# BUMBLE BEE NICHE OVERLAP ALONG AN ELEVATION GRADIENT: HOW TRAITS CAN INFORM NOVEL COMPETITIVE PRESSURES UNDER CLIMATE CHANGE

by

KAITLYN MARIE BARTHELL

B.A., University of Colorado, 2021

A thesis submitted to the
Faculty of the Graduate School of the
University of Colorado in partial fulfillment
of the requirement for the degree of
Master of Arts
Department of Ecology and Evolutionary Biology
2023

Committee Members:
Dr. Julian Resasco
Dr. Deane Bowers
Dr. Adrian Carper

Barthell, Kaitlyn Marie (M.A., Ecology and Evolutionary Biology)
Bumble Bee Niche Overlap Along an Elevation Gradient: How Traits
Can Inform Novel Competitive Pressures Under Climate Change
Thesis directed by Assistant Professor Dr. Julian Resasco

# ABSTRACT

Climate change-induced range shifts can disrupt interactions among species by moving them in and out of ecological communities. These disruptions can include impacts on competition for shared resources. Bumble bees (*Bombus spp.)* are important pollinators shifting their range upwards in elevation in response to climate change. These shifts could lead to altered competition among species and threaten co-existence. These impacts are particularly worrying at the tops of mountains where bumble bees are "trapped" and can no longer move up to higher elevations to track climate change. To better understand this issue, we investigated changes in diet niche overlap among bumble bee species along a 2296m elevation gradient in the southern Rocky Mountains. Additionally, we investigated how morphological and phenological traits impact diet composition (flower species visited) among bumble bee species and explored how the continued upward movement of bumble bee species under climate change into the mountaintop may affect trait overlap of newly co-occurring species. We found that diet niche overlap among bumble bee species increased with elevation. We also found that differences in morphological and phenological traits (body size, tongue length, date of activity) were correlated with differences in diet composition among bumble bee species. Finally, we described how the movement of bumble bee species from lower elevations into the mountain top would lead to increased trait overlap and likely more species sharing similar flowers. These shifts could have implications for future competition for resident high-elevation species on mountaintops and exacerbate the effects of climate change on high-elevation bumble bees.

# ACKNOWLEDGEMENTS

It has taken a village of supportive scientists, friends, and family members to complete this research. Foremost, I would like to thank my advisor, Dr. Julian Resasco. I have grown tremendously as a scientist over the last three years thanks to his guidance. Thank you to Andrew Gaier, Anna Paraskevopoulos, Asia Kaiser, Jess Mullins, Virginia Scott, Isabella Manning, Nicole Perez, and Chloe Sommer for their field and laboratory work contributions. I'd also like to thank my committee members, Dr. Deane Bowers and Dr. Adrian Carper for their wisdom and intellectual guidance of this research. Thank you to members of the Resasco and Bowers lab for their thoughtful contributions to my learning and for their camaraderie in the field and in the lab.

Without financial support and access to field sites, this research would not have been possible. Thank you to the National Science Foundation (Award: NSF-DEB 2102974), the National Geographic Society, and CanBee Cocktails for their financial support. Thank you to the United States Department of Agriculture Forest Service, The Pikes Peak Ranger District, and the City of Manitou Springs for fieldwork permissions.

I extend my gratitude to my friends, as well as to my teammates, coaches, and staff on the University of Colorado cross country and track program for being a valuable support network. Finally, thank you to my parents Denise and John Barthell and my partner Austin Miller for their encouragement and love as I have pursued my interests in ecology.

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# INTRODUCTION

Climate change is causing many species to move their ranges upwards in elevation and towards the poles to track changing environmental conditions (Hughes 2000; Walther et al. 2002). Range shifts can modify community structure and disrupt ecosystems through the turnover of species in and out of ecological communities (Wardle et al. 2011). The rearrangement of competing species in communities may threaten coexistence, considering the ability of species competing for a shared resource to co-exist is facilitated by each competitor having distinct patterns of resource use, or a distinct "niche" (Chesson 2000). To fully account for the effects of climate change on species, it is important to study how biotic interactions like competition may change in addition to the impacts of abiotic factors.

Insects are critical to the pollination of over 85% of flowering plant species (Ollerton et al. 2011). Insect pollinator communities may be especially vulnerable to species' range shifts that may occur under climate change, considering their strong dependence on host flower species for food (Michener 2007) and their high sensitivity to changing environmental variables (Kingsolver 2013). Range-shift caused spatial mismatches between pollinators and their host plants threaten pollination success (Gomez-Ruiz and Lacher 2019) and affect competition for both groups (Richman et al. 2020). Understanding how pollinator species partition their diet and how range changes may impact these interactions is important for understanding the impacts of climate change on plant-pollinator communities.

Mountain elevation gradients provide an opportunity to study how plant-pollinator communities change with elevation and how range shifts under climate change may alter competitive interactions. Mountains are climatically heterogeneous, making them hotspots for biodiversity and 'natural experiments' for understanding ecological responses to climatic change

(Körner 2007). As elevation changes, different factors such as temperature, precipitation, productivity, area, and species interactions also change, supporting different numbers of species (McCain and Grytnes 2010). For many plant and pollinator taxa, species richness tends to peak at middle elevations along mountain ranges and decline into higher elevations (Wohlgemuth et al. 2008; Gallou et al. 2017; Chesshire et al. 2021; Sponsler et al. 2022b). As the richness of host flowers and their associated pollinators change, the spectrum of available resources and the number of species competing for those resources also change, potentially altering the competitive pressures within a community (Doublet et al. 2022). As plant and pollinator species move in and out of communities to track climate change, competitive pressures will likely be affected. This is particularly worrying at the tops of mountains, where species can no longer move upwards in elevation in response to climate change.

Bumble bees (*Bombus spp*.) are shifting their ranges in response to climate change (Kerr et al. 2015; Marshall et al. 2020). Bumble bees are essential pollinators of native plants in mountain ecosystems (Bingham and Orthner 1998; Gorenflo et al. 2017; Minachilis et al. 2021); however, studies have documented dramatic bumble bee declines across the Northern Hemisphere (Kosior et al. 2007; Cameron et al. 2011). Climate change is a distinct driver of these losses, and its effects are independent of other important drivers such as land-use change and pesticide use (Kerr et al. 2015; Soroye et al. 2020). In response to climate change, bumble bees are moving upwards in elevation to remain within a habitable range of environmental conditions (Kerr et al. 2015). However, at mountaintops, bumble bee species can no longer move upward to track suitable climate. The upward movement of colonizing species into the same environment as resident species at mountaintops will likely cause novel competitive interactions for shared floral resources that could exacerbate the effects of changing climate. Accounting for these novel competitive interactions is important for fully understanding how species will respond to climate change (Alexander et al. 2015*)*.

Competition among co-occurring bumble bee species is heavily influenced by their dependence on the flowers that they visit for pollen and nectar food resources (Heinrich 1976). Exploitative competition, wherein species compete indirectly for a shared resource, is the primary mechanism for resource partitioning among bumble bee species (Inouye 1978). Since closely related species, like bumble bees in the genus *Bombus*, are similar in life history and morphology, they are likely to compete more strongly with each other for limited resources than more distantly related taxonomic groups (Burns and Strauss 2011). Co-existence among bumble bee species may be facilitated by traits that allow for niche partitioning of floral resources. For example, morphological trait variation among species, such as body size and tongue length, also may influence diet niche partitioning. Past research has found that bumble bee morphological traits influence which flowers species visit (Harder 1985; Sponsler et al. 2022a). Longer-tongued bumble bees, for example, are more likely to visit flowers that are more closed in shape (lip, funnel, and flag-shaped flowers) than their shorter-tongued counterparts that prefer more open-shaped flowers (disc, stalk-disc, bell, head-shaped flowers; Sponsler et al. 2022a). This difference in visitation may be attributed to species visiting flowers that are most energetically efficient for their morphology (Balfour et al. 2021). Phenology, or bumble bee activity, differs among species during the season and can also be a mechanism for diet niche partitioning since bumble bees can only access flowers that are open when they are actively foraging. Therefore, variation in morphology and phenology may be important indicators of variation in diet composition and niche partitioning among bumble bee species.

Past research on bumble bee competitive interactions along elevation gradients has shed light on the patterns and mechanisms underlying niche overlap. Using historical data collected between 1966 and 1969 by Macior (1974) in the Colorado Rocky Mountains, Miller-Struttmann and Galen (2014) found high niche overlap in the lowest and highest elevation zones of their study, with niche overlap peaks attributed to disturbance in the lowest zone (1,600-2,700m) and a shortened flowering window in the highest zone (3,500-4,300m). Additionally, they found that long-tongued bumble bees altered their foraging behavior in the alpine by becoming more generalized, suggesting that tongue length plays a role in determining what flowers species visit at different elevations (Miller-Struttmann and Galen 2014). Another study in the Rocky Mountains found that bumble bee species that have recently colonized alpine environments are likely to take better advantage of foraging early and late in the season, potentially making them more likely to outcompete resident species in a warming alpine environment (Miller-Struttmann et al. 2022). Still, patterns of niche overlap along elevation gradients, traits' influence in determining diet, and how climate change may impact competition among mountain bumble bees are poorly understood.

In this study, we investigated bumble bee distributions and host floral diet niche overlap along the elevation gradient of Pikes Peak in the Colorado Front Range. Further, we examined how bumble bee traits relate to host flower diet composition to better understand niche overlap and how it might change with elevational range shifts under climate change. We asked the questions: **Q1:** How does bumble bee diet niche overlap change along the elevation gradient?; **Q2:** Is variation in phenology, body size, and tongue length correlated with variation in bumble bee diet composition?; and **Q3:** How might the upward movement of bumble bee species impact phenological and morphological trait overlap at the top of the mountain? For Q1, we predicted that bumble bee diet niche overlap would increase with elevation, considering high-elevation mountain environments have short flowering seasons and lower plant species diversity (McCain and Grytnes 2010; Stephens et al. 2022). For Q2, we predicted that variation in phenology, body size, and tongue length values would positively correlate with variation in diet composition for species, considering that bumble bees should make foraging choices that are most efficient for their morphology (Balfour et al. 2021) and should only be able to visit flowers that are open during their foraging period. For Q3, we predicted that the simulated upward movement of bumble bee species into the mountaintop would create increased overlap in phenology, body size, and tongue length trait space for resident high-elevation bumble bee species.

To test our questions, we conducted a four-year survey of bumble bee and host flower interactions along a 2295m elevation gradient on Pikes Peak (Figure 1A), a mountain in the southern Front Range of the Rocky Mountains in Colorado, USA. We divided the elevation gradient into five zones of equal elevational length (Figure 1B). We estimated bumble bee and host flower species richness and diet niche overlap within each elevation zone. Next, we assessed if phenology, body size, and tongue length are related to diet composition in bumble bees by testing for correlations between variation in diet composition and trait values among bumble bee species. Finally, as a thought experiment, we explored how trait space overlap may change in the mountaintop if species are to continue moving upwards in elevation under climate change by simulating the upward movement of bumble bee species.

## METHODS

### Study area

The Front Range of Colorado, USA, in the southern Rocky Mountains, is a hotspot for bumble bee diversity; half of the United States' 48 bumble bee species reside in Colorado, most of which occupy counties within or bordering the Front Range (Wright et al. 2017). The Front Range's natural transition from low-elevation short-grass prairie, that begins around 1000m, to high-elevation mountain environments, that peak around 4300m, encompasses suitable habitat for many bumble bee species. The high species richness of the Front Range is useful for understanding bumble bee community patterns in niche partitioning along an elevation gradient as well as making predictions about the consequences of the upward movement of bumble bee species.

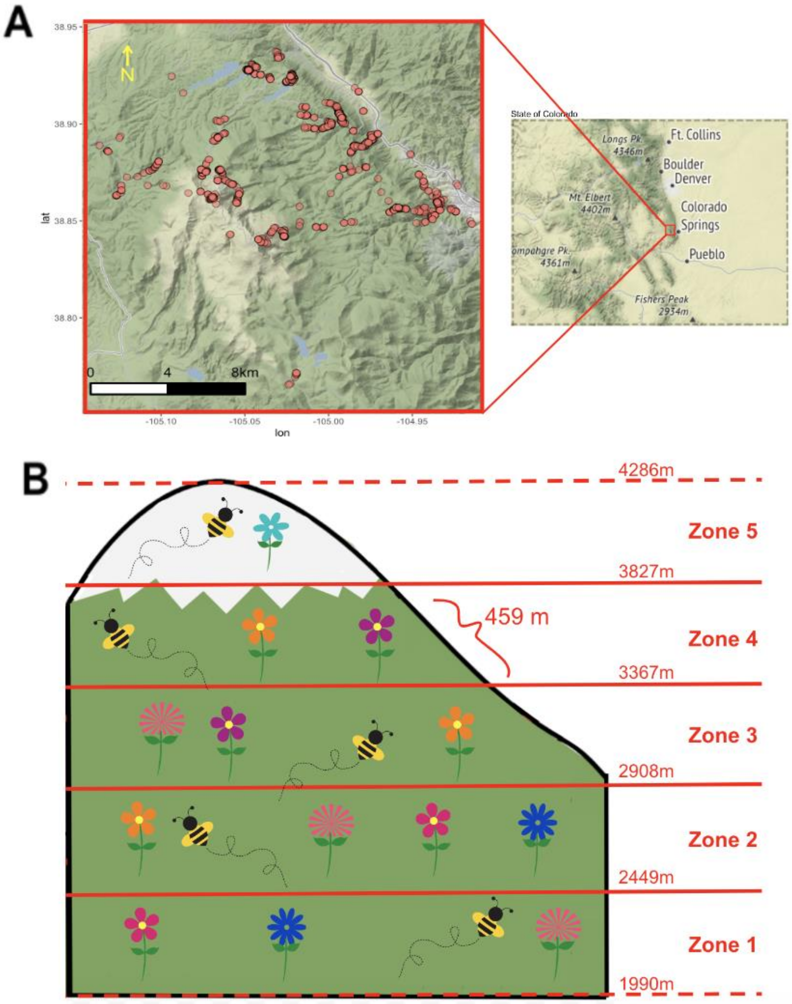

**Figure 1: A)** Map of bumble bee and host flower observations surrounding Pikes Peak (2019-2022). Each point represents a GPS location used to observe bumble bee and host flower interactions. **B)** Figure of the five elevational zones used in this study. Each zone encompassed 459m of the elevational landscape

We carried out our study between May and September for four years (2019-2022) on Pikes Peak, the highest mountain (4302m) in the southern Front Range of Colorado (38° 50' 15.59" N, -105° 02' 23.40 W"). Observations were made along an elevation gradient ranging from 1990m to 4286m on Pikes Peak (Figure 1A). The United States Department of Agriculture Forest Service manages most of the study area. Vegetation varies considerably along the elevation gradient: at the base of the mountain in the foothills (1981m-2438m), Gambel oak shrubs (*Quercus gambelii*) dominate dry, rocky slopes; as elevation increases, ponderosa pine (*Pinus ponderosa*) and aspen (*Populus spp*.) begin to dominate in the montane (2438m-3048m); in the subalpine (3048m-3474m), Engelmann spruce (*Picea engelmannii*) and limber pine (*Pinus flexilis*) predominate; finally, in the alpine (3475-4302m), trees disappear and are replaced by tundra vegetation such as sedges and mat-forming plants (Kelso 2012). High plant species turnover provides a range of floral resources that bumble bees can use along the elevation gradient.

## Study design

Bumble bee and host flower interaction data were used to characterize bumble bee niche overlap along the elevational gradient. The bumble bee interaction data used in this study were a subset of those from a concurrent study on plant-pollinator interactions on Pikes Peak (Resasco 2021). Because of accessibility, we recorded interactions along hiking trails and roadsides that spanned the elevation extent and observed one to several flowers of a single species for at least 1-minute increments. The bumble bee had to be observed touching the flower's stamen or pistil for an interaction to be recorded. Bumble bees found interacting with flowers in the field were usually collected via aspirator or net, frozen, and mounted for later identification. Individuals that could confidently be identified on sight, including the western bumble bee *(Bombus* *occidentalis*), which is declining in western North America, were released. All host flowers observed were identified to species using Ackerfield (2015). Each collected interaction data point had an associated date, WGS84 GPS coordinates, and elevation recorded. We sampled nearly every weekday during the span of the flowering period between late May and mid-August from 2019-2022. Two additional sampling days occurred in September of 2022 to include late-season plant-pollinator interaction data. Observation effort varied by year and elevation (Appendix Figure 1A & B, Appendix Table 1 and 2 for details). Some flower species were observed for long periods of 1-minute increments as "rate observations" for the concurrent study at Pikes Peak, which may have overrepresented some host flower and bumble bee interactions. All collected bumble bees were identified to species and caste in the lab using a Leica Stereozoom S9i microscope, taxonomic keys, and references (Byron 1980; Williams et al. 2014; Wright et al. 2017). In addition to our collected data, we also obtained tongue length (mm) and body size (mm) data from Colorado-based studies and guidebooks (Macior 1974; Wright et al. 2017) for our trait analyses. The tongue trait data were not available for all species and castes. Specifically, *Bombus balteatus*, *B. fervidus*, *B. fraternus*, *B. griseocollis*, *B. huntii*, *B. insularis*, *B. pensylvanicus*, and *B. rufocinctus*, as well as all male tongue length values were excluded from tongue trait analyses due to lack of data in Macior (1974).

We divided observations along the elevation gradient into five zones of equal elevational length (459m), taken from the bumble bee and flower interaction observation at the lowest observation (1990m) to the observation at the highest observation (4286m; Figure 1B). Zone 1 was our lowest zone in the elevation gradient (1990m to 2449m), followed by zone 2 (2449m-2908m), zone 3 (2908m-3367m), zone 4 (3367m-3827m), and zone 5 (3827m-4286m). We pooled data collected from all four years for our analyses since our dataset is too limited to examine temporal patterns. Therefore, generalized results across all years helped us better understand the overall trends among elevation zones.

### Statistical analysis

We conducted all analyses in R version 4.0.3 (R Core Team 2020). We checked the data for GPS errors by mapping data points using the “ggmap” package version 3.0.0 (Kahle and Wickham 2013) and checking elevation correspondence with latitude and longitude using the “elevatr” package version 0.4.2 (Hollister et al. 2021). Bumble bee observations were dropped from the dataset if their GPS points were incorrect (seven points), if their elevations appeared as outliers (>300m different from the elevation points within the USGS Elevation Point Query Service; four points), or if we were unable to identify the host flower species (two points). We used the packages “bipartite” version 2.16, “ggridges” version 0.5.4, and “ggplot2” version 3.4.1 for data visualization (Dormann et al. 2009; Wilke 2022; Wickham 2016).

### Assessing elevational patterns of species richness and sampling completeness

To help inform our understanding of diet niche partitioning among bumble bee species, we calculated the richness of host flower species and bumble bee species within each elevational zone. Host flower and bumble bee species richness estimates give us insight into the breadth of available flower species and the number of bumble bees competing for them. Species richness for host flowers and bumble bees was estimated for each of the five elevational zones using rarefaction from the “iNEXT” package version 3.0.0 (Hsieh et al. 2022). To account for uneven sampling within each zone, we compared rarefied species richness estimates and their confidence intervals at n=1,937 observations, the final observation of the maximum sampled zone (zone 2). All other zone species richness estimates were extrapolated to this point. To assess the thoroughness of sampling at each elevation zone, we estimated sample completeness for bumble bee and host flower species visited and their unique interactions using the “iNEXT” package (Hsieh et al. 2022).

### Q1: How does bumble bee diet niche overlap change along the elevation gradient?

We used Horn’s index (Horn 1966) to calculate niche overlap in diet composition among bumble bee species, where 0 indicates no niche overlap and 1 indicates perfect niche overlap. We estimated niche overlap for bumble bees across the elevation gradient using non-parametric bootstraps that sampled without replacement using the “bootstrapnet” package version 1.0.0 (Stefan and Knight 2023). This approach allowed us to account for uneven sampling across the elevation gradient by comparing niche overlap estimates at equivalent observations and estimating uncertainty within each elevational zone. We chose to bootstrap without replacement to avoid the potential bias of overrepresenting common interactions between bumble bees and host flowers. In our bootstrap, we estimated niche overlap at a starting interval of N=50 observations (approximately 10% of 491 unique flower and bumble bee interactions) and continued at n=1 intervals until no more samples were left in each zone. This sampling procedure was replicated 1000 times. The mean niche overlap values for each zone were compared at n=117 observations. We chose n=117 since our least sampled zone (zone 5) had 118 interactions, and sampling below this value gave us confidence intervals for all zones. To test whether niche overlap increased with elevation (Q1), we ran a linear regression on each elevation zone's mean niche overlap estimates. To visualize differences in host flower diet composition among bumble bee species, we used nonmetric multidimensional scaling (NMDS) of diet compositions of bumble bees across all elevation zones, as well as within each zone using the “vegan” package version 2.5.7 (Oksanen et al. 2020).

### Q2: Is variation in phenology, body size, and tongue length correlated with variation in bumble bee diet composition?

We used Mantel tests to examine the relationship between species traits (phenology, body size, and tongue length) and diet composition (plant species visited and frequency of these interactions) among all bumble bee species. We created a matrix of Bray-Curtis dissimilarity values for the frequency and composition of diet, as well as matrices of Euclidean distances of trait values (phenology, body size, tongue length) between each pairwise combination of bumble bee species. Bray-Curtis dissimilarity values, which range from 0 (complete similarity) to 1 (complete dissimilarity), were calculated using the “vegan” package (Oksanen et al. 2020). Euclidean distance dissimilarity values were calculated using the “stats” package version 4.0.3 (R Core Team 2020). In total, four matrices were created. We tested for correlation between the diet dissimilarity matrix and each trait matrix using Mantel tests from the “vegan” package (Oksanen et al. 2020). Mantel tests determine correlation by comparing observed matrices to a null model, where the matrix values are shuffled at random and tested for significance based on these permutations (Oksanen et al. 2020). The resulting Mantel test statistic value varies between -1 and 1, with -1 indicating perfect negative correlation and 1 indicating perfect positive correlation. To investigate whether body size variation correlates with tongue length variation, we ran Pearson's correlation on available female bumble bee tongue length and corresponding body size values in base R. Pearson’s correlation coefficient varies between -1 and 1, with -1 indicating perfect negative correlation and 1 indicating perfect positive correlation. Our tongue length Mantel test and our Pearson’s correlation test were limited to only female bumble bees and a portion of the bumble bee species observed in the study due to our data limitations. Finally, to visualize diet composition in relation to trait variables, we created NMDS plots of species diet composition pooled across all elevation zones and overlaid with the average trait values for each species in multidimensional space using the “vegan” package (Oksanen et al. 2020).

### Q3: How might the upward movement of bumble bee species impact phenological and morphological trait overlap at the top of the mountain?

To explore the effects that the continued upward movement of bumble bee species may have on the trait overlap of high-elevation mountaintop bumble bee species, we simulated the colonization of bumble bees currently found in zone 4 into zone 5 and examined phenological and morphological trait overlap between current resident and simulated colonizing bumble bee species. To do this, we added 459m to the elevation data for bumble bees found in zone 4, which underlies the highest elevation zone, zone 5. Next, to identify colonizing and resident mountaintop species that may have high trait similarity under simulated climate change, we calculated the percent overlap of phenology, body size, and tongue length ranges between each unique pairing of resident and colonizing bumble bee species, as well as species that we found restricted at high elevations (*Bombus balteatus, B. frigidus, and B. sylvicola*) and colonizing bumble bee species. Percent trait overlap was calculated by dividing the shared trait space by the total trait space for both species. We highlighted bumble bee pairings with high trait overlap (>75%) as more likely to have high competition for floral resources under climate change.

In this exercise, we assumed that bumble bees would move upwards but not their plant resources. This assumption was made on the basis that bumble bees will likely be able to respond to warming temperatures with range shifts more quickly than plants (Pyke et al. 2016). Therefore, plants were not included in this exploratory model. To visualize changes in the mountaintop’s bumble bee community trait overlap under this simulation, line segment graphs representing the range of occupied trait space between each pairing of resident and colonizing bumble bee species were plotted using the “ggplot” package (Wickham 2016).

# RESULTS

We observed 19 out of Colorado's 24 bumble bee species interacting with 148 host flowering plants, totaling in 491 unique bumble bee and host flower interactions (Appendix Figure 2). We observed 15 of the 18 species previously recorded for El Paso County (*B. appositus, B. bifarius, B. centralis, B. fervidus, B. flavifrons, B. fraternus, B. griseocollis, B. huntii, B. insularis, B. melanopygus, B. nevadensis, B. occidentalis, B. pensylvanicus, B. rufocinctus, B. sylvicola*), and four species that were not previously recorded for El Paso County (*B. mixtus, B. californicus, B. frigidus, B. balteatus*) in Wright et al. (2017). *Bombus centralis, B. bifarius,* and *B. flavifrons* were the most dominant bumble bee species in our flower-bumble bee network (Appendix Figure 2). *Geranium caespitosum* (GERCAE), *Chamerion angustifolium* (CHAANG), *Frasera speciosa* (FRASPE), and *Aconitum columbianum* (ACOCOL) were the most dominant host flower species in our network (Appendix Figure 2), however these species were included in the "rate observations" from the concurrent study that this data was subsetted from, so these interactions are overrepresented.

Bumble bee species varied in their elevation range, phenology, as well as in their body size and tongue length values (Figure 2A & 2B). Bumble bee phenology is constrained by changing environmental variables with elevation; therefore, species with a longer phenological window tended to occupy lower elevations. *Bombus griseocollis, B. nevadensis, B. huntii,* and *B. rufocinctus* interacted with flowers for most of the summer study periods, whereas *B. pensylvanicus, B. occidentalis, B. mixtus, B. balteatus,* and *B. sylvicola* had narrow foraging windows (Figure 2A). *Bombus griseocollis* emerged and was abundant relatively early in the summer seasons compared to the rest of the species observed and was also most concentrated at the lowest elevations in the study (Figure 2A&B). *Bombus pensylvanicus,* which is in decline and categorized as vulnerable by the International Union for the Conservation of Nature, appeared to have a narrow foraging window (Hatfield et al. 2015); however, observations of this species were limited to four occurrences, and this limited sample size may be responsible for the narrow foraging window we observed (Figure 2A). *Bombus balteatus*, *B. frigidus*, and *B. sylvicola* were found at the highest elevations within the study extent, while *B. griseocollis* was most found at the lowest elevations (Figure 2B).

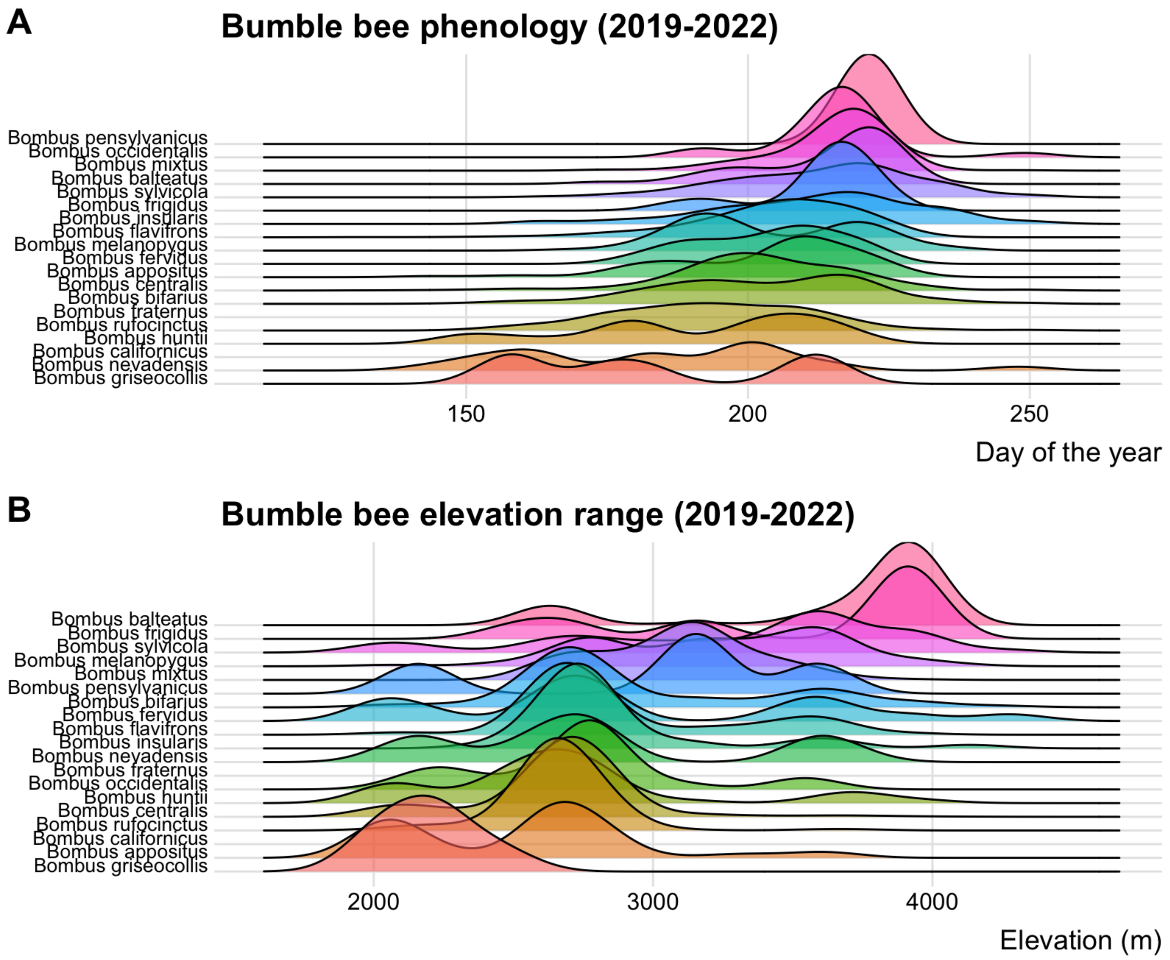

**Figure 2: A)** Bumble bee phenology across all elevational zones pooled for 2019-2022. **B)** Bumble bee elevational range pooled for 2019-2022

### Assessing elevational patterns of species richness and sampling completeness

Bumble bee rarefied species richness estimates were unimodal, with peaks in lower to middle elevational zones. Bumble bee species richness was greatest in zone 2 (21 species, SE: +/- 2.32) and lower in zone 1 (16 species, SE: +/- 3.27), zone 3 (17 species, SE: +/- 5.80), zone 4 (15 species, SE: +/- 2.40), and zone 5 (15 species, SE: +/- 5.05; Figure 3A). Zones 2 and 4 were significantly different from one another. Host flower rarefied species richness estimates peaked in zone 2 (117 species, SE: +/- 10.22) and dropped to its lowest point in zone 5 (19 species, SE: +/- 4.57). All zones were significantly different from one another apart from zone 3 (99 species, SE: +/- 46.76), whose confidence interval overlapped with zone 1 (80 species, SE: +/- 19.78), zone 2 (117 species, SE: +/- 10.22), and zone 4 (51 species, SE: +/- 7.86; Figure 3B). The sample completeness for bumble bee and host flower species in each zone was generally high. Sample completeness for bumble bee and host flower interactions was lower, varying between 70% and 95% for the elevation zones (Appendix Figure 3).

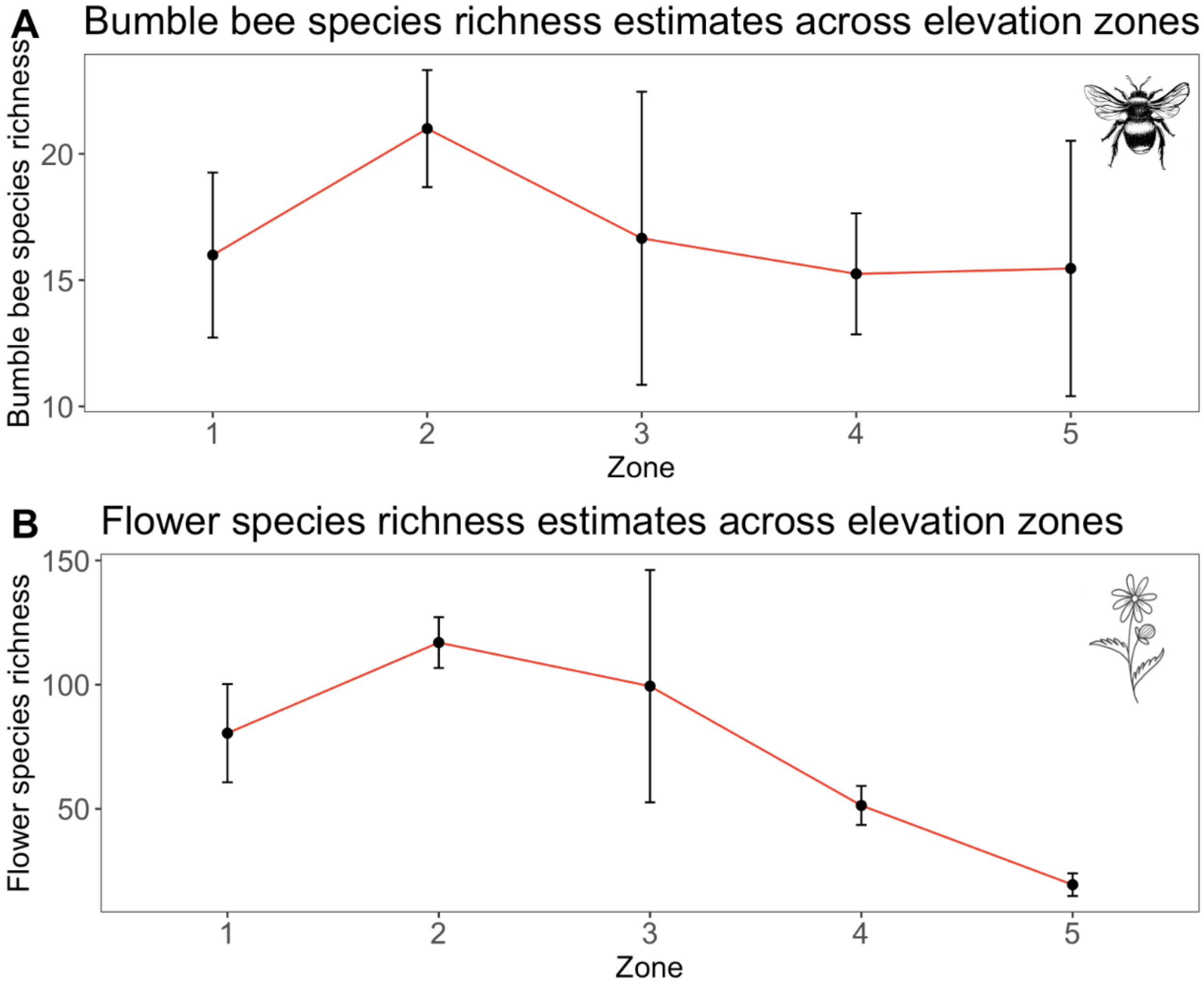

**Figure 3: A)** Bumble bee species richness and **B)** host flower species richness across the elevation gradient

**Q1: How does bumble bee diet niche overlap change along the elevation gradient?**

Bumble bee diet niche overlap increased linearly with elevation zone (t = 13.43, P-value = < 0.001; Figure 4; Appendix Figure 4). Niche overlap values in the highest elevational zone (zone 5) were over three times greater than the lowest elevational zone (zone 1). Zones 1 and 2 were significantly different from zone 5. Niche overlap measurements compared at n=117 included zone 1: 0.11 (lower 95% CI: 0.06, upper 95% CI: 0.18), zone 2: 0.15 (lower 95% CI: 0.08 upper 95% CI: 0.26), zone 3: 0.22 (lower 95% CI: 0.14, upper 95% CI: 0.36), zone 4: 0.26

(lower 95% CI: 0.17, upper 95% CI: 0.39), and zone 5: 0.35 (lower 95% CI: 0.33, upper 95% CI: 0.36). NMDS plots showed differences in bumble bee species' diet composition relative to one another (Figure 5).

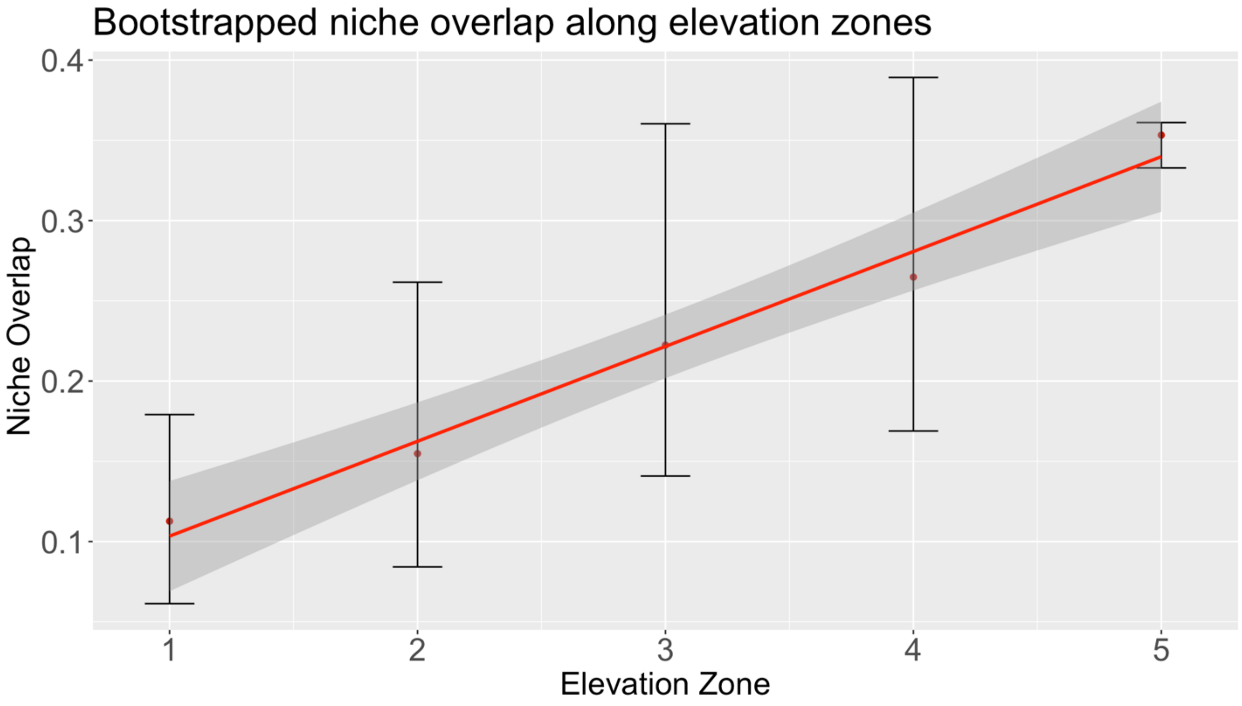

**Figure 4:** Niche overlap (Horn's index) values across the elevation gradient compared at N=117 using a non-parametric bootstrap without replacement.

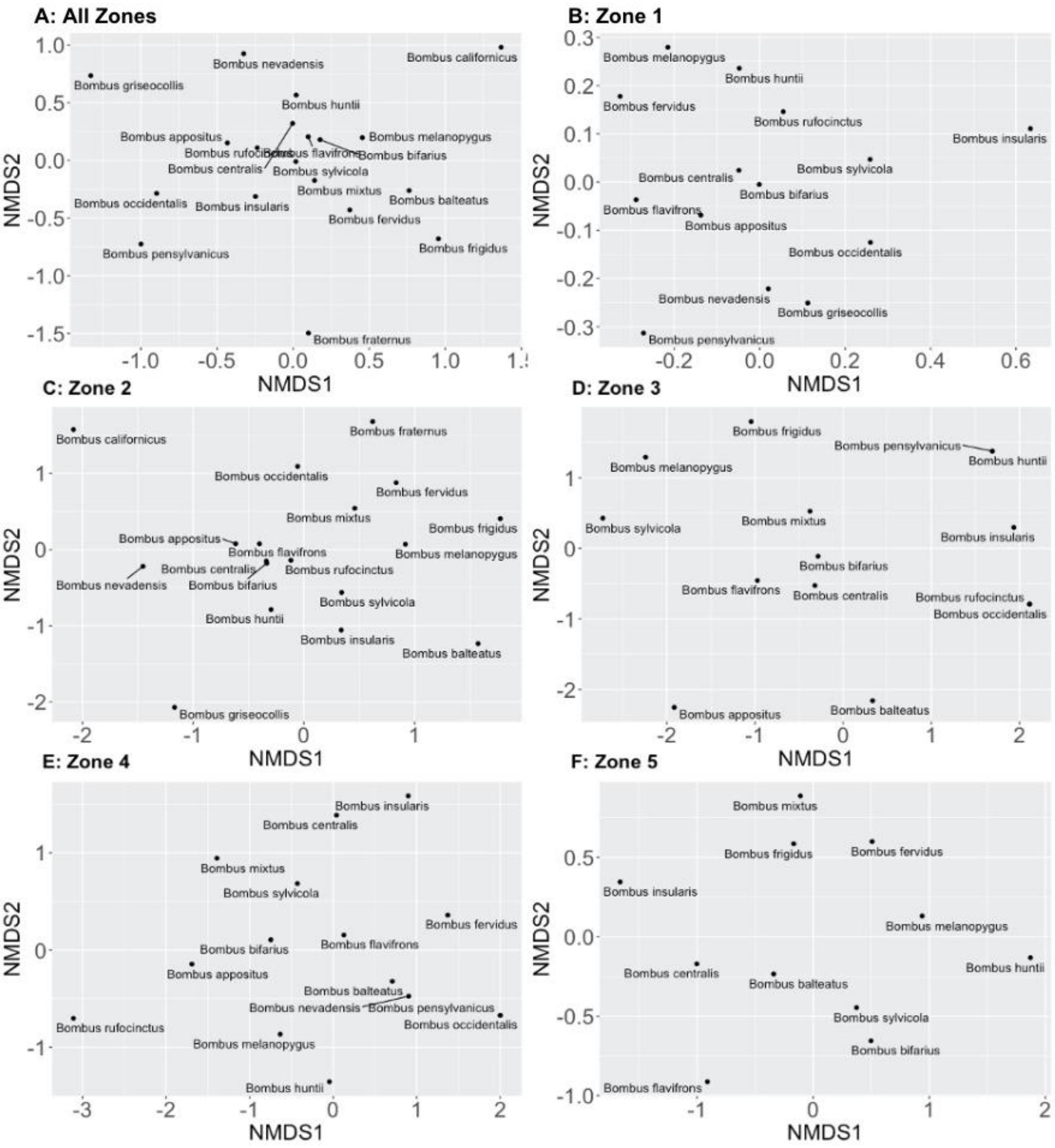

**Figure 5:** NMDS Plots of the dissimilarity of diet among bumble bee species within all zones combined **(A)** and within each individual zone **(B, C, D, E, F).**

### Q2: Is variation in phenology, body size, and tongue length correlated with variation in bumble bee diet composition?

Euclidean distance values in phenology, body size, and tongue length among bumble bees were all positively and significantly correlated with differences in diet composition (Bray-Curtis dissimilarity). The Mantel test statistic showed a positive correlation of 0.35 (P-value = 0.001) for phenology and diet differences, a positive correlation of 0.26 (P-value = 0.014) for body size and diet differences, and a positive correlation of 0.34 (P-value = 0.026) for female tongue length and diet differences (Figure 6). Pearson's product moment correlation coefficient between available female bumble bee tongue length and body size values were positively correlated at 0.78 (P-value < 0.01). NMDS plots for diet overlaid with continuous data for phenology, body size, and tongue length visually illustrated differences in diet as they relate to trait values (Figure 7).

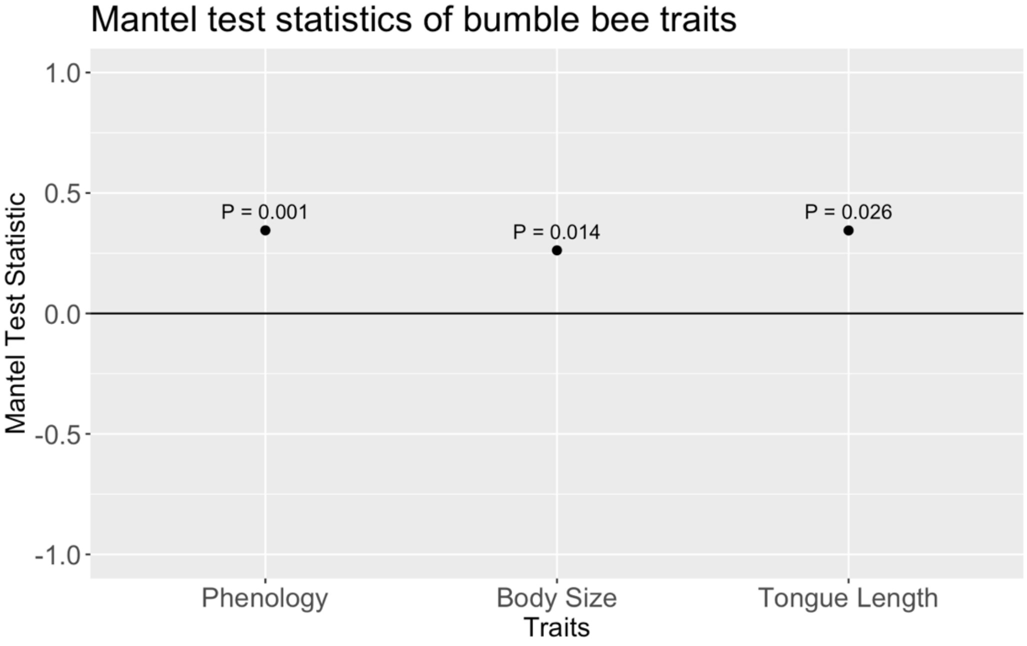

**Figure 6:** Mantel test statistic correlation values of phenology, body size, and tongue length trait Euclidean dissimilarity compared to diet Bray-Curtis dissimilarity values among bumble bee species across the elevation gradient.

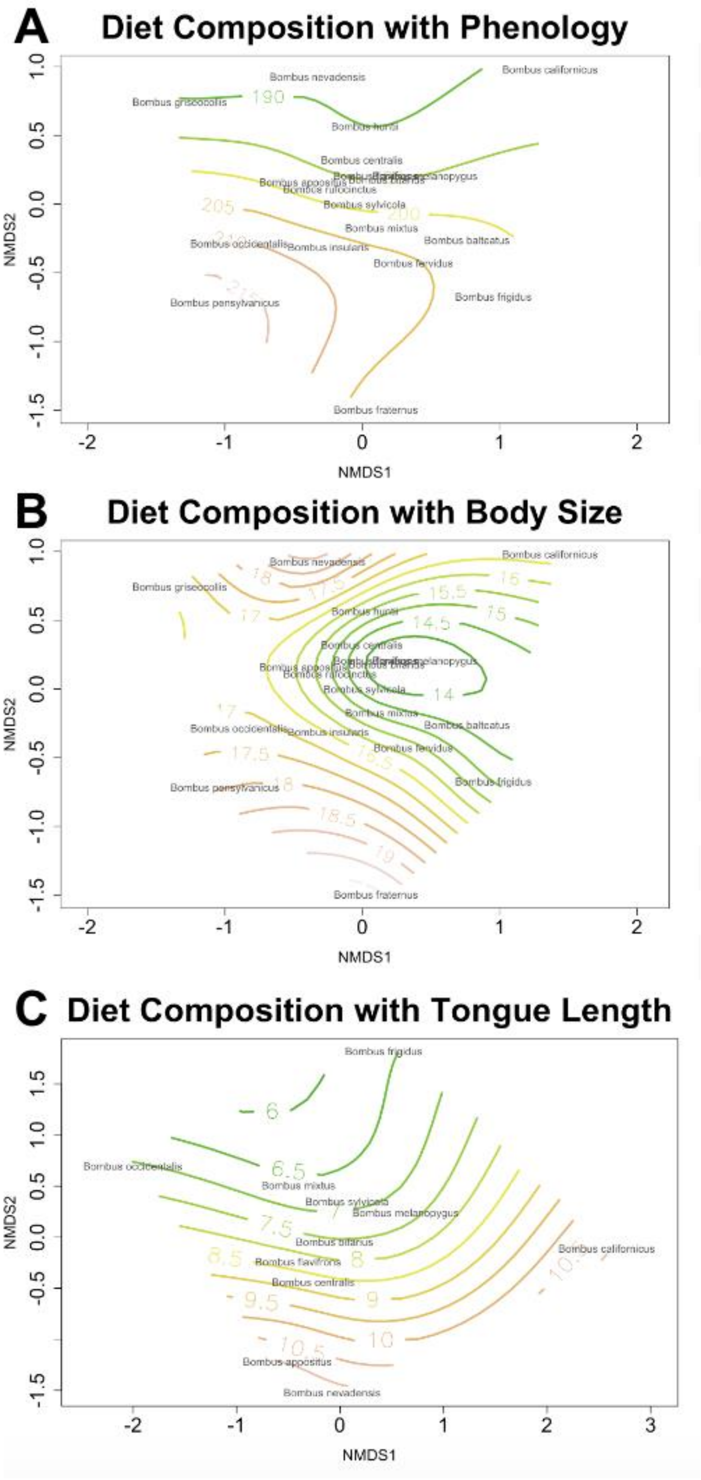

**Figure 7:** NMDS plots showing differences in diet composition of bumble bee species with overlaid continuous data for **A)** phenology, **B)** body size, and **C)** tongue length data.

### Q3: How might the upward movement of bumble bee species impact phenological and morphological trait overlap at the top of the mountain?

Eleven species were recorded in the mountaintop (zone 5) before simulating the colonization of lower elevation species upwards: *Bombus balteatus*, *B. bifarius*, *B. centralis*, *B. fervidus*, *B. flavifrons*, *B. frigidus*, *B. huntii*, *B. insularis*, *B. melanopygus*, *B. mixtus*, and *B. sylvicola*. Five additional bumble bee species, *Bombus appositus*, *B. nevadensis*, *B. occidentalis*, *B. pensylvanicus*, *B. rufocinctus*, colonized the mountaintop under our simulation, resulting in 16 different species residing at the mountaintop (Figure 8). Adding these bumble bee species to the mountaintop created an addition of species occupying high phenological and morphological (body size, tongue length) trait space overlap (Figure 8A-C).

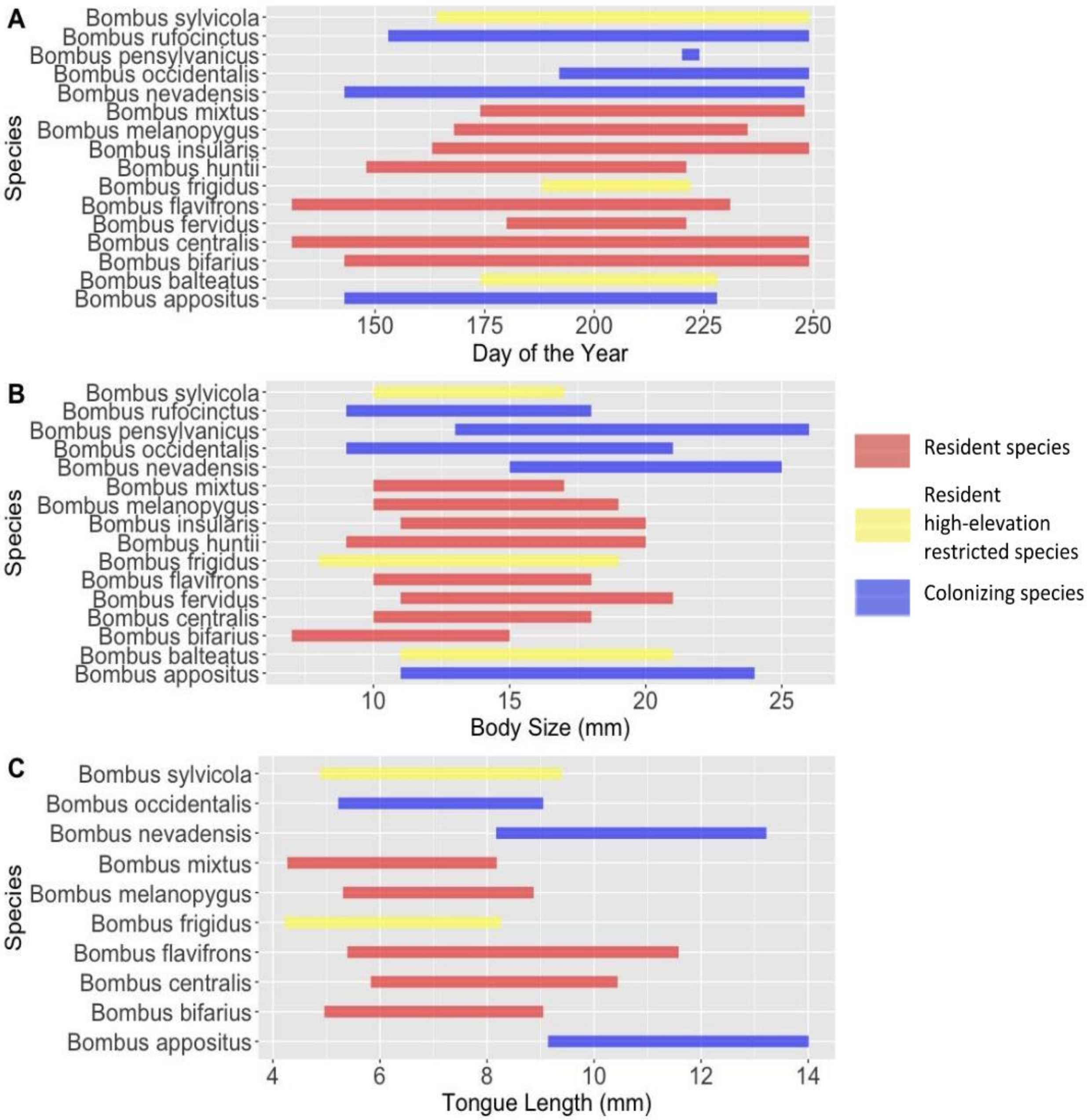

**Figure 8: A)** Bumble bee phenology, **B)** body size, and **C)** tongue length overlap at the mountaintop (zone 5) after simulating shifting bumble bee observations from lower elevations upwards by 459m.

Percent phenological overlap between resident species restricted to high elevations (*Bombus balteatus, B. frigidus, B. sylvicola*) and potential future colonizing bee species at the top of the mountain was greatest (>75%) for the combination of *B. sylvicola* and *B. nevadensis* (79.2%) as well as *B. sylvicola and B. rufocinctus* (88.5%) (Table 1). Out of resident species restricted to high elevations, *Bombus sylvicola* was the only species to have high phenological trait overlap with colonizing bee species (two total). Out of the colonizing bee species, *Bombus nevadensis* and *B. rufocinctus* had the greatest number (one) of high phenological trait overlap (>75%) values with *B. sylvicola* (Table 1). The percentages of phenological overlap between all resident species and potential colonizing bumble bee species were also calculated (Appendix Table 3).

Percent body size overlap between resident species restricted to high elevations (*Bombus balteatus, B. frigidus, B. sylvicola*) and potential future colonizing bee species at the top of the mountain was greatest (>80%) for the combinations of *B. balteatus* and *B. occidentalis* (83.3%) as well as *B. frigidus* and *B. rufocinctus* (81.8%) (Table 1). Out of resident species restricted to high elevations, *Bombus balteatus* and *B. frigidus* had the greatest numbers (two) of high body size trait overlap (>75%), followed by *B. sylvicola* with one interaction of high body size trait overlap (75%; Table 1). Out of colonizing bee species, *Bombus occidentalis* and *B. rufocinctus* had the greatest number of (two) high body size trait overlaps (>75%), followed by *B. appositus* with one interaction of high body size trait overlap (>75%) (Table 1). The percentages of body size overlap between all resident species and potential colonizing bumble bee species were also calculated (Appendix Table 3).

Percent tongue length overlap between resident species restricted to high elevations (*Bombus balteatus, B. frigidus, B. sylvicola*) and potential future colonizing bee species at the top of the mountain was greatest (>84%) for the combination of *B. sylvicola* and *B. occidentalis* (84.7%; Table 1). Out of resident species restricted to high elevations, *Bombus sylvicola* was the only species with high tongue length trait overlap with colonizing bee species (>75%; Table 1). Out of colonizing bee species, *Bombus occidentalis* was the only species with high tongue length trait overlap with resident species (>75%) (Table 1). The percentages of tongue length overlap between all resident species and potential colonizing bumble bee species were also calculated (Appendix Table 3).

| | *B. appositus* | *B. nevadensis* | *B. occidentalis* | *B. pensylvanicus* | *B. rufocinctus* |
|---|---|---|---|---|---|
| Phenology | | | | | |
| *B. balteatus* | *63.5%* | *51.4%* | *48%* | *7.4%* | *56.3%* |
| *B. frigidus* | *40%* | *32.4%* | *49.2%* | *5.6%* | *35.4%* |
| *B. sylvicola* | *60.4%* | ***79.2%*** | *67.1%* | *4.7%* | ***88.5%*** |
| Body Size | | | | | |
| *B. balteatus* | ***76.9%*** | *42.9%* | ***83.3%*** | *53.3%* | *58.3%* |
| *B. frigidus* | *50%* | *23.5%* | ***76.9%*** | *33.3%* | ***81.8%*** |
| *B. sylvicola* | *42.9%* | *13.3%* | *58.3%* | *25%* | ***77.8%*** |
| Tongue Length | | | | | |
| *B. balteatus* | *X* | *X* | *X* | *X* | *X* |
| *B. frigidus* | *0%* | *1%* | *62.9%* | *X* | *X* |
| *B. sylvicola* | *2.8%* | *14.7%* | ***84.7%*** | *X* | *X* |

**Table 1:** Percent of phenology, body size, and tongue length trait overlap for each unique pairing of resident bumble bee species found solely at high elevations and colonizing bumble bee (*Bombus*) species that were moved into the mountaintop. Interactions in bold are over 75% trait overlap.

# DISCUSSION

## Overview

Our results show that (a) bumble bee species diet niche overlap increases with elevation, (b) phenology and morphology are correlated with diet among species, and (c) future mountaintop bumble bee species may have increased competition under climate change due to more species occupying similar morphological and phenological trait space. These results present new patterns of bumble bee niche overlap across an elevation gradient and suggest that morphological and phenological traits can be important for explaining diet composition, which can be used to inform the consequences of future upward movement of bumble bee species into mountaintop environments on competitive interactions.

## Assessing elevational patterns of species richness and sampling completeness

As expected, both bumble bees and host flowers had unimodal peaks in species richness at middle-lower elevations. After peaking in zone 2, species richness for both taxa continued to taper with elevation. The unimodality in our findings is generally consistent with past studies that have investigated bumble bee and host flower species richness along elevation gradients across the globe (Goulson et al. 2008; Miller-Struttmann and Galen 2014; Sponsler et al. 2022b). Our estimates of bumble bee species richness were similar to Miller-Struttmann and Galen (2014), who found that bumble bee communities in the Colorado Front Range generally decreased in species richness with elevation. However, our estimates of nectar and pollen host flower richness were different from Miller-Struttmann and Galen (2014), who found increases in pollen host-flower species richness with elevation. The difference in results may be due to our sampling methodology involving nectar and pollen foraging interactions and Miller-Struttmann and Galen's study involving pollen foraging interactions only. There is evidence that bumble bees vary in their probability of carrying pollen from one or multiple flower species with elevation (Miller-Struttmann and Galen 2014) and tend to visit a broader range of flowers for nectar resources than pollen (Goulson and Darvill 2004), which would explain differences in our results. Our estimates of bumble bee and host flower richness are valuable in informing niche overlap (Costa-Pereira et al. 2019) as they determine the availability of the shared resource.

### Q1: How does bumble bee diet niche overlap change along the elevation gradient?

As predicted, bumble bee diet niche overlap increased with elevation and was greatest at the mountaintop, likely due to a restricted temporal flowering window and low plant species richness. Our results differ from Miller-Struttmann and Galen's (2014) historical niche overlap analysis in the Colorado Front Range, which found high diet niche overlap at the top and bottom of the elevation gradient (Miller-Struttmann and Galen 2014). This may be explained by differences in host flower species richness patterns between our study and Miller-Struttmann and Galen's study, as they had much lower host flower species richness in their lowest elevational zone compared to our lowest zones. Additionally, Miller-Struttmann and Galen (2014) attributed high niche overlap at the bottom of their elevation gradient to disturbance. While there is heavy traffic on designated paths at Pikes Peak, the extent of our study is largely protected from intensive land use and human development. Therefore, disturbance may be less of an influence than in Miller-Struttmann and Galen (2014).

Competition is logistically difficult to measure in the wild as it requires excluding bumble bee species from one another (Goulson et al. 2008; Brosi et al. 2017). Our niche overlap estimates are not a direct measure of competition but rather a statistic that measures the shared use of a resource. Past research has suggested that exploitative competition is the primary mechanism driving niche partitioning among bumble bee species (Inouye 1978). Therefore, niche overlap is a valuable tool for identifying species utilizing a limited resource similarly and identifying potential novel competitive interactions under climate change.

### Q2: Is variation in phenology, body size, and tongue length correlated with variation in bumble bee diet composition?

Differences in both morphological (tongue length and body size) and phenological traits were correlated with differences in diet among species. This supports the idea that morphological and phenological traits mediate diet niche partitioning (Goulson et al. 2008; Balfour et al. 2021; Sponsler et al. 2022a), likely due to resource accessibility and the energetic efficiency of visiting certain flowers depending on morphology (Balfour et al. 2021). We found that tongue length and body size were highly correlated. The non-independence of these traits makes it difficult to determine which trait is driving diet composition in bumble bees (Christmas et al. 2022; de Keyzer et al. 2016). Notwithstanding, our findings indicate the value that morphological variation has in helping to predict the diet of bumble bee species, as bumble bees are more likely to visit different flowers depending on their phenology, body size, and tongue length. Body size and tongue length values used in this analysis were taken from Colorado bumble bees used for past scientific research (Macior 1974; Wright et al. 2017). We assumed that while tongue length and body size may be different at Pikes Peak or may have changed since these past measurements, the relative relationships of these traits among species have likely remained similar, and therefore our findings from these data are still valuable to understanding trait impacts on diet.

### Q3: How might the upward movement of bumble bee species impact phenological and morphological trait overlap at the top of the mountain?

Moving bumble bee observations upwards into the mountaintop created the addition of new species and pairs of co-occurring bumble bee species that may compete with one another under climate change. Of the three resident mountaintop species most restricted to high elevations, *B. sylvicola* had the greatest number of high trait overlap interactions with colonizing species across all three traits. *B. sylvicola* is classified by the International Union for the Conservation of Nature as “least concern” (Hatfield et al. 2015b). However, recent genomic analysis of specimens initially identified as *B. sylvicola* has revealed the presence of a cryptic species, *B. incognitus* (Christmas et al. 2022). Two species being categorized as *B. sylvicola* may be conflating their population status. Additionally, high-elevation resident species such as *B. sylvicola* have lower tolerances for warming temperatures (Oyen et al. 2016) and may be less well adapted to take advantage of a longer foraging window in the alpine under climate change compared to colonizing species (Miller-Struttmann et al. 2022). Increased competitive pressures in addition to these pre-existing disadvantages may exacerbate the effects of climate change on resident alpine species.

Our approach to this simulation was relatively simple and only considered individual comparisons of traits among co-occurring bumble bee species. We did not consider how the range shifts of host flowers might impact potentially novel competitive interactions at the tops of mountains since we assumed bumble bees will be more mobile in their response to warming temperatures than plants. In addition, we assumed that all bumble bees would move upwards in response to climate change at the same rate. This may not be realistic, as past research has documented species variation in bumble bee elevation shifts (Pyke et al. 2016; Marshall et al. 2020). We also assumed that bumble bee species' phenology would not change as they move upwards in elevation. Past research has found that bumble bees that have already colonized the alpine tend to be less genetically constrained, or “canalized,” in their phenology than resident bumble bees (Miller-Struttmann et al. 2022), so it may be reasonable to assume that phenologies will not change drastically between colonizing and resident species. Still, that with even simple models we were able to detect species-specific competitive impacts underscores the need for more complex analyses to measure novel competitive pressures under climate change.

Our findings highlight the value of using trait measurements to predict potential novel competitive interactions under climate change. However, future research is needed to predict the outcomes of these potential novel competitive interactions. Additionally, considering the relative fitness of the different trait values species may have, rather than trait values alone, would also be valuable for predicting competitive outcomes (Funk and Wolf 2016) for future predictive models of novel competitive pressures under climate change.

## Conclusion

In summary, our study shows that bumble bee diet niche overlap increases with elevation, morphological and phenological traits (phenology, body size, tongue length) are correlated with diet composition among bumble bee species, and the upward movement of bumble bees under climate change may lead to new pairs of species with very similar trait values, increasing the likelihood that they will have a similar diet at the mountaintop under climate change. The effects of climate change may be exacerbated by the movement of species to remain within habitable environmental conditions. Our findings highlight the utility of using species traits to predict expected novel competitive interactions under climate change.

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# APPENDICES

**Appendix Table 1:** Bumble bee and host flower interaction observation effort by year (2019-2022).

| **Year** | **Number of data collectors** | **Total *Bombus* and host flower interactions** | **First day of routine observation (used in study)** | **Last day of routine observation (used in study)** | **Additional observation days (used in study)** |
|---|---|---|---|---|---|
| 2019 | 2 | 275 | May 31st | August 8th | August 22nd, 23rd |
| 2020 | 3 | 558 | May 21st | August 14th | |
| 2021 | 3 | 1578 | May 23rd | August 19th | |
| 2022 | 2 | 540 | May 11th | August 10th | September 5th, 6th |
| **Total** | **NA** | **2951** | **NA** | **NA** | **NA** |

**Appendix Table 2:** Bumble bee and host flower interactions by elevational zone by individual year and summarized across all four years.

| Year | Zone 1 | Zone 2 | Zone 3 | Zone 4 | Zone 5 |
|---|---|---|---|---|---|
| 2019 | 99 | 102 | 13 | 59 | 2 |
| 2020 | 31 | 481 | 2 | 38 | 6 |
| 2021 | 82 | 1150 | 130 | 158 | 58 |
| 2022 | 58 | 202 | 122 | 106 | 52 |
| **Total** | **270** | **1935** | **267** | **361** | **118** |

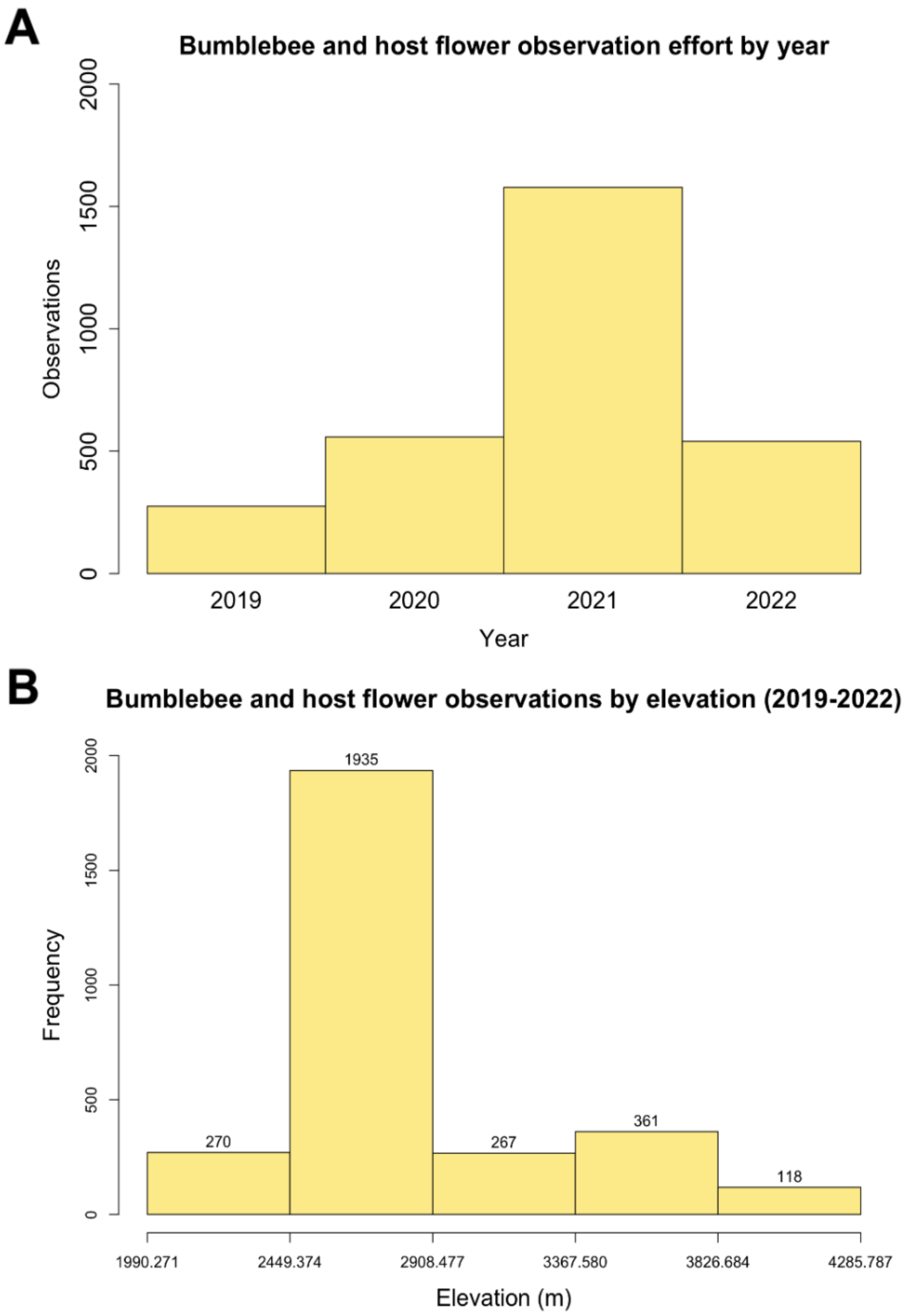
**Appendix Table 3:** Table of the percent of phenological, body size, and tongue length trait overlap for each unique pairing of resident and colonizing species in zone 5 after moving species found zone 4 into the zone 5 community. Interactions in bold are over 75% phenological trait overlap.

| Phenology | *Bombus appositus* | *B. nevadensis* | *B. occidentalis* | *B. pensylvanicus* | *B. rufocinctus* |
|---|---|---|---|---|---|
| *B. balteatus* | 63.5% | 51.4% | 48% | 7.4% | 56.3% |
| *B. bifarius* | **80.2%** | **99%** | 53.8% | 3.8% | **90.6%** |
| *B. centralis* | 72% | **89%** | 48.3% | 3.4% | **81.4%** |
| *B. fervidus* | 48.2% | 39% | 42% | 2.3% | 42.7% |
| *B. flavifrons* | **85%** | **75.2%** | 33.1% | 4% | 66.1% |
| *B. frigidus* | 40% | 32.4% | 49.2% | 5.6% | 35.4% |
| *B. huntii* | **85.9%** | 69.5% | 28.7% | 1.3% | 67.3% |
| *B. insularis* | 61.3% | **80.2%** | 66.3% | 4.7% | **89.6%** |
| *B. melanopygus* | 65.2% | 63.8% | 53.1% | 6% | 69.8% |
| *B. mixtus* | 51.4% | 70.5% | 74.7% | 5.4% | **77.1%** |
| *B. sylvicola* | 60.4% | **79.2%** | 67.1% | 4.7% | **88.5%** |
| Body Size | *B. appositus* | *B. nevadensis* | *B. occidentalis* | *B. pensylvanicus* | *B. rufocinctus* |
| *B. balteatus* | **76.9%** | 42.9% | **83.3%** | 53.3% | 58.3% |
| *B. bifarius* | 23.5% | 0% | 42.9% | 10.5% | 54.5% |
| *B. centralis* | 50% | 20% | 66.7% | 31.3% | **88.9%** |
| *B. fervidus* | **76.9%** | 42.9% | **83.3%** | 53.3% | 58.3% |
| *B. flavifrons* | 50% | 20% | 66.7% | 31.3% | **88.9%** |
| *B. frigidus* | 50% | 23.5% | **76.9%** | 33.3% | **81.8%** |
| *B. huntii* | 60% | 31.3% | 91.7% | 41.2% | **81.8%** |
| *B. insularis* | 69.2% | 35.7% | **75%** | 46.7% | 63.6% |

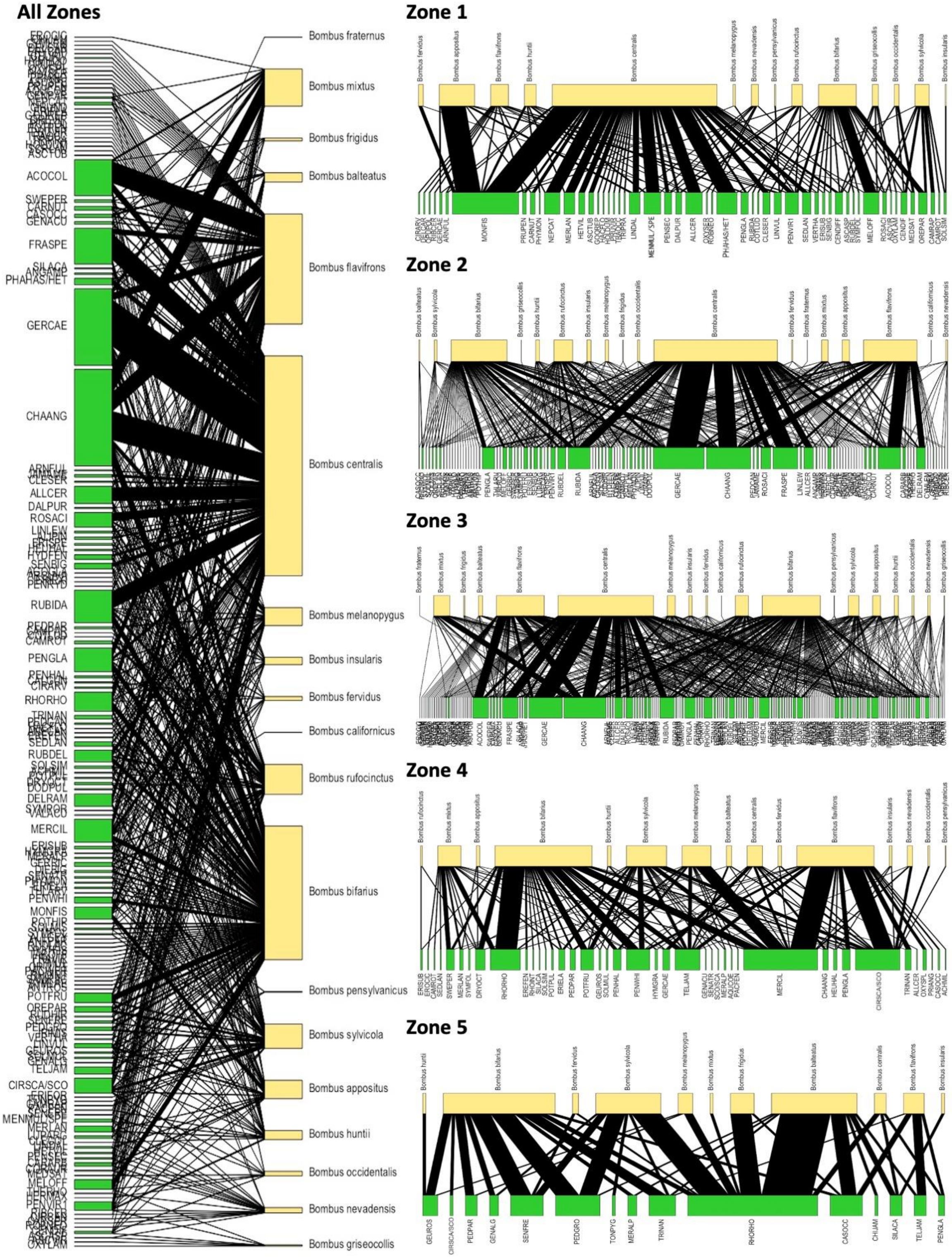
| | | | | | |
|---|---|---|---|---|---|
| *B. melanopygus* | 57.1% | 26.7% | **75%** | 37.5% | 80% |
| *B. mixtus* | 42.9% | 13.3% | 58.3% | 25% | **77.8%** |
| *B. sylvicola* | 42.9% | 13.3% | 58.3% | 25% | **77.8%** |

| Tongue Length | *B. appositus* | *B. nevadensis* | *B. occidentalis* | *B. pensylvanicus* | *B. rufocinctus* |
|---|---|---|---|---|---|
| *B. balteatus* | X | X | X | X | X |
| *B. bifarius* | 1% | 10.7% | **93.6%** | X | X |
| *B. centralis* | 0.159 | 30.7% | 61.7% | X | X |
| *B. fervidus* | X | X | X | X | X |
| *B. flavifrons* | 28.3% | 43.6% | 57.5% | X | X |
| *B. frigidus* | 0% | 1% | 62.9% | X | X |
| *B. huntii* | X | X | X | X | X |
| *B. insularis* | X | X | X | X | X |
| *B. melanopygus* | 0% | 8.8% | **93%** | X | X |
| *B. mixtus* | 0% | <0.1% | 61.9% | X | X |
| *B. sylvicola* | 2.8% | 14.7% | **84.7%** | X | X |

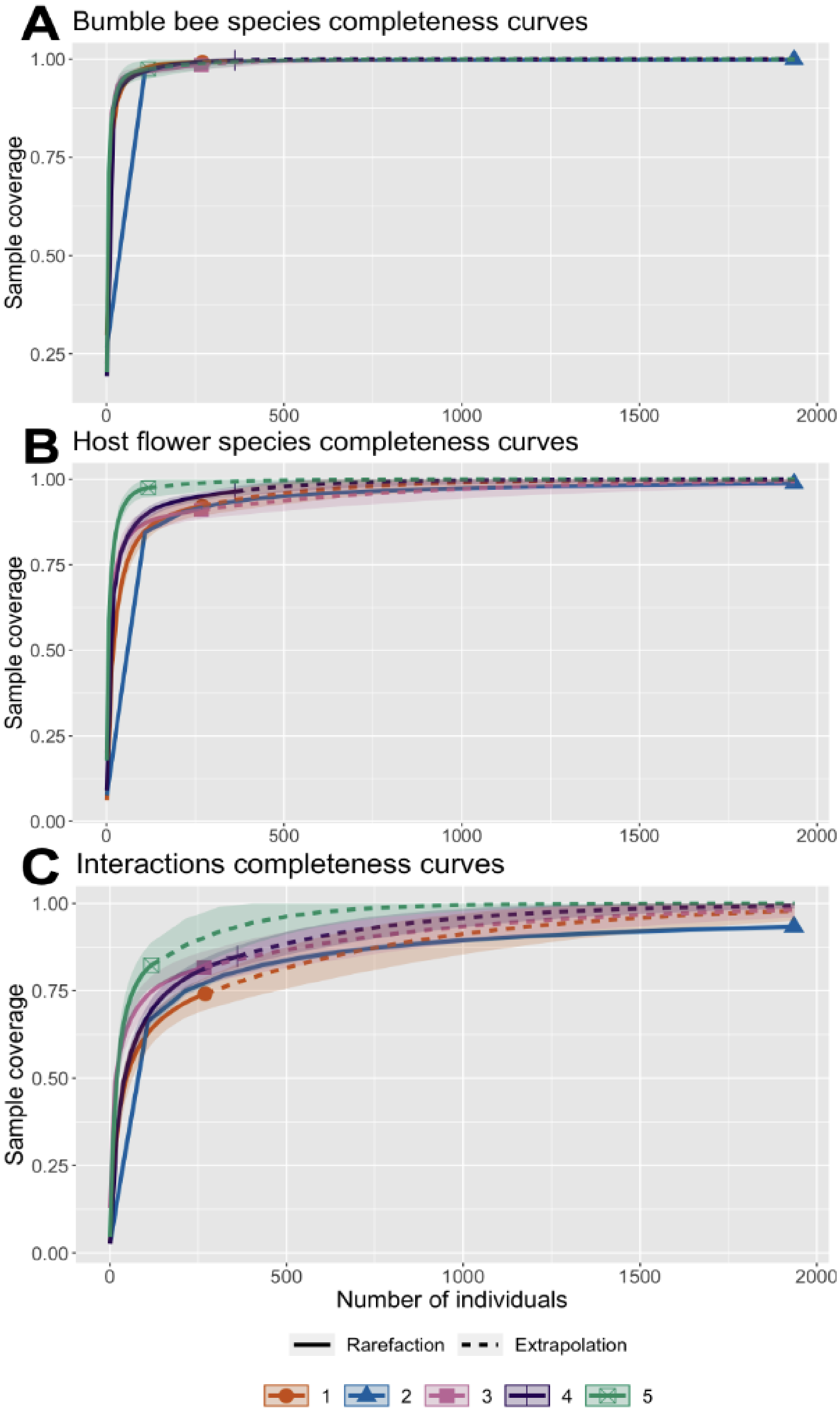
**Appendix Figure 1:** A) Bar plot of the total number of 1-minute bumble bee and host flower observations for all four years of the study (2019-2022). B) Histogram of the total number of 1-minute bumble bee and host flower observations across the elevation gradient totaled across all four years of the study.



**Appendix Figure 2:** Bumble bee and host flower interaction webs for the entire elevation study extent as well as each zone.



**Appendix Figure 3:** Sample completeness curves for rarefied and extrapolated A) bumble bee species richness, B) host flower species richness, and 3) unique host flower and bumble bee interaction "richness" estimates using hill number q=0.



**Appendix Figure 4:** Niche overlap values, bootstrapped for each elevational zone using the "bootstrapnet" package. Calculations started at N=50 and continued at N=1 until all observations were used in each elevational zone.