

Behaviour of Foraging Bumble Bees Across Morphological and Environmental Contexts

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Abstract

Bumble bees as pollinators are being produced at an industrial scale and are used throughout the world to pollinate agricultural crops in glasshouses. The agricultural crops they are exposed to are often monocultural and mass-produced. The lack of floral variety they are provided with is thought to have negative effects on pollination performance and bee health, and has been suggested to influence worker drift in a glasshouse. Bumble bees are unique due to the large size differentiation among workers within a colony, which are thought to lead to consistent differences among individuals. Larger workers are more likely to be foragers, and smaller workers are more likely to perform in-nest tasks. These size differences are thought to cause differences among workers in foraging efficiency, the types of flowers they visit, and the size of their foraging ranges. Worker size also affects circadian rhythm strength, and individuals with stronger circadian rhythms are thought to anticipate potential activity cues such as sunrise. Bumble bees also show communication among foragers, but it is unknown whether they have 'scouting' bees that recruit inactive bees early in the day to forage. Understanding more about the behaviour of these populations and the effect environments can have on their activity is important for understanding how to aid in the conservation of these populations.

Chapter 3 investigates bumble bee foragers and their activity among individuals of different sizes and within different colonies. I hypothesized (1) if bumble bees differ intrinsically from one another, then consistent differences in behaviour will be observed among foragers from the same colony and (2) if body size causes differences in anticipation of sunrise and foraging activity, then larger foragers will be seen initiating foraging earlier. To answer these questions, the nest entrance of two colonies were recorded that had access to a semi-natural floral environment in a glasshouse with tomato, cornflower and snapdragon flowers. Each bumble bee was marked with a coloured number tag to allow identification of each forager. Individuals differed from one another in foraging activity within both colonies. Additionally, larger workers initiated foraging earlier than smaller ones. The results support previous studies that show that individuals within a nest vary intrinsically from one

another in foraging behaviour and show consistent differences within the same colony, as well as from other colonies. Moreover, larger bees appear to anticipate sunrise and potential food availability, suggesting the presence of strong diurnal foraging circadian rhythms, with larger bees leaving the nest for the first time earlier than smaller bees. These differences among individuals could facilitate the temporal division of workers, and morphological differences could account for some of the variation seen among colonies.

In chapter 4 I assessed bumble bee foraging activity across two different environments, a simple monofloral glasshouse, and an enriched polyfloral glasshouse. I hypothesised that (1) if simplified monocultures have a detrimental effect on bumble bee colonies, then decreased foraging activity will be observed in the simple environment compared to the enriched and (2) if nest switching behaviour is influenced by the availability of natural nectar sources, then lower levels of nest switching will be observed in the enriched environment. The methods of this chapter were the same as chapter 3, except that there were two glasshouses and four colonies. One glasshouse had 3 flower types including tomatoes, and the other only had tomatoes and artificial sugar syrup as a nectar source. I found that in the simple environment, bumble bees showed significantly decreased foraging activity, initiated foraging later, and spent less time out of the nest. Nest switching occurred at high rates and there was no difference among colonies or environments. These results show that bumble bee performance is affected by a monocultural glasshouse environment, and that nest switching occurs within a glasshouse independent of the environment they are exposed to.

The research done in this thesis contributes to understanding bumble bee size differentiation and suggests that it may persist to separate workers temporally in activity. It shows differences among bumble bee foragers, and these foragers may differ from one another intrinsically or in thresholds to foraging tasks. It also provides insights into how monocultures and glasshouses can be affecting bumble bee colonies. This adds to literature about how monocultures may be having detrimental effects on pollinator species and reiterates the importance of providing a variety of floral resources to bees to enhance these populations.

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Chapter One

General Introduction

1.1 Global Effects on Pollination Services

1.1.1 *Human-Induced Rapid Environmental Change*

Human-induced environmental change is shaping the world rapidly as agricultural intensification and urban sprawl increase and spread out across what used to be rural landscapes (Terando, Costanza et al. 2014). This environmental change is having profound effects on species as they are either dying out or being forced to adapt to this new ecosystem. This is causing some of the greatest local extinction rates of native species in recent times (McKinney 2002, Walther, Post et al. 2002). Land use change can cause pollution, habitat loss, increased noise, climate change, introduction of invasive species, and reduction of natural resources (Barnes, Morgan Iii et al. 2001, Johnson and Klemens 2005, Sih, Ferrari et al. 2011). These initial adaptive responses are often behavioural, changing the actions of individuals to increase survival of populations (Tuomainen and Candolin 2011).

These behavioural changes are crucial for the future survival of populations (Sih, Ferrari et al. 2011). Because this land use change is so rapid, animals who do not have the tools to adapt, either physiologically or behaviourally, are struggling in these new environments (Sinervo, Méndez-de-la-Cruz et al. 2010). As the climate shifts, and the environment around them changes, species are being forced to adjust to these novel situations (Bradshaw and Holzapfel 2006, Hoffmann and Sgrò 2011). Habitat loss and fragmentation can also have downstream effects by increasing competition between species due to a higher density of animals in a smaller area (Dolman and Sutherland 1995).

1.1.2 Pollinators: An Ecosystem Service

Pollination of plants provided by insects is a vital ecosystem service in the environment (Klein, Vaissière et al. 2007). This service is performed by many insect species such as wasps, bees, butterflies, flies, beetles and moths, and is crucial for plants such as fruit, vegetables, and nuts (Delaplane, Mayer et al. 2000, Klein, Vaissière et al. 2007). Pollination is essential for approximately 87% of flowering plants worldwide, is necessary for 35% of commercial crop production, and its economic value was estimated to be €153 billion in 2005 (Klein, Vaissière et al. 2007, Potts, Biesmeijer et al. 2010, Hanley, Breeze et al. 2015). Maintaining these services is not only important for the commercial production of crops, but also for biodiversity of species. Not only do they increase plant yield for human consumption, they also sustain native and introduced plants in the wild, producing food for animals in the environment (Potts, Biesmeijer et al. 2010).

1.1.3 Effects of Environmental Change on Bee Pollinators

Many pollinator species are threatened by rapid human-induced land changes (Leonhardt and Blüthgen 2012). The greatest of these threats is changing land-use, such as agricultural intensification and increasing urban sprawl (Johnson and Klemens 2005, Vanbergen and Initiative 2013, Hanley, Breeze et al. 2015). As the population of the world grows the demand for food increases and more farmland is needed to sustain this demand (Bommarco, Kleijn et al. 2013). Farmland has become increasingly specialized and while it increases crop yield it decreases the biodiversity that once existed there (Tilman, Fargione et al. 2001, Ollerton, Erenler et al. 2014). This land is intensively managed, resulting in the loss of field margins and edge habitats as flowering weeds are removed and crop fields expand (Tilman, Fargione et al. 2001, Benton, Vickery et al. 2003). This causes the loss of wild flowers that previously provided variety in the floral species foraging bees had access to (Goulson, Nicholls et al. 2015). These changes are reducing floral resources for species, destroying nesting sites, and fragmenting habitats (Kells and Goulson 2003, Potts,

Biesmeijer et al. 2010). Bee species require an abundance of floral resources, as they gather their entire caloric intake from flowers (Leonhardt and Blüthgen 2012).

Cropland that is used to produce flowering crops often yield only mass-produced singular species of flowers, which results in a monotonous diet for pollinators (Di Pasquale, Salignon et al. 2013). Agricultural environments can cause a lack of adequate nutrition in pollinators as flower species vary in the amount of carbohydrates, lipids, protein, and micronutrients they provide for foraging insects (Haydak 1970, Goulson, Nicholls et al. 2015, Vaudo, Tooker et al. 2015). While the full effects of a monotonous diet are unknown, previous studies have shown if bee species do not get the diversity of nutrients they require it can affect brood production, colony success, increase susceptibility to disease, increase the effects of parasites, and influence the longevity and physiology of the individuals (Carvell, Roy et al. 2006, Rundlöf, Nilsson et al. 2008, Di Pasquale, Salignon et al. 2013, Goulson, Nicholls et al. 2015)

1.2 Bees as Pollinators

Bees (Family: Apidae) are considered some of the most important pollinators worldwide, especially honey bees and bumble bees (*Apis* and *Bombus*) which are widely used for commercial purposes (Velthuis and Van Doorn 2006, Aizen and Harder 2009). These commercial colonies are distributed worldwide, and the need for insect pollination of crops is only growing (Aizen, Garibaldi et al. 2008, Breeze, Bailey et al. 2011).

1.2.1 Social Bee Colony Behaviour

Social bees form eusocial colonies, deemed 'superorganisms' where workers each contribute to the colony by performing differing tasks in and around the nest (Wilson and Sober 1989, Hölldobler and Wilson 2009). However, behaviour can vary greatly among these individuals leading to division of labour. Workers perform one or a few tasks in the nest more than other tasks, all contributing to the functionality of the colony (Dornhaus 2012, Charbonneau and Dornhaus 2015). This can be

facilitated by mechanisms such as temporal polyethism, where as they age they transition from in-nest to foraging tasks, or morphological polyethism, where the size of the individuals predisposes them to perform certain tasks (Beshers and Fewell 2001, Jandt, Huang et al. 2009, Baracchi and Cini 2014). Each individual varies slightly in their morphology and physiology, resulting in differing behaviours and the adoption of diverse strategies within a singular colony (Slater 1981, Amdam, Norberg et al. 2004).

Individual thresholds are defined as the level of stimulus required to trigger an individual to action in a specific nest task. They are considered a key factor in the behavioural variation observed within colonies (Beshers and Fewell 2001, Jandt and Dornhaus 2014). Small intrinsic differences between individuals get amplified over time as individuals are more likely to perform certain tasks, developing into large disparities among individual's behaviour (Beshers and Fewell 2001). This causes a feedback loop, as the performance of a task reduces the triggering stimuli, meaning individuals with a higher threshold are unlikely to perform that task and individuals with a low threshold will consistently perform it (Bonabeau, Dorigo et al. 2000, Beshers and Fewell 2001). This can lead to consistent differences among workers as they become 'specialists' at these tasks (Merkle and Middendorf 2004). Variation between workers can arise through factors such as genetics, hormones, or experience (Beshers and Fewell 2001, Weidenmüller 2004).

While different individuals perform various tasks within the nest, if a stimulus becomes too intense, an increasing number of individuals will switch tasks to deal with it as their threshold is reached. For instance, if a nest becomes too hot, workers will stop performing other tasks and switch to fanning behaviour to thermoregulate the nest (Weidenmüller 2004, Jandt and Dornhaus 2014). These thresholds also extend to foraging activities outside of the nest, and individuals can vary in the effort they expend foraging (Fewell and Page Jr 2000). Variation in thresholds not only affects the probability of an individual to perform a specific task, but also the time spent on it (Weidenmüller 2004). This mechanism allows maximum colony efficiency and flexibility, matching the number of individuals allocated and needed for a specific activity (Fewell and Winston 1992, Dreller and Page 1999). These thresholds could play a large role in division of labour within insect colonies resulting in the complex organization we observe (Bonabeau, Theraulaz et al. 1997).

1.2.2 Consistent Differences Among Workers

The intrinsic inter-individual differences and thresholds described above can result in consistent individual differences among workers within a colony across time and situations (Gordon 1996, Whitfield, Cziko et al. 2003, Réale, Reader et al. 2007, Jandt, Bengston et al. 2014). Individuals perform the same tasks but in differing ways. Honey bee in-nest workers can vary in the pace they perform and the amount of effort they expend on tasks (Oster and Wilson 1979). Honey bee foragers can vary in the distances they forage from the nest, with some foraging close and others several kilometres from the hive (Winston 1991, Beekman and Ratnieks 2000). There are also differences among foragers in whether they will forage for nectar or pollen, or on the type of floral species they will visit (Winston 1991). This suggests that not only do individuals differ in their task repertoire and probability to perform these tasks, but they also differ in the energy and effort they expend to perform these tasks.

1.2.3 Forager Recruitment in Bees

Communication is an important part of social bee colonies allowing the coordination of activities in the nest (Ingrid, Alicia et al. 2005, Leonhardt, Menzel et al. 2016). One such form of communication is the recruitment of nest mates to available food sources (Leonhardt, Menzel et al. 2016). The most common example of this is the honey bee waggle dance, where foragers ‘dance’ to show the location of nearby food sources (von Frisch 1967). Additionally, in honey bees there is also a specialized group within the foraging caste known as scouts. Food scouts make up approximately 23% of a honey bee population, although this is variable, and these foragers leave the nest without being enlisted by others to find a food source and then return to recruit inactive workers (Seeley 1983). Non-scouts rely on scouts to come back and relay the location of resources and do not actively search for food themselves (Liang, Nguyen et al. 2012). Research has suggested that honey bee scouts could be individuals with a lower threshold to the foraging activity or food stores in the nest (Jones, Myerscough et al. 2004, Beekman, Gilchrist et al. 2007).

In contrast, bumble bees have a primitive form of food communication. They perform irregular runs and vibrations, releasing pheromones as they move within the nest to inform other workers of their successful foraging bout and to recruit others to forage (Dornhaus and Chittka 2001, Molet, Chittka et al. 2008). This method of communication does not advertise the location of resources. Bumble bee foragers instead rely on personal information to locate floral resources (Dechaume-Moncharmont, Dornhaus et al. 2005). This may be because bumble bees evolved in temperate habitats where resources are spread more evenly than in the tropical areas where honey bees evolved (Dornhaus and Chittka 1999, Heinrich 2004). Floral resources vary in both time and space and take a considerable effort to find and the risks of energetic shortfall versus rewards of finding a floral patch has trade-offs (Dechaume-Moncharmont, Dornhaus et al. 2005, Dornhaus 2012). However, because bumble bees evolved in areas that were not patchy, the costs of communicating the locations of floral patches likely outweighed the benefits (Dornhaus and Chittka 1999, Heinrich 2004). Still, it is unclear whether bumble bees have a specific scouting group within the foraging caste that begin foraging earlier and return to recruit others. Research suggests that bumble bee foragers rely on personal information to locate floral resources (Dechaume-Moncharmont, Dornhaus et al. 2005).

1.2.4 Circadian Rhythms in Bees

Organisms anticipate expected changes in the environment, allowing them to predict potential activity periods in the form of a circadian rhythm (Panda, Hogenesch et al. 2002). An animal's circadian rhythm allows it to exploit its environment effectively to benefit its survival (West and Bechtold 2015). Most social insect colonies show diurnal rhythms, and they are considered to be important in the colony to improve efficiency (Bloch, Herzog Erik et al. 2013). There is also evidence for circadian rhythms to vary among workers within a social insect colony (Charbonneau and Dornhaus 2015). Research has shown that foraging honey bees have strong diurnal rhythms: they make strong associations between food availability and time of day, coinciding their flights with nectar availability of plants that they have previously visited (Aschoff 1986, Moore and Doherty 2009, Van Nest and Moore 2012). This is

the opposite of in-nest workers, which do not show specific activity periods, and are often active throughout the night as well as the day (Klein, Olzowy et al. 2008).

1.3 Bumble Bee Colony Behaviour (*Bombus spp.*)

1.3.1 Bumble Bee Colony Lifecycle

Bumble bee colonies produce a single generation over one summer season, and their life cycle can be defined in three separate parts (Plowright and Lavery 1984, Duchateau and Velthuis 1988). First, a solitary queen emerges from hibernation after the winter and founds a nest when she finds a suitable site. These nests can vary in depth, with some species nesting subterraneously, such as *Bombus terrestris*, and others just under or on the surface (Kells and Goulson 2003). Second, the queen begins producing workers, laying diploid (female) eggs, and ceases foraging. These workers maintain the nest and look after the queen over the summer (Duchateau and Velthuis 1988). Third, at the end of the summer season reproductive individuals are produced. The queen begins laying haploid eggs, creating males (drones), and the colony starts rearing queens from diploid eggs (Duchateau and Velthuis 1988, Bloch 1999). After mating, these new queens find a small cavity to hibernate in for 6 to 9 months, and the cycle begins again the next summer (Alford 1969, Beekman, van Stratum et al. 1998).

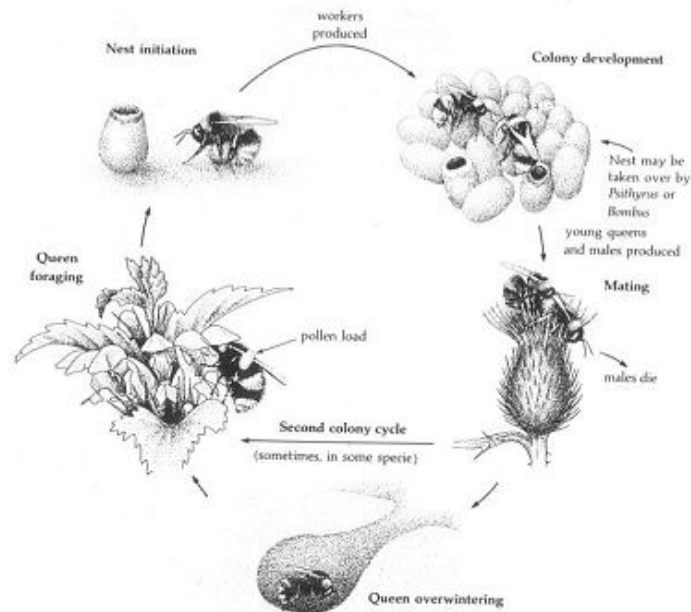


Figure 1.1. The bumble bee colony life cycle, from the queen emerging and initiating the nest, the growth of the nest, to mating and the new queens hibernating. Source: (Prysjones and Corbet 2011)

1.3.2 Bumble Bee Colony Division of Labour

Bumble bees show morphological polyethism to an extent (Jandt and Dornhaus 2009, Jandt, Huang et al. 2009). These morphological differences are determined by how much they are fed by workers in their larval phase and adult body size is fixed after emergence (Couvillon and Dornhaus 2009, Couvillon, Jandt et al. 2010). This size variation in workers is maintained throughout the life of the colony, is fixed around a single mean point and persists independently of resource availability, suggesting it is adaptive and not due to lack of food (Couvillon, Jandt et al. 2010). Workers can vary in body mass by 10-fold, leading to consistent differences among individuals within a nest, with larger bees being more likely to be foragers, and smaller bees tending to be nurses and cleaners in the nest (Jandt and Dornhaus 2009, Couvillon, Jandt et al. 2010). There is significant variation among the foraging caste as well, as forager body size (thorax width) can vary from 3 to 6.9 mm (Goulson, Peat et al. 2002). Bumble bees exhibit more size variation than seen in other bee species, still, there is no conclusive explanation for why this significant size difference persists among bumble bee workers within a colony (Goulson, Peat et al. 2002, Peat, Tucker et al. 2005).

Bumble bee foragers leave the nest to gather food for the colony's reproduction and survival (Westphal, Steffan-Dewenter et al. 2006). There are many explanations as to why larger bumble bees are more likely to forage. Larger bumble bees are more efficient foragers, bringing back more nectar and pollen in one trip, and larger nectar foragers bring back more food per unit time (Goulson, Peat et al. 2002, Spaethe and Weidenmüller 2002). They visit more flowers per unit of time as well and are understood to maintain greater foraging ranges (Cresswell, Osborne et al. 2000, Greenleaf, Williams et al. 2007). Larger bumble bees have better image sensitivity, visual resolution and acuity compared to smaller bees, allowing them to locate flowers better (Spaethe and Chittka 2003). This can affect the time spent foraging and permits them to forage under poorer light conditions (Spaethe, Tautz et al. 2001, Kapustjanskij, Streinzer et al. 2007). A one third increase in a bumble bee's body size can double its ability to detect targets (Spaethe and Chittka 2003, Chittka and Spaethe 2007).

Larger bees are less susceptible to predation when out of the nest. Foraging is an inherently dangerous task and likely increases worker mortality. Due to their size, they are less likely to fall prey to predators such as spiders (Van Doorn 1986, Cartar 1991, Verdolin 2006). Moreover, they can probe deeper flowers, forage in lower temperatures, learn faster than their smaller counterparts, and have higher antennal sensitivity (Heinrich 1975, Peat, Tucker et al. 2005, Worden, Skemp et al. 2005, Spaethe, Brockmann et al. 2007). Large workers are also better at in-nest care of brood, which leads to the question of why is this size dimorphism so prominent in bumble bee colonies if larger individuals are more effective at all tasks (Cnaani and Hefetz 1994)?

1.3.3 Types of Bumble Bee flights

There are several reasons a bumble bee will leave the nest. The first, as discussed above, is exploitation flights to gather food and ensure the success of the nest (Woodgate, Makinson et al. 2016). This is however not the only reason that bumble bees will leave the nest. Workers can leave the nest for defecation flights, when weather permits, to ensure that waste doesn't build up within the nest (Nicolson 2009). Foragers also perform orientation flights to familiarise themselves

with the environment around the nest, and they can make these throughout their lifetime (Osborne, Smith et al. 2013, Woodgate, Makinson et al. 2016).

1.3.4 Bumble Bee Circadian Rhythms

Studies on bumble bees have found that larger individuals have stronger circadian rhythms (Yerushalmi, Bodenheimer et al. 2006). Stronger activity patterns in larger bumble bees mean that they are less active during the night and have a strong diurnal pattern of activity that anticipates sunrise (Yerushalmi, Bodenheimer et al. 2006). This could facilitate foraging, and leaving the nest earlier is thought to be advantageous as flowers often have larger loads of nectar and pollen early in the morning (Kapustjanskij, Streinzer et al. 2007). This assists in division of labour within a bumble bee nest, with larger individuals typically being foragers and smaller individuals providing around the clock brood care (Yerushalmi, Bodenheimer et al. 2006, Stelzer, Stanewsky et al. 2010). The nest is a dark and constant environment, and light patterns do not have any effect on the tasks performed (Yerushalmi, Bodenheimer et al. 2006, Klein, Olzowy et al. 2008). However, the strength of circadian rhythms and likelihood to be active can differ among the foraging caste of individuals. This is likely due to the size difference among the group of foraging bees (Goulson, Peat et al. 2002, Yerushalmi, Bodenheimer et al. 2006).

1.4 Bumble Bees as Commercially Used Pollinators

1.4.1 Commercial Use of Bumble Bees

Bumble bees are an important part of crop success in temperate regions and are used extensively to pollinate crops in glasshouses making them of great agricultural, environmental, and economic importance (Knight, Martin et al. 2005, Ings, Ward et al. 2006, Otterstatter and Thomson 2008, Brown and Paxton 2009). Many plants are specifically pollinated by bumble bees, such as tomatoes and berries, due to need for buzz-pollination behaviour (buzzing at a high frequency, stimulating flowers to release pollen) (De Luca and Vallejo-Marín 2013). Bumble bees also have longer

tongues which allow them to probe flowers other pollinators cannot (Velthuis and Van Doorn 2006, Cameron, Lozier et al. 2011). Bumble bees are generalist pollinators, meaning that they visit a number of plant species and are highly linked to them within the ecosystem (Waser and Ollerton 2006, Patrício-Roberto and Campos 2014).

Bumble bee species have been introduced in many countries to stimulate growth of agriculturally important plants (Ings, Ward et al. 2006). Bumble bee pollination as a commercial industry alone is worth up to €12 billion a year, and they are important for glasshouse pollination in temperate zones because of their ability to forage in relatively low temperatures and low light conditions (Heinrich 1975, van Heemert, de Ruijter et al. 1990, Velthuis and Van Doorn 2006, Kapustjanskij, Streinzer et al. 2007). There are over 30 producers of 5 species of commercial bumblebee stock worldwide, with *Bombus terrestris* being the most common, and these bees have been used for pollination since their value was discovered in 1987 (Goka, Okabe et al. 2006, Velthuis and Van Doorn 2006). A large part of this industry is the pollination of tomato plant species. Tomatoes need to be manually shaken to release pollen and increase fruit set (Neiswander 1954, Free 1970, Buchmann 1983, Matteoni, Dogterom et al. 1998, Goulson 2010, De Luca and Vallejo-Marín 2013). Bumble bees are beneficial for farmers as without them they would be forced to hand pollinate all of their tomato crops which is extremely time consuming (Haigh, Bell et al. 2006).

Although bumble bee pollination has spread rapidly over the world, there has been very little research on the effects of commercial glasshouses on bumble bees, and farmers have voiced concerns over inadequate pollination in glasshouses (Morandin, Kevan et al. 2001). These commercial environments are highly artificial, with colonies often being supplied just one species of plant as a pollen supply and supplemented with the sugar substitute that commercial suppliers provide as a nectar source (Morandin, Kevan et al. 2001, Morandin, Laverty et al. 2001).

1.4.2 Worker Drift Among Bee Colonies

In natural systems, social insects have incentive to distinguish between their nest mates and outsiders. Outsiders may enter other nests when they are attempting to rob the colony of food and resources, or to predate upon the colony (Nunes, Nascimento et al. 2008). Insects moving among colonies can also cause the spread of

disease (Richards and Dunn 2003, Cremer, Armitage et al. 2007). Fidelity to, and defence of nest sites are very common in social insects (Blacher, Yagound et al. 2013). These insects have complex recognition systems allowing them to discriminate between those who belong in the nest, and those that should be kept outside (Crowley, Provencher et al. 1996, Wiley 2013). These recognition systems are seen throughout a variety of social insect species and are thought to be facilitated by nest odours and cuticular hydrocarbons (Gamboa, Reeve et al. 1986, Smith and Breed 1995, Vander Meer and Morel 1998, Singer 2015).

Guard workers at the nest entrance monitor the incoming workers and compare the chemical profile of the bee to the profile of the colony. The role of these guards is important to prevent non-nest insects from entering and reducing the fitness of the colony. If an approaching bee is detected and considered a threat they will signal the rest of the nest (Torres, Breed et al. 1992, Nunes, Nascimento et al. 2008, Nouvian, Reinhard et al. 2016).

Despite this, worker drift (nest switching) has been described a great deal in honey bee colonies where conspecifics are able to enter and assimilate to another nest without repercussions. This can happen often in commercial contexts where there are a large number of nests at high densities (Free 1958, Free and Spencer-Booth 1961, Currie and Jay 1991, Pfeiffer and Crailsheim 1998, Jürgen, Benjamin et al. 2002). This phenomenon has been observed in bumble bees as well (Birmingham, Hoover et al. 2004, Birmingham and Winston 2004). Commercial bumble bee colonies kept in glasshouses unusually do not appear to exclude these conspecifics when they enter the nest, signifying this is a consequence of the confined environment (Free and Butler 1959). It has been suggested that workers may be drifting into other colonies in tomato plant glasshouses, as they solely produce pollen, and while there are syrup feeders provided they may enter nests looking for a familiar sugar source (Birmingham and Winston 2004), however it has not been explored in great detail.

1.5 Sensitivity of Bumble Bee Species to Land-Use Change

The decline in bumble bee species seen globally is thought to be attributed mostly to agricultural land-use change (Goulson, Lye et al. 2008). Bumble bee nest sites are found in uncultivated edges and field boundaries which have been removed

(Tilman, Fargione et al. 2001). Colonies will fail if they do not find a suitable nesting site after emerging from hibernation (Kells and Goulson 2003). If colonies establish, they are faced with the degradation and loss that agricultural intensification causes by breaking up the natural habitats that may remain (Saville, Dramstad et al. 1997, Potts, Biesmeijer et al. 2010).

Bumble bee colonies require a supply of food continuously for the successful establishment, growth, and production of sexuals at the end of their cycle (Westphal, Steffan-Dewenter et al. 2009, Williams, Regetz et al. 2012). The degraded environment bumble bees are faced with when nesting around agricultural farmland, where wild plants and natural habitats have been removed, do not account for this constant need of resources. While there are many early flowering crops there are considerably less late in the season when colonies are at their largest (Rundlöf, Nilsson et al. 2008). Bumble bee reproduction and success may be affected by this resource scarcity in agriculturally degraded habitats in the late summer if they cannot gather enough food to produce new drones and queens (Westphal, Steffan-Dewenter et al. 2009). Bumble bee nesting sites are fixed for the summer and downstream effects can have large effects on the colony as the season progresses. This is also influenced by their relatively limited foraging range. Bumble bees only forage at a maximum of 2km away from the nest, compared to honey bees that on average forage 5.5km from the nest, and up to 9.4km (Beekman and Ratnieks 2000, Osborne, Martin et al. 2008). The consequences of environmental change interacting with bumble bee populations are not known in depth yet (Cresswell, Osborne et al. 2000, Walther-Hellwig and Frankl 2000).

1.6 Thesis Aims

The overall aim of this thesis is to examine foraging patterns of individual bumble bees and the effect environmental enrichment can have on these patterns. Through this research, I anticipated to increase understanding of morphological differences in bumble bee colonies, and answer questions about how simplified monocultural environments can influence colony performance. Moreover, a majority of research on bumble bees is performed in the lab, and through this research I aim to show whether observed differences persist in a semi-natural environment.

In Chapter 3, I investigated (1) worker behaviour within colonies to explore whether they differ from one another and (2) the effect of thorax width on bumble bee foraging patterns. Through this, it allowed the exploration of whether bumble bee colonies have a foraging sub-caste of 'scouts' that leave consistently earlier in the day, and begin foraging earlier, activating the remaining foragers in the colony after they perform a successful foraging trip. These results will provide insight into why such large size dimorphism continues to exist in bumble bee colonies, and how bumble bee workers, while genetically very similar, vary in their foraging activity.

In Chapter 4, I aimed to discover whether colony foraging behaviour was affected by their surrounding environment. This was explored through (1) how bumble bee activity is affected by a simplified or enriched environment and (2) how this environmental enrichment can affect drifting behaviour among bumble bee colonies in a glasshouse. These results will provide insight into how exposure to monofloral environments could be influencing colony level foraging patterns, and how colonies may be interacting with each other when unnaturally confined inside a glasshouse.

Chapter 2

Methods of Glasshouse Field Study

2.1 Glasshouse Set-Up

This experiment took place at Invermay Agricultural Research Centre in Mosgiel, Dunedin in a glasshouse (Fig. 2.1). The glasshouse was translucent polycarbonate (12m wide x 42m long), separated by a wall in the middle (each side 12m wide x 21m long). It was orientated north to south and was sealed extensively with aluminium screens on all openings to limit bees from escaping. Each side of the glasshouse contained either an enriched (South) or simple (North) environmental set-up.

To control climate in the glasshouse, fans were programmed to turn on at 30°C though temperatures could rise above this. They would turn off again when temperatures fell below 26°C.

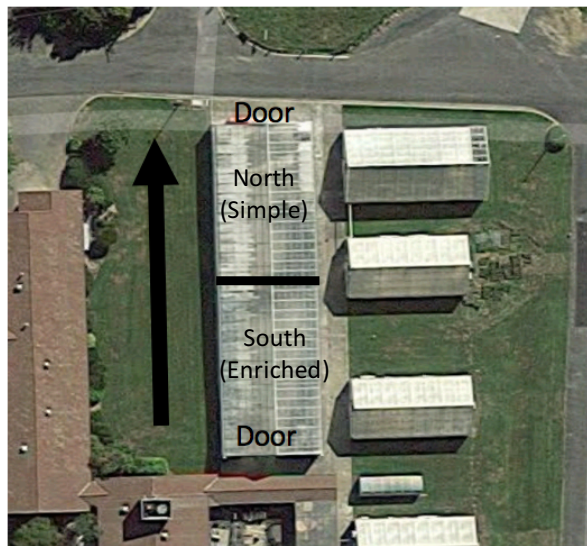


Figure 2.1. Birdseye view of the glasshouse. The arrow indicates north, and the line shows the bisecting wall. The enriched and simple environments are indicated. Image was obtained from Google Maps (<https://maps.google.com>)

2.2 Colony Set-Up

Four *Bombus terrestris* queenright colonies were purchased from Biobees Limited (www.biobees.co.nz, located in Havelock North, Bay of Plenty, New Zealand) and arrived on the 23rd February 2018. The colony (queen, workers, and brood) were transferred to a wooden nest box (20x20cm) with an entrance chamber (10x20cm). The nest box had removable transparent Plexiglas covers, with holes in the middle that allowed access to the box with minimal disturbance (Fig. 2.2a). Before they were transferred, workers were tagged with a coloured number tags approximately 2.2mm in diameter (sourced from www.ecrotek.co.nz), with different colours (green, yellow, orange, or white) designated for each colony (Fig. 2.3). This allowed the identification of each individual worker for data collection. To tag individuals, they were placed inside a queen marking tube in the dark under red light conditions to reduce stress for the bees and tags were attached using glue to ensure they stayed on (Fig. 2.4). Once they were tagged, they were then transferred to the inner nest box. It is unknown whether these tags affect the bumble bee's activity. However, if it does have an influence, all bees were tagged, and it should therefore affect all bees in the same way and any patterns observed would still be valid. The nest comb and queen were transferred as well. The bottom of the box contained a layer (approximately 3cm deep) of BreederCelect™ paper pellet cat litter (FibreCycle Pty. Ltd) to absorb moisture.

Colonies were arranged on either side of the glasshouse 25th February 2018, in the afternoon. Two colonies, randomly selected, were placed on separate tables at the rear of each glasshouse side (North or South). Each nest entrance was marked with a colour to provide a visual cue to orientate returning foragers. During a pilot study, we determined that temperatures remained most consistent near the centre of the glasshouse (or the rear of each side). Nest boxes were covered with a (approximately 40x50x10cm) sheet of polystyrene for additional insulation (Fig. 2.2b). For the duration of the study, the bumble bees were free to forage in the glasshouse, and the nest box entrance was open at all times. Next to each colony on the table, a low percentage (10-25%) sugar solution was placed to provide a constant source of food and avoid starvation in the event of the glasshouse becoming too hot, or in the possibility of low flower availability.

Every 1-3 days, depending on how many new bumble bees were in the nest when it was checked (approximately 5 or more), unmarked workers were pulled from the nest and tagged in the afternoon after data collection. Each day, unmarked foragers were caught each day while they were foraging on flowers or syrup and were tagged with a blue number tag. This meant that while their original colony was unknown, foraging data could still be collected on them. Bumble bees found dead during the experiment were collected and stored in a -4°C freezer. When the experiment was over, the remaining bees were placed on dry ice to quickly euthanize them. Thorax width was measured in all collected individuals post-mortem with callipers to determine their body size.

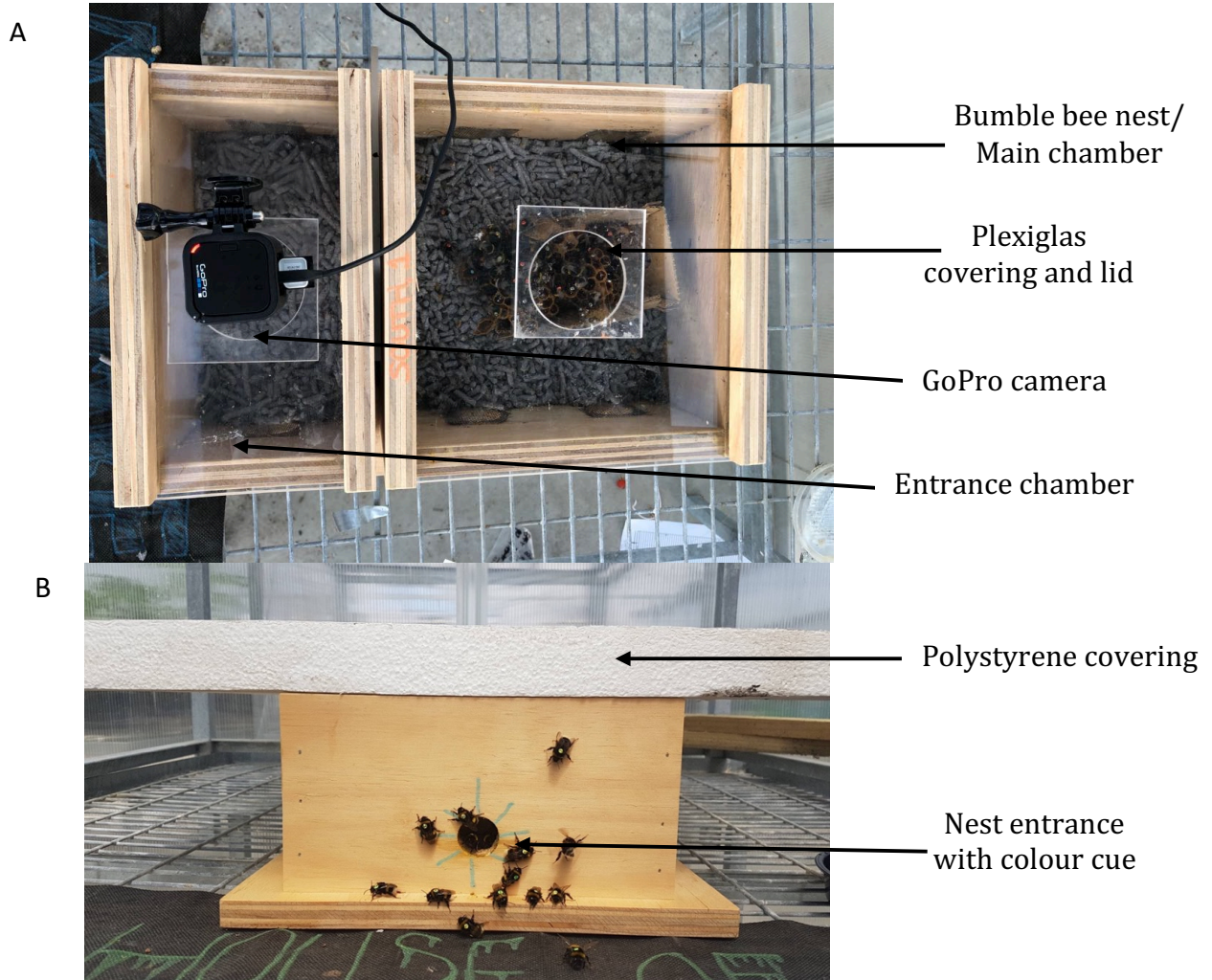


Figure 2.2. Each colony was provided with a nest box (a) Overhead view of the nest box, with the Plexiglas top, holes in the lid, and a GoPro in its filming location (b) Front view of the nest box showing the nest entrance and the polystyrene insulating cover on top of the nest.



Figure. 2.3. A bee marked with a coloured number tag, used to distinguish individuals. This particular bee was callow (newly emerged and has not sclerotized yet) and could not sting.

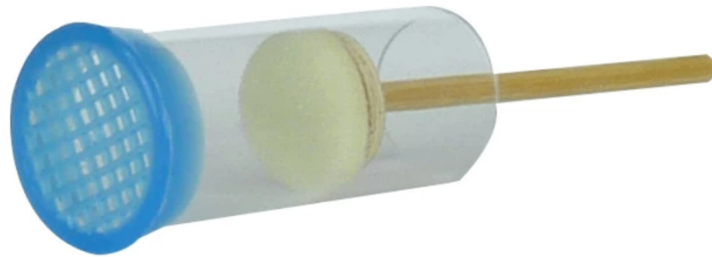


Figure 2.4 The queen marking tubes that were used in this experiment to mark bumble bees. The sponge tipped plunger is used to push the individual towards the grate at the end. The thorax is then positioned at a hole in the grid, and the number tag is glued to the thorax through the hole using a toothpick (Imaged sourced from www.nz-bee-equipment.glopalstore.com).

2.3 Observation of Nest Entrance Activity

Foraging activity was recorded 3-4 days each week for four weeks (n = 14 observations on each colony). Recording began at sunrise (time of sunrise was obtained from the official MetService New Zealand website (www.metservice.com)) and continued uninterrupted for 5 hours. This time was chosen as sufficient to cover the main foraging activity of the colony which peaks at approximately 10-11am (Free 1955). To record activity, GoPro Hero4 Sessions (gopro.com) were placed directly on top of each nest entrance box just before sunrise (Fig. 2.2a), so that each bumble bee that entered or left the nest could be identified. The time (hh/mm/ss) was recorded from the New Zealand Time and Date site (www.timeanddate.com/worldclock/new-zealand) when each GoPro was turned on to enable the conversion of video time to real time. All time stamp data was converted from (hh/mm/ss) to minutes for a standardized measure.

From the video, individual bee IDs and time were recorded for each departure and arrival. As bumble bees are known to switch colonies periodically in a commercial environment (Birmingham, Hoover et al. 2004). I also noted whether the bee was entering or leaving the colony from which it originated (unknown for the workers marked while foraging).

2.5 Data Sorting

For each bumble bee, the data from each day they were observed were calculated across a number of measures (Table. 2.1). To determine whether some foragers act as scouts and whether larger individuals were leaving earlier, the time of their first departure of the day after sunrise was calculated. To explore whether some individuals were putting more effort into foraging than others, I counted the number of foraging bouts a bee performed, the average bout time, and the total time they spent foraging each day. Separately, I also calculated the first bout length of the day to explore whether some individuals foraged on their first bout (longer bout time) or performed very short defecation or exploratory flights.

Individual foraging bouts were calculated as the minutes between an individual leaving the nest and their subsequent return. This return could be to their 'home' nest

or it could be to the other nest in the glasshouse. The data from both colonies within the glasshouse were combined into one data set for the following calculations. The length of an individual's first foraging bout was the time between their very first leave and return of the day. The number of bouts they performed was calculated as the number of times they were observed leaving and returning to complete a foraging trip throughout the data collection period. The total time an individual spent foraging was calculated by adding all of the complete bouts an individual performed. Average bout duration for the entire 5-hour trial was calculated for each bumble bee.

After data from both colonies were assembled over the 14 days of observation, each day was looked at individually. Individuals were average foraging bouts of less than 60 seconds were removed. This was to exclude bees continuously departing for defecation or orientation flights over the 5-hour period rather than foraging (Capaldi, Smith et al. 2000, Westphal, Steffan-Dewenter et al. 2006). This exclusion was not applied to the measurement of the first foraging bout, which could be under 60 seconds. Only individuals with four or more days of foraging data were included in the analyses to ensure reliability of the results.

For each forager, the average and standard deviation of all foraging behaviours were calculated, and all 14 days of data for each individual were condensed to singular data point for each measure. Standard deviation was also assessed to determine whether some individuals were more consistent or variable in their foraging activity.

Table 2.1. Summary of the individual foraging behaviours being observed in this experiment as described above.

Foraging Behaviour	Description
Time of First Leave After Sunrise	The time that an individual leaves the nest for the first time in the day
Bouts Per Day	The number of times an individual leaves and returns, completing a foraging bout, in a day
Average Bout Length	The total time spent foraging divided by the number of bouts an individual performed in a day
Total Time Spent Foraging	The time of every bout an individual performed in a day added together
First Bout Length	The time between an individual leaves the nest for the first time and returns

Chapter Three

Individual Differences in *Bombus terrestris* Behaviour

3.1 Introduction

3.1.1 Aims and Hypotheses

Individuals within social insect colonies can vary consistently in behaviour (Jandt, Bengston et al. 2014). These differences can be facilitated by a number of factors, such as morphological and physiological differences (Slater 1981, Amdam, Norberg et al. 2004, Dall, Houston et al. 2004). Morphological variation among bumble bee workers has been found to be linked to consistent individual differences. Bumble bee colonies show morphological polyethism, with larger workers having a higher probability to be foragers, and smaller workers being more likely to stay in the nest, facilitating division of labour (Jandt and Dornhaus 2009, Jandt, Huang et al. 2009). It is also thought that body size is linked to foraging behaviour. Larger individuals are considered more efficient foragers, carrying larger loads of nectar, have better visual acuity allowing them to forage in lower light conditions, can fly further from the nest, and can forage in cooler temperatures (Cresswell, Osborne et al. 2000, Spaethe, Tautz et al. 2001, Goulson, Peat et al. 2002, Heinrich 2004).

The first aim of this chapter is to explore whether individuals within a bumble bee (*Bombus terrestris*) colony varied consistently among one another in their foraging patterns. This way, I explored the question of whether bumble bees have 'scouts' or 'activator' bees that are leaving earlier and initiating foraging before other workers. I tested the hypothesis that if bumble bees differ intrinsically from one another, then differences in behaviour will be observed among foragers from the

same colony. Furthermore, I tested the hypothesis that if a group of foragers are acting as 'activators', then some individuals would be observed initiating foraging consistently earlier than others from the same nest.

Secondly, I aimed to explore whether body size could be a mechanism behind any observed individual differences, and whether small and large foraging bumble bees would differ in their general daily foraging activity and the consistency of that activity. I tested the hypothesis that if larger bees initiate foraging earlier in the day, anticipating sunrise, then it would suggest that they have strong circadian foraging rhythms (Yerushalmi, Bodenheimer et al. 2006).

For this chapter, the data of the colonies in the enriched (South) glasshouse were used. This was due to the greater applicability of this glasshouse to the natural environment, a larger sample size, and to ensure there was no environmental influence on the results.

3.2 Methods

3.2.1 Experiment Set-Up

In this chapter behaviour was compared among foraging bumble bees from the two South glasshouse colonies (South 1 and South 2) and individuals of unknown origin in the enriched environment glasshouse (South) (Fig. 2.1). In this glasshouse foragers had access to tomato plants which only produce pollen (n = 20) (Fig. 3.2.1a) and two types of nectar producing flowers (Cornflower (*Centaurea cyanus*) n = 20, Snapdragon (*Antirrhinum majus*) n = 20) (Fig. 3.2.1b,c) providing a natural nectar source. Two bumble bee (*Bombus terrestris*) colonies were placed at the rear of the glasshouse with each bumble bee individually marked with a number tag for observation (Fig. 2.6). Plants and colonies were placed on 1x1m tables to lift them off the ground to allow easier observation and maintenance (Fig. 3.2.2). These tables were arranged in a 5x5 grid, with flowers on the two outer rows and tomatoes on the three inner rows (Fig. 3.2.3). Each table of tomatoes had 2 plants, and each table of flowers had 4. The middle row and 2nd and 4th tables of the outer rows were covered in nets for another experiment and are therefore excluded from these methods.



Figure 3.2.1. Images of the flowers foragers had access to within the enriched glasshouse (a) tomato flowers (b) cornflower (c) Snapdragon (Images used courtesy of JM Jandt).



Figure 3.2.2. Image showing the south glasshouse enriched set up. 4 flowering plants are located on each outer table, and 2 tomato plants on each inner table. This image was taken from close to the door facing the rear of the glasshouse. This image was taken before the bumble bees were in the glasshouse, and therefore none of the flowers were covered with nets yet.

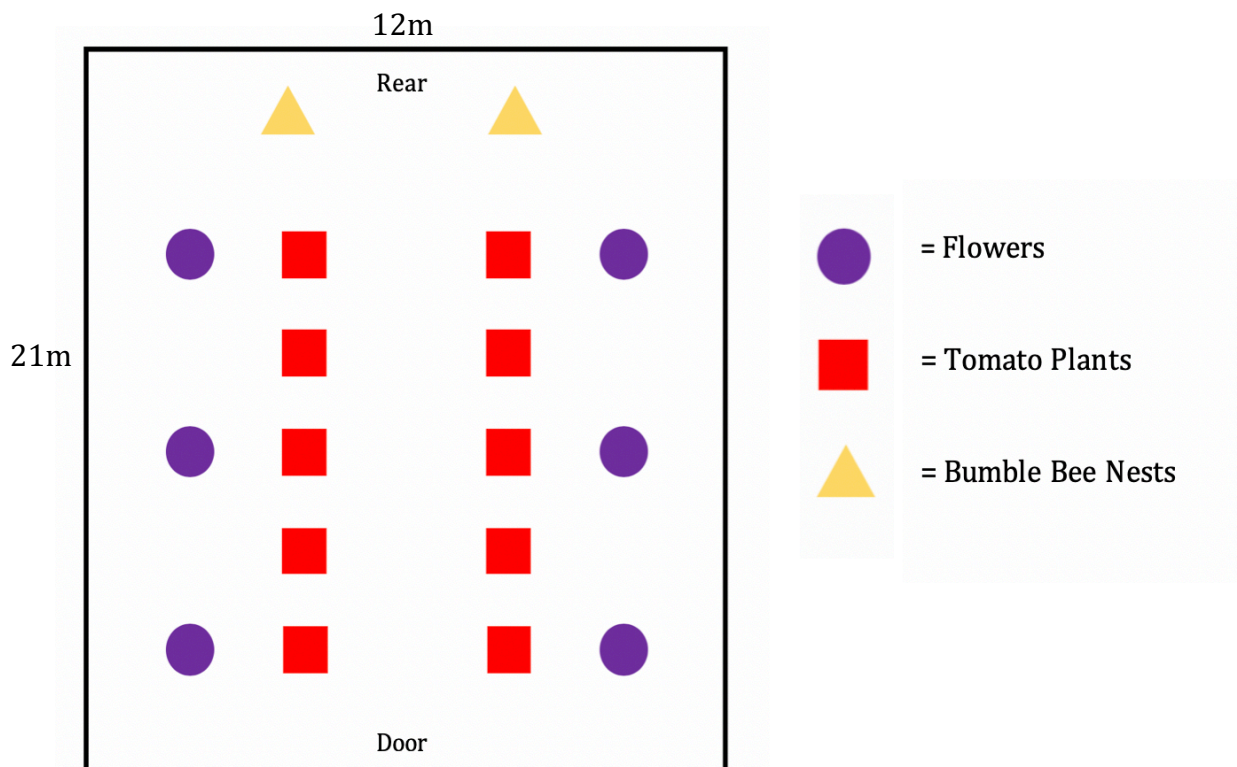


Figure 3.2.3. Diagram of the Enriched Glasshouse set-up in the south glasshouse. Two bumble bee colonies were located at the rear of the glasshouse, 20 tomato plants on the inner table rows, and 40 flowers on the outer table rows

3.2.2 Data Analysis

Data on foragers from both colonies and from unknown colony origin were pooled for this analysis, as thorax widths were measured on only 17 bees total. All statistical analyses were performed in JMP v. 14. The distribution of the variables measured (Table 2.1) was not normal, therefore non-parametric tests were used.

A Wilcoxon/Kruskal-Wallis (rank sums) test was performed to analyse whether individuals within a colony differed from one another in the observed variables: average time of first leave after sunrise, average first bout length, average bouts per day, average of average bout time, and average total time spent foraging. The analysis was run with the predictor being bee ID number, the response was the measured variable, and by colony origin (where the bumble bee was found and marked) was used to separate individuals into their respective colonies (excluding unknown colony origin). A second analysis was run with the same predictor and response, without colony origin and including all observed bees (including unknown colony origin) to observe whether individuals within the glasshouse differed from one another.

A multivariate model Spearman's ρ test was used to determine whether body size was correlated with any of the measured variables (Table 2.1). The same analysis was also run for the standard deviation of the measured variables to determine whether body size was correlated with variability of foraging activity. Individuals from the unknown colony origin group were included as they are from both colonies, and while their home nest is unknown this is not included as a factor in this analysis and therefore is not expected to influence the results

3.3 Results

3.3.1 Summary of Data

The data below are a summary of the dataset used in the analysis (Table 3.3.1). This data covers all five of the measured variables in this chapter and shows the average and standard deviation of each colony for and all the observed foragers in the glasshouse, including unknown origin individuals for each measured variable.

Table 3.3.1. Summary of foraging data of average \pm standard deviation over all variables observed across both colonies and all individuals observed (including unknown origin individuals)

Foraging Variable	South 1	South 2	All Observed
Number of Bees Observed	23	30	64
Bouts Per Day	9.43 \pm 5.63	8.6 \pm 5.47	8.63 \pm 5.25
Bout Length	21.08 \pm 16.01	18.8 \pm 5.47	21.07 \pm 15.81
First Bout Length	13.39 \pm 57.03	10.74 \pm 48.32	12.61 \pm 52.66
Time of First Leave	69.89 \pm 18.52	70.29 \pm 14.51	70.84 \pm 17.42
Total Time Spent Foraging	123.69 \pm 58.36	112.3 \pm 54.73	121.79 \pm 57.01

3.3.2. Individual Foraging Differences within Colonies

South 1 Colony

Individuals in the colony South 1 varied significantly from each other in their bouts per day ($\chi^2 = 67.85$, $df = 22$, $p = <0.0001$; Fig. 3.3.1c), average bout length ($\chi^2 = 63.87$, $df = 22$, $p = <0.0001$; Fig. 3.3.1d), and total time spent foraging ($\chi^2 = 46.61$, $df = 22$, $p = 0.002$; Fig. 3.3.1e). South 1 individual foragers did not differ from one another in the time of their first leave after sunrise ($\chi^2 = 32.03$, $df = 22$, $p = 0.08$; Fig. 3.3.1a), or the length of their first bout ($\chi^2 = 23.17$, $df = 22$, $p = 0.39$; Fig. 3.3.1b).

South 2 Colony

Individuals in the colony of South 2 varied significantly from one another in all the foraging activities measured: Time of first leave after sunrise ($\chi^2 = 47.12$, $df = 29$, $p = 0.02$; Fig. 3.3.2a); First bout length ($\chi^2 = 62.25$, $df = 29$, $p = 0.0002$; Fig. 3.3.2b); Bouts per day ($\chi^2 = 61.94$, $df = 29$, $p = 0.0004$; Fig. 3.3.2c); Average bout length ($\chi^2 = 61.92$, $df = 29$, $p = 0.0004$; Fig. 3.3.2d); Total time spent foraging ($\chi^2 = 63.37$, $df = 29$, $p = 0.0002$; Fig. 3.3.2e).

All Foragers Observed Combined

With all the observed foragers combined, individuals differed significantly over all measured variables: Time from sunrise ($\chi^2 = 107.72$, $df = 63$, $p = 0.0004$), first bout length ($\chi^2 = 99.65$, $df = 63$, $p = <0.002$; Fig. 3.3.4b, 3.3.5b, 3.3.6b), bouts per day ($\chi^2 = 148.92$, $df = 63$, $p = <0.0001$), average bout length ($\chi^2 = 114.54$, $df = 63$, $p = <0.0001$), total time spent foraging ($\chi^2 = 125.03$, $df = 63$, $p = <0.0001$).

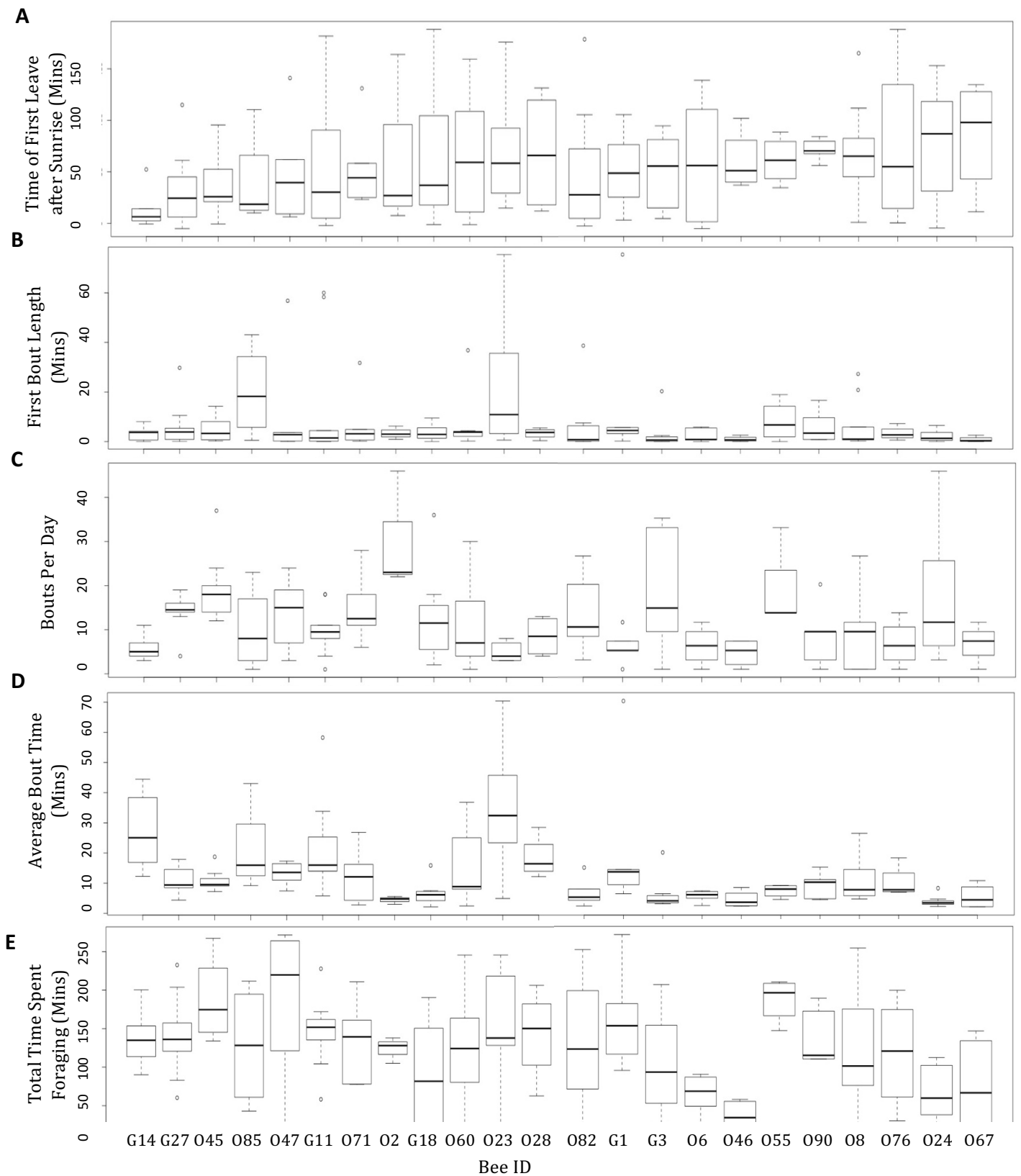


Figure 3.3.1. Individual distribution of activity within the South 1 colony over the days they were observed over the measured foraging patterns: (a) Time of first leave (b) first bout length (c) bouts per day (d) average bout time (e) total time spent foraging (N = 23)

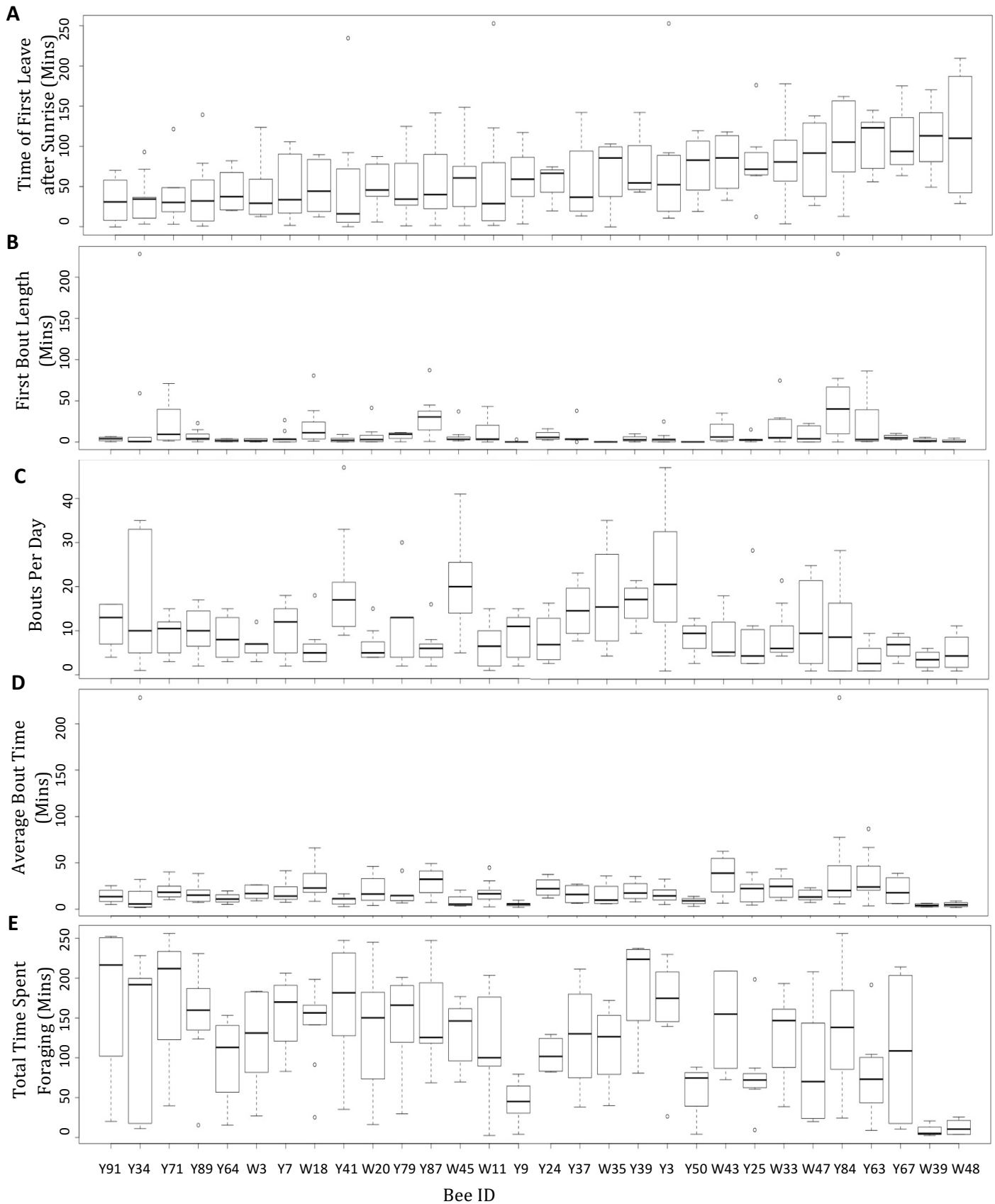


Figure 3.3.2. Distribution of individual activity in the colony South 2 over the days they were observed over the measured foraging patterns: (a) Time of first leave; (b) first bout length; (c) bouts per day; (d) average bout time (e) total time spent foraging (N = 30)

3.3.3 Body Size Differences

Thorax width was recorded for 17 of the 64 foraging bees in the two south glasshouse colonies (ave \pm se: 4.49 ± 0.58 ; min = 3mm; max = 5.3mm). There was no significant difference between individuals that were seen foraging, and individuals that were not recorded leaving the nest (Appendix 1). Larger bees were found to leave significantly earlier than their smaller counterparts (Spearman $\rho = -0.55$, $P = 0.02$; Fig 3.3.3).

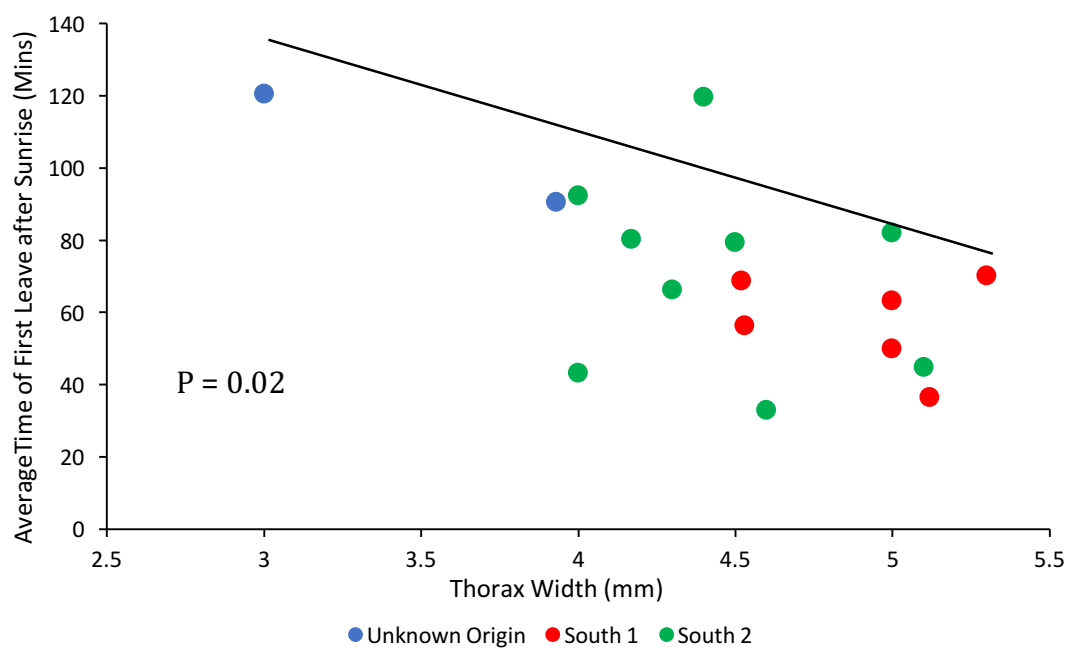


Figure 3.3.3. Relationship between the average time after sunrise each individual first left over the time they were observed from each colony group and their corresponding thorax width ($n = 17$ observations pooled over 3 groups)

There was no evidence that body size could be used to predict any of the other foraging patterns recorded: average first bout length (Spearman $\rho = 0.27$, $P = 0.30$; Fig. 3.3.4a), average number of bouts per day (Spearman $\rho = 0.04$, $P = 0.9$; Fig. 3.3.4b), average of average bout length (Spearman $\rho = -0.22$, $P = 0.4$; Fig. 3.3.4c), average total time spent foraging (Spearman $\rho = 0.19$, $P = 0.46$; Fig. 3.3.4d).

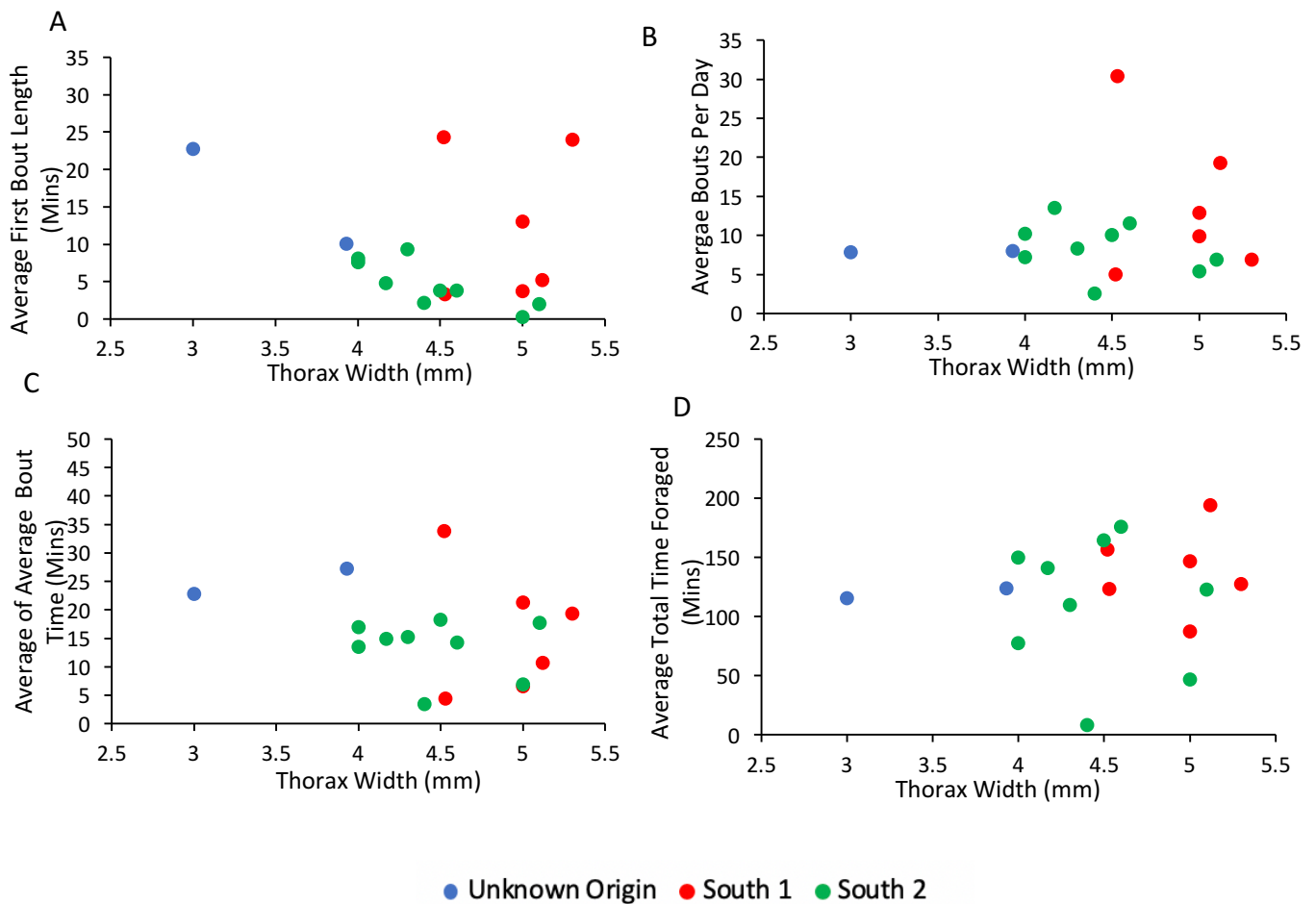


Figure 3.3.4. There was no relationship between thorax width of individuals from three colony groups and measured foraging patterns (a) average first bout of the day; (b) average number of foraging bouts; (c) average of average bout time; (d) average total foraging effort. ($N = 17$ observations pooled over 3 groups)

Body size did not correlate with variability (calculated as standard deviation) in any foraging patterns observed: time of first leave after sunrise (Spearman $\rho = -0.03$, $P = 0.91$; Fig. 3.3.5a); first bout length (Spearman $\rho = -0.27$, $P = 0.29$; Fig. 3.3.5b); bouts per day (Spearman $\rho = -0.03$, $P = 0.91$; Fig. 3.3.5c); average bout time (Spearman $\rho = -0.35$, $P = 1.6$; Fig. 2.3.5d); total time spent foraging (foraging effort) (Spearman $\rho = 0.06$, $P = 0.83$; Fig. 3.3.5e).

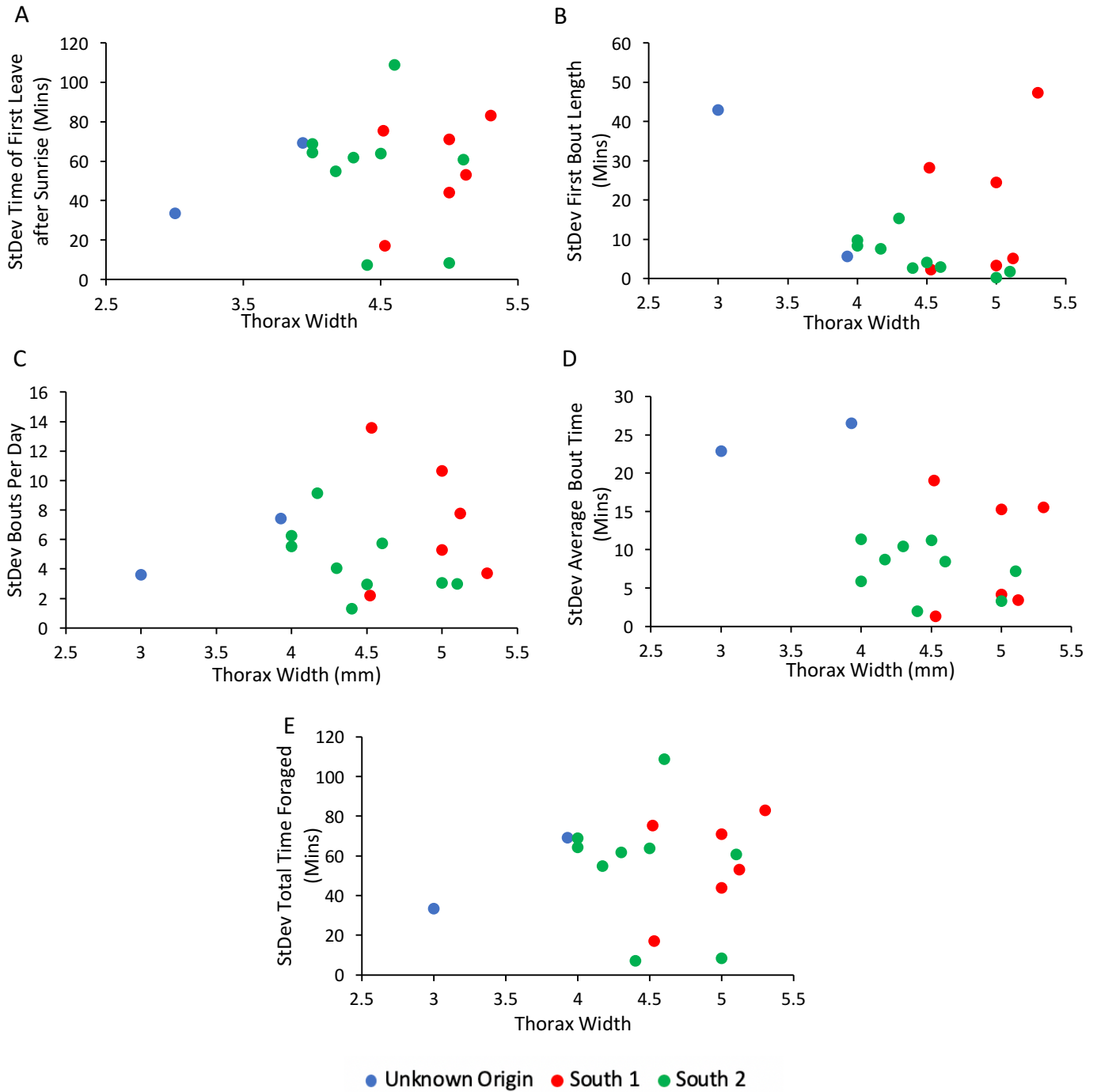


Figure 3.3.5. There was no relationship between thorax width of individuals over all colonies and the standard deviation of measured foraging patterns: (a) Time of first leave after sunrise (b) First bout length(c) Bouts per day (d) Average bout time (e) Total foraging effort. (N = 17 observations over 3 groups)

3.4 Discussion

The main aims of this chapter were to explore whether individuals differ from one another in their foraging activity, and how body size correlates with foraging bumble bee behaviour. The results showed that individuals differed significantly from one another in all of the measured foraging patterns in South 2. In South 1 bumble bees differed in bouts per day, average bout length, and total time spent foraging, but not in their time of first leaving and first bout length. All observed individuals differed significantly from one another in every activity measured. Differences among individuals that were found in every analysis were bouts performed per day, average bout length, and total time spent foraging. Thorax width correlated with a foragers first time leaving the nest for the day; however, it had no effect on first bout length, bouts per day, average bout length, and total time spent foraging (total foraging effort). It also was not correlated with variability (measured as standard deviation) of the above foraging patterns.

3.4.1 Individual Differences Within Colonies

Individuals differed from one another within a colony to varying degrees in different foraging patterns. The results of this study show that individuals differ in their foraging strategies to some extent. Small differences among workers, physiological or morphological, may induce differences in behaviour of individual bumble bees despite being closely related to one another (Slater 1981, Amdam, Norberg et al. 2004). Individuals differed in both colonies in the bouts they performed per day, average bout length, and the total time they spent foraging. When not accounting for colony, individuals differed from one another in every foraging pattern measured. This suggests that there are consistent differences among bumble bee workers in their foraging activity. Some observed individuals were performing more bouts per day than others, others had a longer average bout time, and some were putting more effort into foraging than others with a longer total time spent foraging.

Oster and Wilson (1979) described that honey bees can vary from one another consistently in their pace they perform tasks, and the effort they expend on the

activities they engage in. This research is consistent with what is seen here in bumble bees, with some individuals performing foraging tasks at a greater level than others. Winston (1991) described that honey bee foragers can vary in their foraging tasks outside the nest, which aligns with this research showing that bumble bee foragers can show consistent differences in the tasks they perform.

Furthermore, in these results it does not appear that bumble bees have an 'activator' group of foragers equivalent to honey bee scouts. Individuals differed from one another in their first time of departure and their first bout length in only one of the observed colonies. Moreover, there did not appear to be foragers with very little variation in their leaving times who consistently leave earlier than others (Fig. 3.3.1a, 3.3.2a). This aligns with previous research that bumble bees do not have complex foraging recruitment systems and have not evolved to communicate the location of food sources (Dornhaus and Chittka 1999), making scouts much less effective. Research has suggested that this is because they evolved in an environment where floral resources are evenly distributed and the costs would outweigh the benefits (Dornhaus and Chittka 1999, Heinrich 2004).

3.4.2 Body Size and Behaviour

Larger foragers left the nest on average significantly earlier than smaller foragers (Fig. 3.3.3). These results correspond with previous research showing that foraging bees anticipate sunrise, demonstrating strong circadian foraging rhythms. Moore and Doherty (2009) found that foraging honey bees show robust time-keeping and circadian food anticipatory behaviour. Stelzer, Stanewsky et al. (2010) showed that bumble bee colonies show strong circadian rhythms both at the colony level, and in many individual workers. They also found that workers anticipated the coming of daylight, increasing their activity 1 to 3 hours before. Yerushalmi, Bodenheimer et al. (2006) found larger bumble bee (*Bombus terrestris*) foragers show more developed circadian rhythms under laboratory conditions compared to their smaller nest mates, anticipating sunrise and decreasing their activity overnight. They showed that a greater number of larger bumble bees developed these rhythms than smaller bees, and they also did so earlier.

The results of this study support the hypothesis that larger bumble bees have stronger circadian foraging rhythms, anticipating sunrise and begin foraging earlier in the morning. This experiment is the first to show this pattern for individual bumble bees in a semi-natural environment over an extended period of time. These results suggest that the main difference between larger and smaller foragers is the time they leave the nest for the first time to initiate foraging for the day. This provides support to evidence suggesting that temporal differences in activity could play a role in division of labour (Yerushalmi, Bodenheimer et al. 2006, Stelzer, Stanewsky et al. 2010).

However, it needs to be taken into consideration that there were no significant differences found among the foraging group that had corresponding thorax widths and the workers that were not observed leaving the nest in this experiment (Appendix 2). This could be due to the small number of bees that had a thorax width recorded. It also needs to be considered that due to the small sample size used in the analysis (17 individuals) the data were pooled, and colony differences were not considered. This could potentially confound the results as there could be differences among colonies such as average colony body size. With a larger sample size colony differences could be accounted for in future analyses.

There is yet to be a conclusive explanation for the existence of this size differential within a bumble bee colony and the purpose of smaller workers (Couvillon and Dornhaus 2010), especially when larger foragers appear to be better at every nest task (Goulson, Peat et al. 2002). While this study focused on the activity of larger foraging workers, there is previous evidence showing that smaller bees develop circadian rhythms later, if at all, and therefore serve a functional role of providing around the clock brood care (Yerushalmi, Bodenheimer et al. 2006). This size difference that manifests in bumble bee colonies could provide a purpose both through larger individuals solely foraging and anticipating sunrise to ensure access to larger nectar and pollen loads on flowers, but also to facilitate the temporal division of larger and smaller workers, influencing the tasks they perform in the nest (Yerushalmi, Bodenheimer et al. 2006, Kapustjanskij, Streinzer et al. 2007).

3.4.3. Conclusions

This study aimed to explore whether individual bumble bee foragers varied from one another in foraging activity, and whether body size was a potential mechanism for differences observed in bumble bee behaviour. I found that (1) there was variation in individual workers within colonies in the measured foraging activities: bouts performed per day, average bout length, and total time spent foraging and (2) individual size influenced the time a bee initiated foraging in the morning, with larger bumble bees leaving significantly earlier than smaller ones. Overall the results of this study suggest that individuals vary significantly from one another in the bouts per day they performed, their average bout length, and the total time they spent foraging. These results also preliminarily suggest that bumble bee colonies do not have scouts. Additionally, these results suggest that body size correlated with the first time a bumble bee leaves to forage for the day. It does not correlate with any of the other foraging patterns measured, suggesting that differences in body size does not account for all the observed variation among foraging bumble bees. These findings contribute to understanding size differences in bumble bee colonies and how these seemingly similar individual workers can vary considerably in behaviour from one another.

Chapter 4

Environmental Effects on Bumble Bee (*Bombus terrestris*) Behaviour

4.1 Introduction

4.1.1. Hypotheses and Aims

Bumble bees are used intensively within glasshouse environments for pollination purposes, where they are exposed to mass-produced monocultural floral environments with very little resource variety (Di Pasquale, Salignon et al. 2013). These environments are thought to have negative effects on these populations, as they are not getting the variety of nutrients needed to support colony health and growth (Haydak 1970, Goulson, Nicholls et al. 2015, Vaudo, Tooker et al. 2015). These glasshouse environments are also thought to have an effect on drifting behaviour of bumble bee foragers, where they move freely among nests without consequences (Free and Butler 1959). It is thought that these individuals may be seeking out known nectar sources in a tomato dominated glasshouse, where foragers do not have access to nectar producing plants and only artificial sugar feeders (Birmingham and Winston 2004).

The first aim of this chapter is to explore the effect that environment can have on foraging activity of bumble bee (*Bombus terrestris*) colonies. I aim to explore the consequences of simplified habitats in the context of an experimental glasshouse divided in two, with two differing environments; simple and an enriched habitat. This experiment focuses on the foraging activity and patterns of *B. terrestris* foragers and whether they vary between the glasshouses. I hypothesize that if the environment and type of nectar source available has an influence on bumble bee colonies, then I would observe differences in foraging activity in the simple environment compared to the enriched.

The second aim of this chapter was to explore how the enrichment of a commercial glasshouse environment can affect normal worker and colony behaviour, exploring bumble bee worker drift in a confined glasshouse. It has previously been suggested that workers could be drifting among colonies seeking out a familiar nectar source as in commercial tomato glasshouses there are no nectar producing flowers (Birmingham and Winston 2004). I hypothesize that if worker drift is a consequence of bumble bee workers looking for nectar from a familiar source, then workers will be observed moving between colonies at higher rates in the simple environment compared to the enriched environment.

4.2 Methods

4.2.1 Experiment Set Up

In this chapter behaviour of foragers was compared among bumble bees from known colony origins from two sides of a glasshouse. Each side of the glasshouse contained either an enriched or simple environmental set up (Fig. 4.2.1). The enriched side was located in the south side of the glasshouse and the simple in the north (Fig. 2.1). Both glasshouses had tomato plants ($n = 20$) available to foraging bumble bees on each side (Fig. 3.2.1a). The glasshouse side with the enriched environment contained cornflower and/or snapdragon flowers ($n = 20$) of each, to provide a natural nectar source (Fig. 3.2.1b,c). Tables were used to lift both the tomato plants, flowers, and colonies off the ground to allow easier maintenance and observation (Fig. 3.2.2). Each table of tomatoes had 2 plants on it, and each table of flowers had 4 plants. These were arranged between the 19th and the 22nd of February. The glasshouse side with the simple environment contained 6 syrup feeders, filled with syrup provided by Biobees Limited (www.biobees.co.nz, located in Havelock North, Bay of Plenty, New Zealand) as a nectar source (4.2.2). The syrup feeders were refilled as needed and were placed on tables in the Simple glasshouse corresponding to the location of flowers in the enriched glasshouse. The middle row of tables and 2nd and 4th outer tables in the 5x5 grid were excluded from these methods as they were covered and being used for another experiment.

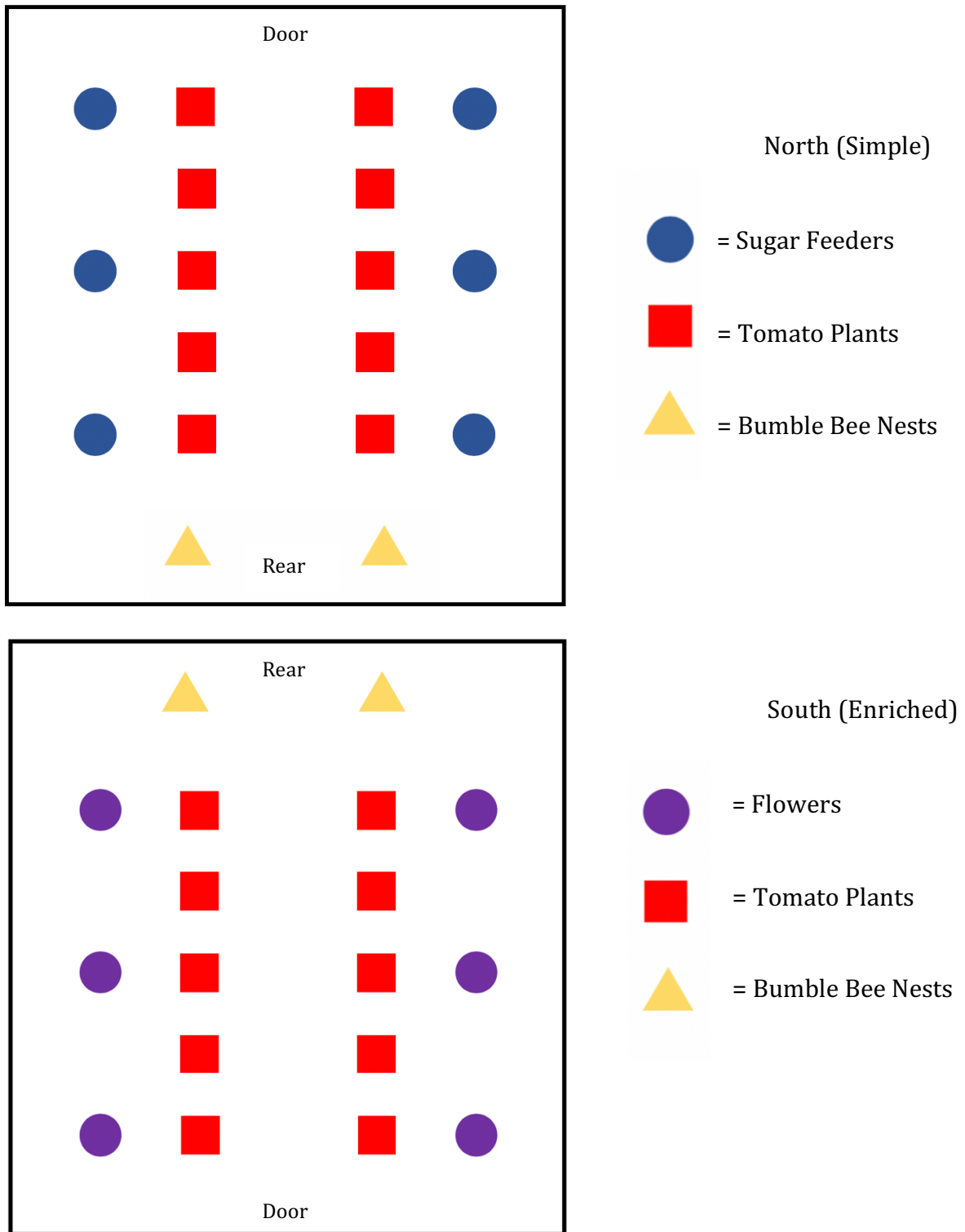


Figure 4.2.1. Diagram of the double glasshouse set-up with the enriched environment (south), and the simple environment (north). There were two colonies in each environment at the rear of the glasshouse. Tomatoes were placed in both environments. There were flowers in the enriched environment, and syrup feeders in place of these in the simple environment.



Figure 4.2.2. The sugar/syrup feeders that were used to supplement the colonies in the glasshouses. The base and top of the container were coloured as to simulate a flower.

4.2.1 Nest Switching Data Sorting

Worker drift behaviour between colonies was observed: how many days individuals nest switched over all the days they were seen foraging, and the number of bouts where they returned to their home nest was measured. If a forager went between nests during the time it was observed, it was categorized as 'switching'. Those that did not switch between nests were marked as a 'non-switcher'. Additionally, a bumble bee could be in a nest that was not its origin and not categorized as a nest switcher, if it is repeatedly going back to the same nest within an observation period. Colony level nest switching behaviour was calculated as the proportion of bumble bees in each colony that were observed nest switching at least once over all 14 days of observation. The same calculation was made for the two different environments to show total nest switching behaviour.

An individual forager's nest switching behaviour was calculated as the proportion of days they were seen nest switching over all the days they were observed foraging. A home nest proportion was also calculated for individual foragers as the number of bouts they were observed returning home to their origin nest over all bouts they performed and completed (the bee was seen leaving and returning in the observation period). This was over every day they were observed

An individual's home percentage was also calculated as a proportion of the number of bouts they returned home compared to the number of bouts they performed for each day. If an individual performed 20 bouts in the observation period, and for 10 of them they returned to their home nest, their home percentage would be 50% for that day of data.

4.2.2 Data Analysis

All statistical analyses were performed in JMP v. 14. Response variables used in these analyses are the same as in chapter 3 (Table 1). Distributions of data were determined by running a goodness of fit analysis to ensure the correct distribution and link were used (Kolmogorov's D for log-normal distribution and Shapiro-Wilk W Test for normal distribution) (Appendix 1). A separate Generalized Linear Model (GLM) was conducted for each response variable: average time of first leave after sunrise (Distribution: Normal; Link: Log); Average first bout length (Distribution: Normal; Link: Log); Average bouts per day (Distribution: Normal; Link: Log); Average bout length (log-normal distribution); Average total time spent foraging (Distribution: Normal; Link: Identity). Two factors were included in each model: environment, and colony origin (nested within Environment). Distributions were determined by running a goodness of fit analysis (Kolmogorov's D for log-normal distribution and Shapiro-Wilk W Test for normal distribution).

For the nest switching analyses a GLM with binomial distribution was run twice, with the response variable: proportion of days individuals spent nest switching; proportion of bouts individuals returned to their nest origin. The two factors were included: environment and colony origin (nested within environment).

4.3 Results

4.3.1 Environmental Differences

Bumble bees in the enriched environment left significantly earlier than bees in the simple environment (GLM: Environment: $\chi^2=6.388$, DF = 1, P = 0.012; Colony: $\chi^2=0.54$, DF = 2, P = 0.76; Fig. 4.3.1a), and spent more time foraging (GLM: $\chi^2=5.74$, DF = 1, P = 0.017; Colony: $\chi^2=1.06$, DF = 2, P = 0.59; Fig. 4.3.1b). There were no significant differences between colonies in any of the analyses performed.

Environment did not predict the average bouts performed per day (GLM: Environment: $\chi^2=1.56$, DF = 1, P = 0.22; Colony: $\chi^2=2.55$, DF = 2, P = 0.21; Fig. 4.3.2a), average bout time (GLM: $\chi^2=0.28$, DF = 1, P = 0.6; Colony: $\chi^2=0.5$ DF = 2, P = 0.78; Fig. 4.3.2b), and the average first bout length (GLM: $\chi^2=2.186$, DF = 1, P = 0.139; Fig. 4.3.2c).

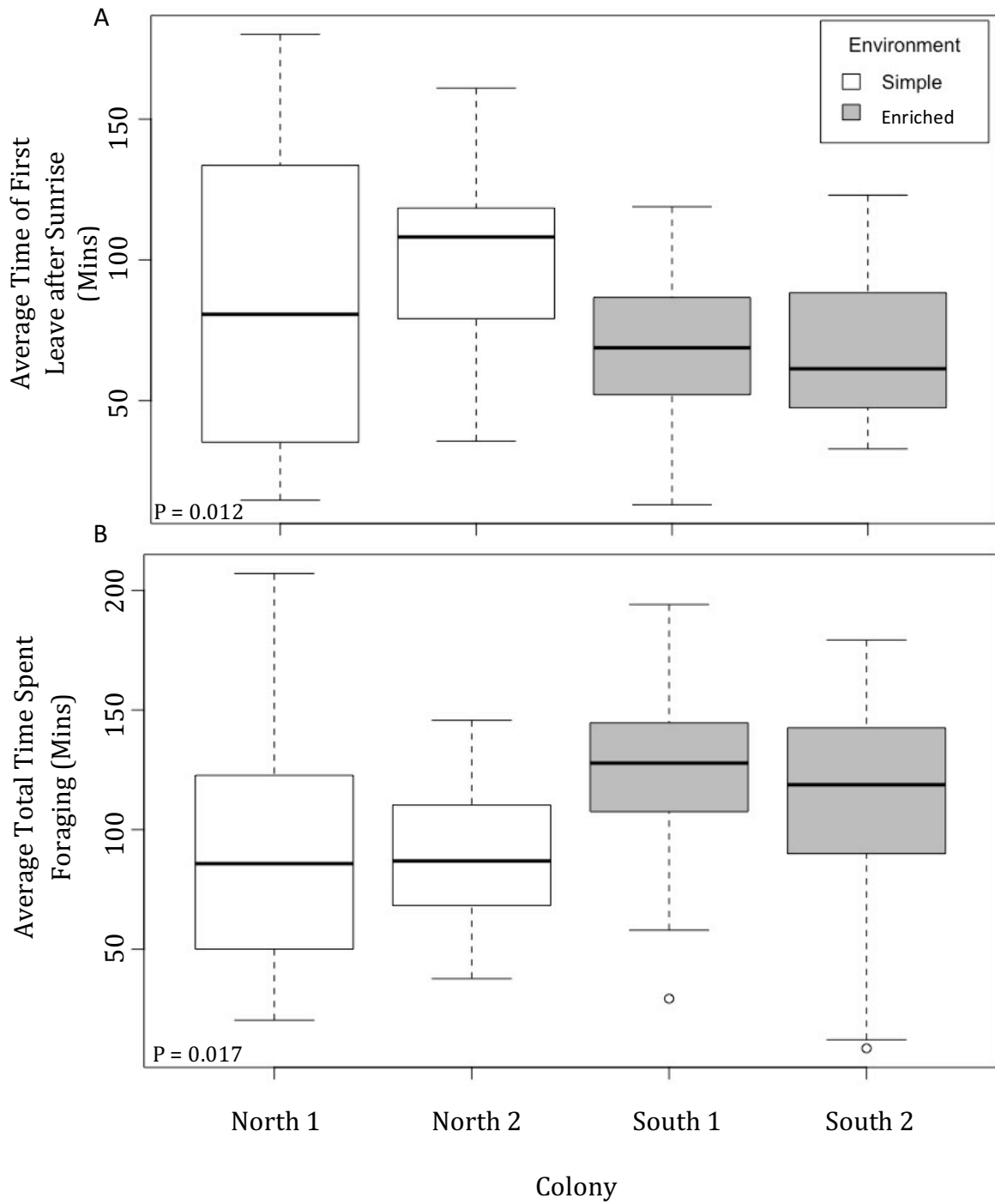


Figure 4.3.1. Distribution of bumble bee foraging patterns amongst the four observed colonies and between both environments (b) Average total foraging effort. (Simple Environment, $N = 20$; Enriched Environment, $N = 53$).

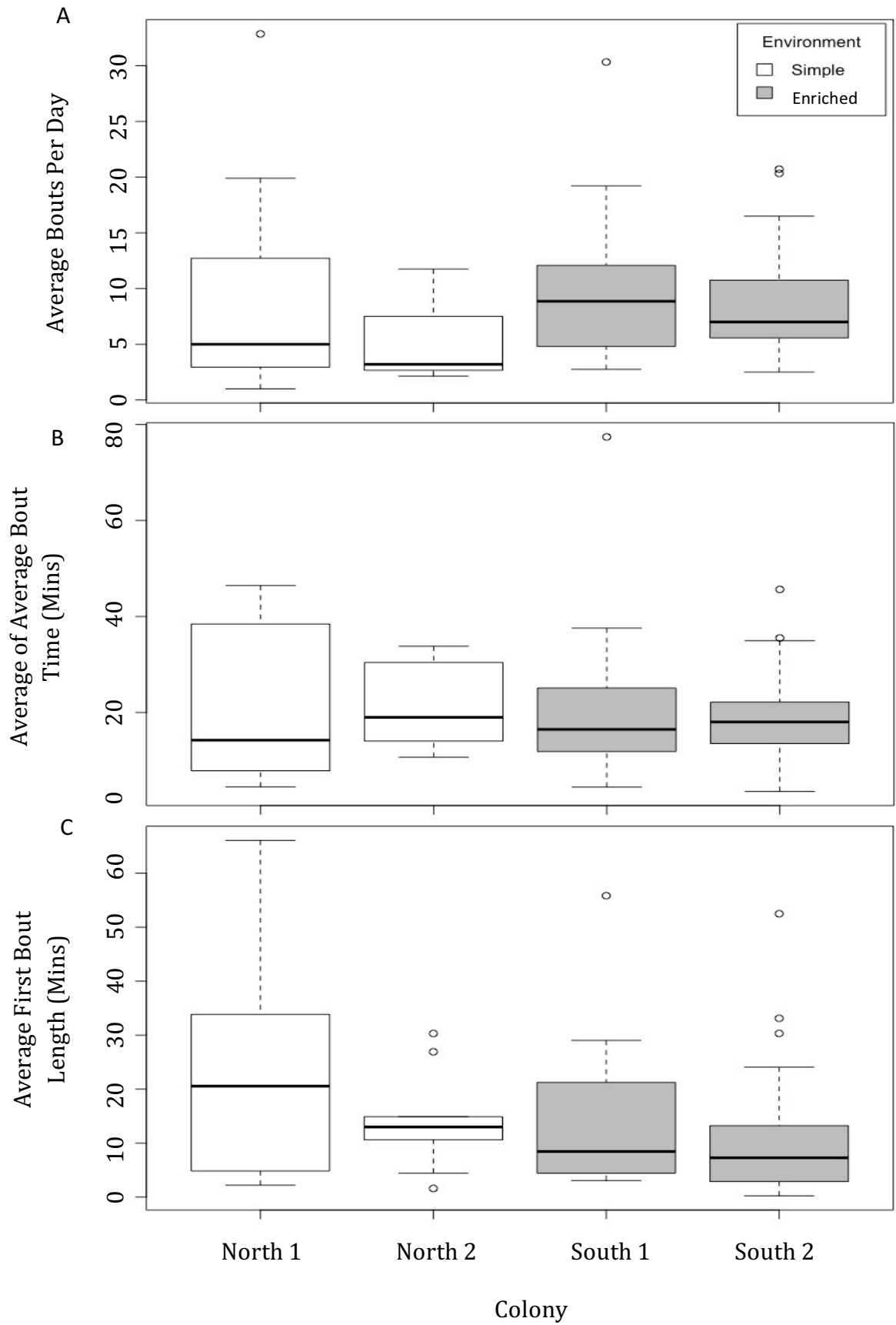


Figure 4.3.2. Distribution of bumble bee foraging patterns amongst the four observed colonies and between both environments (a) Average bouts per day; (b) Average bout time (c) Average first bout length (Simple, N = 20; Enriched, N = 53).

4.3.2 Nest Switching Behaviour

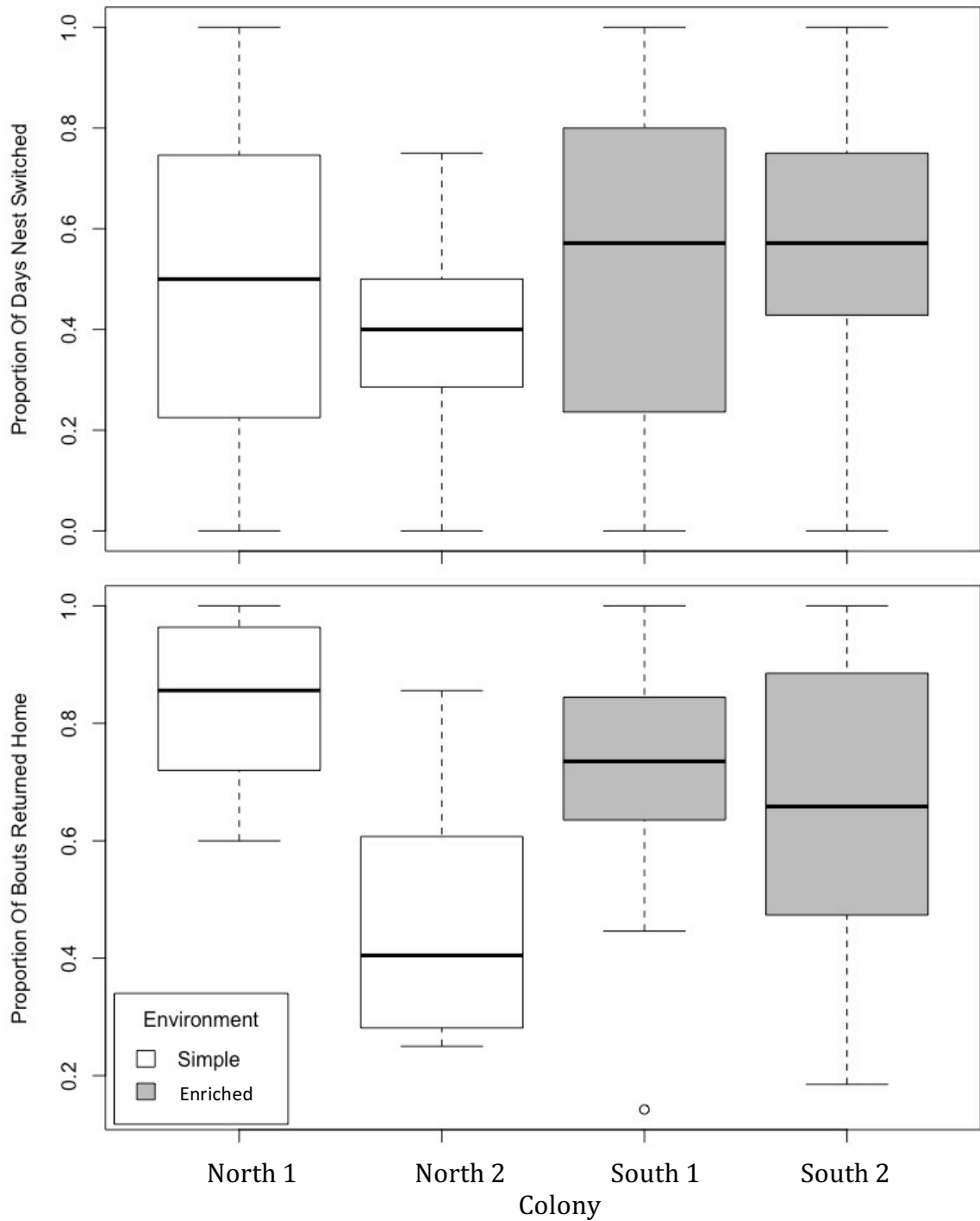
Over 80% of bumble bees from each colony were observed switching (leaving one nest and returning to another) on at least one of the days they were observed (Table 4.3.1).

Table 4.3.1. Average nest switching percentage of bumble bees who switched for at least one day over the time they were observed from each colony and environment over the entire observation period (N = 14 days).

Environment	Origin Nest	Sample Size	% Colony Switching Nests	% Average (Within Environment)
Enriched	South 1	23	91%	91%
	South 2	30	90%	
Simple	North 1	9	89%	85%
	North 2	11	81%	
Overall Average %			87.75%	

There were no observed differences in the proportion of days an individual switched nest between colonies or environments (GLM: Environment: $\chi^2=0.52$, DF = 1, P = 0.47; Colony: $\chi^2=3.14$, DF = 2, P = 0.21; Fig. 4.3.3a).

Environment and colony origin did not predict the number of bouts an individual returned to their original nest (GLM: Environment: $\chi^2=0.004$, DF = 1, P = 0.95; Colony: $\chi^2=3.14$, DF = 2, P = 0.21; Fig. 4.3.3b).



4.3.3. Distribution of foraging bumble bees behaviour amongst the four observed colonies and between both environments: (a) proportion of days each individual switched between nests (b) proportion of bouts that individual foragers returned to their nest of origin (100% = they always returned home).

4.4 Discussion

I aimed through this study to investigate the effect that a simplified environment without natural nectar sources can have on foraging behaviour in bumble bee (*Bombus terrestris*) colonies, and how environmental enrichment in a glasshouse can influence drifting behaviour of foragers. The results of this study show that overall individuals in the simple habitat had lower overall foraging time (effort) and initiated foraging later than those in the enriched environment provided with floral nectar sources. There were no differences between environments in the average bouts performed per day, average of average bout time, and average first bout length. Analyses also showed no differences between colonies within the same environment. Nest drifting behaviour between nests in close proximity from one another was observed to be much higher than previously suggested. At the colony level, 87.75% of foragers were observed switching between nests at least once. There were no differences among colonies and between environments for both the proportion of days individuals spent nest switching, and the number of bouts that they returned to their nest of origin.

4.4.1. Foraging Patterns Between Environments

Bumble bees in the simple environment had significantly less foraging activity than those in the enriched environment. They initiated foraging later and expended less effort on foraging in total. Analyses showed that colonies within the same environment did not differ from one another, therefore the two variances observed were caused by the environment that they were exposed to. In addition, there was a large difference in the number of observations that qualified for analysis between the two environments, with only 20 foragers in the simple environment compared to 53 foragers in the enriched environment. These results suggest that the colonies in the simple environment were spending less time foraging both in terms of how long they were foraging for, and the time over a 24-hour period they were foraging in.

The difference in environmental enrichment could have caused the variance in foraging times between the two glasshouse environments: as the environmental enrichment increases, so does the environmental complexity and foraging difficulty due to a number of differing flowers (Cakmak, Sanderson et al. 2009). Learning new flowers can be a time-consuming activity for foragers. Laverty (1994) found that naïve bumble bees on simple flowers would take 3-10 minutes to learn how to handle the flower, and 5-30 minutes on more complex flowers. With more flowers, they would spend more time out of the nest foraging (Laverty 1994, Raine and Chittka 2007). Moreover, with nectar provided *ad libitum* in a simple sugar feeder versus bees having to forage for nectar on flowers, foraging for nectar could take longer in the enriched environment. However, there was no significant difference between environments for average bout time, suggesting that floral complexity is not the reason for the observed patterns.

Another difference between environments that needs to be considered is the possibility of nectar source quality differing among the glasshouses. The energy content and quality of the nectar sources of the flowers versus the syrup were not measured, and whether they differ from one another is unknown. If one source provided was of a higher quality and energetic reward than another it could have an influence on the observed results due to an energetic shortfall in one of the environments. The sources used within the glasshouse were cornflower, snapdragon and syrup provided by the bumble bee rearing company Biobees. Both sources are regularly used as bee food suggesting that they are adequate for bee nutrition. Cornflower and snapdragon have been shown to be popular flowers for foraging bees, and cornflower is often included in bee forage seed mixes (Carreck and Williams 1997, Niovi Jones and Reithel 2001, Carreck and Williams 2002, Bretagnolle and Gaba 2015, Keshtkar, Monfared et al. 2015).

The syrup used in this study was provided by the bumble bee rearing company Biobees, and these types of syrup are used worldwide for both research studies and providing colonies with a sugar source in supplement to pollen as was done in this study. Approximately one million kg of sugar syrup are used by bumble bee rearing facilities and another two million are used to feed bumble bees in glasshouse environments (Velthuis and Van Doorn 2006). Previous research has suggested that this syrup provides adequate nutrition for colony development (Plowright and Jay

1966, Ribeiro, Duchateau et al. 1996, Rasmont, Regali et al. 2005, Gurel, Karsli et al. 2012). While both nectar sources in this experiment have a history of being used for and by foraging bees, the energetic quality of these sources has not been researched in depth.

An alternative aspect to be considered is that the bumble bees in the simple environment could have needed to forage less due to the *ad libitum* syrup feeders which could account for the results observed in which foragers in the simple environment spent less time foraging and started later. The colonies in the enriched environment could have simply been 'hungrier' and therefore spent more time foraging as the nectar sources were not as readily available. Future research should explore the energetic nutritional quality of this syrup versus flowers as it cannot be ruled out as a contributing factor. Moreover, the behaviour of individuals while out foraging could be observed such as how much time they spend on flowers, syrup feeders or tomato plants and what they are foraging for during their bouts to consider whether the bees were foraging more due to necessity.

The results of this study, along with previous studies, reiterate the importance of providing bee species with multiple flower species instead of a monoculture. In this instance, the simplified environment caused bumble bees to forage later and spend less time foraging overall. These results could have implications for commercial users of bumble bees as economically they may not be getting the most out of the colonies they use if they continue providing them with existing environments.

4.4.2 Nest-Switching (Drift) Behaviour Among Bumble Bee Colonies

Bumble bees were observed changing between nests at a very high rate in both environments and among all of the observed colonies. The level of worker drift did not vary between environments or colonies. These results do not support the hypothesis that environmental enrichment would reduce the number of bees entering other nests, and workers do not appear to be drifting between nests due to a lack of natural nectar sources. The results of this experiment align with previous research that when bee colonies are in close confinement from one another workers will move between nests (Free 1958, Free and Spencer-Booth 1961, Currie and Jay 1991, Pfeiffer and Crailsheim 1998, Jürgen, Benjamin et al. 2002). This is a topic that has not

been observed in great detail between bumble bee colonies, and this experiment provides some of the first data of this behaviour over a constant 5-hour observation period in commercial *Bombus terrestris* nests.

A study performed by Birmingham, Hoover et al. (2004) investigated worker drifting behaviour between bumble bee colonies in a commercial glasshouse. They discovered similar results where bumble bees would drift into different colonies and remain there. In a second study Birmingham and Winston (2004) found that 28% of their marked bumble bees drifted into foreign nests and remained there. The number of bees they found nest switching was much lower than the percentage observed in my own results (87.75%). This disparity may have been due to differences between methods. While Birmingham and Winston (2004) do not specify their exact methods of colony observation, it appears that they only surveyed the marked workers present in a colony, and did not watch the colonies for an extended period of time within a day. This could cause differences in results as through extended video footage each trip bumble bees performed and each individual nest switching in the 5-hour period was observed. The glasshouse set-up between experiments also differed. Birmingham and Winston (2004) performed their experiment in a 6-hectare glasshouse, while the one used in this experiment was much smaller (21x12m). While they also used more colonies, the increased space available could have reduced the number of bees entering conspecific nests.

These high levels of drift behaviour appear to be a result of the glasshouse environment commercially colonies are placed in. O'Connor, Park et al. (2013) found that nest drifting is an uncommon occurrence in wild bumble bee (*Bombus terrestris*) colonies. Only six workers throughout 14 colonies were thought to be drifters. Zanette, Miller et al. (2014) found a similar result with only 3% of workers found in wild colonies were unrelated to nestmate workers and were most likely the result of drifting. The results of this experiment show that worker drift occurs within a glasshouse environment, despite the level of environmental enrichment, which could be of concern for commercial growers, but also people looking to repeat experiments similar to this one.

4.4.3 Conclusions

The results of this chapter provide support to the idea that simple monocultural environments decrease the foraging effort of bumble bee (*Bombus terrestris*) foragers in a glasshouse experiment. These results add to the growing evidence that the environment colonies are provided with can have significant effects on individuals, and bumble bees in the simple environment were less active than those in the enriched. This reiterates the importance of supplementing pollinators with multiple floral resources. These results could also have implications for commercial bumble bee users, who may need to consider supplementing their glasshouse environments with flowers to get the most out of their colonies.

This study also showed nest switching occurring between bumble bee colonies in a glasshouse is occurring at unprecedented rates despite environmental enrichment. Bumble bee drifters were recorded at higher levels than any other study and could have implications for future research intending to place colonies in close proximity to one another. Care will need to be taken in future to ensure that colonies are kept separate from one another to ensure separate results for each one.

Chapter 5

General Discussion

The main aim of this thesis was to examine how individual behaviour of bumble bee (*Bombus terrestris*) foragers varied with size and between a simple and enriched glasshouse environment. Chapter 3 showed that workers within a bumble bee colony vary in the amount of effort they expend on foraging activity. It also showed that bumble bee body size is correlated with the time workers initiated foraging in the morning, with larger workers leaving the nest earlier than smaller workers. There was no evidence found of 'activator' bees who began foraging earlier commencing foraging for the colony. Chapter 4 showed that foraging patterns differed across environments, and colonies in the simple glasshouse started foraging later and spent less time foraging overall than those in the enriched environment. Additionally, it showed that environmental enrichment does not affect bumble bee drifting behaviour within a glasshouse.

5.1 Individual Differences Among Bumble Bee Workers

The hypothesis that individuals would vary behaviourally within a colony was mostly supported, supplementing evidence that individuals differ from one another intrinsically resulting in consistent behavioural differences. This can manifest in their activity and possibly cause differences in individual thresholds for foraging activities (Slater 1981, Robinson 1989, Beshers and Fewell 2001, Robinson 2009). Individuals in both colonies differed in the amount of effort they expended on foraging, with workers varying in the bouts they performed per day, their average bout length, and the total time spent foraging. When colony was not accounted for, individuals differed from one another in every activity measured, including first bout length and the time they initiated foraging. This suggests that there are consistent individual differences

among foragers in the amount of effort they put into tasks as was observed in honey bees in Oster and Wilson (1979).

It is possible that the observed differences in variation among colonies could be because the internal environment of the colony differed. The social environment within the nest can influence behaviour within insect colonies. Holbrook, Barden et al. (2011) found that worker task specialization increased as the colony grew in size. Burkhardt (1998) found that in the ant *Pheidole dentata* workers behave like solitary foragers in small groups, spending a long time foraging and recruit weakly. In larger groups they are more likely to recruit and return to the nest sooner (Burkhardt 1998). Cook and Breed (2013) found that honey bee fanning thresholds were influenced by the workers around them. Individuals in a group had a higher propensity to fan and had significantly lower thermal thresholds (Cook and Breed 2013). Differences in social environments with the observed colonies could account for the variation among them seen in the results.

No conclusive evidence was found of a foraging 'activator' or 'scout' sub-caste within the observed foraging bumble bees. However, bumble bee foragers have been shown to communicate when they return to the nest with food. Dornhaus and Chittka (2001) found that successful foragers returned and performed irregular excited runs and vibrated, likely releasing pheromones at the same time, suggesting that there is stimulation of inactive bumble bees occurring.

Workers can also be stimulated to begin foraging by their environment. The nutrition level of the colony has a significant effect on whether bees will leave the nest to forage and also their propensity to recruit others (Cartar 1992, Molet, Chittka et al. 2008). Daugherty, Toth et al. (2011) discovered in *Polistes* wasps reduced lipid stores were associated with low food that resulted in increased foraging at both the individual and colony level. This effect has also been observed in honey bee colonies, where if lipid synthesis was inhibited workers were more likely to forage precociously (Toth, Kantarovich et al. 2005), and if pollen stores are depleted they would account for this by increasing foraging effort and the amount of pollen they were bringing back to the nest (Fewell and Winston 1992).

This effect has been found in bumble bee species as well; when energy stores were experimentally reduced colonies would increase foraging effort and changed the rate with which they collected food (Cartar 1992). Molet, Chittka et al. (2008) found

that this effect in bumble bee (*Bombus terrestris*) colonies, showing that the amount of stored food in the nest (number of full honey pots) affected the response of workers to recruitment pheromones. With less food in the nest, workers would have a lower threshold to respond. They also discovered that when food stores were low workers were more likely to be stimulated into foraging due to nectar arrival in the nest, and successful foragers performed more excited runs releasing pheromones to communicate and recruit others (Molet, Chittka et al. 2008). This literature suggests that the nutritional level of the colony has a large effect on foraging workers. A workers individual threshold could be attuned to these influences instead of the actual time of sunrise and colony nutrition could be influencing the time that individuals leave after sunrise, as if food stores are low within the nest, they may be stimulated to leave the nest earlier than on days where food stores are higher.

Another element that could be influencing these results is temperature. Circadian rhythms have been shown to be highly dependent on temperature (Tomioka, Sakamoto et al. 1998, Yadlapalli, Jiang et al. 2018). This is noteworthy in insects who are ectothermic and are therefore very sensitive to ambient temperatures (Beck 1983). Temperature is of significance to bumble bee species as it affects their ability to fly (Heinrich 1975, Goulson, Peat et al. 2002, Heinrich 2004), and therefore could affect their time of leaving after sunrise as well. Differences in temperature could be influential in the variation seen in the initiation of foraging by individuals.

Future research could control for the nectar stores in the nest and to account for temperature fluctuations in the glasshouse. Foraging individuals could also be observed after they enter the nest, to discern whether they are recruiting others to forage, and whether foraging rate increases after they return.

5.2 Body Size Differences and Individual Behaviour

Overall my results support the hypothesis that larger bumble bees anticipate sunrise. Larger workers initiated foraging earlier than smaller bees, suggesting they show morning food anticipatory behaviour and may demonstrate strong circadian diurnal foraging rhythms. These findings are consistent with previous evidence that under constant laboratory conditions bigger individuals developed stronger and

more developed activity rhythms compared to their smaller counterparts (Yerushalmi, Bodenheimer et al. 2006, Stelzer, Stanewsky et al. 2010). These findings are important as they show this correlation persists within a semi-natural environment where bumble bees can forage in a larger area, instead of in a laboratory context. Size differences among bumble bees is a result of imbalanced larval feeding (Couvillon and Dornhaus 2009). The evidence in this study supports the idea that this size dimorphism persists in colonies because larger and smaller bumble bees could have differing functions with the colony.

There have been many proposals as to why bumble bee colonies have size differences among workers. Goulson, Peat et al. (2002) have previously suggested it may be due to larger bumble bees being more efficient nectar foragers. They showed that larger foragers could bring back more nectar per unit of time, foraging at a greater rate, and this is thought to be either by extracting more nectar per flower or visiting more flowers per unit of time (Goulson, Peat et al. 2002). Peat, Tucker et al. (2005) proposed that size dimorphism allows the colony to exploit a number of different floral resources due to a range of tongue lengths between smaller and larger foragers. They found that different sized bumble bees tend to visit different flowers and that individuals could be making these choices to lower their flower handling times. This study supports the findings of Yerushalmi, Bodenheimer et al. (2006) who found similar findings to this study, with larger workers having stronger circadian rhythms than smaller bumble bees. They suggested that workers differed in temporal function, with smaller workers being active around the clock to take care of brood, and larger workers having strong diurnal rhythms for foraging tasks, further facilitating division of labour within colonies (Yerushalmi, Bodenheimer et al. 2006).

While larger bumble bees are only probabilistically more likely to be foragers, Jandt, Huang et al. (2009) found that if workers do switch to foraging they are more likely to continue performing foraging tasks than to switch to any other behaviour. This likelihood for larger bees with stronger circadian rhythms to be foragers, combined with persisting in this activity after they begin, could be a mechanism to ensure that this group starts early in the day. Stelzer and Chittka (2010) performed a study in the arctic circle in the summer when there is constant sunlight (midnight sun) observing the foraging activity of bumble bees and found that even with endless

daylight these diurnal foraging patterns persisted, suggesting an adaptive advantage to this behaviour.

Flowers are thought to accumulate nectar and pollen overnight and this early activity from bumble bee foragers may be in anticipation of these resources and initiating foraging closer to sunrise could allow them to exploit these reserves (Kapustjanskij, Streinzer et al. 2007). Their stronger circadian rhythm allows them to keep track of this important event in the environment and make the most of the food availability before it runs out (Van Nest and Moore 2012). As larger bumble bees are also able to carry more forage, can see better in lower light conditions, and can maintain their thoracic temperature in cooler conditions they are also the most well equipped to exploit these morning food sources (Goulson, Peat et al. 2002, Spaethe and Chittka 2003, Kapustjanskij, Streinzer et al. 2007).

With such a large size difference among the foraging group (3 to 6.9 mm (Goulson, Peat et al. 2002)) circadian rhythms will differ among these bumble bees as well. This could serve a purpose among these foragers. Floral resources vary in both time and space in the environment (Dechaume-Moncharmont, Dornhaus et al. 2005, Dornhaus 2012). If some foragers are starting earlier, and some are starting later, it could allow bumble bee colonies to efficiently exploit all foraging resources over the day. Herrera (1990) showed that pollen producing plants peak in the late afternoon, while nectar producing plants peak at midday. Differences in circadian rhythms among the foraging group could allow trips to be staggered throughout the day to exploit these temporally variable floral resources.

Honey bees achieve activity differences among workers through temporal polyethism division of labour: younger honey bees are in-nest workers and have less defined circadian rhythms while their elders are foragers with strong diurnal rhythms (Beshers and Fewell 2001). However, the social systems in bumble bee species are less complex than in honey bees (Sadd, Barribeau et al. 2015). In bumble bee species where division of labour is facilitated through morphological differences (Goulson, Peat et al. 2002, Jandt and Dornhaus 2009), an adaptive explanation of maintaining this size differentiation is if it accomplishes the same outcome. This adaptation would be desirable for social colonies, as the difference in activity between foragers and in-nest workers would facilitate the temporal organization of the colony and increase efficiency as a whole (Bloch, Herzog Erik et al. 2013).

5.3 Environmental Effects on Colony Foraging Activity

The results of this study support the hypothesis that the environment colonies were exposed to would influence foraging activity, and that individuals in the simple environment would forage less. Colonies in the simple monocultural environment with syrup for nectar spent less time on overall foraging effort and initiated foraging later in the day than those in the enriched with multiple flower types providing a natural nectar source. While this effect could be due to environmental complexity and longer flower handling times (Lavery 1994, Raine and Chittka 2007), the average bout time between environments was the same, suggesting this is not causing the difference observed. This suggests that the differences observed between glasshouses is due to environmental effects on the colonies, and the difference in environmental enrichment.

Monocultures are thought to have a serious effect on bee health in agricultural environments. Previous research has shown that pollinators living around agricultural monocultures are generally less healthy than those exposed to a variety of flowers, which could possibly account for the differences observed in this study (Rao, Schmidt et al. 1995, Carvell, Roy et al. 2006, Goulson, Nicholls et al. 2015). Girard, Chagnon et al. (2012) conducted a study around monocultures and found that honey bees exposed to blueberry monocultures with intensively managed field margins were less healthy than those exposed to cranberries. Cranberries are low in nectar, but the edges were less maintained providing a variety of floral sources, demonstrating that monocultures are detrimental and suggesting a variety of flowering weeds are essential for bee health in an agricultural environment. Flowers also vary in their amino acid content which again reiterates the importance of a variety of flower sources (Rao, Schmidt et al. 1995, Girard, Chagnon et al. 2012). Cook, Awmack et al. (2003) found that honey bee foragers preferred flowers that had higher proportions of essential amino acids, and the nutritional value of flowers could influence forager behaviour. The foraging bees in the simple environment could have been overall less healthy due to the lack of floral variety, and therefore spent less time foraging, and initiated foraging later in the day.

Another aspect that needs to be considered is the effect of parasitism. Stressors often do not act in isolation, and the reason for these differences may also be due to

an interaction of factors (Goulson, Nicholls et al. 2015). The colonies we used for this experiment were commercially produced and therefore were likely to be parasitized as commercial colonies have much higher levels of parasites (Colla, Otterstatter et al. 2006, Goka, Okabe et al. 2006, Goulson, Lye et al. 2008, Yoneda, Furuta et al. 2008). A study performed by (Graystock, Yates et al. 2013) found pathogens in 77% of the commercial hives they tested and this is thought to be because of poor management of these colonies (Manley, Boots et al. 2015).

The interaction of parasitism and the monoculture environment would cause increased harm to those colonies. Di Pasquale, Salignon et al. (2013) found through their research that not only the abundance of resources matters, but also the quality of those resources when bees are infected with parasites. Honey bees fed a polyfloral blend of pollen tolerated infection and survived longer than those on a monofloral diet (Di Pasquale, Salignon et al. 2013). This research was also supported by Dolezal, Carrillo-Tripp et al. (2016) who found that *Varroa* mite infestations are linked to the landscape around honey bee colonies, however it also depended on the type of landscape, furthering the idea that it is the quality of resources around colonies that matters. They also found that if infection levels were high enough, it obscured any effect of landscape, suggesting that the interactions of stressors are increasingly important (Dolezal and Toth 2018).

Bumble bees likely show similar outcomes, as Roger, Michez et al. (2017) found that a poor diet can decrease immune function in *Bombus terrestris* individuals and decreases larval and pupal masses of developing bumble bees. These factors would account for the differences we see between environments, and the colonies spending less time foraging overall, as well as foraging later. Even at low intensities stressors can cause brain damage, impairing skills needed for foraging such as learning and memory, and could even be disrupting the circadian rhythms of foragers causing them to forage later (Klein, Cabirol et al. 2017).

An aspect of this study that needs to be taken into consideration when interpreting these results is that there is only one repetition of this experiment presented, and the enriched and simple environments are possibly confounded by their location in the glasshouse. The two environments during these observations were in different sides of the glasshouse (north and south), and there could have been differences present between these sides, such as the light intensity, whether they

differ in temperature levels and how quickly they heat up, and the humidity levels of the glasshouse with the north-south orientation. This is due to the angle of the sun in the southern hemisphere, where the sun is angled towards the north in the sky, meaning that the north glasshouse would get more direct sunlight (Jay and Warr 1984). Bumble bees require a minimum temperature to activate their flight muscles (Heinrich 1975, Nieh, León et al. 2006). If the north glasshouse warmed faster, bumble bees may have initiated foraging earlier than those in the south glasshouse. However, this was not observed in the results shown, and colonies in the south initiated foraging earlier than those in the north.

Another effect that the glasshouse orientation could have had is that the temperature rose to a higher level in the north glasshouse due to more direct sun exposure. This could have had an effect on the time bees spent foraging overall. (Kwon and Saeed 2003) found that foraging activity was highest in a glasshouse at 25.7°C, but decreased up to 69% at 37.2 °C. If the temperature was much warmer in the north environment, this could have caused a decrease in the overall time spent foraging as we observed and could contribute to the difference seen between the glasshouses in the number of recorded individuals. A repetition of this experiment with the environments swapped, and accurate temperature recordings over the course of the study would account for any environmental differences that could have influenced the outcome.

These results of this experiment are important for supporting the evidence that wildflowers around agricultural can influence colony performance (Holzschuh, Steffan-Dewenter et al. 2010, Vaudo, Tooker et al. 2015). It also supports conservation plans for providing diverse floral resources around agricultural landscapes to enhance pollination in these plantations (Carvell, Roy et al. 2006, Samnegård, Persson et al. 2011, Williams, Regetz et al. 2012).

This study also has implications for commercial growers who utilize bumble bee colonies for pollination. Commercial users of bumble bees in large scale glasshouse environments should consider the use of supplemental flowers as a natural nectar source. These results have shown that this variety of flowers encourages more time spent out of the nest foraging out on plants and could increase pollination of commercial crops.

5.4 Nest Drift in a Glasshouse Environment

The results of this experiment also showed that nest switching/drift behaviour of bumble bee workers in a glasshouse occurs at a high rate within both the enriched and simple environments. This is different to previous research in bumble bee species that reported the level of drifting as much lower (28% versus 87.5%) (Birmingham, Hoover et al. 2004, Birmingham and Winston 2004). There was no significant difference among colonies and environments in the levels of drift occurring.

The cause and purpose of worker drift is still unknown. Some of the reasons suggested for worker drift are nectar robbing, or workers trying to sneak their own eggs into a different nest (Birmingham, Hoover et al. 2004, Birmingham and Winston 2004). However, this does not account for the lack of nest defences seen among commercial colonies within glasshouses. High levels of drift among commercial colonies and reduction in nest defences could be due to individuals not being able to discern between colony odours. Nests are in such close proximity in a glasshouse where the same type of pollens and nectar are found in both colonies, as well as commercial colonies from the same distributor being potentially related, could cause very similar colony odours (Birmingham and Winston 2004). Conversely, Mullen and Rust (1994) found in a study that isozyme levels in commercially reared bumble bees (*Bombus occidentalis*) are the same in both commercially reared and wild populations. This suggests that workers drifting among colonies and failure to exclude non-nest mates is not due to inbreeding and genetic similarity within commercial populations. However, this does not change that these colonies are foraging on the same resources, which can still produce a similar colony odour profile.

Another reason for the lack of colony defences could be the abundance of available resources within a commercial glasshouse. These colonies may be less aggressive and more accepting of foreign workers as there is less competition (Reeve 1989). Downs and Ratnieks (2000) found that honey bee guard acceptance behaviour increased as resources became more abundant throughout the season, and fights over resources and nectar robbing became less common. Colonies in a commercial glasshouse do not have to compete, and therefore exclusion behaviour may occur at a much lower rate.

This is of concern for commercial users of bumble bees, with these colonies having such high levels of parasites (Graystock, Yates et al. 2013). There is a high potential for pathogens to spread within a glasshouse rapidly through horizontal transmission with foragers going in and out of neighbouring nests (Ingemar and Scott 2001, Manley, Boots et al. 2015). This could cause problems among multiple colonies in a glasshouse. With previous research showing that parasites have a larger effect on bees in monocultures (Di Pasquale, Salignon et al. 2013, Dolezal and Toth 2018), as commercial environments are, growers will need to consider this. This also of concern for future studies considering housing bumble bee colonies in close proximity as the colonies may not be able to be considered separate entities due to the high level of foragers present in both data sets.

While steps can be taken to separate colonies, Birmingham and Winston (2004) found in their study that the use of simple landmarks did not improve the number of workers drifting between nests. This suggests that workers are not switching nests due to orientation problems, but instead are drifting on purpose, or that landmark cues do not outweigh any potential similarity of chemical cues. This makes worker drift a difficult problem to solve within glasshouses.

5.5 These Results in the Context of Rapid Environmental Change

This thesis reiterates the importance of providing flowers to insect populations in the environment, as agricultural land expands and removes these resources, to not only enhance commercial pollination but also wild pollinators (Carvell, Roy et al. 2006). However, the effects of increased flowers has further implications than this in the environment. Holzschuh, Steffan-Dewenter et al. (2010) found that wild species were improved and abundance increased with a higher percentage of non-crop habitats and increased field edge territories for nesting. Williams, Regetz et al. (2012) observed that colony growth was highly influenced by the floral resources available and that early-flowering resources are important, especially to bumble bees, for colonies to grow to a size that they can effectively exploit the environment. Park Mia, Blitzler et al. (2015) discovered that the effects of pesticides in agricultural environments can be buffered by providing a variety of floral resources. Naturally established pollinator populations as well as commercial ones are essential for

agricultural success as the demand for crop pollination is growing faster than the commercial stock of bee species (Aizen and Harder 2009). Therefore, natural pollination services are crucial for supporting this service (Aizen and Harder 2009).

Wild bee species have been shown to enhance crop success whether honey bees are present or not, and even provide sufficient pollination services for high demand crops (Kremen, Williams et al. 2002, Garibaldi, Steffan-Dewenter et al. 2013). The integration of both commercial and natural populations would improve outcomes for agricultural crop pollination (Garibaldi, Steffan-Dewenter et al. 2013). To ensure the continuing diversity of wild pollinator species farmers need to consider whether the environment they are providing is bee-friendly (Osborne, Clark et al. 1999, Bartomeus, Potts et al. 2014). Increasing edge habitats/field margins that host flowers and nesting sites, providing gardens, and reintroducing floral resources could reduce the decline of insects around the world (Klein, Vaissière et al. 2007, Goulson, Lye et al. 2008, Le Féon, Schermann-Legionnet et al. 2010, Samnegård, Persson et al. 2011, Blaauw and Isaacs 2014). There is still a lot to be learned about bee species and the threats they face and the further loss of wild species would resonate throughout the entire ecosystem (Goulson, Hanley et al. 2005, Brown and Paxton 2009)

5.6 How This Study Could Be Improved

This study presents important evidence to support research in this field, however there are ways I would improve this study if I were to perform it again. Firstly, I would measure all the bees as they are marked, to ensure that all body sizes are recorded, and bees would not have to be tracked down within the glasshouse post-mortem. This would ensure that every forager recorded would have corresponding body size, allowing for a larger sample size for body size analyses. The small amount of dead bumble bee foragers found in this experiment is likely because bumble bee workers remove the dead from their nest or they leave when they begin to decline in health to ensure the health of the nest (Rueppell, Hayworth et al. 2010, Munday and Brown 2018, Sun, Haynes et al. 2018). There were also no differences in body size found among the 17 individual foragers with a thorax width and the rest of the bees measured, even though it was expected that there would be due to previous evidence (Goulson, Peat et al. 2002). Although, this is likely because of the small number of bees

recorded, and future research with every bee recorded may show the expected patterns. Additionally, with the sample size in each environment being only two colonies, a repeat of this experiment was performed; however, I discovered that the time to extract data from the videos was extremely long, and therefore the second month could not be included in this thesis. Moreover, in this repetition the enriched and simple environment locations were swapped to account for any confounding variables that the glasshouse location may have presented such as temperature and light differences. In the future, I would enlist a research assistant to help with collecting data from the videos, to allow this to be extracted in a timely manner.

The amount of nest switching that occurred among colonies in the same environment also needs to be considered. In these analyses, they were considered two separate entities. However, they may not be able to be considered two colonies in future experiments with such high numbers of bumble bees going between nests and remaining within the other colony. The high percentage of worker drift observed in this experiment may be why there were no differences observed between colonies in a singular environment, as bees were switching so often it is hard to consider them entirely separate nests. These results will need to be considered for future studies that plan to put bumble bee colonies in close proximity from one another, as they may not be able to be considered as separate entities for analyses. This also needs to be considered with marking individuals. In future, it may be necessary to mark newly emerged (callow) bumble bees with that nest origin colour to ensure that they are from that colony when keeping two nests within the same glasshouse.

Another aspect I would do differently is analysis of temperatures within the glasshouse. Temperature was recorded inside and outside of the bumble bee colonies to check whether it would affect foraging patterns. However, due to error with the technology used in this experiment, temperature data was not recorded properly, and it could not be used in the analyses. In future, I would ensure temperature measurements were being recorded successfully, and would also do manual checks at sunrise and throughout the day to ensure that there would be back up data if necessary.

5.7 Future Applications: Agent Based Modelling

The results presented in this thesis could be used to understand more about bumble bee colonies in the environment. Agricultural intensification is not the only threat presenting habitat fragmentation and loss to the wild pollinators; urban sprawl is causing similar problems for pollinator species (Terando, Costanza et al. 2014). Cities and farms are spreading out and breaking up natural environments, meaning that insects have increasing issues finding food sources around their nest due to the patchiness of available resources (Walther-Hellwig and Frankl 2000, Barnes, Morgan lli et al. 2001). These fragmented environments may be more detrimental to species that do not communicate the location of resources to nest mates, such as bumble bees, as each individual forager has to track it down itself. The full effects of environmental change have not been studied fully and there are many impacts that we may not yet be aware of (Cresswell, Osborne et al. 2000).

Agent-based models are common tools used to predict how environmental factors might be influencing bumble bee (or other species) populations over time. It allows the ability to create a 'virtual world' where one can observe the interactions of autonomous individuals and the results and consequences of those exchanges (An, Mi et al. 2009, Maleki-Dizaji, Kiran et al. 2011). This could be a valuable means of exploring how human-induced rapid environmental change could be impacting pollinator dynamics. Agent-based modelling is especially useful when exploring collective strategies in different environment contexts (Klügl, Oechslein et al. 2004, Oechslein, Puppe et al. 2006, Dornhaus 2012).

This model would explore how the fragmented environments could be influencing bumble bee foraging ability and colony energetics. The implementation of accurate biological evidence in agent-based models is extremely important (Maleki-Dizaji, Kiran et al. 2011), and the results of this thesis could be used to apply real foraging data to the autonomous agents created within the simulation. The measured variables in this experiment could be incorporated in this model to give the simulated colonies real foraging activity parameters of how long they spend out of the nest and how many foraging trips they perform in the day. The hypothesis that would be tested is that colonies would use disproportionately more energy in an irregular environment versus a more uniform environment collecting sufficient resources. Colonies need

adequate energy uptake versus energy use to ensure success. A simulation could explore whether insects are able to gather enough to grow and reproduce in agriculturally intensified and urban environments. It could also be adapted for use in not just bumble bees but other social insect species with the correct parameter information.

5.8 Final Conclusions

The main findings of this study were (1) that individuals differ from one another in foraging patterns both within the same colony and among colonies, (2) body size correlated with initiation of foraging, (3) environment affects the foraging activity of colonies and (4) environmental enrichment does not affect the number of individuals drifting among colonies. This suggests that there are consistent individual differences among workers within a bumble bee colony, and body size could be a factor in the observed differences and that it serves a functional purpose within the nest. It also suggests that simplified monocultural environments have a significant effect on the behaviour of bumble bees, and that workers are not drifting among colonies in a glasshouse to find familiar natural sugar sources. These results contribute to the existing body of literature of consistent behavioural differences among closely related bumble bees, and how body size could contribute to the differences observed. It also contributes to research into how monocultures can affect bumble bee colony activity in a negative way, possibly diminishing effectiveness of pollination. This thesis provides a long-term study of individual data in a semi-natural environment and provides evidence to support studies performed in a lab setting, suggesting these can translate to wild colonies. Furthermore, it provides a new perspective into the use of bumble bees in a glasshouse environment, which has not been studied in depth. More research is needed into how simplified glasshouse environments could reduce activity of bumble bees being used within them. I have also expanded on possibilities for future research in this area, including the use of agent-based modelling to explore how existing bumble bee foraging strategies could be interacting with the changing environment, and through the model researchers could predict the type of environment declining populations need to flourish.

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Appendices

Appendix 1: Distributions of Foraging Data

Analyses were performed to determine the distribution of data for the measured variables for the GLM analysis (Chapter 4). A goodness of fit test was run in the JMP software under distribution analysis. Four of the foraging observations data were found to have a log-normal distribution through the Kolmogorov's D test: First bout length ($D = 0.0787 > 0.1500$ (Prob>D))(Fig. A.1a), average bouts per day ($D = 0.06496 > 0.1500$ (Prob>D))(Fig. A1b), average bout time ($D = 0.0731 > 0.1500$ (Prob>D))(Fig. A.1c), and average time of first leave after sunrise ($D = 0.07105 > 0.1500$ (Prob>D))(Fig. A.1d).

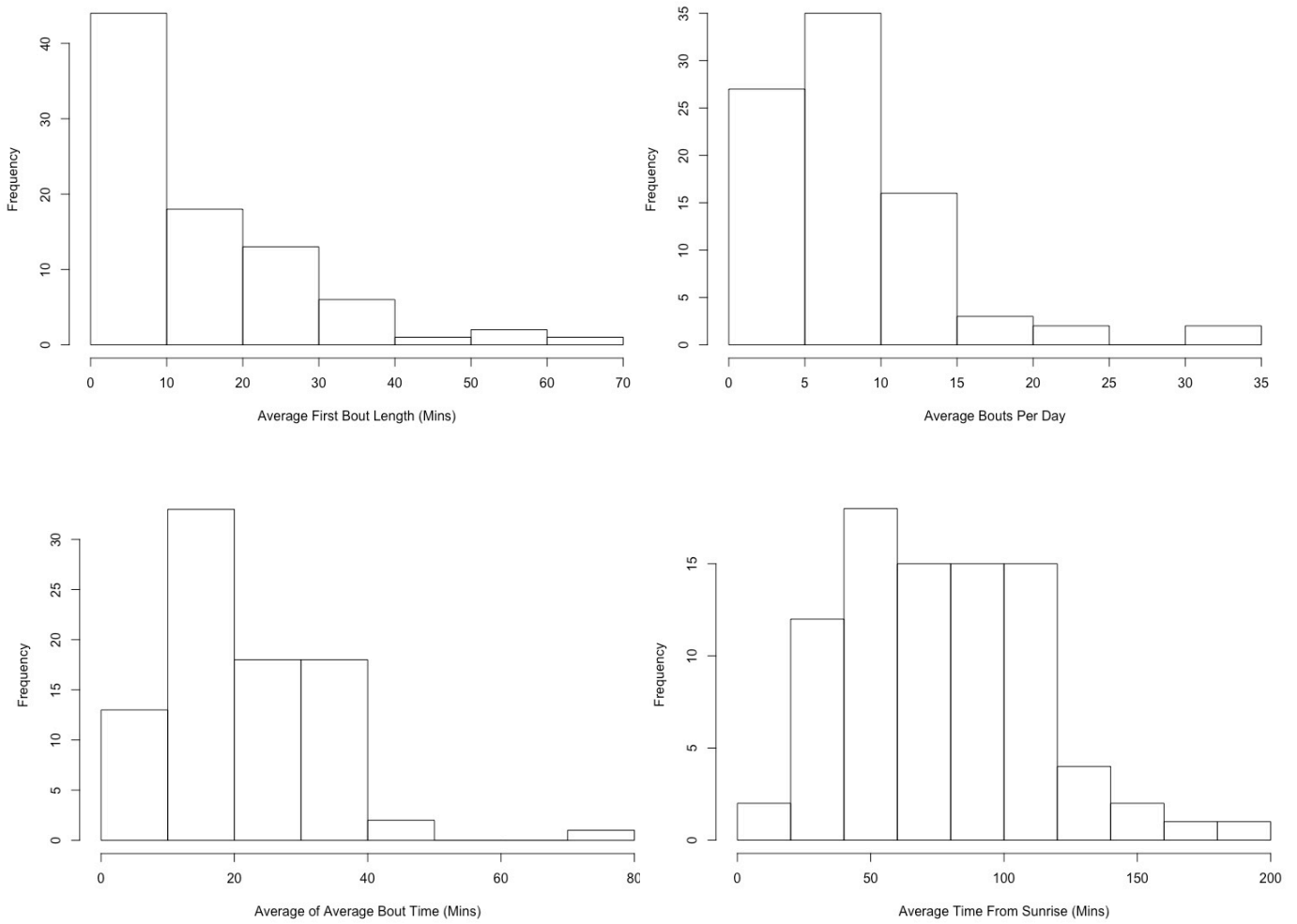


Figure A.1. Distributions of foraging data (a) average first Bout length (b) average bouts per day (c) average of average bout time (d) average time of first leave after sunrise

The data from the foraging observation total time spent foraging was found to be normally distributed through the Shapiro-Wilk W Test ($W = 0.98315 < 0.4386$ (Prob < W)) (Fig. A.2).

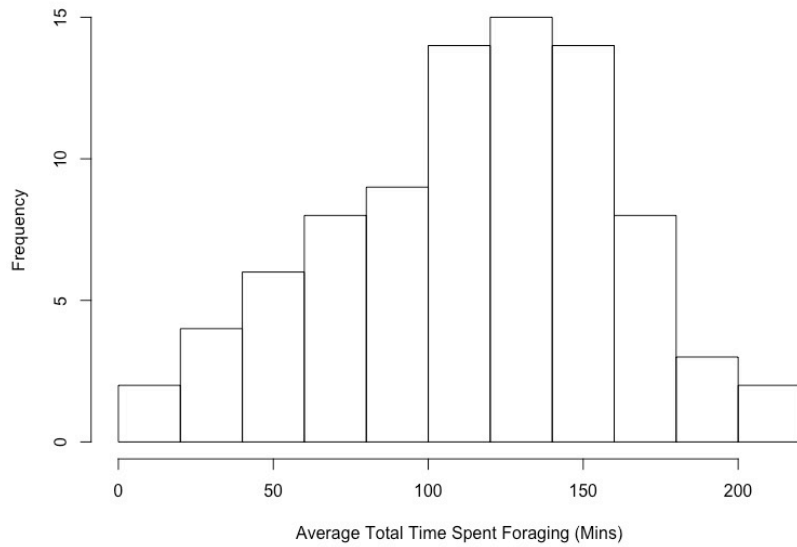


Figure A.2. Distribution of foraging data of the measure average total time spent foraging

Appendix 2: Distributions of Bumble Bee Body Size

A two-tailed t-test was run to determine whether body size varied among the foragers with recorded thorax widths and those who were not observed foraging. Body size between observed foraging workers and those who were not seen leaving the nest consistently did not differ significantly within the South 1 colony ($t = -1.59$, $df = 12.22$, $p = 0.14$; Fig. A.3a), the South 2 colony ($t = 0.02$, $df = 22.53$, $p = 0.98$; Fig. A.3b), or among all individuals in both colonies observed ($t = 0.61$, $df = 30.24$, $p = 0.55$; Fig. A.3c).

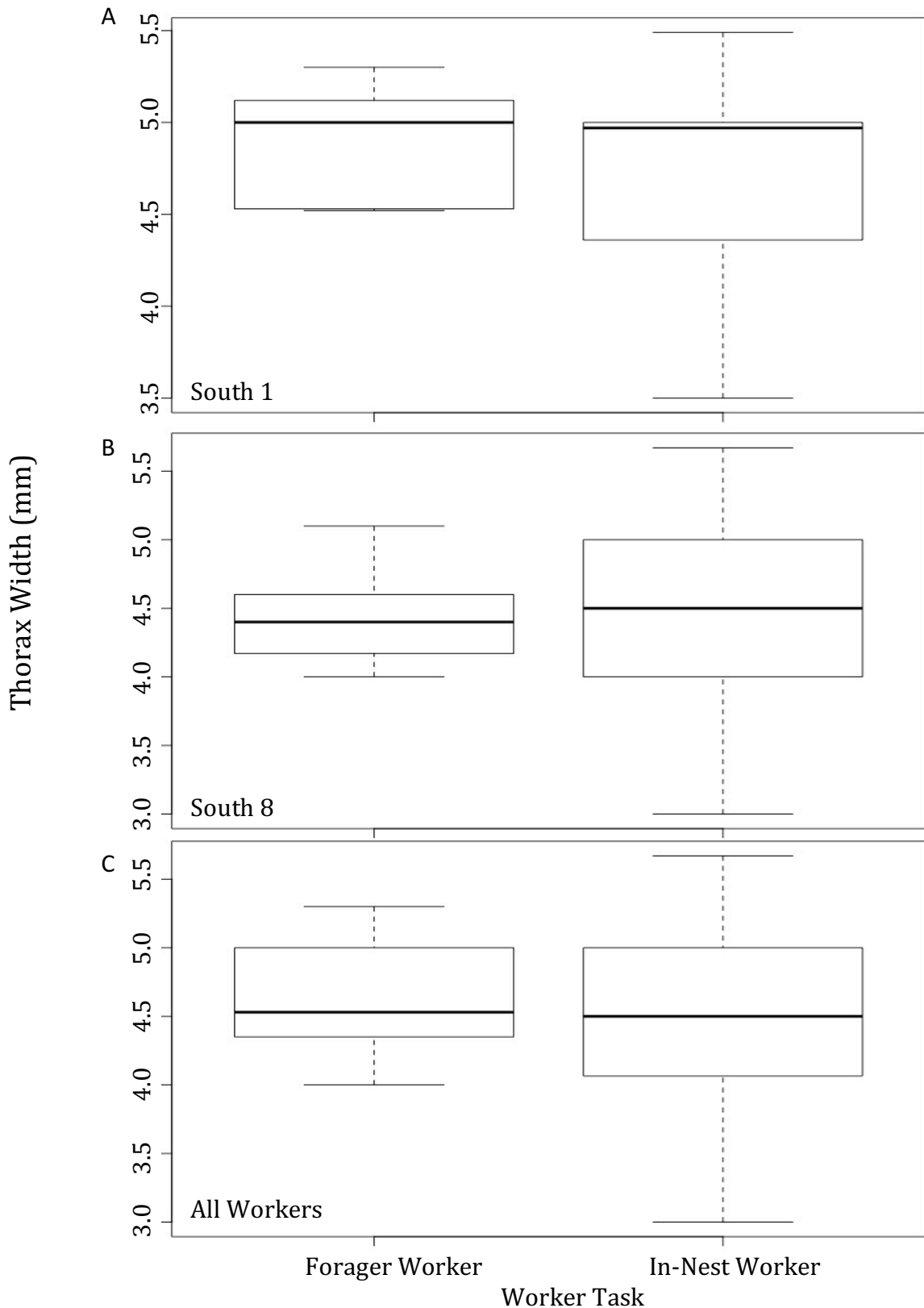


Figure A.3. Distributions of bumble bee body sizes among workers observed foraging and those that were not (a) In the colony South 1 (b) In the colony South 2 (c) All individuals observed in the south glasshouse.