Determining the Hearing Range of Hummingbirds

By

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ABSTRACT

Hummingbirds are one of few creatures that use incredibly high frequency sounds to communicate with each other. However, biologists wonder whether hummingbirds can hear such unique sounds. Using a female Costa hummingbird, an experiment of 3 locations (each with a speaker and feeder attached) were used to permit the movement of a sound source throughout a series of trials. The procedure was to record whether the hummingbird could make the association and have a higher success rate when finding the food. Using 4 different frequencies (200 Hz, 4 kHz, 10 kHz, 14 kHz) at 33 trials each, the results were all greater than the baseline success rate of 11 for 33. After analyzing the trials using a Chi squared test, each of the frequency blocks obtained significant results. Now that this paper has given some insight on the hearing range of hummingbirds, future researchers can apply this experiment to even lower and/or higher sounds and discover whether hummingbirds can identify and respond accordingly to the sound.

ACKNOWLEDGMENTS

First, I’d like to thank my Lord and Savior Jesus Christ for giving me life up until this point. The journey of my capstone project spanned many months, and God gave me grace to wake up and progress each day. I’d also like to thank my parents for being by my side and helping me through this journey. I was initially hesitant about participating in University Honors, but my parents gave me the encouragement I needed to take the step and be in the position I find myself in today. I’d also like to thank my faculty mentor professor Christopher Clark. Although professor Clark had many commitments as a professor, lead faculty advisor, and faculty mentor, he always found time to discuss my project and give me the constructive feedback I needed.

1. Introduction:

Hummingbirds are small birds that are capable of producing beautiful songs with their throats. The ability to vocalize is key to all species, as it is used to express many emotions such as fear, courtship, aggression, and more. Prior discoveries from Robert Dooling in the book “Nature’s Music: The Science of Birdsong " mentioned that the hearing range of most birds falls within 2 to 8 Khz. However, in 2020, authors Duque et al published a paper titled “High-frequency hearing in a hummingbird”, in which they described a species of hummingbird that has been recorded producing a sound of 13.4 kHz! The authors were honest to acknowledge before conducting their study that “it remains unknown whether these hummingbirds can hear these sounds” (Duque 1), but research needs to be done to discover exactly what the hearing range of a hummingbird is. Intuition would argue that all hummingbirds can hear sounds that high, because if not, what’s the point? As brought up by Duque et al, “for these vocal signals to be effective, the intended receiver should be able to detect and discriminate them” (Duque 1). Only a few studies have focused on the hearing range of hummingbirds. Carolyn Pytte and 2 co authors published an article in 2004 titled “Ultrasonic singing by the blue-throated hummingbird; a comparison between production and perception.” In the article they focused on a blue-throated hummingbird that produces ultrasonic songs, or songs that reach up to 50 Khz! In order to determine if the hummingbird could hear a sound this high, Pytte et al constructed an experiment that used sounds from 1 kHz to 50 kHz for testing. The idea was to inspect for an auditory brainstem response and analyze the results to make an estimation on the hearing range for that specific hummingbird. Their findings proved that the blue-throated hummingbird couldn’t hear sound beyond 7 kHz, which is close to the estimate Robert Dooling proposed back in 2004. As a response to their findings, Pytte came to the conclusion that the ultrasonic song was meant to attract the attention of an animal other than a blue-throated hummingbird. The second study was Duque et al's with the Ecuadorian Hillstar hummingbird that produces a 13.4 kHz sound. Their experiment was to expose the Ecuadorian Hillstar hummingbird to a high frequency song and observe physical and neurological changes to it. The hummingbird demonstrated the ability to hear the song by changing its body posture, tilting its head, and approaching the speaker.

Carolyn Pytte stated in the introduction of her paper that "hearing tests have not been previously performed on any hummingbird species" (Pytte 1), and this was true. I wanted to determine the hearing range of a hummingbird, but do so in a different way. Unlike the hearing tests mentioned by Pytte that simply involve the choosing of sounds for the hummingbird to try and hear, this experiment will use operant conditioning to obtain more detailed results. The outcomes from the operant conditioning side of the experiment would simultaneously provide results regarding the hummingbirds ability to hear a specific sound, since the hummingbird would have to use the location of the sound to approach the reward location. The hummingbird used for this study was a Costa hummingbird (Calypte costae).

2. Methods:

2.1 Preparation

The inspiration for this study was B.F. Skinner's operant conditioning. Operant conditioning tries to incentivize the test subject to act a certain way. While Ivan Pavlov's classical conditioning trained dogs to salivate at the sound of a metronome (an object unrelated to dog food), operant conditioning uses reinforcement and punishment to modify the behavior of a test subject. The desired behavior is for the hummingbird to listen to the speaker. A sugared water solution would act as the reinforcement and a saltwater solution as the punishment. Both reinforcement and punishment have positive and negative versions, and a summary of the meaning of each is shown in Table 1.1 below.

	Positive (+)	Negative (-)
Reinforcement	Something is added/given to **increase** a certain behavior	Something is taken away to **increase** a certain behavior
Punishment	Something is added/given to **decrease** a certain behavior	Something is taken way to **decrease** a certain behavior

Table 1.1 details the difference between positive reinforcement, positive punishment, negative reinforcement, and negative punishment

The speaker playing sound will always be located with the sugared water, so if the hummingbird accurately approaches the location with the sounding speaker, it will be rewarded with sugared water. Therefore, the sugared water is the positive reinforcer in this experiment. If the hummingbird decides not to listen to the speaker and approaches another location, it will be dealt with the positive punishment of nasty saltwater.

2.2 Subject and location

A 4 year old female Costa hummingbird was used for this experiment. My faculty mentor does research on hummingbirds, and in order for him to conduct studies, he has to have IACUC approval. A year ago my faculty mentor captured the hummingbird and placed her in her own cage with a male hummingbird next door. In order for me to conduct trials on the hummingbird, I also had to get IACUC approval through their training.

2.3 Materials

Multiple locations were set up with only one of those locations having a speaker playing sound. I chose to use 3 shepherd hooks to establish 3 locations for this experiment. Although one location had sound coming from it, identical speakers still had to be present to keep all 3 locations as identical as possible. Each location had an MP3 player, speaker, and feeder. It was important to establish some distance between the 3 locations because if the shepherd's hooks were bunched up together, the hummingbird would have difficulty determining exactly where the sound was coming from, since sound travels as a longitudinal wave as seen in Figure 2.

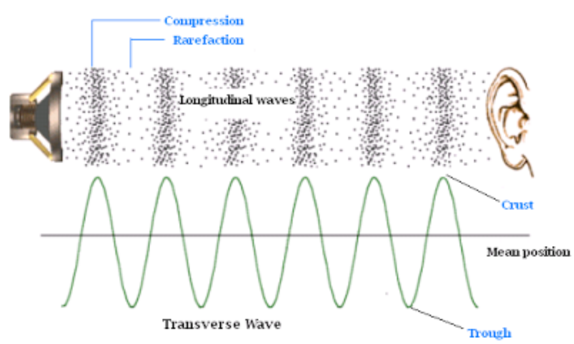
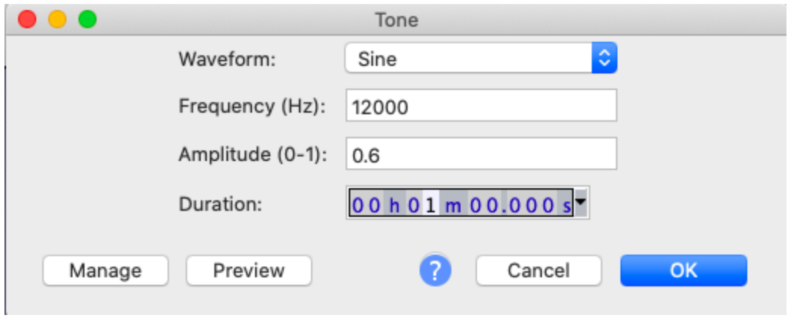


Figure 2 shows the path of sound through the air

The purpose of the feeder was to hold the reward or punishment and allow the hummingbird to discover which liquid was inside by inserting its beak through the hub and inside the feeder. For the reward, a mixture of water and sugar with a 2:1 ratio [1 cup water, ½ cup sugar] was prepared and a 2% saltwater solution [2g salt per 100mL] was prepared as the punishment. The concentration of the salt within the water allowed this solution to be labeled as a punishment, since regular water is merely liquid for the hummingbird. Each feeder was covered in a white fabric with only the hub (or the tip) of the feeder visible. This helped prevent the hummingbird from using any visual cue to determine where the reward was at.

The next step was creating the sound that would be played from the speaker. When deciding which frequencies would be used, I chose a low-level frequency, a couple of frequencies around the hearing range, and a high-level frequency. 200 Hz, 4 kHz, 10 kHz, and 14 kHz were selected. The options for sound were either a continuous pure tone with no silent intervals in between or a pulse tone with silent intervals throughout. In “Continuous Versus Pulsed Tones in Audiometry", the authors state “listener preference, however, indicated that pulsed tones were preferred over continuous tones by 67% of the listeners when listening to low-level or high-frequency tones" (Burk 1). Pulse tones are preferred because it’s easier to detect a pulse tone than a pure continuous tone. The use of pulse tones, as also mentioned by Burk and Wiley, “has been shown to decrease the number of false positives…” (Burk 1) as well as allow the test subjects to distinguish the sound from other noises around them.

Using the Mac application Audacity, the following information was set, and one of the 4 frequencies was inputted.



Setting the duration to 1 minute allowed me to proceed by converting the pure tone into that of a pulse tone, since no option for pulse tone was available. The following table focuses on the first 10 seconds to outline the pattern that occurred between a sounding tone and silence:

Tone was Playing A Sound	**Tone was Silent**
0-1 second	
	1-2 seconds
2-3 seconds	
	3-4 seconds
4-5 seconds	
	5-6 seconds
6-7 seconds	
	7-8 seconds
8-9 seconds	
	9-10 seconds

For the time frames in which the tone was silent, I adapted it to gradually arrive at silence so that the tone wouldn't have a cut-and-dry ending. This pattern continued for the remaining 50 seconds, and soon the pulse tone was created. During the trials, the MP3 player was set to repeat this 1 minute clip over and over until it was stopped by me. Once this process was carried out for all 4 frequencies, I was now ready to begin trials.

2.4 Procedure

Before I could start, negative control trials had to be conducted. More information is located in the 'Results' section, but the procedure was to conduct these trials to get the 'insignificant' result needed to proceed with the experiment. Arriving at the aviary everyday to do trials was not an option since the hummingbird could quickly catch on and memorize the time I arrived. This would be unfortunate because the hummingbird would simply eat ahead of time and be unaffected by any abstinence of food. Because of this, I varied the time of my arrival between the hours of 8am and 2pm. Regardless of time, I took away the hummingbird's food source for 20 to 30 minutes so that the hummingbird would be hungry during the trials and actively search for the reward. During this time I would step away and begin to establish which order I would follow when moving the reward. With about 10 minutes left, I would enter the aviary once again and begin preparing the 3 locations. Using tape, I quickly set up all 3 apparatuses with a covered feeder, MP3 player, and speaker. Once the locations were set up and the reward location was established, it was time to wait for the hummingbird to actively find the food (the reward) to ingest.

As I waited and took note of the hummingbird's attempts, a series of criteria needed to be established to determine whether an attempt would be labeled a success or failure. The following table breaks down the criteria that was used for this experiment:

*A **Successful** Trial*	*A **Failed** Trial*
The hummingbird went straight to the reward location **OR** The hummingbird approached the wrong location but quickly arrived at the right location without placing its beak in the incorrect location	The hummingbird went to the incorrect location and inserted its beak into the feeder **OR** The hummingbird took 3 or more attempts to find the correct reward location

The second option for a successful trial (approaching the wrong location but not placing its beak inside the feeder) was included because this occurrence was likely to occur. The episodic memory of a hummingbird takes note of valuable information such as location of food. Understanding that the hummingbird would still have the location from trial #1 as the location with the sound and reward, the hummingbird most likely would approach it in the following trial. The evidence of acquired knowledge would be the instances when the hummingbird approached that location but refrained from fully committing to the location because of the absence of the sound. When this happened during the trial period, it was evident to me that the hummingbird was clearly using the sound as an indication of where the reward resided.

If the hummingbird failed a trial, it was important to wait until it arrived at the correct location before proceeding to the next trial. I noticed that while I was focused on changing the locations to prepare for the next trial, the hummingbird would approach the feeder still standing and find out that it had saltwater and not the reward. This simple detail could instantly ruin my trials because the hummingbird would uncover that the reward is in 1 of 2 locations and that the third can be ignored. Instead of having a 33% success rate, the probability jumps up to 50%. For this reason, I made sure to place all 3 feeders on the floor while I switched the locations after each trial. This same process was carried out for all 33 trials, and once they were collected, it was time to analyze them using a chi squared test.

2.5 Statistics of Analyses

A chi squared test was calculated for each set of data in this experiment. A chi squared test is a statistical test that compares the data collected from an experiment to its data prediction. With the null hypothesis of this experiment stating that the pulse tone would **not** influence the hummingbird's ability to reach the correct reward location successfully, the chi squared test would show whether the collected results supported or refuted this claim. A component of the chi squared test is comparing the calculated p-value to that of the baseline p-value of 0.05. If the p-value is lower than 0.05, the null hypothesis is rejected; if the calculated p-value is above 0.05, the null hypothesis is accepted. Instead of using the terms 'accepting' and 'rejecting', the terms 'significant' and 'insignificant' were used respectively. 'Significant' was like rejecting the null hypothesis and insignificant was the same as accepting the null hypothesis.

The data prediction is the 33% chance the hummingbird arrives at the correct location with no "outside help". If the success rate of the pulse tone trials proves to be significant, I made the interpretation that the speaker was the reason for the improvement in the hummingbird. Although there is a chance some factor outside of my control could've contributed to the results, the chi squared test gave me an opportunity to prove statistically that my hypothesis (the pulse tone would positively contribute to the hummingbird's success) was correct at the frequency tested. If the result was insignificant for a specific frequency, I could interpret the results as an indication that the hummingbird couldn't hear the tone.

3. Results:

The first sets of data collected were from the negative control trials. The negative control trials consisted of 33 trials in which each apparatus was set up with all 3 materials (MP3 player, speaker, and feeder), but none of the speakers were on. This allowed me to check from the start for anything in the setup that could give a slight advantage to the hummingbird. Naturally with 3 possible locations and nothing to guide the hummingbird, it has a 1/3 chance (33%) of approaching and selecting the correct reward location the first time. This means that the success rate of the negative control trials had to be around this range for me to feel confident and ready to conduct the same trials but with sound. As shown in the table below, the results of the negative control trials ended up having a success rate of 39% (13 out of 33). A chi squared test was used to make sure this 6% difference was insignificant, and indeed it was (See Figure 1.2). This result gave me the green light to proceed.

	Correct	Incorrect	*Marginal Row Totals*
Expected	11 (12) [0.08]	22 (21) [0.05]	33
Observed	13 (12) [0.08]	20 (21) [0.05]	33
Marginal Column Totals	24	42	66 (Grand Total)

The chi-square statistic is 0.2619. The *p*-value is .608815. *Not* significant at $p < .05$.

The chi-square statistic with Yates correction is 0.0655. The *p*-value is .798041. *Not* significant at $p < .05$.

Figure 1.2 shows the insignificant result from the negative control trials that was necessary to proceed with the experiment

The hummingbird was able to hear each sound as shown in Figures 3a to 3d. During each of the trials, the hummingbird exhibited actions that indicated a level of detection from the hummingbird; head tilts and neck extensions were observed. Another observation I made throughout the trials were specific locations throughout the cage that aided and helped with determining the correct locations. I was almost confident each time that if I found the hummingbird in that certain spot, the chances of identifying the correct location would increase. It seemed like these 4 specific locations, along with head tilts and neck extensions, allowed the hummingbird to examine each location from far as to use her ears to find the sound. When analyzing the tables below, note that the goal of the frequency blocks was to obtain 'significant' results as opposed to the 'insignificant' result from the negative control trials. The 'significant' result allowed me to make the assumption that the sound had a positive influence on the hummingbird in the trial.

Figure 3a: Results from trials at 200 Hz

	Correct	Incorrect	*Marginal Row Totals*
Expected	11 (16) [1.56]	22 (17) [1.47]	33
Observed	21 (16) [1.56]	12 (17) [1.47]	33
Marginal Column Totals	32	34	66 (Grand Total)

The chi-square statistic is 6.0662. The p-value is .013779. Significant at $p < .05$.

The chi-square statistic with Yates correction is 4.9136. The p-value is .026646. Significant at $p < .05$.

Figure 3b: Results from trials at 4 kHz

	Correct	Incorrect	*Marginal Row Totals*
Expected	11 (16) [1.56]	22 (17) [1.47]	33
Observed	21 (16) [1.56]	12 (17) [1.47]	33
Marginal Column Totals	32	34	66 (Grand Total)

The chi-square statistic is 6.0662. The p-value is .013779. Significant at $p < .05$.

The chi-square statistic with Yates correction is 4.9136. The p-value is .026646. Significant at $p < .05$.

Figure 3c. Results from trials at 10 kHz

	Correct	Incorrect	*Marginal Row Totals*
Expected	11 (15) [1.07]	22 (18) [0.89]	33
Observd	19 (15) [1.07]	14 (18) [0.89]	33
Marginal Column Totals	30	36	66 (Grand Total)

The chi-square statistic is 3.9111. The p-value is .047968. Significant at $p < .05$.

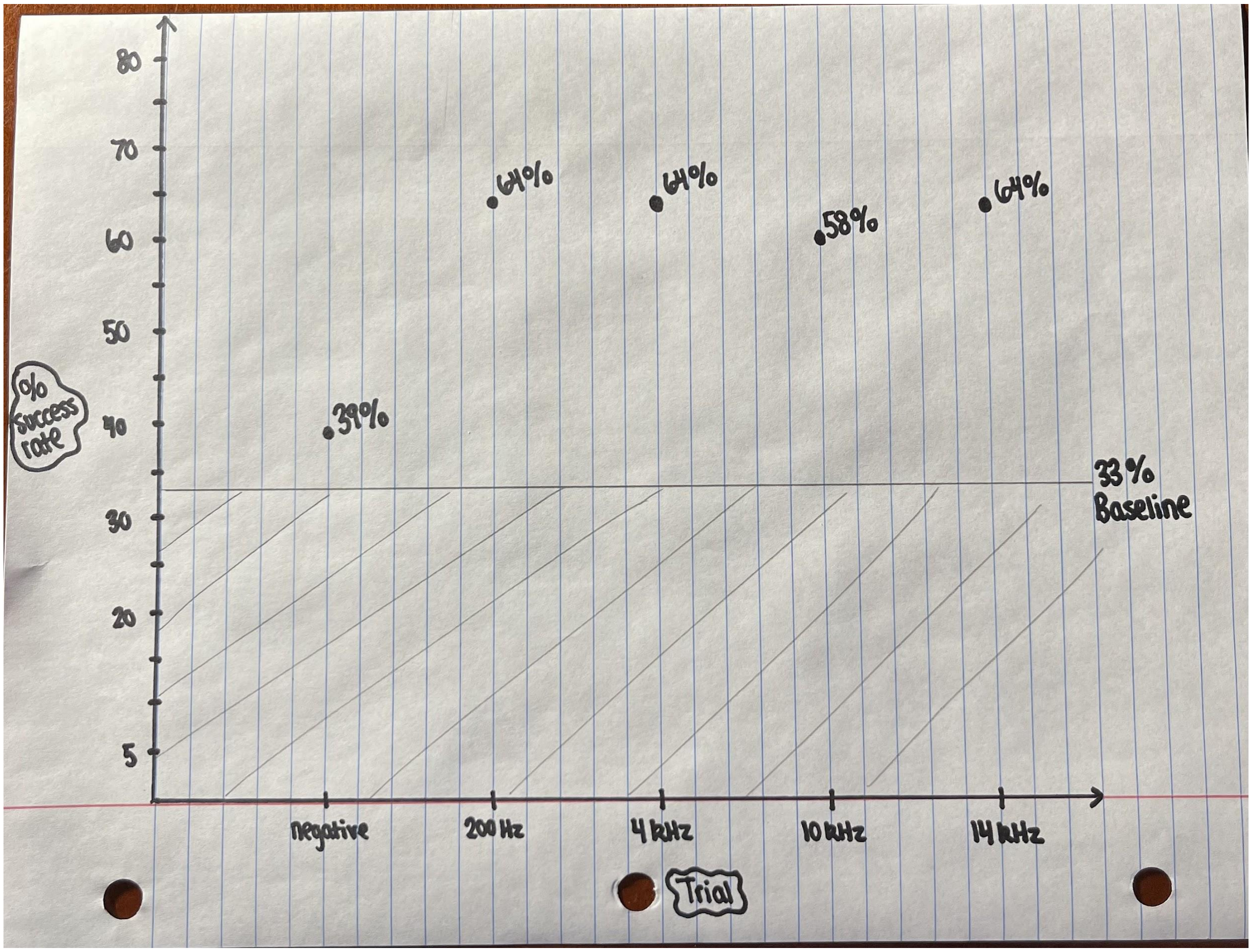
Figure 3d. Results from trials at 14 kHz

	Correct	Incorrect	*Marginal Row Totals*
Expected	11 (16) [1.56]	22 (17) [1.47]	33
Observed	21 (16) [1.56]	12 (17) [1.47]	33
Marginal Column Totals	32	34	66 (Grand Total)

The chi-square statistic is 6.0662. The p-value is .013779. Significant at $p < .05$.

The chi-square statistic with Yates correction is 4.9136. The p-value is .026646. Significant at $p < .05$.

To place all the results obtained into perspective, here is a graph that shows the effect of the pulse tone at each frequency.



The purpose of this graph is to show that the success rate of every trial block was higher than the baseline, indicating that there was a factor that drove the success rate higher. With the exception of the higher negative trial result, in which we showed via a chi squared test the insignificance of the 6% discrepancy, the success rate was almost 2 times better.

One of the observations I made when analyzing the data from all 5 trial blocks is that the success rate for location 3 was very low. Below is a table that breaks down the number of times the reward was at location 3 and how many of them resulted in a failed attempt.

	Reward at location 3	**Failed Result**	**Success Rate**
Trial Block			
Negative	12	8	33% (4/12)
200 Hz	11	10	9% (1/11)
4 kHz	11	8	27% (3/11)
10 kHz	10	9	10% (1/10)
14 kHz	12	11	8% (1/12)

This ended up giving an individual failure rate for location 3 in general of 46 out of 56, or 82%! One of the possible reasons was the presence of the male hummingbird next door. I initially set location 3 considering both location 2 and the neighboring hummingbird's cage, but apparently the space wasn't enough. More will be discussed in the conclusion about changes and modifications another experimenter can implement to improve this experiment. To achieve its high success rate while compensating for location 3's failures, the hummingbird was excellent in determining the reward location whenever it was located between locations 1 and 2. This can be expected however, since the possibility of the hummingbird arriving at the correct location becomes an even 50/50 chance.

4. Discussion:

The purpose of this study was to determine which sounds a hummingbird could hear and use the information obtained to construct a hearing range. Not many studies had been done to answer this question, although a few experiments did so. A 2004 study by Carolyn Pytte et al used a blue-throated hummingbird to expose it to sounds varying in frequency. As the sounds increased from 1 kHz to 50 kHz, Pytte et al observed the brainstem of the hummingbird to find an activation as a result of detecting the sound. A 2020 study conducted by Duque et al used an Ecuadorian Hillstar hummingbird to see if it could hear a high frequency sound of 13.4 kHz. Duque referenced the findings of Robert Dooling, in which he had the hearing range of most birds to be between 2 and 8 kHz (Marler 2004). We are one step closer to determining the hearing range of hummingbirds with this study. Using 3 locations with 1 sugar water reward, trials were conducted with 4 different frequencies to see if the success rate would be higher. The 4 different frequencies were spaced out to represent both the low frequency area and the high frequency area. The results obtained showed an increase in the success rate regardless of frequency. From this study, the conclusion was made that the hummingbirds' hearing range is 200 Hz to 14 kHz (14,000 Hz), which didn't match the results claimed by Robert Dooling. Rather than contradicting the results of Robert Dooling, these results elaborated more on the exact hearing range of hummingbirds. Although this study provided us with information on the hearing range of hummingbirds, a future experimenter could implement changes to improve this study and potentially expand its borders to focus on another aspect.

Isolating the hummingbird during the trials could be a change implemented by another experimenter. The female hummingbird used in this study was in an outside cage with a neighboring male hummingbird. There were many instances throughout the trials when the female hummingbird would approach the end of her cage, and as a result, the male hummingbird would arrive as well to defend his territory. This can be a possible explanation as to why the success rate at location 3 was so low, since location 3 was about 2 ½ feet from the border separating the 2 hummingbirds. Possibly isolating the hummingbird in future studies could eliminate all the noises from being outside and any other hummingbirds.

A future experimenter could also place more importance on the volume of the speakers. Since the motive of this experiment focused on determining if the hummingbird was even physically capable of hearing the sound, the speakers were placed at a somewhat equal level. Now that the assumption could be made that this hummingbird could hear sounds from 200 Hz to 14,000 Hz, a future experimenter could focus on a sole frequency and determine how loud the sound has to be in order for the hummingbird to detect it.

A future experimenter could elaborate on the information gathered to determine if the hummingbird can actually hear beyond the current boundaries. For example, can hummingbirds hear the sounds of their wings flapping? Researchers have discovered that the frequency of a hummingbird's wing flapping is 50 Hz, which is below the 200 Hz mark.

An interesting topic to stem from this experiment would be to conduct an experiment where the hummingbird is exposed to a sound of 50 Hz and see how it responds. The two options could be a generated pulsed tone of 50 Hz or a recording of a hummingbird flying through the air.

Another aspect of this experiment that can be focused on is the time it takes the hummingbird to complete the trials. In the beginning, the time in which the feeder was changed from one location to another would be written down. However, I decided to stop writing this information since the purpose of this experiment was not focusing on the response rate of the hummingbird, but rather her success rate. Just because this study didn't focus on time doesn't mean it can't be the focal point of another person's experiment. Anything from response rate to departure rate could be analyzed.

The final topic an experimenter could detail is the number of minutes it took for the hummingbird to complete trial 1, trial 2, etc. Then the experimenter could calculate the average response time for each frequency and make an analysis on any correlation between frequency and response rate. It's possible that the data could shed light into new information regarding hummingbirds. If the average response rate between 10 kHz and 14 kHz increased 3-fold, the experimenter could look into the possibility of the hummingbird having difficulties hearing the 14 kHz sound.

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