

## Estimating Local Abundance of *Bombus* Queens in Meadows of Rocky Mountains, Colorado, USA

### Introduction

As pollinators, bumblebees (*Bombus* sp.) provide essential ecosystem services in plant communities around the world (Potts et al., 2016). They play particularly key roles in the ecological networks of subalpine meadows through established relationships with multiple wildflower species (CaraDonna & Waser, 2020). In recent decades, bumblebees have faced observed declines globally due to climate change (Cameron & Sadd, 2020). Despite this increased threat and its broader implications for the resilience of these ecosystems, their life cycle and community dynamics represent massive knowledge gaps that negatively impact our understanding of landscape ecology (Ogilvie & CaraDonna, 2022). Bumblebee queens are particularly vulnerable to environmental shifts and stressors since they are solely responsible for the establishment of colonies in the spring (Sarro et al., 2021). Further study of this essential life stage can give important insight into current and future population declines (Goulson et al., 2015).

The CaraDonna lab is currently conducting a long-term study of bumble bee populations via weekly counts at six established sites at Rocky Mountain Biological Laboratory. The relative abundance estimates generated from the project can provide valuable insights into population and community patterns (e.g., Ogilvie & CaraDonna 2022). However, the actual, local abundance (or population size) of bumble bee queens at these long-term monitoring sites still remains unknown; relative abundance estimates are simply estimates and do not tell us how many individual queens might be at these sites (Briggs et al., 2022). Most of the common methods for bumblebee sampling and collection, such as pan traps, vane traps, and trap nests, can yield poor indices of actual species status and abundance since they rely upon capture probabilities, which are highly variable and inconsistent (McKelvey & Pearson, 2001). Additionally, they are all lethal, which poses major detrimental impacts on the bumblebee populations being studied, especially in the context of repeated, long-term sampling. Therefore, accurate representations of bumblebee populations remain largely unknown. Mark-recapture methods are an underutilized yet viable approach that can produce a more population estimate by accounting for some of the biases inherent in index sampling methods (Briggs et al., 2022). This can help to ascertain more precise estimates of local abundance for *Bombus* species within the CaraDonna & Ogilvie long-term bumble bee monitoring project at the RMBL.

Our primary research questions were: (1) How many individual queens of *B. appositus*, *B. bifarius*, *B. flavifrons*, *B. insularis*, and *B. rufocinctus* do the meadows at our RMBL study sites support? (2) Is there a relationship between the mark-recapture local abundance estimates and the observational counts?

### Methods

*Study sites and mark-recapture of bumble bee queens.* Net sampling occurred from June 19 to July 12 at two meadows within the East River Valley. These sites are referred to as Copper Creek (CC) and Frogs Pond (FP). They are each separated by at least 1 km (edge to edge) and are each approximately 500m in diameter, which corresponds with observed bumble bee foraging distances in similar environments (Elliott, 2009). Although possible, previous mark-recapture data from these study sites suggests that individual bees do not commonly travel between sites. Sampling occurred on clear, warm days between 9:30am and 5:00pm. Each site received 40 person-minutes of search time during each visit, divided into 20 person-minutes in the dry meadow and 20 person-minutes in the wet meadow. The sites were visited in different orders during each sampling period. After capturing a queen using insect nets, they were marked on the thorax with a Sharpie oil-based paint pen before release. The color of the paint pen varied by site and sampling day in order to successfully distinguish bees upon recapture. Bees who were recaptured were marked again and then released. For each individual queen that was captured and marked, we recorded: (i) species, (ii) whether the bee was captured in the dry or wet meadow, (iii) whether the bee was a recapture, (iv) the recapture color if applicable (to determine day of initial marking), (v) whether the bee was foraging, nesting, both, or flying through, and (vi) the plant species visited if applicable.

*Data analysis.* If sufficient recapture rates were achieved, we would use a mark-recapture model to estimate the local abundance (e.g., population size) of bumble bee queens at our study sites, following Briggs et al. (2022). In particular, we would use a closed mark-recapture model, which assumes that there are no changes in population while our research is conducted. Since sampling at each site took place over a short period and previous data suggests that individuals do not move regularly between our study sites, the closed model gives the most accurate depiction of these populations. If not, we would compare population size estimates of bumble bee queens across species using simple correlation. We also used generalized linear models to model the relationship with relative abundance estimates of bumble bee queens for each species and the mark-recapture population size estimates. R Studio was utilized for statistical analysis and graphs.

## Results

Figure 1. Mark-Recapture Species Abundance by Site

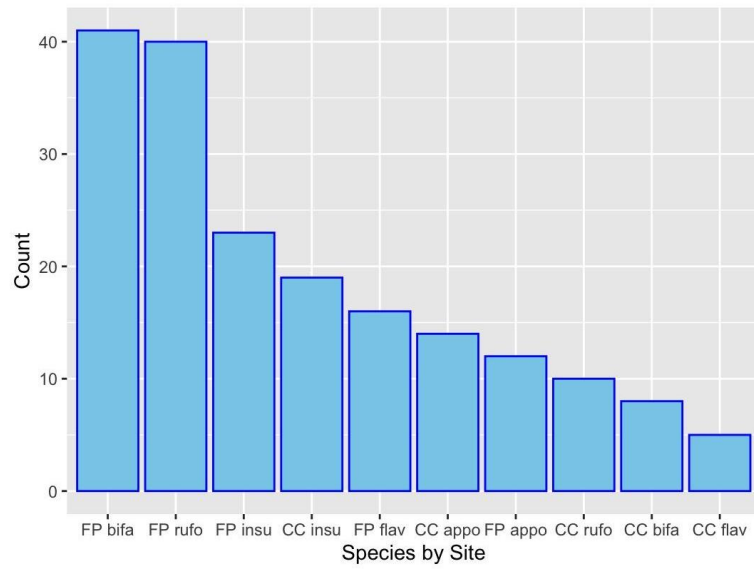


Figure 2. Observational Monitoring Species Abundance by Site

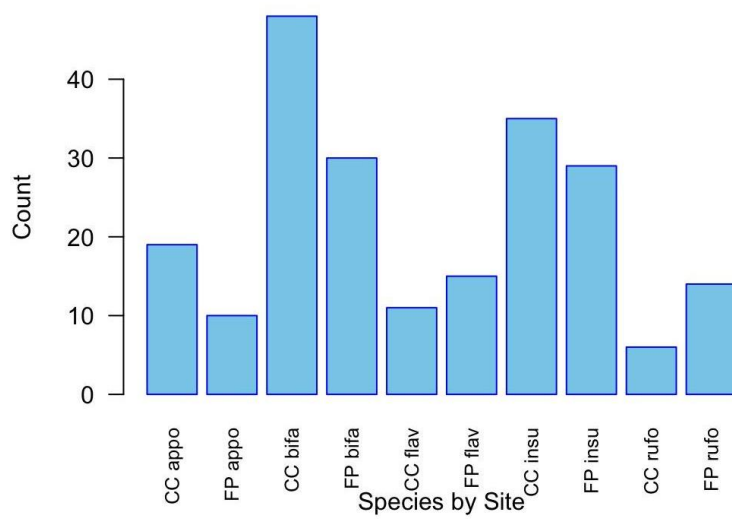
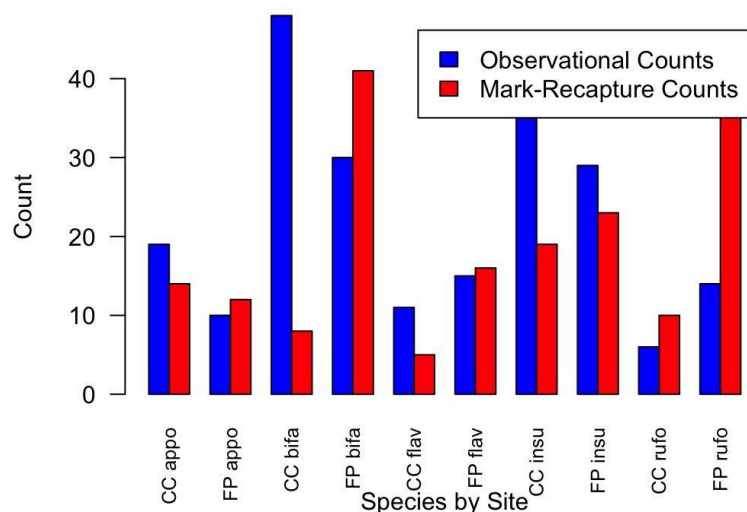


Figure 3. Species Abundance by Site Comparing Observational and Mark-Recapture Abundance



All five species (*B. appositus*, *B. bifarius*, *B. flavifrons*, *B. insularis*, & *B. rufocinctus*) were found at both sites. In total, 188 individual queens were captured and marked between the two sites: 56 individual queens were captured and marked at Copper Creek, and 132 individual queens were captured and marked at Frogs Pond. Overall abundance for each species was as follows: *B. rufocinctus* (50), *B. bifarius* (49), *B. insularis* (42), *B. appositus* (26), and *B. flavifrons* (21). At Copper Creek, abundance was highest for *B. insularis* (19), followed by *B. appositus* (14), *B. rufocinctus* (10), *B. bifarius* (8), and *B. flavifrons* (5) (Fig. 1). Abundance at Frogs Pond was highest for *B. bifarius* (41), *B. rufocinctus* (40), *B. insularis* (23), *B. flavifrons* (16), and *B. appositus* (12) (Fig. 1).

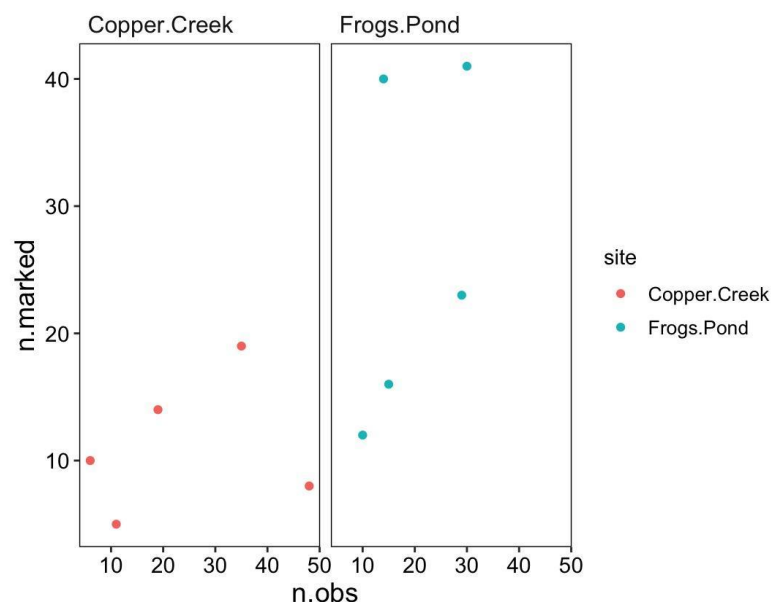
Observational counts from the long-term monitoring project, which were collected this summer by Paul CaraDonna and Jane Ogilvie over a period of nine weeks (when queens are active), yielded 217 individual queen observations across the two sites. Overall count per unit by species was as follows: *B. bifarius* (78), *B. insularis* (64), *B. appositus* (29), *B. flavifrons* (26) and *B. rufocinctus* (20). At Copper Creek, the counts were: *B. bifarius* (48), *B. insularis* (35), *B. appositus* (19), *B. flavifrons* (11), and *B. rufocinctus* (6). At Frogs Pond, the counts were: *B. bifarius* (30), *B. insularis* (29), *B. flavifrons* (15), *B. rufocinctus* (14), and *B. appositus* (10).

During session one, 15 individuals were captured at Copper Creek, and 34 individuals were captured at Frogs Pond. During session two, 12 individuals were captured at Copper Creek, and 36 individuals were captured at Frogs Pond. During session three, 3 individuals were captured at Copper Creek, and 14 individuals were captured at Frogs Pond. During session four, 8 individuals were captured at Copper Creek, and 11 individuals were captured at Frogs Pond. During session five, 2 individuals were captured at Copper Creek, and 9 individuals were captured at Frogs Pond. During session six, 8 individuals were captured at Copper Creek, and 3 individuals were captured at Frogs Pond. During session seven, 3 individuals were captured at

Copper Creek, and 6 individuals were captured at Frogs Pond. During session eight, 0 individuals were captured at Copper Creek, and 7 individuals were captured at Frogs Pond.

In total, five individuals were recaptures, comprising only 2.7% of all captures (far below the threshold of 10% which is needed for mark/recapture models) On June 19th, a *B. bifarius* queen was recaptured at Frogs Pond. On June 20th, a *B. bifarius*, *B. insularis*, and *B. rufocinctus* queen were each recaptured at Frogs Pond. On June 25th, a *B. insularis* queen was recaptured at Copper Creek.

Figure 4. Correlation Between Observational and Mark-Recapture Abundance By Site



Overall, although there was evidence of a positive correlation between the relative abundance of queens from the long-term monitoring counts and the number of captured and marked queens, this relationship was not statistically significant (Copper Creek:  $r = 0.706$ ,  $p = 0.19$ ; Frogs Pond:  $r = 0.695$ ,  $p = 0.19$ ).

## Discussion

Our bumble bee queen recapture rate was very low ( $> 3\%$ ), which complicates the ability to draw conclusions from the data since mark-recapture experiment validity is dependent on meeting the model assumption and having a minimum recapture rate ( $\sim 10\%$ ) (Briggs et al., 2022). While we chose to use a closed mark-recapture model, which assumes that there are no changes in population, in reality, these sites might function as very open systems with frequent movement across the landscape. Bumble bee queen death may also be a factor influencing our recapture

rates. . Mark-recapture has been found to underestimate bumblebee movement by providing only a minimum estimate of foraging range (Mola & Williams, 2019). It can be particularly difficult to ascertain population estimates for bees using mark-recapture models since their populations may act as closed for a few days and then open for longer periods (Briggs et al., 2022).

Bumblebee movement is potentially much more dynamic than is currently understood (Mola & Williams, 2019). Seasonal timing also likely played a prominent role in limiting the amount of recaptures, as our research was conducted from mid-June to early July. During this time bumble bee queens may be successfully establishing colonies and therefore retiring to their nests (instead of actively foraging). This is supported by the fact that many of the individuals we captured were carrying heavy pollen loads, suggesting they were provisioning the first cohort of worker larvae. In other words, we may have marked queens during their last days of active foraging.

We found a positive correlation overall between the mark-recapture counts and observational counts, but the pattern was not statistically significant. This is likely because of our low sample sizes ( $n = 56$  at Copper Creek,  $n = 132$  at Frogs Pond). This differs from the study by Briggs et al. (2022), who found no difference in capture probability between aerial netting and mark-recapture. A consistent relationship between index sample and population size is needed for accurate population estimates (Briggs et al., 2022). Differences in counts, which are often interpreted as proportional differences, actually indicate different capture probabilities (McKelvey & Pearson, 2001). While we anticipated a positive correlation between the counts, there are notable differences in the counts for several species at certain sites, in particular *B. bifarius* at Copper Creek and *B. insularis* at Frogs Pond. Therefore, it is critical that this research continue and be repeated in order to investigate these differences in counts.

While the number of recaptures did not meet the threshold of the chosen closed model, our study still provides a minimum number of queens found at the sites, thus ascertaining a minimum population estimate and giving a direction to future research on local bumblebee abundance. While there are complications to mark-recapture, it provides a viable alternative to other common methods such as vane traps, pan traps, and trap nests, which can lead to mortality and even have the potential to deplete bumblebee populations (Prendergrast et al., 2020). Future iterations of this study should still utilize mark-recapture but shorten the sampling period, increase the person-minutes spent capturing, and conduct the study earlier in the summer season so as to avoid co-occurring with queen succession as much as possible. Potential future directions also include the impact of flexibility of movement and anthropogenic change, including habitat fragmentation and resource availability, on bumblebee counts (Mola & Williams, 2019).

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