

**A COMPARISON OF POLLINATOR BIODIVERSITY BETWEEN
GREEN SPACES, INDUSTRIAL AREAS AND RESIDENTIAL
LAND-USE ZONES IN URBAN, SOUTHERN ONTARIO**

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ABSTRACT

A COMPARISON OF POLLINATOR BIODIVERSITY BETWEEN GREEN SPACES, INDUSTRIAL AREAS AND RESIDENTIAL LAND-USE ZONES IN URBAN, SOUTHERN ONTARIO

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Pollinators, especially bees, have been suffering massive population declines. This project investigates abundance and diversity of bees in the urban setting. Twenty sites in Kitchener, Waterloo, Cambridge and Guelph, in four types of city land-uses (green, industrial, new residential and old residential) were assessed for the summers of 2007 and 2008. Three sampling techniques were used: pan traps, trap nests and netting, with yellow pan traps being the most effective in attracting bees. Over 107 species of bees from 25 genera were collected. DNA barcoding was used with limited success to identify specimens to the species level. Abundance and diversity of bees did not vary based on city land-use. Both abundance and diversity were higher at specific sites with naturalized areas. Strangely, bee diversity and abundance were also negatively correlated with plant diversity. Bee diversity was also higher earlier in the summer.

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CHAPTER 1: INTRODUCTION

1.1 Pollination

Pollination is defined as the transfer of pollen from anther to stigma. Because plants are immobile, this process requires a vector by which the pollen can be moved. This vector can be abiotic – wind, water, gravity – or it can be biotic. Biotic vectors include certain insects, birds, mammals and reptiles (Proctor et al. 1996).

1.2 Bees as Pollinators

Amongst many species of animals that function as biotic vectors for pollination, one order and superfamily stand out in particular. Almost all Hymenoptera: Apoidea, or bees, are completely dependent on pollen and nectar for every stage of their development. Because of this dependence, they are designed to carry pollen in the branched hairs that cover their bodies. Many species also have special “baskets” on their legs or abdomen called scopa in which they collect pollen (Roubik 1995). These features make bees more efficient in general pollination than other insects and, consequently, they are the primary focus of this thesis.

1.3 Biodiversity

Biodiversity, the measure of variety of flora and fauna (Wilson 1992), is important for many reasons, most notably its essential role in ecosystem stability (Tilman 1996).

Biodiversity can be measured at any spatial scale and is defined by three components: species abundance, species richness, and proportional species abundance (Magurran

1988). Species abundance describes the number of individuals of a certain species that are found in a defined area. Richness describes the number of taxa, and is usually measured at the species level, though richness measured at the genera or even family level can also provide interesting information. The measurement of richness, however, is biased by abundance. When sampling random individuals from a pool, one is most likely to collect an individual from a species which is relatively high in abundance, as that species makes up a greater percentage of the total pool of individuals. Similarly, species which are rare make up a lower percentage of the pool, and therefore are less likely to be collected in random sampling. The third measure of biodiversity, the proportional species abundance, exists to try account for this discrepancy. Certain biodiversity indices have been developed to allow the measurement of proportional species abundance, including the Shannon-Weiner Index (Shannon and Weaver 1949). The Shannon-Wiener Index assumes random sampling from an infinitely large community made up of all known species. The index increases with an increasing number of species present in a site, but is restricted by uneven distribution of species. It reaches a maximal value when all species are evenly distributed (Magurran 1988; Shannon and Weaver 1949).

1.4 Bees of Canada

Canada is home to an estimated 970 species of bees (Danks 1979). Thirty-nine genera and six families are found in Canada east of Manitoba (Packer et al. 2007). Different groups of bees have different social structures, habitats, and food preferences (Roubik 1995). For example, honey bees and bumble bees are well-known eusocial insects. These bees are organized in caste systems; a queen, drones, and sterile female workers live

together in a hive, almost as a super-organism. Only the queen produces offspring, while the workers collect all the nectar and pollen needed for the hive (Kevan 2007). Solitary bees, on the other hand, such as the genus *Megachile*, live individually. Each female makes her own nest, collects pollen, and lays eggs on her own. Some of these solitary bees, particularly certain species of ground-nesting bees, live gregariously – in individual nests but in close proximity to others of the same species. Some may live so close together that a single square metre can house up to 300 bees (Packer et al. 2007). Social bees typically continue to care for and feed their young, while solitary bees lay eggs into a pollen ball, and then leave them to survive and develop without care (Kevan et al. 1988).

Different types of bees also show preference for different habitats based on the different resources they use to build their nests. Most bees nest in tunnels they dig into the ground, with soil preferences ranging from sandy soils, to light or densely vegetated soils. Other species nest in hollow plant stems, or holes in trees. Some species, like the carpenter bees, (*Xylocopa* spp.), even excavate holes into solid wood. Bees from the family Megachilidae may line their nests with leaves or petals (*Megachile*) or plant hairs (*Anthidium*), or they may plug their nests with mud or clay (*Anthidiellum*, *Dianthidium*, *Osmia*) (Packer et al. 2007).

Bees may also specialize in the types of flowers from which they harvest pollen or nectar. A species may have long or short tongues which may constrain their feeding to certain types of flower. For example, only species with longer tongues, such as bumble bees

(*Bombus* spp.) can easily stick their tongues down the narrow corolla tubes of the red clover (Free 1993). Specialist bees, known as oligolectic species, may collect pollen only from a type of flower, such as the squash or pumpkin plant (Kevan et al. 1988) in the case of *Peponapis pruinosa* Say, or the thistle plant in the case of *Melissodes desponsa* Smith (Packer et al. 2007). Other species, such as *Halictus ligatus* Say, are generalists, or polylectic species, and collect pollen and nectar from a wide range of plants on a single foraging expedition (Packer et al. 2007). Because of the specific specializations of bees, bee diversity is essential for the continued pollination of diverse flowering plant species.

1.5 Pollinator Decline

Pollinators, especially solitary bees, bumble bees, and honey bees, have been suffering massive population declines in some parts of the world (Steffan-Dewenter et al. 2005). A recent study by Franzén and Nilsson (2010) suggest that solitary bees, which make up most of the Canadian bee species, are at highest risk of extinction. Human disturbances, including pesticide use, habitat destruction, and resource destruction, have devastated pollinator populations in some parts of the world (Kevan and Viana 2003). This phenomenon has been recognized with the Convention on Biological Diversity (FAO 2008) and by The World Conservation Union [IUCN] which is now promoting pollinator resource protection. The North American Pollinator Protection Campaign [NAPPC] was recently founded to work to protect this essential group (NRC 2007). Furthermore, because pollinators serve as good indicator group for biodiversity and conservation (Kevan 1999), documentation of their current distribution may facilitate conservation monitoring on a larger scale.

1.6 Urban Ecology

With the continuing trend of urbanization worldwide (United Nations Population Division 2005), it is increasingly important that society develop an understanding of urban ecology (Wolch 2007). As city limits grow, urbanization takes over more natural habitats. Urban planners must take care to ensure that the biodiversity is not entirely eliminated within the urban setting. Urban areas are capable of supporting highly diverse arthropod populations (Frankie and Ehler 1978). Because type of habitat seems to have a greater effect on bee populations than does relative density of the habitat, scientists believe that urban landscapes can be developed to promote pollinator conservation (McIntyre and Hostetler 2001).

1.7 Pollinators in the City

In Europe, several studies have investigated the presence of bees in the urban setting (Haeseler 1982; Jacob-Remacle 1984; Kratochwil and Klatt 1989; Torres et al. 1989, Saure 1996; Ahrné et al. 2009). Haeseler (1982) found bees living in and around paved roads and lots. Kratochwil and Klatt (1989) showed that ruderal sites could provide homes for bees in the city, but the most diverse groups of bees were restricted to ruderal sites with high flora diversity, which were restricted to the fringes of the city. This finding was very similar to what Saure (1996) found in Berlin: that bees were most often found in ruderal places and disturbed areas such as abandoned railways.

More recently, a study by Ahrné et al. (2009) supported the notion that habitat has a great effect on bee populations. Their study showed that abundance of bumble bees was

primarily affected by local management, including that of the abundance of floral resources. The study also showed, however, that urbanization has a negative effect on bumble bee populations. The richness of bumble bee diversity decreased significantly with increasing urbanization (Ahrné et al. 2009). Specifically, Zanette et al. (2005) showed that loss of vegetation and increased construction of buildings accounted for devastation of eusocial bee populations in Brazil, and that these bees responded even to small-scale changes in their habitats. That study also demonstrated that generalist species of bees were less affected by urbanization, perhaps in part due to their high abundance (Zanette et al. 2005), a finding which supported earlier work by Taura and Laroca (2001) which showed a similar trend of polylectic bees being favoured in an urban area, perhaps due to the diversified floral resources that were available at different times of the year. Taura and Laroca (2001) also showed that the number of species of bees found in the urban setting were significantly lower than those found in neighbouring (non-urban) plateaus. They did, however, collect enough bees to show a linear relationship between the number of bee species and the \log_{10} of the number of individual bees collected.

Although studies have investigated certain aspects of pollinator ecology within the city, no studies have compared city land-use zones within the same region to one another. Several studies have shown the potential of urban gardens to provide habitats for pollinators (Frankie et al. 2009; Owen 1991; Wojcik et al. 2008). A well known example is from Jennifer Owen (1991) of Leicestershire, England who monitored pollinators in her residential backyard, and over a fifteen year period saw 51 species of bees in her small garden. Though residential gardens are considered to be a significant source of

“green space” within the urban environment (Loram et al. 2007), these spaces are highly fragmented, and therefore should be considered separately from actual green spaces. Not only does fragmentation have a significant effect on population ecology of pollinators (Buchmann and Nabhan 1996, Taki and Kevan 2007, Taki et al. 2007, Taki et al. 2008), residential “green spaces” are personally maintained, while zoned green spaces are maintained by the community, or by special interest groups. Furthermore, new residential areas may have more recently experienced significant disturbance as compared to older residential areas. The two types of residential zones may also have different regulations concerning land use.

Research in urban Californian parks has shown that bumble bee abundance and diversity are dependent on resource availability, both in terms of feeding and nesting resources, rather than park size (McFrederik and LeBuhn 2006). This is supported by the fact that diversity and activity of pollinators is directly linked to floral resource richness and abundance (Steffan-Dewenter and Tschardt 1999). Gardens that have a high diversity of plants attract even more pollinators than the sum of the pollinators attracted to each of those plants individually (Frankie et al. 2005). As well as being affected by floral resources, bee diversity is also affected by seasonal patterns of emergence (Wojcik et al. 2008). Those fluctuations occur at both the species and genus level. Furthermore, in terms of nesting resources, even within what may appear to be a heterogeneous landscape, female bees are highly selective in where they build their nests (Potts and Willmer 1997). That selectivity may restrict the nesting habitats of bees. Matteson et al. (2008) found that in urban gardens in New York City, cavity nesting bees were the most

common species, at 33 percent. This is in contrast with typical distributions, which indicate soil-nesting individuals as the most common species in cityscapes, at around 70 percent of solitary bee species (Frankie et al. 2009).

1.8 Collection of Bees

Native bees are known to travel up to 750 metres from their nesting sites to gather food (Gathmann and Tschardtke 2002, Steffan-Dewenter et al. 2002). The quantity and survival rate of bees' offspring are highly dependent on their mother's ability to acquire both pollen and nectar, so it is important for them to find flowers with abundant resources to minimize their workload. In their quest for food, bees are attracted to blue and yellow, and can therefore be sampled using coloured plastic pan traps, often called "bee bowls", filled with liquid in which the bees perish (Potts et al. 2005) and from which they can be collected. Droege et al. (2010) have been working to optimize collection using pan traps. They have shown that the optimal between-bowl distance for maximum capture rate is between three and five metres. They also showed slight clumping in bee collection, suggesting that nesting aggregations may affect relative collection of bees. However, they also noted that relatively little is known about the relationship between the number of bees collected in pan traps and the true number of bees present in the environment.

In areas where human activities have disrupted natural ecosystems, native bees nest primarily in dry grassy areas, meadows and fallow (Steffan-Dewenter 2002). Hollow tubing can be used to mimic the holes found in these environments, providing extra nesting spots. Trap nests, boxes filled with hollow tubes, have been used successfully for

environmental assessment by providing alternative nesting locations from which eggs and larvae can be collected and later hatched (Danks 1971; Frankie et al. 1998; Godfrey and Hilton 1983; Krombein 1967; Taki et al. 2004; Tscharrntke et al. 1998).

1.9 Identification of Specimens Using DNA Barcoding

DNA barcoding refers to the use of a specific mitochondrial DNA sequence, the cytochrome *c* oxidase I [COI] sequence, for species identification (Hebert et al. 2003). The process requires extraction of DNA, isolation and amplification of the COI gene using special primers, and sequencing of the PCR product. These sequences are uploaded to online databases such as GenBank and Barcode of Life Database [BOLD] (Hebert et al. 2003). The COI sequence is approximately 600 base pairs in length, and is present in almost all species. It is highly conserved within a species, but differs from species to species. High levels of similarity in the sequence indicate more closely related species, thus making it a valuable tool for distinguishing species (Hebert et al. 2003). DNA barcoding has been proposed as a means of defining different species from morphologically cryptic species (Hebert et al. 2003) and for identifying specimens that are too damaged to be identified using standard morphology-based taxonomy. For example, the partially-digested and heavily damaged stomach contents of bats have been identified by Clarke et al. (2009) using this technique, demonstrating that it can also be used to indicate multiple sequences from a blended sample. DNA barcoding has long been used on bees (Danforth 1999) including its recent use in evaluation of the bee fauna of Nova Scotia, Canada (Sheffield et al. 2009), which showed two previously undescribed species.

1.10 Hypothesis and Objectives

My research establishes a baseline of abundance and diversity of pollinators (Hymenoptera: Apoidea) in Guelph, Cambridge, Kitchener and Waterloo in southern Ontario. It defines potential study sites in the study region based on city land-use and evaluates the abundance and diversity of pollinators at selected sites using pan traps, trap nests and sweep netting. It also evaluates floral diversity and special features of the selected sites to indicate whether or not these factors directly affect the abundance and diversity of these pollinators. My project also assesses the efficacy of DNA barcoding as a tool for establishing diversity without using formal morphological taxonomic identification.

Based on the differences in land use between urban land-use zones, it is hypothesized that the abundance and diversity of pollinators varies amongst zones. Furthermore, it is hypothesized that specific features of the sites, including floral diversity and abundance, patches of naturalized land, presence of human traffic and presence of large water bodies account for discrepancies in bee biodiversity amongst individual sites. Finally, it is hypothesized that DNA barcoding provides a faster, simpler and more accurate means by which to ascertain the diversity of bees at each site than traditional taxonomy.

CHAPTER 2: METHODS

2.1 Study Site Selection

All surveying and sampling took place in the summer months (May to September, inclusive) in the cities of Guelph, Cambridge, Kitchener and Waterloo, Ontario. Potential study sites were selected using city zoning maps. Five sites were selected for each of the four land-use types: green, industrial, new residential and old residential (Table 1, Figure 1). These four land-use types were selected because of the different by-laws governing potential uses for the properties as well as maintenance requirements. For example, in residential land-use zones, citizens are typically required to maintain yards with grass less than a specified height, whereas conservation areas or industrial areas would not be subject to such bylaws. Green spaces include both open and wooded green spaces, conservation areas, golf courses, and city parks. Industrial areas include all areas zoned for industrial use, regardless of maintenance or level of use. New residential regions include all residential areas established and built within the last ten years. Old residential regions include those residential areas established and built at least thirty years ago. Distinction was made between new and old residential regions because of the relatively recent disturbance of the new residential areas.

Locations that were demarcated by a 500 metre radius of uniformly zoned land based on zoning maps were selected as possible sites. Uniform zoning was defined as a minimum of 90 percent. The minimum radius of 500 metres was used to minimize visitation by bees from other zones. The intention was to include a 750-metre radius based on the

Table 1. List of selected sites described in terms of city, descriptive location, latitude and longitude, and special features, including the presence of a large naturalized area, pedestrian traffic, and the presence of a significant water body.

	Site Name	City	Descriptive Location	Specific Location		Features		
				Latitude (degrees)	Longitude (degrees)	Naturalized	Traffic	Water Body
Green Space	G1	Guelph	Hanlon Creek Conservation Area	43.509	-80.207	Yes	None	None
	G2	Guelph	Guelph Lake Sports Fields	43.588	-80.254	Yes	Low	Yes
	G3	Waterloo	Grey Silos Golf Course	43.516	-80.487	Yes	Low	Yes
	G4	Kitchener	Homer Watson Park	43.405	-80.436	Yes	None	Yes
	G5	Cambridge	Shade's Mills Conservation Area	43.380	-80.284	Yes	None	Yes
Industrial	I1	Guelph	Southgate Drive	43.488	-80.202	Yes	None	None
	I2	Guelph	Massey Drive	43.541	-80.300	Yes	None	Yes
	I3	Waterloo	Kumpf Drive	43.502	-80.543	Yes	None	Yes
	I4	Cambridge	Savage Road	43.366	-80.287	Yes	Low	None
	I5	Cambridge	Franklin Boulevard	43.403	-80.308	Yes	None	None
New Residential	N1	Guelph	Clairfields Drive	43.506	-80.192	No	Low	None
	N2	Guelph	Edinburgh at Rickson	43.511	-80.211	No	Low	None
	N3	Waterloo	Chesapeake Drive	43.508	-80.505	Yes	None	None
	N4	Kitchener	Max Becker Street	43.405	-80.512	No	Low	None
	N5	Cambridge	McNichol Drive	43.342	-80.275	No	Low	None
Old Residential	O1 (2007)	Guelph	Forest Street	43.531	-80.245	No	Low	None
	O1 (2008)	Guelph	Cambridge Street	43.543	-80.255	No	Low	None
	O2	Guelph	Metcalfe Street	43.554	-80.244	No	Low	None
	O3	Waterloo	Thorndale Drive	43.450	-80.551	No	None	None
	O4 (2007)	Kitchener	Becker Street	43.454	-80.466	No	Low	None
	O4 (2008)	Kitchener	Conestoga Parkway	43.454	-80.467	Yes	Low	None
	O5	Cambridge	Chalmers Street	43.355	-80.298	Yes	None	None

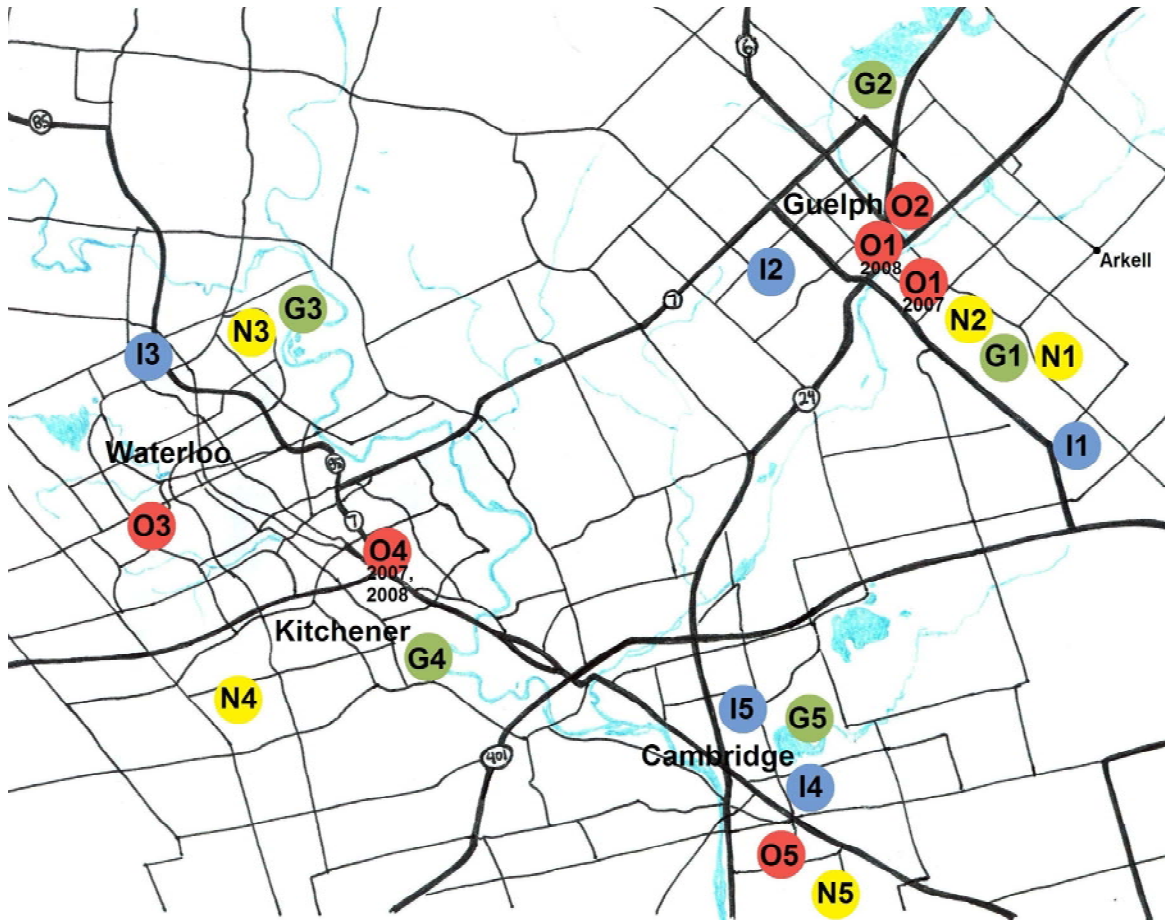


Figure 1. Map of selected collection sites in Guelph, Cambridge, Kitchener and Waterloo.

flight distance of foraging solitary bees (Gathmann & Tschardt 2002, Steffan-Dewenter et al. 2002). However, a 750-metre radius eliminated too many sites to provide a reasonable sample size. Consequently, a slightly smaller radius (500 metres) was used to increase the number of potential sites to allow for more replicates. Following selection, sites were verified by visitation to ensure that the map zoning was not out-of-date, and to evaluate suitability of selected sites. Locations unsuitable for sampling (high human traffic areas and areas with small children or dogs) were discarded.

When twenty final sites had been selected, special features of the land-use zone, including level of human traffic, presence of naturalized areas, and presence of large bodies of water were noted. Human traffic was defined as either low or absent. The presence of a naturalized area was defined as an area of at least 50 square metres of unmanicured property, where all plants were permitted to bloom. The presence of large body of water was defined based on a surface area of 50 square metres of water within a 100-metre radius of the centre of the land-use zone.

The floral richness was evaluated by photographing all plants in bloom within a radius of approximately 10 metres from the site center. Floral abundance was measured by approximating the percentage blossom cover within the same radius. Blossom cover was estimated visually for each plant species based on one square foot and one square metre quadrats. The sum of all plant species at a given site gave a total area of blossom cover which was converted into a percentage based on a ten-metre radius around the site centre (314 square metres). The floral richness and abundance were reevaluated every three

weeks throughout the summer of 2008. Floral richness and abundance evaluations were not conducted during the summer of 2007.

2.2 Pan Traps

Three blue and three yellow bowls from the Solo Cup Company (Chicago, Illinois) were used at each site. Bowls of the two colours were paired with at least five metres between each pair of bowls. The bowls were not placed in maintained grass to prevent disturbance to lawn maintenance routines. Bowls were set slightly set into the ground, and each was individually covered with 2.5-centimetre chicken wire held down with tent pegs (Figure 2). Approximately 200 millilitres of 75% propylene glycol (food grade) was poured into the bowls and was changed based on visual and olfactory assessment.

Specifically, discoloured, highly diluted or malodorous propylene glycol was replaced with fresh. The content of the bowls was retrieved biweekly, and was preserved in sealed, refrigerated containers with 95 percent ethanol. The bees collected in each sample were later pinned and identified for species richness and abundance measurements. Richness, or the alpha diversity, was measured by the number of species and the number of genera collected. The total number of identified species will is referred to as the “minimum number of species”; the potential maximum number of species based on specimens identified only to genus is referred to as the “maximum number of species”. Both the minimum and the maximum number of species collected were recorded to account for specimens that could not be identified to the species level. “Abundance” was measured as the total number of bees collected.



Figure 2. Pan traps were set slightly into the ground to avoid tipping and were covered with 2.5-centimetre chicken wire held down with tent pegs. The bowls were filled with approximately 200 millilitres of 75% propylene glycol.

The Shannon-Wiener Index was calculated using the formula

$$H' = - \sum_{i=1}^s (p_i \ln p_i) - [(S-1) / 2N]$$

where H' is the Shannon Index value, n_i is the abundance of species i , S is the species richness, N is the total number of specimens, and p_i is the relative abundance (n_i/N) (Shannon and Weaver 1949; Magurran 1988).

Due to difficulty in obtaining permissions to collect in certain sites, sampling in 2007 was delayed until late June, rather than beginning in May as planned. Collection with pan traps was constant from the end of June until the first week of September for all sites. Large numbers of bees were collected during the summer of 2007, so sampling was reduced for the summer of 2008. In 2008, sampling started in early May and continued until September, but collections were only performed for the first week of each three-week period to prevent oversampling and potential negative impacts on the pollinator populations.

2.3 Trap Nests

Trap nests were constructed within a two-litre milk carton as described by Taki et al. (2004) (Figure 3). Nine tubes, each of four diameters (3, 5, 7, and 9 millimetre) of paper tubes 15 centimetres in length (Jonesville Paper Tube Corporation, Jonesville, Michigan) were held at equal spacing by Styrofoam. The carton was covered in burlap to naturalize the look of the box. Three trap nesting boxes were allocated to each site, at ground level, at one and at two meters elevation. Established surroundings, such as pre-existing fences, were used preferentially over temporary fixtures for the trap nests. Filled tubes were

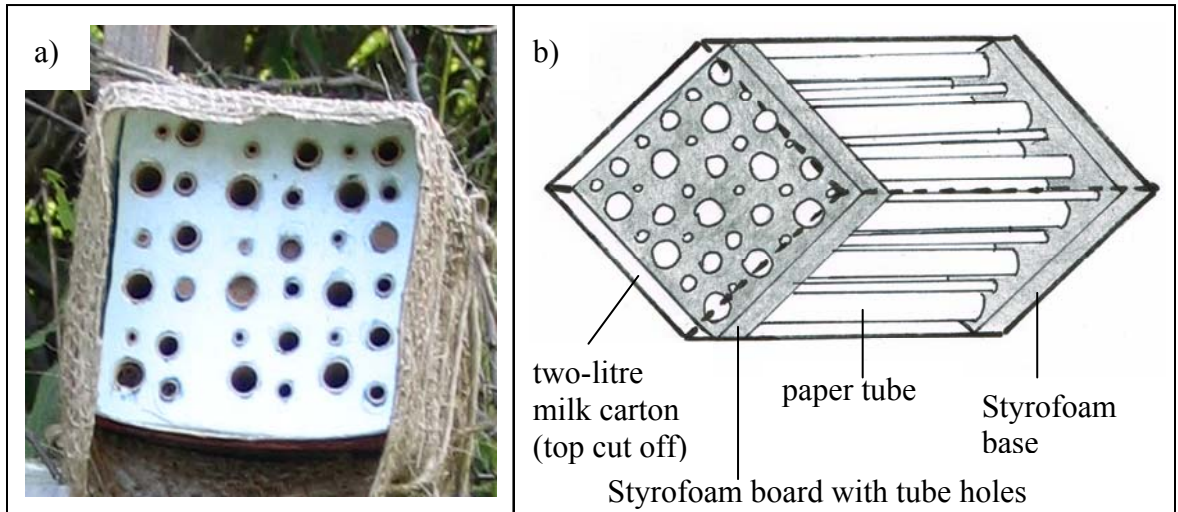


Figure 3. a) Partially filled trap nest box and b) internal view of a trap nest box, constructed from an unused two-litre milk carton, Styrofoam board, 15-centimetre paper tubes with 3, 5, 7 and 9 millimetre diameters, wrapped in burlap.

collected monthly and labeled according to site and trap nest. The filled tubes were placed inside polyvinylchloride [PVC] casings with metal screening on either end to allow the pupae to eclose. This protected the tubes from rain while allowing them to be exposed to temperature changes, but prevented the insects from escaping. Once eclosed, the contents of the trap nest tubes were pinned and identified for abundance and richness measurements. During the summer of 2007, trap nest tubes were collected monthly. During the summer of 2008, the tubes were collected once every three weeks, in synchrony with pan trap collection.

2.4 Sweep Netting

Sweep netting was performed once in June and once in July using a generic 15"-diameter white aerial net. One hundred strokes were made over flowering plants at each site. These sweeps were non-destructive to avoid damage to residential gardens. The bees caught in the sweep nest were preserved in ethanol for subsequent pinning and identification, and were to be used for the purpose of measuring richness only. Due to a negative reaction from some residential site owners who were concerned about destruction of their plants, as well as difficulty in performing sweeps with uniformity from one site to the next, sweep netting was conducted only during the summer of 2007. The richness data were not analyzed statistically because of the impact that the drastic variance in the numbers of bees caught at each site would have had on the measure of richness.

2.5 Efficacy of Surveying Using Pan Traps

There was an extreme discrepancy between the numbers of bees observed on plants and

numbers collected in the pan traps. Consequently, a separate experiment was completed to examine the role of proximity of pan traps to flowers and the level of disparity between bees observed on plants and bees captured in pan traps. This experiment took place at Arkell Research Farm on the outskirts of Guelph. Four small gardens were created using a variety of potted, pollinator-friendly plants from nurseries and from natural growth along roadsides where they were blooming. These plants included shrubby cinquefoil (*Potentilla fruticosa* Linnaeus), Missouri ironweed (*Vernonia missurica* Rafinesque), giant yellow hyssop (*Agastache nepetoides* Kuntze), Culver's root (*Veronicastrum virginicum* Linnaeus), grey-headed coneflower (*Ratibida pinnata* (Ventenat) Barnhart), yellow cup plant (*Silphium perfoliatum* Linnaeus), blue lobelia (*Lobelia inflata* Linnaeus), Russian sage (*Perovskia atriplicifolia* Bentham), tansy (*Tanacetum vulgare* Linnaeus), sweet white clover (*Melilotus alba* Medikus), an unknown species of goldenrod (*Solidago* sp.) and an unknown species of purple thistle. The first six were selected based on a study by Tuell et al. (2008) which showed these plants to be particularly attractive to native bees. The other plants were selected from those growing along the roadsides based on anecdotal observations of pollinator attraction. Almost all of these plants were included in each garden, though not all were available in each case. Pots were arranged with the tallest plants in the centre, and were supported with stakes and string.

Pan traps were placed in blue and yellow pairs at 0, 5, 10, 25, 50 and 100 metres from the gardens on two transects a minimum of 90 degrees apart. All other blooms within a 100-

metre radius of the pan traps were removed manually. The contents of the pan traps were collected twice over a period of one week.

During this same week, visits to the flowers in each garden were observed for two 15-minute periods on each of three separate days. These observations took place only on so-called “good bee days,” which were defined as days where the temperature was above 18 degrees Celsius and there was little wind. The observer sat as still and as quietly as possible in a chair approximately one metre from the garden and observed insect activity on these plants. Particular attention was paid to the number of bees that visited the gardens. This experiment took place over the week of August 1 to 8, 2008, and was repeated three weeks after the first trial from August 20 to 27, 2008.

2.6 Identification and DNA Barcoding

All Apoidea specimens collected underwent two methods of identification. Final identifications were made by Dr. Cory Sheffield using traditional morphological taxonomy. Specimen vouchers were pinned and labeled, after collection of legs for barcoding, to be stored with the Bee Collections at York University, Ontario.

Prior to manual identification, samples were to be identified using DNA barcoding (Hebert et al. 2003) at the Biological Institute of Ontario [BIO] through the Barcode of Life Database [BOLD] Laboratories, University of Guelph. All samples were stored in ethanol prior to their treatment for submission to BIO. Sterilization of all forceps and scissors was performed using ethanol and flame before handling of each specimen. The

specimen was removed from the sample vial and the bottom segment of its mesothoracic left leg was excised and placed in an individual well in the sample plate and was covered with ethanol. Within BIO, DNA was extracted from the samples and was processed using the facility's standard protocols. The COI sequence in each sample was amplified using LepF1 (5'-ATT CAA CCA ATC ATA AAG ATA TTG G-3') and LepR1 (5'-TAA ACT TCT GGA TGT CCA AAA AAT CA-3') polymerase chain reaction [PCR] primers. The PCR products were sequenced, and these sequences were uploaded by BIO to BOLD. Using BOLD, sequences from a subsample of 645 randomly selected specimens were matched to existent sequences to indicate possible identifications. Any sequence matching $\geq 97\%$ to a previously identified sequence was considered a positive match. For each selected sample, the number of positive identifications was recorded.

2.7 Data Analyses

All statistical analyses of the data were performed using SPSS using a Type I error rate of 0.05 for a 95 percent confidence level. Abundance and richness distributions were tested for normality. When data were normally distributed based on a Shapiro-Wilk test for normality, site types were compared using ANOVA. When they were not normally distributed, site types were compared using a Kruskal-Wallis non-parametric test. A Kolmogorov-Smirnov test was used to compare distribution of abundances and diversities between sites with specific features: naturalized versus non-naturalized, with water versus without, and low-traffic versus no-traffic sites. A curve fit test was used to indicate if there were relationships between floral richness or percentage blossom cover and the abundance or richness of bees. If these relationships were linear, a Pearson's correlation

was used to assess the nature of the correlation. Temporal variations for each year were evaluated using ANOVA if the data were normally distributed or a Kruskal-Wallis non-parametric test if they were not. Any significant values for the Kruskal-Wallis non-parametric test were evaluated post-hoc using Mann-Whitney tests to indicate the specific nature of the significant relationships. A paired-samples T-test was used to compare collections from 2007 to those from 2008 in four matching weeks of sampling at the 18 sites that remained the same between the two years. A paired-sample T-test was also used to compare collections from yellow and blue pan traps to indicate whether either was more effective in attracting bees. The number of trap nest tubes filled at each site was tested for normality using a Shapiro-Wilk test, and then compared site-wise using ANOVA or Kruskal-Wallis non-parametric tests. Significant values for the Kruskal-Wallis non-parametric test were evaluated post-hoc using Mann-Whitney tests.

The distribution of the bees acquired at Arkell Farm using pan traps at various distances from a floral centre was tested for normality using a Shapiro-Wilk test. The non-normally distributed results were analysed using a Kruskal-Wallis non-parametric test.

CHAPTER 3: RESULTS

3.1 Abundance and Diversity by City Land-use Zone

A total of 1592 bees were collected in pan traps at twenty sites (Table 1) over the two summers of collection, with 1126 collected in 2007 (Table 2) and 466 collected in 2008 (Table 3). The bees included over 94 species from 23 genera. Sixty-seven species from 23 genera were collected in 2007 (Table 4), and 69 species from 21 genera were collected in 2008 (Table 5). The most abundant species in 2007 was *Halictus ligatus*, with 453 specimens collected, and in 2008 it was *Megachile rotundata* Fabricius with 52 specimens collected. The number of specimens collected at individual sites varied from 1 to 413 in 2007 (Figure 4) and from 0 to 88 in 2008. The minimum number of species varied from 1 to 21 in 2007 and from 0 to 28 in 2008. The maximum number of species in 2007 was between 1 and 50, and in 2008 it was between 0 and 36 (Figure 5). The accumulation curve acquired in this study (Figure 6) does not fit with linear, logarithmic, inverse, quadratic or cubic curves ($p = 0.000$, $p = 0.000$, $p = 0.000$, $p = 0.000$ and $p = 0.000$, respectively).

The minimum number of species, maximum number of species, number of genera, abundance, Shannon-Wiener value based on the minimum numbers of species, and the Shannon-Wiener value based on the maximum numbers of species were all tested using the Shapiro-Wilk test for normality. Except for the minimum number of species in 2007 ($p = 0.025$), abundance per site in 2007 ($p = 0.000$) and 2008 ($p = 0.013$), and minimum ($p = 0.003$) and maximum ($p = 0.004$) Shannon-Wiener values for 2007, all data sets

Table 2. Description of collection of bees by yellow and blue pan traps at each site in 2007 by total number of identified species (listed as minimum number of species), potential maximum number of species based on specimens identified only to genus, number of genera, abundance of bees, rank in terms of abundance of bees, Shannon-Wiener value for minimum number of species, and Shannon-Wiener value for maximum number of species.

Site	Minimum # of Species	Maximum # of Species	# of Genera	Abundance	Rank by Abundance	Shannon-Wiener for Min # of Species	Shannon-Wiener for Max # of Species
O1	16	17	10	50	5	2.0860	2.1138
O2	7	8	7	18	12	1.4571	1.5341
O3	6	6	4	12	15	1.3498	1.3498
O4	7	8	6	10	16	1.8344	1.9730
O5	20	32	13	78	3	2.4495	2.8496
N1	1	1	1	1	19	0.0000	0.0000
N2	1	1	1	1	19	0.0000	0.0000
N3	16	19	9	52	4	2.4195	2.5096
N4	2	2	2	2	7	0.6931	0.6931
N5	7	20	5	23	9.5	1.3255	2.9319
I1	17	25	11	41	6	1.8605	3.0073
I2	21	50	11	282	2	2.3098	2.6040
I3	1	1	1	1	19	0.0000	0.0000
I4	14	22	10	413	1	0.8192	0.8751
I5	11	12	8	18	12	2.2931	2.3701
G1	17	17	10	38	7	2.2654	2.2654
G2	12	14	7	18	12	2.3222	2.5053
G3	8	10	6	17	14	1.7873	1.9504
G4	9	11	8	23	9.5	1.8686	2.0119
G5	15	16	12	28	8	2.5123	2.5618

Table 3. Description of collection of bees by yellow and blue pan traps at each site in 2008 by total number of identified species (listed as minimum number of species), potential maximum number of species based on specimens identified only to genus, number of genera, abundance of bees, rank in terms of abundance of bees, Shannon-Wiener value for minimum number of species, and Shannon-Wiener value for maximum number of species.

Site	Minimum # of Species	Maximum # of Species	# of Genera	Abundance	Rank by Abundance	Shannon-Wiener for Min # of Species	Shannon-Wiener for Max # of Species
O1	6	6	5	7	14	1.7479	1.7479
O2	2	2	2	2	16	0.6931	0.6931
O3	8	8	6	12	11	1.6957	1.6957
O4	20	23	14	59	2	2.3015	2.3574
O5	12	12	9	29	8	1.9604	1.9604
N1	1	1	1	1	18	0.0000	0.0000
N2	2	2	2	2	16	0.6931	0.6931
N3	25	32	15	88	1	2.8913	3.0458
N4	0	0	0	0	19.5	---	---
N5	8	8	7	9	12	2.0432	2.0432
I1	19	20	10	35	6	2.7560	2.7956
I2	12	17	9	34	7	2.3952	2.6553
I3	0	0	0	0	19.5	---	---
I4	19	20	12	39	5	2.7165	2.7521
I5	15	16	11	20	10	2.6262	2.6892
G1	28	36	13	49	3	3.1794	3.3491
G2	15	15	9	26	9	2.4844	2.4844
G3	7	7	6	8	13	1.9062	1.9062
G4	2	2	2	2	16	0.6931	0.6931
G5	21	30	13	44	4	2.8172	3.2010

Table 4. Abundance of each bee species collected using yellow and blue pan traps in 2007 by site (continued next page).

Family	Species	Site															Total									
		G1	G2	G3	G4	G5	I1	I2	I3	I4	I5	N1	N2	N3	N4	N5		O1	O2	O3	O4	O5				
Andrenidae	<i>Andrena hirticincta</i> (Provancher)																				1			2		
	<i>Andrena nasonii</i> (Robertson)								1																1	
	<i>Andrena wilkella</i> (Kirby)						3											1							4	
	Unknown <i>Andrena</i> species	1	1																						2	
	<i>Calliopsis andreniformis</i> (Smith)					3																				3
	<i>Apis mellifera</i> (Linnaeus)	1			1			36	1									1	4	1	1					47
	<i>Bombus fervidus</i> (Fabricius)																1									1
	<i>Bombus impatiens</i> (Cresson)						1		1									1								3
	<i>Bombus rufocinctus</i> (Cresson)						4		4		3															9
	Unknown <i>Bombus</i> species						1																			1
Apidae	<i>Melissodes druriella</i> (Kirby)				3													1							6	
	<i>Ceratina calcarata</i> (Robertson)	1	1	1																						6
	<i>Ceratina dupla</i> (Say)	1				2	2	1										1								13
	<i>Ceratina</i> sp.	4				1	1	1					1													8
	<i>Holcoposites calliposidis</i> (Linsley)					1																				1
	<i>Nomada lepida</i> (Cresson)	1																								1
	<i>Nomada</i> species1					1																				2
	<i>Nomada</i> species6																									1
	<i>Xylocopa virginica</i> (Linnaeus)																									1
	<i>Hylaeus annulatus</i> (Linnaeus)	1																								1
Colletidae	Unknown <i>Hylaeus</i> species	1				2	4	20	3								1	2								36
	<i>Agapostemon virescens</i> (Fabricius)																									3
	<i>Augochlorella aurata</i> (Smith)	1	1		2	1	3	48	36	2							1									109
	<i>Augochlorella pura</i> (Say)																									1
	<i>Halictus confusus</i> (Smith)	13	2	7	9	5	2	21	27	2	1						3	22	2	7						134
	<i>Halictus ligatus</i> (Say)	6			2	4	3	76	329								5	1	2	5						453
	<i>Halictus rubicundus</i> (Christ)																1									2
	<i>Lasioglossum anomalum</i> (Robertson)	1				1	6	10																		23
	<i>Lasioglossum coriaceum</i> (Smith)		1																							1
	Halictidae	<i>Lasioglossum cressonii</i> (Robertson)																	2							
<i>Lasioglossum divergens</i> (Lovell)							1			1											3					5
<i>Lasioglossum ellisiae</i> (Sandhouse)																								1		1
<i>Lasioglossum leucozonium</i> (Schrank)																								1		2
<i>Lasioglossum lineatulum</i> (Crawford)				2									1													3

Table 4. (continued)

Family	Species	Site																		Total		
		G1	G2	G3	G4	G5	I1	I2	I3	I4	I5	N1	N2	N3	N4	N5	O1	O2	O3		O4	O5
Halictidae	<i>LasioGLOSSUM oenotherae</i> (Stevens)	1																				1
	<i>LasioGLOSSUM versatum</i> (Robertson)		1													1						2
	Unknown <i>LasioGLOSSUM</i> species	1	2	3	3	5	10	9	2	2	3	14	2	2	1	2	1	2	1	2	12	68
	<i>Sphecodes carolinus</i> (Mitchell)	1		1								1										3
	<i>Sphecodes pimpinellae</i> (Robertson)					1																1
	<i>Sphecodes species1</i>					1																1
	<i>Sphecodes species2</i>	1				1	2															1
	Unknown <i>Sphecodes</i> species	1	3					1		1												1
	Unknown <i>Sphecodes</i> species	1						1		1						2					4	11
	<i>Anthidium manicatum</i> (Linnaeus)				1											2		10				10
<i>Chelostoma campanularum</i> (Kirby)																					1	
Unknown <i>Chelostoma</i> species																					1	
<i>Coelioxys rufitarsis</i> (Smith)								10				1									1	
Unknown <i>Coelioxys</i> species																					1	
<i>Heriades leavitti</i> (Crawford)																					1	
Unknown <i>Heriades</i> species																					1	
<i>Hoplitis anthocopoides</i> (Schenck)	1					4	1														2	
<i>Hoplitis producta</i> (Cresson)							1														1	
<i>Hoplitis spoliata</i> (Provancher)						1	1														2	
Unknown <i>Hoplitis</i> species						2															2	
<i>Megachile brevis</i> (Say)						5										5					10	
<i>Megachile campanulae</i> (Roberts)						1															1	
<i>Megachile centuncularis</i> (Linnaeus)									1												1	
<i>Megachile frigida</i> (Smith)										1											1	
<i>Megachile inermis</i> (Provancher)	1															1					6	
<i>Megachile latimanus</i> (Say)						3	1	22	2	3					1						1	
<i>Megachile relativa</i> (Cresson)	1						1								4	1					7	
<i>Megachile rotundata</i> (Fabricius)						9									1						9	
<i>Megachile texana</i> (Cresson)	1					1									1						5	
<i>Osmia coerulea</i> (Linnaeus)																					1	
<i>Osmia conjuncta</i> (Cresson)	5					1															6	
<i>Osmia similissima</i> (Smith)	1					1															2	
<i>Stelis trypetina</i> (Robertson)						1															1	
Unknown <i>Stelis</i> species									1												1	
Total by Site		38	18	17	23	28	41	282	1	413	18	1	1	52	2	23	50	18	12	10	78	1126

Table 5. Abundance of each bee species collected using yellow and blue pan traps in 2007 by site (continued next page).

Family	Species	Site															Total							
		G1	G2	G3	G4	G5	I1	I2	I3	I4	I5	N1	N2	N3	N4	N5		O1	O2	O3	O4	O5		
Andrenidae	<i>Andrena cressonii</i> (Robertson)					3																	3	
	<i>Andrena imitatrix</i> (Cresson)	1																					1	
	<i>Andrena integra</i> (Smith)													1							1		2	
	<i>Andrena melanothroa</i> (Cockerell)													1									1	
	<i>Andrena miserabilis</i> (Cresson)				1																		1	
	<i>Andrena nasonii</i> (Robertson)	1	2			6			1	1													11	
	<i>Andrena nivalis</i> (Smith)			1																	1		1	
	<i>Andrena persimulata</i> (Viereck)									1													1	
	<i>Andrena rugosa</i> (Robertson)																						1	
	<i>Andrena wheeleri</i> (Graenicher)	1					2																3	
	<i>Andrena wilkella</i> (Kirby)										1			3									4	
	Unknown <i>Andrena</i> species	4	1			4					2			1									12	
	<i>Calliopsis andreniformis</i> (Smith)					2																	2	
	Apidae	<i>Apis mellifera</i> (Linnaeus)	1				4				2												1	9
		<i>Bombus impatiens</i> (Cresson)	5	3	1		1			3		1				1						2	1	18
		<i>Bombus rufocinctus</i> (Cresson)	1												1									2
		<i>Melissodes desponsa</i> (Smith)																						1
<i>Ceratina calcarata</i> (Robertson)		1								2				4	2	2						2	1	15
<i>Ceratina dupla</i> (Say)		4	1	1	1	4	1		2	1	1			7					1			1	25	
<i>Ceratina</i> sp.		1	6				1	1	5	1				2								2	1	20
<i>Nomada articulata</i> (Smith)		1																				1	2	
<i>Nomada denticulata</i> (Robertson)		1								1				1									4	
<i>Nomada</i> species2																							1	
<i>Nomada</i> species3																							1	
<i>Nomada</i> species4		1													2								3	
<i>Nomada</i> species6		1	1			1																	3	
<i>Nomada</i> species7		1	1				1		1													2	6	
Unknown <i>Nomada</i> species							1																2	
Colletidae		<i>Xylocopa virginica</i> (Linnaeus)																						1
		<i>Hylaeus modestus</i> (Say)					2			1													1	5
	Unknown <i>Hylaeus</i> species	2	1			3	3		1	1			5										16	
Halictidae	<i>Agapostemon virescens</i> (Fabricius)								1													2	6	
	<i>Augochlorella aurata</i> (Smith)	2				1	2	5	6	1			5							1		1	24	
	<i>Halictus confusus</i> (Smith)	4	1			3	3	3	3				1									3	22	
	<i>Halictus ligatus</i> (Say)	1							6	3			1								1	2	14	

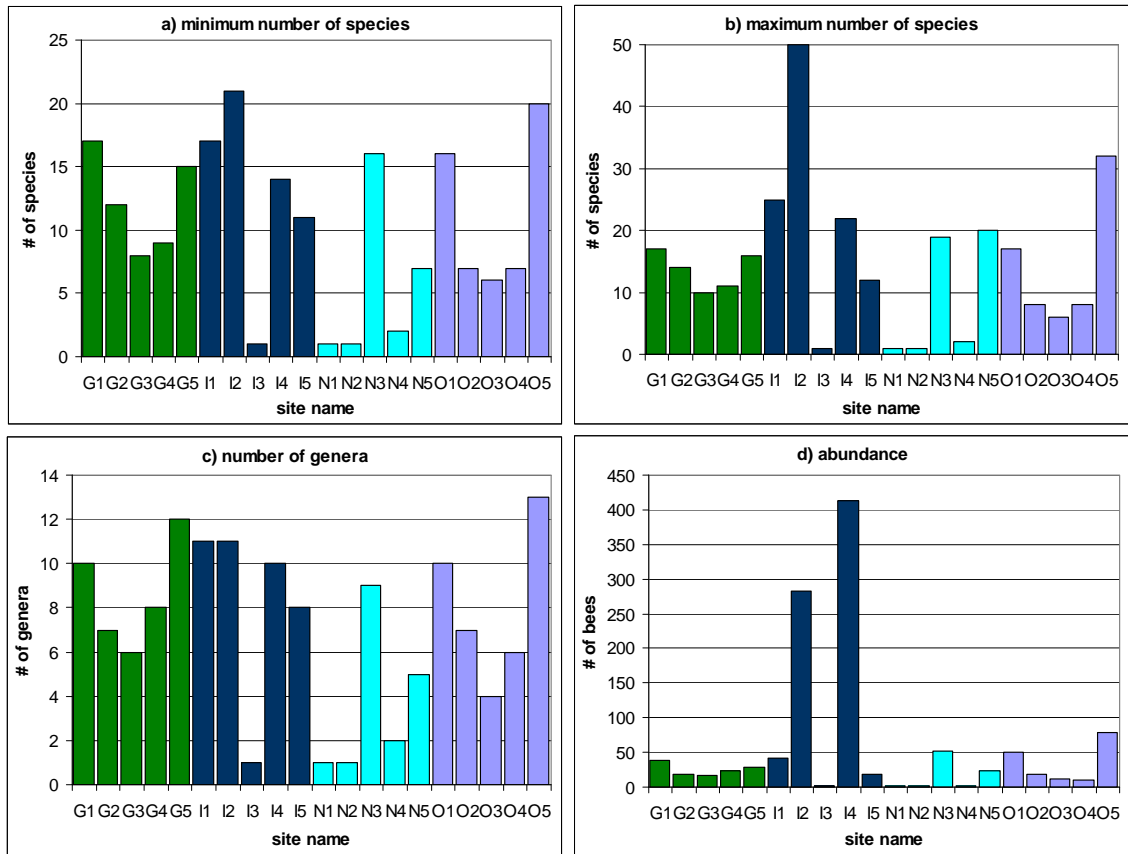


Figure 4. Specimens collected in blue and yellow pan traps in 2007, by site: a) the total number of identified species (listed as minimum number of species), b) the potential maximum number of species (based on specimens identified only to genus), c) the number of genera, and d) the abundance of bees.

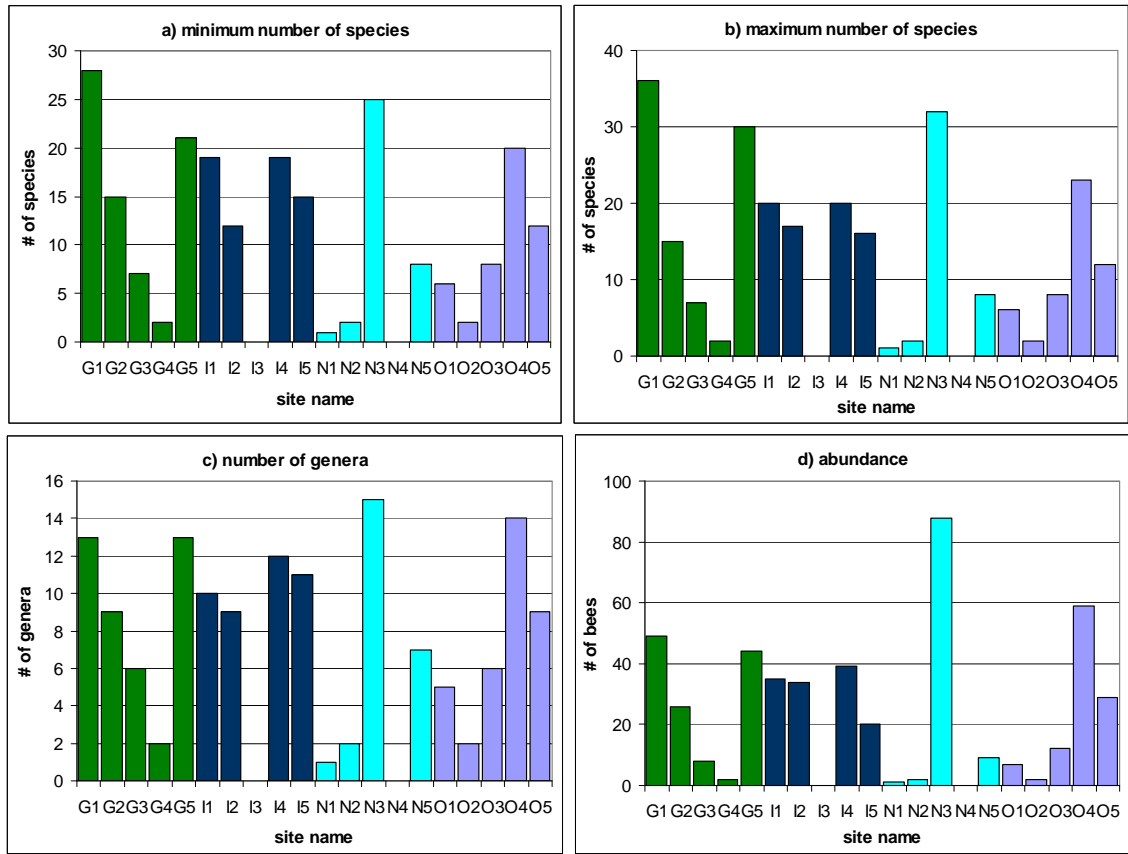


Figure 5. Specimens collected in blue and yellow pan traps in 2008, by site: a) the total number of identified species (listed as minimum number of species), b) the potential maximum number of species (based on specimens identified only to genus), c) the number of genera, and d) the abundance of bees.

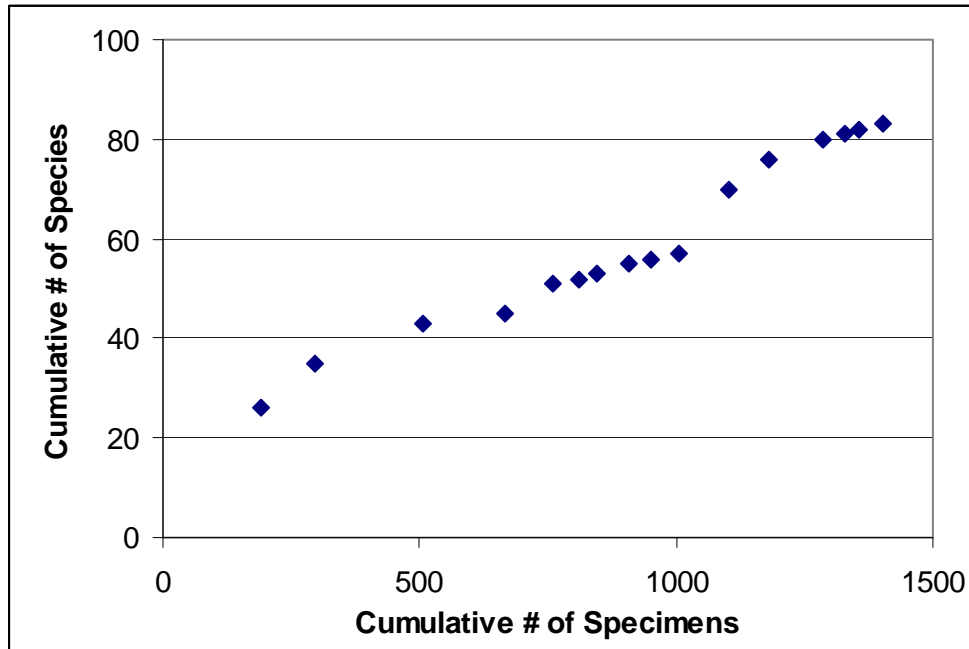


Figure 6. Accumulation curve showing cumulative number of species relative to the cumulative number of specimens collected in blue and yellow pan traps over the summers of 2007 and 2008. No significant correlations were found ($\alpha \leq 0.05$).

were normally distributed (Table 6). ANOVAs of the normally distributed data sets and Kruskal-Wallis non-parametric tests of the non normal data sets showed no significant variation by site type for any value of richness or abundance of bees (Table 6).

3.2 Abundance and Diversity of Bees by Feature

Various site-specific features were evaluated to assess their potential effects on abundance and diversity of bees (Figure 7, Figure 8). The minimum and maximum number of bee species and the number of genera in naturalized areas were significantly higher than those of non-naturalized areas in 2007 ($p = 0.028$, $p = 0.005$, and $p = 0.047$, respectively) and in 2008 ($p = 0.009$, $p = 0.009$, and $p = 0.009$, respectively). The abundance in naturalized areas was also significantly higher than that of non-naturalized areas but only in 2008 ($p = 0.009$). However, there were no significant differences between areas with light traffic and no traffic in 2007 or 2008, or between areas with and without bodies of water in either 2007 or 2008 (Table 7).

The minimum and maximum number of bee species ($p = 0.001$, $p = 0.002$), the number of bee genera ($p = 0.002$), and the abundance of bees ($p = 0.018$) were all negatively correlated to the floral richness (Figure 9) according to Pearson's correlation. However, comparison between the percentage blossom cover and the number of bee species, genera, and the abundance of bees (Figure 10) showed no apparent trends using fits to linear, logarithmic, inverse, quadratic and cubic curve (Table 8).

Table 6. P-values for Shapiro-Wilks test for normality of the total number of identified species (listed as minimum number of species), the potential maximum number of species (based on specimens identified only to genus), the number of genera, the abundance of bees, the Shannon-Wiener value for minimum number of species, and the Shannon-Wiener value for maximum number of species, and for comparison by site type (green, industrial, new residential and old residential) using ANOVA or Kruskal-Wallis non-parametric tests. * denotes significance ($\alpha \leq 0.05$).

	2007			2008		
	Shapiro-Wilks Normality	ANOVA	Kruskal-Wallis Non-Parametric	Shapiro-Wilks Normality	ANOVA	Kruskal-Wallis Non-Parametric
Minimum # of Species	0.025*	---	0.255	0.072	0.577	---
Maximum # of Species	0.214	0.380	---	0.158	0.571	---
# of Genera	0.199	0.116	---	0.182	0.673	---
Abundance	0.000*	---	0.492	0.013*	---	0.659
Shannon-Weiner for Min # of Species	0.003*	---	0.216	0.052	0.213	---
Shannon-Weiner for Max # of Species	0.004*	---	0.160	0.133	0.184	---

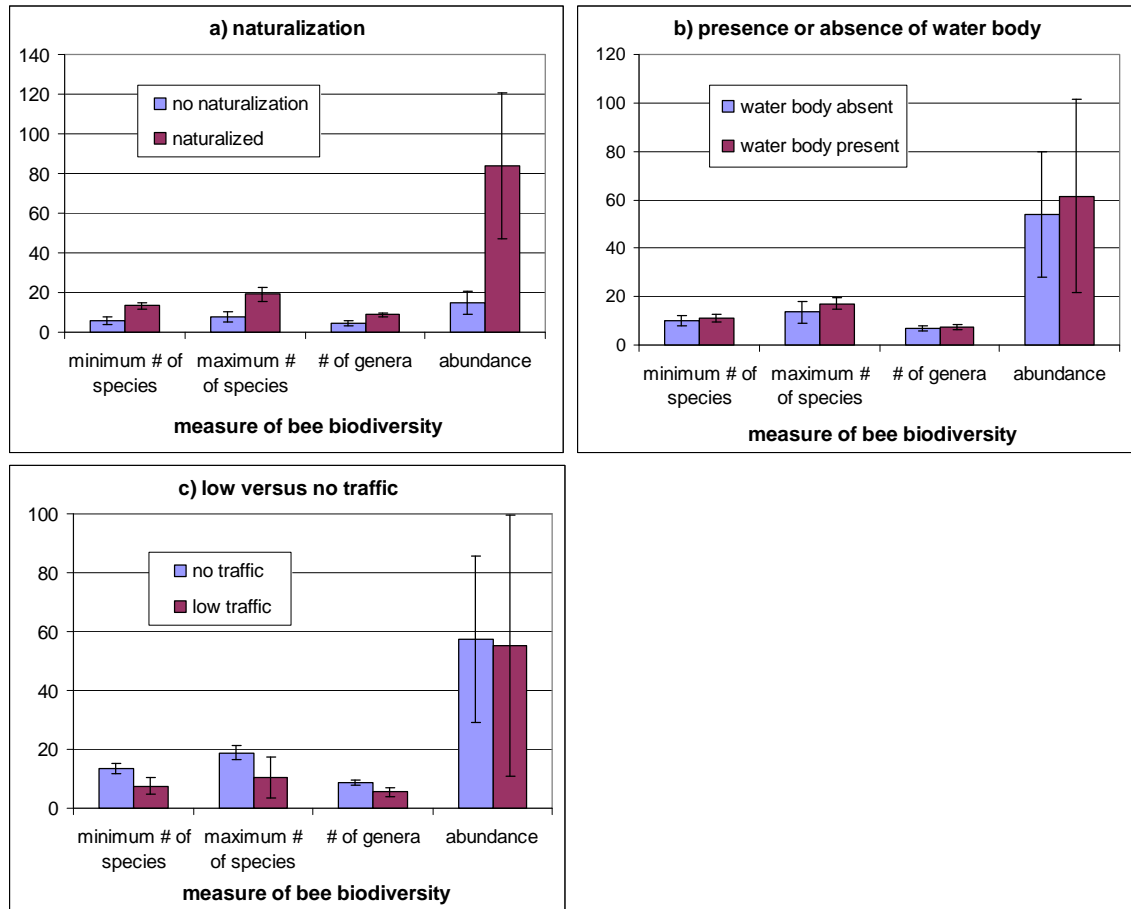


Figure 7. Comparison between a) areas with and without a naturalized area of at least 50 square metres, b) presence and absence of a water body of at least 50 square metres, and c) low versus no human traffic in terms of average number of identified species (listed as minimum number of species), average potential maximum number of species (based on specimens only identified to genus), average number of genera, and average abundance of bees collected in blue and yellow pan traps in 2007, all +/- standard error.

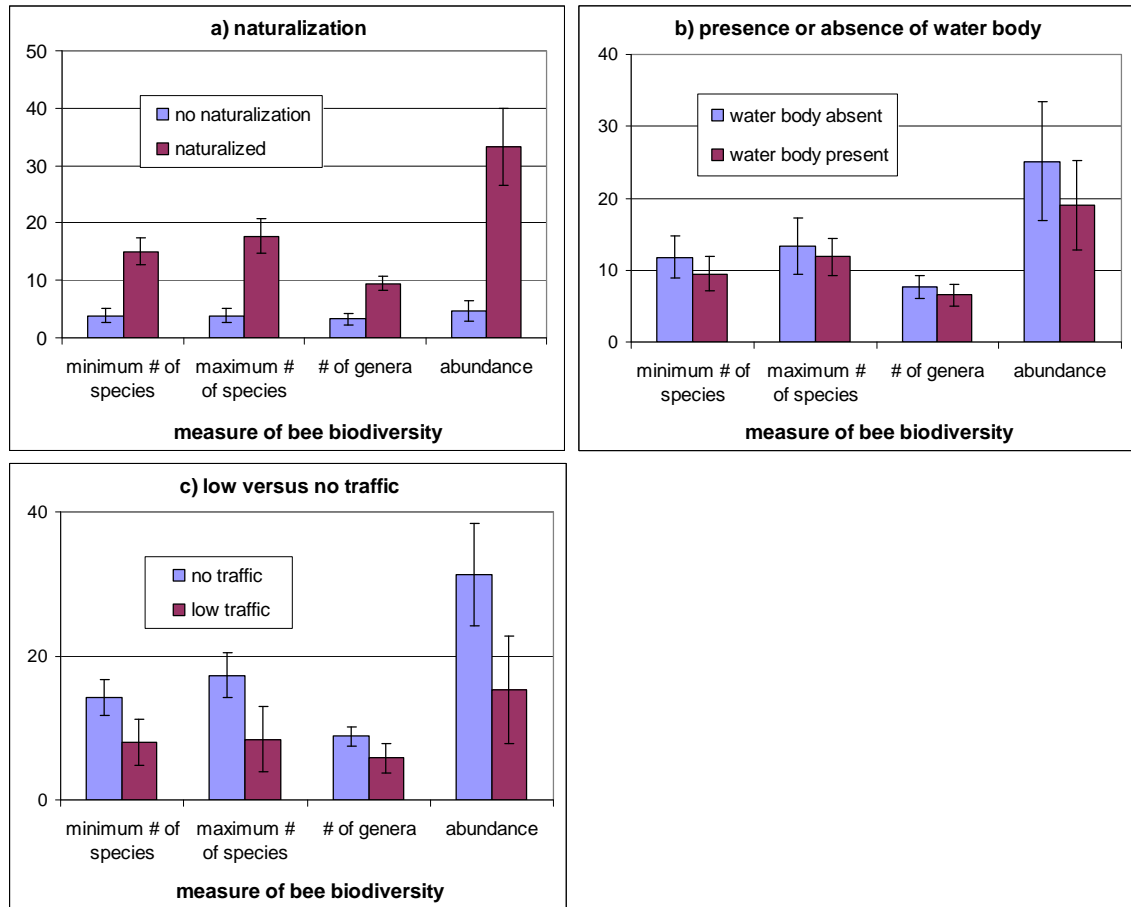


Figure 8. Comparison between a) areas with and without a naturalized area of at least 50 square metres, b) presence and absence of a water body of at least 50 square metres, and c) low versus no human traffic in terms of average number of identified species (listed as minimum number of species), average potential maximum number of species (based on specimens only identified to genus), average number of genera, and average abundance of bees collected in blue and yellow pan traps in 2008, all +/- standard error.

Table 7. P-values from Kolmogrov-Smirnov test between areas with and without a naturalized area of at least 50 square metres, with and without a water body of at least 50 square metres, and with low versus no human traffic in terms of average number of identified species (listed as minimum number of species), average potential maximum number of species (based on specimens only identified to genus), average number of genera, and average abundance of bees collected in blue and yellow pan traps in 2007 and 2008. * denotes significance ($\alpha \leq 0.05$).

	2007			2008		
	Presence and Absence of Naturalized Areas	Low and No Traffic	Presence and Absence of Water bodies	Presence and Absence of Naturalized Areas	Low and No Traffic	Presence and Absence of Water bodies
Minimum # of Species	0.028*	0.739	0.400	0.009*	0.998	0.400
Maximum # of Species	0.005*	0.739	0.164	0.009*	0.998	0.400
# of Genera	0.047*	0.998	0.055	0.009*	0.936	0.400
Abundance	0.120	0.936	0.400	0.009*	0.900	0.164

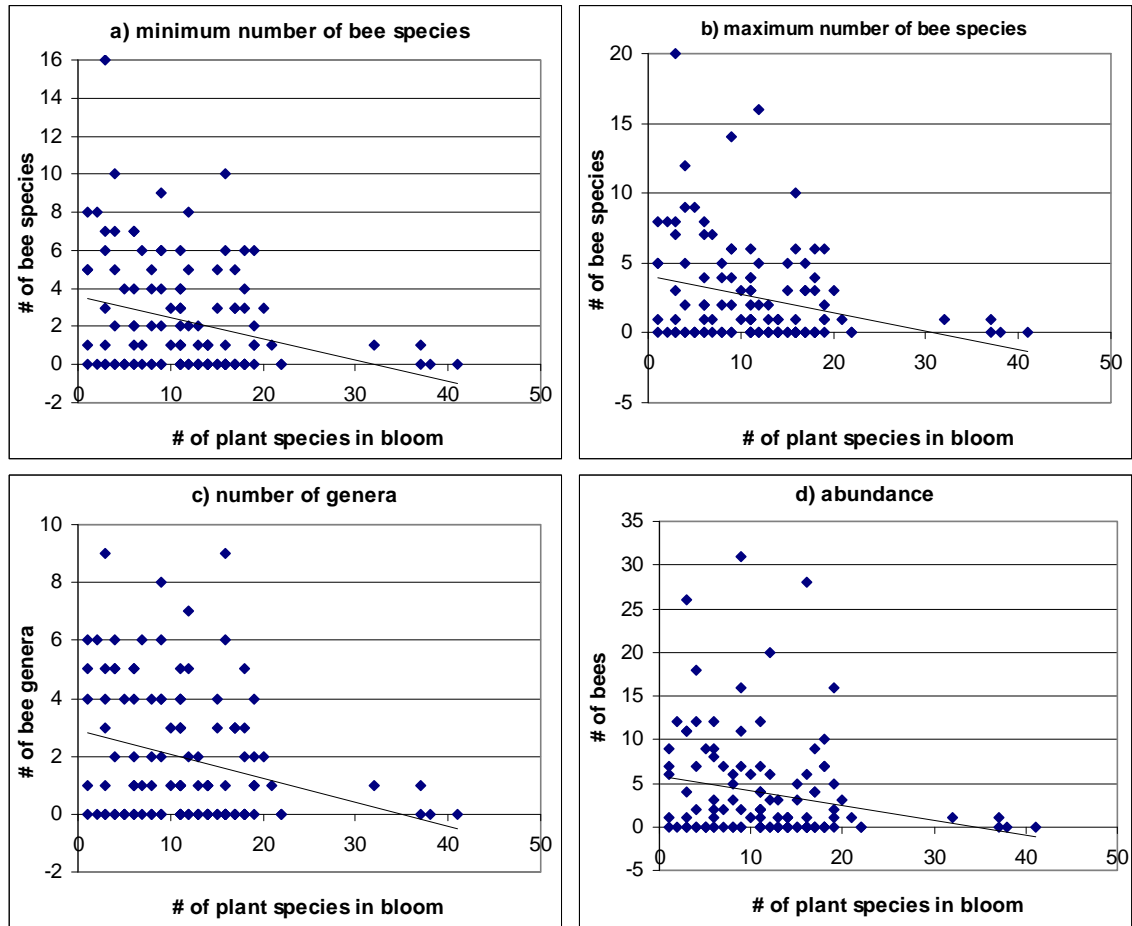


Figure 9. Relationship between a) total number of identified species (listed as minimum number of species), b) potential maximum number of species (based on specimens only identified to genus), c) number of genera, and d) abundance of bees collected in blue and yellow pan traps and the number of plant species in bloom in a 10-metre radius around the bowls, measured in weekly increments during the summer of 2008. Linear regression for a) is $y = -0.133x + 5.126$, $R^2 = 0.035$ and $p = 0.026$, for b) is $y = -0.159x + 5.897$, $R^2 = 0.034$, $p = 0.030$, for c) is $y = -0.091x + 3.753$, $R^2 = 0.040$, $p = 0.017$, and for abundance d) is $y = -0.217x + 9.193$, $R^2 = 0.017$, $p = 0.128$.

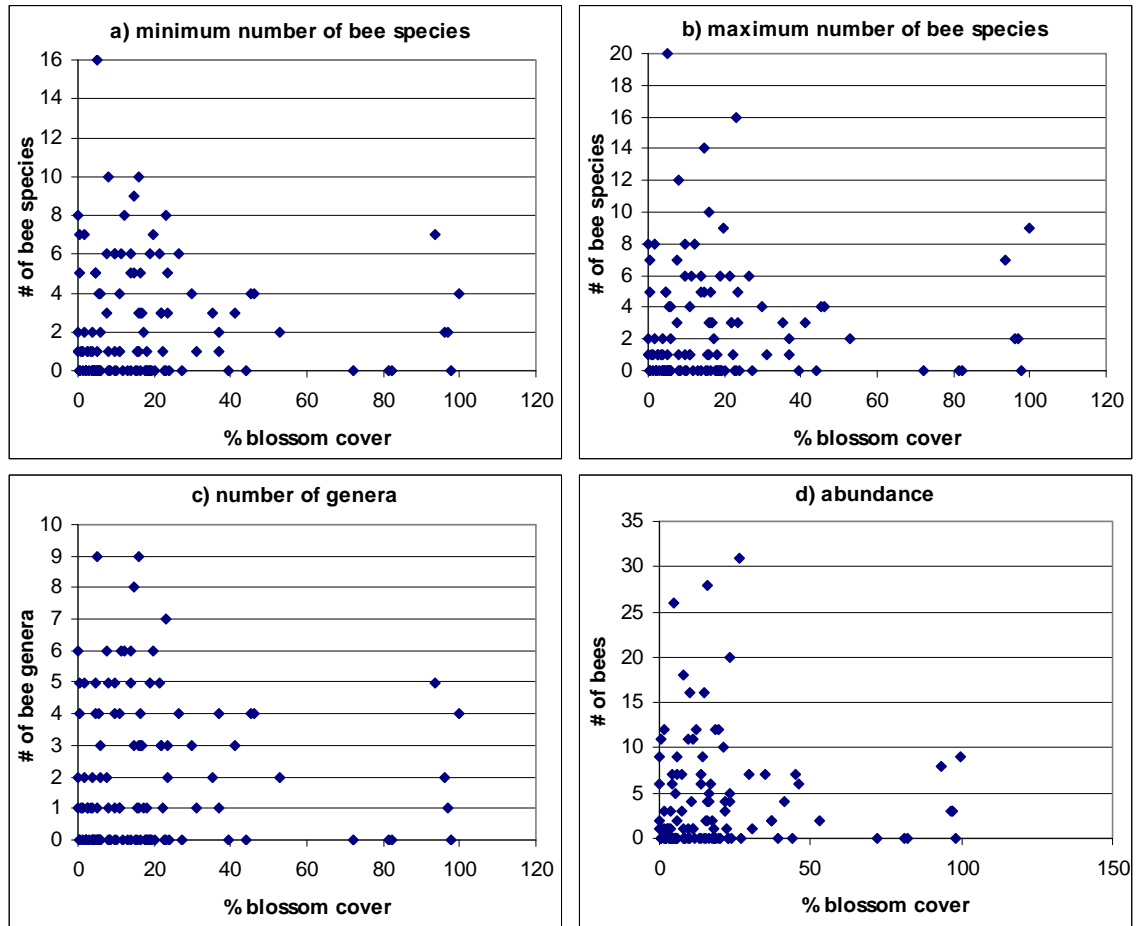


Figure 10. Relationship between a) total number of identified species (listed as minimum number of species), b) potential maximum number of species (based on specimens only identified to genus), c) number of genera, and d) abundance of bees collected in blue and yellow pan traps and the percentage of blossom cover in a 10-metre radius around the bowls measured in week-long increments during the summer of 2008. No significant correlations were found ($\alpha \leq 0.05$).

Table 8. P-values of linear, logarithmic, inverse, quadratic and cubic curve fit tests between the percentage of blossom cover in a 10-metre radius around the bowls, and the number of identified species (listed as minimum number of species), the potential maximum number of species (based on specimens only identified to genus), the number of genera, and the abundance of bees collected in blue and yellow pan traps in week-long increments during the summer of 2008.

	Curve Fit				
	Linear	Logarithmic	Inverse	Quadratic	Cubic
Minimum # of Species to Plant Cover	0.668	0.203	0.860	0.129	0.132
Maximum # of Species to Plant Cover	0.529	0.153	0.984	0.156	0.127
# of Genera to Plant Cover	0.505	0.154	0.780	0.060	0.076
Abundance to Plant Cover	0.641	0.112	0.777	0.069	0.058

3.3 Temporal Variations

In 2007, there was no significant variation between weeks in minimum number of species ($p = 0.115$), maximum number of species ($p = 0.124$), number of genera ($p = 0.126$), or abundance ($p = 0.264$) (Figure 11). ANOVA showed significant differences between weeks in minimum number of species ($p = 0.001$), maximum number of species ($p = 0.000$), number of genera ($p = 0.001$), and abundance ($p = 0.019$) in 2008 (Figure 12). Although the Tukey's HSD post hoc analysis showed no significant differences in abundance, it did show some significant differences in numbers of species and genera (Table 9). The minimum number of species was significantly different between the weeks of May 15 to 23 and August 8 to 16 ($p = 0.004$), May 15 to 23 and August 28 to September 5 ($p = 0.027$) and between the weeks of June 5 to 13 and August 8 to 16 ($p = 0.023$). The maximum number of species was different between the weeks of May 15 to 23 and August 8 to 16 ($p = 0.007$), May 15 to 23 and August 28 to September 5 ($p = 0.034$), between June 5 to 13 and August 8 to 16 ($p = 0.007$) and between June 5 to 13 and August 28 to September 5 ($p = 0.034$). The number of genera was different between the weeks of May 15 to 23 and August 8 to 16 ($p = 0.022$), between June 5 to 13 and August 8 to 16 ($p = 0.007$) and between June 5 to 13 and August 28 to September 5 ($p = 0.033$). In week-by-week comparisons, significantly more bees were collected in 2007 than in 2008 in terms of maximum and minimum numbers of species ($p = 0.009$, $p = 0.002$), number of genera ($p = 0.008$), and abundance ($p = 0.045$) (Figure 13).

3.4 Yellow Versus Blue Pan Traps

Over the two summers, 1331 bees were collected in yellow pan traps and 263 were

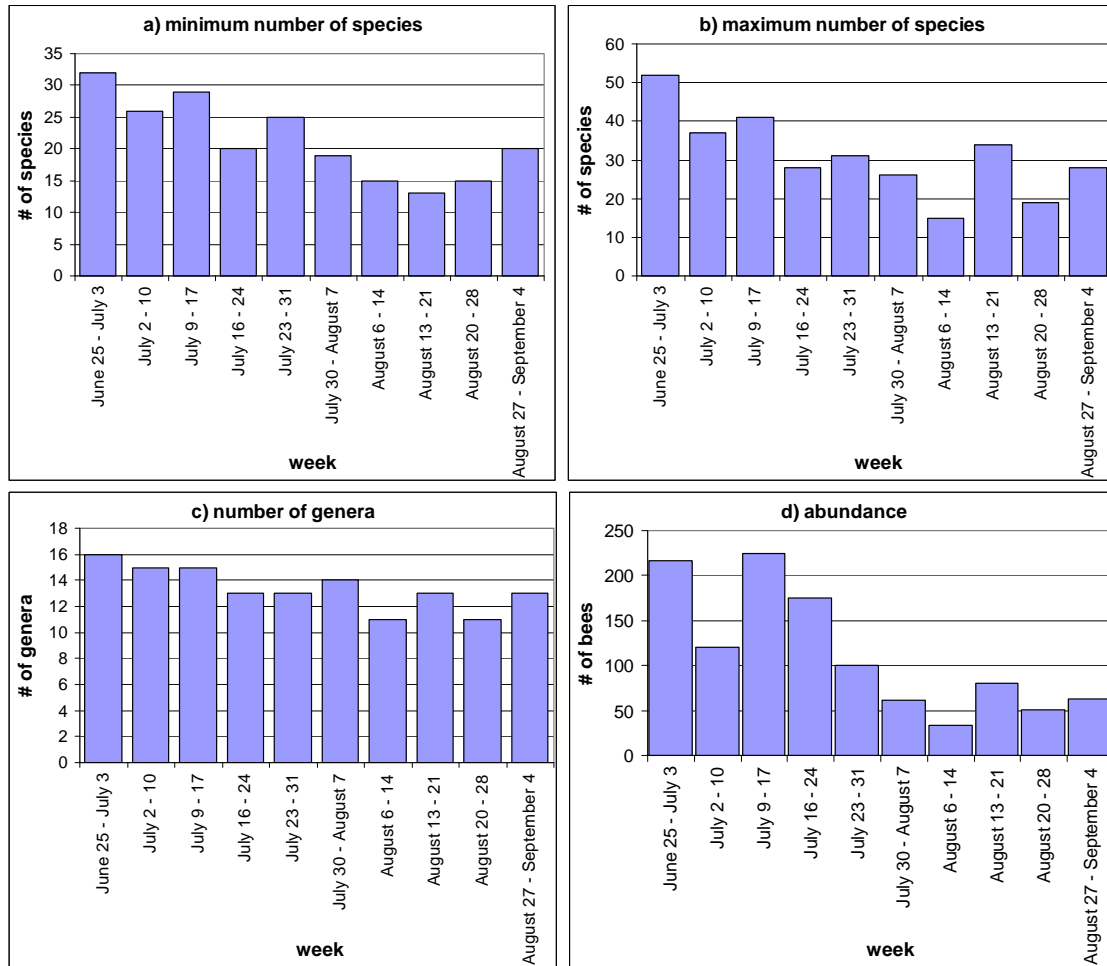


Figure 11. Variation in a) the number of identified species (listed as minimum number of species), b) the potential maximum number of species (based on specimens only identified to genus), c) the number of genera, and d) the abundance of bees collected in yellow and blue pan traps from one week to the next in the summer of 2007.

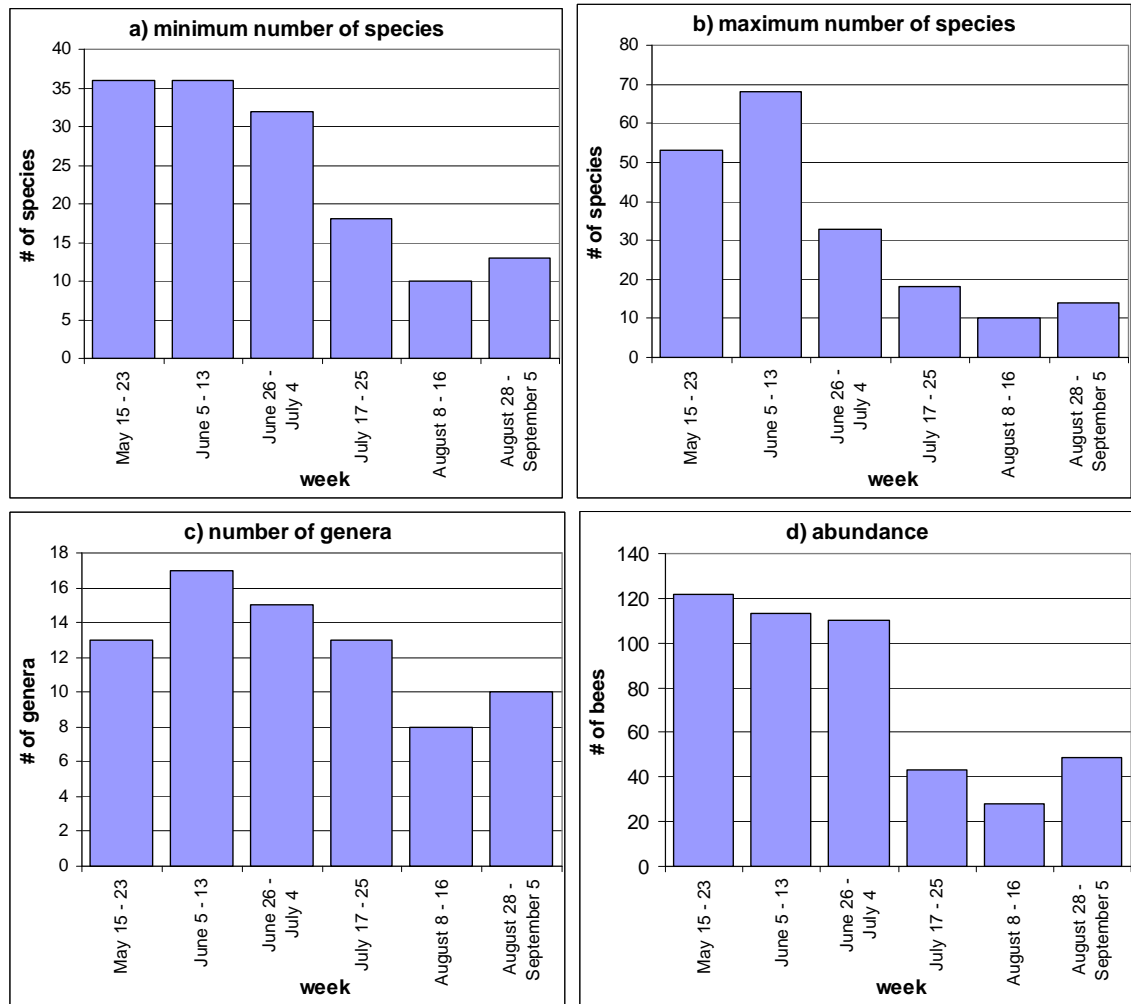


Figure 12. Variation in a) the number of identified species (listed as minimum number of species), b) the potential maximum number of species (based on specimens only identified to genus), c) the number of genera, and d) the abundance of bees collected in yellow and blue pan traps from one week to another in the summer of 2008.

Table 9. P-values from Tukey’s HSD post-hoc analysis of variations of the number of identified species (listed as minimum number of species), potential maximum number of species (based on specimens only identified to genus), number of genera and abundance of bees collected in yellow and blue pan traps between weeks in 2008. * denotes significance $\alpha \leq 0.05$.

		Date					
	Date	May 15 - 23	June 5 - 13	June 26 - July 4	July 17 - 25	August 8 - 16	August 28 - September 5
Minimum # of Species	May 15 - 23	---	0.992	0.700	0.060	0.004*	0.027*
	June 5 - 13	0.992	---	0.954	0.222	0.023*	0.120
	June 26 - July 4	0.700	0.954	---	0.735	0.198	0.550
	July 17 - 25	0.060	0.222	0.735	---	0.939	1.000
	August 8 - 16	0.004*	0.023*	0.198	0.939	---	0.988
	August 28 - September 5	0.027*	0.120	0.550	1.000	0.988	---
Maximum # of Species	May 15 - 23	---	1.000	0.557	0.064	0.007*	0.034*
	June 5 - 13	1.000	---	0.557	0.064	0.007*	0.034*
	June 26 - July 4	0.557	0.557	---	0.863	0.406	0.739
	July 17 - 25	0.064	0.064	0.863	---	0.973	1.000
	August 8 - 16	0.007*	0.007*	0.406	0.973	---	0.995
	August 28 - September 5	0.034*	0.034*	0.739	1.000	0.995	---
# of Genera	May 15 - 23	---	0.999	0.804	0.201	0.022*	0.088
	June 5 - 13	0.999	---	0.577	0.088	0.007*	0.033*
	June 26 - July 4	0.804	0.577	---	0.903	0.387	0.719
	July 17 - 25	0.201	0.088	0.903	---	0.948	0.999
	August 8 - 16	0.022*	0.007*	0.387	0.948	---	0.995
	August 28 - September 5	0.088	0.033*	0.719	0.999	0.995	---
Abundance	May 15 - 23	---	1.000	0.999	0.248	0.084	0.274
	June 5 - 13	1.000	---	1.000	0.397	0.159	0.430
	June 26 - July 4	0.999	1.000	---	0.448	0.189	0.483
	July 17 - 25	0.248	0.397	0.448	---	0.996	1.000
	August 8 - 16	0.084	0.159	0.189	0.996	---	0.994
	August 28 - September 5	0.274	0.430	0.483	1.000	0.994	---

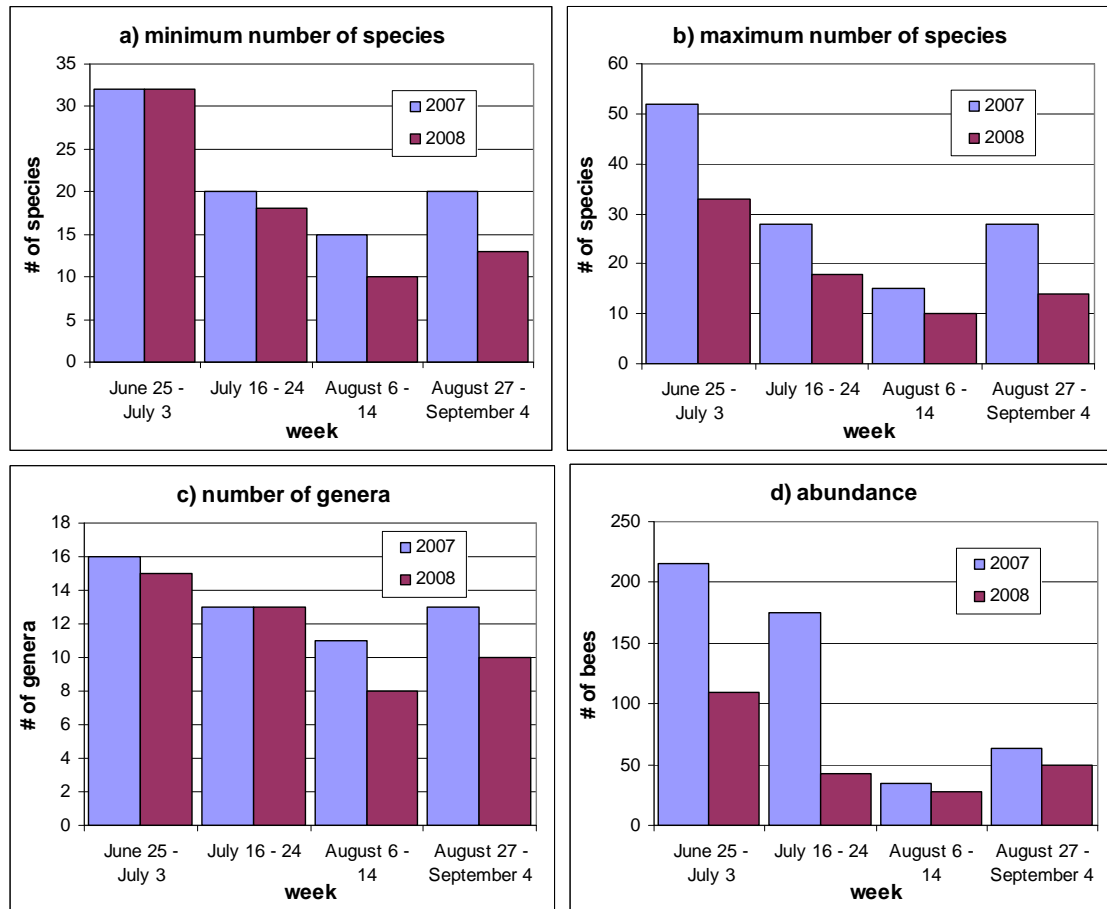


Figure 13. Week-by-week comparison between 2007 and 2008 in terms of a) number of identified species (listed as minimum number of species), b) potential maximum number of species (based on specimens only identified to genus), c) number of genera and d) abundance of bees collected in yellow and blue pan traps.

collected in blue pan traps. A paired-samples T-test showed that yellow pan traps collected significantly more species ($p = 0.000$), genera ($p = 0.000$) and total bees ($p = 0.004$) (Figure 14).

3.5 Trap Nests Collections

Over the two summers, 1944 filled tubes were collected from trap nests, 686 in 2007 and 1258 in 2008 (Table 10). The numbers of filled trap nest tubes were not normally distributed in either 2007 ($p = 0.006$) or 2008 ($p = 0.041$). A Kruskal-Wallis non-parametric test showed significant differences in number of trap nest tubes filled in between site types in 2008 ($p = 0.011$), but not in 2007 ($p = 0.055$) and. In 2008, significantly more nest-filled tubes were collected in the green spaces than in new residential areas ($p = 0.008$) or in old residential areas ($p = 0.008$) (Figure 15). There were no significant differences between industrial spaces and green spaces ($p = 0.095$), new residential ($p = 0.222$), or old residential areas ($p = 0.548$). The difference between new and old residential areas was almost, but not quite, significant ($p = 0.056$).

Of approximately 550 tubes from which insects had hatched by February 2009, only 28 contained bees; the rest contained wasps. These tubes contained 143 bees from eight species: *Hylaeus annulatus* Linnaeus, *Hylaeus modestus* Say, *Megachile brevis* Say, *Megachile campanulae* Roberts, *Megachile centuncularis* Linnaeus, *Megachile pugnata* Say, *Megachile relativa* Cresson and *Megachile rotundata*. Bees hatched from tubes collected from only seven of the twenty sites. Each site, except the two green sites, produced only a single species of bees. Six of the tubes were from green spaces,

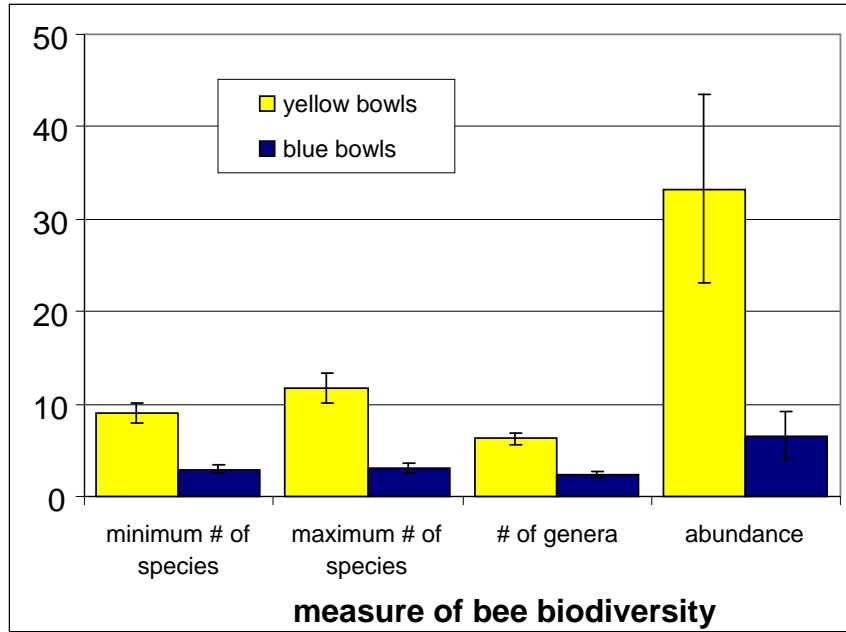


Figure 14. Comparison between bees collected in yellow and blue pan traps based on site averages (+/- standard error) of the number of identified species (listed as minimum number of species), the potential maximum number of species (based on specimens only identified to genus), the number of genera, and the abundance of bees in 2007 and 2008.

Table 10. Number of nest-filled tubes collected by site in 2007 and in 2008. A Shapiro-Wilks test for normality of the data showed that number of filled tubes at each site was not normally distributed in either 2007 ($p = 0.006$) or 2008 ($p = 0.041$). A stem-and-leaf plot showed two outliers in 2007 (G1 and G5) but none in 2008. The numbers of filled tubes collected in 2007 cannot be compared to the number collected in 2008 due to different sampling durations.

Site Number	Site Type							
	2007				2008			
	Green	Industrial	New Residential	Old Residential	Green	Industrial	New Residential	Old Residential
1	100	29	19	62	161	43	19	39
2	40	37	25	14	94	47	33	40
3	22	17	15	39	120	32	60	79
4	78	13	3	2	83	32	14	60
5	97	47	16	11	98	144	13	47
Total	337	143	78	128	556	298	139	265

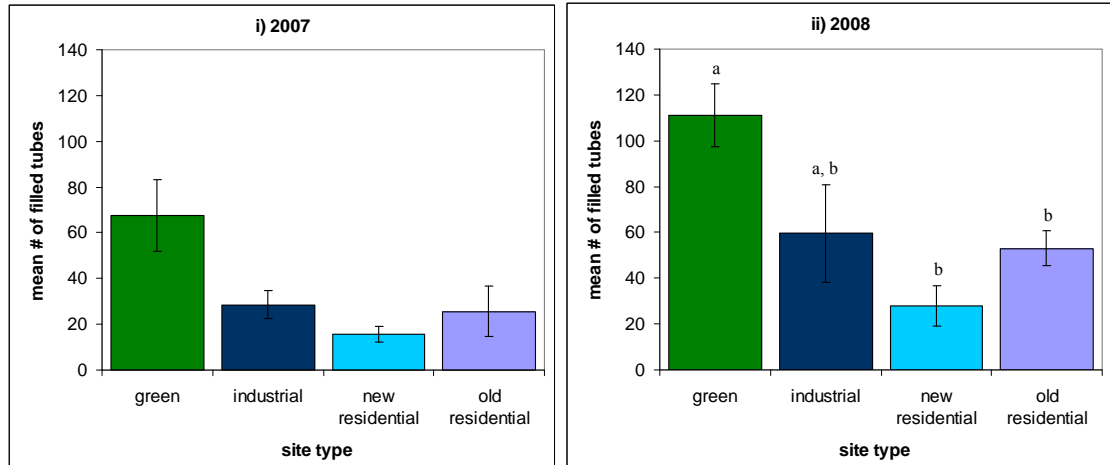


Figure 15. Mean number of trap nest tubes filled by site (+/- standard error) in i) 2007 and ii) 2008. a and b denote significance ($p \leq 0.05$).

specifically G3 in 2008 and G5 in both 2007 and 2008. These green space tubes contained 18 bees. Only two bees hatched from residential land-use zones: one from a tube from N1 in 2007 and one from a tube from O1 in 2008. The remaining 20 tubes were from industrial sites I2, I3, I4 and I5. Interestingly, 88 bees hatched from the tubes from the site at which only a single bee was collected in pan traps over the two years combined, the fewest from any individual site. Unfortunately, many of the tubes collected in 2007 were destroyed by an animal (presumably a squirrel, based on teeth marks in remaining tubes) while the bees were left to overwinter outdoors. Also, not all of the insects had hatched out of their tubes. Because of great reduction in available data from these two factors, data from 2007 and 2008 were pooled. A Shapiro-Wilks test revealed a non-normal distribution of abundance by site ($p = 0.000$) and a Kruskal-Wallis non-parametric test indicated that there was no significant difference between site types ($p = 0.082$).

3.6 Sweep Netting

Sweep netting resulted in the collection of 310 specimens from between 52 and 137 species and 18 genera (Table 11). These bees included 12 species, 2 genera and 1 family that were not collected through the extensive use of pan traps in the same sites.

3.7 Efficacy of Surveying Using Pan Traps

Over the course of the two weeks of experimenting at Arkell Farm, a total of 39 bees were collected from the 96 bowls. A Kruskal-Wallis non-parametric test showed no

Table 11. Bee species collected via sweep netting by site in 2007 (continued next page).

Family	Species	Site															Total								
		G1	G2	G3	G4	G5	I1	I2	I3	I4	I5	N1	N2	N3	N4	N5		O1	O2	O3	O4	O5			
Andrenidae	<i>Andrena cressonii</i> (Robertson)								2													1	3		
	<i>Andrena dunnigi</i> (Cockerell)																					1	1		
	<i>Andrena melanochroa</i> (Cockerell)									1													1	1	
	<i>Andrena nasonii</i> (Robertson)										2							2			1	7	12		
	<i>Andrena vicina</i> (Smith)									1													1	1	
	<i>Andrena wheeleri</i> (Graenicher)																	1					1	1	
	Unknown <i>Andrena</i> species									2								1				1	4	4	
	<i>Apis mellifera</i> (Linnaeus)				2		3	1	2										1					8	8
	<i>Bombus bimaculatus</i> (Cresson)																		1					1	1
	<i>Bombus citrinus</i> (Smith)		3																					3	3
<i>Bombus impatiens</i> (Cresson)			1	1		1											1						4	4	
Unknown <i>Bombus</i> species																1							1	1	
<i>Ceratina calcarata</i> (Robertson)																	1						2	2	
<i>Ceratina dupla</i> (Say)					1	2															1	1	5	5	
<i>Ceratina</i> sp.		2									1												4	4	
<i>Melissodes druriella</i> (Kirby)				4																			4	4	
<i>Nomada denticulata</i> (Robertson)																	1						1	1	
<i>Nomada</i> species5									1														1	1	
<i>Nomada</i> species7																1							2	2	
Unknown <i>Nomada</i> species									4								3						10	10	
<i>Colletes compactus</i> (Cresson)				2																			2	2	
<i>Colletes inaequalis</i> (Say)									1														1	1	
<i>Colletes simulans</i> (Cresson)				1																			1	1	
<i>Hylaeus annulatus</i> (Linnaeus)		1	1																				2	2	
<i>Hylaeus modestus</i> (Say)			1	1		1																	3	3	
Unknown <i>Hylaeus</i> species		3	1			2	7	2										1					16	16	
<i>Agapostemon virescens</i> (Fabricius)																							1	1	
<i>Augochlora aurata</i> (Smith)		2			2	1	2	2															2	14	
<i>Augochlora pura</i> (Say)																		1					1	2	
<i>Halictus confusus</i> (Smith)						3	5		1	1	1	2	3					1	1				1	19	
<i>Halictus ligatus</i> (Say)		3	2		1	8	7	1	13		2		1										3	43	
<i>Halictus rubicundus</i> (Christ)																								2	2
<i>Lasioglossum anomalum</i> (Robertson)		1						15	2	3													6	28	
<i>Lasioglossum coriaceum</i> (Smith)			1																				1	1	

Table 11. (continued)

Family	Species	Site															Total						
		G1	G2	G3	G4	G5	I1	I2	I3	I4	I5	N1	N2	N3	N4	N5		O1	O2	O3	O4	O5	
	<i>Lasioglossum ellisiae</i> (Sandhouse)						1																1
	<i>Lasioglossum leucozonium</i> (Schränk)						1		5													2	8
	<i>Lasioglossum lineatulum</i> (Crawford)									1													1
	<i>Lasioglossum versans</i> (Lovell)																			3			3
	Unknown <i>Lasioglossum</i> species	1	2	1	4	1	7	1	5	5	3	1			1							23	59
	<i>Sphexcodes ranunculi</i> (Robertson)								1							1							2
Megachilidae	<i>Chelostoma campanularum</i> (Kirby)																					16	17
	Unknown <i>Heriades</i> species			1																			1
	<i>Megachile brevis</i> (Say)						1																1
	<i>Megachile latimanus</i> (Say)					1	1																2
	<i>Megachile mendica</i> (Cresson)															1							1
	<i>Megachile rotundata</i> (Fabricius)													1								2	3
	<i>Megachile texana</i> (Cresson)															1							1
	Unknown <i>Megachile</i> species															1							1
	<i>Osmia conjuncta</i> (Cresson)								1													1	2
	Unknown <i>Osmia</i> species										1												1
Melittidae	<i>Macropis nuda</i> (Provancher)																						1
	Total by Site	10	12	7	17	18	48	11	14	40	3	9	4	7	0	4	8	16	5	2	75	310	

differences in the numbers or types of bees collected at each distance from the gardens (Figure 16). Over the twelve hours of observation, a total 344 bees visited the gardens.

3.8 DNA Barcoding

Of the 1995 samples submitted, almost sixty-five percent of the samples, 1291 samples, returned COI sequences within a year of submission. Of the 645 randomly selected COI sequences, 214, or one third, matched at ≥ 97 percent with a single previously identified sequence from BOLD (Figure 17). Seventeen (2.6 percent) came back without any positive identifications. Thirty-one (4.8 percent) came back with two positive identifications. Most of the samples (338, or 52.4 percent) came back with three identifications. The rest (45, or 7 percent) came back with 4 to 20 positive identifications.

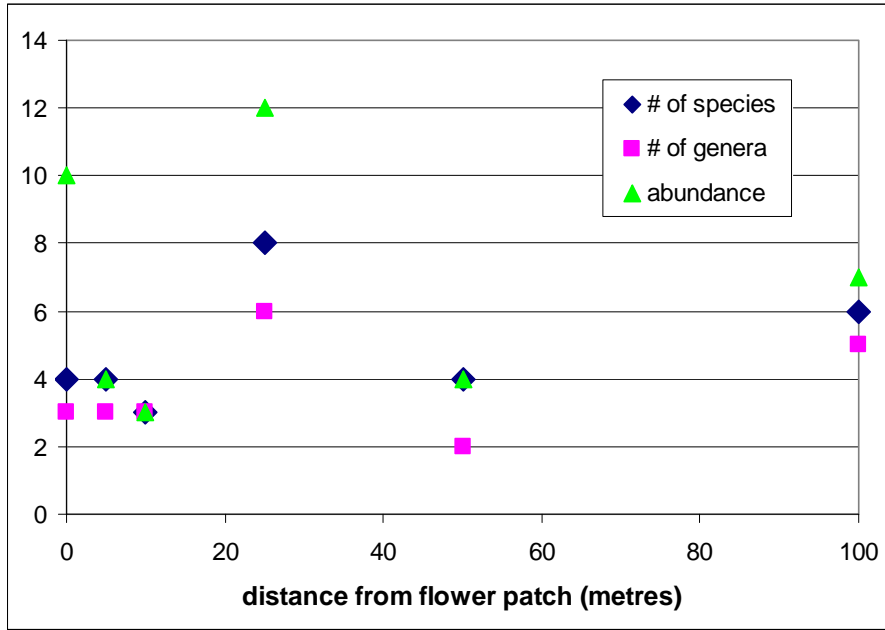


Figure 16. Number of species, number of genera and abundance of bees collected in yellow and blue pan traps by distance from floral centre at Arkell Farm in 2008.

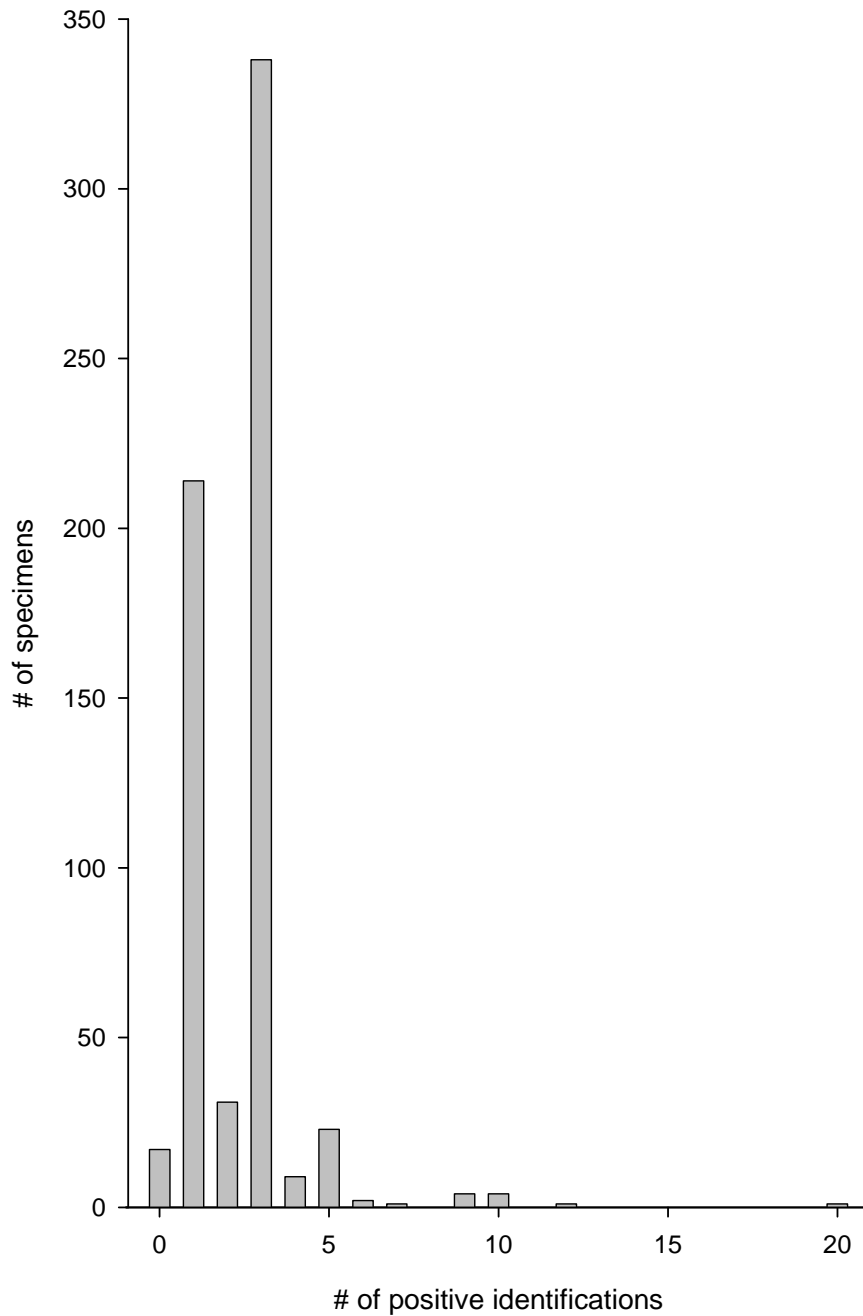


Figure 17. Number of positive identifications (greater than 97 percent match of COI sequence) produced per specimen by the Barcode of Life Database [BOLD] (n= 645 of 1995 specimens submitted).

CHAPTER 4: DISCUSSION

4.1 Abundance and Diversity by City Land-use Zone

A total of 1592 bees were collected in the blue and yellow pan traps across the four cities over two summers. Many more bees were collected in 2007 than in 2008 due to a combination of the more intensive collection (ten weeks in 2007, rather than six weeks in 2008) and warmer drier weather in the summer of 2007 than in 2008. Similar numbers of species and genera were collected in both years. A total of 94 species were collected in the pan traps. Although between 66 and 69 species were collected each year, 25 of these species were unique to the sampling year, which suggests that the evaluation of the diversity of bees in the study area is far from complete. In order to obtain accurate baseline data for the area one would clearly need to continue to sample intensively for several years.

The most abundant species collected in 2007 was *Halictus ligatus*, with 453 specimens, while only 14 specimens of *Halictus ligatus* were collected the following year. The high number of *Halictus ligatus* that were collected in 2007 suggests that the pan traps may have been in close proximity to nesting grounds for this species. *Halictus ligatus* is not a solitary bee, but rather a primitive eusocial sweat bee (Richards and Packer, 1994), meaning that although they have a caste system, their nests are typically small, with no more than a dozen workers (Packer et al. 2007). *Halictus ligatus* may also nest in aggregations. Both eusociality and aggregate nesting would contribute to the presence of many individuals of the same species in a small space. In fact, 303 of the 453 specimens

of *Halictus ligatus* collected in 2007 were from a single site (I4). The near absence of the species the following year in the same places suggests that extensive collection of specimens may have a significant negative effect on the population of *Halictus ligatus*. If that is true, widespread efforts to use pan traps to collect as many bees as possible to evaluate diversity and abundance may negatively affect the populations of bees, and therefore careful consideration should be made before implementing an intensive destructive sampling collection technique.

In 2008 *Megachile rotundata* was the most common species collected, with 52 specimens. However, unlike *Halictus ligatus* in 2007, *Megachile rotundata* was not found predominately at one particular site, and its values were not as extremely different from those from other species. Additionally, *Megachile rotundata* was found in higher abundance in 2008 than in 2007. This increase may be a consequence of the shift in location of site O4 although the new site was only an estimated 50 metres west. Out of 59 specimens collected at O4 in 2008, 15 specimens were *Megachile rotundata*; in 2007, none of the ten bees at O4 were *Megachile rotundata*. Such a large increase was not noted at any other site from 2007 to 2008, which suggests that the shifted location, despite the modesty of the physical distance, may be responsible for this difference. Zanette et al. (2005) found that eusocial bees responded to very small-scale changes in their habitat. The present study suggests that this response is not only true of eusocial bees, but may also be true of solitary bees. If a location shift of only 50 metres can cause a six-fold increase in the number of bees collected, then the precise location of the pan traps might have had a much stronger impact on collections than previously anticipated.

This would severely limit the applicability of any single site collection to a larger scale unit, such as, for example, a city land-use zone. Although the effects of city land-use could be measured despite local variation, the level of replication would have to be high to account for variable bee distribution on scales as small as 50 metres within zones. This finding is congruent with Zanette et al.'s (2005) finding that bees responded to small-scale changes in their habitat.

Statistical analysis indicated that there were no significant differences between site type in terms of minimum number of species, maximum number of species, number of genera, abundance, minimum Shannon-Wiener value, or maximum Shannon-Wiener value in either 2007 or 2008. That is contrary to the primary hypothesis of this thesis, which proposed that differences in the land use and maintenance of different city land-use zones would result in differences in the populations of pollinators. A number of factors could explain the lack of statistical difference between the sites, including discrepancies at a small scale (as discussed earlier), an inadequate number of sites, and variation in maintenance techniques despite land-use.

In 2007, the number of specimens collected at each of the five industrial sites varied from 1 to 413. This was also the maximal range of abundance for all twenty sites. Such a drastic variation amongst so few points makes finding statistical significance extremely unlikely. Therefore, even if trends existed in these differences, they would be unlikely to show statistical significance. Having a significantly higher number of sites, such as 50 or

more, would considerably increase the probability of accurately representing trends, and thus of finding any existing statistically significant differences.

In 2008, the number of specimens at an individual site varied from 0 to 88, but unlike in 2007, this maximal range was in the new residential areas, rather than the industrial areas. This brought to light differences in maintenance techniques at different sites. In particular, while most new residential sites had relatively low abundance and diversity of bees, site N3 had highest abundance of any site that year, and the second highest minimum diversity by species and the highest diversity of any site by genera of any site in either year (Table 2, Table 3). This site had one unique characteristic when compared to other new residential sites: a patch of naturalized land of about 100 square metres. This observation led to the evaluation of abundance and diversity in each site based on specific site features, rather than land-use zone type.

4.2 Abundance and Diversity of Bees by Feature

Overall, the abundance and diversity of bees were found to be significantly increased by the presence of naturalized areas at any given site (with the exception of general abundance in 2007). This finding supports the hypothesis that special features may account for discrepancies between individual sites. Naturalized areas provide a variety of soil textures (sandy, vegetated) and a wide range of plant resources, both of which are important for encouraging the presence of bees.

The presence or absence of significant water bodies, however, had no significant effect on bee biodiversity, suggesting that the water requirements of bees can be met by pools of water that are sufficiently small that they would not necessarily be considered significant from the human perspective. Furthermore, the majority of described bee species worldwide are found in semi-arid or desert environments (Michener 2000). There was also no significant difference between sites with low levels of traffic, and those with no traffic. Though all potential sites with medium or high levels of traffic were eliminated before the experiment began, there was still a noticeable difference in the amount of human activity at some sites, particularly the residential and some industrial sites, as compared to those in certain conservation areas and other private industrial sites. However, these minor disturbances did not affect the bee biodiversity at these sites.

The biodiversity of bees was not affected by the percentage blossom cover. The numbers of bee species, the number of genera of bees, and the abundance of bees were compared to the percentage blossom cover. However, no trends appeared using linear, logarithmic, inverse, quadratic or cubic correlations. In contrast, the minimum and maximum number of bee species, the number of bee genera, and the abundance of bees were all significantly affected by the number of species of plants in bloom at a given site. These relationships were all shown to be negative correlations, meaning that the number of bee species and genera and the abundance of bees decreased in the presence of an increasing floral diversity.

This finding is somewhat counter-intuitive, in that the presence of more species of plants in bloom would be expected to entice more species of bees (Frankie et al. 2005). This theory assumes that all sites are equal except for the number of plant species. However, in reality it is entirely possible that these sites were not pollinator friendly in some way. For example, site G2 was sprayed with insecticide part-way into the summer of 2008 in an effort to control the mosquito population and prevent the spread of the West Nile virus. Bees simply could not survive in this site regardless of the plant species. With this in mind, it seems likely that many of the sites in this experiment had few bees despite having many plant species.

Another contributing factor may be that bees have a strong preference for flowers as compared to pan traps. If there is a high number of plant species in bloom, the bees may be less likely to land in pan traps because of their strong attraction to the flowers. It is also possible that the most accurate correlation would take into account both abundance and diversity of plants, but this correlation would be complex and consequently difficult to predict.

4.3 Temporal Variations

In 2007, no significant variation was recorded in numbers of species or genera or in abundance from week to week. This may be because sampling in 2007 took place continuously over a period of little more than two months. This short time span combined with continuous sampling can explain the lack of variation throughout the summer of 2007. Unlike in the summer of 2007, in 2008 sampling took place over almost four

months, and was spread out to one week out of every three. The extended season, as well as the breaks between sampling periods, allowed for significant variations in 2008 despite their absence in 2007.

In 2008, though abundance did not vary significantly between weeks, the numbers of species and genera at the beginning of the summer were significantly greater than those at the end. Many factors could explain the differences between the beginning of the summer and the end. Different species may hatch at different times, and therefore the number of a given species may vary across the season (Ginsberg 1983). This parallels the findings of Wojcik et al. (2008) which showed that different family and genus groups have seasonal fluctuations. Certain species on bees depend on different floral resources which may be more or less abundant depending on the season, and which may vary differently within sites over a given time period. The weather may also significantly affect the amount of activity during any given period of time. These significant intra-annual variations suggest that researchers should make an concerted effort to span the entire bee season, rather than truncating it, as this will allow a more accurate sampling of diversity as shown by Grixti and Packer (2006) and MacKay and Knerer (1979).

Four weeks of sampling were common to both 2007 and 2008. For these four weeks, a paired sample test was done on the 18 sites that were consistent in the two years. It showed that, at the site level, significantly more bees were collected in 2007 than in 2008 in terms of maximum and minimum numbers of species, number of genera and abundance. This is most likely a result of the much warmer and drier weather

experienced in 2007 than in 2008, but the drastic decrease in the presence of *Halictus ligatus* at site I4 suggests that the previous year's collection efforts may also have had an impact on the following year's collections. The significant inter-annual variations suggest that sampling should take place over a period of several years to ensure that these variations do not significantly bias collections.

4.4 Yellow Versus Blue Pan Traps

Significantly more bees, in terms of species, genera, and total bees, were collected in yellow bowls than blue bowls over the two summers. This may be because the blue bowls were somewhat darker than expected (more navy than cerulean), and may not have been an optimal colour to attract the bees. The blue bowls also absorbed more heat from the sun, and were often noticeably warmer to the touch than their yellow counterparts. Future studies should be careful to acquire light blue bowls, or to stick to yellow and white instead. However, collectors must remain aware that whatever colours they choose may present a colour preference-based taxonomic bias (Westphal et al. 2008). Ongoing work by Droege et al. (2010) to standardize collection methods should help to minimize the biases of collection using pan traps.

4.5 Trap Nests Collections

Almost two thousand filled tubes were collected over the two summers, significantly more of which were collected in 2008 than in 2007, reflecting the extended 2008 collection season. In both 2007 and 2008, significantly more nest-filled tubes were collected in the green spaces than in the new or the old residential areas. There was,

however, a lack of significant difference between industrial, new residential and old residential sites, and between green and industrial spaces. The numbers of tubes filled proved not to be highly indicative of the presence of bees, as only 28 of 550 hatched tubes contained bees. However, these numbers are still interesting in that they reflect the biodiversity at each site, as proposed by Taki et al. (2008). Those tubes that were not filled with bees were filled with wasps. Most of these wasps are predatory and many lay their eggs in or on the larvae of insects. In order to support a large and diverse population of wasps, a site would have to host a large and diverse population of prey insects for them. Therefore one could assume that a large and diverse population of wasps in trap nest tubes probably is indicative of a site with a high general insect biodiversity (Taki et al. 2008).

Bees hatched out of only 28 tubes. One hundred and forty-three bees from eight species, all of which were either Megachilidae or Colletidae, hatched from these tubes. However, these bees-filled tubes came from only seven of the twenty sites. Green sites were the only sites to produce more than a single species of bees. Therefore, it is possible that one very industrious female could have been responsible for the entire trap nest bee population at each other site. Six of the 28 tubes were from green spaces, two were from residential spaces (one old and one new) and the remaining 20 were from industrial sites. Perhaps the most interesting finding from the bees that hatched from the tubes, however, was that 88 of the 143 bees that hatched came from site I3, an industrial site whose pan traps only collected a single bee over two years of collection! The presence of bees in these nesting in tubes, particularly in cases like site I3, may well suggest a need for more

appropriate locations in which bees from neighbouring areas can establish nests, allowing bees to shift from simply foraging in the area, to inhabiting it.

4.6 Sweep Netting

Though the bees collected by sweep netting were not used for any statistical evaluation of sites, this collection was interesting as it indicated that a high diversity to abundance ratio can be achieved with a sweep net. The 310 specimens collected included at least 52 species from 18 genera, while the 1592 specimens collected over the two summers included a minimum of 94 species and 23 genera – a much lower abundance to species ratio. Some of this can be accounted for based on the accumulation curve of sampling (Pielou 1969), which would predict that the pan traps may have come closer to sampling all species than the sweep netting. The accumulation curve acquired in this study did not fit with tested curves (Figure 6). However, if collection for this study continued, it might be possible in this study to show a linear relationship similar to that presented by Taura and Laroca (2001) between the number of species collected and the \log_{10} of the total specimens collected.

Regardless of the accumulation curve, these numbers do suggest that sweep netting may be more effective, especially considering that twelve species were collected in the sweep nets that were not collected in the pan traps. Taking this into consideration, the most effective means of establishing diversity and abundance should be to use sweep netting to establish diversity and to use pan traps as a means of measuring relative abundance. However, this would work only in a non-urban setting, as residents sometimes seem not

to appreciate the use of sweep netting because of potential damage to plants. Also interesting from the sweep netting collection was the collection of 11 bees from 5 species at site I3, the previously mentioned site where the pan traps only collected a single bee over the two years of collection. Between the 5 species collected in the sweep net, the species that hatched out of the trap nest tubes and the species collected in the pan traps, this site hosted 7 species, despite the pan traps showing only a single species. It is surprising that there is such a large discrepancy between the collection using three methods and the collection using pan traps, and it supports the hypothesis that pan traps may not be the most effective means of establishing diversity at a site.

4.7 Efficacy of Surveying Using Pan Traps

Statistical tests showed that there was no significant difference in the number of bees collected at any distance from the flowers. This was contrary to the hypothesis that prefaced this experiment, which was that, when in close proximity to the flowers, bees would chose to land on the blossoms rather than the pan traps due to the olfactory cues that flowers provide in addition to the visual cues offered by both the blossoms and the pan traps. These results do not support this hypothesis. However, the pronounced difference between the number of bees observed visiting the flowers and the number collected in the pan traps clearly shows that bees do indeed prefer flowers to pan traps. Three-hundred and forty-four bees were observed visiting the flowers over twelve hours of observation. Over the corresponding two weeks of collection using the pan traps, 39 bees were collected. The number of bees observed outweighs the number of bees collected by a factor of almost nine. Additionally, the twelve hours of observation only

make up about seven percent of the daylight hours for which the pan traps were collecting. Furthermore, 96 bowls were used at the Arkell Farm, which amounts to sixteen times the number of bowls at any given site in the main experiment in this thesis. From these numbers, one can approximate that only one bee will be collected for every two thousand bees that visit the flowers. While this suggests that pan traps are not likely to kill any population that they are used to sample entirely, they can do significant damage, as mentioned earlier in the case of *Halictus ligatus*, which was prominently present in 2007 collections, but was conspicuously sparse the following year. The study at Arkell Farm also suggests limitations in the use of pan traps for sampling diversity, due to the proportion of species that will never get sampled. This observation is also supported by the taxonomic colour-preference bias found by Westphal et al. (2008).

4.8 DNA Barcoding

DNA barcoding has great potential for the naïve ecologist. Theoretically, a scientist who has no knowledge of the local flora or fauna could collect a tissue fragment from each of a large number of random specimens, barcode them collectively and, without knowing a single species present, indicate the diversity and abundance of species collected. This project attempted to demonstrate this potential by submitting a large number of unidentified samples to BIO and then using BOLD to acquire identifications. Unfortunately, success was limited, suggesting that there is room for significant improvement in the DNA barcoding system.

Less than 65 percent of the samples submitted to BIO came back with COI sequences. The rest did not provide sequences for undefined reasons. Probable problems include processing failure, contamination, too little or too much tissue for DNA extraction, and destruction of samples. Contamination and the amount of tissue are both controlled by the ecologist, rather than the barcoding facility, and therefore human error would have contributed to these. In this case the ecologist was carefully trained on two separate occasions in sample collection and preparation. However, in the case of the naïve ecologist, this extensive training might not be possible, giving room for an even higher percentage of samples to fail.

Of the samples that produce COI sequences, 645 were randomly selected and their identifications were acquired from the BOLD. In an ideal system, all of these samples should have returned a single identification, but less than one third came back with a single, positive, species identification. A small percentage (2.6 percent) came back with no matching identifications, indicating that samples of these species have not yet been entered in the database. Sixty-four percent came back with multiple positive identifications, including a sequence that was a positive match at 97 percent to twenty different species. Many of these matches included species from more than one genus.

There are several possible explanations for the fact that barcoding only provided useful results for twenty percent of the samples. The most likely explanation is that the screening process allowing users to submit identifications to the BOLD is inefficient. If anyone is permitted to upload “identified” sequences, sequences incorrectly identified

may pollute the pool of correctly identified sequences, providing inaccurate identifications. If this is the case, then measures must be put in place to ensure that individuals who are contributing to the identification of specimens for the database are qualified to do so.

The next possible explanation is contamination. Samples which may have been correctly identified in theory may be contaminated by an unidentified source, and their identities may therefore falsely represent the COI sequences they provide. This is supported by several samples acquired from bees in this experiment that came back with obviously incorrect single positive identifications as Lepidoptera. This requires not only that a contaminant was present but also that this contaminant contributed the majority of the DNA extracted from the sample. It would be beneficial to examine the process and ascertain when such contamination may occur so that it can be minimized.

It has also been suggested that infections by *Wolbachia* and other bacteria that change the DNA sequences of their hosts (Nikoh et al. 2008, Ochman et al. 2000) could result in natural contamination that is beyond the control of either ecologists or barcoding technologists. Complications caused by pseudogenes have also been considered in relation to barcoding of bees (Schaefer and Renner 2008).

The final explanation is the level of divergence in the COI sequence that occurs within a species. According to a recent publication by Sheffield et al. (2009) some species show remarkable variability in their COI sequences. For example, *Andrena miserabilis* Cresson

showed a 5.91 percent mean distance variability between 4 samples of the same species, including a maximum distance of 8.9 percent (Sheffield et al. 2009). Typically, a divergence of more than two or three percent is considered to show that two specimens are not the same species. However, Sheffield's work suggests that perhaps this generalization should not be made lightly. It might be better to indicate what level of variability indicates a different species on a case-by-base basis based on intraspecies variations and interspecies variations of closely related species.

Despite the apparent short-comings of DNA barcoding at present, after the major flaws in the system are corrected, this may still become a useful tool for ecologists to measure biodiversity. Once the databases are cleaned up and expanded and the margin for error in acquiring sequences is minimized, barcoding should provide an easy alternative for scientists who are not taxonomically inclined to approximate species richness without rigorous evaluation of samples.

CHAPTER 5: CONCLUSION

5.1 Distribution of Bees in Urban southern Ontario

The Guelph, Cambridge, Kitchener and Waterloo area is home to at least 107 species of bees from 25 genera, and all six of the bee families found in Canada. The distribution of these species and their abundances did not vary by city land-use. Diversity and abundance of bees do, however, seem to depend on relatively small changes in site location (50 metres), and therefore any future investigations into this matter should replicate to a higher degree to ensure that they account for slight variations within a land-use zone. Although city land-use zones do not affect the bee biodiversity, the presence of naturalized areas did have a significant impact on the abundance and diversity of bees. This supports the suggestion that even slight variations in habitat and resources within an area may impact its biodiversity, and thus any city that wishes to improve its pollinator populations may do so by introducing naturalized areas. Though plant diversity was negatively correlated with bee diversity and abundance, it is unlikely that the presence of plants actually damages the bee populations. Bee diversity was observed to be higher in May and early June than in late August and early September.

5.2 Collection of Bees

Collection of bees should include the entire season of bee activity in order to maximize observation of diversity. Pan traps are the most effective means by which to measure relative abundance of bees in a variety of locations. Yellow pan traps are more effective than blue in attracting bees, in terms of both abundance and diversity. Collection with pan

traps, however, should not continue indefinitely, as anecdotal evidence suggests that it may be detrimental to the bee populations. Further work to optimize and standardize collection of bees, particularly using pan traps, is ongoing (Droege et al. 2010). Sweep netting may be a positive alternative to pan traps for assessing bee diversity. However, it cannot be used for assessing abundance, and should be used only with great care in residential areas because of gardener's concern for damage to their plants. Trap nests may indicate the general level of insect biodiversity in an area, but do not provide a consistent or accurate means by which to evaluate populations of bees in an area, as they are far more attractive to wasps than to bees.

5.3 Use of DNA Barcoding

Despite the potential that DNA barcoding offers, many improvements need to be made to the system before it will be functional for the naïve ecologist. First, the percentage of samples that provide COI sequences must be improved, perhaps by making the collection method less prone to contamination. Secondly, the database should be cleaned up to eliminate inaccurate submissions. Future submissions should only be accepted from individuals with taxonomic expertise to avoid further introduction of incorrectly labeled individuals. Third, a system needs to be put in place to either minimize contamination or maximize results despite contamination. Finally, the degree of divergence used to evaluate species should be carefully examined on a case-by-case level.

EPILOGUE

Although my thesis focuses on statistically significant differences in the data collected there are a number of anecdotes and insights gained by my research which may be valuable in their own right.

This thesis describes a preliminary investigation of the effects of land-use on bee biodiversity in the urban setting. The data indicate that land-use zones do not, in themselves, explain the abundance and diversity of bees. That is encouraging, as it suggests that any area of a city could be made bee-friendly. My findings also indicate that naturalized areas of at least 50 square metres can significantly improve bee biodiversity, regardless of land-use. Given that a relatively small area of naturalization seems to be needed to improve biodiversity of bees, anyone from a residential gardener to a park planner can develop a naturalized area to help the bees. Naturalized areas should include patches of bare ground, diverse soil textures and plants allowed to grow freely. Projects that aim to develop small patches of pollinator friendly habitat are being advocated by several organizations, including the North American Pollinator Protection Campaign (NAPPC), Pollinator Gardeners of Canada and the Ontario Horticultural Society.

Even at small scales, floral diversity is critical to the attraction of diverse bee species. Though my study indicated that bee diversity and abundance were negatively correlated with floral diversity, I would like to emphasize my belief that this trend is typical. Conversely, most studies, like those by Frankie et al. (2005, 2009) and Wojcik et al.

(2008) indicate that diverse floral resources, even when offered in small quantities, have the potential to draw in diverse bee taxa.

My study also suggests that scale is an important consideration when studying pollinators in the urban setting. Future studies would benefit from considering both large and small scales. Although large scale studies can provide more accurate baseline data, small scale studies may provide more insight into the specific habitat preferences of groups of bees. Specifically, it would be beneficial to study biodiversity on a small scale based on typical species-specific foraging distance. Various species of bees have a tendency to travel various distances from their nests to forage. The assumption that bees typically travel further than they do might cause studies to investigate on a scale that is too broad to accurately assess their distribution.

Additionally, future studies should span the full bee season, but should also be careful to not over-sample, as this could have devastating effects. My works indicated that abundance and diversity were higher earlier in the season, so investigations should begin as soon as the bees come out in the spring.

Although there is still much to be learned about bees in the urban setting, the creation of naturalized areas within the city may allow us to increase the biodiversity of bees even without knowing all there is to know about the pollinators in our cities.

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