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The Effects of Environmental Changes and Human Disruption on Western Honey bees' (*Apis mellifera*) Behaviors

Abstract

For this project, I will be examining the Western Honey bees' behavior in response to different environmental factors. These factors include playing different predator sounds, human disruptions, and how their responses are affected by different climate changes such as rainy weather and cold days. To better understand the interaction of honey bees towards these environmental changes, I observed how these would prompt aggressive and defensive honey bee behaviors, which is distinctly noticed by their buzzing noises and their body movements. These observations and experiments will take place during the daytime since honeybees are diurnal and will be compared in two locations, which are Hidden Lake Park in Tracy, CA and the Las Positas College Campus in Livermore, CA. For one experiment, I played different frequencies to mimic the frequency of predator noises and recorded qualitative observations of the honey bees' responses. For another experiment, I played predator noises (Oriental hornet and California Scrub Jay) and recorded whether the honey bees would move or display aggressive behavior, and I recorded the honey bees' frequencies in response to these sounds each over a period of 180 seconds in 30 second intervals. Lastly, I stood in front of a rose bush to demonstrate how human activity disrupts honey bee pollination and I recorded their movement, behavior, and frequencies. The results of this study support that environmental changes cause aggressive and defensive

behaviors to emerge, and the most apparent is playing predator sounds of the Oriental hornet, since this is one of the honey bees most noxious predators. Another significant trigger was the extremely high frequencies played as this caused increased buzzing noises, rapid wing movements, antennae to be pointed forward, and hissing noises. Playing California scrub jay sounds and interfering with the environment also agitated honey bees as well causing their buzzing noises to increase, but these were not as prominent as playing the Oriental hornet sounds and frequencies.

Introduction

Background:

Western honey bees have complex social lives and most create colonies with a single fertile female, with many normally non-reproductive females and a few fertile males. Honey bees are specialized to be pollinators, engaging in behaviors with physical attributes that are specifically directed to gather nectar and pollen to feed the brood. Their individual colonies can house ten to thousands of bees and their colony activities are organized by complex communication between individuals, through pheromones and the dance language (Evans and Butler 2010).

Typical Defensive Behaviors:

Guards and stingers are two populations that usually perform nest defense. When encountering large predators, some guards may fly towards it immediately, while others extrude their sting, raise their abdomen and release alarm pheromones produced by their stinger, and this alerts other honey bees of a possible threat. Of course, guards cannot defend against large predators alone, and therefore, they rely on the large recruitment of bees in their colony. Honey bees also produce piping or hissing sounds when hornets are around, and this is described as 'shimmering.' Hissing is an innate response to harmful stimuli, and this behavior is also produced in response to electric shocks. It is still unknown if these hissing sounds are used as an alarm to signal the colony, used to pose as a threat to hornets (which are known to use high-frequency sounds for communication) or are just distress sounds. Another key element in the defensive behavior of honeybees is a pheromonal, alarm blend that sends signals of threats to the whole colony. People such as beekeepers recognize the characteristic banana-like scent exuding from the hive when the bees are disturbed. The sting apparatus itself carries this alarm pheromone which can alert and attract bees and provoke stinging attacks (Nouvian et al. 2016).

Causes of Pollinator Loss

Because of honey bees' wide cultivation, this species is the single most important pollinator for agriculture globally. In the process of foraging, they fertilize numerous plants, which makes it possible for plants to reproduce and form fruit. This is significant because the number of young that survive adulthood is determined largely by the amount of pollen collected, since bees enable plants to breed with individuals in entirely separate areas, keeping the plant's offspring healthy and more resilient (Wilson and Carril 2016). Unfortunately, there is evidence in pollinators declining and the most notable is *Apis mellifera*, the semi-domesticated species whose pollination is managed and used to purchase. Today, there are around 3,500 species of bees that are left to pollinate crops and the wild plants in North America, and their contribution to crops is estimated to be around $3 billion annually (Blaylock and Richards 2009). These bees are part of the family, Apidae, and considered to be the most economically significant bee pollinators in the world since they are responsible for nearly the entire almond crop of North America and numerous other fruits and vegetables (Wilson and Carril 2016).

When the weather turns cold, these colonies tend to die out and only some survive the winter since there are no flowers to provide nectar. Unlike other social insects such as wasps or bumblebees, they are not hibernators since they consume honey and shiver to generate heat that allows them to withstand the winter temperatures (Evans and Butler 2010). Another reason why this species of honey bees is dying out is due to human activity. As of 2006, colony collapse disorder or CCD of honeybees was 32% and has been increasing in following years. Researchers have found that this is likely to result from a variety of pathogens that include Varroa mites, viruses, and issues related to nutrition, such as malnourishment due to poor forage availability. Furthermore, climate change may affect bee nutrition as flowering seasons fluctuate, and these disrupt the sync with the bees' need for food resources since bees synchronize their foraging with the plants' cycle. Most bees exhibit "floral constancy," which means that they show a preference for going to the same flower species. The pollen carrying structures are modified according to the location of pollen within their favorite plants and the particular qualities of the pollen they carry (Watanabe 2014). These declines are "associated with habitat loss, nontarget pesticide exposure, and invasive species, [and] changes in the distribution of pollinators and the plant species they visit [have] lead to loss of synchrony and disruption of migratory routes" (Blaylock and Richards 2009). This loss of pollinators has recently received much attention and has influenced researchers to increase public awareness about the negative effects of pollinator loss and what society can do to protect these insects. However, pollinating insects like bees often induce anxiety among people, and negative emotions of fear (such as the fear of being stung) and disgust prompt a considerable lack of support for conservation and appropriate plans for protection (Schönfelder and Bogner 2017). That is why due to my interest in raising awareness to protect pollinators, I want to conduct these experiments and observe how honey bees react to these environmental disruptions because colony collapse disorder is a serious issue with the number of honey bees declining in North America, since our continent extremely depends on these insects for food and growing economy.

Question

What is the effect of environmental factors (predator noises, human disruption, climate change) on Western Honey bee behavior in terms of aggression and defense (buzzing noises/ body movements)?

Materials and Methods

- Ink Pen
- Field Guide notebook: 100 pg labelled composition book
- Frequency Detector from Apple store app: “Audio Spectrum Analyzer dB RTA” by developer Elena Polyanskaya
- Frequency Generator from Apple store app: “Sonic v” by developer Von Bruno
- Video recorder iPhone 7 camera
- YouTube video: “Asian giant hornet sound” by Francis Eble
- YouTube video: “Sounds of a Scrub Jay” by Saunie H.

1. To conduct experiments, go to location: 1355 Eastlake Cir, Tracy, CA 95304 Hidden Lake Park to observe honey bees between the times 11:00am-4:00pm for one or more hours.
2. Bring field guide notebook and record with an ink pen the weather measurements (temperature, wind speed, air quality, etc.), and physical description of the area (vegetation, topography, physical features, presence of other organisms, etc.)
3. Draw location with bees, sketches of bees (posture, population, distribution, etc)

4. Find an area where there is a fairly large population of bees such as a rose bush to conduct observations and experiments.
5. Conduct experiment with a frequency generator to play different frequencies. Record qualitative observations to each played frequency such as body movement, buzzing noises, as well as detected frequency from honeybees with the frequency detector
    a. These frequencies will be 0 Hz, 250 Hz, 1500 Hz, 2750 Hz, 4000 Hz, and 6000Hz
    b. If these need to be increased play frequencies: 4000 Hz, 6000 Hz, 10,000 Hz, 12,000 Hz, 15,000 Hz, and 18,000 Hz
    c. Each of these frequencies will be played for 2 minutes each.
6. Conduct experiment witnessing behaviors in response to different predator sounds
    a. Play California scrub jay (*Aphelocoma californica*) sounds for around 10 minutes using the YouTube video: "Sounds of a Scrub Jay"
    b. Record how many bees (moved away/ stayed) and how many created a hissing noise and create a pie chart
    c. Record Frequency (Hz) using the Frequency Detector in intervals of 30 seconds for a total of 180 seconds and created a scatter plot
7. Repeat Step 6 instead with Oriental hornet (*Vespa orientalis)* sounds using the YouTube video: "Asian giant hornet sound"
8. Conduct experiment by standing in front of rose bush
    a. Record frequency of bees using the frequency detector in intervals of 30 seconds for a total of 180 seconds and create a scatter plot
    b. Record how many bees (moved away/ stayed) and how many created a hissing noise and create a pie chart

9. Repeat these experiments at 3000 Campus Hill Dr, Livermore, CA 94551 Las Positas College behind the pool area where there are rose bushes and compare honey bee behaviors with those in Tracy CA.
10. After experiments are collected, write questions to address, facts that need to be clarified, and plans of what to do next.
11. Research scholarly articles that correlate with experiments conducted

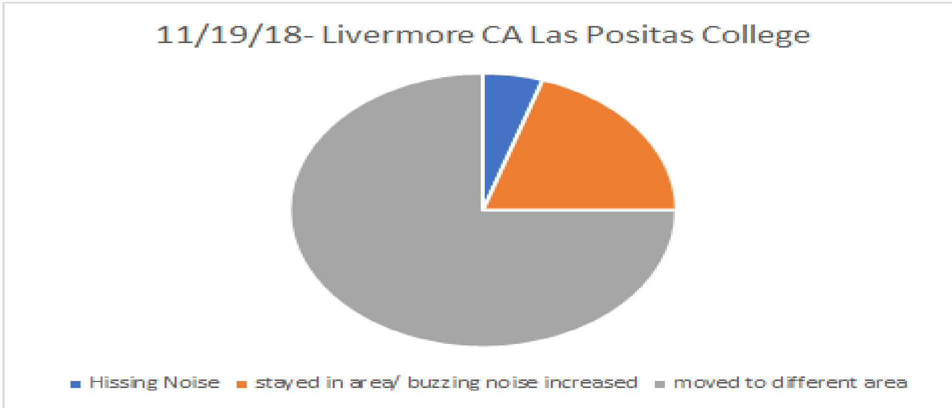
Results

Table #1: Qualitative observations of how honey bees respond to different frequencies 11/19/2018, Livermore, CA Las Positas College

0 Hz	Control; buzzing noise, no distinct effect on behavior
250 Hz	No distinct effect on behavior, bee moved away from rose bush
1500 Hz	Bee stays in rose bush buzzing noise remains constant
2750 Hz	Buzzing noise slightly increases, movement from one rose to another increases
4000 Hz	Buzzing noise increases, movement from one rose to another increases
6000 Hz	Extremely increased buzzing noise, movement stays in one place, antennae pointed forward

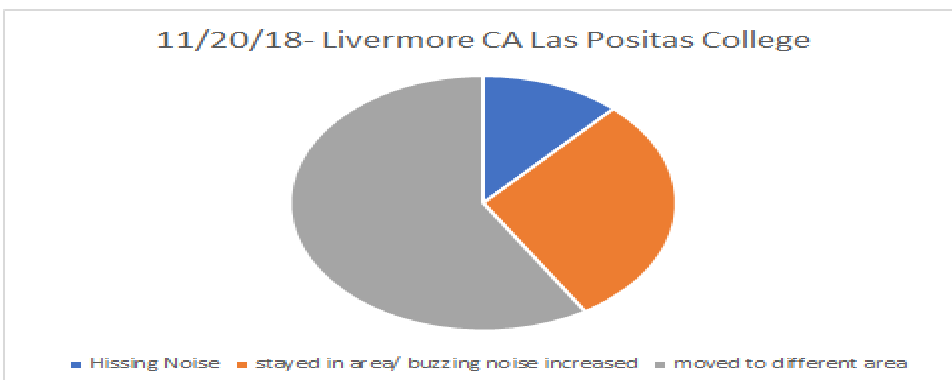
Summary of Table #1: Overall, the buzzing noise remained constant, and a trend of increasing defensive behaviors with increasing frequency is observed. A limitation to this experiment is that the frequencies played are not great enough since bees have a dominant frequency of 6 kHz when disturbed and later experiments should test higher frequencies to prompt defensive behaviors.

Graph #1: Pie Graph demonstrating ratios of bees' responses to Oriental hornet predator sounds



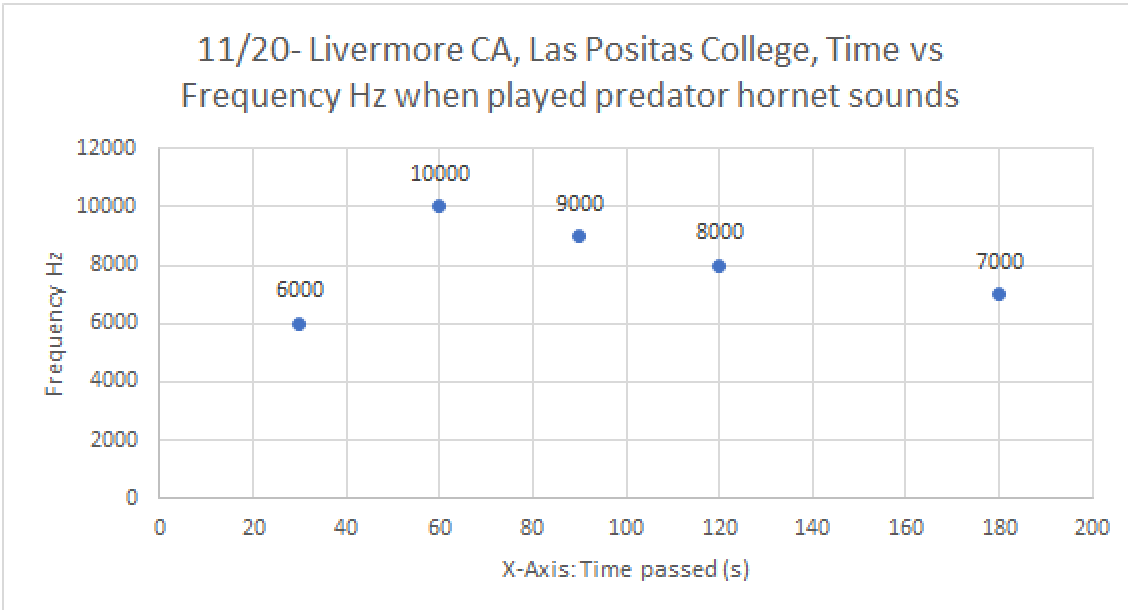
Summary of Graph #1: This demonstrates that 5% or 1 bee of the present bee population of 20 created a distinct hissing noise, 25 % or 4 stayed in the area and increased their buzzing noise, 75% or 15 of the bees simply moved to a different area over the course of around 15 minutes when the sounds were played.

Graph #2: Pie Graph demonstrating ratios of bees responses to Oriental hornet predator sounds



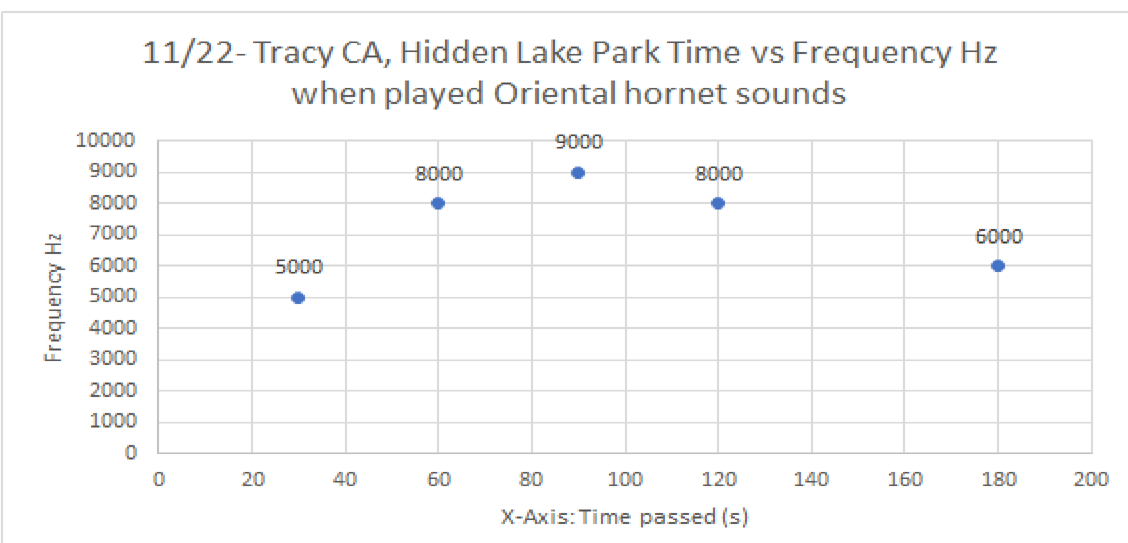
Summary of Graph #2: This demonstrates 12 % or 2 bees of the present honey bee population of 17 made a hissing sound, 29% or 5 bees stayed in the area and increased their buzzing noise, and 59% or 10 bees moved to a different area over the course of around 10 minutes. This demonstrates defensive and aggressive behavior with the hissing noises created by the bees.

<u>Graph #3:</u> Scattered Plot demonstrating Frequency (Hz) recorded from honey bees when played Oriental hornet predator sounds



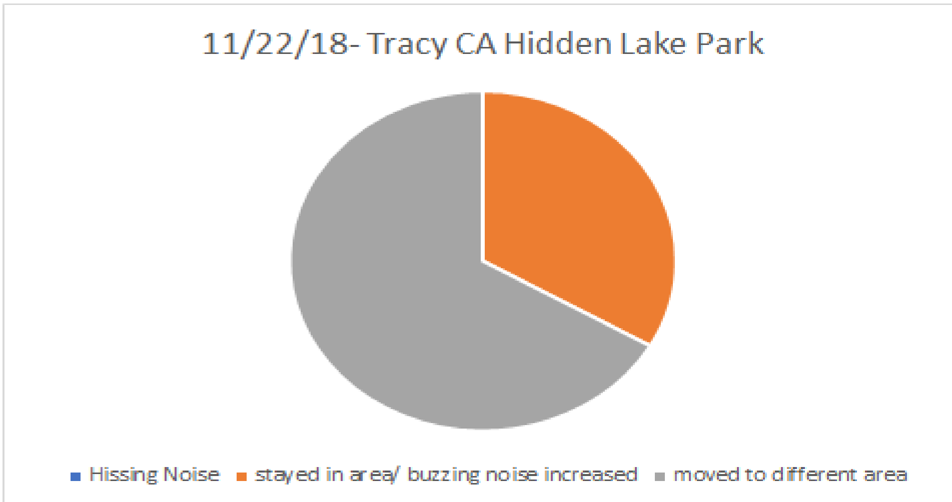
<u>Summary of Graph #3:</u> This demonstrates how honey bees were affected playing the predator hornet sounds as their frequencies increased after 60 seconds but this decreased after 90 seconds.

<u>Graph #4:</u> Scattered Plot demonstrating Frequency (Hz) recorded from honey bees when played Oriental hornet predator sounds



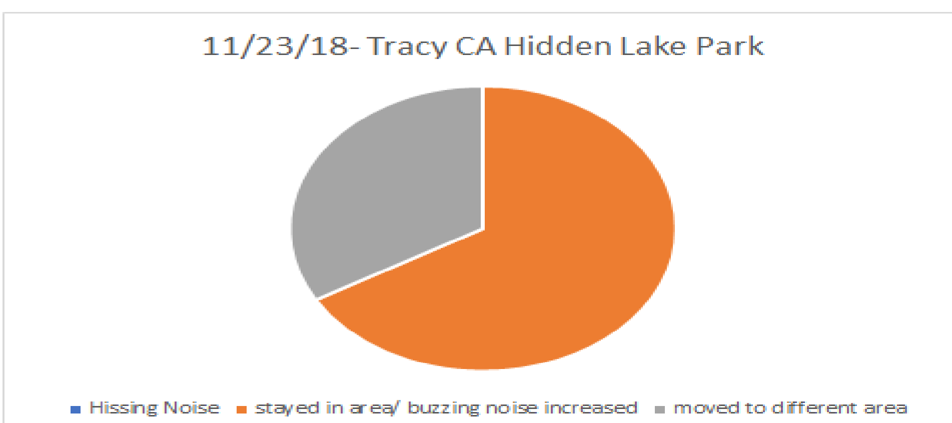
Summary of Graph #4: This demonstrates how honey bees were affected playing the predator hornet sounds as their frequencies increased after 60 seconds but this decreased after 120 seconds.

Graph #5: Pie Graph demonstrating ratios of bees' responses when I stand in front of rose bush



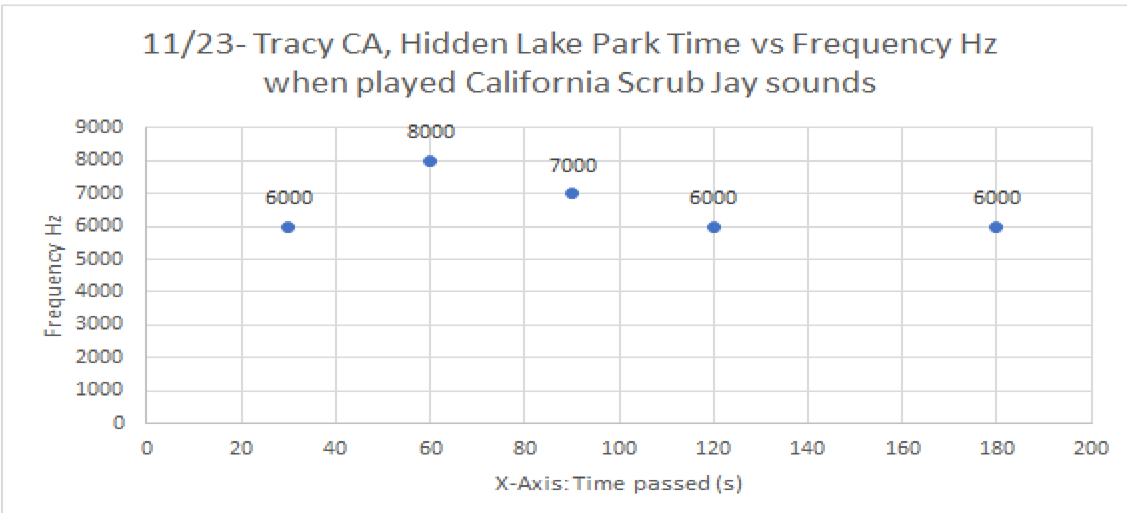
Summary of Graph #5: This demonstrates 33% or 4 bees of the present honey bee population of 12 stayed in the area and buzzing noise increased, 66% or 8 bees moved to a different area while none of the bees made a distinct hissing sound. I stood in front of the rose bush for around 10 minutes.

Graph #6: Pie Graph demonstrating ratios of bees responses to California Scrub Jay sounds



Summary of Graph #6: This demonstrates 66% or 10 bees of the present honey bee population of 15 stayed in the area and buzzing noise increased, 33% or 5 bees moved to a different area while none of the bees made a distinct hissing sound. The bird sounds were played for around 15 minutes.

Graph #7: Scattered Plot demonstrating Frequency (Hz) recorded from honey bees when played California Scrub Jay sounds



Summary of Graph #7: This demonstrates how honey bees were affected playing the predator California scrub jays as their frequencies increased after 60 seconds but this decreased after 90 seconds.

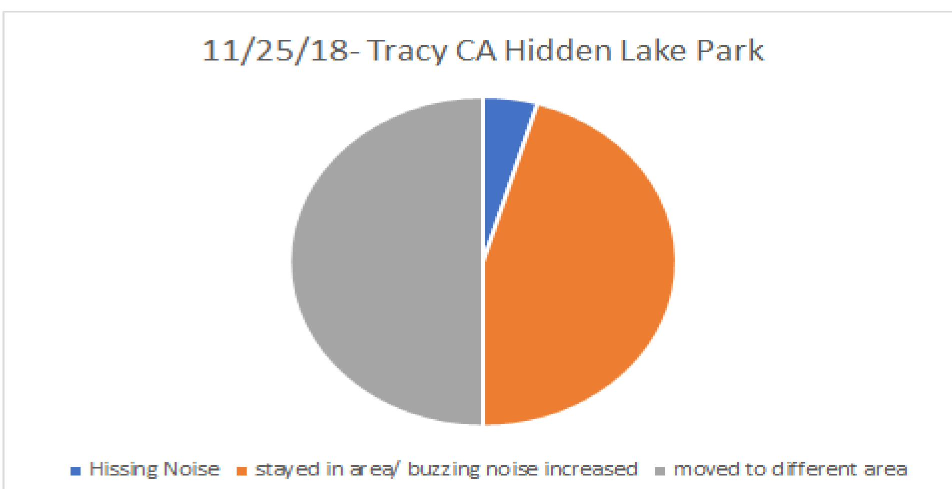
Table #2: Qualitative observations of how honey bees respond to different frequencies

11/23/2018, Tracy CA Hidden Lake Park

0 Hz	Control; buzzing noise, no distinct effect on behavior
2500 Hz	Buzzing noise increased, bee moved away from rose bush
4000 Hz	Bee stays in rose bush buzzing noise stays constant
6000 Hz	Buzzing noise increases, movement from one rose to another increases
10,000 Hz	Extremely increased buzzing noise, movement stays in one place, antennae pointed forward, bee created hissing sound
12,000 Hz	Extremely increased buzzing noise, movement stays in one place, antennae pointed forward, a couple bees created a hissing sound, rapid wing movement

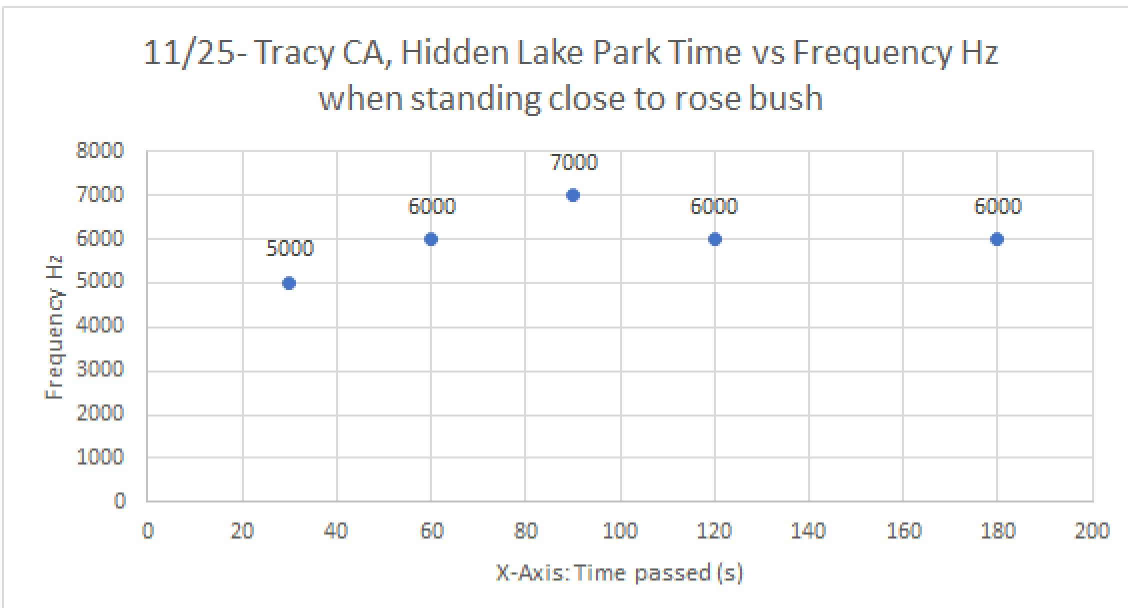
Summary of Table #2: Overall, the buzzing noise increases, and a trend of increasing defensive behaviors with increasing frequency is observed. Bees were more affected by the larger frequencies tested.

Graph #8: Pie Graph demonstrating ratios of bees responses to Oriental Hornet sounds



Summary of Graph #8: This demonstrates that 5% or 1 bee of the present bee population of 22 created a distinct hissing noise, 45 % or 10 stayed in the area and increased their buzzing noise, 50% or 11 of the bees simply moved to a different area over the course of around 15 minutes when the sounds were played.

Graph #9: Scattered Plot demonstrating Frequency (Hz) recorded from honey bees when I stood in front of rose bush as bees were pollinating



Summary of Graph #9: This demonstrates how honey bees were affected while I stood in front of the rose bush, as their frequencies increased after 60 seconds but this decreased after 120 seconds.

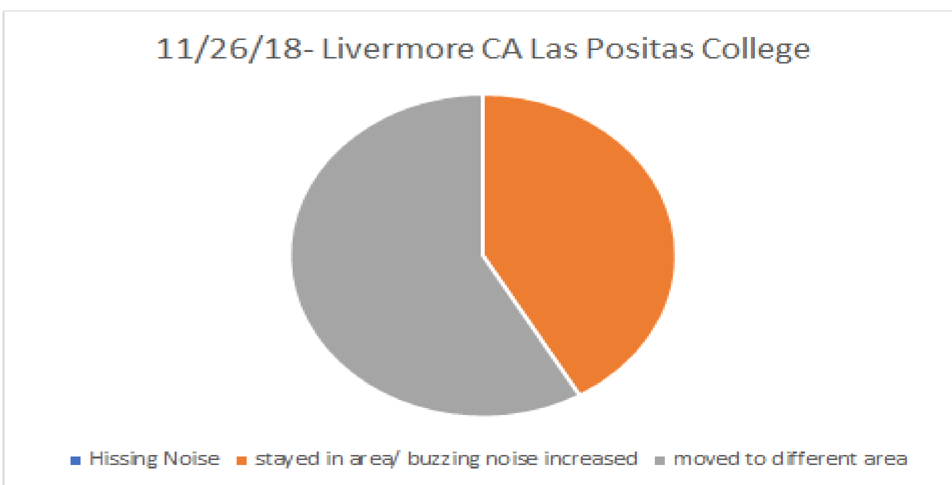
Table #3: Qualitative observations of how honey bees respond to different frequencies

11/25/2018, Tracy CA Hidden Lake Park

0 Hz	Control; buzzing noise, no distinct effect on behavior
4000 Hz	Buzzing noise increased, bee moved away from rose bush
6000 Hz	Bee stays in rose bush buzzing noise stays constant
10,000 Hz	Extremely increased buzzing noise, movement increases from one rose bush to another, antennae pointed forward,
15,000 Hz	Extremely increased buzzing noise, movement stays in one place, antennae pointed forward, some bees created hissing sound
18,000 Hz	Extremely increased buzzing noise, movement stays in one place, antennae pointed forward, some bees created hissing sound

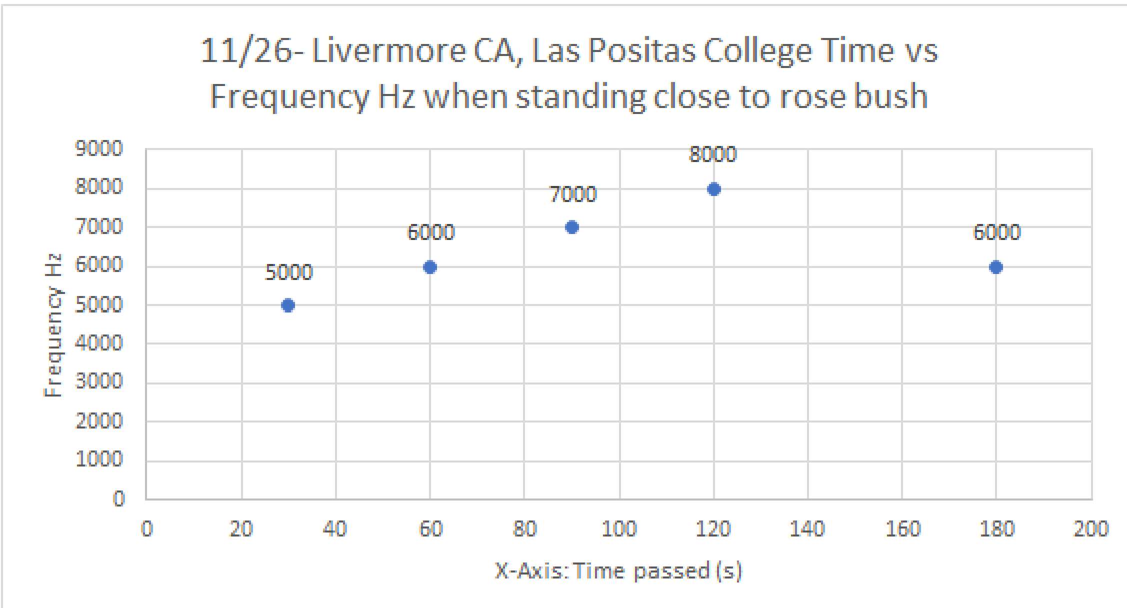
Summary of Table #3: Overall, the buzzing noise increases, and a trend of increasing defensive behaviors with increasing frequency is observed. Bees were more affected by the larger frequencies tested.

Graph #10: Pie Graph demonstrating ratios of bees responses to California Scrub Jay sounds



Summary of Graph #10: This demonstrates 42% or 5 bees of the present honey bee population of 12 stayed in the area and buzzing noise increased, 58% or 7 bees moved to a different area while none of the bees made a distinct hissing sound. These sounds were played for around 10 minutes.

Graph #11: Scattered Plot demonstrating Frequency (Hz) recorded from honey bees when I stood in front of rose bush as bees were pollinating



Summary of Graph #11: This demonstrates how honey bees were affected while I stood in front of the rose bush, as their frequencies increased after 120 seconds demonstrating an increasing trend of frequency as time passed but this decreased after 180 seconds.

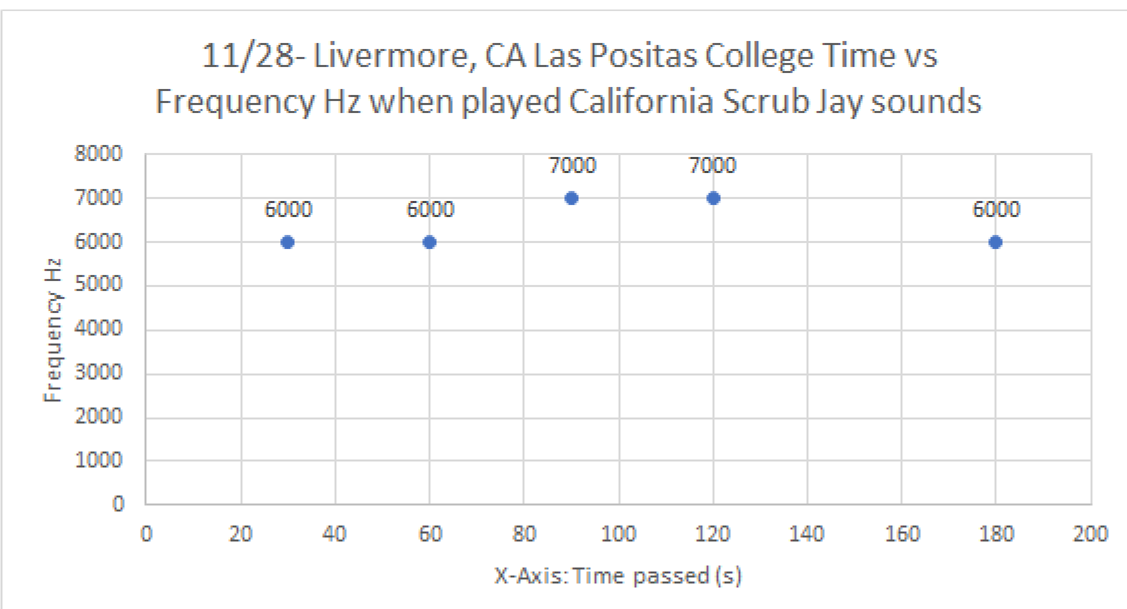
Table #4: Qualitative observations of how honey bees respond to different frequencies

11/26/2018, Livermore CA, Las Positas College

0 Hz	Control; buzzing noise, no distinct effect on behavior
4000 Hz	Buzzing noise increased, bee moved away from rose bush
6000 Hz	Bee stays in rose bush buzzing noise stays constant
10,000 Hz	Extremely increased buzzing noise, movement increases from one rose bush to another, antennae pointed forward
15,000 Hz	Extremely increased buzzing noise, rapid wing movement, movement stays in one place, antennae pointed forward, hissing noise created
18,000 Hz	Extremely increased buzzing noise, rapid wing movement, movement stays in one place, antennae pointed forward, hissing noise created

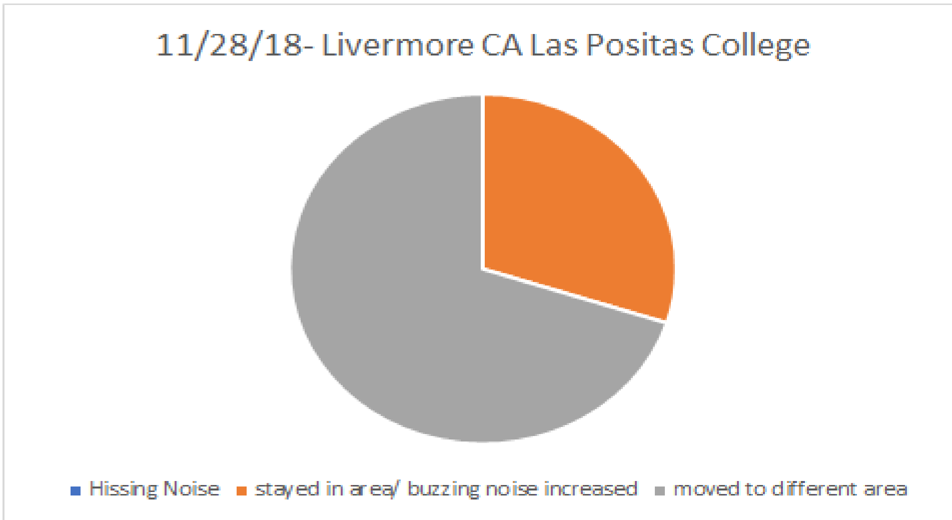
Summary of Table #4: Overall, the buzzing noise increases, and a trend of increasing defensive behaviors with increasing frequency is observed. Bees were more affected by the larger frequencies tested. These are the same for both honey bees in Livermore and Tracy.

Graph #12:



Summary of Graph #12: This demonstrates how honey bees were affected playing the predator California scrub jays as their frequencies increased after 90 seconds but this remained constant and decreased after 180 seconds.

Graph #13: Pie Graph demonstrating ratios of bees responses when I stand in front of rose bush



Summary of Graph #13: This demonstrates 30% or 3 bees of the present honey bee population of 12 stayed in the area and buzzing noise increased, 70% or 7 bees moved to a different area while none of the bees made a distinct hissing sound. I stood in front of the rose bush for around 10 minutes.

Conclusion/ Discussion

In conclusion, it is demonstrated that environmental factors (predator noises, human disruption, climate change) provoke defensive and aggressive Western Honey bee behavior as shown by their recorded frequencies, increased buzzing noises, hissing sounds, and antennae pointed forward. This is seen with the comparison between Tables #3 and #4, in which the higher frequencies played induced increased buzzing noises, rapid wing movement, antennas pointed forward, and hissing noises. Predator noises of the Oriental hornets as seen in Graphs #2 and 8 demonstrated a few bees created hissing noises which is an innate behavior to harmful stimuli, and further demonstrates how these hornets are a major predator to honey bees. Furthermore, Graphs #3 and #4 support that honey bees were agitated by Oriental hornet sounds due to the high frequencies produced. This is because “hornets are particularly known to induce serious damages by killing many individual honeybees or even by destroying entire colonies, occupying the beehive, using its resources (honey, pollen, brood and adult honeybees) to feed their brood” (Papachristoforou et al. 2008). Predator noises of the California scrub jay demonstrated in Graphs #6 and #10 demonstrate no hissing noises, but the honey bees either moved to a different area or stayed in the area and increased their buzzing noises, and this varied between the two locations. This demonstrates that the bees either knew the noises were artificial since they stayed in the same location and continued pollination, or they viewed the noises as a threat that cannot be combatted alone, and therefore moved to a different area. Another possible reason why the honey bees moved was to release alarm pheromones and this is typical of honey bee behavior to recruit other bees to handle bigger predators such as the blue jay. Graphs #7 and #12 demonstrate the scrub jay sounds were a threat for the first minute due to the increased frequency, however this decline in frequency of the honey bees support how the honey bees may have come to realize the predator was not real. The experiments, in which I stand in front of the rose bush, mimic the disruption caused by human activity, and this is demonstrated by Graphs #9 and #11. These graphs demonstrate the bees were triggered by my presence due to the increased frequencies over a period of almost two minutes, however the frequencies returned to normal once they did not view me as a threat since I stood still. Graphs #5 and #13 demonstrate how the honey bees mainly moved to a different area and continued pollinating, and this accentuates how honey bees are extremely busy workers and are focused on collecting nectar from the flowers due to the shortage of time in the day. This is because some plants only offer nectar and pollen at specific times of the day, and many species of bees learn to adapt their foraging to the availability of their local flowers (Evans and Butler 2010).

With this information that honey bees become easily disrupted from their everyday work, awareness can be raised about the importance of animal conservation and at the same time foster a pro-environmental behavior in the education of young children, so they are ingrained to help the environment. This education should focus on positive attitudes toward animals, since negative emotions may prohibit successful learning, such as fear knowing that bees can sting people (although this happens very rarely). People, especially those in school, should understand the interrelations with the environment and key position held by pollinating animals, which is most notably honey bees. Educational programs that focus on conserving pollinators can reduce the danger perceived through false information and instead support a newfound interest (Schönfelder and Bogner 2017).

A study conducted that relates to my experiment is one by Nouvian et al. and they completed a brief overview of the methods used to study honey bee aggression and examine how these could be used to gain further knowledge of these behaviors. Nouvian also studied defense against other insects and large predators and discovered that honey bees would fan their wings at a very high frequency and release pheromones that signal a threat to the whole colony. (Nouvain et al 2016). This was useful to my project as it was necessary to differentiate between the different aggressive and defensive behaviors and why these were displayed. However, this analysis required controlled laboratory assays to study the aggression of individuals, and this is different from my experiment because the honey bees were mostly observed as a populace.

Another study conducted that relates to my experiment is one by Papachristoforou et al. and they discovered both the Oriental hornet and European honey bee engage in distinct acoustic behavior, producing a hissing sound of unexpectedly high frequency. When analyzing these hissing sounds during an extended sample of artificial attacks by hornets, it was discovered that honeybees can produce sounds of a wide frequency range with a dominant frequency of around 6 kHz (Papachristoforou et al. 2008). This was useful information as it influenced me to choose to play Oriental hornet sounds and determine what frequencies to play to provoke high frequency hissing sounds from the honey bees, which are indicative of their defensive and aggressive behaviors.

Strengths	
Comparing Honey bee behavior in two locations	Having two locations is useful because this allows one to compare and view the similarities and differences in honey bee behavior between the two areas. This also allows one to acquire more information and observations to convey a more detailed, accurate representation of honey bee behavior. It was observed in this project there were no distinct differences between the two areas since Tracy and Livermore had somewhat similar weather patterns. It would be interesting to expand this research to different states where the different weather patterns can impact honey bees differently, and this would allow one to gather more information to demonstrate Honey bee behaviors of the United States.
Using a variety of experiments	In this project, I used a variety of experiments which include playing different frequencies, playing different predator sounds, and standing in front of the rose bush where the honey bees were collecting nectar. Having a variety of experiments allows one to evoke different behaviors of honeybees and learn

	what and why honey bees are triggered by those behaviors. For example, studying the wing movements is an "efficient way to transfer information about foraging distance as it was demonstrated that the fundamental frequency of sounds can be easily modulated through opening and closing the wings" (Łopuch and Tofilski 2017).
Weaknesses	
Only performing experiment during cold weather	The rainy period of weather "keeps the bees inside the colony, forcing them to use up food that had been stored for the winter...and the pollen may not contain the usual proteins, vitamins, and other substances required by the bees." When bees have poor health, this means they are more susceptible to disease, including about twenty known viruses to which bees are vulnerable (Evans and Butler 2010). This was a disadvantage to my study because the weather correlates to why a small population of bees were observed. Since the weather is not warm, the flowers available have a nectar shortage since flowers do not flourish this time of year. To resolve this solution, it is better to observe the honey bees during the day and on warmer climates such as the months of September and beginning of October.
Bees may be aware of artificial environment	This was observed with the predator noises of California scrub jay. This is because I noticed that the frequencies started to decrease after a minute or two, which signifies that the bees may be aware that the predator is not real. To resolve this solution, it is better to conduct this experiment in a laboratory-

	controlled environment to isolate the bee's frequencies from foreign noises and this would allow one to study individual honey bee behavior.
Air Quality	Due to the wildfires of Butte County, this had a major impact on the air quality of both Tracy and Livermore CA. This air quality may have contributed to why there were very few honey bees around when experiments were conducted. To resolve this solution, it is important to wear protective gear such as a mask and try to not conduct experiments when there are poor weather conditions. During the September, or the beginning of October of the Fall semester is a better option.
Limitations	
Ethical/ Safety Issues	One ethical issue of this project is possibly agitating the honey bees since the frequencies recorded were extremely high, and defensive/aggressive behaviors were displayed. Since these behaviors were displayed, this would disrupt their routine collecting nectar and pollinating the flowers. It is also a safety issue because I stood directly in front of the rose bush where there were numerous bees and they were disturbed by my presence. To resolve this issue, I should have stood a safe distance away from the bees to not put myself at risk.
Only using 2 predator sounds.	In this study, the California scrub jay and Oriental hornet predator sounds were utilized. To resolve this solution to collect more information on honey bee behavior, a variety of predator sounds should be used to gather more observations of how honey bees would respond to specific threats.

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