Call Quality and Distribution of Male *Hyla versicolor*

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Abstract:

Many male anurans use vocal calls to attract females, who can choose from among calling males. This sexual selection has the potential to alter the evolution of anuran species. The lek mating system of the Gray Tree Frog (*Hyla versicolor*) was examined to discover patterns in the distribution of males on the mating ground with regard to call quality. A vernal pond located in Michigan's Upper Peninsula was used as a sample mating ground. The location of each calling male was documented along with a two-minute recording of his call and his length and cloacal temperature. Larger males were found to have lower frequency calls. In addition, temperature was correlated to several parameters of call quality. The distribution of males did not show the typical lek mating system arrangement, where the best quality males are located in the middle of the breeding site. Therefore, I conclude that male location is not strongly influenced by the physical distribution on the breeding site. Other factors, such as biotic conditions may have more of an influence.

Introduction:

Organisms use a variety of methods to seek adequate mates. Males use many different methods, depending on the taxa and species, to attract females to them and convince them they are worth mating with. Likewise, several different methods of choosing the right mate have evolved in females. Both male efforts and female choices can be limited by environmental and social constraints (such as high predation risk or low density), but they often have a genetic basis (Widemo and Saether, 1999). These processes of sexual selection, if based on heritable traits, have the potential to affect the evolution of species.

One such example of sexual selection is the advertisement calls of frogs and toads. Males call to advertise their position to females. These vocalizations elicit phonotaxis by females ready to mate (Gerhardt, 1994). The specific acoustic properties preferred by the females differs by species and the sound quality of a male calls varies by individual (Gerhardt, 1994). Females may use different methods, such as a 'best-of-n' tactic (where she evaluates all options and chooses the best) or threshold-based tactics (where she mates with the first male that surpasses a predetermined threshold), or some other tactic, to choose a mate (Gibson and Langen, 1996).

Despite the tactic used by the female, males possessing calls of higher quality will have a greater chance of reproducing successfully. An advertisement call includes several individual calls separated by silence. In several frog species, such as the gray tree frog (*Hyla versicolor*), each call is composed of many pulses as well. Sound quality can be determined by several different acoustic properties such as pulse rate, pulse number, call rate, and sound pressure level (Gerhardt et al. 1996). For example, a female gray tree frog prefers calls with higher-than-average pulse rates and those that are of long duration (Gerhardt, 1994).

Hyla veriscolor have a 'lek' mating system, where males aggregate and advertise collectively, but compete for the best sites on the mating ground. The males provide no resources or parental care; in fact they provide nothing for the female except gametes (Gerhardt et al. 1996). The female's choice is based solely on the male's presentation.

In a typical lek mating system, the best sites are those in the middle of the mating ground. Males located there have the greatest chance of reproducing. However, male tree frogs found in the middle of the mating ground may actually be at a disadvantage. It has been found that females choose the louder call when presented with two calls differing only in sound pressure level (Fellers, 1979a and b). Generally, males that are closer to the female will have louder calls because the calls will have less degradation and interference from vegetation. The minimum distance between *H. veriscolor* males is 70cm, but they are often found even farther apart

(Fellers, 1979b). Males located on the outer edges of the mating ground would be closer to approaching females and thus have an advantage. On the other hand, females have been known to bypass calling males to get to a more distant male who is producing a call with different (and presumably better) acoustic properties (Gerhardt et al. 1996).

This experiment seeks to discover if there is a pattern in the distribution of male *H. veriscolor*, particularly to see if high-quality callers are more common in the middle of the mating ground. My null hypothesis is that males are not distributed according to call quality. In addition, I'm interested in discovering whether call quality varies according to snout-vent length and temperature of the male.

Methods:

Study site

A population of *H. veriscolor* located in vernal pond V at the University of Notre Dame Environmental Research Center in the Upper Peninsula of Michigan was used in this experiment (Appendix A). The pond is located at a latitude and longitude of 46.2511 and -89.5177 and is at an elevation of 513 meters above sea level. It is located in an aspen (*Populus tremuloides*) stand and is surrounded primarily by grasses and fallen logs and branches. Prior to collecting data the pond was marked with stakes, creating a 20x20 meter grid, with stakes at every meter interval.

Data collection

Data collection began when the *H. versicolor* males began calling and continued to the end of the mating season. Males were observed calling on eight nights between May 30 and June 9, 2008. Air temperature was recorded every night. On each night, the calling males were located and their call was recorded with a M-Audio Microtrack 24/96 for two minutes. The snout-vent length and cloacal temperature were also taken for every frog. Each male was numbered and marked by toe clipping and a flag with the respective number was placed in the exact location the frog was calling from. Males were released back at their original location after each sampling period. Individual males continued to get recorded in this manner until the entire population was accounted for. When it was not possible to sample every male in the population, a random number generator was used to locate a coordinate and the closest male was sampled. This process was repeated until the frogs stopped calling for the night. The next day, all flags were collected and the location of each frog was recorded with an x,y coordinate according to the grid system. This entire process was repeated every night for the duration of the mating season. Several males were found more than once over the course of the season, but never was the same frog recorded more than three times. A total of 57 males were recorded over the extent of the experiment.

Analyses

The recordings of the calls were then analyzed with the program RavenPro1.3 (Appendix B). Seven acoustic properties were examined: call rate, intercall period, call period, call length, number of pulses, pulse rate, and frequency. Call rate is the number of calls per minute, the intercall period is the length of silence between two calls, the call period is the length of time from the start of one call to the start of the next, and the call length is the length of the call, all in seconds. The number of pulses in each call, the pulse rate, in pulses per second, and the frequency, in Hertz, were also recorded. Average numbers for each acoustic property were calculated for each frog.

SYSTAT 12 was used to analyze the length and temperature data. A plug-in for Excel, called “SpPack,” was used to perform statistics on the distribution data.

Results:

Effects of male physical attributes

Regression analyses were preformed on the length and temperature data. Several of the call characteristics showed a significant correlation to cloacal temperature, including call rate, intercall period, call period, call length, and pulse rate (Table 1). Call rate and pulse rate both increased as temperature increased, while intercall period, call period, and call length decreased with temperature (Figure 1). Interestingly, there was only a slight relationship between cloacal temperature and air temperature ($p=0.116$). A significant relationship was found between length and call frequency ($p<0.001$). The larger the frog the lower the call frequency was likely to be (Figure 2).

Spatial distribution

The distribution of the calling male *H. versicolor* can be seen in Figure 3. A first-order nearest neighbor analysis was used to determine the distribution of the calling males. A Clark-Evan's R-value of 0.727 and a z-value of -3.84 were found. Both the Clark-Evan's R-value ($R<1$) and the z-value ($z<0$) indicate that the males were not evenly distributed but rather, were aggregated. They tended to be present near the edges of the pond and on a large branch in the southern section of the pond, as the distribution shows.

The quality of each frog call was determined according to assumed female preferences. For every call, each acoustic property was awarded a value representing a ranking of high, medium or low (with values of 0, 5, and 10, respectively). The rankings were determined by setting the upper and lower limits at half the standard deviation for that variable. Thus, the three rankings were distributed roughly evenly within each variable. Higher call rates, shorter intercall periods, longer call lengths, higher pulse numbers, higher pulse rates, and lower frequencies were considered advantageous. Call periods were no taken into consideration because it includes both call lengths and intercall periods. By adding up the values given for each variable, every male was given a value representing the quality of their call. These values ranged between 0 and 55. The distribution of the frogs along with their call quality could then be visually represented on a coordinate grid (Figure 4).

In addition, each frog was designated a ‘quality ranking’ of low, medium, or high based off of the quality value given (0-20 represented low, 25-35 medium, and 40-55 high). After calculating the distance of each male to the middle (coordinates 10,10) an ANOVA was preformed. No significant relationship was found between the quality of a male’s call and his distance from the middle of the pond (p-value=0.926, F-ratio=0.077, df=2).

Discussion:

Effects of male physical attributes

The relationship between size and call frequency has been observed in previous literature (Marquez-M. de Orense, and Tejedo-Madueno, 1990). Frogs with larger snout-vent lengths also have larger air sacs, which are capable of creating calls of lower frequencies. Since females prefer lower frequency calls, this creates an advantage to larger frogs. However, no other call variables were correlated to frog size. So although size may assist an individual male in creating a high quality call, it is not the only factor that needs to be considered.

Cloacal temperature also had an effect on the quality of a male’s call. Relationships between acoustic call properties and ambient air temperature have been observed in the literature. Blair (1958) found air temperature to be inversely related to call length and directly related to pulse rate in *H. versicolor*. Gerhardt (1978) found similar results with respect to pulse rate. Because intercall period is partially dependent on call length, and a smaller call length may allow for more calls per minute, it makes sense for these to follow the same pattern as call length, as they did in my experiment.

However, the literature data were all based on ambient air temperatures, not the actual cloacal temperature of the frog. Because *H. versicolor* are ectotherms, their body temperature should vary with air temperature. Ectotherms cannot regulate their own body temperature and thus, their heat comes from the environment around them. In my experiment, only a slight trend was observed between air temperature and the average cloacal temperature for that night. This may be due to the variability in time of the air temperature measurements and the drop in temperature during the night. The cloacal thermometer could also be at fault. The process of taking cloacal temperatures was a lengthy one. The frogs were held in the hands of the researchers for some time before the temperature steadied enough to take a reading, and this warming may have skewed the results.

Spatial distribution

The spatial distribution analysis showed that the distribution of calling males in the pond was aggregated. This is fairly obvious by simply looking at the mapped distribution. Males seem to prefer places to perch while calling. Therefore, they were more common around the edge of the pond, in the vegetation and on partially submerged logs and branches, where they were able to find a perch. The distribution shows the outer edges of the vernal pond as well as the large aspen branch that crosses the pond in the lower region. No males were found calling while floating in open water, located in the upper portions of the pond. Besides these patterns, though, no patterns emerged with regard to the individual male quality. For example, both high and low quality males were found on the main branch crossing near the middle of the pond.

Other lek mating systems often show a “king-of-the-hill” pattern, where the highest quality males are found in the center and the quality of the males degrade as you travel away from the middle. The tree frogs I sampled did not follow this pattern, as there was no association between the quality of a male’s call and his distance from the center of the pond. Therefore, I conclude that male *H. versicolor* do not arrange themselves on the breeding ground according to call quality in the same manner as other lek mating systems.

This pattern – or lack thereof – may not be indicative of *H. versicolor* behavior every year. Due to cooler weather and the delayed onset of summer, the breeding season was later this year than usual. Perhaps more importantly however, the breeding season was broken up. The breeding season ranged over an eleven-day period (May 30 to June 9), but on only eight of those days were *H. versicolor* observed calling. This variability may have prevented high quality males from establishing good territories and returning to them multiple nights, as would be expected if the males were arranged in a lek system. If males were given the opportunity to set up territories and return to them, a lek pattern might be observed.

However, because tree frogs prefer perches instead of open water, a distribution according to biotic conditions is much more probable than a “king-of-the-hill” pattern. For example, males may prefer perching on partially submerged branches because fewer obstacles that would degrade the quality of sound reaching the females surround them. For the same reason, areas with more grass coverage or small shrubs may be less preferred. If this were true, you would expect to find males with higher quality calls located on the preferred perches. Future research exploring the distribution of male calls with regard to biotic conditions would be beneficial to test this idea.

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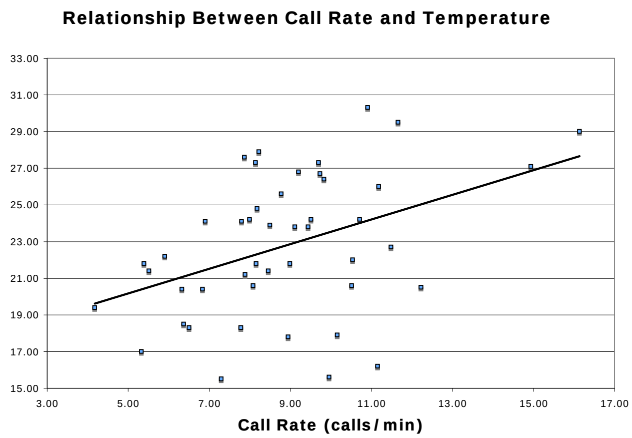
a.)



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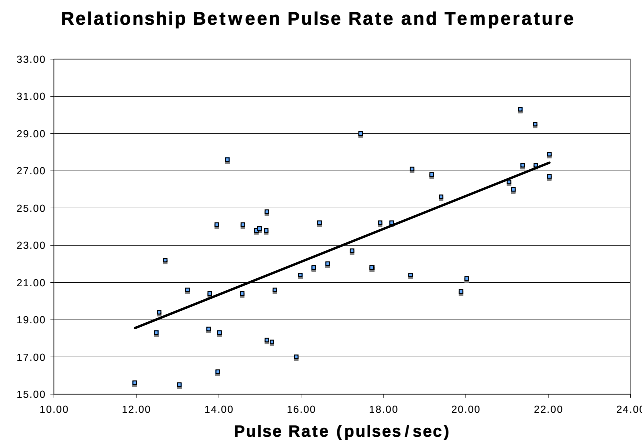

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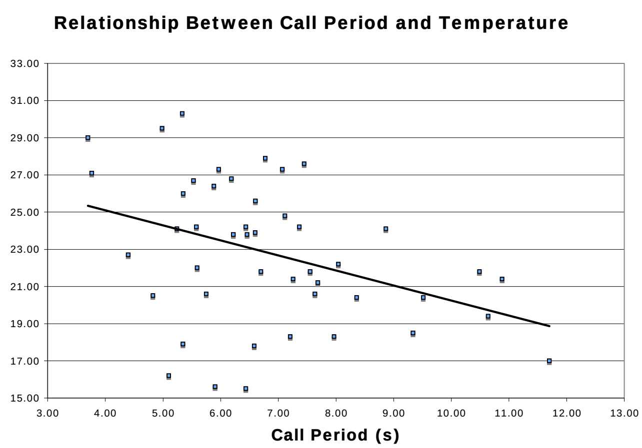

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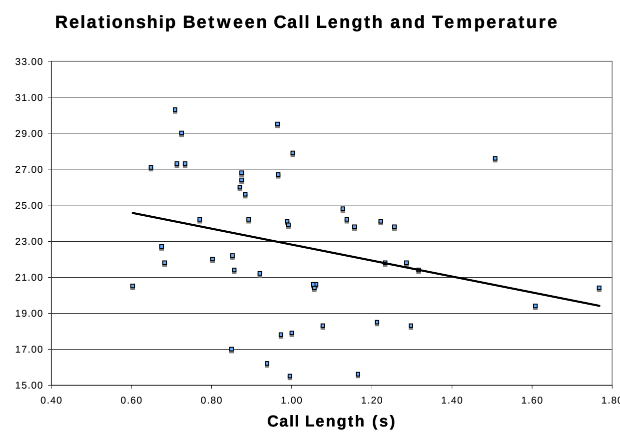


Figure 1: Regression analyses showed significant relationships between a. call rate (p-value=0.005), b. pulse rate (p-value<0.001), c. call period (p-value=0.009), and d. call length (p-value=0.052).

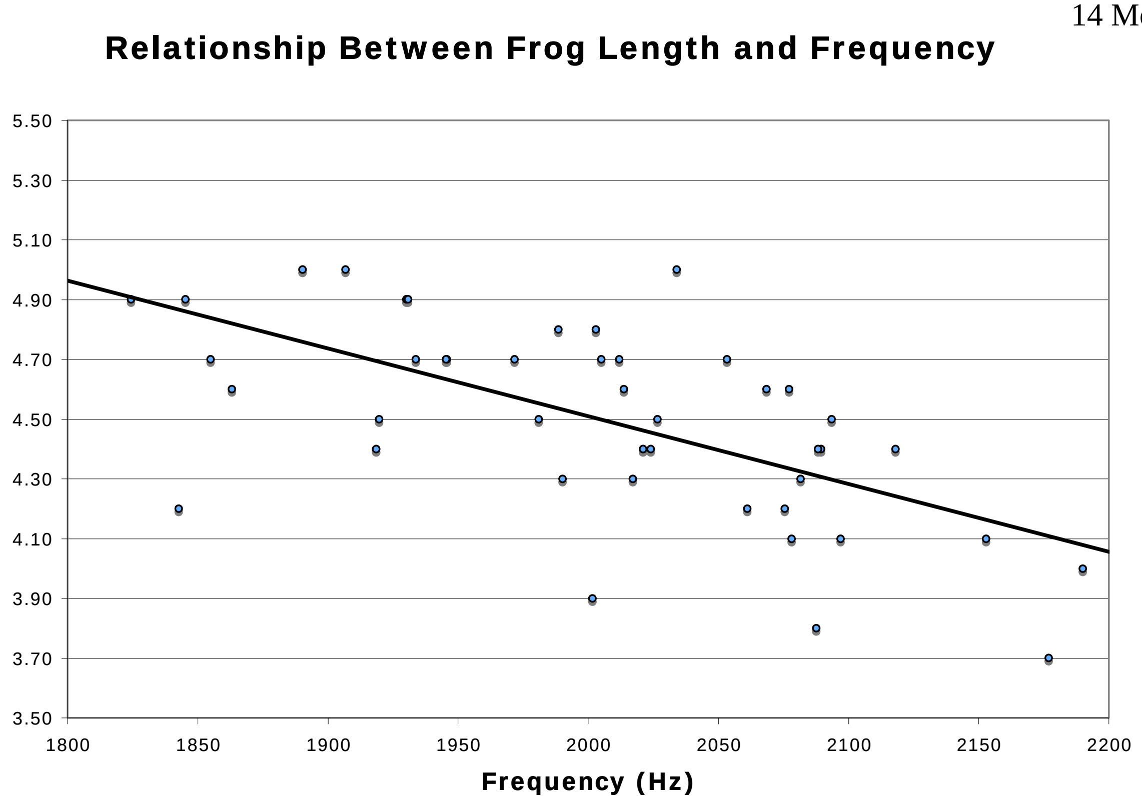


Figure 2: A regression analysis showed the significant negative relationship between frog length and frequency of the call (p-value<0.001).

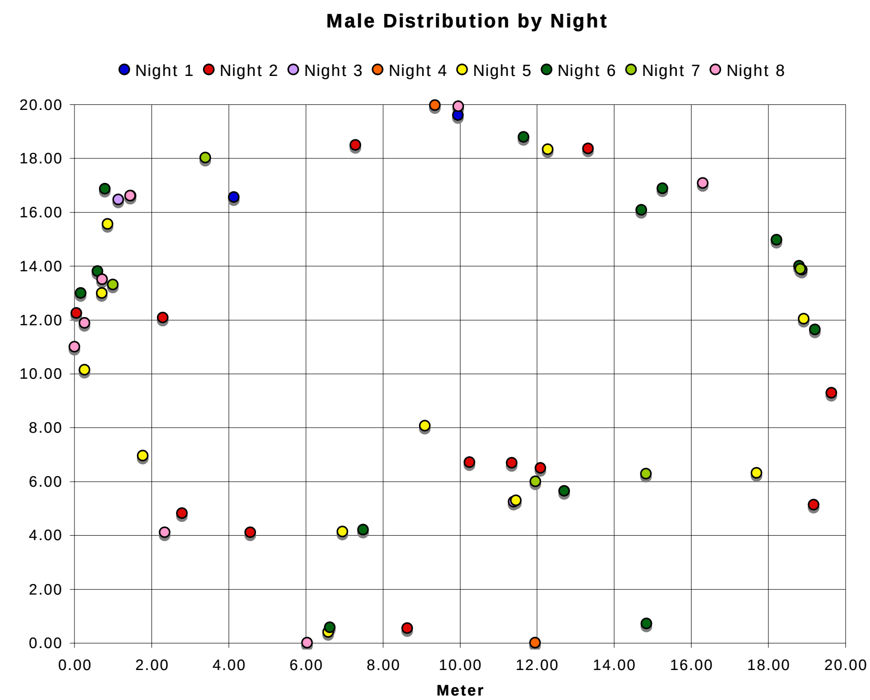


Figure 3: Distribution of calling male *Hyla versicolor* at vernal pond V, across eight nights between May 30 and June 9, 2008. North is up.

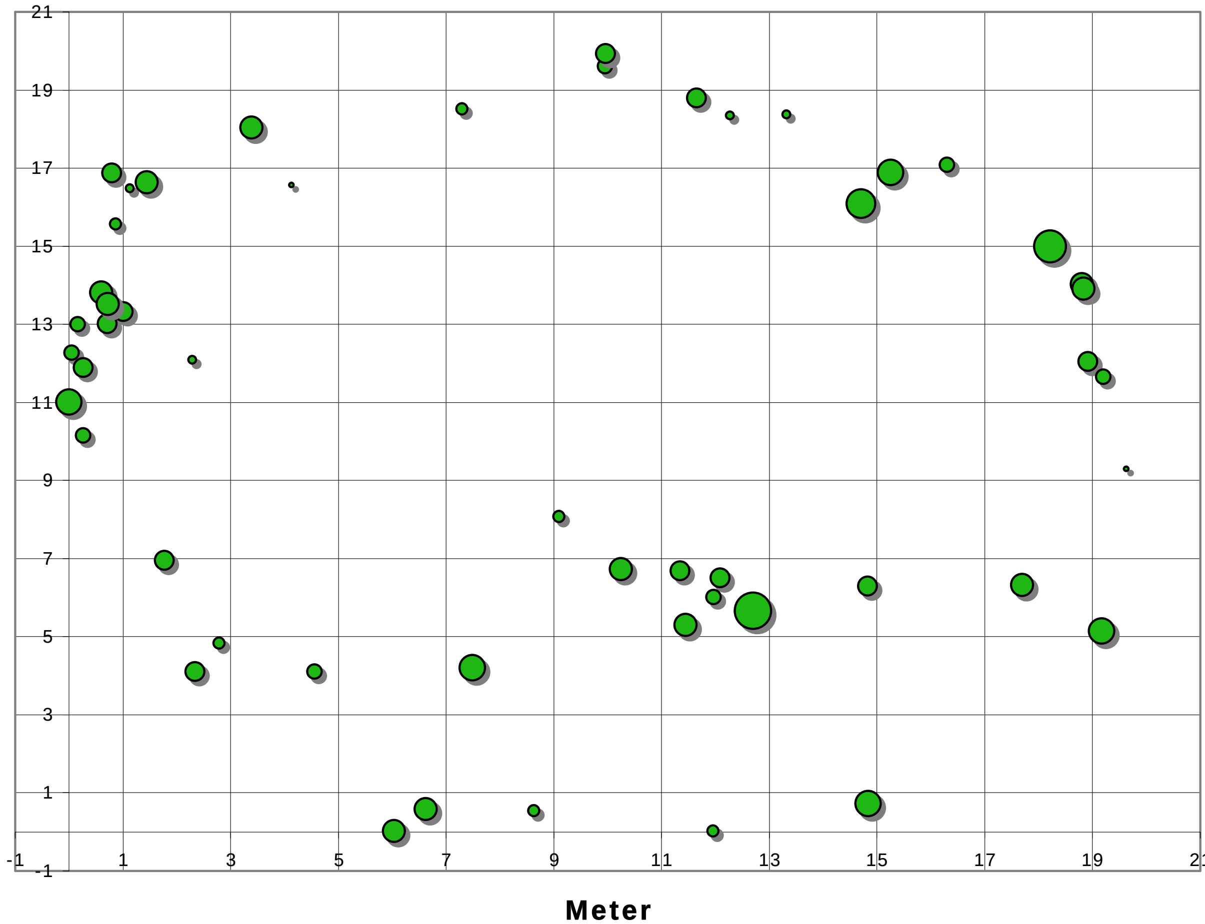
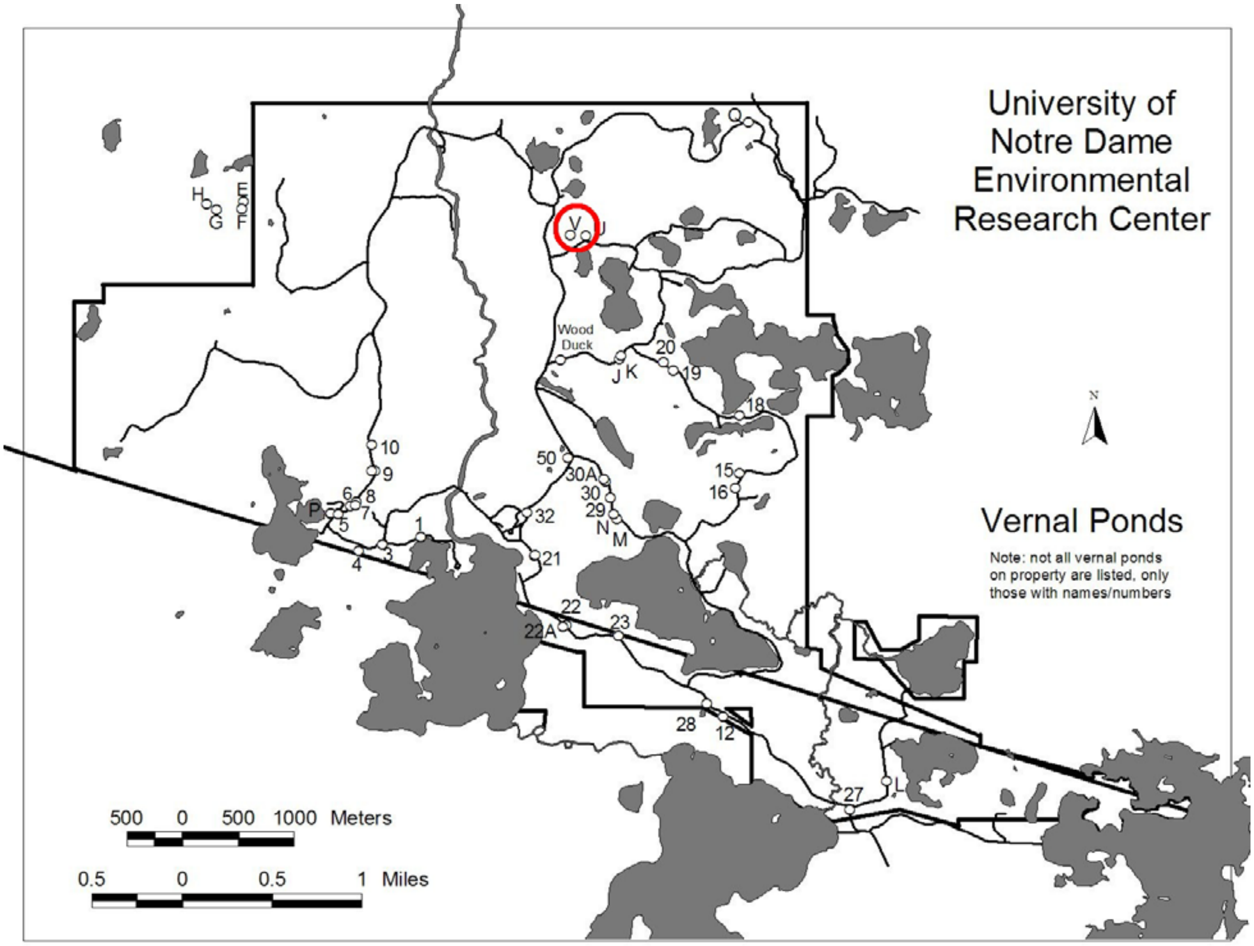


Figure 4: Male distribution at vernal pond V according to call quality. Width of circles represents call quality based on six variables: call rate, intercall period, call length, pulse rate, pulse number, and frequency.

Table 1: P-values for regression analyses. * indicates significant results.

	Length	Temp
Length	-	
Temp	0.903	-
Call Rate	0.909	*0.005
Intercall	0.723	*0.013
Call Period	0.558	*0.009
Call Length	0.071	*0.052
Pulses	0.066	0.115
Pulse Rate	0.811	*0.000
Frequency	*0.000	0.751

Appendix A: Map of vernal ponds located on UNDERC's property. Experimental site is circled in red.



Appendix B: Sample spectograms from RavenPro.