampled the greater overall abundance.

One of the advantages of transect-based survey methods is that the relationships between insect pollinators and their preferred forage plants can be observed. Of the seven insects sampled during the standardised transect surveys (Table 3.6), four of the hoverflies were sampled mid-flight: *S. scripta* (n = 2), *E. tenax* (n = 1), and *Chrysotoxum* sp. (n = 1). Of the remaining three individuals, a single unknown solitary bee and one *E. corollae* individual were sampled whilst foraging on *Cirsium* sp., while the remaining *E. corollae* individual was sampled whilst foraging on *Crepis* sp.

Table 3.6 Abundance of insect pollinator species sampled by pan trapping and standardised transect surveys.

				Pan traps	
Order	Family	Species	Pan traps	22– 23 April[†]	Transect surveys
Hymenoptera	~	Unknown solitary bee sp.	0	0	1
	Apidae	*Anthophora plumipes*	1	0	0
		Apis mellifera	1	0	0
		Bombus terrestris	1	0	0
		Ceratina parvula	5	5	0
		Eucera sp.	1	0	0
	Halictidae	*Halictus* sp.	1	0	0
		Halictus phryganicus	7	5	0
		Lasioglossum nitidulum	10	6	0
		Lasioglossum leucozonium	2	2	0
Diptera	Syrphidae	*Chrysotoxum* sp.	0	0	1
		Eristalis tenax	1	0	1
		Eupeodes corollae	3	1	2
		Sphaerophoria scripta	0	0	2

[†]The dates that coincide with the standardised transect surveys.

The population estimates for *B. terrestris*, *H. phryganicus*, *E. corollae*, and *S. scripta* together provide a picture of the relative abundance of these four bee and hoverfly species within the wider insect community present on Prassológos on the final day of the MRR experiment ($\widehat{N}_{14}$). The results of the Pearson's chi-square test show that there were significant differences between these expected values and the counts observed within the samples collected by both pan trapping and the standardised transect surveys, with significantly fewer individuals sampled by both survey methods than were estimated to be active by the MRR method (Table 3.7). This result remained consistent when the relative abundance of each of the four individual species was compared to the counts observed within the samples collected by both pan trapping and the standardised transect surveys (Table 3.7). The results concerning pan trapping also remained consistent regardless of whether the analysis used counts collected over the full four-day sampling period, or over the two days where both pan traps and transect surveys were deployed in concert (p = <0.001 for all species).

Table 3.7 Results of Chi-square tests comparing the samples from pan trapping and standardised transect walks to population estimates generated by mark-release-recapture analysis.

		Pan trapping			Transect walks		
Family	Species	χ^2	DF	p	χ^2	DF	p
All species		650.12	3	<0.001	663.96	3	<0.001
Apidae	*Bombus terrestris*	13.29	1	<0.001	15.22	1	<0.001
Halictidae	*Halictus phryganicus*	514.02	1	<0.001	428.91	1	<0.001
Syrphidae	*Eupeodes corollae*	152.36	1	<0.001	154.33	1	<0.001
	Sphaerophoria scripta	69.45	1	<0.001	65.51	1	<0.001

In terms of the rank abundance of these four species, a combination of both pan trapping and standardised transect surveys, when deployed in concert, provided samples that were the closest match to the rank abundance of the community estimated on the final day of the MRR experiment ($\widehat{N}_{14}$) (Fig. 3.4A & E). However, together the samples from both survey methods still

underestimated the relative abundance of *H. phryganicus* and *B. terrestris* (not a single *B. terrestris* individual was captured) and overestimated the relative abundance of *S. scripta*.

Over the full four-day sampling period, pan trapping provided the closest match to the estimated rank abundance of *H. phryganicus* and *E. corollae* (Fig. 3.4A & B). Pan trapping overestimated the relative abundance *B. terrestris* and underestimated the relative abundance of *S. scripta* (not a single individual was captured), swapping the ranks of these two species when compared to their rank abundances, as estimated by the MRR experiment. If we constrain the pan trap samples to those collected when pan trapping was deployed in concert with standardised transect surveys), the rank of both *H. phryganicus* and *E. corollae* is the same, but the relative abundance of the former is overestimated and that of the latter is underestimated (Fig. 3.4A & C). The relative abundances of both *B. terrestris* and *S. scripta* were incalculable, as not a single individual of either species was captured during this period.

The standardised transect surveys collected samples that were the least similar, in terms of rank abundance, to the community estimated on the final day of the MRR experiment (Fig. 3.4A & D), massively overestimating the relative abundance of both *E. corollae* and *S. scripta*. Meanwhile, the relative abundances of *H. phryganicus* and *B. terrestris* were incalculable, as not a single individual of either species was captured.

The ternary plots also indicate some interesting trends in the raw abundance data collected by each method. In terms of relative abundance, Figure 3.5 indicates that that the samples collected via pan trapping contained a higher proportion of solitary bees (84.4%) than the standardised transect surveys (14.3%), or the variable transect surveys carried out as part of the MRR experiment (10.4%). Conversely, the proportion of hoverflies was greater in the samples collected via the variable (81.6%) and standardised transect surveys (85.7%) than in those collected via pan trapping (12.5%). The combined samples of both pan trapping and standardised transect surveys, when deployed in tandem, were more similar to those collected by pan trapping than those collected by either transect method, with samples dominated by a high proportion of solitary bees (73.1%). In terms of relative abundance, neither bumblebees nor honeybees were prevalent in the samples collected using any of these three sampling methods (Figure 3.6).

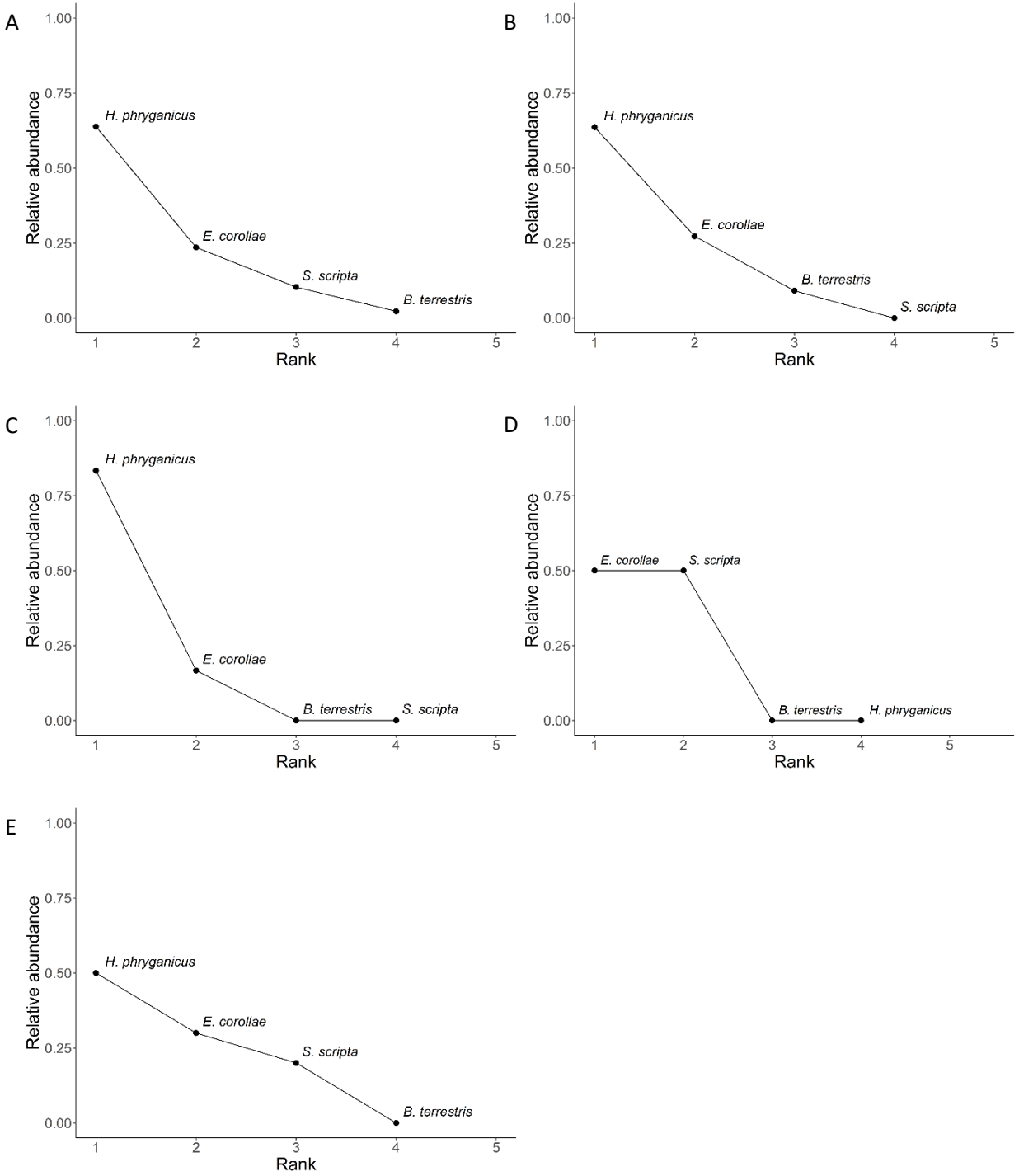

Figure 3.4 Rank abundance curves showing the relative abundance of *Bombus terrestris*, *Halictus phryganicus*, *Eupeodes corollae*, and *Sphaerophoria scripta* against their rank within the community for: A) the Jolly-Seber population estimates for the final day of the MRR experiment ($\widehat{N}_{14}$); B) the samples collected via pan trapping across the full four-day sampling period; C) the samples collected via pan trapping between the 22nd and 23rd April; D) samples collected via standardised transect surveys between the 22nd and 23rd April; and E) the combined samples collected by both pan trapping and standardised transect surveys between the 22nd and 23rd April.

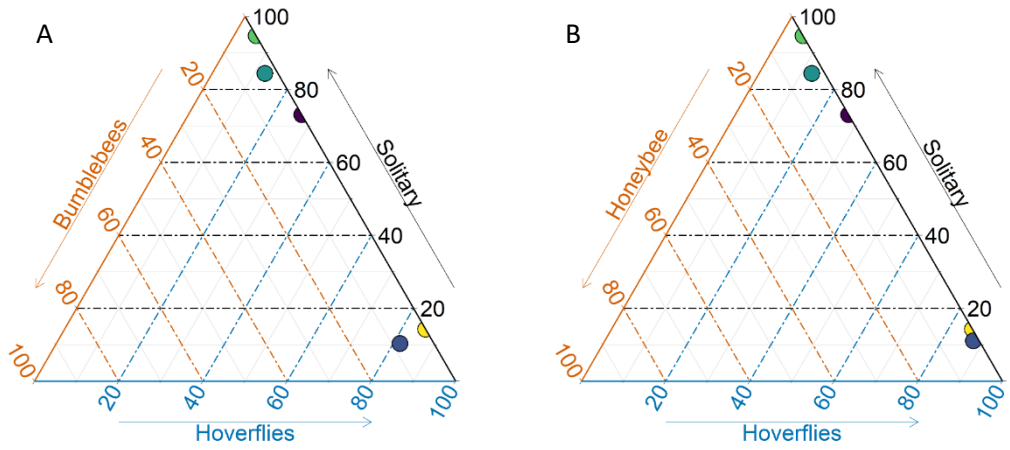

Figure 3.5 Ternary plots showing the proportional composition of samples generated by each different survey method (blue: MRR variable transect surveys; yellow: standardised transect surveys; cyan: pan trapping; green: pan trapping between the 22nd-23rd; and purple: the combined samples of pan trapping and standardised transect surveys between the 22nd-23rd) in terms of the abundance of: A) Bumblebees (orange), solitary bees (black), and hoverflies (blue); and B) Honeybees (orange), solitary bees (black), and hoverflies (blue).

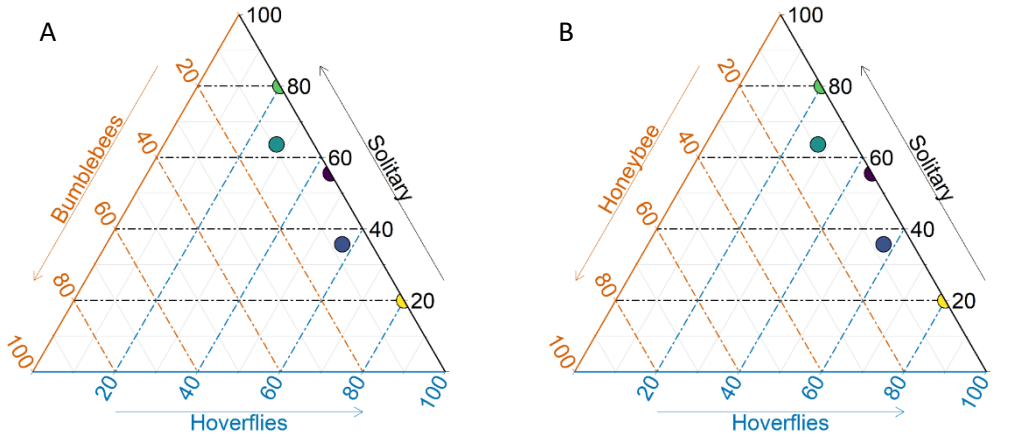

Figure 3.6 Ternary plots showing the proportional composition of samples generated by each different survey method (blue: MRR variable transect surveys; yellow: standardised transect surveys; cyan: pan trapping; green: pan trapping between the 22nd-23rd; and purple: the combined samples of pan trapping and standardised transect surveys between the 22nd-23rd) in terms of the species richness of: A) Bumblebees (orange), solitary bees (black), and hoverflies (blue); and B) Honeybees (orange), solitary bees (black), and hoverflies (blue).

These trends were mirrored in the species richness data, but to a lesser degree (Figure 3.6). Samples collected via pan trapping still contained a greater proportion of solitary bee species (63.6%) than either the variable (35.7%) or standardised transect surveys (20.0%), while both variable and standardised transect surveys sampled a greater proportion of hoverfly species

(57.1% and 80.0%, respectively) than pan trapping (27.3%). A combination of the samples collected by tandem pan trapping and standardised transect surveys, sampled solitary bee and hoverfly species in proportions that were much closer to one-another.

3.3.2.4 Correlating bee and hoverfly abundance and species richness sampled by pan trapping and standardised transect surveys with local floral abundance

There were no significant correlations between the total abundance ($r_{(8)} = 0.09$, $p = 0.798$) or species richness ($r_{(8)} = 0.11$, $p = 0.754$) of bees and hoverflies sampled by pan trapping and the floral abundance present within a 2m radius of each of the pan trapping stations. This remained consistent when only samples collected by both pan trapping and transect surveys, when deployed in concert, were considered: abundance ($r_{s(8)} = 0.18$ $p = 0.630$) and species richness ($r_{(8)} = 0.06$, $p = 0.872$). Similarly, there was no significant correlation between the total abundance ($r_{s(8)} = 0.04$, $p = 0.869$) or species richness ($r_{s(8)} = 0.04$, $p = 0.869$) of bees and hoverflies sampled by the standardised transect surveys and the floral abundance present along the standardised transects.

3.4 Discussion

In light of recent evidence concerning insect pollinator declines (Biesmeijer et al., 2006; Ollerton et al., 2014; Powney et al., 2019), the design of standardised monitoring protocols is key to being able to accurately and reliably monitor changing population trends (Potts et al., 2016; Powney et al., 2019). One aspect of this process that has been investigated extensively by previous studies is the quantification of sampling bias within different survey methods, in particular pan trapping and transect surveys (Cane, Minckley, & Kervin, 2000; Roulston, Smith, & Brewster, 2007; Westphal et al., 2008; Wilson, Griswold, & Messinger, 2008; Grundel et al., 2011; Nielsen et al., 2011; Popic, Davila, & Wardle, 2013; Prendergast et al., 2020). Most previous research has focused on direct comparisons of the abundance or species richness sampled by these methods, in order to assess which method best represents insect pollinator communities (albeit usually whilst focusing solely on bees). As of yet, there has been no attempt to ground truth these findings with data concerning the "true" relative abundance of pollinator species within a given community. The aim of this Chapter was to use a mark release recapture (MRR) experiment to

estimate the relative abundance of bee and hoverfly species within a semi-closed island community in Greece. I then compare these population estimates to samples collected via pan trapping and standardised transect surveys to see whether the rank abundance of bee and hoverfly species within these samples matched the estimated rank abundance of species within the island community.

Of the fifteen bee and hoverfly species surveyed during the MRR experiment (and twenty-one bee and hoverfly species surveyed across Prassológos), only six species had any associated recapture events: *Bombus terrestris*, *Halictus phryganicus*, *Episyrphus balteatus*, *Eupeodes corollae*, *Sphaerophoria scripta*, and *Xylocopa violacea*. Even within a small, semi-closed island ecosystem, the MRR protocol used in this Chapter would not have been capable of providing estimates of relative abundance for more than a third of the taxa surveyed. I was primarily unable to provide population estimates for smaller solitary bee or hoverfly species (*A. hesperia*, *L. malachurum*, *L. nitidulum*, and *P. quadrifasciatus*) or larger species that would potentially have been capable of migrating to and from the mainland (*A. mellifera*, *A. plumipes*, *E. aeneus*, *S. pyrastri*, and *X. violacea*); many of these species were also surveyed in relatively small numbers, which may, in the larger species, be indicative of transitory populations. The variable transect surveys that formed the basis of the MRR experiment were open to collector bias (Westphal et al., 2008; Nielsen et al., 2011), and although both surveyors had extensive experience in terms of hand netting and transect surveys, neither had prior experience with the bee and hoverfly fauna of Prassológos prior to beginning the MRR surveys. This may explain why several smaller solitary bee species, e.g., *L. leucozonium* and *C. parvula*, were not sampled by the variable transect surveys, despite the presence of both species on the island being confirmed during the pan trapping surveys (see Nielsen et al., 2011). This may also be evidence that transect-based surveys do underestimate the relative abundance of smaller insect species (Potts, Evan, & Boone, 2005; Rhoades et al., 2017), but since providing evidence for the presence of these sampling biases is the main point of this Chapter (and the use of the MRR protocol itself), I will not hypothesise further about their presence without available data.

However, despite only recapturing individuals for six species, we were still able to sample fifteen of the twenty-one species found on the islet using this protocol. This would appear to agree with Nielsen et al. (2011) who, in their study of phryganic and other Mediterranean habitats, found that variable transects, like those used here as the basis of the MRR experiment, sampled the greatest number of bee species, including the greatest number of unique bee species, out of the five survey methods they tested. Although, admittedly, in our experiment, the variable transects were sampled over seven days, compared to the pan traps' four-day protocol, and the standardised transect surveys' two-day protocol. In order to generate reliable estimates for a greater share of the species present, I would recommend that future researchers working within this kind of experimental island system use more surveyors, to ensure that a greater proportion of each species' population can be captured and marked. I would also recommend surveyors spend a short period of time prior to beginning the MRR surveys acquainting themselves with the focal fauna. The length of the sampling period could be extended to provide additional time to recapture marked individuals but, given that many insects have relatively short lifespans and that several of the species present on Prassológos are highly dispersive (Beekman & Ratnieks, 2000; Steffan-Dewenter & Kuhn, 2003; Osborne et al., 2008; Rotheray & Gilbert, 2011; Carvell et al., 2012), shorter sampling periods do have the advantage that marked individuals are less likely to leave the population via death or emigration before being recaptured (Henderson & Southwood, 2016). I was able to meet most of the assumptions necessary for reliable population estimation in the four species for which estimates were made. The seven-day marking period, combined with a simple marking strategy, ensured that marks were retained over the course of the experiment and read correctly by both surveyors, and that the marking process itself was carried out within three minutes of capture with marked individuals being allowed time to recover prior to release. However, for two of the four species: *E. corollae* and *H. phryganicus*, tests for the primary assumptions of equal probability of survival and equal probability of capture between periods i and $i+1$ could not be estimated. This, combined with sparse recapture data, means that our population estimates for *H. phryganicus* are almost certainly overestimated, while estimates for *E. corollae* should be treated with caution.

It is challenging to say, based upon these data, whether samples collected by pan trapping or by standardised transect surveys best represent the bee and hoverfly community present on Prassológos during this experiment. If I focus solely on the smaller community formed by the four species for which we were able to estimate relative abundance: *B. terrestris*, *H. phryganicus*, *E. corollae*, and *S. scripta*, then the rank abundance of these four species appears to be best reflected by the combined samples from both survey methods, when deployed together over the two-day period. However, this still results in underestimations of the relative abundance of *H. phryganicus* and *B. terrestris*, and overestimations of the relative abundance of *S. scripta*. Samples collected over the full four days of pan trapping appear to best represent the relative abundance of *H. phryganicus*, which would appear to confirm the hypothesis that pan trapping is well-suited to sampling populations of smaller solitary bee species, such as the Halictidae (Roulston, Smith, & Brewster, 2007; Westphal et al., 2008; Wilson, Griswold, & Messinger, 2008; Grundel et al., 2011), while casting doubt upon the idea that they overestimate the relative abundance of these species in samples (Portman, Bruninga-Socolar, & Cariveau, 2020). But, since the estimated population size of *H. phryganicus* presented in Section 3.3.2.2 was most likely itself an overestimation (due to poor recapture rates), I cannot definitively say whether either survey method, in fact, provides an accurate picture of the relative abundance of this species. Pan trapping also overestimated the relative abundance of *B. terrestris*, potentially bringing into question the view than pan traps under-sample larger insect species such as bumblebees (Potts, Evan, & Boone, 2005; Roulston, Smith, & Brewster, 2007), although the relative abundance of this species within the pan trap samples was still very low overall.

The standardised transect surveys overestimated the relative abundance of both hoverfly species and underestimated the relative abundance of both bee species. Most of the literature that has focused on comparing pan trapping and transect surveys has done so solely in terms of either method's ability to sample bees (but see Popic et al., 2013). Both *E. corollae* and *S. scripta* share bold black and yellow markings, and *S. scripta* has a distinctive body shape, so their increased abundance in the samples collected by the standardised transect surveys relative to their estimated abundance in the community may have been a result of collector bias. This may also explain why

the transect surveys failed to sample any specimens of the relatively abundant (but visually dull) *H. phryganicus*, or any of the smaller solitary bee species that were relatively abundant in the pan trapping samples (i.e., *C. parvula* or *L. nitidulum*). This result may provide some confirmation of the hypothesis that transect-based methods are less well-suited to sample the populations of smaller solitary bees (Potts, Evan, & Boone, 2005; Templ et al., 2019).

If we focus away from the population estimates for these four species, however, and concentrate on the relative abundance of broad taxonomic groups sampled across the variable transect surveys (as shown in Figures 3.5 & 3.6), then the standardised transect surveys actually provide the closest match, regardless of the wide disparity in total sample sizes. Both transect-based methods sampled a lower proportion of solitary bees, honeybees, and bumblebees, and a higher proportion of hoverflies (in terms of both abundance and species richness) than pan trapping. This would appear to disagree with the findings of O'Connor et al. (2019b), who found that pan trapping and transect surveys sampled a similar species richness (and an approximately equivalent rate of species accumulation) of hoverflies; although, this decreased in transects if the surveys weren't carried out by acknowledge experts. Pan traps, meanwhile, sampled a high overall proportion of solitary bees and low proportions of hoverflies, honeybees, and bumblebees (again, in terms of both abundance and species richness). This provides further evidence that pan traps may be a good choice for sampling solitary bee populations, although it tells us nothing regarding how well the relative abundance of these solitary bee species compares with the relative abundance of these species in the focal community. It also shows that the samples collected via pan trapping were not representative of the samples generated by the variable transect walks in terms of taxonomic diversity, especially if we only consider the samples generated when both pan traps and standardised transect surveys were deployed together. The low abundance sampled by the standardised transect surveys means that a combination of these samples, and those collected by pan trapping between the 22nd and 23rd, were also a poor match for the relative abundance of bee and hoverfly taxa sampled during the variable transect surveys. However, these combined samples were a much closer match to the proportional species richness sampled during the variable transect surveys. If considered only across the two days where both survey methods were

used in tandem, both pan trapping and standardised transect surveys also sampled the same number of species (n = 5). This would appear to indicate that, if the aim of a survey is to generate a comprehensive species inventory for an area, a combination of both pan trapping and transect surveys is appropriate, and that both standardised and variable transect protocols should be considered; a view that agrees with the findings of Grundel et al. (2011), Nielsen et al. (2011), O'Connor et al. (2019b), and Prendergast et al. (2020).

The relative abundance of *B. terrestris* was poorly represented in samples collected by both pan trapping and standardised transect surveys. Considering the abundance of evidence that suggests transects are particularly well-suited to sampling bumblebee populations, this may be further evidence that the present population was transitory rather than one native to Prassológos. Bumblebees are noted as being more robust to higher wind speeds than smaller bee species (Peat & Goulson, 2005; Crall et al., 2017), but travelling over approximately 1km of open water in search of forage is unlikely to present a sufficient reward in the inclement weather that occurred during the methods comparison portion of this experiment to warrant the energetic effort required (Ravi et al., 2013). This would also likely have been the case for local honeybees, which would certainly have been travelling across from mainland Lesvos since there was no colony or hive on Prassológos. Honeybees were captured rarely enough during the MRR surveys that I assume Prassológos either did not present an attractive enough resource for scouts from local hives, or that the islet had not been located by local foragers.

Both pan trapping and standardised transect surveys sampled significantly fewer insects than the numbers estimated by the MRR survey. This, to an extent, agrees with the results of Gezon et al. (2015), who showed that repeated pan trapping, whether over the course of a season or across multiple years, did not affect local bee community composition or abundance. This indicates that, even in a small, semi-closed island ecosystem, regular systematic destructive sampling by both pan trapping and standardised transect surveys only removes a relatively small proportion of the bee and hoverfly community present, even when used in tandem.

The unexpected high winds present on and around Prassológos when deploying and comparing methods in tandem, may have affected the sampling ability of both the pan traps and standardised

transect surveys, making comparisons between the two methods problematic. There has been relatively little research concerning the effects of local weather conditions on the efficacy of different insect pollinator survey methods, an absence which has been commented on by past authors (see Saunders & Luck, 2013; Crall et al., 2020). Results from **Chapter 2** show that increased wind speeds decreased the abundance of solitary bees captured by pan trapping, whereas bumblebee abundance and species richness in the same traps was observed to increase under higher wind speeds. In the field, fewer bees and hoverflies are typically observed on transect surveys during periods of higher winds (personal observation), which can also make it more difficult to sample with a net. A more informative approach for this Chapter may have been to continue with the transect walks during the period of higher winds, and then use these data as part of a generalised linear mixed-effects model to assess the effect of changing wind speeds upon the efficacy of both pan trapping and standardised transect surveys. Regardless, the sampling bias caused by different weather conditions is clearly an area of research that would benefit from further study.

In contrast to the results of **Chapter 2**, I found no correlation between the floral abundance present around the pan traps or along the standardised transects and the number of bees and hoverflies sampled along them. While much of the current literature considers pan traps to be less effective in areas with high floral abundance (Roulston, Smith, & Brewster, 2007; Wilson, Griswold, & Messinger, 2008; Baum & Wallen, 2011; O'Connor et al., 2019b; Westerberg et al., 2021), transect surveys are noted for their ability to sample at the plant-pollinator interface (Potts, Evan, & Boone, 2005; Popic, Davila, & Wardle, 2013) and are considered to have a positive sampling bias towards areas with a higher floral abundance (Popic, Davila, & Wardle, 2013; O'Connor et al., 2019b). Both O'Connor et al. (2019b) and Templ et al. (2019) found that increased floral density had opposing effects on the abundance of bee and hoverfly samples collected via pan trapping and transect surveys, decreasing the former while increasing the latter, within two homogeneous crop monocultures. The abundance of flowers found surrounding the pan traps and along the transect surveys in this experiment were similar to those encountered within O'Connor et al. (2019b) (see O'Connor et al., 2019a), although our island site is likely to have been

significantly more heterogeneous in terms of floral density. I hypothesise that the lack of a relationship between floral resource abundance and bee and hoverfly diversity may be due to the size of the islet in question. In a semi-closed island site less than one hectare in area, resident insect species may be under less behavioural pressure to visit high-resource floral patches, since the floral resources present across the island are effectively within reach, even for smaller solitary bee species with smaller foraging ranges (Gathmann & Tscharntke, 2002; Greenleaf et al., 2007).

3.5 Conclusions

The use of a MRR experiment to ground truth samples collected via different survey methods in order to quantify specific sampling biases is a novel approach, that has not been previously tested within insect pollinator communities. Due to low recapture rates, I was only able to generate population estimates for four of twenty-one species sampled on the islet of Prassológos, and, therefore, was unable to provide concrete evidence for the existence of the often-hypothesised sampling biases associated with pan trapping and transect surveys; namely that pan traps overestimate the relative abundance of smaller solitary bee species, and that transect surveys underestimate the relative abundance of these smaller species, while overestimating the abundance of larger, slower moving taxa, such as bumblebees. I was, however, able to identify some interesting trends in my data that suggest that these proposed biases may, in reality, exist. Based upon these results, I recommend that future studies emphasise differences in relative abundance and the composition of samples from different survey methods, when attempting to assess their relative efficacy, rather than concentrating purely on differences in the raw abundance or species richness sampled (see Cane, Minckley, & Kervin, 2000; Roulston, Smith, & Brewster, 2007; Westphal et al., 2008; Nielsen et al., 2011; Popic, Davila, & Wardle, 2013).

Furthermore, this experiment did provide an interesting proof of concept for future experimental work in this area. Within a semi-closed island system, a greater number of surveyors may increase the number of species for which reliable population estimates can be generated. By replicating this experiment over a number of years, one could provide enough data to answer questions concerning the sampling bias inherent to a range of survey methods. In addition, enough small island ecosystems exist, even around the UK (e.g. the Isles of Scilly and the Outer Hebrides), that

this concept could be explored in more detail across a wider geographic range with some effort. There also exists the potential to create closed artificial populations using a large experimental flight chamber, wherein reared populations of insects like *Bombus terrestris*, *Apis mellifera*, and *Osmia bicornis*, could be used to assess questions relating to sampling bias (Lihoreau, Chittka, & Raine, 2016).

Chapter 4

Acoustic differentiation between flower-visiting insect taxa based on wing beat frequencies: a future monitoring tool?

4.1 Introduction

There is growing evidence concerning insect pollinator declines across the globe (Biesmeijer et al., 2006; Ollerton et al., 2014; Teichroew et al., 2015; Koh et al., 2016; Powney et al., 2019), The scale of these declines, combined with the relative lack of centralised, standardised monitoring protocols (but see Carvell et al., 2016), makes quantifying these trends in pollinator populations problematic (Isaac et al., 2014; Powney et al., 2019). Citizen science, defined as "the involvement of volunteers in research" by Roy et al. (2016), has a long history within the UK (Pocock et al., 2015), where several long-term, national-scale biodiversity monitoring projects and organisations rely upon citizen scientists to increase the spatio-temporal scales over which they can gather data (see Roy et al., 2012: Appendix II).

The use of citizen scientists in biodiversity surveys brings with it some methodological issues. Survey methods need to be standardised, easy to use, and ideally non-destructive, in order to encourage willingness and motivation to participate across a range of potential stakeholder groups (Knapton, 2017; Barkham, 2017). The UK's national Pollinator Monitoring Scheme (PoMS)[8] has integrated citizen science into its data collection protocol through the use of Flower-Insect Timed Counts (FIT Counts), which are analogous to the focal floral resource observations described in Roy et al. (2016) (see also Westphal et al., 2008; Nielsen et al., 2011). This non-destructive, visual survey method is simple and quick to carry out but does rely upon individuals identifying insects from a wide range of taxa to broad groups by sight (Carvell, 2017). Misidentified observations can be a major source of bias within citizen science surveys, responsible for both Type I and Type

[8] https://ukpoms.org.uk/home

II errors (Dennis et al., 2006; Isaac et al., 2014); while research has shown that, even with training, volunteers are still liable to make identification errors when surveying insect communities (Roy et al., 2016; Falk et al., 2019; but see Kremen, Ullman, & Thorp, 2011; Ratnieks et al., 2016).

The use of sound to classify organisms may present a more reliable method of non-destructively surveying insect pollinators across large spatial scales. There are many applications of acoustics within biological monitoring, one of the most common of which is bioacoustics, defined as the study of the sounds produced by animals (Ozga, 2017), often with special reference to sounds used for communication (Laiolo, 2010; Ozga, 2017). Bioacoustics has been used to recognise and successfully classify organisms from a wide range of different taxa, including amphibians (Acevedo et al., 2009; Huang et al., 2009), birds (Briggs et al., 2012; Cheng et al., 2012), marine mammals (Oswald, Barlow, & Norris, 2000; Moore et al., 2006; Soldevilla et al., 2008), bats (Herr, Klomp, & Atkinson, 1997; Armitage & Ober, 2010; Zamora-Gutierrez et al., 2016), and insects (Chesmore & Nellenbach, 2001; Chesmore, 2004; Chesmore & Ohya, 2004).

This bioacoustic revolution in biological recording has been applied broadly to biodiversity monitoring as well as being used to detect invasive and pest species (Chesmore, 2008), and to monitor the effects of anthropogenic activity upon biodiversity and animal behaviour (see Laiolo, 2010). This has partly been made possible due to the extensive uptake of machine learning techniques within the fields of ecology and conservation science. Machine learning is a form of artificial intelligence and is primarily used within ecology to detect patterns in large, complex datasets (Olden, Lawler, & Poff, 2016). Supervised machine learning, where pattern-recognition algorithms are trained using known data (Olden, Lawler, & Poff, 2016), have been used to classify animal acoustic signals with well-above eighty per cent accuracy. For instance, Chesmore & Ohya (2004) used artificial neural networks (ANNs) to identify four UK grasshopper species with between eighty and one hundred per cent accuracy, while Huang et al. (2009) achieved similar rates of accuracy classifying five frog species using support vector machine models (SVMs).

The widespread application of machine learning techniques, in conjunction with the proliferation of personal, hand-held technology (August et al., 2015), has led to the creation of smartphone

applications such as Cicada Hunt, designed for the New Forest Cicada Project[9]. This app uses the microphone present in all current smartphones to detect the call of the rare New Forest cicada (*Cicadetta montana s. str.*), encouraging further citizen scientist-participation in this monitoring scheme (Pantidi et al., 2014; August et al., 2015). Whereas, plug-in microphones, such as the Echo Meter Touch[10], have been developed that can combine with smartphone applications to allow the general public to detect and identify bat species.

All of which begs the question: can we apply bioacoustic technology to monitor insect pollinator communities? One potential barrier to doing so is the issue of actively vs. passively generated sound. Many of the insect taxa that have been the focus of bioacoustic research produce active sound, i.e., sounds that are produced for a specific purpose, such as the stridulation signals in the Orthoptera (see Chesmore, 2004; Chesmore & Nellenbach, 2001; Chesmore & Ohya, 2004). These signals are typically species-specific and are thus easier targets for acoustic classification. Most insect pollinator species do not produce sound in this way, rather the sounds they produce are the passive by-product of their wingbeats, creating the buzz commonly associated with flying insects (Ganchev & Potamitis, 2007). These sounds do vary in frequency as a function of body size, with larger insects tending to generate lower frequencies. This has been relatively well studied in bumblebees (*Bombus* spp.). Miller-Struttmann et al. (2017), for instance, found that the characteristic frequency associated with the wing beats of two alpine bumblebee species fell from approximately 200Hz to nearer 125Hz as both wing length and tongue length increased. And van Roy et al. (2014) showed a similar relationship between both body mass and wing dimensions and the wing beat frequencies of *Bombus terrestris* and *B. ignitus*. Whereas Burkart, Lunau, & Schlindwein (2011) found that, in various neotropical bee species, wing beat frequency was also negatively associated with increasing body size (intertegular distance). As a rule, larger insects tend to have larger wings, which are more subject to inertia and thus beat more slowly (while providing more lift), which leads to lower wing beat frequencies (Byrne, Buchmann & Spangler,

[9] http://www.newforestcicada.info
[10] https://www.wildlifeacoustics.com/products/echo-meter-touch-2-android

1988; van Roy et al., 2014). These frequencies are also liable to vary with temperature. Unwin & Corbet (1984) found that the wing beat frequencies of bumblebee foragers decreased by approximately 20Hz as the ambient air temperature rose between 14 and 24.5°C. This is assumed to be an adaptation on behalf of the bee, where the wing-beat frequency is modulated down in warmer temperatures to decrease the rise in body temperature associated with maintained use of the flight muscles (Unwin & Corbet, 1984). A similar adaptation has also been observed in an anthophorid solitary bee (Spangler & Buchmann, 1991), but not in honeybees (*Apis mellifera*), where Woods, Heinrich, & Stevenson (2005) observed in increase in wing beat frequency in response to increasing ambient air temperature. Smaller insects, especially dipteran species, also increase their frequency of their wing beats in response to increasing temperature, since their smaller size means that the resulting increase in air movement across their body surface can have a net cooling effect (Unwin & Corbet, 1984).

One notable exception where insect pollinator species do produce active sound is sonication, or buzz pollination, a behaviour exhibited primarily by bumblebees. Sonication refers to the use of intense thoracic muscle vibration to cause flowers with poricidal (tubular/conical) anthers to release their concealed pollen (Burkart, Lunau, & Schlindwein, 2011; De Luca & Vallejo-Marín, 2013). These vibrations occur at higher frequencies than those related to flight and have been observed to negatively correlate with bee body size in a similar fashion to flight (Burkart, Lunau, & Schlindwein, 2011). Research by De Luca, Cox, & Vallejo-Marín (2014) also indicates that there may a species-specific element to sonication frequencies.

Some authors have investigated whether we can classify flower-visiting insect taxa using the passively generated sounds of their wing beats. Initial research by Burkart, Lunau, & Schlindwein (2011) showed that buzz pollination and flight frequencies were significantly different between two neotropical bee species. Moore & Hassall (2016) appear to be the first to contrast the wing-beat frequencies of different pollinator species, finding no significant pairwise differences between mimetic hoverflies (Diptera: Syrphidae) and their Hymenopteran models using an unsupervised classification technique. The most compelling research to-date has been carried out by Gradišek et al. (2016) and Kawakita & Ichikawa (2019). Gradišek et al. (2016) tested the

performance of four machine learning algorithms in terms of their ability to classify nine bumblebee species: their overall rate of classification accuracy was 82.7 per cent, with the classification accuracy for individual species varying between fifty and eighty-five per cent. Their best performing algorithm has since been incorporated into an open-access internet-based application (animal-sounds.ijs.si), where the public can upload their own bumblebee recordings for classification. Meanwhile, Kawakita & Ichikawa (2019) focused on the classification of a broader selection of taxa: *Apis mellifera*, *Bombus ardens*, the solitary bee *Tetralonia nipponensis*, and the Japanese yellow hornet (*Vespa simillima xanthoptera*). The precision of their support vector machine (SVM) classification algorithm differed by species, between seventy-three per cent (*Bombus ardens*) and one hundred per cent (*Vespa simillima xanthoptera*), indicating that classification based upon insect wing beat frequencies may also be possible between common flower-visiting insect taxa. But these groups only scratch the surface of the sheer diversity of taxa that can be encompassed by the term "pollinator" (see Ollerton, 2017). However, there remains no research concerning the performance of bioacoustic classification techniques aimed at insect pollinator species as a community, using multiple representatives of key taxonomic groups, in an attempt to simulate the diversity that may be encountered as part of a biodiversity survey.

4.1.1 Aims

This chapter investigates whether it is possible to reliably classify flower-visiting insects to different levels of taxonomic resolution using the passively generated sound of their wing beats; with the overall aim of assessing the potential of bioacoustics as a future survey tool for insect pollinator communities, including by citizen scientists. The study aims to answers the following questions:

1. Can we reliably differentiate between flower-visiting insect taxa at different levels of taxonomic resolution using wing beat frequency?
2. Can this process be automated using machine-learning algorithms?

4.2 Materials & Methods

4.2.1 Data collection

Acoustic data collection took place between June-September 2016 and June-August 2018 in multiple sites surrounding Leeds, West Yorkshire, and Wimborne Minster, Dorset. Sites varied in terms of the diversity of insect and plant species present, including wildflower meadows, brownfield sites, and urban parks and gardens.

Acoustic data were collected using an omnidirectional Sony microphone attached to a Sony ICD-PX312 Dictaphone. The microphone was placed near to flower-visiting insects, no-more than 5cm away, while they were foraging from or visiting flowers (Fig. 4.1). I endeavoured to record at least ten seconds of flight sounds from each individual. Each individual was identified by eye to genus, and to species where possible, whilst being recorded. Once flight sounds from an individual had been recorded, I immediately moved on to another individual, preferably from a different insect taxon, in an attempt to reduce the likelihood of recording the same individual multiple times. Insect audio was recorded at 32 kbps/44.1 kHz in .MP3 format.

Insects from two common flower-visiting insect Orders were sampled: the Hymenoptera and the Diptera. Within these two Orders, I chose six groups of insects to take recordings from: honeybees (*Apis mellifera*), bumblebees (*Bombus* spp.), solitary bees (any non-*Apis* and non-*Bombus* bee species), hoverflies (Syrphidae), social wasps (*Vespula vulgaris*), and non-syrphid Diptera. Due to the diversity of UK Diptera, I concentrated on just one morphotype of non-Syrphid: the "green bottle". This term can refer to a number of species from several genera of Calliphoridae, as well as several species of Tachinid mimic (Tachinidae Recording Scheme, 2021). I did not differentiate between these species in the field, but all are of an approximately similar size (personal observation) and are common floral visitors (Kevan & Baker, 1983; Bräuer, Neinhuis, & Voigt, 2017). Two species of bumblebee: *Bombus terrestris* and *B. lucorum*, were also aggregated together under the name "*Bombus terrestris*" during this experiment, due to difficulties in visually differentiating workers from these two species in the field.

Figure 4.1 Pictures of acoustic recording taking place in the field.

4.2.2 Signal processing

Insect audio recordings were processed using the free Audacity software (Audacity Team, 2017). Periods of flight noise were removed from the original recordings in sections lasting between 0.1 and 10 seconds and converted to .wav files for later analysis. Sections containing high-amplitude background noise capable of potentially interfering with the insect flight audio were not used for further analysis. In some cases, where background noise was present but did not interfere with the insect flight audio (i.e., where the frequency of background noise was constant and distinct from the frequencies occupied by the insect flight audio), Audacity's equalisation tool was used to reduce the amplitude of this noise. Where possible, background noise was left within recordings to simulate the kinds of real-world recordings that would be generated as part of a national-scale, citizen scientist-led acoustic monitoring scheme.

4.2.3 Can we differentiate between flower-visiting insects at different levels of taxonomic resolution using simple classifiers?

Initially, I was interested in whether simple acoustic features could be used to define different flower-visiting insect groups. Past studies have often passed over this approach, citing the close

visual proximity of different insect groups in terms of acoustic descriptors like natural frequency, but carrying out no statistical analysis to confirm this (see Gradišek et al., 2016).

Data analysis was carried out within R, version 3.5.1 (R Core Team, 2018). The *analyzeFolder* function in the soundgen package (Anikin, 2019) was used to extract two acoustic features from each insect flight audio recording: the lowest dominant frequency (Hz), which should approximate to the fundamental frequency, and the frequency with the highest spectral energy (Hz), which should approximate to the dominant frequency. These two features were calculated using a short-time Fourier Transform (STFT). An STFT involves splitting an audio recording into multiple segments, in this case lasting 50ms. A moving window passes along these segments and carries out a Fast Fourier Transform (FFT), where a time-domain signal (all sounds start as a time-domain signal, measured in terms of amplitude over time) is split into a series of sine and cosine waves that represent the harmonic structure of the acoustic signal (Clements, 1998). This transforms the signal from the time-domain into the frequency-domain. The values for the fundamental and dominant frequencies were calculated within each of these 50ms segments by the moving window as it passed across the recording. The values for these two features were then mean averaged across each recording. Where there was more than one recording per individual, these values were mean averaged across all of these recordings.

To test whether these simple acoustic features could be used to distinguish between different flower-visiting insect taxa, I used a series of Gamma-distributed generalised linear mixed-effects models (GLMMs) and Gamma-distributed general linear models (GLMs). The GLMMs, using the *glmer* function in the lme4 package (Bates et al., 2015), were fitted to test whether I could distinguish between insects at higher levels of taxonomic resolution, as follows:

1. Order-level, using the random effect term (1|Order/Group/Taxon).
    a. Hymenoptera vs. Diptera.
2. Group-level within the Hymenoptera, using the random effect term (1|Group/Taxon).
    a. Honeybees vs. bumblebees vs. solitary bees vs. social wasps
3. Group-level within the Diptera, using the random effect term (1|Group/Taxon)
    a. Hoverflies vs. non-syrphid Diptera

The GLMs, using the *glm* function, were fitted to test whether I could distinguish between insects at the "Taxon-level" - the lowest level of taxonomic resolution available:

1. Between bumblebee species
2. Between solitary bee genera (and one Family: the Halictidae)
3. Between hoverfly genera (and one tribe: the Bacchini)

Pair-wise comparisons between insect classes in both GLMMs and GLMs were calculated via General Linear Hypothesis testing, using the *glht* function in the multcomp package (Hothorn, Bretz, & Westfall, 2008).

4.2.4 Can we use machine learning algorithms to train models to automatically classify flower-visiting insects at different levels of taxonomic resolution?

The second aspect of this chapter is to automate the acoustic classification process using machine learning classification algorithms. These algorithms will use a selection of the acoustic data I have processed to train models to classify different insect classes. These models can then be validated, in terms of classification accuracy, using "new" data (processed acoustic data that were not used for model training).

4.2.4.1 Advanced feature extraction for machine learning

Feature extraction is the process of taking an acoustic signal and, using a form of Fourier analysis, extracting a set of features that represent specific portions or characteristics of the signal (Gradišek et al., 2016). There are a wide variety of features that are used to classify acoustic data, including the fundamental and dominant frequencies (see Raman, Gerhardt, & Wilkerson, 2007; Rashed et al., 2009; De Luca, Cox, & Vallejo-Marín, 2014; Moore & Hassall, 2016; De Luca et al., 2018), and Mel-frequency Cepstral Coefficients (MFCCs) (Zhu, 2011; Kawakita & Ichikawa, 2019). But since there has been relatively little research concerning the classification of insect taxa using passively generated wingbeat signals, there is no standard or accepted set of features that has been well-tested on this group. I therefore chose to use the same feature extraction methodology as Gradišek et al. (2016) in their paper concerning bumblebee classification. This involved the use of the open-source Speech and Music Interpretation by Large-space Extraction

toolkit (openSMILE), version 2.1 (Eyben et al., 2013), compiled in Linux via the open-source Ubuntu operating system. I used the openSMILE toolkit to extract the INTERSPEECH 2010 Paralinguistic Challenge feature set (see Schuller et al., 2010), which contains 1582 numerical features, including features relating to the fundamental frequency and MFCCs, for each insect flight audio recording. Data were entered into openSMILE in .wav format and the resulting feature sets were saved as .csv files.

4.2.4.2 Feature selection

Large feature sets are often pared down after extraction, a process referred to as feature selection (Gradišek et al., 2016). The current set of 1582 features would pass on a lot of extraneous data to the machine learning algorithms - potentially interfering with any patterns in the dataset and reducing processing speed (Gradišek et al., 2016).

I used information gain theory to select the best 100 features for each machine learning model at the Order-, group-, and taxon-level. Information gain is based on information entropy (H): features that distinguish between two classes well represent a higher "information gain" and were selected for the final feature set (Gradišek et al., 2016). The *information.gain* function in the FSelector package in R (Romanski & Kotthoff, 2018) was used to calculate the information gain value for each feature within the INTERSPEECH 2010 Paralinguistic Challenge feature set.

4.2.4.3 Machine learning algorithms

The performance of two common machine learning algorithms was tested as part of this analysis: random forest models and support vector machine models (SVMs). SVMs are a supervised classification technique that aims to separate data classes using a hyperplane: a line drawn in multi-dimensional space (Patel, 2017). The hyperplane is drawn so as to maximise the margin between the two or more data classes using support vectors (see Fig. 4.2): the data points that lie closest to the hyperplane (Patel, 2017). The best hyperplane is one that maximises the margin between the hyperplane and the surrounding support vectors (Patel, 2017). This works well for classes that are linearly separable, but where classes are not SVMs use what is called the "kernel trick" to separate them (Patel, 2017; Ippolito, 2019). The kernel trick involves transforming the data by adding extra dimensions; hyperplanes can then be added to separate classes within multi-

dimensional space, before reducing the number of dimensions back to two to test the performance of the separator (Patel, 2017; Ippolito, 2019) (see Fig. 4.3). Different kernels exist to achieve this.

SVM models have been successfully used to classify both amphibian and bird species (Acevedo et al., 2009; Huang et al., 2009; Zhao et al., 2017), as well as four insect pollinator species in the study by Kawakita & Ichikawa (2019).

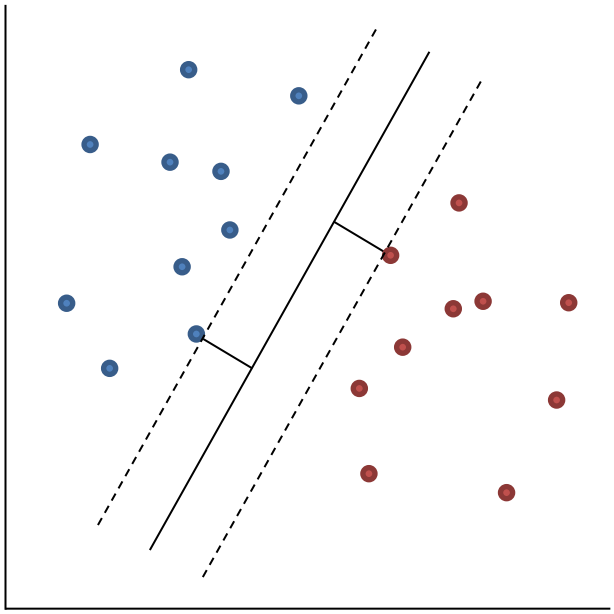

Figure 4.2 A visual representation of a Support Vector Machine (SVM) model separating two data classes using a hyperplane (solid black line) fitted to maximise the margin (dotted black line) between the hyperplane and the nearest data points from each class, known as support vectors.

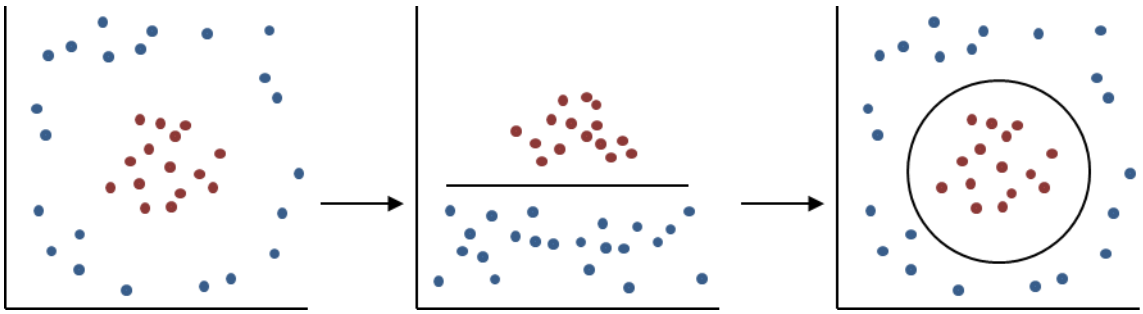

Figure 4.3 A visual representation of the kernel trick for classifying non-linearly separable data (left) classes using Support Vector Machine (SVM) models. Data are transformed by inserting additional dimensions using a non-linear kernel (centre) where a hyperplane can be applied to separate the two classes; the data can then be transformed back into two dimensions (right) where the non-linear hyperplane can be viewed.

Random forest models are also supervised classification models but are based upon the decision tree algorithm. A decision tree classifies data based upon a tree-like decision structure (see Fig. 4.4), where decisions made at nodes, based upon extracted features, split data along branches which lead to further decision nodes, eventually leading to leaf nodes where the data cannot be split any further (Cutler et al., 2007). Each leaf nodes represents a class into which data can be assigned (Gradišek et al., 2016). The random forest algorithm is an ensemble method that combines many decision trees, where each tree is trained using a random subset of data and a random subset of features (Cutler et al., 2007; Gradišek et al., 2016). An individual is passed through each tree and classified based upon the number of votes for each data class (Cutler et al., 2007; Gradišek et al., 2016). Random forest models have been used to successfully classify bird and bat species (Armitage & Ober, 2010; Kampichler et al., 2010), as well as the nine bumblebee species in the study by Gradišek et al. (2016).

Machine learning classification models were built and tested using R, version 3.6.1 (R Core Team, 2019). Both random forest and SVM models require data to be partitioned into training and testing sets: the training set trains the initial model, which is then validated using the testing set to assess the model's performance. I tested two data partition sizes: seventy per cent training and thirty per cent testing data, and eighty per cent training and twenty per cent testing data. The aim was to maximise the amount of data available to train the models, which should generate more accurate predictions, while leaving enough testing data to validate them properly. A small testing set may result in inaccurate predictions of classification accuracy if the testing set includes any outliers or anomalous data, or if some classes are too similar.

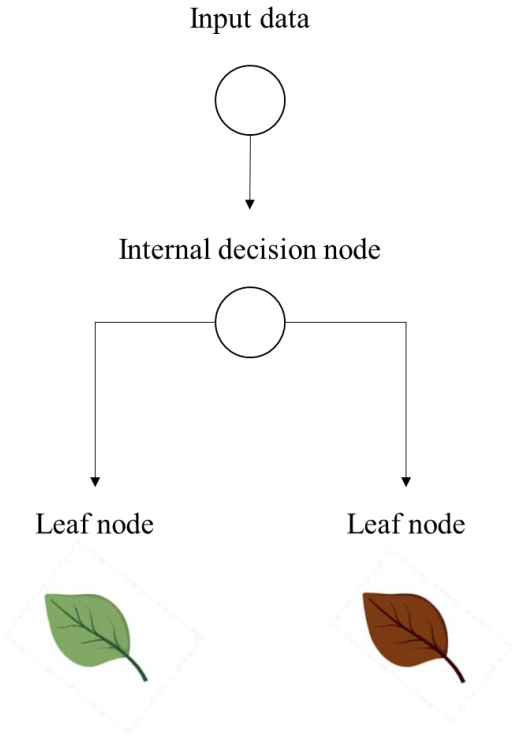

Figure 4.4 A visual representation of a decision tree algorithm, where data is run through and split at a series of internal decision nodes based upon extracted data features, before reaching leaf nodes, representing classified data classes, where the data cannot be split any further. Created using Biorender.com.

The SVM models were trained using the *train* function in the caret package (Kuhn et al., 2019). Since my data were not linearly separable, I used the Radial Basis Function kernel to create hyperplanes between my data classes by specifying "SvmRadial" in the *method* argument within the *train* function (Karatzoglou et al., 2004). As part of the training process, SVM models require a process called *K*-fold cross-validation. *K*-fold cross-validation involves further splitting the training set into *k* subsets; a different selection of *k*-1 subsets are then used across *k* iterations to train the model, while the remaining subset is used to validate model performance (Gradišek et al., 2016; Gupta, 2017). This process helps to reduce any bias introduced into the model training process by the prior removal of a portion of the data for future model validation, i.e., under-fitting, or failing to accurately classify a data class due to lack of training data (Gupta, 2017). I used 10-fold cross validation (Gupta, 2017) via the *trainControl* function in the caret package, which was integrated into the SVM model using the *trControl* argument in the *train* function. After the SVM model was trained, I validated it using the testing set via the *predict* function.

The random forest models were trained using the *randomForest* function in the randomForest package (Liaw & Wiener, 2002). The optimal number of acoustic features to pass on to each decision tree was calculated using the *tuneRF* function in the randomForest package. The optimal number of trees to include in the forest was estimated by trial and error: I ran the training model with 2001, 1501, 1001, 751, and 501 trees, and picked the model which provided the greatest classification accuracy. Random forest models do not require *k*-fold cross-validation, since they already incorporate a number of decision trees, each of which uses different subsets of both the training set and the available acoustic feature set (Breiman & Cutler, 2004). The best training model was validated using the testing set via the *predict* function.

The results from both SVM and random forest models were presented as confusion matrices: tables where the rows represent the known data classes, and the columns represent the predicted data classes. Confusion matrices allow for the inspection of rates of classification accuracy for individual classes, in addition to the overall rate of classification accuracy that is provided by the model.

4.3 Results

I recorded 1096 instances of insect flight from 540 individual flower-visiting insects, spanning two orders: Hymenoptera and Diptera, and six common groups: honeybees (*Apis mellifera*), bumblebees (*Bombus* spp.), solitary bees, social wasps (*Vespula vulgaris*), hoverflies (Syrphidae) and one morphotype of non-syrphid fly: the green bottle (Table 4.1). These groups included eight bumblebee species, three solitary bee genera and one family, and six hoverfly genera and one tribe. The Halictidae and the Bacchini were grouped at the family- and tribe-level, respectively, due to difficulties differentiating their constituent genera in the field.

Table 4.1 A summary of the number of individuals (N) and recordings (*n*) sampled per class, and the mean average values for the fundamental and dominant frequencies (Hz.) ± standard error.

Order	Group	Taxon	N(*n*)*	Fundamental frequency (Hz.) ± SE	Dominant frequency (Hz.) ± SE
Hymenoptera	~	~	451 (954)	208.74 ± 1.15	328.64 ± 0.90
	Bumblebees	~	391 (734)	205.59 ± 1.40	329.98 ± 0.89
		Bombus hortorum	20 (58)	197.96 ± 1.32	331.13 ± 2.18
		Bombus hypnorum	20 (32)	190.50 ± 1.67	335.96 ± 3.32
		Bombus jonellus	7 (28)	157.31 ± 1.85	324.72 ± 4.40
		Bombus lapidarius	104 (241)	224.67 ± 4.12	330.11 ± 1.77
		Bombus pascuorum	73 (187)	205.70 ± 0.77	341.30 ± 1.72
		Bombus pratorum	19 (37)	197.44 ± 1.62	297.79 ± 3.47
		Bombus terrestris	64 (127)	190.03 ± 0.99	328.75 ± 2.13
		Bombus vestalis	12 (23)	163.06 ± 2.13	261.48 ± 2.38
	Honeybee	*Apis mellifera*	47 (89)	225.73 ± 1.34	328.64 ± 0.90
	Solitary bees	~	71 (109)	219.01 ± 1.66	280.71 ± 3.27
		Colletes spp.	19 (32)	212.85 ± 2.50	300.65 ± 6.04
		Halictidae spp.	16 (31)	202.52 ± 3.18	253.07 ± 4.73
		Hylaeus spp.	20 (27)	240.42 ± 3.79	250.61 ± 6.94
		Megachile spp.	16 (19)	217.99 ± 3.34	325.12 ± 7.55
	Social wasps	*Vespula vulgaris*	14 (23)	208.54 ± 2.48	340.89 ± 6.38
Diptera	~	~	87 (141)	247.34 ± 5.35	318.62 ± 5.31
	Hoverflies	~	69 (118)	246.80 ± 5.42	319.16 ± 5.52
		Bacchini spp.	12 (18)	225.43 ± 4.60	269.45 ± 5.94
		Episyrphus sp.	11 (21)	229.16 ± 2.04	355.06 ± 14.47
		Eristalis spp.	13 (20)	249.39 ± 20.07	300.40 ± 13.49
		Merodon sp.	4 (14)	222.48 ± 1.76	372.45 ± 8.46
		Sphaerophoria spp.	8 (16)	280.89 ± 2.69	304.73 ± 8.78
		Syritta sp.	7 (12)	225.95 ± 4.16	331.38 ± 8.30
		Volucella spp.	10 (11)	183.17 ± 4.13	332.48 ± 9.69
	Non-syrphid flies	Green bottle spp.	18 (23)	259.82 ± 30.78	306.10 ± 11.68

4.3.1 Can we differentiate between flower-visiting insects at different levels of taxonomic resolution using simple classifiers?

At the order-level, there were significant differences between Hymenopteran and Dipteran insects in terms of the fundamental frequency (Hz.) of their wing beats (Table 4.2), with Hymenopteran wing beats having a significantly lower fundamental frequency than Dipteran wing beats (parameter est. -0.168 ± SE 0.073, $p = 0.022$).

Table 4.2 Output from the Gamma-distributed GLMMs and GLMs concerning differences between flower-visiting insect classes based upon the fundamental and dominant frequencies (Hz.).

Taxonomic resolution	Fundamental frequency (Hz.)			Dominant frequency (Hz.)		
GLMM (Gamma)	X^2	DF	*P*	X^2	DF	*P*
Order-level	5.290	1	0.022	0.073	1	0.787
Hymenoptera: group-level	1.179	3	0.758	12.419	3	0.006
Diptera: group-level	3.111	1	0.078	0.026	1	0.872
GLM (Gamma)	*F*	DF	*P*	*F*	DF	*P*
Bumblebees: taxon-level	1.510	7	0.163	4.890	7	<0.001
Solitary bees: taxon-level	1.860	3	0.145	1.647	3	0.187
Hoverflies: taxon-level	2.170	6	0.059	0.688	6	0.660

At the group-level, there were significant differences between Hymenopteran insect groups based upon the dominant frequency of their wing beats (Table 4.2). Solitary bee wing beats had a significantly lower dominant frequency than those of honeybees (-62.90 ± 23.24, $p = 0.031$) and bumblebees (-34.51 ± 13.81, $p = 0.054$), while social wasp wing beats had a significantly higher dominant frequency than those of solitary bees (74.15 ± 29.95, $p = 0.058$). There were no differences between Dipteran groups based upon either the dominant or fundamental frequencies of their wing beats.

At the taxon-level, there were significant differences between bumblebee species based upon the dominant frequency of their wing beats (Table 4.2). The wing beats of *Bombus vestalis* had a significantly lower dominant frequency than those of *B. hortorum* (-0.265 ± 0.065, $p = 0.001$), *B. jonellus* (-0.249 ± 0.0.085, $p = 0.057$), *B. lapidarius* (-0.221 ± 0.054, $p = 0.001$), *B. pascuorum* (-0.284 ± 0.056, $p = <0.001$), and *B. terrestris* (-0.222 ± 0.056, $p = 0.002$). The wing beats of

Bombus pratorum had a lower dominant frequency than those of *B. pascuorum* (-0.160 ± 0.046, p = 0.010). There were also significant differences between hoverfly species based upon the fundamental frequency of their wing beats (Table 4.2), with the wing beats of *Volucella* species having a significantly lower fundamental frequency than those of *Sphaerophoria* species (-0.359 ± 0.103, p = 0.008).

A note regarding the GLMMs: the number of data points was often not sufficient to handle the complex nested random effect terms included in each model. Since these terms were necessary to account for the potential variation associated with a nested dataset, I was forced to lower the number of points used to evaluate the adaptive Gauss-Hermite approximation to the log-likelihood from one to zero via the *nAGQ* argument in the *glmer* function (D. Bates et al., 2015, 2019). This allowed the models to run but reduced the accuracy of the parameter estimates generated by the GLMMs, so the results of these models should be viewed as pointing towards evidence of potential trends rather than as representing actual effects.

4.3.2 Can we use machine learning algorithms to train models to automatically classify flower-visiting insects at different levels of taxonomic resolution?

Since my dataset is inherently unbalanced (see Table 1), I chose to artificially balance the number of recordings from different insect classes passed on to each decision tree within the random forest models during model training; a process known as down-sampling (Thapliyal, 2019). This was carried out using the *sampsize* argument in the *randomForest* function in the randomForest package (Liaw & Wiener, 2002). I was unable to carry out a similar process within the SVM models, as the *weight* argument in the *train* function in the caret package (Kuhn et al., 2019) is unavailable to SVM model types.

4.3.2.1 Order-level classification: Hymenoptera vs. Diptera

The random forest model with the highest overall classification accuracy at the order-level used a 70% - 30% data partition between the training and testing sets. The training model classified flower-visiting insects to order with an overall accuracy rate of 83.83%, classifying Dipteran insects with 75.79% accuracy, and Hymenopteran insects with 84.97% accuracy (Table 4.3).

When the training model was validated using the testing dataset, the overall accuracy of the model was 86.63%; Dipteran insects were classified with 74.47% accuracy, and Hymenopteran insects with 88.65% accuracy (Table 4.3).

The SVM model with the highest overall classification accuracy at the order-level used an 80% - 20% data partition between the training and testing sets. The training model classified flower-visiting insects to order with an overall accuracy rate of 89.96% but, due to the cross-validation process, I cannot produce a confusion matrix for the training set. When the training model was validated using the testing data, the overall accuracy of the model was 90.00% (Table 4.4), and Dipteran insects were classified with 33.33% accuracy, and Hymenopteran insects with 97.93% accuracy.

Table 4.3 The rates of classification accuracy for two flower-visiting insect Orders generated by the random forest model, using a 70% - 30% training – testing data partition. Values in bold indicate accurate classifications.

Training data			
	Diptera	Hymenoptera	% Classification accuracy
Diptera	**72**	23	75.79
Hymenoptera	101	**571**	84.97
Testing data			
	Diptera	Hymenoptera	% Classification accuracy
Diptera	**35**	12	74.47
Hymenoptera	32	**250**	88.65

Table 4.4 The rates of classification accuracy for two flower-visiting insect Orders generated by the SVM model, using an 80% - 20% training – testing data partition. Values in bold indicate accurate classifications.

Testing data			
	Diptera	Hymenoptera	% Classification accuracy
Diptera	**9**	18	33.33
Hymenoptera	4	**189**	97.93

4.3.2.2 Hymenopteran group-level classification: Bumblebees vs. Honeybees vs. Solitary bees vs. social wasps

The random forest model with the highest overall classification accuracy at the group-level within the Hymenoptera used a 70% - 30% data partition between the training and testing sets. The training model classified flower visiting Hymenoptera to groups with an overall accuracy rate of 70.91%, classifying bumblebees with 72.23% accuracy, honeybees with 69.84% accuracy, solitary bees with 73.63% accuracy, and social wasps with 18.75% accuracy (Table 4.5). When the training model was validated using the testing dataset, the overall accuracy of the model was 72.47%; bumblebees were classified with 74.15% accuracy, honeybees with 65.39% accuracy, solitary bees with 72.22% accuracy, and social wasps with 42.86% accuracy (Table 4.5).

The SVM model with the highest overall classification accuracy at the group-level within the Hymenoptera used a 70% - 30% data partition between the training and testing sets. The training model classified flower visiting Hymenoptera to groups with an overall accuracy rate of 80.83%. When the training model was validated using the testing data, the overall accuracy of the model was 83.62%; bumblebees were classified with 95.76% accuracy, honeybees with 34.62% accuracy, solitary bees with 27.78% accuracy, and social wasps with 0.00% accuracy (Table 4.6).

Table 4.5 The rates of classification accuracy for the four Hymenopteran insect groups generated by the random forest model, using a 70% - 30% training – testing data partition. Values in bold indicate accurate classifications.

Training data					
	Bumblebee	Honeybee	Solitary bee	Social wasp	% Classification accuracy
Bumblebee	**359**	38	63	37	72.23
Honeybee	8	**44**	10	1	69.84
Solitary bee	11	10	**67**	3	73.63
Social wasp	6	1	6	**3**	18.75
Testing data					
	Bumblebee	Honeybee	Solitary bee	Social wasp	% Classification accuracy
Bumblebee	**175**	15	31	15	74.15
Honeybee	3	**17**	4	2	65.39
Solitary bee	4	1	**13**	0	72.22
Social wasp	1	1	2	**3**	42.86

Table 4.6 The rates of classification accuracy for the four Hymenopteran insect groups generated by the SVM model, using a 70% - 30% training – testing data partition. Values in bold indicate accurate classifications.

Testing data					
	Bumblebee	Honeybee	Solitary bee	Social wasp	% Classification accuracy
Bumblebee	**226**	4	6	0	95.76
Honeybee	14	**9**	3	0	34.62
Solitary bee	13	0	**5**	0	27.78
Social wasp	6	0	1	**0**	0.00

4.3.2.3 Dipteran group-level classification: Hoverflies vs. non-Syrphid flies

The random forest model with the highest overall classification accuracy at the group-level within the Diptera used an 80% - 20% data partition between the training and testing sets. The training model classified flower visiting Diptera to groups with an overall accuracy rate of 84.07%, classifying hoverflies with 86.32% accuracy, and non-Syrphid flies with 72.22% accuracy (Table 4.7). When the training model was validated using the testing dataset, the overall accuracy of the model was 93.10%; hoverflies were classified with 91.67% accuracy, and non-Syrphid flies with 100% accuracy (Table 4.7).

Table 4.7 The rates of classification accuracy for the two Dipteran insect groups generated by the random forest model, using an 80% - 20% training – testing data partition. Values in bold indicate accurate classifications.

Training data			
	Non-syrphid fly	Hoverfly	% Classification accuracy
Non-syrphid fly	**13**	5	72.22
Hoverfly	13	**82**	86.32
Testing data			
	Non-syrphid fly	Hoverfly	% Classification accuracy
Non-syrphid fly	**5**	0	100
Hoverfly	2	**22**	91.67

The SVM model with the highest overall classification accuracy at the group-level within the Diptera used an 80% - 20% data partition between the training and testing sets. The training model classified flower visiting Diptera to groups with an overall accuracy rate of 85.85%. When the

training model was validated using the testing data, the overall accuracy of the model was 82.76%; hoverflies were classified with 100% accuracy, and non-Syrphid flies with 16.67% accuracy (Table 4.8).

Table 4.8 The rates of classification accuracy for the two Dipteran insect groups generated by the SVM model, using an 80% - 20% training – testing data partition. Values in bold indicate accurate classifications.

Testing data			
	Non-syrphid fly	Hoverfly	% Classification accuracy
Non-syrphid fly	**1**	5	16.67
Hoverfly	0	**23**	100

4.3.2.4 Bumblebee taxon-level classification

The random forest model with the highest overall classification accuracy at the taxon-level within the bumblebees used a 70% - 30% data partition between the training and testing sets. The training model classified bumblebees to species with an overall accuracy rate of 58.28%, classifying different species with between 28.57% accuracy (*Bombus jonellus*) and 91.67% accuracy (*B. vestalis*) (see Table 4.9). When the training model was validated using the testing dataset, the overall accuracy of the model was 56.36%; with different species classified with between 35.71% accuracy (*B. jonellus*) and 72.73% accuracy (*B. vestalis*) (see Table 4.9).

The SVM model with the highest overall classification accuracy at the taxon-level within the bumblebees used an 80% - 20% data partition between the training and testing sets. The training model classified bumblebees to species with an overall accuracy rate of 52.76%. When the training model was validated using the testing dataset, the overall accuracy of the model was 55.78%; with different species classified with between 12.50% accuracy (*B. jonellus*) and 77.36% accuracy (*B. lapidarius*) (see Table 4.10).

Table 4.9 The rates of classification accuracy for the eight bumblebee species generated by the random forest model, using a 70% - 30% training – testing data partition. Values in bold indicate accurate classifications.

Training data									
	B. hortorum	*B. hypnorum*	*B. jonellus*	*B. lapidarius*	*B. pascuorum*	*B. pratorum*	*B. terrestris*	*B. vestalis*	% Classification accuracy
B. hortorum	**24**	0	0	8	1	0	7	0	60.00
B. hypnorum	1	**9**	0	2	4	5	6	0	33.33
B. jonellus	0	0	**4**	0	0	0	10	0	28.57
B. lapidarius	6	2	0	**96**	36	2	19	1	59.26
B. pascuorum	4	1	0	36	**80**	2	12	0	59.26
B. pratorum	2	1	0	1	4	**21**	2	0	67.74
B. terrestris	4	1	1	17	12	1	**54**	2	58.70
B. vestalis	0	0	0	0	0	0	1	**11**	91.67
Testing data									
	B. hortorum	*B. hypnorum*	*B. jonellus*	*B. lapidarius*	*B. pascuorum*	*B. pratorum*	*B. terrestris*	*B. vestalis*	% Classification accuracy
B. hortorum	**7**	0	0	7	2	0	2	0	38.39
B. hypnorum	0	**3**	0	0	0	1	1	0	60.00
B. jonellus	0	0	**5**	1	0	0	8	0	35.71
B. lapidarius	6	0	0	**53**	8	1	10	1	67.09
B. pascuorum	1	0	0	11	**31**	1	8	0	59.62
B. pratorum	0	0	0	0	3	**3**	0	0	50.00
B. terrestris	1	1	0	9	9	1	**14**	0	40.00
B. vestalis	3	0	0	0	0	0	0	**8**	72.73

Table 4.10 The rates of classification accuracy for the eight bumblebee species generated by the SVM model, using a 70% - 30% training – testing data partition. Values in bold indicate accurate classifications.

Testing data									
	B. hortorum	*B. hypnorum*	*B. jonellus*	*B. lapidarius*	*B. pascuorum*	*B. pratorum*	*B. terrestris*	*B. vestalis*	% Classification accuracy
B. hortorum	**4**	0	0	6	1	0	1	0	33.33
B. hypnorum	0	**1**	0	0	0	2	0	0	33.33
B. jonellus	0	0	**1**	0	0	0	7	0	12.50
B. lapidarius	2	0	0	**41**	4	0	6	0	77.36
B. pascuorum	0	0	0	10	**21**	1	4	0	58.33
B. pratorum	0	0	0	0	3	**1**	1	0	20.00
B. terrestris	1	0	0	7	5	0	**10**	0	43.48
B. vestalis	1	0	0	0	0	0	3	**3**	42.86

4.3.2.5 Solitary bee taxon-level classification

The random forest model with the highest overall classification accuracy at the taxon-level within the solitary bees used an 80% - 20% data partition between the training and testing sets. The training model classified solitary bees to Family or genus with an overall accuracy rate of 71.26%, classifying Colletes spp. with 80.00% accuracy, Halictidae spp. with 72.00% accuracy, Hylaeus spp. with 66.67% accuracy, and Megachile spp. with 62.50% accuracy (Table 4.11). When the training model was validated using the testing dataset, the overall accuracy of the model was 90.91%; Colletes spp. were classified with 71.43% accuracy, Halictidae spp. with 100% accuracy, Hylaeus spp. with 100% accuracy, and Megachile spp. with 100% accuracy (Table 4.11).

The SVM model with the highest overall classification accuracy at the taxon-level within the solitary bees used an 80% - 20% data partition between the training and testing sets. The training model solitary bees to Family or genus with an overall accuracy rate of 66.20%. When the training model was validated using the testing data, the overall accuracy of the model was 81.82%; Colletes spp. were classified with 100% accuracy, Halictidae spp. with 100% accuracy, Hylaeus spp. with 60.00% accuracy, and Megachile spp. with 60.00% accuracy (Table 4.12).

Table 4.11 The rates of classification accuracy for the three solitary bee genera/one solitary bee Family generated by the random forest model, using an 80% - 20% training – testing data partition. Values in bold indicate accurate classifications.

Training data					
	Colletes	Halictidae	Hylaeus	Megachile	% Classification accuracy
Colletes	**17**	0	3	4	80.00
Halictidae	4	**12**	1	2	72.00
Hylaeus	5	1	**11**	2	66.67
Megachile	2	1	3	**8**	62.50
Testing data					
	Colletes	Halictidae	Hylaeus	Megachile	% Classification accuracy
Colletes	**5**	0	0	2	71.43
Halictidae	0	**6**	0	0	100
Hylaeus	0	0	**6**	0	100
Megachile	0	0	0	**3**	100

Table 4.12 The rates of classification accuracy for the three solitary bee genera/one solitary bee Family generated by the SVM model, using an 80% - 20% training – testing data partition. Values in bold indicate accurate classifications.

Testing data					
	Colletes	Halictidae	Hylaeus	Megachile	% Classification accuracy
Colletes	**6**	0	0	0	100
Halictidae	0	**6**	0	0	100
Hylaeus	2	0	**3**	0	60.00
Megachile	1	1	0	**3**	60.00

4.3.2.6 Hoverfly taxon-level classification

The random forest model with the highest overall classification accuracy at the taxon-level within the hoverflies used a 70% - 30% data partition between the training and testing sets. The training model classified hoverflies to tribe or genus with an overall accuracy rate of 61.54%, classifying different tribes/genera with between 0.00% accuracy (*Volucella* spp.) and 80.00% accuracy (*Sphaerophoria* spp.) (see Table 4.13). When the training model was validated using the testing dataset, the overall accuracy of the model was 67.65%; with different tribes/genera classified with between 50.00% accuracy (*Syritta* sp. and *Volucella* spp.) and 100% accuracy (*Eristalis* spp. and *Merodon* sp.) (see Table 4.13).

The SVM model with the highest overall classification accuracy at the taxon-level within the hoverflies used a 70% - 30% data partition between the training and testing sets. The training model classified hoverflies to Family or genus with an overall accuracy rate of 47.72%. When the training model was validated using the testing dataset, the overall accuracy of the model was 52.94%; with different tribes/genera classified with between 0.00% accuracy (*Syritta* sp. and *Volucella* spp.) and 83.33% accuracy (*Eristalis* spp.) (see Table 4.14).

Table 4.13 The rates of classification accuracy for the six hoverfly genera/one hoverfly tribe generated by the random forest model, using a 70% -30% training – testing data partition. Values in bold indicate accurate classifications.

Training data								
	Bacchini	Episyrphus	Eristalis	Merodon	Sphaerophoria	Syritta	Volucella	% Classification accuracy
Bacchini	**4**	1	1	0	0	2	0	50.00
Episyrphus	1	**11**	1	0	0	1	0	78.57
Eristalis	0	3	**10**	0	1	2	1	58.82
Merodon	0	0	3	**9**	0	0	0	75.00
Sphaerophoria	1	0	0	0	**8**	1	0	80.00
Syritta	0	0	1	0	1	**6**	0	75.00
Volucella	0	1	6	1	0	1	**0**	0.00
Testing data								
	Bacchini	Episyrphus	Eristalis	Merodon	Sphaerophoria	Syritta	Volucella	% Classification accuracy
Bacchini	**6**	2	2	0	0	0	0	60.00
Episyrphus	1	**4**	0	0	2	0	0	57.14
Eristalis	0	0	**3**	0	0	0	0	100
Merodon	0	0	0	**2**	0	0	0	100
Sphaerophoria	0	0	0	1	**5**	0	0	83.33
Syritta	0	0	1	0	1	**2**	0	50.00
Volucella	0	0	1	0	0	0	**1**	50.00

Table 4.14 The rates of classification accuracy for the six hoverfly genera/one hoverfly tribe generated by the SVM model, using a 70% -30% training – testing data partition. Values in bold indicate accurate classifications.

Testing data								
	Bacchini	Episyrphus	Eristalis	Merodon	Sphaerophoria	Syritta	Volucella	% Classification accuracy
Bacchini	**2**	5	0	0	0	1	0	25.00
Episyrphus	0	**4**	1	0	0	0	0	80.00
Eristalis	0	0	**5**	0	0	1	0	83.33
Merodon	0	0	2	**5**	0	0	0	71.43
Sphaerophoria	0	1	1	0	**2**	0	0	50.00
Syritta	0	0	1	0	0	**0**	0	0.00
Volucella	0	1	1	1	0	0	**0**	0.00

4.4 Discussion

Bioacoustic technology has been widely employed within the fields of ecology and conservation to monitor the occurrence of species from a wide range of taxa (Chesmore & Nellenbach, 2001; Chesmore & Ohya, 2004; Huang et al., 2009; Armitage & Ober, 2010; Briggs et al., 2012; Cheng et al., 2012). Bioacoustics also provides a novel and accessible method by which citizen scientists can involve themselves in large-scale, long-term standardised monitoring schemes (Gradišek et al., 2016), thanks to widespread ownership of personal hand-held technology with built-in sound recording capabilities (August et al., 2015; Gradišek et al., 2016). The focus of most bioacoustics research to date has been on taxa that produce active, species-specific sound, i.e., mating or alarm calls, but our ability to identify species by passively generated sound has yet to be extensively explored (but see Burkart, Lunau, & Schlindwein, 2011; Moore & Hassall, 2016; Gradišek et al., 2016; Kawakita & Ichikawa, 2019). I show that, using machine learning techniques, it is possible to differentiate flower-visiting insects, with varying rates of accuracy and at different levels of taxonomic resolution, based solely upon acoustic features extracted from their wing-beat frequencies. I explore the limitations of this method with regard to its potential for future use as a survey tool for monitoring insect pollinator taxa and provide recommendations for future studies regarding the development of this research.

I was unable to consistently differentiate between flower-visiting insects at either the group- or the taxon-level using either of the simple acoustic features (the fundamental and dominant frequencies), but I was able to distinguish between Hymenopteran and Dipteran insects using the fundamental frequency of their wing beats. This would appear to be contrary to results presented by Moore & Hassall (2016), who found no differences between Hymenopteran and Dipteran insects based upon similar acoustic features. One of the key physiological differences between these two insect orders is their number of wings: the extra pair of wings present in Hymenopteran insects would increase total wing surface area, which has been linked to lower wing-beat frequencies (Corben, 1983; Ha et al., 2013). Past studies have also observed that greater body size, body mass, and wing length are all associated with decreasing wing beat frequencies (Byrne, Buchmann, & Spangler, 1988; Molloy et al., 1988; Burkart, Lunau, & Schlindwein, 2011;

Gradišek et al., 2016; Miller-Struttmann et al., 2017). Most of the Hymenopteran insects sampled as part of this study were larger and more robust than the Diptera (personal observation), which was reflected in the fundamental frequencies of their wing beats (Tables 4.1 & 4.2). If this result remains consistent in a larger, more diverse dataset, then this simple classifier may provide a quick method of differentiating between these two orders.

It is similarly unsurprising that *Bombus vestalis* can be reliably distinguished from most other bumblebee species recorded during this study, based upon the significantly lower dominant frequency of their wing beats. *Bombus vestalis* is a cuckoo bumblebee and is therefore only represented by males and reproductive females, which are analogous to queens in other *Bombus* species in terms of size (Goulson, 2010). Since the other bumblebee species recorded during this study were almost entirely represented by smaller workers and males, it is logical that *B. vestalis* individuals would be easily separable from them by virtue of their body mass alone. What is surprising is that the solitary bees, as a group, appear to beat their wings with a lower dominant frequency than honeybees, social wasps, and bumblebees, all of which are, on average, larger than the solitary bee genera recorded as part of this study. This would run counter to the existing evidence showing that wing-beat frequencies in insects are related to body size (Unwin & Corbet, 1984; Byrne, Buchmann & Spangler, 1988). Alternatively, this may indicate that the dominant frequency may not be an effective method of representing complex acoustic signals since it is largely defined by amplitude, which can be affected by other factors than those relating to morphology: proximity to the microphone, for example (De Luca et al., 2018).

The results of the machine learning classification algorithms were far more promising. Of the two algorithms compared here, the random forest models performed better in all insect classes, mirroring the results of Gradišek et al. (2016), as well as those of Kampichler et al. (2010) and Kawakita & Ichikawa (2019). Although the SVM models often presented with higher overall levels of classification accuracy than the random forest models, in terms of individual insect classes they were always biased towards the most abundant insect classes. This was likely due to my being unable to specify class weights in the SVM models during the model training process, which was possible within the random forest models.

Within the random forest models, a seventy per cent/thirty per cent partition between the training and testing sets performed best in terms of classification accuracy within models with a large amount of available data (see Tables 4.3, 4.5 & 4.9), whereas an eighty per cent/twenty per cent partition performed better in models with less available data (see Tables 4.7 & 4.11). Future data collection should, therefore, focus on generating a much more balanced dataset, with enough data in each insect class to provide an adequate training set; I would recommend collecting recordings from at least 100 individuals per class.

Combining the random forest models with the more comprehensive feature set extracted using the openSMILE toolkit, I was able to classify flower-visiting insects to order with 84.97 per cent accuracy, which increased to 86.63 per cent when the training model was validated with new data. This supports my earlier finding that Hymenopteran and Dipteran insects are distinguishable from one another based upon the sound of their wing beats.

An even higher level of classification accuracy was reached at the group-level within the Diptera: 84.07 per cent, rising to 93.10 per cent when the training model was validated. Differences in flight kinematics may account for this. No distinctions were made between hovering and non-hovering flight during the field recordings, and research suggests that hovering flight not only has different mechanics than regular flight (Ellington, 1984b; Lei Mou, Peng Liu, & Sun, 2011), but may also be associated with specific wing morphometrics (Ellington, 1984a); all of which may affect the wing-beat frequency of hoverflies in relation to non-syrphids. Research by Rashed et al. (2009) also indicates that hoverflies and non-syrphid flies may be separable in terms of flight acoustic signals, although Moore & Hassall (2016) found no differences between the two groups.

Classification accuracy within the Hymenopteran groups was slightly lower: 70.91 per cent, rising to 72.47 per cent after validation. This is lower than the rates of classification accuracy found by Kawakita & Ichikawa (2019), especially within the “wasp” category. In the absence of differing flight mechanics, distinguishing between Hymenopteran insects is likely to be a function of the size variation between groups. This could explain why the honeybee and social wasp groups achieved lower levels of classification accuracy, as both have a fairly generic body form common to many Hymenopteran insects, combined with a body size that overlaps with that of many other

Hymenopteran groups. This, combined with the fewer recordings available for model training within the social wasp group, may be the reason why these two groups were more likely to be misclassified than the solitary bees (which represent a wider range of body sizes) and bumblebees (which are, on average, larger and more robust than other UK Hymenoptera).

The wide range of body sizes present within insect taxa, especially within the UK bee fauna (Falk, 2015), makes this kind of group-level analysis impractical. It was computed here due to high levels of data imbalance between the bumblebees and the other hymenopteran genera, but it would be far more informative to focus on collecting a more diverse dataset, with a balanced number of recordings from a range of genera and body size classes, to test the extent to which we can classify individuals at the genus- or family-level. Indeed, the taxon-level random forest models show that solitary bee genera and Families can be classified to a surprisingly high level of accuracy: 100 per cent within the Halictidae, *Hylaeus*, and *Megachile* during after model validation. These taxa represent a range of size classes, from the smaller *Hylaeus* spp. and Halictidae spp.[1] to the larger *Megachile* spp., which could explain this result if body size or mass is strongly related to wing beat frequency.

This level of classification accuracy is analogous to those from other studies focused on classifying Orthoptera based upon stridulation signals (see Chesmore, 2001, 2004; Chesmore & Nellenbach, 2001; Chesmore & Ohya, 2004), which makes this result all the more remarkable. Although, the testing set was small enough within all solitary bee taxa that further data would be required to truly validate this finding. The results concerning bumblebee species classification add further credence to the hypothesis that Hymenopteran wing-beat frequencies are strongly related to body size. The rates of classification accuracy within different bumblebee species ranged from between 28 and 91 per cent, where the highest level of accuracy was found in *Bombus vestalis*, validating earlier results concerning the dominant frequency feature. This wide disparity in classification accuracy would be expected if wing-beat frequency was strongly related to body

[1] Most of the Halictidae recorded during this study were the smaller, metallic green species, typified by Lasioglossum morio or Halictus tumulorum.

size or mass. Most of the bumblebees recorded as part of this study were workers, and many bumblebee species in the UK overlap in terms of worker size (Falk, 2015). In addition, intraspecific bumblebee worker size can vary by as much as ten times over the course of a year (Peat, Tucker, & Goulson, 2005) based upon a number of factors, including the quantity and quality of forage in the surrounding area (Persson & Smith, 2011), the foraging requirements of the colony (Peat, Tucker, & Goulson, 2005), and colony age (Couvillon et al., 2010). Classifying bumblebees to species based solely upon their wing beat frequencies is likely, therefore, to present a significant challenge. This makes the results of Gradišek et al. (2016) all the more surprising, since they were able to classify many bumblebee species to a high degree of accuracy using the same method as presented here, and with fewer individuals to train their random forest models.

Classification accuracy at the genus-level was higher for the hoverflies, at levels comparable to the models for solitary bee genera. There were also clear size differences between the hoverfly genera recorded as part of this study (Stubbs & Falk, 2002), though these do not appear to relate to any kind of pattern regarding classification accuracy. For instance, both *Merodon* and *Volucella* species are similar in terms of body size and shape, both being bumblebee mimics (Stubbs & Falk, 2002; Rotheray & Gilbert, 2011), and yet individuals from the genus *Merodon* were classified with 100 per cent accuracy after validation compared to the 50 per cent accuracy found in the *Volucella*. The genus *Sphaerophoria*, which represents a fairly unique, elongate body shape among the hoverfly taxa recorded here (Stubbs & Falk, 2002), was classified quite well. Other morphological features may be more important predictors of wing beat frequency in hoverflies, wing length for instance (Ottenheim & Volmer, 1999; but see Cheng & Sun, 2016), or the ratio of wing surface area or wing length to body mass (Corben, 1983; Ha et al., 2013). This would benefit from further research (as in Ha et al., 2013), which could be extended to other flower-visiting insect taxa and then used to inform a more relevant acoustic feature set, aimed at better representing the wing beat frequencies of specific taxa. However, as with the solitary bee genera, the size of our testing set for different hoverfly genera was small enough that we would need to collect more data to validate this and our findings regarding classification accuracy.

4.5 Conclusions

These results suggest that accurate and reliable classification of flower-visiting insects using wing beat frequencies may be possible, especially at the higher levels of taxonomic resolution. Our ability to classify individuals to genus- or species-level is highly likely to be reliant on the extent of the size variation between taxa. The bumblebee model found was unable to reliably classify different species due to overlapping size classes within the bumblebee group, likely also combined with high levels of intraspecific size variation; but this may be just as true for solitary bee or social wasp species. The Halictidae are, on average, smaller than most other bee families, but contain species that range from 4mm to over 1cm in length (Falk, 2015), and many species from different bee families or genera overlap in terms of size. In addition, many insect species are sexually dimorphic (Falk, 2015), which may cause further intraspecific differences in terms of wing beat frequency. This requires further research in the form of a more diverse, balanced dataset that includes recordings representing the full range of the UK's flower-visiting insect taxa. Since this would involve sampling ca. 6000 species in total (Falk, S. 2018, personal communication, 01 November), a dataset that aimed to characterise the level of size variation present within key flower-visiting insect taxa may be more practical. This would still allow us to answer questions concerning our ability to classify individuals at different levels of taxonomic resolution, and those concerning the effects of size variation between taxa on wing beat frequency. If recorded individuals were also lethally sampled, then morphometric data could also be generated concerning body mass or wing surface area etc. The relationship between this functional diversity and different acoustic features, like the fundamental frequency, would allow us to evaluate inter- and intraspecific differences in wing beat frequency as it relates to morphology (see Gradišek et al., 2016; Miller-Struttmann et al., 2017).

In terms of the machine learning classification methods, the random forest models provided a high level of classification accuracy within several groups, allowing an inherently unbalanced dataset to be reweighted through down-sampling. There are, however, other classifiers that could be explored, specifically the artificial neural networks (ANNs) used in Chesmore (2001), Chesmore & Nellenbach (2001), and Chesmore & Ohya (2004), which achieved higher levels of

classification accuracy than those found here. And, whereas the feature set extracted using openSMILE contained many frequency-domain features that have previously been used to successfully classify insect taxa using wing beat frequency (Raman, Gerhardt, & Wilkerson, 2007; Gradišek et al., 2016; Kawakita & Ichikawa, 2019), the performance of alternative feature extraction tools also deserves investigation, including the time-domain signal coding (TDSC) methods used by Chesmore (2001) and the more pared-down feature sets of Raman et al. (2007) and Kawakita & Ichikawa (2019). There are also alternative feature sets available through the openSMILE toolkit that could be explored (Eyben et al., 2013).

Regarding the use of bioacoustics as a potential survey tool for citizen scientists - this would depend largely on what the monitoring was aiming to achieve. It is clear from my results that the reliable acoustic classification of flower-visiting insects to any level of taxonomic resolution would require both more testing and more data before being applied to any kind of standardised survey protocol. Despite achieving high levels of classification accuracy between Hymenopteran and Dipteran insects, there are many other insect orders that I have not recorded and tested, and therefore have no data concerning how their acoustic signals may overlap with those of the two orders tested here. Otherwise, at the moment, its greatest utility would be as a classification tool between Hymenopteran and Dipteran insects during surveys like the Flower-Timed Insect Counts (FIT Counts) employed by the UK PoMS (UK Pollinator Monitoring Scheme, 2021). Individuals could make recordings and then classify them using machine learning algorithms based in software on their smartphone (similar to Gradišek et al., 2016, and Mukundarajan et al., 2017). My results were generated using recordings made by an affordable, mid-range Dictaphone and a lapel microphone; equivalent data should be achievable using a modern smartphone with an in-built microphone (August et al., 2015). This would reduce the collector bias typically associated with visual surveys, but would run the risk of misclassifying “other” insects as members of the Hymenoptera or Diptera. Alternatively, it is possible that I could adapt the random forest models (or explore other classification methods) to simply recognise insect wing beats in general (e.g., Heise et al., 2017), and then test the effectiveness of this within a FIT Count-type survey to detect flower-visiting insect activity without any further taxonomic classification. This may still yield

interesting data that could be tied to overall insect decline (Penone et al., 2013), but would provide no species-level population data and would be difficult to tie to measures of insect abundance, since it would be possible to record individuals multiple times (Gibb et al., 2019).

Chapter 5

Acoustic pan trapping: testing the performance of a novel survey method for flower-visiting insects

"…for hundreds, maybe thousands of years, physicians have listened to our hearts and our lungs and our intestines to check their health. And in the last few years, ecologists have started doing the same thing to assess the health of ecosystems." (Eldridge, 2019)

5.1 Introduction

Advances in technology are transforming the ways in which we survey and monitor organisms (August et al., 2015). From the advent of the internet and smartphone technology, enabling wide-spread citizen science involvement in biological monitoring through applications like iRecord[2], to advances in molecular techniques providing us with the ability to monitor the environment non-invasively through the use of eDNA (Barnes & Turner, 2016; Thomsen & Sigsgaard, 2019), technology is increasing the spatial and temporal scales over which we can monitor ecosystem change. This comes at a time when the need to record long-term ecological data at large spatial scales is more relevant than ever. Declines within insect pollinator populations in response to anthropogenic drivers are challenging to monitor at large spatio-temporal scales due to a shortage of both trained, field-capable personnel (Carvell et al., 2016) and taxonomic skills (Agnarsson & Kuntner, 2007; Drew, 2011; Timms et al., 2013), especially when combined with the relative lack of standardised monitoring protocols for groups like insect pollinators (Westphal et al., 2008; Popic, Davila, & Wardle, 2013).

There are several responses to this: 1) the use of citizen scientists to gather records, which has the potential to provide long-term, large-scale, continuous data, but with the joint caveats that the taxonomic resolution of these data is often low, and that effort must be expended to maintain and motivate volunteers (Roy et al., 2012); and 2) the development and introduction of novel passive

[2] https://www.brc.ac.uk/iRecord/

monitoring tools. Passive monitoring techniques allow ecological communities to be surveyed without the direct involvement of surveyors. Recent developments in drone technology (Koh & Wich, 2012; Ivošević et al., 2015; Hodgson et al., 2018), weather radar (Chilson et al., 2012; Frick et al., 2017; Shamoun-Baranes et al., 2019), light detection and ranging (LIDAR) (Malmqvist et al., 2018), and the use of recorded video footage (Steen, Lene, & Orvedal, 2011; Gilpin, Denham, & Ayre, 2017; Steen, 2017), have provided novel tools for the non-invasive, passive monitoring of animal communities, including insect pollinators. But research regarding how many of these methods compare to more traditional survey techniques is on-going.

Passive acoustic monitoring is an extension of the bioacoustic survey method explored within **Chapter 4**, using autonomous acoustic sensors to provide long-term soundscape recordings across varied spatial scales (Gibb et al., 2019). The term "soundscape" refers to the sum of all sound that emerges from a landscape (Pijanowski et al., 2011). These sounds are not just biological in nature (what is termed "biophony"), but also include sounds relating to human activity ("anthrophony", sometimes also called technophony), and ambient noise, such as wind, rain, and thunder ("geophony") (Pijanowski et al., 2011; Sueur & Farina, 2015; Farina, 2018). The collection and analysis of sound at a landscape scale enables not only the exploration of species interactions and diversity (Aide et al., 2013; Gasc et al., 2013; Campos-Cerqueira & Aide, 2017), but also their reactions of drivers of change, including anthropogenic activity (Penone et al., 2013; Lecchini et al., 2018; Lopez-Tello & Muthukumar, 2018) and natural phenomena (Galen et al., 2019); a field referred to as **ecoacoustics** (Sueur & Farina, 2015). Passive acoustic survey techniques are non-destructive and non-invasive (Gibb et al., 2019), and are often combined with machine learning classification methods to aid in the identification of different taxa (Aide et al., 2013).

Passive acoustic sensors have been used to provide data within a number of systems, including freshwater (Linke et al., 2018; Desjonquères, Gifford, & Linke, 2019), terrestrial (Chesmore, 2001; Payne, Thompson, & Kramer, 2003; Kalan et al., 2015; Wrege et al., 2017), and marine environments (Simon et al., 2010; Parks et al., 2011; Stimpert et al., 2011; Lecchini et al., 2018). Past uses include the detection and monitoring of species populations (Chesmore, 2001; Payne,

Thompson, & Kramer, 2003; Kalan et al., 2015), monitoring the effects of anthropogenic disturbance or activity upon species behaviour or community composition (Penone et al., 2013; Lecchini et al., 2018), and observing the effects of acoustic diversity upon human enjoyment of, or the mental health benefits associated with, urban greenspaces (Watts, Miah, & Pheasant, 2013; Medvedev, Shepherd, & Hautus, 2015; Moscoso, Peck, & Eldridge, 2018). In relation to insect taxa, passive acoustic monitoring has been used to explore the effects of urbanisation and agricultural intensification upon Orthopteran communities, showing that community diversity was negatively affected by human activity (Penone et al., 2013). And more recently, it has also been used to monitor and investigate flight activity in bees: Miller-Struttmann et al. (2017) used microphones set at flower height to monitor bumblebee activity in alpine meadows, showing that measures of acoustic activity were positively correlated with the results of visual transect surveys and with local pollination service provision, indicating that passive acoustic monitoring could provide a novel method for quantifying certain ecosystem services. Galen et al. (2019) also used passive acoustic sensors to monitor "bee" activity during a solar eclipse, showing that complete darkness at the height of the eclipse disrupted flight activity. However, since the authors did not ground truth their acoustic observations in any way, it is impossible to know whether the sounds that they were detecting came solely from foraging bees or were, as I suspect, representative of a broader range of insect taxa.

The recent uptake of acoustic monitoring within ecology has been facilitated by the proliferation and affordability of recording devices (Gibb et al., 2019). Autonomous acoustic sensors are now routinely capable of recording for periods in excess of several weeks (Whytock & Christie, 2016; Hill et al., 2018, 2019), and make use of single-board computers like the Raspberry Pi or Arduino to employ open-source operating systems that are programmable by the user (Whytock & Christie, 2016). These, in turn, allow for a variety of different recording schedules to be pre-programmed prior to data collection, while giving practitioners the option to focus on specific sound thresholds, such as ultrasound, or to alter microphone sensitivity in response to perceived or predicted background noise (Whytock & Christie, 2016). The cost of these sensors is variable, but the use of passive acoustic monitoring is still invariably cheaper, in terms of both time and

expense, than employing surveyors to collect data in the field (Gibb et al., 2019). Data storage on these devices often requires the use of micro-SD cards, which do require continual replacement when recording for long periods of time (Whytock & Christie, 2016). Although, technology now enables wireless internet connectivity between some acoustic sensors and a central storage hub, so that audio files can be transferred directly to the user without the need for internal storage (Sheng et al., 2019).

Notable advances have also been made in developing automated detection and classification tools for acoustic signals within soundscapes (Gibb et al. 2019), although manual counts are still often used, particularly when attempting to quantify classification or detection errors (Gibb et al. 2019). A wide range of different supervised and unsupervised tools for ecoacoustic analyses can be accessed via open-source software, such as R (Sueur, Aubin, & Simonis, 2008; Katz, Hafner, & Donovan, 2016; Ligges et al., 2018; Anikin, 2019; R Core Team, 2021), Python (van Rossum & Drake, 2009; Giannakopoulos, 2015; Fonseca et al., 2019), and Biosounds (Darras et al., 2020), but the use of these tools assumes a familiarity with both programming and acoustic analysis. Many researchers have also developed specific algorithms for their own projects. Heise et al. (2017) used MATLAB to create an automated detection tool based upon Auditory Scene Analysis, which was later used successfully by Miller-Struttmann et al. (2017). There is also a range of commercial software tools that facilitate automated acoustic detection and analysis. Kaleidoscope Pro (© Wildlife Acoustics, Inc., 2019), for instance, provides an intuitive supervised cluster analysis tool based upon Hidden Markov Models, that is capable of identifying and aggregating similar acoustic signals. And while this programme has been used to detect both bat and avian acoustic signals (Abrahams & Denny, 2018; Ross et al., 2018), it has yet to be tested with insect acoustic signals. These commercial tools are often expensive, but do not require extensive programming ability or in-depth prior knowledge of acoustic analysis, creating a low barrier to entry for conservation practitioners.

In light of recent results by Heise et al. (2017), Miller-Struttmann et al. (2017), and Galen et al. (2019), it is clear that autonomous passive acoustic sensors can be used to generate soundscape recordings from which insect flight sounds can be extracted and analysed to answer relevant

ecological questions at a range of spatial scales. By combining an autonomous acoustic recording device with the design of a traditional passive survey method aimed at insect pollinator species, such as the pan trap, I aim to create a template for a non-destructive, passive acoustic survey technique focused on attracting and sampling flower-visiting insect communities through the passively generated sound of their wing beats. The addition of a visual attractant to the simpler recording method employed by Miller-Struttmann et al. (2017) would standardise the technique, potentially allowing it to be used within a range of habitats with differing levels of floral diversity. However, the performance of this novel "acoustic pan trap" would still need to be compared with that of more traditional survey methods beyond the visual transects used by Miller-Struttmann et al (2017), in order to assess its utility in terms of wider scale insect pollinator monitoring.

Since pan trapping will form the basis of the design off this new passive acoustic survey method, it would make sense to use this method as one basis for methodological comparison. Pan trapping is a similar passive survey method aimed at flower-visiting insect species, that uses brightly coloured bowls filled with water to attract and then destructively sample insects (Westphal et al., 2008). A comparison with this method would also allow me to assess how well with measures of acoustic animal activity relate to measures of animal abundance – a facet of ecoacoustic monitoring that is still poorly understood due to the issue surrounding the assumed non-independence of acoustic signals (Marques et al., 2013; Gibb et al., 2019). That is, it can be difficult to assess whether ten approximately identical bird calls come from ten individuals of the same species or a single individual calling multiple times.

Of the three standardised survey techniques currently employed by the UK Pollinator Monitoring Scheme (PoMS): pan trapping, standardised transect surveys, and Flower-Insect Timed Counts (FIT Counts), the FIT Count (a timed focal floral resource observation) would provide another logical source of comparison. Focal floral resource observations (FFOs) involve observing a focal plant or vegetative plot and counting the number of insect individuals that visit during a specific time frame (Potts, Evan, & Boone, 2005; Westphal et al., 2008; Roy et al., 2016). If individuals are not destructively sampled upon visiting the focal resource, then FFOs provide a measure of flower-visiting insect activity that would be conceptually similar to the acoustic insect activity

recorded by an acoustic pan trap. FFOs can also be carried out at intervals throughout the day, providing a measure of diel activity within local insect communities that could be compared to the diel acoustic activity recorded by an acoustic pan trapping station.

Indeed, this novel acoustic pan trapping method occupies an interesting middle-ground between these two established survey methods. As a passive survey method, it lacks the collector bias associated with active observation-based methods like FFOs, while providing a non-destructive alternative to traditional pan trapping. However, the relative success of this novel method would be heavily reliant upon matching this survey technique with suitable automated detection software that could reliably extract individual instances of insect flight sound from whole soundscape recordings. The acoustic data would also be unable to provide any form of taxonomic resolution until classification algorithms (like those discussed in **Chapter 4**) have been more thoroughly tested on a broad range of flower-visiting insect taxa.

5.1.1 Aims

In this chapter, I will test the performance of a novel passive acoustic survey method: an acoustic pan trap, in relation to two common survey techniques: traditional water-based pan trapping and focal floral resource observations. I will also investigate the reliability and accuracy of the commercially available Kaleidoscope Pro acoustic analysis software, in terms of its ability to detect and identify instances of insect flight sound from whole soundscape recordings. I will address the following questions:

1. What are the Type 1 (false positive) and Type 2 (false negative) error rates associated with the Kaleidoscope Pro cluster analysis tool?
2. Are there differences between the number of instances of insect flight sound clustered by Kaleidoscope Pro and the number of instances of insect flight sound present within the soundscape recordings?
3. Is there a relationship between the number of instances of insect sound recorded by the acoustic pan traps and the number of insects sampled by traditional water-based pan trapping or observed during hourly focal floral resource observations?

4. Is there a relationship between the number of instances of insect sound recorded per hour by the acoustic pan traps and the number of insects observed during hourly focal floral resource observations?
5. Is the number of instances of insect sound recorded per hour by the acoustic pan traps affected by local environmental variables, specifically hourly temperature (°C), hourly wind speed (mph), hourly rainfall (mm), and local floral abundance?

5.2 Materials & Methods

5.2.1 Data collection

Acoustic data were collected from thirteen sites between August and October 2017, and from seven sites between June and September 2018, of which six were repeat visits to sites from 2017. These sites were a mixture of urban and rural areas within Leeds, West Yorkshire, and its surrounding suburbs (see Table 5.1), each covering an area of approximately 100 x 100m and separated by a minimum of 1km. The sites were primarily a mixture of amenity grassland and semi-natural grassland. The amenity grassland sites consisted of urban parks and playing fields, where the grass sward was short and routinely mown and the flora consisted of a mixture of hardy species like *Bellis perennis* and *Taraxacum* spp. Some of these amenity sites contained areas of grass left unmown to promote biodiversity, which contained a slightly broader range of floral species, including Red Clover (*Trifolium pratense*) and Common Ragwort (*Senecio jacobaea*). The semi-natural grassland sites were defined by a flora consisting of Common Bird's-foot trefoil (*Lotus corniculatus*), Red Clover, Common Ragwort, and Common Hogweed (*Heracleum sphondylium*). Site visits were carried out during fair weather, where temperatures were between 13-25°C, wind speeds were not more than 10mph, and there was no sustained rainfall. The hourly temperature (°C), hourly wind speed (mph), and hourly rainfall (mm) were recorded for each sampling date using data from the UK Met Office MIDAS station at Bramham (SE 448416), accessed via the Centre for Environmental Data Analysis (CEDA) archive (Met Office, 2020). Each site was visited and sampled once per year, with the following three sampling protocols being deployed in parallel (Fig. 5.3).

5.2.1.1 Acoustic pan trap design

The design of the acoustic pan trap was based upon the SOLO acoustic recorder (Whytock & Christie, 2016). SOLO is an open-source, autonomous recording tool comprised primarily of a Raspberry Pi single board computer, a sound card, an external battery pack, a microphone, and a waterproof container (see Fig. 5.1), and is designed to record sounds of up to 22.05 kHz. for up to forty days at a time (Whytock & Christie, 2016). The Unix-based operating system is open-source, and, through the Raspberry Pi, various aspects of the software can be altered to suite individual project requirements (Whytock & Christie, 2016). The SOLO recorder was chosen because it is affordable (ca. £110 per recorder), versatile, and designed for self-assembly; it has also been extensively field-tested in the UK by its designers (Whytock & Christie, 2016).

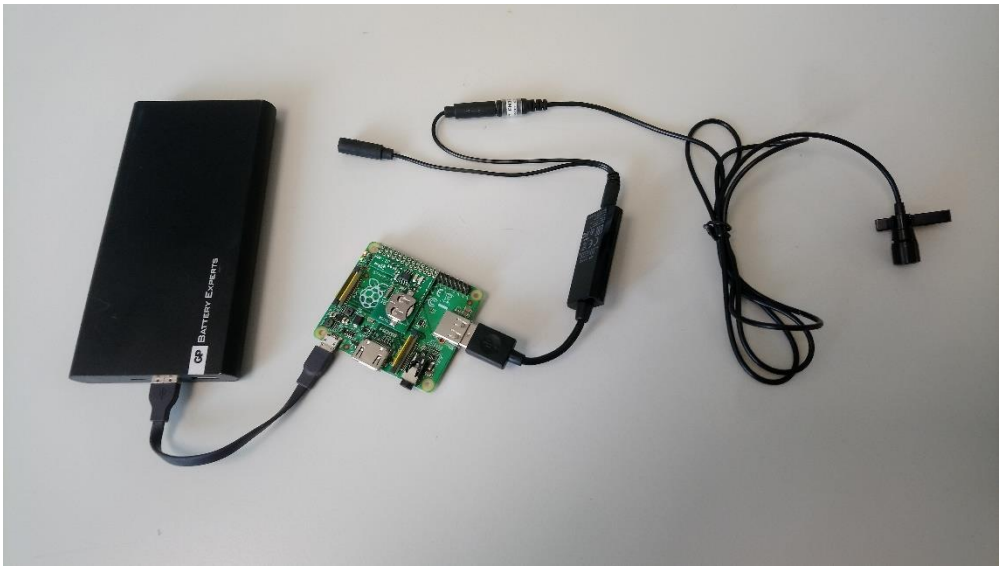

Figure 5.1 The internal components of a single SOLO acoustic recorder. From left to right: the external battery pack, the Raspberry Pi single board computer, the USB soundcard, and the microphone.

A plastic picnic plate (433.74cm^2) was used in place of the traditional water-filled trio of bowls, and, as per the results from **Chapter 2**, plates with a wider surface area were chosen to create an attractive, non-destructive surface for foraging insects. These plates were painted with UV-fluorescent yellow paint (Wilson et al., 2016). This colour has been shown to be attractive to a range of different insect pollinator taxa, as seen in **Chapter 2** (Bowie et al., 1999; Laubertie, Wratten, & Sedcole, 2006; Gollan, Ashcroft, & Batley, 2011; Vrdoljak & Samways, 2011; Heneberg & Bogusch, 2014). A single colour was chosen over the traditional trio of UV-

fluorescent yellow, blue, and white associated with pan trapping due to the placement of the microphone on the plates. If the plates were painted in thirds radiating from the centre, this would have left at least one colour behind the microphone, potentially biasing the resulting recordings towards those insects attracted to the remaining colours.

The picnic plate was attached to a stand on a wooden stake, set at approximately the same level as the surrounding flowering vegetation (Westphal et al., 2008; Tuell & Isaacs, 2009). There were six heights along the wooden stake to which the stand could be raised, starting at approximately 10cm above ground level and increasing in 10cm increments to approximately 60cm above ground level. The SOLO acoustic recorder was set underneath the plate and the microphone was attached to the centre of the plate, tilted slightly upwards (see Fig. 5.2).

Figure 5.2 A single passive acoustic pan trapping station, as deployed in the field.

5.2.1.2 Pan trapping protocol

The pan trapping protocol used here was based upon the results from **Chapter 2**. Five pan trapping stations were set out at each site at regular intervals along either a 50m or a 100m transect (Fig. 5.3), depending upon the area of the site in question. A distance of 10m (on a 50m transect) or 20m (on a 100m transect) was left between neighbouring pan trapping stations, so as to avoid competition between them for insects (Droege et al., 2010). Each pan trapping station consisted of three 296ml bowls, painted UV-fluorescent yellow, blue, and white (Sparvar Leuchtfarbe). These bowls were filled with ca. 180ml of water and approximately one drop of un-scented detergent and attached to a stand on a wooden stake at approximately the same level as the surrounding flowering vegetation. As with the acoustic pan traps, the bowls could be raised to one of six heights along the wooden stake, starting at approximately 10cm above ground level and increasing in 10cm increments to 60cm above ground level. Each pan trapping station was set out between 08:30 - 09:30 AM and was left active for approximately seven hours. Specimens collected from the pan traps were strained through muslin and preserved in 70% ethanol for identification. Samples from each pan trapping station were stored separately by bowl colour.

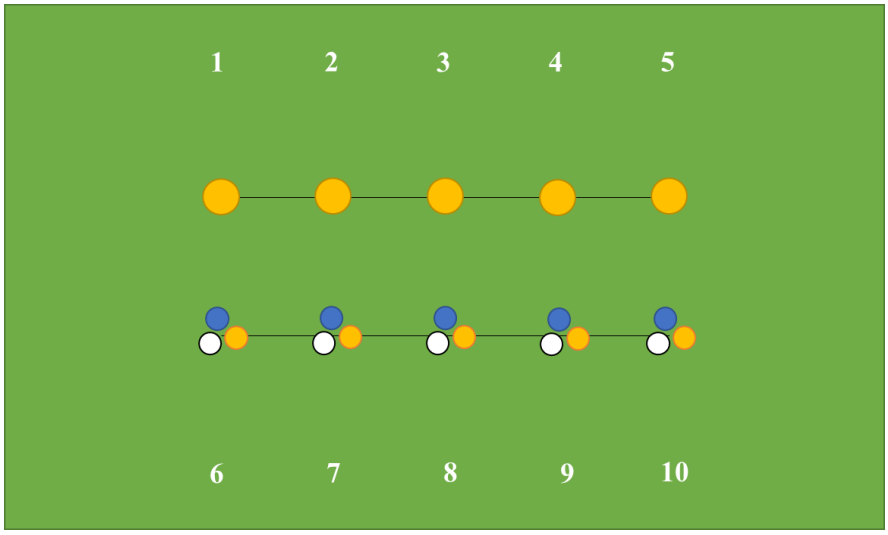

Figure 5.3 A visual representation of the experimental set-up during each survey: two parallel 50m transects, set at least 10m away from one another, with either traditional pan trapping stations (the trio of white, yellow, and blue circles) or acoustic pan trapping stations (large yellow circles) set at equal distances (10m). The numbers in white indicate the potential positions for the random placement of the focal floral resource observations.

5.2.1.3 Acoustic pan trapping protocol

Five acoustic pan trapping stations were set out along a second 50m or 100m transect, run in parallel to the pan trapping transect (Fig. 5.3). A distance of 10m (on a 50m transect) or 20m (on a 100m transect) was left between neighbouring acoustic pan trapping stations, so as to avoid competition between them for insects (Droege et al., 2010) (Fig. 5.3). A 10m distance was left between the acoustic and traditional pan trapping transects. Each acoustic pan trapping station was set out between 08:30 - 09:30 AM and was left active for approximately seven hours. Acoustic data were exported from the 64GB micro-SD cards using the Linux Reader 2.7 software (DiskInternals) and stored securely on University of Leeds' servers. After each use, the SOLO Operating System Image (SOSI) was flashed onto the micro-SD card using Win32 Disk Imager 1.0 software[3].

5.2.1.4 Floral abundance counts

In addition to local weather variation, the floral abundance present within a two meter radius of each pan trapping and acoustic pan trapping station was also recorded. As in **Chapters 2**, each flower was counted as a single floral unit and then multiplied by the estimated volume of nectar sugar produced per flower per 24 hours (µl) found in Baude, Kunin, & Memmott (2015), following the method presented in Baude et al. (2016) and O'Connor et al. (2019). For species with where the flowers comprise multiple florets, e.g., the racemes belonging to members of the Fabaceae, I counted each flower head as a single floral unit (Carvell et al., 2007). I then counted the number of florets belonging to representative three flower heads of each of these species and calculated the average number of florets per head. This value was then multiplied by the volume of nectar sugar produced per floret per day to provide the volume of nectar sugar produced per floral unit. This process allowed me to account for the differing levels of floral reward produced by different plant species and brought this analysis into line with the findings from **Chapter 2**. . For information concerning how each floral species was classified in terms of what constituted a floral unit, the average number of florets per floral unit, and the estimated volume of nectar sugar

[3] https://sourceforge.net/projects/win32diskimager/

produced per floret per day and per floral unit per day, see Supplementary Table 3.1. For a summary of the relative abundance of the floral species within a two-meter radius of each acoustic pan trapping station per site, see Supplementary Table 3.2.

5.2.1.5 Focal floral resource observation protocol

I designed the focal floral resource observation (FFO) protocol specifically for this experiment, although aspects were based upon the protocol used by the UK's PoMS Flower-Insect Timed Counts (FIT Counts) (UK Pollinator Monitoring Scheme, 2021), as well as the method used by Roy et al. (2016). The primary advantage of this protocol is that it does not rely on existing on-site floral resources to survey insect activity, and instead uses a portable potted plant to attract insect visitors. This was advantageous because most of my survey sites were urban in nature and several had little in the way of existing floral resources (either in terms of abundance or species richness).

The focal floral resource I chose comprised a single potted *Rudbeckia fulgida* var. 'Little Goldstar', purchased from B&Q[4]. *Rudbeckia fulgida* is an herbaceous perennial plant native to North America (Campbell & Seymour 2013) while the "Little Goldstar" cultivar is commonly found in garden centres across the UK. A member of the Asteraceae, the inflorescences (capitula) of this plant are formed of yellow ray florets surrounding a conical dark brown disk. The florets produce a faint scent, and the stamens produce small quantities of pollen (Uebelhart, 2011). I can find no data concerning nectar production by either *R. fulgida* or the "Little Goldstar" cultivar. *Rudbeckia fulgida* also has ultraviolet-absorbing nectar guide patterns present on its ray florets that are visible to insects, the size of which have been shown to enhance visitation rates by insects in Rudbeckia cultivars (Horth, Campbell, & Bray, 2014).

Rudbeckia fulgida has been shown to be attractive to flower-visiting insect taxa. Rollings & Goulson (2019) ranked *R. fulgida* 26th out of 111 plant cultivars in terms of attractiveness to insect pollinators, noting that it was attractive to a wide variety of pollinator groups (Simpson's D =

[4] https://www.diy.com/

0.75), in particular - solitary bees. While Harris et al. (2016) found that *R. fulgida*, along with two similar species: *R. hirta* and *R. triloba*, were visited by a wide range of beneficial insects, including pollinator taxa such as bees and hoverflies. Rudbeckia spp. are also listed on the Royal Horticultural Society's list of "Plants for Pollinators"[5], although Garbuzov, Alton, & Ratnieks (2017) showed that, while plants with this label are often more attractive to insect pollinators than plants that are not, many ornamental cultivars found in UK garden centres exhibit lower visitation rates when compared to known attractive plant species[6]. But two of the three Rudbeckia cultivars tested by Garbuzov, Alton, & Ratnieks (2017) (*R. hirta* "Toto Gold" and *R. triloba* "Prairie Glow") did compare favourably with other ornamental cultivars in terms of insect pollinator visitation; although, neither *R. fulgida* nor the "Little Goldstar" cultivar were included in their study.

The use of a Rudbeckia cultivar as the focal floral resource will naturally have biased the results of the FFOs in favour of those insects that are attracted to flowers with a similar shape, scent, and colour. However, a short period of ad-hoc observation in the garden centre where I purchased the plants demonstrated that our chosen cultivar attracted a diverse range of insect taxa, including bumblebees, honeybees, hoverflies, and non-syrphid Diptera. Another plant species that has been used in FFOs is lavender (*Lavendula* spp.) (Roy et al., 2016), but evidence suggests that this plant is primarily a source of forage for longer-tongued insect species (Balfour, Garbuzov, & Ratnieks, 2013).

FFOs were carried out once per hour over the seven-hour survey period during which the pan traps and acoustic pan traps were active. Once per hour at approximately ten minutes past the hour, a single potted *R. fulgida* var. 'Little Goldstar' plant was placed at least 10m away from an acoustic/pan trapping station, the position of which was decided at random with a ten-sided die (Fig. 5.3), at a perpendicular angle to the station in question. The plant was then left undisturbed for ten minutes to give insects foraging in the local area time to acclimatise to its presence. After

[5] https://rhs.org.uk/plantsforpollinators
[6] Garbuzov, Alton, & Ratnieks (2017) used marjoram (*Origanum vulgare*).

the acclimation period, the plant was observed for twenty minutes and each insect visitation to the plant was recorded along with the identity of the visitor. Insects were identified to species where possible, otherwise insect identity was marked using the system used by the PoMS FIT Counts, where insects are labelled as either bumblebees, honeybees, solitary bees, wasps, hoverflies, other flies, butterflies and moths, beetles (larger than 3mm), small insects (smaller than 3mm), or as other insects.

A single potted *R. fulgida* var. 'Little Goldstar' plant, sold in a 2l. pot, was in use for FFOs at any one time, although I cycled through several plants over the course of each survey season due to wear. The number of inflorescences per plant varied, but when choosing replacements I chose plants with a similar number of capitula to the original. If more were present than the original, I trimmed the capitula to remove any excess. The original plant purchased in 2017 had thirty-nine inflorescences. The average number of inflorescences per plant was 38 (N = 4 plants) in 2017, and 35 (N = 3 plants) in 2018. The choice to move and then observe a single plant multiple times over the course of each survey, as opposed to leaving seven plants in static locations for the duration of each day's sampling, was made primarily due to logistic concerns regarding the movement of equipment to and from each site, which was predominantly carried out via two people using either taxis or public transport.

5.2.2 Data analysis

5.2.2.1 Signal processing

The SOLO recorders record sound in .wav format audio files lasting ten minutes, except for those audio files representing the beginning and end of a given sampling period, which may be shorter. These audio files were grouped in one-hour bins, i.e., 09:00-10:00, and labelled according to the hour at the start of the bin, i.e., 09:00. The binned files were organised by trapping station and then by survey site. No further signal processing was carried out on the audio files, e.g., noise reduction, since the aim was to use field-realistic soundscape recordings representing a range of habitats, inclusive of the variety of biophonic, geophonic, and anthrophonic noise present in each.

5.2.2.2 Automated detection of insect flight sounds

The cluster analysis tool present in the Kaleidoscope Pro 5.4.2 software (Non-bat Analysis Mode) (Wildlife Acoustics Inc., 2021) was used to detect and cluster instances of insect sound (**buzzes**) present in the binned audio files, with each hour of audio recordings from each survey being run through Kaleidoscope Pro individually.

I based the clustering protocol upon the advanced classifier tutorial present on (Wildlife Acoustics Inc., 2019b). The cluster analysis tool aims to detect vocalisations within a series of recordings based upon a common set of signal parameters: the signal frequency (Hz.), the signal length (seconds), and the inter-syllable gap (the minimum length of time separating individual vocalisations, measured in seconds). Vocalisations that meet these parameters are clustered into similar sound classes. The parameters I used were based upon visual inspections of spectrograms of the soundscape files, and of the insect audio recordings made during **Chapter 4**. Spectrograms were generated using Audacity (Audacity Team, 2019).

Signal parameters:

- Signal frequency: 90 – 3000 Hz.
- Signal length: 0.1 – 7 seconds
- Inter-syllable gap: 0.1 second

The cluster analysis tool was initially run using the signal parameters alone to guide the selection and clustering of individual sounds. Then, using the 12:00-13:00, 13:00-14:00, and 14:00-15:00 audio files from the first acoustic pan trapping station from each survey as an exemplar sound set, each clustered sound was listened to and each insect buzz was labelled “insect”, leaving all non-insect sounds with their original cluster labels. These labels were then used to train the cluster analysis tool to create stronger classifiers. Only clear examples of insect buzzes were labelled as such. Instances where insect buzzes intersected with other types of biophony (i.e., bird song or human speech) were not labelled, as the competing acoustic signals may confuse the clustering tool. For the same reasons, any insect buzz that was identifiable but overlaid with high-amplitude geophonic or anthrophonic sound (i.e., wind or traffic noise) was also not labelled. I chose to use the audio files from between 12:00 and 15:00 as exemplar sound sets because insect flight activity

often peaks after midday towards the mid-afternoon (Steen, 2017), and I wanted to maximise the number of buzzes available for labelling.

The ability of the cluster analysis tool to accurately and reliably cluster (classify) unknown insect buzzes should increase with the number of known buzzes used to train the classifier. When I refer to accuracy, I describe the software's ability to determine whether a given sound belongs to the insect cluster or not. I set an arbitrary minimum acceptable number of insect buzzes per survey at 15. If there were not at least 15 instances of insect flight sound present within the three exemplar audio files from the first trapping station, then the equivalent audio files from the second acoustic pan trapping station were used and so on. The cluster analysis tool was then re-run using these manual labels to create a KCS file representing a Specialist Insect Classifier (**SIC**) (Wildlife Acoustics Inc., 2019b). This classifier was then used to detect and cluster instances of insect flight sound within the rest of the audio recordings from a given site.

A SIC was generated independently for each site visit using the same set of signal parameters, enabling me to consider the unique diversity of sound present within the soundscapes recorded from different sites at different times. There were two exceptions to this: Allerton Grange Fields (2017) and Farnley Hall Park (2018), where no more than two and fourteen buzzes, respectively, were found across any of the exemplar sound sets for these two sites. The Allerton Grange Fields site was not included in any further analysis, since I could not realistically create an SIC for the site with only two training buzzes. In the case of Farnley Hall Park (2018), I used the SIC created for the same site in 2017; while the soundscapes will be different, the mixture of sounds present within the soundscapes at the site should be similar enough to allow the application of the 2017 SIC to the 2018 soundscape data.

5.2.2.3 What are the Type 1 (false positive) and Type 2 (false negative) error rates associated with the Kaleidoscope Pro cluster analysis tool?

In order to test the performance of the supervised cluster analysis tool in Kaleidoscope Pro, specifically the tool's ability to reliably recognise and accurately cluster insect buzzes, I started by quantifying the proportion of false positive and false negative errors within my soundscape

recordings. **False positive errors** (FP) are defined as instances of non-insect sound incorrectly classified as insect buzzes; the false positive rate (FPR) is calculated as:

$$FPR = \frac{FP}{FP + TN}$$

Where TN represents the number of true negatives, or the number of instances of correctly classified non-insect sound.

False negative errors (FN) are defined as insect buzzes incorrectly classified as instances of non-insect sound; the false negative rate (FNR) is calculated as:

$$FNR = \frac{FN}{FN + TP}$$

Where TP represents the number of true positives, or the number of correctly classified insect buzzes. Neither the false positive rate nor the false negative rate are true rates, but instead represent proportions of error within a given soundscape recording.

I began by identifying a subset of my existing soundscape recordings. I binned all my hour-long recording segments into three groups, according to the number of insect buzzes clustered per hour by the cluster analysis tool. Due to the non-Gaussian distribution of these data (Fig. 5.4), I separated the groups based upon their distance from the median. Those recordings within the first quartile (0-25%: 0-7 buzzes) were defined as “low estimated activity”, those within the second and third quartiles (26-75%: 8-35 buzzes) were defined a “medium estimated activity”, and those within the fourth quartile (>75%: >36 buzzes) were defined as “high estimated activity”. Those recordings used to train the SICs at each site were not binned, since I would expect the false positive and false negative rates derived from an analysis of these recordings to be artificially low. Twenty-five hour-long recording segments were then randomly selected from each of these three groups, seventy-five hours in total, representing approximately 10.20 per cent of the total number of hours of soundscape data recorded during this experiment.

I re-ran the appropriate site-specific SIC on each of these recording segments and listened to each instance of sound detected and clustered by the cluster analysis tool, using the Kaleidoscope Pro Viewer window. I identified each instance of sound as either a true positive, a true negative, a

false positive, or a false negative. The number of true positives plus the number of false negatives provided a count of insect buzzes detected by the SICs, referred to here as **detected buzzes**. This approach to quantifying false positive and false negative error rates, using a subset of the original data to inspect and verify the identity of individual classified signals, is commonplace within ecoacoustic research (Heinicke et al., 2015; Abrahams & Denny, 2018; Ross et al., 2018; Pérez-Granados & Schuchmann, 2020).

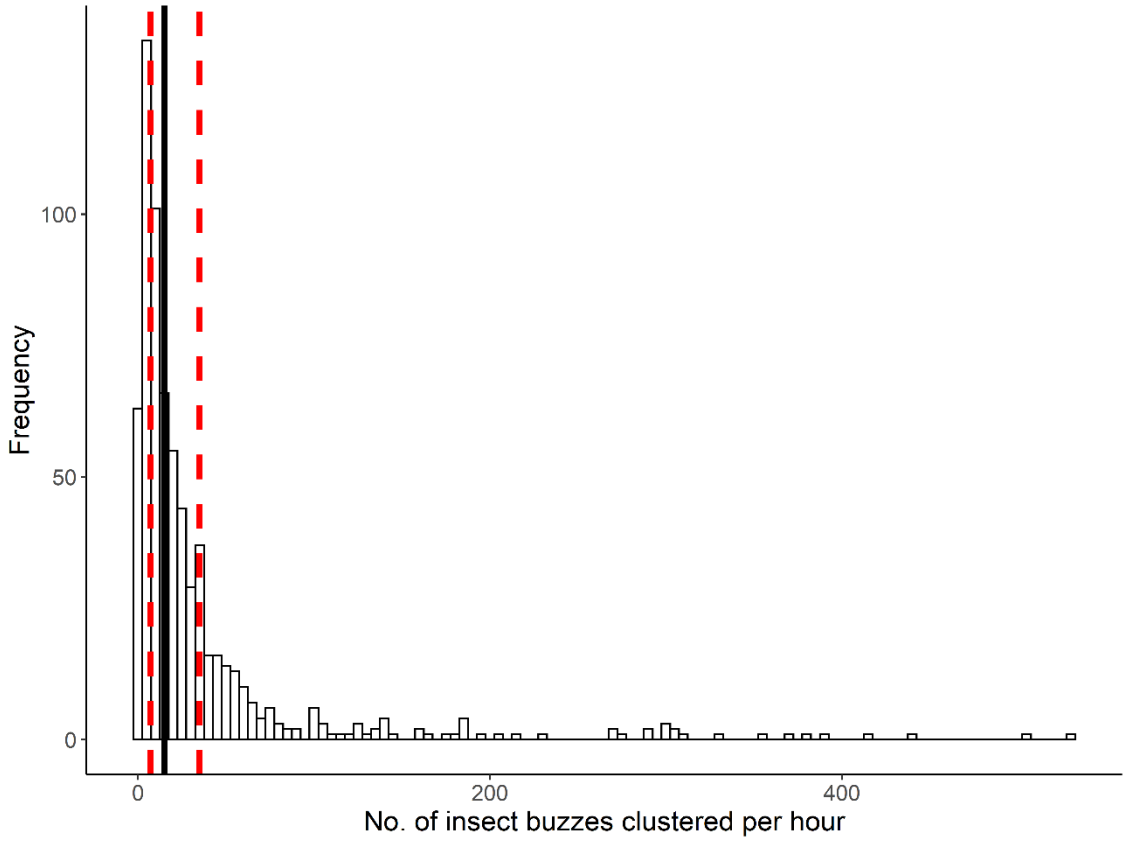

Figure 5.4 A histogram showing the distribution of the number of clustered insect buzzes per hour across all acoustic pan trapping stations during this experiment. The black vertical line shows the median (15), while the red dashed vertical lines show the first (7) and third (35) quantiles.

Once I had quantified the false negative and false positive error rates within this subset of soundscape recordings, I wanted to explore whether the number of insect buzzes used to train the relevant SICs effected these two measures of error. All subsequent statistical analyses were carried out in R, version 4.0.5 (R Core Team, 2021).

I used a generalised linear model to test for this, with a quasibinomial error distribution to account for the fact that my dependant variables were expressed as proportions. I included the estimated

activity level of each recording (low, medium, or high) as an additional explanatory variable, to test whether either measure of error differed between them. Results were plotted using the *geom_smooth* and *geom_boxplot* functions in the ggplot2 package (Wickham, 2009).

Global model example:

$$False\ positive\ rate \sim No.of\ training\ buzzes + Estimated\ activity\ level$$

I was also interested in whether there was a relationship between the false positive and false negative error rates, e.g., did recordings with low false positive errors also have low false negative errors. I used a Spearman's rank (non-parametric) correlation to test for this. Data normality was assessed using the *shapiro.test* function, and the results were plotted using the *geom_point* and *geom_smooth* functions in the ggplot2 package (Wickham, 2016).

5.2.2.4 Is there a difference between the number of instances of insect flight sound clustered by the Kaleidoscope Pro cluster analysis tool and the number of instances of insect flight sound present within the soundscape recordings?

Using the same subset of seventy-five hour-long recordings, representing recordings with low, medium, and high estimated activity, I loaded each hour-long soundscape recording segment into Audacity and listened to them manually; counting and labelling the number of insect buzzes present using the same criteria as those applied by the SICs:

- Signal frequency: 90 – 3000 Hz.
- Signal length: 0.1 – 7 seconds
- Inter-syllable gap: 0.1 second

Insect buzzes were labelled using the Add Label at Selection function within Audacity and the annotations for each segment were exported and saved as text files. Any sounds where I was unsure of their identity, either due to their amplitude or the presence of other overlapping sounds or background noise, were ignored. Any instances where the flight sounds from multiple insects overlaid one another along a single stretch of recording were labelled and counted as a single insect buzz. The difference between these manual counts of insect buzzes within each hour-long soundscape recording segment and the total number of detected buzzes (true positives + false

negatives) provided an estimate for a third form of error: the number of insect buzzes recorded by the acoustic pan trap but not detected by the SICs, hereafter termed **undetected buzzes**.

This process left me with four measures of insect activity from within each hour-long recording from the subset: the number of buzzes clustered by Kaleidoscope Pro, the number of buzzes detected by Kaleidoscope Pro, the number of true positives, and my manual counts. I used a generalised linear mixed-effects model (GLMM) to test whether there were significant differences between these four measures of insect activity. Since the data were overdispersed, I used a negative binomial distribution via the *glmer.nb* function in the lme4 package (Bates et al., 2015). Each hour-long soundscape recording was allocated a unique identifier, which was used as a random effect within each model (1|ID) to account for the fact that I was comparing multiple measures of acoustic activity from within each recording. I initially ran the GLMM on all seventy-five recordings within the data subset, I then ran it a further three times: once on each sub-group of twenty-five recordings, representing soundscapes with low, medium, and high estimated insect activity. Overdispersion was tested for using the *overdisp.glmer* function in the RVAideMemoire package (Hervé, 2019), and pairwise comparisons were carried out via General Linear Hypothesis testing, using the *glht* function in the multcomp package (Hothorn, Bretz, & Westfall, 2008). The results were plotted using the *geom_boxplot* function in the ggplot2 package (Wickham, 2009).

Following the calculation of the number of undetected buzzes present within each soundscape recording, I used a quasibinomial GLM (via the *glm* function) to investigate whether the number of signals used to train each SIC affected the proportion of undetected buzzes within each recording.

$$Proportion\ of\ undetected\ buzzes = \frac{Manual\ counts - detected\ insects}{Manual\ counts}$$

I also included the estimated activity level of each hour-long recording (low, medium, or high) as an additional explanatory variable, to test whether the proportion of undetected buzzes differed between recordings with different levels of estimated activity. Results were plotted using the *geom_smooth* function in the ggplot2 package (Wickham, 2009).

Finally, I tested whether there was a relationship between the number of buzzes clustered by each SIC and my manual counts of insect activity. I also tested whether there was a similar relationship between the number of true positives and my manual counts. In both cases I used a Spearman's rank (non-parametric) correlation. Data normality was assessed using the *shapiro.test* function, and the results of these correlations were plotted using the *geom_point* and *geom_smooth* functions in the ggplot2 package (Wickham, 2009).

5.2.2.5 Is there a relationship between the number of instances of insect sound detected by the acoustic pan traps and the number of insects sampled by traditional water-based pan trapping or observed during hourly focal floral resource observations?

I summed the number of clustered insect buzzes from each of the acoustic pan trapping stations deployed per site, and then averaging these totals to create twenty site-level means. I performed the same set of calculations for the traditional pan trapping stations. I then used Pearson's (parametric) and Spearman's rank (non-parametric) correlations to test whether there was a relationship between the site-level means of the total number of insect buzzes clustered per acoustic pan trapping station and the total number of insects sampled per traditional pan trapping station. I also summed the number of insect visitors observed during the FFOs at each site and correlated these values against the mean total number of insect buzzes clustered per acoustic pan trapping station per site. Data normality was assessed using the *shapiro.test* function, and the results of these correlations were plotted using the *geom_point* and *geom_smooth* functions in the ggplot2 package (Wickham, 2009).

5.2.2.6 Is there a relationship between the number of instances of insect sound detected per hour by the acoustic pan traps and the number of insects observed during hourly focal floral resource observations?

I calculated the mean number of insect buzzes clustered per hour across all five of the acoustic pan trapping stations per site and correlated these values with the number of insect visitations recorded during each hourly FFO per site using Pearson's and Spearman's rank tests. Data normality was assessed using the *shapiro.test* function, and the results of these correlations were

plotted using the *geom_point*, *geom_smooth*, and *geom_line* functions in the ggplot2 package (Wickham, 2009).

5.2.2.7 Is the number of instances of insect sound detected per hour by the acoustic pan traps affected by local environmental variables, specifically maximum day-time temperature (°C), wind speed (mph), rainfall (mm), and local floral abundance?

I used a GLMM to test for the effects of local environmental variables on the number of clustered buzzes per hour. I used a negative binomial distribution via the *glmer.nb* function in the lme4 package (Bates et al., 2015). The following variables were included as explanatory variables: hourly temperature (°C), hourly wind speed (mph), and the volume of nectar sugar produced per day within a two meter radius of each acoustic pan trapping station. Hourly rainfall (mm) data were collected but were not included in the GLMM since there were no instances of rainfall during this study. The year in which each sampling visit took place, as well as the hour of the day were also included as additional fixed effects, to account for temporal variation in insect activity across this experiment. The date of each sampling visit and the location of the acoustic pan trapping station (1-5) were used within a nested random effect (1|Date/Station) to account for any natural variation in insect acoustic activity between sites or between trapping stations within sites.

Both hourly temperature and hourly wind speed showed signs of non-linear relationships with the number of clustered buzzes per hour. Hourly temperature during this study ranged between 10 and 25°C, with a median value of 17°C. The number of clustered buzzes peaked at approximately 17°C (see Supplementary Fig. 3.1). Hourly wind speed ranged between 3 and 17mph, with a median speed of 9mph. The number of clustered buzzes per hour was greatest between 3 and 4mph, dropping as wind speeds rose to 10mph, before rising again as wind speeds increased (see Supplementary Fig. 3.2). In response to these patterns, I fitted second-order polynomial terms to both hourly temperature and hourly wind speed, using the poly function in R. I also applied a natural log transformation to the nectar sugar variable to correct for a strong right-hand skew in the data. For a summary of the total volume of nectar sugar produced per day within a two-meter radius of each acoustic pan trapping station per site, see Supplementary Table 3.3.

Global model example:

$$No.\,of\ clustered\ buzzes\ per\ hour \sim poly(hourly\ temperature, degree = 2) + poly(hourly\ wind\ speed, degree = 2) + \log(daily\ nectar\ sugar\ production + 1) + hour + year + (1|date/station)$$

Overdispersion was tested for using the *overdisp.glmer* function in the RVAideMemoire package (Hervé, 2019). Backwards stepwise regression was used to find the minimum adequate model. Pairwise comparisons were carried out via General Linear Hypothesis testing, using the *glht* function in the multcomp package (Hothorn, Bretz, & Westfall, 2008). Results were plotted using the *geom_smooth* and *geom_bar* functions in the ggplot2 package (Wickham, 2009).

5.3 Results

Approximately 735 hours of acoustic soundscape data were recorded across thirteen site visits in 2017, and seven site visits in 2018 (Table 5.1). Kaleidoscope Pro's cluster analysis tool clustered 27,481 acoustic signals as insect buzzes from a total 289,039 detected acoustic signals across all acoustic pan trapping stations successfully deployed during this experiment. In contrast, the pan trapping surveys sampled 5808 insects, and the FFOs observed 1170 instances of insect visitation to the focal floral resources.

5.3.1 What are the Type 1 (false positive) and Type 2 (false negative) error rates associated with the Kaleidoscope Pro cluster analysis tool?

The false positive and false negative error rates associated with in Kaleidoscope Pro's cluster analysis tool were quantified across a subset of seventy-five hours of soundscape recordings, which was further split into three subsets of twenty-five hours, representing recordings with high, medium, and low estimated acoustic insect activity. The mean values shown in Table 5.2 show that false positive errors were universally low, with fewer than ten per cent of detected non-insect sounds incorrectly clustered as insects per hour, regardless of the estimated acoustic activity. The false negative errors were much higher, with an average sixty per cent of detected insect buzzes incorrectly clustered as non-insect sounds across the whole subset.

Table 5.1 A summary of the sites, locations, and dates of the surveys made during this Chapter, together with brief habitat classifications for each site. A date in bold indicates a site where a full set of soundscape recordings was made, all other sites made only partial sets of recordings due to errors associated with the SOLO recorders.

		Date		
Site name	Coordinates	2017	2018	Habitat
Allerton Grange fields	SE313383	08/09/17	-	Amenity grassland
Asket Hill	SE338370	01/09/17	-	Semi-natural grassland
Bramley Fall Park	SE244363	-	04/07/18	Semi-natural grassland
Farnley Hall Park	SE247322	**26/08/17**	**02/07/18**	Amenity grassland
Halton Moor	SE336330	**19/09/17**	-	Amenity grassland
Killingbeck fields	SE340342	17/09/17	-	Unmown amenity grassland
Kirkstall Abbey	SE261361	**28/08/17**	31/07/18	Amenity grassland
Meanwood Grove	SE280381	-	**27/06/18**	Semi-natural grassland
Meanwood road	SE292362	**28/08/17**	-	Amenity grassland
Meanwood farm	SE291365	28/08/17	-	Semi-natural grassland
Primrose Valley	SE346340	29/09/17	-	Amenity grassland
Rothwell Country Park	SE354296	**12/09/17**	-	Low-lying scrub
Rothwell Pastures	SE340283	**15/09/17**	**03/08/18**	Semi-natural grassland
Skelton Woods	SE361382	**06/09/17**	-	Unmown amenity grassland border
Temple Newsam	SE356319	**20/09/17**	**03/07/18**	Amenity grassland
Water Haigh	SE367284	04/09/17	**01/09/18**	Unmown amenity grassland

There was a weak negative correlation between the false positive and false negative rates ($r_{s(59)}$ = -0.31, P = 0.017; Fig. 5.5A), where recordings with higher false positive rates tended to have lower false negative rates. For a full summary of the false positive and false negative error rates across this subset of recordings, see Supplementary Table 3.4.

Table 5.2 The mean false positive and false negative error rates (± SE) across a subset of seventy-five hours of soundscape recordings, and within each further subset of twenty-five hours, representing recordings with high, medium, and low estimated acoustic insect activity.

	Mean false positive rate	N	Mean false negative rate	N
All subset data	0.058 ± 0.007	75	0.601 ± 0.039	61
High activity	0.078 ± 0.008	25	0.472 ± 0.051	22
Medium activity	0.062 ± 0.011	25	0.720 ± 0.057	22
Low activity	0.034 ± 0.016	25	0.613 ± 0.092	17

The quasibinomial GLMs showed no significant differences in terms of either the false positive ($\chi^2_{(2)}$ = 2.331, p = 0.312) or false negative ($\chi^2_{(2)}$ = 2.282, p = 0.319) error rates between the three estimated activity classes. However, both classes of error were affected by the number of insect buzzes used to train the relevant SICs. As the number of training buzzes increased, there was a weak but significant increase in the false positive rate (parameter estimate: 0.002, 95% CI [0.001, 0.003]; Fig. 5.5B), and a weak but significant decrease in the false negative rate (-0.003 [-0.005, -0.002]; Fig. 5.5C).

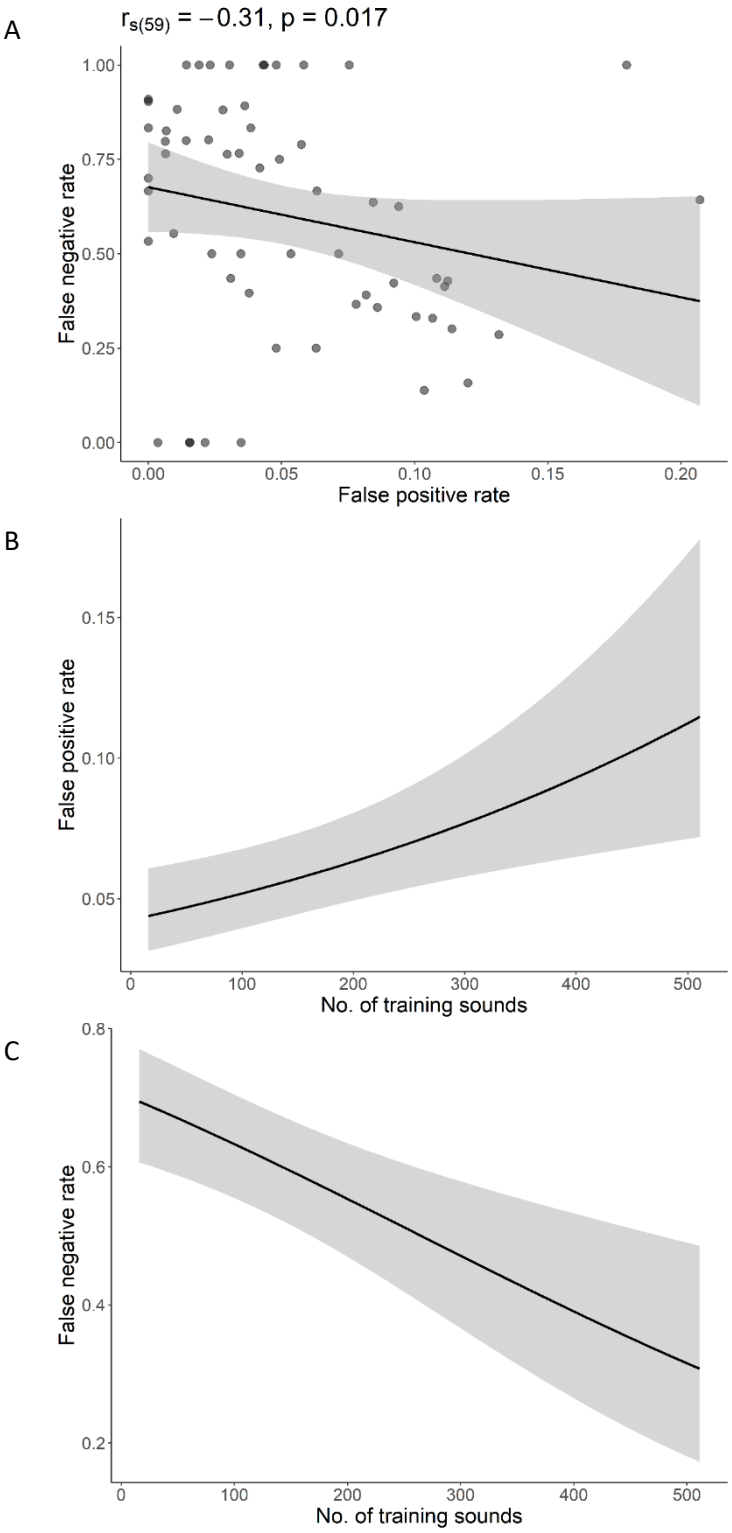

Figure 5.5 A) A scatterplot showing the relationship between the false positive rate and the false error rate (± SE); B) The false positive rate ± 95% confidence intervals plotted against the number of insect sounds used to train the specialist insect classifiers (SICs); C) The false negative rate ± 95% confidence intervals plotted against the number of insect sounds used to train the specialist insect classifiers (SICs).

5.3.2 Are there differences between the number of instances of insect flight sound clustered by the Kaleidoscope Pro cluster analysis tool and the number of instances of insect flight sound present within the soundscape recordings?

The number of insect buzzes present within each of the seventy-five hour-long soundscape recordings were counted manually and compared to three measures of estimated insect activity derived using Kaleidoscope Pro: the number of clustered insect buzzes (clustered insects), the number of true positives, and the number of detected insects (true positives + false negatives). These four measures of acoustic activity (known or estimated) differed significantly from one another ($\chi^2_{(3)}$ = 199.120, p = <0.001; Table 5.3, Fig. 5.6A). Pairwise comparisons indicate that that there were no significant differences between the number of insect buzzes clustered by Kaleidoscope Pro and the manual counts of insect buzzes taken from the soundscapes themselves. However, the number of true positives was significantly lower than all other measures of insect activity, while the number of total detected buzzes was significantly lower than both the number of clustered buzzes and the manual counts.

There remained significant differences between the four measures of acoustic activity (known or estimated) within each of the three activity subgroups: low ($\chi^2_{(3)}$ = 102.380, p = <0.001), medium ($\chi^2_{(3)}$ = 109.360, p = <0.001), and high ($\chi^2_{(3)}$ = 43.768, p = <0.001). Within the medium and high activity groups, these comparison tests showed the same pattern of pairwise differences as within the whole subset of seventy-five recordings (Fig. 5.6B & 5.6C). Within the low activity group, all three measures of estimated insect activity were significantly lower than the manual counts of insect activity, while the number of total detected insect buzzes and clustered insect buzzes were both significantly greater than the number of true positives. There were no significant differences between the number of clustered insect buzzes and the number of total detected insect buzzes (Fig. 5.6D). For further details concerning the pairwise comparisons within each of the three subgroups, see Supplementary tables 3.5, 3.6, and 3.7.

Table 5.3 Pairwise comparisons between four measures of acoustic insect activity: manual counts of insect buzzes per hour, the number of insect buzzes clustered per hour by Kaleidoscope Pro (clustered insects), the number of true positives therein, and the number of total detected buzzes (true positives + false negatives). Bold values indicate significant pairwise comparisons.

Pairwise comparison	Parameter estimate	95% CI
True positives -- Clustered insects	**-1.816**	**(-2.230, -1.402)**
Detected insects -- Clustered insects	**-0.871**	**(-1.271, -0.471)**
Manual count -- Clustered insects	0.134	(-0.250, 0.518)
Detected insects -- True positives	**0.945**	**(0.565, 1.326)**
Manual count -- True positives	**1.950**	**(1.564, 2.337)**
Manual count -- Detected insects	**1.005**	**(0.643, 1.368)**

There was a moderately strong positive correlation between the number of insect buzzes clustered per hour by the cluster analysis tool and the manual counts of insect buzzes during each hour-long recording ($r_{s(73)}$ = 0.56, P = <0.001; Fig. 5.7A). There was a much stronger positive correlation relationship between the no. of true positives clustered per hour and the manual counts of insect buzzes during each hour-long recording ($r_{s(73)}$ = 0.88, P = <0.001; Fig. 5.7B).

The proportion of undetected buzzes was quantified within each hour-long soundscape recording within the subset of seventy-five recordings. The mean values shown in Table 5.4 indicate that the detection rate was relatively low across the whole subset, regardless of estimated activity - never rising above 50%. Quasibinomial GLMs showed no significant differences in terms of the proportion of undetected buzzes between the three activity classes ($\chi^2_{(2)}$ = 3.431, p = 0.180). The proportion of undetected buzzes was, however, affected by the number of insect buzzes used to train the relevant SICs. As the number of training buzzes increased, there was a weak but significant decrease in the proportion of undetected buzzes (-0.002 [-0.004, -0.001]; Fig. 5.8A). For a breakdown of manual counts and the number of undetected insect buzzes, see Supplementary Table 3.9.

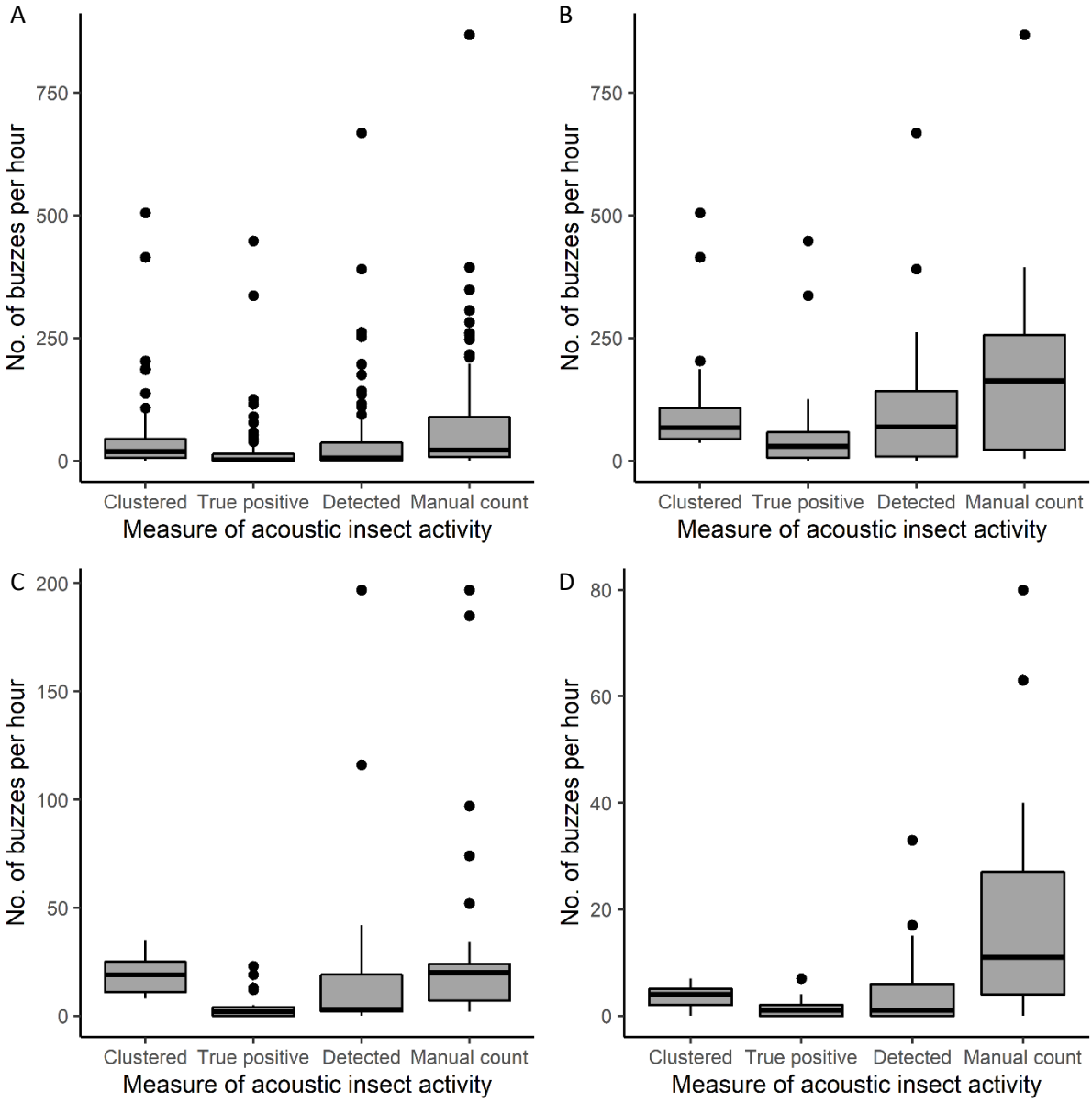

Figure 5.6 A) A boxplot showing the number of buzzes per hour clustered by Kaleidoscope Pro (Clustered), the number of true positives, the number of buzzes per hour detected by Kaleidoscope Pro (true positives + false negatives), and the manual counts of insect buzzes across all seventy-five hour-long recordings within the data subset; B) A boxplot showing the number of buzzes per hour clustered by Kaleidoscope Pro (Clustered), the number of true positives, the number of buzzes per hour detected by Kaleidoscope Pro (true positives + false negatives), and the manual counts of insect buzzes across the twenty-five recordings with high estimated acoustic insect activity; C) A boxplot showing the number of buzzes per hour clustered by Kaleidoscope Pro (Clustered), the number of true positives, the number of buzzes per hour detected by Kaleidoscope Pro (true positives + false negatives), and the manual counts of insect buzzes across the twenty-five recordings with medium estimated acoustic insect activity; D) A boxplot showing the number of buzzes per hour clustered by Kaleidoscope Pro (Clustered), the number of true positives, the number of buzzes per hour detected by Kaleidoscope Pro (true positives + false negatives), and the manual counts of insect buzzes across the twenty-five recordings with low estimated acoustic insect activity.

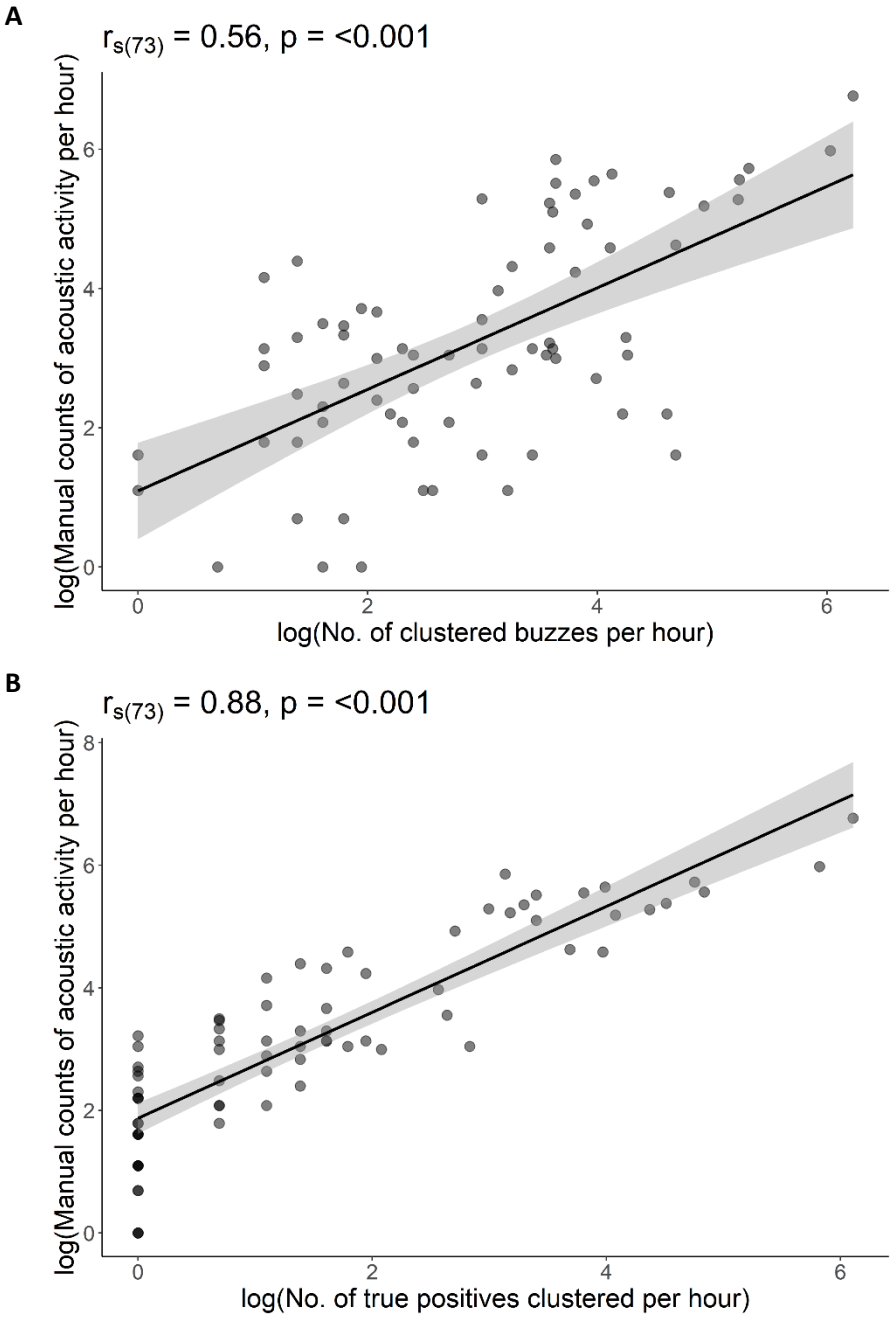

Figure 5.7 A) A scatterplot showing the relationship between the number of buzzes clustered per hour by Kaleidoscope Pro and the manual counts of insect buzzes per hour (±SE); B) A scatterplot showing the relationship between the number of true positives clustered per hour by Kaleidoscope Pro and the manual counts of insect buzzes per hour (±SE).

Table 5.4 The mean proportions of the manual counts that were either not detected or not correctly clustered the cluster analysis tool in Kaleidoscope Pro (± SE) across a subset of seventy-five hours of soundscape recordings, and within each further subset of twenty-five hours, representing recordings with high, medium, and low estimated acoustic insect activity.

	Mean undetected	N	Mean un-clustered	N
All subset data	0.620 ± 0.035	72	0.849 ± 0.022	72
High activity	0.527 ± 0.067	25	0.736 ± 0.048	25
Medium activity	0.597 ± 0.051	25	0.889 ± 0.022	25
Low activity	0.753 ± 0.055	22	0.932 ± 0.021	22

Out of interest, I repeated this test using the proportion of the manual counts that were **not correctly clustered** by Kaleidoscope Pro as the dependant variable:

$$\frac{Manual\ counts - True\ positives}{Manual\ counts}$$

This proportion was universally high across the whole data subset, and never dropped below 70%. Again, the quasibinomial GLMs showed no significant differences between the three activity classes ($\chi^2_{(2)} = 2.169$, $p = 0.338$), but there was a weak, but significant decrease in the proportion of manual counts not correctly clustered by the cluster analysis tool in response to an increasing number of training buzzes (-0.004 [-0.006, -0.003]; Fig. 5.8B).

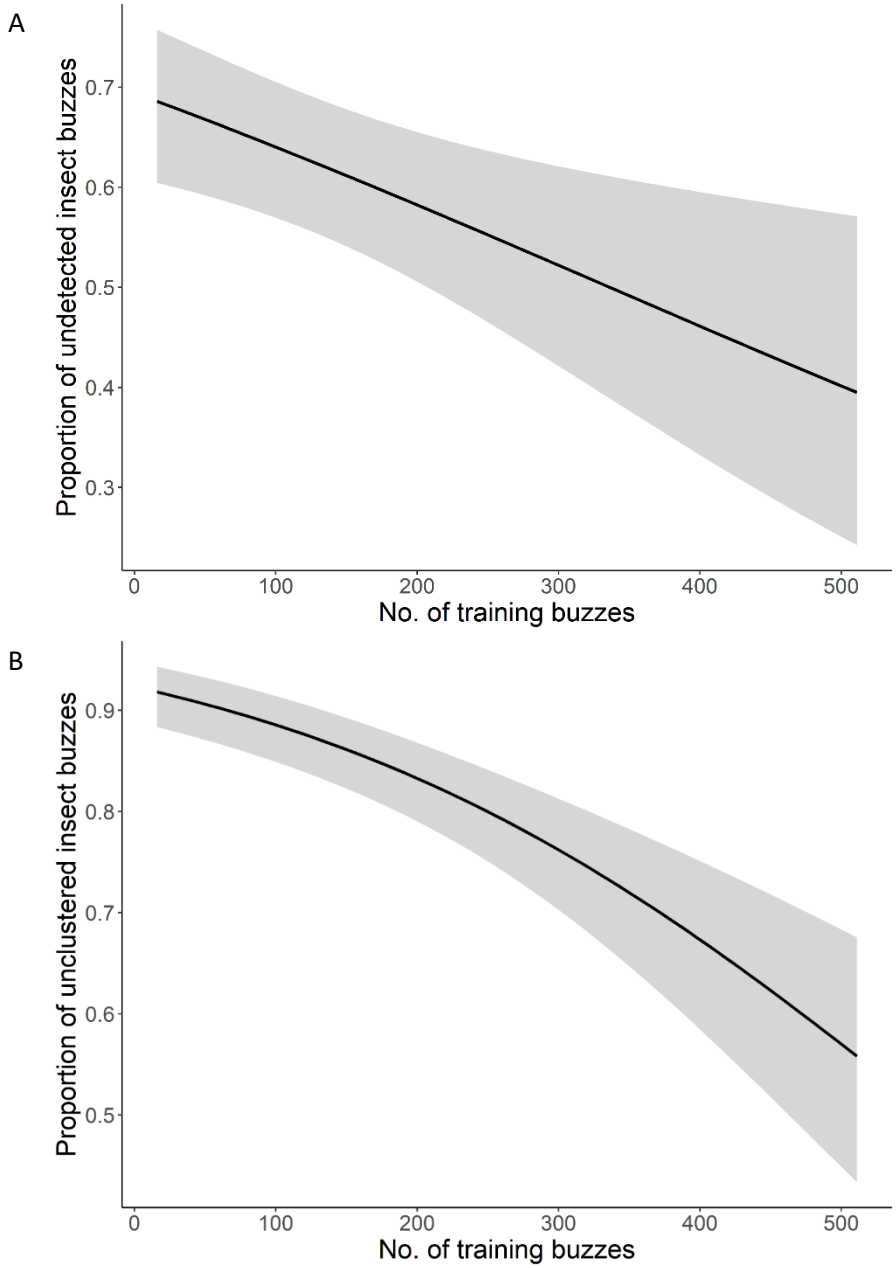

Figure 5.8 A) The proportion of buzzes not detected by Kaleidoscope Pro per hour ± 95% confidence intervals plotted against the number of insect sounds used to train the specialist insect classifiers (SICs); B) The proportion of buzzes not correctly clustered by Kaleidoscope Pro per hour ± 95% confidence intervals plotted against the number of insect sounds used to train the specialist insect classifiers (SICs).

5.3.3 Is there a relationship between the number of instances of insect sound detected by the acoustic pan traps and the number of insects sampled by traditional pan trapping or observed during focal floral resource observations?

There were seven site visits where one or more of the acoustic pan trapping stations did not record any audio data during the sampling period due to technical problems (see Table 5.1). Data from these site visits were not used when testing for a relationship between the number of insect buzzes and the total number of insects sampled by the pan traps or observed during the FFOs, since the audio data would not be representative of the sum of acoustic insect activity present at these sites. These data were, however, used to test for relationships between the number of buzzes detected per hour by individual acoustic pan trapping stations and the number of insects observed per hour during the FFOs.

There was no correlation between the site-level means of insect buzzes detected by the acoustic pan trapping stations and insects sampled by the pan trapping stations ($r_{s(11)} = -0.291$, $p = 0.334$; Fig. 5.9A). In case this was an artefact of only one colour being represented by the acoustic pan trapping stations versus the three colours present at each of the traditional pan trapping stations, the correlation was run again using only the number of insects sampled by the yellow pan traps; however, there was no correlation present ($r_{s(11)} = -0.143$, $p = 0.641$; Fig. 5.9B). Similarly, there was no correlation between the site-level mean number of insect buzzes detected by the acoustic pan trapping stations and the total number of insects visitations observed during the focal floral resource observations per site[1] ($r_{s(14)} = 0.021$, $p = 0.940$; Fig. 5.9C).

[1]The correlation between the acoustic pan trapping stations and the FFOs used data from three sites that were not included in the correlations between the acoustic pan trapping stations and the traditional pan traps. At these three sites, all five acoustic pan trapping stations were recording but ceased doing so before the end of the seven hour survey period due to battery issues, and therefore were not representative of the sum of acoustic insect activity present at these sites. But I **was** able to correlate them with the summed insect visitations during the FFOs up until the point when they ceased recording.

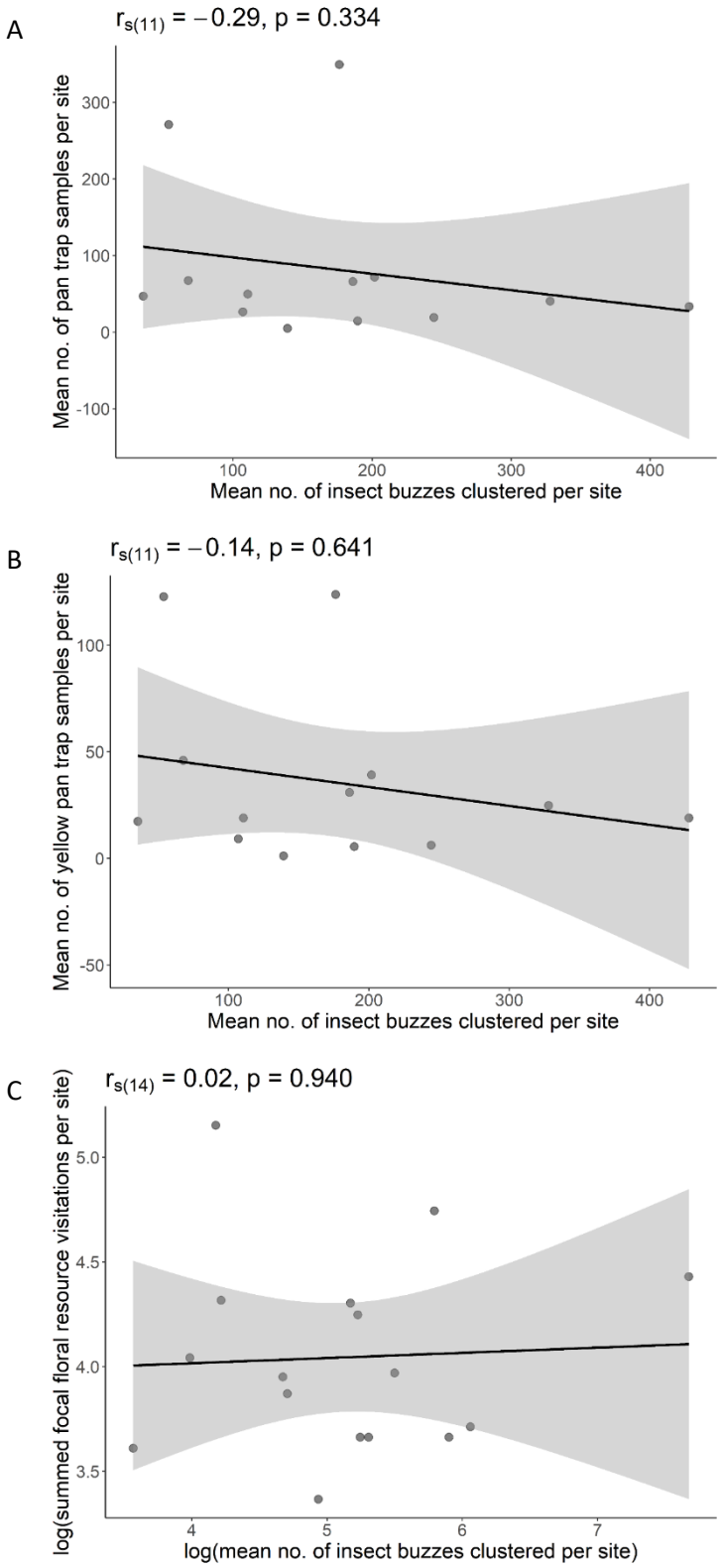

Figure 5.9 A) A scatterplot showing the relationship between the mean number of insect buzzes clustered per site by Kaleidoscope Pro and the mean number of insects sampled per site by water-based pan trapping; B) A scatterplot showing the relationship between the mean number of insect buzzes clustered per site by Kaleidoscope Pro and the mean number of insects sampled per site by water-based pan trapping (yellow bowls only); C) A scatterplot showing the relationship between the mean number of insect buzzes clustered per site by Kaleidoscope Pro and the mean number of insects observed per day during the hourly FFOs.

5.3.4 Is there a relationship between the number of instances of insect sound detected per hour by the acoustic pan traps and the number of insects observed during hourly focal floral resource observations?

There were only two sites with significant correlations between the mean number of insect buzzes recorded per hour across all five acoustic pan trapping stations and the number of insects observed per hour during the FFOs: Bramley Fall Park (2018) ($r_{s(5)}$ = -0.89, p = 0.007) and Farnley Hall Park (2018) ($r_{(5)}$ = -0.81, p = 0.028). In both cases, the mean number of insect buzzes recorded per hour by the acoustic pan traps fell as the number insect visitations during the FFOs increased (Fig. 5.10).

5.3.5 Is the number of instances of insect sound recorded per hour by the acoustic pan traps affected by local environmental variables, specifically average hourly temperature (°C), average hourly wind speed (mph), hourly total rainfall (mm), and local floral abundance?

The following variables had significant effects upon the number of clustered insect buzzes recorded per hour by the acoustic pan traps: average hourly temperature (°C), average hourly wind speed (mph), and the hour of the day. The effects of temperature and wind speed were tested for with linear and second-order polynomial terms, and the coefficients presented below represent orthogonal polynomial terms. Regarding temperature, the linear term had a significant negative effect on the number of clustered insect buzzes recorded per hour (-6.896 ± SE 3.243, p = 0.034) (Fig. 5.11A). Regarding wind speed, the second order term had a significant negative effect on the number of clustered insect buzzes recorded per hour (-2.759 ± SE 1.252, p = 0.028) (Fig. 5.11B). The hour of the day had a significant effect upon the number of clustered insect buzzes recorded per hour ($\chi^2_{(9)}$ = 184.32, p = <0.001; Table 5.4, Fig. 5.12), with significantly fewer clustered insect buzzes recorded between 8:00-09:00 and 16:00-17:00 than at other times of day (Table 5.4). For a full breakdown of all pairwise comparisons between different times of day, see Supplementary Table 3.10.

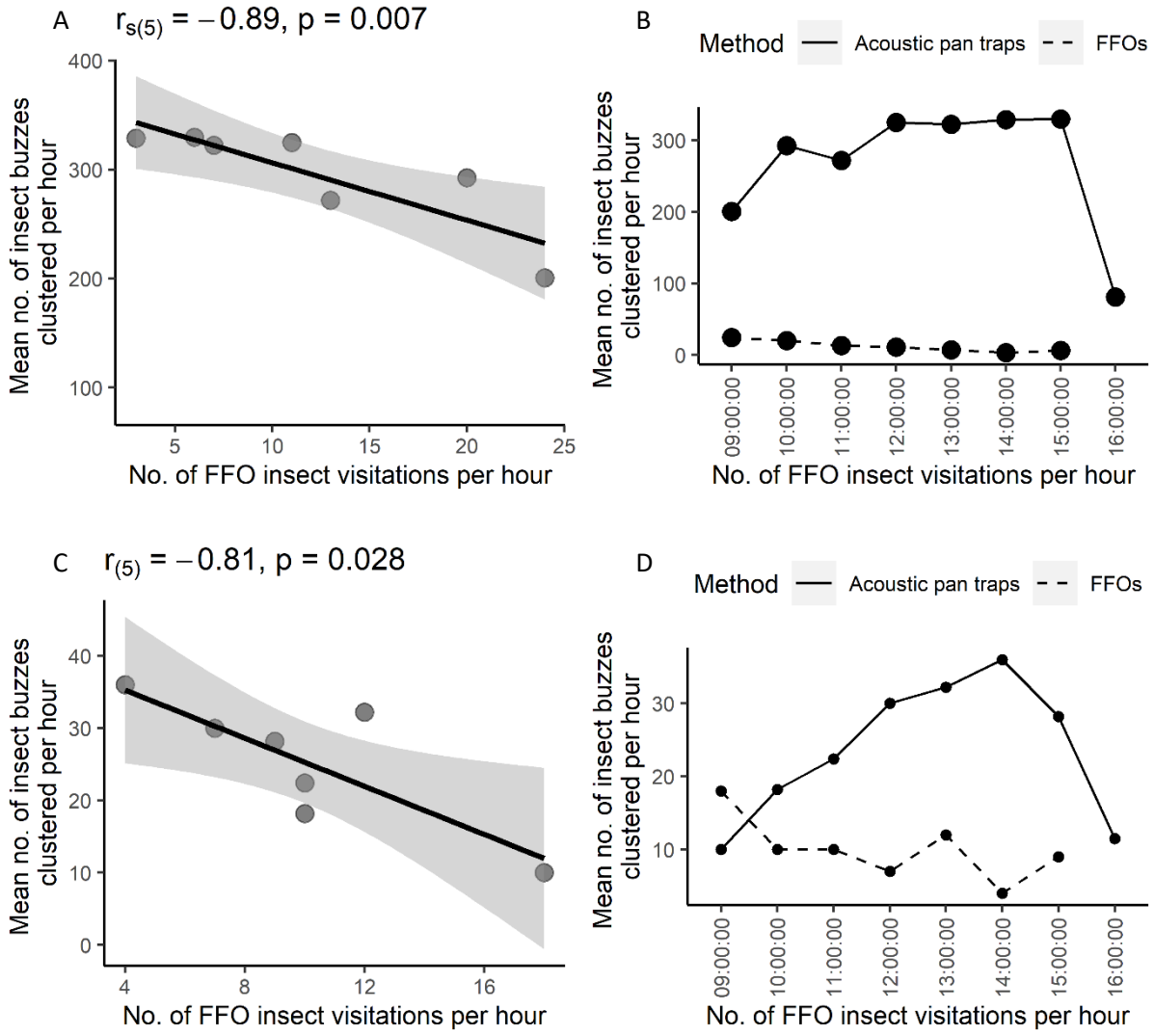

Figure 5.10 A) A scatterplot showing the relationship between the number of insect buzzes clustered per hour by Kaleidoscope Pro and the number of insects observed during the hourly FFOs at Bramely Fall Park (2018); B) A line chart showing the number of insect buzzes clustered per hour by Kaleidoscope Pro and the number of insects observed during the hourly FFOs at Bramely Fall Park (2018); C) A scatterplot showing the relationship between the number of insect buzzes clustered per hour by Kaleidoscope Pro and the number of insects observed during the hourly FFOs at Farnley Hall Park (2018); B) A line chart showing the number of insect buzzes clustered per hour by Kaleidoscope Pro and the number of insects observed during the hourly FFOs at Farnley Hall Park (2018).

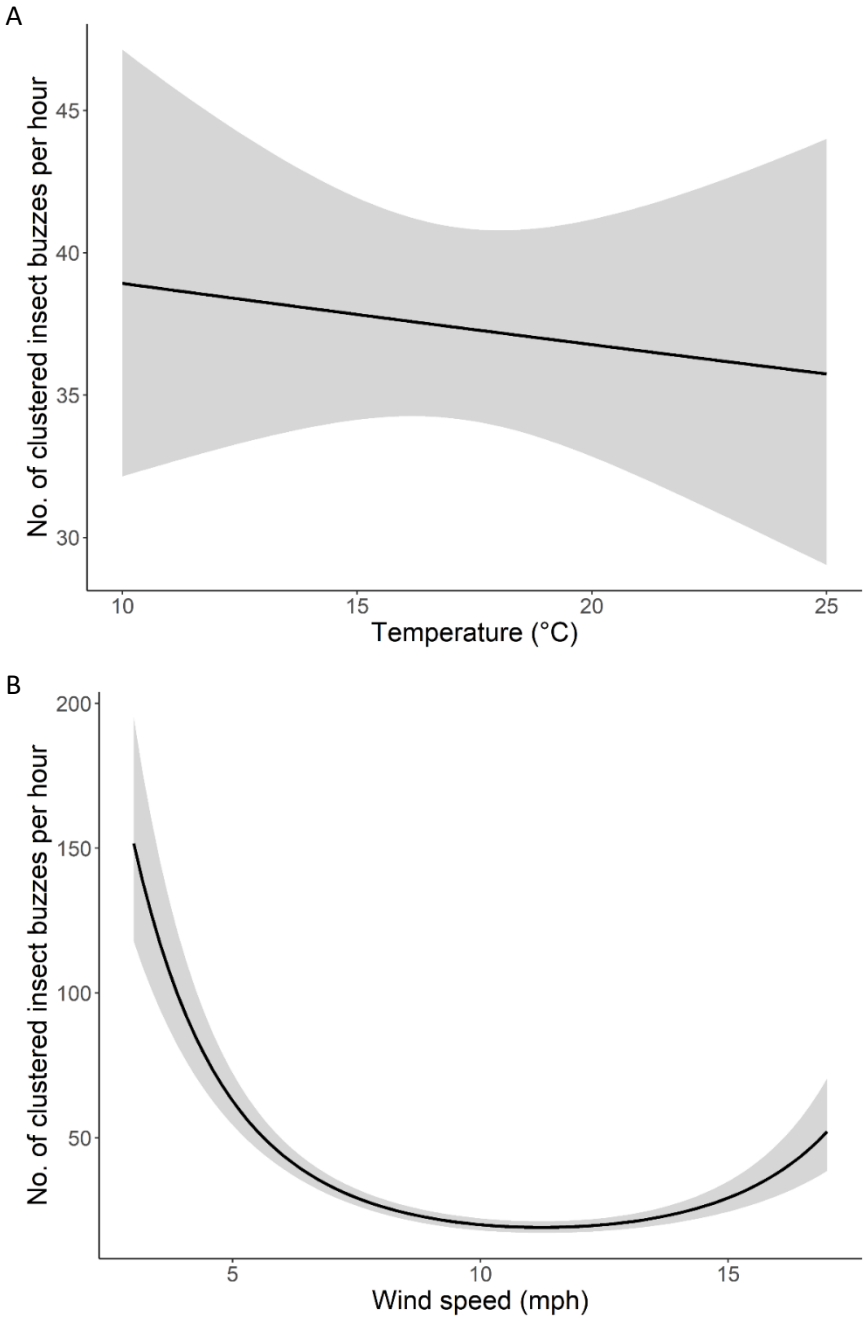

Figure 5.11 A) The number of insect buzzes clustered per hour by Kaleidoscope Pro ±95% confidence intervals plotted against the hourly temperature (°C); B) The number of insect buzzes clustered per hour by Kaleidoscope Pro ±95% confidence intervals plotted against the hourly wing speed (mph).

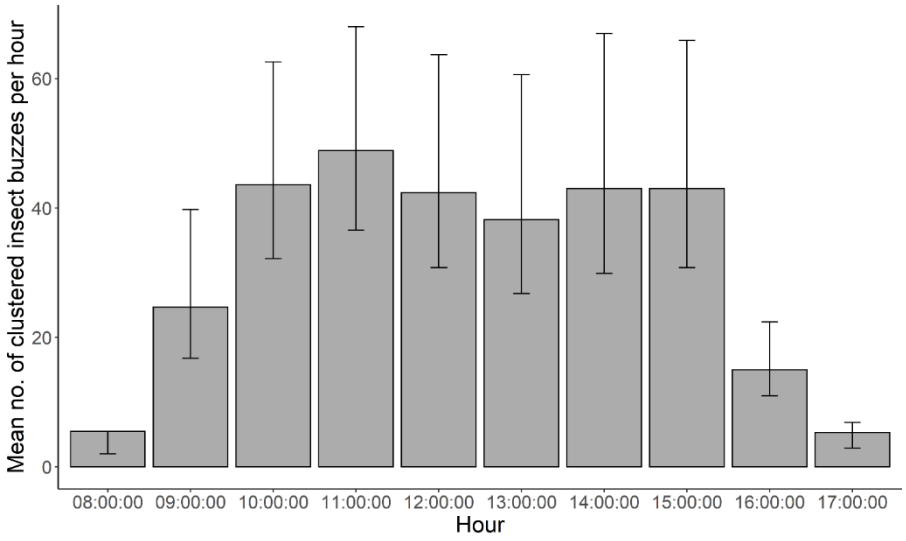

Figure 5.12 A bar chart showing the mean number of insect buzzes clustered per hour by Kaleidoscope Pro ±95% confidence intervals plotted against the hours of the day over which the surveys were carried out. Means were calculated over both 2017 and 2018.

5.4 Discussion

Passive acoustic monitoring has been increasingly used to provide a low-cost, non-destructive, autonomous alternative to traditional sampling methods within many ecological systems, and with a focus on numerous different taxonomic groups (Browning et al., 2017; Gibb et al., 2019). In terms of insect monitoring, low-cost passive acoustic sensors have already been used to detect and count mosquitos, with the aim to quantify the spread of disease vectors (Raman, Gerhardt, & Wilkerson, 2007; Mukundarajan et al., 2017), and to investigate the effects of habitat loss, degradation, and fragmentation on Orthopteran communities (Penone et al., 2013). More recently, these techniques have been used to measure "bee" activity during a solar eclipse (Galen et al., 2019), and to predict pollination services in relation to the density of bumblebee flight sounds (Miller-Struttmann et al., 2017); suggesting that this technique may have promising applications with regard to monitoring flower-visiting insects. Research by Miller-Struttmann et al. (2017) also shows that acoustic bumblebee activity in alpine meadows is a positive predictor of bumblebee abundance sampled along visual transect surveys, but we have no evidence aside from this regarding how well passive acoustic monitoring techniques perform in relation to traditional insect pollinator survey methods, especially those commonly used alongside transect surveys as part of standardised insect pollinator monitoring schemes, such as pan trapping or focal floral resource observations (FFOs) (see Carvell et al., 2016).

In an attempt to answer this question, I designed a standardised passive acoustic monitoring tool: an acoustic pan trap. Emulating traditional pan trap design, this is, as far as I am aware, the first instance of a passive acoustic sensor combined with a visual attractant aimed at surveying flower-visiting insect communities. I trialled this novel survey method alongside traditional pan trapping and FFOs across twenty-one site visits to compare the results of the three survey methods, using a commercially available, established method of automated soundscape analysis that had yet to be tested in regard to its ability to detect and identify insect wing beat signals: the cluster analysis tool from the Kaleidoscope Pro software (non-bat analysis mode), version 5.4.2 (Wildlife Acoustics Inc., 2021). I discuss the utility of the Kaleidoscope Pro software in terms of its ability to detect and identify insect buzzes from soundscape data, and present evidence that, while passive acoustic monitoring does provide a wealth of data concerning insect activity, these data are not predictive of insect abundance sampled via pan trapping or FFOs. I also explore whether local environmental variables, such as wind speed and temperature, affect the number of insect buzzes detected and clustered from soundscape data by Kaleidoscope Pro. I finish by providing recommendations for the future development and testing of this novel survey method in relation to insect pollinator monitoring.

The cluster analysis tool within the Kaleidoscope Pro software did not perform as well as expected in terms of its ability to reliably and accurately detect and cluster insect wing beat signals, or "buzzes", from within soundscape recordings collected by the acoustic pan traps. In terms of the cluster analysis tool's ability to detect insect buzzes from within the soundscape data, Kaleidoscope Pro never outperformed the human ear. Within a subset of seventy-five hour-long recordings, the number of insect buzzes manually counted per hour was significantly greater than the total number buzzes detected by the cluster analysis tool (true positives + false negatives). This remained consistent regardless of whether a recording was perceived as being high, medium, or low in terms of insect acoustic activity. The mean proportion of undetected insect buzzes was 0.62 (± SE 0.035) which indicates that my SICs were only detecting, on average, approximately forty per cent of the available insect signals present within the soundscape data. The proportion of undetected insect buzzes did decrease significantly when greater numbers of insect buzzes were

used to train the SICs, although our results indicate that between 300 and 400 training signals would have been required to raise the detection rate to more than fifty per cent. This might be considered prohibitive if I am solely using insect buzzes generated from individual sites to create site-specific classifiers (to account for the individual acoustic footprint of different areas, in terms of background noise), especially in areas with relatively low acoustic insect activity. However, repeated visits to the same site over a short period of time, e.g., several months (so that the acoustic footprint of the site remains relatively constant), should provide enough data to make this possible.

It is possible that this low detection rate may have been due to an issue with the microphone used, as opposed to an issue with Kaleidoscope Pro. Recent research suggests that the signal-to-noise ratio (the power of a given signal relative to the power of the background noise) inherent to each microphone[2] can affect its ability to detect acoustic signals of interest (Darras et al., 2016, 2018; Darras et al., 2020). However, Darras et al. (2020) indicates that microphones equivalent to those used as part of this study (with a high signal-to-noise ratio of ca. 80dB at 1kHz) were capable of detecting bird calls over an area in excess of 9000m^2, which suggests that they should be capable of detecting insect wing-beats within a 433.74cm^2 plate.

In terms of the cluster analysis tool's ability to accurately classify those insect buzzes it has detected; the number of true positives was significantly lower than both the number of total number buzzes detected by the cluster analysis tool and the manual counts of buzzes per hour, a pattern which remained consistent regardless of the perceived activity level of each recording. The mean proportion of the manual counts that were not correctly clustered by the algorithm was 0.85 (± 0.022), indicating that my SICs only accurately clustered fifteen per cent of the insect signals present. And, while this proportion decreased significantly as the number of insect signals used to train the SICs increased, it was initially so high that our results suggest that more than 500

[2] Defined in relation to the self-noise associated with a microphone. That is, the noise produced by a microphone in a perfectly silent environment, which itself defines the lowest sound pressure level that can be detected by a given microphone (Darras et al., 2020).

training signals would be required raise the number of accurately clustered availably signals to more than fifty per cent.

The SICs created and trained using the cluster analysis tool had very low associated false positive rates (mean: 0.058 ± SE 0.007; range: 0 – 0.4), indicating that they are capable of both accurately and reliably clustering non-insect acoustic signals. These values are comparable with those of Raman, Gerhardt, & Wilkerson (2007), whose mosquito detection program had an associated false positive rate of 6.5%, and are considerably lower than those linked with the Computational Auditory Scene Analysis (CASA) tool developed by Heise et al. (2017) (and later used by Miller-Struttmann et al. (2017)), where the false positive rate was much more variable, ranging from 0.047 to 0.946, with an overall false positive rate of 0.386. The false negative rates associated with the SICs, however, were both high and highly variable (mean: 0.601 ± 0.039; range: 0 – 1); on average, only forty per cent of detected insect buzzes were correctly classified as such. This compares far less favourably with the results of the CASA tool developed by Heise et al. (2017), where the false negative rate ranged between 0.062 and 0.805, with an overall false negative rate of 0.320 – approximately half that associated with my SICs. In analytical terms, my SICs have high **specificity** (a low false positive rate) but low **sensitivity** (a high false negative rate) (Heise et al., 2017).

Increasing the number of insect signals used to train the SICs did decrease the false negative rate, but my results indicate that approximately 500 training signals would be required to match the rates shown by Heise et al. (2017). In addition, Figure 5.5B suggests that increasing the number of training signals to 500 would effectively double the false positive rate. I am not sure why this should be so, since logic suggests that a better trained algorithm should experience lower Type I and Type II errors. It is possible that background noise (usually either anthrophony or geophony) present within the training signals may be affecting the false positive rate. If the signal-to-noise ratio is low, then the cluster analysis tool may begin to associate the background noise with the insect cluster. I avoided using insect signals with any high amplitude background noise to train the SICs, but with more than 300 training signals it's possible that geophonic or anthrophonic signals with even moderate power relative to the insect signal may begin to effect the accuracy of

the cluster analysis tool, potentially increasing the false positive rate (Darras et al., 2016; Darras et al., 2020). The CASA detection tool developed by Heise et al. (2017) actually incorporates the signal-to-noise ratio of the soundscape recordings into its analytical approach through the application of their focal template. This "filter" (for want of a better term) takes 10ms sections of sound and looks for the four time-frequency bins that contain the highest spectral energy. Insect wing beats are highly harmonic signals (Heise et al., 2017), and if these four bins are in a harmonic relationship with one another then the signal is passed on for further classification, if not the sound is rejected as background noise. The mosquito detection tool by Raman, Gerhardt, & Wilkerson (2007) also used the harmonic "shape" of a wing beat to decide if a sound belonged to a mosquito or not: using the difference (in terms of decibels) between the first four harmonics of a given sound, as well as the differences between these peaks and the five inter-harmonic frequencies between them. If the shape of the signal described by the differences between these peaks did not meet the threshold associated with a mosquito's wing beats, then the sound segment in question would be discarded. By comparison, Kaleidoscope Pro describes each sound using Discrete Cosine Transform (DCT) coefficients extracted following a Fast Fourier Transform (FFT). These coefficients describe the shape of (or the pattern of energy within) each signal that meets the signal parameters described in section 5.2.2.2 by summing the cosine functions that comprise the waveform. The cluster analysis then uses a combination of Hidden Markov Models, K-means clustering, and Fisher scores to determine the similarity of different signals (Wildlife Acoustics Inc., 2019a). This may lead to a less focused analysis, as the cluster analysis tool is not focusing specifically on the harmonic nature of the candidate signals, but on differences in their overall shape. Heise et al. (2017) use a similar technique, initially, to describe the shape of each of their signals – a Discrete Fourier Transform (DFT) – which sums both the sine and cosine functions of the signal. But the analytic process applied after this transformation, specifically their use of a high-pass filter to remove high-energy low frequency background noise from each 10ms section of sound, and the application of their focal template which filters out non-harmonic signals, probably explains why their detection rate and classification accuracy was much higher than those associated with Kaleidoscope Pro.

There is an additional source of error that my current analysis has not considered: the number of insects that came into contact with the acoustic pan trapping stations but were not recorded by the microphones. The signal-to-noise ratio associated with the microphone indicates that I should be able to pick up any insect signal produced within the bounds of the UV-fluorescent plate (Darras et al., 2016; Darras et al., 2020). But in an environment with high levels of background noise, smaller insects, e.g., pollen beetles (*Meligethes* spp.) or smaller dipteran species, may produce flight sounds with a signal-to-noise ratio that is lower than the self-noise associated with the microphone (see footnote 18, page 163). I could attempt to solve this issue by collecting video footage of the acoustic pan trapping stations and comparing the number of visible insect visitations to manual counts of insect signals within the soundscapes.

Kaleidoscope Pro's cluster analysis tool has been used by other studies to detect and classify both bat and bird signals. The bat analysis mode within Kaleidoscope Pro operates very differently from the non-bat analysis mode used within this Chapter, with an automated identification function based upon pre-built libraries of recordings of known bat species. In addition, it appears to be common practise within bat acoustic studies to manually verify all identifications made by automated classifiers like Kaleidoscope Pro (see Braun de Torrez, Ober, & McCleery, 2018; Gorresen et al., 2018; Layng et al., 2019; Nocera et al., 2019), so discussions of error rates or classification accuracy using these types of commercial software aren't common. In terms of birds, however, Ross et al. (2018) used Kaleidoscope Pro to detect the calls of five bird species within soundscapes from five sites across Okinawa, Japan. Classification accuracy ranged between zero and one hundred per cent, depending on the site and the species, with an average accuracy of 76.8% (indicating a false negative rate of 33.2%, approximately similar to Heise et al. (2017)). Abrahams & Denny (2018) used Kaleidoscope Pro to detect Capercaillie (*Tetrao urogallus*) mating calls at lek sites near Aviemore, Scotland. The authors used a similar process to generate a supervised classifier and achieved comparable levels of error to those within this Chapter, with a false positive rate of 16.4% and a false negative rate of 64.9%. Pérez-Granados & Schuchmann (2020), meanwhile, achieved remarkably low false negative rates, between 0.015 and 0.09, whilst monitoring two nocturnal Neotropical bird species. The authors also manually

counted the number of bird calls within a subset of their soundscape data and found that their supervised classifier was detecting between 73.6 and 85.2% of the bird calls known to be present. By comparison my SICs were detecting thirty-eight per cent of the insect signals known to be present. This would suggest that our SICs are not as accurate or as reliable as those created by previous studies to detect and cluster bird calls.

Overall, although both the number of clustered insect buzzes and the number of true positives were positively correlated with the number of manual counts, which enabled me to carry on with my intended program of methods comparisons, I would not recommend Kaleidoscope Pro as an effective automated detection and classification tool for insect wing beat signals. The initial logic behind this choice of program was its low barrier to entry – it requires no programming ability and only a basic knowledge of acoustic concepts like frequency and amplitude. But my results suggest that the detection software developed by teams like Raman, Gerhardt, & Wilkerson (2007) and Heise et al. (2017) appear to be much better suited to the detection and classification of insect wing beat signals than commercially available programs aimed primarily at analysing bird and bat calls. These tools focus on known aspects of insect wing beat signals, e.g., their harmonic structure, that distinguish them from the majority of other biophonic, geophonic, or anthrophonic signals within a soundscape. Future studies should focus on adapting these tools and making them not only open source, but also more openly accessible to a non-expert audience, via the addition of a menu-driven graphical user interface (GUI) for example.

The methods comparisons show that the number of insect buzzes clustered by Kaleidoscope Pro from the acoustic pan trap recordings were not predictive of the number of insects sampled by the traditional pan traps or the number of insect visitations observed during the FFOs. With regard to the pan trapping samples, this is not necessarily surprising. Pan trap surveys provide a measure of abundance, and since the method is destructive – each insect can only be sampled once. Indeed, the results from **Chapter 2** indicate that, depending on the bowl size used, as the number of insects sampled increases, the number of new individuals attracted into the bowls appears to decrease. The acoustic pan traps, meanwhile, provide a measure of acoustic activity where each insect can be sampled multiple times. I had assumed, however, (and perhaps naively) that there might be a

link between the results of the two methods, in that areas with greater insect abundance might also have higher levels of acoustic insect activity, but this was not the case. Several previous studies have looked at methods by which passive measures of acoustic activity can be successfully transformed into estimates of animal density (Thompson, Schwager, & Payne, 2010; Marques et al., 2013; Bader et al., 2015), using prior knowledge concerning the behaviour of animal species, such as call rates, average group size, or range size, combined with the detection space associated with their microphones. However, these techniques are unlikely to be useful in regard to insect flight signals since these sounds are a passive by-product of their movement, rather than an active process, such as a mating call. My results also indicate that this lack of a link between the two methods is unlikely to be the result of colour bias, introduced by the combination of UV-fluorescent yellow, blue, and white bowls used during the pan trapping surveys, compared to the UV-fluorescent yellow plates used by the acoustic pan trapping stations. However, if attempting to compare the two methods again in future I would use yellow pan traps only, and try to match the approximate surface area of the bowls to that of the plates used as part of the acoustic pan traps.

What is more surprising is that there was no link between the number of clustered insect buzzes and the number of insect visitations recorded during the FFOs. Like the acoustic pan traps, the FFOs provide a measure of insect activity, since each insect visiting the focal floral resource was not marked in any way, or captured, and could visit the plant multiple times within the twenty minute survey period. In contrast, Miller-Struttmann et al. (2017) found a positive relationship between bumblebee activity recorded along visual transect surveys and acoustic bumblebee activity recorded by microphones placed at flower height within flower-rich alpine meadows. I also found no overall link between the average acoustic activity clustered per hour across all acoustic pan trapping stations per site and the insect activity recorded per hour by the FFOs, with significant correlations at just two sites visited in 2018. In both cases, the correlation was negative, with the number of clustered buzzes falling as the number of insect visitations to the focal floral resource increased. Since the pattern of diel activity described by the number of clustered buzzes per hour compares positively to past studies of temporal patterns in flower-visiting insect activity

(Heinrich, 1979; Gilbert, 1985; Corbet et al., 1993; Steen, 2017), although diel activity is far from constant between insect taxa (Willmer & Stone, 2004), it's likely that our FFO protocol was simply not as effective at sampling insect activity as the visual transect surveys used by Miller-Struttmann et al. (2017). Both Westphal et al. (2008) and Nielsen et al. (2011) note that 1 x 2m floral observation plots performed poorly in comparison to both variable and standardised transect surveys in terms of sampled bee abundance. The FFO protocol used here was designed so that it could be carried out in any habitat regardless of the floral abundance present, whereas the FIT counts used by UK PoMS (upon which my protocol was partly based) relies on existing floral resources. I used a single plant and moved it randomly between a set of ten pre-defined locations within each site. It is entirely possible that this constant movement may not have allowed flower-visiting insects enough time to grow accustomed to either the presence or the position of the plant, or the level of resource that it represented in terms of pollen or nectar. If this was the case, then the FFOs would only have measured chance visitations by insects that happened to be come into contact with the plant, rather than a more representative measure of insect foraging activity within each site. By comparison, the acoustic pan traps remained in static locations throughout the day. If I were to carry out this experiment again, I would change the protocol concerning the FFOs to either leave a single plant in one randomly chosen location throughout each survey, or five plants in a third stationary transect parallel to the transects containing the acoustic pan trapping stations and traditional pan trapping stations.

As previously discussed, both the detection and classification rates associated with Kaleidoscope Pro were poor in comparison to those used by other authors (see Raman, Gerhardt, & Wilkerson, 2007; Heise et al., 2017; Miller-Struttmann et al., 2017), indicating that the number of clustered insect buzzes that I am correlating with the results of alternative survey methods is not necessarily representative of the acoustic insect activity present across each site. Although, within my subset of seventy-five hour-long recordings, the number of clustered buzzes and the number of true positives were both positively correlated with the number of buzzes manually counted from each soundscape. I am, therefore, hesitant to say that the results of the novel acoustic pan trapping are definitively not predictive of measures of insect abundance or activity provided by more

traditional methods. To do so, I would need to apply a more accurate and reliable detection and classification tool, as well as redesign the FFO protocol to be more representative of flower-visiting insect activity within each site.

This doesn't, however, mean that the value of the acoustic pan traps as a survey tool can't currently be discussed. Within the subset seventy-five hour-long recordings, the mean number of insect buzzes manually counted per hour was 77.09 ± 15.30 (range: 0 – 868). This was also, in a way, an underestimate of the true number of insect buzzes present, since I was only counting the number of buzzes that met the signal parameters listed in section 5.2.2.1 and that, therefore, could have been detected by Kaleidoscope Pro. The total number of insect buzzes that met these parameters across all seventy-five recordings was 5782, which is approximately comparable to the total number of insects sampled by the pan traps throughout this experiment (N = 5808). This promises a large quantity of available data that could eventually be used to predict pollination services within a given area (Miller-Struttmann et al., 2017), and study insect activity in relation to anthropogenic activity, e.g., urbanisation or pesticide use (Penone et al., 2013), or natural phenomena (Galen et al., 2019).

Increases in hourly temperature and hourly wind speed both had significant negative effects upon the number of insect buzzes clustered per hour. The fact that there was an effect of temperature indicates that I am observing at least some form of biological pattern in the number of clustered insects. Insects are poikilotherms and many rely (to varying extents) on ambient air temperature to increase the internal temperature of their flight muscles and therefore maintain flight (Heinrich, 1974; Heinrich, 1975). Extremes (high or low), therefore, will result in decreases in flight activity. Either due to an inability to maintain the internal thoracic temperature necessary to initiate flight, or due to overheating (Heinrich, 1974). However, given the weak nature of the effect and the fact that the number of clustered buzzes only started to drop as temperatures rose past 21°C (similar to the effect I saw upon bumblebees and hoverflies sampled via pan trapping in **Chapter 2**), it is unlikely that this drop is due to insects overheating, but rather (potentially) individuals seeking shade during the warmer portions of the day (Rotheray & Gilbert, 2011).

Increasing wind speeds may also depress insect flight activity (Digby, 1958; Juillet, 1964; Walters & Dixon, 1984; Vicens & Bosch, 2000), but the number of clustered insect buzzes dropped sharply at relatively low wind speeds of between 3 and 5mph. During the analysis for **Chapter 2**, the abundance of bees and hoverflies sampled by traditional pan trapping peaked between approximately 7 and 10mph. This suggests that it is more likely that the low-frequency background noise caused by higher wind speeds interfered with the ability of the SICs to detect insect buzzes. Wind, a source of geophonic noise, exists at relatively low frequencies (less than 2kHz, based upon my own observations), which have been shown to interfere with the detection of low-frequency animal calls by significantly lowering their signal-to-noise ratio (Lin et al., 2014; Priyadarshani, Castro, & Marsland, 2018). All of the wing-beat signals associated with the flower-visiting insect taxa recorded during Chapter 4 had fundamental frequencies between 150 and 300 Hz. In this manner, increasing wind speeds may decrease the detection rate of the cluster analysis tool or increase the false negative rate by interfering with its ability to classify insect wing-beats. Heise et al. (2017) dealt with this type of issue by passing 10ms segments of sound through a high-pass filter, reducing the spectral energy associated with low-frequency signals.

As in **Chapter 3**, there was no effect of local floral abundance (expressed here as the volume of nectar sugar produced per day within a two meter radius of each acoustic pan trapping station) upon the number of clustered buzzes. O'Connor et al. (2019b) observed no effect of increasing volumes of nectar sugar production surrounding their pan trapping stations on sampled bee and hoverfly abundance. If I compare the sites visited during this Chapter to those visited in O'Connor et al. (2019b) and **Chapter 2**, both the number of florets and the volume of nectar sugar produced per day surrounding each trapping station were greater within the latter two studies, often by an order of magnitude. It's probable that the floral density at many of my sites was so low that I should expect to see no effect of either floral abundance or nectar sugar production per day on insect flight activity. The sites used within this study were predominately urban in nature, representing mostly areas of amenity grassland along with some semi-natural grassland sites, chosen in an attempt to test the acoustic pan traps in areas with a diverse range of background

soundscapes[3]. By comparison, Miller-Struttmann et al. (2017) carried out their study in clover-rich alpine meadows during peak flowering, actively placing their acoustic recorders in areas of high floral abundance. The acoustic survey protocol used by Miller-Struttmann et al. (2017) incorporated no standardised attractant, but simply placed microphones at the same height as the surrounding clover racemes. In this way they may have biased the results of their acoustic surveys towards those of their visual transects, which tend to be positively correlated with floral abundance (Popic, Davila, & Wardle, 2013; O'Connor et al., 2019b). This acoustic survey protocol would also be of limited use within habitats with low levels of floral abundance.

In terms of time spent in the field, the acoustic pan traps were comparable to the traditional pan traps and required less effort (but more equipment) than the FFOs. In terms of data analysis, the time needed to sort through and identify the physical specimens sampled by the pan traps was little different to the time required to create the site-specific SICs for the cluster analysis, while all of the identifications for the FFO data were completed in the field. It is important to note, however, that the computational time spent training any detection and classification algorithm should be thought of as an investment. Once an algorithm has been trained, tested, and found to meet the accuracy requirements of a given study, it can then be continuously re-applied to new data, reducing future data analysis time in comparison to more traditional methods. The algorithm may still require updating as new training data containing different taxa or new sites with different acoustic fingerprints are collected, but this doesn't need to happen each time the algorithm is applied. Which means that acoustic pan trapping data would require less effort to analyse in the long-term than samples from traditional pan trapping. In addition, the technology fuelling the current peak in interest in passive acoustic monitoring is constantly being updated. Since starting this body of research in 2017, the AudioMoth recorders, fast becoming an industry standard, have become more affordable than the SOLO recorders used in this Chapter, and their open source

[3] The sites used in 2017 were also originally chosen as part of a separate thesis chapter, focusing on how experimental additions of floral resources in areas around Leeds, as part of Buglife's Urban Buzz scheme (https://www.buglife.org.uk/projects/urban-buzz-leeds), might affect pan trapping efficacy. The acoustic pan traps were also tested within these sites so as not to duplicate sampling effort during a limited survey season.

operating system makes them equally easy to use and apply to novel survey methods like the acoustic pan trap. These recorders are also significantly smaller than the SOLO recorders, and would reduce the amount of equipment needed to carry out future acoustic surveys.

There is also a move towards more open sharing of data, citing the huge amounts of information inherent to soundscape recordings relating to different biophonic, geophonic, and anthrophonic sources of sound, which most studies only scratch the surface of. My soundscapes contain data concerning bird song and orthopteran stridulation in urban and non-urban environments, traffic noise, human speech that could be linked to people's use of urban greenspace, all in addition to the insect wing beat signals that I was primarily interested in. There have been moves towards the creation of open-source, citable libraries where practitioners and researchers could lodge their data so that others can access them (Kasten et al., 2012; Browning et al., 2017; Gibb et al., 2019). This feeds into the concept that soundscape recordings are multipurpose datasets, and that the data they contain can be re-purposed by researchers with different expertise, focusing on different aspects of the soundscape, to answer a variety of ecological questions. The creation of these libraries for soundscape data could become key to the concept of whole ecosystem monitoring through sound.

5.5 Conclusions

Acoustic pan trapping provides a novel method of passively monitoring flower-visiting insect activity that has the potential to collect long-term data concerning insect activity. I was unable to generate accurate estimates of acoustic insect activity throughout this experiment due to the limitations associated with the cluster analysis tool in Kaleidoscope Pro. Namely, higher rates of Type II errors than those associated with other algorithms used to investigate acoustic insect activity (Raman et al., 2007; Heise et al., 2017). Due possibly in part to this, I was also not able to find any link between the number of insect buzzes clustered by Kaleidoscope Pro and either the abundance of flower-visiting insects sampled by pan trapping or observed during hourly focal floral resource observations. I can, therefore, make no comparisons between the efficacy of the acoustic pan trap in relation to these two common survey methods. Despite this, my data indicate that the acoustic pan traps were recording large quantities of acoustic insect activity per hour.

Acoustic pan trapping is clearly not a replacement for either visual surveys or passive surveys based upon methods like pan trapping, but the data it can provide would still allow us to explore the effects of drivers of insect pollinator decline, e.g., agricultural intensification and urbanisation, by using acoustic activity as a proxy for community health. Future research should focus on the creation, adaptation, or optimisation of novel or existing detection software tools to allow us to extract these acoustic signals with greater levels of accuracy, so that we can use these data moving forward.

Chapter 6

General discussion: towards a standardised set of monitoring protocols for insect pollinators

"What is a university for if it isn't to tell you that everything you think you know is wrong?"

Terry Pratchett, Collegiate Casting-Out of Devilish Devices.

6.1 Introduction

Insect pollinators account for nearly 350,000 species, and are responsible for reproduction in 87.5 per cent of flowering plant species, including 75 per cent of the world's leading food crops, or 35 per cent of global food-crop production by volume (Ollerton, Winfree, & Tarrant, 2011; IPBES, 2016; Ollerton, 2017). Environmental change driven by human activity, including habitat loss and fragmentation, agricultural intensification and pesticide-use, and climate change, has led to declines in insect pollinator populations worldwide (Potts et al., 2010; Gonzalez-Varo et al., 2013; Goulson et al., 2015). In the UK, a third of our native wild bee and hoverfly species have experienced declines since 1980 (Powney et al., 2019), changes that appear to primarily affect rarer and more specialist species, with generalist species and crop specialists displaying rare positive trends (Biesmeijer et al., 2006; Powney et al., 2019). If these trends are allowed to continue then we risk not only the ecosystem services provided by insects in terms of agriculture, but also the pollination services provided to wild plant species, and the knock-on effects this would have upon plant-pollinator interaction and terrestrial ecosystem stability (Biesmeijer et al., 2006; Burkle, Marlin, & Knight, 2013; Christmann, 2019).

It is concerning, therefore, that the greater part of our knowledge concerning insect pollinator decline is limited to records of species occupancy (Powney et al., 2019), trends in species richness

(Biesmeijer et al., 2006; Carvalheiro et al., 2013; Ollerton et al., 2014), and changes in range size (see Williams & Osborne, 2009). Even in countries with a well-studies insect fauna, such as the UK, we lack long-term abundance data at the species level which would allow us to quantify population trends and pinpoint targets for future conservation.

6.2 Standardised sampling protocols for current survey methods

The systematic collection of long-term abundance data is only possible through the development and use of standardised survey methods and monitoring protocols (Lebuhn et al., 2013; O'Connor et al., 2019b; Powney et al., 2019). Historical data collection by specialist recording schemes, such as the Bees, Wasps, and Ants Recording Society (BWARS) and the Hoverfly Recording Society (HRS), provide valuable long-term datasets, but the lack of standardised survey protocols means that these data are open to sampling bias and are difficult to compare and contrast over space and time (Powney et al., 2019). It is vital, therefore, that that the biases of established survey methods are explored, and the performance of different sampling protocols are tested, enabling population data from future monitoring schemes to be compared across wider spatio-temporal scales, both nationally and internationally (Lebuhn et al., 2013).

My first two chapters focus on two of the most common methods used to survey insect pollinator communities: pan trapping and transect surveys (Westphal et al., 2008; Nielsen et al., 2011; Popic, Davila, & Wardle, 2013). In **Chapter 3**, I focus on attempting to quantify the sampling biases inherent within these two methods with regard to bees and hoverflies, but first, in **Chapter 2**, I take a closer look at the varied range of protocols employed by users of pan trapping, in an attempt to standardise future usage of this method.

Pan trapping is a popular method for sampling insect pollinators (Westphal et al., 2008; Lebuhn et al., 2013), primarily due to its lack of collector bias. Surveyors currently employ a range of protocols based upon differences in bowl size, bowl colour, and trap duration (Carboni & Lebuhn, 2003; Moreira et al., 2016; Gonzalez et al., 2020), which may bias the data collected and make it challenging to compare results between studies (Lebuhn et al., 2013; Powney et al., 2019). And, while there has been a lot of focus upon comparing the performance of this method to others, such

as transect walks (see **Chapter 3**), there has been comparatively little published comparing the performance of different pan trapping protocols (but see Droege et al., 2010; Wilson et al., 2016).

Chapter 2 presents what is, to the best of my knowledge, the first published study to compare multiple aspects of pan trapping methodology, based primarily around bowl size, bowl colour, and trap duration, in combination with the effects of local environmental variability, upon the abundance and species richness of bee and hoverfly samples captured by pan traps. The results form the basis for recommendations regarding future best practise concerning this method.

While past studies have explored the effects of bowl size on pan trap samples (Droege, 2002; Wilson et al., 2016; Gonzalez et al., 2020), they have often used volume as their sole measurement (see Droege, 2002; Wilson et al., 2016), but here I show that bowl surface area and bowl depth are potentially more likely to influence the number of bees and hoverflies sampled, with significantly greater abundance and species richness captured in bowls with larger surface areas and greater surface area-to-depth ratios. However, while total sample size did increase with bowl surface area, the proportion of bumblebees sampled (relative to the abundance of all non-bumblebees) actually decreased. Whether this is due to olfactory signals left by previous foragers, or increased sample size blocking out the attractive colours of the bowls, is unclear, but this is the first evidence that pan traps may have size-specific carrying capacities.

This concept can also be linked with time. Of the three different trap durations tested as part of this study: seven hours, 24 hours, and 48 hours, the bowls left out for 48 hours sampled the most bees and hoverflies. However, as trap duration increased, the rate of capture within these groups decreased, indicating that shorter trap durations are sampling more bees and hoverflies per hour than long longer surveys.

These results open up interesting avenues for further research. For instance, manipulating the number of insects present in pan traps by emptying them at regular intervals and replacing the samples with different numbers of insects, i.e. laboratory-raised Calliphoridae, would allow for an examination of whether pan trap carrying capacities are a response to visual stimuli, based upon an aversion to the sight of dead insects or a blockage of bowl colour. Any aversive effects

related to scent-marks left by previous foragers could be eliminated by regularly wiping the rim of the bowls with ethanol or another quick-drying solvent.

In terms of how the local environment variables affects the sampling ability of pan traps, the primary finding was that nectar sugar production at two spatial scales: within a two meter radius of the pan trapping stations, and as an average across the experimental site, had a positive effect on bee and hoverfly abundance and species richness within the pan traps. This contrasts with the findings of several past studies (Roulston, Smith, & Brewster, 2007; Baum & Wallen, 2011; Wilson et al., 2016; O'Connor et al., 2019b; Westerberg et al., 2021), all of which suggest that pan traps sample fewer bees in the presence of greater floral diversity. This is the first evidence to contradict these past findings, and indicates that pan traps may be suitable for sampling in a wider variety of habitats than previously supposed. Alternatively, this result may be the product of a density-visitation relationship (see Rathcke, 1983), where, at relatively low levels of floral density, the presence of more flowers is facilitating visits to the pan traps. This would also agree with the findings of O'Connor et al. (2019b), who found than bee and hoverfly samples decreased in pan traps placed in high floral density crop monocultures. In order to test for this, and for the repeatability of my own results, I recommend that future research focuses on testing pan traps in habitats representing a much wider range of floral densities than those tested here. In addition, future studies should fully quantify their chosen measure of floral abundance, diversity, or density, since many previous studies have used only broad estimates of floral diversity at a site-level scale (Roulston, Smith, & Brewster, 2007; Baum & Wallen, 2011; Popic, Davila, & Wardle, 2013).

This chapter also provides a synthesis of initial evidence concerning how local weather affects pan trap sampling. Many sampling protocols include caveats regarding what is and is not suitable weather, but there is, to my knowledge, very little research informing these suggestions (Saunders & Luck, 2013). I show that solitary bee species were caught less often during extremes of wind (>10 mph), and that fewer pollinators and bumblebees were sampled when temperatures were in excess of 25-30°C; providing the first evidence to suggest an upper temperature limit in relation pan trapping surveys. This evidence shows that more attention should be paid to "ambient" effects

like weather in relation to the sampling ability of different survey methods, especially in light of results like those found in **Chapter 3**, where the sampling ability of both pan traps and transect surveys may have been negatively impacted by extreme wind speeds, and **Chapter 5**, where increased wind speeds decreased the ability of an automated acoustic detection software to detect and cluster instances of insect flight sounds from soundscape recordings.

Studies like this have the potential to being applied to any sampling method, and the creation of standardised protocols for individual survey methods is an important first step towards the systematic collection of data concerning insect pollinator population trends. The second is the exploration of methods-based sampling bias. There has been a lot of focus on comparing the performance of different methods in an attempt to discern or quantify their inherent sampling bias (Westphal et al., 2008; Nielsen et al., 2011; Popic, Davila, & Wardle, 2013), with pan trapping and transect surveys being the most commonly compared within the literature (Cane, Minckley, & Kervin, 2000; Roulston, Smith, & Brewster, 2007; Westphal et al., 2008; Wilson, Griswold, & Messinger, 2008; Nielsen et al., 2011; Popic, Davila, & Wardle, 2013; O'Connor et al., 2019b).

The primary biases of both pan trapping and transect surveys are well-known: colour bias, based upon the bowl colours used, and collector bias, respectively (Potts, Evan, & Boone, 2005). Evidence for other potential biases has also been presented by previous studies, primarily that pan trap samples contain a greater proportion of smaller solitary bees than samples from transect surveys, but fewer bumblebees (*Bombus* spp.) or honeybees (*Apis mellifera*) (Roulston, Smith, & Brewster, 2007; Wilson, Griswold, & Messinger, 2008; Grundel et al., 2011), and that transect surveys are biased towards larger, slower-flying insect species (Potts, Evan, & Boone, 2005). This grey area in our knowledge-base has led to disagreements over which method best represents insect pollinator communities in its samples. For instance, several past studies indicate that pan trapping should always be pared with an active sampling method like transect surveys as their respective sampling biases offset one another (Grundel et al., 2011; Nielsen et al., 2011), whereas other studies indicate that transect surveys or pan trapping alone are sufficient to survey insect pollinator communities in a representative manner (Westphal et al., 2008; Lebuhn et al., 2013; Popic, Davila, & Wardle, 2013; LeBuhn et al., 2016).

Chapter 3 is an attempt to quantify these biases, but with one critical difference. Instead of simply comparing the samples from the two methods, I used a mark-release-recapture (MRR) experiment in a closed island ecosystem in Greece (Prassológos, Lesvos) to provide estimates of the relative abundance of local bee and hoverfly species. Thus allowing for comparisons between the rank abundance of species in samples collected by pan trapping and transect surveys and the rank abundance of species estimated to be present within the local bee and hoverfly community. This is the first study of sampling bias in relation to insect pollinators to include comparisons with an independent source of data concerning the relative abundance of species within a community, and, to my knowledge, the first study to use MRR methods to estimate population size in bee and hoverfly species using a closed island ecosystem. The pan trapping protocol used during this study was based upon initial results from **Chapter 2**.

The MRR experiment estimated population size for four insect pollinator species on the islet of Prassológos: two bees and two hoverflies. In comparison to their estimated population size, the relative abundance of each species as sampled by both pan trapping and transect surveys was significantly smaller than expected. This suggests that the subsample of the community being collected by both methods is smaller than has been supposed by some studies (see Tepedino et al., 2015; Wilson et al., 2016). Indicating that both methods can be used fairly intensively, even in quite isolated systems, without adversely affecting local pollinator community composition or diversity (as in Gezon et al., 2015).

It may be possible to quantify exactly how large this subsample of the population is, at least with regard to pan trapping, through the use of repeated observational studies. An observer would set up a pan trapping station and record the number of insects from different taxa that are sampled by the bowls in relation to the number that approach but do not land, or which approach and land in the water but escape etc. This type of experiment would explore the behaviour of different insect pollinator taxa in relation to the pan traps, and allow an exploration of taxonomic sampling bias within the method. Although, it would be require high levels of concentration and taxonomic ability from the surveyor, but this could be mitigated through the use of video footage, which has been shown to provide an accurate alternative to in-person surveys (Steen, Lene, & Orvedal, 2011;

Gilpin, Denham, & Ayre, 2017; Steen, 2017); with the added advantage that instances of challenging or cryptic taxa, or smaller, fast-flying individuals, can be dealt with by replaying footage or seeking confirmation from additional surveyors (Gilpin, Denham, & Ayre, 2017).

In terms of sampling bias, the rank abundance of the four species I was able to produce population estimates for was most closely matched by a combination of the samples from both pan trapping and transect surveys (employed in tandem). This would appear to confirm the results of several past studies who suggest that the two survey methods should be employed together in order to balance their respective sampling biases (Roulston, Smith, & Brewster, 2007; Grundel et al., 2011; Nielsen et al., 2011; Prendergast et al., 2020). On its own, pan trapping samples represented the relative abundance of one solitary bee species (*Halictus phryganicus*) better than either transect surveys or a combination of pan trapping and transect surveys; evidence contrary to the common hypothesis that pan trapping overestimates solitary bee abundance (Roulston, Smith, & Brewster, 2007; Westphal et al., 2008; Wilson, Griswold, & Messinger, 2008; Grundel et al., 2011; Portman, Bruninga-Socolar, & Cariveau, 2020). The transect surveys, meanwhile, overestimated the relative abundance of the two hoverfly species, possibly due to the effects of collector bias. Bumblebees and honeybees were poorly sampled by both methods; but data from the MRR surveys show that honeybees were rare visitors to Prassológos, while bumblebee foragers, although common, may have been discouraged by the presence of high winds during the pan trapping/transect survey portion of the experiment. The effects of wind upon sampling by either survey method were not quantified as part of this study, but may have provided a key advance to the findings from **Chapter 2** if they had been. This drives home the message that local weather variables should be recorded and accounted for in analyses relating to the performance of different survey methods, as weather can be a source of sampling bias in and of itself (Tyre et al., 2003).

The data generated as part of this chapter didn't lend themselves to a rigorous statistical analysis, in part due to the small number of samples collected. However, in the context of the system in which we were working, neither pan trapping nor transect surveys collected a representative sample of the species richness encountered during the MRR surveys, although pan trapping

collected the most diverse and abundant set of samples. This supports the view of Grundel et al. (2011) and Nielsen et al. (2011), who suggest that the two methods should be used in tandem to generate more complete species inventories.

Mark-release-recapture provides an exciting novel tool to ground-truth the findings of different survey methods, although limited in its use with regard to insect pollinator communities by the availability of closed population systems like islands. Open-population analysis methods exist and were used during this study, but landscapes that are truly open to unlimited immigration and emigration would contain communities that are too large and mobile to accurately quantify using MRR methods.

6.3 The development and testing of novel survey methods

The evidence for insect pollinator declines crosses international boundaries (Biesmeijer et al., 2006; Goulson, Lye, & Darvill, 2008; Carvalheiro et al., 2013; IPBES, 2016) and both survey methods and sampling protocols need to be applicable to nationwide surveys if we are to quantify their population trends moving forward (Lebuhn et al., 2013). However, survey methods, such as those described and tested in **Chapters 2** and **3**, are not necessarily applicable to monitoring at a nationwide scale (but see Carvell et al., 2016).

For instance, the standardised pan trapping protocol defined in **Chapter 2** would have to involve equipment being sent to surveyors, and would generate samples that would need to be sent back for identification if individual surveyors did not have the taxonomic experience necessary to identify samples themselves. Whereas transect surveys, if the goal is to provide data at the highest level of taxonomic resolution possible, would require surveyors either with previous experience or to undergo extensive training regarding insect field identification. Prior research suggests that there are not enough trained personnel, either in terms of field experience or traditional taxonomy skills, to supply volunteers for a full-time, nationwide pollinator monitoring scheme (Pocock et al., 2015).

In response to this, current monitoring schemes, like the UK's national Pollinator Monitoring Scheme (PoMS), often incorporate citizen scientists into their sampling protocols in an effort to

expand the spatial and temporal scales over which they can gather data. However, citizen science data collection still requires standardised survey protocols to enable reliable data comparisons between individuals. While the use of volunteers can introduce bias into data collection through collector bias, temporally-patchy records, and misclassification of taxa in the field (Isaac et al., 2014; Falk et al., 2019).

In **Chapter 4**, I explore a novel, non-destructive classification method for insect pollinator taxa that can be applied to future monitoring surveys, including those using citizen scientist volunteers. Bioacoustics is a discipline devoted to the study of sounds made by animals, and has been used extensively to classify and monitor a range of taxonomic groups (Chesmore, 2001, 2004; Acevedo et al., 2009; Armitage & Ober, 2010; Briggs et al., 2012). Its use within pollinator monitoring may reduce the bias associated with misclassification errors by citizen scientists in the field, while providing an exciting new avenue through which volunteer participants could involve themselves in long-term, large-scale standardised monitoring surveys.

In terms of insect pollinator taxa, bioacoustic classification has been explored by two previous publications: 1) by Gradišek et al. (2016) focuses on species-level identification in bumblebees using their wing beat frequencies, and 2) by Kawakita & Ichikawa (2019) used wing beat frequency to classify four Hymenopteran pollinator species. However, here I present the first study to use bioacoustic classification methods to identify a range of Hymenopteran and Dipteran taxa at multiple levels of taxonomic resolution.

By comparing two different machine learning methods: random forest and support vector machine (SVM) models, I was able to reliably classify between Hymenopteran and Dipteran insects with an 86 per cent accuracy rate, between syrphid and non-syrphid flies with a 93 per cent accuracy rate, and between bumblebees, honeybees, solitary bees, and social wasps, with a 72 per cent accuracy rate. This indicates that bioacoustic classification at lower levels of taxonomic resolution is theoretically possible, and could present a reliable method of surveying insect pollinator species analogous to the Flower-Timed Insect Counts (FIT Counts) used by PoMS (Carvell, 2017). The implementation of such a method would, however, require a more balanced dataset incorporating

a wider range of flower-visiting insect taxa, so as to better explore and quantify the differences between them.

In contrast to the results of Gradišek et al. (2016), I found that bioacoustic classification was less accurate at higher levels of taxonomic resolution. Within the bumblebee genus, for example, classification accuracy for individual species ranged between 28 and 91 per cent. This was likely due the relationship between wing beat frequency and body size (Byrne, Buchmann, & Spangler, 1988; Molloy et al., 1988; Burkart, Lunau, & Schlindwein, 2011; Gradišek et al., 2016; Miller-Struttmann et al., 2017), considering the high level of overlap in terms of worker size between bumblebee species (Peat, Tucker, & Goulson, 2005; Goulson, 2010; Falk, 2015). Whereas different solitary bee and hoverfly genera were classified with greater accuracy (between fifty and 100 per cent). Future data collection should aim to better quantify the effects of body size on the inter- and intra-specific variation in insect wing beat frequencies, to drive further development of this potential survey tool.

The dataset collected and used to train the machine learning algorithms is, to my knowledge, the largest collection of insect pollinator flight recordings in the UK, containing 1196 instances of insect flight sounds. It is my intention to publish this dataset in an open-access format, so that it can be used and built upon by future researchers and conservation practitioners for the further test and develop this methodology. However, it is also inherently unbalanced, with insect classes like the non-syrphid Diptera and the social wasps containing far fewer recordings than the bumblebee class. Future data collection should focus upon generating a more balanced dataset, as well as a more diverse one. As mentioned in **Chapter 1**, the term "insect pollinator" can refer to more than 350,000 species, approximately 6000 of which may be found in the UK (Falk, S. 2017, personal communication), including bees, flies, beetles, wasps, moths, and butterflies (Ollerton, 2017). Any collection of future recordings should aim to include examples of as many of these different taxa as possible, to enable classification between multiple taxonomic groups.

In **Chapter 5**, I expand on the technology explored in **Chapter 4**, by exploring the application of acoustic research to the passive monitoring of insect pollinator communities. There has been increasing interest in recent years in the exploration of passive, non-invasive survey methods

capable of exploring community interactions and species diversity at a landscape scale (August et al., 2015; Gibb et al., 2019). These include eDNA and the use of metabarcoding (Thomsen & Sigsgaard, 2019), Light Detection and Ranging (LiDAR) (Malmqvist et al., 2018), weather radar networks (Chapman, Reynolds, & Smith, 2004), and passive acoustic methods (Miller-Struttmann et al., 2017; Galen et al., 2019).

In terms of insect monitoring, autonomous acoustic sensors have been used by past studies to explore the effects of human activity upon community composition (Penone et al., 2013), as well as changes in animal activity in relation to natural phenomena (Galen et al., 2019). More recently, Miller-Struttmann et al. (2017) used passive acoustic sensors to successfully predict pollination services based upon the density of local bee "buzzes", showing that acoustic measures of bumblebee activity were positively correlated with visual observations of bumblebee abundance. As an extension of this I wanted to test the performance of passive acoustic surveys in relation to other traditional survey methods, in order to assess its future utility in terms of monitoring flower-visiting insect community activity.

As part of this chapter, I designed the first example of a standardised passive acoustic trap aimed at sampling flower-visiting insects, based upon the autonomous SOLO acoustic recorder (Whytock & Christie, 2016) and the methods used in Miller-Struttmann et al. (2017). I also provided the first test of the commercially available Kaleidoscope Pro automated detection software (© Wildlife Acoustics, Inc., 2019) with regard to its ability to detect and cluster instances of insect flight sound from soundscape recordings. The number of instances of insect sound recorded by the acoustic pan traps and detected by Kaleidoscope Pro would be correlated with insect samples collected using two traditional survey techniques, both used by the UK PoMS as part of their standardised survey protocol: pan trapping and hourly focal floral resource observations (FFOs). The sampling protocols governing the pan trapping and acoustic pan trapping were designed based upon the findings from **Chapter 2**.

The supervised cluster analysis tool employed by Kaleidoscope Pro did not provide an effective automated method of either detecting or classifying instances of insect flight sound. Using a randomly-selected subset of seventy-five hours of soundscape recordings, I quantified the false

positive and false negative errors. I found that, although the algorithm had very low false positive errors and was, therefore, very effective at classifying instances of non-insect sound, it performed much more poorly in terms of its ability to correctly classify instances of insect flight sound, especially in relation to more specialist classifiers previously developed to classify acoustic insect signals (Raman et al., 2007; Heise et al., 2017). I also manually quantified the number of insect acoustic signals within each of these recordings and compared these data to the number of insect signals detected by Kaleidoscope Pro, finding that it was detecting only 40 per cent of the acoustic insect signals present. Based upon this, I would not recommend the use of the Kaleidoscope Pro software for future work aiming to quantify acoustic insect activity from within soundscape data. Instead, I would focus on the creation or adaption of algorithms that emphasise the qualities that separate insect wing beats from other signals within a soundscape, namely their highly harmonic nature.

The number of instances of insect sound clustered by Kaleidoscope Pro did not correlate with the samples collected by traditional pan trapping. This was not necessarily unexpected, since the two methods are sampling insects in fundamentally different ways: the pan traps provide a measure of abundance, while the acoustic pan traps provide a measure of activity. Although, it may be expected that insect activity may be greater in areas with higher overall insect abundance. This suggests that the future utility of this method would lie in measuring insect pollinator activity in relation to drivers of change in insect communities or populations, like urbanisation (see Penone et al., 2013) or agricultural intensification, rather than as a method of quantifying changing population trends. Acoustic recordings could be combined with video footage, in a similar fashion to Steen, Lene, & Orvedal (2011), Gilpin, Denham, & Ayre (2017), and Steen (2017), to compare the species attracted to the acoustic pan traps to those sampled by the water-based pan traps or observed during the FFOs. This would also provide some data concerning the acoustic detection space associated with the acoustic pan traps (Darras et al., 2016). That is, up to what spatial extent can the microphone record insect wing beats? And is this affected by the size of the insect in question or the level of ambient background noise? This method could also be combined with more comprehensive versions of the classification tools initially tested in **Chapter 4**, potentially

allowing us to generate some estimate of the taxonomic diversity surrounding the acoustic pan traps, even if only at a low level of taxonomic resolution.

There were also no correlations between the number of instances of insect sound detected per hour by the acoustic pan traps and the number of insect observed per hour using the FFOs in most of the experimental field sites. This was a more surprising result since both the acoustic pan trap and the FFOs provide a measure of insect activity. However, it may be that this result is due to issues concerning the FFO protocol combined with the relatively poor detection and classification rates associated with Kaleidoscope Pro.

Across the random subset of seventy-five hours of recordings, the acoustic pan traps recorded an average 77.09 ± 15.30 insect signals per hour. Multiplied across the 735 hours of soundscape recordings generated throughout this study, this provides a library of nearly 57,000 instances of acoustic insect activity. This is, to my knowledge, the only soundscape dataset focused specifically on recording flower-visiting insects and insect pollinators. As with the data informing **Chapter 4**, it is my intention to make this dataset open-access so that it is available to other researchers or conservation professionals etc. The soundscapes themselves contain a wealth of data in addition to the sounds relating to insects, including sounds relating to bird diversity, human activity, and urbanisation. All of which can be explored in future, along with their relationship to different levels of insect activity. Soundscape data like this could also form the basis for measures of habitat quality in urban greenspaces, or the pace of recovery in areas receiving conservation action, where insect activity is just one aspect of the diversity of sounds being measured (Gibb et al., 2019). Measures of insect activity could also be used as a proxy to test people's enjoyment or appreciation of greenspaces, in terms of their effects on human health (Millennium Ecosystem Assessment, 2005).

6.4 Concluding remarks

It is clear from the results of this thesis that neither the novel acoustic survey methods explored in **Chapters 4** and **5**, nor the traditional survey methods tested in **Chapters 2** and **3**, are the sole foci around which we should base future standardised insect pollinator monitoring. Both sets of techniques have a place in future monitoring strategies, alongside the many other methods

currently used or being investigated in relation to insect pollinators (**Chapter 1**). The more traditional survey methods, like pan trapping and transect surveys, provide data with a high level of taxonomic resolution that informs population trends and our knowledge of decline within different species. While the novel, non-invasive acoustic technology may enthuse and motivate citizen scientists to become involved with schemes like PoMS, providing data at a lower taxonomic resolution but with less inherent bias relating to misidentifying insects in the field. Or by providing measures of flower-visiting insect activity in relation to drivers of decline. The key area of future research is the continued focus on standardisation of monitoring protocols, as in **Chapter 2**, and the focus on quantifying sampling bias, as in **Chapters 3**, **4** and **5**, without which, data concerning insect pollinator populations and communities cannot be reliably compared between studies, or across space and time.

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Appendix 1.

Supplementary Table 1.1. List of studies cited by Gonzalez et al. (2020) that used pan traps to survey bees, published between January 2014 and December 2018, showing the duration (in hours) over which the pan traps were left active. Instances of multiple rows per study indicate multiple treatments within the same article.

Year	Author	Reported trap duration			Country	Comments
		Hours	Days	Weeks		
2018	Amy et al.	7			Belgium	Set out between 09:00-17:00.
2018	Campbell et al.	72			USA	
2018	Choate et al.	48			USA	
2018	Davis et al.	24			Ireland	
2018	Halinski et al.	24			Brazil	
2018	Happe et al.		7		Germany	
2018	Kehinde et al.		5		Italy, South Africa	
2018	Milam et al.	24			USA	
2018	Sircom et al.		7-10		Canada	
2018	Perrot et al.		4		France	
2018	Pfiffner et al.	27-37			Switzerland	Only sampled during hours of sunshine.
2018	Sivakoff et al.	48			USA	
2018	Stein et al.	72			Burkina Faso	
2018	Stephenson et al.	11			USA	
2018	Talašová et al.	48			Czech Republic	
2017	Andersson et al.		~4		Stockholm	
2017	Bukovinszky et al.		2		Netherlands	
2017	Gervais et al.	48			Canada	
2017	Harris et al.		6-7		USA	
			5			
2017	Heneberg et al.	*			Czech Republic	*“The Moericke traps were exposed for several days in each of the following three

						periods: 6–10 May 2013, 6–10 June 2013, and 8–15 August 2013.”
2017	Lagucki et al.	24			USA	
2017	Landaverde-González et al.	*			Mexico	*“…we sampled the bee community in chilli-growing areas for at least 1 day every week from May to June in 2010 and from May to August in 2011.”
2017	Lucas et al.		4		Wales	
2017	McCravy and Ruholl	9			USA	
2017	McKechnie et al.	24			Canada	
2017	Meyer et al.	11			Swiss Alps	
2017	Morrison et al.	7			Spain	
2017	Normandin et al.	48			Canada	
2017	Pascarella	24			USA	
2017	Plascencia and Philpott	11			USA	
2017	Rhoades et al.	24			USA	
2017a	Zou et al.		10		China	
2017b	Zou et al.		10		China	
2016	Basu et al.	24			India	
2016	Campbell et al.	72			USA	
2016	Elwell et al.	8.5			USA	
2016	Féon et al.	48			Argentina	
2016a	Geslin et al.	24			France	
2016b	Geslin et al.	24			South Africa	
2016	Gonzalez et al.		2		Turkey	
2016	Gostinski et al.	48			Brazil	
2016	Hall				USA	I could not access this article

2016	Heneberg et al.		2-8		Czech Republic	
2016	Hevia et al.	~8			Spain	Left active between sunrise and sunset
2016	Kovacic et al.	8			Croatia	
2016	Lazarina et al.	48			Greece	
2016	Love and Cane	~4-6			USA	
2016	Moreira et al.	24			Brazil	
2016	Mouga and Warkentin	7			Brazil	
2016	Quistberg et al.	~7-8			USA	
2016	Ritchie et al.	24			USA	
2016	Rodrigo et al.	~30			Spain	
2016	Ruttan et al.	8			Canada	
2016	Sahli et al.				Hawaii	I could not access this article
2016	Sing et al.	9			China, Malaysia, Thailand, Singapore	
2016	Todd	24			USA	
2016	Torne-Noguera et al.	~9-10			Spain	
2016	Wheelock et al.	24			USA	
2015	Classen et al.	48			Tanzania	
2015	Connelly et al.	72			USA	
2015	Geslin et al.	24			France	
2015	Gezon et al.	~9			USA	
2015	Gill and O'Neal	24			USA	
2015	González et al.	72			Argentina	
2015	Halinski et al.	24			Brazil	
2015	Hanula et al.		5		USA	
2015	Joshi et al.	24			USA	
2015	Marshall et al.	24			Netherlands	
2015	Meindl and Ashman		~2-3		USA	Bowls were left for 2 weeks, but were collected three times a week

2015	Miller et al.	24			Hawaii	
2015	Moisan-DeSerres et al.	~72			Canada	
2015	Richards et al.	~6			Canada	
2015	Rodríguez and Kouki	72			Finland	
		96				
2015	Rubene et al.	*			Sweden	Pan traps were emptied three times between 1st of June and 22nd of August, 2011.
2015	Sammegård et al.		2-3		Ethiopia	
			3-4			
2015	Saunders et al.	8			Australia	
2015	Schlueter and Stewart	~8-10			USA	
2015	Tang et al.	24			England	
2015	Wood et al.	96			United Kingdom	
2014	Alvarez et al.	~8			Argentina	
2014	Buri et al.	11			Switzerland	
2014	Cruz-Sánchez et al.	48			Spain	
2014	Fortel et al.	24			France	
2014	Geroff et al.	9			USA	
2014	Hall and Ascher	~24-26			USA	
2014	Heneberg and Bogusch		2-7		Czech Republic	
2014	Jackson et al.		2-3		USA	
2014	Larsen et al.		7		New Zealand	
2014	Pardee and Philpott	~6-7			USA	
2014	Ramírez et al.	10			Mexico	
2014	Rogers et al.	8			USA	
2014	Sardiñas and Kremen	4			USA	
2014	Shapiro et al.	8-10			USA	
		7-9				
		48				

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Supplementary Table 1.2. The sampling date and site identity of each survey carried out during Chapter 2.

Survey number	Date	Site
1	30/07/2014	St. George's Field
2	03/08/2014	Spen farm: agroforestry plot
3	03/08/2014	Spen farm: agroforestry plot
4	02/09/2014	Spen farm: fallow field plot
5	10/09/2014	Spen farm: agroforestry plot
6	10/09/2014	Spen farm: agroforestry plot
7	16/06/2015	Spen farm: fallow field plot
8	24/06/2015	Spen farm: agroforestry plot
9	24/06/2015	Spen farm: agroforestry plot
10	01/07/2015	Bramham Park
11	01/07/2015	Bramham Park
12	09/07/2015	Spen farm: agroforestry plot
13	11/07/2015	Spen farm: fallow field plot
14	15/07/2015	Spen farm: agroforestry plot
15	17/07/2015	Spen farm: fallow field plot
16	21/07/2015	Spen Farm: fallow field plot
17	05/08/2015	Bramham Park
18	29/09/2015	St. George's Field
19	03/10/2015	Meanwood Grove

Supplementary table 1.3. The identity of each floral species found during each survey, along with each species' definition of a floral unit, the mean number of florets per floral unit, the mean volume of nectar sugar produced per floret per 24-hours (µl) (taken primarily from Baude, Kunin, & Memmott (2015)), and the mean volume of nectar sugar produced per floral unit per 24-hours (µl) (mean number of florets per floral unit multiplied by the mean volume of nectar sugar produced per floret per 24-hours).

Survey	Date/site	Plant species	Floral unit definition	Mean no. of florets/floral unit	Mean vol. of nectar sugar produced/day/floret (µl)	Mean vol. of nectar sugar produced/day/floral unit (µl)
1	30/07/2014	*Bellis perennis*	Single capitulum	220.8	0.84	185.472
	St. George's Field	*Leontodon autumnalis*	Single capitulum	45.3	13.70236274	620.7170321
		Trifolium repens	Single raceme	27	48.97	1322.19
		Veronica agrestis	Single flower	1	14.42	14.42
2	03/08/2014	*Cirsium vulgare*	Single capitulum	76.26	76.51	5834.6526
	Spen farm: agroforestry plot	*Prunella vulgaris*	Single flower	1	138.62	138.62
		Senecio jacobaea	Single capitulum	45.9	22.6	1037.34
		Trifolium repens	Single raceme	35.6	48.97	1743.332
3	03/08/2014	*Cirsium vulgare*	Single capitulum	76.26	76.51	5834.6526
	Spen farm: agroforestry	*Senecio jacobaea*	Single capitulum	45.27	22.6	1023.102
4	02/09/2014	*Anagallis arvensis*	Single flower	1	0	0
	Spen farm: fallow field plot	*Borago officinalis*	Single flower	1	72	72
		Ranunculus acris	Single flower	1	78.83	78.83
		Sherardia arvensis	Single flower	1	9.48	9.48
		Sinapis sp.	Single flower	1	55.6	55.6
		Trifolium repens	Single raceme	32	48.97	1567.04
		Veronica persica	Single flower	1	31.5897447	31.5897447
5	10/09/2014	*Cirsium vulgare*	Single capitulum	76.26	76.51	5834.6526

	Spen farm: agroforestry plot	*Senecio jacobaea*	Single capitulum	39.5	22.6	892.7
6	10/09/2014	*Cirsium vulgare*	Single capitulum	76.26	76.51	5834.6526
	Spen farm: agroforestry plot	*Leontodon autumnalis*	Single capitulum	45.3	13.70236274	620.7170321
		Senecio jacobaea	Single capitulum	43.9	22.6	992.14
7	16/06/2015	*Anagallis arvensis*	Single flower	1	0	0
	Spen farm: fallow field plot	*Bellis perennis*	Single capitulum	120	0.84	100.8
		Geranium columbinum	Single flower	1	2.69	2.69
		Leucanthemum vulgare	Single capitulum	172.4285714	15.81	2726.095714
		Myosotis stricta	Single spike	5	23.36	116.8
		Papaver rhoeas	Single flower	1	5.35	5.35
		Ranunculus acris	Single flower	1	78.83	78.83
		Raphanus raphanistrum	Single spike	3.7	115.08	425.796
		Sherardia arvensis	Single flower	1	9.48	9.48
		Sinapis arvensis	Single spike	3	55.6	166.8
		Sonchus asper	Single capitulum	304	0.13	39.52
		Veronica persica	Single flower	1	31.59	31.59
8	24/06/2015	*Cruciata laevipes*	Single stem	220	3.58	787.6
	Spen farm: agroforestry plot	*Daucus carota*	Single umbel	385.5	7.35	2833.425
		Heracleum sphondylium	Single umbel	390	98.17	38286.3
		Plantago major	Single stem	30	0	0
		Ranunculus acris	Single flower	1	78.83	78.83

		Trifolium repens	Single raceme	24	48.97	1175.28
		Vicia sativa	Single flower	1	300.34	300.34
9	24/06/2015	*Geranium columbinum*	Single flower	1	2.69	2.69
	Spen farm: agroforestry plot	*Myosotis stricta*	Single spike	5	23.36	116.8
		Ranunculus acris	Single flower	1	78.83	78.83
		Sonchus asper	Single capitulum	304	0.13	39.52
		Trifolium repens	Single raceme	30	48.97	1469.1
10	01/07/2015	*Bellis perennis*	Single capitulum	120	0.84	100.8
	Bramham Park	*Centaurea nigra*	Single capitulum	46	198.99	9153.54
		Dactylorhiza fuchsii	Single stem	35	0	0
		Daucus carota	Single umbel	132	7.35	970.2
		Lamiastrum galeobdolon	Single whorl	3.444444444	440.16	1516.106666
		Leontodon hispidus	Single capitulum	35	6.15	215.25
		Lotus corniculatus	Single flower	1	61.82	61.82
		Ranunculus repens	Single flower	1	104.51	104.51
		Trifolium pratense	Single raceme	25	116.86	2921.5
11	01/07/2015	*Daucus carota*	Single umbel	75	7.35	551.25
	Bramham Park	*Galium aparine*	Single stem	80	9.48	758.4
		Lotus corniculatus	Single flower	1	61.82	61.82
		Ranunculus acris	Single flower	1	78.83	78.83
		Trifolium repens	Single raceme	20	48.97	979.4
		Vicia cracca	Single flower	1	484.4	484.4
		Vicia sativa	Single flower	1	300.34	300.34

12	09/07/2015	*Cirsium arvense*	Single capitulum	55.6	76.22	4237.832
	Spen farm: agroforestry plot	*Ranunculus acris*	Single flower	1	78.83	78.83
		Trifolium repens	Single raceme	28.8	48.97	1410.336
13	11/07/2015	*Anagallis arvensis*	Single flower	1	0	0
	Spen farm: fallow field plot	*Borago officinalis*	Single stem	8.958333333	72	645
		Cardamine pratensis	Single stem	4.477777778	58.68	262.756
		Phacelia tanacetifolia	Single stem	14	75.64	1058.96
		Plantago major	Single stem	58	0	0
		Ranunculus repens	Single flower	1	104.51	104.51
		Sherardia arvensis	Single flower	1	9.48	9.48
		Sinapis arvensis	Single spike	5.35	55.6	297.46
		Veronica persica	Single flower	1	31.59	31.59
14	15/07/2015	*Cirsium arvense*	Single capitulum	35.9	76.22	2736.298
	Spen farm: agroforestry plot	*Cirsium vulgare*	Single capitulum	74.6	76.51	5707.646
		Ranunculus repens	Single flower	1	104.51	104.51
		Senecio jacobaea	Single capitulum	41.53333333	22.6	938.6533333
		Trifolium repens	Single raceme	19.37142857	48.97	948.6188571
15	17/07/2015	*Bellis perennis*	Single capitulum	120	0.84	100.8
	Spen farm: fallow field plot	*Borago officinalis*	Single stem	7.2	72	518.4
		Cardamine pratensis	Single stem	3.3	58.68	193.644
		Cirsium arvense	Single capitulum	72.2	76.22	5503.084
		Cirsium vulgare	Single capitulum	76.4	76.51	5845.364

		Daucus carota	Single umbel	1023.6	7.35	7523.46
		Leucanthemum vulgare	Single capitulum	201	15.81	3177.81
		Papaver rhoeas	Single flower	1	5.35	5.35
		Phacelia tanacetifolia	Single stem	14.8	76.09	1126.132
		Prunella vulgaris	Single spike	5	138.62	693.1
		Ranunculus repens	Single flower	1	104.51	104.51
		Senecio jacobaea	Single capitulum	159	22.6	3593.4
		Sinapis arvensis	Single spike	2	55.6	111.2
		Trifolium repens	Single raceme	25.55	48.97	1251.1835
16	21/07/2015	*Borago officinalis*	Single stem	6.714285714	72	483.4285714
	Spen Farm: fallow field plot	*Cardamine pratensis*	Single stem	15.45833333	58.68	907.0949998
		Cirsium arvense	Single capitulum	55.25	76.22	4211.155
		Papaver rhoeas	Single flower	1	5.35	5.35
		Phacelia tanacetifolia	Single stem	6.6	75.64	499.224
		Sinapis arvensis	Single spike	2	55.6	111.2
17	05/08/2015	*Centaurea nigra*	Single capitulum	46	198.9882969	9153.461657
	Bramham Park	*Filipendula ulmaria*	Single flower	1	0	0
		Gallium verum	Single flower	1	0.656449056	0.656449056
		Leontodon autumnalis	Single capitulum	35	13.70236274	479.5826959
		Leucanthemum vulgare	Single capitulum	201	15.81	3177.81
		Lotus corniculatus	Single flower	1	61.82363977	61.82363977
		Prunella vulgaris	Single flower	1	138.6185614	138.6185614

		Ranunculus acris	Single flower	1	78.83013231	78.83013231
		Rhinanthus minor	Single flower	1	108.8977655	108.8977655
		Stachys officinalis	Single flower	1	311.106	311.106
		Trifolium pratense	Single raceme	22	116.8559138	2570.830104
18	29/09/2015	NA	NA	-	-	-
	St. George's Field					
19	03/10/2015	*Lotus corniculatus*	Single flower	1	61.82363977	61.82363977
	Meanwood Grove	*Trifolium pratense*	Single raceme	1	116.8559138	116.8559138

Supplementary table 1.4. The relative abundance of floral species within a two-meter radius of each pan trapping station per survey visit, together with the total floral abundance surrounding each pan trapping station. Relative abundance is measured as the total number of florets present per species.

		7-hour pan trapping stations				24-hour pan trapping stations				48-hour pan trapping stations			
Survey	Plant species	28ml bowls	57ml bowls	156ml bowls	284ml bowls	28ml bowls	57ml bowls	156ml bowls	284ml bowls	28ml bowls	57ml bowls	156ml bowls	284ml bowls
1	*Bellis perennis*	0	0	662.4	0	0	0	662.4	0	1104	220.8	0	0
1	*Leontodon autumnalis*	906	0	0	0	951.3	0	0	0	0	0	0	0
1	*Trifolium repens*	0	0	0	0	0	0	0	27	0	135	0	0
1	*Veronica agrestis*	0	0	0	0	0	0	0	0	0	0	2	0
1	Total	906	0	662.4	0	951.3	0	662.4	27	1104	355.8	2	0
2	*Cirsium vulgare*	76.26	0	152.52	152.52	76.26	0	152.52	152.52	228.78	0	152.52	0
2	*Prunella vulgaris*	0	0	0	2	0	0	0	0	0	0	0	15
2	*Senecio jacobaea*	23638.5	12943.8	59073.3	13311	23638.5	12943.8	59073.3	13311	10143.9	33461.1	36582.3	9639
2	*Trifolium repens*	1281.6	1673.2	0	356	1281.6	1673.2	0	356	1495.2	676.4	213.6	1281.6
2	Total	24996.36	14617	59225.82	13821.52	24996.36	14617	59225.82	13819.52	11867.88	34137.52	36948.42	10935.6
3	*Cirsium vulgare*	0	76.26	228.78	0	0	76.26	228.78	0	0	0	0	0
3	*Senecio jacobaea*	497.97	0	0	0	497.97	0	0	0	679.05	543.24	0	0
3	Total	497.97	76.26	228.78	0	497.97	76.26	228.78	0	679.05	543.24	0	0
4	*Anagallis arvensis*	0	7	0	0	0	7	0	0	0	3	0	0
4	*Borago officinalis*	33	24	262	4	33	24	262	4	49	46	9	39
4	*Ranunculus acris*	0	2	0	0	0	2	0	0	1	0	0	0

4	*Sherardia arvensis*	0	34	0	0	0	24	0	0	9	65	0	0
4	*Sinapis sp.*	2647	2619	3877	3433	2647	2619	3877	3433	6478	1760	2903	3880
4	*Trifolium repens*	0	0	0	32	0	0	0	32	0	0	0	0
4	*Veronica persica*	0	25	0	43	0	25	0	43	49	23	54	0
4	Total	2680	2711	4139	3512	2680	2701	4139	3512	6586	1897	2966	3919
5	*Cirsium vulgare*	0	0	0	0	0	0	0	0	152.52	0	0	0
5	*Senecio jacobaea*	0	0	434.5	0	0	0	434.5	0	0	434.5	0	632
5	Total	0	0	434.5	0	0	0	434.5	0	152.52	434.5	0	632
6	*Cirsium vulgare*	228.78	76.26	76.26	0	228.78	76.26	76.26	0	0	0	0	0
6	*Leontodon autumnalis*	0	0	0	5073.6	0	0	0	5073.6	0	0	0	0
6	*Senecio jacobaea*	3994.9	6453.3	6189.9	0	3994.9	6453.3	6189.9	0	18262.4	13960.2	13345.6	8692.2
6	Total	4223.68	6529.56	6266.16	5073.6	4223.68	6529.56	6266.16	5073.6	18262.4	13960.2	13345.6	8692.2
7	*Anagallis arvensis*	0	0	0	0	0	0	0	0	0	22	12	2
7	*Bellis perennis*	8280	0	1680	30240	207000	0	201000	0	0	0	0	0
7	*Geranium columbinum*	0	183	0	0	0	183	0	321	0	21	39	77
7	*Leucanthemum vulgare*	0	1608	6030	0	0	1608	0	0	3417	402	7	1407
7	*Myosotis stricta*	16120	5160	7750	4830	16120	5160	14925	0	875	2625	0	1350
7	*Papaver rhoeas*	9	0	0	0	9	0	0	0	1	1	0	0
7	*Ranunculus acris*	192	732	720	440	192	732	166	208	659	364	111	374
7	*Raphanus raphanistrum*	1904	132	0	0	1904	33	2088	780	1072	972	312	664
7	*Sherardia arvensis*	0	47123	0	95521	0	47123	0	0	150796	150796	188495	28274
7	*Sinapis arvensis*	960	36	360	1584	960	48	723	525	0	648	0	8532

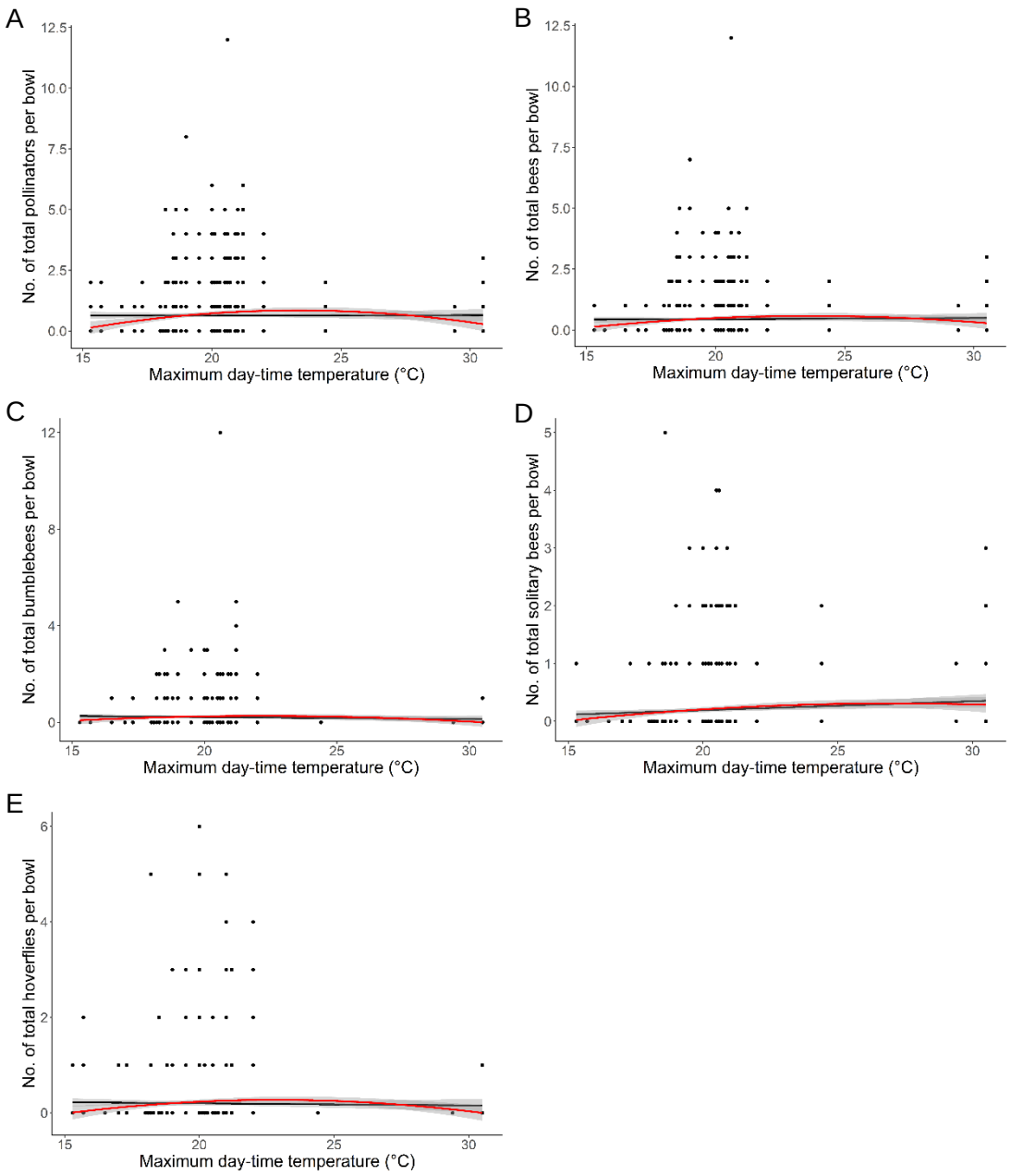
7	*Sonchus asper*	59280	0	0	0	59280	0	70832	24320	0	0	0	0
7	*Veronica persica*	0	4	0	0	0	4	0	0	0	9	7	83
7	Total	86745	54978	16540	132615	285465	54891	289734	26154	156820	155860	188983	40763
8	*Cruciata laevipes*	0	0	19800	0	0	0	52800	0	0	0	0	0
8	*Daucus carota*	2760	1590	1640	720	2760	1590	1640	720	3168	10400	4598	1440
8	*Heracleum sphondylium*	9750	1560	0	0	9750	1560	0	0	0	0	0	0
8	*Plantago major*	0	0	2132	1664	0	0	2132	1664	0	0	0	68
8	*Ranunculus acris*	0	0	18	18	0	0	18	18	15	0	118	7
8	*Trifolium repens*	0	0	0	0	0	0	0	0	0	0	0	120
8	*Vicia sativa*	26	7	5	16	26	7	5	16	1	0	0	6
8	Total	12536	3157	23595	2418	12536	3157	56595	2418	3184	10400	4716	1641
9	*Geranium columbinum*	0	0	0	0	0	0	0	0	0	0	2	0
9	*Myosotis stricta*	0	0	0	0	0	0	0	0	0	20	0	0
9	*Ranunculus acris*	0	0	0	0	0	0	0	0	0	0	0	1
9	*Sonchus asper*	0	0	0	0	0	0	0	0	0	0	1520	0
9	*Trifolium repens*	60	0	510	120	60	0	510	120	0	0	120	390
9	Total	60	0	510	120	60	0	510	120	0	20	1642	391
10	*Bellis perennis*	3840	6600	600	0	3840	6600	600	0	5160	4080	2400	3480
10	*Centaurea nigra*	92	138	0	506	92	138	0	506	0	92	0	0
10	*Dactylorhiza fuchsii*	418	190	595	736	418	190	595	736	224	612	68	544
10	*Daucus carota*	576	0	0	0	576	0	0	0	120	0	1560	0
10	*Lamiastrum galeobdolon*	126	6	0	110	126	6	0	110	27	180	0	9

10	*Leontodon hispidus*	0	0	420	1960	0	0	420	1960	385	0	0	4620
10	*Lotus corniculatus*	100	52	16	27	100	52	16	27	78	33	0	0
10	*Ranunculus repens*	13	14	0	0	13	14	0	0	0	38	12	3
10	*Trifolium pratense*	22	0	40	0	22	0	40	0	544	120	2800	36
10	Total	5187	7000	1671	3339	5187	7000	1671	3339	6538	5155	6840	8692
11	*Daucus carota*	0	0	0	0	0	0	0	0	0	5850	0	0
11	*Galium aparine*	0	0	0	0	0	0	0	0	320	0	0	0
11	*Lotus corniculatus*	0	0	0	3	0	0	0	3	0	17	0	0
11	*Ranunculus acris*	0	2	0	0	2	2	0	0	0	4	0	0
11	*Trifolium repens*	0	0	0	0	0	0	0	0	0	20	0	0
11	*Vicia cracca*	0	0	0	0	0	0	0	0	0	14	0	0
11	*Vicia sativa*	42	0	0	14	0	0	0	14	17	0	0	0
11	Total	42	2	0	17	2	2	0	17	337	5905	0	0
12	*Cirsium arvense*	0	58	330	0	0	58	330	0	104	0	0	0
12	*Ranunculus acris*	0	0	0	0	0	0	0	0	0	0	8	0
12	*Trifolium repens*	832	660	0	2820	832	660	0	2820	924	884	120	2752
12	Total	832	718	330	2820	832	718	330	2820	1028	884	128	2752
13	*Anagallis arvensis*	8064	21312	8512	9728	8064	21312	8512	9728	0	0	8025	0
13	*Borago officinalis*	147	497	800	2744	147	497	800	2744	1520	2688	1230	1728
13	*Cardamine pratensis*	26	93.6	70	43.2	29.9	93.6	70	43.2	24	0	0	0
13	*Phacelia tanacetifolia*	360	481	288	0	360	481	288	0	0	0	0	84
13	*Plantago major*	0	0	0	0	0	0	0	0	0	174	0	0

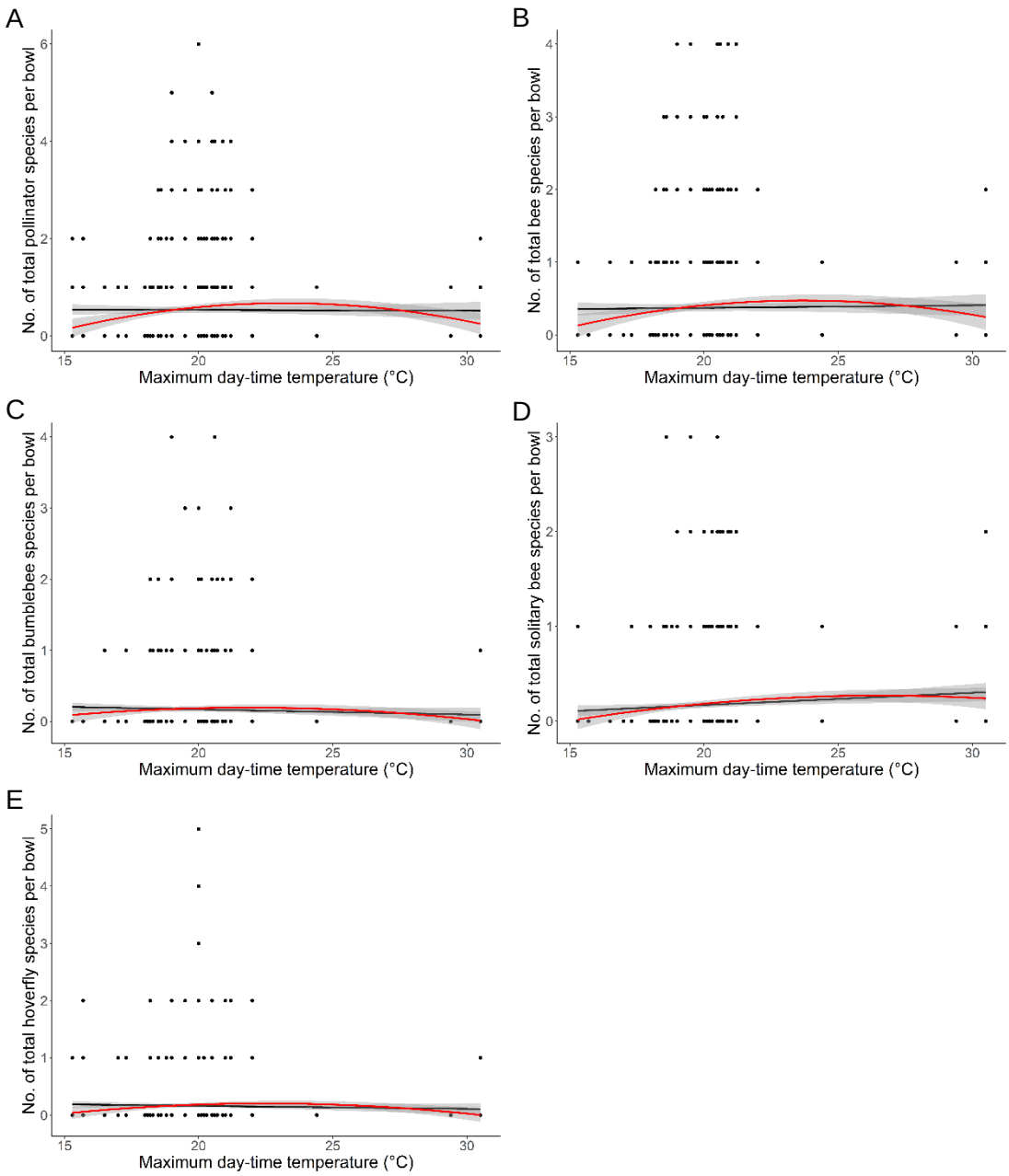
13	*Ranunculus repens*	227	66	31	116	227	66	31	116	600	440	208	0
13	*Sherardia arvensis*	0	0	0	0	0	0	0	0	0	0	7539	0
13	*Sinapis arvensis*	56	300	1168	110	117.6	300	1168	110	117	68	374	1400
13	*Veronica persica*	12032	11020	11430	0	12032	11020	11430	0	0	0	0	0
13	Total	20912	33769.6	22299	12741.2	20977.5	33769.6	22299	12741.2	2261	3370	17376	3212
14	*Cirsium arvense*	128.4	45.6	0	0	128.4	45.6	0	0	0	0	0	0
14	*Cirsium vulgare*	0	0	0	0	0	0	0	0	223.8	0	0	0
14	*Ranunculus repens*	0	0	0	0	0	0	0	0	9	0	0	0
14	*Senecio jacobaea*	0	0	0	0	0	0	0	0	342	588.8	0	589.6
14	*Trifolium repens*	0	0	1612.8	725.4	0	0	1612.8	725.4	0	91	432.6	84.8
14	Total	128.4	45.6	1612.8	725.4	128.4	45.6	1612.8	725.4	574.8	679.8	432.6	674.4
15	*Bellis perennis*	0	1080	0	0	0	0	0	0	0	0	0	0
15	*Borago officinalis*	26.4	0	70.2	0	26.4	0	70.2	0	0	0	0	0
15	*Cardamine pratensis*	118.8	0	122.1	0	118.8	0	122.1	0	0	0	0	0
15	*Cirsium arvense*	0	0	1155.2	0	0	0	1155.2	0	0	0	0	0
15	*Cirsium vulgare*	1528	3896.4	2139.2	1833.6	1528	3896.4	2139.2	1833.6	3972.8	10390.4	916.8	4507.6
15	*Daucus carota*	0	0	0	5140	0	0	0	5140	12336	0	1023	17187
15	*Leucanthemum vulgare*	0	0	0	4623	0	1809	0	4623	8844	5427	0	6633
15	*Papaver rhoeas*	0	4	0	1	0	4	0	1	2	0	1	0
15	*Phacelia tanacetifolia*	473.6	0	784.4	0	473.6	0	784.4	0	0	0	0	0
15	*Prunella vulgaris*	0	0	0	0	0	0	0	0	0	0	0	30
15	*Ranunculus repens*	0	18	0	48	0	18	0	48	33	21	75	61

15	*Senecio jacobaea*	0	1016	0	1470	0	1480	0	441	0	0	0	630
15	*Sinapis arvensis*	142	0	138	0	142	0	138	0	0	0	0	0
15	*Trifolium repens*	0	471.2	0	0	0	471.2	0	0	0	211.2	1100.4	0
15	Total	2288.8	6485.6	4409.1	13115.6	2288.8	7678.6	4409.1	12086.6	25187.8	16049.6	3116.2	29048.6
16	*Borago officinalis*	0	38.4	16.8	0	2	140	6.6	0	9	0	12.6	0
16	*Cardamine pratensis*	37.4	30.8	47.3	8.4	16.8	36	941.2	509.6	25.2	101.2	4	99.2
16	*Cirsium arvense*	436.8	0	0	0	0	0	218.4	15.6	0	0	0	364
16	*Papaver rhoeas*	1	5	0	0	0	10	0	0	0	7	0	0
16	*Phacelia tanacetifolia*	0	12.6	52.8	0	0	140.8	0	76.8	25.2	408	0	244.2
16	*Sinapis arvensis*	0	0	0	0	0	0	0	0	0	0	0	18
16	Total	475.2	86.8	116.9	8.4	18.8	326.8	1166.2	602	59.4	516.2	16.6	725.4
17	*Centaurea nigra*	9706	2438	6118	2254	9706	2438	6118	2254	3496	2944	2530	1656
17	*Filipendula ulmaria*	0	33	0	0	0	33	0	0	0	0	0	0
17	*Gallium verum*	0	0	0	0	0	0	0	0	150	0	0	0
17	*Leontodon autumnalis*	70	0	0	0	70	0	0	0	0	0	0	0
17	*Leucanthemum vulgare*	0	0	201	0	0	0	201	0	0	0	0	0
17	*Lotus corniculatus*	3	0	7	22	3	0	7	22	2	1	3	0
17	*Prunella vulgaris*	0	29	29	0	0	29	29	0	28	0	95	9
17	*Ranunculus acris*	0	6	0	0	0	6	0	0	0	0	0	0
17	*Rhinanthus minor*	63	4	57	3	63	4	57	3	14	22	11	12
17	*Stachys officinalis*	0	0	0	0	0	0	0	0	234	0	0	24

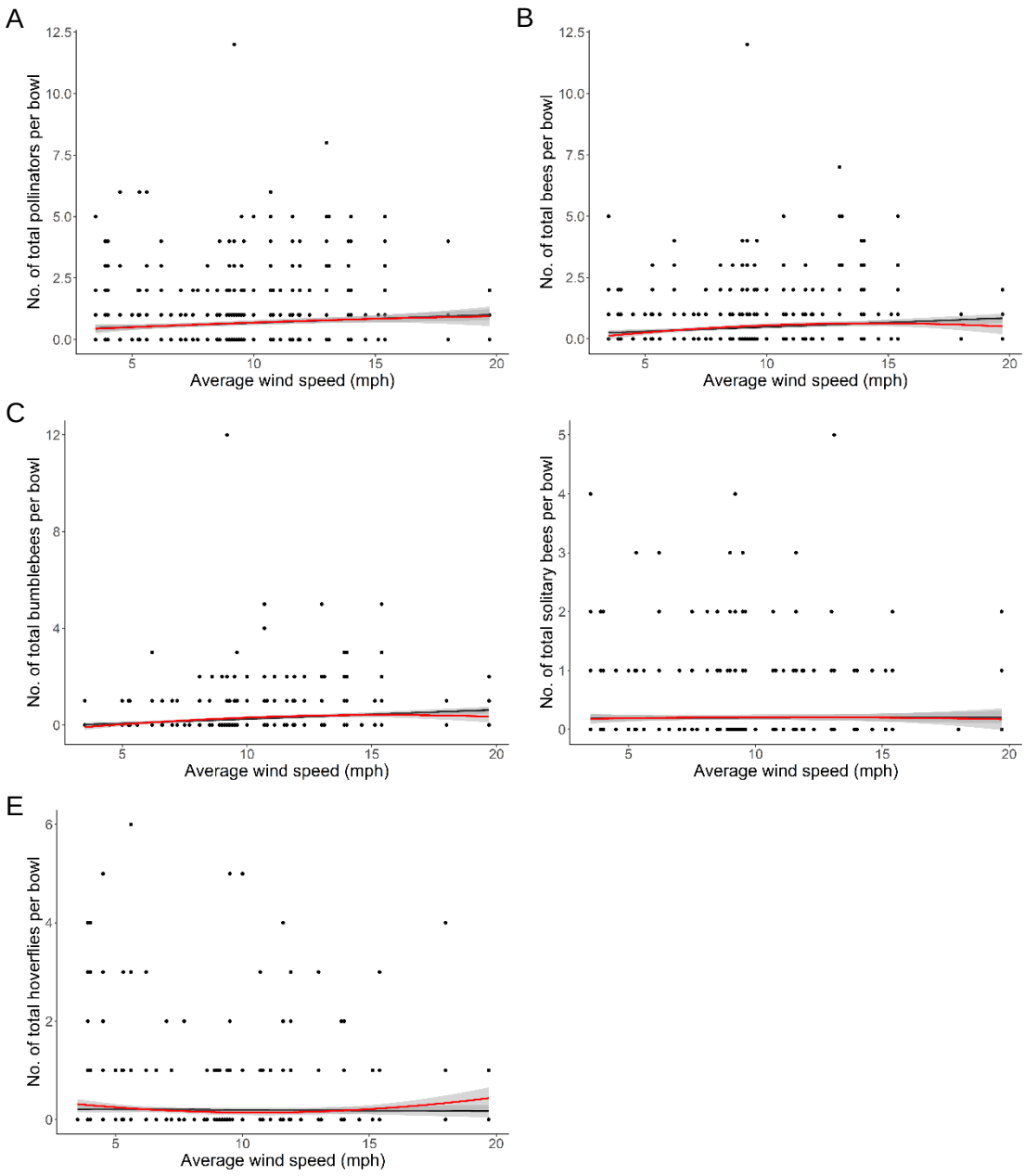
17	*Trifolium pratense*	66	0	0	88	66	0	0	88	0	0	396	0
17	Total	9908	2510	6412	2367	9908	2510	6412	2367	3924	2967	3035	1701
18	NA	0	0	0	0	0	0	0	0	0	0	0	0
19	*Lotus corniculatus*	0	114	207	150	0	114	207	150	0	0	14	0
19	*Trifolium pratense*	62	0	0	0	62	0	0	0	0	0	0	0
19	Total	62	114	207	150	62	114	207	150	0	0	14	0



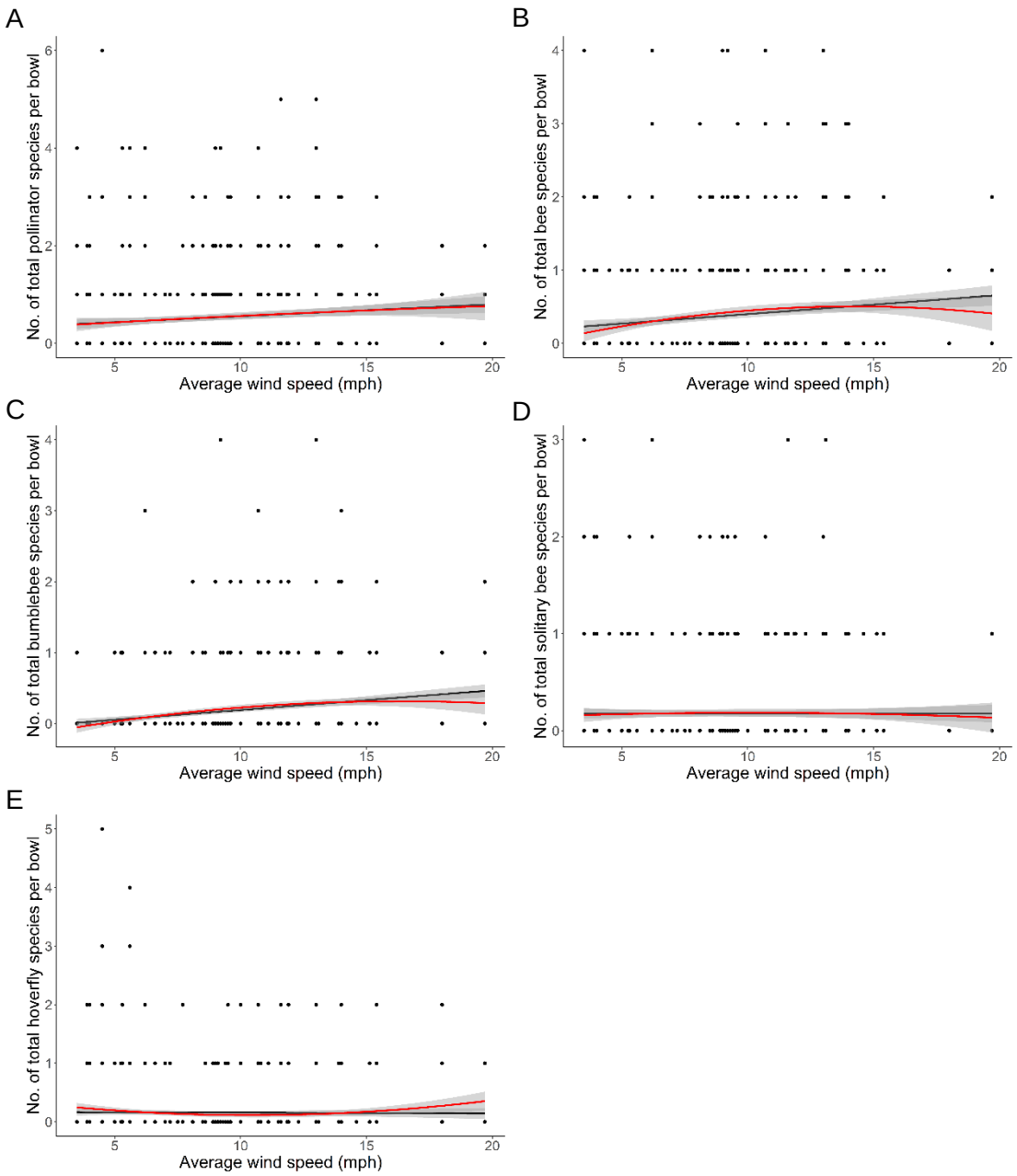
Supplementary Figure 1.1. A) Total pollinator abundance plotted against maximum day-time temperature (°C); B) Total bee abundance plotted against maximum day-time temperature (°C); C) Bumblebee abundance plotted against maximum day-time temperature (°C); D) Solitary bee abundance plotted against maximum day-time temperature (°C); E) Hoverfly abundance plotted against maximum day-time temperature (°C). The black line displays a linear relationship (± 95% confidence intervals), the red line displays a second-order polynomial relationship (± 95% confidence intervals).



Supplementary Figure 1.2. A) Total pollinator species richness plotted against maximum day-time temperature (°C); B) Total bee species richness plotted against maximum day-time temperature (°C); C) Bumblebee species richness plotted against maximum day-time temperature (°C); D) Solitary bee species richness plotted against maximum day-time temperature (°C); E) Hoverfly species richness plotted against maximum day-time temperature (°C). The black line displays a linear relationship (± 95% confidence intervals), the red line displays a second-order polynomial relationship (± 95% confidence intervals).



Supplementary Figure 1.3. A) Total pollinator abundance plotted against average wind speed (mph);B) Total bee abundance plotted against average wind speed (mph); C) Bumblebee abundance plotted against average wind speed (mph); D) Solitary bee abundance plotted against average wind speed (mph); E) Hoverfly abundance plotted against average wind speed (mph). The black line displays a linear relationship (± 95% confidence intervals), the red line displays a second-order polynomial relationship (± 95% confidence intervals).



Supplementary Figure 1.4. A) Total pollinator species richness plotted against average wind speed (mph); B) Total bee species richness plotted against average wind speed (mph); C) Bumblebee species richness plotted against average wind speed (mph); D) Solitary bee species richness plotted against average wind speed (mph); E) Hoverfly species richness plotted against average wind speed (mph). The black line displays a linear relationship (± 95% confidence intervals), the red line displays a second-order polynomial relationship (± 95% confidence intervals).

Supplementary table 1.5. The total volume of nectar sugar produced per 24 hours (µl) within a two-meter radius of each pan trapping station per survey visit. Along with the mean volume of nectar sugar produced per 24 hours (µl) across all pan trapping stations per survey visit.

Survey	7-hour pan trapping stations				24-hour pan trapping stations				48-hour pan trapping stations				
	28ml bowls	57ml bowls	156ml bowls	284ml bowls	28ml bowls	57ml bowls	156ml bowls	284ml bowls	28ml bowls	57ml bowls	156ml bowls	284ml bowls	Survey mean
1	12414.34	0	556.42	0	13035.06	0	556.42	1322.19	927.36	6796.42	28.84	0	2969.75
2	602824.70	374466.48	1346725.89	330208.47	602824.70	374466.48	1346725.89	329931.23	319976.04	789344.17	848889.28	282680.65	629088.66
3	11254.12	5834.65	17503.96	0	11254.12	5834.65	17503.96	0	15346.53	12277.22	0	0	8067.43
4	149549.20	148614.13	234425.20	194088.21	149549.20	148519.33	234425.20	194088.21	365416.77	102510.77	163760.66	218536.00	191956.91
5	0	0	9819.70	0	0	0	9819.70	0	11669.31	9819.70	0	14283.20	4617.63
6	107788.70	151679.23	145726.39	69520.31	107788.70	151679.23	145726.39	69520.31	412730.24	315500.52	301610.56	196443.72	181309.53
7	678896.63	668200.47	354559.10	1166525.08	845821.43	657474.75	820267.78	139374.13	1679328.93	1674148.53	1832024.40	904922.03	951795.27
8	985252.34	166934.08	85858.64	11516.38	985252.34	166934.08	203998.64	11516.38	24767.59	76440.00	43097.24	18814.25	231698.50
9	2938.20	0	24974.70	5876.40	2938.20	0	24974.70	5876.40	0	467.20	6079.38	19177.13	7775.19
10	91337.99	40323.36	8750.52	162829.68	91337.99	40323.36	8750.52	162829.68	87862.27	120997.72	341944.12	39818.13	99758.78
11	12614.28	157.66	0	4390.22	157.66	157.66	0	4390.22	8139.38	52124.76	0	0	6844.32
12	40743.04	36740.96	25152.60	138095.40	40743.04	36740.96	25152.60	138095.40	53175.16	43289.48	6507.04	134765.44	59933.43
13	446268.33	449358.75	512746.23	218342.14	449922.14	449358.75	512746.23	218342.14	180059.52	243301.20	202562.20	208609.76	340968.12
14	9786.65	3475.63	78978.82	35522.84	9786.65	3475.63	78978.82	35522.84	25792.73	17763.15	21184.42	17477.62	28145.48
15	169497.57	346959.61	331649.54	289401.20	169497.57	385139.10	331649.54	266145.80	537911.70	893307.55	139393.61	600840.37	371782.76

16	35492.88	5551.96	7978.96	492.91	1129.82	22896.09	72351.26	36901.51	4032.86	36836.99	1141.92	53037.22	23153.70
17	1947098.10	490061.98	1231248.09	460489.75	1947098.10	490061.98	1231248.09	460489.75	774089.89	588279.12	564267.44	339545.50	876998.15
18	0	0	0	0	0	0	0	0	0	0	0	0	0
19	7245.07	7047.89	12797.49	9273.55	7245.07	7047.89	12797.49	9273.55	0	0	865.53	0	6132.79

Supplementary table 1.6. The relative abundance of bee and hoverfly species sampled by pan trapping during each survey visit.

Survey	Date	Site	Group	Species	Abundance
1	30/07/2014	St. George's Field	Honeybee	*Apis mellifera*	1
			Bumblebee	*Bombus soroeensis*	1
			Hoverfly	*Episyrphus balteatus*	5
			Solitary bee	*Lasioglossum smeathmanellum*	1
			Hoverfly	*Melanostoma mellinum*	1
2	03/08/2014	Spen farm: agroforestry plot	Honeybee	*Apis mellifera*	2
			Bumblebee	*Bombus hortorum*	1
			Bumblebee	*Bombus lapidarius*	2
			Bumblebee	*Bombus pascuorum*	1
			Bumblebee	*Bombus terrestris/lucorum*	8
			Hoverfly	*Episyrphus balteatus*	4
			Hoverfly	*Eristalis arbustorum*	1
			Hoverfly	*Eristalis tenax*	3
			Solitary bee	*Halictus rubicundus*	1
			Hoverfly	*Helophilus pendulus*	3
			Solitary bee	*Hylaeus communis*	1
			Solitary bee	*Lasioglossum calceatum/albipes*	6
			Solitary bee	*Lasioglossum fulvicorne*	1
			Solitary bee	*Lasioglossum leucopus*	3
			Hoverfly	*Melanostoma mellinum*	2
			Hoverfly	*Neoascia podagrica*	1
			Hoverfly	*Platycheirus manicatus*	1
			Hoverfly	*Platycheirus nielseni*	1
			Hoverfly	*Rhingia campestris*	1
			Hoverfly	*Syritta pipiens*	7
3	03/08/2014	Spen farm: agroforestry plot	Bumblebee	*Bombus lapidarius*	1
			Bumblebee	*Bombus terrestris/lucorum*	1

			Bumblebee	*Bombus vestalis*	3
			Hoverfly	*Dasysyrphus albostriatus*	1
			Hoverfly	*Episyrphus balteatus*	18
			Hoverfly	*Helophilus pendulus*	2
			Solitary bee	*Lasioglossum calceatum/albipes*	1
			Solitary bee	*Lasioglossum smeathmanellum*	1
			Hoverfly	*Melanostoma mellinum*	2
			Hoverfly	*Neoascia podagrica*	2
			Hoverfly	*Sphaerophoria rueppellii*	1
4	02/09/2014	Spen farm: fallow field plot	Honeybee	*Apis mellifera*	8
			Bumblebee	*Bombus pascuorum*	1
			Hoverfly	*Dasysyrphus albostriatus*	1
			Hoverfly	*Eristalis arbustorum*	7
			Hoverfly	*Eristalis pertinax*	2
			Hoverfly	*Eristalis tenax*	3
			Solitary bee	*Halictus rubicundus*	1
			Solitary bee	*Halictus tumulorum*	5
			Hoverfly	*Helophilus pendulus*	2
			Hoverfly	*Helophilus trivittatus*	1
			Solitary bee	*Lasioglossum calceatum/albipes*	4
			Solitary bee	*Lasioglossum leucopus*	4
			Hoverfly	*Neoascia podagrica*	1
			Hoverfly	*Parasyrphus nigritarsus*	1
			Hoverfly	*Xylota segnis*	1
5	10/09/2014	Spen farm: agroforestry plot	Honeybee	*Apis mellifera*	1
			Solitary bee	*Halictus tumulorum*	1
			Hoverfly	*Helophilus pendulus*	8
			Solitary bee	*Lasioglossum calceatum/albipes*	1
			Solitary bee	*Lasioglossum leucopus*	1
			Hoverfly	*Rhingia campestris*	1

6	10/09/2014	Spen farm: agroforestry plot	Honeybee	*Apis mellifera*	2
			Hoverfly	*Eristalis arbustorum*	1
			Hoverfly	*Eristalis pertinax*	2
			Hoverfly	*Eristalis tenax*	1
			Solitary bee	*Halictus tumulorum*	4
			Hoverfly	*Helophilus pendulus*	22
			Solitary bee	*Lasioglossum leucopus*	3
			Hoverfly	*Melanostoma scalare*	1
			Hoverfly	*Neoascia podagrica*	4
			Hoverfly	*Syrphus ribesii*	4
7	16/06/2015	Spen farm: fallow field plot	Solitary bee	*Andrena haemorrhoa*	1
			Bumblebee	*Bombus hortorum*	1
			Bumblebee	*Bombus terrestris/lucorum*	3
			Solitary bee	*Halictus tumulorum*	7
			Solitary bee	*Lasioglossum calceatum/albipes*	7
			Solitary bee	*Lasioglossum leucopus*	2
			Solitary bee	*Sphecodes ephippius*	1
8	24/06/2015	Spen farm: agroforestry plot	Bumblebee	*Bombus hortorum*	1
			Bumblebee	*Bombus lapidarius*	1
			Hoverfly	*Cheilosia albitarsis*	2
			Solitary bee	*Halictus tumulorum*	1
			Solitary bee	*Lasioglossum calceatum/albipes*	1
			Solitary bee	*Lasioglossum leucopus*	1
			Solitary bee	*Osmia aurulenta*	1
9	24/06/2015	Spen farm: agroforestry plot	Solitary bee	*Andrena chrysosceles*	1
			Honeybee	*Apis mellifera*	1
			Bumblebee	*Bombus hortorum*	1
			Bumblebee	*Bombus hypnorum*	1
			Bumblebee	*Bombus pratorum*	2

			Bumblebee	*Bombus sylvestris*	2
			Solitary bee	*Halictus tumulorum*	1
			Solitary bee	*Hylaeus hyalinatus*	1
			Solitary bee	*Lasioglossum calceatum/albipes*	5
			Solitary bee	*Lasioglossum leucopus*	1
			Solitary bee	*Lasioglossum smeathmanellum*	1
			Solitary bee	*Osmia leaiana*	1
			Solitary bee	*Stelis punctulatissima*	1
			Hoverfly	*Xylota segnis*	1
10	01/07/2015	Bramham park	Solitary bee	*Andrena pascuorum*	1
			Bumblebee	*Bombus pascuorum*	1
			Bumblebee	*Bombus terrestris/lucorum*	1
			Solitary bee	*Lasioglossum calceatum/albipes*	2
			Solitary bee	*Lasioglossum leucopus*	12
			Solitary bee	*Lasioglossum morio*	1
			Solitary bee	*Megachile willughbiella*	1
			Hoverfly	*Merodon equestris*	1
			Solitary bee	*Osmia leaiana*	1
11	01/07/2015	Bramham park	Solitary bee	*Andrena bicolor*	1
			Solitary bee	*Andrena semilaevis*	1
			Bumblebee	*Bombus pratorum*	1
			Solitary bee	*Lasioglossum calceatum/albipes*	3
			Solitary bee	*Lasioglossum leucopus*	3
12	09/07/2015	Spen farm: agroforestry plot	Solitary bee	*Andrena bicolor*	4
			Honeybee	*Apis mellifera*	2
			Bumblebee	*Bombus lapidarius*	1
			Bumblebee	*Bombus terrestris/lucorum*	1
			Bumblebee	*Bombus vestalis*	2
			Hoverfly	*Episyrphus balteatus*	1
			Solitary bee	*Halictus tumulorum*	2

			Solitary bee	*Lasioglossum calceatum/albipes*	2
			Solitary bee	*Lasioglossum leucopus*	1
			Solitary bee	*Lasioglossum villosulum*	2
13	11/07/2015	Spen farm: fallow field plot	Solitary bee	*Andrena semilaevis*	3
			Honeybee	*Apis mellifera*	3
			Bumblebee	*Bombus lapidarius*	8
			Bumblebee	*Bombus terrestris/lucorum*	14
			Bumblebee	*Bombus vestalis*	1
			Hoverfly	*Eristalis arbustorum*	1
			Hoverfly	*Eristalis tenax*	1
			Solitary bee	*Halictus tumulorum*	16
			Solitary bee	*Lasioglossum calceatum/albipes*	1
			Solitary bee	*Lasioglossum leucopus*	1
			Solitary bee	*Lasioglossum villosulum*	3
			Hoverfly	*Syrphus ribesii*	1
14	15/07/2015	Spen farm: agroforestry plot	Solitary bee	*Andrena minutula*	1
			Solitary bee	*Andrena subopaca*	1
			Honeybee	*Apis mellifera*	1
			Bumblebee	*Bombus hortorum*	2
			Bumblebee	*Bombus lapidarius*	1
			Bumblebee	*Bombus pascuorum*	1
			Bumblebee	*Bombus pratorum*	2
			Bumblebee	*Bombus terrestris/lucorum*	1
			Bumblebee	*Bombus vestalis*	1
			Hoverfly	*Episyrphus balteatus*	3
			Hoverfly	*Eupeodes corollae*	1
			Solitary bee	*Halictus rubicundus*	1
			Solitary bee	*Halictus tumulorum*	2
			Solitary bee	*Lasioglossum calceatum/albipes*	4
			Solitary bee	*Lasioglossum smeathmanellum*	1

			Solitary bee	*Lasioglossum villosulum*	1
			Hoverfly	*Syrphus ribesii*	4
15	17/07/2015	Spen farm: fallow field plot	Solitary bee	*Andrena minutula*	1
			Solitary bee	*Andrena subopaca*	1
			Honeybee	*Apis mellifera*	2
			Bumblebee	*Bombus hortorum*	3
			Bumblebee	*Bombus lapidarius*	19
			Bumblebee	*Bombus pascuorum*	2
			Bumblebee	*Bombus pratorum*	1
			Bumblebee	*Bombus terrestris/lucorum*	15
			Hoverfly	*Episyrphus balteatus*	4
			Hoverfly	*Eristalis arbustorum*	1
			Hoverfly	*Eristalis tenax*	1
			Solitary bee	*Halictus tumulorum*	4
			Solitary bee	*Lasioglossum leucopus*	1
			Solitary bee	*Lasioglossum morio*	1
			Hoverfly	*Syrphus ribesii*	3
16	21/07/2015	Spen farm: fallow field plot	Solitary bee	*Andrena bicolor*	1
			Honeybee	*Apis mellifera*	1
			Bumblebee	*Bombus lapidarius*	12
			Bumblebee	*Bombus pascuorum*	1
			Bumblebee	*Bombus pratorum*	1
			Bumblebee	*Bombus terrestris/lucorum*	10
			Hoverfly	*Episyrphus balteatus*	2
			Hoverfly	*Eristalis tenax*	1
			Solitary bee	*Lasioglossum calceatum/albipes*	1
			Solitary bee	*Lasioglossum villosulum*	1
			Hoverfly	*Syrphus ribesii*	3
17	05/08/2015	Bramham park	Solitary bee	*Andrena bimaculata*	1
			Solitary bee	*Andrena falsifica*	1

			Honeybee	*Apis mellifera*	4
			Bumblebee	*Bombus lapidarius*	24
			Bumblebee	*Bombus pascuorum*	2
			Bumblebee	*Bombus rupestris*	8
			Bumblebee	*Bombus soroeensis*	2
			Bumblebee	*Bombus terrestris/lucorum*	6
			Bumblebee	*Bombus vestalis*	10
			Hoverfly	*Episyrphus balteatus*	6
			Solitary bee	*Heriades truncorum*	1
			Solitary bee	*Lasioglossum calceatum/albipes*	2
			Solitary bee	*Lasioglossum fulvicorne*	2
			Solitary bee	*Lasioglossum smeathmanellum*	14
			Hoverfly	*Melanostoma mellinum*	1
			Hoverfly	*Platycheirus albimanus*	1
			Hoverfly	*Syrphus vitripennis*	3
18	29/09/2015	St. George's Field	Honeybee	*Apis mellifera*	1
			Hoverfly	*Episyrphus balteatus*	1
			Hoverfly	*Eristalis pertinax*	1
			Hoverfly	*Eristalis tenax*	2
			Hoverfly	*Helophilus hybridus*	1
			Solitary bee	*Lasioglossum smeathmanellum*	1
19	03/10/2015	Meanwood Grove	Honeybee	*Apis mellifera*	2
			Bumblebee	*Bombus hortorum*	1
			Bumblebee	*Bombus pascuorum*	8
			Hoverfly	*Episyrphus balteatus*	1
			Hoverfly	*Eristalis tenax*	2
			Hoverfly	*Helophilus pendulus*	6

Supplementary table 1.7. The relative abundance of bycatch (identified to order) sampled by pan trapping during each survey visit.

Transect	Non-Syrphid Diptera	Coleoptera	Hemiptera	Lepidoptera	Non-bee Hymenoptera	Other
1	80	0	0	0	25	12
2	609	3	3	2	26	6
3	432	0	0	1	21	13
4	647	3	1	1	33	146
5	595	0	3	4	10	11
6	257	4	0	4	17	7
7	146	20	8	0	33	19
8	303	14	54	5	71	16
9	391	7	56	2	121	23
10	52	0	22	1	2	4
11	195	12	74	2	33	18
12	291	27	74	9	76	25
13	255	8	28	0	29	31
14	203	26	23	5	91	35
15	218	37	8	1	34	54
16	1030	87	0	0	13	94
17	466	69	7	0	37	101
18	82	3	1	0	9	0
19	99	3	6	0	6	4

Supplementary Table 1.8. Model averaged parameter estimates ± 95% confidence intervals for the effects of trap duration on sampled abundance and species richness within each pollinator group. Asterisks refer to statistically significant parameter estimates (where the respective 95 percent confidence intervals do not include zero).

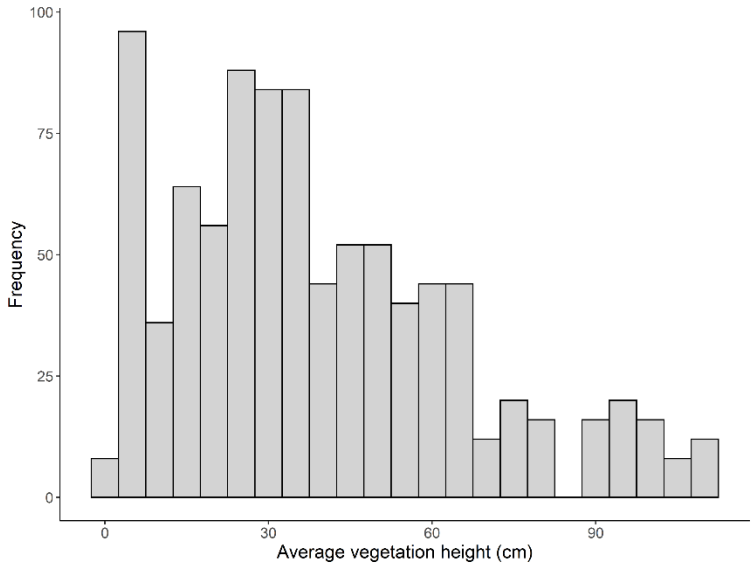
			Parameter estimates ± 95% CI	
Group	Parameter		Abundance	Richness
Total pollinators	Intercept		-4.964 [-6.830, -3.097]	-3.862 [-5.720, -2.005]
	Trap duration†	24	0.057 [-0.213, 0.327]	0.010 [-0.244, 0.264]
		48	0.531 [0.279, 0.783] *	0.459 [0.227, 0.692] *
Total bees	Intercept		-4.842 [-6.697, -2.986]	-4.087 [-5.648, -2.527]
	Trap duration†	24	-0.009 [-0.317, 0.299]	0.005 [-0.293, 0.304]
		48	0.600 [0.318, 0.882] *	0.598 [0.333, 0.862] *
Bumblebees	Intercept		-6.498 [-8.538, -4.458]	-5.353 [-7.317, -3.389]
	Trap duration†	24	0.693 [-0.435, 1.821]	0.883 [0.309, 1.457] *
		48	1.479 [0.143, 2.816] *	1.226 [0.701, 1.752] *
Solitary bees	Intercept		-4.051 [-6.794, -1.308]	-3.506 [-5.946, -1.065]
	Trap duration†	24	-0.778 [-2.561, 1.006]	-0.686[-2.149, 0.778]
		48	-1.362 [-3.251, 0.526]	-0.779[-2.928, 1.370]
Hoverflies	Intercept		-6.782 [-10.156, -3.407]	-6.809 [-10.522, -3.097]
	Trap duration†	24	0.001 [-0.271, 0.273]	NA††
		48	-0.045 [-0.421, 0.330]	NA††
† 7 hours was the reference category				
†† trap duration did not feature in any of the component models of the model averaging				

Supplementary Table 1.9. Model averaged parameter estimates ± 95% confidence intervals for the effects of trap duration on the ratio of sampled pollinator abundance to the abundance of bycatch. Asterisks refer to statistically significant parameter estimates (where the respective 95 percent confidence intervals do not include zero).

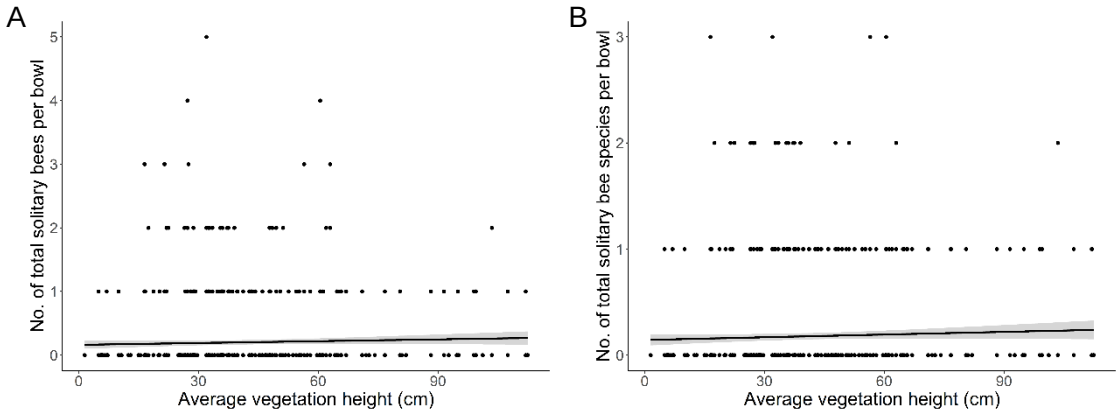
			Parameter estimates ± 95% CI
Group	Parameter		Abundance
Total pollinators	Intercept		-1.790 [-4.152, 0.573]
	Trap duration†	24	-0.368 [-0.642, -0.095]*
		48	-0.234 [-0.498, 0.029]
Total bees	Intercept		-3.240 [-6.536, 0.056]
	Trap duration†	24	-0.520 [-4.568, 3.528]
		48	0.918 [-2.314, 4.150]
Bumblebees	Intercept		-4.625 [-8.573, -0.676]
	Trap duration†	24	-0.046 [-1.952, 1.859]
		48	2.459 [0.799, 4.119]*
Solitary bees	Intercept		-5.625 [-7.861, -3.389]
	Trap duration†	24	-1.125 [-1.599, -0.651]*
		48	-0.787 [-1.215, -0.360]*
Hoverflies	Intercept		-4.293 [-6.333, -2.253]
	Trap duration†	24	-0.183 [-0.865, 0.499]
		48	-0.714 [-2.046, 0.617]
† 7 hours was the reference category			

Supplementary Table 1.10. Model averaged parameter estimates ± 95% confidence intervals for the effects of trap duration on the abundance and species richness sampled per hour within each pollinator group. Asterisks refer to statistically significant parameter estimates (where the respective 95 percent confidence intervals do not include zero).

			Parameter estimates ± 95% CI	
Group	Parameter		Abundance	Richness
Total pollinators	Intercept		-6.942 [-8.807, -5.076]	-5.763 [-7.614, -3.913]
	Trap duration†	24	-0.733 [-1.007, -0.459] *	-0.780 [-1.035, -0.525] *
		48	-0.963 [-1.218, -0.708] *	-1.037 [-1.270, -0.804] *
Total bees	Intercept		-6.920 [-8.735, -5.106]	-6.076 [-7.603, -4.549]
	Trap duration†	24	-0.821 [-1.132, -0.510] *	-0.808 [-1.107, -0.508] *
		48	-0.921 [-1.206, -0.637] *	-0.928 [-1.193, -0.662] *
Bumblebees	Intercept		-8.612 [-10.558, -6.665]	-7.772 [-9.873, -5.672]
	Trap duration†	24	-0.047 [-0.723, 0.629]	0.022 [-0.369, 0.413]
		48	-0.022 [-0.818, 0.773]	-0.144 [-0.616, 0.327]
Solitary bees	Intercept		-5.907 [-8.701, -3.114]	-5.173 [-7.477, -2.870]
	Trap duration†	24	-1.649 [-3.543, 0.245]	-1.538 [-3.207, 1.306]
		48	-3.151 [-4.732, -1.570] *	-2.640 [-4.867, -0.414] *
Hoverflies	Intercept		-8.833 [-12.240, -5.425]	-8.819 [-12.588, -5.050]
	Trap duration†	24	-0.743 [-1.463, -0.023] *	-0.519 [-1.491, 0.452]
		48	-1.600 [-2.509, -0.691] *	-1.651 [-2.654, -0.648] *
† 7 hours was the reference category				



Supplementary Figure 1.5. A histogram of the average vegetation height (cm) present within a 1m radius of each pan trapping station.



Supplementary Figure 1.6. A) Solitary bee abundance plotted against the average vegetation height (cm) present within a 1m radius of each pan trapping station; B) Solitary bee species richness plotted against the average vegetation height (cm) present within a 1m radius of each pan trapping station.

References cited in Appendix 1.

Baude, M., Kunin, W. E., & Memmott, J. (2015). *Nectar sugar values of common British plant species [AgriLand].* NERC Environmental Information Data Centre. https://doi.org/https://doi.org/10.5285/69402002-1676-4de9-a04e-d17e827db93c

Appendix 2.

Supplementary figure 2.1. Adapted aquarium equipment used to partially anaesthetise pollinators prior to marking.

In supplementary Figure 2.1, the clear plastic tube is used to contain the insect while the compressed CO_2 gas was gradually released from the canister in short bursts. After approximately one minute, the insect was released from the tube and was gently pressed down using a cardboard tube covered with string or thread mesh at one end. The insect was marked on the appropriate spot on the wing through the mesh and released.

Supplementary table 2.1. Each floral species found along each survey visit, along with each species' definition of a floral unit, and the mean number of florets per floral unit.

Floral species	Floral unit definition	Mean no. of florets/floral unit
Cirsium sp.	Single capitulum	57.75483
Crepis sp.	Single capitulum	21.33333
Geranium sp.	Single flower	1
Anagallis arvensis	Single flower	1
Unknown	Single flower	1
Silene sp.	Single flower	1
Calendula sp.	Single capitulum	66

Supplementary table 2.2. The relative abundance of each floral species within a two-meter radius of each pan trapping station, together with the total number of flowers present within a two-meter radius of each pan trapping station. Measured on 21/04/2016. Relative abundance is measured as the number of florets, not the number of floral units.

Station	*Cirsium* sp.	*Crepis* sp.	*Geranium* sp.	*Anagallis arvensis*	Unknown	*Silene* sp.	*Calendula* sp.	Station total
1	8663.225	0	30	7	0	0	0	8700.225
2	9240.773	0	19	0	0	0	0	9259.773
3	3465.29	85.33333	16	140	0	60	0	3766.623
4	1501.626	256	17	62	0	8	0	1844.626
5	2425.703	85.33333	7	48	0	35	0	2601.036
6	1097.342	0	6	0	0	0	0	1103.342
7	1039.587	917.3333	0	6	2	25	0	1989.92
8	16748.9	0	6	0	0	0	0	16754.9
9	1848.155	192	6	0	0	0	5082	7128.155
10	5659.974	682.6667	14	0	0	0	0	6356.64

Supplementary table 2.3. The relative abundance of each floral species along each transect (within one meter to either side of the central line), together with the total number of flowers present along each transect. Measured on 22/04/2016. Relative abundance is measured as the number of florets, not the number of floral units.

Transect	*Cirsium* sp.	*Crepis* sp.	*Anagallis arvensis*	*Silene* sp.	Unknown	*Geranium* sp.	*Calendula* sp.	Transect total
1	6526.296	64	60	5	0	10	0	6665.296
2	5890.993	0	14	2	0	7	0	5913.993
3	2310.193	149.3333	44	6	0	24	0	2533.527
4	1039.587	554.6667	17	12	0	29	0	1652.254
5	1443.871	170.6667	73	3	1	14	0	1705.537
6	9240.773	469.3333	1	0	0	10	0	9721.107
7	519.7935	0	10	42	0	11	0	582.7935
8	5197.935	106.6667	35	19	0	5	0	5363.602
9	1905.909	170.6667	2	0	2	55	0	2135.576
10	5197.935	0	0	5	0	10	330	5542.935

Supplementary Table 2.4. A table summarising the fit of each Jolly-Seber (superpopulation approach) model run for each species during the MRR analysis, as described using AIC_C, ΔAIC_C, and Akaike weights.

Model	AIC_C	ΔAIC_C	Akaike weight	No. of parameters
Bombus terrestris				
$\phi_i \sim \cdot, p_i \sim \cdot, b_i \sim \cdot, N \sim \cdot$	167.13	0.00	0.9993025792	4
$\phi_i \sim \cdot, p_i \sim t, b_i \sim \cdot, N \sim \cdot$	183.14	16.01	0.0003341437	17
$\phi_i \sim t, p_i \sim \cdot, b_i \sim \cdot, N \sim \cdot$	184.00	16.87	0.0002172350	16
$\phi_i \sim \cdot, p_i \sim \cdot, b_i \sim t, N \sim \cdot$	184.79	17.66	0.0001460421	16
$\phi_i \sim t, p_i \sim \cdot, b_i \sim t, N \sim \cdot$	236.63	69.50	0.0000000000	28
$\phi_i \sim t, p_i \sim t, b_i \sim \cdot, N \sim \cdot$	252.28	85.15	0.0000000000	29
$\phi_i \sim \cdot, p_i \sim t, b_i \sim t, N \sim \cdot$	255.76	88.63	0.0000000000	29
$\phi_i \sim t, p_i \sim t, b_i \sim t, N \sim \cdot$	478.40	311.27	0.0000000000	41
Halictus phryganicus (unconstrained)				
$\phi_i \sim t, p_i \sim t, b_i \sim t, N \sim \cdot$	-272.38	0.00	1.0000000000	41
$\phi_i \sim \cdot, p_i \sim \cdot, b_i \sim \cdot, N \sim \cdot$	98.35	370.73	0.0000000000	4
$\phi_i \sim \cdot, p_i \sim t, b_i \sim \cdot, N \sim \cdot$	108.82	381.20	0.0000000000	17
$\phi_i \sim t, p_i \sim \cdot, b_i \sim \cdot, N \sim \cdot$	131.35	403.73	0.0000000000	16
$\phi_i \sim \cdot, p_i \sim \cdot, b_i \sim t, N \sim \cdot$	145.25	417.63	0.0000000000	16
$\phi_i \sim t, p_i \sim \cdot, b_i \sim t, N \sim \cdot$	498.14	770.52	0.0000000000	28
$\phi_i \sim t, p_i \sim t, b_i \sim \cdot, N \sim \cdot$	666.89	939.27	0.0000000000	29
$\phi_i \sim \cdot, p_i \sim t, b_i \sim t, N \sim \cdot$	669.37	941.75	0.0000000000	29
Halictus phryganicus (constrained)				
$\phi_i \sim \cdot, p_i \sim \cdot, b_i \sim \cdot, N \sim \cdot$	98.35	0	1.0000000000	4
Eupeodes corollae				
$\phi_i \sim \cdot, p_i \sim t, b_i \sim \cdot, N \sim \cdot$	303.35	0.00	0.999602	17
$\phi_i \sim t, p_i \sim \cdot, b_i \sim \cdot, N \sim \cdot$	324.40	21.05	0.00002673418	16
$\phi_i \sim \cdot, p_i \sim \cdot, b_i \sim t, N \sim \cdot$	326.19	22.84	0.00001094446	16
$\phi_i \sim t, p_i \sim t, b_i \sim \cdot, N \sim \cdot$	330.68	27.33	0.000001159879	29
$\phi_i \sim \cdot, p_i \sim \cdot, b_i \sim \cdot, N \sim \cdot$	333.09	29.74	0.0000003471298	4
$\phi_i \sim t, p_i \sim \cdot, b_i \sim t, N \sim \cdot$	333.27	29.92	0.0000003168987	28
$\phi_i \sim \cdot, p_i \sim t, b_i \sim t, N \sim \cdot$	333.76	30.41	0.0000002482734	29
$\phi_i \sim t, p_i \sim t, b_i \sim t, N \sim \cdot$	364.75	61.40	0.0000000000000	41
Sphaerophoria scripta				
$\phi_i \sim \cdot, p_i \sim t, b_i \sim \cdot, N \sim \cdot$	543.03	0.00	0.9992643	17
$\phi_i \sim \cdot, p_i \sim \cdot, b_i \sim t, N \sim \cdot$	558.54	15.51	0.0004278014	16
$\phi_i \sim t, p_i \sim t, b_i \sim \cdot, N \sim \cdot$	559.74	16.71	0.0002352059	29

$\phi_i \sim \cdot$, $p_i \sim t$, $b_i \sim t$, $N \sim \cdot$	562.63	19.60	0.00005546717	29
$\phi_i \sim t$, $p_i \sim \cdot$, $b_i \sim t$, $N \sim \cdot$	565.37	22.34	0.00001410018	28
$\phi_i \sim t$, $p_i \sim \cdot$, $b_i \sim \cdot$, $N \sim \cdot$	568.40	25.37	0.000003088310	16
$\phi_i \sim \cdot$, $p_i \sim \cdot$, $b_i \sim \cdot$, $N \sim \cdot$	577.97	34.94	0.00000002584792	4
$\phi_i \sim t$, $p_i \sim t$, $b_i \sim t$, $N \sim \cdot$	584.28	41.25	0.000000001101937	41

Supplementary Table 2.5. The estimated population size ($\widehat{N}_i$) of *Bombus terrestris*, with 95% confidence intervals, for each sampling period during the seven-day MRR experiment.

Sampling period	$\widehat{N}_i$	- 95% CI	+ 95% CI
1	18.38981	7.335439	46.10289
2	21.93581	10.31011	46.67067
3	14.25879	7.082498	28.70639
4	18.02232	10.09693	32.1686
5	12.52032	6.657191	23.54723
6	16.3754	9.527884	28.14409
7	11.78872	6.348654	21.89028
8	15.68232	9.140322	26.90661
9	11.48084	6.206107	21.23869
10	15.39065	8.962471	26.42933
11	11.35127	6.153235	20.94042
12	15.26791	8.892972	26.21272
13	11.29674	6.136774	20.79535
14	15.21625	8.868403	26.10778

Supplementary Table 2.6. The estimated population size ($\widehat{N}_i$) of *Sphaerophoria scripta*, with 95% confidence intervals, for each sampling period during the seven-day MRR experiment.

Sampling period	$\widehat{N}_i$	- 95% CI	+ 95% CI
1	434.93	250.77	754.33
2	432.71	258.09	725.45
3	234.89	152.30	362.25
4	241.62	163.51	357.05
5	138.78	94.78	203.20
6	149.82	106.89	209.99
7	92.61	62.27	137.73
8	105.71	73.75	151.54
9	70.42	45.40	109.24
10	84.52	56.27	126.97
11	59.77	37.18	96.09
12	74.34	47.70	115.88
13	54.65	33.78	89.74
14	69.45	43.61	110.60

Supplementary Table 2.7. The estimated population size ($\widehat{N}_i$) of *Eupeodes corollae*, with 95% confidence intervals, for each sampling period during the seven-day MRR experiment.

Sampling period	$\widehat{N}_i$	- 95% CI	+ 95% CI
1	58.68	8.39	410.22
2	92.53	26.73	320.29
3	89.65	44.32	181.33
4	122.41	74.94	199.95
5	106.99	61.42	186.37
6	139.13	81.88	236.40
7	116.70	58.69	232.02
8	148.49	77.02	286.25
9	122.13	55.66	267.95
10	153.72	73.48	321.61
11	125.17	53.73	291.58
12	156.66	71.30	344.18
13	126.87	52.54	306.34
14	158.30	69.98	358.08

Supplementary Table 2.8. The estimated population size ($\widehat{N}_i$) of *Halictus phryganicus* (unconstrained), with 95% confidence intervals, for each sampling period during the seven-day MRR experiment.

Sampling period	$\widehat{N}_i$	- 95% CI	+ 95% CI
1	9.00	9.00	9.00
2	8.17	8.17	8.17
3	0.99	0.99	0.99
4	0.52^{-14}	0.52^{-14}	0.52^{-14}
5	3.99	3.99	3.99
6	0.52^{-12}	0.52^{-12}	0.52^{-12}
7	3.00	3.00	3.00
8	3.01	3.01	3.01
9	54.25	54.25	54.25
10	54.25	54.25	54.25
11	7.99	7.99	7.99
12	0.56^{-12}	0.56^{-12}	0.56^{-12}
13	3.99	3.99	3.99
14	0.86^{-12}	0.86^{-12}	0.86^{-12}

Supplementary Table 2.9. The estimated population size ($\widehat{\boldsymbol{N}}_{\boldsymbol{i}}$) of *Halictus phryganicus* (constrained), with 95% confidence intervals, for each sampling period during the seven-day MRR experiment.

Sampling period	$\widehat{N}_i$	- 95% CI	+ 95% CI
1	499.89	99.27	2517.26
2	499.11	99.21	2511.00
3	487.42	97.82	2428.85
4	486.66	97.70	2424.14
5	475.26	95.53	2364.54
6	474.52	95.36	2361.28
7	463.41	92.46	2322.68
8	462.68	92.24	2320.77
9	451.85	88.71	2301.49
10	451.14	88.46	2300.80
11	440.58	84.43	2299.01
12	439.88	84.15	2299.44
13	429.59	79.78	2313.26
14	428.91	79.48	2314.66

Appendix 3.

Supplementary Table 3.1. The identity of each floral species found during each survey, along with each species' definition of a floral unit, the mean number of florets per floral unit, the mean volume of nectar sugar produced per floret per 24-hours (µl) (taken primarily from Baude, Kunin, & Memmott (2015)), and the mean volume of nectar sugar produced per floral unit per 24-hours (µl) (mean number of florets per floral unit multiplied by the mean volume of nectar sugar produced per floret per 24-hours).

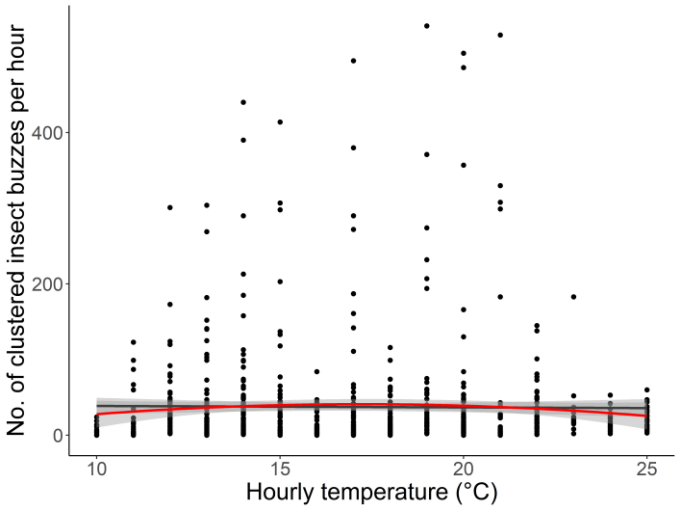
Date	Site	Binomial	Floral unit definition	Mean no. of florets/floral unit	Mean vol. of nectar sugar produced/day/floret (µl)	Mean vol. of nectar sugar produced/day/floral unit (µl)
26/08/2017	Farnley Hall Park	*Bellis perennis*	Single capitulum	95	0.841424157	79.93529492
27/08/2017	Kirkstall Abbey	*Taraxacum spp.*	Single capitulum	206.4	22.57258633	4658.981819
28/08/2017	Meanwood farm	*Senecio jacobaea*	Single capitulum	24.4	22.59852245	551.4039478
28/08/2017	Meanwood road	*Bellis perennis*	Single capitulum	95	0.841424157	79.93529492
		Taraxacum spp.	Single capitulum	206.4	22.57258633	4658.981819
		Trifolium repens	Single raceme	22.7	48.96555613	1111.518124
01/09/2017	Asket Hill	NA	NA	NA	NA	NA
02/09/2017	Temple Newsam	*Taraxacum spp.*	Single capitulum	206.4	22.57258633	4658.981819
04/09/2017	Water Haigh	*Ranunculus repens*	Single flower	1	104.5103816	104.5103816
		Taraxacum spp.	Single capitulum	206.4	22.57258633	4658.981819
		Trifolium pratense	Single raceme	24.1	116.8559138	2816.227523
06/09/2017	Skelton wood	*Cirsium vulgare*	Single capitulum	195.2	76.51305191	14935.34773
		Lotus corniculatus	Single flower	1	61.82363977	61.82363977
		Ranunculus repens	Single flower	1	104.5103816	104.5103816
		Senecio jacobaea	Single capitulum	24.4	22.59852245	551.4039478
12/09/2017	Rothwell Country Park	*Anagallis arvensis*	Single flower	1	0	0
		Lotus corniculatus	Single flower	1	61.82363977	61.82363977
		Trifolium pratense	Single raceme	24.1	116.8559138	2816.227523
15/09/2017	Rothwell pastures	*Lotus corniculatus*	Single flower	1	61.82363977	61.82363977
		Taraxacum spp.	Single capitulum	206.4	22.57258633	4658.981819
		Trifolium pratense	Single raceme	24.1	116.8559138	2816.227523

17/09/2017	Killingbeck fields	*Heracleum sphondylium*	Single umbel	14.66666667	98.16742963	1439.788968
		Trifolium pratense	Single raceme	24.1	116.8559138	2816.227523
19/09/2017	Halton Moor	*Taraxacum spp.*	Single capitulum	206.4	22.57258633	4658.981819
20/09/2017	Primrose Valley	NA	NA	NA	NA	NA
27/06/2018	Meanwood Grove	*Lotus corniculatus*	Single flower	1	61.82363977	61.82363977
		Ranunculus repens	Single flower	1	104.5103816	104.5103816
		Trifolium dubium	Single raceme	14.8	0	0
02/07/2018	Farnley Hall Park	NA	NA	NA	NA	NA
03/07/2018	Temple Newsam	*Leucanthemum vulgare*	Single capitulum	135.2	15.81	2137.512
04/07/2018	Bramley Fall Park	*Heracleum sphondylium*	Single umbel	14.66666667	98.16742963	1439.788968
31/07/2018	Kirkstall Abbey	*Plantago lanceolata*	Single stem	3	2.666406005	7.999218015
		Taraxacum spp.	Single capitulum	206.4	22.57258633	4658.981819
03/08/2018	Rothwell pastures	*Lotus corniculatus*	Single flower	1	61.82363977	61.82363977
		Senecio jacobaea	Single capitulum	24.4	22.59852245	551.4039478
01/09/2018	Water Haigh	*Leontodon autumnalis*	Single capitulum	37.2	13.70236274	509.7278939

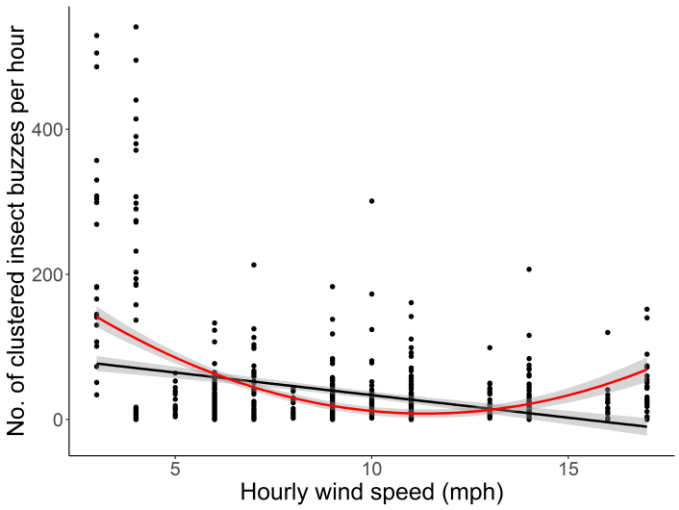
Supplementary Table 3.2. The relative abundance of floral species within a two-meter radius of each acoustic pan trapping station per survey visit. Relative abundance is measured as the total number of florets present per species.

Date	Site	Binomial	Station 1	Station 2	Station 3	Station 4	Station 5
26/08/2017	Farnley Hall Park	*Bellis perennis*	0	95	0	190	0
27/08/2017	Kirkstall Abbey	*Taraxacum spp.*	0	0	619.2	206.4	619.2
28/08/2017	Meanwood farm	*Senecio jacobaea*	561.2	0	902.8	0	0
28/08/2017	Meanwood road	*Bellis perennis*	570	380	0	190	380
		Taraxacum spp.	412.8	0	206.4	0	0
		Trifolium repens	0	0	0	0	68.1
01/09/2017	Asket Hill	NA	0	0	0	0	0
02/09/2017	Temple Newsam	*Taraxacum spp.*	1857.6	825.6	0	206.4	825.6
04/09/2017	Water Haigh	*Ranunculus repens*	0	0	0	0	3
		Taraxacum spp.	825.6	0	0	0	0
		Trifolium pratense	1397.8	2482.3	313.3	24.1	48.2
06/09/2017	Skelton wood	*Cirsium vulgare*	0	0	0	585.6	0
		Lotus corniculatus	10	16	2	0	2
		Ranunculus repens	0	0	1	0	1
		Senecio jacobaea	0	0	0	0	536.8
12/09/2017	Rothwell Country Park	*Anagallis arvensis*	0	0	3	0	0
		Lotus corniculatus	8	11	0	15	0
		Trifolium pratense	0	144.6	0	120.5	0
15/09/2017	Rothwell pastures	*Lotus corniculatus*	3	0	0	0	1
		Taraxacum spp.	0	0	0	0	206.4
		Trifolium pratense	0	0	0	0	168.7
17/09/2017	Killingbeck fields	*Heracleum sphondylium*	220.0000001	0	0	0	0
		Trifolium pratense	0	0	361.5	0	0
19/09/2017	Halton Moor	*Taraxacum spp.*	0	0	0	619.2	0
20/09/2017	Primrose Valley Park	NA	0	0	0	0	0

27/06/2018	Meanwood Grove	*Lotus corniculatus*	166	4	0	0	0
		Ranunculus repens	3	1	0	0	5
		Trifolium dubium	236.8	0	0	0	0
02/07/2018	Farnley Hall Park	NA	0	0	0	0	0
03/07/2018	Temple Newsam	*Leucanthemum vulgare*	135.2	2704	811.2	0	0
04/07/2018	Bramley Fall Park	*Heracleum sphondylium*	220.0000001	29.33333334	29.33333334	0	0
31/07/2018	Kirkstall Abbey	*Plantago lanceolata*	0	0	0	0	3
		Taraxacum spp.	4953.6	2683.2	5985.6	1032	1857.6
03/08/2018	Rothwell pastures	*Lotus corniculatus*	84	0	10	6	0
		Senecio jacobaea	0	0	0	0	268.4
01/09/2018	Water Haigh	*Leontodon autumnalis*	0	74.4	37.2	0	0



Supplementary Figure 3.1. The number of clustered buzzes per hour plotted against hourly temperature (°C). The black line displays a linear relationship (± 95% confidence intervals), the red line displays a second-order polynomial relationship (± 95% confidence intervals).



Supplementary Figure 3.2. The number of clustered buzzes per hour plotted against hourly wind speed (mph). The black line displays a linear relationship (± 95% confidence intervals), the red line displays a second-order polynomial relationship (± 95% confidence intervals).

Supplementary Table 3.3. The total volume of nectar sugar produced per 24 hours (µl) within a two-meter radius of each acoustic pan trapping station per survey visit. Along with the mean volume of nectar sugar produced per 24 hours (µl) across all acoustic pan trapping stations per survey visit.

Date	Site	Station 1	Station 2	Station 3	Station 4	Station 5	Total
26/08/2017	Farnley Hall Park	0	79.93529492	0	159.8705898	0	239.8058847
28/08/2017	Kirkstall Abbey	0	0	13976.94546	4658.981819	13976.94546	32612.87273
28/08/2017	Meanwood farm	12682.2908	0	20401.94607	0	0	33084.23687
28/08/2017	Meanwood road	9797.575407	319.7411797	4658.981819	159.8705898	3654.295552	18590.46455
01/09/2017	Asket Hill	0	0	0	0	0	0
02/09/2017	Temple Newsam	41930.83637	18635.92727	0	4658.981819	18635.92727	83861.67273
04/09/2017	Water Haigh	181977.1236	290071.4348	36610.95779	2816.227523	5945.98619	517421.7299
06/09/2017	Skelton wood	618.2363977	989.1782363	228.1576611	44806.0432	12359.04451	59000.66001
12/09/2017	Rothwell Country Park	494.5891182	17577.42517	0	15008.49221	0	33080.5065
15/09/2017	Rothwell pastures	185.4709193	0	0	0	24434.39812	24619.86904
17/09/2017	Killingbeck fields	21596.83452	0	42243.41284	0	0	63840.24736
19/09/2017	Halton Moor	0	0	0	13976.94546	0	13976.94546
20/09/2017	Primrose Valley Park	0	0	0	0	0	0
27/06/2018	Meanwood Grove	10576.25535	351.8049407	0	0	522.551908	11450.6122
02/07/2018	Farnley Hall Park	0	0	0	0	0	0
03/07/2018	Temple Newsam	2137.512	42750.24	12825.072	0	0	57712.824
04/07/2018	Bramley Fall Park	21596.83452	2879.577936	2879.577936	0	0	27355.9904
31/07/2018	Kirkstall Abbey	111815.5636	60566.76364	135110.4727	23294.90909	41938.83558	372726.5447
03/08/2018	Rothwell pastures	5193.185741	0	618.2363977	370.9418386	6065.443426	12247.8074
01/09/2018	Water Haigh	0	1019.455788	509.7278939	0	0	1529.183682

Supplementary Table 3.4. The site name, acoustic pan trapping station, hour of the day, and estimated activity level associated with each of the seventy-five hour-long soundscape recordings within the data subset used to quantify the number of true positives (TP), false positives (FP), and false negatives (FN), and therefore the true positive rate (TPR), false positive rate (FPR), and the false negative rate (FNR). Also displayed are the number of insect signals used the train the relevant site-specific Specialist Insect Classifier (SIC), and the number of clustered buzzes present.

Date	Site	Station	Hour	Activity	Training buzzes	Clustered buzzes	TP	FP	FN	TPR	FPR	FNR
20/09/2017	Primrose Valley Park	4	10:00:00	Low	224	2	0	2	0	NA	0.011	NA
28/08/2017	Meanwood road	5	16:00:00	Low	33	7	3	4	3	0.500	0.071	0.500
27/08/2017	Kirkstall Abbey	1	16:00:00	Low	16	5	0	5	3	0.000	0.030	1.000
19/09/2017	Halton Moor	5	12:00:00	Low	30	5	1	4	0	1.000	0.016	0.000
28/08/2017	Meanwood road	4	08:00:00	Low	33	2	2	0	4	0.333	0.000	0.667
26/08/2017	Farnley Hall Park	4	09:00:00	Low	31	6	2	4	15	0.118	0.011	0.882
31/07/2018	Kirkstall Abbey	1	09:00:00	Low	48	4	0	4	1	0.000	0.075	1.000
19/09/2017	Halton Moor	3	15:00:00	Low	30	4	0	4	0	NA	0.022	NA
27/08/2017	Kirkstall Abbey	3	12:00:00	Low	16	3	3	0	7	0.300	0.000	0.700
19/09/2017	Halton Moor	5	13:00:00	Low	30	2	1	1	0	1.000	0.004	0.000
31/07/2018	Kirkstall Abbey	5	15:00:00	Low	48	7	7	0	8	0.467	0.000	0.533
02/09/2017	Temple Newsam	4	15:00:00	Low	22	5	1	4	0	1.000	0.015	0.000
19/09/2017	Halton Moor	3	13:00:00	Low	30	7	4	3	13	0.235	0.006	0.765
27/08/2017	Kirkstall Abbey	2	16:00:00	Low	16	0	0	0	0	NA	0.000	NA
12/09/2017	Rothwell Country Park	3	09:00:00	Low	59	0	0	0	0	NA	0.000	NA

03/08/2018	Rothwell pastures	5	11:00:00	Low	28	2	2	0	10	0.167	0.000	0.833
27/08/2017	Kirkstall Abbey	1	12:00:00	Low	16	4	1	3	5	0.167	0.038	0.833
20/09/2017	Primrose Valley Park	5	16:00:00	Low	224	5	0	5	0	NA	0.029	NA
27/08/2017	Kirkstall Abbey	5	16:00:00	Low	16	4	1	3	0	1.000	0.021	0.000
01/09/2017	Asket Hill	2	17:00:00	Low	114	6	0	6	0	NA	0.400	NA
27/08/2017	Kirkstall Abbey	4	12:00:00	Low	16	3	1	2	4	0.200	0.014	0.800
17/09/2017	Killingbeck fields	2	15:00:00	Low	47	3	0	3	3	0.000	0.014	1.000
03/08/2018	Rothwell pastures	1	11:00:00	Low	28	3	3	0	30	0.091	0.000	0.909
19/09/2017	Halton Moor	2	09:00:00	Low	30	1	0	1	0	NA	0.037	NA
17/09/2017	Killingbeck fields	2	10:00:00	Low	47	3	0	3	0	NA	0.029	NA
06/09/2017	Skelton wood	5	16:00:00	Medium	178	11	0	11	0	NA	0.212	NA
20/09/2017	Primrose Valley Park	5	11:00:00	Medium	224	9	2	7	2	0.500	0.024	0.500
20/09/2017	Primrose Valley Park	4	16:00:00	Medium	224	24	0	24	1	0.000	0.048	1.000
28/08/2017	Meanwood road	2	11:00:00	Medium	33	25	3	22	5	0.375	0.094	0.625
19/09/2017	Halton Moor	1	14:00:00	Medium	30	19	0	19	1	0.000	0.019	1.000
01/09/2018	Water Haigh	1	11:00:00	Medium	54	9	4	5	15	0.211	0.057	0.789
27/08/2017	Kirkstall Abbey	4	15:00:00	Medium	16	10	1	9	1	0.500	0.035	0.500
26/08/2017	Farnley Hall Park	2	14:00:00	Medium	31	18	2	16	0	1.000	0.035	0.000
03/07/2018	Temple Newsam	5	10:00:00	Medium	33	35	5	30	37	0.119	0.028	0.881

28/08/2017	Meanwood road	1	10:00:00	Medium	33	30	0	30	1	0.000	0.180	1.000
02/07/2018	Farnley Hall Park	2	13:00:00	Medium	31	35	0	35	2	0.000	0.044	1.000
01/09/2018	Water Haigh	4	11:00:00	Medium	54	10	0	10	3	0.000	0.023	1.000
03/07/2018	Temple Newsam	1	16:00:00	Medium	33	10	0	10	3	0.000	0.043	1.000
27/06/2018	Meanwood Grove	3	12:00:00	Medium	78	35	23	12	93	0.198	0.023	0.802
12/09/2017	Rothwell Country Park	3	16:00:00	Medium	59	19	13	6	10	0.565	0.031	0.435
03/08/2018	Rothwell pastures	3	10:00:00	Medium	28	19	19	0	178	0.096	0.000	0.904
04/09/2017	Water Haigh	3	14:00:00	Medium	145	19	2	17	1	0.667	0.101	0.333
19/09/2017	Halton Moor	1	09:00:00	Medium	30	14	1	13	1	0.500	0.053	0.500
31/07/2018	Kirkstall Abbey	4	10:00:00	Medium	48	12	0	12	0	NA	0.023	NA
01/09/2017	Asket Hill	5	15:00:00	Medium	114	34	5	29	9	0.357	0.207	0.643
12/09/2017	Rothwell Country Park	4	12:00:00	Medium	59	14	3	11	8	0.273	0.042	0.727
12/09/2017	Rothwell Country Park	4	16:00:00	Medium	59	22	12	10	24	0.333	0.063	0.667
03/07/2018	Temple Newsam	4	09:00:00	Medium	33	25	4	21	33	0.108	0.036	0.892
27/08/2017	Kirkstall Abbey	4	13:00:00	Medium	16	8	0	8	0	NA	0.036	NA
15/09/2017	Rothwell pastures	4	12:00:00	Medium	30	30	4	26	7	0.364	0.084	0.636
27/06/2018	Meanwood Grove	4	12:00:00	High	78	44	26	18	84	0.236	0.030	0.764
02/07/2018	Farnley Hall Park	5	13:00:00	High	31	53	0	53	1	0.000	0.058	1.000

28/08/2017	Meanwood farm	4	15:00:00	High	364	49	14	35	9	0.609	0.082	0.391
01/09/2017	Asket Hill	2	16:00:00	High	114	107	0	107	0	NA	0.132	NA
27/06/2018	Meanwood Grove	4	15:00:00	High	78	37	22	15	72	0.234	0.034	0.766
28/08/2017	Meanwood farm	4	14:00:00	High	364	70	16	54	3	0.842	0.120	0.158
28/08/2017	Meanwood farm	2	11:00:00	High	364	36	6	30	2	0.750	0.048	0.250
15/09/2017	Rothwell pastures	2	10:00:00	High	30	67	0	67	0	NA	0.092	NA
02/07/2018	Farnley Hall Park	2	14:00:00	High	31	37	1	36	3	0.250	0.049	0.750
04/07/2018	Bramley Fall Park	4	09:00:00	High	511	107	39	68	30	0.565	0.108	0.435
04/07/2018	Bramley Fall Park	1	16:00:00	High	511	101	90	11	52	0.634	0.078	0.366
04/07/2018	Bramley Fall Park	3	10:00:00	High	511	185	78	107	57	0.578	0.092	0.422
03/07/2018	Temple Newsam	2	11:00:00	High	33	37	29	8	36	0.446	0.009	0.554
28/08/2017	Meanwood farm	3	13:00:00	High	364	44	6	38	2	0.750	0.063	0.250
04/07/2018	Bramley Fall Park	2	11:00:00	High	511	414	336	78	54	0.862	0.104	0.138
28/08/2017	Meanwood road	5	12:00:00	High	33	60	52	8	29	0.642	0.086	0.358
27/06/2018	Meanwood Grove	4	11:00:00	High	78	61	53	8	209	0.202	0.006	0.798
04/07/2018	Bramley Fall Park	3	11:00:00	High	511	137	58	79	25	0.699	0.114	0.301
15/09/2017	Rothwell pastures	5	10:00:00	High	30	99	0	99	0	NA	0.131	NA

04/07/2018	Bramley Fall Park	3	12:00:00	High	511	187	125	62	50	0.714	0.132	0.286
04/07/2018	Bramley Fall Park	5	14:00:00	High	511	505	448	57	220	0.671	0.107	0.329
04/07/2018	Bramley Fall Park	4	11:00:00	High	511	203	115	88	81	0.587	0.111	0.413
28/08/2017	Meanwood farm	3	15:00:00	High	364	69	4	65	3	0.571	0.112	0.429
01/09/2017	Asket Hill	3	13:00:00	High	114	36	29	7	19	0.604	0.038	0.396
27/06/2018	Meanwood Grove	3	10:00:00	High	78	52	44	8	208	0.175	0.007	0.825

Supplementary table 3.5. Pairwise comparisons between four measures of acoustic insect activity: manual counts of insect buzzes per hour, the number of insect buzzes clustered per hour by Kaleidoscope Pro (clustered insects), the number of true positives therein, and the number of total detected insects (true positives + false negatives), within the low estimated activity category. Bold values indicate significant pairwise comparisons, where the 95% confidence intervals do not overlap zero.

Pairwise comparison	Parameter estimate	95% CI
True positives -- Clustered insects	**-1.4191**	**[-2.1822, -0.6559]**
Detected insects -- Clustered insects	-0.1221	[-0.7610, 0.5168]
Manual count -- Clustered insects	**1.2157**	**[0.6237, 1.8077]**
Detected insects -- True positives	**1.2970**	**[0.5702, 2.0238]**
Manual count -- True positives	**2.6348**	**[1.9307, 3.3389]**
Manual count -- Detected insects	**1.3378**	**[0.7747, 1.9010]**

Supplementary table 3.6. Pairwise comparisons between four measures of acoustic insect activity: manual counts of insect buzzes per hour, the number of insect buzzes clustered per hour by Kaleidoscope Pro (clustered insects), the number of true positives therein, and the number of total detected insects (true positives + false negatives), within the medium estimated activity category. Bold values indicate significant pairwise comparisons, where the 95% confidence intervals do not overlap zero.

Pairwise comparison	Parameter estimate	95% CI
True positives -- Clustered insects	**-2.3193**	**[-2.9475, -1.6911]**
Detected insects -- Clustered insects	**-1.0204**	**[-1.5882, -0.4525]**
Manual count -- Clustered insects	-0.2072	[-0.7385, 0.3241]
Detected insects -- True positives	**1.2989**	**[0.7025 1.8954]**
Manual count -- True positives	**2.1121**	**[1.5183 2.7058]**
Manual count -- Detected insects	**0.8131**	**[0.2914 1.3348]**

Table 3.7. Pairwise comparisons between four measures of acoustic insect activity: manual counts of insect buzzes per hour, the number of insect buzzes clustered per hour by Kaleidoscope Pro (clustered insects), the number of true positives therein, and the number of total detected insects (true positives + false negatives), within the high estimated activity category. Bold values indicate significant pairwise comparisons, where the 95% confidence intervals do not overlap zero.

Pairwise comparison	Parameter estimate	95% CI
True positives -- Clustered insects	**-1.46609**	**[-2.16375, -0.76841]**
Detected insects -- Clustered insects	**-0.80659**	**[-1.50983, -0.10335]**
Manual count -- Clustered insects	-0.06355	[-0.73982, 0.61272]
Detected insects -- True positives	**0.65950**	**[0.05298, 1.26602]**
Manual count -- True positives	**1.40254**	**[0.78558, 2.01950]**
Manual count -- Detected insects	**0.74304**	**[0.13529, 1.35080]**

Supplementary Table 3.8. The site name, acoustic pan trapping station, hour of the day, and estimated activity level associated with each of the seventy-five hour-long soundscape recordings within the data subset used to manually quantify the number of insect buzzes present within each recording (manual counts) and calculate the number and proportion of buzzes that were either not detected by Kaleidoscope Pro (undetected) or not correctly clustered by Kaleidoscope Pro (unclustered). Also displayed are the number of insect signals used the train the relevant site-specific Specialist Insect Classifiers (SIC), and the number of clustered buzzes present.

Date	Site	Station	Hour	Activity	Training buzzes	Detected buzzes	Manual counts	Undetected buzzes	Prop. undetected	Unclustered buzzes	Prop. unclustered
20/09/2017	Primrose Valley Park	4	10:00:00	Low	224	0	5	5	1.000	5	1.000
28/08/2017	Meanwood road	5	16:00:00	Low	33	6	10	4	0.400	7	0.700
27/08/2017	Kirkstall Abbey	1	16:00:00	Low	16	3	13	10	0.769	13	1.000
19/09/2017	Halton Moor	5	12:00:00	Low	30	1	27	26	0.963	26	0.963
28/08/2017	Meanwood road	4	08:00:00	Low	33	6	17	11	0.647	15	0.882
26/08/2017	Farnley Hall Park	4	09:00:00	Low	31	17	40	23	0.575	38	0.950
31/07/2018	Kirkstall Abbey	1	09:00:00	Low	48	1	9	8	0.889	9	1.000
19/09/2017	Halton Moor	3	15:00:00	Low	30	0	0	0	NA	0	NA
27/08/2017	Kirkstall Abbey	3	12:00:00	Low	16	10	26	16	0.615	23	0.885
19/09/2017	Halton Moor	5	13:00:00	Low	30	1	22	21	0.955	21	0.955
31/07/2018	Kirkstall Abbey	5	15:00:00	Low	48	15	19	4	0.211	12	0.632
02/09/2017	Temple Newsam	4	15:00:00	Low	22	1	31	30	0.968	30	0.968
19/09/2017	Halton Moor	3	13:00:00	Low	30	17	38	21	0.553	34	0.895
27/08/2017	Kirkstall Abbey	2	16:00:00	Low	16	0	2	2	1.000	2	1.000
12/09/2017	Rothwell Country Park	3	09:00:00	Low	59	0	4	4	1.000	4	1.000
03/08/2018	Rothwell pastures	5	11:00:00	Low	28	12	63	51	0.810	61	0.968
27/08/2017	Kirkstall Abbey	1	12:00:00	Low	16	6	32	26	0.813	31	0.969

20/09/2017	Primrose Valley Park	5	16:00:00	Low	224	0	1	1	1.000	1	1.000
27/08/2017	Kirkstall Abbey	5	16:00:00	Low	16	1	7	6	0.857	6	0.857
01/09/2017	Asket Hill	2	17:00:00	Low	114	0	0	0	NA	0	NA
27/08/2017	Kirkstall Abbey	4	12:00:00	Low	16	5	11	6	0.545	10	0.909
17/09/2017	Killingbeck fields	2	15:00:00	Low	47	3	5	2	0.400	5	1.000
03/08/2018	Rothwell pastures	1	11:00:00	Low	28	33	80	47	0.588	77	0.963
19/09/2017	Halton Moor	2	09:00:00	Low	30	0	0	0	NA	0	NA
17/09/2017	Killingbeck fields	2	10:00:00	Low	47	0	1	1	1.000	1	1.000
06/09/2017	Skelton wood	5	16:00:00	Medium	178	0	2	2	1.000	2	1.000
20/09/2017	Primrose Valley Park	5	11:00:00	Medium	224	4	7	3	0.429	5	0.714
20/09/2017	Primrose Valley Park	4	16:00:00	Medium	224	1	2	1	0.500	2	1.000
28/08/2017	Meanwood road	2	11:00:00	Medium	33	8	16	8	0.500	13	0.813
19/09/2017	Halton Moor	1	14:00:00	Medium	30	1	4	3	0.750	4	1.000
01/09/2018	Water Haigh	1	11:00:00	Medium	54	19	22	3	0.136	18	0.818
27/08/2017	Kirkstall Abbey	4	15:00:00	Medium	16	2	5	3	0.600	4	0.800
26/08/2017	Farnley Hall Park	2	14:00:00	Medium	31	2	13	11	0.846	11	0.846
03/07/2018	Temple Newsam	5	10:00:00	Medium	33	42	97	55	0.567	92	0.948
28/08/2017	Meanwood road	1	10:00:00	Medium	33	1	4	3	0.750	4	1.000
02/07/2018	Farnley Hall Park	2	13:00:00	Medium	31	2	24	22	0.917	24	1.000
01/09/2018	Water Haigh	4	11:00:00	Medium	54	3	20	17	0.850	20	1.000

03/07/2018	Temple Newsam	1	16:00:00	Medium	33	3	12	9	0.750	12	1.000
27/06/2018	Meanwood Grove	3	12:00:00	Medium	78	116	185	69	0.373	162	0.876
12/09/2017	Rothwell Country Park	3	16:00:00	Medium	59	23	34	11	0.324	21	0.618
03/08/2018	Rothwell pastures	3	10:00:00	Medium	28	197	197	0	0.000	178	0.904
04/09/2017	Water Haigh	3	14:00:00	Medium	145	3	22	19	0.864	20	0.909
19/09/2017	Halton Moor	1	09:00:00	Medium	30	2	7	5	0.714	6	0.857
31/07/2018	Kirkstall Abbey	4	10:00:00	Medium	48	0	2	2	1.000	2	1.000
01/09/2017	Asket Hill	5	15:00:00	Medium	114	14	20	6	0.300	15	0.750
12/09/2017	Rothwell Country Park	4	12:00:00	Medium	59	11	20	9	0.450	17	0.850
12/09/2017	Rothwell Country Park	4	16:00:00	Medium	59	36	52	16	0.308	40	0.769
03/07/2018	Temple Newsam	4	09:00:00	Medium	33	37	74	37	0.500	70	0.946
27/08/2017	Kirkstall Abbey	4	13:00:00	Medium	16	0	8	8	1.000	8	1.000
15/09/2017	Rothwell pastures	4	12:00:00	Medium	30	11	22	11	0.500	18	0.818
27/06/2018	Meanwood Grove	4	12:00:00	High	78	110	211	101	0.479	185	0.877
02/07/2018	Farnley Hall Park	5	13:00:00	High	31	1	14	13	0.929	14	1.000
28/08/2017	Meanwood farm	4	15:00:00	High	364	23	137	114	0.832	123	0.898
01/09/2017	Asket Hill	2	16:00:00	High	114	0	4	4	1.000	4	1.000
27/06/2018	Meanwood Grove	4	15:00:00	High	78	94	348	254	0.730	326	0.937
28/08/2017	Meanwood farm	4	14:00:00	High	364	19	20	1	0.050	4	0.200

28/08/2017	Meanwood farm	2	11:00:00	High	364	8	22	14	0.636	16	0.727
15/09/2017	Rothwell pastures	2	10:00:00	High	30	0	8	8	1.000	8	1.000
02/07/2018	Farnley Hall Park	2	14:00:00	High	31	4	19	15	0.789	18	0.947
04/07/2018	Bramley Fall Park	4	09:00:00	High	511	69	101	32	0.317	62	0.614
04/07/2018	Bramley Fall Park	1	16:00:00	High	511	142	216	74	0.343	126	0.583
04/07/2018	Bramley Fall Park	3	10:00:00	High	511	135	195	60	0.308	117	0.600
03/07/2018	Temple Newsam	2	11:00:00	High	33	65	247	182	0.737	218	0.883
28/08/2017	Meanwood farm	3	13:00:00	High	364	8	68	60	0.882	62	0.912
04/07/2018	Bramley Fall Park	2	11:00:00	High	511	390	394	4	0.010	58	0.147
28/08/2017	Meanwood road	5	12:00:00	High	33	81	97	16	0.165	45	0.464
27/06/2018	Meanwood Grove	4	11:00:00	High	78	262	282	20	0.071	229	0.812
04/07/2018	Bramley Fall Park	3	11:00:00	High	511	83	178	95	0.534	120	0.674
15/09/2017	Rothwell pastures	5	10:00:00	High	30	0	8	8	1.000	8	1.000
04/07/2018	Bramley Fall Park	3	12:00:00	High	511	175	260	85	0.327	135	0.519
04/07/2018	Bramley Fall Park	5	14:00:00	High	511	668	868	200	0.230	420	0.484
04/07/2018	Bramley Fall Park	4	11:00:00	High	511	196	306	110	0.359	191	0.624
28/08/2017	Meanwood farm	3	15:00:00	High	364	7	26	19	0.731	22	0.846

01/09/2017	Asket Hill	3	13:00:00	High	114	48	163	115	0.706	134	0.822
27/06/2018	Meanwood Grove	3	10:00:00	High	78	252	256	4	0.016	212	0.828

Table 3.9. Pairwise comparisons between the different hours of the day during which the experiment was conducted, across both 2017 and 2018, in terms of the number of insect buzzes clustered per hour by Kaleidoscope Pro. Bold values indicate significant pairwise comparisons, where the 95% confidence intervals do not overlap zero.

Pairwise comparison	Parameter estimate	95% CI
09:00 -- 08:00	1.435	-0.389, 3.258
10:00 -- 08:00	**2.168**	**0.349, 3.987**
11:00 -- 08:00	**2.274**	**0.448, 4.100**
12:00 -- 08:00	**2.151**	**0.310, 3.992**
13:00 -- 08:00	**2.085**	**0.239, 3.931**
14:00 -- 08:00	**2.270**	**0.411, 4.129**
15:00 -- 08:00	**2.239**	**0.373, 4.105**
16:00 -- 08:00	1.347	-0.529, 3.222
17:00 -- 08:00	0.204	-1.851, 2.260
10:00 -- 09:00	**0.733**	**0.368, 1.099**
11:00 -- 09:00	**0.839**	**0.424, 1.255**
12:00 -- 09:00	**0.716**	**0.229, 1.204**
13:00 -- 09:00	**0.650**	**0.138, 1.163**
14:00 -- 09:00	**0.835**	**0.273, 1.397**
15:00 -- 09:00	**0.804**	**0.214, 1.394**
16:00 -- 09:00	-0.088	-0.703, 0.527
17:00 -- 09:00	**-1.230**	**-2.265, -0.195**
11:00 -- 10:00	0.106	-0.233, 0.445
12:00 -- 10:00	-0.017	-0.408, 0.374
13:00 -- 10:00	-0.083	-0.494, 0.327
14:00 -- 10:00	0.102	-0.352, 0.556
15:00 -- 10:00	0.071	-0.409, 0.551
16:00 -- 10:00	**-0.821**	**-1.330, -0.312**
17:00 -- 10:00	**-1.964**	**-2.946, -0.981**
12:00 -- 11:00	-0.123	-0.468, 0.222
13:00 -- 11:00	-0.189	-0.547, 0.169
14:00 -- 11:00	-0.004	-0.395, 0.387
15:00 -- 11:00	-0.035	-0.448, 0.378
16:00 -- 11:00	**-0.927**	**-1.371, -0.484**
17:00 -- 11:00	**-2.070**	**-3.026, -1.113**
13:00 -- 12:00	-0.066	-0.397, 0.265

14:00 -- 12:00	0.119	-0.227, 0.465
15:00 -- 12:00	0.088	-0.273, 0.449
16:00 -- 12:00	**-0.804**	**-1.195, -0.413**
17:00 -- 12:00	**-1.946**	**-2.887, -1.006**
14:00 -- 13:00	0.185	-0.154, 0.524
15:00 -- 13:00	0.154	-0.196, 0.505
16:00 -- 13:00	**-0.738**	**-1.118, -0.359**
17:00 -- 13:00	**-1.880**	**-2.816, -0.945**
15:00 -- 14:00	-0.031	-0.367, 0.305
16:00 -- 14:00	**-0.923**	**-1.286, -0.560**
17:00 -- 14:00	**-2.066**	**-2.998, -1.133**
16:00 -- 15:00	**-0.892**	**-1.251, -0.533**
17:00 -- 15:00	**-2.035**	**-2.966, -1.103**
17:00 -- 16:00	**-1.142**	**-2.080, -0.205**

References cited in Appendix 3.

Baude, M., Kunin, W. E., & Memmott, J. (2015). *Nectar sugar values of common British plant species [AgriLand].* NERC Environmental Information Data Centre. https://doi.org/https://doi.org/10.5285/69402002-1676-4de9-a04e-d17e827db93c