Rusty Patched Bumble Bee (*Bombus affinis)* Species Status Assessment

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Executive Summary

This report summarizes the results of a species status assessment (SSA) completed for the rusty patched bumble bee, *Bombus affinis*, to assess the species' overall viability. Historically, the species was widely distributed across areas of Quebec, North Dakota, South Dakota, Minnesota, Wisconsin, Iowa, Missouri, Illinois, Kentucky, Tennessee, Indiana, Michigan, Ontario, Ohio, Pennsylvania, New York, Vermont, Maine, Massachusetts, New Hampshire, Delaware, Rhode Island, Connecticut, New Jersey, Maryland, Virginia, District of Columbia, West Virginia, North Carolina, South Carolina, and Georgia.

To assess the species' viability, we used the three conservation biology principles of resiliency, representation, and redundancy. Specifically, we identified the species' ecological requirements for survival and reproduction at the individual, population, and species levels, and described the beneficial and risk factors influencing *B. affinis'* viability. We evaluated the changes in representation, resiliency, and redundancy from historical to the current time, and forecasted changes into the future.

Bombus affinis is a colonial species with an annual cycle that starts in early spring when colonies are initiated by solitary queens, progresses with the production of workers throughout the summer, and ends with the production of males and new queens in late summer and early fall. Survival and successful recruitment require floral resources (for food) from early spring through fall, undisturbed nest sites in proximity to foraging resources, and overwintering sites for the next year's queens. Populations of *B. affinis* consist of tens to hundreds of colonies, and the health (long-term productivity) of populations is affected by the quantity of nectar and pollen available and the proximity of floral resources to nesting sites. In addition to proximity, the permeability of the landscape is important to ensure reproductive individuals are able to disperse to find unrelated mates. At the species level, *B. affinis* needs a sufficient number and distribution of healthy populations to withstand environmental stochasticity (resiliency), catastrophes (redundancy), and biological and physical changes in its environment (representation).

We evaluated the change in resiliency, representation, and redundancy from the past until the present, and projected the anticipated future states of these conditions. To forecast the biological condition into the future, we devised risk scenarios by eliciting expert information on 5 stressors: pathogens, pesticides, habitat loss and degradation, climate change, and small population dynamics. To assess resiliency, we evaluated the trend in *B. affinis* occurrences (populations) over time and the trend in *B. affinis* abundance relative to all *Bombus* spp. over time. To forecast anticipated future abundance, we used a population model to project the number of populations expected to persist based on plausible future risk scenarios. To assess the adaptive capacity (representation) of *B. affinis*, we evaluated the spatial extent of occurrences over time. At a coarse scale, we tallied the number of ecoregions, states, and counties occupied by *B. affinis*. At a finer scale, we calculated the extent of occurrence (EoO) within each ecoregion (within the historically occupied range) over time. To assess redundancy, we calculated the risk of ecoregion-wide extirpations given the number of populations present historically, currently, and forecasted for 5 to 50 years into the future.

Based on input received from species experts, we developed three future risk scenarios: 1) Status Quo - the rate of decline continues unchanged into the future; 2) Most Likely - the effect from pathogens, pesticides, and habitat loss and degradation will be constant into the future, but the negative impact from small population size and climate change will increase; and 3) Better-case – same as the Most Likely scenario with the exception of no impact from pathogens by Year 10.

Our analyses indicate that the resiliency, representation, and redundancy of *B. affinis* have all declined since the late 1990s and are projected to continue to decline over the next several decades. Historically, *B. affinis* was an abundant and wide-spread species, with hundreds of populations across an expansive range encompassing 31 states and 15 ecoregions. Although not yet analyzed, the species' wide distribution most certainly encompassed high spatial heterogeneity (occurrences distributed across an array of climatic conditions), allowing *B. affinis* to fluctuate asynchronously in response to varying environmental conditions. Due to the large number of populations within the ecoregions, the risk of losing areas of adaptive diversity (representing the species' ability to adapt to changing conditions) due to catastrophic events was extremely unlikely prior to late 1990s.

Since the late 1990s, *B. affinis* distribution and abundance has declined. Five percent of the historical locations (grids) are currently (the last 2 decades) occupied by *B. affinis*, and the relative abundance of *B. affinis* declined from 8% historically to 1% currently. Although we could not assess the health of all existing populations, analyzing data from 25 of the 69 current grid occurrences suggest that many of the persisting populations are affected by at least one stressor (many with high severity) over a broad extent of the population. Along with the loss of populations, a marked decrease in the spatial extent has occurred in recent times; the spatial extent is currently 8% of its historical extent. This expansive reduction has likely led to loss of spatial heterogeneity and adaptive diversity. Similarly, the loss of occurrences has increased the risk of ecoregion-wide extirpations due to catastrophic events (*i.e.,* severe drought and prolonged, high temperatures). Furthermore, many of the existing populations continue to face the effects of past on ongoing stressors, including pathogens, pesticides, habitat loss and degradation, climate change, and small population dynamics. It is likely that several of these risk factors are acting synergistically on the species, and the combination of multiple stressors is likely more harmful than a single stressor acting alone.

The abundance of *B. affinis* is forecasted to decline over time under all three risk scenarios evaluated, with extinction predicted in all but one ecoregion within 5 years; Ecoregion 220 is forecasted to be extinct by Year 30. These projections, however, are likely optimistic due to continued loss of spatial heterogeneity and the consequent increased risk of population extirpation due to environmental stochasticity. As fewer and fewer populations persist, the ability to withstand normal environmental stochasticity is diminished, and thus the decline to extinction is accelerated. Furthermore, as fewer populations persist and the spatial extent of the species declines, the species' ability to withstand catastrophic events and changes in its environment is likely to be greatly reduced.

Thus, the species' ability to sustain populations over time has declined, and is expected to continue to decline into the future. All measures of its historical biological condition indicate that *B. affinis* was abundant and widely distributed; it was the fourth ranked *Bombus* species in our relative abundance analysis prior to the late 1990s, with an expansive geographical range covering most of the Midwestern and eastern U.S. and areas of Quebec and Ontario, representing 15 ecoregions. Since the late 1990s, marked and precipitous declines have been recorded in spatial extent and in the number of extant populations. Although the ultimate source of the acute and widespread decline is debated, and despite that the relative role and synergistic effects of the primary stressors are unknown, the decline in *B. affinis* is undisputable. Regardless of the uncertainty in the causative factors, at least one if not all, stressors are likely to continue affecting the remaining populations. The magnitude and extent of losses to date have greatly reduced the ability of *B. affinis* to adapt to changing environmental conditions and to guard against further losses of adaptive diversity and potential extinction due to catastrophic events. In reality, the few populations persisting and the limited spatial heterogeneity associated with these populations have substantially reduced the ability of *B. affinis* to withstand normal environmental variation and recover from transient, stochastic perturbations. Coupled with the increased risk of

extirpation due to the interaction of reduced population size and its reproductive system, *B. affinis* may lack the resiliency required to sustain populations into the future, even without further exposure to stressors.

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Chapter 1. Introduction, Data, and Analytical Framework

This report summarizes the results of a species status assessment (SSA) conducted for the rusty patched bumble bee (*Bombus affinis*). We, the U.S. Fish and Wildlife Service, were petitioned to list *B. affinis* as endangered under the Endangered Species Act (ESA) on January 31, 2013, by the Xerces Society for Invertebrate Conservation. A subsequent complaint for not meeting the statutory petition finding deadlines was filed on May 13, 2014. The Service committed to a deadline of September 30, 2015 for submitting to the Federal Register a 90-day finding on the rusty patched bumble bee petition. In September of 2015, the Service found that the petition presented substantial scientific or commercial information indicating that the petitioned action may be warranted. Therefore, a review of the status of the species was initiated to determine if the petitioned action is warranted. Based on the status review, the Service will issue a 12-month finding on the petition, which will address whether the petitioned action is warranted. Thus, we conducted a SSA to compile the best available data regarding the species' biology and factors that influence the species' viability. The SSA will be the biological underpinning of the Service's forthcoming decision on whether *B. affinis* warrants protection under the ESA.

The SSA assesses the ability of *B. affinis* to maintain populations over time (*i.e*., viability). To assess *B. affinis* viability, we used the three conservation biology principles of resiliency, representation, and redundancy (or the "3Rs", Shaffer and Stein 2000, pp. 308-311). These principles are generally described later in this chapter, and more specifically for *B. affinis* in Chapter 2. Our approach for assessing *B. affinis* viability involved 3 stages. In Stage 1, we described the species' ecology in terms of the 3Rs. Specifically, we identified the ecological requirements for survival and reproduction at the individual, population, and species levels. In Stage 2, we determined the baseline condition of the species using the ecological requirements identified in Stage 1. That is, we assessed the species' historical and current condition in relation to the 3Rs and identified past and ongoing factors (beneficial and risk factors) that led to the species' current condition. In Stage 3, using the baseline conditions established in Stage 2 and the predictions for future risk and beneficial factors, we projected the likely future condition of *B. affinis*.

The species' ecology (Stage 1) is summarized in Chapter 2; risk and beneficial factors in Chapter 3; the historical and current conditions (Stage 2) in Chapters 4 and 5, respectively; and the future condition (Stage 3) in Chapter 6. Lastly, the viability of *B. affinis* over time is described through a synthesis of historical, current, and future conditions analyses and is provided in Chapter 7.

1.1 Occurrence Data

The occurrence data used for our analyses is primarily based on the *Bombus* of North America (BBNA) database developed by Leif Richardson (University of Vermont) that compiles data from over 125 sources, including museum collections, published reports, state natural heritage records and citizen science observation networks where the sightings have been verified to the species level. We imported the data into ESRI ArcGIS version 10.3 where geographic coordinates were projected and an ESRI feature class was created. The database includes 113,199 *Bombus* species records within a rectangle that overlaps with the known range of *B. affinis*. Additional *Bombus* records were continually added to the

database as new occurrence data was received through coordination efforts with local and state agencies and species experts. We obtained additional data from one site in Tennessee and one site in North Carolina from expert elicitation questionnaires (see Chapter 4) that have not been included in this current analysis, but will be included in future analyses. We received the data from those two sites after much of the analyses had been completed (after March 10, 2016) and we do not expect that the addition of those two sites would have a significant impact on our analyses or conclusions.

We refined the *B. affinis* range by selecting all U.S. counties and equivalent-sized polygons in Canada with *B. affinis* occurrences and then adding all adjacent counties to those records to account for likely dispersal distances. County equivalent polygons for Canada were generated by taking the mean area of *B. affinis* U.S. counties and creating a grid layer of equal size that overlaps with the Canadian *Bombus* records in the database. This initial spatial screening of the data produced "holes" in the species' range map that were surrounded by other occurrence records. Given the data and the species' life history characteristics, it was logical to assume the species likely also occurred in these "hole" areas. Therefore, we included those areas as a part of the species' refined range map. In addition, any locations that were outside of the main occurrence boundary were given a county-sized corridor towards the nearest *B. affinis* detection to account for likely dispersal distance. We used all *Bombus* points within the refined occurrence boundary in our analyses.

We used a series of quality control checks to remove duplicative data and verify the spatial locations of *Bombus* records. The database was intersected with county layers to verify spatial locations and any records not matching written descriptions were corrected by species experts or through other data sources.

Although the database is comprised of an impressive number of records spanning more than 100 years, the data were generally collected through unsystematic, opportunistic surveys and reporting, and there are very few repeated surveys in any one location. Thus, it is difficult to compare the number of occurrences over time. Additionally, because bumble bee nest locations vary year-to-year (see Chapter 2), tracking colonies, and thus populations, over time is difficult. Furthermore, more targeted surveys were conducted in recent years by those interested in the species' apparent decline. We also have very little sampling data for 2014 and 2015. We followed published methods used by others (Colla *et al.* 2012, p. 3587; Hatfield *et al.* 2014; Fitzpatrick *et al.* 2007; Williams 2005) to account for the sampling bias and the movement of colonies over time. We created a post hoc systematic sampling method by overlaying a 10 kilometer (km) x 10 km grid across the range of *B. affinis* and assigning a unique numerical identifier and a textual description of the year(s) *B. affinis* and all other *Bombus* species were detected within that grid. All *B. affinis* occurrences falling within a grid and within the same decade were tallied as a single grid occurrence.

We chose a 10 km x 10 km grid size for the following reasons. Based on studies of a closely related species, *B. terrestris* (Kraus *et al.* 2009, p. 249; Lepais *et al.* 2010, pp. 826-827), the maximum dispersal distance of *B. affinis* is likely to be 1 to 10 km. Individuals concurrently visiting a site are often from different colonies (J. Strange, USDA, pers. comm. 2016), and limited information suggests populations are densely aggregated (L. Richardson, University of Vermont, pers. comm. 2016). It is, therefore, reasonable to assume that multiple detections over time within a 10 km x 10 km area likely represent a single population (D. Goulson, University of Sussex, pers. comm. 2016, indicated that a 10 km x 10 km might be able to support a small population of bumble bees). Thus, tallying the number of 10 km x 10 km grids occupied by *B. affinis* is a reasonable proxy for the number of populations present on the landscape.

We analyzed data in 10-year increments to capture a range of detections for each grid and allow variation in historical trends to be assessed. The data prior to the 1950s is particularly sporadic; we lumped all occurrences from 1900-1949 into a single time period. Thus, the time period categories are: pre-1950, 1950s, 1960s, 1970s, 1980s, 1990s, 2000s, and 2010s. Grid occurrences were assigned as historical or current:

Historical = occurrences from 1900-1999;
Current = occurrences from 2000-2015.
Total occurrences = historical + new occurrences recorded in the current time period[1]

We define current as occurrences in the last two decades (2000-2010 and 2011-2015) because there was not sufficient sampling across the range in the last 4 years of available data to adequately compare it to the previous decades.

All maps were generated using ESRI ArcGIS Version 10.3, and data used for external analysis were queried using ESRI ArcGIS, exported and summarized using Microsoft Access, and saved as Microsoft Excel Workbooks for further analysis.

We intended to use the U.S. Department of Agriculture (USDA) Plant Hardiness Zone Map, which identifies growing zones or regions according to the average annual extreme minimum winter temperature (1976 to 2005), to analyze the species' distribution and resiliency because the species' breeding females must hibernate during winter and have sufficient floral resources to feed upon after emergence (see Chapter 2). The map is divided into 10-degree (°) Fahrenheit zones and is available as a *.TIFF raster image or as an ESRI ArcGIS shapefile. The image can be georeferenced over the current *B. affinis* records for visual interpretation; however, the specific values cannot be obtained and used for analysis without a functioning ESRI ArcGIS shapefile. Dr. Leif Richardson (pers. comm. 2016) suggested that we use species distribution modeling based on data from Worldclim, Climond, or other spatial data providers. In the next version of the SSA, we will evaluate both the species distribution modeling and hardiness zone mapping approaches to assess whether either is useful for analyzing *B. affinis* resiliency.

We are currently creating a habitat connectivity model that identifies barriers to *B. affinis* movement based on the most recent 2011 National Land Classification Database (NLCD) maps and the likelihood of bumble bee movement across the different natural, agricultural and urban/suburban habitats, following methods in Jha and Kremen (2013). Due to the complexities of completing this analysis, the full results are not yet available but these analyses will be available for future conservation planning. We will classify the 2011 NLCD raster images into four different model scenarios using different land use cost values for land classification types grouped into strong, moderate, weak or no movement limitations. Current *B. affinis* records will be used as a starting point for movement across these weighted land classifications by using ESRI cost distance tools under Spatial Analyst. The final output will provide numerical values of resistance measures at a 30 meter pixel level and represented as a heat map identifying the most likely travel corridors and the potential for connectivity to other known populations. The resistance measures can then be analyzed at the grid level to rank the resiliency of those populations based on available habitat and opportunities for recruitment. The four-scenario model will be run off of the entire *B. affinis* dataset using the 1988 NLCD database map to assess the

[1] Current occurrence consist of grids documented during the historical period as well as new occurrences (i.e., those that have bumble bee occurrences from 2000 and beyond but not before).

potential change in connectivity at a time prior to the apparent significant population decline identified in this SSA analysis to present conditions.

1.2 Analytical Framework

To assess the viability of *B. affinis*, we applied the conservation biology principles of resiliency, representation, and redundancy (henceforth, 3Rs). Viability is the ability to sustain populations over time; to do this, a species must have a sufficient number and distribution of healthy populations to withstand changes in its biological (*e.g.*, novel diseases, predators) and physical (*e.g.,* climate change) environment, environmental stochasticity (*e.g.,* wet or dry, warm or cold years), and catastrophes (*e.g.,* severe and prolonged droughts).. Viability is not a single state—viable or not viable; rather, there are degrees of viability--less to more viable or low to high viability. Generally speaking, the more resiliency, representation, and redundancy a species has, the more protected it is against the vagaries of the environment, the more it can tolerate stressors (one or more factors that may be acting on the species or its habitat, causing a negative effect), the better able it is to adapt to future changes, and thus, the more viable it is. The 3Rs framework (assessing the health, number, and distribution of *B. affinis* populations relative to frequency and magnitude of environmental stochasticity and catastrophic events across its historical range of adaptive diversity) is useful for describing a species' degree of viability through time.

1.2.1 Resiliency

Resiliency is the ability to sustain populations in the face of environmental variation and transient perturbations. Environmental variation includes normal year-to-year variation in rainfall and temperatures, as well as unseasonal weather events. Perturbations are stochastic events such as fire, flooding, and storms. Simply stated, resiliency is having the means to recover from "bad years" and disturbances. To be resilient, a species must have healthy populations; that is, populations that are able to sustain themselves through good and bad years. The healthier the populations and the greater number of healthy populations, the more resiliency a species possesses. For many species, resiliency is also affected by the degree of connectivity among populations and the diversity of ecological niches occupied. Connectivity among populations increases the genetic health of individuals (heterozygosity) within a population and bolsters a population's ability to recover from disturbances via rescue effect (immigration). Diversity of climate niches improves a species' resiliency by guarding against disturbances and perturbations affecting all populations similarly (*i.e*., decreases the chance of all populations experiencing bad years simultaneously or to the same extent).

1.2.2 Representation

Species-level representation is the ability of a species to adapt to near and long-term changes in the environment; it's the evolutionary capacity or flexibility of a species. Representation is the range of variation found in a species, and this variation--called adaptive diversity--is the source of species' adaptive capabilities. Representation can, therefore, be measured through the breadth of adaptive diversity of the species. The greater the adaptive diversity, the more responsiveness and adaptable the species will be over time, and thus, the more viable the species is. Maintaining adaptive diversity includes conserving both the ecological diversity and genetic diversity of a species. By maintaining these two sources of adaptive diversity across a species' range, the responsiveness and adaptability of a species over time is preserved. Ecological diversity is the physiological, ecological, and behavioral

variation exhibited by a species across its range. Genetic diversity is the number and frequency of unique alleles within and among populations.

In addition to preserving the breadth of adaptive diversity, maintaining evolutionary capacity requires maintaining the evolutionary processes that drive evolution; namely, gene flow, genetic drift, and natural selection. Gene flow is expressed through the physical transfer of genes or alleles from one population to another through immigration and breeding. The presence or absence of gene flow can directly affect the size of the gene pool available. Gene flow will generally increase genetic variation within populations by bringing in new alleles from elsewhere, but decrease genetic variation among populations by mixing their gene pools (Hendry *et al.* 2011, p. 173). Genetic drift is the change in the frequency of alleles in a population due to random, stochastic events. Genetic drift always occurs, but is more likely to negatively affect populations that have a smaller effective population size (N_e) and populations that are geographically spread and isolated from one another. Natural selection is the process by which heritable traits can become more (selected for) or less (not selected for) common in a population based on the reproductive success of an individual with those traits. Natural selection influences the gene pool by determining which alleles are perpetuated in particular environments. This selection process generates the unique alleles and allelic frequencies, which reflect specific ecological, physiological, and behavioral adaptations that are optimized for survival in different environments.

1.2.3 Redundancy

Species-level redundancy is the ability of a species to withstand catastrophic events. Redundancy protects species against the unpredictable and highly consequential events for which adaptation is unlikely. In short, it is about spreading the risk. Redundancy is best achieved by having multiple populations widely distributed across the species' range. Having multiple populations reduces the likelihood that all populations are affected simultaneously, while having widely distributed populations reduces the likelihood of populations possessing similar vulnerabilities to a catastrophic event. Given sufficient redundancy, single or multiple catastrophic events are unlikely to cause the extinction of a species. Thus, the greater redundancy a species has, the more viable it will be. Furthermore, the more populations and the more diverse or widespread that these populations are, the more likely it is that the adaptive diversity of the species will be preserved. Having multiple populations distributed across the range of the species, will help preserve the breadth of adaptive diversity, and hence, the evolutionary flexibility of the species.

Chapter 2. Species Ecology

In this chapter, we briefly describe the *B. affinis* taxonomy and discuss the species' life history characteristics at the individual, population, and species levels. This is not an exhaustive review of the species natural history; rather, it provides the ecological basis for the SSA analyses conducted in Chapters 3-7.

2.1 Species description and taxonomy

All bumble bees, including, *B. affinis*, belong to the genus *Bombus* (within the family Apidae), which includes approximately 250 species found primarily in temperate regions of North America, Central America, South America, Europe, and Asia. There are 23 *Bombus* species in the eastern U.S. *Bombus affinis* belongs to the subgenus, *B. sensu stricta,* which also includes 3 other species in the U.S. (Williams *et al.* 2008, p. 53).

2.2 Individual-level ecology

Bombus affinis is a eusocial (highly social) organism forming colonies consisting of a single queen, female workers, and males. Colony sizes of *B. affinis* are considered large compared to other bumble bees, and healthy colonies may consist of up to 1000 individual workers in a season (Macfarlane *et al.* 1994, pp. 3-4). Queens and workers differ slightly in size and coloration; queens are larger than workers (Plath 1922, p. 192, Mitchell 1962, p. 518). All *B. affinis* have entirely black heads, but only workers and males have a rusty reddish patch centrally located on the abdomen.

Bombus affinis annual cycle begins in early spring with colony initiation by solitary queens and progresses with the production of workers throughout the summer and ending with the production of reproductives, males and new queens, in mid to late summer and early fall (Macfarlane *et al.* 1994, p.4; Colla and Dumesh 2010, p. 45; Plath 1922, p. 192). The males and new queens disperse to mate and the original founding queen, males, and workers die. The new queens go into diapause (a form of hibernation) over winter. The following spring, the queen, or foundress, searches for suitable nest sites and collects nectar and pollen from flowers to support the production of her eggs, which are fertilized by sperm she has stored since mating the previous fall. She is solely responsible for establishing the colony. As the workers hatch and the colony grows, they assume the responsibility of food collection, colony defense, and care of the young, while the foundress remains within the nest and continues to lays eggs. During later stages of colony development, in mid-July or August to September, the new queens and males hatch from eggs. At the end of the season the foundress dies and the new queens (gynes, or reproductive females) mate before hibernating.

Bombus affinis has been observed and collected in a variety of habitats, including prairies, woodlands, marshes, agricultural landscapes, and residential parks and gardens (Colla and Packer 2008, p. 1381; Colla and Dumesh 2010, p. 46 ; USFWS RPBB unpublished geodatabase 2016). *Bombus affinis* requires areas that support sufficient food (nectar and pollen from diverse and abundant flowers), undisturbed nesting sites in proximity to floral resources, and overwintering sites for hibernating queens (Goulson *et al.* 2015, p. 2; Potts *et al.* 2010, p. 349).

Bumble bees are generalist foragers, meaning they gather pollen and nectar from a wide variety of flowering plants (Xerces 2013, pp. 27-28). *Bombus affinis* is a short-tongued species (Medler 1962, p. 214), so they are not able to easily access the nectar in flowers with deep corollas (all of the petals of a flower). The species is one of the first to emerge early in the spring and the last to go into hibernation, so to meet its nutritional needs, *B. affinis* requires a constant and diverse supply of flowers that bloom throughout the colony's long life cycle, from April through September (MacFarlane *et al.* 1994, p. 5). The nectar from flowers provides carbohydrates and the pollen provides *B. affinis* with protein. The number of queens that a colony can produce is directly related to the amount of pollen that is available (Burns 2004, p. 150). It has been suggested that *B. affinis* needs floral resources in close proximity to its nest sites, because studies of other *Bombus* species typically exhibit foraging distances of less than 1 km from their nesting sites (Knight *et al.* 2005, p. 1816; Wolf and Moritz 2008, p. 422; Dramstad 1996, pp. 163-182; Osborne *et al.* 1999, pp. 524-526; Rao and Strange 2012, pp. 909-911). *Bombus affinis* may also be dependent on woodland spring ephemeral flowers because of the species' early emergence in the spring and is often associated near woodland habitats (Colla and Dumesh 2010, p. 45-46). The availability of floral resources is dependent on the proper soil and precipitation conditions to sustain them. Extended periods of drought, for instance, may lessen the availability and diversity of flowering plants in a given area because plant phenology is primarily driven by temperature, precipitation, and the timing of snowmelt in the spring (Inouye and Wielgolaski 2003, p. 207; Wielgolaski and Inouye 2003, pp. 179-181; Pyke *et al*. 2016, p. 12).

Bombus affinis nests are typically in abandoned rodent nests or other similar cavities, one to four feet below ground (Plath 1922, pp. 190-191; Macfarlane *et al.* 1994, p. 4). *Bombus affinis* nests have also been occasionally observed above ground (Plath 1922, p. 190). Little is known about the overwintering habitats of *B. affinis* foundress queens, but other species of *Bombus* typically form a chamber in soft soil, a few centimeters deep and sometimes use compost or mole hills to overwinter (Goulson 2010, p. 11).

Bombus affinis live in temperate climates, and are not likely to sustain prolonged periods of high temperatures (over 35 ^{o} Celsius (C) (95° Fahrenheit (F)) (Goulson, pers. comm. 2016). *Bombus* are able to fly in cool temperatures and low light levels, particularly in comparison to other bees, which can extend their daytime foraging times (Corbet *et al.* 1993, p. 20). Table 2.1 summarizes ecological requirements at the individual level.

Table 2.1. The ecological requisites for survival and reproductive success of *B. affinis* individuals.

Life Stage	Winter	Spring	Summer	Autumn
Queen		Diverse floral resources; suitable nest habitat	Diverse floral resources; suitable nest habitat	Diverse floral resources; suitable nest habitat
Worker females		Diverse floral resources in close proximity to nest	Diverse floral resources in close proximity to nest	Diverse floral resources in close proximity to nest
Males			Diverse floral resources	Diverse floral resources; suitable dispersal habitat
Gynes (new foundress queens)	Suitable diapause sites		Diverse floral resources	Diverse floral resources; suitable dispersal habitat

2.3 Population-level ecology

Population viability requires healthy demographics and sufficient habitat to support a healthy demography; specifically, viability is a function of population size (N) and its population growth rate (lambda, λ) (Table 2.2). The population structure of *B. affinis* operates similarly to a metapopulation. A metapopulation is an assemblage of interacting subpopulations; a population of *B. affinis* is a collection of interacting colonies. But, whereas a subpopulation is composed of many reproductive individuals, a *B. affinis* colony is founded by a single queen, and thus, a colony represents one reproductive unit. The population size of *B. affinis* is, therefore, the number of successful nests or colonies—not individuals--comprising a given geographical area.

The size of a population influences population viability through the processes of demographic and environmental stochasticity. The number of colonies required to ensure long-term persistence of the population is unknown and likely varies across spatial scales. As small populations are inherently more vulnerable to extirpation due to environmental and demographic stochasticity (Goulson and Darvill 2008, pp. 197-198), generally speaking, the larger the population, the higher the likelihood of persistence over time (Hanski 1999, p. 36). The number of colonies comprising a population is determined by the number of foundresses, which is, in turned, determined by the number of mated gynes. The number of mated gynes and their overwinter survival is influenced by habitat quality and quantity, specifically, quality and density of the floral resources and the proximity of these resources to nest sites and overwintering sites. Given that several kilos of food are needed to support a single nest (Goulson, pers. comm. 2016) and supposing that hundreds of nests represent a minimum population size, the amount of habitat needed to support a population is likely quite large. This is particularly true for *B. affinis,* as their colonies are large relative to most *Bombus* species, typically numbering in the hundreds of individuals (Macfarlane *et al.* 1994, pp. 3-4).

In addition to habitat availability, the number of mated gynes, and hence the number of colonies, is also influenced by the number of fertile males and whether the landscape matrix is conducive to dispersal of reproductives. *Bombus affinis* typically disperse over 1 km distances, but the landscape must be permeable and free of hazards in order for unrelated gynes and males to successfully find and mate with each other. Thus, connectivity among colonies is also essential for successful recruitment of next year's queens, and therefore, is influential in determining population size.

Population size also affects population viability through genetic health. Small populations have lower levels of genetic diversity (heterozygosity), which reduces the capacity of a population to respond to environmental change and may lead to reduced population fitness, such as longevity and fecundity, via inbreeding depression (Darvill *et al.* 2006, p. 608). Populations of monoandrous (colonies headed by a single queen who mates with a single male) social species, such as *B. affinis*, are especially vulnerable to inbreeding depression, because the rate of genetic drift in a population is determined by the effective population size, Ne, which is much lower than the number of individuals occupying an area (Goulson and Darvill 2008, pp. 197-198; Darvill *et al.* 2006, p. 602). The Ne in bumble bees is 1.5 times the number of successful nests, not 2 times, as is the case with diploid-diploid organisms (Goulson and Darvill 2008, pp. 197-198).

The reproductive system renders bumble bees particularly sensitive to loss of genetic diversity. *Bombus* species are haplodiploidy (*i.e.*, males are haploid and females are diploid) and exhibited a single locus complementary sex determination (sl-CSD) system (Zayed 2009, p.238). Typically, heterozygotes at the

sex-determining locus develop into diploid females from fertilized eggs, while hemizygotes develop into haploid males from unfertilized eggs (Zayed 2009, p. 239). In cases, however, where females mate with haploid males that share a sex-determining allele in common (called "matched mating"), half of the females' progeny will be homozygous at the sex-determining locus and will consequently develop into diploid males instead of females. As males do not contribute resources to the colony, homozygosity at the sex-determining locus imposes a cost to the colony by decreasing the number of females produced (Ellis *et al.* 2006, p. 4376). Additionally, diploid males are unviable, or if viable and mate, produce diploid sperm, which will lead to unviable fertilized eggs or sterile triploid daughters (Zayed 2009, p. 239 and references within), so those males that are produced are unable to contribute to next year's cohort. Matched mating occurs most often when allele diversity at the sex-determining locus is low (Ellis *et al.* 2006, p. 4376; Zayed 2009, pp. 239-241). Thus, as Ne decreases, the likelihood of producing diploid males increase, which will further reduce the population size, potentially resulting in a negative, reinforcing downward cycle (*i.e*., extinction vortex). Zayed and Packer (2005, pp. 10743-10744) found, through modeling simulations, that extinction risks in haplodiploid populations were an order of magnitude higher than probabilities of extinction due to inbreeding depression in diploid populations. They attributed this high extinction risk to the effects of the "diploid male vortex"; a phenomenon where diploid males initiate a positive feedback cycle that leads to rapid extinction. Several species of bumble bee in England have demonstrated a dynamic consistent with this negative, reinforcing pattern. *Bombus subterraneous*, for example, following reduction in population size due to habitat loss eventually went extinct despite continued suitability of habitat (Darvill *et al.* 2006, p. 608). Maintaining genetic diversity within populations, thus, requires large Ne and gene flow within and among populations.

The viability of a population is also determined by its long-term lambda; in order for any population to persist over time, its growth rate, λ, must exceed 1.0. Species that fluctuate greatly with environmental conditions, require strong lambdas over time to avoid extirpation. The minimum λ needed to sustain a *B. affinis* population over time is unknown, but insects are particularly susceptible to environmental stochasticity. Although bumble bees, because of their relatively larger body size and fuzzy bodies, are not as strongly influenced by environmental conditions, climatic conditions affect the availability of requisite resources, and hence, bumble bee numbers. Pollen and nectar availability, especially in spring and fall when floral resources are scarcer, are influenced by environmental conditions (Holm 1966, pp. 156-157); in years with unfavorable weather, the supply of food is limited, leading to smaller and fewer colonies. Thus, population viability requires occupying areas with a diversity of environmental conditions (spatial heterogeneity) to ensure floral resources are available throughout the season and year-to-year despite variations in climatic variables, such as temperature and precipitation. Similarly, spatial heterogeneity increases the likelihood of asynchrony among colonies, a pre-requisite for metapopulation long-term persistence (Hanski 1999, p. 28). In spatially heterogeneous populations, it is unlikely that the entire population will contemporaneously experience the same environmental conditions, thus ensuring that not all colonies comprising a population will fail due to unfavorable conditions.

In summary, the significant determinants of population-level viability for *B. affinis* are a healthy demography and sufficient quality habitat to support this demography. The demography of *B. affinis* populations is a function of its population size (the number of successful nests) and its population growth rate over time. The population size required to support a viable population is likely variable across spatial scales and is unknown, but generally speaking, the larger the population, the more genetically healthy and thus the more robust to extirpation. Similarly, the minimum long-term λ required to sustain a population over time is unknown, but it must exceed 1.0 and likely must be higher,

given the susceptibility to environmental stochasticity. Both of these variables, N and λ, are dependent upon the amount and quality of floral resources, nest sites, and overwinter sites across temporal scales (within and among years). A precise estimate of the area of habitat required to support a viable population is dependent on the density and quality of floral resources, but given the large amount of food needed to support successful colonies, it is reasonable to assume a large area is required. Another important aspect of population viability is connectivity among colonies to ensure mating of unrelated reproductives and connectivity among populations to maintain within population genetic diversity. Lastly, the degree of spatial heterogeneity across the population area reduces the chances of all colonies failing concurrently due to poor environmental conditions, and thus, is important for long-term persistence.

Table 2.2. The requisites for survival and reproductive success of *B. affinis* populations.

Population Health (fitness)	Element	Importance
Healthy demography	Large Ne	multiple, successful colonies
	Permeable matrix	to find unrelated mates
Habitat to support a healthy demography	Sufficient floral resources	quantity of nectar & pollen
	Nesting & overwinter sites	
	Permeable matrix	to safely & efficiently find food
	Heterogeneity	diverse environmental conditions

2.4 Species-level ecology

In this section, we describe the ecological requirements at the species-level in terms of the 3Rs. The species level ecological requirements are discussed below and summarized in Table 2.4.

2.4.1 Resiliency

Resiliency is the ability to sustain populations in the face of environmental variation and transient perturbations. *Bombus affinis* resiliency is a function of the number of populations and the distribution of these populations relative to degree and spatial extent of environmental stochasticity (Figure 2.1). Generally speaking, the greater number of healthy populations and spatial heterogeneity occupied by the species, the greater likelihood of sustaining populations through time. Healthy populations (*i.e.*, large N, positive growth rates, and spatially heterogeneous) are better able to recover from stochastic events and withstand variation in the environment. Thus, the greater number of healthy populations, the more resiliency the species possesses. Environmental stochasticity acts at local and regional scales, and thus, populations can fluctuate in synchrony over broad geographical areas (Hanski 1999, p. 372). Additionally, over longer periods, landscape and habitat changes can be synchronized over large areas, leading to correlated extinction risks among populations at a larger regional scale (Hanski 199, pp. 381-382). For example, analyses of butterfly, moth, and aphid dynamics over wide areas in Britain indicate that populations can fluctuate in synchrony over areas of at least 10^5 km^2 (Hanski 1999, pp. 381-382). Thus, having populations distributed across a diversity of environmental conditions helps guard against concurrent losses of populations at local and regional scales by inducing asynchronatic fluctuations

among populations. The greater degree of spatial heterogeneity (specifically, the diversity of temperature and precipitation conditions occupied by *B. affinis*), the greater resiliency the species will possess. Lastly, resiliency is also influenced by the degree of connectivity among populations. Movement among populations is essential for genetic health via gene flow and demographic rescue. Thus, connectivity among *B. affinis* populations is also a requisite for species level resiliency.

Given the above, *B. affinis* resiliency can be described as having healthy populations distributed across an array of climatic conditions (spatial heterogeneity). As described under the Population-level Ecology section, a healthy *B. affinis* population is one that includes multiple, successful colonies; has suitable floral resources to support a large population size; has sufficient connectivity among colonies; and occupies a spatially heterogeneous area. Spatial heterogeneity means occupying a diversity of climates suitable for *B. affinis* activity and for long growing seasons for floral resources.

2.4.2 Representation

Representation is the ability of the species to adapt to physical (*e.g*., climate conditions, habitat conditions or structure across large areas) and biological (*e.g*., novel diseases, pathogens, predators) changes in its environment presently and into the future; it is the evolutionary capacity or flexibility of the species. Representation is the range of variation found in a species, and this variation--called adaptive diversity--is the source of species' adaptive capabilities. *Bombus affinis* adaptive diversity is a function of the amount and spatial distribution of genetic and phenotypic diversity (Figure 2.1). By maintaining these two sources of adaptive diversity across a species' range, the responsiveness and adaptability of a species over time is preserved.

Genetic diversity is the primary fuel for adapting to changing environmental conditions (Hendry *et al*. 2011, pp. 164-165); for adaptation to occur, there must be variation upon which to act (Lankau *et al.* 2011, p. 320). The genetic diversity of *B. affinis* is determined by its allele diversity (size of its gene pool), which is influenced by the level of gene flow among populations and the rates of genetic drift within populations. Gene flow is influenced by the degree of connectivity and landscape permeability (Lankau *et al.* 2011, p. 320). *Bombus affinis* was included as a target species in a genetic study of several *Bombus* species (Cameron *et al.* 2011b, p. 664), however, an insufficient number of *B. affinis* individuals were captured to complete an analysis. Thus, we looked to the broader literature on *Bombus* species to gain insights on *B. affinis* genetic variation across the range.

Many bumble bee species exhibit high levels of gene flow at multiple spatial scales (Woodard *et al.* 2015 and refs within, p. 2924, Lozier *et al*. 2011, pp. 4880-4882; Cameron *et al*. 2011b, pp. 664-665), and as such, show little genetic structure at local or regional scales. Although, natural barriers (*e.g.,* large water bodies) and elevation gradients can limit dispersal (Woodard *et al.* 2015, p. 2924), and hence, lead to genetic structuring. Based on genetic studies of other bumble bee species with similar ecologies, and given the dispersal capabilities of *B*. *affinis*, it is unlikely that the species exhibits much genetic differentiation across its broad range. To preserve the breadth of genetic diversity, it is important to maintain high levels of gene flow among populations. Genetic variation can be negatively affected by genetic drift, which is driven by Ne; populations with small Ne experience stronger drift than those with large Ne (Zayed 2009, p. 246). Thus, preserving the genetic diversity of *B. affinis* requires maintaining large populations and connectivity among the populations.

Phenotypic diversity (the physiological, ecological, and behavioral variation expressed by *B. affinis*) is also important for adapting to changes in environmental conditions. Phenotypic variation determines how organisms interact with their environment and how they respond to selection pressures (Hendry *et al.* 2011, p. 161). The degree of phenotypic variation is determined by the diversity of physical and biological pressures to which organisms are exposed, which vary across spatial and temporal scales. As such, species that span environmental gradients are expected to harbor the most phenotypic and genetic variation (Lankau *et al.* 2011, p. 320). Thus, preserving the breadth of phenotypic diversity of *B. affinis* requires maintaining populations across historical latitudinal, longitudinal, and elevational gradients, as well as climatic gradients; doing so, increases the likelihood that the species will retain the potential for adaptation over time. *Bombus affinis* representation is, therefore, described as having healthy populations widely distributed across a breadth of ecological conditions.

2.4.3 Redundancy

Species-level redundancy reflects the ability of a species to withstand catastrophic events, and is best achieved by having multiple, widely distributed populations relative to the spatial occurrence of catastrophic events. In addition to guarding against a single or series of catastrophic events extirpating all populations of *B. affinis*, redundancy is important to protect against losing irreplaceable sources of adaptive diversity. Having multiple populations distributed across the range of the species, will help preserve the breadth of adaptive diversity, and hence, the evolutionary flexibility of the species. Thus, *B. affinis* redundancy is described as having multiple, healthy populations widely distributed across the breadth of adaptive diversity relative to the spatial occurrence of catastrophic events.

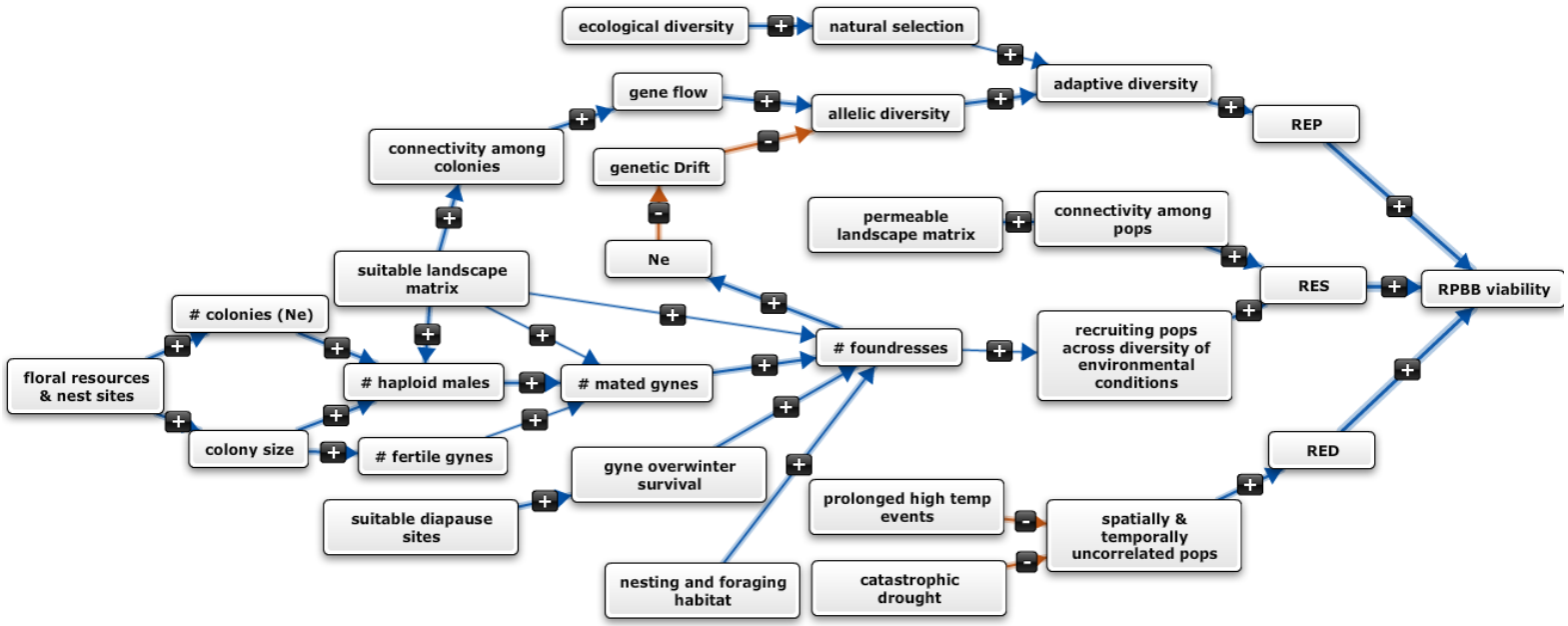
2.5 Synopsis

Viability is the ability to sustain populations over time. To do this, *B. affinis* needs a sufficient number and distribution of viable populations to withstand environmental stochasticity (resiliency), catastrophes (redundancy), and changes in its environment (representation) (Table 2.3).

Table 2.3. Ecological requirements for species-level viability

3 Rs	**Requisites of long-term viability**	**Description**
Resiliency *(able to withstand stochastic events)*	Interconnected, healthy populations across a diversity of climatic conditions	Populations with 1) large Ne, sufficient floral resources in close proximity to nesting and overwintering sites, 2) permeability among colonies, and 3) spatial heterogeneity; high connectivity among populations dispersed across diverse climatic conditions (spatial heterogenity)
Representation *(to maintain evolutionary capacity)*	Maintain adaptive diversity of the species	Healthy populations distributed across areas of unique adaptive diversity (*i.e* ., ecoregions)
	Maintain evolutionary processes	Maintain evolutionary drivers--gene flow, natural selection, genetic drift--to mimic historical patterns
Redundancy *(to withstand catastrophic events)*	Sufficient distribution of healthy populations	Sufficient distribution to guard against catastrophic events wiping out portions of the species adaptive diversity, i.e., to reduce covariance among populations
	Sufficient number of healthy populations	Adequate number of healthy populations to buffer against catastrophic losses of adaptive diversity

Figure 2.1 A conceptual model of the relationships among the ecological requirements of *B. affinis*. RPBB = *B. affinis*, REP = representation, RES = resiliency, RED= redundancy.



Chapter 3. Methods and Analysis of Historical Condition

In this Chapter, we describe the methods used to assess resiliency, representation, redundancy over time, and our analysis of the historical condition of *B. affinis*. Specifically, we report the trend in grid occupancy and relative abundance of *B. affinis*, and describe the spatial distribution of *B. affinis* populations over the historical period of 1900-1999.

For the purposes of our analyses, the historical condition is the reference condition; it provides the context for the current and future conditions. That is, historical condition is the baseline from which the current and future degrees of resiliency, representation, and redundancy are measured.

3.1 Methods

3.1.1 Resiliency

This analysis assesses the ability of the species to withstand the natural variation in environmental conditions and transient perturbations. Resiliency, thus, requires healthy populations distributed across an array of environmental conditions (namely, temperature and precipitation gradients). As explained in Chapter 1, we assumed that occurrences of *B. affinis* falling within 10 x 10 km^2 grids comprise a population. To assess the health of *B. affinis*, populations, we sought population-specific data pertaining to the population size (*i.e.,* number of colonies) and the habitat suitability of each population area (grid cell). To measure the degree of spatial heterogeneity occupied by *B. affinis* across the range, we intended to use the USDA's growing season zones as a proxy to characterize the diversity of climates occupied. We were unable, however, to obtain the USDA growing season shapefiles in time to complete an analysis of spatial heterogeneity. Thus, we relied on the trend in occurrences alone to assess *B. affinis* resiliency over time. Specifically, we analyzed the:

1. trend in number of grid occurrences over time
2. trend in % occupancy of bumble bee grids over time
3. trend in relative abundance of *B. affinis* over time

To assess trends in occurrence, we assigned grid occurrences as follows:

Historical populations = grid occurrences reported from 1900-1999
Current populations = grid occurrences reported from 2000-2015
Total populations = historical grid occurrences + new grid occurrences discovered since 1999

We calculated "trend in number of grid occurrences" by tallying the number of *B. affinis* grid occurrences within 10-year time periods beginning in 1950. Because the number of animals observed is influenced by sampling effort, we also evaluated "trend in % Occupancy" by dividing the # of *B. affinis* grid occurrences by the total number of *Bombus* grids surveyed in each 10-year time period. The number of *Bombus* grids are the grids with at least 1 *Bombus* species recorded. Information on grids that were sampled but had no *Bombus* occurrences was not available, and thus, the analysis does not fully represent sampling effort. We calculated "trend in relative abundance" by dividing the number of *B. affinis* specimens sampled by the total number of *Bombus* species sampled for each 10-year time

period. We used the *z* tests of equal proportions to evaluate whether the change in trends are statistically significant:

$$z = (P_h - P_c) \div \sqrt{[(P_h(1-P_h)/n_h) + (P_c(1-P_c)/n_c)]},$$

where P_h is the estimated historical relative abundance of *B. affinis*, P_c is the estimated current relative abundance of *B. affinis*, n_h is the total historical abundance across all bumble bee species, and n_c is the total current abundance of all bumble bee species.

The fundamental assumptions applied in our resiliency analysis include:

1. Each 10 km x 10 km grid cell represents a single population
2. A grid occurrence represents a healthy population
3. Trend in occurrences is an indicator of the species' ability to withstand environmental stochasticity

3.1.2 Representation

This analysis assesses the species' ability to adapt to physical and biological changes in its environment. Its adaptive capacity is influenced by the species' degree of adaptive diversity. To best capture the breadth of adaptive diversity, we selected the Bailey Ecoregions (Bailey 1983, Bailey *et al.* 1994) and the equivalent Canadian Ecoregions (Ecological Stratification Working Group, 1996) to delineate the unique areas of adaptive diversity (henceforth referred to as ecoregions). Both data sources identify a hierarchy of ecosystems that characterize the landscape by relatively homogeneous biophysical and climatic conditions. Bailey's ecoregions include four geographic levels; domains, divisions, provinces, and subregions. In the U.S., the division scale was selected because boundaries are differentiated based on precipitation levels and patterns as well as temperature, which are two important determinants of *B. affinis* survival and reproductive success. Domains proved to be too large and did not adequately parse out the data, while subregions broke the data out at too fine a scale and many subregions were not represented by the data. The subregions and provinces are differentiated based on terrain features and we felt this would not significantly influence *B. affinis* adaptive capability. In Canada, Ecoregions were the most equivalent classification order to Bailey's divisions in the U.S. The classification codes for each unique U.S. division and Canadian ecoregion were appended to the *Bombus* database and used in evaluating representation (Figure 3.1, Table 3.1).

To assess *B. affinis* representation over time, we evaluated the changes in adaptive diversity over several spatial scales. We used spatial extent as a proxy for adaptive diversity. Specifically, we evaluated *B. affinis* spatial extent by:

1. trend in the number of ecoregions, states, and counties occupied
2. trend in the extent of occurrence (EoO) rangewide and within the ecoregions

We defined spatial extent as the proportion of the range/ecoregion occupied by *B. affinis*. We multiplied the number of *B. affinis* grid occurrences reported for the unit of interest (*i.e.*, rangewide or ecoregion) by 10,000 (area of a 10 km x 10 km grid) to calculate the extent of occurrence (EoO) for that unit. Using the number of grid occurrences reported for each decade, we calculated the rangewide and ecoregion EoOs. We then calculated the historical and current spatial extents by dividing the time

period EoO by the total EoO. "Total" EoO was derived by multiplying the number of total occurrences from 1900 through 2015 (n=894) by 10,000.

The fundamental assumptions applied in our representation analysis are:

1. Ecoregions accurately capture the full spectrum of *B. affinis* adaptive diversity
2. Gains or losses in spatial extent represent commensurate gains or losses of adaptive diversity

Figure 3.1. The representation units delineated for *B. affinis*. Units were derived using the Bailey's Ecoregions in the U.S. and the Ecological Stratification Working Group Ecoregions in Canada.

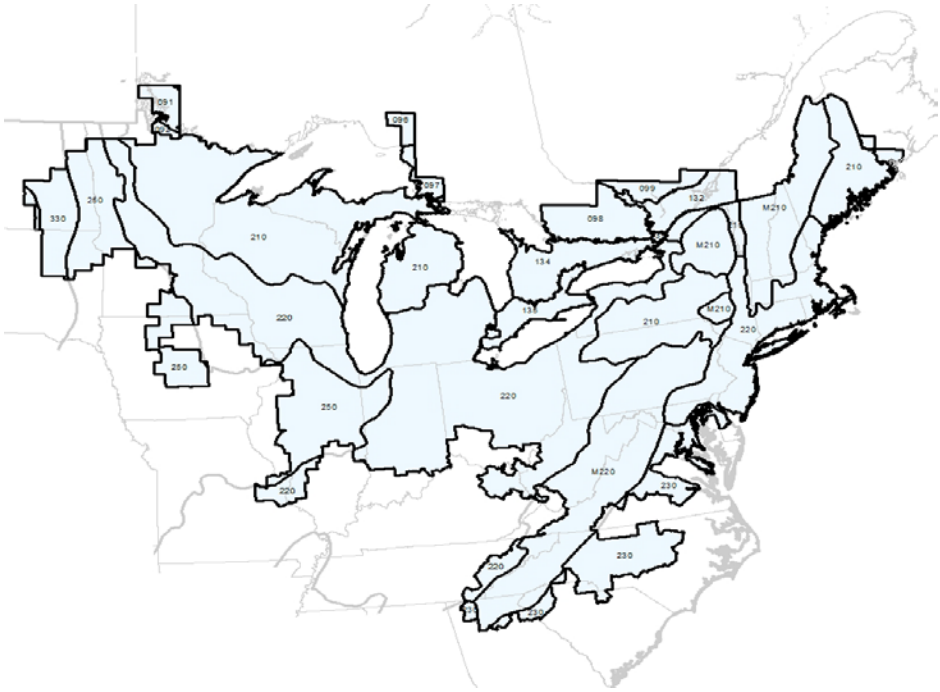


Table 3.1. The Bailey's division/Canada ecoregion name, corresponding number, and states/provinces falling within the ecoregions (Bailey *et al.* 1994).

Ecoregion Name	**Ecoregion #**	**U.S. State/Canada Province**
Lake of the Woods	91	ON
Abitibi Plains	96	ON
Algonquin-Lake Nipissing	98	ON
Southern Laurentians	99	QU
St. Lawrence Lowlands	132	ON, QU
Frontenac Axis	133	ON
Manitoulin-Lake Simcoe	134	ON
Lake Erie Lowland	135	ON
Warm Continental Division	210	ME, MI, MN, NY, PA, VT, WI
Hot Continental Division	220	CT, DE, GA, IL, IN, IA, KY, ME, MD, MA, MI, MN, MO, NH, NJ, NY, OH, PA, RI, TN, VA, WV, WI
Subtropical Division	230	DE, DC, GA, MD, NC, SC, VA
Prairie Division	250	IL, IN, IA, MN, MO, ND, SD, WI
Temperate Steppe Division	330	ND, SD
Warm Continental Division - Mountain Provinces	M210	CT, ME, MA, NH, NY, VT
Hot Continental Division - Mountain Provinces	M220	GA, KY, MD, NC, PA, SC, TN, VA, WV

3.1.3 Redundancy

This analysis assesses the ability of the species to withstand catastrophic events. Its ability to do so is a function of the number of populations and the distribution of those populations relative to the spatial occurrence of the catastrophic events. We analyzed two sources of catastrophic events: 1) severe drought and 2) prolonged, high temperatures. Other events, such as novel disease outbreaks, are plausible, but due to time constraints and lack of consistent data we did not include these in our analysis. Specifically, we evaluated *B. affinis* vulnerability to catastrophic events by:

1. calculating the likelihood of ecoregion-wide extirpation
2. assessing the spatial overlap of B. affinis occurrences and catastrophic events

To calculate the probability of ecoregion-wide extirpation, we used the following equation (Ruckelshaus *et al.* 2002, p.312):

$$p(X) = \left(1 - e^{-\lambda * t}\right)^{n},$$

where λ is the annual rate (frequency) of catastrophic events, t is the number of years of concern, and n is the number of grid occurrences in the ecoregion. We used the total number of grids occupied in the ecoregion for n and 25 years as the duration of time, t.

The p(X) is the probability of all grid occurrences within an ecoregion being extirpated (*i.e.,* no individuals survive) by a catastrophic event. We calculated the probabilities of extirpation for each grid within an ecoregion and then multiplied these grid-specific probabilities to obtain the p(X) ecoregion-wide.

We determined λ by calculating the frequencies of high temperature events and drought events. We defined a catastrophic temperature event as 14 or more days in which the maximum diurnal temperature is greater or equal to 35° C[2]. We defined a catastrophic drought event as a D3+ level or D4+ level drought intensity for a minimum of 14 days. A D3 level drought is characterized by major crop/pasture losses; D4 level drought is characterized as exceptional and widespread crop/pasture losses and shortage of water in reservoirs and streams (U.S. Drought Monitor, 2016).

We used the 2004–2014[3] Daymet daily maximum temperature data tiles from the Oak Ridge National Laboratory Distributed Active Archive Center (Thorton *et al.* 2014) to derive the frequency (λ) of high temperature events. Daymet tiles cover 2 degree longitude x 2 degree latitude areas, with a spatial resolution of 1 km. Each tile provides daily maximum temperatures for each pixel for an entire year. Tiles with known *B. affinis* grid occurrences were downloaded and loaded into yearly mosaic datasets in

[2] A peer reviewer indicated that our high temperature threshold may too high and that the risks are underestimated as we did not consider the synergistic effects of co-occurrence of high temperature and drought. A quick scan of the suggested literature indicates that we may have underestimated the risks; thus, subsequent analyses will evaluate the need to revise our definition of catastrophic event.

[3] The intent of describing the historical condition is not to predict the future vulnerability to catastrophic events based on its historical condition; but rather, it is to set the baseline from which we can measure changes in redundancy from the historical period to the current time period. For future conditions, however, we are interested in assessing the change in the degree of redundancy as well as the future vulnerability due to catastrophic events based on its current condition. To ensure redundancy analyses were comparable between time periods, we used the recent temperature and drought datasets for our historical and current redundancy analyses. The only difference, therefore, between the historical and current condition is the number of occurrences and the spatial dispersion.

a file geodatabase using ArcGIS (version 10.3.1). The maximum daily temperature in each *B. affinis* grid was calculated for each day from April 1 through September 29 for each of the 11 years (2004-2014).

We used the 2000–2014 weekly Vegetation Drought Response Index (VegDRI) data from the Earth Resources Observation and Science Center (Brown *et al.* 2008, 2012) to derive the frequency (λ) of drought events. VegDRI data cover two week periods, and combine satellite-based observations of vegetation conditions with climate and environmental data to provide drought information, and thus, are assumed to provide reasonable estimates of the availability of foraging sources for *B. affinis*. The data are at a 1 km spatial resolution and cover the entire lower 48 states; we used the closest U.S. grids to assess risk in Canada. We downloaded data for April through September from 2000 through 2012; the 2013-2015 data were in a different format and could not be processed in time for this analysis.

Both the Daymet and VegDRIdata were overlaid with *B. affinis* grid occurrences to calculate median λ values for each occurrence grid. The VegDRI values were scaled to translate to categorical drought intensities; drought values <96 were assigned to the moderate (D3+) drought category and values <81 assigned to the severe (D4+) drought category.

This model assumes identical and independent risks (*i.e.,* catastrophic events are uncorrelated). Spatial dispersion of occurrences (along with life history and genetic diversity), however, influence the likelihood of a single event extirpating multiple populations (Ruckelshaus *et al.* 2002, p. 314). Thus, to account for covariance among grids, we also incorporated a spatial dispersion analysis.

To evaluate vulnerability due to covariance among grid occurrences of an ecoregion, we overlaid the distribution of grid occurrences with drought and temperature λ values and visually evaluated the potential to lose geographical areas within an ecoregion.

The fundamental assumptions applied in our redundancy analysis include:

1. Drought frequencies in 2000-2012 represent the true risk of drought over the next 25 years; and the frequency of temperatures from 2004-2014 represent the true risk of prolonged high temperatures over the next 25 years.
2. Daily maximum temperature exceeding 35° C for 14 or more days will curtail *B. affinis* activity sufficiently such that the population collapses; conversely, temperatures below 35° C or for less than 14 days will not cause population collapses
3. There are not synergistic effects when high temperatures and drought co-occur
4. Moderate drought intensities will sufficiently reduce floral resources such that the population collapses. Conversely, drought intensities below the moderate level do not impose population level fitness problems.
5. No autocorrelation among grid occurrences. For example, the probability of a drought occurring in one grid does not affect the probability of drought occurring in another even if in close proximity to each other.

3.2 Analysis of Historical Condition

3.2.1 Resiliency

Resiliency is the ability to sustain populations in the face of environmental variation and transient perturbations. Bombus affinis resiliency is described as having healthy populations distributed across an array of climatic conditions.

Results

Trend in Occurrence - There are 845 *B. affinis* grid occurrences reported from the historical period of 1900-1999 (a total of 894 grid occurrences reported from 1900-2015). The number of grids occupied by *B. affinis* varied from a high of 337 grids pre-1950 to a low of 98 grids in the 1990s (Table 3.1.1). The proportion of bumble bee grids occupied by *B. affinis* (% Occupancy) varied by decade, with a low of 20% pre-1950 to a high of 28% in the 1980s; the median % Occupancy for the historical time period was 23% (Table 3.1.1). Abundance of *B. affinis* relative to all other *Bombus* species (Relative Abundance) varied from 7 to 10%, with a decade median of 8% overall (Table 3.2). *Bombus affinis* was among the top 4 *Bombus* species in Relative Abundance.

Table 3.2. *Bombus affinis* grid occurrence statistics from the historical time period. "Number of Grids" are the total number of grids having at least one *B. affinis* record during the 10-year time period. "% Occupancy" is the proportion of all *Bombus* grids having at least one *B. affinis* record. "Relative Abundance" is the proportion of the total number of bumble bee specimens sampled that were *B. affinis*. Historical: Number = total number of grids (not # of specimens) occupied by *B. affinis* from 1900 to 1999; % Occupancy = median % occupancy spanning 1900-1999 time period; Relative Abundance = median the Relative Abundance of *B. affinis* spanning 1900-1999 time period.

Time-period	Number	% Occupancy	Relative Abundance
Pre-1950s	337	20%	7%
1950s	137	21%	7%
1960s	209	25%	10%
1970s	171	23%	9%
1980s	146	28%	7%
1990s	98	23%	7%
Historical	845	23%	8%

3.2.2 Representation

Representation is the ability to adapt to changing environmental conditions; it's the species evolutionary capacity or flexibility. Bombus affinis representation is described as having healthy populations distributed across a wide breadth of ecological conditions (i.e., having populations distributed widely across the ecoregions).

Results

Distribution - *B. affinis* historically occupied large areas of Midwestern and Eastern U.S., with reaches up into southern Quebec and Ontario (Figure 3.2). The species was recorded from 31 states, 367 counties,

and 15 ecoregions (Table 3.3), but documented occurrences varied over time at all spatial scales. For example, the number of states occupied varied from 22 to 26; the number of counties varied from 49 to 91; and the number of ecoregions varied from 8 to 14. This variation is likely a reflection of both natural fluctuations in *B. affinis* abundance due to environmental stochasticity and sampling inconsistencies.

Figure 3.2. The historical range of *B. affinis*. Dots represent the counties occupied by *B. affinis* during the historical period (1900-1999). Black lines delineate the 15 ecoregions occupied by the species.

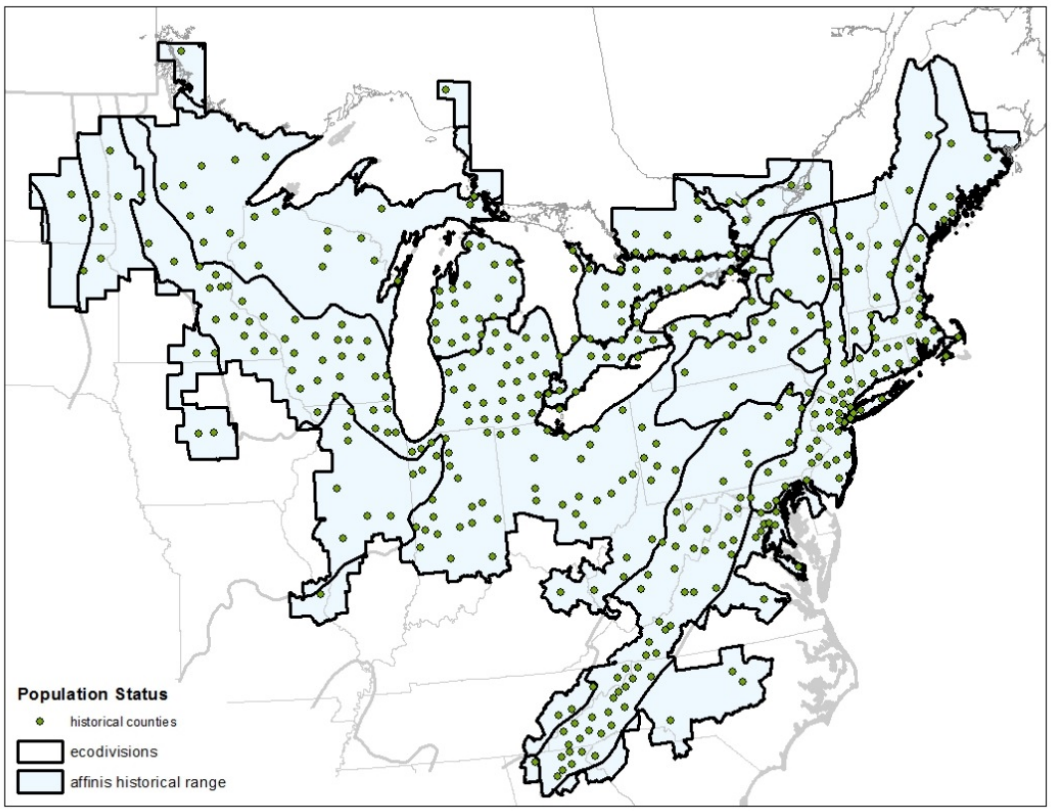


Table 3.3. The number of states, counties, and ecoregions occupied by *B. affinis* during the historical period. "Number of Units" is the number of states/counties/ecoregions having at least one *B. affinis* grid occurrence. "Historical" is the total number of units having at least one *B. affinis* grid occurrence over the time span of 1900-1999.

	Number of Units						
Unit	Pre-1950s	1950s	1960s	1970s	1980s	1990s	Historical
States	26	22	24	24	22	22	31
Counties	75	53	67	67	49	91	186
Ecoregions	12	10	14	13	10	8	15

Spatial Extent – The extent of occurrence (EoO) of *B. affinis* varied over time. For the historical time period, EoO was 95% of the total EoO for *B. affinis* (Table 3.4).

Table 3.4. The rangewide Extent of Occurrence for the historical time period. Extent of Occurrence (EoO) is the percent of the total spatial extent (*i.e.,* area occupied by 894 grids) occupied by *B. affinis* in each historical decade. "Historical" is the EoO over the time span of 1900-1999.

	Historical Extent of Occurrence						
Decade	pre-1950	1950s	1960s	1970s	1980s	1990s	Historical
EoO	38%	15%	23%	19%	16%	11%	95%

Similarly, the spatial extent within and among ecoregions varied over the historical time period. Among ecoregions, the number of grids occurrences reported for the historical period ranged from 1 grid occurrence in Ecoregions 91 and 96 to 445 grid occurrences in Ecoregion 220. Although the % of EoO occupied during the historical period was greater than 94% for all ecoregions, no ecoregion—as indicated by the average % Occupancy--was fully occupied in every decade (Table 3.2.3). The average % of the total EoO occupied ranged from a low of 15% in ecoregions 210, 250 and 330 to a high of 23% in Ecoregion 134. The median % of EoO occupied within ecoregions was 18%.

Table 3.5. The number of *B. affinis* occurrences and Extent of Occurrence (EoO) per ecoregion for the historical period. The "Number" is the number of grids within the ecoregions to have at least one *B. affinis* occurrence. Extent of Occurrence is the proportion of the total spatial extent of the ecoregion occupied during a decade; the % of the total occupied EoO and the average EoO for the historical period are provided. For example, in Ecoregion 132, 100% of the grids were occupied at some point during the period of 1900-1999, but the average # of grids occupied in any one decade was only 22%.

Ecoregion	Number	Extent of Occurrence	
	Historical	Historical	Average
132	10	100%	22%
133	4	100%	20%
134	39	100%	23%
135	36	97%	19%
210	105	96%	15%
220	445	94%	17%
230	24	96%	17%
250	18	56%	15%
330	4	100%	15%
91	1	100%	20%
98	8	100%	18%
99	1	100%	20%
M210	36	100%	16%
M220	113	98%	16%
96	1	100%	20%

3.3.3 Redundancy

Redundancy is the ability of a species to guard against losses of adaptive diversity due to catastrophic events. Bombus affinis redundancy is described as having multiple, healthy populations widely distributed across the breadth of adaptive diversity relative to the spatial occurrence of catastrophic events.

Results

Probability of Ecoregion Extirpation - The frequency or chance (λ) of a catastrophic event occurring varies by grid, but in most cases the probabilities are low. The frequency of temperatures exceeding 35° C (95 ^{o} F) is 0.0 for 800 of the 802 historical grids; the remaining 2 have frequencies less than 0.10 (Table 3.6). The frequency of a catastrophic drought occurring varies among grids and with drought intensity (D3+ or D4+ level drought), but the chances of a catastrophic event occurring is low for most grids. For example, of the 802 historical grids analyzed, 67% (536) and 96% (771) of the grids have frequencies of less than 0.30 for D3+ and D4+ intensities, respectively. Due to the low frequencies of a catastrophic event in the majority of the grids, the risk of ecoregion-wide extinction is zero for all ecoregions (Table 3.7).

Table 3.6. The cumulative frequency (λ) distribution for the three types of catastrophic events: high temperature, D3+ level drought, and D4+ level drought. Column 1 is the drought frequency (λ) values; the Catastrophic Event columns provide the tally of grids with frequencies equal to or less than the specified λ value. For example, there are 802 grids that had high temperature events λ of 0.10 or less. Similarly, there are 240 and 634 grids that had D3+ and D4+ drought event λ of 0.10 or less, respectively.

	Catastrophic Events		
λ	High Temp	D3+ Drought	D4+ Drought
0.00	800	47	365
0.10	802	240	634
0.20	802	391	738
0.30	802	536	771
0.40	802	696	797
0.50	802	744	802
0.60	802	777	802
0.70	802	801	802
0.80	802	802	802
0.90	802	802	802
1.00	802	802	802

Table 3.7. The probability of ecoregion-wide extirpation. Ecoregion extirpation, p(X), is the probability that all populations within an ecoregion are extirpated due to either 1) a high temperature or D3+ drought event or 2) high temperature or D4+ drought event.

Ecoregion	Temp/D3+ p(X)	Temp/D4+ p(X)
91	0.00	0.00
96	0.00	0.00
98	0.00	0.00
99	0.00	0.00
132	0.00	0.00
133	0.00	0.00
134	0.00	0.00
135	0.00	0.00
210	0.00	0.00
220	0.00	0.00
230	0.00	0.00
250	0.00	0.00
330	0.00	0.00
M210	0.00	0.00
M220	0.00	0.00

Spatial dispersion – The frequencies of catastrophic drought varied by grid, and thus, the risk of a catastrophic event wiping out all populations is a function of both the number of grid occurrences and the spatial overlap between the grid occurrences and areas at risk of a catastrophic event. Furthermore, within ecoregion losses can, especially for the large ecoregions, lead to losses of adaptive diversity. Suppose, for example, that an ecoregion has 10 grid occurrences, of which a small subset have high p(X) values. The low probabilities, especially those with no risk, of the remaining occurrences mathematically yield a low risk for the ecoregion as a whole. Although the chance of losing all occurrences within an ecoregion may be low, losing a subset of the occurrences could result in large reductions in spatial extent, and thus, lead to losses of adaptive diversity. Thus, to fully describe the degree of redundancy, it is important to evaluate risk as a function of both the number of occurrences and the extent of the ecoregion at risk to catastrophic events. Due to time constraints, however, we were unable to assess the degree of spatial overlap within ecoregions.

3.4 Synopsis

Bombus affinis occurrence varies over time, as is expected due to inherent fluctuations in insect populations and sampling inconsistencies. Despite these limitations, clearly *B. affinis* was an abundant species with hundreds of occurrences across an expansive range. It occurred in 31 states and occupied 15 different ecological regions. Although not analyzed specifically, the species' wide distribution most certainly encompassed high spatial heterogeneity allowing *B. affinis* to fluctuate asynchronously in response to varying environmental conditions. Due to the large number of occurrences within the ecoregions, historically, the risk of losing areas of adaptive diversity due to a catastrophic event was extremely unlikely.

Chapter 4. Analysis of Current Condition

In this Chapter, we describe our analysis of the current condition of *B. affinis*. Specifically, we report the trend in grid occupancy and relative abundance, and describe the number and spatial distribution of *B. affinis* during the 2000-2015 time period.

4.1 Resiliency

Resiliency is the ability to sustain populations in the face of environmental variation and transient perturbations. Bombus affinis resiliency is described as having healthy populations distributed across an array of climatic conditions.

Methods - See Chapter 3 for an explanation of the methods.

Results

Trend in Occurrence - There are 69 *B. affinis* grid occurrences reported for the current time period of 2000 through 2015 (Table 4.1.1). The percent of bumble bee grids occupied by *B. affinis* is 5%, and the abundance of *B. affinis* relative to total number of *Bombus* specimens is 1% (Table 4.1.)[4].

Table 4.1. *B. affinis* grid occurrence statistics from the current time period. "Number of Grids" is the total number of grids having at least one *B. affinis* record. "% Occupancy" is the proportion of all *Bombus* grids sampled having at least one *B. affinis* record. "Relative Abundance" is the proportion of the total number of bumble bee specimens sampled that were *B. affinis*. Current: Number = total number of grids occupied by *B. affinis* from 2000-2015; % Occupancy = % occupancy spanning 2000-2015 time period; Relative Abundance = the relative abundance of *B. affinis* spanning 2000-2015 time period.

Time period	Number	% Occupancy	Relative Abundance
2000	42	4%	0.01
2010	32	5%	0.01
Current	69	5%	0.01

Population health - We received information for 25 of the 69 current populations (Appendix 2). Of these, 18 (72%) have at least one high severity stressor affecting 50% or more of the 25 km^2 area analyzed. Two (8%) of the 25 populations have no or only low severity stressors occurring within the 25 km^2 area. Pathogens and small population effects are the two most commonly reported stressors in terms of high severity and broad extent (Table 4.2).

[4] Through peer review, we learned of recent survey efforts in Maine and Vermont. There are 9000+ additional *Bombus* records since 2010; none of which were *B. affinis*. Undoubtedly, including these records in our analyses would decrease the % Occupancy and Relative abundance estimates.

Table 4.2. A summary of the severity and extent of stressors at a subset of *B. affinis* populations (see Chapter 3 for methods).

Severity	Pathogen	Pesticide	Habitat Loss/ Degradation	Small Popn Size
High (3) Level Stressor	16	9	4	19
Medium (2) Level Stressor	1	8	12	1
Low (1) Level Stressor	0	4	1	0
Extent				
Broad (>=75%) Extent	18	6	14	17
Moderate (26-74%) Extent	0	0	0	1
Small (<=25%) Extent	1	2	1	0

4.2 Representation

Representation is the ability to adapt to changing environmental conditions; it's the species evolutionary capacity or flexibility. Bombus affinis representation is described as having healthy populations distributed across a wide breadth of ecological conditions *(i.e., having populations distributed widely across the ecoregions).*

Methods - See Chapter 3 for an explanation of the methods.

Results

Distribution - Recent records (2000-2015) indicate that *B. affinis* range has declined (Table 4.2.1); it currently exists in 13 states and 1 province (IL, IN, MA, MD, ME, MN, NC, OH, PA, TN, VA, WI, and Ontario), 41 counties, and 6 ecoregions (Figure 4.1). The number of states occupied has declined by 68%, the number of counties occupied by 89%, and the number of ecoregions occupied by 60% (Table 4.3).

Figure 4.1. *Bombus affinis* range map showing the current distribution. Dots represent counties with *B. affinis* at least 1 record since 2000. X represent counties with historical occurrences only (*i.e.*, no *B. affinis* records since 2000).

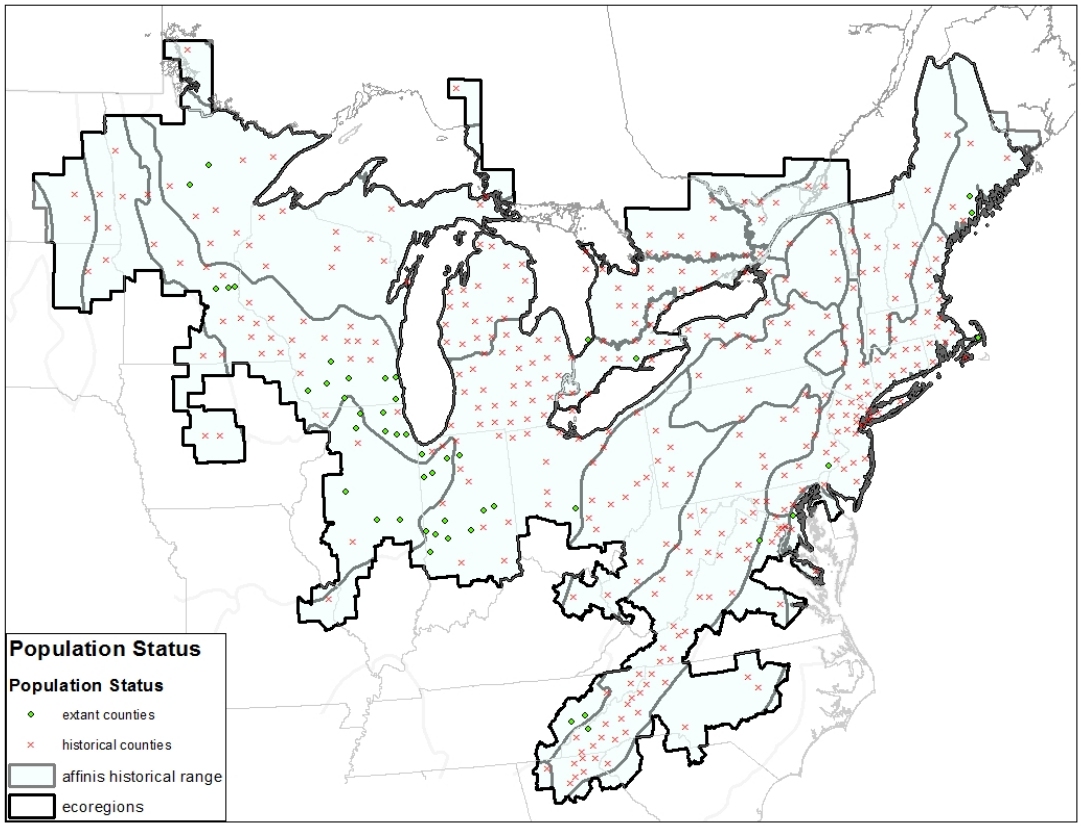


Table 4.3. The number of states, counties, and ecoregions occupied by *B. affinis* during the current time period. "Number of Units" is the number of states/counties/ecoregions having at least one *B. affinis* grid occurrence. "Current" is the total number of units having at least one *B. affinis* grid occurrence over the time span of 2000-2015. % Occupancy is the percent of historical units occupied during the Current time period (2000-2015).

	Number of Units			
Unit	2000s	2010s	Current	% Occupancy
States	10	4	10	32%
Counties	25	16	42	11%
Ecoregions	6	3	6	40%

Spatial Extent- The current spatial extent, EoO, of *B. affinis* is 8% (*i.e.*, 8% of the total documented range was occupied during the time period of 2000-2015) (Table 4.4). The number of grid occurrences within the ecoregions varies from 0 to 42 (Table 4.5). The percent of the total EoO occupied in the current time period varies from 0% to 50% among the ecoregions (Table 4.5).

Table 4.4. The rangewide Extent of Occurrence (EoO) for the current time period. Extent of Occurrence is the percent of the total spatial extent (*i.e.,* area occupied by 894 grids) occupied by *B. affinis* from 2000-2009 and 2010+ time periods. "Current" is the EoO over the time span of 2000-2015.

	Current Extent of Occurrence		
Decade	2000	2010	Current
EoE	5%	4%	8%

Table 4.5. The number of *B. affinis* occurrences and extent of occurrence (EoO) per ecoregion for the current period. The "Number" columns refer to the number of grids within the ecoregions to have at least one *B. affinis* occurrence during the specified timeframe. The "Extent of Occurrence" columns give the proportion of the total spatial extent of the ecoregion occupied during the specified timeframe.

Ecoregions	Number			Extent of Occurrence		
	2000	2010	Current	2000	2010	Current
91	0	0	0	0%	0%	0%
96	0	0	0	0%	0%	0%
98	0	0	0	0%	0%	0%
99	0	0	0	0%	0%	0%
132	0	0	0	0%	0%	0%
133	0	0	0	0%	0%	0%
134	0	0	0	0%	0%	0%
135	3	0	3	8%	0%	8%
210	3	1	4	3%	1%	4%
220	16	29	42	3%	6%	8%
230	1	0	1	4%	0%	4%
250	16	2	16	50%	6%	50%
330	0	0	0	0%	0%	0%
M210	0	0	0	0%	0%	0%
M220	3	0	3	3%	0%	2%

4.3 Redundancy

Redundancy is the ability of a species to guard against losses of adaptive diversity due to catastrophic events. Bombus affinis redundancy is described as having multiple, healthy populations widely distributed across the breadth of adaptive diversity relative to the spatial occurrence of catastrophic events.

Methods - See Chapter 3 for an explanation of the methods.

Results

Probability of Ecoregion Extinction - The frequency of a catastrophic event occurring varies by grid and by event type, but in most cases the frequencies are low. No grids had a high temperature event (>35° C for 14 or more days) (Table 4.6). The frequency of a catastrophic drought occurring varied with among grids and with drought intensity. The frequency of high temperatures was zero for all 66 grids analyzed (Table 4.6). For D3+ intensity drought, all 66 grids had frequencies less than 0.60, and for D4+ intensity drought, all grids had frequencies less than 0.50 (Table 4.6).

Table 4.6. The cumulative frequency (λ) distribution for the three types of catastrophic event: high temperature, D3+ level drought, and D4+ level drought. Column 1 is the frequency (λ) values; the Catastrophic Event columns provide the tally of grids with frequencies equal to or less than the specified λ values. For example, all 66 grids had high temperatures frequencies of 0.10 or less, while only 10 grids for D3+ drought and 56 grids for D4+ drought had frequency values equal to 0.10 or less.

	Catastrophic Events		
λ	High Temp	D3+	D4+
0.00	66	5	36
0.10	66	10	56
0.20	66	17	60
0.30	66	41	64
0.40	66	63	66
0.50	66	66	66
0.60	66	66	66
0.70	66	66	66
0.80	66	66	66
0.90	66	66	66
1.00	66	66	66

Due to the low frequency of D4+ intensity drought and high temperature events, the risk of ecoregion-wide extirpation is zero for all current ecoregions, except for Ecoregion 230 (Table 4.3.2). Given the few number of grids occupied and the grid-specific catastrophic event frequencies for Ecoregion 230, the probability of ecoregion-wide extirpation within 25 years is 0.854. Considering a D3+ intensity and high temperature scenario, 4 of the 6 ecoregions have high risk of extirpation due to D3+ intensity drought or high temperature events (Table 4.7).

Table 4.7. Probability of Ecoregion-wide extirpation, p(x), due high temperatures or a moderate (D3+ intensity) to severe (D4+ intensity) drought.

	p(x)	
Ecoregion	High temp/ D3+ drought	High temp/ D4+ drought
135	0.000	0.000
210	0.996	0.000
220	0.000	0.000
230	1.000	0.854
250	0.990	0.000
M220	0.997	0.000

Spatial Dispersion – Due to time constraints, we were unable to complete this analysis.

4.3 Synopsis

The number of occurrences, % occupancy of grids, and relative abundance has declined. Data available on 25 of the 69 extant populations suggest that many of the persisting populations are experiencing threats. Along with the loss of populations, there has been a marked loss of range and spatial extent, with occurrences currently in 13 states/provinces and 6 ecoregions. This expansive reduction has likely led to loss of spatial heterogeneity (ability to withstand environmental stochasticity) and adaptive diversity (ability to adapt to physical and biological changes in its environment). Similarly, the loss of occurrences within ecoregions has increased the risk of extirpation due catastrophic events.

Chapter 5. Risk and Conservation Factors

In this chapter, we review the negative and beneficial factors affecting *B. affinis* and describe future scenarios applied to forecast the future condition of the species. Factors that have a negative impact on *B affinis* individuals are referred to as risk factors (also as stressors or threats); factors that have a beneficial effect are referred to as conservation factors. We begin with describing generally the most prominent risk factors and conservation actions based on the best available data. Next, we describe the analyses undertaken to develop future factor scenarios for forecasting the abundance and distribution of *B. affinis* into the future.

5.1 Risk Factors

We focused on four exogenous stressors (stressors originating outside an organism or system), including pathogens, pesticides, habitat loss and degradation, and climate change, and one endogenous stressor (stressor originating from inside an organism or system), small population dynamics. We fully acknowledge that our risk factor analysis is not a thorough evaluation of all stressors affecting the species and its habitat. We chose to focus on these five stressors for our analysis because, according to the best available data, these five stressors are the leading factors attributed to the decline of *B. affinis* and other *Bombus* species. Below we describe each of these five risk factors and our rationale and available evidence of how they may be affecting *B. affinis*.

5.1.1 Pathogens

Natural pathogen loads in Bombus species

A large number of pathogens, including parasites, are known to attack and infect bumble bees. For the most part, *Bombus* species have co-evolved with these pathogens and do not exhibit effects at the colony or population level. Pathogens and parasites are widespread generalists in the host genus, but affect species differentially according to host susceptibility and tolerance to infection (Kissinger *et al.* 2011, p. 221; Malfi and Roulston 2014, p. 18). The host species' life history plays a role in the virulence of a given pathogen; for instance, parasites may have relatively smaller effects on species with shorter colony life cycles and smaller colony sizes (Rutrecht and Brown 2009, entire).

Pathogens as a B. affinis stressor

The precipitous decline of certain *Bombus* species from the mid-1990s to present—particularly species in the subgenus *B. sensu stricto ,* including *B. affinis*—was contemporaneous with the collapse of commercially bred *B. occidentalis*, which were raised primarily to pollinate greenhouse tomato and sweet pepper crops beginning in the late 1980s (Szabo *et al.* 2012, pp. 232-233, and others). This collapse was attributed to the microsporidium *Nosema bombi*. Around the same time, several North American wild bumble bee species—*B. affinis, B. franklini, B. occidentalis*, *B. terricola*, and *B. pensylvanicus* (of these, only *B. pensylvanicus* is not in the subgenus *sensu stricto*)—also began to decline rapidly (Szabo *et al*. 2012, p. 232). The temporal congruence and speed of these declines led to the suggestion that they were caused by spillover of *N. bombi* from the commercial colonies to wild populations.

Transmission of *N. bombi* most likely occurs when spores are fed to larvae (Eijnde and Vette 1993 and Rutrecht 2007, as cited in Meeus *et al.* 2011, p. 666), and can have large effects on individual bees; infected animals may have crippled wings, and queens may have distended abdomens and be unable to mate (Otti and Schmid-Hempel 2007, pp. 122-123). Murray *et al.* (2013, p. 274, citing Rutrecht *et al*. 2007) noted that *N. bombi* spreads slowly through novel populations, as transmission primarily occurs via contaminated pollen or nectar fed to the larvae, with subsequent inter-colony infections through drift of infected adults into non-natal colonies. Brown (2011, p. 169) cited two possible interpretations of the contemporaneous collapse of native bumble bees with commercial breeding of *B. occidentalis*: (1) a high parasite prevalence represents the moving edge of a wave of infections, indicating that these *Bombus* populations are on the verge of extinction, or (2) the high prevalence may simply indicate that the declining species naturally support high populations of the parasite.

Notwithstanding earlier studies postulating *N. bombi* spillover around commercial greenhouses (e.g., Colla *et al.* 2006, entire), as well as the timing of commercialization and *Bombus* declines, Szabo *et al.* (2012, p. 237) found that pathogen spillover in this form cannot fully account for these declines. Further, Cameron *et al.* (2016, p. 4386) conclusively show that there is no evidence for the importation of an exotic *N. bombi* strain, as the strain previously thought to be European was present and widespread in North America prior to the importation of European bumble bees in the 1990s. These authors do conclude that *N. bombi* prevalence has increased since the 1990s, particularly in declining species such as *B. affinis*. Overall, although results of recent work show both a higher prevalence of *N. bombi* in rapidly declining North American bumble bee species than in stable species (Cameron *et al.* 2011b, entire; Cordes *et al.* 2011, p. 2) and a high infection intensity (*i.e.*, number of spores per bee) in declining species, it remains debatable as to whether pathogen spillover of *N. bombi* is driving bumble bee declines. It is also worth noting that evidence of pathogen spillover is lacking in European *Bombus* despite widespread commercial production and transport of hives since early the 1980s.

Nosema bombi has been found to be part of the natural pathogen load in North American bumble bee populations—for instance, it has been reported in Canada since the 1940s (Cordes *et al.* 2011, p. 7) and appears to have a broad host range in North America (Kissinger *et al.* 2011, p. 222). It is thus not clear if its recent prevalence is indicative of natural trends or of the rapid spread of an invasive strain (Brown 2011, p. 169; Cameron et al. 2011b, p. 665; Meeus *et al.* 2011, p. 666). Although Cordes *et al.* (2011, p. 7) found a new allele in *N. bombi*, the recent study by Cameron *et al*. (2016) found no evidence of an exotic *N. bombi* strain. Malfi and Roulston (2014, p. 24) found that *N. bombi* infections are more frequent and more severe in rare species (albeit *B. affinis* was not included in the sample), and also that the species with the highest percentage of infected individuals were rare species. They concluded that the evidence linking *N. bombi* to the *Bombus* decline is correlative but does suggest species undergoing range reductions are more susceptible to *N. bombi* infections, while noting that it is nonetheless possible that elevated levels of *N. bombi* are natural in the host species.

Patterns of losses observed, however, cannot be completely explained by exposure to *N. bombi*. Several experts have surmised that *N. bombi* may not be the culpable (or only culpable) pathogen in the precipitous decline of wild *Bombus* in North America (*e.g.*, D. Goulson pers. comm. 2016; J. Strange and A. Tripodi, USDA, pers. comm., 2016), and the evidence for chronic pathogen spillover from commercial bumble bees as a main cause of decline remains debatable (see various arguments in Colla *et al.* 2006; Otterstatter and Thomson 2008; Szabo *et al.* 2012; Manley *et al.* 2015).

Szabo *et al.* (2012, p. 237) noted that other pathogens may be involved in the ongoing decline of *B. affinis*, *e.g.* some viruses can be transmitted from honey bees (*Apis mellifera* Linnaeus) to bumble bees, as shown in Singh *et al.* (2010). Deformed wing virus, DWV, is just such an emerging disease, with its

prevalence in honey bees linked to its growing prevalence in sympatric bumble bees (Furst *et al*. 2014, p. 364). Tripodi (pers. comm. 2016) also notes that DWV has been detected in wild and commercially-sourced bumble bees. Although virological research focuses on honey bees, many of the 24 viruses isolated in *Apis* have a broad host range, infecting some *Bombus* species (Manley *et al.* 2015, p. 2).

In addition to fungi such as *N. bombi* and viruses such as DWV, other viruses, bacteria, and parasites are being investigated for their effects on *Bombus* in North America. Those potentially of greatest concern for *B. affinis* are described briefly below, noting that studies for this particular species have not been conducted.

Acute Bee Paralysis was the first honey bee virus to be detected in bumble bee hosts, although its occurrence in natural populations and effects on bumble bee health are unknown. The Black Queen Cell Virus (BQCV) has been the most commonly detected bumble bee pathogen in ongoing surveys, having been found in 31 percent of 559 samples tested to date (Tripodi, pers. comm. 2016). It should be noted that although 12 *Bombus* species across the U.S. have tested positive for BQCV, *B. affinis* has not been evaluated. The effects of this virus, which occurs not only in honey bees and bumble bees but a number of other arthropods, are unknown (Tripodi, pers. comm. 2016).

Little is known about bacterial diseases in bumble bees, although early reports speculated that pathogenic bacteria were responsible for some larval mortality (Tripodi, pers. comm. 2016). *Spiroplasma melliferum* and *S. apis*, pathogenic bacteria that are known to cause mortality in honey bees, have been found in numerous flower-visiting insects, including in the hemolymph and guts of some *Bombus* species (various studies cited by Tripodi, pers. comm. 2016). Other bacteria have been found to cause sterility in honey bee queens, but such effects have not been recorded in bumble bees.

With regard to parasites, the trypanosomatid, *Crithidia bombi* is found in species throughout the genus *Bombus*, and *C. expoeki*, assumed to be a similar pathogen, is known from *Bombus* samples collected in both Europe and North America (Tripodi, pers. comm. 2016). *Crithidia* pathogens generally affect the reproduction of colonies; although acute mortality is rarely observed, under experimental conditions of nutritional stress the host mortality rate increased by 50% (Brown *et al.* 2000, p. 1). Experimental evidence shows that bumble bees can contract *C. bombi* while feeding on flowers that have been previously visited by infected bees (Durrer and Schmid-Hempel 1994, as cited by Tripodi, pers. comm. 2016), and bees from commercial rearing facilities have tested positive for this pathogen upon delivery (Otterstatter *et al*. 2005, p. 388; Murray *et al*. 2013, p. 274).

The neogregarine *Apicystis bombi*, is a widely distributed parasite of multiple bumble bee species (Tripodi, pers. comm. 2016). It decimates the body fat of infected individuals, and infected spring queens die before founding colonies. Although commercially-sourced colonies of *B. terrestris* were found to harbor this parasite (Graystock *et al.* 2013, p. 4), little is currently known about its biology.

Goulson *et al.* (2015, p. 3) found that the African honey bee parasite *Aethina tumida* recently invaded North America, and it is likely that it also attacks wild bumble bee species. They also noted that commercial trade in bumble bee colonies is redistributing bee diseases throughout the world, and that "High-density populations of managed bees may provide conditions for the rapid multiplication of parasites that then spill over into wild populations."

With regard to vulnerability to pathogens, Manley *et al.* (2015, p. 1) noted a heightened risk in populations that lack evolved resistance to novel pathogens, while Whitehorn *et al.* (2014, p. 667) found the prevalence of a parasite higher in populations with lower genetic diversity at the population level, postulating that population-level genetic homogeneity may facilitate parasite transmission and elevate prevalence. Generally, Cordes *et al.* (2011, p. 7) and others consider that pathogen spread could be a

consequence of rather than the cause of a *Bombus* population declines, and Brown (2011, p. 169) asserts that considerable work is needed to know the causal direction of correlations between patterns of decline, parasite prevalence, and loss of genetic diversity.

Cameron *et al.* (2011b, p. 662) sum up the likelihood of pathogen spread being a primary cause of *B. affinis* declines by stating that higher pathogen prevalence and reduced genetic diversity are realistic predictors of patterns of decline in North American bumble bees, although cause and effect remain uncertain. Szabo *et al.* (2012, p. 232) found no evidence that pathogen spillover caused the near disappearance of the previously widespread *B. affinis* despite the temporal association between bumble bee declines and the onset of commercial bumble bee use. However, even without taking pathogen spillover into account, D. Goulson (pers. comm. 2016) notes that no other cause aside from an epizootic of *N. bombi* or another unknown pathogen or pathogens adequately explains the rate and magnitude of the ongoing decline of *B. affinis* and other North American *Bombus* species.

5.1.2 Pesticides

A variety of pesticides are widely used in agricultural, urban, and even natural environments. Native bees are simultaneously exposed to multiple pesticides, including insecticides, fungicides, and herbicides. The pesticides with greatest effects on bumble bees are insecticides and herbicides: insecticides are specifically designed to directly kill insects, including bumble bees, and herbicides reduce available floral resources, thus indirectly affecting bumble bees. Herbicides can also have direct effects on bees (Moffett and Morton 1975, p. 179). For a more detailed discussion of the indirect impacts of herbicides, see Sanchez-Bayo (2015, entire).

Bumble bees are exposed to multiple pesticides throughout their lives, from development to adults (Sanchez-Bayo and Goka 2014, p. 5; Goulson *et al.* 2015, p. 4). For example, bees collected from a research area in northeastern Colorado in both grasslands and wheat fields exhibited 19 pesticides in 54 samples; the neonicotinoid insecticide, thiamethoxam, was the most frequently detected pesticide (present in 46% of the samples) (Hladik *et al.* 2016, pp. 472-473). Pesticides known to affect bumble bees include (but not limited to): acetamiprid, buprofezin, clothianidin, clyhalothrin, cyromazine, deltamethrin, diflubenzuron, flucycloxuron, flufenoxuron, imidacloprid, lufenuron, novaluron, spinosad, teflubenzuron, thiacloprid, and thiamethoxam (Elton *et al.* 2013, pp. 5-6; Gill *et al.* 2012, p. 107; Mommaerts *et al.* 2006, pp. 3-4; Sanchez-Bayo and Goka 2014, pp. 7-8; Scott-Dupree *et al.* 2009, p. 179).

Bumble bees are exposed to pesticides when they consume contaminated nectar or gather contaminated pollen, and also absorb toxins directly through their exoskeletons. Although the overall toxicity of pesticides to bumble bees is unknown, pesticides have been documented to have both lethal and sub-lethal effects on bumble bees (Appendix 3). Lab experiments have documented direct contact mortality of two bumble bee species, *B. impatiens* or *B. terrestris*, exposed to several pesticides, including imidacloprid, clothianidin, clyhalothrin, acetamiprid, deltamethrin, spinosad, thiacloprid, or thiamethoxam (Appendix 3) (Bortolotti *et al.* 2002, pp. 68-70; Gill *et al.* 2012, p. 107; Marletto *et al.* 2003, pp. 156-157; Mommaerts *et al.* 2006, pp. 3-4; Sanchez-Bayo and Goka 2014, pp. 7-8; Scott-Dupree *et al.* 2009, p. 179). Documented sub-lethal effects to individual bumble bees and colonies include reduced or no male production (Fauser-Misslin *et al.* 2014, pp. 453-454; Feltham *et al.* 2014, p. 320; Gill *et al.* 2012, p. 107; Mommaerts *et al.* 2006, pp. 3-4;Mommaerts *et al.* 2010, pp 2111-212; Scholer and Krischik 2014, p.7), reduced or no egg hatch (Elton *et al.* 2013, pp. 6-7; Mommaerts *et al.* 2006, pp. 3-4), reduced queen production (Fauser-Misslin *et al.* 2014, pp. 453-454; Feltham *et al.* 2014, p. 320;

Whitehorn *et al.* 2012, p. 352), reduced queen longevity (Fauser-Misslin *et al.* 2014, pp. 453-454), reduced colony weight gain (Feltham *et al.* 2014, p. 320; Whitehorn *et al.* 2012, p.351; Scholer and Krischik 2014, p. 6), reduced brood (Elton *et al.* 2013, p. 6; Feltham *et al.* 2014, p. 320; Gill *et al.* 2012, p. 107; Laycock *et al.* 2012, p. 3), reduced feeding (Fauser-Misslin *et al.* 2014, pp. 453-454; Feltham *et al.* 2014, p. 320; Gill *et al.* 2012, p. 107; Gill and Raine 2014, pp. 6-7; Larson *et al.* 2013, pp. 2-3; Laycock *et al.* 2012, p. 4; Mommaerts *et al.* 2010, pp. 211-212; Scholer and Krischik 2014, p. 5; Thompson *et al.* 2014, pp. 2-3), impaired ovary development (Laycock *et al.* 2012, pp. 4-5), increased number of foragers or foraging trips or duration (risky behaviors) (Gill *et al.* 2012, p. 107; Gill and Raine 2014, pp. 5-8; Feltham *et al.* 2014, p. 320). Pyrethroids also have documented affects to bumble bees, for example chronic lab exposure to the pyrethroid, k-cyhalothrin, had significant impact on *B. terrestris* worker size, a key aspect of colony function, particularly under limited food conditions (Baron *et al*. 2014, p. 464).

Neonicotinoids are a class of insecticides used to target pests of agricultural crops, turf, gardens, and pets (Cox 2001, p15: Goulson 2014, p. 2); the neonicotinoid, imidacloprid, is also used to treat forest pests, such as emerald ash borer (U.S. Forest Service, 2016). Neonicotinoids are systemic insecticides that act as an insect neurotoxin, affecting the central nervous system of insects. Laboratory data indicates that neonicotinoids kill insects by interfering with receptors of the insect's nervous system, causing overstimulation, paralysis, and death. The neonicotinoid family includes acetamiprid, clothianidin, imidacloprid, nitenpyram, nithiazine, thiacloprid and thiamethoxam. The typical neonicotinoid application methods include: foliar spray, granular, seed coating, soil furrow, and drench or drip irrigation.

Neonicotinoids have been strongly implicated as the cause of the decline of bees (European Food Safety Authority 2015, p. 4211; Pisa *et al.* 2015, p. 69; Goulson 2013, p. 7-8), and for *B. affinis* declines due to the contemporaneous introduction of neonicotinoid use and the precipitous decline of the species (Colla and Packer 2008, p. 10). Imidacloprid became widely used in the United States starting in the early 1990s and clothianidin and thiamethoxam entered the market beginning in the early 2000s (Douglas and Tooker 2015, pp. 5091-5092) (Figure 5.1.2). The use of neonicotinoids rapidly increased as seed-applied products were introduced in field crops, marking a shift toward large-scale, preemptive insecticide use. For example, 34−44% of soybeans and 79−100% of corn hectares were treated with neonicotinoids in 2011 (Douglas and Tooker 2015, pp. 5088, 5092). As of 2013, nearly all corn planted in the United States was treated with neonicotinoids and various fungicides (Stokstad 2013, p. 675) and, as of 2014, approximately one-third of U.S. soybean acreage was planted with neonicotinoid-treated seeds[5] (Douglas and Tooker 2015, p. 5092). Nearly 2 million pounds of imidacloprid were used in the United States in 2013, primarily for the agricultural crops of soybeans, wheat, orchards and grapes, vegetables, and fruit (Douglas and Tooker 2015, p. 5090; U.S. Geological Survey National Pesticide Synthesis 2016). If current trends continue, Douglas and Tooker (2015, p. 5093) predict that neonicotinoid use will increase further through application to more soybeans and other crop species and through an increase of per-seed rates.

Neonicotinoids persist and accumulate in soils, and owing to their systemic property, are found in nectar and pollen of treated crops and landscapes (Goulson 2013, pp. 979-981) and in guttation droplets (drops

[5] A peer reviewer indicated that most available data on neonicotinoids underestimates their application, because the most common use is as a seed treatment. Treated seeds, classified as “treated article pesticides”, are specifically exempted from regulation by EPA by the Federal Insecticide, Fungicide, and Rodenticide Act. Based on this information, we may have underestimated the risks; thus, subsequent analyses will consider this issue.

of xylem sap on the tip or edges of leaves) (Girolami *et al.* 2009, pp. 1811-1814). Reported levels of neonicotinoids in soils, waterways, field margins, and floral resources overlap substantially with concentrations that are sufficient to control pests in crops, and commonly exceed the LC50 (the concentration that kills 50% of individuals) for non-target insects (Goulson 2013, p. 985). Similarly, neonicotinoids are present at toxic levels in guttation droplets (Girolami *et al.* 2009, p. 1811-1814).

Most studies examining the effect of neonicotinoids on bees have been conducted using the European honey bee (*Apis mellifera*), followed by *B. terrestris* and *B. impatiens* (Lundin *et al.* 2015, p. 7). Only a handful of other *Bombus* species have been studied, and there have been no studies on *B. affinis* (Lundin *et al.* 2015, p. 7). We infer, however, that studies of the effect of pesticides to other *Bombus* species will likely reflect their effects on *B. affinis* because these species have similar life history traits (e.g., generalist foragers collecting pollen from same food sources). Bumble bees may, in fact, be more vulnerable to pesticide exposure. Bumble bees are more susceptible to pesticides applied early in the year than are honey bees, because for 1 month every year the entire bumble bee population depends on the success of the queens to forage and establish new colonies. Also, because most bumble bees have smaller colonies (N=~ several hundred to a thousand) than honey bees (N=~30,000), a single bumble bee worker is more important to the survival of the colony than a single honey bee worker (Thompson and Hunt 1999, p. 155). Furthermore, since bumble bees nest underground, they are additionally exposed to pesticide residues in the soil (Arena and Sgolastra 2014, p. 333). Moreover, bumble bee larvae consume large amounts of unprocessed pollen, and therefore, are much more exposed to pesticide residues in pollen (Arena and Sgolastra 2014, p. 333). Given life-history of bumble bees, it seems likely that bumble bees are more susceptible than honey bees to pesticides, specifically when the application of a pesticide overlaps with colony establishment in the spring (Arena and Sgolastra 2014, p. 333). Many studies finding detrimental effects of neonicotinoid exposure on bees were conducted using the honey bees, studies (*e.g*., Piiroinen and Goulson 2016, entire) are now emerging that have simultaneously documented effects to bumble bees and honey bees at field-realistic levels.

While most of the research on the effects of pesticides has been based on honey bees, as generalist foragers, both honey bees and bumble bees are often collecting from the same pollen sources (E. Evans, University of MN, pers. comm. 2016). Based on detected concentrations in the wild and the results of toxicity test, as well as the frequency of hives across the landscape, Sanchez-Bayo and Goka (2014, pp. 12-14) predicted that exposure to thiamethoxam, imidacloprid, and clothianidin (along with two organophosphates--phosmet and chloropyrifos) pose the greatest risk to honey bees at a global scale. However, the additive and synergistic effects of exposure to multiple pesticides and multiple times may exacerbate the toxicity of exposure to any single pesticide, and thus, additional pesticides in combination with others, may pose risks to bees as well. Several studies have revealed that bees are often chronically exposed to a cocktail of pesticides throughout their lifetime (Sanchez-Bayo and Goka 2014, p. 5; Chauzat *et al.* 2006, pp. 256-257; Mullin *et al.* 2010, pp. 3-8; Krupke *et al.* 2012, pp. 3-5). For example, Sanchez-Bayo and Goka (2014, p. 5) detected 161 different pesticides at honey bee colonies. The effects of chronic exposure to multiple pesticides are poorly understood and are not regularly examined in risk assessments (Goulson 2016, p. 4), and thus, the toxicity results, may underestimate the actual risks posed to bees.

Furthermore, pesticide formulations typically contain less than 50 percent active ingredients with the remainder being surfactants (surface active agent that reduces the surface tension of water) and solvents (collectively, referred to as adjuvants). As bees forage, they are exposed to many adjuvants as well as active ingredients (Mullin *et al.* 2015, p. 7). Adjuvants, however, are not typically included in risk

assessments that are required for pesticide registration (Mullin *et al.* 2015, p. 2), and are therefore, less studied, but can be as or more toxic to bees as the active ingredients (Mullin *et al.* 2015, p.4). For example, bumble bees are highly susceptible to emulsifiers such as perfluorooctane sulfonic acid (Mommaerts *et al*. 2011, pp. 450-452). Goodwind and McBrydie (2010, p.232) found that 4 of 11 commercially available spray adjuvants were toxic to honey bees at field rates. Furthermore, active ingredients and inert ingredients may interact synergistically, causing impacts that would not occur by exposure to the active ingredients alone (Mullin *et al.* 2015, p. 3).

Lastly, bees are exposed to a number of significant and interacting stressors (Goulson *et al.* 2015, entire), which can compound the effects of pesticides. For example, exposure to fungicides greatly increased the toxicity of insecticides in honey bees (Schmuck *et al.* 2003, pp. 82-85; Iwasa *et al.* 2004, p. 376; Piling and Jepsen 1993, pp. 295-296; Mullin *et al.* 2015, p. 4 citing Zhu *et al.* 2015). Similarly, honey bees exposed to fungicides had reduced colony nutrition and higher virus levels to fungicides (DeGrandi-Hoffman *et al.* 2015, pp. 2523-2524). Pettis *et al.* (2013, p.4), for example, found increased probability of *Nosema* infection in honey bees feeding on pollen with high fungicide loads. Several studies found exposure to insecticides reduced resistance to diseases (Fauser Misslin *et al.* 2014, pp. 454-455, Pettis *et al.* 2013, p. 4), and exposure to dietary related stresses (*e.g*., short-term starvation) reduced the ability of bees to cope with toxins (Brown *et al.* 2000, p. 424; Tyler *et al.* 2006, p. 2; Moret and Hempel 2000, p. 1167). Piiroinen and Goulson (2016, pp. 3-5) found that exposure to *N. caranae* reduced learning in honeybees and bumble bees, but both species reacted differently to the combination of pathogen plus pesticide exposure.

Determining the extent bee fatality caused by pesticides is difficult due to the myriad of other potential stressors (*e.g*., pathogens, parasitoids, and diseases) and possible synergistic effects of these sources (see Synergistic Effects Ch. 5.1.6). There are known instances where neonicotinoids such as clothianidin have adverse effects to the immunity and promotes replication of viral pathogens in bees (*e.g.,* DiPrisco *et al.* 2013). The interruption or disruption of endocrine functions is related to the immune systems of animals and the application of neonicotinoids that may potentiate the increase of pathogens. However, it is the end result of these interactions that are the crux of the decline of bees. It is a very important point as to which factors are having the effects, but it is also known that the corrective measures leading to recovery of species will have to address potential pathways for each of these agents and the declines are cumulative impacts of these agents. It almost becomes academic as to what is causing the decline because they are so interrelated (see Chapter 5) - the important fact is that there is a decline in *B. affinis* (Chapter 7).

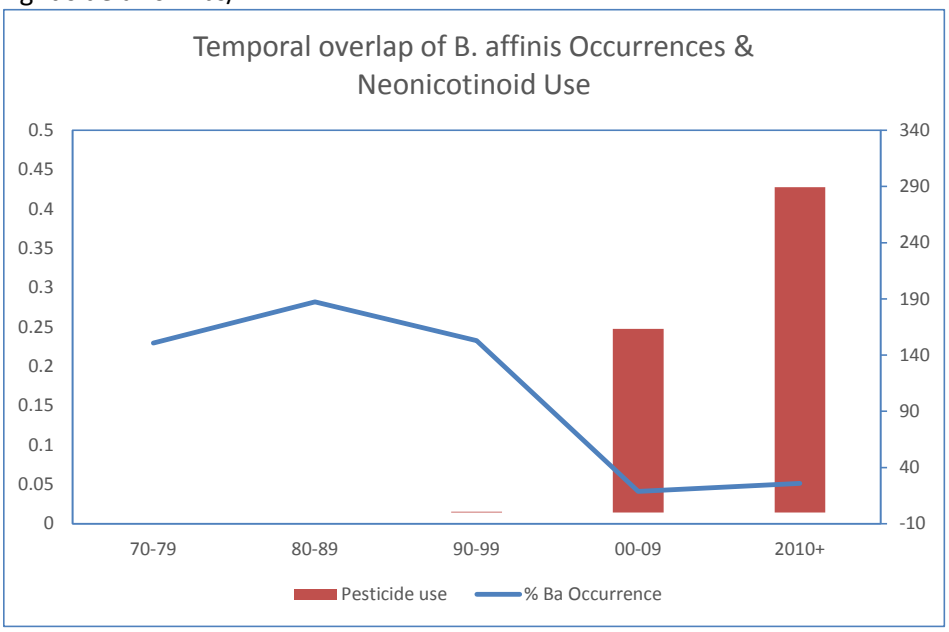
To assess the perceived cause and effect relationship between neonicotinoid application levels and *B. affinis* declines, and to obtain insights on the future risk, we gathered available data on pesticide use for a subset of chemicals and charted the application trend over time throughout the range of *B. affinis*. Specifically, using pesticide application rate data collected from 1995 to 2013 (USGS National Pesticide Synthesis, accessed February 2016), we examined the trend in use of three prevalent neonicotinoids; imidacloprid, clothianidin, and thiamethoxam over time in 43 grids[6] with recent (since 2000) occurrences. Because USGS data are provided at the county level, we overlaid grids and counties and used the county application rate that corresponded to the majority of the grid area to avoid double counting. If a grid had equal area in two counties, we used the average of those counties. All three chemicals were added for each year to get a total application rate of imidacloprid, clothianidin, and

[6] *The analysis was completed using 43 grids. For the final SSA report, we will incorporate the information from the remaining grids.*

thiamethoxam combined. We also examined trends of neonicotinoid application rates (imidacloprid, clothianidin, and thiamethoxam) at the ecoregion scale currently occupied by *B. affinis*. While we chose to focus these trend analyses on three commonly used and studied neonicotinoids, we recognize that there are a myriad of pesticides, herbicides, fungicides, inactive ingredients, and other chemicals that have documented negative effects on bees (as discussed above) and could be similarly analyzed for application rate trends in our study area.

The results of overlaying *B. affinis* trend over neonicotinoid use trend provides a striking picture (Figure 5.1.), and hence, explains why neonicotinoid use has been implicated in the precipitous decline that occurred in the mid-1990s. Just as striking, however, is that as neonicotinoid use exponentially increased, a commensurate increase in the rate of decline of *B. affinis* was observed. Although neonicotinoids might be primarily responsible for the decline of *B. affinis*, other possible explanations include: 1) the timing of neonicotinoid appearance and precipitous decline is merely coincidental, 2) neonicotinoid use is not the ultimate cause of the decline, 3) neonicotinoid use in concert with another stressor (*e.g.*, pathogens) is the cause of the decline, 4) the rate of decline is increasing but the increased effort in surveys is obscuring the losses, or 5) the remaining populations are in locations where exposure to neonicotinoids is minimal. Given the evidence of neonicotinoid toxicity on bumble bees, it is unlikely that neonicotinoid use hasn't contributed to the loss of *B. affinis* populations.

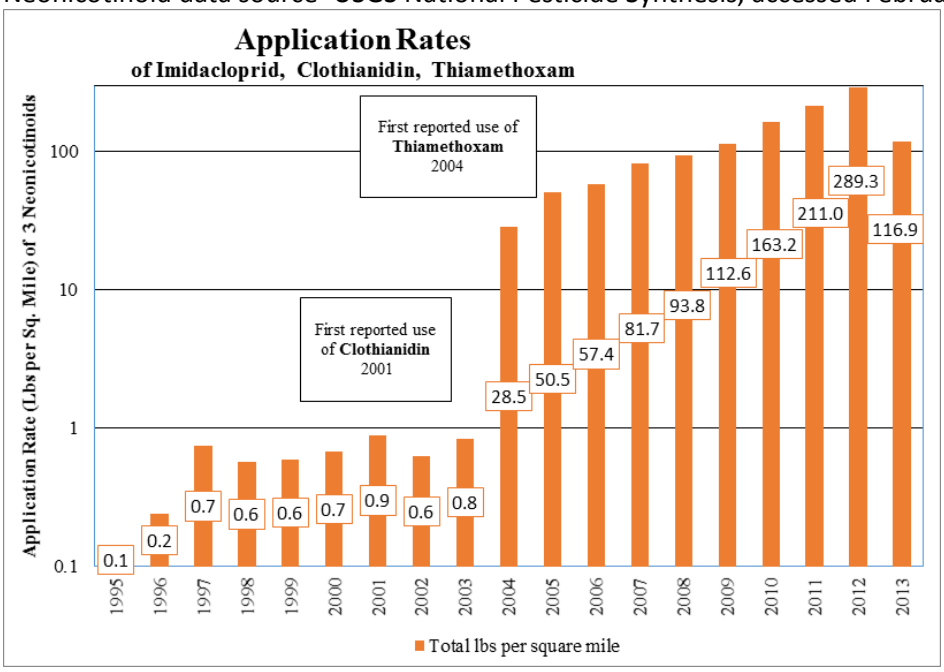
Figure 5.1. The trend in *B. affinis* % Occupancy and neonicotinoid (imidacloprid, clothianidin, and thiamethoxam) use over time. Neonicotinoid data source -USGS National Pesticide Synthesis, accessed February 2016. Left side axis = % of *Bombus* grids occupied by *B. affinis*; right side axis = lbs/mi^2.



Our analysis also shows an increasing trend of application rates of imidacloprid, clothianidin, and thiamethoxam over time in 43 grids with recent (since 2000) occurrences (Figure 5.2). Use of imidacloprid was first reported in 1995, use of clothianidin was first reported in 2001, and use of thiamethoxam was first reported in 2004. The application rate fits an exponential growth rate curve ($y = 0.078e^{0.4644x}$, $R^2 = 0.88$). Specifically, the application rate dramatically increased from less than 1

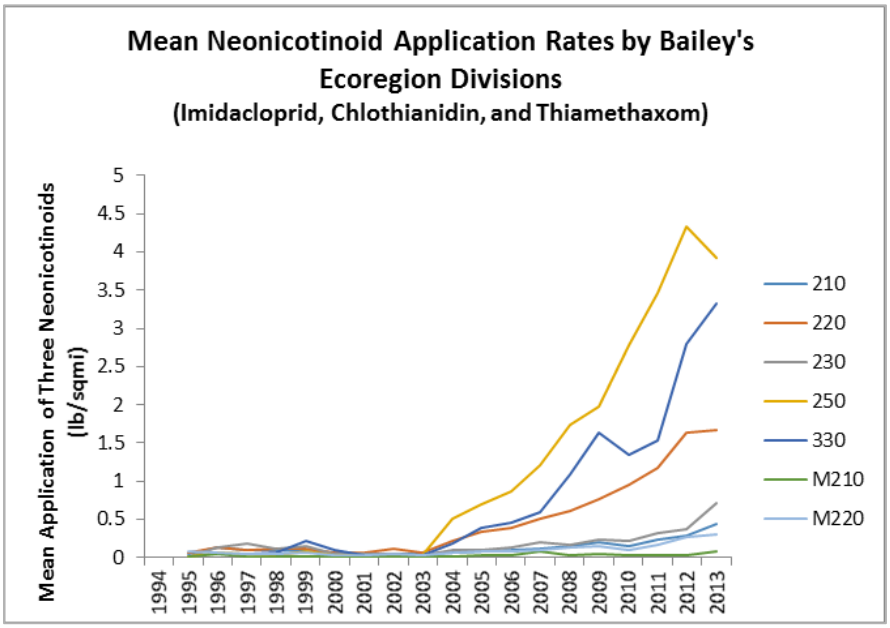
pound/square mile (lbs/mi^2) in the 1990s to 28 lbs/mi^2 in 2004, due to the introduction of thiamethoxam use. The application rate nearly doubled in the next year to 50.5 lbs/mi^2 in 2005 and nearly doubled again by 2008, with an application rate of 93.8 lbs/mi^2. By 2012, the application rate more than tripled from its 2008 rate of 93.8 lbs/mi^2, to 289.3 lbs/mi^2.

Figure 5.2. Combined total reported application (lbs/mi^2) of three prevalent neonicotinoids at 43 grid locations from 1995 to 2013. Use of imidacloprid was first reported in 1995, use of clothianidin was first reported in 2001, and use of thiamethoxam was first reported in 2004. Neonicotinoid data source -USGS National Pesticide Synthesis, accessed February 2016.



Application rates of neonicotinoids are increasing in all ecoregions currently occupied by *B. affinis* (Figure 5.3). Since 2002, the rates of application increased most dramatically in five ecoregions. Specifically, the average rates of application increased from less than 0.5 lbs/m^{i2} in 2002 to over 4.3 lbs/mi^2 in 2009. The most pronounced increases occurred in the Prairie Division (250), the Warm Continental Division (210), and the Hot Continental Division (220).

Figure 5.3. Average (arithmetic mean) application rate of three prevalent neonicotinoids, imidacloprid, clothianidin, and thiamethoxam, over time. Neonicotinoid data source -USGS National Pesticide Synthesis, accessed February 2016.



5.1.3 Habitat loss and degradation

Bombus affinis historically occupied native grasslands of the Northeast and upper Midwest; however, much of this landscape has now been lost or fragmented. Estimates of native grassland losses since European settlement of North America are as high as 99.9% (Samson and Knofp 1994, p. 418). Habitat loss is commonly cited as a long-term contributor to bee declines through the 20th century, and may continue to contribute to current declines, at least for some species (Goulson *et al.* 2015, p. 2; Goulson *et al.* 2008; Potts *et al.* 2010, p. 348; Brown and Paxton 2009, pp. 411-412), *B. affinis* may not be as severely affected by habitat loss compared to habitat specialists, such as native prairie endemics. Still, loss or degradation of habitat reduces both bee diversity and abundance (Potts *et al.* 2019, pp. 348-349), and small, isolated patches of habitat may not be sufficient to support healthy bee populations (Hatfield and LeBuhn 2007, p. 154-156; Öckinger and Smith 2007, pp. 55-56).

The habitat requirements of *B. affinis* are discussed in Chapter 2. Bee diversity is strongly linked to floral diversity and abundance availability over their entire active season (Hines and Hendrix 2005; others – see Chapter 2). This seems particularly relevant for short-tongued species like *B. affinis*, as they have limitations on the types of flowers they can access. Thus, the greatest impact of habitat loss on bees is the loss of floral resources necessary as food and nectar. Loss or degradation of floral resources has occurred primarily through conversion of lands to agriculture and urbanization, but also from factors such as intensive livestock grazing and suppression of natural fire regimes. Conversion of natural habitat that is rich in flowers to farmlands, urban and suburban areas, and other uses is the primary cause of bumble bee habitat loss (Goulson *et al.* 2015, p. 2).

In addition to direct conversion of native landscapes to agricultural lands, intensification of agricultural practices also affects the quality of floral resources available to bees. Over time, farm sizes have grown and new technologies allow previously non-arable lands to be used. Additionally, the wide-spread use of herbicides in agricultural, urban, and even natural landscapes has led to decreases in flowering plants (Potts *et al.* 2010; p. 350). For example, the increasing, widespread use of the herbicide glyphosate in conjunction with increasing use of crops that are genetically modified to be resistant to glyphosate, has reduced the flowering plants in agricultural areas (Pleasants and Oberhauser 2013). Herbicide use can also lead to a loss of floral diversity through an increase in pervasive invasive plant species that outcompete wildflowers (R. Jean, Environmental Solutions & Innovations, Inc., pers. comm. 2016). For example, in some areas, floral diversity has been greatly reduced by the invasion of garlic mustard (*Alliaria petiolata*) and Japanese (*Lonicera japonica*) and Amur (*L. maackii*) honey suckle (R. Jean, Environmental Solutions & Innovations, Inc., pers. comm. 2016). Because of drift from agricultural plots, loss of flowering plants and reductions in floral diversity occur in surrounding natural areas as well (Potts *et al.* 2010, p. 350).

Some agricultural crops increase floral availability (*e.g.,* sunflower crops), but, at a large scale, these crops are grown as monocultures (only one species of plant). Monocultures typically do not support bumble bees, as the flowers are only available for a short period of time. Bumble bees require floral resources throughout their foraging period (spring through fall); areas with high floral diversity have variation in flowering times.

Ongoing urbanization also contributes to the loss and fragmentation of natural habitats. Bees, however, may be more resilient to loss due to urbanization, as many urban areas have gardens that can support bees (Goulson *et al.* 2010, p. 1207; Goulson *et al.* 2015, p. 2; Frankie *et al.* 2005, entire). Highly urbanized areas, however, have few bees, and roads and human infrastructure associated with urban areas add to habitat fragmentation and may lead to direct mortality (*e.g*., from vehicle collisions) (Goulson *et al.* 2015; p. 2).

Although habitat loss has established negative effects on bumble bees (Goulson *et al.* 2008; Williams and Osborne 2009, pp. 371-373), many feel it is unlikely to be a main driver of the recent, widespread North American bee declines (Szabo *et al.* 2012; p. 236; Colla and Packer 2008, p. 1388; Cameron *et al.* 2011b, p. 665). Szabo *et al.* (2012, p. 237) "…consider it unlikely that such effects [of habitat loss] could have simultaneously triggered population declines among multiple species living in different areas, including areas that still retain extensive potential habitat." Furthermore, the bee species examined in studies by Colla and Packer (2008, entire) and Cameron *et al.* (2011b, entire) were recently common in both rural and urbanized regions, indicating they persisted despite extreme habitat loss that had already occurred. However, the past effects of habitat loss and degradation may continue to have impacts on bumble bees that are stressed by other factors. If there is less food available or if the bumble bees must extend more energy and time to find food, they are less healthy overall, and, thus, less resilient to other stressors. For example, nutritional stress may decrease the ability to survive parasite infection (Brown *et al.* 2000, pp. 425-426) or cope with pesticides (Goulson *et al.* 2015, p. 5). Furthermore, bumble bees may be more vulnerable to extinction than other animals because their colonies have long cycles, where reproductive individuals are primarily produced near the end of those cycles. Thus, even slight changes in resource availability could have significant cumulative effects on colony development and productivity (Colla and Packer 2008, p. 1380).

5.1.4 Small population dynamics

The social organization of bees has huge impacts on their population biology and genetics (reviewed in: Pamilo *et al.* 1997, entire; Chapman and Bourke 2001, entire; Zayed 2009, entire). *Bombus affinis* is a eusocial bee species and a population is made up of colonies, rather than individuals. Consequently, the effective population size is much smaller than the census population size. Genetic effects of small population sizes depend on the effective population size (rather than the actual size) and in *B. affinis* the effective population sizes are inherently small due to their eusocial, haplodiploid life history (see Chapter 2 for further discussion).

Diploid male production is probably one of the most significant genetic consequences of small population sizes in eusocial bees. *Bombus affinis* exhibits a single-locus complementary sex determination; a diploid female mates with a haploid male sharing one of her sex determining alleles. They produce a brood of diploid females, diploid males, and haploid males (Crook and Crozier 1995, p. 281). Diploid males produce diploid sperm and are effectively sterile. The production of diploid males is a necessary by-product of complementary sex determination, and is a large threat to the short-term viability of small populations especially when interacting synergistically with inbreeding depression.

The production of diploid males is caused by low allelic variation at the sex determination locus and results in a positive feedback cycle (Zayed 2009, p. 241). Diploid male production causes smaller population sizes and decreased population growth. In turn, declining populations are expected to produce higher frequencies of diploid males because of the overall reduced number of reproductive individuals. This phenomenon is termed the "diploid male vortex" and may interact synergistically with other deterministic factors causing faster extinction rates than would be expected; Carvalho 2001 (as cited in Zayed 2009, p. 242) demonstrated a direct causal link between diploid male production, and extinction in bees.

In small populations, inbreeding can reduce individual fitness, and loss of genetic variability from genetic drift can diminish the ability for populations to adapt to a changing environment (Frankham 2005, p. 131). In haplodiploids, recessive lethal and mildly deleterious alleles are more likely to be purged in haploid males and occur less often in haplodiploid populations, versus diploid populations (Werren 1993, as cited in Zayed 2009, p. 243). However, female-limited genes likely constitute a significant source of inbreeding depression. In small populations, bees are expected to suffer from reduced fitness due to inbreeding depression.

Many populations are extirpated or reduced due to deterministic factors like habitat loss, overexploitation, and climate change. However, even when the habitat and conditions are favorable, populations may become extinct as a result of various stochastic events and natural catastrophes. Random events like drought, floods, and fires exacerbate each other and become more likely to cause extirpation or extinction in small populations (Shaffer 1981, p. 131). Bee populations and communities commonly experience large annual fluctuations in population size (Multiple sources in Murray *et al.* 2009, p. 211-212), which exposes populations to extirpation due to natural variation in environmental conditions (see Chapter 2 for further discussion of inherent vulnerability to environmental stochasticity).

Populations require genetic diversity to adapt with changing environments. Populations with low effective population numbers are susceptible to greater genetic loss, and maintain low genetic variation. Consequently, small populations have limited potential evolutionary responses to changes in their environment such as novel pathogens, pesticides, and contaminants. The nature of eusocialism and

complementary sex determination makes *B. affinis* highly susceptible to extinction in small population sizes and isolated populations.

5.1.5 Climate change

Global climate change is broadly accepted as one of the most significant risks to biodiversity world-wide. Specific impacts of climate change on pollinators are not well understood, however. Most of the existing information on climate change impacts on pollinators comes from studies on butterflies--studies specifically related to bumble bees are scant.

The changes in climate likely to have the greatest effects on bumble bees include: increased drought, increased flooding, increased storm events, increased temperature and precipitations, early snow melt, late frost, and increased variability in temperatures and precipitation. These climate changes may lead to decreased resource availability (due to mismatches in temporal and spatial co-occurrences), decreased availability of nesting habitat (due to changes in rodent populations or increased flooding or storms), increased stress from over-heating (due to higher temperatures), and increased pressures from pathogens and non-native species, (Goulson *et al.* 2015, p. 4; Goulson, pers. comm. 2016; Kerr *et al.* 2015, pp. 178-179; Potts *et al.* 2010, p. 351; Cameron *et al.* 2011a, pp. 35-37; Williams and Osborne 2009, p. 371).

Climate variability may lead to range shifts, such that there is spatial mismatch among plants and their pollinators (Memmott *et al.* 2007, p.712). While this has been demonstrated in butterflies (Forister *et al.* 2010, pp. 2088-2089; Hickling *et al.* 2006, p. 452), it may be less of a factor for bumble bees. As generalists, they do not require synchrony with a particular plant species. That being said, elevational range shifts have been documented in some bumble bees (*e.g*., Pyke *et al*. 2016, pp. 8-10). Temporal mismatches may be more of an issue for bumble bees, especially *B. affinis*, due to their long active season during which they require consistent access to floral resources. Also, floral resource availability in early spring is a critical for bumble bees, as that is when they first emerge from diapause and initiate nests. Thus, temporal asynchrony could lead to diminished resource availability at times that are critical to bee development and colony success. For example, as the climate in the Rocky Mountains has become warmer and drier in the past 30 years, researchers have observed a mid-season period of low floral resources (Aldridge *et al.* 2011, pp. 908-909); in other words, climate change and drought may compress bloom times. One study (Miller-Struttmann *et al*. 2015, pp. 1541-1544) argues that declining availability of floral resources due to warmer summers may be favoring bumble bees that are generalists, and thus may be prompting evolution of bumble bees toward features that allow for more generalist foraging (shorter-tongues). Other potential effects from climate change include increased flooding and storm events, which may directly reduce available nesting habitat and hibernating habitat (Goulson *et al.* 2015, p. 4) by inundating those areas. Changes in rodent populations due to climate change may also reduce nesting habitat, as bumble bees often use rodent burrows as nesting areas. Furthermore, bumble bees are poorly adapted to high temperatures (see Chapter 2.2), thus are vulnerable to increased stress from over-heating. Finally, effects from climate change may add increased stress in the future, further compounding pressures from other factors, including pathogens, non-native species, and habitat loss (Goulson *et al.* 2015, p. 4-5; Kerr *et al.* 2015, pp. 178-179; Williams and Osborne 2009, p. 371).

5.1.6 Synergistic Effects

It is likely that several of these risk factors are acting additively and synergistically on *Bombus* species (Goulson 2015, p. 5) and the combination of multiple stressors is likely more harmful than a stressor acting alone (Gill *et al.* 2012; Coors and DeMeester 2008; Sih *et al.* 2004). There is recent evidence that the interactive effects of pesticides and pathogens could be particularly harmful for bumble bees (Fauser-Misslin *et al.* 2014, pp. 453-455; Baron *et al.* 2014, pp. 463-465) and other bees (Alaux *et al.* 2010, pp. 775-777; Pettis *et al.* 2012, pp. 155-156; Vidau *et al.* 2011, pp. 3-5; Aufavre *et al.* 2012, pp. 2-3). Nutritional stress may compromise the ability of bumble bees to survive parasitic infections as evidenced by a significant difference in mortality in bumble bees on a restricted diet than well fed bees infected with *Crithidia bombi* (Brown *et al.* 2000, pp. 424-425). Bumble bees with activated immunity may have metabolic costs, such as increased food consumption (Tyler *et al.* 2006, p. 2; Moret and Schmid-Hempel 2008, pp. 1166-1167). Furthermore, exposure to pesticides may increase with increased food consumption in infected bees (Goulson 2015, p. 5). There is evidence that activating immunity impairs learning in bumble bees (Riddelland Mallon 2006, Alghamdi *et al.* 2008, p. 480). Impaired learning is thought to reduce the ability of bees to locate floral resources and extract nectar and pollen, therefore, exacerbating nutritional stresses (Goulson 2015, p. 5).

If *B. affinis* has low genetic diversity, it may be more susceptible to disease and other stochastic stressors. For example, evidence of the relationship between low genetic diversity and disease susceptibility was discussed in Cameron *et al.* (2011b, p. 665), who stated that declining North American species with low genetic diversity have higher prevalence of the pathogen *N. bombi*.

5.2 Beneficial Factors

We are aware of only a few specific measures for bumble bee conservation at any of the current locations of *B. affinis* in the United States. In Canada, *B. affinis* was listed as Endangered on Schedule 1 of the Species at Risk Act in 2012 and a recovery strategy has been proposed for the species there (Environment and Climate Change Canada 2016, entire). However we are aware of only 9 current occurrences (3 grids, see Ch. 6) in Canada. *Bombus affinis* is listed as state endangered in Vermont and Special Concern in Connecticut, Michigan, and Wisconsin. Of those four states, there are only 18 grids with current records in one state, Wisconsin. A few agencies and others have or may soon start monitoring programs, such as Bumble Bee Watch (www.bumble beewatch.org), a collaborative citizen science effort to track North American bumble bees, the Xerces Society, and the IUCN Conservation Breeding Specialist Group (CBSG) has developed some general conservation guidelines for bumble bees (Hatfield *et al.* 2014b, pp.11-16; Cameron *et al*. 2011a, entire).

There is an increased awareness on pollinators, in general, and thus efforts to conserve pollinators may have a fortuitous effect on *B. affinis*. For example, planting appropriate flowers may contribute to pollinator conservation; however there is a need to develop regionally appropriate and bumble bee - specific recommendations based on evidence of use (Goulson 2015, p. 6).

5.3 Future Scenarios

In this section, we examined the risk factors discussed in the previous sections of this chapter to identify future risk scenarios, and later use this to forecast the future condition of *B. affinis* (Chapter 6). Specifically, we sought to quantify which factors are contributing to the decline of the species, the relative roles of each stressor, and how each stressor will contribute to the species population trend into

the future (at 10, 20, 30, 40 and 50 years). We used this information to determine likely future scenarios and how those scenarios will play out into the future.

Expert Identification – Through informal conversations and correspondence, we consulted with experts on the risk factors identified in this chapter and *Bombus* ecology. Experts were identified through those that were lead or co-authors on scientific literature on one or more of these risk factors or an expert on *Bombus* ecology that was familiar with the risk factors affecting *B. affinis* or other *Bombus* species.

Expert Elicitation Questionnaires –In order to quantify what factors are contributing to the decline of the species, the relative roles of each stressor, and how each stressor will contribute to the species' status into the future, we consulted with subject-matter experts. Questionnaires were sent to 6 pathogen experts, to 11 pesticide experts, to 6 bumble bee population experts, 6 *Bombus* habitat experts, and to 9 *Bombus* experts familiar with the potential effects of climate change effects on the species. Several experts were asked about more than one stressor.

Experts were asked about the factors that are currently contributing to the decline of the species (see Appendix 1.2 for an example questionnaire). For each stressor, experts were asked the following three questions (tailored to each stressor):

1. What percent of the rate of decline of *B. affinis* can be attributed to this stressor?
2. Which scenario is the mostly likely to occur in the future at the following time-periods?

3a. How will your selected scenarios in question 2 effect the current rate of decline?
3b. What is the magnitude of change expected?

Responses were compiled, and the relative weights of each stressor (calculated from the median percent decline attributed to each stressor) and the most likely future effects to the status of *B. affinis* trajectory were calculated for each stressor individually. Weights of each stressor were based on the relative contribution of each stressor to the current decline. The relative weights of the stressors were normalized (to add to one) to adjust for data measured on different scales. We requested that responses to questions 1 and 3B be assigned to one of four "bins" – each representing a range of values (*e.g.*, bin 1 ranged from 1-25%). The magnitude of change due to each stressor alone was calculated by determining the median of all expert's expected future impacts on the current rate of decline (25%, see Chapter 4) at the various timeframes.

We determined the most likely future scenario by taking the median of all responses to question two for each individual stressor at each of the five time periods. For example, five experts provided responses regarding pathogens - - for year ten, four experts said that the most likely scenario is continued exposure to and continued impacts of pathogen[s] as experienced over the past 20 years with no additional epizootics emerging (scenario 2) and one expert said continued exposure to current pathogen[s] and minimal natural resistance among extant colonies with additional epizootics highly likely (scenario 3) is the most likely scenario. Thus, the median is scenario 2. We used this same method to determine the most likely scenario for subsequent years and all stressors.

Using the most likely future scenario for each stressor, we determined the most likely future rate of decline for each of the five time periods by summing the rates of decline due to each stressor. We then used this information to estimate how many grids will remain at future time periods (Chapter 6).

Results

Responses - We received responses from five pathogen experts, four pesticide experts, three bumble bee population experts, three habitat experts, and five *Bombus* experts familiar with climate change (a total of 8 experts responded, with some individuals having expertise in multiple areas). A few experts declined to provide information in the questionnaires, stating that the uncertainties into the future were too speculative to quantify. Others were familiar with a particular stressor (*e.g.*, effects of pesticides on bumble bees) but did not feel familiar with the species specifically, or familiar with the application rates in the geographic region of recent *B. affinis* occurrences (*e.g.,* experts from overseas). A few experts provided some information, but did not complete the magnitude of change of stressors into the future.

Because there were some uncertainties around the most likely future scenario, the individual responses may not fully comport with the description of the scenario. For example, although the median most likely future scenario for pathogens is that their contribution to the rate of decline remains unchanged over time, because experts' views on this varied, the actual values that appear for later time periods may be different.

The responding experts believed that pathogens have had the greatest effect on the *B. affinis* current rate of decline (weighted higher than all the other stressors), with a relative weight of 0.31 (*i.e*., 31% of the decline to date is attributable to pathogens, Table 5.1). Based on expert's responses, as well as informal conversations with other species experts, the most likely future scenario for pathogens is continued exposure to current pathogen[s], minimal natural resistance among extant colonies, and additional epizootics are highly likely. Taking the median of the expert's responses, the degree to which pathogens are likely to affect the rate of decline in the future varied by decade, increasing the rate of decline by 38% in 10 years, 6.2% in 20 years, and 12.5% in 30, 40, and 50 years. Some experts indicated more uncertainty the further into the future that effects were estimated.

Relative to the other stressors, experts believe that 15% of the rate of decline to date is due to the effects of pesticides (relative weight of 0.15). Based on expert's responses, as well as informal conversations with other species experts, the most likely future scenario for pesticides is no change in trends of pesticide application rates and extent of use and no new potentially detrimental chemicals used. One expert believes, however, that, given our increased understanding of how important pesticides are as stressors, more detrimental compounds are less likely to be used as widely in the future. That expert believes that public and regulatory scrutiny of pesticide use will eventually reverse the decline, but not soon. Thus, taking the median of the expert's responses, the degree to which pesticides are likely to affect the rate of decline in the future varied by decade are: no effect to the rate of decline by years 10 and 20, and decreasing the rate of decline by 6.2% in 30 years, 6.2% in 40 years, and 12.5% in 50 years. Some experts indicated more uncertainty the further into the future that effects were estimated.

The relative weight of habitat loss or degradation as a stressor is 0.23 (23% of the decline to date is attributable to habitat loss or degradation), however, the expert responses varied greatly, with two experts attributing no or only a small percentage of the decline to this stressor, while one expert attributed a high percentage to habitat loss. Based on expert's responses, as well as informal conversations with other species experts, the most likely future scenario for habitat loss and degradation is that the rate of habitat loss and degradation will continue into the future, with continued loss of floral resources and connectivity. Taking the median of the expert's responses, the degree to which habitat loss and degradation are likely to affect the rate of decline stayed constant into the future, increasing the rate of decline by 12.5% in each decade.

The relative weight of climate change as a stressor is 0.15 (15% of the decline to date is attributable to the effects of climate change). Taking into consideration the most recent information on climate change impacts to bumble bees, (per Kerr *et al.* 2015) and based on information from species experts, the most likely future scenario is that climate change will have an increasing negative effect on *B. affinis*. Taking the median of the expert's responses, the degree to which climate change is likely to affect the rate of decline in the future varied by decade: increasing the rate of decline by 12.5% in 10 and 20 years, and by 25% in 30, 40, and 50 years.

The relative weight of the effects of small population size as a stressor is 0.15 (15% of the decline to date is attributable to the effects of small population size. Based on expert's responses, as well as informal conversations with other species experts, the most likely future scenario for small population effects is increased vulnerability to demographic, genetic or environmental stochasticity due to smaller population sizes. Taking the median of the expert's responses, the degree to which small population effects are likely to affect the rate of decline in the future increased over time: increasing the rate of decline by 12.5% in 10 and 20 years, and by 38% in 30, 40, and 50 years.

Table 5.1. Relative weights attributed to each stressor into future years (10, 20, 30, 40 and 50 years).

Stressor	Weights	Rate of decline per stressor	10+ years	20+ years	30+ years	40+ years	50+ years
Pathogen	0.308	0.077	0.380	0.062	0.125	0.125	0.125
Pesticide	0.154	0.038	0.000	0.000	-0.062	-0.062	-0.125
Habitat	0.231	0.058	0.125	0.125	0.125	0.125	0.125
Climate Change	0.154	0.038	0.125	0.125	0.252	0.252	0.252
Small Pop	0.154	0.038	0.125	0.125	0.380	0.380	0.380

The grid resiliency ranking resulted in nearly all of the grids having identical habitat conditions rankings (22 of 25) and very similar stressors acting influencing the current condition of those locations (see Appendix 2). All evaluated grids had at least one identified stressor and twenty (83%) had one or more severe stressor. Eighteen grids (75%) had one or more stressor with high severity and broad extent (>75% of the area). We believe that the grids with one or more broad and severe stressor would have less resiliency into the future.

5.4 Synopsis

Based our analyses above, we assume that pathogens, pesticides, and habitat loss and degradation will continue to affect the species at their current rate into the future (at all timeframes). However, small population size and climate change are likely to have an increasing negative effect on the species into the future. Small population size is expected to increase by (by 38% in years 30, 40, and 50) and therefore play a larger role in the rate of the decline of the species. Similarly, climate change is expected to increase into the future (by 25% in year 50), and therefore contribute more to the species decline. Information received in informal conversations with other species experts also supports that this is a likely future scenario.

Description of Future Scenarios

As discussed above, the current rate of decline is 25%. We summarized the future rate of decline in three scenarios: "status quo" rate of decline, where the current rate of decline continues unchanged into the future; the "most likely" scenario as calculated based on the expert responses (see above); and the "better case" scenario (described below).

The most likely scenario for the future rates of decline are as follows: in year 10, the rate of decline is 30%, 27% in year 20, 29% in years 30, 40, and 50 (Table 5.5).

Based on expert responses and discussions, although the most likely future scenario is as described above, there is another potential future scenario, which we are calling the "better case" scenario. Some experts believe that the spread and resulting effects of current pathogens may have played out (or will soon play out), and will have no further impacts on the species into the future. Although we do not believe this is a plausible situation, as novel pathogens are likely on the horizon, we conducted an analysis for this potential future "better case" scenario. In this scenario, we changed only the change in rate of decline due to pathogens (such that there is no future effect), while maintaining the change in rate of decline due to all other stressors as was used in the most likely future scenario. The better case scenario for the future rates of decline are as follows: In year ten, the rate of decline is 19% in year 10, 19% in year 20, 16% in year 30 and 40, and 15% in year 50 (Table 5.5).

Table 5.2. Future rate of decline under three future risk scenarios: a) status quo, b) most likely, and c) better-case. The "current" rate of decline was calculated based on change in % Occupancy from 1980 to 2010.

	Status Quo	Most Likely	Better-case
Current	25%	25%	25%
Year 10+	25%	30%	19%
Year 20+	25%	27%	19%
Year 30+	25%	29%	19%
Year 40+	25%	29%	19%
Year 50+	25%	29%	19%

Chapter 6. Analysis of Future Conditions

In this chapter, we describe our analysis of the future condition of *B. affinis*. Specifically, we forecast the number of populations likely to persist over time and use this information to infer the future distribution of *B. affinis* occurrence over the next 50 years.

6.1 Resiliency

Resiliency is the ability to sustain populations in the face of environmental variation and transient perturbations. Bombus affinis resiliency is described as having healthy populations distributed across an array of climatic conditions.

Methods

To assess the future degree of resiliency of *B. affinis*, we forecasted number of *B. affinis* grid occurrences for next 50 years using the three future risk scenarios described in Chapter 5 to forecast the number of populations expected to persist in future decades. We calculated the expected number of populations using the equation:

$$N_{t+1}=N_t*e^{(r+\varepsilon)},$$

where, *Nt* is the number of populations currently, *r* is the intrinsic rate of growth, and *ε* is the effect of environmental stochasticity. The effect of environment stochasticity is a randomly generated deviation from the intrinsic population growth rate and is selected from a normal distribution with a mean of 0 and variance equal to σ^2 (*i.e.*, $(\log(\lambda_t) - mu)^2$). We calculated mu by simulating population numbers over time using the change in % Occupancy from the pre-decline decades (1950-1980) as mu. We ran the model for 5000 iterations and used the average variance in λ as our starting mu. We held ε constant for all iterations. The intrinsic rate of growth, r, was calculated for each decade by first calculating r for the current time period:

$$r_c = \ln(\text{product}(10\text{-year } \lambda)^{1/n}$$

The future decade intrinsic rates of growth, r_t, were then calculated as:

$$r_t= r_c + D_t^{1/10},$$

where, the D_t is the annual future rate of decline derived from the future risk scenarios (Table 5.3.2). We projected N_{t+1} for 100 years and ran the model for 5000 iterations to obtain probabilities of extirpation for the three future risk scenarios.

Fundamental assumptions applied in our future resiliency analyses are:

1. The magnitude of environmental stochasticity, ε, stays constant over time
2. The 69 populations have normal vital rates
3. Future rates of decline are captured in one of the 3 risk scenarios
4. There are no colony or population level Allee effects (vital rates do not change as N declines)

Results

The number of populations is forecasted to decline to zero for all three future risk scenarios (Table 6.1). Although the randomness of environmental stochasticity (Ɛ) causes some temporal variation within a

simulation (a single risk scenario), the trajectory is unchanged across the 5000 iterations; all iterations end with no occurrences persisting.

Table 6.1. The forecasted number of populations for the future time periods. The number of grids are mean number of populations projected to persist for the three future risk scenarios: a) status quo, b) most likely, and c) better-case.

	Projected Number of Grids Rangewide		
Time	Status Quo	Most Likely	Better-case
Year 5	36	36	36
Year 10	22	22	22
Year 20	6	6	7
Year 30	0	0	0
Year 40	0	0	0

6.2 Representation

Representation is the ability to adapt to changing environmental conditions; it's the species evolutionary capacity or flexibility. Bombus affinis representation is described as having healthy populations distributed across a wide breadth of ecological conditions (i.e., having populations distributed widely across ecoregions).

Methods

We used the forecasted number of populations expected to persist in each ecoregion to calculate future spatial extent. The spatial extent is the proportion of an ecoregion occupied by *B. affinis* occurrences, and calculated by multiplying the total number of *B. affinis* grids predicted to be occupied in an ecoregion by 10,000 (area of a 10 km x 10 km grid). We calculated the % of the total extent of occurrence (EoO) of *B. affinis* occupied during future decades by dividing the forecasted future EoO by the total EoO. "Total" EoO was derived by multiplying the number of total occurrences (n=894) by 10,000. We were unable to assess the distribution of grid occurrences within an ecoregion, and therefore, we could not predict the number of states nor counties that will be occupied into the future.

The fundamental assumption applied in forecasting distribution and spatial extent of *B. affinis* is:

1. The spatial dispersion (or connectivity) of populations within an ecoregion has not effect on population persistence.

Results

Distribution – Based on the population projections, marked losses in *B. affinis* range are expected. By year 10, 4 of 6 currently occupied ecoregions will no longer be occupied under all three future risk scenarios (Table 6.2).

Table 6.2. The forecasted number of ecoregions occupied in future decades under the three future risk scenarios. The columns represent the number of ecoregions projected to have at least one *B. affinis* population in each of the future decades.

	Forecasted Number of Ecoregions Occupied				
Risk Scenario	Year 10	Year 20	Year 30	Year 40	Year 50
Most Likely	2	1	0	0	0
Status Quo	2	1	0	0	0
Better-case	2	1	0	0	0

Spatial Extent- With losses of populations and ecoregions, the future rangewide spatial extent, EoO, of *B. affinis* declines as well. Within 10 years, the % EoO is forecasted to be reduced to 2.0% of historical extent under all three scenarios (Table 6.3).

Table 6.3. The forecasted rangewide Extent of Occurrence rangewide for the future time period under the three future risk scenarios. Extent of Occurrence (EoO) is the percent of the historical range occupied by *B. affinis* in each of the future decades.

	Future Rangewide Extent of Occurrence				
Risk Scenario	Year 10	Year 20	Year 30	Year 40	Year 50
Most Likely	2%	1%	0%	0%	0%
Status Quo	2%	1%	0%	0%	0%
Better-case	2%	1%	0%	0%	0%

As future losses were modeled on patterns of past declines, the spatial distribution of the loss of populations is projected to occur throughout the range. The forecasted number of populations, and thus the spatial extent within the ecoregions, declines in all ecoregions for all three future risk scenarios (Table 6.4a-c). Under all future risk scenarios, *B. affinis* is extirpated from all current ecoregions by Year 30. In all but Ecoregion 250, the % total EoO is reduced to less than 5% by Year 10; Ecoregion 250 is reduced to 13% by Year 10 and 0% of its original EoO by Year 30.

Table 6.4a-c. The forecasted number of *B. affinis* populations and the extent of occurrence (EoO) by ecoregion for the future time period under the three future risk scenarios. The "Number" is the forecasted number of grids within the ecoregions to have at least one *B. affinis* record. The projected Extent of Occurrence, (EoO) is the proportion of the total spatial extent of the ecoregion occupied during a decade.

a. "Status Quo" Future Risk Scenario

	Number					Extent of Occurrence				
Ecoregion	Yr 10	Yr 20	Yr 30	Yr 40	Yr 50	Yr 10	Yr 20	Yr 30	Yr 40	Yr 50
135	0	0	0	0	0	0%	0%	0%	0%	0%
210	0	0	0	0	0	0%	0%	0%	0%	0%
220	18	6	0	0	0	4%	1%	0%	0%	0%
230	0	0	0	0	0	0%	0%	0%	0%	0%
250	4	0	0	0	0	13%	0%	0%	0%	0%
M220	0	0	0	0	0	0%	0%	0%	0%	0%

b. “Most Likely” Future Risk Scenario

	Number					Extent of Occurrence				
Ecoregion	Yr 10	Yr 20	Yr 30	Yr 40	Yr 50	Yr 10	Yr 20	Yr 30	Yr 40	Yr 50
135	0	0	0	0	0	0%	0%	0%	0%	0%
210	0	0	0	0	0	0%	0%	0%	0%	0%
220	18	6	0	0	0	4%	1%	0%	0%	0%
230	0	0	0	0	0	0%	0%	0%	0%	0%
250	4	0	0	0	0	13%	0%	0%	0%	0%
M220	0	0	0	0	0	0%	0%	0%	0%	0%

c. “Better-case” Future Risk Scenario

	Number					Extent of Occurrence				
Ecoregion	Yr 10	Yr 20	Yr 30	Yr 40	Yr 50	Yr 10	Yr 20	Yr 30	Yr 40	Yr 50
135	0	0	0	0	0	0%	0%	0%	0%	0%
210	0	0	0	0	0	0%	0%	0%	0%	0%
220	18	7	0	0	0	4%	1%	0%	0%	0%
230	0	0	0	0	0	0%	0%	0%	0%	0%
250	4	0	0	0	0	13%	0%	0%	0%	0%
M220	0	0	0	0	0	0%	0%	0%	0%	0%

6.3 Redundancy

Redundancy is the ability of a species to guard against losses of adaptive diversity due to catastrophic events. Bombus affinis redundancy is described as having multiple, healthy populations widely distributed across the breadth of adaptive diversity relative to the spatial occurrence of catastrophic events.

As we are unable to predict which specific populations persist, we cannot use the methodology for historical and current conditions to derive probabilities of ecoregion extirpation. However, as the number of populations decline, the risk of ecoregion-wide extirpation increases.

6.4 Synopsis

The abundance of *B. affinis* is forecasted to decline over time under all three risk scenarios considered. The rate of decline may be exacerbated by continued loss of spatial heterogeneity and consequent increased risk of population extirpation due to environmental stochasticity. Further, as fewer populations persist and the spatial extent of the species declines, the species ability to withstand catastrophic events and changes in its environment is likely to be greatly reduced.

Chapter 7. Synthesis

This Chapter synthesizes the results from our historical, current, and future analyses and discusses the consequences for the future viability of *B. affinis*. We assessed the viability of *B. affinis* by evaluating the ability of the species to maintain a sufficient number and distribution of healthy populations to withstand environmental stochasticity (resiliency), catastrophes (redundancy), and changes in its environment (representation).

Prior to the mid- to late 1990s, *B. affinis* was widely distributed across areas of 31 states/provinces, including Quebec, North Dakota, South Dakota, Minnesota, Wisconsin, Iowa, Missouri, Illinois, Kentucky, Tennessee, Indiana, Michigan, Ontario, Ohio, Pennsylvania, New York, Vermont, Maine, Massachusetts, New Hampshire, Delaware, Rhode Island, Connecticut, New Jersey, Maryland, Virginia, District of Columbia, West Virginia, North Carolina, South Carolina, and Georgia. Since 2000, *B. affinis* has been reported from Illinois, Indiana, Massachusetts, Maryland, Maine, Minnesota, North Carolina, Ohio, Pennsylvania, Tennessee, Virginia, Wisconsin, and Ontario (Figure 4.1). The primary causes of the decline include habitat loss and degradation, pathogens, pesticides, and small population dynamics.

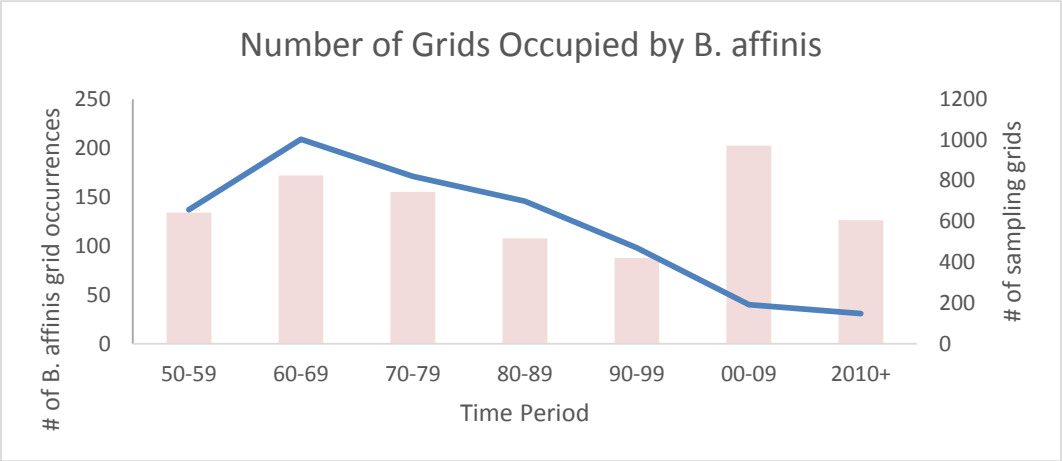
Our analyses indicate that the viability of *B. affinis* has declined since the mid- to late 1990s, and this decline is projected to continue. The magnitude and the implications of reductions in resiliency, representation, and redundancy are discussed below.

7.1 Resiliency

> *Resiliency is the ability to sustain populations in the face of environmental variation and transient perturbations. Bombus affinis resiliency is described as having healthy populations distributed across an array of climatic conditions.*

Bombus affinis abundance has markedly declined over time as indicated by the number of grid occurrences (populations), % occupancy, and relative abundance. Historically, the median number of grid occurrences per decade was 146; since 2000, there are 69 grid occurrences; 42 documented from the 2000 decade and 32 from the 2010 decade. The current number of grid occurrences represents a 91% decline from the historical period (Figure 4.1).

Figure 7.1. The number of *B. affinis* grid occurrences from 1950–2015. The line represents the number of *Bombus* grids with at least 1 *B. affinis* record during the 10-year period. The bars represent the number of grids with a *Bombus* record; they represent partial sampling effort as negative occurrence data are not included.



The pattern of loss holds for % Occupancy. *Bombus affinis* abundance, when taking survey effort into account, shows the same precipitous decline beginning just prior to 2000 (Figure 7.2). From 2010, it appears that % Occupancy increases, but this is more likely due to an increase in search intensity rather than an increase in *B. affinis* abundance. Dr. Dave Goulson (pers. comm. 2016) cautioned that as interest in a species of concern increases, so too does the search intensity for the species. Thus, an increase in the number of *B. affinis* grid occurrences may not be an indication of an increasing or stabilizing trend, but instead, increased searcher interest. Presumably in response to concern for loss of *B. occidentalis*, bumble bee sampling efforts increased greatly in the 2000s decade, but despite this increase in sampling, *B. affinis* occurrence continued to drop. The slight uptick in % Occupancy in 2010, from 4% to 5%, is likely due to an increased effort to search specifically for *B. affinis*. This becomes more apparent when the data are examined based on mid-decade periods; occupancy declines through 2015 despite the highest (more than double) search effort conducted in any decade (Figure 7.3). Note, our analyses do include an additional 9000+ *Bombus* records (from yet to be determined number of grids) from Vermont in 2013 (5000 *Bombus* specimens) and Maine in 2015 (>4000 *Bombus* specimens); none of those 9000+ records were *B. affinis* (Richardson, pers. comm. 2016).

Figure 7.2. Historical and current *B. affinis* % Occupancy. The % Occupancy is the proportion of *Bombus* occupied grids with *B. affinis* presence. Red bars represent # of *Bombus* grids; blue line represents the % of *Bombus* grids occupied by *B. affinis*.

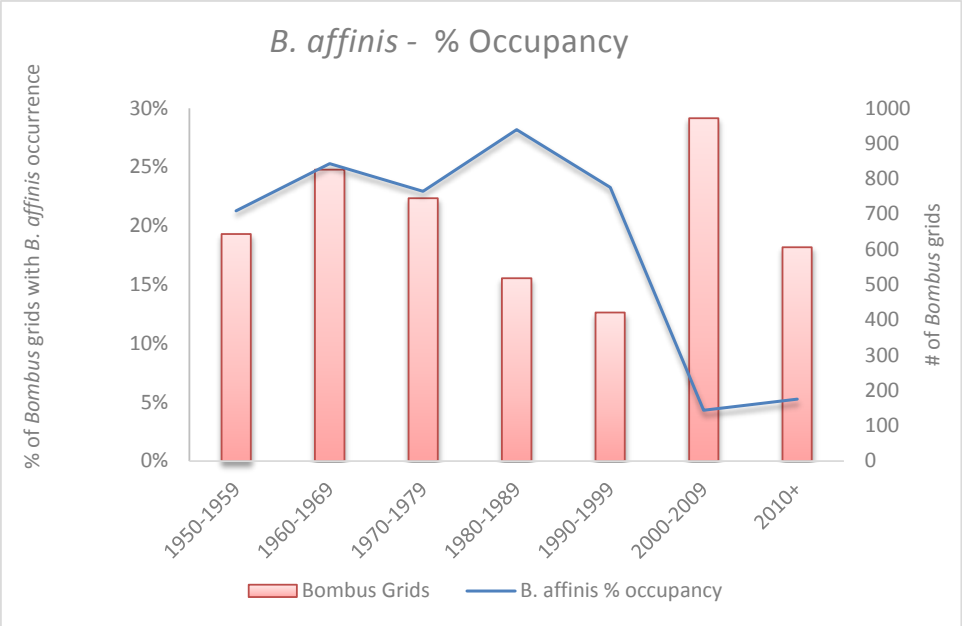
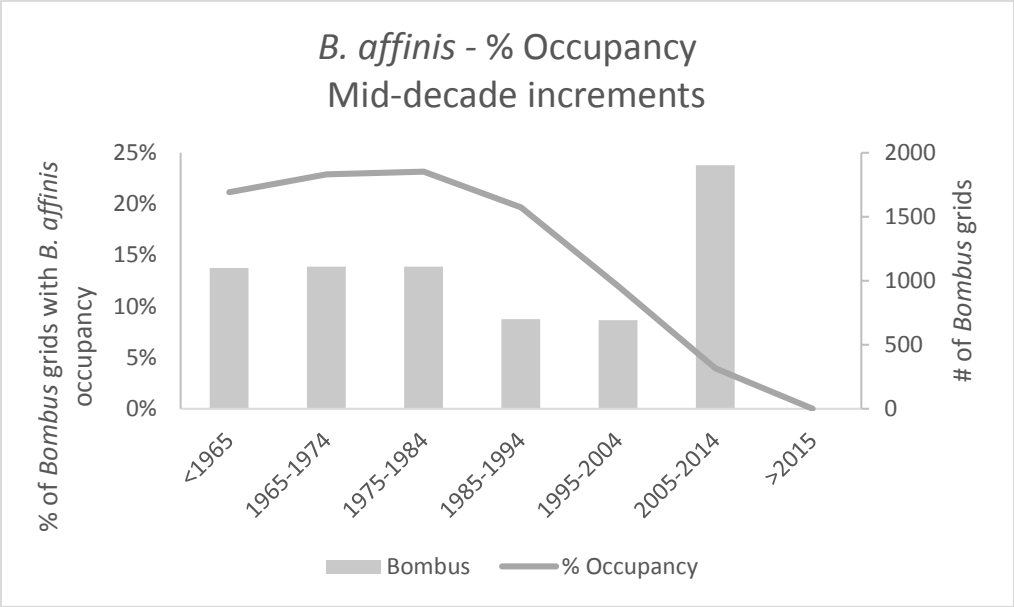
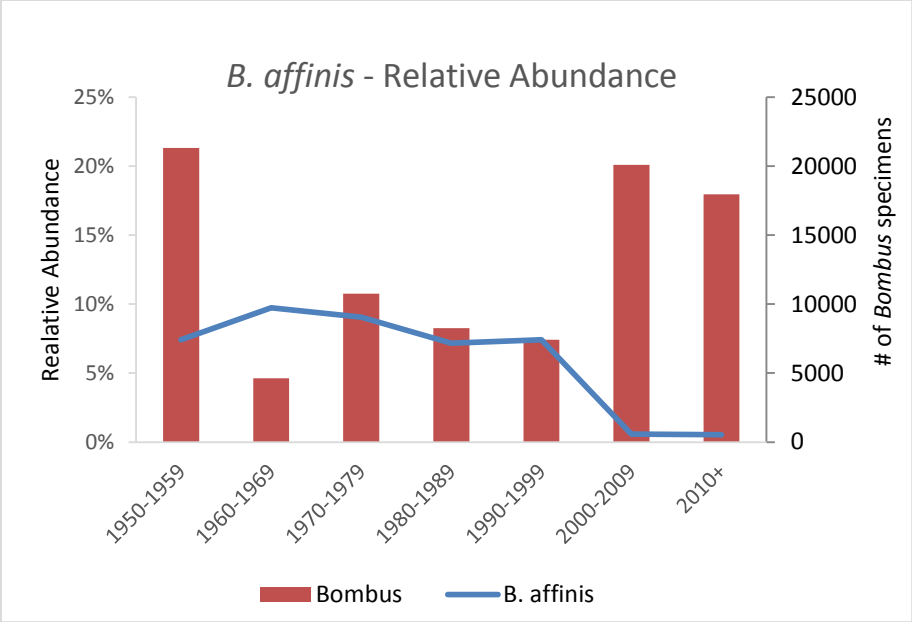


Figure 7.3. Historical and current *B. affinis* % Occupancy trend by mid-decade. The % Occupancy is the proportion of *Bombus* occupied grids with *B. affinis* presence. The bars represent # of *Bombus* grids; the line represents the % of *Bombus* grids occupied by *B. affinis*.



As a secondary measure of trend in occurrence, we evaluated the abundance of *B. affinis* relative to all *Bombus* species sampled. The results mirror the trend in grid occurrence data, with *B. affinis* relative abundance markedly declining over time (Figure 7.4). Notably, most other *Bombus* species showed no change or an increase in relative abundance over time, with the exception of two other species (*B. pensylvanicus* and *B. terricola*), which appear to have similar decline patterns as *B. affinis*.

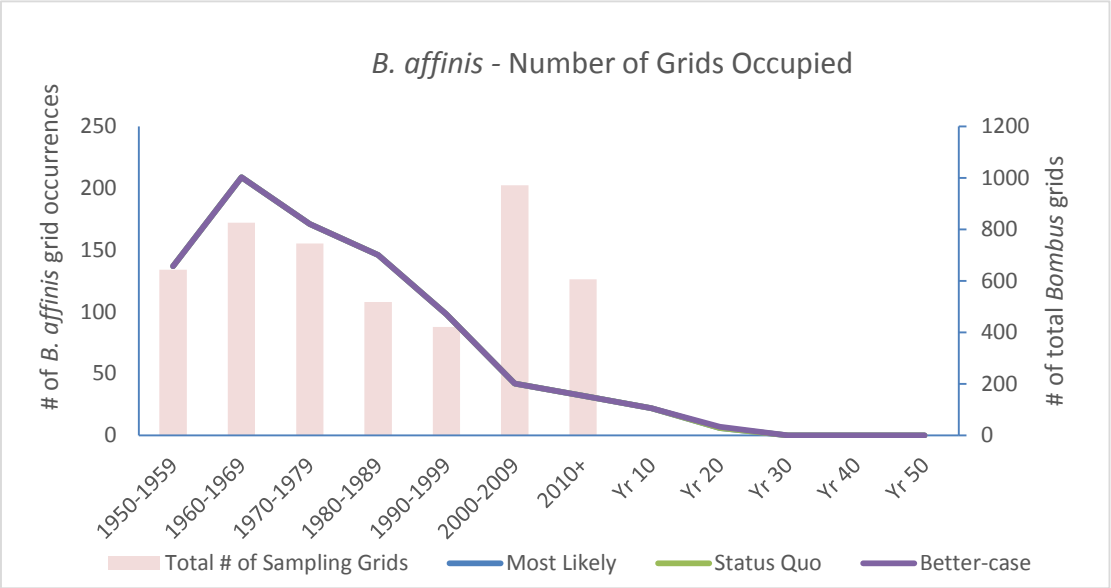
Figure 7.4. *B. affinis* Relative Abundance over time. Relative Abundance is the ratio of *B. affinis* number of specimens relative to the total number of specimens of all *Bombus* species. The bars represent the number of *Bombus* spp specimens; the line represents the trend in *B. affinis* numbers.



The declines in both % Occupancy and Relative Abundance are highly statistically significant ($p<0.001$, *z* tests of equal proportions). Our results comport with previously reported findings. Colla and Packer (2008, pp. 1379, 1389), for example, found that *B. affinis* in particular has declined drastically in abundance throughout its native range. Hatfield *et al.* (2014, p. 8) concluded that the species merits critically endangered status by IUCN standards. There are similar findings from others (*e.g.,* Cameron *et al.* 2011b; Grixti *et al.* 2009).

Forecasting into the future, our population model predicts a continued decline under all three future risk scenarios (Figure 7.5), with the median number of *B. affinis* occupied grids declining to 0 by Year 30. Despite differences in rates of decline in the scenarios, the results are nearly indistinguishable among the three risk scenarios.

Figure 7.5. The forecasted number of *B. affinis* grid occurrences over time. Although indistinguishable, the future trajectories under three future risk scenarios are displayed.



The above projections are based on scenarios that assume that the magnitude of decline stays constant through time (Status Quo), increases (Most Likely), or decreases (Better-case). Although we did not have sufficient information to assess the health of all current grid occurrences, such information available for a subset of sites suggests at least 18 sites (72% of the sites for which we have stressor information) are impacted by severe and broad extent stressors, and thus, we believe an increase in the rate of decline is a reasonable most likely future scenario. Additionally, the projections assume that all 69 current grid occurrences represent extant populations, *i.e.,* multiple colonies within each grid. Many of the current populations, however, have not been reconfirmed since the early 2000s and may no longer persist. For example, none of the previously occupied grid occurrences revisited in 2015 had *B. affinis*. Also, many of the current populations are documented by 5 or fewer individuals (93%); only 2 populations are documented by more than 10 individuals (Table 7.1). These small populations are at an increased risk of extinction due to environmental and demographic stochasticity; the latter being particularly important given the haplodiploidy biology of *B. affinis*. Given these data, some of the 69 current populations may no longer persist and others are likely already quasi-extinct; if either supposition is true, our projections underestimate the time to range-wide extinction.

Resiliency of *B. affinis* is primarily influenced by the degree of spatial heterogeneity (distribution across an array of climatic conditions) and connectivity among populations. Having populations distributed across a diversity of environmental conditions helps guard against concurrent losses of populations at local and regional scales by inducing asynchronatic fluctuations among populations. The greater degree of spatial heterogeneity (specifically, the diversity of temperature and precipitation conditions occupied by *B. affinis*), the greater resiliency the species will possess. Connectivity is essential for genetic health via gene flow and demographic rescue. Although we were unable to evaluate the distribution of

Table 7.1. The number of *B. affinis* specimens documented in each grid during three time periods: current (2000-2015), 2000 to 2009, and 2010 to 2015. For example, during the Current period, 39 grids (populations) were documented by 1 *B. affinis* specimen, 25 grids had 2 to 5 specimens, 3 grids had 6 to 10 specimens, 1 grid had 11 to 20 specimens, and 1 grid had 1 specimen.

	Current		2000-09		2010+	
# of individuals	# of grids	% of total	# of grids	% of total	# of grids	% of total
1	39	56%	27	68%	13	42%
2-5	25	36%	10	19%	16	33%
6-10	3	4%	2	5%	1	3%
11-20	1	1%	1	2%	0	0%
>20	1	1%	0	0%	1	0%

populations across spatial heterogeneity gradients and the degree of connectivity among populations, it is clear by the magnitude of geographic loss (see Representation section below) that the species no longer occupies the breadth of spatial heterogeneity it once did. Given the natural history of *B. affinis*, and insects in general, reducing the number of populations and the abundance of those populations greatly increase the species vulnerability to extinction from natural environmental variation alone. Although our future projections accounted for environmental stochasticity, ε, we derived its value from the historical period prior to the precipitous decline. Given that the effect of environmental stochasticity likely increases (*i.e.*, amplitude of population fluctuations increase) as population size decreases, the time to extinction would likely be shortened if we used ε values that are more closely comport with the current condition rather than the historical condition.

7.2 Representation

Representation is the ability to adapt to changing environmental conditions; it's the species evolutionary capacity or flexibility. Bombus affinis representation is described as having healthy populations distributed across a wide breadth of ecological conditions (i.e., having populations distributed widely across ecoregions).

The widespread losses in grid occurrences have led to reductions in distribution and spatial extent of *B. affinis* (Figure 7.6). Historically, *B. affinis* was broadly distributed across the eastern U.S., upper Midwest, and southern Quebec and Ontario, comprising 15 ecoregions, 31 states/provinces, and 378 counties (Figure 7.7). Since 2000, *B. affinis* distribution has declined across its range, with current records occurring in 6 ecoregions, 13 states/provinces, and 41 counties (Figure 7.7).

Figure 7.6. *Bombus affinis* range map showing current distribution. Dots represent counties with *B. affinis* at least 1 record since 2000, and X*s* represent counties with historical occurrences only (*i.e.*, no *B. affinis* records since 2000).

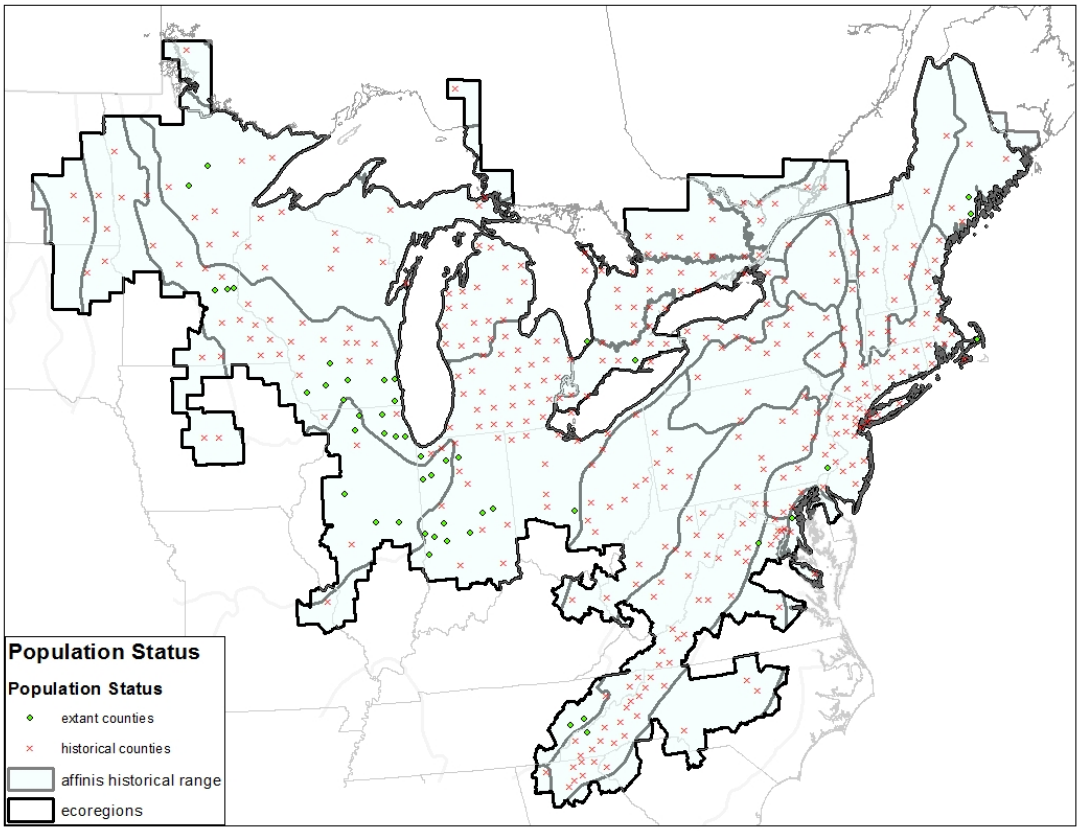
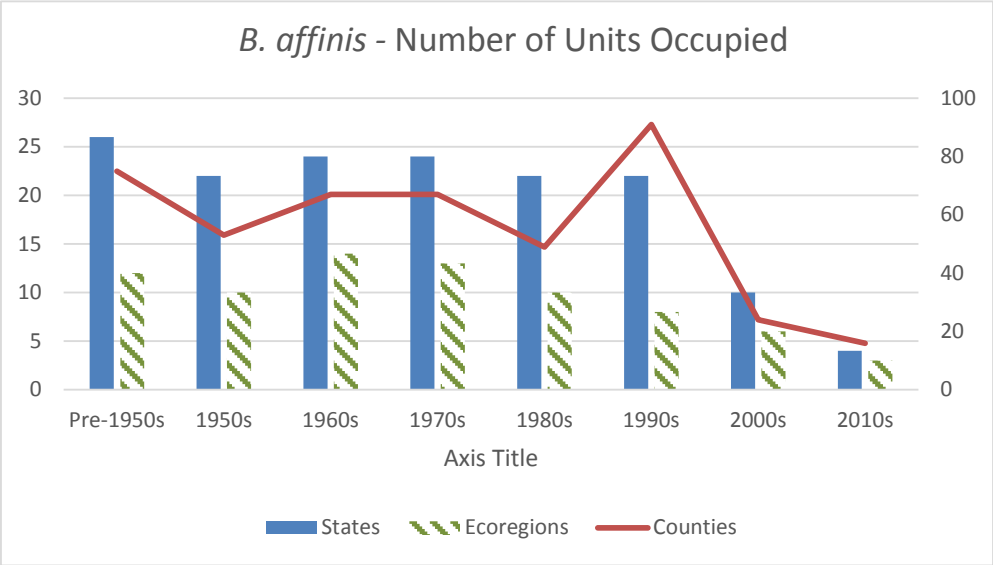
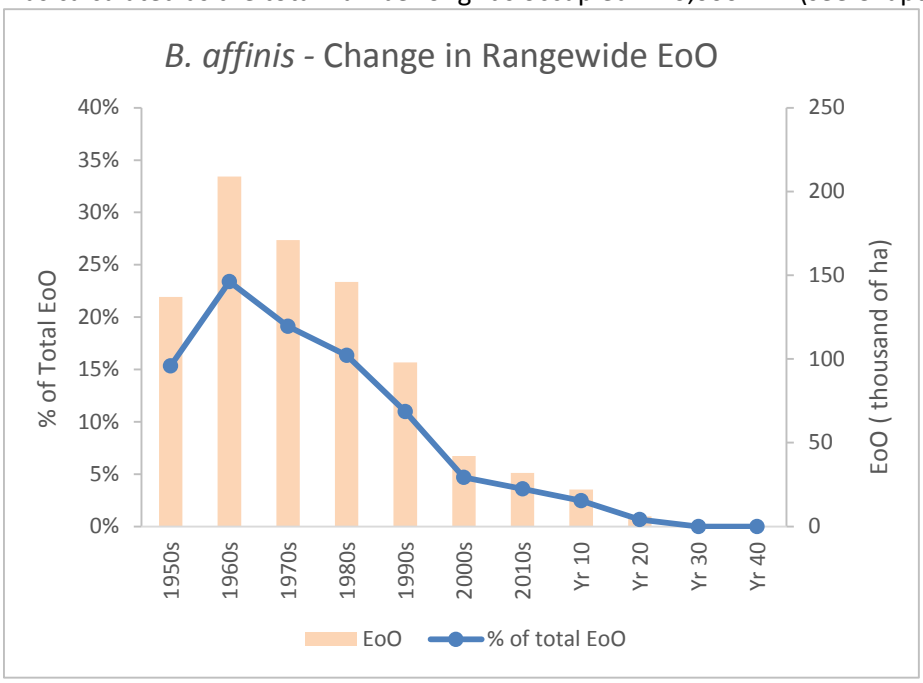


Figure 7.7. The tally of ecoregions, states, and counties occupied by *B. affinis* over time. We graphed counties on the secondary axis to better distinguish the trend in number of states and ecoregions.



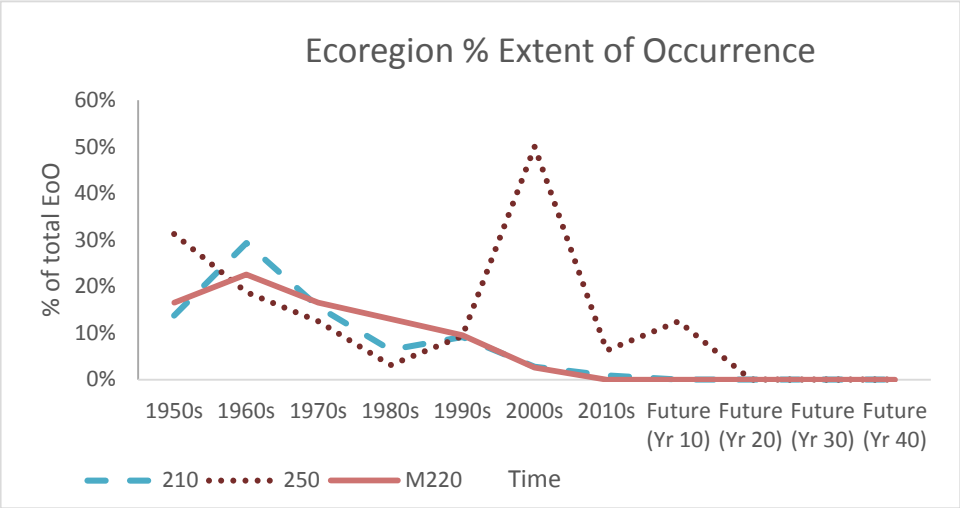
The contraction of the range has led to reductions in rangewide and within ecoregion spatial extent (EoO). Prior to year 2000, the rangewide EoO was 95% of its total EoO; since 2000, EoO is 8% and is projected to decline to 0% by Year 30 (Figure 7.8).

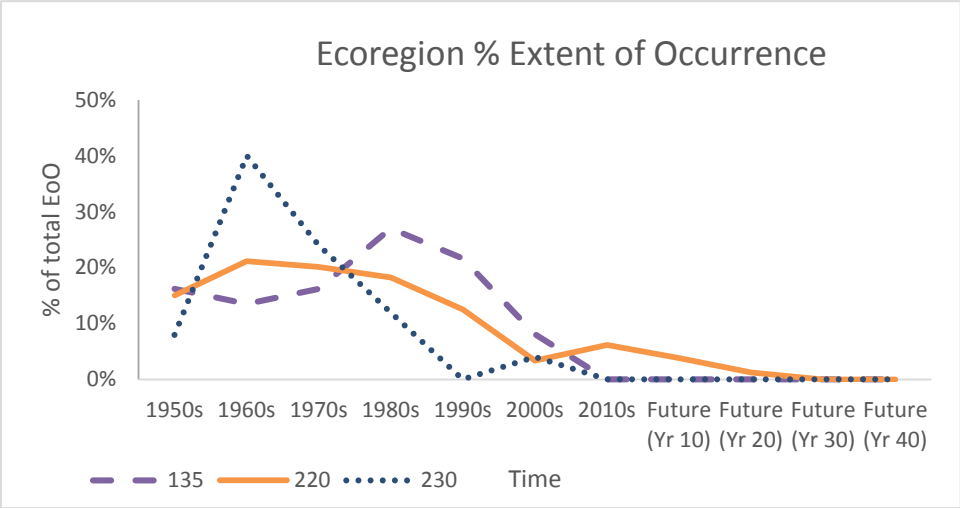
Figure 7.8. Rangewide Extent of Occurrence (EoO) over time. Primary axis gives the % of the decade's EoO relative to the historical total EoO. Secondary axis shows the EoO for each decade. Total EoO is maximum the spatial extent of B. affinis from historical to current time. It was calculated as the total number of grids occupied * 10,000 km^2 (see Chapter 3 for methods).



Reductions in EoO are apparent in all ecoregions (Figure 7.9). In the 1980s, the EoO of Ecoregion 250 (one of the 15 historically occupied ecoregions) dropped greatly, then spiked to a historical high in 2000s and dropped again in the 2010 decade. Sampling effort in the 2000s was extraordinary high, with 84% of all historical grids sampled during this period; thus, the unusual % Occupancy and EoO for this Ecoregion are likely explained by increased sampling effort. As the 2010 decade is not yet over, it is premature to conclude conclusively that spatial extent has declined from the 2000s decade, but thus far, the data suggest reductions are continuing, and are likely to continue (Figure 7.9). Looking into the future, by year 10, the EoO continues to decline in all ecoregions, with 4 of the 6 current ecoregions declining to 0% of its original EoO; the remaining 2 ecoregions, Ecoregion 220 and 250, decline to 4% and 13%, respectively.

Figure 7.9. Ecoregion % of total Extent of Occurrence (EoO) over time. Note, in Ecoregion 250, sampling effort in the 2000s was extraordinary high, with 84% of all historical grids sampled during this period; this likely explains the extraordinary peak in the 2000s decade.





Representation is influenced by the amount of adaptive diversity possessed by a species. Having populations distributed across a diversity of ecological conditions helps to ensure that the breadth of adaptive diversity is maintained. The loss of spatial extent as predicted by our analyses suggests that *B. affinis* will continue to lose adaptive diversity, and thus, be less able to adapt to physical and biological changes in its environment.

7.3 Redundancy

Redundancy is the ability of a species to guard against losses of adaptive diversity due to catastrophic events. Bombus affinis redundancy is described as having multiple, healthy populations widely distributed across ecoregions relative spatial occurrence of catastrophic events.

Reductions in the number of grid occurrences and spatial extent have increased the risk of future losses of adaptive diversity due to catastrophic events. We evaluated two catastrophic events: prolonged high temperatures and drought. The former preclude bumble bees from foraging, and thus, can lead to population collapse if temperatures are too hot for too long. Given the very low frequency of high temperature events, this event had no influence on our redundancy analyses. Droughts affect the availability of floral resources; if flowers are lacking at any point during their active season, colonies will starve and the population will collapse. As the redundancy analysis is concerned about losses of adaptive diversity, we evaluated the risks of ecoregion-wide extirpations. Prior to the 1990s, the large number of grid occurrences within the ecoregions, and presumably the spatial dispersion of these occurrences, ensured low likelihoods of catastrophic events causing extirpation of ecoregions. Since the late 1990s, however, the risks of ecoregion extirpations has markedly increased for 4 of the 6 extant ecoregions under D3+ level droughts and for 1 ecoregion under D4+ level droughts (Table 7.2).

Table 7.2. Extinction probabilities, p(X), for the ecoregions based on historical and current number of grid occurrences. Extinction probabilities are provided for D3+ and D4+ drought frequencies.

	D3+ Intensity		D4+ Intensity	
Ecoregion	Historical	Current	Historical	Current
135	0.000	0.000	0.000	0.000
210	0.000	0.996	0.000	0.000
220	0.000	0.000	0.000	0.000
230	0.000	1.000	0.000	0.854
250	0.000	0.990	0.000	0.000
M220	0.000	0.997	0.000	0.000

As we were unable to predict which specific grids will be occupied in the future, we were unable to calculate estimates of p(X). However, as the number of populations decrease, the chances of catastrophic events causing ecoregion extirpation increase.

7.4 Viability Synopsis

To understand the overall viability, we calculated probabilities of ecoregion extirpations given future risk scenarios and catastrophic events. We applied the current probabilities of extirpation due to catastrophic events (*i.e.*, we assumed that the probability of ecoregion-wide extirpation due to catastrophic events does not change with decreasing number of populations). The probabilities of ecoregion extirpation are nearly certain for all ecoregions except Ecoregion 220 (Table 7.3). Even so, Ecoregion 220 is predicted to decline to 0 (median N) by Year 30.

Table 7.3. The projected median number of grids persisting over time and probabilities of ecoregion-wide extinction given "Most Likely" risk scenario and current risk of ecoregion extirpation due to catastrophic events.

	Median Number of Grids (populations)								Prob of Ecoregion Extinction					
Ecoregion	Hist	Curr	Yr 5	Yr 10	Yr 20	Yr 30	Yr 40	Yr 50	Yr 5	Yr 10	Yr 20	Yr 30	Yr 40	Yr 50
91	1	0	0	0	0	0	0	0	1.00	1.00	1.00	1.00	1.00	1.00
96	1	0	0	0	0	0	0	0	1.00	1.00	1.00	1.00	1.00	1.00
98	8	0	0	0	0	0	0	0	1.00	1.00	1.00	1.00	1.00	1.00
99	1	0	0	0	0	0	0	0	1.00	1.00	1.00	1.00	1.00	1.00
132	10	0	0	0	0	0	0	0	1.00	1.00	1.00	1.00	1.00	1.00
133	4	0	0	0	0	0	0	0	1.00	1.00	1.00	1.00	1.00	1.00
134	39	0	0	0	0	0	0	0	1.00	1.00	1.00	1.00	1.00	1.00
135	36	3	0	0	0	0	0	0	0.91	1.00	1.00	1.00	1.00	1.00
210	105	4	0	0	0	0	0	0	1.00	1.00	1.00	1.00	1.00	1.00
220	445	42	27	18	6	0	0	0	0.00	0.00	0.10	0.64	0.93	0.99
230	24	1	0	0	0	0	0	0	1.00	1.00	1.00	1.00	1.00	1.00
250	18	16	9	4	0	0	0	0	0.99	0.99	1.00	1.00	1.00	1.00
330	4	0	0	0	0	0	0	0	1.00	1.00	1.00	1.00	1.00	1.00
M210	36	0	0	0	0	0	0	0	1.00	1.00	1.00	1.00	1.00	1.00
M220	113	3	0	0	0	0	0	0	1.00	1.00	1.00	1.00	1.00	1.00

Based on our analyses, *B. affinis* viability has declined over time. All measures of biological condition indicate that *B. affinis* was historically abundant and widely distributed; it was the fourth ranked *Bombus* species in our relative abundance analysis prior to mid-1990s, with an expansive geographic range covering most of the Midwestern and eastern U.S. and areas of Quebec and Ontario, representing 15 ecoregions. Since the late 1990s, marked and precipitous declines have been recorded in the number of populations (91% reduction) and in spatial extent (92% reduction from historical average). Given the acute decline, it is clear that *B. affinis* was exposed to one or more novel, pervasive stressors. Although the ultimate source or cause of the widespread decline is debated, and despite that lack of information about the relative role and synergistic effects of the primary stressors, the response of *B. affinis* is undisputable. Regardless of the uncertainty in the causative factors, at least one if not all stressors are likely to continue affecting the remaining poulations. Given that all of the future scenarios analyzed forecast the same result regardless of degree or magnitude, being able to pinpoint the exact degree or magnitude is immaterial.

The magnitude and extent of population losses to date have greatly reduced the evolutionary capacity of *B. affinis* to adapt to changing environmental conditions over time and to guard against further losses of adaptive diversity and potential extinction due to catastrophic events. In reality, the few number of populations persisting and the limited spatial heterogeneity associated with these populations have substantially reduced the ability of *B. affinis* to withstand normal environmental variation and recover from transient, stochastic perturbations. Coupled with the increased risk of extirpation due to the interaction of reduced population size and its sl-CSD (single locus-complimentary sex determination) system, *B. affinis* may lack the resiliency required to sustain populations even without further exposure to exogenous stressors.

Under the most likely future risk scenario, all but 1 ecoregion is projected to be extirpated within 5 years; the remaining ecoregion, is projected to decline to extinction in 30 years. These projections assume all 69 populations are extant, which is uncertain. They also do not account for high severity threats occurring at least 18 sites nor do they fully account for the "male diploid extinction vortex"; both of which will increase the rate of decline. Thus, *B. affinis*' ability to withstand environmental variation, catastrophic events, and changes in physical and biological conditions in its environment has been severely reduced, rendering it vulnerable to extinction.

To increase the *B. affinis* viability (ability to sustain populations into the future), it is necessary to:

1. prevent further declines by protecting the remaining populations and sufficient habitat needed to support them (this is paramount);
2. improve its resiliency by increasing the number of healthy populations and ensuring the populations are distributed across an array of environmental gradients;
3. improve its representation by restoring populations throughout the breadth of adaptive diversity (ecoregions); and
4. improve its redundancy by restoring multiple, healthy populations in each of its ecoregions.

7.5 Uncertainty

Inherently, predicting the future condition requires us to make plausible assumptions. Our analyses are predicated on multiple assumptions, which could lead to over- and underestimates of viability. In total, however, we believe our predictions are optimistic, especially given that some of the remaining populations are almost certain to be imperiled if not quasi-extinct (given that most populations are documented by one or a few individuals). In Table 7.4, we identify the key sources of uncertainty and indicate the likely effect of our assumptions on the viability assessment.

Table 7.4. Key assumptions made in the analysis and the impact on our viability assessment if such assumptions are incorrect. "Overestimated" means the viability of the species is optimistic; "Underestimate" means the viability of the species is pessimistic. Text in italics is the more likely result of the two outcomes.

Assumption	**Degree of viability is:**
A 10 x 10 Km grid occurrence represents a healthy population	*Overestimated* /Underestimated
No Allee effects	Overestimated
Ɛ stays constant over time	Overestimated
Ecoregions capture the full breadth of adaptive diversity	Overestimated/Underestimated
Gains or losses in spatial extent represent commensurate gains or losses of adaptive diversity	Overestimated/Underestimated
Frequencies from 2000-2012 represent the true risk of drought over time	*Overestimated* /Underestimated
Frequency from 2004-2014 represent the true risk of prolonged temperature events	*Overestimated* /Underestimated
A D2 or lesser intensity drought will not cause population to collapse	*Overestimated* /Underestimated
No autocorrelation among grids	Overestimated
Temperatures <35° C will not cause population collapse	Overestimated
There are no synergistic effects from co-occurrence of drought and high temperatures	Overestimated
Risk scenarios reflect plausible extremes	Overestimated/Underestimated

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Appendix 1. Sample questionnaires

Figure A.1.1: Example Location – Specific Questionnaire. We provided each "location expert" with a specific list of occurrences and associated grids on which to comment.

Our intent is to assess the quality of the remnant populations of B. affinis across the range.

Instructions: Please answer the following 2 questions to the best of your knowledge. If you are not familiar with a particular subject(s), feel free to leave blank(s). Examples are given (in red).
Please refer to the "locations" tab for the locations we are asking you to evaluate. You may complete one questionnaire for multiple occurences within the same grid. If you are including multiple locations in one assessment, please indicate that.

Your Name:	
Sample Location and Description (county, state):	
GRID Number (refer to locations tab):	
Occurrence Number(s) (refer to locations tab):	
Sample Location size (acres):	
Brief Habitat Description:	

1. Please rate the habitat suitablility (at the majority of the location(s), as defined in the blue box below).	

Habitat Suitability Rating	**Definition**	
Good	abundant and diverse floral resources throughout adult flight season, sufficient nesting habitat in appropriate proximity that could support a healthy population	
Moderate	limited abundance or seasonal availability of floral resources and nesting habitat; may support a population	
Poor	insufficient flora resources and insufficient nesting habitat and connectivity; will not support a healthy population	

2. Please provide the potential stressors to the species or its habitat within a 25km radius of this location. Please indiciate the extent, severity, frequency of occurrence, if applicable. Add additional plausible stressors to the species, if relevant.

	yes or no?	Extent (% of area impacted within 25km radius)	Severity (high, medium, low)	Brief description
Example (Pesticide)	yes	70	high	25km radius from prairies is largely agricultural land planted in corn & soy
Pathogens				
Pesticides				
Loss/Degradation of floral resources				
Small population effects*				

*small population(s) vulnerable to demographic, genetic or environmental stochasicity

Figure A.1.2: Sample of the stressor questionnaire used to query experts.

QUESTIONNAIRE: GENERAL EFFECTS OF PESTICIDE(S) ON BOMBUS AFFINIS (with a focus on the future)

Your Name:

Instructions: Based on the reductions of occurrences and range of B. affinis as desribed by Colla and Packer (2008)* and Cameron et al. (2011)**, please answer the following 3 questions, in the yellow boxes, to the best of your knowledge. Examples are given in red text.
Notes: Examples are illustrative only -- please do not anchor on them!

1. What percent of the rate of decline of B. affinis can be attributed to pesticides? Please insert the bin number that corresponds with your best estimate.

Answer	Percent of decline	Percent of decline-example	Bins (% of decline):	Comments: I think extant populations are probably more threatened by pesticides now as
		2	Bin 0 (Zero Impact)	
			Bin 1 (1-25%)	
			Bin 2 (26-50%)	
			Bin 3 (51-75%)	
			Bin 4 (76-100%)	

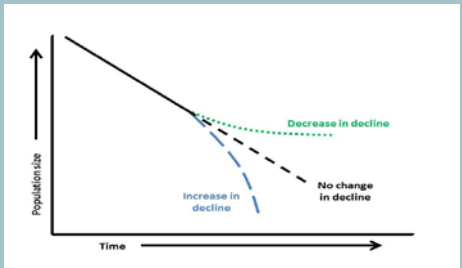
2. Which scenario is the mostly likely to occur in the future at the following time-periods? (see definitions in blue box below)

Future Years	Pesticide scenario	Pesticide scenario - example	Scenarios	Pesticides	I don't feel I can comment on this one
10 yrs (2016-2026)		2	1	Decreased application rates and extent of use; stop use of potentially detrimental pesticides	
20 yrs (2027-2036)		3	2	application rates and extent of use; no new potentially detrimental chemicals used	
30 yrs (2037-2046)		3	3	Increased application rates and extent of use and/or new potentially detrimental pesticides	
40 yrs (2047-2056)		3			
50 yrs (2057-2066)		3			

3. A) How will your selected scenarios in question 2 effect the current rate of decline? Refer to the chart below for an illustration of the direction represented by each scenario (it is not an indication of the magnitude change).
3. B) Please indicate the magnitude to change expected by inserting the bin number that corresponds to your belief.

	A	Question 3 (A) - example	B	Question 3 (B) - example		
Future Years	Increase/No Change/Decrease	Increase/No Change/Decrease	Magnitude of Change	Magnitude of Change	Bins (Magnitude of Change):	Comments:
10 yrs (2016-2026)		No change		2	Bin 0 (Zero Change)	
20 yrs (2027-2036)		Increase		2	Bin 1 (1-25%)	
30 yrs (2037-2046)		Increase		3	Bin 2 (26-50%)	
40 yrs (2047-2056)		Increase		3	Bin 3 (51-75%)	
50 yrs (2057-2066)		Increase		4	Bin 4 (76-100%)	

Chart to illustrate Question 3A



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Appendix. 2. Site-specific stressor information.

Table A. 2.1. Summary of site-specific habitat and stressor questionnaires. Habitat suitability was ranked as follows: good =1, moderate =2, and poor =3. Extent for each stressor (pathogens, pesticides, loss or degradation of floral resources, and small population effects) is given as the proportion (expressed in decimal, 1=100%) of the 25km radius from a site affected by the stressor; "Broad extent" is defined as extent >50%. Severity of each stressor was ranked as: low =1, moderate =2, or high =3. "NA" means the category is not applicable to the specific grid. Blank cells indicate no response provided for the specific grid.

Grid #	Habitat Suitability	Pathogens		Pesticides		Loss/Degradation of floral resources		Small population effects*		Total # sites with >2 severe and broad extent
		Extent	Severity	Extent	Severity	Extent	Severity	Extent	Severity	
1389	1	100%		100%	1	100%	1			
1654	1	100%		100%	1	100%	2			
13947	1									
9002	2			60%	3	90%	2	100%	3	1
9518	2			70%	3	70%	3	100%	3	1
25715	1	100%	3	100%	2	50%	2	100%	3	1
25724	1	100%	3	100%	2	50%	2	100%	3	1
14342	1	100%	3	22%	1	100%	2	100%	3	1
14799	1	100%	3	70%	3	80%	3	100%	3	1
12112	1	100%	3	60%	2	80%	2	100%	3	1
12113	1	100%	3	60%	2	80%	2	100%	3	1
15847	2	100%	3	100%	3	90%	3	100%	3	1
12661	1	100%	3	60%	3	80%	2	100%	3	1
12298	1	100%	3	68%	3	80%	2	100%	3	1
12299	1	100%	3	75%	2	80%	2	100%	3	1
12840	1	100%	3	60%	3	50%	2	100%	3	1
12478	1	100%	3	60%	3	50%	2	100%	3	1
15675	1	100%	3	50%	2	50%	2	100%	3	1
14615	1	100%	3	100%	3	100%	3	100%	3	1
15329	1	100%	3	50%	2	25%	2	100%	3	1
1806	1	25%	2	15%	1	NA	NA	50%	3	
7003	1	100%	3							1
8664	3				3	95%			3	
8831	2				2	90%			3	
22136	1								2	

Appendix 3 – Pesticide information

Table A.3.1: Summary of the lethal effects of pesticides on *Bombus.*

Active Substance	Chemical Class	Species	Exposure	End Point	Time	Value (µg/bee) (unless stated)	Citation
Clothianidin	Neonicotinoid	*B.impatiens*	Acute Contact	LC50	48h	0.39 (wt:vol) x 10-3	Scott-Dupree *et al.* 2009
Clothianidin	Neonicotinoid	*B.terrestris*	Acute oral	100% mortality	96h	100 µg/L	Thompson *et al.* 2014
Imidacloprid	Neonicotinoid	*B. terrestris*	Acute contact	LD50	24h	0.554	Bortolotti *et al.* 2002
Imidacloprid	Neonicotinoid	*B. terrestris*	Acute contact	LD50	48h	0.014	Bortolotti *et al.* 2002
Imidacloprid	Neonicotinoid	*B. terrestris*	Acute oral	LD50	48h	0.014	Bortolotti *et al.* 2002
Imidacloprid	Neonicotinoid	*B. terrestris*	Acute oral	LD50	72h	0.006	Bortolotti *et al.* 2002
Imidacloprid	Neonicotinoid	*B. terrestris*	Acute oral	LD50	24h	0.04	Marletto *et al.* 2003
Imidacloprid	Neonicotinoid	*B.impatiens*	Acute oral	LC50	72h?	59ppb	Mommaerts *et al.* 2010
Imidacloprid	Neonicotinoid	*B.impatiens*	Acute Contact	LC50	48 h	3.22 (wt:vol) x 10-3	Scott-Dupree *et al.* 2009
Thiacloprid	Neonicotinoid	*B.terrestris*	Acute oral	LC50	72h?	18ppb	Mommaerts *et al.* 2010
Thiamethoxam	Neonicotinoid	*B.terrestris*	Acute oral	LC50	72h?	0.12ppb	Mommaerts *et al.* 2010
Thiamethoxam	Neonicotinoid	*B.terrestris*	Acute oral	100% mortality	96h	100 µg/L	Thompson *et al.* 2014

Table A.3.2. Summary of the sublethal effects of pesticides on *Bombus*. B.t. = *Bombus terrestris*, B.i.= *Bombus impatiens*, and B.o.= *Bombus occidentalis.* x=significant change, 0=no detected effect at 5% significance level.

Chemical	Species	Exposure	Exposure Duration	Avoidance of treated areas	Decreased brain function (mitochondrial)	Decreased bee weight	Increased # Foragers	Foraging bout frequency	Decreased pollen collected	More time to access flowers	Incr. duration of foraging bouts	Reduced feeding	Reduced ilnitial time to defense response	Decr. duration of defense response	decr # with defense response	Decr # of queens	Decr queen weight	Decr Number of males (drones)	Decr Worker weight	Decr Initial worker weight	Decr Worker production	Decr # Workers	Incr Worker mortality (increase loss)	Incr Worker mortality & loss	Decr number of wax cells/honey pots	Decr Number of brood chambers	Decr Brood number	Decr Number of eggs	Decr Number of larvae produced	Decr Nest structure mass	Nest building delay	Colony Failure (n failed / n survived)	Decr Colony density	Decr Colony weight	Decr Colony size (fewer births, more deaths)	Exposure Rates	Citation
Carbaryl	*B. i.*	sprayed turf	14-d	0																																	Gels et al. 2002
Chitinase	*B. o.*	Oral - pollen	52-d													0		0				0					0									0.6µg[AI])/g	Morandin and Winston 2003
Chlorantraniliprole	*B. i.*	sprayed turf	6-d, 42-d	0																																0.23 kg AI /ha	Larson et al. 2013
Chlorpyrifos	*B. i.*	sprayed turf	14-d	0																																	Gels et al. 2002
Clothianidin	*B. t.*	Oral - sugar water										x																								1ppb	Fauser-Misslin et al. 2014
Clothianidin	*B. i.*	Oral - sucrose water/pollem	85-d?									0				0		0		0							0									6ppb	Franklin et al. 2004
Clothianidin	*B. i.*	Oral - sucrose water/pollem	85-d?									0				0		0		0							0									36ppb	Franklin et al. 2004
Clothianidin	*B. i.*	sprayed turf	6-d, 42-d	0												x							x											x		0.45 kg AI/ha	Larson et al. 2013
Clothianidin	*B. t.*	Oral - sugar water	3-d		x																															10 nM	Moffat et al. 2015
Clothianidin	*B. i.*	Oral - sugar water	11wk?			x						0													x									x		10ppb	Scholer and Krischik 2014
Clothianidin	*B. i.*	Oral - sugar water	11wk?			x						x													x									x		20ppb	Scholer and Krischik 2014
Clothianidin	*B. i.*	Oral - sugar water	11wk?			x						x						x							x		x							x		50ppb	Scholer and Krischik 2014
Clothianidin	*B. i.*	Oral - sugar water	11wk?			x						x						x							x		x							x		100ppb	Scholer and Krischik 2014
Clothianidin	*B. t.*	Oral - sugar water	4-d																																	1 g/L	Thompson et al. 2014
Clothianidin	*B. t.*	Oral - sugar water	4-d									x																								10 g/L	Thompson et al. 2014
Clothianidin	*B. t.*	Oral - sugar water	4-d																																	100 g/L	Thompson et al. 2014
Clothianidin & b-cyfluthrin	*B. t.*	Field test - coated seeds	?													x					x												x	x		recom. dose	Rundolf et al. 2015
Clothianidin/bifenthrin	*B. i.*	sprayed turf	6-d, 42-d	0																																0.45 kg AI/ha	Larson et al. 2014

Table A.3.2. Con't. Summary of the sublethal effects of pesticides on *Bombus*. B.t. = *B. terrestris*, B.i.= *B. impatiens*, and B.o.= *B. occidentalis.* x=significant change, 0=no detected effect at 5% significance level.

Chemical	Species	Exposure	Exposure Duration	Avoidance of treated areas	Decreased brain function (mitochondrial)	Decreased bee weight	Increased # Foragers	Foraging bout frequency	Decreased pollen collected	More time to access flowers	Incr. duration of foraging bouts	Reduced feeding	Reduced initial time to defense response	Decr. duration of defense response	decr # with defense response	Decr # of queens	Decr queen weight	Decr Number of males (drones)	Decr Worker weight	Decr Initial worker weight	Decr Worker production	Decr # Workers	Incr Worker mortality (increase loss)	Incr Worker mortality & loss	Decr number of wax cells/honey pots	Decr Number of brood chambers	Decr Brood number	Decr Number of eggs	Decr Number of larvae produced	Decr Nest structure mass	Nest building delay	Colony Failure (n failed / n survived)	Decr Colony density	Decr Colony weight	Decr Colony size (fewer births, more deaths)	Exposure Rates	Citation
Clothianidin/bifenthrin	*B. i.*	sprayed turf	6-d, 42-d	0																																0.45 kg AI/ha	Larson et al. 2014
Cry1Ac: control plus Cry1Ac	*B. i.*	Oral - pollen	52-d													0		0				0					0									11 ng ([AI])/g	Morandin and Winston 2003
Cry1Ac: control plus Cry1Ac	*B. i.*	Oral - pollen	52-d																			0					0									11 ng ([AI])/g	Morandin and Winston 2003
Cyfluthrin	*B. i.*	sprayed turf	14-d	0																																	Gels et al. 2002
Imidacloprid	*B. t.*	Oral - sucrose water	42-d																																x	10ppb	Bryden et al. 2013
Imidacloprid	*B. t.*	Oral - sucrose water/pollem	28-d					x	x																											0.7 ppb/6ppb	Feltham et al. 2014
Imidacloprid	*B. i.*	turf not- irrigated	28-30d	0				x					x	x	x		0		x			x	x		x	x								x		0.336 kg [AI]/ha	Gels et al. 2002
Imidacloprid	*B. i.*	turf irrigated	28-30d	0									0	0	0		0		0			0			0	0								0		0.336 kg [AI]/ha	Gels et al. 2002
Imidacloprid	*B. t.*	Oral -sucrose water	28-d				x		x																											10 ppb	Gill and Raine 2014
Imidacloprid	*B. t.*	Oral - sucrose water	28-d				x	0	x		x										x		x	0			x			0		0/10				10 ppb	Gill et al 2012
Imidacloprid	*B. t.*	Oral - sugar water	28-d																								x									98.43 µg/kg	Laycock and Cresswell 2015
Imidacloprid	*B. t.*	Oral - sugar water	28-d																																	39.37 µg/kg	Laycock and Cresswell 2015
Imidacloprid	*B. t.*	Oral - sugar water	28-d																																	15.75 µg/kg	Laycock and Cresswell 2015
Imidacloprid	*B. t.*	Oral - sugar water	28-d																																	6.30 µg/kg	Laycock and Cresswell 2015
Imidacloprid	*B. t.*	Oral - sugar water	28-d																																	2.52 µg/kg	Laycock and Cresswell 2015
Imidacloprid	*B. t.*	Oral - sugar water	28-d																																	1.01 µg/kg	Laycock and Cresswell 2015
Imidacloprid	*B. t.*	Oral - sugar water	28-d																																	0.40 µg/kg	Laycock and Cresswell 2015
Imidacloprid	*B. t.*	Oral - sugar water	28-d																																	0.16 µg/kg	Laycock and Cresswell 2015
Imidacloprid	*B. t.*	Oral - sugar water	28-d																																	0.06 µg/kg	Laycock and Cresswell 2015
Imidacloprid	*B. t.*	Oral - sugar water	14-d																								x									98.43 µg/kg	Laycock and Cresswell 2015

Table A.3.2. Con't. Summary of the sublethal effects of pesticides on *Bombus*. B.t. = *B. terrestris*, B.i.= *B. impatiens*, and B.o.= *B. occidentalis.* x=significant change, 0=no detected effect at 5% significance level.

Chemical	Species	Exposure	Exposure Duration	Avoidance of treated areas	Decreased brain function (mitochondrial)	Decreased bee weight	Increased # Foragers	Foraging bout frequency	Decreased pollen collected	More time to access flowers	Incr. duration of foraging bouts	Reduced feeding	Reduced initial time to defense response	Decr. duration of defense response	decr # with defense response	Decr # of queens	Decr queen weight	Decr Number of males (drones)	Decr Worker weight	Decr Initial worker weight	Decr Worker production	Decr # Workers	Incr Worker mortality (increase loss)	Incr Worker mortality & loss	Decr number of wax cells/honey pots	Decr Number of brood chambers	Decr Brood number	Decr Number of eggs	Decr Number of larvae produced	Decr Nest structure mass	Nest building delay	Colony Failure (n failed / n survived)	Decr Colony density	Decr Colony weight	Decr Colony size (fewer births, more deaths)	Exposure Rates	Citation
Imidacloprid	*B. t.*	Oral - sugar water	7-49d									x										x	x													200ppm	Mommaerts et al. 2009
Imidacloprid	*B. t.*	Oral - sugar water	7-49d									x										x	x													20ppm	Mommaerts et al. 2009
Imidacloprid	*B. t.*	Oral - sugar water	7-49d									x										x	x													2ppm	Mommaerts et al. 2009
Imidacloprid	*B. t.*	Oral - sugar water	7-49d									x						x				x	x													0.2 ppm	Mommaerts et al. 2009
Imidacloprid	*B. t.*	Oral - sugar water	7-49d									x						x				x	x													20ppb	Mommaerts et al. 2009
Imidacloprid	*B. t.*	Oral - sugar water	7-49d									x										x	?													10ppb	Mommaerts et al. 2009
Imidacloprid	*B. t.*	Oral - sugar water	7-49d																				0													2ppb	Mommaerts et al. 2009
Imidacloprid	*B. o.*	Oral - pollen	52-d													0		0				0					0									7ng ([AI])/g	Morandin and Winston 2003
Imidacloprid	*B. i.*	Oral - pollen	52-d																			0					0									7 ng ([AI])/g	Morandin and Winston 2003
Imidacloprid	*B. i.*	Oral - pollen	52-d?							x	x											0					0									30 ng ([AI])/g	Morandin and Winston 2003
Imidacloprid	*B. i.*	Oral - sugar water	11wk?			x						x						x																x		10ppb	Scholer and Krischik 2014
Imidacloprid	*B. i.*	Oral - sugar water	11wk?			x						x						x									x							x		20ppb	Scholer and Krischik 2014
Imidacloprid	*B. i.*	Oral - sugar water	11wk?			x						x						x							x		x							x		50ppb	Scholer and Krischik 2014
Imidacloprid	*B. i.*	Oral - sugar water	11wk?			x						x						x							x		x							x		100ppb	Scholer and Krischik 2014
Imidacloprid	*B. t.*	Field - seed coating	9-d																																	0.7 mg/seed	Tasei et al. 2000
Imidacloprid	*B. t.*	Oral - sugar water	4-d																																	1 g/L	Thompson et al. 2014
Imidacloprid	*B. t.*	Oral - sugar water	4-d									x																								10 g/L	Thompson et al. 2014
Imidacloprid	*B. t.*	Oral - sugar water	4-d									x																								100 g/L	Thompson et al. 2014
Imidacloprid/L-cyhalothin mix	*B. t.*	Oral/sprayed filter paper	28-d				x		x																											10ppb/37.5ppm	Gill and Raine 2014
Imidacloprid/L-cyhalothin mix	*B. t.*	Oral/Spray	28-d				x	x	x		x										x		x	x			x			0		2of8				10ppb/Label guidance	Gill et al 2012
Lambda-cyhalothrin	*B. t.*	Sprayed filter paper	28-d				x				x																									37.5 ppm	Gill and Raine 2014
Lambda-cyhalothrin	*B. t.*	Field Spray	28-d				0	0	0		0										0		x	x			0			0		0/10				850:3030 Label guidance	Gill et al 2012

Table A.3.2. Con't. Summary of the sublethal effects of pesticides on *Bombus*. B.t. = *B. terrestris*, B.i.= *B. impatiens*, and B.o.= *B. occidentalis.* x=significant change, 0=no detected effect at 5% significance level.

Chemical	Species	Exposure	Exposure Duration	Avoidance of treated areas	Decreased brain function (mitochondrial)	Decreased bee weight	Increased # Foragers	Foraging bout frequency	Decreased pollen collected	More time to access flowers	Incr. duration of foraging bouts	Reduced feeding	Reduced iinitial time to defense response	Decr. duration of defense response	decr # with defense response	Decr # of queens	Decr queen weight	Decr Number of males (drones)	Decr Worker weight	Decr Initial worker weight	Decr Worker production	Decr # Workers	Incr Worker mortality (increase loss)	Incr Worker mortality & loss	Decr number of wax cells/honey pots	Decr Number of brood chambers	Decr Brood number	Decr Number of eggs	Decr Number of larvae produced	Decr Nest structure mass	Nest building delay	Colony Failure (n failed / n survived)	Decr Colony density	Decr Colony weight	Decr Colony size (fewer births, more deaths)	Exposure Rates	Citation
Propiconazole	*B. t.*	Oral -honey water	28-d									x													x											23mg/kg	Elton et al 2013
Propiconazole	*B. t.*	Oral -honey water	28-d									x																								230mg/kg	Elton et al 2013
Thiacloprid	*B. t.*	Oral - sugar water	7-49d																			x	x													120ppm	Mommaerts et al. 2009
Thiacloprid	*B. t.*	Oral - sugar water	7-49d																			x	x													60ppm	Mommaerts et al. 2009
Thiacloprid	*B. t.*	Oral - sugar water	7-49d															x				x	x													12ppm	Mommaerts et al. 2009
Thiacloprid	*B. t.*	Oral - sugar water	7-49d																				0													1.2ppm	Mommaerts et al. 2009
Thiacloprid	*B. t.*	Oral - sugar water	7-49d																				0													12ppb	Mommaerts et al. 2009
Thiamethoxam	*B. t.*	Oral -honey water	28-d																						x			x	x		x					10 µg/kg (field maximum)	Elston et al 2013
Thiamethoxam	*B. t.*	Oral -honey water	28-d																						x											1 µg/kg (field realistic mean)	Elton et al 2013
Thiamethoxam	*B. t.*	Oral - sugar water	4-d																																	1 g/L	Thompson et al. 2014
Thiamethoxam	*B. t.*	Oral - sugar water	4-d																																	10 g/L	Thompson et al. 2014
Thiamethoxam	*B. t.*	Oral - sugar water	4-d																																	100 g/L	Thompson et al. 2014
Thiomethoxam	*B. t.*	Oral - sugar water	7-49d																			x	x													100ppm	Mommaerts et al. 2009
Thiomethoxam	*B. t.*	Oral - sugar water	7-49d																			x	x													10ppm	Mommaerts et al. 2009
Thiomethoxam	*B. t.*	Oral - sugar water	7-49d																			x	x													1ppm	Mommaerts et al. 2009
Thiomethoxam	*B. t.*	Oral - sugar water	7-49d																			x	x													0.5ppm	Mommaerts et al. 2009
Thiomethoxam	*B. t.*	Oral - sugar water	7-49d															x				x	x													0.1ppm	Mommaerts et al. 2009
Thiomethoxam	*B. t.*	Oral - sugar water	7-49d																				0													10 ppb	Mommaerts et al. 2009