A comparison of blue vane trap, timed targeted netting, and timed photographic collection methods for evaluating Canadian bumble bee diversity

By
Jocelyn Jane Armistead, B.Sc.
Biological Sciences (Ecology & Evolution)

A thesis
submitted in partial fulfillment
of the requirements for the degree of
Master of Science

Faculty of Mathematics and Science,
Brock University
St. Catharines, Ontario

ABSTRACT

Bumble bee (genus *Bombus*) populations across Canada are experiencing increases and decreases in abundance; some species are becoming more common while others are at risk of extirpation or extinction. It is important to monitor population changes so that extirpation and extinction can be prevented. Current population assessments for bumble bees, when conducted, use many different collection methods, but this limits our ability to compare across studies and understand trends. There is a call within the scientific community to create a national standard method for collecting bees. The goal of this research was to provide a recommendation for which collection methods could be used across Canada for bumble bee assessments, including assessments of species at risk. Three collection methods, blue vane traps (BVTs), timed targeted netting, and timed targeted photography, were compared with the objective of determining which method provided good diversity information, detected at-risk species, and required low sampling effort. To assess the universality of method performances across the country, surveys were conducted in three different regions of Canada, the Carolinian portion of the Mixedwood Plains Ecozone (southern Ontario), the Prairies Ecozone (Saskatchewan), and the Boreal Shield Ecozone (Newfoundland and Labrador). With some exceptions, the general structure of surveys was that BVTs were deployed for 1 week at a time, and multiple 30-minute netting and photographic surveys were conducted during each collection week. Regional differences were apparent. In the Prairies Ecozone BVTs collected the most specimens while in the other regions BVTs collected the fewest. BVTs detected the most species in the Carolinian and Prairies Ecozones, but netting detected the most species in the Boreal Shield Ecozone. For all regions, BVTs were the most efficient method at low sample sizes when compared using rarefaction. BVTs also detected the most species at risk. Distinct species compositions produced by BVTs compared to netting and photos demonstrated complementarity between these methods. Netting and photo species distributions also differed from each other in most regions. The overall recommendation when assessing Canadian bumble bee populations is to use BVTs in week-long durations with either netting or photo surveys to complement them.

ACKNOWLEDGEMENTS

This research was a collaborative work and I am grateful to the many people who were involved. Firstly, I would like to thank my supervisor, Dr. Miriam Richards, who gave me the chance to complete this research even though I had a long break between my undergraduate and graduate studies. Coming back into science and particularly starting a master's degree during the COVID-19 pandemic has been challenging in ways I could not have imagined. You have supported me throughout this learning process and pushed me to do my best. Completing this degree required me to refresh previous knowledge, learn many new skills, and go beyond what I thought were my limits. I would not have reached this point without your patience and guidance.

I would also like to thank my other committee members Dr. Cory Sheffield and Dr. Gary Pickering, and my external examiner Dr. Jessica Forrest for the valuable advice they provided towards improving my work. Additionally thank you to Dr. Sheffield for proposing this bumble bee project, and for verifying many of my bumble bee identifications. I would like to acknowledge and thank the Faculty of Graduate studies at Brock University, the Friends of the Royal Saskatchewan Museum, the Saskatchewan Ministry of Agriculture, and the Canada-Saskatchewan Growing Forward 2 bi-lateral agreement for the funding provided.

This research could not have been completed without the help of all the other surveyors from Saskatchewan and Newfoundland and Labrador. Thank you for your time and effort in collecting bumble bees from your regions. From SK the surveyors include Dr. Sheffield, Kirsten Palmier, and Ryan Oram, all from the Royal Saskatchewan Museum. From NL the surveyors include Dr. Carolyn Parsons, Brendan Meaney, Danica Brockwell, and Tara Ryan, all from Agriculture and Agri-food Canada, and Leah Madore of the Government of Newfoundland and Labrador. Especially thank you, Dr. Sheffield, and Dr. Parsons, for identifying bumble bees, organizing the data from your regions, and answering my many questions regarding how the surveying took place in each region.

Thank you as well to all the private and public property owners who allowed us to survey on their properties. From Ontario, thank you to Dr. Miriam Richards and Dr. Adonis Skandalis, George Melvin, the Maretzki family, Barb and George Mitges, Brock University, and Niagara Region. From Saskatchewan, thank you to the provincial government and the Nature Conservancy of Canada. From Newfoundland and Labrador, thank you to the provincial government, Agriculture and Agri-Food Canada, Memorial University, and Pye Centre for Northern Boreal Food Systems.

Thank you to the past and present members of the Brock Bee Lab, Lyllian Corbin, Lyndon Duff, James Mesich, Jessie de Haan, Alex Proulx, Leslie Proctor, Leonardo Giontella, Jesse Maretzki, and Nora Romero. I will be forever grateful to you for all your help, including always being willing to lend an ear when I need to run through ideas, for reading and providing feedback on my many drafts, for offering advice to help improve my scientific communication, and much more. Nora Romero thank you specifically for providing me with lessons on how to identify bees and being willing to review some of my specimens when I needed a second opinion on species identification.

Thank you to Dr. Jess Vickruck for discussing blue vane traps with me and providing advice on their use. Thank you to Dr. Erica Oberndorfer for providing certain flower identifications for the Newfoundland and Labrador surveys.

In addition, thank you to Jim Niece and Ronni Huffman who allowed me to conduct practice surveys on their properties in early June and to Patty Moss for connecting me with them. While these sites were not used in the later surveys, I am still very grateful you were willing to be a part of this project.

Finally, a big thank you to my friends, family, and partner for always believing in me and for reminding me I have the strength to do anything I put my mind to. With special thanks to my dad for helping with BVT platform design and letting me borrow his tools all summer. The love and support I receive from you all mean the world to me.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SUPPLEMENTARY TABLES

LIST OF SUPPLEMENTARY FIGURES

INTRODUCTION

The importance of bees and the concern about population declines

Bees are essential pollinators of wild and agricultural plants and important members of ecosystems, but there is concern that bees are declining across the world, and many studies have been conducted to better understand this trend (Biesmeijer *et al*. 2006; Bartomeus *et al*. 2013; Zattara & Aizen, 2021; Turley *et al*. 2022). Despite the number of bee records (specimens and observations) being at an all-time high, the number of species being detected in many regions is lower than historically documented (Zattara & Aizen, 2021).

Bumble bees (Hymenoptera: Apidae, Apinae, Bombini; *Bombus* spp.) are a genus of bees with approximately 250 species worldwide (Michener, 2007; Williams *et al.* 2014). They are one of only a handful of bee genera that are capable of buzz pollination, which is when they vibrate their bodies when in contact with a flower to release pollen from plant species that require this form of pollination, and this makes them much more efficient pollinators than honey bees for crops like blueberries and tomatoes, among others (Michener, 2007; Williams *et al*. 2014; Colla, 2016).

Unfortunately, long-term studies of population trends in North America suggest bumble bees are experiencing more dramatic population declines than most other wild bees (Bartomeus *et al*. 2013; Turley *et al*. 2022). Since this genus seems to be particularly vulnerable to decline, it is important to monitor their populations and take steps to prevent declines from becoming extinctions.

The need for a standardized bee collection protocol in Canada

In order to monitor bee populations, specimens must first be collected or detected (recorded but not removed from the environment). There are a number of different methods used for collecting and detecting bees, some of which are known to work better for bumble bees than others. Despite years of deploying an array of collection methods, scientists still do not fully understand how all methods compare to each other. This is a limitation because it reduces comparability between studies, and so there are calls from the scientific community to standardize bee collection protocols (Nielsen *et al*. 2011; Strange & Tripodi, 2019; Woodard *et al*. 2020).

Previous researchers (Lebuhn *et al.* 2013; Strange & Tripodi, 2019) have attempted to devise a standard large-scale bee monitoring program and a rapid assessment protocol, respectively. However, Lebuhn *et al.* (2013) were met with debate by Tepedino *et al*. (2015) who questioned the methods of their power analysis, criticized their suggested use of pan traps which would result in a large number of bee deaths, and commented on the lack of detail provided in regard to program design (including lack of or unrealistic consideration of costs, and regional differences in pan trap effectiveness and length of monitoring time). Lebuhn *et al.* (2015) responded to these criticisms defending the coefficients of variation used in their power analyses, mentioning that pan trap sampling is not known to contribute to overall bee declines, and clarifying that they were attempting to show the possibility of the program, not the details. Even so, no standard national protocol to collect bees has been accepted or implemented anywhere in North America (Portman *et al*. 2020; Woodard *et al.* 2020). The United States National Native Bee Monitoring Research Coordination Network has been hosting discussions with experts to gather diverse perspectives in order to develop a national standard bee monitoring protocol and create a national network in the United States (National Native Bee Monitoring RCN, 2023). This is an important initiative as reducing the inconsistency in collection methodologies across studies will help to improve replicability and comparability (Packer & Darla-West, 2021). This would also result in improved baseline data for future researchers to use in conservation assessments and lead to a better understanding of the status of native bees, including species at risk. While it is good that the United States is working on a native bee monitoring program, Canada's bee species would also benefit from developing nationwide monitoring protocols, but how collection methods work across different Canadian landscapes needs to be better understood to do this.

Research purpose and objectives

The purpose of this research was to improve our understanding of how commonly used bumble bee collection or detection methods compare to each other in different regions of Canada to assist in developing a standard method for monitoring bumble bees that could be used across the country, including for COSEWIC assessments. The three methods chosen for comparison were blue vane traps (BVTs), timed targeted netting, and timed targeted photographs. The surveys took place at locations in Ontario, Saskatchewan, and Newfoundland and Labrador. The objectives were to assess whether the methods produce different bumble bee diversity results (i.e., species detection, evenness), whether they detect at-risk species, and to evaluate the level of sampling effort required for each, so as to provide a recommendation for which method(s) should be used in Canadian bumble bee population or community assessments. The timed aspect of the netting and photo surveys was included as a way to standardize the surveys and determine how long sampling should take place (survey effort). Additionally, I assessed whether the intensive photo surveys could have been replaced by community science data from the website iNaturalist.

LITERATURE REVIEW

Bumble bee life history

There are 49 bumble bee species known in Canada and the USA (Sheffield *et al*. 2020; Williams, 2021). Their life history, including that of the parasitic species, has been summarized thoroughly by Laverty & Harder (1988), Michener (2007), Williams *et al.* (2014), and Colla (2016); the following two paragraphs are a synthesis of the information contained within these four sources. Eusocial bumble bee colonies consist of the female castes, queens (reproducing females) and workers (non-reproducing females), and males, each of which have different roles in the annual cycle of an individual colony. In early spring, gynes (potential-queens who have not laid eggs) emerge from their place of hibernation and begin to search for a site to build their nest. Common locations for nest sites include old rodent nests, under clumps of grass, and cavities such as in hollow wood. Once a nest site is chosen, the gyne forages for pollen and nectar, which is brought back to the nest. The pollen is molded into a rough ball shape which the gyne then lays eggs on. The gyne also creates nectar storage containers called honey pots. Once the eggs hatch, the larvae feed on the pollen and develop into workers over the course of a few of weeks. The fully developed workers then take on the roles of foraging for nectar and pollen, nest maintenance, and defense. At this point the queen no longer leaves the nest and focuses on laying eggs. In late summer the queen starts producing eggs that develop into gynes and males. The males fly in search of conspecific mates from other colonies, and as the temperatures drop in late summer or autumn the mated gynes look for places in the soil to hibernate, while the old queen, workers, and males die. Gynes that survive the winter start new colonies and become queens the following spring.

Continuing the synthesis of Laverty & Harder (1988), Michener (2007), Williams *et al.* (2014), and Colla (2016)'s descriptions of bumble bee lifestyles, North American cuckoo bumble bees, subgenus *Psithyrus* and one *Alpinobombus* species, *B. natvigi* (formerly *B. hyperboreus* (Williams *et al.* 2015)), exhibit a parasitic lifestyle. Emerging slightly later than gynes of other bumble bees, female cuckoos search for established bumble bee nests that contain workers. The cuckoo bee kills or subdues the nest queen and takes over. The female cuckoo now lays her eggs, and only females and males are ever produced. The original workers from the nest forage and feed the new cuckoo offspring that are not related to them. Like other bumble bees, the new females and males search for conspecific mates and in late summer or fall, all bumble bees except for the mated females die. The female cuckoos find a place beneath the soil to hibernate for the winter and the cycle begins again the following spring.

Status of bumble bees in Canada

In Canada, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) assesses species thought to be in decline, and as of June 2023 they had assessed 6 bumble bee species: *B. affinis*, *B. bohemicus*, both subspecies of *B. occidentalis* (now *B. occidentalis s. str*. and *B. mckayi* (Williams, 2021)), *B. terricola*, *B. pensylvanicus*, and *B. suckleyi* (COSEWIC, 2010, 2014a, 2014b, 2015, 2018, 2019, 2022). Three candidate species were also noted as possibly requiring assessment: *B. vandykei*, *B. morrisoni*, and *B. variabilis* (COSEWIC, 2023). To understand how bumble bee species are faring in Canada it is necessary to consider assessments both from Canada and the United States, as bumble bee populations do not recognize country borders. In addition to COSEWIC, scientific papers from Canada and the USA also indicate declines or rarity for many of these species mentioned above (Grixti *et al.* 2009;

Cameron *et al.* 2011; Colla *et al.* 2012; Bartomeus *et al.* 2013; Sheffield *et al*. 2016; Jacobson *et al*. 2018; MacPhail *et al.* 2019; Strange & Tripodi, 2019; Simon *et al*. in press). Many of these studies (COSEWIC and other) make use of changes in relative abundance to indicate decline, but this measure must be interpreted with caution since the relative abundance of one species is linked to all other species in the collection.

While some species are at-risk, it is important to note there are likely still regional differences in how well populations are doing, and not all species are declining (Colla, 2016). For example, *B. terricola* is only declining in its southern range (Bartomeus *et al.* 2013; COSEWIC, 2015). Within Canada specifically, *B. terricola* is noted to be in decline in Ontario, Quebec, and the Maritimes, but stable in other regions (COSEWIC, 2015). There are also mixed results regarding *B. fervidus* and *B. vagans*, with some studies indicating declines in the abundance or distribution of one or both species (Colla & Packer, 2008; Grixti *et al.* 2009; Colla *et al.* 2012; Jacobson *et al.* 2018; Turley *et al.* 2022). However, *B. fervidus* and or *B. vagans* also appear abundant or stable in some regions (Colla *et al.* 2012; Novotny *et al.* 2021; Christman *et al*. 2022). These mixed results may be due to differences in the province, state or region studied since few papers considered the whole range of *B. fervidus* and *B. vagans* (e.g., Colla & Packer, 2008; Grixti *et al.* 2009; Colla *et al.* 2012; Jacobson *et al.* 2018; Novotny *et al.* 2021; Christman *et al*. 2022; Turley *et al.* 2022)

Three species that appear to be doing particularly well are *B. impatiens*, *B. griseocollis*, and *B. bimaculatus* (Colla & Packer, 2008; Grixti *et al.* 2009; Colla *et al*. 2012; Colla, 2016; Jacobson *et al*. 2018; Strange & Tripodi, 2019; Novotny *et al.* 2021; Jackson *et al*. 2022). *Bombus impatiens* was almost universally noted as increasing in relative abundance and distribution, except for Turley *et al*. (2022) which found a significant decrease in *B. impatiens* and *B. bimaculatus* in Pennsylvania. Even abundant species can undergo sudden declines, so it is still important to monitor them. For example, *B. affinis*, *B. pensylvanicus*, and *B. occidentalis* were once widespread, but they, as well as other bumble bee species, underwent declines in the mid-to-late 20th century (Colla & Packer, 2008; Grixti *et al*. 2009; Cameron *et al*. 2011; Colla *et al*. 2012; Williams *et al*. 2014; Jacobson *et al.* 2018).

Measurements to detect population changes

To detect population trends like those mentioned above, bee monitoring studies typically look for changes in a species abundance and distribution, and changes in species composition and diversity for the bee community (see Supplementary Table S1.1 for a list of studies). For a given location, time, and species, *abundance* is the count of individuals of a species, and *distribution* is the geographical area where it exists (Molles & Cahill, 2008). If the population is stable, abundance and distribution will remain relatively the same over time; for instance, unless the population is already small, the International Union for Conservation of Nature (IUCN) Redlist Guidelines for determining declining status only consider population reductions of 30% or greater (IUCN Standards and Petitions Committee, 2022). Trends of increase in abundance and distribution are important because they indicate a population is doing particularly well. Trends of decreasing abundance and distribution are of concern to scientists because there is the possibility the species may become a species at risk, and ultimately become extirpated or even extinct.

Species composition and diversity are measures of how all species in a community are distributed. *Species composition* refers to the mixture of species of interest present in an area and their *relative abundances* (the proportion they each comprise of the total abundance (Magurran, 2004)) (Billheimer *et al*. 2001). *Diversity* is a function of the number of species in an area, *species richness*, and how similar or different the species' abundances are, *evenness* (Smith & Smith, 2001; Molles & Cahill, 2008; Magurran, 2004). Communities in which species are similarly abundant are more even than communities in which a few species are dominant (Smith & Smith, 2001; Magurran, 2004). Diversity is highest when both species richness and evenness are high (Molles & Cahill, 2008).

The challenges of monitoring bee populations and communities

Sampling bee populations and communities to detect trends is expensive and time consuming, and there is often limited funding available (Magurran, 2004). Bees, like most insects, are challenging to sample because of high inter-annual variation in their abundance (Kimoto *et al.* 2012; Gezon *et al.* 2015; Onuferko *et al.* 2018; Didham *et al.* 2020; Woodard *et al.* 2020; Packer & Darla-West, 2021; Turley *et al.* 2022). Even within years, different bee species within a community will emerge, be active and overwinter at different times of the season (Colla & Dumesh, 2010; Kimoto *et al*. 2012; Onuferko, 2013; Williams *et al.* 2014). This high variability makes it difficult to determine when to assess baseline population size, which then limits the ability to detect population trends (Didham *et al*. 2020). High variability also means that long periods of time (i.e., years) are needed to be sure that trends are real (Fox *et al.* 2019; Didham *et al.* 2020; IUCN Standards and Petitions Committee, 2022). For instance, the IUCN requires 10 years of data for assessing population trends of insects, and some suggest even 10 years may not be long enough (Fox *et al.* 2019; IUCN Standards and Petitions Committee, 2022).

Many studies make use of museum collections or databases to provide the historical data needed to detect trends (Grixti *et al*. 2009; Cameron *et al*. 2011; Colla *et al.* 2012; Bartomeus *et al*. 2013; Kerr *et al.* 2015; Jacobson *et al.* 2018; Bell, 2019; MacPhail *et al.* 2019; Soroye *et al.* 2020; Hemberger *et al*. 2021; Zattara & Aizen, 2021; Jackson *et al.* 2022; Simon *et al*. in press). These types of data often have varying or unknown sampling effort (Grixti *et al*. 2009; Bartomeus *et al*. 2013, Welti *et al.* 2021). Varying sampling effort prevents direct comparisons of abundances, which is why relative abundance is usually used instead (Magurran, 2004). However, relative abundance also has drawbacks when used for species conservation assessments, etc., as the proportion of any one species is dependent on all the species counted in a sample. So, it is important to consider that any apparent trends in relative abundance may be simply due to changes in other species in the collection.

Overview of common bee collection methods

Bees are collected for study in various ways. Common passive collection methods include pan traps, Malaise traps, trap nests, baits, and vane traps. Pan traps are blue, yellow, and white bowls or cups filled with soapy water (Westphal *et al*. 2008; Nielson *et al*. 2011; Richards *et al*. 2011; Geroff *et al*. 2014; Droege, 2015; McCravy, 2018; Portman *et al*. 2020; Packer & Darla-West, 2021; Tronstad *et al*. 2022). While pan traps are one of the most common methods for collecting bees in general, they have been shown to be less likely to collect large bees, like bumble bees, possibly because big bees can climb out of the bowl (Westphal *et al*. 2008; Droege, 2015; Bell 2019; Prendergast *et al.* 2020; Packer & Darla-West, 2021; Pei *et al*. 2022; Tronstad *et al*. 2022). Other passive methods include Malaise traps (funnel-shaped, mesh fabric traps), trap nests (which provide tubular nesting structures for cavity nesting bees), and baits (mainly used for attracting orchid bees) (Sheffield *et al*. 2008; Westphal *et al*. 2008; Nielson *et al*. 2011; McCravy, 2018; Prendergast *et al*. 2020; Packer & Darla-West, 2021). Vane traps have colourful vanes, which are attractive to bees and especially bumble bees, and when bees intercept these vanes during flight, they fall into a collection jar (Stephen & Rao, 2005; Geroff *et al*. 2014; Packer & Darla-West, 2021).

Active methods for collecting or recording incidence data from bees include netting, bee vacuums, visual observations, and photographs (Stephen & Rao, 2007; Westphal *et al*. 2008; Nielson *et al*. 2011; Droege, 2015; McCravy, 2018; Portman *et al*. 2020; Prendgergast *et al*. 2020; Packer & Darla-West, 2021). Unlike passive methods, active methods require manipulation by a surveyor, who either detects bees through visual or photo observations or uses a tool to collect bees.

Scientists generally recommend using a combination of active and passive methods when collecting bees because these methods have different strengths and weaknesses, including biases, which produce different results (Wilson *et al*. 2008; Nielson *et al*. 2011; Rhoades *et al*. 2017; McCravy, 2018; Prendergast *et al*. 2020; Mundy-Heisz, 2021; Prendergast & Hogendorn, 2021; Packer & Darla-West, 2021; Pei *et al*. 2022; Tronstad *et al*. 2022). For example, pan traps, vane traps, and Malaise traps have many advantages over active methods, in that they reduce physical labour, they are good for surveying places that are hard to survey by net, and they collect bees continuously throughout the day including mornings and evenings (Westphal *et al.* 2008; Prendergast *et al.* 2020; Packer & Darla-West, 2021; Prendergast & Hogendoorn, 2021; Montero-Castaño *et al.* 2022). Passive methods are also easier to standardize and lack the variation that occurs due to differences in collectors' skills at detecting, identifying, and catching bees (Westphal *et al.* 2008; Prendergast *et al.* 2020; Packer & Darla-West, 2021; Prendergast &

Hogendoorn, 2021; Montero-Castaño *et al.* 2022). However, active collection methods also have advantages over passive traps. Species bias is thought to be much lower, they permit the collection of floral associations and other ecological data along with bee specimens, and they reduce bycatch (Westphal *et al.* 2008; Portman *et al*. 2020; Prendergast *et al.* 2020; Packer & Darla-West, 2021; Prendergast & Hogendoorn, 2021; Montero-Castaño *et al.* 2022). Reducing bycatch is dually beneficial in that fewer insects are killed, and less lab space is taken up storing unneeded specimens (Portman *et al.* 2020). However, bycatch can also be useful for monitoring populations and more if kept for other entomologists studying different insects (McCravy, 2018; Packer & Darla-West, 2021).

In addition to their benefits, all methods have drawbacks as well. Some commonly mentioned concerns for pan, Malaise, and vane traps are that the catch rate can be affected by trap visibility, height, and the abundance of surrounding flowers, and they differ in attractiveness to species (Kimoto *et al.* 2012; Geroff *et al*. 2014; Onuferko *et al*. 2015; Droege, 2015; McCravy, 2018; Portman *et al*. 2020; Prendergast *et al.* 2020; Packer & Darla-West, 2021; Prendergast & Hogendoorn, 2021). There are also concerns that some traps may cause declines to local populations if overused (Gibbs *et al*. 2017; McCravy, 2018; Prendergast *et al*. 2020; Packer & Darla-West 2021; Prendergast & Hogendoorn, 2021). While most often discussed with traps, oversampling is a concern that can apply to any type of collection method. However, Gezon *et al*. (2015) showed that at their sample sites collections done every other week with either pan traps or netting were not detrimental to bee populations. Another study, Sheffield *et al*. (2008), continued to collect more cavity nesting bees in trap nests over a three-year period despite any offspring from the nests being removed from the community. These examples illustrate that bee communities can withstand some level of lethal sampling. With blue vane traps though, some researchers have indicated that these traps may oversample bumble bees, and caution is recommended when using them in spring before queens establish their nests (Gibbs *et al*. 2017; Packer & Darla-West, 2021). By contrast, the most common concerns for active methods are collector bias, and that they require more labour from the surveyor (Westphal *et al*. 2008; Nielson *et al*. 2011; Cane *et al*. 2013; Rhoades *et al*. 2017; McCravy, 2018; Prendergast *et al.* 2020; Packer & Darla-West, 2021; Krahner *et al*. 2021; Prendergast & Hogendoorn, 2021).

Criteria for evaluating the best collection method for bumble bee assessments

When choosing the method(s) for a national monitoring program for bumble bees, an ideal method should collect the target species, provide an accurate representation of species composition and richness, be consistent across landscapes, times, weather patterns and individual surveyors, be repeatable, contain little collector bias, and minimize cost, sampling effort, processing time and bee death (Westphal *et al.* 2008; Nielsen *et al*. 2011; Lebuhn *et al*. 2013; Woodard *et al*. 2020; Packer & Darla-West, 2021; Mundy-Heisz, 2021). All methods, whether passive or active, provide a representation of bee abundance in each area, but it is difficult to know how accurate these representations are (Portman *et al*. 2020; Packer & Darla-West, 2021). Due to this, it may be necessary to use more than one method, but if using multiple methods, it is important to choose those which complement each other, meaning that the species compositions produced are not the same (Westphal *et al*. 2008; Nielson *et al*. 2011). This will result in compensation for each method's weaknesses and produce the most complete species composition possible.

Monitoring populations or communities takes time and money, so understanding how to prevent oversampling and save funding resources is important. When working with at-risk species, non-lethal methods are preferable, but even when not working with at-risk species sampling should not be detrimental to bee populations (Gezon *et al*. 2015; Prendergast & Hogendoorn, 2021; Packer & Darla-West, 2021). To prevent oversampling and impact on species, collecting as few individuals as possible is recommended (Woodard *et al*. 2020; Montero-Castaño *et al.* 2022). Collecting fewer specimens also has the added benefits of saving the surveyor time and effort in the field, and time processing specimens.

Collection methods selected for this thesis

Since this research was focused on testing collection methods for use in a national bumble bee monitoring protocol, the methods tested needed to be ones that are effective for bumble bees. The three methods chosen were BVTs, timed targeted netting, and timed targeted photographs.

Blue vane traps (BVTs)

Vane traps consist of two perpendicular, plastic vanes set into a funnel, connected to a collection jar. The coloured vanes act as both an attractant and a flight-intercept trap where bees and other insects fall into the collection jar once they hit the vanes. Stephen & Rao (2005) discovered that unscented blue and yellow vane traps were effective for collecting bees and particularly bumble bees. Research comparing the two colours of vanes consistently showed blue to be more attractive to bees in general than yellow (Stephen & Rao, 2005, 2007; Geroff *et al*. 2014; Joshi *et al*. 2015; Prendergast *et al*. 2020). BVTs are now a commonly deployed collection method for bumble bee research (Stephen & Rao, 2007; Rao & Stephen, 2010; Kimoto *et al.*

2012; Joshi *et al*. 2015; Gibbs *et al*. 2017; Prendergast *et al.* 2020; Mundy-Heisz, 2021; Tronstad *et al.* 2022; Turley *et al.* 2022).

There is no standard number of BVTs recommended for use in evaluating bumble bee populations. There is also no standard field placement, although they are often suspended at heights between 0.75 and 2 meters (Stephen & Rao, 2005, 2007; Kimoto *et al.* 2012; Geroff *et al*. 2014; Joshi *et al.* 2015; Gibbs *et al*. 2017; Rhoades *et al*. 2017; McCravy, 2018; Prendergast *et al*. 2020; Turley *et al*. 2022). BVTs can also be placed into the ground at a depth of half of the collection jar height, eliminating the need for a hanging device, although this can sometimes result in the capture of small rodents (Packer & Darla-West, 2021). The collection jars do not require any scent or trapping agent to function (Stephen & Rao, 2005, 2007; Kimoto *et al.* 2012; Tronstad *et al*. 2022). However, a preservative such as dry insecticide, soapy water, propylene glycol or ethylene glycol solution can be added to prevent specimen decay when traps are deployed for long periods (Joshi *et al*. 2015; Prendergast *et al*. 2020; Packer & Darla-West, 2021; Turley *et al*. 2022). BVTs can be deployed for up to months at a time, but in areas of high precipitation they may fill with rainwater (Prendergast *et al*. 2020; Packer & Darla-West, 2021).

There are certain drawbacks to keep in mind when using BVTs. For instance, some studies suggest that BVT attractiveness is reduced when flowers are abundant, although others refute this (Stephen & Rao, 2007; Kimoto *et al*. 2012; Bell, 2019; Prendergast & Hogendoorn, 2021). The vanes and collection jars are known to lose colour over time, including some change over a single field season, and this could potentially affect catch rates (Joshi *et al.* 2015; Packer & Darla-West, 2021). Importantly, BVTs are known to be particularly attractive to large bees like bumble bees and less attractive to honey bees (Stephen & Rao, 2005, 2007; Kimoto *et al.* 2012; Geroff *et al*. 2014; Joshi *et al*. 2015; McCravy, 2018; Prendergast *et al.* 2020; Packer &

Darla-West, 2021; Prendergast & Hogendoorn, 2021). The bias towards bumble bees makes them useful for studying this genus. Vane traps are also known to collect species not associated with the vegetation they are placed in (Rao & Stephen, 2010; Gibbs *et al.* 2017; Rhoades *et al.* 2017). Gibbs *et al.* (2017) suggest that this may indicate BVTs are an extreme attractant to certain species. Several studies using BVTs found that they collected fewer bees over time, and based on this some researchers have suggested that BVTs may oversample species that are highly attracted to them, causing local population declines (Kimoto *et al*. 2012; Joshi *et al.* 2015; Gibbs *et al*. 2017; McCravy, 2018; Prendergast *et al*. 2020; Packer & Darla-West, 2021; Prendergast & Hogendoorn, 2021; Tronstad *et al*. 2022).

Targeted netting

Bumble bees are ideal specimens to collect by net because they are large and relatively slow, so easy to detect and catch (Prendergast *et al*. 2020; Pei *et al*. 2022). Targeted netting, using a net to collect bees detected visually is different from sweep netting which uses continuous, “blind” swings of a net, often in a figure eight pattern, to collect all insects from vegetation encountered (Westphal *et al*. 2008; Richards *et al*. 2011; McCravy, 2018; Packer & Darla-West, 2021). Sweep netting is usually done by walking a transect, and the continuous swinging nature controls the area and rate of sampling better than targeted netting (Westphal *et al*. 2008; Packer & Darla-West, 2021). Westphal *et al.* (2008) and Nielson *et al*. (2011) suggest that the meandering path of targeted netting may intersect with more flowers which in theory could reveal more species. However, both studies found little difference between netting done in a straight transect or a meandering path.

There is no standard amount of time for targeted netting collections; in studies it ranges from multiple 5-minute collections to hours or even to a certain number of specimens collected (Stephen & Rao, 2007; Wilson *et al*. 2008; Westphal *et al*. 2008; Nielson *et al*. 2011; Richards *et al*. 2011; Cane *et al.* 2013; Gezon *et al*. 2015; Gibbs *et al*. 2017; Rhoades *et al.* 2017; Strange & Tripodi, 2019; Prendergast *et al*. 2020; Krahner *et al*. 2021; Mundy-Heisz, 2021; Montero-Castaño *et al.* 2022; Pei *et al*. 2022; Tronstad *et al*. 2022). Netting is typically done during sunny, low wind, conditions, in temperatures above 15°C, no earlier than 09:00 h and no later than 18:00 h. Since it is restricted to a certain period of the day, crepuscular species may be missed in net collections (Rhoades *et al*. 2017; Packer & Darla-West, 2021).

Since individual surveyors vary in how efficiently they collect bees, collector bias is a concern, and while this can be reduced through training it can never be fully eliminated (Westphal *et al*. 2008; Nielson *et al*. 2011; Cane *et al*. 2013; McCravy, 2018; Prendergast & Hogendoorn, 2021; Krahner *et al*. 2021). Capture success can also be influenced by weather and vegetation, since windy conditions, and tall, dense or rigid vegetation are difficult to use a net in (Stephen & Rao, 2007; Cane *et al*. 2013; Droege, 2015; Prendgergast *et al*. 2020; Packer & Darla-West, 2021).

Photographs

Bumble bees can often be identified from well-taken photographs because they are large and their colour pattern is often diagnostic of which species they are (MacPhail *et al.* 2020b; Packer & Darla-West, 2021). Identification keys typically include details about the colour patterns on the head, thorax, and abdomen of bumble bees (Laverty & Harder, 1988; Williams *et al.* 2014; Ascher & Pickering, 2022). Photos are not commonly chosen as a main method for bumble bee documentation by professional scientists, except sometimes to support visual or catch-and-release observations (e.g., Thomson & Zung, 2015; MacPhail *et al*. 2019). Interestingly, Thomson & Zung (2015) designed a device to hold bumble bees still for photograph by digital camera to support catch and release netting surveys. However, photos are very popular in community science projects, which can produce data for scientific bumble bee research and population assessments (Stafford *et al*. 2010; COSEWIC, 2018, 2019; MacPhail *et al.* 2019; Flaminio *et al.* 2021; Hemberger *et al.* 2021; Suzuki-Ohno *et al*. 2021; Zattara & Aizen, 2021; Simon *et al.* in press). Community science is when non-expert and expert members of the community collect scientific data (MacPhail & Colla, 2020; MacPhail *et al*. 2020a; Flaminio *et al.* 2021). Despite how often photos in the form of community science data are used in bumble bee research, collection method comparison studies do not usually include them. One exception is a recent study by Simon *et al.* (in press) which offers comparison of photographic, community science collected bumble bee records to BVT collected records. In this study the community science iNaturalist records produced similar rarefaction results to BVT records, and while many of the species' proportional abundances were similar between these two data sets, *B. vosnesenskii* was much more common in iNaturalist records. It would be beneficial to learn more about the strengths and weaknesses of this collection method, including how it compares to other methods. It would also be useful to learn more about how community science data and scientific data compare.

Photographic bee documentation is discussed briefly in some review papers and books (Packer & Darla-West, 2021; Montero-Castaño *et al.* 2022). Studies recommend photos as a way to reduce killing bumble bees in surveys (Woodard *et al*. 2020; Montero-Castaño *et al.* 2022). Non-lethality is one of the reasons that photos are popular with community scientists, as is their ease of use since most people have cellphone cameras (Flaminio *et al.* 2021). While simple to take, poor-quality photos are common in community science data, since good quality photos require some knowledge of the angles to shoot, where to stand and may require multiple shots (Packer & Darla-West, 2021; Bumble Bee Watch, 2022; Suzuki-Ohno *et al*. 2022). Certain bumble bee species look very similar to each other and require taxonomists to look at small characteristics to distinguish species and these characteristics might not be visible in photographs (Williams *et al*. 2014; Ascher & Pickering, 2022; MacPhail *et al*. 2020b; Suzuki-Ohno *et al*. 2022). For instance, when Stafford *et al*. (2010) used community scientists to collect photos of bees, they found two bumble bee species were particularly hard to distinguish in photos that did not include a good shot of the tip of the abdomen. Similarly, Suzuki-Ohno *et al*. (2021) found it too difficult to distinguish between *B. deuteronymus deuteronymus* and *B. pseudobaicalensis* with community scientist photographs. Since photographs are an active method, they likely contain biases that are common to these types of methods, such as collector's bias.

Traditionally specimens have been collected by professional scientists, but advancements in technology have made community science increasingly popular in recent years (MacPhail & Colla, 2020; Callaghan *et al.* 2022). By involving more people in the data collection process, community science can help to greatly increase survey range and coverage (MacPhail & Colla, 2020; Dubaić *et al*. 2022). Community science data has been used to measure population and distribution changes, discover new species, monitor the loss and introduction of new species to an area, and help track invasive species (MacPhail & Colla, 2020; Flaminio *et al.* 2021; Callaghan *et al.* 2022; Simon *et al.* in press). Some limitations are that this type of data often only provides presence information, this data is usually geographically biased to cities, and rare species may be over or under-represented (Stafford *et al*. 2010; MacPhail & Colla, 2020). Also, there are concerns about data quality and accuracy, and only a few studies evaluating this (MacPhail *et al*. 2020b; Flaminio *et al*. 2021; Suzuki-Ohno *et al*. 2022).

Popular community science websites with a focus on bumble bee observations

There are some popular community science websites that host bumble bee observations. Bumble Bee Watch (https://www.bumblebeewatch.org/) focuses specifically on bumble bees, and all submissions are subject to expert review (MacPhail *et al*. 2020b). Since all records are considered accurate after expert review, MacPhail *et al*. (2020b) were able to compare the accuracy of the initial species identifications offered by the community scientists to the ones after expert review. Users accurately identified bumble bee species about 53% of the time which was lower than the 80% threshold considered by the authors as the point at which expert verification would not be needed. This has implications for the quality of other community science databases that do not guarantee expert review.

Another popular community science website for uploading photos and other media is iNaturalist (www.inaturalist.org). This website covers many taxa, but there are projects on the site that are specific to bumble bees. On iNaturalist, a machine learning algorhithm suggests identifications when pictures are uploaded and then experts and non-experts voluntarily provide additional identification by either agreeing with the initial identification or suggesting alternative possibilities. To reach research grade status at species level at least two thirds of identifiers need to agree on the identification (Spiesman *et al*. 2021; Callaghan *et al*. 2022). iNaturalist conducted an internal study on the accuracy of records identified to species (records which are likely research grade), and of all the taxa tested, insects were the least accurately identified based on expert review, 65.3% compared to 77% or higher for all other taxa (Ueda, 2019). The sample size of this study was small though, just over 3000 records total for all taxa and 30 experts, and it is difficult to know how results for all insects extrapolate to bumble bees. A recent study by Simon *et al*. (in press) offers some evidence that iNaturalist records can provide similar information to BVT sampling on Galiano island, British Columbia. Here, iNaturalist records produced a rarefaction curve that was equivalent to the curve produced by BVT sampling. The species proportional abundances were also generally similar across the iNaturalist records and BVT records except for one species known to be associated with disturbed habitats, like urbanization, which was detected in a higher proportion in the iNaturalist records. It would be useful to assess whether the similarity in results between iNaturalist and scientific data demonstrated in Simon *et al*. (in press) on Galiano Island is true in other regions of Canada. If so, then iNaturalist records could replace more intensive surveying which would greatly reduce survey effort.

Objectives

The main objectives of this research were to evaluate three collection or detection methods to assess whether they produce different bumble bee diversity results (i.e. species detection, evenness), whether they detect at-risk species, and to evaluate the level of sampling effort required for each. The methods compared were blue vane traps (BVTs), timed targeted netting, and timed targeted photographs. Comparisons were made for the Carolinian (southern Ontario), Prairies (Saskatchewan), and Boreal Shield (Newfoundland and Labrador) Ecozones. The timed aspect of the netting and photo collections was a way to standardize collections and to evaluate how many or how long these collections should be in order to detect the species in the area, including at-risk ones. BVTs were chosen as they are the passive collection method best suited to collecting bumble bees and still a relatively new method in need of further study. Targeted netting is a classic method for bee collection, useful for collecting large bees like bumble bees, and one that also reduces unnecessary bycatch. Photographs were chosen for comparison because of their increased use in recent bumble bee assessments and the lack of information currently available about this method. Photos from the Prairies and Boreal Shield Ecozones were uploaded to iNaturalist. These methods were assessed in three different regions of Canada because I was interested in finding a collection method that would be effective at a national level. An additional objective was to assess whether iNaturalist data could replace the more intensive scientific photo surveys and thereby reduce survey effort. Based on the outcomes of the comparison regarding diversity, species at risk, level of survey effort, and regional differences, I provide a recommendation for which methods could be effective in Canadian bumble bee population or community assessments including COSEWIC assessments.

MATERIALS AND METHODS

Study Regions and Sites

Bumble bees were collected in three ecozones within three provinces: Ontario, Saskatchewan, and Newfoundland and Labrador (Figures 1.1 to 1.3). Sites were surveyed with permission from the private and public property owners, and contained vegetation that made it likely bumble bees would be present. Site names are listed in Table 1.1, and detailed site descriptions and maps are provided in the Appendix and Supplementary Figures S1.1 to S1.14.

The eight sites in Ontario lie within a specific region of the Mixedwood Plains Ecozone called the Carolinian Zone (Government of Canada, 2013; Ontario Biodiversity Council, 2021; Figure 1.1). Carolinian Zone sites consisted of agricultural properties, naturalized former landfills, home gardens, and wildflower fields. Four of the areas surveyed in the Carolinian Zone had been used for bee research in previous Brock Bee Lab studies: Elm Street Naturalization Site, Station Road Naturalization Site, Phys Ed Hydro, and Quarry Southwest (Richards *et al*. 2011; Rutgers-Kelly & Richards, 2013; Kutby, 2013; Onuferko, 2013; Onuferko *et al*. 2015, 2018; de Haan, 2021).

The three Saskatchewan sites surveyed are in the Prairies Ecozone (Government of Canada, 2013; Figure 1.2). The Prairies Ecozone had three sites, of which two were nature conservation sites and one was a public trail system.

The five Newfoundland and Labrador sites are in the Boreal Shield Ecozone (Government of Canada, 2013; Figure 1.3). The Boreal Shield Ecozone had five sites which consisted of a mixture of agricultural research properties, an area of natural vegetation across from a botanical garden, and a former gravel pit.

Figure 1.1: Survey sites (red box) in the Carolinian Zone which is located in southern Ontario within the Mixedwood Plains Ecozone (Ontario Biodiversity Council, 2021), represented in beige. Maps are modified from Government of Canada map of ecozones (Government of Canada, 2013), and Google Maps (© 2022 Google).

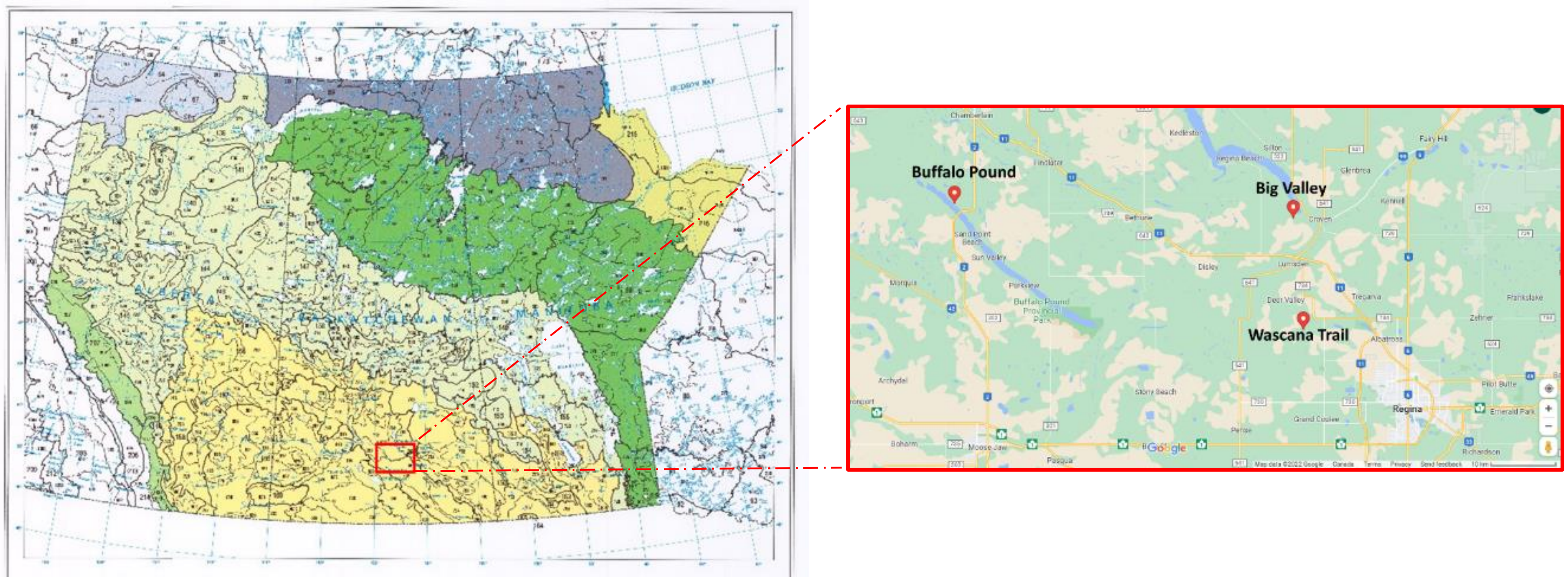


Figure 1.2: Survey site locations (red box) in Saskatchewan, within the Prairies Ecozone (represented in yellow). Maps are modified from Government of Canada map of ecozones (Government of Canada, 2013), and Google Maps (© 2022 Google).

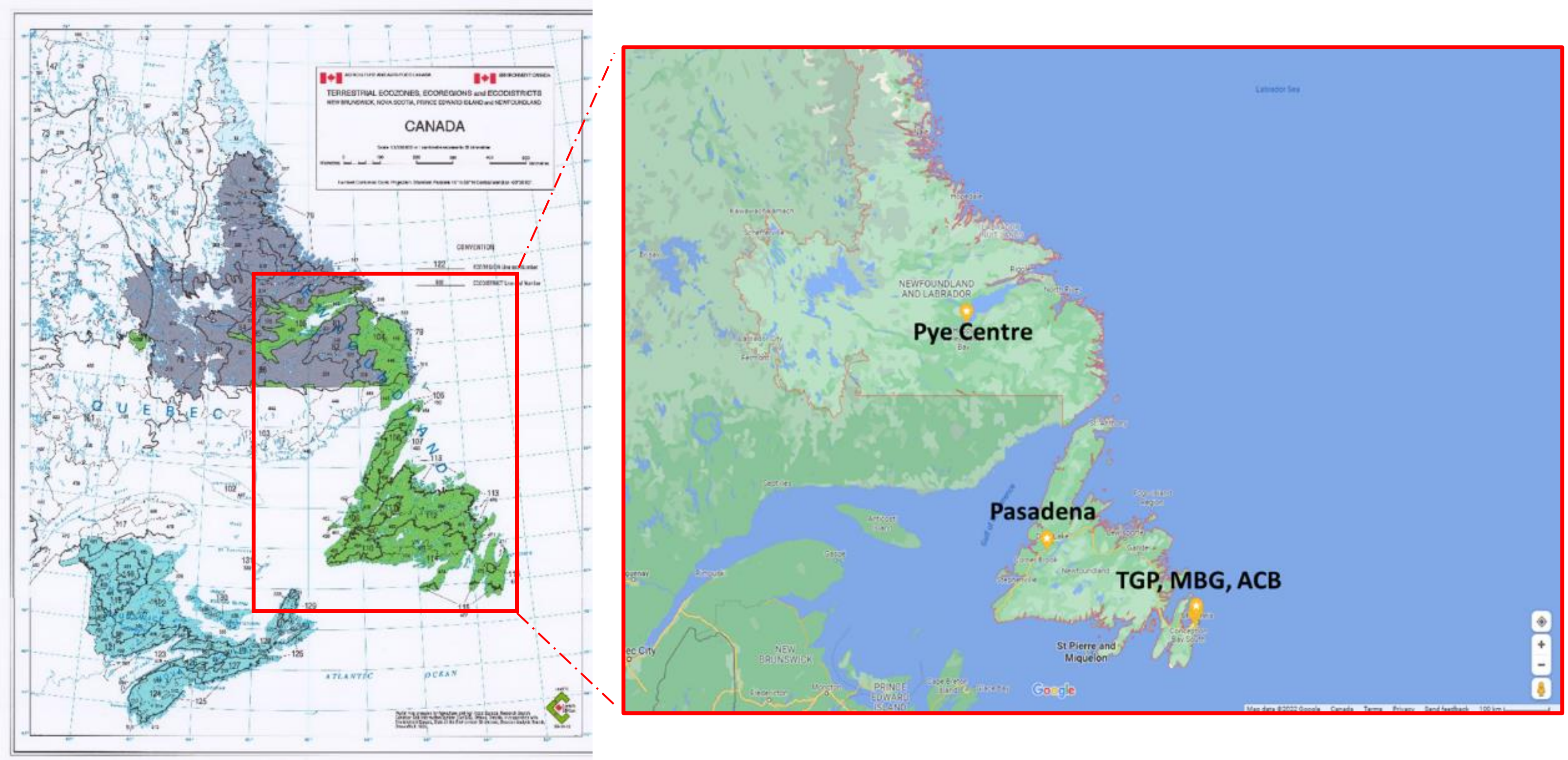


Figure 1.3: Survey site locations (red box) in Newfoundland and Labrador, within the Boreal Shield Ecozone (represented in green). Maps are modified from Government of Canada map of ecozones (Government of Canada, 2013), and Google Maps (© 2022 Google).

Table 1.1: Survey site locations for each of the three regions studied.

Region	Site	City/Town/Municipality	Latitude	Longitude
Carolinian Zone (ON)	Elm St.	Port Colborne	42.9279	-79.2588
	Station Rd.	Wainfleet	42.8844	-79.3777
	Konkle Rd.	Beamsville	43.1450	-79.5025
	Victoria Ave.	Vineland	43.1377	-79.3911
	Farr Rd.	Welland	42.9963	-79.2068
	Townline Rd.	Fort Erie	42.9644	-79.0150
	PEH	St. Catharines	43.1186	-79.2559
	QSW	St. Catharines	43.1196	-79.2380
Prairies Ecozone (SK)	Buffalo Pound	Dufferin	50.7302	-105.6073
	Big Valley	Lumsden	50.7107	-104.8684
	Wascana Trail	Lumsden	50.5563	-104.8463
Boreal Shield Ecozone (NL)	Pye Centre	Happy Valley-Goose Bay	53.3206	-60.2299
	Pasadena	Pasadena	49.0190	-57.5990
	Torbay Gravel Pit	Torbay	47.6538	-52.7563
	MUN Bot Garden	St. John’s	47.5695	-52.7580
	AAFC Cranberry Bog	St. John’s	47.5133	-52.7857

Bumble bee collection methods

Survey timing

All surveys were conducted in the summer and fall of 2021 using BVTs, netting and photos in all three regions. Surveys were begun in summer to avoid collecting spring emerging queens which would have affected colony establishment. The general structure was that survey periods were a week in length with BVTs deployed for the full week, while multiple 30-minute netting and photo surveys were conducted throughout the week. There was some variation by region (Table 1.2).

In the Carolinian Zone, surveys were conducted at seven sites following the schedule outlined in Table 1.3. An eighth site, QSW, was added to the study in July to avoid disruptions to bee behavioural studies conducted in the area prior to this time, and no BVTs were deployed there. Two sites were surveyed each day with four sites surveyed per week. A random number generator was used each day to decide which of the two sites to visit first. BVTs were deployed between 07:00h and 09:30h on Day 1, and trapped specimens were collected between 15:30h and 17:30h on Days 1, 3 and 7. Netting and photographic surveys were conducted between the hours of 10:00 h and 15:30 h, on the same days that BVTs were emptied. Both surveys (netting and photo) done at the same site, on the same day, followed the same path, and were separated by 30 minutes to prevent collecting the same specimens. The order in which the netting and photo surveys were conducted, and at which end of the field to begin was decided randomly. Rainy weather caused occasional delays to the schedule, and most of the Day 3 surveys in September were conducted on Day 4 due to other commitments. At each site, consecutive survey periods were separated by approximately two weeks.

Table 1.2: Comparison of BVT, netting and photo survey methods in the Carolinian Zone, the Prairies Ecozone, and the Boreal Shield Ecozone. Overall survey structure was similar with some differences in details based on region.

	Carolinian Zone	**Prairies Ecozone**	**Labrador Boreal Shield Ecozone**	**W. Newfoundland Boreal Shield Ecozone**	**E. Newfoundland Boreal Shield Ecozone**
No. sites	8	3	1	1	3
Collection timing					
No. days in a survey period	7	Period 1: 8 Period 2: 15	7	7	7 or 8
No. survey periods	5	2	1	1	2
Collection days - BVT	1, 3, and 7	Period 1: 2, 4 and 8 Period 2: 15	7	7	7 or 8
Collection days – netting and photo	1, 3 and 7	Period 1: 1, 2, 4 and 8 Period 2: 1 and 15	1 and 7	1 and 7	1 and 7 or 8
BVT surveys					
No. BVTs per site	2 or 3	3*	1**	4	2
BVT placement	On platforms	In the ground	In the ground	In the ground	In the ground
Minimum BVT spacing	48 m	500 m	20 m	20 m	20 m
Netting surveys					
No. surveyors	1	1	1 or 2	1	1 or 2
Survey length (min)	30	30	30 or 15	30	30 or 15
Handling time (time to place bee in vial)	Included	Included	Excluded	Excluded	Included or excluded
Photo surveys					
No. surveyors	1	1	1 or 2	1	1 or 2***
Survey length (min)	30	30	30 or 15	30	30 or 15

* BVTs 1 and 3 at Wascana Trail site were lost during the second survey period

**BVT 2 in this region was excluded due to a labelling mix-up

*** For survey period 1, a phone error resulted in the photos of one surveyor being lost

Table 1.3: Start dates for bumble bee survey periods in Carolinian Zone in 2021. Surveys periods were one week in duration, repeated 5 times, with the general structure that sampling was carried out on the first, third and seventh days (not full 24-hour durations).

Site	**Survey dates**				
	Period 1	**Period 2**	**Period 3**	**Period 4**	**Period 5**
Victoria, Konkle	22 June	13 July	3 August	24 August	14 September
Elm St., Station Rd.	23 June	12 July	4 August	23 August	13 September
Townline, Farr	30 June	21 July	12 August	1 September	25 September
PEH, QSW	1 July*	20 July	13 August	31 August	21 September

*PEH only

In the Prairies Ecozone, surveys were conducted following the schedule outlined in Table 1.4. All three sites were surveyed on the same days, but the order in which sites were visited was alternated. BVT placement was from mid-morning to mid-day, and trap contents were collected after 24 hours, 3 full days and 7 full days in the first survey period. The second survey period was extended by one week due to a long period of rain, with BVTs left on site for 14 full days. Netting and photo surveys were conducted on the day of BVT placement and on the days BVTs were emptied. This means netting and photo surveys were conducted four times per site in the first survey period and twice in the second survey period. Netting and photo surveys were conducted simultaneously, by two surveyors (one per method), during peak bumble bee flight hours, with surveyors walking in opposite directions to prevent collection of the same specimens.

In the Boreal Shield Ecozone, surveys were conducted following the schedule outlined in Table 1.4. Two sites (PC and PAS) were only surveyed for a single week, whereas three sites (TGP, MBG and ACB) were surveyed twice. BVTs were set out between 10:30h and 14:45h, and collection occurred on the final day of the week, on day 7 or 8, also between 10:30h and 14:45h. Netting and photo surveys were conducted during peak bumble bee flight hours on the first and last days of the survey week. At each site a coin was flipped to determine whether the netting or the photo survey would be completed first, and a period of 30-minutes separated the two surveys.

Blue vane trap (BVT) deployment

The same brand of BVT (BanfieldBio Inc., 2L container) was used in all regions. These consist of two, blue-coloured vanes set perpendicularly into a funnel that leads into a clear collection jar (Figure 1.4).

Table 1.4: Start and end dates for 2021 bumble bee survey periods in both the Prairies Ecozone and the Boreal Shield Ecozone. In the Prairies the first survey period was one week (8 days) in duration, with BVT contents checked after 24 hours, 3 full days and 7 full days, and netting and photo surveys carried out on those same days plus the day of BVT set up. The second survey period was two weeks in duration with BVTs collected on the last day and netting and photo surveys carried out on the first and last day. Boreal Shield surveys were one week in duration (7 or 8 days), with BVTs collected on the last day, and netting and photo collections carried out on the first and last days.

Sites	Period 1	Period 2
Prairies Ecozone		
Buffalo Pound, Big Valley, Wascana Trail	27 July - 3 August	17 August - 31 August
Boreal Shield Ecozone		
PC	-	20 August - 26 August
PAS	30 July - 5 August	-
TGP, ACB	19 July - 26 July	20 August - 26 August
MBG	19 July - 26 July	23 August - 30 August

Figure 1.4: A depiction of the ways blue vane traps (BVTs) were installed in different regions. A) diagram of a BVT and platform used in the Carolinian Zone B) photograph of BVT set at Station Rd. site, in the Carolinian Zone dated 17 June 2021 C) photograph of BVT set into the ground at TGP site, in the Boreal Shield Ecozone, dated 19 July 2021 (provided by Dr. Carolyn Parsons). When not actively surveying at sites in the Carolinian Zone, BVTs and steel strapping were removed, while wooden stakes remained in place all field season.

In the Carolinian Zone, two or three BVTs were placed at each site, separated by a distance of at least 48 m. Locations and number of traps at each site (Supplementary Table S1.2) were determined based on considerations such as the layout of the site, the advice of property owners, the level of public traffic, and to be as far as possible from any pan trapping being conducted by other researchers at Elm St. and Station Rd. In June the height of the vegetation at some sites obscured the visibility of the traps when placed in the ground. On the advice of Dr. Jess Vickruck (Research Scientist for Agriculture and Agri-Food Canada), the traps were raised on platforms so that the vanes were at approximately the same height as the surrounding flowers. The height of the traps was then adjusted over the summer when the vegetation grew (Supplementary Table S1.3). A propylene glycol solution (500 ml; Hood Chemical, PIPEMATE, 95% propylene glycol and 5% dipotassium phosphate) was added to the collection jars. Since this is mildly toxic, permission for use was obtained from the private and public property owners, and warning signs were added to the platforms at public sites identifying the chemical and warning that it should not be consumed. The propylene glycol solution was reused until sufficient dilution by rainfall warranted replacement, as indicated by a colour change from bright to very light pink.

In the Prairies Ecozone, three BVTs were placed at each site, separated by at least 500 m. Traps were placed in the ground at a height of about half the collection jar, because vegetation at these sites was short. Undiluted propylene glycol was added to the jar and reused throughout the survey periods. During the second survey period, two of the BVTs at the Wascana Trail site were lost, leaving information from only one BVT at that site.

In the Boreal Shield Ecozone, two BVTs were placed at each site, with a couple of exceptions (at PC site, a labelling error occurred which meant that information could only be retained from one BVT, and at PAS site, four BVTs were used because only one site was obtained when it was initially anticipated there would be two). Traps were placed at least 20 m apart, in the ground so that about half of the collection jar was buried to hold the trap in place. Vegetation height was mixed and when necessary, longer vegetation around the trap was cleared. A 50% propylene glycol, 50% water solution was added to the collection jars and reused throughout the collection periods.

Targeted netting procedures

In the Carolinian Zone, I used a BioQuip, 15” diameter, collapsible insect net to collect bumble bees. I started at one end of a site, as determined by BVT placement, and walked towards the other end in a meandering fashion actively searching for bumble bees on flowers and catching them with the net. Once captured, bees were transferred from the net to vials containing 70% ethanol. Vial labels containing the site, date, and floral association were completed. Floral associations are the flower the bumble bee was on when collected, and all bumble bees collected on the same type of flower (within the same 30-minute survey) were placed in the same vial. Handling time, the time that it took to transfer each bee to the correct vial, take pictures of the floral association and complete the vial label, was included in the 30-minute survey. Taking pictures of the flowers (flower, leaf and overall plant) and completing the vial labels only occurred for the first specimen added to that vial. Since cloud cover and light rain did not seem to deter bumble bees, surveys were continued in these conditions. A few surveys were conducted during rainy weather when it could not be avoided or if it started raining during the middle of a timed survey.

In the Prairies Ecozone, one netting surveyor (who was alternated) actively looked for bumble bees on flowers for 30 minutes collecting any encountered with a net. The netting surveyor walked in the opposite direction of the photo surveyor, following a meandering path in the general vicinity of BVT placement. Handling time was included in the 30 minutes since all surveyors were experienced with collecting bees. Bumble bees successfully caught were placed in 70% ethanol within a labelled vial.

In the Boreal Shield Ecozone, netting surveys were conducted either by 2 surveyors for 15 minutes each or 1 surveyor for 30 minutes. Surveyors followed a meandering path in the general vicinity of the BVTs actively looking for bumble bees and collecting them with a net. Dr. Carolyn Parsons included handling time (the time to transfer bees to vials) in the 30-minute time frame when conducting the netting surveys since she was experienced with transferring bees quickly. The other surveyors were less experienced in collecting bees and likely would have taken longer to transfer bees from the net to the vial, reducing the amount of time spent surveying. To prevent this they used stopwatches, pausing each time a bumble bee was caught and restarting after the bee was transferred to the labelled vial with 70% ethanol. The flowers the bees had been collected on were recorded after the 30-minute survey was complete.

Photographs

In the Carolinian Zone I actively searched for bumble bees on flowers following a meandering path from one end of the site, as determined by BVT placement, towards the opposite end. Photo and netting surveys conducted at the same site on the same day followed the same path, but which type of survey was done first was random. When I saw a bumble bee on a flower, I used the burst shot function of my Samsung Galaxy S8 cell phone (resolution 12 MP, 4032 x 3024) to take 100 photographs of the bee, focusing on the abdomen hair pattern. The abdomen was often covered by wings while the bee was on a flower, and so I continued taking burst shots until the abdomen became visible, usually as the bee switched flowers, or until I felt I had the best image I was likely to get. Additional photos of the side or face of the bee were taken if possible, since these help with identification (Bumble Bee Watch, 2022). Floral associations were captured in the images taken.

In the Prairies Ecozone, 30-minute photo surveys were performed by one surveyor (who this was alternated). Using an iPhone XR cellphone camera (resolution 326 ppi, 1792 x 828 (Apple Inc., 2022)), the surveyor walked in the opposite direction of the netting surveyor and in a meandering fashion actively looked for bumble bees. When a bumble bee was encountered, the surveyor captured images of it with the camera. The photo surveys at Big Valley site on 30 July 2021 and at Wascana Trail on 17 August 2021 could not be completed because the wind was too strong.

In the Boreal Shield Ecozone, photo surveys were conducted by either 2 surveyors for 15 minutes or 1 surveyor for 30 minutes. Surveyors actively searched for bumble bees in a meandering fashion in the general vicinity of the BVTs. When a bumble bee was encountered the surveyor photographed it using a cellphone camera. Floral associations were recorded in the images. Photos from one of the surveyors who had surveyed sites TGP, MBG and ACB in the first survey period were lost due to a phone malfunction.

Specimen processing and identification

In the Carolinian Zone, netting specimens were fluffed using a hairdryer, pinned, and labelled with information such as the date, site, collection method, and floral association. BVT specimens were processed similarly but were first washed in soapy water to remove the propylene glycol before drying. All photographs were reviewed and only the images most useful for identifying each specimen were kept. Bumble bees were identified as species when possible. The main source used for identification was Williams *et al.* (2014), with additional sources consulted including Discover Life (Ascher & Pickering, 2022), the bumble bee specimens in the Brock Bee Lab collection, and the Bees of Canada guide on Dr. Laurence Packer's website (Miklasevskaja *et al*. n.d., https://www.yorku.ca/bugsrus/resources/galleries/boc#Bombus) when identifications were difficult. Dr. Cory Sheffield, a bee taxonomist with the Royal Saskatchewan Museum, verified voucher specimens from the netting and BVT collection methods (all but 10 BVT specimens and almost 250 netting specimens). Examples of each species (females and males) were included in the voucher specimens as well as any specimens that were difficult to distinguish. Photographic identifications were not verified due to time constraints.

In the Prairies Ecozone, specimens collected in BVTs and nets were identified to species by Dr. Sheffield, based on the Williams *et al.* (2014) identification guide. Photographic records were uploaded to iNaturalist.org and verified by Dr. Sheffield.

In the Boreal Shield Ecozone, BVT and netting identifications were completed by Dr. Carolyn Parsons, Entomology Research Assistant with Agriculture and Agri-Food Canada, using Williams *et al.* (2014) and voucher specimens were verified by Dr. Sheffield. Photo records were uploaded to iNaturalist for identification. Photos were identified by Dr. Parsons or various iNaturalist users and some photos were verified by Dr. Sheffield.

All voucher specimens were returned to their respective regions after verification. Specimens are stored in the collections of Dr. Richards (the Brock Bee Lab), Dr. Sheffield, and Dr. Parsons. Floral association identification is described in the Appendix.

Data Analysis

Analyses were completed in R version 4.0.2, R Studio version 1.3.1073 (R Core Team, 2022). The *tidyverse* package (version 1.3.0) was consistently used. Visualizations used a combination of packages such as *ggplot2* (version 3.3.5), *cowplot* (version 1.1.0), *ggpubr* (version 0.4.0), *lemon* (version 0.4.5), *effects* (version 4.2-0), *gridGraphics* (version 0.5-1), and *gridExtra* (version 2.3). Other packages and functions are described below.

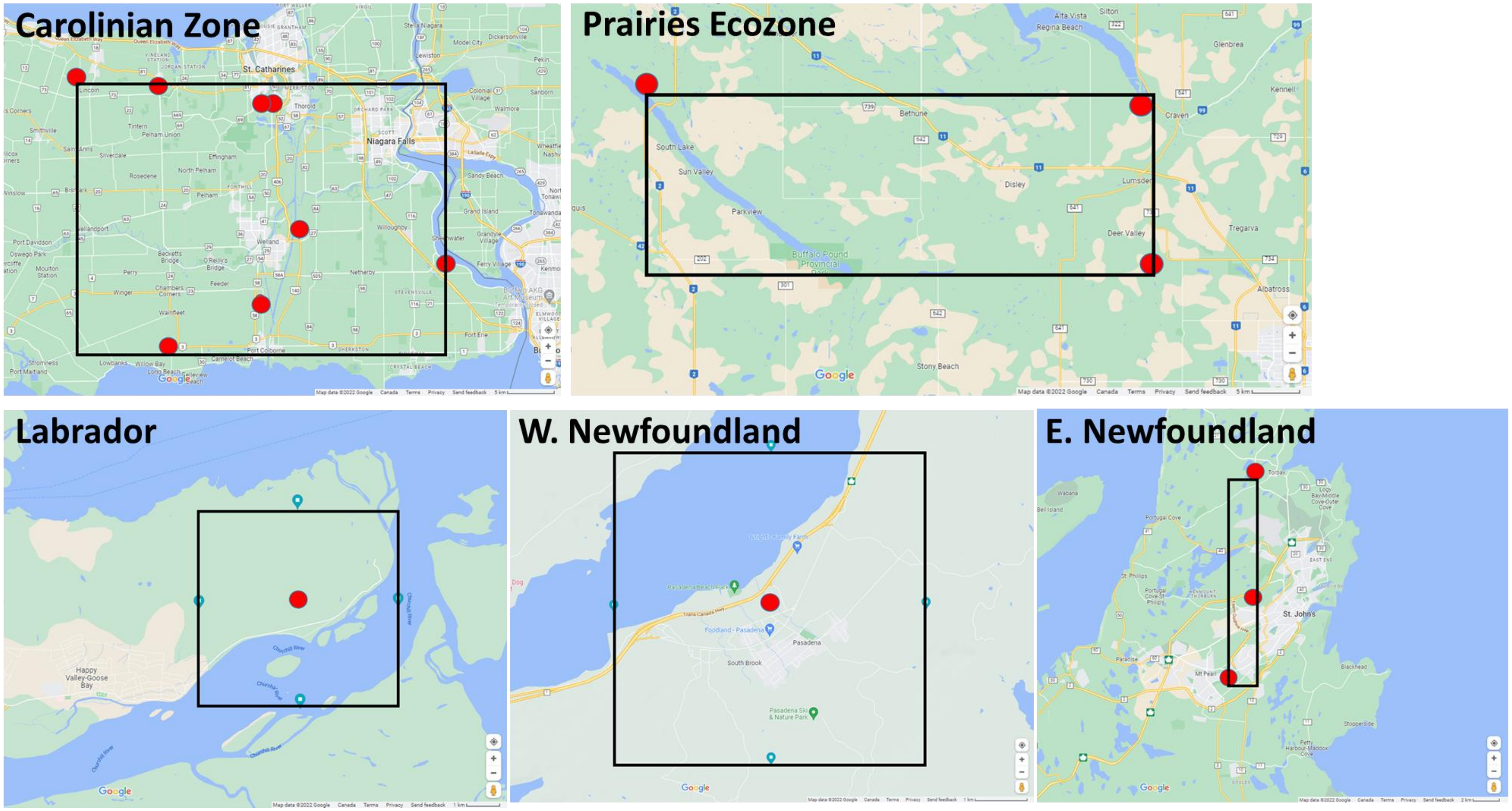
Variation in bee abundance among sites and collection weeks

Bee species counts are known to vary throughout the season, with different peak timings depending on the species (Colla & Dumesh, 2010; Kimoto *et al*. 2012; Onuferko, 2013; Williams *et al.* 2014). To visualize the effect of week on number of specimens collected, *calendar week* numbers were assigned to the collection periods. Since collection periods did not always align with the start of a week, the numbers used are approximations based on the calendar week each survey period started in, unless commenced on a Friday or Saturday, in which case the following calendar week was used. To be consistent with previous Brock Bee Lab studies, 16 was subtracted from calendar week, so that calendar week 1 corresponds to mid-April, the time when most bees in Niagara begin their flight seasons (Richards *et al*. 2011; Rutgers-Kelly & Richards, 2013; Kutby, 2013; Onuferko, 2013; Onuferko *et al*. 2015, 2018). In all regions, site and calendar week variation was present (Supplementary Figures S1.15 to S1.17), so these factors were included in later linear modeling.

Evaluating species composition

A list of species known to be in each of the regions surveyed was created by combining records from the BVT, netting and photo methods, with records from the *Bumble Bees of Canada and Alaska Monitoring Program* project on iNaturalist.org (iNaturalist, 2022, note that as of 7 April 2023 this project is no longer accessible). The Carolinian list of known species was also supplemented with records collected by the Brock Bee Lab in previous studies from 2003-2019 (Richards *et al*. 2011; Rutgers-Kelly & Richards, 2013; Kutby, 2013; Onuferko, 2013; Onuferko *et al*. 2015, 2018). These iNaturalist records were downloaded on 17 October 2022, and contained 83,273 observations dating back to 1969. The iNaturalist dataset was limited to research grade records up to and including 2021. To choose records most relevant to the field sites used, I created a set of boundaries defined by the latitude and longitude coordinates of the most northern, southern, eastern, and western field sites in each region (Figure 1.5). There was only one site in Labrador and in western Newfoundland, so for these areas the boundary limits were set to 3 km from the survey site in all four directions. Species collected at field sites, using the BVT, netting and photo methods, were compared to the list of known species. At-risk and parasitic species were indicated in the list. I considered a species as being “at risk” when it had been assessed by COSEWIC and given a status of special concern, threatened, endangered or extirpated, as these are the status levels at which the Canadian government will consider protection under the *Species at Risk Act* (COSEWIC, 2022).

Within each region, the species’ relative abundances were calculated for each collection method, and the proportions were compared using chi-squared tests. Specimens that could not be identified to species were excluded from analyses, unless otherwise stated. Chi-squared tests were calculated in Excel and run using the *chisq.test* function in R, pooling data across species


Carolinian Zone

Prairies Ecozone

Labrador

W. Newfoundland

E. Newfoundland

Figure 1.5: The boundaries used to create a subset of the iNaturalist data from the *Bumble Bees of Canada and Alaska Monitoring Program* for the Carolinian, Prairies, and Boreal Shield Ecozones. Red circles represent survey sites. The outermost site locations in each region were used as the edges of the boundaries. In Labrador and western Newfoundland there was only one site, so boundary limits were set to 3km from the site in all four directions. The images are modified Google Maps (© 2022 Google).

where necessary. Chi-squared analyses require that no expected value be below one and no more than 20% of expected values be below five (Whitlock & Schluter, 2020). In the Carolinian Zone, the grouped category consisted of *B. citrinus, B. perplexus, B. borealis, B. auricomus, B. pensylvanicus* and *B. terricola*. In the Prairies Ecozone, there were two grouped categories, a eusocial grouping of *B. huntii, B. centralis, B griseocollis, B. fervidus, B. terricola,* and *B. nevadensis,* and a parasitic grouping of *B. insularis, B. suckleyi,* and *B. flavidus*. In the Boreal Shield Ecozone there was a eusocial grouping of *B. sandersoni* and *B. perplexus,* and a parasitic grouping of *B. citrinus, B. flavidus,* and *B. bohemicus.*

Evenness is a parameter that represents how similar or dissimilar the relative abundances of species are in an assemblage, and the more similar the species' relative abundances are to each other, the more even the distribution (Smith & Smith, 2001; Magurran, 2004; Molles & Cahill, 2008). Bee collections produced with the three methods were compared using Pielou's Evenness (J), which was done by first calculating Shannon's Diversity (H') using the *vegan* package (version 2.5-7) and the *diversity* function, and then by using the formula J = H'/log(S), where S is the species count for each collection method (Oksanen *et al*. 2020; Oksanen, 2022). The *specnumber* function was used to calculate S. Pielou's evenness ranges from 0 to 1, where 1 is the most even (Smith & Smith, 2001).

Comparison of intensive and community science photo collection

To assess whether iNaturalist data could provide similar information to the more intensive photo surveys from this thesis, the iNaturalist records from the *Bumble Bees of Canada and Alaska Monitoring Program* described above were subsetted further for the years 2019, 2020, and 2021 to provide a comparison with field photo collections from 2021. The Carolinian Zone subset was filtered for 1 June to 1 October of each year, and the Prairies and Boreal Shield Ecozone subsets were filtered for 1 July to 31 August, since these dates encompassed the 2021 survey dates from the present study. Additionally, since in the Prairies and Boreal Shield Ecozones some of the photo records collected for this thesis had been added to the iNaturalist project, these were removed to avoid duplication. Three bumble bee records I had photographed in the Carolinian Zone in early June (one research grade *B. fervidus* and two non-research grade *Bombus*) had been added to the project but were not filtered out as I knew these records were from before 22 June 2021 and so were not present in the thesis data set. After filtering, only the Carolinian Zone had enough research grade records for comparison. Each record was treated as one specimen. A chi-squared test was then conducted using the research grade iNaturalist subset and the photo survey records, grouping *B. fervidus, B. citrinus, B. perplexus,* and *B. vagans* because of low counts.

Comparing netting and photo methods

Since the BVT records produced the most distinct species compositions, analysis was conducted to determine if there was also a significant difference between netting and photo methods. The number of specimens collected (including ones not identified to species) and the species richness detected for netting and photo surveys conducted at the same site on the same day were compared for each region. To test the effect of collection method on the number of specimens collected per survey and the species richness collected per survey linear models (model 1: number of specimens ~ site * collection date *collection method; model 2: species richness ~ site * collection date * collection method) were run in R using the *aov* function. Site and collection date were included in the model because previous analyses suggested these variables may have had effects on the number of specimens collected (Supplementary Figures S1.15 to S1.17). Site and collection method were treated as factors and collection date was treated as a continuous date format. This was done separately for each region. In the Prairies Ecozone there were two netting surveys that did not have a matching photo survey (30 July 2021 at Big Valley site, and 17 August 2021 at Wascana Trail site) due to strong winds making photographs difficult to take, so these were removed from the paired netting and photo comparisons.

Chi-squared tests were conducted to determine if there was a significant difference between the netting and photo species compositions. In the Carolinian Zone a grouped category was necessary for *B. perplexus* and *B. borealis*. In the Boreal Shield Ecozone *B. flavidus* and *B. perplexus* were grouped. In the Prairies Ecozone a chi-squared test comparing the netting and photo records could not be completed because too many species had low counts, so a Fisher's Exact Test was conducted instead using the *fisher.test* function.

Evaluating sampling effort

Species accumulation curves illustrate the pattern of increase in observed species richness as individuals are added to a sample (Gotelli & Colwell, 2001; Magurran, 2004). Such curves allow us to determine the point at which sampling fails to detect new species. Continued sampling past this point is considered oversampling because it results in further bee death but does not produce additional information and is a waste of surveyor effort. For each collection method, species accumulation curves were used to visualize the relationship between cumulative species richness and the collection date. This allowed me to assess the length of sampling time (in weeks or months) required to maximize the number of bumble bee specimens collected and minimize effort.

Rarefaction curves are a useful tool for comparing species richness from samples with different levels of sampling effort (Gotelli & Colwell, 2001). Using all individuals, repeated random subsampling is used to create the curves and comparison is done at the lowest sample size (Gotelli & Colwell, 2001; Magurran, 2004; Hsieh *et al*. 2016). Curves can also be extended to predict the number of species that would likely be detected with increased sampling of individuals (Chao *et al.* 2014; Hsieh *et al.* 2016). The package *iNEXT* (version 2.0.20) was used to produce rarefaction curves for each region and collection method. Information on the *iNEXT* package can be found in Chao *et al.* (2014) and Hsieh *et al.* (2016) and (2020). The default settings of the *iNEXT* function (40 knots with 50 bootstrap replications) were used to calculate the standard error and 95% confidence intervals. Rarefaction curves were set to run to double the reference sample size, as recommended as the maximum reliable estimate for rarefaction (Chao *et al.* 2014; Hsieh *et al.* 2016).

Species accumulation curves were also used to assess how many sites were needed for each region. These curves were created for each region and collection method using the *vegan* package (version 2.5-7) and the *specaccum* function (Oksanen *et al*. 2020). Since sites were not added to the study in a linear fashion, permutations were run on the order of the sites to create curves with confidence intervals. For the Carolinian Zone sites, 1000 permutations were used to create the curves. However, the Prairies and the Boreal Shield Ecozones did not have enough sites to run 1000 permutations. Since the Prairies Ecozone had only three sites, the maximum number of permutations possible was 5, and since the Boreal Shield Ecozone had five sites, the maximum number of permutations was 119.

Site quality was assessed in the Carolinian Zone, with the intent of providing recommendations for which sites to survey in future bumble bee studies in this region. Sites were considered high quality if they had efficient species detection (high number of species detected per specimen collected), the presence of many species, and the presence of rare, parasitic, and at-risk species. Sites were considered low quality if they were inefficient in detecting species, few species were detected, and if no rare, parasitic, or at-risk species were detected.

To assess the optimal length of BVT duration (per site and collection week) in the Carolinian Zone, the increase in species richness over successive collections was analysed using a linear model (cumulative species richness ~ site * collection day) conducted in R with the *aov* function. While the main purpose of this analysis was to test the effect of collection day, site was also included since it may have been responsible for some of the variation. Site was treated as a factor and collection day was treated as an ordered factor. Within each site and collection week, the cumulative species richness represented the increase in the number of species detected with each successive collection day (Day 7 species richness = additive species richness from Days 1, 3, and 7). Some collections broke protocol and were done on Days 4 (n = 5) or 8 (n = 2) due to weather or scheduling delays and these were excluded from these analyses. If collection day was significant then a post-hoc Tukey's Honestly Significant Difference test (Tukey HSD) was conducted using the *TukeyHSD* function, to determine which collection days were significantly different from each other. Letters representing significant differences were calculated using the *multcompView* package (version 0.1-8) with the *multcompLetters4* function (Graves *et al.* 2019). These were added to figures.

A similar analysis was conducted in the Prairies Ecozone. A linear model (cumulative species richness ~ site + duration) was run to test the effect of BVT duration (a duration of 1 represents a 24-hour period) on the cumulative species richness collected per site and week. The interaction between site and duration was not included in the model because including it resulted in the model being overfitted and unable to provide predictions. Site was treated as a factor and duration was treated as an ordered factor. While the second survey period in this region did not match the one-week duration protocol, it was included in the analysis to allow comparison of a one-week duration and a two-week duration. If duration was significant, a Tukey HSD test was conducted to determine which durations were significantly different from each other. BVT duration could not be assessed in the Boreal Shield Ecozone, since BVTs were only emptied on the last day of each week in that region.

Similar analyses were conducted to determine how many netting and photo surveys were needed per site per week. For each region, linear models (cumulative species richness ~ site * number of surveys) were used to test the effect of the number of surveys (within site and week) on cumulative species richness. Site and the interaction between site and number of surveys were included in the model because they may have also affected the cumulative species richness. Site was treated as a factor and number of surveys was treated as an ordered factor. In the Prairies Ecozone, including the interaction effect resulted in overfitting, so this was removed from the linear models (cumulative species richness ~ site + number of surveys) for this region. If the number of surveys was significant, then a Tukey HSD test was conducted to determine which number of surveys were significantly different from each other. The netting and photo surveys from the second survey period in the Prairies Ecozone were excluded from these analyses because they were conducted as only one survey per week per method, so they were not useful in comparing whether multiple surveys should be conducted within a week. Letters representing significant differences were calculated as previously done and added to figures. The Carolinian

Tukey HSD comparison of one netting survey compared to two resulted in a p value of 0.052, and while this was greater than the significance level of 0.05, I felt that this was likely a true significant difference. One survey compared to three was significant ($p < 0.001$) while two compared to three was not ($p = 0.169$), so the significant increase in species detection likely occurred between one and two surveys not two and three. This result also matched the pattern seen in the photo surveys of the same region. Acknowledging this, letter calculation was adjusted to a threshold of 0.052 for this comparison.

RESULTS

Bumble bee species and floral associations

From all surveys and regions together, 22 bumble bee species were collected (Table 1.5), representing almost half of the species occurring in Canada (Williams *et al.* 2014). In total, 12 species were detected in the Carolinian Zone, 12 species in the Prairies Ecozone and 10 species in the Boreal Shield Ecozone. Almost half of the species (10) were detected in multiple regions. Four species that had been assessed by COSEWIC were detected (*B. bohemicus*, *B. pensylvanicus, B. suckleyi*, and *B. terricola*). Most species were eusocial, but five parasitic species were detected (*B. bohemicus. B. citrinus*, *B. flavidus, B. insularis*, and *B. suckleyi*).

From the netting and photo surveys 45 plant species were associated with 9 bumble bee species in the Carolinian Zone (Supplementary Table S1.4). In the Boreal Shield Ecozone, bumble bees were detected on 28 plant species (Supplementary Table S1.5).

Assessing species composition

When comparing patterns of species composition by collection method for all three regions, netting and photo methods produced species distributions more similar to each other than to the BVT species distribution (Figure 1.6). BVTs also collected a lower proportion of the most common species in each region compared to the other methods: *B. impatiens* in the Carolinian Zone, *B. rufocinctus* in the Prairies Ecozone, and *B. vagans* in the Boreal Shield Ecozone.

Species composition for the Carolinian Zone is detailed in Table 1.6. The netting and photo methods collected far more bumble bee specimens than the BVT method. In this region 12 of the

Table 1.5: A complete list of the bumble bee species collected in this study and the regions they were found in. COSEWIC status comes from the respective assessment reports dated 2014a, 2015, 2018, and 2019. Sources for species names, social habit, and nesting habit were Richards *et al.* (2011), Onuferko (2013), and Williams *et al.* (2014). When nesting habit differed by source, Williams *et al.* (2014) was given preference due to its more recent publication date and specialization on bumble bees.

Bombus species	**COSEWIC status**	**Social habit**	**Nesting habit**	**Ecozone**
auricomus (Robertson)		Eusocial	Ground surface	Carolinian
bimaculatus (Cresson)		Eusocial	Mainly underground	Carolinian
bohemicus (Seidl)	Endangered	Parasitic	Nests of other *Bombus*	Boreal Shield
borealis (Kirby)		Eusocial	Underground	Carolinian, Prairies, Boreal Shield
centralis (Cresson)		Eusocial	Underground	Prairies
citrinus (Smith)		Parasitic	Nests of other *Bombus*	Carolinian, Boreal Shield
fervidus (Fabricius)		Eusocial	Mainly ground surface or above ground	Carolinian, Prairies
flavidus (Eversmann)		Parasitic	Nests of other *Bombus*	Prairies, Boreal Shield
griseocollis (DeGeer)		Eusocial	Underground or ground surface	Carolinian, Prairies
huntii (Greene)		Eusocial	Underground	Prairies
impatiens (Cresson)		Eusocial	Underground	Carolinian
insularis (Smith)		Parasitic	Nests of other *Bombus*	Prairies
nevadensis (Cresson)		Eusocial	Underground and ground surface	Prairies
pensylvanicus (DeGeer)	Special concern	Eusocial	Mainly ground surface	Carolinian
perplexus (Cresson)		Eusocial	Underground	Carolinian, Boreal Shield
rufocinctus (Cresson)		Eusocial	Ground surface or above ground	Carolinian, Prairies, Boreal Shield
sandersoni (Franklin)		Eusocial	Underground	Boreal Shield
suckleyi (Greene)	Threatened	Parasitic	Nests of other *Bombus*	Prairies
ternarius (Say)		Eusocial	Underground	Prairies, Boreal Shield
terricola (Kirby)	Special concern	Eusocial	Underground	Carolinian, Prairies, Boreal Shield
vagans (Smith)		Eusocial	Mainly underground	Carolinian, Boreal Shield

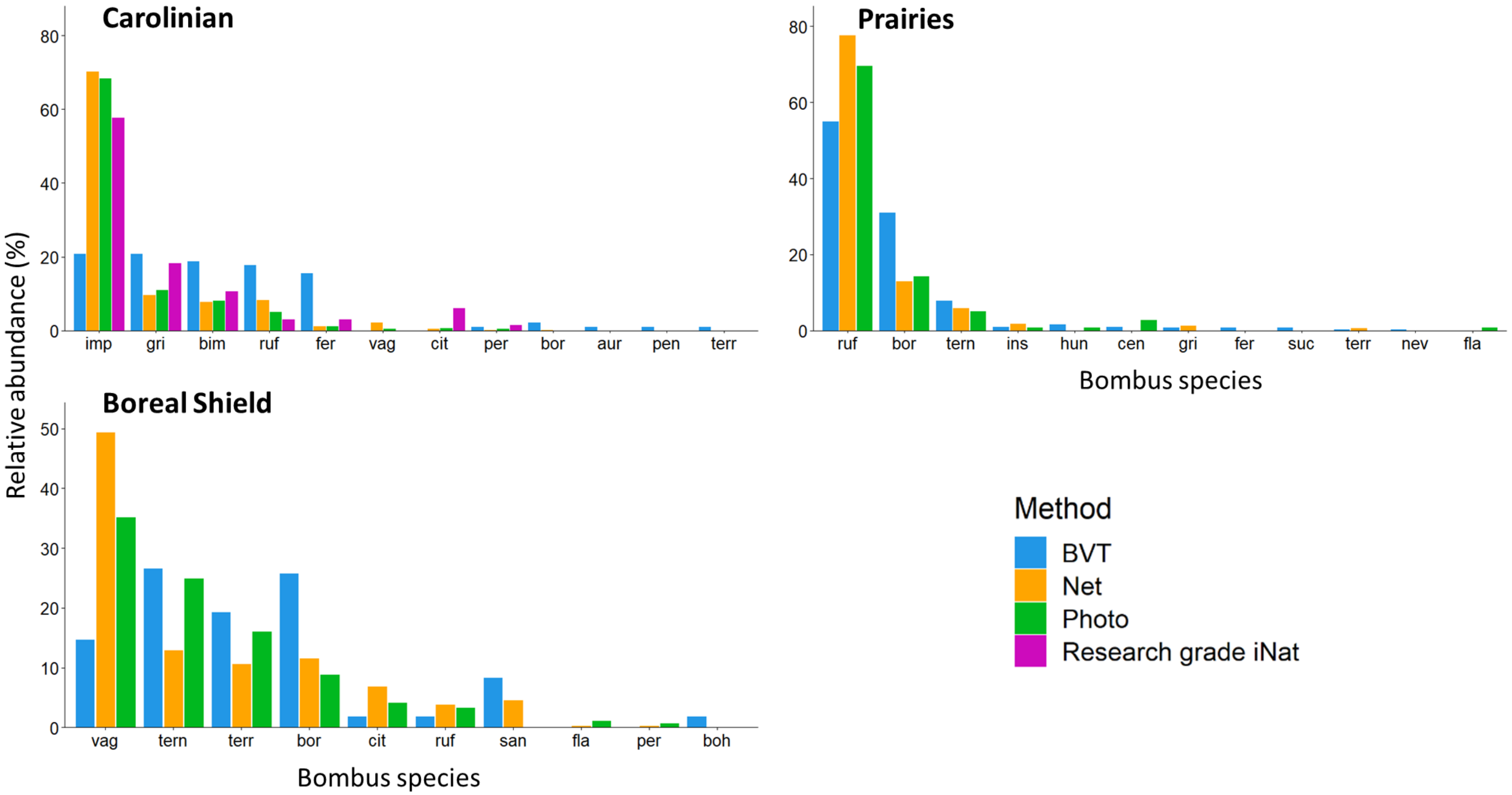


Figure 1.6: Comparing the relative abundances for species collected by blue vane traps (BVTs), netting and photographs within regions, reveals that bumble bee species composition differed by collection method, and that BVTs produced the most distinct distribution. In the Carolinian Zone, research grade iNaturalist records produce a distinct distribution to that of the photo records from this thesis. Species legend: *aur = B. auricomus, bim = B. bimaculatus, boh = B. bohemicus, bor = B. borealis, cen = B. centralis, cit = B. citrinus, fer = B. fervidus, fla = B. flavidus, gri = B. griseocollis, hun = B. huntii, imp = B. impatiens, ins = B. insularis, nev = B. nevadensis, pen = B. pensylvanicus, per = B. perplexus, ruf = B. rufocinctus, san = B. sandersoni, suc = B. suckleyi, tern = B. ternarius, terr = B. terricola, and vag = B. vagans.*

Table 1.6: Number of specimens and relative abundance (%) for each bumble bee species by collection method, compared with a list of species known to be in the Carolinian Zone. The species distribution in BVTs differed significantly from the netting and photo methods (X^2, grouping *B. citrinus, B. perplexus, B. borealis, B. auricomus, B. pensylvanicus* and *B. terricola*, $X^2 = 216.16$, df = 12, **$p < 0.001$**), as did the distribution of netting and photo records from each other (X^2, grouping *B. perplexus, and B. borealis*, $X^2 = 20.95$, df = 7, **$p = 0.004$**). The species distribution of research grade iNaturalist records was significantly different from photo records (X^2, grouping *B. fervidus*, *B. citrinus*, *B. perplexus*, and *B. vagans*, $X^2 = 16.05$, df = 4, **$p = 0.003$**). One species present in the region was absent from the iNaturalist research grade records but present in the other grade records.

Species known	**Total**	**Method**				
in locality as of 2021	**(*sans* iNat)**	**BVT**	**Net**	**Photo**	**iNat research grade**	**iNat other**
B. impatiens	1468	20 (20.8%)	685 (70.2%)	763 (68.4%)	38 (57.6%)	
B. griseocollis	235	20 (20.8%)	94 (9.6%)	121 (10.9%)	12 (18.2%)	
B. bimaculatus	183	18 (18.8%)	76 (7.8%)	89 (8.0%)	7 (10.6%)	
B. rufocinctus	154	17 (17.7%)	81 (8.3%)	56 (5.0%)	2 (3.0%)	
B. fervidus	39	15 (15.6%)	11 (1.1%)	13 (1.2%)	2 (3.0%)	
B. vagans	26		21 (2.2%)	5 (0.4%)		2 (5.4%)
*B. citrinus***	13		5 (0.5%)	8 (0.7%)	4 (6.1%)	
B. perplexus	8	1 (1.0%)	2 (0.2%)	5 (0.4%)	1 (1.5%)	1 (2.7%)
B. borealis	3	2 (2.1%)	1 (0.1%)			
B. auricomus	1	1 (1.0%)				
*B. pensylvanicus**	1	1 (1.0%)				
*B. terricola**	1	1 (1.0%)				
B. mixtus						
B. ternarius						
B. rufocinctus/vagans	6			6 (0.5%)		
B. griseocollis/bimaculatus	2			2 (0.2%)		
B. rufocinctus/mixtus	1			1 (0.1%)		
B. impatiens/bimaculatus	1			1 (0.1%)		
Unknown	45			45 (4.0%)		34 (91.9%)
Total count	2187	96	976	1115	66	37
No. species	12	10	9	8	7	2
% of known species	86	71	64	57	50	14
Pielou's evenness		0.80	0.48	0.48	0.67	

*Species at-risk

**Parasitic species

14 bumble bee species known to be present were detected. BVTs detected 10 species, netting detected 9 species and photos detected 8 species. Two species, *B. mixtus* and *B. ternarius,* were missed by all three collection methods. BVT species composition was significantly different from the netting and photo methods ($X^2 = 216.16$, df = 12, $p < 0.001$), and this was mainly due to BVTs collecting fewer *B. impatiens* and more *B. fervidus,* among other differences (Supplementary Table S1.6). This resulted in a more even species distribution as confirmed by Pielou's evenness scores being highest for the BVT method (Table 1.6). Three species (*B. auricomus, B. pensylvanicus,* and *B. terricola*) were only collected by BVTs but as singletons. *Bombus auricomus* is uncommon in Canada (Dr. Cory Sheffield, personal communication) and the other two are species at risk. BVTs were more effective than netting and photos in this region for detecting species at risk. The single parasitic species detected, *B. citrinus,* and the eusocial species, *B. vagans,* were only present in netting and photo collections. The photo method was the only method to contain records that could not be identified to species.

The species composition produced by the research grade iNaturalist records was significantly different from the photo records ($X^2 = 16.05$, df = 4, $p = 0.003$). Most of this difference (10.23 of the 16.05 X^2 value) stemmed from the grouped category (*B. fervidus, B. citrinus, B. perplexus,* and *B. vagans*) containing more records than expected in the iNaturalist data (Supplementary Table S1.7). Of those grouped species, the most distinct proportional difference was *B. citrinus* being higher in relative abundance in the iNaturalist records (Table 1.6). Other differences in proportion such as fewer *B. impatiens* and more *B. griseocollis* in the iNaturalist records, contributed to the significant difference between these two methods as well, but to a lesser degree (Supplementary Table S1.7). Overall, research grade iNaturalist records detected 7 of the 14 species known to be in the area (Table 1.6). The one species present in photo records but not in research grade iNaturalist records, *B. vagans,* was present in the other grade iNaturalist records.

Species composition for the Prairies Ecozone is detailed in Table 1.7. BVTs collected more bumble bee specimens than the netting and photo methods. All 12 bumble bee species known to be in this area were detected in the present study. BVTs detected 11 species, netting detected 6 species, and photos detected 7 species. Only four species, *B. rufocinctus, B. borealis, B. ternarius,* and *B. insularis,* were detected by all three methods. The species composition produced by the BVT method was significantly different from the other collection methods in this region ($X^2 = 38.10$, df = 8, $p < 0.001$). BVTs collected a lower proportion of *B. rufocinctus* and a higher proportion of *B. borealis*, while netting had the opposite pattern (Supplementary Table S1.8). Pielou's evenness calculations were similar for all collection methods, though once again BVTs produced the greatest evenness (Table 1.7). Two at-risk species were detected, *B. suckleyi* and *B. terricola*. *B. suckleyi* was only detected by BVTs and *B. terricola* was detected by BVTs and netting. Photos did not detect at-risk species. Three parasitic species were detected, *B. insularis, B. suckleyi,* and *B. flavidus*. *Bombus flavidus* and *B. nevadensis* were singletons, collected by photos and BVTs respectively. Photos were the only collection method to contain species not able to be identified to species.

The species compositions for the Boreal Shield Ecozone are detailed in Table 1.8. Netting and photo methods collected more specimens than the BVT method. All 10 species known to be in the area were detected. Netting detected 9 species, while BVTs and photos each detected 8. Once again, BVT distributions were significantly different from the other methods ($X^2 = 94.24$, df = 12, $p < 0.001$), collecting proportionally fewer *B. vagans* and more *B. borealis*

Table 1.7: Number of specimens and relative abundance (%) for each bumble bee species by collection method, compared with a list of species known to be in the Prairies Ecozone. The species distribution in BVTs differed significantly from the other methods (X^2 with a eusocial grouping of *B. huntii, B. centralis, B griseocollis, B. fervidus, B. terricola*, and *B. nevadensis,* and a parasitic grouping of *B. insularis, B. suckleyi,* and *B. flavidus,* $X^2 = 38.10$, df = 8, $\mathbf{p < 0.001}$). The species distribution of netting and photos did not differ significantly (Fisher's Exact Test with 10,000 replicates, $p = 0.169$)

Species known in locality as of 2021	**Total**	**Method**		
		BVT	**Net**	**Photo**
B. rufocinctus	466	236 (55.0%)	132 (77.6%)	98 (69.5%)
B. borealis	175	133 (31.0%)	22 (12.9%)	20 (14.2%)
B. ternarius	51	34 (7.9%)	10 (5.9%)	7 (5.0%)
*B. insularis***	8	4 (0.9%)	3 (1.8%)	1 (0.7%)
B. huntii	8	7 (1.6%)		1 (0.7%)
B. centralis	8	4 (0.9%)		4 (2.8%)
B. griseocollis	5	3 (0.7%)	2 (1.2%)	
B. fervidus	3	3 (0.7%)		
*B. suckleyi****	3	3 (0.7%)		
*B. terricola**	2	1 (0.2%)	1 (0.6%)	
B. nevadensis	1	1 (0.2%)		
*B. flavidus***	1			1 (0.7%)
Unknown	9			9 (6.4%)
Total count	740	429	170	141
No. species	12	11	6	7
% of known species	100	92	50	59
Pielou's evenness		0.49	0.44	0.45

*Species at-risk

**Parasitic species

***Both at-risk and parasitic

Table 1.8: Number of specimens and relative abundance (%) for each bumble bee species by collection method, compared with a list of species known to be in the Boreal Shield Ecozone. The species distribution in BVTs differed significantly from the other methods (X^2 with a eusocial group of *B. sandersoni* and *B. perplexus,* and a parasitic group of *B. citrinus, B. flavidus,* and *B. bohemicus,* $X^2 = 94.24$, df = 12, $\mathbf{p < 0.001}$). The species distributions of netting and photos also differed significantly from each other (X^2 grouping *B. flavidus* and *B. perplexus,* $X^2 = 52.60$, df = 7, $\mathbf{p < 0.001}$).

Species known in locality as of 2021	**Total**	**Method**		
		BVT	**Net**	**Photo**
B. vagans	362	16 (14.7%)	219 (49.4%)	127 (35.1%)
B. ternarius	176	29 (26.6%)	57 (12.9%)	90 (24.9%)
*B. terricola**	126	21 (19.3%)	47 (10.6%)	58 (16.0%)
B. borealis	111	28 (25.7%)	51 (11.5%)	32 (8.8%)
*B. citrinus***	47	2 (1.8%)	30 (6.8%)	15 (4.1%)
B. rufocinctus	31	2 (1.8%)	17 (3.8%)	12 (3.3%)
B. sandersoni	29	9 (8.3%)	20 (4.5%)	
*B. flavidus***	5		1 (0.2%)	4 (1.1%)
B. perplexus	3		1 (0.2%)	2 (0.6%)
*B. bohemicus****	2	2 (1.8%)		
Unknown	22			22 (6.1%)
Total count	914	109	443	362
No. species	10	8	9	8
% of known species	100	80	90	80
Evenness		0.83	0.72	0.76

*Species at-risk
**Parasitic species
***Both at-risk and parasitic

compared to netting and photo methods (Supplementary Table S1.9). Netting also detected fewer *B. ternarius* than expected. Pielou's evenness was highest for BVTs in this region as well (Table 1.8). *Bombus terricola* was detected by all three methods, while *B. bohemicus* (parasitic) was only detected by BVTs, again displaying that BVTs were the most effective method for detecting at-risk species. *Bombus flavidus* (parasitic) and *B. perplexus* were absent from BVT records and *B. sandersoni* was absent from photo records. Only the photo method contained records not able to be identified to species.

Comparing netting and photo methods

The BVT method clearly produced different results from the other two methods, but comparison of the netting and photo methods was also conducted. In the Carolinian Zone, the photo method (mean = 9.5, sd = 8.9) collected more bumble bee specimens per survey compared to the netting method (mean = 8.3, sd = 7.2), though this only approached significance. Both methods produced a similar number of species per survey (Supplementary Figure S1.18 and Supplementary Table S1.10). Site and the interaction between site and collection date affected the number of specimens collected. Site, collection date and their interaction significantly affected the species richness detected per survey. Chi-squared analysis revealed that the netting and photo species compositions were significantly different from each other ($X^2 = 20.95$, df = 7, p = 0.004), with photos having proportionally fewer *B. rufocinctus* and *B. vagans* than netting records (Table 1.6, Supplementary Table S1.11).

In the Prairies Ecozone, netting and photo collection methods collected similar numbers of bumble bee specimens and species richness per survey conducted (Supplementary Figure S1.19 and Supplementary Table S1.12). The variation seen in the number of specimens collected per survey was due to site. The variation seen in the number of species collected per survey was due to site and collection date approached significance. A Fisher's Exact Test of the species compositions revealed the netting and photo distributions were not significantly different from each other (Fisher's Exact Test with 10,000 replicates, $p = 0.169$) (Table 1.7).

In the Boreal Shield Ecozone, the netting method (mean = 27.7, sd = 8.8) collected more specimens per survey than the photo method (mean = 22.6, sd = 15.9) at a level approaching significance and greater species richness per survey (netting: mean = 3.9, sd = 1.3; photo: mean = 2.9, sd = 1.3) at a level that is possibly significant ($p = 0.053$)(Supplementary Figure S1.20 and Supplementary Table S1.13). Site, collection date, and some two-way interactions had significant effects on the number of specimens collected per survey. Chi-squared analysis of the species compositions produced by netting and photo methods revealed significant differences in the distributions ($X^2 = 52.60$, df = 7, $p < 0.001$) (Table 1.8). These differences were mainly due to netting records containing more *B. sandersoni* and *B. vagans*, and photo records containing more *B. ternarius* and *B. terricola* (Supplementary Table S1.14).

Evaluating sampling effort

Species accumulation curves depicting the increase in species richness at each collection date demonstrate that in all regions, BVTs continue to detect new species throughout all or most of the field season, while the netting and photo methods stop collecting new species (Figure 1.7). This pattern is most distinct in the Carolinian Zone, which had the longest field season (22 June to 1 October) and the most collection dates. Here the netting and photo methods both stopped detecting new species after approximately a month of use (late July), while the BVT method detected new species into early September. One month of sampling corresponded to two survey

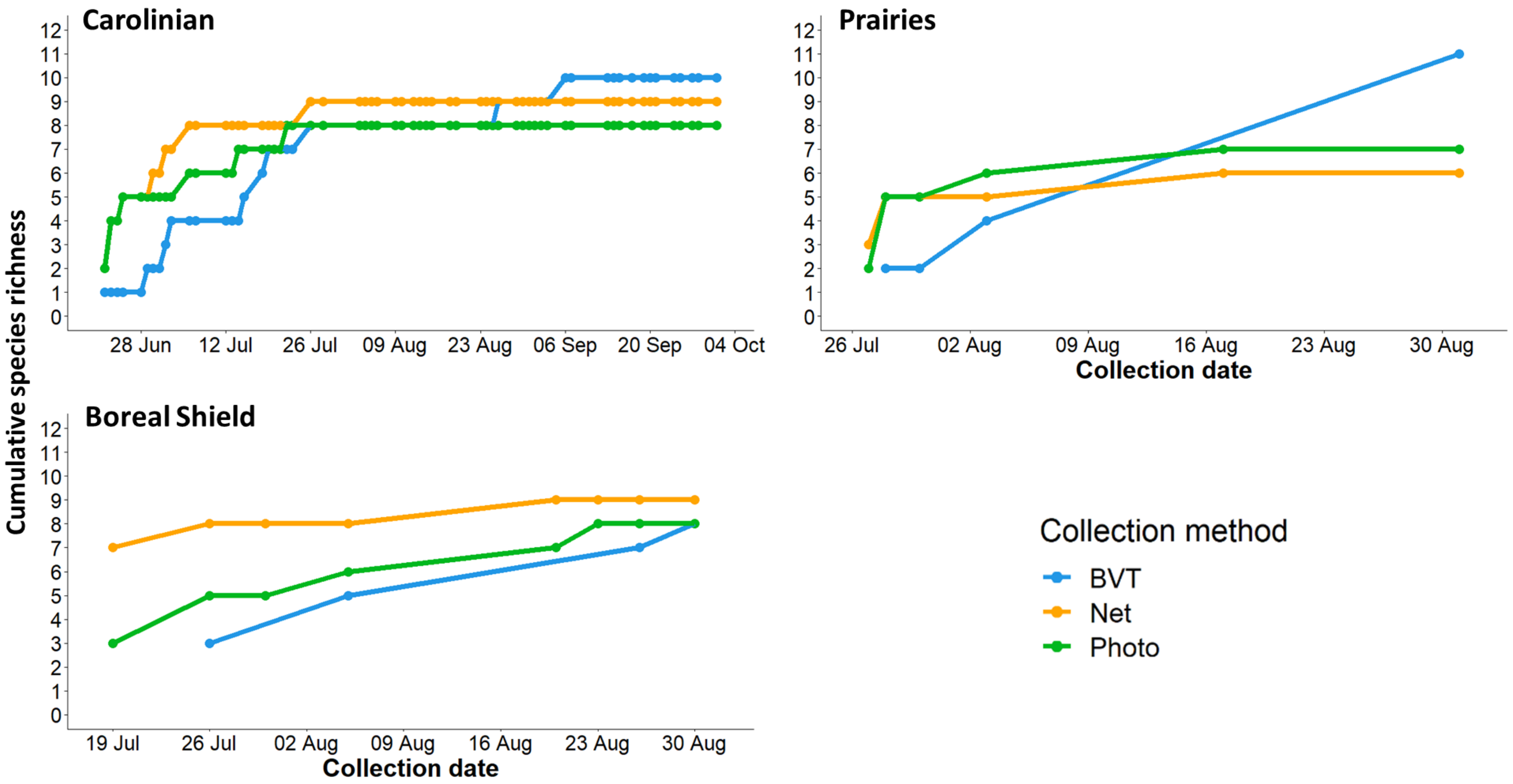


Figure 1.7: Species accumulation curves depicting cumulative bumble bee species richness based on collection date for the three collection methods show that across all regions BVTs are the only method that continues to detect new species throughout the entire or most of the field season. Points represent collection dates. The number of sites surveyed on each collection date was not always consistent, particularly in the Boreal Shield Ecozone. No species were collected by the netting and photo methods on the final collection day in the Prairies Ecozone because no specimens were captured by those methods on that day.

periods per site. The specimens collected after late July for netting and photo methods and after early September for the BVT method are examples of oversampling (sampling past the point when new species information stops being produced). The BVT method in the Prairies and Boreal Shield Ecozones continued to collect new species throughout the whole field season. The netting and photo methods in these regions appear to stop detecting new species near the end of the field season (late August). This corresponded to about three weeks of netting and photo surveying in the Prairies Ecozone and approximately 1 month of netting and photo surveys in the Boreal Shield Ecozone.

BVTs in the Carolinian Zone collected far fewer individuals (identified to species) than the netting and photo methods but detected more species. BVTs collected 96 individuals and 10 species, netting 976 individuals and 9 species, and photos 1060 individuals and 8 species (Figure 1.8). In the Prairies Ecozone a different pattern emerged where BVTs collected the most individuals and species (429 individuals, 11 species), compared to the netting method (170 individuals, 6 species) and photo method (132 individuals, 7 species). The Boreal Shield Ecozone also had a unique pattern to collections where netting collected more individuals and species (443 individuals, 9 species) compared to BVTs (109 individuals, 8 species) and photos (340 individuals, 8 species). When comparing rarefaction curves at the level of the smallest sample, BVTs were the most efficient (produced more species with fewer individuals) in that their curve lay above the curves of the netting and photo methods. None of the collection methods detected all 14 species known to be in the Carolinian Zone, but based on the extended curves, the BVT method was predicted to come the closest to doing so with double the sampling effort. Extrapolation of rarefaction curves was illustrated as possible by Chao *et al*. (2014) and then built into the R iNEXT package as explained in Hsieh *et al*. (2016). Netting and photo

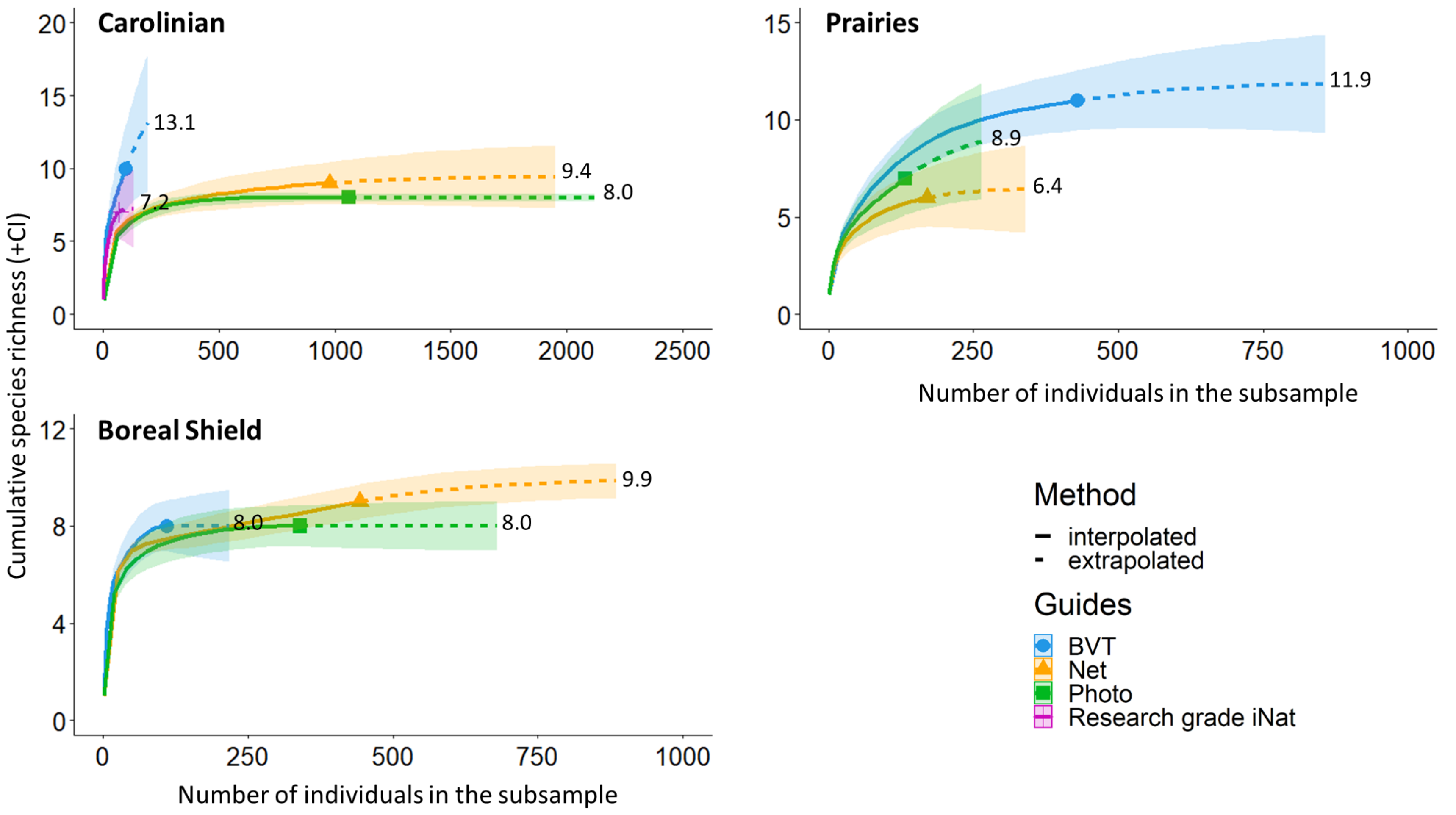


Figure 1.8: Rarefaction curves and confidence intervals depicting cumulative bumble bee species richness estimates based on the number of individuals sampled for each collection method used in the Carolinian, Prairies, and Boreal Shield Ecozones indicate that BVTs were more efficient than netting and photos at low sample sizes, but that the method that detected the most species varied by region. Numbers at the end of each curve provide the extrapolated species richness estimate at double the sample size taken in this thesis.

methods were predicted to not find any additional species with increased sampling effort. In the Prairies Ecozone, none of the collection methods caught all 12 bumble bee species known to be in the area. At double the sampling effort, the extended rarefaction curve predicted that BVTs could detect approximately 12 species. Photos were also predicted to find approximately two more species, and netting was not predicted to find additional species. In the Boreal Shield Ecozone, none of the collection methods detected all 10 species known to be in the area, but the netting method was predicted to find approximately 10 species if sampling effort was doubled. BVTs and photos were not expected to find any additional species.

For all regions and methods, species accumulation curves created using permutations on the order of the sites indicate that increasing the number of sites increases the number of species collected by each method (Figure 1.9). In the Carolinian Zone, the region with the most sites, the curves for all collection methods continue to increase, especially the BVT curve. This suggests that additional sites would be beneficial for all methods, but particularly for the BVT method. For all regions, the curves are steepest between 1 and 2 sites and often between 2 and 3 sites, suggesting that surveying fewer than 3 sites will greatly reduce the number of species that can be detected with any of the collection methods used.

Sites in the Carolinian Zone did not provide information of equal value, meaning some sites were more useful than others (Table 1.9). At PEH the number of species detected per specimen collected was higher than most other sites. The greatest species richness of any site was detected at PEH, including the at-risk species, *B. pensylvanicus* and *B. terricola*, the parasitic species *B. citrinus*, and the uncommon species, *B. vagans*. At Konkle the number of

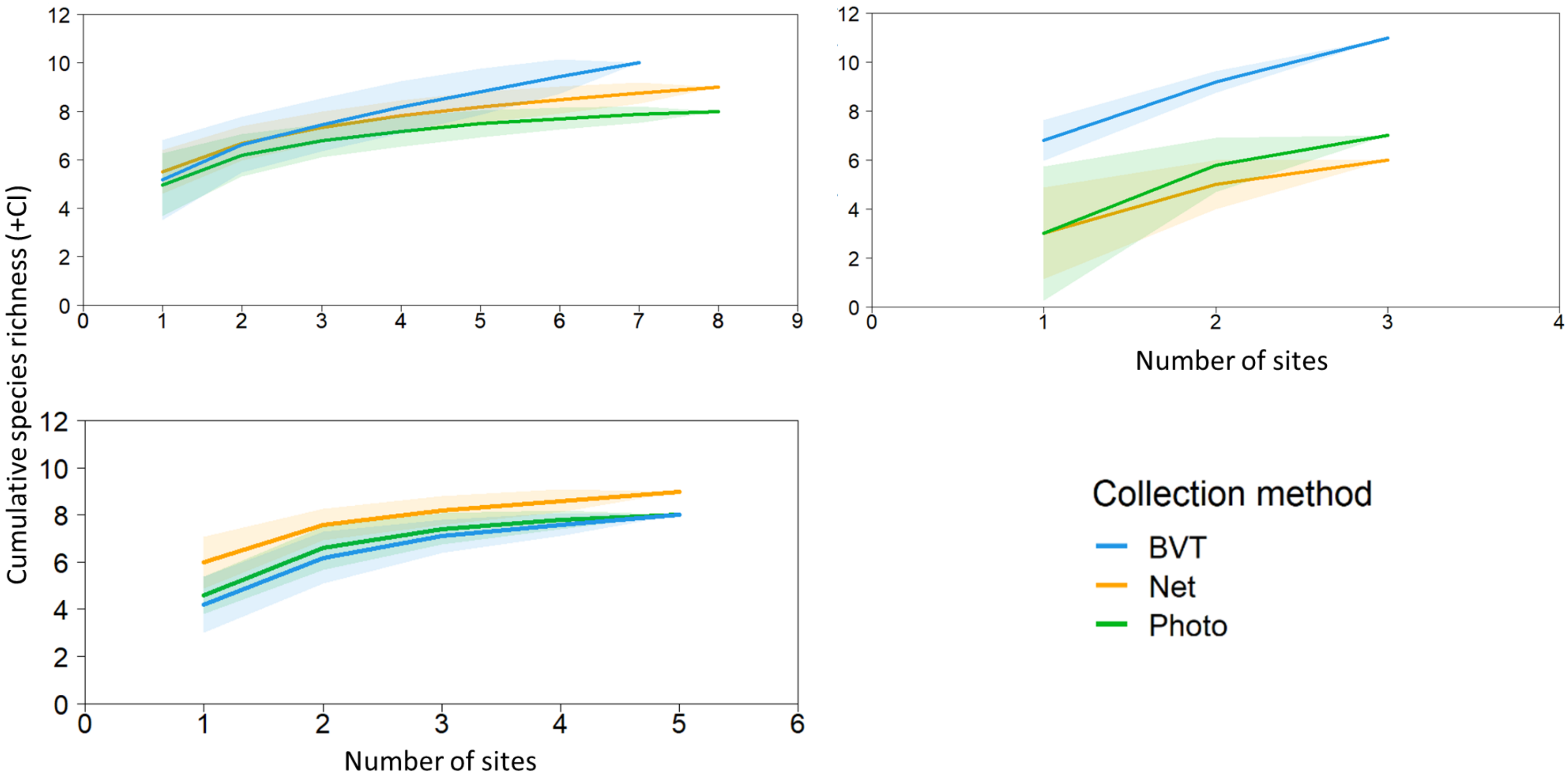


Figure 1.9: Species accumulation curves and confidence intervals (order of the sites was permutated), for each collection method and region, indicate that the more sites surveyed the more species detected by BVTs, netting and photos.

Table 1.9: Assessment of site quality for the Carolinian Zone. Qualities that make a site high quality are efficient species detection (high number of species detected per specimen collected), the presence of many species, and the presence of rare, parasitic, and at-risk species (these qualities are noted in bolded blue). Qualities that make a site low quality are inefficiency in detecting species, few species (noted in bolded red), and no rare, parasitic, or at-risk species detected. Based on these criteria Konkle and PEH are high quality sites that should be included in future sampling, whereas Elm St. and QSW are low quality and less valuable for future sampling.

***Bombus* species**	**Total count**	**Number of specimens detected**							
		Elm St.	**Farr**	**Konkle**	**PEH**	**QSW**	**Station Rd.**	**Townline**	**Victoria**
B. auricomus	**1**			**1**					
B. terricola*	**1**				**1**				
B. pensylvanicus*	**1**				**1**				
B. borealis	**3**		**1**	**1**				**1**	
B. perplexus	**8**	**4**					**3**		**1**
B. citrinus**	**13**				**11**				**2**
B. vagans	**26**			**2**	**1**		**6**	**17**	
B. fervidus	39	3	2	1	3	1	16	8	5
B. rufocinctus	154	19	3	5	8	71	3	29	16
B. bimaculatus	183	25	3	13	26	9	61		46
B. griseocollis	235	71	5	16	9	57	64	6	7
B. impatiens	1468	466	82	107	118	303	138	175	79
Total count	2132	588	96	146	178	441	291	236	156
Species richness		6	6	**8**	**9**	**5**	7	6	7
Species/specimen		**0.01**	**0.06**	**0.05**	**0.05**	**0.01**	**0.02**	0.03	0.04

*At-risk species

**Parasitic species

species detected per specimen collected was also high. Surveys detected the second highest species richness, including three uncommon species, *B. auricomus*, *B. borealis*, and *B. vagans*. These two sites would be particularly valuable for future studies. QSW was the least valuable site since collection was less efficient (produced few species per specimen collected), surveys produced the lowest species richness of any site, and only common species were detected. This result may have been affected by the late start at QSW compared to the other sites as well as the lack of BVTs. Collections at Elm St. also showed low efficiency in detecting species, and although one uncommon species, *B. perplexus*, was detected there it was not unique to that site. The lack of unique species at QSW and Elm St., along with low efficiency, make these sites less valuable for future studies.

The cumulative increase in the number of species collected by BVT for each site and week combination in the Carolinian Zone is depicted in Figure 1.10. Based on a linear model, the BVT collection day (1, 3 or 7) had a significant effect on species richness (Table 1.10). Based on a post-hoc Tukey HSD test, BVTs deployed for 3 and 7 days collected significantly more species than BVTs deployed for 1 day, and BVTs deployed for 7 days collected significantly more species than 3 days (Tukey HSD: 1 and 3, $p = 0.018$; 1 and 7, $p < 0.001$; 3 and 7, $p = 0.026$). Similar analysis was conducted on the data from the Prairies Ecozone, as seen in Figure 1.11. A linear model revealed that BVT duration had a significant effect on cumulative species richness (Table 1.11). BVTs deployed for 14 days resulted in significantly more species than shorter durations (Table 1.12).

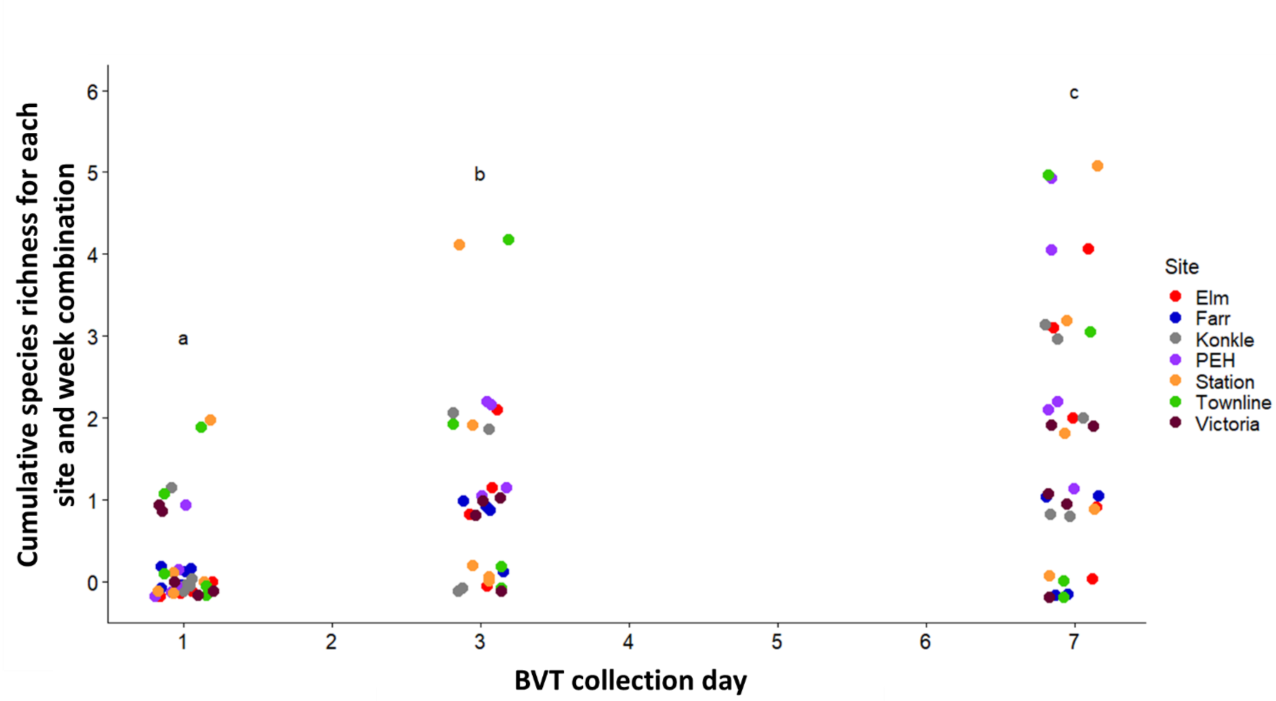


Figure 1.10: BVTs deployed in the Carolinian Zone detected significantly more species when deployed for seven days instead of three days or one day. Each point represents the cumulative species richness reached by the end of each collection day for each site and collection week combination. The points are jittered to reduce overlap. Five collections done on Day 4 and two on Day 8 were excluded. Days not sharing the same letter represent significant differences based on a Tukey HSD test.

Table 1.10: A linear model reveals that BVT collection day significantly impacted the species richness collected in the Carolinian Zone. The colons represent interactions, and the variable of greatest interest is bolded. This analysis supports Figure 1.10.

Linear Model: Cumulative Species Richness ~ Site * Collection Day

	d.f.	**Sum Sq.**	**Mean Sq.**	**F**	**P**
Site	6	11.34	1.89	1.40	0.224
Collection Day	**2**	**42.50**	**21.25**	**15.78**	**<0.001**
Site:Collection Day	12	8.30	0.69	0.51	0.900
Residuals	77	103.70	1.35		

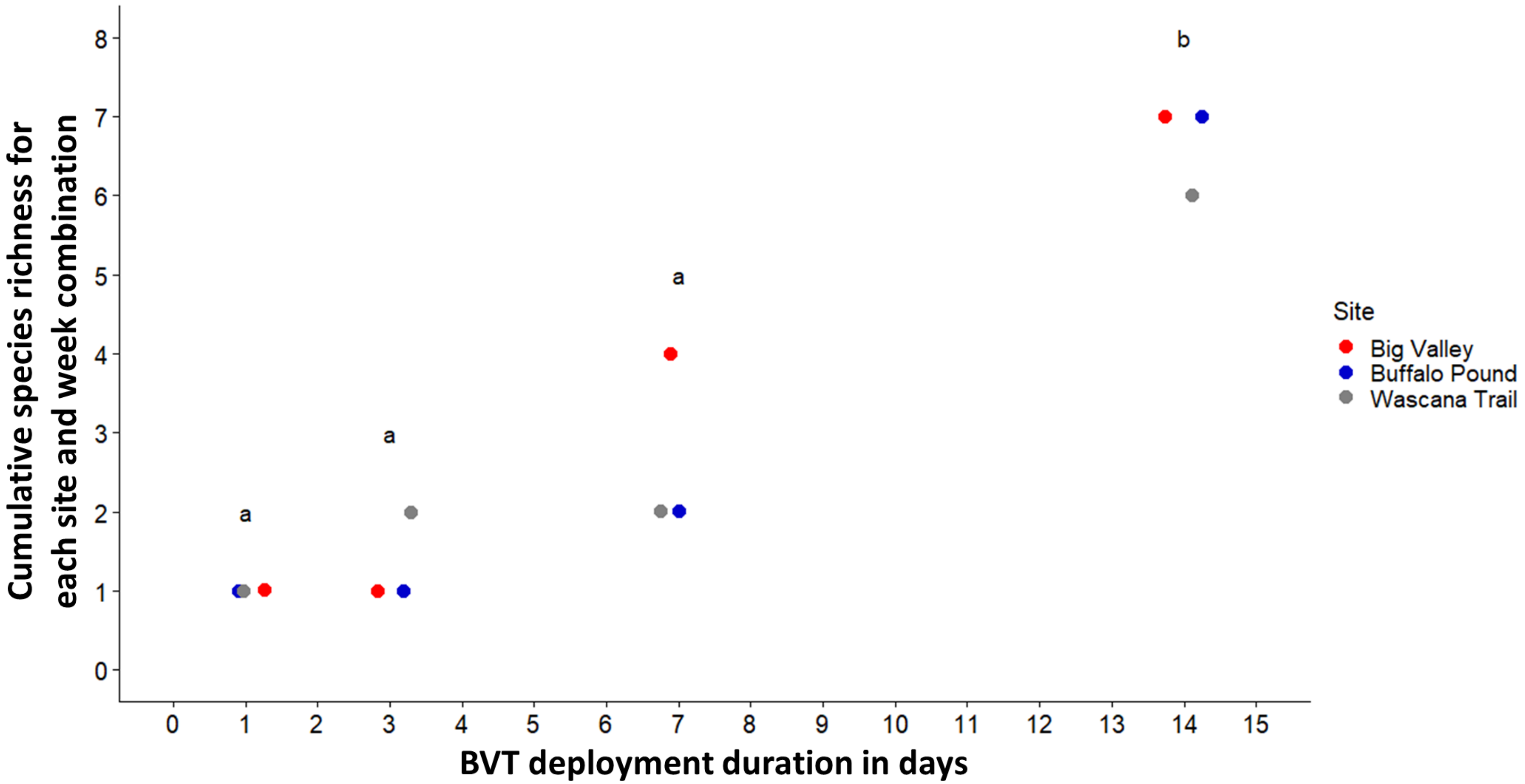


Figure 1.11: BVTs deployed for a 14-day duration in the Prairies Ecozone resulted in significantly greater species richness than shorter durations from a separate survey period. Each point represents the cumulative species richness detected for each BVT deployment duration within site and collection week. The points are jittered to reduce overlap. Durations not sharing the same letter represent significant differences based on a Tukey HSD test.

Table 1.11: A linear model reveals that BVT duration significantly impacted the species richness detected in the Prairies Ecozone. The variable of greatest interest is bolded. This analysis supports Figure 1.11.

Linear Model: Cumulative Species Richness ~ Site + Duration					
	d.f.	**Sum Sq.**	**Mean Sq.**	**F**	**P**
Site	2	0.67	0.33	0.60	0.579
Duration	**3**	**60.92**	**20.31**	**36.55**	**<0.001**
Residuals	6	3.33	0.56		

Table 1.12: A post-hoc Tukey HSD test comparing the different BVT deployment durations in the Prairies Ecozone revealed that only the 14-day duration caught significantly more species than the other durations. Significance is bolded. These results support Figure 1.11.

Tukey HSD	
Duration comparison	**P**
1 and 3	0.944
1 and 7	0.118
1 and 14	**<0.001**
3 and 7	0.228
3 and 14	**<0.001**
7 and 14	**0.002**

The cumulative number of species detected after 1, 2, and 3 netting surveys within weeks and sites in the Carolinian Zone is depicted in Figure 1.12. Based on a linear model, the number of netting surveys conducted had a significant effect on cumulative species richness, as did site (Table 1.13). A post-hoc Tukey HSD test revealed that conducting a second survey per week significantly increased species richness but conducting three surveys was not significantly different from only doing two (Tukey HSD: 1 and 2, $p = 0.052$; 1 and 3, $p < 0.001$; 2 and 3, $p = 0.169$). Similar analysis was completed for the netting surveys in the Prairies Ecozone as seen in Figure 1.13. In this region a linear model indicated that the number of netting surveys conducted per week did not have a significant effect on the cumulative species richness, only site did (Table 1.14). In the Boreal Shield Ecozone a linear model revealed that within sites conducting two netting surveys per week increased the cumulative species richness compared to only doing one survey per week at a level that approached significance (Figure 1.14 and Table 1.15).

The cumulative number of species detected after 1, 2, and 3 photo surveys per week in Carolinian Zone sites is depicted in Figure 1.15. Based on a linear model, the number of netting surveys conducted had a significant effect on cumulative species richness, as did site (Table 1.16). A post-hoc Tukey HSD test revealed that conducting more than one survey per week significantly increased species richness but conducting three surveys was not significantly different from two (Tukey HSD: 1 and 2, $p = 0.018$; 1 and 3, $p < 0.001$; 2 and 3, $p = 0.463$).

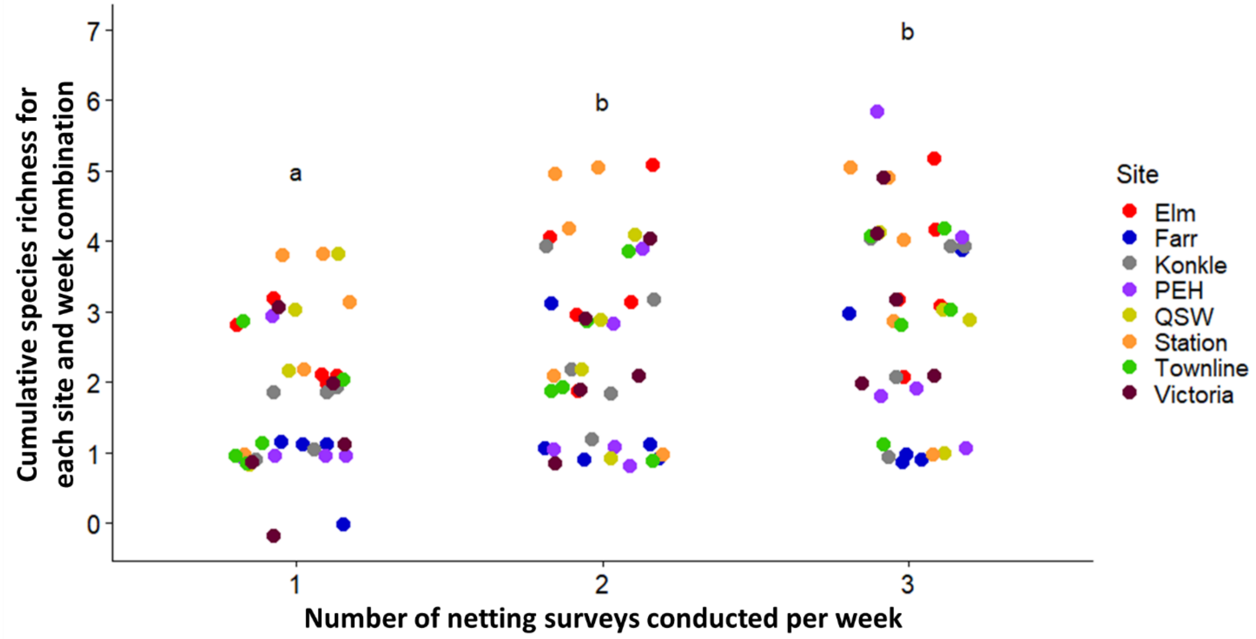


Figure 1.12: In the Carolinian Zone, conducting two netting surveys per week resulted in significantly greater species richness than only one survey per week, and similar species richness to three surveys per week. Each point represents the cumulative species richness detected for each additional netting survey conducted within site and week. The points are jittered to reduce overlap. Number of netting surveys not sharing the same letter represent significant differences based on a Tukey HSD test. The Tukey comparison of 1 and 2 surveys per week approached significance at p = 0.052 and letters on this figure were calculated at a significance level of 0.052 to demonstrate that I am reasonably confident this is a true significant difference.

Table 1.13: A linear model reveals that the number of netting surveys conducted significantly impacted the species richness detected in the Carolinian Zone, as did site. Colons represent interactions and the variable of greatest interest is bolded. This analysis supports Figure 1.12.

Linear Model: Cumulative Species Richness ~ Site * Number of Surveys

	d.f.	**Sum Sq.**	**Mean Sq.**	**F**	**P**
Site	7	32.52	4.646	3.001	0.007
Number of surveys	**2**	**27.28**	**13.641**	**8.813**	**<0.001**
Site:Number of surveys	14	5.02	0.358	0.232	0.998
Residuals	93	143.95	1.548		

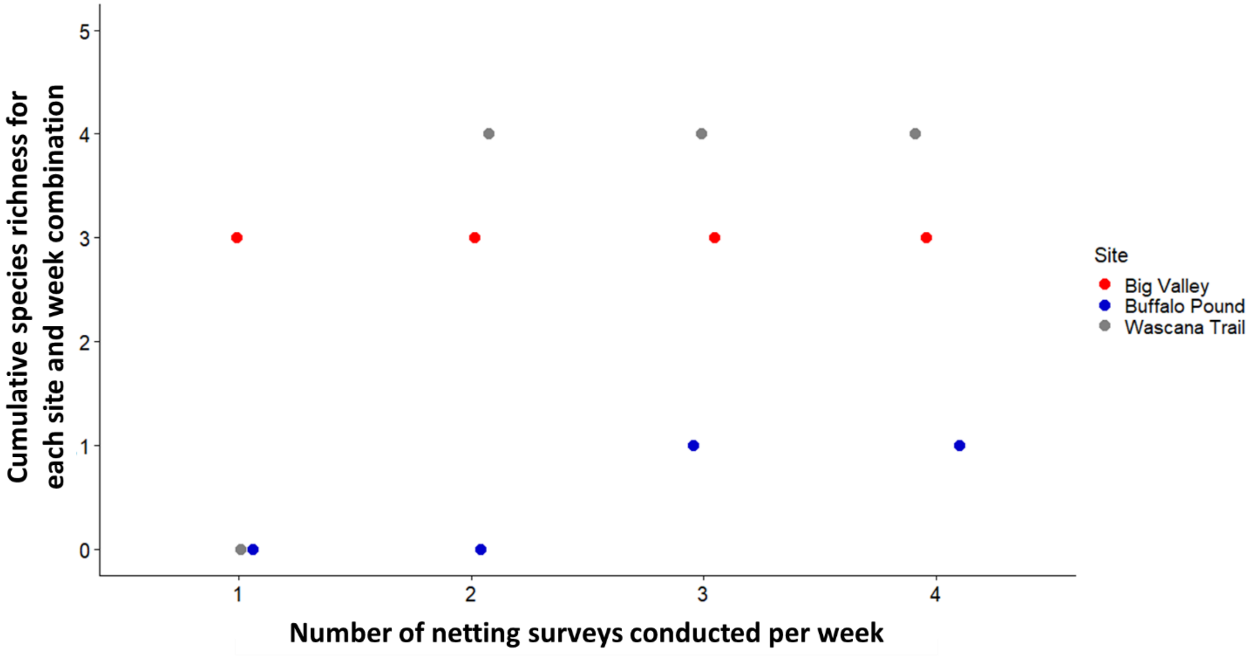


Figure 1.13: In the Prairies Ecozone, conducting more than one netting survey in a week increased the species richness in some sites but did not significantly increase species richness overall. Each point represents the cumulative species richness detected for each additional netting survey per week at each site. Only netting surveys from the first survey period were compared. Points are jittered to reduce overlap.

Table 1.14: A linear model reveals that the number of netting surveys conducted did not significantly impact the species richness detected in the Prairies Ecozone, but site did. Colons represent interactions and the variable of greatest interest is bolded. These results support Figure 1.13.

Linear Model: Cumulative Species Richness ~ Site + Number of Surveys

	d.f.	**Sum Sq.**	**Mean Sq.**	**F**	**P**
Site	2	16.67	8.33	6.82	0.029
Number of surveys	**3**	**5.67**	**1.89**	**1.55**	**0.297**
Residuals	6	7.33	1.22		

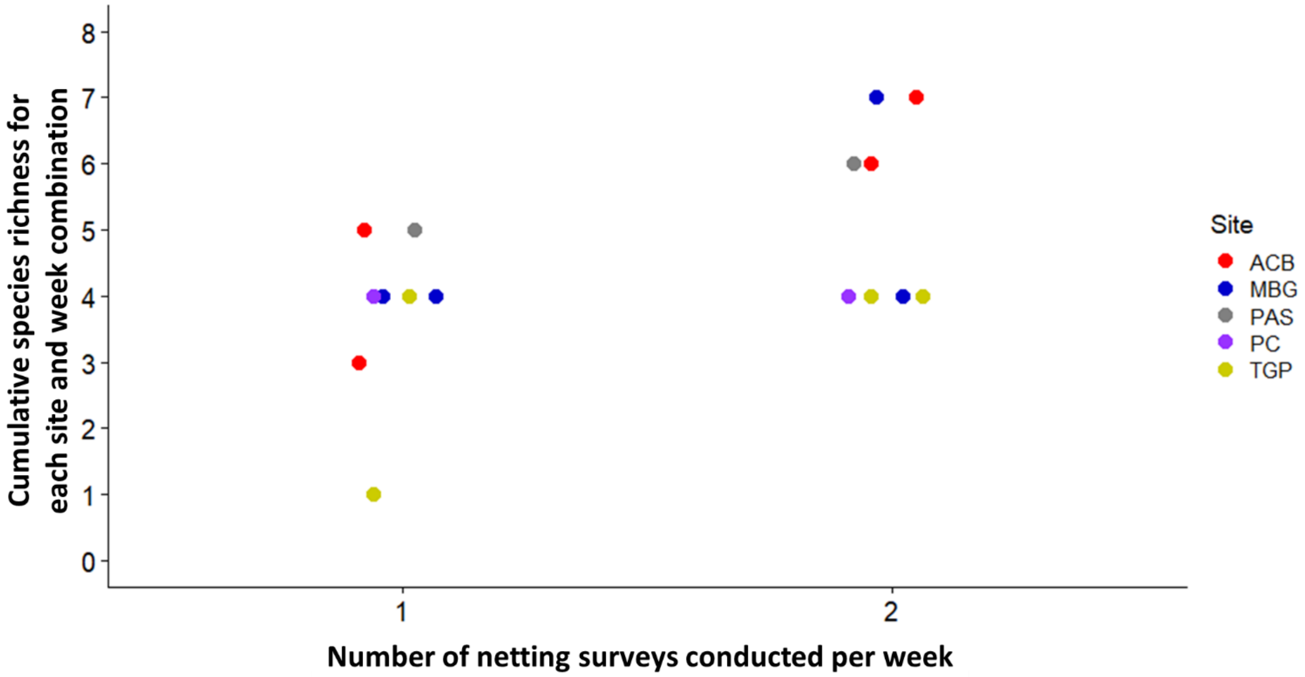


Figure 1.14: In the Boreal Shield Ecozone, there is an increase in the species richness detected that approaches significance when a second netting survey is conducted per week. Each point represents the cumulative species richness detected for each additional netting survey conducted within site and collection week. The points are jittered to reduce overlap.

Table 1.15: A linear model reveals that the impact of the number of netting surveys conducted per site per week on cumulative species richness in the Boreal Shield Ecozone approaches significance. Colons represent interactions and the variable of greatest interest is bolded. This analysis supports Figure 1.14.

Linear Model: Cumulative Species Richness ~ Site * Number of Surveys

	d.f.	**Sum Sq.**	**Mean Sq.**	**F**	**P**
Site	4	11.25	2.81	1.47	0.321
Number of surveys	**1**	**9.00**	**9.00**	**4.70**	**0.073**
Site:Number of Surveys	4	2.25	0.56	0.29	0.872
Residuals	6	11.50	1.92		

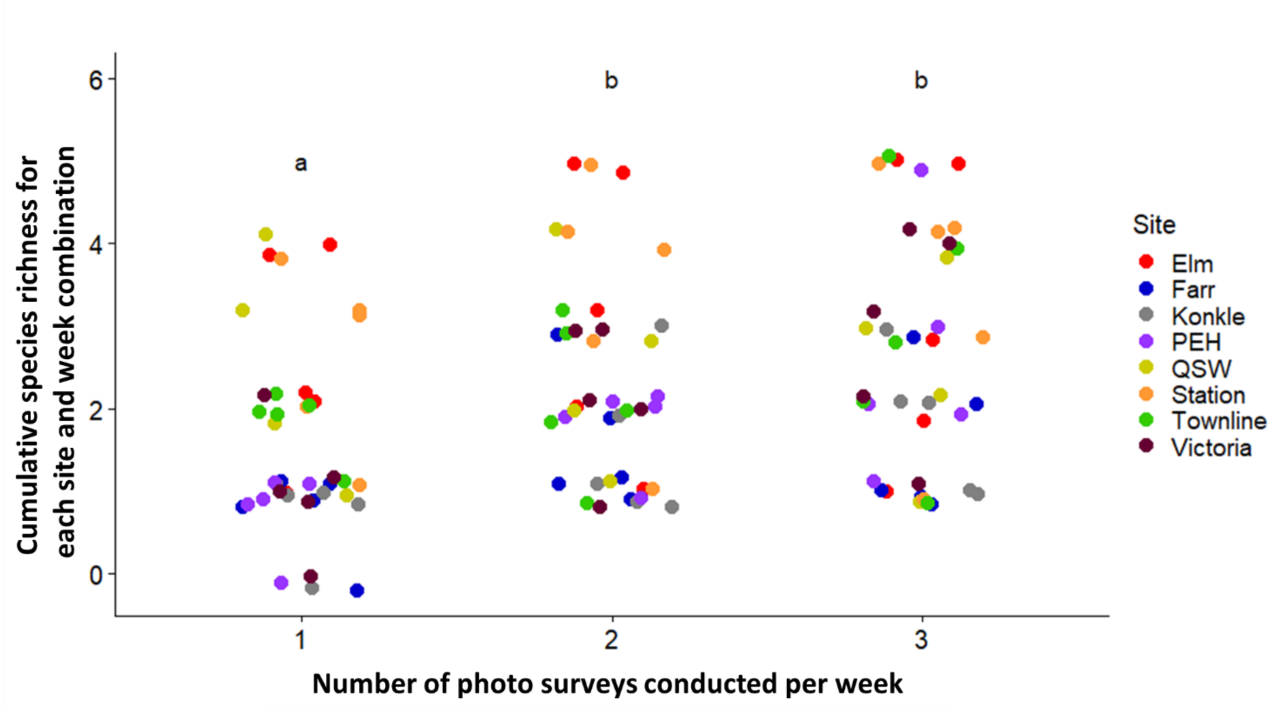


Figure 1.15: In the Carolinian Zone, conducting two photo surveys per week resulted in significantly more species being detected than conducting one survey per week, and a similar number of species as three surveys per week. Each point represents the cumulative species richness detected for each additional photo survey conducted within site and collection week. The points are jittered to reduce overlap. Number of photo surveys not sharing the same letter represent significant differences based on a Tukey HSD test.

Table 1.16: A linear model reveals that the number of photo surveys conducted per site per week significantly impacted the species richness detected in the Carolinian Zone, as did site. Colons represent interactions and the variable of greatest interest is bolded. These results support Figure 1.15.

Linear Model: Cumulative Species Richness ~ Site * Number of Surveys

	d.f.	**Sum Sq.**	**Mean Sq.**	**F**	**P**
Site	7	48.65	6.95	5.32	<0.001
Number of surveys	**2**	**21.61**	**10.80**	**8.28**	**<0.001**
Site:Number of surveys	14	6.93	0.50	0.38	0.978
Residuals	93	121.40	1.31		

Similar analysis was completed for the photo surveys in the Prairies Ecozone as seen in Figure 1.16. In this region the number of photo surveys conducted per week did not have a significant effect on the cumulative species richness, only site did (Table 1.17). In the Boreal Shield Ecozone a linear model revealed that conducting two photo surveys per week rather than one did not result in increased species richness (Figure 1.17 and Table 1.18).

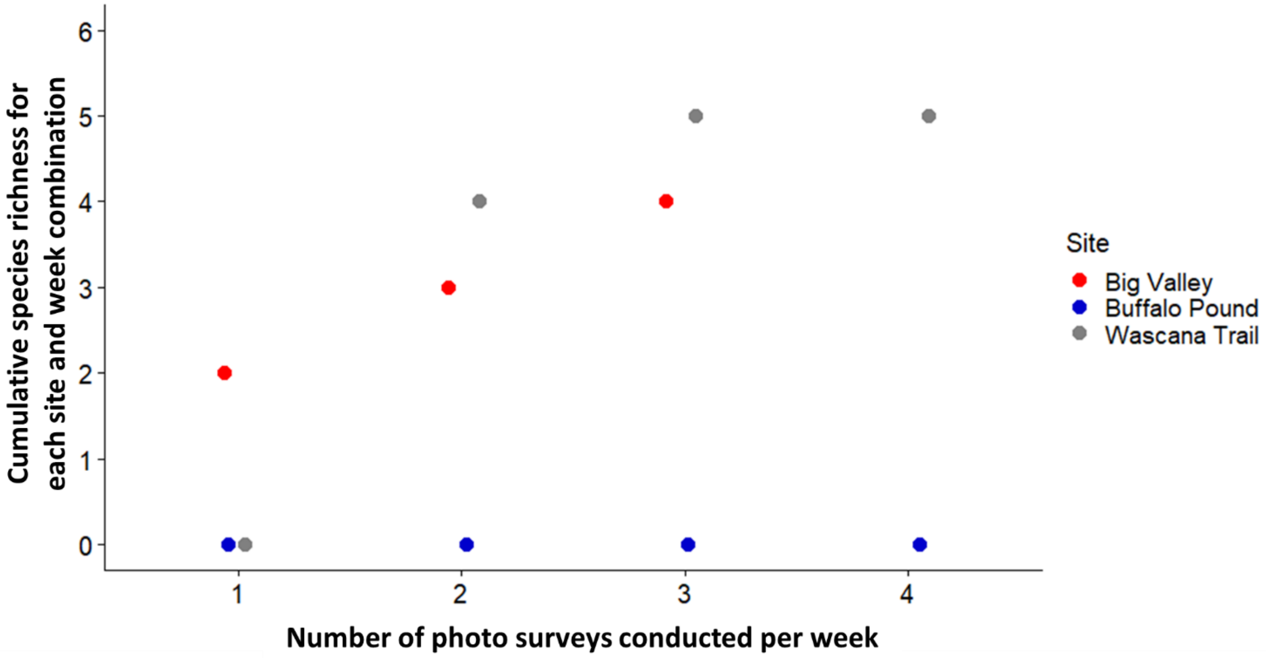


Figure 1.16: In the Prairies Ecozone, conducting more than one photo survey per week increased the species richness in some sites but did not significantly increase species richness overall. Each point represents the cumulative species richness detected for each additional photo survey conducted per site per week. Only photo surveys from the first survey period were compared. The points are jittered to reduce overlap.

Table 1.17: A linear model reveals that the number of photo surveys conducted per site per week did not significantly impact the species richness detected in the Prairies Ecozone, but site did. The colons represent interactions, and the variable of greatest interest is bolded. These results support Figure 1.16.

Linear Model: Cumulative Species Richness ~ Site + Number of Surveys

	d.f.	**Sum Sq.**	**Mean Sq.**	**F**	**P**
Site	2	27.91	13.96	7.90	0.028
Number of surveys	**3**	**10.17**	**3.39**	**1.92**	**0.245**
Residuals	5	8.83	1.77		

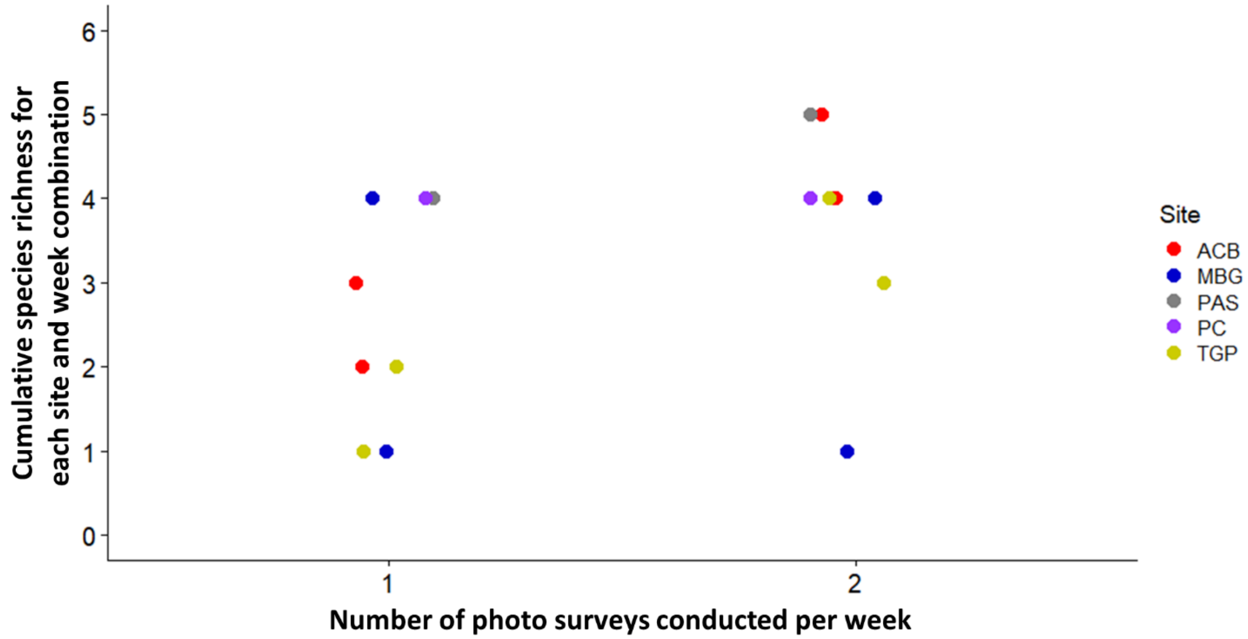


Figure 1.17: In the Boreal Shield Ecozone, conducting a second photo survey did not significantly increase the number of species detected. Each point represents the cumulative species richness detected for each additional photo survey conducted within site and collection week. The points are jittered to reduce overlap.

Table 1.18: A linear model reveals that the number of photo surveys conducted per site per week did not significantly impact the species richness detected in the Boreal Shield Ecozone. Colons represent interactions and the variable of greatest interest is bolded. These results support Figure 1.17.

Linear Model: Cumulative Species Richness ~ Site * Number of Surveys

	d.f.	Sum Sq.	Mean Sq.	F	P
Site	4	8.94	2.23	1.22	0.394
Number of surveys	**1**	**5.06**	**5.06**	**2.76**	**0.148**
Site:Number of surveys	4	3.44	0.86	0.47	0.758
Residuals	6	11.00	1.83		

DISCUSSION

Performance of collection methods regarding abundance and number of species

The goal of this study was to provide a recommendation for which collection method of blue vane traps (BVTs), timed targeted netting, timed targeted photographs, or combinations thereof work best for Canadian bumble bee population and community assessments, including COSEWIC assessments. In the Carolinian and Boreal Shield Ecozones, netting and photo methods collected more specimens than the BVT method, but the opposite was true in the Prairies Ecozone. The method that collected the most specimens in each region did not always correspond to the method that collected the most species. BVTs collected the most species in the Carolinian and Prairies Ecozones, and netting collected the most species in the Boreal Shield Ecozone. The ideal collection method would collect as many species as possible but with the fewest number of specimens required to do so.

Patterns of species composition

Species composition varied by method used, and in all regions the BVT distribution was significantly different from the netting and photo methods. In all regions as well, BVTs collected lower proportions of the most common species, compared to the netting and photo methods, which resulted in Pielou’s evenness values being highest for BVTs. BVTs may have been better at representing the evenness of the community because these traps sampled continuously throughout the survey period.

Bombus fervidus was collected in much higher proportions in BVTs in the Carolinian Zone compared to netting and photos, potentially suggesting that this species is particularly attracted to BVTs, or active outside of the times netting and photo collections were conducted. BVTs collected proportionally fewer *B. impatiens* than the other methods in the Carolinian Zone. BVTs also missed collecting *B. vagans* in the Carolinian Zone and while this species was not particularly common even in the netting and photo collections in this region, *B. vagans* was collected in much lower proportions in the Boreal Shield Ecozone where it is the most common species. While BVTs collected fewer *B. rufocinctus* than the other methods in the Prairies Ecozone, they collected proportionally more of this species than the other methods in the Carolinian Zone, so there does not appear to be a consistent pattern regarding that species. However, the pattern of *B. borealis* capture in BVTs compared to netting and photos is one of the most striking and consistent patterns across regions. *Bombus borealis* was collected in much higher proportions in BVTs in the Prairies and Boreal Shield Ecozones. It was a very rare species in the Carolinian Zone collections, but even in this region 2 of the 3 individuals collected were from BVTs. This suggests that BVTs are highly attractive to *B. borealis* and possibly that the Carolinian Zone does not provide as ideal a habitat for this species compared to the other two regions.

In the Carolinian Zone an additional comparison was made between the photo method and research grade iNaturalist records. The iNaturalist records produced only one fewer species than the intensively collected photo records but a significantly different species distribution. The iNaturalist records had a higher proportion of *B. citrinus* and *B. griseocollis*, and a lower proportion of *B. impatiens*. While these differences could be due to the unstructured nature of the iNaturalist surveys compared to the structured photo surveys, other explanations are possible.

The choice to include three years of iNaturalist data compared to one year of photo survey data may be a cause of the differences seen. Bee species are known to have high inter-annual variation (Kimoto *et al.* 2012; Gezon *et al.* 2015; Onuferko *et al.* 2018; Didham *et al.* 2020; Woodard *et al.* 2020; Packer & Darla-West, 2021; Turley *et al.* 2022). So, if *B. citrinus* populations were naturally higher in 2019 or 2020 compared to 2021, then the photo data from only 2021 would have a lower frequency of *B. citrinus* by default. Community science data is known to be biased towards cities (Stafford *et al*. 2010; MacPhail & Colla, 2020) so the differences seen could also be due to few urban habitats being sampled in this thesis. The missing species, *B. vagans*, was present in the non-research grade iNaturalist records suggesting that this species is difficult to identify by photograph. This statement is further supported by the netting and photo comparison described below.

Comparing netting and photo methods

After determining BVT records were distinct from netting and photo records, comparisons of the latter two methods were made to see if there was also a significant difference between them. Within site and collection date in the Carolinian and Prairies Ecozones there was no significant difference between the number of specimens and species richness collected per netting and photo survey. In the Boreal Shield Ecozone, netting surveys collected more species per survey than photos did at a level that was possibly significant. While it is possible netting is simply a better method for collecting bumble bees in this region, the difference could also be related to most of the surveyors in this region using stopwatches to exclude handling time from the netting surveys but not the photo surveys. It does take time to capture a good photo of each specimen which likely reduces the 30-minutes slightly, while the netting surveys had a full 30-minutes.

Netting and photo species compositions significantly differed from each other in the Carolinian and Boreal Shield Ecozones, but not in the Prairies Ecozone. Netting records from the Carolinian Zone contained more *B. rufocinctus* and *B. vagans* than photo records from this region, indicating a bias against these species with photographs. One of the colour patterns of *B. rufocinctus* in the Carolinian Zone is quite similar to the colour pattern of *B. vagans*. There are 6 photo records where I was not able to differentiate between the two species and likely some of the 45 unknown records contain these species as well, which could explain why these species appear less frequently in the photo records. In the Boreal Shield Ecozone, netting records contained more *B. sandersoni* and *B. vagans*, and fewer *B. ternarius*, and *B. terricola*. In particular, *B. sandersoni* was not detected by photos at all, while netting detected it at 4.5%. *Bombus sandersoni* and *B. vagans* are known to be particularly difficult to distinguish even with physical specimens, sometimes requiring malar ratio measurements to identify with certainty (Milam *et al*. 2020). The 22 photo records not identified to species likely contain some *B. sandersoni* and *B. vagans* records that could not be distinguished. I am not sure why the netting records contained fewer *B. ternarius* and *B. terricola*; perhaps it has something to do with the behaviour of these species. As a hypothetical example, if these species tended to prefer plants that are difficult to net on then this could explain why they appear less frequently in netting collections. This is something that could be investigated in future research in this region.

Studies comparing bees collected by BVTs and netting often report these different methods produce different species compositions (Gibbs *et al.* 2017; Rhoades *et al*. 2017; Bell, 2019; Mundy-Heisz, 2021). Knowing this, it was not surprising that the BVT method produced different results from the netting and photo methods, but I was surprised that the netting and photo methods produced significantly different results from each other in the Carolinian and Boreal Shield Ecozones. Often when bee species compositions differ between BVTs and netting the explanation given is that the BVTs are biased towards or against certain species (Gibbs *et al.* 2017; Prendergast & Hogendoorn, 2021). My results do support some BVT species bias, but also biases in netting and photo collections. Active methods like netting and photos are subject to collector bias which should not be overlooked, and actual collection time is far shorter for these methods so species that are active outside of the times sampling is done may be missed, such as crepuscular species (Westphal *et al.* 2008; Nielson *et al*. 2011; Cane *et al.* 2013; Rhoades *et al*. 2017; McCravy, 2018; Prendergast *et al*. 2020; Packer & Darla-West, 2021; Prendergast & Hogendoorn, 2021; Krahner *et al*. 2021). All methods, whether passive or active, provide a representation of bee abundance, but it is difficult to know how accurate these representations are (Portman *et al*. 2020; Packer & Darla-West, 2021). Since the BVT, netting and photo collections produce different species composition results, a national protocol would require multiple methods be used together.

Method effectiveness at detecting at-risk and parasitic species

For methods to be useful for COSEWIC conservation assessments, they need to be able to detect at-risk species. If a collection method cannot detect at-risk species, then there is no way to distinguish true absence from detection failure. When combining the records from all regions, all collection methods used were able to detect some at-risk species; however, BVTs detected the most at-risk species within regions and overall. BVTs detected *B. terricola, B. pensylvanicus, B. suckleyi,* and *B. bohemicus,* while netting and photos only detected *B. terricola*. A possible reason BVTs outperformed netting and photos regarding collecting at-risk species is the continuous sampling of the traps compared to shorter sampling periods by the other methods. At-risk species were collected in low abundances, including as singletons within regions (except for *B. terricola,* in the Boreal Shield Ecozone where it is a common species) so there did not seem to be any evidence of the collection methods oversampling them.

In the Carolinian Zone the only parasitic species detected, *B. citrinus,* was present in netting, photo, and iNaturalist records, but not in BVTs. In the Prairies Ecozone BVTs and photos each detected two of three parasitic species, and netting detected one. In the Boreal Shield Ecozone BVTs, netting, and photos each detected two of three parasitic species. Overall, there is no clear single best method for collecting parasitic species and likely there are species specific preferences towards one method or the other.

The literature is mixed on whether parasitic species are more likely to be caught in nets than in traps. Netting collected more parasitic bee specimens compared to pan traps in Krahner *et al.* (2021). However, Rhoades *et al.* (2017) found proportionally no difference in parasitic bees in net collections compared to BVTs and pan traps, and the number of parasitic bees per collection event was lowest for netting.

Regional differences in how methods performed

The variation in method performance across ecozones suggests that there are regional differences that need to be taken into consideration when monitoring bumble bees in Canada. In particular, the performance of BVTs and netting appear to vary between regions.

In the Carolinian Zone, the proportion of the total specimens collected by BVTs was low in comparison to another Ontario study (Mundy-Heisz, 2021) in which BVTs and netting each collected about half (54% and 45%) of the total bumble bee specimens. In my study, the difference between BVTs and netting was much greater, with BVTs collecting only 4% of the total specimens compared to netting collecting 45%. A possible explanation could be that the height of trap placement in the Carolinian Zone may have been lower than optimal. Height has been shown to affect performance of other trap types like pan traps (Geroff *et al*. 2014; Packer & Darla-West, 2021). BVTs are typically hung at heights between 0.75 meters and 2 meters (Stephen & Rao, 2005, 2007; Kimoto *et al*. 2012; Geroff *et al*. 2014; Joshi *et al*. 2015; Gibbs *et al*. 2017; Rhoades *et al*. 2017; Prendergast *et al*. 2020; Turley *et al*. 2022). BVT heights in the Carolinian Zone were aligned with the height of the surrounding flowers but this was often below 0.75 meters (Supplementary Table S1.3), which may have affected trap performance. Future researchers may wish to assess the effect of height on BVT performance in this region.

There was a clear distinction between how BVTs performed in the Prairies compared to the other regions. Firstly, this was the only region in which BVTs collected the most specimens. Secondly, and possibly due to collecting more specimens, BVTs also collected more species, 11 compared to 6 for netting and 7 for photos. This was a strong contrast to the Carolinian and Boreal Shield Ecozones where the number of species collected by the three different methods varied by only 1 or 2.

A possible explanation for the difference between BVT effectiveness in the Prairies compared to the other regions could be that western bumble bee species are more attracted to BVTs than eastern species. In support of this, the only article I found that compared BVT bumble bee catch rate in an eastern location to a western location, Mundy-Heisz (2021), found the bumble bee catch rate in Ontario was lower than the bumble bee catch rate reported in an Oregon study, Stephen & Rao (2005). When assembling the results from several western and eastern bee studies involving BVTs to assess for an east vs. west difference, the results were not clear (Supplementary Table S1.15). Many of the western studies indicated high BVT effectiveness (collecting more specimens, more species, and outperforming other collection methods) while some eastern studies appeared to show less extreme results, but not all. For example, Gibbs *et al*. (2017), an eastern study, is one of the key studies in suggesting that BVTs may oversample and deplete species. To evaluate whether or not BVTs are differentially attractive to eastern bumble bees compared to western bumble bees studies need to be specifically designed for this purpose.

In the Boreal Shield Ecozone, netting collected more specimens and more species overall than the other methods. This may be due to the other methods underrepresenting certain species. BVTs collected a lower proportion of *B. vagans* and failed to detect *B. flavidus* and *B. perplexus*, suggesting these species could be less attracted to BVTs. Photos did not detect *B. sandersoni* likely due to it being a species that is more difficult to identify by photograph. While netting also collected some species in lower proportions it only failed to detect the at-risk species *B. bohemicus*.

Sampling effort

Sampling effort is important to assess because population or community monitoring programs often have limited funding available and by reducing the time needed on-site, labour costs can be reduced. As well, bumble bee population assessments are done to conserve bees, and therefore, it is best practice to minimize the lethal impact of surveying (Montero-Castaño *et al.* 2022; Woodard *et al*. 2020)

In all regions, BVTs were the only method to detect new species throughout the whole or most of the field season, and this method continued to find new species as more individuals were collected. On the other hand, netting and photo methods plateaued after approximately 1 month of use in all regions though this was most distinct in the Carolinian Zone where surveying took place over 3 months. This means that in the Carolinian Zone more netting and photo surveys were done than were necessary for detecting the species richness levels reached. Regardless of collection method used, surveying past mid-September in this region was not useful for increasing the species richness detected.

Based on rarefaction curves the BVT method is most efficient at low sample sizes. In both the Carolinian and Prairies Ecozones, the BVT method detected the greatest species richness, but in the Boreal Shield Ecozone netting detected the most species. In the Carolinian Zone BVTs detected 10 of 14 known species, but extrapolated curves suggest BVTs could potentially detect 13 species if sampling effort was doubled. Netting and photo method curves plateaued at 9 and 8 species respectively and were not expected to find additional species. In the Prairies Ecozone the BVT method detected 11 out of 12 known species and was predicted to be able to detect 12 species with double the survey effort. The netting method plateaued at 6 species, while the photo method detected 7 species and was predicted to detect two more with a doubling of sample effort. In the Boreal Shield Ecozone netting detected 9 of 10 known species and was predicted to be able to detect 10 species if sampling effort was doubled. BVT and photo methods both detected 8 species and were not expected to find more with continued surveying. In the Carolinian and Boreal Shield Ecozones BVTs collected the fewest specimens, but in the Prairies Ecozone BVTs collected the most specimens.

These results are both similar and dissimilar to other bee collection method comparisons. In Rhoades *et al*. (2017) rarefaction curves for BVTs, netting and pan traps demonstrated that BVTs had the highest extrapolated bee species richness, and netting stopped detecting species well below BVTs which is like the Carolinian results. In contrast though, they found netting was the most efficient method when few individuals were sampled. Similarly to the Boreal Shield results, Mundy-Heisz (2021) demonstrated BVTs were more efficient than netting at lower numbers of individuals, and that netting detected higher species richness. Overall BVTs appear to be the most efficient method for detecting species at low sample sizes, though there are regional differences in terms of the collection method that detects the most species and that collects the most specimens.

Previous studies have reported concerns about BVTs potentially oversampling certain species that are highly attracted to these traps such as bumble bees (Gibbs *et al.* 2017; McCravy, 2018; Prendergast *et al*. 2020; Packer & Darla-West, 2021; Prendergast & Hogendoorn, 2021). This is due to studies using BVTs demonstrating either high catch rates or that bee abundance declined in later sampling years (Kimoto *et al*. 2012; Joshi *et al*. 2015; Gibbs *et al*. 2017; Prendergast *et al*. 2020; Tronstad *et al*. 2022). This research provides no indication of oversampling with BVTs in the Carolinian and Boreal Shield Ecozones, but in the Prairies Ecozone BVTs did catch far more specimens than the other methods. With only one year of data, it is not possible to determine if the level of sampling was detrimental to local populations in this region. If additional years of sampling were done, then the possibility of BVTs oversampling bumble bees is something that should be assessed. However, oversampling did clearly occur with both the netting and photo collection methods in the Carolinian Zone. This is evidenced by the plateaued species accumulation and rarefaction curves for these methods in this region. Netting and photo surveying continued two months past the point when these methods stopped detecting new species, and the netting and photo rarefaction curves reached asymptote before sampling individuals ceased. This means that netting and photo surveys should be conducted either less frequently or for a shorter period during the field season or both.

Based on species accumulation curves estimating species richness per number of sites surveyed, no region included enough sites to maximize species richness detection. The curves for all regions were steepest when fewer than 3 sites were included. These results suggest no fewer than 3 sites should be used when sampling or the risk of missing many species is high. The region with the most sites surveyed, 7 for BVTs and 8 for netting and photos, was the Carolinian Zone, and the results from that region suggest that future surveys should include more than 8 sites to increase the likelihood of detecting more species. However, these results should be interpreted cautiously as these results could differ depending on the type of landscapes surveyed. More heterogeneous landscapes may require additional sites and less heterogeneous landscapes may require fewer sites. While increasing the number of sites would also increase the number of specimens collected, if netting and photo surveys were done less frequently, more species could be detected while fewer specimens were.

Using multiple sites when surveying is also suggested by Strange & Tripodi (2019) who noted that more species were detected when more than one site was sampled per ecoregion. Another study (sampling from 96 sites in Wyoming, USA) used power analysis to show that 8 to 9 BVTs are needed to detect changes in bumble bee abundance and species richness in this area, and they recommended using 3 BVTs per site, for a minimum of 3 to 4 sites (Tronstad *et al*. 2022). My results support Tronstad *et al*. (2022)'s recommended minimum number of sites but suggest the optimal number of sites is higher, potentially even higher than eight sites per region.

This difference could stem from differences in landscape heterogeneity between regions or from the slightly different questions Tronstad *et al*. (2022) and I were addressing. While Tronstad *et al*.'s study was trying to determine the minimum number of sites to detect change in populations, my analyses addressed the question of how many sites are needed to maximize species richness detected.

I also assessed the quality of sites in the Carolinian Zone, by comparing sites for efficiency (number of species detected per specimen collected), the species richness detected, and the number of uncommon (within the collection), at-risk and parasitic species found at the site. Some sites like Konkle and PEH produced a lot of species, including uncommon, at-risk or parasitic ones, with few specimens. Other sites like Elm St. and QSW produced few species with many specimens collected and none of the species were unique to those sites. If sampling had been done in all sites except for Elm St. and QSW the number of specimens collected would have been reduced by almost half, without the loss of any species, and with only slight changes in the species composition.

Sites that underperform should be replaced. Researchers cannot know in advance which sites will be more useful than others, but a pilot study before a major research project may help in assessing site quality, or site quality may be assessed after the first year of surveying in multi-year studies.

Increasing the length of BVT deployment increased species richness in the Carolinian and Prairies Ecozones. Seven-day durations produced more species than one and three-day durations in the Carolinian Zone. While the fourteen-day duration in the Prairies collected significantly more species than seven days from an earlier survey period, there were no shorter durations within the same survey period to compare with, so it is possible this effect may be due to the time of year rather than duration. My results were similar to the those of Mundy-Heisz (2021) who concluded that BVT deployment for six days was the ideal length of time. In their study, the rarefaction curves produced for BVTs deployed for six days were similar to those from seven-day durations and detected more species than two and four-day durations.

At sites in the Carolinian Zone, conducting a second netting or photo survey per collection week increased the species richness detected, but conducting a third survey per week did not produce more species than two surveys. In the Prairies Ecozone species richness did not increase significantly with the addition of a second, third or fourth netting or photo survey per week per site. Since only one survey period in this region contained multiple netting and photo collections within a week timeframe there was little data for this analysis and the results should be interpreted with caution. In the Boreal Shield Ecozone sites, netting surveys detected greater species richness when two surveys were conducted per week compared to one at a level that approached significance. Photo survey species richness did not significantly increase with a second survey. As noted in the species composition analysis, the photo records in this region appear to be biased against *B. vagans* and *B. sandersoni* which might depress actual species richness levels, including potentially changes in species richness between one survey and two. Overall, the general trend based on the largest dataset suggests that there is a benefit in terms of species richness detection to doing more than one netting or photo survey per site per week, but any more than two is unjustified. This also offers a solution to the oversampling issue in the Carolinian netting and photo surveys. By reducing the number of netting and photo surveys per collection week, the number of specimens collected will be reduced.

Another way to interpret these results is that 1 hour of surveying will detect significantly more species than 30 minutes. Researchers should consider conducting longer (1 hour) surveys if they are only conducting 1 survey per site per week.

Methodological limitations of this study

While the overall structure of surveys in all three regions was similar, there were differences in survey protocols. If redone, this study could be improved by standardizing the protocols across regions, including things such as using a standard number of BVTs per site, setting traps a standard distance apart, and being consistent with whether surveyors include or exclude handling time in netting surveys. Training can help to reduce collector bias, but there will always be some difference between collectors in terms of experience and skill at spotting and sampling bees (Westphal *et al*. 2008; Nielsen *et al*. 2011; Cane *et al*. 2013; Rhoades *et al*. 2017; McCravy, 2018; Prendergast *et al*. 2020; Packer & Darla-West, 2021; Prendergast & Hogendoorn, 2021; Krahner *et al*. 2021). Large surveys like the ones from this study cannot be done without the help of many surveyors, and even at the individual level each surveyor may be biased towards certain species.

The field season when these surveys were conducted was during the COVID-19 pandemic and there were limitations as to how research could be conducted. Sites were chosen with considerations such as being able to obtain permission to survey, the likelihood of bees being present, and safety. While a diverse array of habitats was represented in the Carolinian and Boreal Shield Ecozones, the Prairies sites were all natural areas. Future studies may want to design their studies with more heterogeneous landscapes, including urban areas. The Prairies and Boreal Shield Ecozones had fewer sites and collection weeks than the Carolinian Zone, which limited some of the analysis to the Carolinian Zone only. Not only would increasing the number of sites and the number of BVT survey periods in these regions increase species richness detection, it would also help with improving analysis of regional differences and universal patterns.

While this thesis assessed BVTs, netting and photographs in the Carolinian, Prairies, and Boreal Shield Ecozones, there are many other understudied regions of Canada where method comparison would improve our understanding of regional differences and aid in developing a national protocol. My results show regional differences such as BVTs being more effective at collecting bumble bees in the Prairies, and netting outperforming other methods in the Boreal Shield Ecozone. Canada is a large country with a number of different ecozones, so additional differences are likely.

Site independency is of some concern. When measured in the field, bumble bee foraging distance differs by species, with the maximum foraging distance typically being below 2.5 km, though one study using microsatellite analysis of sisters reported bumble bee foraging distance at 11.6 km (Walther-Hellwig & Frankl, 2000a, 2000b; Wolf & Moritz, 2008; Hagen *et al.* 2011; Rao & Strange, 2012). Out of all sites used in this study, only PEH and QSW were less than 2.5 km apart. At approximately 1.5 km apart, PEH and QSW are likely still independent for most bumble bee workers, and the noticeable difference in results from these two sites supports independence. PEH produced the highest species richness of all sites, including at-risk and parasitic species, while QSW produced the lowest species richness and only common species.

While different people completed the species identifications for each region, differences were minimized for the netting and BVT records by having all identifiers use the Williams *et al*. (2014) identification guide, and by having Dr. Cory Sheffield verify voucher specimens for the

Carolinian and Boreal Shield Ecozones. With the photo records there was less control over the species identification, and it is possible that some of the specimens that were not identified to species for the Carolinian and Boreal Shield Ecozones could be if viewed by an expert, and there is also the possibility that some identifications could be incorrect.

Ferrari & Polidori (2023) recommend adjusting abundance values by sociality because every individual is potentially reproductive in solitary species, but in eusocial species, like bumble bees, this is not the case. Eusocial bee species may appear to be abundant based on the number of individuals, but their effective population size (the number of reproductive individuals) could be low, putting them at higher risk for extinction (Zayed *et al*. 2004; Zayed & Packer, 2005). This difference between the population size based on number of individuals sampled and the effective population size can affect measures of diversity (Ferrari & Polidori, 2023). While this needs to be considered when measuring communities with a mixture of solitary and eusocial species, my study was focused only on bumble bees. There likely is some difference between the parasitic and non-parasitic bumble bees, but Ferrari & Polidori's equation did not consider parasitic species. Even in terms of there being differences between queens, workers and males, my methods reduced this concern by beginning sampling in summer to avoid catching queens.

Recommendation

The goal of this research was to aid in the development of a national bumble bee monitoring protocol, including something that could generate data useful for COSEWIC. I was looking for a collection method or combination of complementary collection methods that would meet the ideals of providing an accurate representation of species composition and richness, consistency across landscapes, times, weather patterns and individual surveyors, be repeatable, contain little collector bias, and minimize cost, sampling effort, processing time and bee death (Westphal *et al.* 2008; Nielsen *et al*. 2011; Lebuhn *et al*. 2013; Woodard *et al*. 2020; Packer & Darla-West, 2021; Mundy-Heisz, 2021). With these criteria in mind, I set out my recommendations.

Due to the differences in species composition produced by BVTs, netting and photos, multiple methods are required to provide the best understanding of species composition. To choose how many and which methods are needed, I must consider which methods are complementary. Complementary methods are methods that provide information that each single method misses (Westphal *et al*. 2008; Nielson *et al*. 2011). In the Carolinian Zone, no collection method detected all twelve species, but a combination of BVTs and netting or BVTs and photos did. In the Prairies Ecozone, netting and photo methods produced a subset of the species detected by BVTs, except for a singleton species detected by photos. In the Boreal Shield Ecozone, netting detected all species except one which was a doubleton, at-risk species collected by BVTs. While photos only collected a subset of species detected by netting in this region, they were still complementary to BVTs. Overall, BVTs and netting are complementary as are BVTs and photos, therefore one of those combinations should be used in bumble bee surveys.

Considering iNaturalist records

Simon *et al*. (in press) provided evidence that iNaturalist records could produce similar rarefaction curves to BVT bumble bee sampling on Galiano Island British Columbia. They also demonstrated the proportional species abundances were similar between iNaturalist records and BVTs, with the exception of one species, *B. vosnesenskii*, which appeared in higher abundances in the iNaturalist observations, but is a species known to do well in urban areas. Community science records are known to be biased towards cities (Stafford *et al*. 2010; MacPhail & Colla, 2020). Contrary to Simon et al.'s results the results from this thesis, do not support that iNaturalist records in the Carolinian Zone produce the same results as more intensive scientific collections. The research grade iNaturalist data produced a species composition that was higher in *B. citrinus* and *B. griseocollis* and lower in *B. impatiens* than the intensive photo collections. As discussed, this may have been a true difference or due to the different number of years included in the two datasets being compared. Unfortunately, there were not enough iNaturalist data after filtering to complete this comparison in the Prairies and Boreal Shield Ecozones. This further demonstrates the spatial bias of community science records. Since they do not always provide adequate coverage in areas away from cities, and my results suggest they do not always produce the same species compositions as more intensive photographic surveys, I do not recommend that iNaturalist records be used to replace scientific surveys for a national protocol in Canada. However, further research should be conducted comparing datasets of the same length of time. There may be regions, like Galiano Island (Simon *et al.* in press) where iNaturalist records can provide good data for bumble bee assessments and further research on this as well as on the accuracy of iNaturalist bumble bee identifications should be conducted.

Which is better, a netting or a photo survey?

In some ways netting and photo methods are similar. These methods are both active collection methods that require significant surveyor effort and attendance on site. They also both detected new species for about the same amount of time (approximately 1 month of use).

However, there are differences between the two methods that need to be considered when deciding between them.

An important benefit of netting is that it produces physical specimens, which is helpful when learning how to identify species, when building a reference collection, and when studying traits that require physical specimens. Small characteristics are sometimes necessary for identifying and distinguishing species with certainty (Williams *et al*. 2014; Ascher & Pickering, 2022). In the Boreal Shield Ecozone, the netting method was the best method overall (even considering BVTs) for detecting species richness. In this region netting produced more species on average than photo surveys from the same sites on the same days at a level that was possibly significant. Based on rarefaction curves netting detected the most species and was also the only method predicted to find additional species in this region with more sampling. Outside of this region, in the Carolinian Zone, netting detected the same number of species on average as photo surveys conducted at the same sites on the same days, but overall netting detected 9 species while photos only detected 8.

One of the main advantages of photos is that they are non-lethal. This is especially important when working with at-risk species, but even when not working with at-risk species it is important that sampling not harm populations (Gezon *et al.* 2015; Prendergast & Hogendoorn, 2021; Packer & Darla-West, 2021). In the Prairies Ecozone photos detected one more species than netting and based on rarefaction curves, photos were predicted to find additional species while netting was not. Considering BVTs collect more specimens in this region, using a complementary method that would not result in further bee mortality would be ideal. For these reasons, photos are the preferable method to complement BVTs in the Prairies.

Unlike with netting where the individual is physically collected, with photos there is the potential to accidentally survey the same individual twice (Montero-Castaño *et al.* 2022). As noted in Stafford *et al.* (2010), not all bumble bee photo records can be identified to species. Not all photographs are good quality, and some species are difficult to identify from photos because they are rare or look similar to others (Montero-Castaño *et al.* 2022; Suzuki-Ohno *et al*. 2022). This was true for all regions studied in this thesis: only 94% to 95% of photo records could be identified to species (Supplementary Table S1.16). This is slightly lower than the percentage (98%) of photographic bumble bee records could be identified to species by experts in Flaminio *et al.* (2021). As demonstrated from the results of this thesis, species that are difficult to identify by photograph because they require visibility of small or hidden characteristics (such as malar ratio or genitalia) may be underrepresented in photo records.

While processing specimens is time consuming for both methods, recent progress has been made towards speeding this up for photo records using artificial intelligence. Ärje *et al.* (2020) developed a machine that could photograph and identify arthropods. Suzuki-Ohno *et al.* (2022) tested the accuracy of a convolutional neural network, Xception, to identify honey and bumble bees from photographs collected by community scientists in Japan, and the accuracy of species identification by the network was around 84%. Another program, BeeMachine (https://beemachine.ai/), also uses a convolutional neural network to identify North American bumble bee species from photos and boasts an accuracy of over 91% (Spiesman *et al*. 2021). This technology holds great potential for speeding up and improving the quality of photo identifications, especially for community science submissions. However, some species are still difficult for BeeMachine to identify. For example, the precision of *B. sandersoni* identifications was low and error rate was high because there were few *B. sandersoni* photographs for the network to train with and this species looks similar to *B. vagans* (Spiesman *et al.* 2021). Using BeeMachine to complete species identifications can make specimen processing time lower than netting, but it is not likely to solve the issue of underrepresentation in photo records of species that are difficult to distinguish because they look similar to each other.

To summarize, netting is preferable to photos in situations such as when a researcher is unfamiliar with bumble bee identification and looking to build a reference collection, and when the region being surveyed contains species that are difficult to distinguish without fine details, such as the Carolinian and Boreal Shield Ecozones. Netting is also preferable in the Boreal Shield Ecozone because it detects the most species there. In other situations, photos are preferable due to their non-lethal nature and reduced processing time when using BeeMachine for identifications. Photos are also preferable to netting in the Prairies Ecozone because they are predicted to detect more species and they are non-lethal, which is particularly important in a region where BVTs collect many specimens.

Most efficient collection method and lowest sampling effort

Another aspect that needs to be considered when providing collection method recommendations is the efficiency of the methods. BVTs were the most efficient method at low sample sizes, producing the highest species richness with the fewest specimens based on rarefaction. This again supports the usefulness of BVTs in bumble bee sampling but does not explain how much sampling should be done.

To minimize sampling effort while still providing adequate species richness information, my results suggest 3 survey sites as the minimum number needed but that potentially more than 8 sites should be used to maximize species richness coverage. These site number recommendations should be interpreted with some caution as differences in landscape heterogeneity could affect these. As species richness was still increasing at 8 sites for the Carolinian Zone, future studies should assess what the maximum number of sites should be for each region. In the Carolinian Zone, week-long surveys were separated by two weeks at any given site, and I suggest following a similar pattern. When considering how long to survey, BVTs were the only method that continued to find new species throughout the entire summer, and I recommend that they be used for this period. However, netting and photo methods should be used for a shorter time as they only collected new species for about 1 month, which corresponded to two sampling periods per site. These two, week-long periods per site of netting or photo sampling should take place when most bumble bee species are active or be split if certain species are active in early summer and different species active in late summer.

For each site I recommend 7-day deployment of BVTs since this period produced greater species richness than shorter periods in the Carolinian Zone. Further research on the optimal length of BVT deployment in other regions would be beneficial. For instance, there was some evidence that a two-week duration was effective in the Prairies, and this should be tested further.

The results suggest that 2 netting or photo surveys should be conducted per collection week at any given site. This could also be interpreted as a single 1-hour survey per week per site. Reducing the number of surveys per week or the total survey time will help to prevent oversampling and offset the collection of additional specimens through the inclusion of more survey sites. Conducting two netting or photo surveys would also coincide well with the days of BVT deployment and collection and therefore minimize the number of trips the surveyor needs to make to sites, as would a single 1-hour survey.

Occasional additional trips to sites may be needed if BVTs require maintenance due to loose platforms, platform adjustment, or if traps fill with water after heavy rainfall, which happened occasionally in the Carolinian Zone. To prevent rain overflow, plastic covers can be added to traps to stop rainfall from entering, as suggested by Packer & Darla-West (2021), or a mesh covered hole could be added near the top of the trap to allow excess water to drain (James Mesich, personal communication).

Conclusion

In Canada, some bumble bee populations such as *B. affinis*, *B. bohemicus*, *B. occidentalis s. str*. and *B. suckleyi* are at high risk of becoming extirpated or extinct (COSEWIC, 2010, 2014a, 2014b, 2019). Other species such as *B. impatiens*, *B. griseocollis,* and *B. bimaculatus* are generally stable or even increasing in abundance (Colla & Packer, 2008; Grixti *et al.* 2009; Colla *et al*. 2012; Colla, 2016; Jacobson *et al*. 2018; Strange & Tripodi, 2019; Novotny *et al.* 2021; Jackson *et al*. 2022). It is important to monitor population changes so that declines can be detected and reversed before they become extinctions and so that the factors allowing certain populations to increase are better understood. Current population assessments for bumble bees employ a variety of different collection methods, but how these methods compare is still not fully understood. There are calls from the scientific community to standardize bee collection protocols (Nielsen *et al*. 2011; Strange & Tripodi, 2019; Woodard *et al.* 2020). Reducing the inconsistency in collection methodologies across studies would help to improve replicability and comparability of results (Packer & Darla-West, 2021). If results can be easily compared, scientists will be better able to interpret true patterns of decline, stability, or increase. Working together at a national level would improve scientists' ability to respond effectively to concerns and make any required changes to protect bumble bee populations. My research assists the efforts of other researchers in trying to develop a national standard protocol for bumble bee collection in Canada. Improving our understanding of the strengths and weaknesses of different bumble bee collection or detection methods, including how efficient they are at detecting at-risk species and how they compare in different regions of the country, is one step towards this goal.

When conducting bumble bee population or community assessments in Canada, I recommend using BVTs together with either netting or photo collection methods as these combinations maximize species richness detection and produce the most complete species composition results. While the choice between netting and photo surveys will depend on the purpose of the study, regionally, netting is the preferable method for the Carolinian and Boreal Shield Ecozones, and photos are preferable for the Prairies Ecozone. If working with at-risk species (such as in COSEWIC assessments) photographs are preferable to netting due to their non-lethal nature, however the researcher must keep in mind that species that require details not visible by photograph (ex. malar ratio and genitalia) to identify correctly will likely be underrepresented in photo collections. I recommend deploying BVTs for 7-day durations and conducting either a 30-minute netting or photo survey on the days of trap placement and collection or a single 1-hour netting or photo survey per week. BVTs should be deployed for the summer period, but netting and photo surveys need only be done for 1 month (2 survey periods per site). This month-long period should correspond to the time when most bumble bee species are active. BVTs appear to be especially attractive to bumble bees in the Prairies Ecozone, so I recommend using them with some caution in that region so as not to oversample.

Fortunately, this is an opportune time to develop a national standard protocol for monitoring bumble bee populations, since the United States National Native Bee Monitoring

Research Coordination Network is already gathering advice from experts to develop a national standard bee monitoring protocol for the USA (National Native Bee Monitoring RCN, 2023). The workshops they host are also open to Canadian bee researchers and with several providing input, the resulting protocol should be compatible in Canada. It is important that a national protocol considers which collection methods work well for different bee genera. My study helps to clarify what methods work well for bumble bees in the Carolinian, Prairies, and Boreal Shield Ecozones. These ecozones are large though and similar studies should be conducted in other locations within these ecozones and within Canada’s other ecozones as well. Once comparability between collection methods is clarified for all ecozones, scientists will be in a better position to know which methods best suit a national bumble bee monitoring protocol.

REFERENCES

Appixi. (2022). LeafSnap Plant Identification (Version 2.3.2(328)) [Mobile App]. Google Play Store. https://play.google.com/store/apps/details?id=plant.identification.snap&hl=en

Apple Inc. (2022). *iPhone XR - Technical Specifications*. Retrieved April 10, 2023, from https://support.apple.com/kb/SP781?locale=en_US

Ärje, J., Melvad, C., Jeppesen, M. R., Madsen, S. A., Raitoharju, J., Rasmussen, M. S., Iosifidis, A., Tirronen, V., Gabbouj, M., Meissner, K., & Høye, T. T. (2020). Automatic image-based identification and biomass estimation of invertebrates. *Methods in Ecology and Evolution*, *11*(8), 922–931. https://doi.org/10.1111/2041-210X.13428

Ascher J. & Pickering J. (2022). *Bombus identification guide*. Discover Life. Retrieved January 10, 2022, from https://www.discoverlife.org/mp/20q?guide=Bombus

Barker, J. (2013). *The ultimate guide to wild flowers of North America*. Parragon.

Bartomeus, I., Ascher, J. S., Gibbs, J., Danforth, B. N., Wagner, D. L., Hedtke, S. M., & Winfree, R. (2013). Historical changes in northeastern US bee pollinators related to shared ecological traits. *Proceedings of the National Academy of Sciences*, *110*(12), 4656–4660. https://doi.org/10.1073/pnas.1218503110

Bell, C. (2019). *Sampling methods and distribution modeling of bees: the status of the western bumble bee (Bombus occidentalis) in Wyoming*. [MSc thesis, University of Wyoming].

Biesmeijer, J. C., Roberts, S. P. M., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T., Schaffers, A. P., Potts, S. G., Kleukers, R., Thomas, C. D., Settele, J., & Kunin, W. E. (2006). Parallel Declines in Pollinators and Insect-Pollinated Plants in Britain and the Netherlands. *Science*, *313*(5785), 351–354. https://doi.org/10.1126/science.1127863

Billheimer, D., Guttorp, P., & Fagan, W. F. (2001). Statistical Interpretation of Species Composition. *Journal of the American Statistical Association*, *96*(456), 1205–1214.

Bumble Bee Watch. (2022). *Tips for photographing bees*. Retrieved August 25, 2022, from https://www.bumblebeewatch.org/photo-tips/

Callaghan, C. T., Mesaglio, T., Ascher, J. S., Brooks, T. M., Cabras, A. A., Chandler, M., Cornwell, W. K., Ríos-Málaver, I. C., Dankowicz, E., Dhiya'ulhaq, N. U., Fuller, R. A., Galindo-Leal, C., Grattarola, F., Hewitt, S., Higgins, L., Hitchcock, C., Hung, K.-L. J., Iwane, T., Kahumbu, P., … Young, A. N. (2022). The benefits of contributing to the citizen science platform iNaturalist as an identifier. *PLOS Biology*, *20*(11), e3001843. https://doi.org/10.1371/journal.pbio.3001843

Cameron, S. A., Lozier, J. D., Strange, J. P., Koch, J. B., Cordes, N., Solter, L. F., & Griswold, T. L. (2011). Patterns of widespread decline in North American bumble bees. *Proceedings of the National Academy of Sciences*, *108*(2), 662–667. https://doi.org/10.1073/pnas.1014743108

Cane, J. H., Kervin, L. J., & McKinley, R. (2013). Sensitivity of Systematic Net Sampling for Detecting Shifting Patterns of Incidence and Abundance in a Floral Guild of Bees at Larrea tridentata. *Journal of the Kansas Entomological Society*, *86*(2), 171–180. https://doi.org/10.2317/JKES121126.1

Chao, A., Gotelli, N. J., Hsieh, T. C., Sander, E. L., Ma, K. H., Colwell, R. K., & Ellison, A. M. (2014). Rarefaction and extrapolation with Hill numbers: A framework for sampling and estimation in species diversity studies. *Ecological Monographs*, *84*(1), 45–67. https://doi.org/10.1890/13-0133.1

Christman, M. E., Spears, L. R., Strange, J. P., Pearse, W. D., Burchfield, E. K., & Ramirez, R. A. (2022). Land cover and climate drive shifts in Bombus assemblage composition. *Agriculture, Ecosystems & Environment*, *339*, 108113. https://doi.org/10.1016/j.agee.2022.108113

Colla, S. R. (2016). Status, Threats and Conservation Recommendations for Wild Bumble Bees (Bombus spp.) in Ontario, Canada: A Review for Policymakers and Practitioners. *Natural Areas Journal*, *36*(4), 412–426. https://doi.org/10.3375/043.036.0408

Colla, S. R., & Dumesh, S. (2010). The Bumble Bees of Southern Ontario: Notes on Natural History and Distribution. *JESO 141*, 39-68.

Colla, S. R., Gadallah, F., Richardson, L., Wagner, D., & Gall, L. (2012). Assessing declines of North American bumble bees (Bombus spp.) using museum specimens. *Biodiversity and Conservation*, *21*(14), 3585–3595. https://doi.org/10.1007/s10531-012-0383-2

Colla, S. R., & Packer, L. (2008). Evidence for decline in eastern North American bumblebees (Hymenoptera: Apidae), with special focus on Bombus affinis Cresson. *Biodiversity and Conservation*, *17*(6), 1379. https://doi.org/10.1007/s10531-008-9340-5

COSEWIC. (2010). COSEWIC assessment and status report on the Rusty-patched Bumble Bee Bombus affinis in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. vi + 34 pp. (www.sararegistry.gc.ca/status/status_e.cfm).

COSEWIC. (2014a). COSEWIC assessment and status report on the Gypsy Cuckoo Bumble Bombus bohemicus in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. ix + 56 pp. (www.registrelep-sararegistry.gc.ca/default_e.cfm).

COSEWIC. (2014b). COSEWIC assessment and status report on the Western Bumble Bee Bombus occidentalis, occidentalis subspecies (Bombus occidentalis occidentalis) and the mckayi subspecies (Bombus occidentalis mckayi) in Canada. Committee on the Status of

Endangered Wildlife in Canada. Ottawa. xii + 52 pp. (www.registrelep-sararegistry.gc.ca/default_e.cfm).

COSEWIC. (2015). COSEWIC assessment and status report on the Yellow-banded Bumble Bee Bombus terricola in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. ix + 60 pp. (www.registrelep-sararegistry.gc.ca/default_e.cfm).

COSEWIC. (2018). COSEWIC assessment and status report on the American Bumble Bee *Bombus pensylvanicus* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. x + 52 pp. (http://www.registrelep-sararegistry.gc.ca/default.asp?lang=en&n=24F7211B-1).

COSEWIC. (2019). COSEWIC assessment and status report on the Suckley's Cuckoo Bumble Bee Bombus suckleyi in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xi + 70 pp. (https://www.canada.ca/en/environment-climate-change/services/species-risk-public-registry.html).

COSEWIC. (2022). *Canadian Wildlife Species at Risk.* Committee on the Status of Endangered Wildlife in Canada. Retrieved June 9, 2023, from https://species-registry.canada.ca/index-en.html#/documents?documentTypeId=33&sortBy=documentTypeSort&sortDirection=asc&pageSize=10

COSEWIC. (2023). *COSEWIC candidate wildlife species*. Retrieved June 9, 2023, from https://www.cosewic.ca/index.php/en-ca/reports/candidate-wildlife-species.html#toc1

de Haan, J.L. (2021) *The ecological and physiological consequences of sun vs. shade nesting for the small carpenter bee, Ceratina calcarata, and their offspring*. [MSc thesis, Brock University] Brock University Digital Repository.

Didham, R. K., Basset, Y., Collins, C. M., Leather, S. R., Littlewood, N. A., Menz, M. H. M., Müller, J., Packer, L., Saunders, M. E., Schönrogge, K., Stewart, A. J. A., Yanoviak, S. P., & Hassall, C. (2020). Interpreting insect declines: Seven challenges and a way forward. *Insect Conservation and Diversity*, *13*(2), 103–114. https://doi.org/10.1111/icad.12408

Droege, S. (2015). *The very handy manual: how to catch and identify bees and manage a collection*. Retrieved August 20, 2022, from http://bio2.elmira.edu/fieldbio/beemanual.pdf

Dubaić, J. B., Lanner, J., Rohrbach, C., Meimberg, H., Wyatt, F., Čačija, M., Galešić, M., Ješovnik, A., Samurović, K., Plećaš, M., Raičević, J., & Ćetković, A. (2022). Towards a real-time tracking of an expanding alien bee species in Southeast Europe through citizen science and floral host monitoring. *Environmental Research Communications*, *4*(8), 085001. https://doi.org/10.1088/2515-7620/ac8398

Ferrari, A., & Polidori, C. (2023). Eusociality as a neglected aspect of wild bee α-diversity and its potential impact on diversity estimation. *Insect Conservation and Diversity*, *16*(1), 1–15. https://doi.org/10.1111/icad.12614

Flaminio, S., Ranalli, R., Zavatta, L., Galloni, M., & Bortolotti, L. (2021). Beewatching: A Project for Monitoring Bees through Photos. *Insects*, *12*(9), 841. https://doi.org/10.3390/insects12090841

Fourcade, Y., Åström, S., & Öckinger, E. (2019). Climate and land-cover change alter bumblebee species richness and community composition in subalpine areas. *Biodiversity and Conservation*, *28*(3), 639–653. https://doi.org/10.1007/s10531-018-1680-1

Fox, R., Harrower, C. A., Bell, J. R., Shortall, C. R., Middlebrook, I., & Wilson, R. J. (2019). Insect population trends and the IUCN Red List process. *Journal of Insect Conservation*, *23*(2), 269–278. https://doi.org/10.1007/s10841-018-0117-1

Gezon, Z. J., Wyman, E. S., Ascher, J. S., Inouye, D. W., & Irwin, R. E. (2015). The effect of repeated, lethal sampling on wild bee abundance and diversity. *Methods in Ecology and Evolution*, *6*(9), 1044–1054. https://doi.org/10.1111/2041-210X.12375

Geroff, R. K., Gibbs, J., & McCravy, K. W. (2014). Assessing bee (Hymenoptera: Apoidea) diversity of an Illinois restored tallgrass prairie: methodology and conservation considerations. *Journal of Insect Conservation*, *18*(5), 951–964. https://doi.org/10.1007/s10841-014-9703-z

Gibbs, J., Joshi, N. K., Wilson, J. K., Rothwell, N. L., Powers, K., Haas, M., Gut, L., Biddinger, D. J., & Isaacs, R. (2017). Does Passive Sampling Accurately Reflect the Bee (Apoidea: Anthophila) Communities Pollinating Apple and Sour Cherry Orchards? *Environmental Entomology*, *46*(3), 579–588. https://doi.org/10.1093/ee/nvx069

Google Maps. (2022). Retrieved July 20, 2022, from https://www.google.ca/maps

Gotelli, N. J., & Colwell, R. K. (2001). Quantifying biodiversity: Procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters, 4*(4), 379–391. https://doi.org/10.1046/j.1461-0248.2001.00230.x

Government of Canada. (2013). *National Ecological Framework for Canada, Ecozones, Ecoregions, and Ecodistricts*. Retrieved December 14, 2022, from https://sis.agr.gc.ca/cansis/publications/maps/eco/all/districts/index.html

Graves, S., Piepho, H. & Selzer, L., with help from Dorai-Raj, S. (2019). multcompView: Visualizations of Paired Comparisons. R package version 0.1-8. https://CRAN.R-project.org/package=multcompView

Grixti, J. C., Wong, L. T., Cameron, S. A., & Favret, C. (2009). Decline of bumble bees (Bombus) in the North American Midwest. *Biological Conservation*, *142*(1), 75–84. https://doi.org/10.1016/j.biocon.2008.09.027

Hagen, M., Wikelski, M., & Kissling, W. D. (2011). Space Use of Bumblebees (*Bombus spp*.) Revealed by Radio-Tracking. *PLOS ONE*, *6*(5), e19997. https://doi.org/10.1371/journal.pone.0019997

Hemberger, J., Crossley, M. S., & Gratton, C. (2021). Historical decrease in agricultural landscape diversity is associated with shifts in bumble bee species occurrence. *Ecology Letters*, *24*(9), 1800–1813. https://doi.org/10.1111/ele.13786

Hsieh, T. C., Ma, K. H., & Chao, A. (2016). iNEXT: An R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods in Ecology and Evolution*, *7*(12), 1451–1456. https://doi.org/10.1111/2041-210X.12613

Hsieh, T. C., Ma, K. H. & Chao, A. (2020). *A quick introduction to iNEXT via examples*. The Comprehensive R Archive Network. Retrieved February 21, 2022, from https://cran.r-project.org/web/packages/iNEXT/vignettes/Introduction.pdf

iNaturalist. (2022). *Bumble bees of Canada and Alaska monitoring program*. Retrieved October 17, 2022, from https://www.inaturalist.org/projects/bumble-bees-of-canada-and-alaska-monitoring-program

IUCN Standards and Petitions Committee. (2022). *Guidelines for Using the IUCN Red List Categories and Criteria*. Version 15.1. Prepared by the Standards and Petitions Committee. Retrieved August 24, 2022, from https://www.iucnredlist.org/resources/redlistguidelines

Jackson, H. M., Johnson, S. A., Morandin, L. A., Richardson, L. L., Guzman, L. M., & M'Gonigle, L. K. (2022). Climate change winners and losers among North American bumblebees. *Biology Letters*, *18*(6), 20210551. https://doi.org/10.1098/rsbl.2021.0551

Jacobson, M. M., Tucker, E. M., Mathiasson, M. E., & Rehan, S. M. (2018). Decline of bumble bees in northeastern North America, with special focus on Bombus terricola. *Biological Conservation*, *217*, 437–445. https://doi.org/10.1016/j.biocon.2017.11.026

Joshi, N. K., Leslie, T., Rajotte, E. G., Kammerer, M. A., Otieno, M., & Biddinger, D. J. (2015). Comparative Trapping Efficiency to Characterize Bee Abundance, Diversity, and Community Composition in Apple Orchards. *Annals of the Entomological Society of America*, *108*(5), 785–799. https://doi.org/10.1093/aesa/sav057

Kerr, J. T., Pindar, A., Galpern, P., Packer, L., Potts, S. G., Roberts, S. M., Rasmont, P., Schweiger, O., Colla, S. R., Richardson, L. L., Wagner, D. L., Gall, L. F., Sikes, D. S., & Pantoja, A. (2015). Climate change impacts on bumblebees converge across continents. *Science*, *349*(6244), 177–180. https://doi.org/10.1126/science.aaa7031

Kimoto, C., DeBano, S. J., Thorp, R. W., Rao, S., & Stephen, W. P. (2012). Investigating Temporal Patterns of a Native Bee Community in a Remnant North American Bunchgrass Prairie using Blue Vane Traps. *Journal of Insect Science*, *12*(108), 1–23. https://doi.org/10.1673/031.012.10801

Krahner, A., Schmidt, J., Maixner, M., Porten, M., & Schmitt, T. (2021). Evaluation of four different methods for assessing bee diversity as ecological indicators of agro-ecosystems. *Ecological Indicators*, *125*, 107573. https://doi.org/10.1016/j.ecolind.2021.107573

Kutby, R.A. (2013). *Bee communities in Restored Landfill Sites of Niagara Region*. [MSc thesis, Brock University]. Brock University Digital Repository.

Laverty, T. M., & Harder, L. D. (1988). THE BUMBLE BEES OF EASTERN CANADA. *The Canadian Entomologist*, *120*(11), 965–987. https://doi.org/10.4039/Ent120965-11

Lebuhn, G., Droege, S., Connor, E. F., Gemmill-Herren, B., Potts, S. G., Minckley, R. L., Griswold, T., Jean, R., Kula, E., Roubik, D. W., Cane, J., Wright, K. W., Frankie, G., & Parker, F. (2013). Detecting Insect Pollinator Declines on Regional and Global Scales. *Conservation Biology*, *27*(1), 113–120. https://doi.org/10.1111/j.1523-1739.2012.01962.x

Lebuhn, G., Droege, S., Connor, E. F., Gemmill-Herren, B., Potts, S. G., Minckley, R. L., Jean, R. P., Kula, E., Roubik, D. W., Wright, K. W., Frankie, G., & Parker, F. (2015). Evidence-based conservation: Reply to Tepedino et al. *Conservation Biology*, *29*(1), 283–285. https://doi.org/10.1111/cobi.12438

MacPhail, V. J., & Colla, S. R. (2020). Power of the people: A review of citizen science programs for conservation. *Biological Conservation*, *249*, 108739. https://doi.org/10.1016/j.biocon.2020.108739

MacPhail, V. J., Gibson, S. D., & Colla, S. R. (2020a). Community science participants gain environmental awareness and contribute high quality data but improvements are needed: Insights from Bumble Bee Watch. *PeerJ*, *8*, e9141. https://doi.org/10.7717/peerj.9141

MacPhail, V. J., Gibson, S. D., Hatfield, R., & Colla, S. R. (2020b). Using Bumble Bee Watch to investigate the accuracy and perception of bumble bee (*Bombus* spp.) identification by community scientists. *PeerJ*, *8*, e9412. https://doi.org/10.7717/peerj.9412

MacPhail, V. J., Richardson, L. L., & Colla, S. R. (2019). Incorporating citizen science, museum specimens, and field work into the assessment of extinction risk of the American Bumble bee (*Bombus pensylvanicus* De Geer 1773) in Canada. *Journal of Insect Conservation*, *23*(3), 597–611. https://doi.org/10.1007/s10841-019-00152-y

Magurran, A. E. (2004). *Measuring Biological Diversity*. Blackwell Science Ltd.

Marshall, L., Perdijk, F., Dendoncker, N., Kunin, W., Roberts, S., & Biesmeijer, J. C. (2020). Bumblebees moving up: Shifts in elevation ranges in the Pyrenees over 115 years. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1938), 20202201. https://doi.org/10.1098/rspb.2020.2201

McCravy, K. W. (2018). A Review of Sampling and Monitoring Methods for Beneficial Arthropods in Agroecosystems. *Insects*, *9*(4). https://doi.org/10.3390/insects9040170

Memorial University of Newfoundland. (2021). *Pye Centre for Northern Boreal Food Systems.* Retrieved June 20, 2022, from https://www.mun.ca/labradorcampus/pye-centre/

Michener, C. D. (2007). *The Bees of the World* (2nd ed.). The Johns Hopkins University Press.

Miklasevskaja, M. Musafija, J. & Packer, L. (n.d.). *Bees of Canada.* Packer Lab - York University. Retrieved January 15, 2022, from https://www.yorku.ca/bugsrus/resources/galleries/boc#Bombus

Milam, J., Johnson, D. E., Andersen, J. C., Fassler, A. B., Narango, D. L., & Elkinton, J. S. (2020). Validating Morphometrics with DNA Barcoding to Reliably Separate Three Cryptic Species of Bombus Cresson (Hymenoptera: Apidae). *Insects*, *11*(10), Article 10. https://doi.org/10.3390/insects11100669

Molles, M.C. & Cahill, J. F. (2008) *Ecology: concepts & applications*. (Canadian ed.) McGraw-Hill Ryerson Limited.

Montero-Castaño, A., Koch, J. B. U., Lindsay, T.-T. T., Love, B., Mola, J. M., Newman, K., & Sharkey, J. K. (2022). Pursuing best practices for minimizing wild bee captures to support biological research. *Conservation Science and Practice*, *4*(7), e12734. https://doi.org/10.1111/csp2.12734

Muma, W. (n.d.) *Ontario wildflowers*. Retrieved January 15, 2022, from https://www.ontariowildflowers.com/

Mundy-Heisz, K.A. (2021). *Pressure on pollinators: an examination of bumblebees (Bombus spp.) and systemic insecticides, parasites, and agricultural habitats*. [PhD thesis, The University of Guelph]. The Atrium.

National Native Bee Monitoring RCN. (2023). *Coming together to conserve our native bees*. Retrieved April 8, 2023, from https://www.nativebeemonitoring.org

Nature Conservancy of Canada. (2022a). *Big Valley*. Retrieved July 20, 2022, from https://www.natureconservancy.ca/en/where-we-work/saskatchewan/featured-projects/big-valley.html

Nature Conservancy of Canada. (2022b). *Buffalo Pound*. Retrieved July 20, 2022, from https://www.natureconservancy.ca/en/where-we-work/saskatchewan/featured-projects/buffalo-pound.html

Nielsen, A., Steffan-Dewenter, I., Westphal, C., Messinger, O., Potts, S. G., Roberts, S. P. M., Settele, J., Szentgyörgyi, H., Vaissière, B. E., Vaitis, M., Woyciechowski, M., Bazos, I., Biesmeijer, J. C., Bommarco, R., Kunin, W. E., Tscheulin, T., Lamborn, E., & Petanidou, T. (2011). Assessing bee species richness in two Mediterranean communities: Importance of habitat type and sampling techniques. *Ecological Research*, *26*(5), 969–983. https://doi.org/10.1007/s11284-011-0852-1

Newcomb, L. (1977). *Newcomb's wildflower guide*. Little, Brown and Company (Inc.)

Novotny, J. L., Reeher, P., Varvaro, M., Lybbert, A., Smith, J., Mitchell, R. J., & Goodell, K. (2021). Bumble bee species distributions and habitat associations in the Midwestern USA, a region of declining diversity. *Biodiversity and Conservation*, *30*(3), 865–887. https://doi.org/10.1007/s10531-021-02121-x

Oksanen, J. F., Blanchet, G, Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E. & Wagner, H. (2020). vegan: Community Ecology Package. R package version 2.5-7. https://CRAN.R-project.org/package=vegan

Oksanen, J. (2022). *Vegan: ecological diversity*. The Comprehensive R Archive Network. Retrieved April 25, 2022, from https://cran.r-project.org/web/packages/vegan/vignettes/diversity-vegan.pdf

Ontario Biodiversity Council. (2021). *State of Ontario's Biodiversity.* Retrieved July 20, 2022, from https://sobr.ca/ontarios-ecozones/

Onuferko, T.M. (2013). *Restoration and succession of a bee community in southern St. Catharines, Ontario, within a ten-year study period*. [MSc thesis, Brock University]. Brock University Digital Repository.

Onuferko, T. M., Kutby, R., & Richards, M. H. (2015). A list of bee species (Hymenoptera: Apoidea) recorded from three municipalities in the Niagara region of Ontario, including a new record of *Lasioglossum furunculum* Gibbs (Halictidae) in Canada. *The Journal of the Entomological Society of Ontario, 146*, 3-22. https://journal.lib.uoguelph.ca/index.php/eso/article/view/3692

Onuferko, T. M., Skandalis, D. A., Cordero, R. L., & Richards, M. H. (2018). Rapid initial recovery and long-term persistence of a bee community in a former landfill. *Insect Conservation and Diversity*, *11*(1), 88–99. https://doi.org/10.1111/icad.12261

Packer, L., & Darla-West, G. (2021). Bees: How and Why to Sample Them. In J. C. Santos & G. W. Fernandes (Eds.), *Measuring Arthropod Biodiversity: A Handbook of Sampling*

Methods (pp. 55–83). Springer International Publishing. https://doi.org/10.1007/978-3-030-53226-0_3

Pei, C. K., Hovick, T. J., Duquette, C. A., Limb, R. F., Harmon, J. P., & Geaumont, B. A. (2022). Two common bee-sampling methods reflect different assemblages of the bee (Hymenoptera: Apoidea) community in mixed-grass prairie systems and are dependent on surrounding floral resource availability. *Journal of Insect Conservation*, *26*(1), 69–83. https://doi.org/10.1007/s10841-021-00362-3

Peterson, R.T. & McKenny, M. (1996). *A field guide to wildflowers of Northeastern and North-central North America*. Houghton Mifflin Company. (Original work published 1968)

Portman, Z. M., Bruninga-Socolar, B., & Cariveau, D. P. (2020). The State of Bee Monitoring in the United States: A Call to Refocus Away from Bowl Traps and Towards More Effective Methods. *Annals of the Entomological Society of America*, *113*(5), 337–342. https://doi.org/10.1093/aesa/saaa010

Prendergast, K. S., & Hogendoorn, K. (2021). FORUM: Methodological shortcomings and lack of taxonomic effort beleaguer Australian bee studies. *Austral Ecology*, *46*(5), 880–884. https://doi.org/10.1111/aec.12998

Prendergast, K. S., Menz, M. H. M., Dixon, K. W., & Bateman, P. W. (2020). The relative performance of sampling methods for native bees: An empirical test and review of the literature. *Ecosphere*, *11*(5), e03076. https://doi.org/10.1002/ecs2.3076

Pye Centre for Northern Boreal Food Systems. (2022). Retrieved July 20, 2022, from https://pye-centre.ca/

Rao, S., & Stephen, W. P. (2010). Abundance and Diversity of Native Bumble Bees Associated with Agricultural Crops: The Willamette Valley Experience. *Psyche*, *2010*, e354072. https://doi.org/10.1155/2010/354072

Rao, S., & Strange, J. P. (2012). Bumble Bee (Hymenoptera: Apidae) Foraging Distance and Colony Density Associated With a Late-Season Mass Flowering Crop. *Environmental Entomology*, *41*(4), 905–915. https://doi.org/10.1603/EN11316

R Core Team. (2022). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.r-project.org/

Rhoades, P., Griswold, T., Waits, L., Bosque-Pérez, N. A., Kennedy, C. M., & Eigenbrode, S. D. (2017). Sampling technique affects detection of habitat factors influencing wild bee communities. *Journal of Insect Conservation*, *21*(4), 703–714. https://doi.org/10.1007/s10841-017-0013-0

Richards, M. H., Rutgers-Kelly, A., Gibbs, J., Vickruck, J. L., Rehan, S. M., & Sheffield, C. S. (2011). Bee Diversity in Naturalizing Patches of Carolinian Grasslands in Southern Ontario, Canada. *The Canadian Entomologist*, *143*(3), 279–299. https://doi.org/10.4039/n11-010

Rutgers-Kelly, A. C., & Richards, M. H. (2013). Effect of meadow regeneration on bee (Hymenoptera: Apoidea) abundance and diversity in southern Ontario, Canada. *The Canadian Entomologist*, *145*(6), 655–667. https://doi.org/10.4039/tce.2013.42

Sheffield, C. S., Kevan, P. G., Westby, S. M., & Smith, R. F. (2008). Diversity of cavity-nesting bees (Hymenoptera: Apoidea) within apple orchards and wild habitats in the Annapolis Valley, Nova Scotia, Canada. *The Canadian Entomologist*, *140*(2), 235–249. https://doi.org/10.4039/n07-058

Sheffield, C. S., Oram, R., & Heron, J. M. (2020). *Bombus (Pyrobombus) johanseni* Sladen, 1919, a valid North American bumble bee species, with a new synonymy and comparisons to other “red-banded” bumble bee species in North America (Hymenoptera, Apidae, Bombini). *ZooKeys*, 984, 59–81. https://doi.org/10.3897/zookeys.984.55816

Sheffield, C. S., Richardson, L., Cannings, S., Ngo, H., Heron, J., & Williams, P. H. (2016). Biogeography and designatable units of *Bombus occidentalis* Greene and *B. terricola* Kirby (Hymenoptera: Apidae) with implications for conservation status assessments. *Journal of Insect Conservation*, *20*(2), 189–199. https://doi.org/10.1007/s10841-016-9853-2

Simon, A. D. F., Best, L. R., & Starzomski, B. M. (in press). Evidence of bumble bee extirpation and colonization: Galiano Island, British Columbia, Canada [Manuscript accepted for publication 16 September 2022]. *Northwest Science*, available at https://groups.google.com/g/beemonitoring/c/bxhHZIibRRo/m/4Kqud64fBAAJ

Smith, R.L., & Smith, T.M. (2001). *Ecology & field biology* (6th ed.). Benjamin Cummings.

Soroye, P., Newbold, T., & Kerr, J. (2020). Climate change contributes to widespread declines among bumble bees across continents. *Science*, *367*(6478), 685–688. https://doi.org/10.1126/science.aax8591

Spiesman, B. J., Gratton, C., Hatfield, R. G., Hsu, W. H., Jepsen, S., McCornack, B., Patel, K., & Wang, G. (2021). Assessing the potential for deep learning and computer vision to identify bumble bee species from images. *Scientific Reports*, *11*(1), 7580. https://doi.org/10.1038/s41598-021-87210-1

Stafford, R., Hart, A. G., Collins, L., Kirkhope, C. L., Williams, R. L., Rees, S. G., Lloyd, J. R., & Goodenough, A. E. (2010). Eu-Social Science: The Role of Internet Social Networks in the Collection of Bee Biodiversity Data. *PLOS ONE*, *5*(12), e14381. https://doi.org/10.1371/journal.pone.0014381

Stephen, W. P., & Rao, S. (2005). Unscented Color Traps for Non-Apis Bees (Hymenoptera: Apiformes). *Journal of the Kansas Entomological Society*, *78*(4), 373–380.

Stephen, W. P., & Rao, S. (2007). Sampling Native Bees in Proximity to a Highly Competitive Food Resource (Hymenoptera: Apiformes). *Journal of the Kansas Entomological Society*, *80*(4), 369–376.

Strange, J. P., & Tripodi, A. D. (2019). Characterizing bumble bee (Bombus) communities in the United States and assessing a conservation monitoring method. *Ecology and Evolution*, *9*(3), 1061–1069. https://doi.org/10.1002/ece3.4783

Suzuki-Ohno, Y., Yokoyama, J., Nakashizuka, T., & Kawata, M. (2021). Bee occurrence data collected in citizen science program "Hanamaru-Maruhana national census" in Japan. *Ecological Research*, *36*(6), 1028–1036. https://doi.org/10.1111/1440-1703.12261

Suzuki-Ohno, Y., Westfechtel, T., Yokoyama, J., Ohno, K., Nakashizuka, T., Kawata, M., & Okatani, T. (2022). Deep learning increases the availability of organism photographs taken by citizens in citizen science programs. *Scientific Reports*, *12*(1), 1210. https://doi.org/10.1038/s41598-022-05163-5

Tepedino, V. J., Durham, S., Cameron, S. A., & Goodell, K. (2015). Documenting bee decline or squandering scarce resources. *Conservation Biology*, *29*(1), 280–282. https://doi.org/10.1111/cobi.12439

Thomson, J. D., & Zung, J. L. (2015). A restraining device to aid identification of bees by digital photography. *Journal of Pollination Ecology*, *17*, 145–147. https://doi.org/10.26786/1920-7603(2015)20

Tourism Saskatchewan Canada. (n.d.). *Wascana Valley Natural Area Recreation Site*. Retrieved July 20, 2022, from https://www.tourismsaskatchewan.com/listings/1003/wascana-valley-nature-recreation-site

Tronstad, L., Bell, C., & Crawford, M. (2022). Choosing collection methods and sample sizes for monitoring bees. *Agricultural and Forest Entomology*, *24*(4), 531–539. https://doi.org/10.1111/afe.12518

Turley, N. E., Biddinger, D. J., Joshi, N. K., & López-Uribe, M. M. (2022). Six years of wild bee monitoring shows changes in biodiversity within and across years and declines in abundance. *Ecology and Evolution*, *12*(8), e9190. https://doi.org/10.1002/ece3.9190

Ueda, K. [kueda]. (2019, October 25). *Identification Quality On iNaturalist*. [Online forum post]. iNaturalist Community Forum. Retrieved December 14, 2022, from https://forum.inaturalist.org/t/identification-quality-on-inaturalist/7507

Venning, F.D. & Saito, M.C. (1984). *A guide to field identification wildflowers of North America*. Western Publishing Company, Inc.

Walther-Hellwig, K., & Frankl, R. (2000a). Foraging habitats and foraging distances of bumblebees, Bombus spp. (Hym., Apidae), in an agricultural landscape. *Journal of Applied Entomology*, *124*(7–8), 299–306. https://doi.org/10.1046/j.1439-0418.2000.00484.x

Walther-Hellwig, K., & Frankl, R. (2000b). Foraging Distances of Bombus muscorum, Bombus lapidarius, and Bombus terrestris (Hymenoptera, Apidae). *Journal of Insect Behavior*, *13*(2), 239–246. https://doi.org/10.1023/A:1007740315207

Welti, E. A. R., Joern, A., Ellison, A. M., Lightfoot, D. C., Record, S., Rodenhouse, N., Stanley, E. H., & Kaspari, M. (2021). Studies of insect temporal trends must account for the complex sampling histories inherent to many long-term monitoring efforts. *Nature Ecology & Evolution*, *5*(5), 589-591. https://doi.org/10.1038/s41559-021-01424-0

Westphal, C., Bommarco, R., Carré, G., Lamborn, E., Morison, N., Petanidou, T., Potts, S. G., Roberts, S. P. M., Szentgyörgyi, H., Tscheulin, T., Vaissière, B. E., Woyciechowski, M., Biesmeijer, J. C., Kunin, W. E., Settele, J., & Steffan-Dewenter, I. (2008). Measuring Bee Diversity in Different European Habitats and Biogeographical Regions. *Ecological Monographs*, *78*(4), 653–671. https://doi.org/10.1890/07-1292.1

Whitlock, M. C., & Schluter, D. (2020). *The analysis of biological data*. (3rd ed.) W. H. Freeman and Company.

Williams, P. H. (2021). Not just cryptic, but a barcode bush: PTP re-analysis of global data for the bumblebee subgenus Bombus s. str. supports additional species (Apidae, genus Bombus). *Journal of Natural History*, *55*(5/6), 271–282. https://doi.org/10.1080/00222933.2021.1900444

Williams, P. H., Byvaltsev, A. M., Cederberg, B., Berezin, M. V., Ødegaard, F., Rasmussen, C., Richardson, L. L., Huang, J., Sheffield, C. S., & Williams, S. T. (2015). Genes Suggest Ancestral Colour Polymorphisms Are Shared across Morphologically Cryptic Species in Arctic Bumblebees. *PLOS ONE*, *10*(12), e0144544. https://doi.org/10.1371/journal.pone.0144544

Williams, P., Thorp, R., Richardson, L. & Colla, S. (2014). *An identification guide bumble bees of North America*. Princeton University Press.

Wilson, J. S., Griswold, T., & Messinger, O. J. (2008). Sampling Bee Communities (Hymenoptera: Apiformes) in a Desert Landscape: Are Pan Traps Sufficient? *Journal of the Kansas Entomological Society*, *81*(3), 288–300. https://doi.org/10.2317/JKES-802.06.1

Wolf, S., & Moritz, R. F. A. (2008). Foraging distance in *Bombus terrestris* L. (Hymenoptera: Apidae). *Apidologie*, *39*(4), 419–427. https://doi.org/10.1051/apido:2008020

Woodard, S. H., Federman, S., James, R. R., Danforth, B. N., Griswold, T. L., Inouye, D., McFrederick, Q. S., Morandin, L., Paul, D. L., Sellers, E., Strange, J. P., Vaughan, M., Williams, N. M., Branstetter, M. G., Burns, C. T., Cane, J., Cariveau, A. B., Cariveau, D. P., Childers, A., … Wehling, W. (2020). Towards a U.S. national program for monitoring native bees. *Biological Conservation*, *252*, 108821. https://doi.org/10.1016/j.biocon.2020.108821

Zattara, E. E., & Aizen, M. A. (2021). Worldwide occurrence records suggest a global decline in bee species richness. *One Earth*, *4*(1), 114–123. https://doi.org/10.1016/j.oneear.2020.12.005

Zayed, A., & Packer, L. (2005). Complementary sex determination substantially increases extinction proneness of haplodiploid populations. *Proceedings of the National Academy of Sciences*, *102*(30), 10742–10746. https://doi.org/10.1073/pnas.0502271102

Zayed, A., Roubik, D. W., & Packer, L. (2004). Use of Diploid Male Frequency Data as an Indicator of Pollinator Decline. *Proceedings: Biological Sciences*, *271*(Supplement 3), S9–S12.

Zichmanis, Z. & Hodgins, J. (1982). *Flowers of the wild Ontario and Great Lakes Region.* Toronto Oxford University Press.

APPENDIX

Site descriptions

Carolinian Zone

Elm Street Naturalization Site (Elm St.) is in northern Port Colborne (Supplementary Figure S1.1). It is a former landfill, now naturalization site, open to the public by 2011 (Kutby, 2013). The naturalization site contained wildflowers and grasses as well as walking trails, small ponds, and an off-leash dog park. Surveys took place in the most northern wildflower area to be as far away as possible from the bee pan trapping that was occurring in the south. The landscape surrounding the naturalization site included conservation areas, farmland, a rail line, industrial properties, and subdivisions.

Station Road Naturalization Site (Station Rd.) is in southern Wainfleet (Supplementary Figure S1.2). It is another former landfill that was converted into a naturalization site open to the public by 2011 (Kutby, 2013). The southern half of the naturalization site contains a wildflower field that was being used for pan trapping, and so bumble bee surveys were conducted in the most northern section near ponds and a walking trail. The landscape surrounding Station Rd. is a mixture of agricultural and wooded areas.

PhysEd Hydro (PEH) is in the northeast area of Brock University Campus, in St. Catharines (Supplementary Figure S1.3). The areas surveyed were the unmown sections of a field of wildflowers and grasses, and the connecting hydro line corridor. A large central area of the field and walking paths were consistently maintained as lawn, while a section of natural vegetation in the southwest was removed in June but began to regrow throughout the summer.

The wildflower field became heavily dominated by *Centaurea* sp. (knapweed) in mid-summer. Hydro lines with a mown walking path were bordered on either side by natural vegetation and forest. The surrounding areas contain multiple athletic fields, university buildings, a large, forested area that is part of the Bruce Trail and a nearby lake.

The Glenridge Quarry Naturalization Site (GQNS) is in southern St. Catharines (Supplementary Figure S1.4). This area was a former stone quarry and then a landfill, until its conversion to a naturalization site between 2001 and 2003 (Richards *et al*. 2011). GQNS contains large open areas with wildflowers, grasses, and a few trees, as well as a large hill, a pond, and wooded areas. The site surveyed for bumble bees in this study is in the southwest, close to Sir Isaac Brock Way, and was designated as Quarry Southwest (QSW). This area was chosen because of the high density of wildflowers present in late July when this site was added to the study (bee behavioural studies were being conducted at GQNS by other researchers in June). Since this site was heavily used by the public, BVTs were not placed here and only netting and photo surveys were conducted. The surrounding area contains natural areas, subdivisions, highways and is near Brock University.

Konkle Road (Konkle) is a private agricultural property, located southwest of Beamsville (Supplementary Figure S1.5). The areas surveyed included the wildflowers growing between and around two collapsed buildings in the north, the edge of the nearby hayfield, another area of wildflowers located further south near some buildings, and a clover field which also contained other wildflowers as well. Surrounding these areas was lawn, a vegetable garden, large hayfields, and vineyards though these were not surveyed. The northern hayfield was harvested in August 2021, and the clover field was mowed once between 19 July 2021 and 3 August 2021 but began to regrow afterwards. The surrounding properties are agricultural.

Victoria Avenue (Victoria) is a private property located south of Vineland (Supplementary Figure S1.6). The main area surveyed was the wildflower field in the western half of the property. To the east there was an additional grassy, wildflower section that was surveyed starting around August when a reduction in blooming wildflowers in the western field provided time for the eastern area to be surveyed. The two areas were separated by a section of trees, lawn, and a house. This eastern field was mowed once between 17 September 2021 and 20 September 2021. The surrounding areas include vineyards, a rock quarry, agricultural properties, and a conservation area.

Farr Road (Farr) is a private property located in eastern Welland (Supplementary Figure S1.7). The vegetation at this site was a mixture of mowed lawn, planted flower and vegetable gardens, and natural vegetation such as trees, thick shrubs, and a wildflower meadow. Surveys were conducted on flower and vegetable gardens, on natural vegetation growing on the property, and in the wildflower meadow located near a pond and behind a donkey enclosure. Access to the wildflower meadow was improved on 7 September 2021 when vegetation was cleared to create walking paths in this area. The surrounding areas included a mixture of suburbs, agriculture, and woodland, with the city located to the west on the other side of Welland Canal and the 406 highway.

Townline Road (Townline) is a private agricultural property located in northern Fort Erie (Supplementary Figure S1.8). This site contained a large hayfield and two smaller fallow fields to the north. At the southern edge of the hayfield was an area of water drainage with natural vegetation, including wildflowers, as was a section at the northern edge of the hayfield, separating the two fallow fields. The fields were enclosed by trees on three sides. Of the two fallow fields only the northeastern one was surveyed because it contained more wildflowers and fewer dense shrubs than the northwestern field. Fields and drainage areas were surveyed, but the route was adjusted once the hayfield was harvested on 4 August 2021. The surrounding area contains large sections of woodland and farmland, with a creek, some suburban properties, the Queen Elizabeth Way highway, and Niagara River.

Prairies Ecozone

Buffalo Pound is located at the northwest edge of the Municipality of Dufferin (Supplementary Figure S1.9). It is a large natural area that consists of grasslands and gentle hills along the north side of Buffalo Pound Lake (Nature Conservancy of Canada, 2022b). The area surrounding Buffalo Pound is agricultural with a scattering of small lakes.

Big Valley is located north of Lumsden (Supplementary Figure S1.9). It is another Nature Conservancy of Canada (NCC) property (Nature Conservancy of Canada, 2022a). The area surveyed was a recently restored section where the dominant vegetation was purple prairie clover and goldenrod. The surrounding properties are natural areas, agriculture, and to the north is Last Mountain Lake.

Wascana Trail is located south of Lumsden Town, Saskatchewan (Supplementary Figure S1.9). It is a hilled area with grass and wooded sections, all part of a public trail system that runs along Wascana Creek (Tourism Saskatchewan Canada, n.d.). Areas with wildflowers and the sandy shoreline of this creek were surveyed. The area surrounding Wascana Trail is agricultural.

Boreal Shield Ecozone

Pye Centre (PC) site is in Happy Valley-Goose Bay in central Labrador (Supplementary Figure S1.10). PC is an agricultural research centre (Memorial University of Newfoundland, 2021; Pye Centre for Northern Boreal Food Systems, 2022). The area surveyed was a clover field. This field was enclosed by trees, the surrounding area is mainly undeveloped, and located close to Churchill River.

Pasadena (PAS) site is in northwest Pasadena, in western Newfoundland (Supplementary Figure S1.11). It is a provincially owned agricultural research site. Some of the crops being grown were apples, blueberries, and mixed vegetables. The site directly bordered the Trans-Canada Highway, is located near Deer Lake, and is separated from subdivisions and industrial properties by trees.

Torbay Gravel Pit (TGP) is in southwest Torbay in eastern Newfoundland (Supplementary Figure S1.12). It is a former gravel pit that has been out of use long enough for natural vegetation to reestablish in most of the area and leave only a few patches of rocky soil visible. The surrounding landscape is a mixture of subdivisions and forest, and to the northwest is Whiteway Pond.

Memorial University of Newfoundland Botanical Garden (MBG) is in western St. John’s, in eastern Newfoundland (Supplementary Figure S1.13). The site surveyed was across the road from the main garden centre and was an area used by staff for composting, storage, and parking. The vegetation consisted of semi-cleared areas for compost piles, etc. surrounded by natural grasses, wildflowers, shrubs, and forest. Although not far from the city, the surrounding area of this site is relatively undeveloped and located near Oxen Pond.

Agriculture and Agri-Food Canada Cranberry Bog (ACB) is in southwestern St. John’s, in eastern Newfoundland (Supplementary Figure S1.14). Surveys were conducted in the section being used for cranberry research. Blueberries were also being grown in nearby fields, though no bumble bee surveys were conducted there. The cranberry plots are surrounded by naturally established wildflowers and grasses with forest beyond. The surrounding area is agricultural, with a river and small lake to the north, and subdivisions to the north and west.

Floral associations

In the Carolinian Zone, floral associations (sometimes more general plant associations) were initially identified using the phone application LeafSnap Plant Identification (Appixi, 2022). LeafSnap often suggested multiple possible identifications, so additional sources such as Peterson's and Newcomb's wildflower guides (Peterson & McKenny, 1968/1996; Newcomb, 1977), and the website Ontario Wildflowers (Muma, n.d., https://www.ontariowildflowers.com/) were heavily relied upon. Other sources include Zichmanis & Hodgins (1982), Venning & Saito (1984), Barker (2013), and iNaturalist (iNaturalist.org). Identifications were made to the species level if possible.

In the Boreal Shield Ecozone, each individual surveyor identified the flowers (or sometimes plants in general) from which they had collected bumble bees during the netting surveys; the identification of *Melilotus officinalis* (sweet yellow clover) was done by Dr. Erica Oberndorfer (Agriculture and Agri-food Canada). Dr. Parsons completed the floral identifications for photo records posted on iNaturalist.

SUPPLEMNTARY TABLES AND FIGURES

Supplementary Table S1.1: Studies measuring changes in bee populations over multiple years typically consider four broad categories of population or community measurement: abundance, distribution, community composition, and diversity. Occasionally other measures such as phylogenetic structure, temperature index, and sex ratio are used but these are rarer (Kimoto *et al*. 2012; Fourcade *et al.* 2019; Zattara & Aizen, 2021; Turley *et al.* 2022).

	Population measurement			
Study	**Abundance** *Including relative abundance*	**Distribution** *Including range, elevation, occupancy, occurrence, persistence, and latitude*	**Community composition** *Including species and taxonomic composition*	**Diversity** *Including biodiversity, species richness, evenness and diversity indices*
Biesmeijer *et al*. 2006		✓		✓
Colla & Packer, 2008	✓			✓
Grixti *et al.* 2009	✓	✓		✓
COSEWIC, 2010	✓	✓		
Cameron *et al.* 2011	✓	✓		
Colla *et al.* 2012	✓	✓		
Kimoto *et al*. 2012	✓		✓	✓
Bartomeus *et al*. 2013	✓	✓	✓	✓
COSEWIC, 2014a	✓	✓		
COSEWIC, 2014b	✓	✓		
COSEWIC, 2015	✓	✓		
Kerr *et al.* 2015		✓		
COSEWIC, 2018	✓	✓		
Fourcade *et al.* 2019		✓		✓
Jacobson *et al.* 2018	✓	✓		✓
Onuferko *et al.* 2018	✓			✓
COSEWIC, 2019	✓	✓		
MacPhail *et al*. 2019	✓	✓		
Marshall *et al*. 2020	✓	✓	✓	✓
Soroye *et al*. 2020		✓		✓
Novotny *et al*. 2021	✓	✓	✓	✓
Zattara & Aizen, 2021				✓
Jackson *et al.* 2022		✓		
Turley *et al*. 2022	✓		✓	✓
Simon *et al*. in press	✓		✓	✓

Supplementary Table S1.2: Numbers and locations of BVTs placed at each Carolinian Zone site. Two or three BVTs were placed at each site.

Site	BVT #	Latitude	Longitude	Distance (m) between BVTs
Farr	1	42.996293	-79.206900	87, 102 and 114
	2	42.996493	-79.205678	
	3	42.995608	-79.206387	
Victoria	4	43.137707	-79.390765	51
	5	43.137735	-79.391388	
PEH	6	43.118696	-79.255202	48, 121 and 165
	7	43.117921	-79.256941	
	8	43.118599	-79.255773	
Station Rd.	9*	42.884291	-79.378382 or - 79.378351	113 or 110
	10	42.884162	-79.377006	
Elm St.	11	42.927926	-79.258094	116
	12	42.927812	-79.259508	
Konkle	13	43.144341	-79.502772	113
	14**	43.145347	-79.502509	
Townline	19***	42.964334	-79.014831	103, 109 and 207
	20	42.965315	-79.014940	
	21	42.963461	-79.015243	

*BVT #9 was moved at the end of the day on 18 July 2021 to the nearest dry area because it was in a location prone to flooding

**BVT #14 was also moved on 5 August 2021, but only by about half a meter

***BVTs #15-18 were tested at sites in early June that did not end up being used in the sampling period

Supplementary Table S1.3: BVT platform installation dates and heights BVTs were set at, measured in centimeters from ground to base of the collection jar. BVTs were raised throughout the season as the vegetation grew.

Site	BVT #	Initial height (cm)	Height adjustments
Farr	1	59	
	2	51	
	3	61	
Victoria	4	41	13 July 2021 - raised to 58 cm; 24 August 2021 - raised to 69 cm
	5	41	13 July 2021 - raised to 59 cm; 24 August 2021 - raised to 69 cm
PEH	6	71	20 July 2021 - raised to 88 cm
	7	59	20 July 2021 - raised to 65 cm; 13 August 2021 - raised to 84 cm
	8	61	20 July 2021 - raised to 85 cm
Station Rd.	9	66	18 July 2021 – BVT was moved; platform height not adjusted but may have been slightly different depending on how far into the ground the post went
	10	61	
Elm St.	11	48	04 August 2021 - raised to 60 cm
	12	51	04 August 2021 - raised to 71 cm
Konkle	13	48	13 July 2021 - raised to 64 cm; 24 August 2021 - raised to 69 cm
	14	36	13 July 2021 - raised to 66 cm; 05 August 2021- moved trap, height now 64 cm
Townline	19	64	
	20	71	
	21	Not recorded, but would have been similar to BVTs #19 and #20	

Supplementary Table S1.4: Flower associations for bumble bee species collected by netting and photo methods in the Carolinian Zone.

Species	Total count	Floral associations
B. impatiens	1468	*Arctium* sp.; *Centaurea* sp; *Cichorium intybus*; *Cirsium* sp.; *Daucus carota*; *Dipsacus fullonum*; *Echinacea purpurea*; *Eupatorium* sp.; *Euthamia graminifolia*; Grass; Hawksbeard or Hawkweed; *Helianthus annuus*; *Helianthus maximilianii*; *Heliopsis helianthoides*; *Hosta* sp.; *Hypericum perforatum*; *Impatiens capensis*; *Leonurus cardiaca*; *Linaria vulgaris*; *Lotus corniculatus*; *Lythrum salicaria*; *Prunella vulgaris*; *Rhamnus cathartica*; *Rosa palustris*; *Rudbeckia laciniata*; *Securigera varia*; *Solanum dulcamara*; *Solidago* sp.; *Sonchus arvensis*; *Sonchus oleraceus*; *Spiraea* sp.; *Symphyotrichum novae-angliae*; *Symphyotrichum* sp.; *Tanacetum vulgare*; *Trifolium pratense*; *Vicia cracca*
B. griseocollis	235	*Arctium* sp.; *Asclepias syriaca; Centaurea* sp; *Cichorium intybus*; *Cirsium* sp.; *Dipsacus fullonum*; *Echinacea purpurea*; *Hypericum perforatum*; *Lotus corniculatus*; *Lythrum salicaria*; *Monarda fistulosa*; *Rosa palustris*; *Securigera varia*; *Solanum dulcamara*; *Solidago* sp.; *Spiraea* sp.; *Vicia cracca*
B. bimaculatus	183	*Centaurea* sp; *Cichorium intybus*; *Cirsium* sp.; *Cornus* sp.; *Dipsacus fullonum*; *Hypericum perforatum*; *Leonurus cardiaca*; *Lythrum salicaria*; *Monarda didyma*; *Rosa palustris*; *Securigera varia*; *Stachys* sp.; *Trifolium repens*; *Trifolium* sp.; *Vicia cracca*
B. rufocinctus	154	*Arctium* sp.; Betulaceae; *Centaurea* sp; *Cichorium intybus*; *Cirsium* sp.; *Daucus carota*; *Dipsacus fullonum*; Hawksbeard or Hawkweed; *Lotus corniculatus*; *Lythrum salicaria*; *Prunella vulgaris*; *Rhamnus cathartica*; *Securigera varia*; *Solidago* sp.; *Sonchus arvensis*; *Sonchus oleraceus*; *Symphyotrichum* sp.; *Tanacetum vulgare*; *Vicia cracca*
B. fervidus	39	*Campanula* sp.; *Cirsium* sp.; *Dipsacus fullonum*; *Vicia cracca*
B. vagans	26	*Centaurea* sp; *Dipsacus fullonum*; *Euthamia graminifolia*; *Lotus corniculatus*; *Securigera varia*; *Solidago* sp.; *Symphyotrichum novae-angliae*; *Vicia cracca*
B. citrinus	13	*Centaurea* sp; *Dipsacus fullonum*; Hawksbeard or Hawkweed; *Monarda fistulosa*; *Symphyotrichum novae-angliae*
B. perplexus	8	*Securigera varia*; *Vicia cracca*
B. borealis	3	*Vicia cracca*

Supplementary Table S1.5: Bumble bees were detected on many of the same flowers across multiple sites in the Boreal Shield Ecozone.

Scientific or common name	**Site**
Alchemilla sp.	MBG
Anaphalis margaritacea	PAS
Aster sp.	PC, ACB
Centaurea nigra	TGP, MBG, ACB
Chamaenerion angustifolium	TGP, MBG, ACB
Convolvulus arvensis	MBG
Digitalis purpurea	MBG
Grass	MBG
Hawkweed	TGP
Hieracium sp. or *Pilosella* sp.	PC, TGP, MBG
Hypericum perforatum	PAS, TGP, ACB
Hypericum sp.	TGP, MBG
Linaria vulgaris	MBG, ACB
Lupinus polyphyllus	TGP
Melilotus albus	PC
Melilotus officinalis	PC
Rubus idaeus	TGP, MBG
Rubus sp.	MBG
Securigera varia	ACB
Solidago sp.	PAS, TGP, MBG, ACB
Stachys sp.	MBG
Symphytum officinale	MBG
Symphytum sp.	MBG
Taraxacum officinale	PC
Trifolium hybridum	PC
Trifolium pratense	PC, PAS, TGP, MBG, ACB
Trifolium repens	PC, PAS, TGP, MBG, ACB
Vicia sp.	PAS, TGP, MBG, ACB

Supplementary Table S1.6: Species contribution to the chi-squared value for the Carolinian Zone BVT, netting, and photo comparison (X^2 = 216.16, df = 12, $p < 0.001$). BVTs had proportionally fewer *B. impatiens* and more *B. fervidus* than expected. The grouped category contains *B. citrinus, B. perplexus, B. borealis, B. auricomus, B. pensylvanicus,* and *B. terricola*. The largest contributions to the chi-squared value are indicated in bold.

***Bombus* species**	**BVT**	**Net**	**Photo**	**BVT** $\frac{(\boldsymbol{obs.-exp.})^2}{\boldsymbol{exp.}}$	**Net** $\frac{(\boldsymbol{obs.-exp.})^2}{\boldsymbol{exp.}}$	**Photo** $\frac{(\boldsymbol{obs.-exp.})^2}{\boldsymbol{exp.}}$
B. impatiens	20	685	763	**32.2**	0.3	1.5
B. griseocollis	20	94	121	8.4	1.7	0.1
B. bimaculatus	18	76	89	11.6	0.7	0.0
B. rufocinctus	17	81	56	14.6	1.6	5.5
B. fervidus	15	11	13	**99.9**	2.6	2.1
B. vagans	0	21	5	1.2	7.0	4.9
Grouped	6	8	13	18.8	1.5	0.0

Supplementary Table S1.7: Species contribution to the chi-squared value for the Carolinian Zone photo and research grade iNaturalist record comparison (X^2 = 16.05, df = 4, $p = 0.003$). Most of the X^2 value came from the grouped category. The grouped category contains *B. fervidus*, *B. citrinus*, *B. perplexus*, and *B. vagans*. The largest contributions to the chi-squared value are indicated in bold.

***Bombus* species**	**Photo**	**Research grade iNat**	**Photo** $\frac{(\boldsymbol{obs.-exp.})^2}{\boldsymbol{exp.}}$	**iNat** $\frac{(\boldsymbol{obs.-exp.})^2}{\boldsymbol{exp.}}$
B. impatiens	763	38	0.1	**1.7**
B. griseocollis	121	12	0.1	**2.3**
B. bimaculatus	89	7	0.0	0.3
B. rufocinctus	56	2	0.0	0.6
Grouped	31	7	0.6	**10.2**

Supplementary Table S1.8: Species contribution to the chi-squared value for the Prairies Ecozone BVT, netting, and photo comparison ($X^2 = 38.10$, df = 8, $p < 0.001$). BVTs have proportionally fewer *B. rufocinctus* and more *B. borealis*, while netting shows the opposite pattern. The eusocial grouped category contains *B. huntii, B. centralis, B. fervidus, B. griseocollis, B. nevadensis,* and *B. terricola.* The parasitic grouped category contains *B. insularis, B. suckleyi*, and *B. flavidus*. The largest contributions to the chi-squared value are indicated in bold.

***Bombus* species**	**BVT**	**Net**	**Photo**	**BVT** $\frac{(obs.-exp.)^2}{exp.}$	**Net** $\frac{(obs.-exp.)^2}{exp.}$	**Photo** $\frac{(obs.-exp.)^2}{exp.}$
B. rufocinctus	236	132	98	**5.1**	**5.2**	**2.3**
B. borealis	133	22	20	**8.9**	**8.6**	**4.3**
B. ternarius	34	10	7	0.6	0.3	0.5
Eusocial group	19	3	5	0.6	1.7	0.0
Parasitic group	7	3	2	0.0	0.0	0.0

Supplementary Table S1.9: Species contribution to the chi-squared value for the Boreal Shield Ecozone BVT, netting, and photo comparison ($X^2 = 94.24$, df = 12, $p < 0.001$). Most of the difference stems from BVTs having fewer *B. vagans* and more *B. borealis* than netting and photos, and netting having fewer *B. ternarius* than expected. The eusocial grouped category contains *B. sandersoni* and *B. perplexus.* The parasitic grouped category contains *B. citrinus, B. flavidus,* and *B. bohemicus*. The largest contributions to the chi-squared value are indicated in bold.

***Bombus* species**	**BVT**	**Net**	**Photo**	**BVT** $\frac{(obs.-exp.)^2}{exp.}$	**Net** $\frac{(obs.-exp.)^2}{exp.}$	**Photo** $\frac{(obs.-exp.)^2}{exp.}$
B. vagans	16	219	127	**18.0**	8.6	0.9
B. ternarius	29	57	90	2.6	**10.6**	7.8
B. borealis	28	51	32	**15.4**	0.3	2.5
B. terricola	21	47	58	2.0	3.9	2.1
B. rufocinctus	2	17	12	0.8	0.2	0.0
Eusocial group	9	21	2	6.6	1.6	8.5
Parasitic group	4	31	19	1.0	0.7	0.1

Supplementary Table S1.10: Linear model analysis reveals that collection method affected the number of specimens collected per netting and photo survey in the Carolinian Zone at a level approaching significance. Site and the interaction between site and collection date were significant. Linear model analysis also reveals that collection method did not affect the species richness detected per netting and photo survey, but that site, collection date, and their interaction did. The colons represent interactive effects, and the variable of greatest interest is bolded. These results support Supplementary Figure S1.18.

Linear Model: Number of Specimens ~ Site * Collection Date * Collection Method

	d.f.	**Sum sq.**	**Mean sq.**	**F**	**P**
Site	7	8172.5	1167.5	43.9	<0.001
Collection Date	1	59.7	59.7	2.2	0.136
Collection Method	**1**	**82.6**	**82.6**	**3.1**	**0.080**
Site:Collection Date	7	1398.0	199.7	7.5	<0.001
Site:Collection Method	7	75.2	10.7	0.4	0.899
Collection Date:Collection Method	1	3.9	3.9	0.1	0.704
Site:Collection Date:Collection Method	7	110.8	15.8	0.6	0.759
Residuals	202	5369.4	26.6		

Linear Model: Species Richness ~ Site * Collection Date * Collection Method

	d.f.	**Sum sq.**	**Mean sq.**	**F**	**P**
Site	7	50.6	7.2	10.9	<0.001
Collection Date	1	34.3	34.3	51.6	<0.001
Collection Method	**1**	**0.7**	**0.7**	**1.1**	**0.299**
Site:Collection Date	7	30.6	4.4	6.6	<0.001
Site:Collection Method	7	1.6	0.2	0.4	0.928
Collection Date:Collection Method	1	0.3	0.3	0.4	0.509
Site:Collection Date:Collection Method	7	3.4	0.5	0.7	0.653
Residuals	202	134.3	0.7		

Supplementary Table S1.11: Species contribution to the chi-squared value for the Carolinian Zone netting and photo comparison ($X^2 = 20.95$, df = 7, $p = 0.004$). Photos had proportionally fewer *B. rufocinctus* and fewer *B. vagans* than netting. The largest contributions to the chi-squared value are indicated in bold.

Bombus species	**Net**	**Photo**	**Net** $\frac{(obs.-exp.)^2}{exp.}$	**Photo** $\frac{(obs.-exp.)^2}{exp.}$
B. impatiens	685	763	0.1	0.1
B. griseocollis	94	121	0.8	0.7
B. bimaculatus	76	89	0.1	0.1
B. rufocinctus	81	56	**3.6**	**3.3**
B. fervidus	11	13	0.0	0.0
B. citrinus	5	8	0.2	0.2
B. perplexus/B. borealis	3	5	0.2	0.2
B. vagans	21	5	**5.8**	**5.4**

Supplementary Table S1.12: Linear model analysis reveals that collection method did not affect the number of specimens collected per netting and photo survey in the Prairies Ecozone, but site did. Linear model analysis also reveals that collection method did not affect the species richness detected per netting and photo survey, but site did, and collection date approached significance. The colons represent interactive effects, and the variable of greatest interest is bolded. These results support Supplementary Figure S1.19.

Linear Model: Number of Specimens ~ Site * Collection Date * Collection Method

	d.f.	**Sum sq.**	**Mean sq.**	**F**	**P**
Site	2	3305.5	1652.8	11.5	<0.001
Collection Date	1	355.5	355.5	2.5	0.132
Collection Method	**1**	**0.3**	**0.3**	**<0.0**	**0.965**
Site:Collection Date	2	424.3	212.2	1.5	0.253
Site:Collection Method	2	77.4	38.7	0.3	0.767
Collection Date:Collection Method	1	19.8	19.8	0.1	0.715
Site:Collection Date:Collection Method	2	21.9	10.9	0.1	0.927
Residuals	20	2883.8	144.2		

Linear Model: Species Richness ~ Site * Collection Date * Collection Method

	d.f.	**Sum sq.**	**Mean sq.**	**F**	**P**
Site	2	34.6	17.3	9.7	0.001
Collection Date	1	6.5	6.5	3.7	0.070
Collection Method	**1**	**0.5**	**0.5**	**0.3**	**0.601**
Site:Collection Date	2	2.8	1.4	0.8	0.467
Site:Collection Method	2	0.1	<0.0	<0.0	0.977
Collection Date:Collection Method	1	<0.0	<0.0	<0.0	0.881
Site:Collection Date:Collection Method	2	<0.0	<0.0	<0.0	0.998
Residuals	20	35.5	1.8		

Supplementary Table S1.13: Linear model analysis reveals that collection method affected the number of specimens collected per netting and photo survey in the Boreal Shield Ecozone at a level approaching significance. Site, collection date and all two-way interactions were also significant or approaching significance. Linear model analysis also reveals that collection method affected the species richness detected per netting and photo survey at a level that was possibly significant. Colons represent interactive effects, and the variable of greatest interest is bolded. These results support Supplementary Figure S1.20.

Linear Model: Number of Specimens ~ Site * Collection Date * Collection Method

	d.f.	**Sum sq.**	**Mean sq.**	**F**	**P**
Site	4	938.7	234.7	4.8	0.016
Collection Date	1	1239.3	1239.3	25.1	<0.001
Collection Method	**1**	**205.0**	**205.0**	**4.2**	**0.064**
Site:Collection Date	4	664.9	166.2	3.4	0.046
Site:Collection Method	4	1058.5	264.6	5.4	0.010
Collection Date:Collection Method	1	217.8	217.8	4.4	0.058
Site:Collection Date:Collection Method	4	237.2	59.3	1.2	0.360
Residuals	12	592.8	49.4		

Linear Model: Species Richness ~ Site * Collection Date * Collection Method

	d.f.	**Sum sq.**	**Mean sq.**	**F**	**P**
Site	4	9.5	2.4	1.4	0.302
Collection Date	1	0.4	0.4	0.2	0.649
Collection Method	**1**	**8.0**	**8.0**	**4.6**	**0.053**
Site:Collection Date	4	6.4	1.6	0.9	0.482
Site:Collection Method	4	6.6	1.7	1.0	0.467
Collection Date:Collection Method	1	5.5	5.5	3.2	0.101
Site:Collection Date:Collection Method	4	2.7	0.7	0.4	0.816
Residuals	12	20.8	1.7		

Supplementary Table S1.14: Species contribution to the chi-squared value for the Boreal Shield Ecozone netting, and photo comparison ($X^2 = 52.60$, df = 7, $p < 0.001$). Most of the difference stems from netting having fewer *B. ternarius* and *B. terricola*, and photos having fewer *B. vagans* and *B. sandersoni*. The largest contributions to the chi-squared value are indicated in bold.

***Bombus* species**	**Net**	**Photo**	**Net** $\frac{(obs.-exp.)^2}{exp.}$	**Photo** $\frac{(obs.-exp.)^2}{exp.}$
B. vagans	219	127	**2.8**	**3.6**
B. ternarius	57	90	**8.2**	**10.7**
B. terricola	47	58	**2.6**	**3.4**
B. borealis	51	32	0.3	0.5
B. citrinus	30	15	0.8	1.1
B. rufocinctus	17	12	0.0	0.0
B. sandersoni	20	0	**6.7**	**8.7**
B. flavidus/B. perplexus	2	6	1.4	1.8

Supplementary Table S1.15: Comparison of BVT effectiveness in Western and Eastern North American studies. Some studies support a trend of BVTs being highly attractive to bees and especially bumble bees in the west and that this attractiveness becomes less extreme as you move further east, but other studies do not.

Study	Location	BVT performance
Western studies		
Stephen & Rao (2005)	Oregon	• The initial BVT study that demonstrates these traps are useful for collecting bees and especially bumble bees
Stephen & Rao (2007)	Oregon	• BVTs collected more bee specimens and species than yellow vane traps, sweep netting, and vacuums
Kimoto *et al*. (2012)	Oregon	• BVTs collected over 7000 bees in 17 collection days in the first year, and over 2000 in 24 collection days in the second year
Rhoades *et al*. (2017)	Washington	• Extrapolated species richness estimates were significantly higher for BVTs than netting
Bell (2019)	Wyoming	• Similar bumble bee catch rates for BVTs and netting • Netting collected a greater proportion (95% compared to 85%) of the total species compared to BVTs
Simon et al. (in press)	British Columbia	• Collected over 47,000 bumble bees with BVTs in a single field season
Eastern studies		
Geroff *et al*. (2014)	Illinois	• BVTs collected the most bumble bee species • Elevated pan traps collected the most bumble bee specimens • In terms of bees in general, malaise traps caught the most specimens and BVTs provided the best sample coverage
Joshi *et al.* (2015)	Pennsylvania	• BVTs collected significantly more bees than pan traps
Gibbs *et al*. (2017)	Pennsylvania and Michigan	• BVTs were so effective at collecting certain bee species that authors suggest these traps may oversample and deplete populations
Mundy-Heisz (2021)	Ontario	• BVT bumble bee catch rate was lower than Stephen & Rao (2005)'s bumble bee catch rate

Supplementary Table S1.16: Comparison among collection methods of the proportion of identifiable specimens. Photo records were the only records that were not able to be completely identified to species. However, photo records were identified by different people. I identified the photos in the Carolinian Zone. Dr. Cory Sheffield verified the photo records for the Prairies as well as some of the Boreal Shield Ecozone records. The remaining Boreal Shield photos were identified by Dr. Carolyn Parsons and or various iNaturalist users.

		Collection Method		
		BVT	**Net**	**Photo**
Carolinian	**Total specimens**	96	976	1115
	Specimens identified to species	100%	100%	95%
Prairies	**Total specimens**	429	170	141
	Specimens identified to species	100%	100%	94%
Boreal Shield	**Total specimens**	109	443	362
	Specimens identified to species	100%	100%	94%

Elm Street Naturalization Site (Elm St.)

4 August 2021 at BVT #12

13 September 2021 near BVT #11

Supplementary Figure S1.1: Images of Elm Street Naturalization Site in the Carolinian Zone. The top image is a modified Google Map (© 2022 Google), with BVT locations and an example survey path in blue. The bottom left and right images are pictures taken at the site.

Station Road Naturalization Site (Station Rd.)

18 July 2021 at BVT #10

10 August 2021 at BVT #9

Supplementary Figure S1.2: Images of Station Road Naturalization Site in the Carolinian Zone. The top image is a modified Google Map (© 2022 Google), with BVT locations and an example survey path in blue. The bottom left and right images are pictures taken at the site.

PhysEd Hydro (PEH)


13 August 2021 at BVT #6

6 September 2021 at BVT #7

Supplementary Figure S1.3: Images of PhysEd Hydro site in the Carolinian Zone. The top image is a modified Google Map (© 2022 Google), with BVT locations and an example survey path in blue. The bottom left and right images are pictures taken at the site.

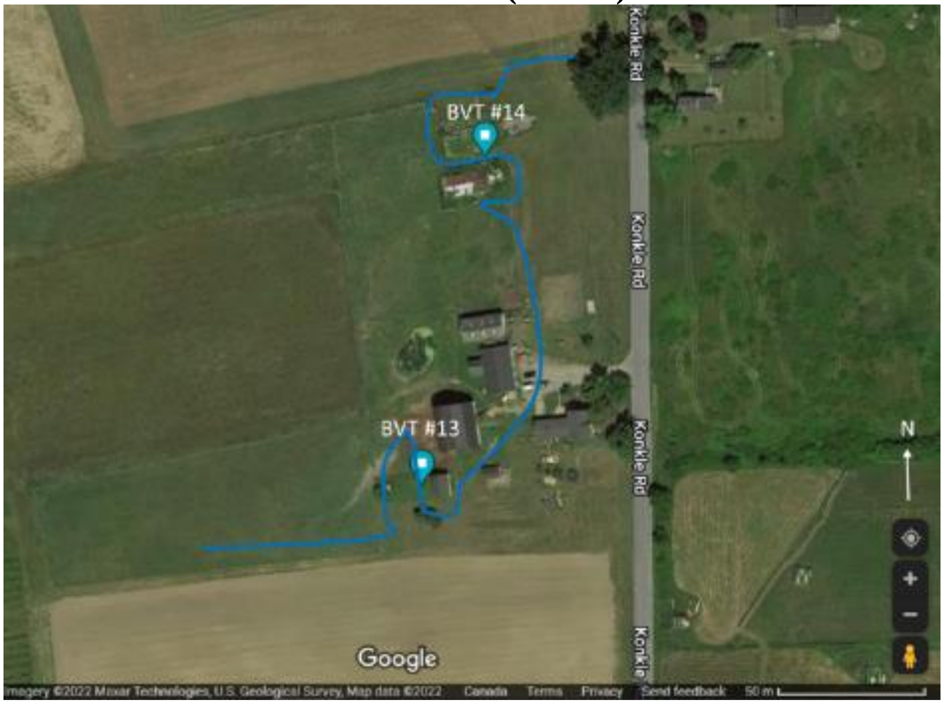
Quarry Southwest (QSW)

20 July 2021 - taken while surveying

15 August 2021 - at the entrance to the naturalization site

Supplementary Figure S1.4: Images of Quarry Southwest site in the Carolinian Zone. The top image is a modified Google Map (© 2022 Google), with an example survey path in blue. The bottom left and right images are pictures taken at the site.

Konkle Road (Konkle)



22 June 2021 at BVT #14

5 August 2021 at BVT #13

Supplementary Figure S1.5: Images of Konkle Road site in the Carolinian Zone. The top image is a modified Google Map (© 2022 Google), with BVT locations and an example survey path in blue. The bottom left and right images are pictures taken at the site.

Victoria Avenue (Victoria)

13 July 2021 at BVT #5

20 September 2021 at BVT #4

Supplementary Figure S1.6: Images of Victoria Avenue site in the Carolinian Zone. The top image is a modified Google Map (© 2022 Google), with BVT locations and an example survey path in blue. The bottom left and right images are pictures taken at the site.

Farr Road (Farr)

2 July 2021 at BVT #3

12 August 2021 at BVT #2

Supplementary Figure S1.7: Images of Farr Road site in the Carolinian Zone. The top image is a modified Google Map (© 2022 Google), with BVT locations and an example survey path in blue. The bottom left and right images are pictures taken at the site.

Townline Road (Townline)

3 July 2021 at BVT #21

1 September 2021 at BVT #20

Supplementary Figure S1.8: Images of Townline Road site in the Carolinian Zone. The top image is a modified Google Map (© 2022 Google), with BVT locations and an example survey path in blue. The bottom left and right images are pictures taken at the site.

Buffalo Pound

Big Valley

Wascana Trail

Supplementary Figure S1.9: Images of Buffalo Pound, Big Valley and Wascana Trail sites in the Prairies Ecozone. Images are modified Google Maps (© 2022 Google), with site locations in blue.

Pye Centre (PC)

19 August 2021 – Northern area, BVT #1 19 August 2021 – general overview of site

Supplementary Figure S1.10: Images of Pye Centre site in the Boreal Shield Ecozone. The top image is a modified Google Map (© 2022 Google), with the site location represented by a blue point. The bottom left and right images are pictures of the site taken by surveyors and were provided by Dr. Carolyn Parsons.

Pasadena (PAS)

Supplementary Figure S1.11: Modified Google Maps (© 2022 Google) image of Pasadena site in the Boreal Shield Ecozone. The site location represented by a blue point.

Torbay Gravel Pit (TGP)

19 July 2021
location of BVT #1 for this site

19 July 2021

Supplementary Figure S1.12: Images of Torbay Gravel Pit site in the Boreal Shield Ecozone. The top image is a modified Google Map (© 2022 Google), with the site location represented by a blue point. The bottom left and right images are pictures of the site taken by surveyors and were provided by Dr. Carolyn Parsons.

MUN Bot Garden (MBG)

19 July 2021 - BVT #2

19 July 2021

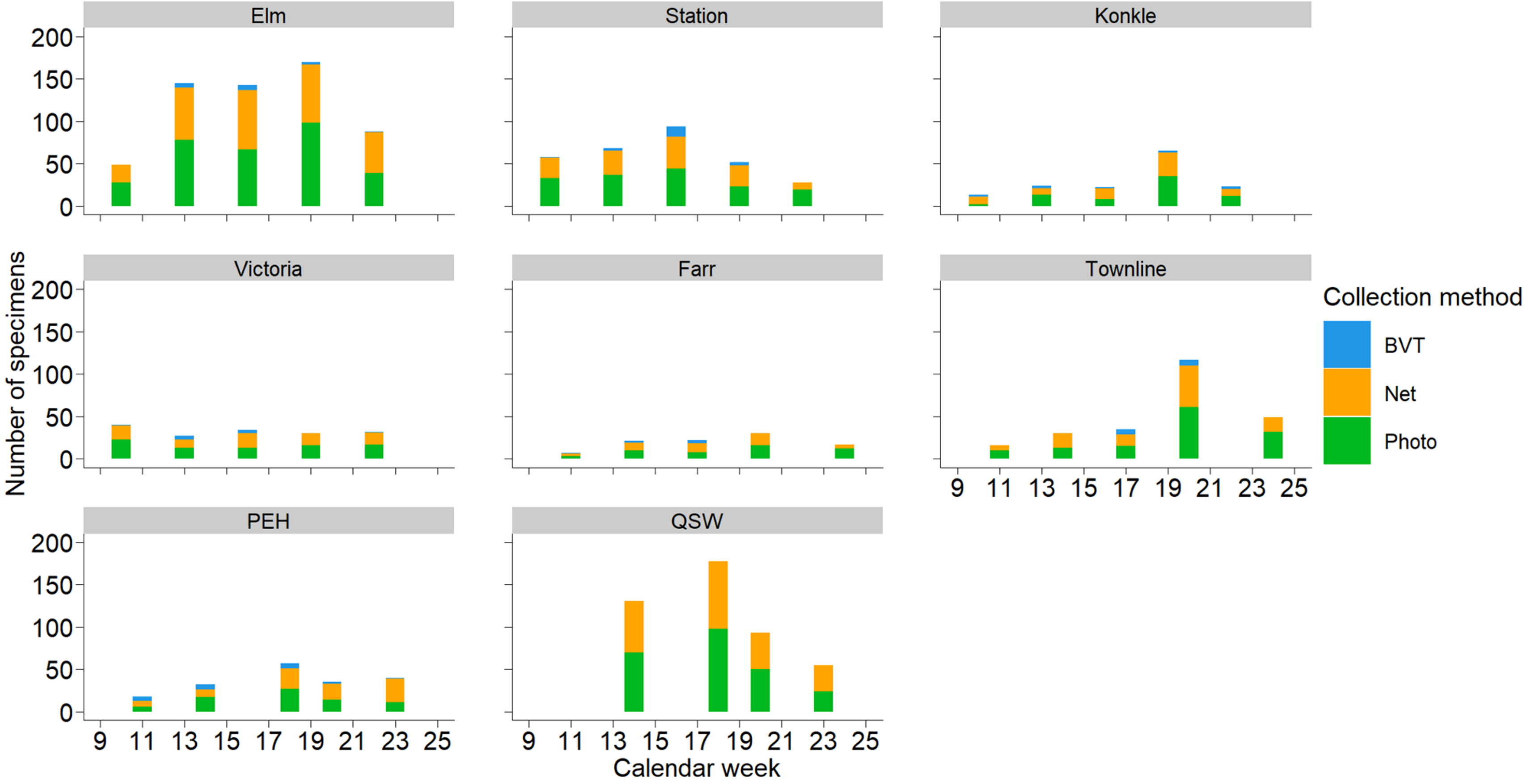
Supplementary Figure S1.13: Images of Memorial University of Newfoundland Botanical Garden site in the Boreal Shield Ecozone. The top image is a modified Google Map (© 2022 Google), with the site location represented by a blue point. The bottom left and right images are pictures of the site taken by surveyors and were provided by Dr. Carolyn Parsons.

AAFC Cranberry Bog (ACB)

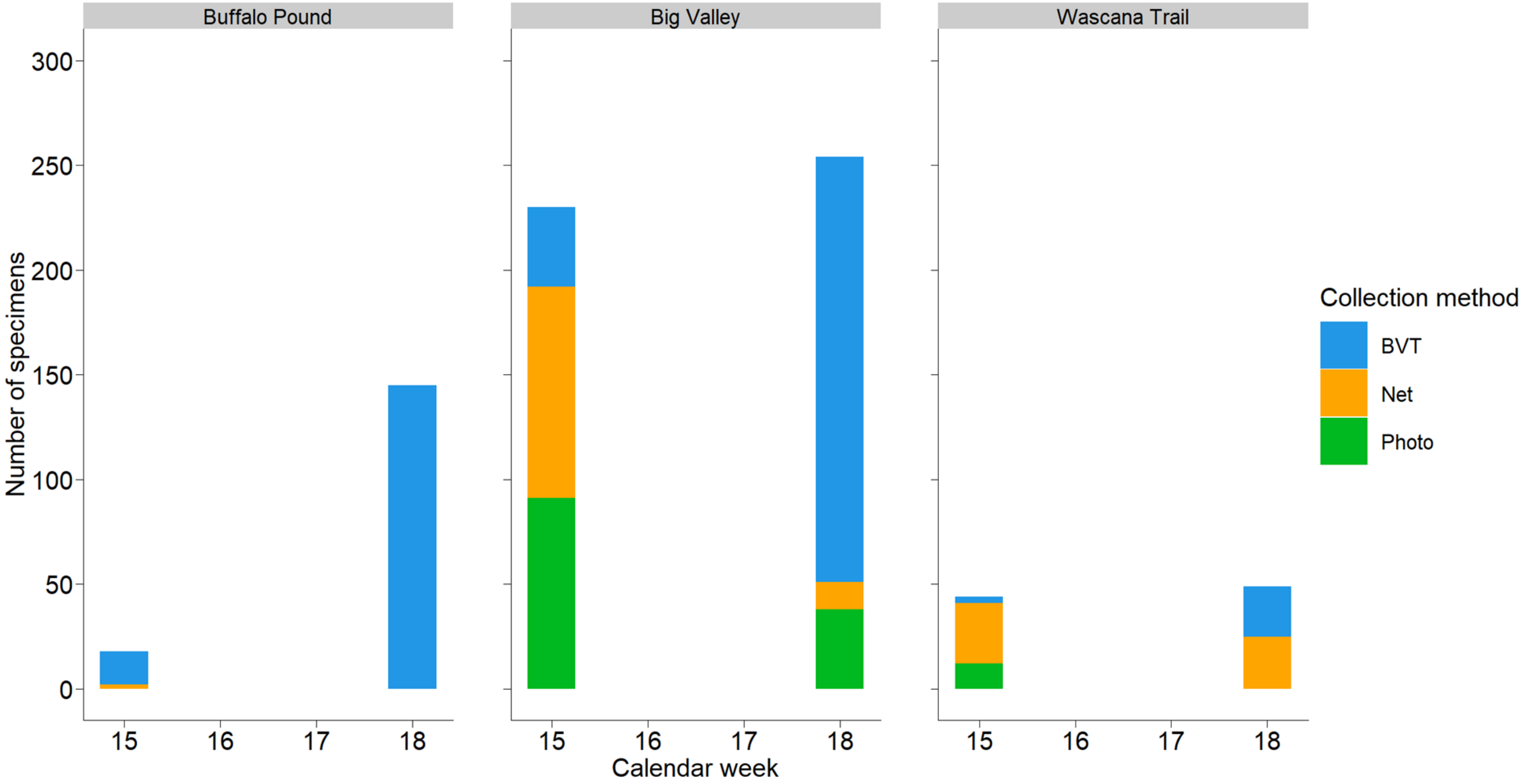

26 July 2021


20 August 2021 - cranberry plots and surrounding vegetation

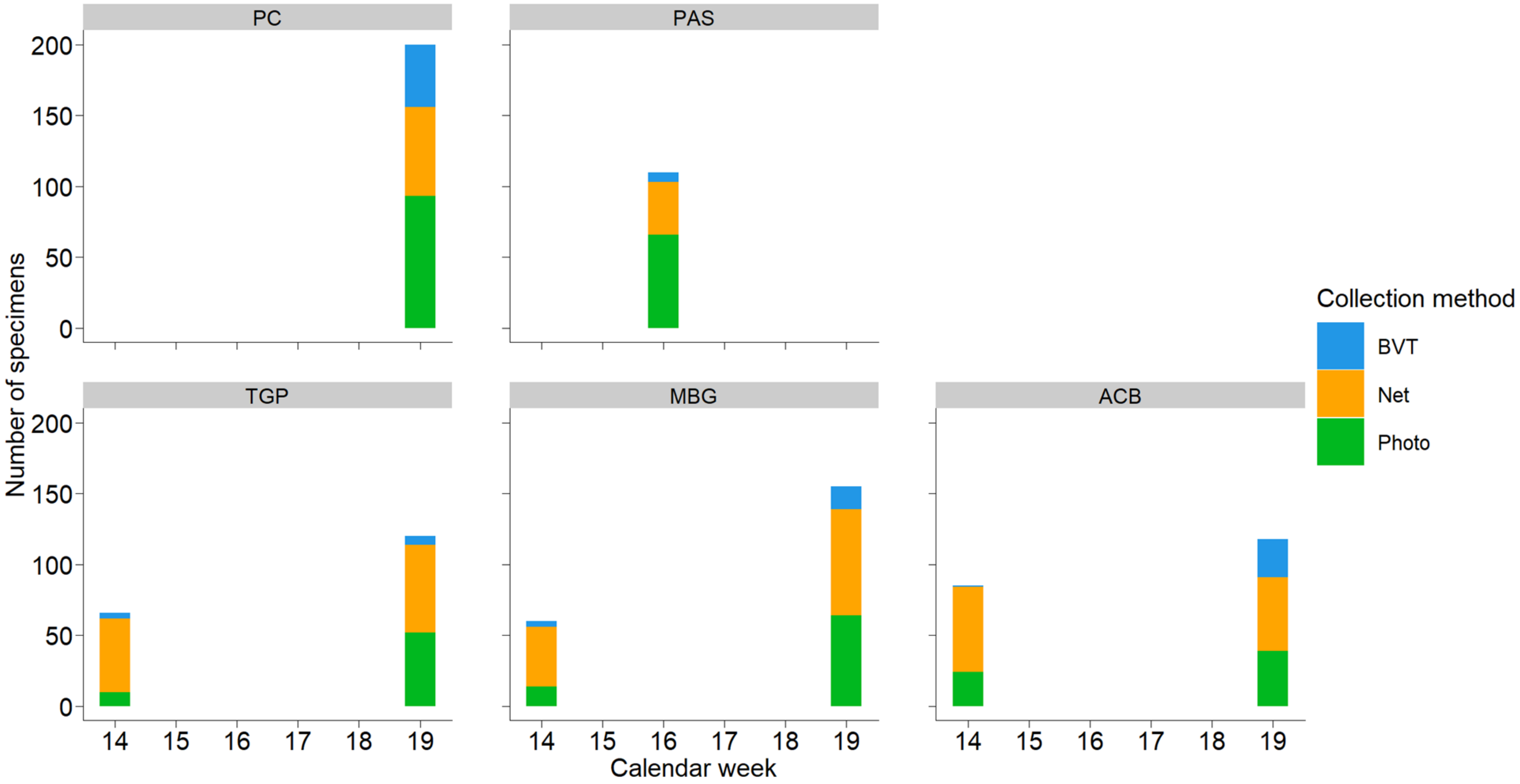
Supplementary Figure S1.14: Images of Agriculture and Agri-Food Canada Cranberry Bog site in the Boreal Shield Ecozone. The top image is a modified Google Map (© 2022 Google), with the site location represented by a blue point. The bottom left and right images are pictures of the site taken by surveyors and were provided by Dr. Carolyn Parsons.



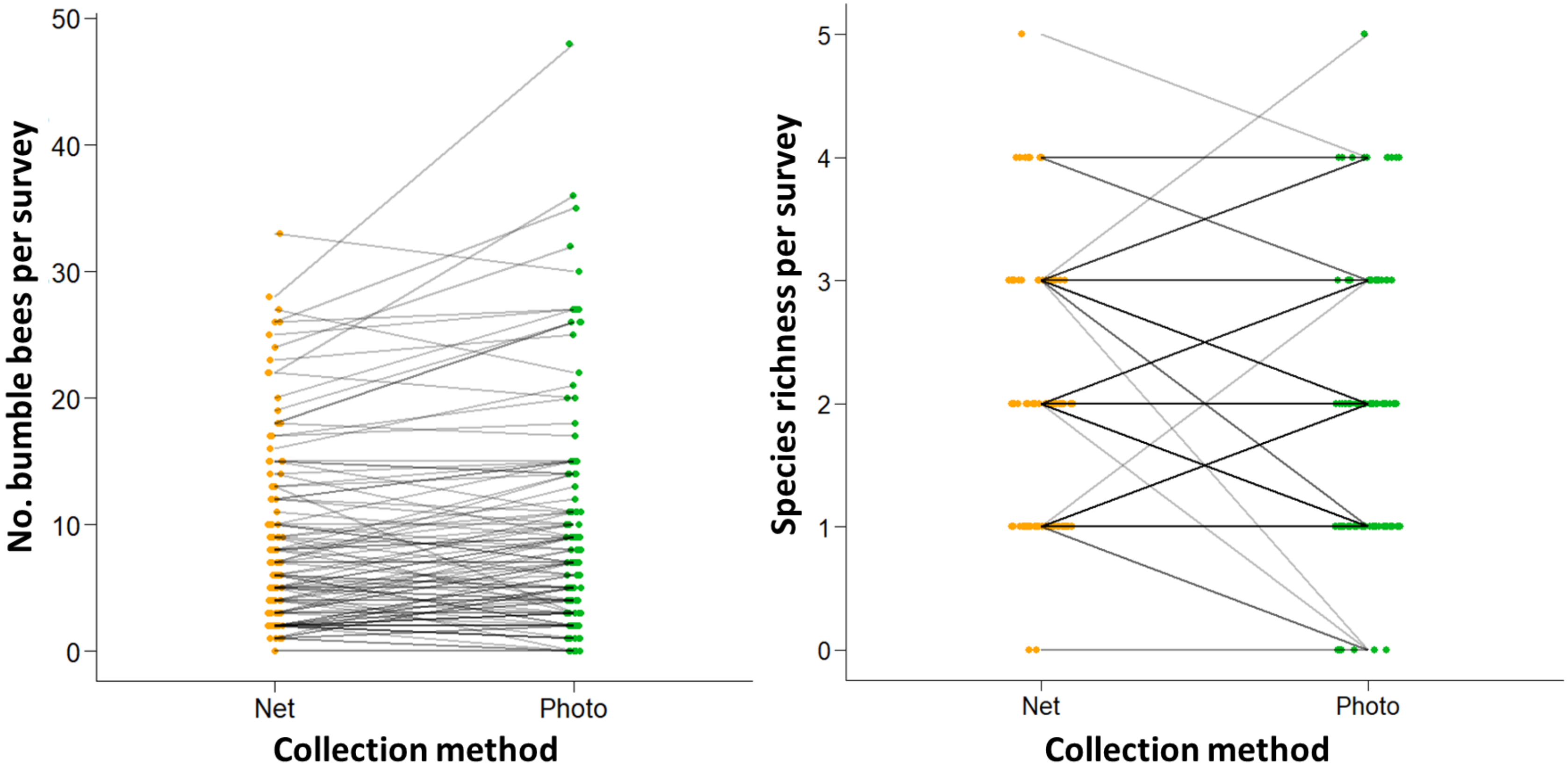
Supplementary Figure S1.15: In the Carolinian Zone, the number of bumble bee specimens collected (including specimens not identified to species) was affected by collection method, site, and week.



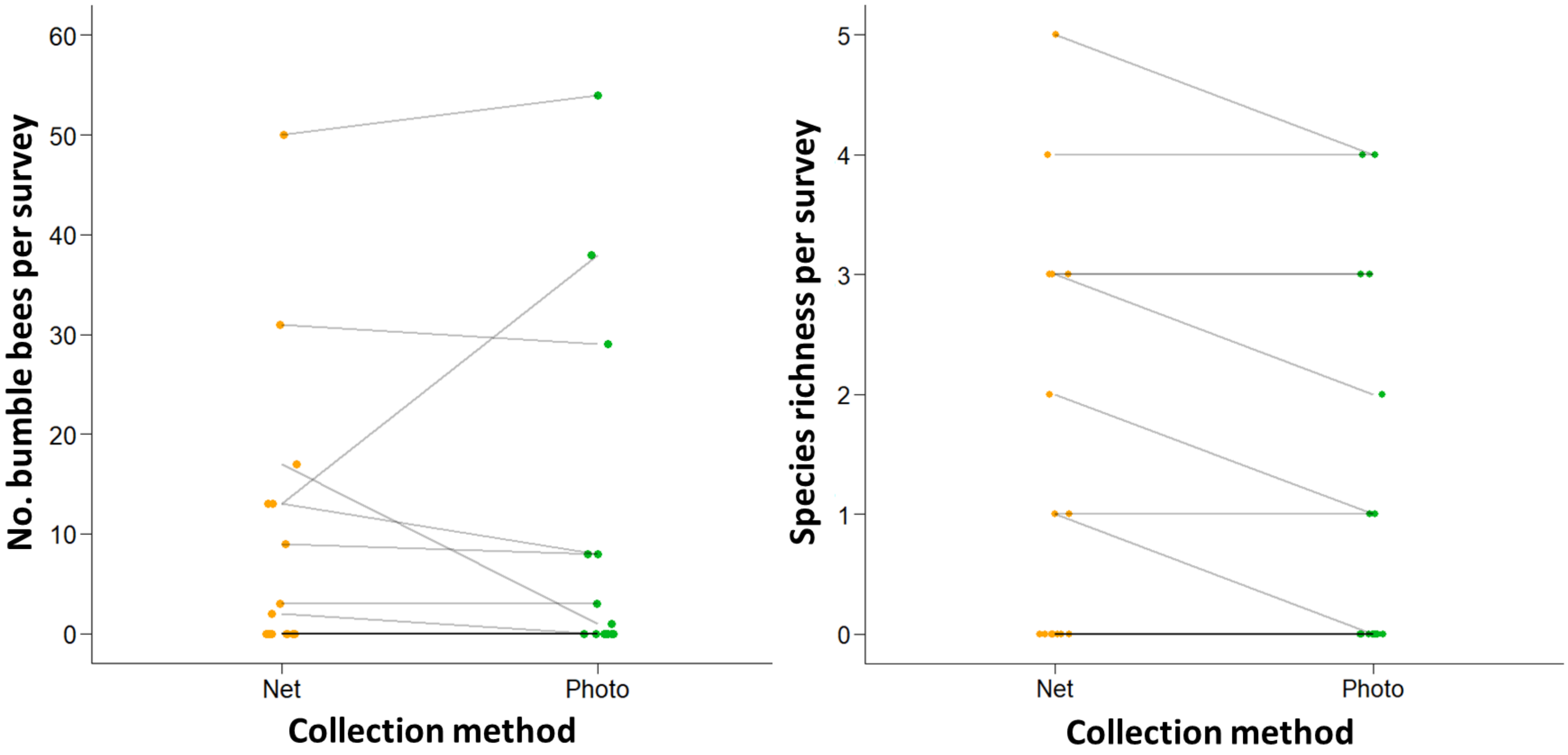
Supplementary Figure S1.16: In the Prairies Ecozone, the number of bumble bee specimens collected (including specimens not identified to species) was affected by collection method, site, and week. Week 18 was the start of a two-week survey period with fewer netting and photo surveys than week 15 and only 1 BVT at Wascana Trail site instead of 3.



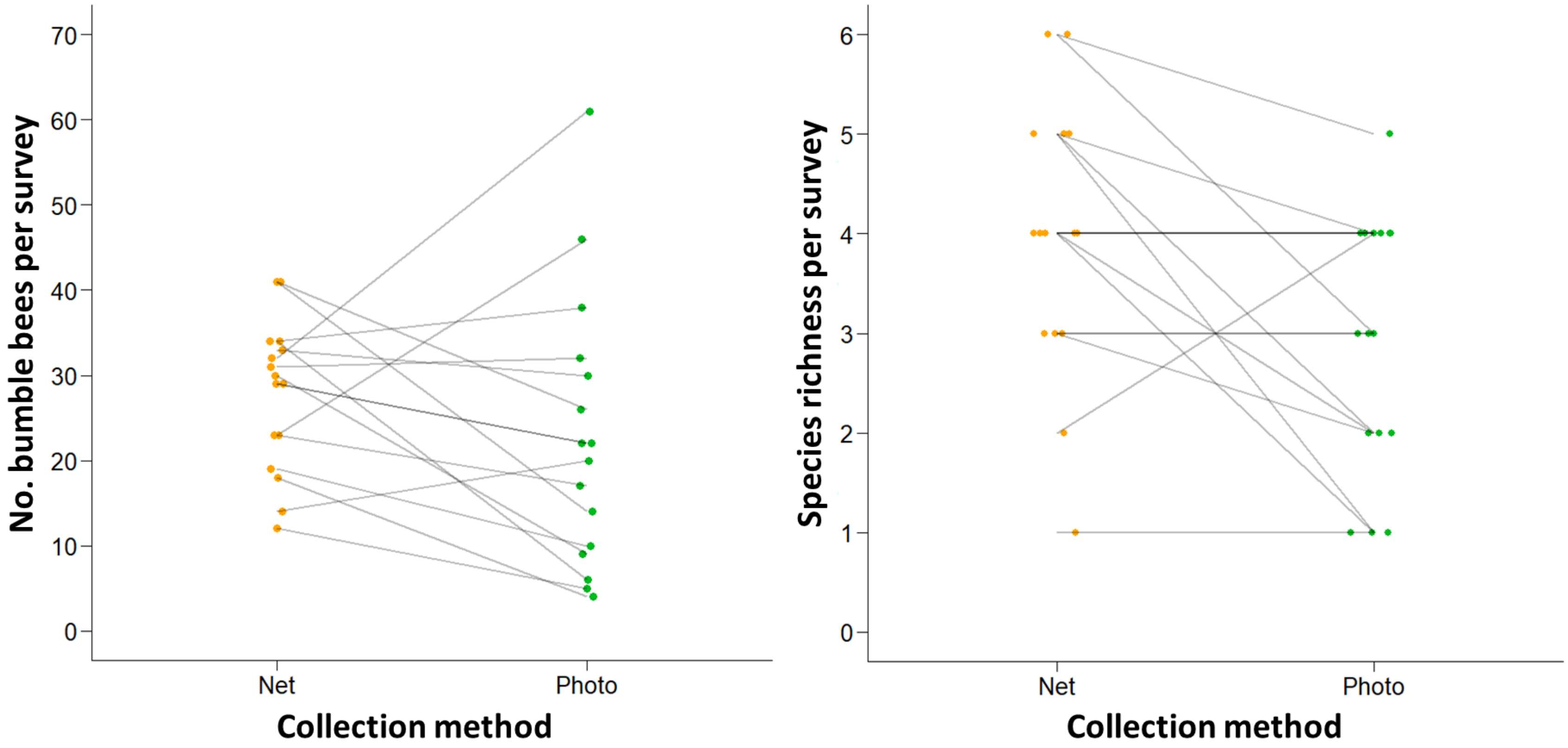
Supplementary Figure S1.17: In the Boreal Shield Ecozone, the number of bumble bee specimens collected (including specimens not identified to species) was affected by collection method, site, and week. Site PC was located in central Labrador. Site PAS was in western Newfoundland. Sites TGP, MBG and ACB were in eastern Newfoundland.



Supplementary Figure S1.18: In the Carolinian Zone, the photo method (mean = 9.5, sd = 8.9) collected more bumble bee specimens per survey compared to the netting method (mean = 8.3, sd = 7.2) at a level approaching significance. Both methods produced a similar number of species per survey (Netting: mean = 1.9, sd = 1.0; Photo: mean = 1.8, sd = 1.1). Points represent the number of specimens (including specimens not identified to species) or species richness collected per survey. Lines connect netting and photo surveys conducted at the same site on the same day. Points are jittered to reduce point overlap and line color intensifies with overlapping lines.



Supplementary Figure S1.19: In the Prairies Ecozone, netting (mean = 8.6, sd = 14.1) and photo (mean = 8.8, sd = 16.5) collection methods collected similar numbers of bumble bee specimens and species richness (Netting: mean = 1.4, sd = 1.7; Photo: mean = 1.1, sd = 1.5) per survey. Points represent the number of specimens (including specimens not identified to species) or species richness collected per survey. Lines connect netting and photo surveys conducted at the same site on the same day. Points are jittered to reduce point overlap and line color intensifies with overlapping lines.



Supplementary Figure S1.20: In the Boreal Shield Ecozone, the netting method (mean = 27.7, sd =8.8) collected more specimens per survey than the photo method (mean =22.6, sd =15.9) at a level that approached significance. The netting method also collected greater species richness per survey than photos at a level that was possibly significant (Netting: mean = 3.9, sd = 1.3; Photo: mean = 2.9, sd = 1.3). Points represent the number of specimens (including specimens not identified to species) or species richness collected per survey. Lines connect netting and photo surveys conducted at the same site on the same day. Points are jittered to reduce point overlap and line color intensifies with overlapping lines.