Examining Phenological Patterns for the Emergence Times of Common *Bombus* Species in the U.S. Mid and South Atlantic

Brianna G. Handford
Middleburg, Virginia

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David E. Carr,
Thesis Advisor

Robert E. Davis,
Director of Distinguished Major Program

ABSTRACT

The goal of this study is to examine the phenology of queen bumblebee (genus *Bombus*) emergence times and its connection to temperature and precipitation across the states of Virginia, West Virginia, Maryland, Delaware, and Pennsylvania. Growing degree days (GDD), cumulative precipitation (∑ppt) and days accumulated (∑day) were the metrics used to study connections between climate and emergence times. It was hypothesized the GDD for queen bee emergence times will vary between species yet remain constant within a species across latitudinal and elevational gradients, and the number of days it takes to reach this threshold will increase with elevation, latitude, and precipitation. Observations were gathered from a citizen science database and a museum database to gather current and historical trends. Data were split between high and low elevations and latitudes to compare the geographic difference in GDD and ∑day values. Results suggest that queen bee emergences currently occur most frequently in the week of March 29th-April 4th; *B. bimaculatus* emerges first, *B. impatiens* emerges second, and *B. griseocollis* emerges third. However, as GDD varied significantly across elevational and latitudinal gradients, temperature was not determined to be the primary factor in queen bumblebee emergence times. Phenology differences at high elevations and latitudes are not very pronounced, as emergence times only differ a couple of days between species. This suggests that day accumulation, especially at high elevations and latitudes, drives *Bombus* emergence times more than temperature accumulation.

ACKNOWLEDGEMENTS

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

APPENDIX

INTRODUCTION

The genus *Bombus* includes about 250 species, with 46 species found in temperate regions of North America. Bumblebees follow annual life histories, meaning their colony begins anew each year with the emergence of a solitary queen bee. The mated queen bee emerges from diapause in the spring (around March or April) after overwintering and begins gathering resources, such as nectar and pollen, to support her future colony. This time is the most vulnerable for a fledgling colony. After determining a suitable nest site and acquiring the necessary resources, eggs hatch after approximately 4 days (Colla et al., 2011).

Since insects are poikilothermic and do not have a well-developed homeostatic thermal regulating mechanism, a certain amount of heat accumulation is required before they can reach certain life stages, such as birth and flight (Sridhar & Reddy 2013). Therefore, bumblebee development is inherently dependent on temperature and climatic conditions. The emergence time of a queen bumblebee (i.e. the beginning of a new colony) is one of the species' most important life history traits, as the timing of this event ideally should align with the presence of partner flora to ensure that the queen bee can gather enough resources to support her colony.

The emergence time of a queen bee is an example of phenology, or the timing of recurrent events in an organism's life cycle in relation to seasonal and interannual variations in climate (Stemkovski et al., 2020). As this event is understood to be dependent on temperature, climate change will likely have a profound impact on bee phenology. As a result of warming temperatures, phenological changes in the emergence times of queen bumblebees have been observed to occur (Bartomeus et al., 2011, Belitz et al., 2021, & Hegland et al., 2009, Pawlikowski et al., 2020). Since queen bee emergence times determine the timing of the entire colony, shifts in these dates can be consequential, especially in regard to mismatches between flora that bees depend on for resources (Kehrberger et al., 2019).

For species that provide critical ecological functions, such as bumblebees, understanding their response to climate change is especially important (Bartomeus et al., 2011). Bumblebees are vital pollinators and a keystone species in most terrestrial ecosystems, necessary for the reproduction of countless plants that many animals (including us) depend on for survival (Goulson et al., 2008). Bumblebees are found to be superior in their pollination abilities, because they have been observed to forage faster and pollinate a larger number of flowers than honeybees (Nayak et al., 2020). Additionally, bumblebees are more effective at low temperatures and have a stronger ability to regulate their body temperature than most insects (protected by their fuzzy exteriors and the ability to generate heat through muscle contractions), making them valuable assets in colder areas that deter other pollinating insects (Keaveny et al., 2022). As temperatures increase and become higher at the beginning of the year, this heat accumulation may lead to early emergence times of queen bumblebees, which could occur prior to the flowering of host plants and lead to the starvation and death of the queen. If not fatal, rising temperatures are certainly not advantageous; higher temperatures reduce the foraging range of bumblebees due to reduced energy distribution and wing beat frequency during heat stress, reducing the ability to collect pollen and resources (Kuo et al., 2023).

Fortunately, there is a mathematical metric used to predict the impact of temperature on insect life stages. Degree days can be used to measure the timing of biological events in bumblebees and are a measurement of heat accumulation over time, calculated from daily minimum temperatures, daily maximum temperatures, and a critical minimum temperature threshold (CT_{min}) (McMaster & Wilhelm, 1997). CT_{min} represents the minimum temperature in which bees regain muscle control; a previous study by Oyen & Dillon (2018) determined this threshold to be 3.7±1.6℃ for *B. impatiens*, and is the value used in this study. Degree days are a common metric used to evaluate and estimate the timing of events in an insect's life cycle and have been used to study *Bombus* species previously (Marshall et al., 2018, Miller-Struttmann & Galen, 2022).

Growing degree days (GDD) are the cumulative measure of degree days since the first day of the year (January 1st). GDD can be used to retrospectively calculate the current growth state of an insect or to help predict the date at which an insect will reach a predetermined growth stage (McMaster & Wilhelm, 1997). Insects have a predictable pattern of development based on heat accumulation; therefore, calculating their GDD may shed insight into how much heat accumulation is necessary for queen bees to become active after diapause. The time it takes to reach this threshold of temperature accumulation is subject to change as climate changes, leading to shifts in phenology.

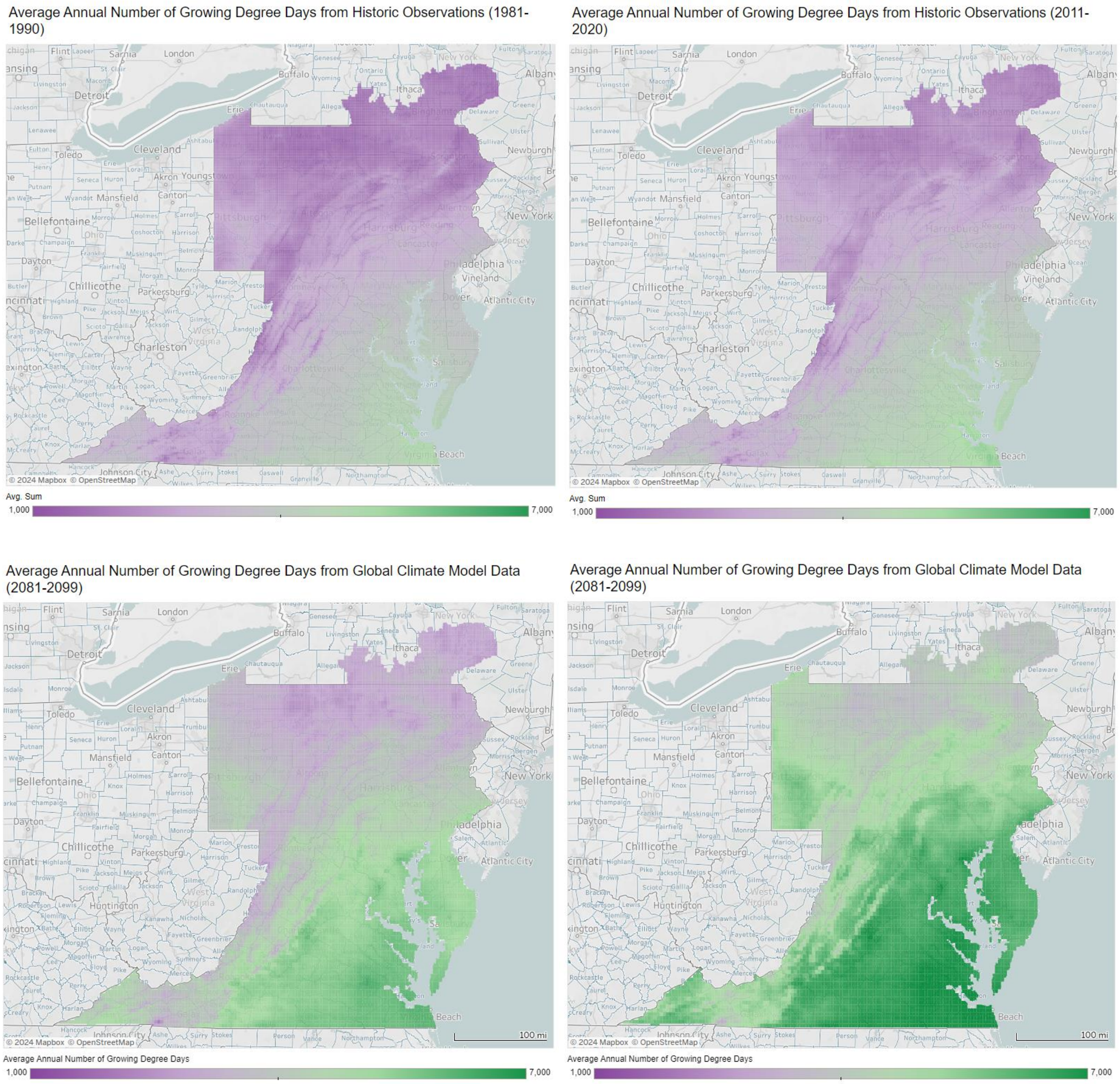


Figure 1: Average annual number of growing degree days from historic observations (top row) and future predictions using global climate model data (bottom row). Historic observations of GDD spanning 30 years are shown on the top row, with the years 1981-1990 on the left and 2011-2020 on the right. Future predictions of GDD between the years 2081-2099 based on RCP emission scenarios 4.5 (left) and 8.5 (right) are shown on the bottom row. Dark purple indicates fewer GDD, while dark green indicates more GDD. All maps were generated with gridded temperature estimates from the Parameter-Elevation Regressions on Independent Slopes Model (PRISM) Climate group at Oregon State University, the same source used to pull climate data for this study. Source: Mid-Atlantic Regional Integrated Sciences and Assessments (MARISA) climate data tools.

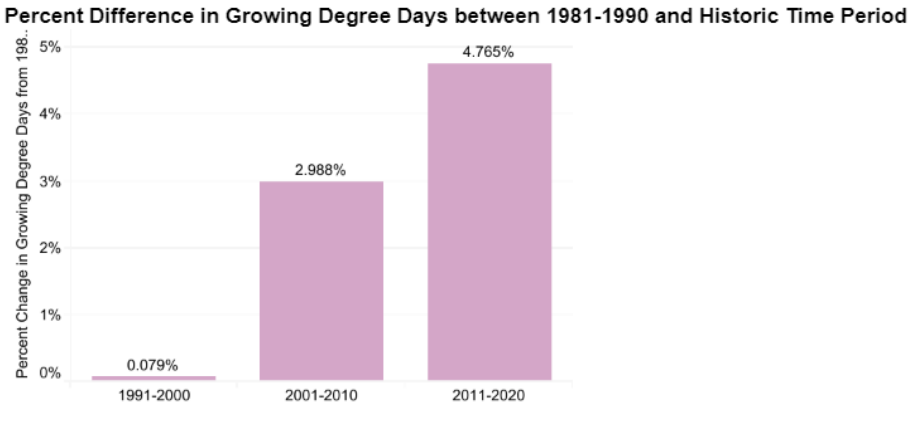


Figure 2: Percent difference in annual growing degree days between 1981-1990 and historic time period by decade in Charlottesville, Virginia. Source: Mid-Atlantic Regional Integrated Sciences and Assessments (MARISA) climate data tool "Average Annual Number of Growing Degree Days from Historic Observations."

As climate change progresses, maximum and minimum daily temperatures can be expected to rise across the Mid-Atlantic United States. This will have a direct impact on growing degree days, as the calculation depends on these temperatures. Fig. 1 shows changes in GDD in the Mid-Atlantic region based on both historic observations from 1981-2020 and projected future changes based on a low emissions (RCP 4.5) and high emissions (RCP 8.5) scenario. In all cases, GDD are increasing with time, which could lead to more rapid heat accumulation and emergence times for queen bees. GDD have increased from 1981-2020, with eastern Maryland and central and eastern Virginia experiencing the largest GDD for the observed historic observations. Additionally, portions of eastern Virginia and coastal Maryland may be expected to experience annual average GDD increases of over 40% in a high emissions future (RCP 8.5) (MARISA, 2021).

Fig. 2 describes the percent difference in growing degree days between 1981-1990 and historic time period by decade in a sample region of Charlottesville, Virginia. The years 1991-2000 yielded a 0.079% increase in GDD since 1981, 2001-2010 yielded a 2.988% increase in

GDD since 1981, and 2011-2020 yielded a 4.765% increase in GDD since 1981. It is clear that GDD is increasing and will likely continue to increase as temperatures increase over time.

As queen bees overwinter near or in the ground, other environmental factors that can affect their emergence time must be taken into account. Precipitation affects moisture availability and temperature near the surface, and therefore may have an impact on queen bee development. A study by Sanderson et al. (2015) looked at the short-term effects of meteorological conditions on bumblebees and found a positive correlation between air temperature and foraging activity, suggesting that bees are more active during warmer weather. The same study also revealed that foraging activity was negatively linked to rainfall, humidity, and wind-speed. However, another study by Karbassioon et al. (2023) found that bumblebees were more resilient to changes in weather conditions than honeybees, making them more predisposed to future changes in day-to-day weather. Both studies show that bumblebees are both reliant yet resilient to weather conditions. Given these studies, it is predicted that bumblebees will face a negative correlation between emergence time and precipitation, though not dramatically due to their observed adaptability.

This study collects observations from a citizen science database, meaning the data were collected by the general public and backed by scientists. iNaturalist, a popular citizen science database, produced the bulk of observations for this study (the other observations are pulled from a museum database). Citizen science is a useful tool for environmental researchers, as it allows data collection to span temporal and spatial scales far beyond the capacities of a research team, allowing for more comprehensive studies. The databases are free and easily accessible, making them a logical choice if one doesn't have the means or funding for a research team. These databases encourage the public to become invested and actively involved in projects, raising awareness and general interest in environmental studies. However, data biases are inevitable due to human error and inconsistencies in collection; it is beyond the control of the scientist to determine the volume of data within a certain temporal and spatial range, making citizen science inherently variable. Additionally, the observations are “presence-only” observations, meaning that corresponding absence data are absent. Nevertheless, citizen science databases are a valuable asset for environmental researchers and have been used in countless scientific inquiries and publications.

Research examining the trends between bumblebee and plant phenology is extensive, yet there is limited research examining the phenological impact of climatic and geographic variables on queen bumblebee emergence times. The goal of this study is to examine the phenology of queen bumblebee emergence times and its connection to temperature (GDD) and precipitation across the states of Virginia, West Virginia, Maryland, Delaware, and Pennsylvania. I hypothesize that the GDD for queen bee emergences will vary between species yet remain constant within a species across latitudinal and elevational gradients. Additionally, I hypothesize that the number of days it takes to reach this threshold will increase with elevation and latitude (as well as with increased precipitation) and will differ between species.

This study is important because it determines the GDD at which common queen *Bombus* species emerge in this geographic region (novel research within this field), allowing for more accurate emergence predictions to occur in the future. This study also examines a relationship not previously studied between temperature, precipitation, elevation, latitude, and the emergence time of queen bumblebees, a crucial pollinator in temperate ecosystems.

METHODS

Data Collection

The study covers a geographic region of the Mid and South Atlantic U.S. and takes observations from the states of Virginia, West Virginia, Maryland, Delaware, and Pennsylvania. This was to ensure a variety of elevations and latitudes in addition to a relatively wide range of climate histories and environments. Bee data are presence-only points, meaning species data is presented as a list of presence locations without corresponding absence data. This becomes relevant in the analysis phase, as this factor is accounted for to accurately calculate emergence times. Bee data were obtained using the citizen science database iNaturalist and the Global Biodiversity Information Facility (GBIF); this is to ensure the data reflect both current and historical trends, respectively.

iNaturalist, a citizen science database with observations from the general public, was used to gather more current bumblebee data. To ensure only queen bees were being studied, the results were filtered to only show results from the months of March through April, which are the estimated emergence times of queen bees (Pawlikowski et al., 2020). Results were also restricted to the geographic range of the study. iNaturalist produced 2,304 observations within these parameters using the keyword *Bombus*, the genus name for bumblebees. Within these observations, 12 species, 284 identifiers, and 1,318 observers were found with dates ranging from 2010-2023 (a majority of them more recent). The data were cleaned and narrowed down by restricting the dataset to “research grade” only (meaning the identification was agreed upon by a member of the community) and by restricting the positional accuracy to 1km to ensure a more accurate geographical location. With these restrictions, 1,550 observations remained. Each entry included the date of the observation, the user who observed it, the quality grade, the number of identification agreements and disagreements, the latitude and longitude, the positional accuracy, the state and county, and the scientific name.

GBIF (Global Biodiversity Information Facility) data were used to gather historical data on *Bombus* species. Founded in 2001, GBIF is an international museum database and online organization that hosts a plethora of scientific data, geared towards research in biodiversity. Using the same search criteria as iNaturalist (keeping geographic boundaries, observance windows, positional accuracy, and genus the same), a general search was made and data were downloaded within these parameters. GBIF produced 378 observations, with the earliest observation occurring in 2002. Each entry included the date of the observation, the user who identified it, the latitude and longitude, the positional accuracy, and the institution where it was recorded (USGS for all observations).

Out of the 1,928 bee observations produced by both iNaturalist and GBIF, only three species had more than 200 total observations. A high number of observations per species is necessary to draw accurate conclusions in the analysis phase. The three species were *B. bimaculatus* (843 observations), *B. griseocollis* (264 observations), and *B. impatiens* (579 observations), leaving a total of 1,686 observations to conduct an analysis. Fig. 3 spatially represents each observation point generated by iNaturalist and GBIF on a mapframe, produced in ArcGIS Pro 3.1.4.

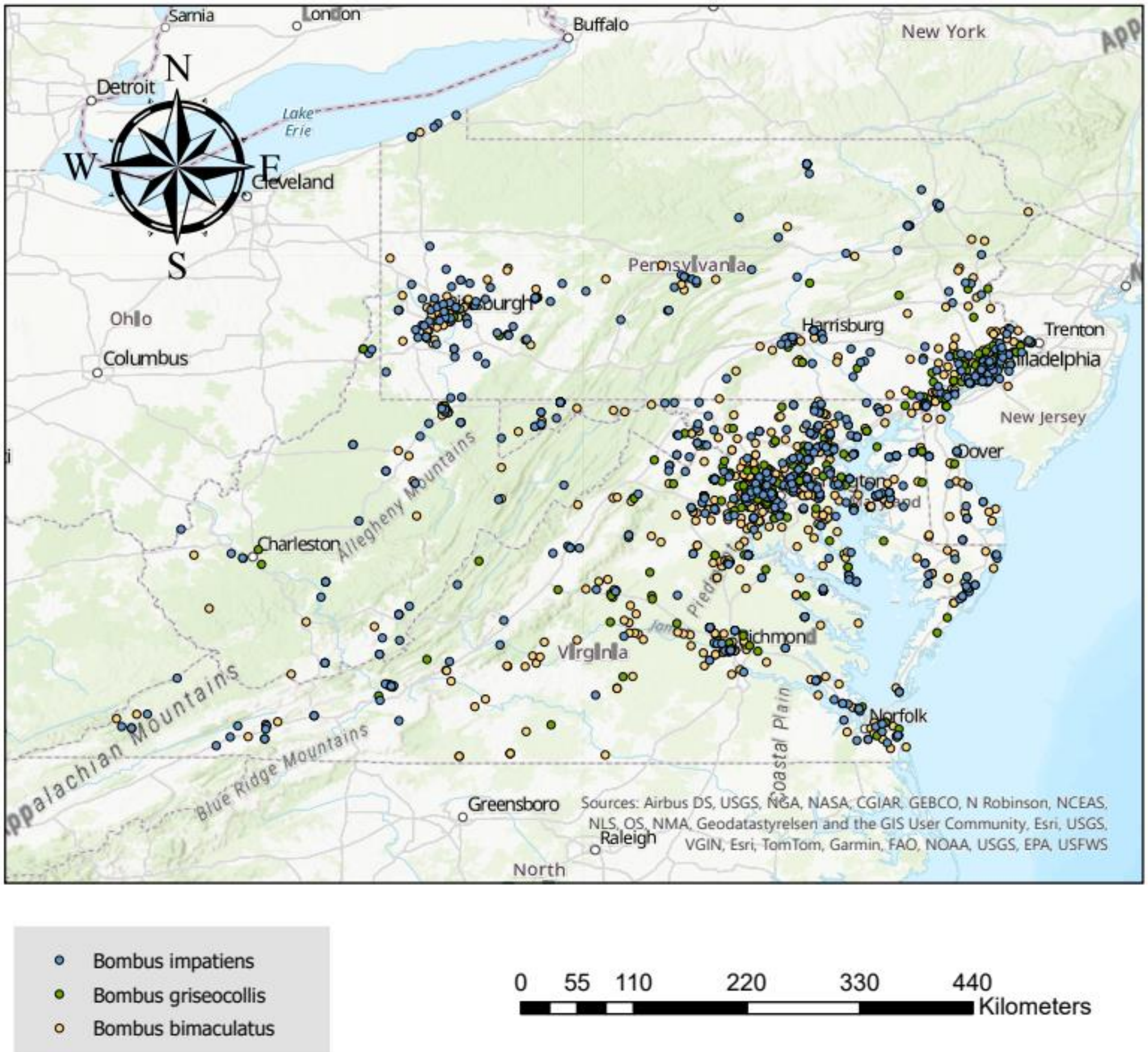


Figure 3: Species distribution of *Bombus* species within the spatial resolution of this study. Point data were generated in ArcGIS Pro 3.1.4 and pulled from iNaturalist and GBIF. *B. impatiens* observations are in blue, *B. griseocollis* observations are in green, and *B. bimaculatus* observations are in yellow.

Weather data was obtained using the Parameter-Elevation Regressions on Independent Slopes Model (PRISM) developed by the Northwest Alliance for Computational Science & Engineering (NACSE), based at Oregon State University. PRISM is a climate database that gathers weather data from a wide range of monitoring networks and applies sophisticated quality control measures to produce climate datasets that show short and long-term climate trends. Using a spatial resolution of 4km, PRISM calculates a climate-elevation regression, assigning weights based on physiographic similarity of the station to each cell. Location, elevation, coastal proximity, and other related variables are considered in the interpolation of the weather data (Daly et al., 2008).

Weather data were downloaded for each observation within the three species chosen. Using their data explorer, a time series containing daily values for maximum and minimum temperatures, mean temperatures, and precipitation were downloaded for each bee observation using the corresponding latitude and longitude values provided by iNaturalist and GBIF. Daily values beginning on January 1st and ending on April 30th were downloaded for the years 2000-2023 for each individual location in order to calculate the accumulation of temperature and precipitation for each observation and to observe long-term trends in climate patterns. As a result, over 4.8 million rows of weather data were generated.

Elevation data for each observation were obtained using ArcGIS Pro 3.1.4. Elevation values were pulled from a raster dataset provided by ESRI. XY point data for each observation within the three main species were downloaded into a map frame, allowing each observation to be represented by a point on a map. An elevation source layer called Terrain 3D was added to the map and allowed each point to pull the corresponding elevation to five decimal places.

Data Analysis

Microsoft Excel was used to organize and analyze bumblebee data; iNaturalist data, GBIF data, and PRISM datasets were all stored in Excel files. The PRISM datasets for each species first underwent three distinct calculations in Excel, as detailed below:

1) **Growing degree days (GDD):** Growing degree days (*eq. 1*) can be used to retrospectively calculate the current growth state of an insect, or to help predict the date at which an insect will reach a predetermined growth stage. First, degree days were calculated for each observation by taking the average daily temperature of that day ((maxTemp - minTemp)/2) and subtracting the critical minimum temperature (CT_{min}), or the temperature bumblebees regain motor control (McMaster & Wilhelm, 1997). *B. impatiens* have a critical minimum temperature of 3.7±1.6 °C (Oyen & Dillon, 2018), so this value was used in calculations. If the degree day was negative, it was omitted from the final summation. Daily degree day values starting on January 1st were added sequentially until the date of the observation. GDD represents the total cumulative number of degree days from January 1st up until the date of an observation; this value represents the total heat the insect has accumulated. This formula was applied to each bee observation across the years of 2000-2023 to gain both short term and long-term perspective. Units are in days.

$$GDD \ = \sum \frac{(maxTemp - minTemp\,)}{2} - CTmin \qquad\qquad (eq.\ 1)$$

2) **Cumulative precipitation (∑ppt):** Daily precipitation values starting on January 1st were added sequentially until the date of the observation, providing insight into each location's cumulative near-ground moisture content and availability at the time of the sighting. This process was repeated for each bee observation. Units are in millimeters.

3) **Days Accumulated (∑day):** A simple calculation determining how many days had passed since January 1st until the date of the observation was conducted. This provides insight as to how far in the year an observation was recorded, which has the ability to

affect GDD and ∑ppt values. This value will be used to estimate when bumblebee emergences occur for each species, as well as provide an explanation for varying values of GDD and ∑ppt values. Units are in days.

Other values, such as mean temperature dew point and vapor pressure deficit, were also calculated. However, they were not as relevant to this study and were later omitted. As there was no way to automate the summation of GDD and ∑ppt variables until a particular date in Excel, this had to be done manually. This was completed by highlighting each row that contained the date of each observation; with it came the corresponding GDD and ∑ppt value. The observations were checked in Excel against a unique number called a reference ID to ensure the data pulled was accurate. With these calculations, each individual observation in Excel contained a corresponding GDD, ∑ppt, and ∑day value, as well as its original latitude, longitude, and elevation.

Analyses of these data were conducted in both Excel and RStudio. In Excel, the cumulative percentage of observations per week was calculated for each species. This was to see how observations accumulated on a weekly basis, and to provide insight as to when the most observations were occurring and where they were accelerating. The interval of week 0-1 indicates the days March 1st-7th, and each following interval denotes the subsequent week. The percent difference between intervals denotes how the observations were changing from week to week (i.e. growing or declining from the previous week), and the percent slope of each interval denotes the quantity of observations per weekly interval as compared to the total number of observations (i.e. how many observations were made during that week).

The cumulative percentage of GDD for each species was calculated in Excel using similar metrics. This analysis demonstrates the distribution of GDD across observations and provides insight into which GDD values were more common than others. A larger slope between GDD values indicates more GDD observations falling within this range, indicating a common GDD. The percent difference between intervals denotes how GDD was changing between intervals (i.e. growing or declining in number from the previous interval), and the percent slope of each interval denotes the volume of observations within that GDD range as compared to the total number of observations (i.e. how many observations were made with that GDD range). Both metrics are used to describe emergence trends and are discussed in greater detail in the following sections.

The data were then analyzed in RStudio to run statistical tests. A Pearson correlation analysis (*eq. 2*) was run for each of the three variables (GDD, ∑ppt, and ∑day). This test was to determine the strength of the linear relationship between the variables with values ranging between -1 to 1. A negative value indicates a negative correlation, 0 denotes no correlation, and 1 indicates a perfect positive correlation. r represents the correlation coefficient, X_i and Y_i represent individual data points, where $\hat{X}$ and $\hat{Y}$ represent their respective means. The R packages *Hmisc* and *readxl* were used to conduct this analysis. Table 3 displays the results from the Pearson correlation analysis using the collective 1,686 observations.

$$r = \frac{\sum(X_i - \bar{X})(Y_i - \bar{Y})}{\sqrt{\sum(X_i - \bar{X})^2 \sum(Y_i - \bar{Y})^2}} \qquad \textit{(eq. 2)}$$

RStudio was then used to estimate phenological metrics; the R package *phenesse* was designed to generate parameterized estimates of phenology for presence only data such as this for any percentile of a distribution (Belitz et al., 2020). Within this package, the “weib_percentile_ci” component was used to calculate a percentile estimate within a 95%

confidence interval (5% uncertainty) using non-parametric bootstrapping. For this study, this package was used to estimate when the first 10% (percentile = 0.1) of individuals within each *Bombus* species were present. This percentile was chosen to generate an estimate of the emergence time for the three species, as the first 10% of sightings are likely closer to the actual emergence time of the species. For each species, this code was run to generate the estimated GDD, ∑ppt, and ∑day (as well as the low and high confidence intervals) corresponding to the 10th percentile of emergences. This analysis tests the hypothesis by mathematically demonstrating the relationship between temperature (GDD), ∑ppt, and ∑day. The results are displayed in Table 4.

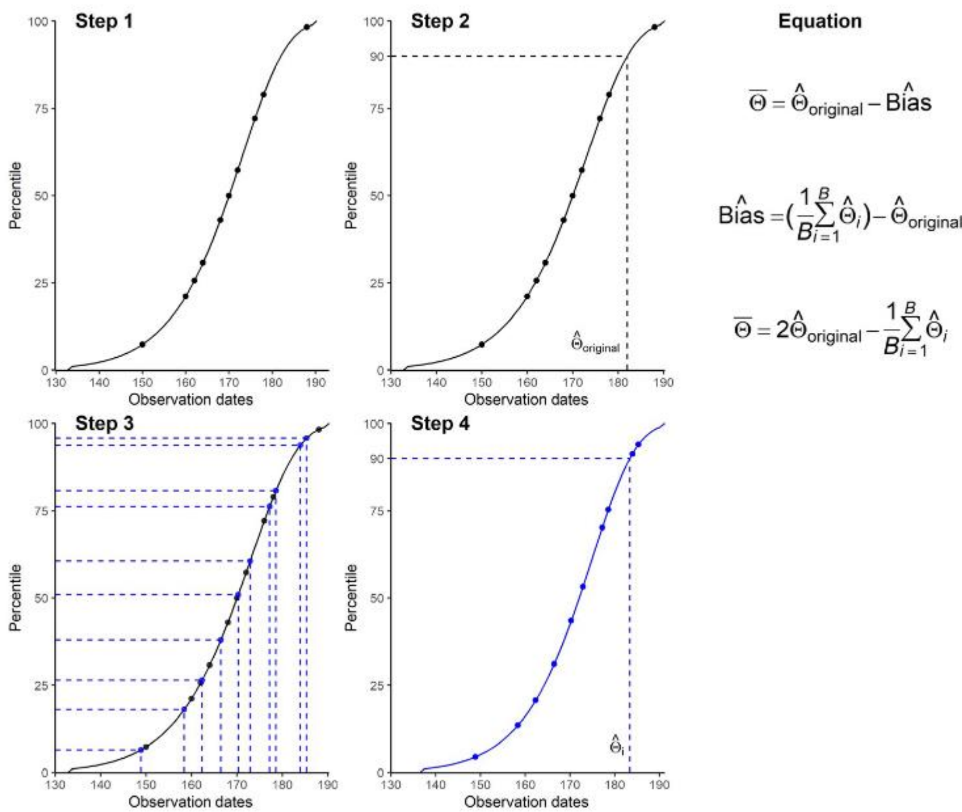


Figure 4: Workflow showing the calculation of a percentile estimate from observation dates using the Weibull-parameterized point estimator. Source: Figure 1, Belitz et al., 2020.

Since the *phenesse* package is designed specifically for presence-only data, adjustments within this package can properly account for biases in the dataset. Belitz et al. (2020) details this process, visualized in Fig. 4. The package uses a Weibull-parameterized point estimator to determine a percentile estimate and account for biases. First, the program calculated a cumulative frequency curve, similar to the cumulative percentage curves generated in Excel for observation and GDD data. The software fits a curve to the point data based on the Weibull distribution, a continuous probability distribution function that has both a shape (k) and scale (λ) parameter. From this Wiebull curve, a GDD value corresponding to the percentile of interest (10th percentile) was calculated.

But since this curve represents presence-only data, this base estimate is assumed to be biased. The program calculates bias estimates by randomly resampling points along the Weibull curve and calculating new estimates of the percentile date. This was done 25 times by setting the "iterations" variable to 25. The program generates 95% confidence intervals by generating a new dataset using random resampling with replacement and repeating the Weibull cumulative distribution function estimate and bias estimate procedures (see equations to the right of Fig. 4). This was done 500 times by setting the "bootstraps" variable to 500. From these iterative and bootstrapped estimations, a distribution of 500 bias-corrected estimates of the 10th percentile date was generated and normally distributed to generate the 95% confidence interval. The 95% confidence limits are the 2.5 and 97.5 percentiles along this distribution.

To test the hypothesis that GDD remains constant for queen emergence times across latitudinal and elevational gradients, a comparison between low and high latitudes as well as low and high elevations was conducted. The 100 lowest and highest values for both latitude and elevation were tested independently and then directly compared against each other to see if these geographic variables affected queen bee emergence times. Appendices D, E, F, and G show the geographic distributions for each species in their respective tests. Each latitude and elevation value were run through the *phenesse* code to calculate GDD and ∑day values for the first 10th percentile of observations. ∑ppt values were excluded, as precipitation is relatively independent of latitude and elevation. These analyses also provided insight into the number of days it takes to reach this threshold, testing the second part of the hypothesis that predicts that the number of days it takes to reach this threshold will increase with elevation and latitude.

RESULTS

Fig. 5 displays the results from the Excel analysis; Table 1 and Table 2 provide corresponding numerical information. The left-hand side of Fig. 5 describes the cumulative percentage of observations per week for each species. Lines of best fit were generated for each curve; the positive trend of the curves is explained by the fact that the percentage is cumulative and compounded every week to generate a new percentage of the total amount of observations. Results from the Excel analysis show that *B. bimaculatus* emerges first, *B. impatiens* follows, and *B. griseocollis* emerges last. As shown in Table 1, the percent difference between intervals denotes how the observations were changing from week to week (i.e. growing or declining from the previous week). The largest percent difference between intervals for each species all occur during the 5th week, or between March 29-April 4th. This suggests that queen bee emergences currently occur most frequently in the week of March 29th-April 4th for this dataset. The R analyses look into these emergence times in more detail. The percent slope of each interval denotes the quantity of observations per weekly interval as compared to the total number of observations (i.e. how many observations were made during that week). As the largest percentages of observations occur later in the year, this likely corresponds to more observations occurring as time progresses and as more and more queen bees emerge.

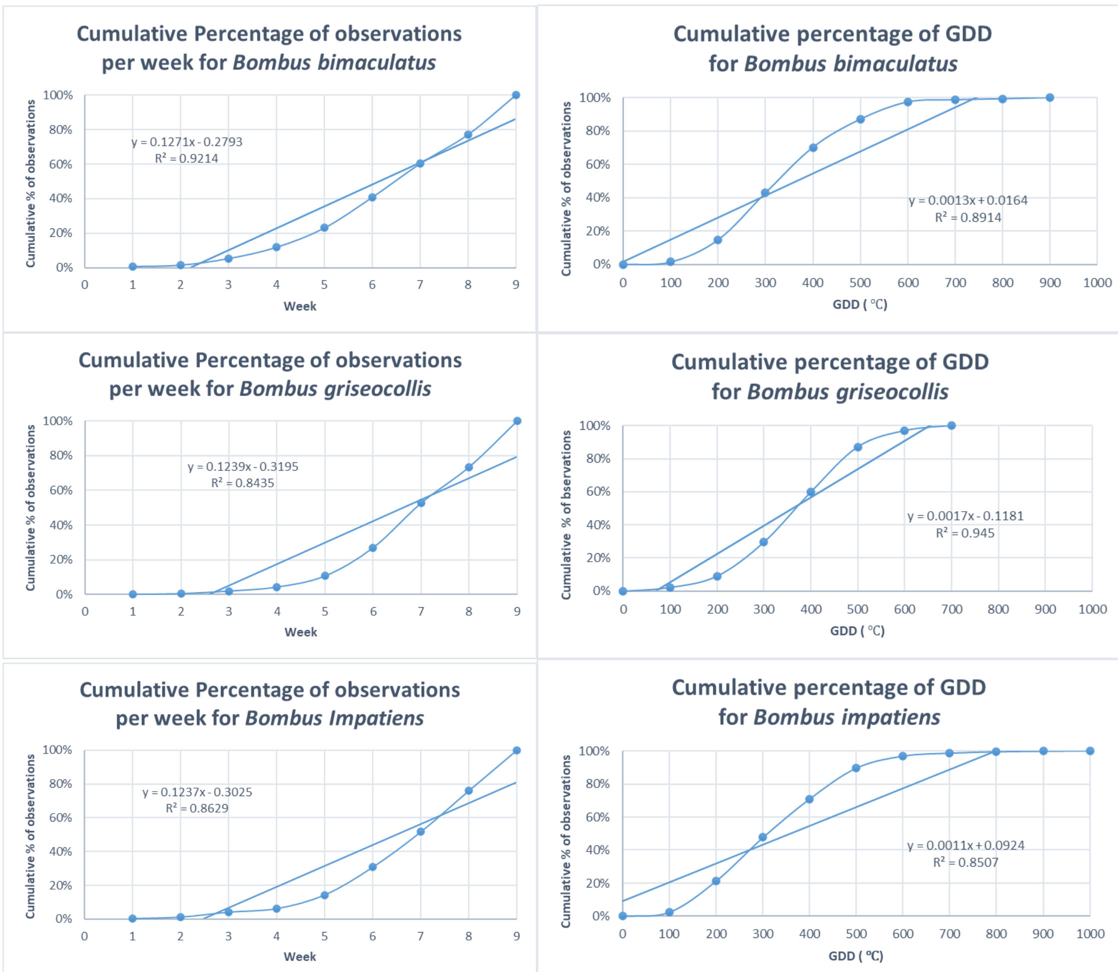


Figure 5: Cumulative Percentage of Observations per week and GDD for each of the three species. Generated using Excel.

The right hand of Fig. 5 describes the cumulative percentage of GDD for each species. The S-shaped nature of the curves is explained by the fact that few species are found with low and high values of GDD, while many species are found to have GDD in the mid-range. The slope between intervals denotes the volume of observations made within that GDD range. As shown in Table 2, the percent difference between intervals denotes how GDD was changing between intervals (i.e. growing or declining in number from the previous interval). The interval of 201-300 GDD contains the largest percent increase from the previous week for *B. bimaculatus* and *B. griseocollis*, indicating that this GDD range is common for queen bee emergences for those species. The percent slope of each interval denotes the volume of observations within that GDD range as compared to the total number of observations (i.e. how many observations were made with that GDD range). The interval of 201-300 GDD also contained the largest percent slope (volume) of observations for *B. bimaculatus* and *B. impatiens*, further supporting the conclusion that 201-300 GDD is a common GDD range for *Bombus* species to emerge. Fig. 5 shows general patterns between emergence times and GDD, and later R analyses will look into these trends in greater detail.

Table 1: Cumulative Percentage of Observations per week

	Percent difference between intervals			Percent slope of each interval		
Week	*B. bimaculatus*	*B. griseocollis*	*B. impatiens*	*B. bimaculatus*	*B. griseocollis*	*B. impatiens*
1 (March 1-7)	0.12%	0.38%	0.52%	0.83%	0.38%	0.86%
2 (March 8-12)	2.97%	1.14%	2.07%	3.80%	1.52%	2.94%
3 (March 15-21)	2.73%	0.76%	-0.86%	6.52%	2.27%	2.07%
4 (March 22-28)	4.63%	4.17%	6.04%	11.15%	6.44%	8.12%
5 (March 29-April 4)	**6.64%**	**9.85%**	**8.29%**	17.79%	16.29%	16.41%
6 (April 5-11)	1.66%	9.47%	4.49%	19.45%	25.76%	20.90%
7 (April 12-18)	-2.61%	-4.92%	3.45%	16.84%	20.83%	**24.35%**
8 (April 19-25)	6.05%	5.68%	-0.35%	**22.89%**	**26.52%**	24.01%

Note: The percent difference between intervals denotes how the observations were changing from week to week (i.e. growing or declining from the previous week), and the percent slope of each interval denotes the quantity of observations per weekly interval as compared to the total number of observations (i.e. how many observations were made during that week compared to the overall total).

Table 2: Cumulative Percentage of GDD for Each Species

	Percent difference between intervals			Percent slope of each interval		
GDD	*B. bimaculatus*	*B. griseocollis*	*B. impatiens*	*B. bimaculatus*	*B.griseocollis*	*B. impatiens*
0-100	1.54%	2.27%	2.59%	1.54%	2.27%	2.59%
101-200	11.74%	4.55%	**16.23%**	13.29%	6.82%	18.83%
201-300	**14.83%**	**13.64%**	7.43%	**28.11%**	20.45%	**26.25%**
301-400	-0.83%	9.85%	-2.94%	27.28%	**30.30%**	23.32%
401-500	-10.44%	-3.03%	-4.66%	16.84%	27.27%	18.65%
501-600	-6.52%	-17.42%	-11.40%	10.32%	9.85%	7.25%
601-700	-9.02%	-6.82%	-5.53%	1.30%	3.03%	1.73%
701-800	-0.71%	-3.03%	-0.86%	0.59%	-100.00%	0.86%
801-900	0.12%	NA	-0.52%	0.71%	NA	0.35%
901-1000	-0.71%	NA	-0.17%	-100.00%	NA	0.17%

Note: The percent difference between intervals denotes how GDD was changing between intervals (i.e. growing or declining in number from the previous interval), and the percent slope of each interval denotes the volume of observations within that GDD range as compared to the total number of observations (i.e. how many observations were made with that GDD range).

Table 3: Results of the Pearson correlation analysis

	GDD	**∑ppt**	**∑day**
GDD	1.00	0.18 <.0001	0.54 <.0001
∑ppt	0.18 <.0001	1.00	0.59 <.0001
∑day	0.54 <.0001	0.59 <.0001	1.00

Note: This test was to determine collinearity and the strength of the linear relationship between the variables with values ranging between -1 to 1. A negative value indicates a negative correlation, 0 denotes no correlation, and 1 indicates a perfect positive correlation.

The results of the Pearson correlation analysis are shown in Table 3; they demonstrate strong significance given the large volume of data (1,686 points) included in the analysis. A negative value indicates a negative correlation, 0 denotes no correlation, and 1 indicates a perfect positive correlation. Table 3 shows that GDD and ∑ppt are very loosely yet positively correlated with a value of 0.18; thus, temperature accumulation and precipitation share a weak positive relationship, just as predicted. This is not surprising, as there is likely to be a lot of year-to-year variation in precipitation. As GDD increases, there is more of an opportunity for precipitation to increase as well, explaining the positive correlation. GDD and ∑day have a value of 0.54, indicating a stronger positive correlation, also just as expected. As the date progresses farther into the year, there is more opportunity for heat (GDD) to accumulate. The correlation is intermediate due to year-to-year variation in temperature, as well as the effects of latitude and elevation on the relationship between date and temperature.

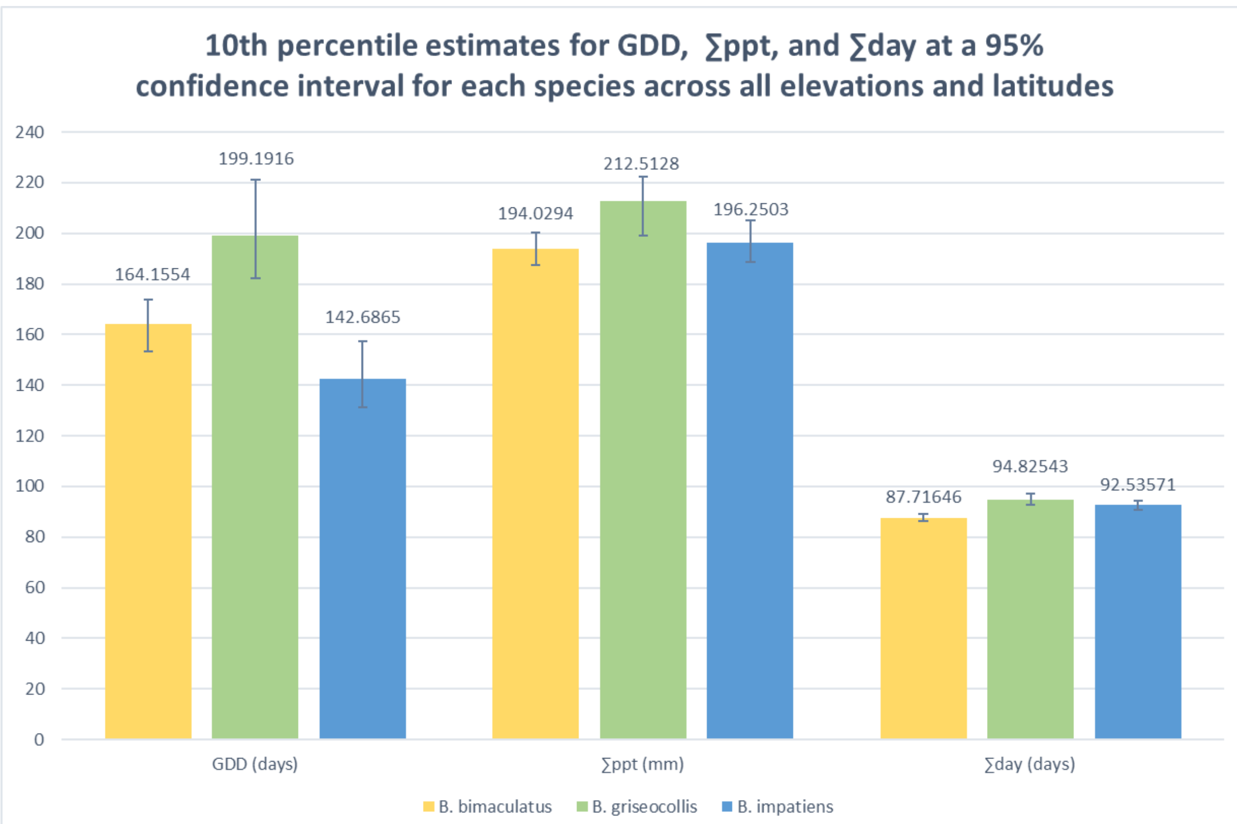


Figure 6: 10th percentile estimates for GDD, ∑ppt, and ∑day at a 95% confidence interval for each species across all elevations and latitudes.

Fig. 6 displays the results from the phenological analysis conducted in RStudio using the R package *phenesse* to determine the estimated GDD, ∑ppt, and ∑day (as well as the low and high confidence intervals) corresponding to the 10th percentile of emergences at a 95% confidence interval for each species across all elevations and latitudes. *B. bimaculatus* emerges first, *B. impatiens* second, and *B. griseocollis* third. This finding is consistent with the Excel analysis as well as with previous research and field observations by T'ai Roulston, an ecological researcher at Blandy Experimental Farm, the University of Virginia's research facility. For the purpose of this study, estimates were regarded as being significantly different if neither estimate was contained within the 95% confidence interval (i.e. if the bounds of the error bar did not overlap with the mean) of the other estimate.

The GDD difference between all species at the 10th percentile of emergence was statistically significant, which aligns with the hypothesis. *B. impatiens* was observed to accumulate the least GDD by the 10th percentile at about 143 days, while *B. griseocollis* was observed to have the most GDD at about 199 days, suggesting that *B. griseocollis* requires more heat accumulation than the other two species to emerge. ∑ppt values, having a weak yet positive correlation with GDD as demonstrated by the results of the Pearson correlation analysis, demonstrate a similar trend to GDD; ∑ppt was the highest for *B. griseocollis*, which was also the last species to emerge. However, the ∑day values for all three species are curiously close given the large differences in GDD seen for each species, especially since the two variables have a Pearson correlation coefficient of 0.54. GDD inevitably increases in weight as it gets warmer into the year, as both maximum and minimum temperatures increase; however, the earliest and latest emergence dates are separated only by about a week (between March 29th-April 5th), which is shorter than expected given the difference in GDD. The confidence intervals are very small for ∑day, suggesting low uncertainty and more accurate values. An analysis looking more closely into the differences in ∑day values between species and across different elevations and latitudes was conducted; the results are displayed in Fig. 7.

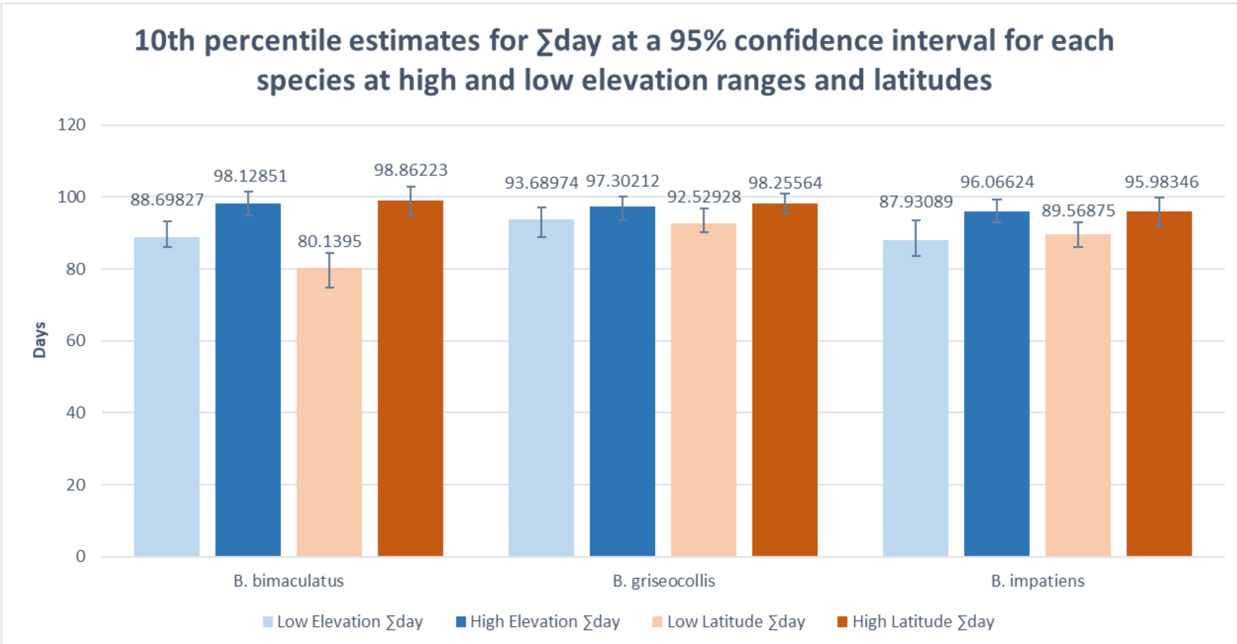


Figure 7: 10th percentile estimates for ∑day at a 95% confidence interval for each species at high and low elevation ranges and latitudes.

Fig. 7 displays the 10th percentile estimates for ∑day at a 95% confidence interval for each species at high and low elevations and latitudes. Appendix B and C specify low and high elevation and latitude ranges, respectively, while Appendices D, E, F, and G show the geographic ranges of the observations tested. High elevations resulted in more ∑day values than at low elevations within a species for all three species, with *B. bimaculatus* and *B. impatiens* demonstrating statistical significance. Between high and low elevation emergence times within a species, *B. bimaculatus* has a difference of about 9 days, *B. griseocollis* with about 3 days, and *B. impatiens* with about 8 days. This supports the hypothesis, which states that the number of days it takes to reach the emergence time will increase with elevation. However, between all three species the emergence times for high and low elevation are notably close to each other and do not differ in a statistically significant manner, which was unexpected. At high elevations, the three species only differ in one or two days at most (April 7-8th). At low elevations, the days differ a bit more, with about 2-5 days separating the species (March 29-April 4th). This goes against the hypothesis that ∑day values will differ between species at both high and low elevations and latitudes.

High latitudes resulted in more ∑day values than low latitudes within a species for all three species, with all three species demonstrating statistical significance. Between high and low latitude emergence times within a species, *B. bimaculatus* has a difference of about 19 days, *B. griseocollis* with about 5 days, and *B. impatiens* with about 5 days. This supports the hypothesis, which states that the number of days it takes to reach the emergence time will increase with latitude. However, emergence days are very close for all three species at high latitudes, and the differences are not statistically significant. At high latitudes, the three species differ only in a few days, one or two days at most (April 6-8th). This goes against the hypothesis that ∑day values will differ between species. At low latitudes, the days differ a bit more (March 21st-April 3rd) and significant differences are seen between *B. bimaculatus* and both *B. griseocollis* and *B. impatiens,* though not between *B. griseocollis* and *B. impatiens*. This suggests that the three species at high latitudes do not experience a significant difference in ∑day, while at low latitudes there is more a bit more interspecies variation.

As the geographic range and sample size for this study were relatively large, this finding is puzzling, as only half of the hypothesis was supported. High elevations and latitudes resulted in later emergence times than low elevations and latitudes, just as expected. But between species, the emergence times at high elevations and latitudes are very close and do not differ significantly, going against the hypothesis. As variation in GDD values are dependent on location and ∑day values are not, tests were run to determine if a relationship between geographic location and GDD is present; results are displayed in Fig. 7 and Fig. 8.

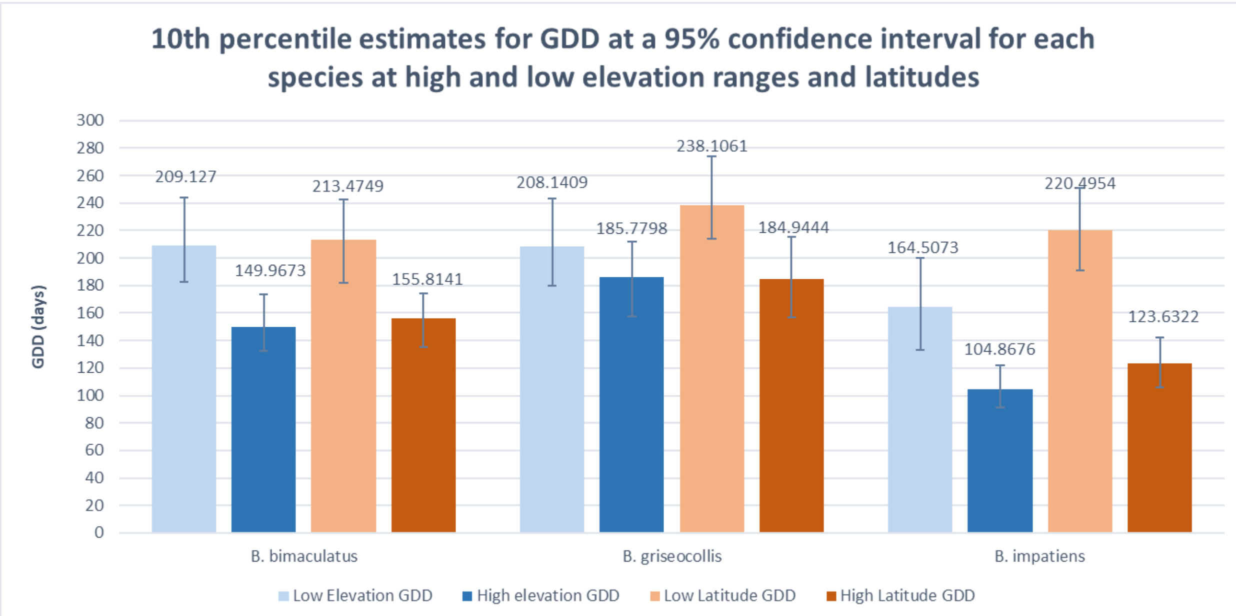


Figure 8: 10th percentile estimates for GDD at a 95% confidence interval for each species at high and low elevation ranges and latitudes.

Fig. 8 displays the 10th percentile estimates for GDD at a 95% confidence interval for each species at high and low elevation ranges and latitudes. Appendix B and C specify low and high elevation and latitude ranges, respectively, while Appendices D, E, F, and G show the geographic ranges of the observations tested. Within a species, high elevations resulted in fewer GDD than low elevations across all three species, with *B. bimaculatus* and *B. impatiens* showing significantly lower GDD at high elevations. Between high and low elevation observations within a species, *B. bimaculatus* has a difference of about 59 days, *B. griseocollis* with about 22 days, and *B. impatiens* with about 60 days. This goes against the hypothesis, as it shows GDD variation within a species across elevations despite the prediction that GDD would remain constant across latitudinal and elevational gradients. What's additionally puzzling is that GDD values at low elevations between species are not statistically significant between *B. bimaculatus* and *B. griseocollis*; rather, they are a mere day apart in GDD. However, at high elevations, the difference in GDD between the three species is statistically significant, suggesting interspecies variation at high elevations. At low elevations the results go against the hypothesis that GDD will vary significantly between species, while at high elevations the results support this hypothesis.

Similarly, high latitudes resulted in fewer GDD than low latitudes within each species, with all three species demonstrating statistical significance. Between high and low latitude observations within a species, *B. bimaculatus* has a difference of about 57 days, *B. griseocollis* with about 53 days, and *B. impatiens* with about 96 days. This also goes against the hypothesis, as this finding demonstrates a variation in the temperature accumulation necessary for queen bees to emerge across different environments, despite the prediction that species emerged at the same measure of heat accumulation. Between species, the difference in low latitudes is not statistically significant for all three species, while high latitudes are. This suggests that the three species at low latitudes do not experience a significant difference in GDD, while at high latitudes there is more interspecies variation.

DISCUSSION

The results show that the cumulative percentage of observations for all species were accelerating the most in week 5, suggesting that queen bee emergences currently occur most frequently in the week of March 29th-April 4th. *B. bimaculatus* emerges first, *B. impatiens* emerges second, and *B. griseocollis* emerges third. Emergence dates occur later at high latitudes and elevations, aligning with the hypothesis. Interestingly, species differences in emergence dates are seen to be much less pronounced at higher elevations and latitudes. GDD responds similarly to high elevation and latitude within a species; higher elevations and latitudes result in fewer GDD while lower elevations and latitude result in more GDD. This goes against the hypothesis that the GDD for queen bee emergences remain constant within a species across latitudinal and elevational gradients.

The finding that *B. bimaculatus* emerges first, *B. impatiens* emerges second, and *B. griseocollis* emerges third is consistent with previous field studies by T'ai Roulston, an ecological researcher at Blandy Experimental Farm, the University of Virginia's research facility. Confirming this trend increases the validity of the other findings. The predicted date of emergence times (March 29th-April 4th) is a novel contribution in this field and can be used in future estimates to predict emergence times.

Emergence times occur later at high elevations and latitudes across all three species, aligning with the hypothesis (as these areas are historically colder and therefore hypothesized to slow emergence time). However, curiously enough, species differences in emergence dates are much less pronounced at higher elevations and latitudes, only differing in their ∑day value by one or two days. Additionally, at lower elevations, the emergence date between species was not found to be significantly different. This finding, combined with the significant variation in GDD across all elevations and latitudes, suggests that temperature is not the driving factor of queen bee emergence times, as previously believed (Bartomeus et al., 2011, Belitz et al., 2021, & Hegland et al., 2009, Pawlikowski et al., 2020). However, since emergence times still occur later at high elevations and latitudes, temperature still likely plays a role, though a less relevant one than previously believed. This finding suggests that perhaps bumblebees have an internal regulating mechanism to determine emergence time based on day length and year progression as opposed to heat accumulation. This combined with a predetermined minimum threshold temperature for emergence is a likely explanation.

GDD is shown to vary considerably between species across all latitudes and elevations. This result was expected, but was unexpected in conjunction with the ∑day values being very close (4-6 days apart) between species. As GDD and ∑day have a Pearson correlation coefficient of 0.54, the trends for these variables were expected to be more similar. As temperature accumulation is somewhat dependent on the number of days passed, the fact that these values are so close suggests that location played a role in emergence times, going against the hypothesis.

GDD varies considerably within a species for high and low elevations and latitudes. This disproves the hypothesis that GDD will remain constant for a species, regardless of the elevation and latitude. This suggests that temperature accumulation is not the driving factor of emergence times, as was widely believed (Bartomeus et al., 2011, Belitz et al., 2021, & Hegland et al., 2009, Pawlikowski et al., 2020). GDD was considerably lower at higher elevations and latitudes, which is likely due to the colder average temperatures at these locations. Logically, this makes sense as to why the GDD accumulation was so different in different locations. However, this does not explain the difference in *emergence* times. Since it was believed that bumblebees are reliant on a certain amount of heat accumulation to emerge, the statistically significant results proving otherwise disproves this hypothesis.

It is only logical to assume that GDD would drive queen bee emergence, as temperature is widely known to determine the completion of many insect life stages (Damos & Savopoulou-Soultani 2012). However, since queen bees overwinter as fully mature and fertile adults, perhaps GDD are not a suitable metric for measuring emergence time. This will require further studies;

however, an interesting paper by Keaveny et al. (2022) found that pre-wintering queen bees are the most freeze tolerant, freezing at significantly lower temperatures than queen bees lacking ovary development. This suggests that queen bumblebees are resilient to extreme temperatures. This idea is supported by a 2021 study by Maebe et al., which states that bumblebees exhibit plastic and adaptive responses to climate change. Bees are surprisingly resilient to thermal stress, so this may also be a contributing factor (Quinlan et al., 2023)

Nevertheless, these findings are an interesting contribution to the literature surrounding *Bombus* phenology and emergence times of queen bees. Previous studies surrounding shifts in phenology for pollinators point to temperature as a driving factor (Bartomeus et al., 2011, Belitz et al., 2021, & Hegland et al., 2009, Pawlikowski et al., 2020). However, this study reveals that day accumulation, especially at high elevations and latitudes, drives *Bombus* emergence times more than temperature accumulation. Temperature accumulation may still play a role, though a less significant one than previously believed. As queen bees overwinter in the ground, perhaps a future study could examine soil degree days (or heat accumulation in soil) as opposed to GDD. The relationship between air temperature and soil temperature is very complex and would require a new study.

However, this research is not without error. A variety of factors could have contributed to biases in the data. The most notable example is geographic bias; as observation data for iNaturalist and GBIF are reliant on outside observers, observations were clustered in areas with lots of people, such as major cities. Notable examples include DC, Philadelphia, Pittsburgh, Richmond, and Norfolk. This means that observations were pulled disproportionately across the geographic extent of this study. For example, there were hardly any observations in northwest Pennsylvania, which may have affected the results. When splitting the points into high and low elevation, Appendix E shows a large concentration of observations on the coast for low elevation observations; while extending almost the full latitudinal gradient, the observations are all on one side of the geographic range. The methodology attempted to account for these biases by running multiple iterations and randomly sampling the data with replacement, though whether it was sufficient or not is hard to say. Additionally, as observers are also impacted by climatic variables, it's safe to assume that not many observations were taken when the weather was unpleasant, leading to inconsistencies in the dataset. Observers are subject to personal error and differing objectives. Or perhaps an observer may have tried to observe a bee but could not get the necessary support to obtain the "research grade" status.

Another important factor to consider is the distribution of the observations throughout time. Although this study focused on the ∑day variable (the number of days that had passed from January 1st until the date of the observation), observations were taken at different years, which has the ability to affect this variable. As observations span the years 2002-2023, climate change within this window was not taken into account, as it was assumed to be relatively minor. However, this undeniably increases heterogeneity into the dataset, and its effect on the results remains uncertain. It's also important to note that a large majority of the dataset occurred more recently, in the last 10 years (93.12% of *B. bimaculatus* observations, 89.02% of *B. griseocollis* observations, and 94.99% of *B. impatiens* observations). Additionally, the ratio between iNaturalist and GBIF data was not evenly distributed, with iNaturalist observations taking up the bulk of the dataset (84.10% of *B. bimaculatus* observations, 76.52% of *B. griseocollis* observations, and 82.73% of *B. impatiens* observations). Both of these factors may have contributed to unintentional bias within the dataset.

Lastly, an additional source of bias could be related to the time constraints on the analyses. As the initial 100 iterations took upwards of 30 hours to complete just one analysis, it had to be reduced to 25, which may have impacted the accuracy of the results. Additionally, as observation dates likely occur a bit after the actual emergence time of a queen bee, the estimations of GDD and ∑day are likely slight overestimates.

Despite these challenges, this research was conducted with the highest level of academic integrity and precision. By using citizen science data (as other professional researchers do), this study was able to span a large spatial and temporal extent, increasing the sample size. Not only did this improve the accuracy of the results, but it also allowed for a more comprehensive study. Additionally, as bumblebees are a charismatic and keystone species, data was readily available. Using the *phenesse* package (specifically designed for presence only data) eliminated much bias from the results and allowed the study to be more plausible.

CONCLUSION

It was expected that GDD would remain constant across varying climatic conditions, as GDD was predicted to be the driver of queen bee emergences. However, as GDD varied significantly across elevational and latitudinal gradients, temperature was determined to have less influence on queen bumblebee emergence times. Curiously, phenology differences at high elevations and latitudes are not very pronounced, as emergence times only differ by a couple of days between species. This suggests that day accumulation in these environments plays a larger role than temperature in determining emergence times. Future research examining the mechanism that triggers queen bee emergence, the amount of time queen bees overwinter, or the impact on soil degree days on queen bee emergence times would supplement this study.

REFERENCES

Balmaki, B., Rostami, M. A., Christensen, T., Leger, E. A., Allen, J. M., Feldman, C. R., ... & Dyer, L. A. (2022). Modern approaches for leveraging biodiversity collections to understand change in plant-insect interactions. *Frontiers in Ecology and Evolution, 10,* 924941.

Bartomeus, I., Ascher, J. S., Wagner, D., Danforth, B. N., Colla, S., Kornbluth, S., & Winfree, R. (2011). Climate-associated phenological advances in bee pollinators and bee-pollinated plants. *Proceedings of the National Academy of Sciences*, *108*(51), 20645-20649.

Belitz, M. W., Larsen, E. A., Shirey, V., Li, D., & Guralnick, R. P. (2023). Phenological research based on natural history collections: Practical guidelines and a lepidopteran case study. *Functional Ecology*, *37*(2), 234-247. DOI: 10.1111/1365-2435.14173

Belitz, M. W., Barve, V., Doby, J. R., Hantak, M. M., Larsen, E. A., Li, D., ... & Stucky, B. J. (2021). Climate drivers of adult insect activity are conditioned by life history traits. *Ecology Letters*, *24*(12), 2687-2699. DOI: 10.1111/ele.13889

Belitz, M. W., Larsen, E. A., Ries, L., & Guralnick, R. P. (2020). The accuracy of phenology estimators for use with sparsely sampled presence-only observations. *Methods in Ecology and Evolution, 11(10)*, 1273-1285.

Belitz M.W., Campbell CJ, Li D (2020). "phenesse: Estimate Phenological Metrics using Presence-Only Data." https://CRAN.R-project.org/package=phenesse.

Colla, S., Richardson, L., Williams, P. (2011). "Bumble Bees of the Eastern United States" (PDF). The Xerces Society. *The Xerces Society.* Retrieved October 4, 2021.

Daly, C., Halbleib, M., Smith, J. I., Gibson, W. P., Doggett, M. K., Taylor, G. H., ... & Pasteris, P. P. (2008). Physiographically sensitive mapping of climatological temperature and precipitation across the conterminous United States. *International Journal of Climatology: a Journal of the Royal Meteorological Society,* 28(15), 2031-2064.

Damos, P., & Savopoulou-Soultani, M. (2012). Temperature-driven models for insect development and vital thermal requirements. *Psyche: A Journal of Entomology*, 2012, 1-13.

Dorian, N. N., McCarthy, M. W., & Crone, E. E. (2023). Ecological traits explain long-term phenological trends in solitary bees. *Journal of Animal Ecology*, *92*(2), 285-296.

Ellwood, E. R., Diez, J. M., Ibáñez, I., Primack, R. B., Kobori, H., Higuchi, H., & Silander, J. A. (2012). Disentangling the paradox of insect phenology: are temporal trends reflecting the response to warming?. *Oecologia*, *168*, 1161-1171.

GBIF.org (19 October 2023) GBIF Occurrence Download https://doi.org/10.15468/dl.k83wd6

Goulson, D., Lye, G. C., & Darvill, B. (2008). Decline and conservation of bumble bees. *Annu. Rev. Entomol.*, *53*, 191-208.

Hegland, S. J., Nielsen, A., Lázaro, A., Bjerknes, A. L., & Totland, Ø. (2009). How does climate warming affect plant-pollinator interactions?. *Ecology letters*, *12*(2), 184-195.

Hufkens, K., Basler, D., Milliman, T., Melaas, E. K., & Richardson, A. D. (2018). An integrated phenology modeling framework in R. *Methods in Ecology and Evolution*, *9*(5), 1276-1285.

iNaturalist community. Observations of *Bombus* from Virginia, West Virginia, Maryland, Delaware, and Pennsylvania, USA observed on/between March-April. Exported from https://www.inaturalist.org on October 5th, 2023.

Inouye, B. D., Ehrlén, J., & Underwood, N. (2019). Phenology as a process rather than an event: from individual reaction norms to community metrics. *Ecological Monographs*, *89*(2), e01352.

Jones, V. & Brunner, J. (1993). Degree Day Models. Washington State University. Retrieved from https://treefruit.wsu.edu/crop-protection/opm/dd-models/.

Karbassioon, A., Yearlsey, J., Dirilgen, T., Hodge, S., Stout, J. C., & Stanley, D. A. (2023). Responses in honeybee and bumblebee activity to changes in weather conditions. *Oecologia*, 201(3), 689-701.

Keaveny, E. C., Waybright, S. A., Rusch, T. W., & Dillon, M. E. (2022). Supercooling points of freeze-avoiding bumble bees vary with caste and queen life stage. *Journal of Thermal Biology*, 104, 103196.

Kehrberger, S., & Holzschuh, A. (2019). Warmer temperatures advance flowering in a spring plant more strongly than emergence of two solitary spring bee species. *PLoS One*, *14*(6), e0218824.

Kerr, J. T., Pindar, A., Galpern, P., Packer, L., Potts, S. G., Roberts, S. M., ... & Pantoja, A. (2015). Climate change impacts on bumblebees converge across continents. *Science*, 349(6244), 177-180.

Kuo, Y., Lu, Y. H., Lin, Y. H., Lin, Y. C., & Wu, Y. L. (2023). Elevated temperature affects energy metabolism and behavior of bumblebees. *Insect Biochemistry and Molecular Biology*, 155, 103932.

Maebe, K., Hart, A. F., Marshall, L., Vandamme, P., Vereecken, N. J., Michez, D., & Smagghe, G. (2021). Bumblebee resilience to climate change, through plastic and adaptive responses. *Global change biology*, *27*(18), 4223-4237.

Marshall, L., Biesmeijer, J. C., Rasmont, P., Vereecken, N. J., Dvorak, L., Fitzpatrick, U., ... & Dendoncker, N. (2018). The interplay of climate and land use change affects the distribution of EU bumblebees. *Global Change Biology*, 24(1), 101-116.

MARISA (Mid-Atlantic Regional Integrated Sciences and Assessments). (2021). Average Annual Number of Growing Degree Days from Historic Observations, *NOAA.* https://www.midatlanticrisa.org/data-tools/climate-data-tools/growing-degree-days-historic.html, data accessed 22 March 2024.

MARISA (Mid-Atlantic Regional Integrated Sciences and Assessments). (2021). Average Annual Number of Growing Degree Days from Global Climate Model Data, *NOAA.* https://www.midatlanticrisa.org/data-tools/climate-data-tools/growing-degree-days-future.html, data accessed 22 March 2024.

Miller-Struttmann, N., Miller, Z., & Galen, C. (2022). Climate driven disruption of transitional alpine bumble bee communities. *Global Change Biology,* 28(21), 6165-6179.

McMaster, G. S., & Wilhelm, W. W. (1997). Growing degree-days: one equation, two interpretations. *Agricultural and forest meteorology,* 87(4), 291-300.

Nayak, R. K., Rana, K., Bairwa, V. K., Singh, P., & Bharthi, V. D. (2020). A review on the role of bumblebee pollination in fruits and vegetables. *Journal of Pharmacognosy and Phytochemistry*, 9(3), 1328-1334.

Oyen, K. J., & Dillon, M. E. (2018). Critical thermal limits of bumblebees (Bombus impatiens) are marked by stereotypical behaviors and are unchanged by acclimation, age or feeding status. *Journal of Experimental Biology, 221(8),* jeb165589.

Pawlikowski, T., Sparks, T. H., Olszewski, P., Pawlikowski, K., Rutkowski, L., & Jakubowski, R. (2020). Rising temperatures advance the main flight period of Bombus bumblebees in agricultural landscapes of the Central European Plain. *Apidologie*, 51(4), 652-663.

PRISM Climate Group, Oregon State University, https://prism.oregonstate.edu, data created 4 Feb 2014, accessed 16 Dec 2020.

Pyke, G. H., Thomson, J. D., Inouye, D. W., & Miller, T. J. (2016). Effects of climate change on phenologies and distributions of bumble bees and the plants they visit. *Ecosphere, 7*(3), e01267.

Renner, I. W., Elith, J., Baddeley, A., Fithian, W., Hastie, T., Phillips, S. J., ... & Warton, D. I. (2015). Point process models for presence-only analysis. *Methods in Ecology and Evolution, 6*(4), 366-379.

Roulston, T'ai. Field Studies. Blandy Experimental Farm, UVA.

Sanderson, R. A., Goffe, L. A., & Leifert, C. (2015). Time-series models to quantify short-term effects of meteorological conditions on bumblebee forager activity in agricultural landscapes. *Agricultural and Forest Entomology, 17(3)*, 270-276.

Sridhar, V., & Reddy, P. V. R. (2013). Use of degree days and plant phenology: a reliable tool for predicting insect pest activity under climate change conditions. In Climate-resilient horticulture: Adaptation and mitigation strategies (pp. 287-294). India: Springer India.

Stemkovski, M., Pearse, W. D., Griffin, S. R., Pardee, G. L., Gibbs, J., Griswold, T., ... & Irwin, R. E. (2020). Bee phenology is predicted by climatic variation and functional traits. *Ecology Letters*, 23(11), 1589-1598.

Quinlan, G. M., Feuerborn, C., Hines, H. M., & Grozinger, C. M. (2023). Beat the heat: thermal respites and access to food associated with increased bumble bee heat tolerance. *Journal of Experimental Biology*, 226(17), jeb245924.

APPENDIX

Appendix A: Table of the 10th percentile estimates for GDD, ∑ppt, and ∑day at a 95% confidence interval for each species across all elevations and latitudes.

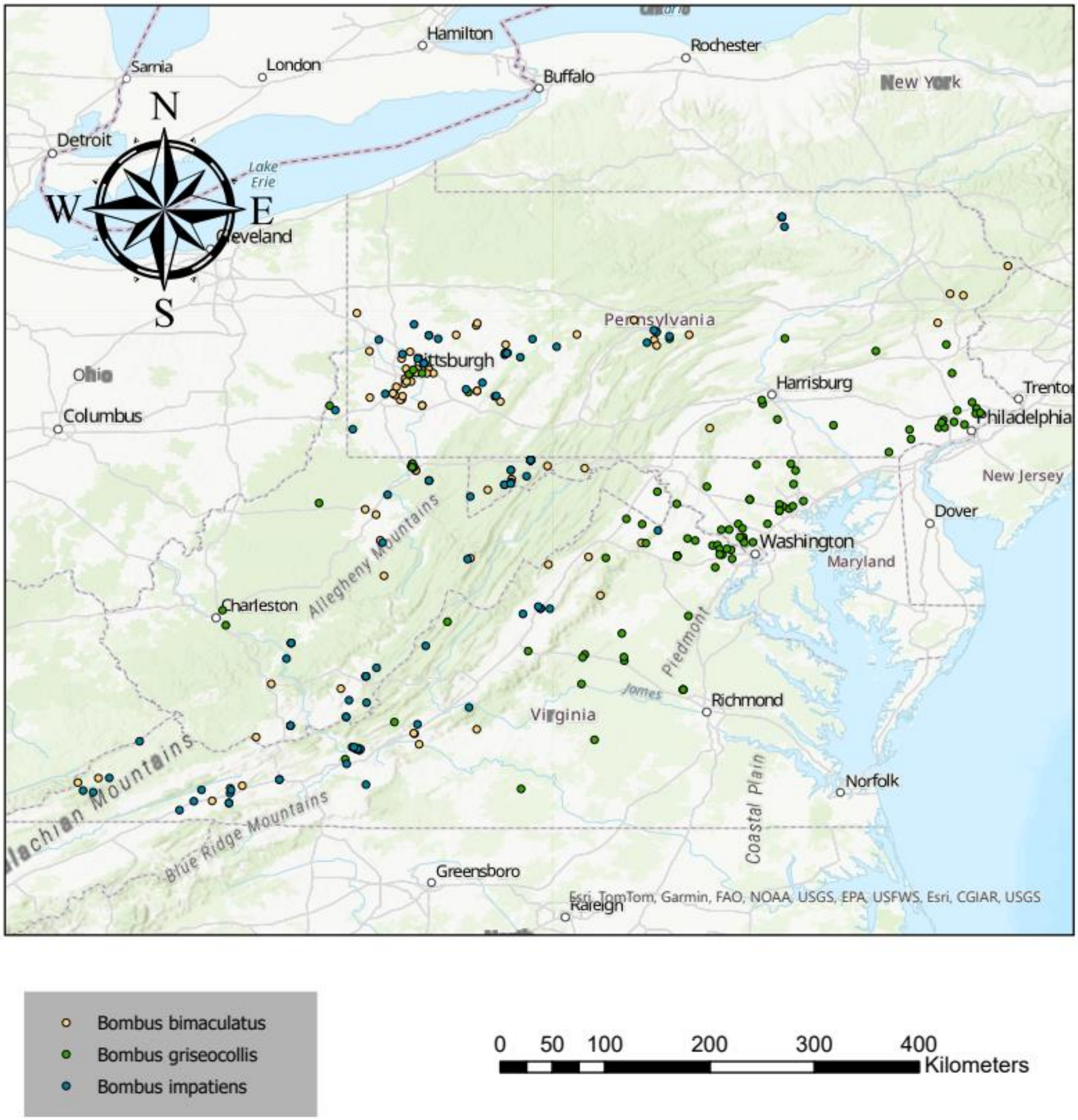
	B. bimaculatus	*B. griseocollis*	*B. impatiens*
GDD (days)	164.1554	199.1916	142.6865
Low confidence interval	153.4666	182.0855	131.1762
High confidence interval	173.7045	221.3157	157.2267
∑ppt (mm)	194.0294	212.5128	196.2503
Low confidence interval	187.5793	199.2048	188.672
High confidence interval	200.2988	222.3053	205.1232
∑day	87.71646	94.82543	92.53571
Low confidence interval	86.1187	92.76699	90.66567
High confidence interval	89.07981	97.10491	94.16812

Appendix B: Table of the 10th percentile estimates for GDD and ∑day at a 95% confidence interval for each species at high and low elevation ranges.

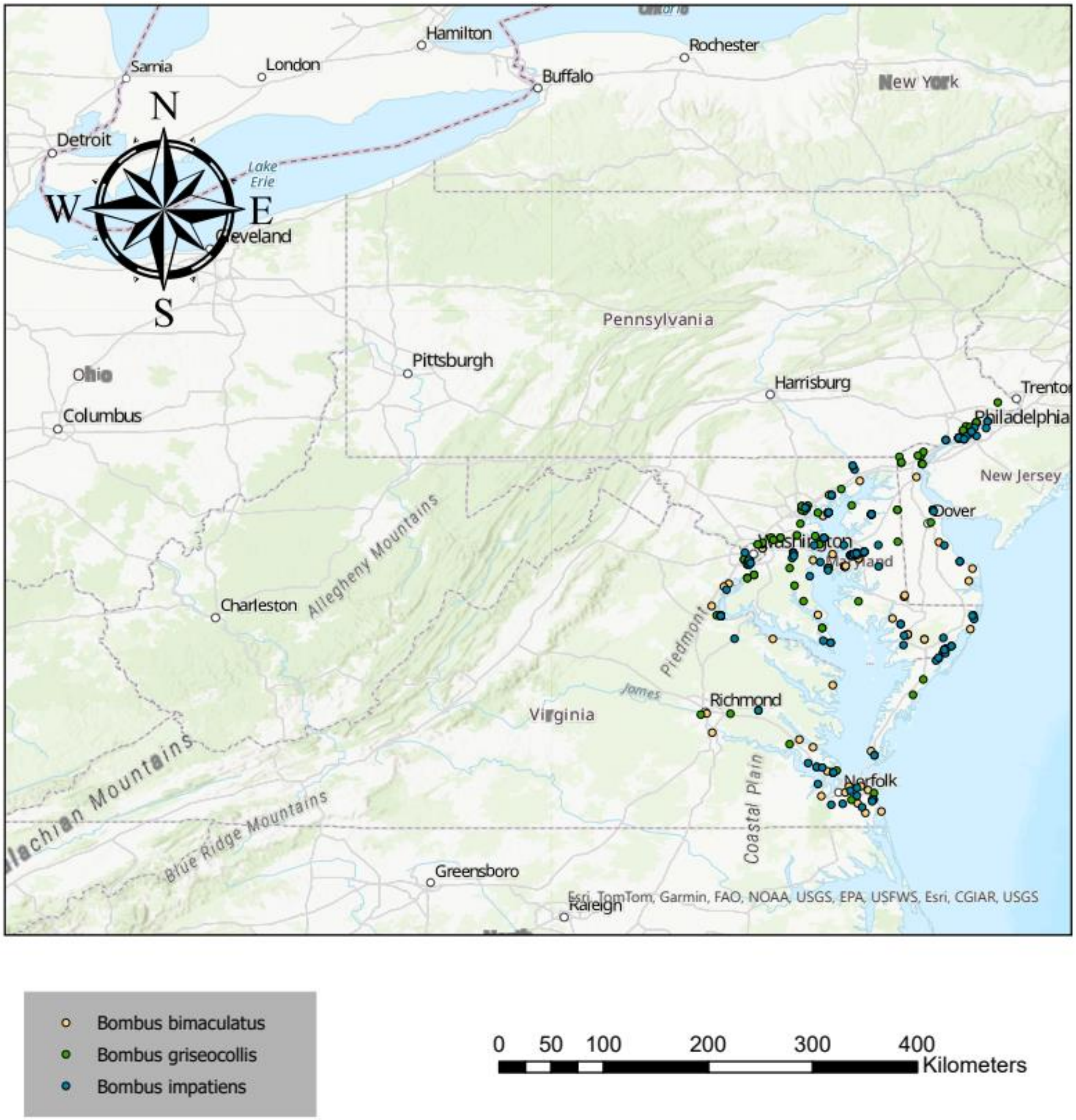
		B. bimaculatus	*B. griseocollis*	*B. impatiens*
GDD	Low elevations	209.127	208.1409	*164.5073*
	Low confidence interval	182.8072	179.6795	133.2312
	High confidence interval	243.9211	243.6296	200.0405
	High elevations	149.9673	185.7798	*104.8676*
	Low confidence interval	132.7204	157.8529	91.22399
	High confidence interval	173.2256	211.9742	122.1987
∑day	Low elevations	88.69827	93.68974	*87.93089*
	Low confidence interval	85.98963	88.85495	83.63153
	High confidence interval	93.21745	97.08884	93.54455
	High elevations	98.12851	97.30212	*96.06624*
	Low confidence interval	94.87101	93.45352	92.90968
	High confidence interval	101.3413	100.1102	99.27751
Elevation ranges (m)	Low elevations lower bound	-0.600000024	-0.5930	-0.7490
	Low elevations upper bound	5.8256187	37.5125	10.1269
	High elevations lower bound	283.5260522	81.7268	361.0634
	High elevations upper bound	941.9396182	739.4696	856.9349

Appendix C: Table of the 10th percentile estimates for GDD and ∑day at a 95% confidence interval for each species at high and low latitudes.

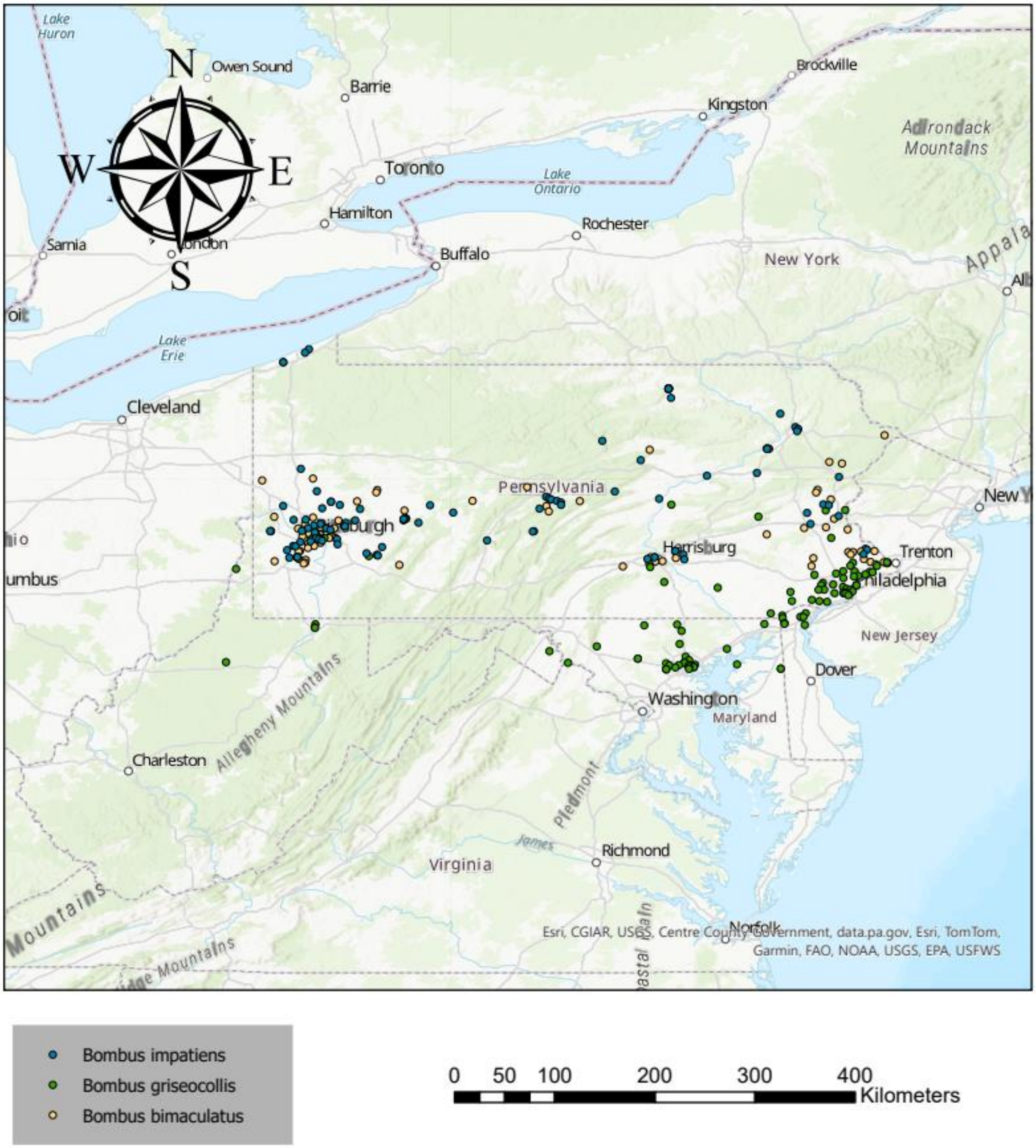
		B. bimaculatus	*B. griseocollis*	*G. impatiens*
GDD	Low latitudes	213.4749	238.1061	220.4954
	Low confidence interval	181.5946	214.2847	191.0432
	High confidence interval	242.8334	274.2964	250.6205
	High latitudes	155.8141	184.9444	123.6322
	Low confidence interval	135.3451	156.9333	106.0799
	High confidence interval	173.9335	215.1187	141.8712
∑day	Low latitudes	80.1395	92.52928	89.56875
	Low confidence interval	74.77131	90.04596	85.93245
	High confidence interval	84.32757	96.72432	93.04042
	High latitudes	98.86223	98.25564	95.98346
	Low confidence interval	94.9104	95.36361	91.80174
	High confidence interval	102.7416	100.8022	99.9454
Latitude Ranges (decimal degrees)	Low latitudes lower bound	36.6072	36.7611	36.6996
	Low latitudes upper bound	37.7411	38.8916	38.3376
	High latitudes lower bound	40.1899	39.2677	40.2541
	High latitudes upper bound	42.0612	40.8001	42.2109



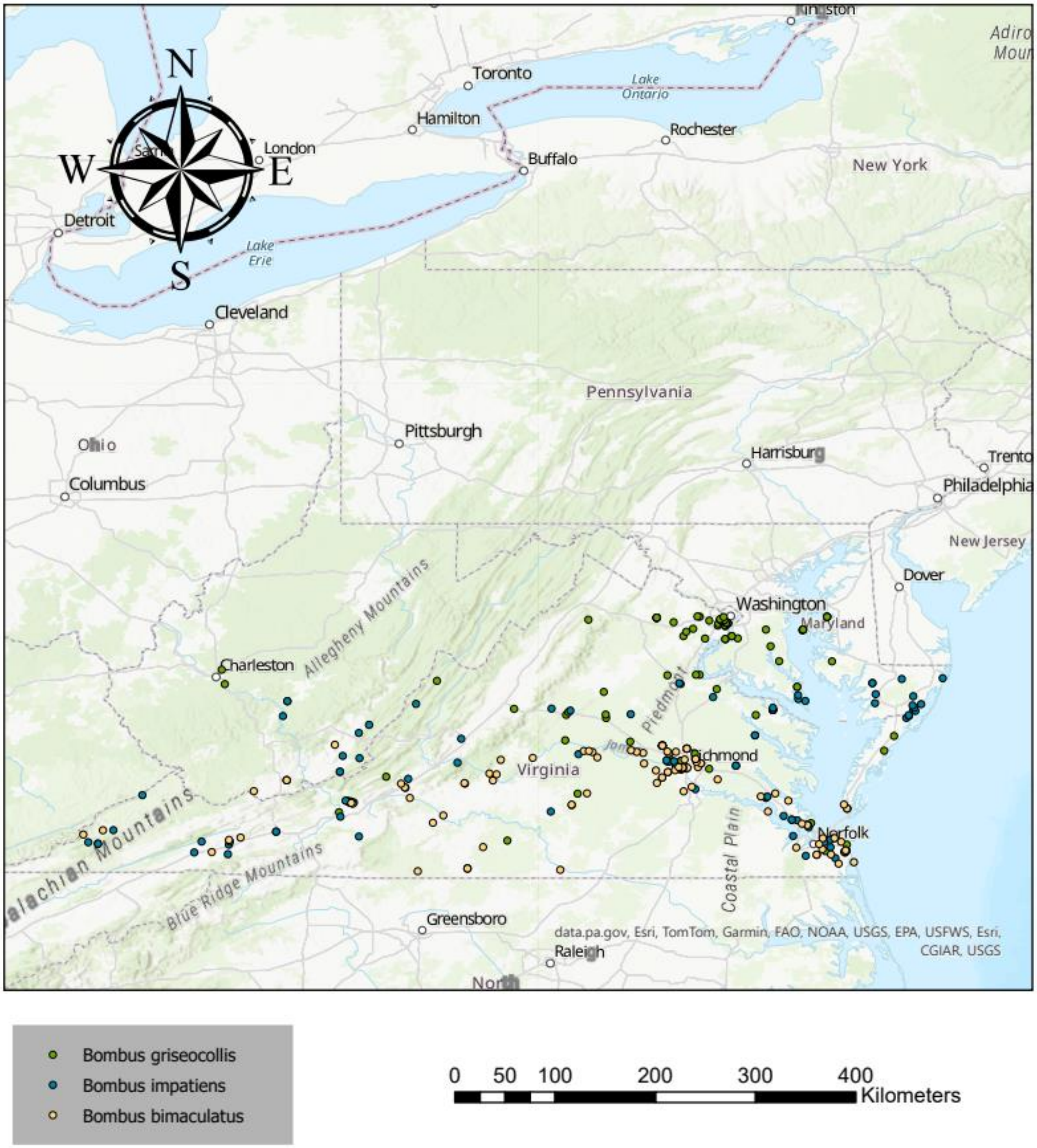
Appendix D: Species distribution of the highest 100 elevations for each *Bombus* species within the spatial resolution of this study. Point data was generated in ArcGIS Pro 3.1.4 and pulled from iNaturalist and GBIF. *B. impatiens* observations are in blue, *B. griseocollis* observations are in green, and *B. bimaculatus* observations are in yellow.



Appendix E: Species distribution of the lowest 100 elevations for each *Bombus* species within the spatial resolution of this study. Point data was generated in ArcGIS Pro 3.1.4 and pulled from iNaturalist and GBIF. *B. impatiens* observations are in blue, *B. griseocollis* observations are in green, and *B. bimaculatus* observations are in yellow.



Appendix F: Species distribution of the highest 100 latitudes for each *Bombus* species within the spatial resolution of this study. Point data was generated in ArcGIS Pro 3.1.4 and pulled from iNaturalist and GBIF. *B. impatiens* observations are in blue, *B. griseocollis* observations are in green, and *B. bimaculatus* observations are in yellow.



Appendix G: Species distribution of the lowest 100 latitudes for each *Bombus* species within the spatial resolution of this study. Point data was generated in ArcGIS Pro 3.1.4 and pulled from iNaturalist and GBIF. *B. impatiens* observations are in blue, *B. griseocollis* observations are in green, and *B. bimaculatus* observations are in yellow.