Assaying the effectiveness of acoustic monitoring for detecting bumblebee (*Bombus* spp.) abundance and diversity

Claire Yu Xue O'Kane, College of Agriculture and Life Sciences, Cornell University, Ithaca NY 14850

Kaitlyn E. Irwin, College of Arts and Sciences, University of Scranton, Scranton PA 18510

Timothy Green, Environmental Protection Division, Brookhaven National Laboratory, Upton NY 11793

Nicole Miller-Struttmann, Biological Sciences Department, State University of New York College at Old Westbury, Old Westbury NY 11568

1. Abstract

Pollinators are essential to global agriculture and the fitness of 85% of angiosperms. Recent bee declines, which threaten these pollination services, have been attributed to insecticides, the intensification of agriculture, and climate change. Our project is designed to determine the effectiveness of acoustic monitoring for surveying native bumblebee species. Acoustic monitoring is a method not used typically for surveying bee populations, but includes many benefits over the traditional and more labor-intensive method of surveying bees. Through this method, our objective is to learn about the effects of fire on bee abundance and activity. A fire gradient was established within the Pine Barrens at Brookhaven National Laboratory (BNL), where four of our recorders were each placed in four 15 acre plots among flowering huckleberry and blueberry plants. Two of the sites within the gradient are unburned, one site was burned once

in the last six years, and one site was burned twice in the last six years. Vibrations (buzzes) created by bees while flying or foraging are recorded in order to analyze bee abundance and diversity. To determine the effectiveness of acoustic monitoring, we collected recordings of buzzes from bees of various species and castes to test for bee abundance and pollination services. Within an enclosure called a flight cage, bees were recorded while foraging in naturally occurring patches of wildflowers, such as *Asclepias syriaca* (Common milkweed) and *Anchusa officinalis* (Common bugloss). Collecting this data helps us to analyze bumblebees' buzzes closely by species. I expect to see variation in frequencies among different species and castes of bumblebees, which would allow us to analyze bumblebee diversity and abundance through acoustic monitoring. This project is important towards helping Brookhaven National Laboratory address pollinator health in its Natural Resource Management Plan. Traditional survey techniques outperformed acoustic methods for monitoring pollination services to blueberries and huckleberries. Preliminary analyses suggest that buzz frequency does not differ by species and cast; however, additional recordings are still being process and will improve our power to test this hypothesis. While simple analyses of acoustic signals are promising in depauperate pollinator communities, such as alpine meadows, more sophisticated approaches may be necessary in more complex communities.

2. Introduction

The success of pollinators is essential to supporting and maintaining our agricultural food security. 70% of the main crops produced for human consumption worldwide are dependent on the ecological services of pollinators (Gallai, et al., 2009). The value of ecological services provided by native insect pollinators in the United States was estimated to be approximately

$3.07 billion annually (Losey and Vaughan, 2006). The majority of insect pollinators are bees. There are 4,500 species of bees that can be found in North America (Calderone, 2012). Recent declines in bee populations pose a major threat to agricultural production and worldwide concern towards the conservation of pollinators has increased. Habitat fragmentation, insecticides, the intensification of agriculture, and the effects of climate change are a few factors that are believed to have impacted bee populations (Calderone, 2012).

Evidence suggests that bumblebee (*Bombus* spp.) populations are at a decline in terms of local abundance and habitat range (Williams, Osborne 2009). Frequent, large scale surveys are necessary to identify the prominent mechanism(s) driving these declines. Traditional surveys of directly collecting bees in the field are time consuming and labor intensive. Acoustic monitoring is an alternative method with less of a direct impact on the bumblebees. Bumblebees create vibrations, or buzzes, as they fly, forage, or act in defense. The buzzes of some species differ in amplitude and duration in accordance with differences in morphological features (De Luca, et al. 2014). Buzzes recorded in the field could provide information concerning population size diversity in an area. Since acoustic surveys reduce the impacts of human activity, acoustic monitoring is a less intrusive method. Recorders could be placed in remote locations to survey bumblebees as they forage for long periods of time, collecting data simultaneously from multiple locations.

Here, we explore the effectiveness of acoustic survey techniques for monitoring bumblebee pollination services to wild blueberry and huckleberries. I predicted that buzz density collected via acoustic monitoring would be similar to the bee density estimates collected using traditional survey techniques. We determined whether species and castes differed in buzz frequency via flight cage experiments. I expect buzz frequency to correlate with bee body size with larger bees, such as queens, producing buzzes with lower frequencies than smaller workers and males.

3. Methods and Study Site

3.1: Study Site:

This research was conducted at Brookhaven National Laboratory in Upton, NY beginning in late May to mid-August, 2016. Brookhaven National Laboratory comprises of 5,300 acres of federal land within the Long Island Central Pine Barrens region. In the undeveloped areas of the laboratory, a fire gradient was established among four 15 acre sites : two of which have never been burned, one which was burned once in the past 6 years, and one which was burned twice in the past 6 years. One 50m circular plot was established at each site. Our sites were primarily comprised of Huckleberry (*Gaylussacia* spp.) and Blueberry (*Vaccinium* spp.) plants. During our collections, we focused on these native plants because they play a significant role in providing for pollination services within this particular ecosystem.

3.2: Traditional and acoustic surveys

Acoustic survey methods were compared with traditional netting methods. A Wildlife Acoustics Song Meter SM4 recorder with two SMM-A2 Acoustic Microphones (one standing 35cm tall and one standing 70cm tall) were placed within each of the sites randomly. Flight buzzes and pollination buzzes were recorded from 0900 to 1500 for three days per week. We conducted traditional surveys concurrently, traversing each 1963.5m^2 area plot at a steady pace for 30 minutes and collecting all bumblebee and blueberry bees observed foraging on Huckleberry and Blueberry plants. At the beginning and end of each collection period, we recorded the start time, air temperature, wind speed, and relative humidity using an anemometer.

To estimate body size, the intertegular space and wing length of each bee were measured. The bees were identified to species and caste, marked with a non-toxic paint pen to avoid repeat measurements and released. Fruiting success was also analyzed after collecting bagged blueberry and huckleberry plants from all of the sites and conducting fruit counts.

In order to begin data analysis of bumblebee abundance and diversity at the sites, the number of buzzes and frequencies were observed from 30 minute clips, usually recorded prior to a bee collection period. These counts were then compiled and analyzed to observe if buzz density and bee density. We used the editing software Audacity (Audacity 2.1.2) to extract the peak frequencies (Hz) of three buzzes from each individual bumblebee. To control for local variation in amplitude, we calculated a sliding average of buzz frequency across three amplitudes.

3.3: Flight cage experiments

In order to determine whether bee species differed in their acoustic signatures, we conducted controlled experiments using two different types of enclosures called flight cages. One rectangular structured flight cage (1m x 1m x 0.5m) created with PVC piping and insect netting was designed for bees to forage and fly around short or low-lying plant species. The other flight cage was structured similarly to a tent (2m x 2.5m x 1.5m) and stood taller in order to surround taller plants. We recorded bees as they foraged from naturally occurring patches of host plants (including *Asclepias syriaca*, *Echium vulgare*, and *3*). Four microphones attached to stakes were placed in the ground equidistant to each other among the plant species and connected to two Wildlife Acoustics Song Meter SM4 recorders. In the 1m x 1m x 0.5m flight cage, shorter microphones (35cm tall) were used, while taller microphones (70cm tall) were used in the 2m x 2.5m x 1.5m flight cage. The fourth microphone was designated as the close-buzz microphone, and used to follow the bumblebees within 25 cm as it foraged for up to 10 minutes. Bees were measured, identified to species and caste, and marked to reduce repeat recordings.

3.4: Data Analysis

To determine the effectiveness of the acoustic monitoring technique, we tested the goodness of fit for buzz density relative to bee density. Then, we tested the relationship between each estimate of bee density (traditional and acoustic) and the number of fruits per stem to see which better predicted pollination services. We ran a general linear model with bee or buzz density as a continuous predictor variable and plant species as a random effect. We used mixed-effect ANOVAs to test for differences in buzz frequency by species and cast. In order to control for variation among individual bees, they were treated as a random factor for this test. Since we were unable to record *B. impatiens* queens in flight, we conducted two separate tests. A one-way mixed effect ANOVA was run for the data on the queen bees, with species set as the fixed factor. A two-way mixed effect ANOVA tested for differences among males and workers, with species and caste both set as fixed factors.

4. Results

4.1: Bumblebee density and abundance analysis via acoustic monitoring

We compared the number of buzzes recorded to the number of bee collected using traditional methods to test the reliability of acoustic survey methods. Unexpectedly, there was no correlation found between traditional and acoustic estimates of bee density (fig. 1). We found a positive correlation between fruit abundance and bee density collected via traditional methods ($F_{1,5}$ = 75.25, P = 0.0003; fig. 2A). In contrast, there was a negative correlation between fruit abundance and buzz density collected via acoustic monitoring ($F_{1,5}$ = 12.84, P = 0.016; fig. 2B), indicating that acoustic signals are not reliable estimates of pollination services in this system.

4.2: Close buzz analysis

The buzz frequencies among workers and males ($F_{1,12}$ = 0.58, P = 0.57) of the three species did not differ significantly (fig. 3A). Similarly, the buzz frequencies of *B. impatiens* and *B. bimaculatus* queens ($F_{1,3}$ = 0.074, P = 0.80) also did not differ significantly (fig. 3B). Our results indicate that buzz frequency averaged at approximately 150Hz for each species that were males and workers, with the exception of worker *impatiens*. The average buzz frequency for *B. impatiens* and *B. bimaculatus* queens were slightly lower, averaging at about 130-140Hz. These results, however, were not significant enough to conclude that they produce different frequencies unique to their species.

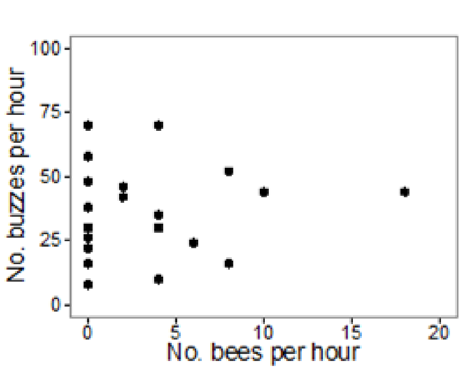

Figure 1. The relationship between buzz density and bee density at four sites located at Brookhaven National Lab, Upton, NY.

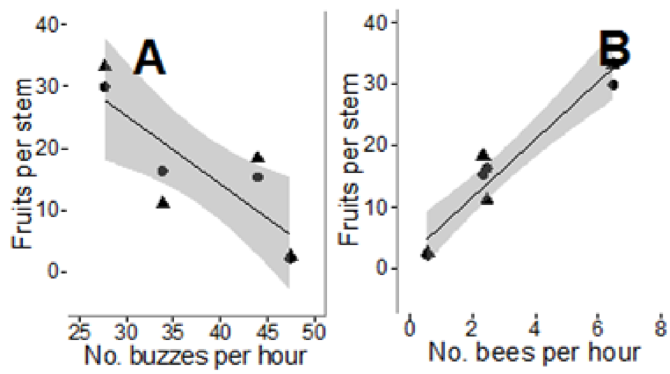

Figure 2. The relationship between reproductive success (i.e., fruits per stem) and bee density estimated by traditional methods (A) and acoustic methods (B).

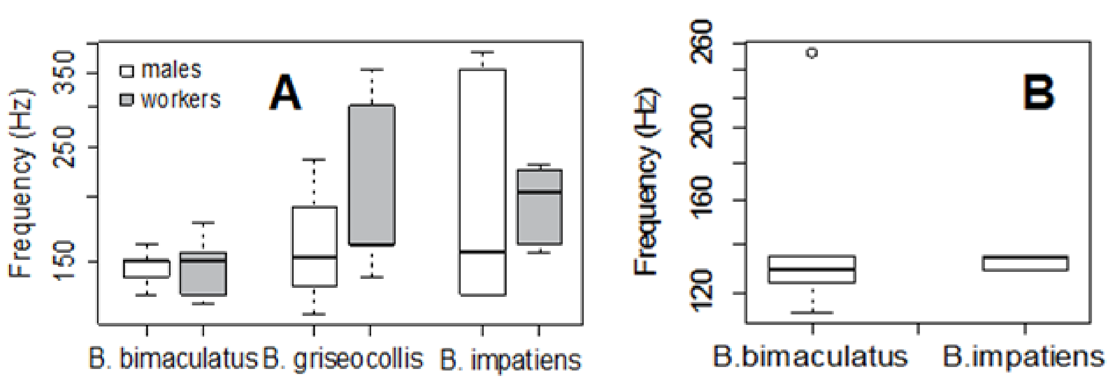

Figure 3. Flight buzz frequency of workers and males (A) and queens (B) did not differ among castes or species.

5. Discussion

Our results indicate that buzz density did not reflect bee activity or population density. These results differ from the results obtained at the alpine study system in Colorado (Miller-Struttmann et al. *in preparation*). There may have not been any correlation with our data because we did not set a specific range of buzz frequencies to count. As a result, we likely included buzzes in our data that did not originate from bees (ie. flies). In order to improve our methods, we would have to limit which buzzes are accepted in our counts based on frequency. As we expected, traditional methods of bee density effectively estimated pollination services, as indicated by a positive relationship between bee density and fruit set (Fig. 2A). On the other hand, estimates resulting from acoustic monitoring did not perform well as predictors of pollination services (Fig. 2B). As suggested earlier, the negative correlation between buzz density and fruit set suggests that buzzes could have originated from other insects that were not effective pollinators.

Acoustic monitoring was also tested to compare buzzes of bumblebees by species and caste. Our results, however, unexpectedly concluded no significant differences between buzz frequencies of *B. bimaculatus*, *B. impatiens*, and *B. griseocollis* bumblebees. However, our results are preliminary, as we only tested 3 individuals per species. Recordings from additional individuals will be analyzed during the fall.

Our collection efforts in the beginning of the summer were hindered by the lack of bumblebees in the field. We believe that the effects of climate change can explain the disappearance of bees in June. This year's winter was much more mild than what is typical, which could have resulted in bumblebees to begin foraging earlier than expected. The bumblebees may have quickly depleted their fat reserves, their source of energy, to begin foraging earlier and thus ran out of energy earlier than usual. The attention and concern of declines in bee populations has risen as of recent years, and the success of acoustic monitoring as the primary method of collecting bee abundance and diversity is hugely significant towards pollinator conservation.

While the results we obtained were not what we expected, the process of acoustic monitoring still in its early stages of being used for detecting bumblebee diversity and abundance. Implementing acoustic survey methods over the traditional methods would be significantly better for bee populations as it provides less of a disturbance to their habitats and behavior. It would not only be beneficial to bee populations, but also to researchers since it would be a much more efficient method when successfully and properly carried out. Population surveys would be conducted more quickly and conveniently. Acoustics is promising for monitoring in some systems (ie. the alpine study system in Colorado), therefore we hope to see if it will work in other buzz pollinating systems in future studies.

6. Acknowledgements

I would like to thank Emma Squires and Emma Hert for assistance with my data collections. This project was supported in part by the U.S. Department of Energy, Office of Science, Office of Workforce Development for Teachers and Scientists (WDTS) under the Science Undergraduate Laboratory Internships Program (SULI).

7. References

Calderone, NW. 2012. Insect Pollinated Crops, Insect Pollinators and US Agriculture: Trend Analysis of Aggregate Data for the Period 1992–2009. PLoS ONE 7(5): e37235. doi:10.1371/journal.pone.0037235

De Luca, P. A., Cox, D. A., & Vallejo-Marín, M. (2014). Comparison of pollination and defensive buzzes in bumblebees indicates species-specific and context-dependent vibrations. *Naturwissenschaften, 101*(4), 331-338. doi:10.1007/s00114-014-1161-7

Gallai, N., Salles, J., Settele, J., & Vaissière, B. E. (2009). Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological Economics, 68*(3), 810-821. doi:10.1016/j.ecolecon.2008.06.014

Losey, J.E., and Vaughan, M. 2006. The economic value of ecological services provided by insects. Bioscience, 56:311–323.

Williams, P. H., & Osborne, J. L. (2009). Bumblebee vulnerability and conservation world-wide. *Apidologie, 40*(3), 367-387. doi:10.1051/apido/2009025

The Xerces Society. (n.d.). Retrieved August 5, 2016, from http://www.xerces.org/