Spring Peepers at UNDERC: Do Different Sized Spring Peepers Yield Different Phonotaxis Responses to Various Conspecific Calls?

BIOS 35502-01: Practicum in Field Environmental Biology

Elisabeth Cafritz

Advisor: Kristin Bahleda

2016

Abstract:

The North American chorus frog, the spring peeper (*Pseudacris crucifer*), is known to demonstrate phonotaxis, the ability to approach sound sources. There have been numerous studies looking at trends associated with calling peepers, but very few focus on the different responses a peeper may have, if any, to a call from a large or small peeper. The goal of this study was to see the type of response spring peepers have to either small peeper calls, with a higher decibel frequency, or large peeper calls, with a lower decibel frequency. The response to another chorus frog, the grey treefrog (*Hyla versicolor*), was also included in this study but for observational purposes only. A chi-squared contingency table was used to determine whether the frogs responded to the calls. The results for both the high and low frequency calls p-value 0.5< 0.26233167540826 and for the treefrog call p-value 0.05<0.07816908582428. These results indicated that the frogs responded. For the low and high frequency results, a Friedman Test and then a Mann-Whitney U Test were used. For the grey treefrog data, a Kruskal-Wallis Test was used. Additionally, there was a significant response type to the high frequency peeper call but not a significant response type to the low frequency peeper call. It was theorized that the size of the peeper calling, large or small, was not a huge factor for mating success in males and therefore did not induce a strong reaction of the frogs tested. However, the p-values found were not very strong. Thus, the hypothesis of the study, which stated that there was a strong correlation between the size of the peeper and the decibel frequency of the call, was not entirely supported. A larger sample size should be taken in order for the concepts of this study to be fully investigated.

Key Words: herpetology; chorus frogs; spring peepers; *Pseudacris crucifer*; phonotaxis; high frequency call, low frequency call

Introduction:

Spring peepers are common in the Wisconsin area and can be found in moist deciduous forest. They are particularly partial to wetlands, where they breed in vernal pools (Wisconsin Department of Natural Resources, 2016). Vernal pools are depressions in landscapes that temporarily fill with snowmelt or heavy rainfall, most commonly after the spring season. Spring peepers congregate in these areas for calling and mating time periods. Peepers advertise their presence by calling throughout early evenings and at night when temperatures are warm; typically starting in late April and ending in mid-July (Contant and Collins 1998). Male peepers sometimes form choruses of three or more when calling in high whistles and in various intervals to attract mates (Rosen and Lemon 1974).

Interactions between groups of frogs can sometimes lead to more aggressive calls to create territorial spacing. These cries are called “trills.” Trills are a passive territorial method to tell other frogs or animals to leave an area. Studies have observed that trilling occurs when spring peepers feel threatened by approaching animals and one another (Rosen and Lemon 1974). They trill when they hear either the peeping (the standard mating call) or trilling from surrounding spring peepers. As expected, trilling tends to subside when the spring peeper feels less threatened. Additionally, calls, whether mating or trilling, can sometimes indicate the size of the frog based on the decibel level potentially because of the energy investment a frog puts forth when calling (Contant and Collins 1998).

Low and high frequency peeper calls have been observed to correlate with the age and size of spring peepers. Frogs with lower call frequencies have been found to be in better body condition, larger and older (3-4 years) (Rosen and Lemon 1974). Some studies have found that female spring peepers might be more receptive to males with lower frequency mating calls rather than higher ones, presumably because these males tend to be better suited mates since they tend to be larger. This is because larger males are thought to be better at fighting off predators (Woodward 1988). However, there have been other studies that argue that size does not matter and that smaller male peepers experience about the same level of reproductive success as lager males do (Forester and Czarnowsky 1985). Males tend to start mating and calling during their second to third year of life. Studies have shown that some males experience a significant body increase and can growth up to 3.9 centimeters long when they start to call (Sullivan and Hinshaw 1990).

The specific hypothesis tested during this experiment is that spring peepers will respond to certain calls depending on their overall size. Additionally, there is a significant correlation between the size of the spring peeper and its response to low frequency peeper calls and high frequency peeper calls. The scientific question being asked is: Do spring peepers have a different phonotaxis response to different conspecific (same species) calls depending on their size?

Methods:

Sampling:

Before testing the frogs, spring peeper and grey treefrog calls were recorded and collected. The sound decibel frequency was taken for each of the calls using a digital sound frequency monitor. First, a small peeper call was collected for 35 seconds near the project sites and had a peak of 92 dBA (Figure 1). The large peeper call was collected from the Internet from a professional wildlife sound recorder, Dr. Lisa Rainsong. The sound decibel range for this call was from 50-80 dBA and it ran for 30 seconds. The grey treefrog (*Hyla versicolor*) chorus call was recorded at a vernal pool located at UNDERC for 50 seconds and its decibel level ranged from 60-70 dBA (Figure 1).

Spring peepers were collected at the University of Notre Dame's Environmental Resource Center (UNDERC) in Land O'Lakes, Wisconsin. Eleven sites were selected on the UNDERC property (Figure 1). The habitats were relatively similar - containing large woody debris, plenty of underbrush and were adjacent to either vernal pools or lakes. Frogs were collected during the weeks of June 12, June 19, June 26, and July 10. Two to three frogs were collected from each site. Once a frog was caught at a site, the site was not revisited for at least five-seven days to avoid recapture.

Frogs were hand-caught at each of the sites and handled minimally to avoid stress. The IACUC (Institutional Animal Care and Use Committee) guidelines state that smaller frogs, like peepers, can be picked up with one hand by placing the index finger between the hind legs while supporting the body with the thumb and middle fingers while the back of the frog should be cradled in the palm of the hand (Figure 2). Before placing the animal in captivity, frogs were measured from vent to snout and the measurements were recorded in centimeters. Frogs were then kept in Tupperware containers with a wet sponge and some leaves for two-three hours and then tested. If absolutely necessary, frogs were kept overnight. Only peepers two centimeters and above were kept overnight since smaller ones are more likely to die in captivity. Frogs kept overnight were tested within eight hours of the next day. Frogs were not sexed.

<u>Experimental Set-Up</u>:

After collecting and measuring the peepers, each frog was subjected to the three different calls: the high and low frequency peeper calls and the grey treefrog call. The frogs were tested in a Y-shaped phonotaxis box made out of plywood (Figure 3). The two ends of the box held two different speakers with saran wrap covering the top of the area of the box to prevent frogs from escaping. One speaker was an actual speaker and the other was a fake speaker in order for both ends of the box to appear similar to avoid bias. The speakers switched sides after every five frogs in case the physical construction of the box and general placement of the speakers influenced the responses of the frogs. The walls of the structure were lined in yoga mat so the frogs could not climb out as easily. The frogs were tested individually and other frogs were kept out of the testing area during test sessions. The frogs were tested in darkness because spring peepers and grey treefrogs call at night. In order to see the frogs but not disrupt them, a red light was used over the testing area since frogs are unable to see red light (J. McLister, personal communication 2016).

To start testing, the frogs were covered under a cup for three minutes in silence then an additional three minutes while one of the three calls played. Then the cup was lifted and for three minutes the frog was observed while the specified call played. The speakers maintained the same volume throughout all of the frogs tested. In order to properly observe the peepers, every three second interval a movement was recorded with a tally mark. The movements recorded were as follows: hop, walk, walk on wall, move (head or body movement in place) or dash (no movement). They were recorded in a table that correlated with the three second interval in which the movement occurred (Table 1). After the session, notes were taken about additional behavior and which way the frog responded (either towards or away from the speaker) (J. McLister, personal communication 2016). Frogs were then released at the same spot where they were captured.

Statistical Analysis:

All of the data analyzed was run using the SYSTAT 13 computer program (SYSTAT 13 2016). The Mann Whitney U Tests, however, were run on *R* (R Studio 2015). The data was first analyzed with a chi-squared contingency table in order to see if the frogs responded. The test calculated if there was a general yes or no response from the peepers tested. It did this by analyzing the proportion of frogs that responded with movement to, away or not at all to the different calls they were subject to. This is the appropriate test to analyze the data because a chi-squared analysis tests independent variables, the high frequency calls, low frequency calls and grey treefrog calls, along with the dependent variable, the possible outcomes. The data was then analyzed further to see if there was any significant correlation with the size of the spring peeper and it's given response to the different recorded calls (Figures 4, 5 and 6).

The recorded frog responses had to be quantified with an index for further testing (Table 2). For each frog, a number was given that corresponded with the index and how the frog responded to the individual call trails. A Shapiro-Wilks Test for normality was run on the high frequency and low frequency call response data. The p-value $0.000040 < 0.05$ and therefore was not normally distributed. A nonparametric test, the Friedman's Test, was used for this data. The grey treefrog call response type data also underwent a Shapiro-Wilks test for normality and had a p-value $0.046186 < 0.05$. Thus a nonparametric test, a Kruskal-Wallis Test, was run with the averages for the response types to the call (Figure 8). The grey treefrog response data was not subject to anymore statistical testing because the spring peeper specific calls were the primary focus of the project.

The Friedman Test required there to be no replicates in the data set. Peepers were placed into size categories and the average size within the category was determined (Table 3). Additionally, the average index response type was calculated within the size categories (Figure 7). The first Freidman Test used the index results as the dependent variable, the average sizes of the frogs as the grouping variable and the call type as the blocking variable. The results were p-value $0.05 < 0.724876$, which was not significant. The second Friedman Test used the same dependent variable but used the call type as the grouping variable and the average frog size as the blocking variable. The p-value 0.05> 0.058782 which was significant, but by too small to fully support. Thus, Mann-Whitney U Tests were run on the low and high frequency call response type data.

Results:

In order to see if there was a response to the call trials, a chi-squared contingency test was used. For the high and low frequency call response types the p-value was 0.26233167540826> 0.05, df=1. The p-value for the grey treefrog call response type was 0.07816908582428 > 0.05, df=1.These results indicated that there was a response to the call trials (Figures 4, 5 and 6). A Shapiro-Wilk Normality Test was then run. For the high and low frequency call response type data the p-value was 0.05>0.00004, meaning the data was not normally distributed. For the grey treefrog call response type the p-value was 0.05>0.046186, meaning that the data was also not normal. For the grey treefrog frog call results the Kruskal-Wallis test results for the p-value 0.05>0.046186, df=6. The response index was the dependent variable and the average sizes of the frogs as the independent variable. The results were not significant. Since the questions asked were primarily focused on spring peeper response types to spring peeper calls no further tests were run on the grey treefrog data.

The first Friedman Test used the averages for high and low frequency call response type. The p-value 0.724876>0.05 and was not significant. The second Friedman Test had a p-value 0.058782 < 0.05 which meant that the data was significant. The p-value for the second Friedman Test was not much smaller than the alpha p-value. Therefore, Mann-Whitney U tests were used on the data corresponding with the size of the frogs and the response type for the high frequency and low frequency call trails. The results for the low frequency trail had a p-value 0.07955>0.05, which was not significant. However, the results for high frequency call trail yielded a p-value 0.03201<0.05, which was significant.

Discussion:

Spring peepers' demonstration of phonotaxis, size range of 1-3.9 centimeters, as well as decibel frequency of calls potentially changing depending on their size may potentially influence a peeper's response to another peeper's call (Wisconsin Department of Natural Resources 2016). However, the different decibels (high for small, low for large) for the different calls yielded different response types from the different frogs tested, regardless the size of the frog tested. The Mann-Whitney U results potentially indicated that there was a significant relationship between the size of the frog and the type of response to smaller spring peeper calls with high decibel recordings but not to larger calls with lower decibel recordings. The size of the frog itself did not influence its response to low frequency call. But a high frequency call might influence more of a phonotaxis response in spring peepers. Meaning that small peeper calls could potentially influence peepers of various sizes.

These results were not expected because during the project it was thought that lower frequency peeper calls would yield more of a response than high frequency peeper calls. This is because low frequency calls can be associated with larger frogs. Larger frogs may make better mates for females and may threaten other smaller males (Lykens and Forester 1987).

Mating, and therefore calling, has been found to be a very random event, however, and is not dictated strictly by frog size (Forester and Czarnowsky 1985), (Lykens and Forester 1987). Perhaps the frequency of the call is not the biggest factor. For instance, location of the calling frog has been proven to have a large influence on success rate, potentially more so than frequency level. For example, spring peepers calling near a crowded vegetated area experienced significantly less mating success, regardless of how large they were, compared to those calling near a flat body of water (Schwartz and Gerhardt 1998).

During mating season, male peepers call in choruses in an effort to attract more females. If size mattered for reproductive success in this case, then it would make sense for males to feel territorial and to move and call more when in close contact with one another i.e. calling near each other (Parris 2002). Since size may not have a huge impact on reproduction success, then perhaps males do not feel the need to react strongly to lower frequency calls (Forester and Lykens 1986). Even satellite males, male peepers that do not call, which are supposedly significantly smaller than calling peepers, do not experience huge losses in mating success. In some studies, it was found that they had rates of higher sexual success potentially because they do not have to expend energy on calling (Forester and Lykens 1986).

If further research is conducted on peeper calls, then much larger sample sizes should be collected. Additionally, sexing the frogs could make understanding the responses more comprehensive since male and female frogs play opposite roles in calling scenarios. It would also be interesting to pay more attention to where the frog was collected since location can be an influential factor during periods of calling (Schwartz and Gerhardt 1998). If this test was furthered a better method to quantify and observe the frogs while they responded to the call trails would make the statistical analysis for the results stronger and easier to run --- therefore yielding more meaningful results.

Acknowledgments:

I would like to thank the University of Notre Dame Environmental Research Center and the Bernard J. Hank Family Endowment for the funding and support to conduct this study. Additionally, I want to thank Gary Belovsky, Michael Cramer and Hannah Madson for doing a fabulous job running the UNDERC-East program. I would also like to extend my sincere thanks to my mentor, Kristin Bahleda, for her guidance and insight during this project I would especially like to thank her for accompanying me during late nights of frog hunting and long hours of frog testing. Additionally, I want to thank Dr. Mcklister for advising the experimental design portion of my study and letting me witness his own work on chorus frogs. Finally, I would like to thank Hallie Harriman and Delaney Martin for their time spent in the field looking for frogs with me.

References

Conant, R. and Collins, J.T. 1998. Reptiles and Amphibians: Eastern: Central North America. Houghton Mifflin Company, New York, New York.

Forester, Don C., and Richard Czarnowsky. "Sexual Selection in the Spring Peeper, Hyla Crucifer (Amphibia, Anura): Role of the Advertisement Call." *Behaviour* 92.1 (1985): 112-27. *JSTOR*. Web. 16 July 2016.

Forester, Don C., and David V. Lykens. "Significance of Satellite Males in a Population of Spring Peepers (Hyla Crucifer)." *Copeia* 1986.3 (1986): 719.*JSTOR [JSTOR]*. Web. 16 July 2016.

Forester, Don C., David V. Lykens, and W. Keith Harrison. "The Significance of Persistent Vocalisation By the Spring Peeper, Pseudacris Crucifer (Anura: Hylidae)." *Behaviour* 108.3 (1989): 197-208. *JSTOR [JSTOR]*. Web. 16 July 2016.

Gerhardt, H. Carl. "Evolutionary and Neurobiological Implications of Selective Phonotaxis in the Green Treefrog, Hyla Cinerea." *Animal Behaviour* 35.5 (1987): 1479-489. *JSTOR [JSTOR]*. Web. 16 July 2016.

Gerhardt, H. Carl. "Selective Responsiveness to Long-Range Acoustic Signals in Insects and Anurans." *American Zoologist Am Zool* 34.6 (1994): 706-14. Web. 16 July 2016.

Lisa Rainsong. *Peeper Soloists Then Pitch Variations in Peepers*. 2013. *SoundCloud*. Web. June 2016. <https://soundcloud.com/lisa-rainsong/peeper-soloist-then-pitch>.

Lykens, David V., and Don C. Forester. "Age Structure in the Spring Peeper: Do Males Advertise Longevity?" *Herpetologcia* 43.2 (1987): 216-23. *JSTOR [JSTOR]*. Web. 3 July 2016. <http://www.jstor.org/stable/3892054>.

McLister, James. Personal communication. How to test phonotaxis in frogs and methods. June 16.

Oxford Dictionary. "Definition of Phonotaxis in English:." *Phonotaxis: Definition of Phonotaxis in Oxford Dictionary (American English) (US)*. Oxford University Press, 2016. Web. 30 June 2016. <http://www.oxforddictionaries.com/us/definition/american_english/phonotaxis>.

Parris, Kristen M. "More Bang for Your Buck: The Effect of Caller Position, Habitat and Chorus Noise on the Efficiency of Calling in the Spring Peeper." *Ecological Modelling* 156.2-3 (2002): 213-24. *Google Scholar*. Web. 16 July 2016.

R-Studio Team (2015). R-Studio: Integrated Development for R. Version: 0.99.902. R-Studio, Inc., Boston, MA. <http://www.rstudio.com/>.

Reinhold, Klaus. "Variation In Acoustic Signaling Traits Exhibits Footprints Of Sexual Selection." *Evolution* 65.3 (2010): 738-45. *JSTOR [JSTOR]*. Web. 16 July 2016.

Rosen, M. and Lemon, R. E. 1974. The Vocal Behavior of Spring Peepers, Hyla crucifer. *Copeia* 4: 940-950.

Schwartz, Joshua J., and Carl H. Gerhardt. "The Neuroethology of Frequency Preferences in the Spring Peeper." *Animal Behaviour* 56.1 (1998): 55-69.*JSTOR [JSTOR]*. Web. 16 July 2016.

Sullivan, B. K. and Hinshaw, S.H. 1990. *Variation in Advertisement Calls and Male Calling Behavior in the Spring Peeper* (Pseudacris crucifer). *Copeia* 4: 1146-1150.

SYSTAT Team (2016). Version: 13. Systat Software, Inc.,n San Jose California USA, </www.sigmaplot.com/>.

Woodward, Bruce D., Joseph Travis, and Sandra Mitchell. "The Effects of the Mating System on Progeny Performance in Hyla Crucifer (Anura: Hylidae)."*Evolution* 42.4 (1988): 784. *JSTOR [JSTOR]*. Web. 16 July 2016.

Wisconsin Department of Natural Resources. "Spring Peeper (Pseudacris Crucifer)." *Spring Peeper (Pseudacris Crucifer)*. Wisconsin Department of Natural Resources, 19 May 2016. Web. 16 July 2016.

Zimmitti, S. J. 1999. Individual Variation in Morphological, Physiological, and Biochemical features with Calling in Spring Peepers. *Physiological and Biochemical Zoology: Ecological and Evolutionary Approaches* 72: 666-676.

Figures

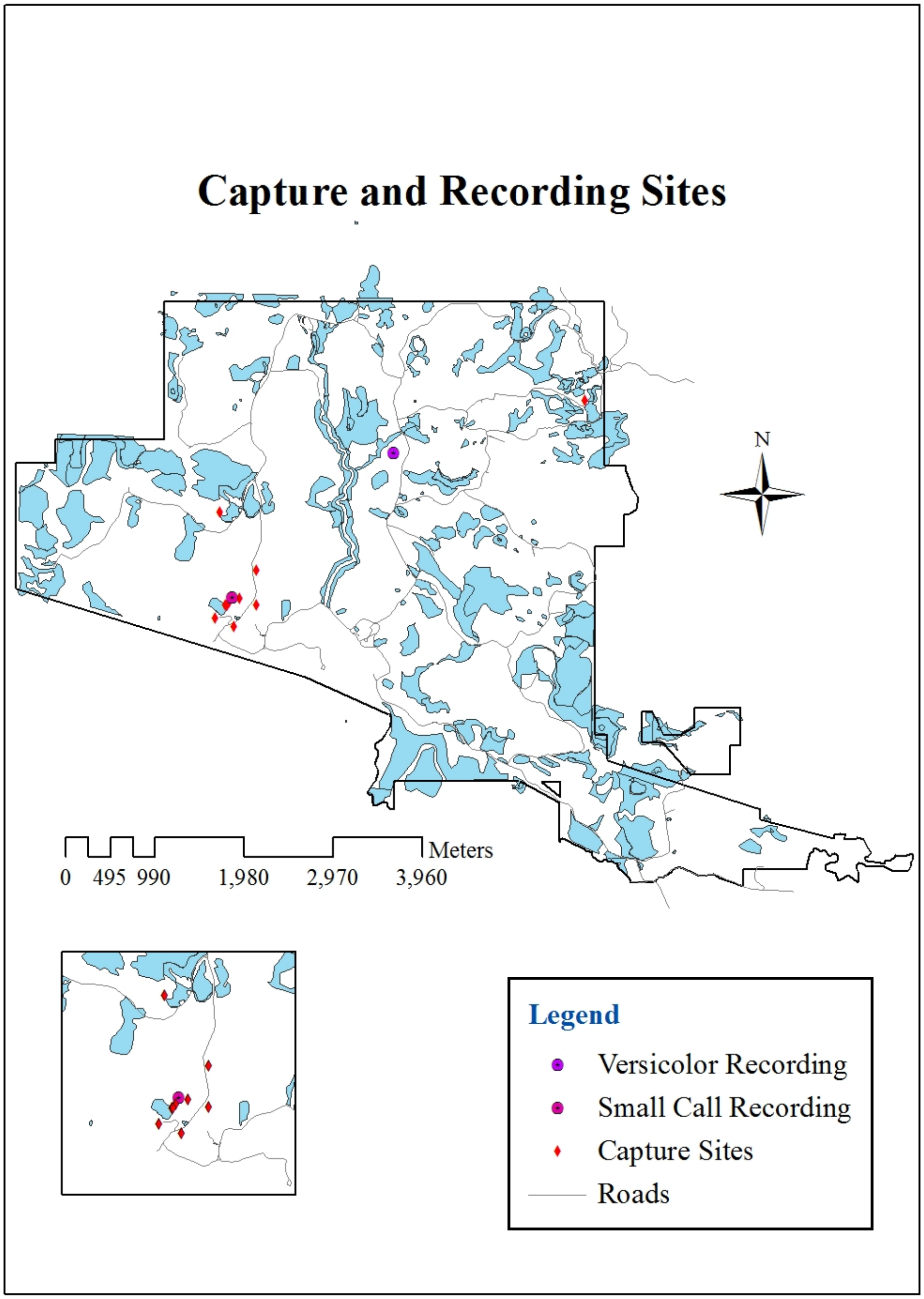

Figure 1. Map of capture and recording sites used on UNDERC's property during project.

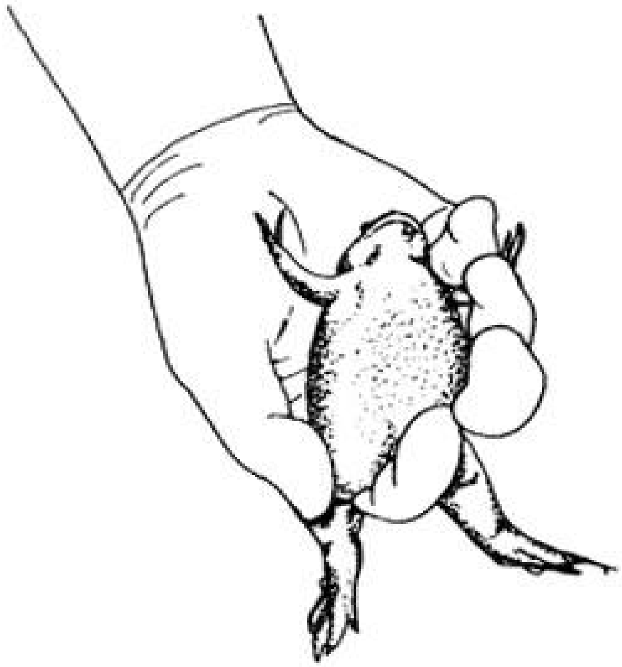

Figure 2. How to hold a small frog properly, image from IACUC Intro to Amphibians Course.

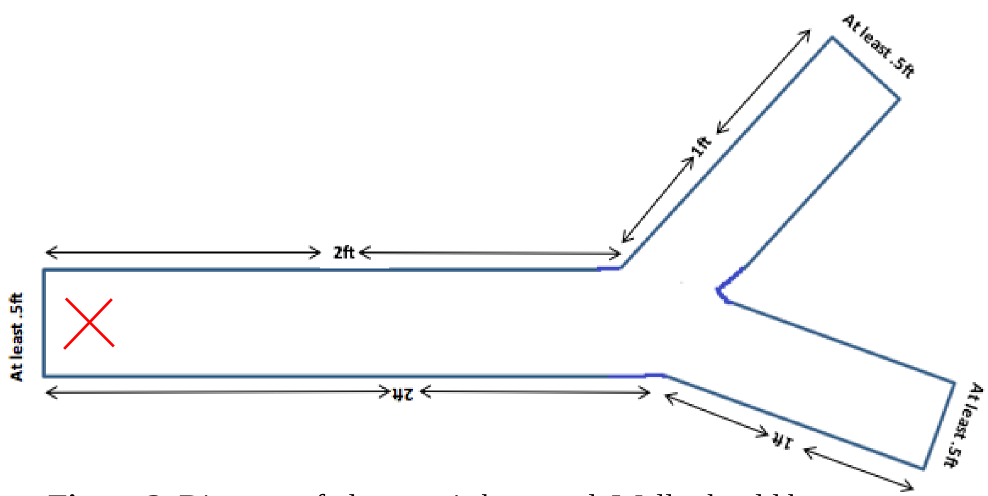

Figure 3. Diagram of phonotaxis box used. Walls should be at least 1 ft. high. The "x" indicates where the frog should start.

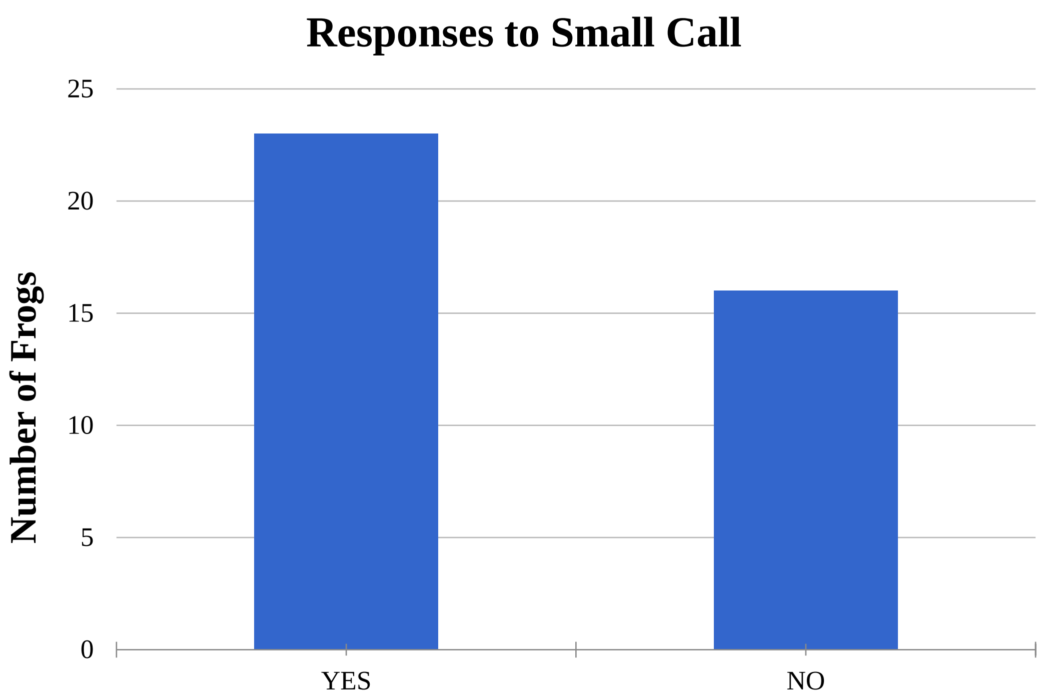

Figure 4. The response and no response numbers to the small peeper call of the frogs collected.

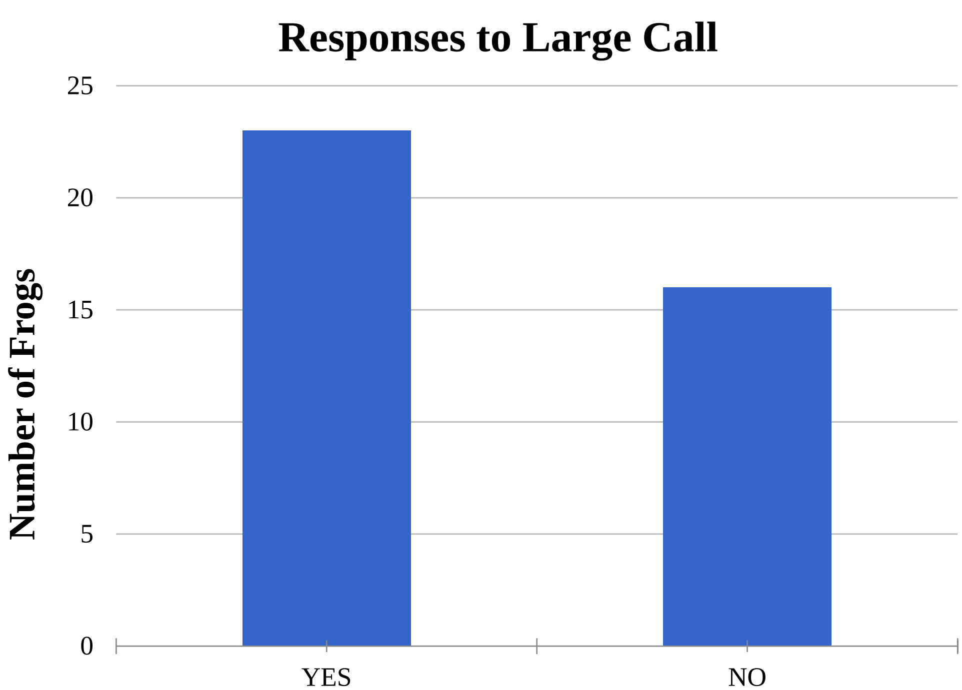

Figure 5. The response and no response numbers to the large peeper call of the frogs collected.

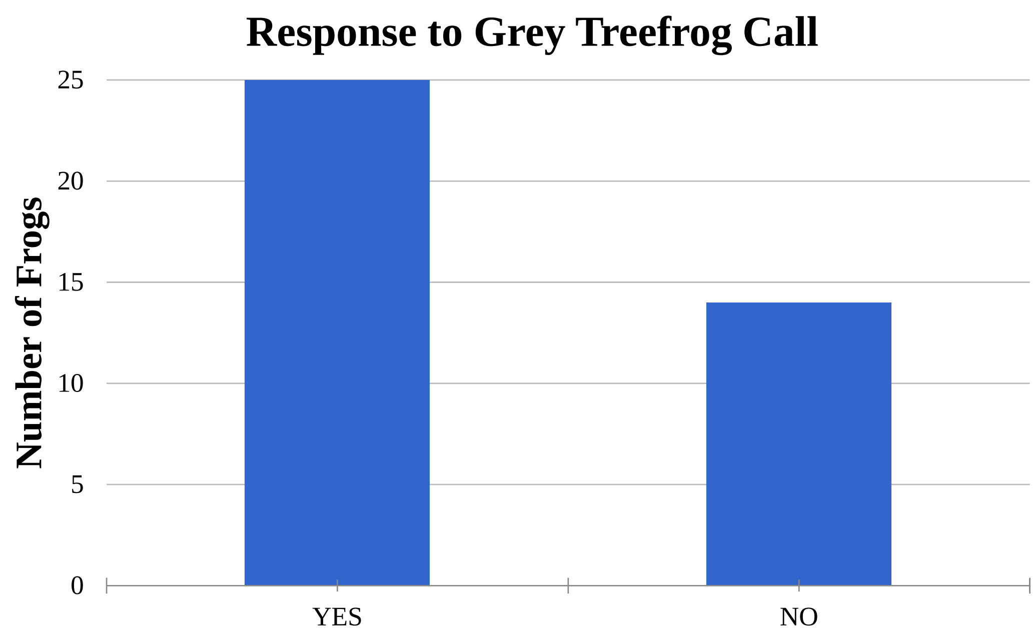

Figure 6. The response and no response numbers to the grey treefrog call of the frogs collected.

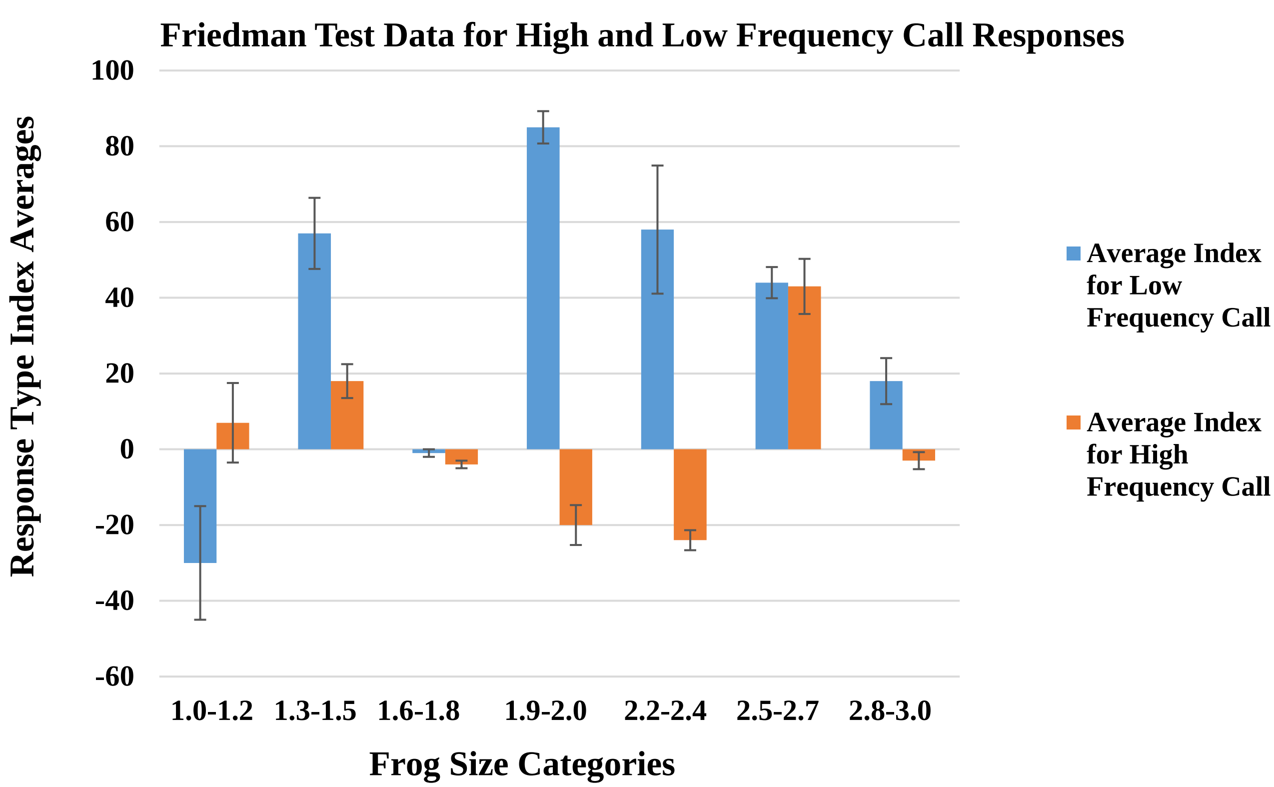

Figure 7. The averages of the response type for each size category for both the high and low frequency calls in order to run the Friedman Tests. The standard errors are in this format: (mean+SE).

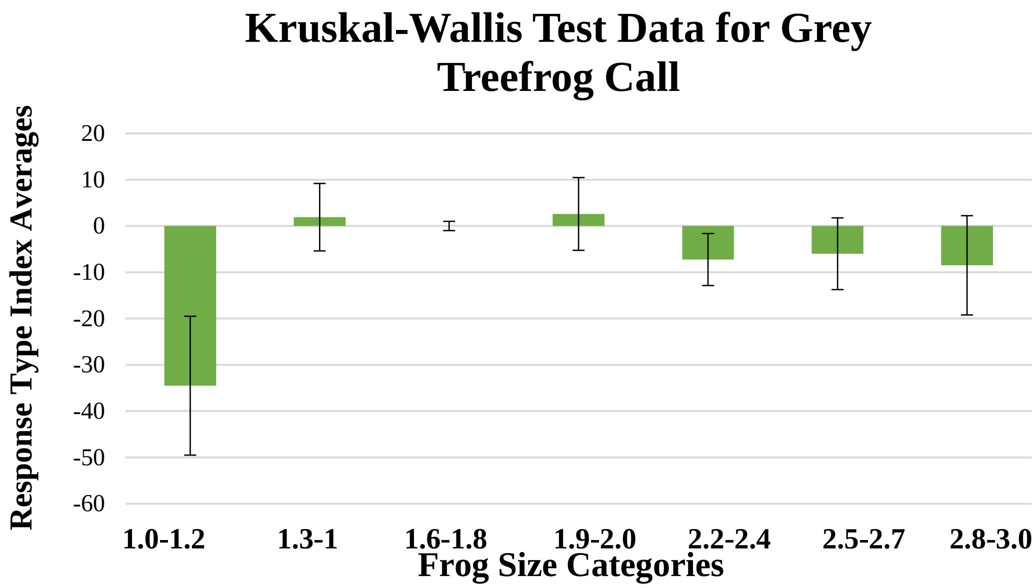

Figure 8. The averages of the response type for each size category for grey treefrog call in order to run the Kruskal Wallis Test. The standard errors are in this format: (mean+SE).

Tables

Call:
Speaker:
Which way did the frog move?
Additional Notes:

Seconds Intervals:	3	6	9
HOP			
WALK			
MOVE: HEAD OR BODY			
WALK ON WALL:			
DASH: NOTHING			

Table 1. The datasheet to properly record the movements of the peeper tested in three second intervals with tally marks.

-3	-2	-1	0	1	2	3
Hop Away	Walk Away	Move Away	Nothing	Move Towards	Walk Towards	Hop Towards

Table 2. The index applied to the response data in order to run the appropriate statistical tests. Each movement the frog made was given a numerical value. Positive numbers were given to movements toward the speakers while movements away had negative values. The movements of each frog were added up using these values so each frog had a quantitative value.

Size Category	Average Size per Category
1.0-1.2	1.1
1.3-1.5	1.4
1.6-1.8	1.7
1.9-2.1	2.0
2.2-2.4	2.3
2.5-2.7	2.6
2.8-3.0	2.9

Table 3. The average size categories and size averages used for running the Friedman Tests for each of the call response types.