

The effects of environmental variation on daily abundance patterns and diet breadth of bumble bee species

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Summary

Bumble bees (*Bombus* spp.) are highly effective essential pollinators for wild and commercialized plants, although a quarter of bumble bee species worldwide are endangered. To aid in conservation efforts, their life history, environmental interactions, and response to abiotic factors must be understood. Although significant data exists regarding bumble bee thermoregulation, dietary breadth, and abundances in various regions, there is a lack of literature synthesizing these data on the scale of daily variation. By studying the daily variation in bumble bee abundance and diet breadth, and the relationship of these factors to temperature fluctuations, we provide a deeper understanding of natural patterns of *Bombus* spp. We found that temperature is not a determining factor of daily abundance in the mid-season at Gothic, Colorado. Bumble bees were most abundant in the first time interval of the day, and we hypothesize that floral resource availability is the contributing factor to this pattern. Diet breadth experiences changes throughout the course of the day, on a habitat and species level.

Introduction

Bumble bees (*Bombus* spp.) are a genus within Hymenoptera categorized by small eusocial colonies, large body size compared to other bees, hairy abdomens which reduce heat loss, and a strong ability to thermoregulate (Goulson 2010). Bumble bees are important pollinators as queens emerge early in the season and can visit short-lived spring flowers, and they visit a wide array of plant species in natural ecosystems. They are also key pollinators for crops, and pollination by *Bombus* spp. can lead to improved crop yield and higher-quality fruits of many crops such as tomato, kiwi, and pepper (Khalifa et al. 2021). There are around 260 species of *Bombus* worldwide, 10-15 of which are native to Gothic, Colorado, including parasitic species that don't have a worker caste and colonize the nests of other bumble bees. This site presents a

unique opportunity to study bumble bee life history and interactions, as only native bees are present, and the high-altitude and largely unmodified habitat allows us to study climate change effects on natural systems.

Understanding the life history and patterns of bumble bee activity is important to conservation efforts, as 25 % of *Bombus* species are considered endangered (IUCN Red List). Several factors are linked to bumblebee decline globally, including introduction of invasive *Bombus* species, pathogens, land use changes that interfere with nesting and foraging, and pesticides (Cameron and Sadd 2020). Warming due to climate change is also linked to detrimental effects on the range and abundance of several bumblebee species, through direct sublethal effects as well as indirect effects such as diminished floral resources (Cameron and Sadd 2020; Jackson et al. 2022).

Floral resources shape pollinator networks, as many insects are dependent on the pollen and nectar supplied by plants. Nectar is a finite resource, and the rate of replenishment following depletion is dependent on the plant species (Luo et al. 2014). Some flowers, such as those of *Aconitum colombianum*, replenish their nectar faster with continued visitation from pollinators, while others, such as *Delphinium barbeyi*, have a similar rate of nectar replenishment regardless of insect visitation (Luo et al. 2014). Flowers also differ in the composition of their resources, and the rewards given to foraging pollinators can vary greatly in nectar concentration (Luo et al. 2014) and protein content (Moerman et al. 2017). Feeding on a variety of flowers (a generalist approach) can be beneficial to bumble bees in some cases while a limited (specialist) diet can be sufficient in others, especially if pollen contains high amino acid and sterol concentrations (Moerman et al. 2017). Fluctuations between generalist and specialist approaches to foraging are not well-known on the diel scale and understanding them would provide important information regarding relative importance of different floral resources and changes in floral resources within a day. The seasonal patterns of abundance and life stage timing of *Bombus* are known in Gothic, Colorado (Ogilvie and CaraDonna 2022, in review), elucidating bumble bee responses to floral resource changes and environmental fluctuations over seasons. However, there lacks an understanding of within-day variation in bumble bee abundance and diet on the local scale, and how it is affected by daily temperature fluctuations.

Although we have a good understanding of how increasing temperatures are affecting bumble bee species across large continental scales, decades, and seasons (Ogilvie et al. 2017; Kudo 2014), we have a poor understanding of how increasing temperatures are affecting bumble bee populations at smaller spatial and temporal scales. Studies of natural bumble bee populations in Sweden (Lundberg, 1980 Bergman et al. 2018) show correlations between diel foraging and temperature peaks, however peak temperatures in these climates remain low ($\sim 15^{\circ}\text{C}$), and highest temperatures in Swedish bumble bee habitats are similar to the lowest daily temperatures experienced during the mid-season in the Rocky Mountains at Gothic, Colorado.

Unlike some insects which are ectothermic and are at the mercy of environmental fluctuations, bumble bees are well-known for their ability to thermoregulate by raising their internal body temperature through contraction of their muscles (Goulson 2010). However, anthropogenic climate change is rapidly modifying bumblebees' natural habitats and causing unpredictable temperature fluctuations. Although research suggests that climate warming may improve bumblebees' foraging potential (Kenna, Pawar, and Gill 2021), extreme heat can place bumblebees above their heat tolerance threshold and result in heat stupor or death (Martinet et al. 2021) and extreme cold or sudden frost may inhibit the ability to produce sufficient heat on an individual or nest basis. The inhibition of thermoregulation presents a danger to bumble bee survival in a changing climate and understanding temperature preferences of bees is therefore crucial to predicting species persistence amidst climate change.

Larger bumblebees can maintain high body temperatures more successfully than small ones (Heinrich 1975; Peat et al. 2005) due to a lower surface area to volume ratio, so queens and large workers may be found in higher abundance during cooler temperatures. Regardless of air temperature, bumble bee queens maintain steady thoracic temperatures when flying (Heinrich 1975), so cool air temperatures would likely result in greater energy loss. While foraging on flowers, worker bees also maintain high thoracic temperatures (Heinrich 1972). Due to the apparent relationship between body size and ability to thermoregulate, we may observe a higher proportion of large *Bombus* species (e.g. *B. nevadensis*) at low temperatures. Furthermore, bees have an upper limit of thoracic temperatures (Heinrich and Heinrich 1983), and if excessive heat is being produced during flight, they may attempt to fly less during

particularly hot periods of the day. Bumble bees may be able to shed heat via dark coloration (Williams 2007) or transferring heat to the abdomen (Heinrich 1976c), although little evidence of heat shedding at high temperatures has been demonstrated (Heinrich 1972).

Mature bumble bees have circadian rhythms that regulate their daily patterns (Yerushalmi, Bodenhaimer, and Bloch 2006; Pandey, Motro, and Bloch 2020) and are modulated by visible light as well as by UV light (Chittka, Stelzer, and Stanewsky 2013). Although bumble bees are known to have diurnal patterns, the literature lacks an understanding of their specific activity throughout the day, which is important in predicting climate-change outcomes and in designing long-term studies. Plant-pollinator interactions can change significantly within a day, as illustrated by Baldock et al. (2011) using network analysis on Kenyan pollinators, and these daily patterns must be considered when sampling to avoid biases. By elucidating bumble bee abundance and diet breadth changes within a day, we hope to address these gaps in knowledge.

To address the relationship between daily temperature fluctuations, bumble bee abundance, and diet breadth, we will observe natural *Bombus* populations in three habitat types at the Rocky Mountain Biological Laboratory over several days throughout the season. We will address the following questions:

Q1: How does bumble bee abundance vary within a day?

Q2: How does daily temperature patterns influence bumble bee abundance within a day?

Q3: How does diet breadth change throughout the day?

Methods

We sampled 6 transects in the Research Meadow, Rocky Mountain Biological Laboratory in Gothic, Colorado. The transects encompassed aspen forest habitat, wet meadow habitat and dry meadow habitat, with two transects per habitat. Each transect was 40 metres long, to ensure that surveying for bumble bees can occur down one pathway to minimize plant trampling, while maintaining a sufficiently large area to observe bee-flower interactions. Each transect was walked at a pace of 10 metres per 5 minutes, looking at either side of the path to observe bumble bee activity.

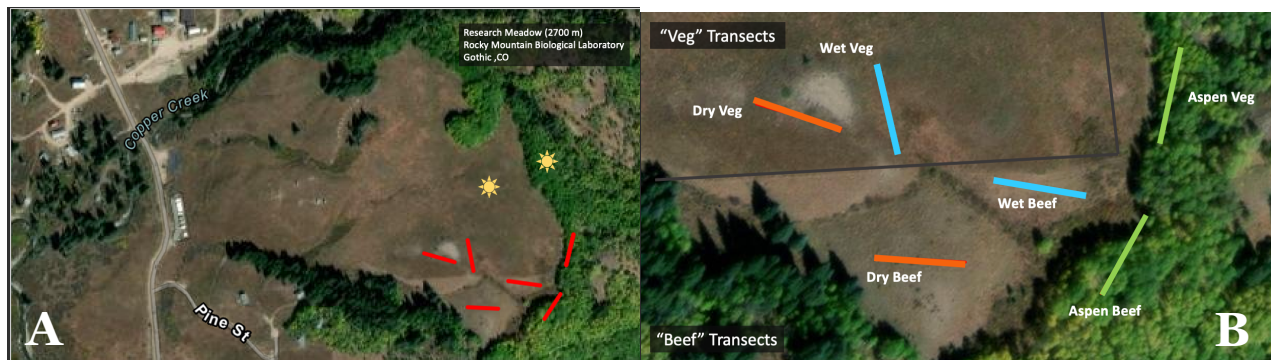
In order to measure bumble bee abundance and diet breadth throughout the day, observational days were divided into five time intervals of two hours each: Time Intervals 1

(8:00-10:00), 2 (10:00-12:00), 3 (12:00-14:00), 4 (14:00-16:00), and 5 (16:00-18:00). This allows for sufficient time within each time interval to adequately sample 6 transects, while also dividing the day into relevant intervals to monitor changes in bee activity over the course of the day.

Two individuals collected data simultaneously, so that within each time interval each transect was observed for twenty minutes, pausing the timer during species identification, data recording or bumble bee catching and releasing. We recorded the identity of the sampled transect; species of observed bumble bees (either identified on the flower or netted and released following identification); species of flowers that the bumble bee was visiting; number of flowers visited per visited plant; whether the bumble bee was a queen, worker, or male; whether pollen baskets were filled on the observed bee; and the time when the observation period began and ended. Temperature data was extracted from HOBO units at the end of the study, and air temperature at the midpoint of each time interval was recorded.

To randomize the order of the transect sampling, one observer would start on a certain habitat in the cycle (the order of which was wet -> dry -> aspen, in a cycle), and the other observer would start on the following habitat in the cycle, in the other set of transects. Transects were divided into two sets: "Veg" (non-cattle grazing side) and "Beef" (cattle-grazing side). For example, in Time interval one, MG may sample WV->DV->AV, and DG would sample DB->AB->WB, with the first letter corresponding to the habitat type, and the second letter corresponding to the set of transects (Veg or Beef). In the next time interval, observers would switch sides (MG would go to Beef and DG would go to Veg), and would start with the next habitat in the cycle on that side (i.e. MG would start with AB and DG would start with DV).

Data analysis was performed using R Statistical Software. To analyze the relationship between temperature and abundance, linear regressions will be used. Comparing abundance across time intervals was done via ANOVA. To analyze changes of diet breadth throughout the day, the diet breadth of individual *Bombus* species was analyzed.



Map1. A) Red lines indicate transects, and yellow suns indicate HOB0 temperature measuring units. **B)** Veg set indicates transects mostly on the non-cattle grazing side of the fence, and the Beef set indicates transects on the cattle-grazing side.

Results

Bumble bee foraging peaked between 8:00-10:00 in dry and wet meadow habitats, as well as in pooled data across all three habitats (Figure 1). Abundance in wet and dry meadow exhibited lowest levels in non-terminal time intervals (intervals 2 and 4 respectively), with a slight resurgence in foraging in the last time interval (interval 5, 16:00-18:00). Abundances across the day did not have strong patterns when analyzed by individual bee species (Figure 2), although foraging in *B. bifarius*, a short-tongued bee, appeared to peak in the first time interval.

Daily temperature exhibited variation throughout the mid-season at Gothic, CO (Figure 3A), however mean temperature was lowest from 8:00-10:00, rising gradually until 14:00-16:00, followed by a slight decrease. If there was a correlation with foraging and temperature, we may see peak foraging in the same time interval, however, peak foraging was during 8:00-10:00 (Figure 1), when temperature was at its lowest (Figure 3A). Moreover, temperature had no correlation to abundance when pooled across habitats (Figure 3B), nor when separated by habitat (Wet, $R^2=0.1107$; Dry, $R^2=0.1086$; Aspen, $R^2=2.987e-06$). When habitats were pooled and foraging-temperature correlation was compared by caste, no pattern emerged: neither queens nor workers showed correlation between abundance of foraging bees and temperature (queens, $R^2=0.0105$; workers, $R^2=0.03676$).

Changes in diet breadth were analyzed by comparing species richness of visited plants across the time intervals. When pooled across all bee species but separated by habitat, diet breadth was lowest in the last time interval for all three habitats (Figure 4A). Diet breadth was highest in Time Interval 1 for Wet Meadow, Time Interval 2 for Dry Meadow, and Time Interval

3 for Aspen (Figure 3A). When diet breadth was analyzed for the three most common *Bombus* species, the patterns were similar across species (Figure 3B). They all exhibited highest diet breadths in Time Interval 3, after which they declined until the last interval. They also all demonstrated a dip in diet breadth after the first time interval. *B. bifarius* demonstrated the highest diet breadth overall, as compared to *B. appositus* and *B. flavifrons* (Figure 4B).

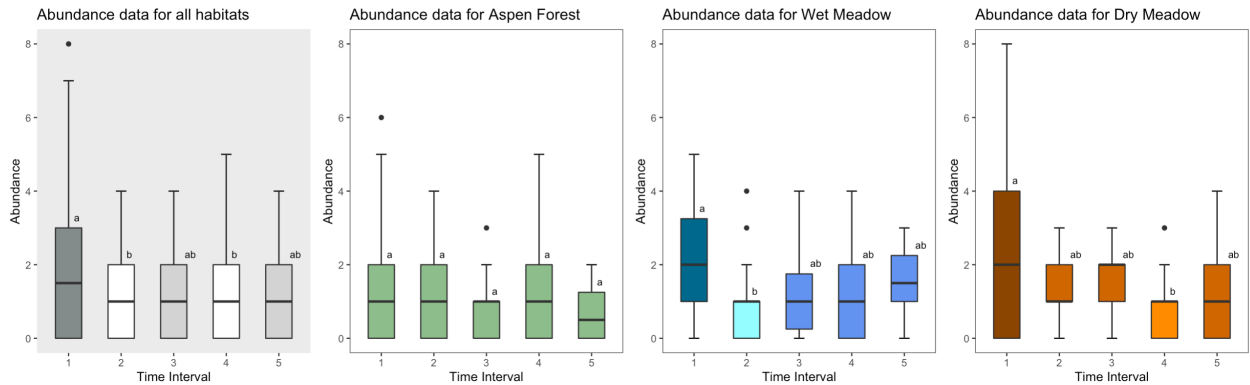


Figure 1. Number of bees observed for each sampling period, compared between time intervals. Each replication is a 20-minute observation period within a certain habitat. Letters indicate groups with significant difference according to the results of a Tukey ANOVA.

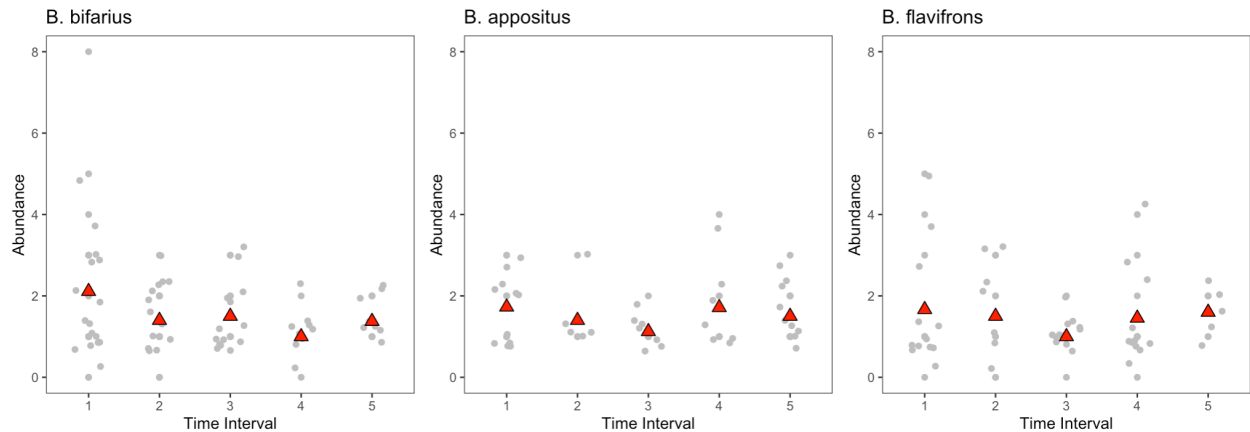


Figure 2. Number of bees observed for each sampling period, compared between time intervals for the three most common *Bombus* species in our study. Each point represents the abundance observed during a 20-minute observation period. Red triangles indicate means. Abundance is relatively scattered, although *B. bifarius* appears to demonstrate a higher abundance during Time Interval 1.

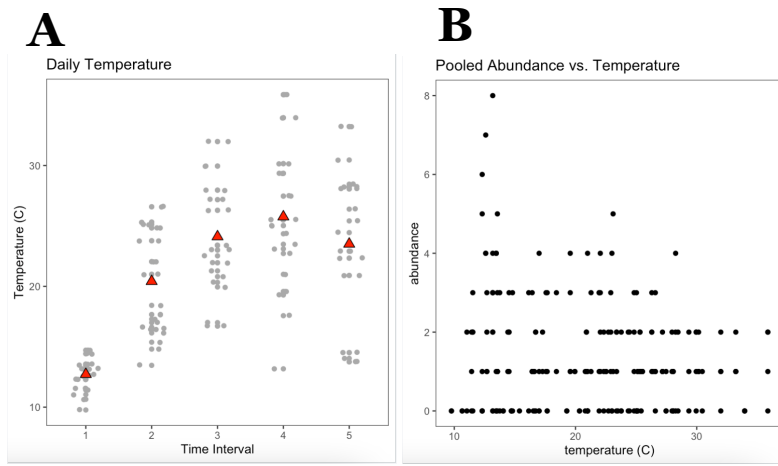


Figure 3. A) Daily temperature at Research Meadow, pooled across temperature measurements taken in aspen and wet meadow habitats between July 5 2022 and August 30 2022. Temperature variation exists across days, although mean temperatures follow a trend of rising throughout the day and peaking in Time Interval 4 (14:00-16:00). Time Interval 1 (8:00-10:00) exhibits the least amount of variation. B) Comparing bee abundance to temperature results in high scatter ($R^2 = 0.03228$) and no significant relationship is evident.

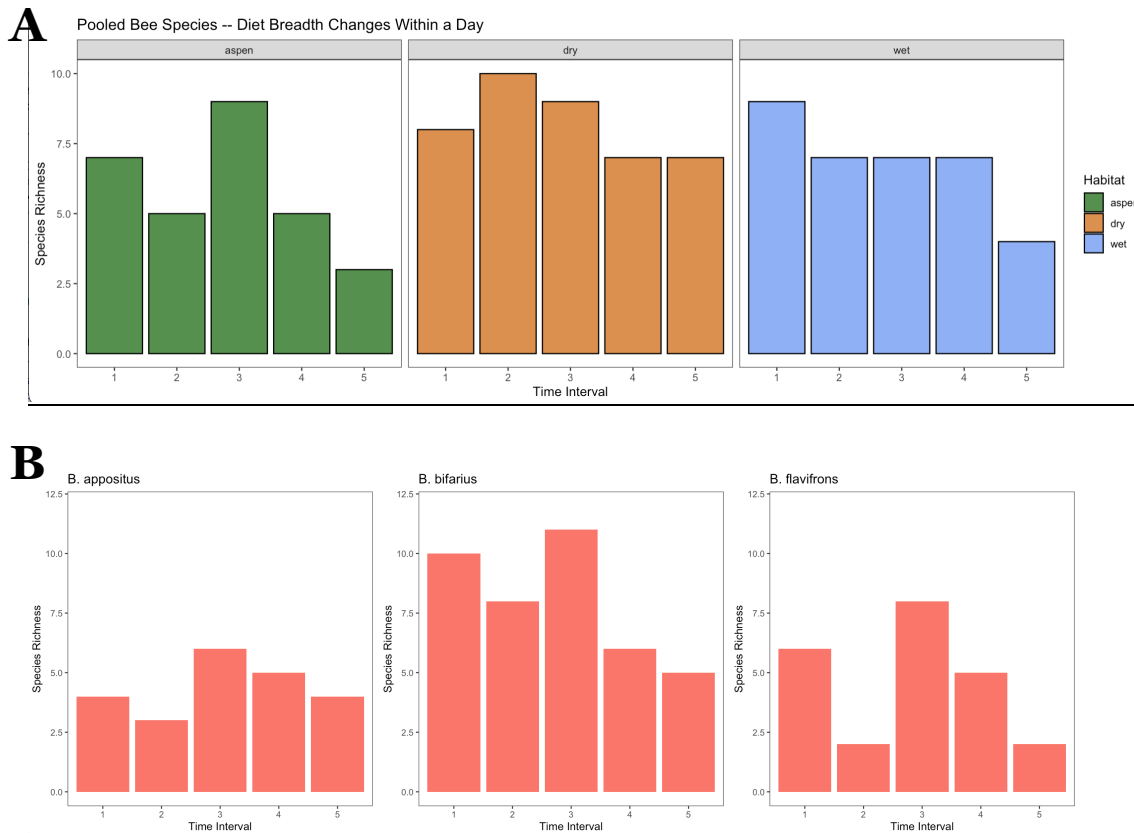


Figure 4. A) Variation in species richness of foraged plants over the day, faceted by habitat. All habitats exhibit peak diet breadth in one of the first three time intervals, and lowest time interval in the last time interval. This suggests a tendency towards bee diet generalization in the early part of the day and specialization in the latter part of the day. B) Species richness across the day faceted by the most common *Bombus* species, with data pooled across habitats. Each species exhibits a dip in diet breadth in the second time interval, a peak in the third time interval, and dropping diet breadth between the third and fifth time intervals.

Discussion

The peak in foraging bumble bee abundance in the first time interval may be related to nectar availability being greatest at the start of the day. Given a lack of correlation between abundance and temperature (Figure 3B), it is likely that temperature is not a limiting factor to bumble bee foraging in the mid-season at Gothic, CO, and that temperatures throughout the day are sufficiently warm to prevent excess energy loss to thermoregulation, and sufficiently cool to prevent overheating during foraging and flight. It may be noted that abundances were low when the temperature was above 35°C (Figure 3B), which may be due to insufficient incidences of high temperatures creating a sampling effect, or due to the difficulty of heat shedding during high ambient temperatures leading to fewer foraging trips during temperatures above 35°C. If temperature is not the key factor influencing foraging rates, it is likely that floral resources play the larger role. One may also notice that foraging increases slightly towards the end of the observed day (Figure 1), which may be due to sufficient time passage for nectar replenishment.

The short-tongued bee *B. bifarius* appeared to have the strongest tendency towards a foraging peak in the first time interval, perhaps due to greater competition from other genera of pollinators which can easily access the smaller flowers visited by *B. bifarius*. Stronger competition may lead to higher foraging in the first time interval as foraging would need to occur before high-quality nectar is depleted from the available floral landscape.

Diet breadth demonstrated peaks in different time intervals according to habitat – diet breadth in Wet Meadow peaked in Time Interval 1, in Dry Meadow in Interval 2, and in Aspen in Interval 3. It is possible that this trend follows the ranking of the bumble bee's preferred foraging sites. A high diet breadth implies generalization and suggests higher competition leading to visitation of less-preferred plants with lower-quality resources. It is possible that after the resources are depleted in wet meadow, bumble bees sequentially move to dry meadow and then aspen, following the floral availability as each habitat has a slightly different species composition (with some overlap). *B. bifarius* demonstrated the highest diet breadth out of the three most common species – it is a short-tongued bee, and thus has a capacity to visit a greater diversity of flower morphologies. However, it was also the most observed bee (number of bees observed overall: $n_{\text{bifarius}}=109$, $n_{\text{flavifrons}}=78$, $n_{\text{appositus}}=62$), and this may be an effect of a greater sample size producing more observations of rare plant species visitation events.

Conclusion

Temperature is not related to abundance during the mid-season in Gothic, Colorado, and foraging occurs at the greatest levels during the coldest observed time interval. This differs from prior studies which indicate a strong correlation between temperature and abundance, although most prior studies of diel patterns occur in colder climates where peak temperatures are comparable to lowest temperatures in Gothic. A stronger correlation between abundance and temperature might be seen in Gothic during the early season when temperatures are much colder and may be a limiting factor to flight.

We suggest that the determining factors of diel abundance patterns in Gothic are floral resources and nectar availability. Diet breadth reductions towards the end of the day follow abundance trends and indicate a tendency towards late-day diet specialization on the community-wide level and on the individual level.

Overall, peak foraging occurs during the earliest time interval observed, and is contrary to expectations of foraging in the mid-day or when temperatures are highest.

Combined, this information is useful to conservation efforts, as it suggests that plants preferred by bumble bees cannot be inferred by taking a snapshot of the day, but may be dynamic. By further investigating the diet breadth changes throughout the day and combining this data with nectar availability and nutritious content, researchers may be better able to elucidate which plants are essential to bumble bee colonies and must be stewarded to protect ecological communities.

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