

Abstract. We performed a study of call dynamics: frequency, pulse, syllable, and song length as they relate to body size, temperature (ambient and cloacal), day of breeding season, and other male call dynamics on a population of gray treefrogs in Gogebic County, Michigan. We found that mean pulses/min increased with total song length separate from any change in mean syllable length or syllables/min. This is significant because individuals calling for longer periods of time and/or using more energy in pulsating are thought to have higher mating success. An inverse relationship exists between frequency and excess attenuation. Production of high-frequency sounds has been demonstrated as being constrained to body size and as increasing with temperature. Our results show mean frequency sharing significant relationships with two variables: positive with cloacal temperature and negative with snout-vent-length. Song length is believed to be controlled by one of two possible variables: day in breeding season or sexual excitability. Our spatial maps of male individual position suggest a non-random distribution and that our population may have been operating under a “hotspot” lek model formation. We found a marginally significant negative relationship between snout-vent-length and day in breeding season which further supports our belief that our lek may be operating under this model formation.

## Introduction

Gray treefrogs, *Hyla versicolor*, follow a lek mating system. A lek mating system is defined as meeting certain conditions: (1) an absence of parental care,

(2) males aggregate and display for mating purposes, (3) females only join males at aggregation locations for mating and no other resources, and (4) a female actively chooses the male she mates with (English, PhD dissertation). Operating within short-term breeding seasons, males form aggregations at traditional mating areas and defend small territories where they advertise themselves as prospective mates (Schwartz et al. 2002). In addition, males may increase their chance of mating success by settling at sites where females' movements are channeled by environmental features. In this way, males can promote female movement through their territories as they move between resource patches (Westcott 1994). Usually, males space themselves throughout the available habitat and defend their calling sites with aggressive calls or physical attacks on intruders. Competition among males in such choruses often takes the form of vocal interactions, with individuals attempting to out-signal their neighbors by increasing the rate, intensity, or complexity of their calls (Pough et al. 2004). Schwartz et al. (2001) found that *H. versicolor* females discriminated in favor of longer calls when the calls were twice as long as the shorter calls. In addition, females in choruses are found to be responsive to differences in calling effort by males, especially when any differences are augmented by differences in call intensity (Schwartz et al. 2001).

Frog vocalizations are energetically expensive to produce when considering the diverse physical, abiotic, and biotic factors which can affect the

propagation and degradation of signals. A male mating call can be broken down by components pulse, syllable, frequency, and decibel. A call is comprised of syllables and silences; one syllable and the silence that comes after is a 'phrase'. A syllable is a single meaningful vocalization that is composed of a certain number of pulses: strums of a vocal chord that make individual clicks that generally occur too fast and close together to hear discriminately. A syllable in the call of one species may include much variation in tone, down-slurs or chucks that make the syllable more complex (birds); or may be much simpler and composed of more constant pitch where differences in song is made up of more pulses which serves to make a longer syllable (insects and frogs). The song will also have loudness or power measured in decibels that will cause the song to travel different distances, and in some species may cause males to have different mating success. Pitch or frequency of the sound waves is measured in kilohertz (kHz). Frequency may also be an aspect of song that is important in species recognition and in other uses of identification and attraction. A single syllable may be consisted of pulses ranging across multiple frequencies, or may be of rather constant frequency. In certain instances, pulse frequency change is believed to be a crucial component in female neuron stimulation and therefore call quality (Ewing 1990, appendix 1).

A large proportion of the variation in metabolic cost of calling is explained by differences in call duration and call rate. Species differences in energetic costs

depend on body size and the type of call produced. For example, each pulse in the calls of *H. versicolor* requires a body wall contraction resulting in much higher metabolic costs per call than in smaller species such as *H. microcephala* and *Pseudacris crucifer* (Gerhardt 1994). Essentially, frogs are generally constrained by their small body size to the production of relatively high-frequency sounds.

Various species of *Hyla* call from trees during daylight hours. These tree-calls are noise-like and can be heard at long distances from any breeding pool. The tree-call is approximately the same length as the mating call (done at the breeding pool) but its function is still unknown. Mating calls contain information, encoded spectrally and/or temporally, important for species recognition and transmission of individual attributes such as body size (Schwartz 1987). Gray treefrog mating calls are shown to be positively affected by temperature and negatively affected by body size. Degree of sexual excitement is also believed to affect *H. versicolor* mating calls (Blair 1958). Other biotic factors, such as other calling males, can affect the transmission of an individual call. Due to the nature of the lek, individuals within a chorus will change the timing of their calls in response to calls of other members in order to preserve signal integrity or reduce acoustic interference (Schwartz 1987).

The calls of other males, followed by a brief time delay, within a chorus can stimulate an individual male to call and thus promote an alternating call

mechanism within a lek. Alternation can also occur as a result of changes males make in the rate and timing of their calls or call components when interacting vocally with other calling males. *H. versicolor* females are able to discriminate male calls at fine levels based on pulse-repetition rate which points to the importance of call clarity during call transmission. Furthermore specific call dynamics, including call length, pulse rate, and number of pulses per minute, all contribute to the fitness of an individual's call as well as to individual recognition by females (Schwartz 1987).

The goal of this study was to analyze male *H. versicolor* call dynamics: frequency, pulse, syllable, and song length as they relate to body size, temperature (ambient and cloacal), and the day of the breeding season. We also explored how individual call dynamics relate to other male call dynamics in the chorus. We hypothesized that call frequency would relate to body size. We also believed that ambient temperature would directly affect cloacal temperature as well as song length. In conjunction, we felt that changes in song length, per minute of calling, would be reflected with changes in number of pulses, per syllable and minute of song; and syllables in terms of syllable length and number of syllables per minute. In relation to lek dynamics, we hypothesized either: (1) a relationship between the day in breeding season and size of males of specific body size or (2) a relationship between ambient temperature and male body size.

## Methods

We sampled vernal pond “V” (max depth = 1 m) located north of Firestone Lake on the University of Notre Dame Environmental Research Center (UNDERC) property in Gogebic County, Michigan (appendix 2A). The pond was located in a mixed aspen stand (*Populus tremuloides*) and largely surrounded by grasses and sedge. The most prominent feature of the pond was a fallen aspen tree which crossed the pond completely, length-wise, and was partially submerged in water. We measured the perimeter of the pond as well as length, width, and max depth. We set up a 3 x 3, 5 m grid outside the perimeter of the pond (appendix 2B). We took UTM coordinates using a Garmin 12 Channel Global Positioning Systems (GPS) unit at each corner of our grid; as well as at locations of logs, major clumps of grass, and the fallen aspen tree.

Once a chorus was established (June 2, 2005), we sampled the entire calling population of males from approximately 2100 h till 2400 h; and ceased sampling once calling had largely stopped (June 6, 2005). We recorded each individual call using a Dan Gibson Model P-350 EPM Parabolic Microphone and an RCA Model No: RP 5012B Digital Voice Recorder. Calls were recorded, directly facing the individual, for 2 min at a distance of approximately 1 m and labeled according to an individual’s toe-clip number. Each male was toe-clipped for identification and their call-site flagged; cloacal temperature (to the nearest 0.5 °C) and snout-vent-length (SVL, to the nearest 0.5 mm) were also recorded. Both

ambient and water temperature were taken at the beginning, middle, and end of each sampling period.

During daylight of the following day, we returned to the pond to record the loci of calling individuals from the previous night's sampling. The location of each male was mapped using a coordinate system within our 3 x 3, 5 m grid and ArcView GIS 3.3 software. Call quality of each male was analyzed using RAVEN 1.2 Bioacoustics software. Regression analysis on call parameters and lek abiotic factors was done using SYSTAT 11.0 software.

## Results

In conjunction with day in breeding season, we found that an increase in ambient temperature had a significant, positive relationship to cloacal temperature (fig. 1B,  $p < 0.001$ ,  $r^2 = 0.2003$ ); ambient temperature significantly decreased during the breeding season (fig. 1A,  $p < 0.001$ ,  $r^2 = 0.2855$ ). Mean frequency was found to have significant relationships with two variables: positively with cloacal temperature (fig. 2A,  $p = 0.057$ ,  $r^2 = 0.0445$ ) and negatively with SVL (fig. 2B,  $p = 0.001$ ,  $r^2 = 0.1583$ ). Total song length significantly increased during the breeding season (fig. 3A,  $p = 0.03$ ,  $r^2 = 0.0775$ ) and pulses/min increased significantly with total song length (fig. 3B,  $p < 0.001$ ,  $r^2 = 0.8495$ ). Both the mean pulses/syllable ( $p = 0.023$ ,  $r^2 = 0.0851$ ) and mean syllable length ( $p = 0.004$ ,  $r^2 = 0.1332$ ) increased significantly during the breeding season (fig. 4A).

Conversely as syllables/min decreased, mean pulses/syllable significantly increased (fig. 4B,  $p = 0.009$ ,  $r^2 = 0.0405$ ).

Although statistical analysis was not available to explore the spatial dynamics of individuals with their morphological and acoustic features comprising our lek, visual representation of all individual positions points towards the existence of a non-random distribution pattern (fig. 5). Using a point system for the theoretically “best quality features” in reference to sexual selection, spatial distribution of the resulting 3 classes of males can be seen within the perimeter of the 225 m<sup>2</sup> grid (fig. 6, larger points are theoretically of better quality). Furthermore, when position of major physical environmental factors are placed on top of the spatial map of all male frogs (and their quality), non-random distribution is increasingly more obvious as well as is the existence of “hotspots” (fig. 7).

Most call parameters (pulse, syllable and song length) were found to hold significant relationships with other call parameters, as expected. Mean pulses/syllable and mean syllable length were both found to significantly increase with total song length. Syllables/min and mean syllable length significantly increased with an increase in pulses/min. Pulses/min and mean syllable length significantly increased with and increased in mean pulses/syllable. Regression analysis of all possible combinations of abiotic and biotic factors can be viewed in table 1.



## Discussion

Vocalization dominates the pre-amplexus reproductive behavior of many frog species. Analysis of anuran mating calls is important because certain characteristics of the call are directly related to the function of finding and attracting appropriate mates and mating calls are therefore generally species specific. Our data show that mean pulses/min increased with total song length, separate from any change in mean syllable length or syllables/min. This is significant in that individuals calling for longer periods of time and/or using more energy in pulsating are thought to have higher mating success (Gerhardt 1978). In response to other calling males, individuals can maintain largely constant calling efforts (energy output) by adjusting call rates through the addition or subtraction of pulses from their calls (thus avoiding call overlap). Laboratory-based two-choice experiments done with *H. versicolor* have shown that call duration and call rate, which together represent calling or pulse effort, help to mediate the discrimination of conspecific males by females. Additionally, increased pulsation is thought to create more stimulation in the female via neuron firing where as syllables in and of themselves are not thought to contribute to female excitability (Schwartz et al. 2001) but probably act as the species recognition or motivation portion of the call. Future repetition of our study should include tracking female movement as well as individuals in amplexus so that a sense of male success in relation to call quality might be established.

Sound frequency is the most important qualitative determinant of maximum communication distance. An inverse relationship exists between frequency and excess attenuation; which is the drop in amplitude with distance in addition to that expected from spherical spreading from a point source (Gerhardt 1994). Mean frequency shared significant relationships with two variables, positive with cloacal temperature and negative with SVL, making it difficult to distinguish whether temperature or body size drives individual call frequency. Production of high-frequency sounds has been demonstrated as being constrained to body size. Dominant frequency is generally well correlated with body size in both intraspecific and interspecific comparisons, so much so that body size may indirectly serve as a general constraint on the evolution of call frequency (Gerhardt 1994). Within that larger trend, W. F. Blair (1958) has shown that frequency increases with temperature in the calls of *Microhyla olivacea* and *M. carolinensis*; and that repetition rate in calls consisting of a series of repeated notes also tended to increase with temperature. Future analysis would entail recording individuals at constant temperature to tease out any possible relationship with body size.

Ambient temperature had a positive effect on cloacal temperature, which follows with relationships ectotherms hold with temperature. Ambient temperature did not significantly affect song length and this might be due to ambient temperature decreasing during the breeding season. It is possible that

total song length might be controlled by other factors such as day in breeding season ( $p = 0.03$ ) or sexual excitability. Male tungara frogs (*Physaleamus pustulosus*) are capable of increasing their own call complexity and length when experiencing increased acoustic stimulation, and therefore sexual excitement (Schwartz et al. 2002). Experimentation is needed to explore both the temporal and acoustic stimulation effects of male calls on females in order to establish which is more significant in relation to total song length and pulses. This might be done by keeping a female in a 12 h daylight/12 h dark environment and allowing for acoustic stimulation, or by allowing for a normal daylight/dark cycle and prohibiting all acoustic stimulation.

Mean pulses/syllable and mean syllable length increased with song length which follows established make up of whole songs: number of syllables is limited by the number of pulses and pauses which summate total song length. It follows then, that total song length did not have a significant relationship with syllables/min but pulses/min. This finding might be the result of the relationship between mean pulses/syllable and mean syllable length ( $p < 0.001$ ) in that syllable length is determined by the number of pulses which can vary with each syllable, regardless of the number of syllables/min. Syllables/min should have decreased as mean pulses/syllable increased, as our results show, because songs comprised of longer syllables (more pulses) will hold less syllables per minute of calling.

Spatial maps of individual position strongly hint towards the existence of “hotspots.” The “hotspot” lek model (an alternative version of the “female-preference model”) formation is the result of attempts by individual males to place their display courts at sites where females can be encountered in the highest numbers or where perches give some other advantage. Males aggregate at “hotspots” independently, taking cues from female ranging habits or some characteristic of the habitat. Initial males presumably settle at the best sites, and to avoid sharing females, successive males settle at sites separated from other males by a distance greater than one female home range (Beehler and Foster 1988). Our results show a marginally significant relationship ( $p = 0.088$ ) between SVL and day in breeding season which further supports our belief that our lek may be operating under a “hotspot” model formation. Future experimentation should involve statistically analyzing both male and female individual spatial positions throughout the breeding season, in reference to the lek, and to surrounding environmental physical factors.

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## Tables and Figures

Table 1. P-values of all regression analysis performed on biotic and abiotic factors (**significant values are bolded**).

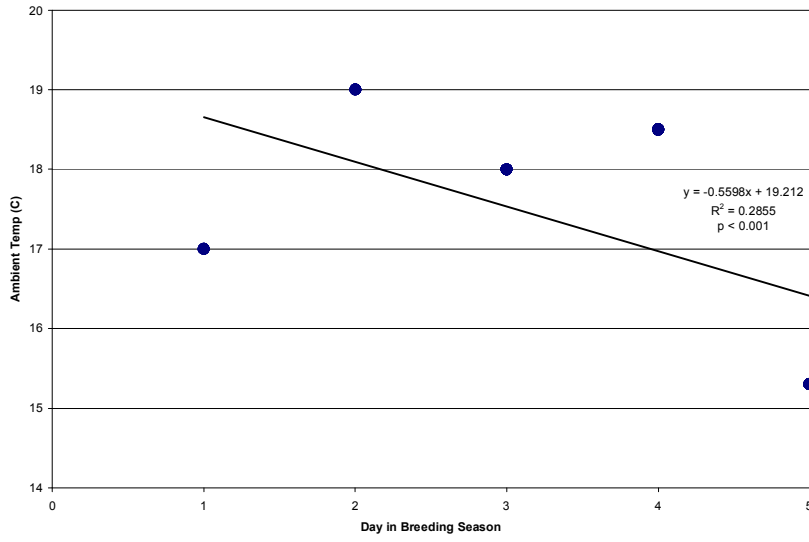
	SVL	Cloacal Temperature	Ambient Temperature	Day in Breeding Season
Mean Pulses/Syllable	0	0.021	0.0149	<b>0.0851</b>
	(p=0.941)	(p=0.269)	(p=0.348)	(p=0.023)
Pulses/Minute	0.001	0.01	0.0007	0.038
	(p=0.819)	(p=0.443)	(p=0.843)	(p=0.131)
Mean Syllable Length	0.005	0.041	<b>0.0828</b>	<b>0.1332</b>
	(p=0.601)	(p=0.118)	*(p=0.024)	(p=0.004)
Syllables/Minute	0.003	0.001	0.0088	0.003
	(p=0.68)	(p=0.769)	(p=0.47)	(p=0.68)
Total Song Length	0.001	0.024	0.0359	<b>0.0775</b>
	(p=0.825)	(p=0.232)	(p=0.143)	(p=0.03)
Mean Frequency	<b>0.1583</b>	<b>0.0445</b>	0.0122	0.031
	(p=0.001)	(p=0.057)	(p=0.395)	(p=0.176)
				X
Day in Breeding Season	<b>0.049</b>	0.001	<b>0.2855</b>	
	*(p=0.088)	(p=0.778)	(p<0.001)	
Ambient Temperature	0.028	<b>0.2003</b>	X	
	(p=0.198)	(p<0.001)		
Cloacal Temperature	0.002	X		
	(p=0.763)			
SVL	X			

	Mean Frequency	Total Song Length	Syllable/Minute	Mean Syllable Length	Pulse/Minute	Mean Pulses/Syllable
Mean Pulses/Syllable	0.027	<b>0.2266</b>	<b>0.0405</b>	<b>0.8161</b>	<b>0.2323</b>	X
	(p=0.203)	*(p<0.001)	(p=0.009)	*(p<0.001)	*(p<0.001)	
Pulses/Minute	0.034	<b>0.8495</b>	<b>0.0533</b>	<b>0.1487</b>	X	
	(p=0.157)	(p<0.001)	*(p<0.001)	*(p<0.001)		
Mean Syllable Length	0.022	<b>0.2972</b>	<b>0.053</b>	X		
	(p=0.258)	*(p<0.001)	*(p=0.074)			
Syllables/Minute	0.013	0.4255	X			
	(p=0.376)	(p=0.734)				
Total Song Length	0.029	X				
	(p=0.191)					
Mean Frequency	X					
Day in Breeding Season						
Ambient Temperature						
Cloacal Temperature						
SVL						

\*significant value not referred to in this study

[A]



[B]

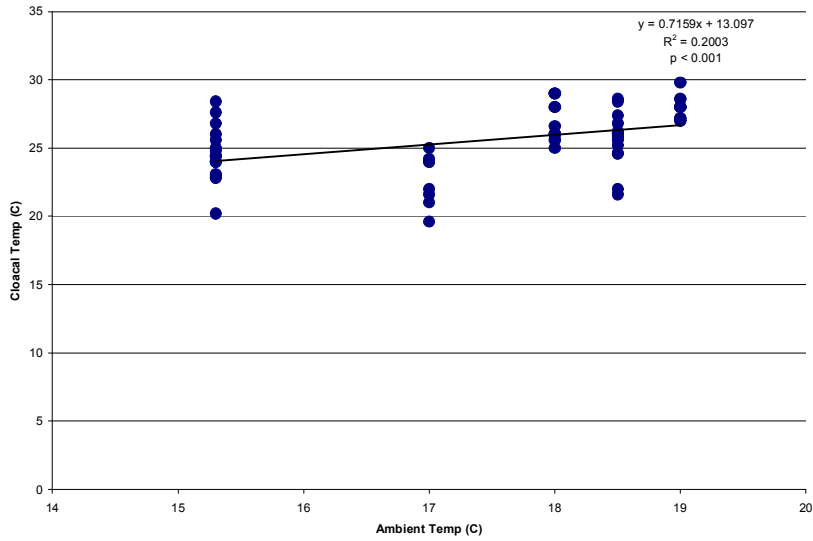
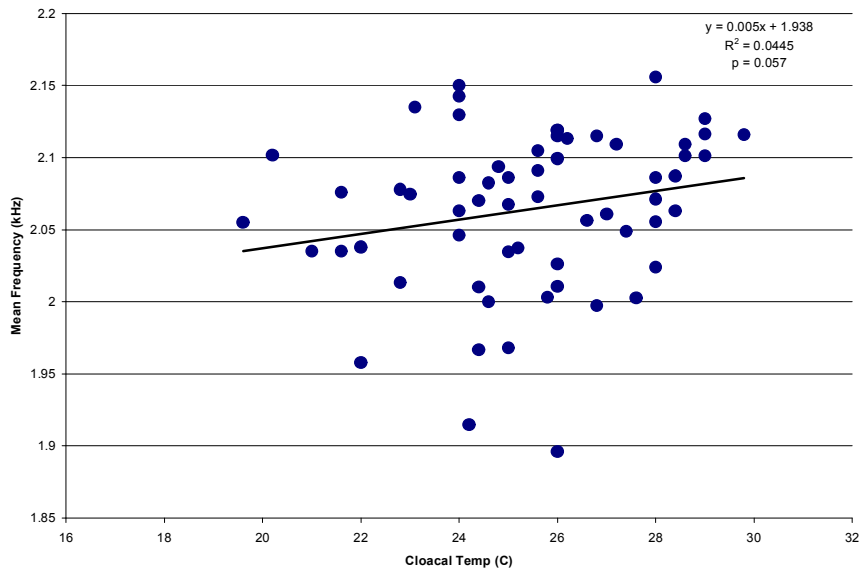


Figure 1. [A] Ambient temperature decreased across the breeding season and [B] cloacal temperature increased with ambient temperature.



[A]



[B]

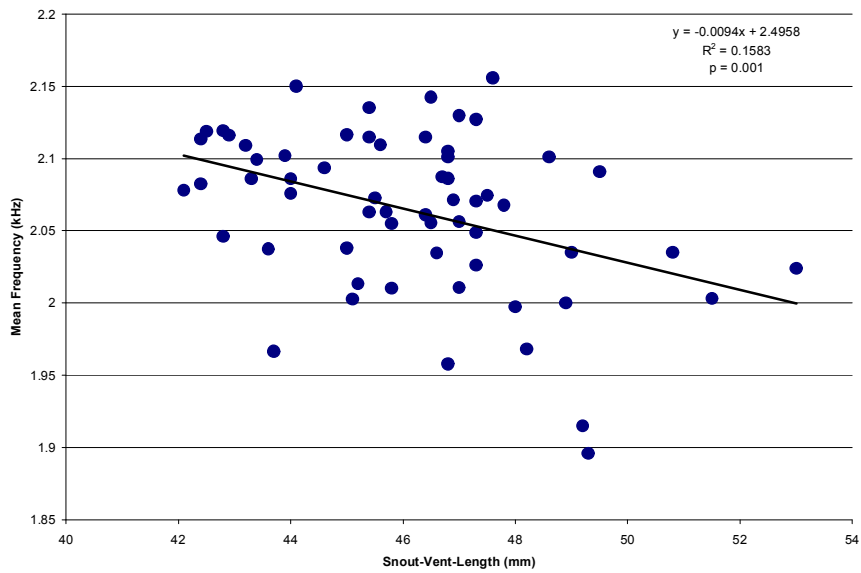
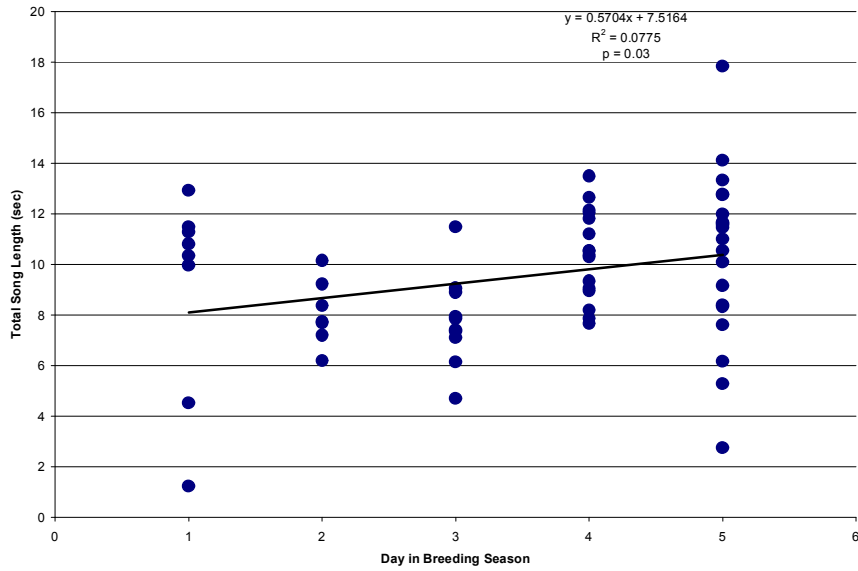


Figure 2. [A] Mean frequency increased with cloacal temperature and [B] decreased with SVL.

[A]



[B]

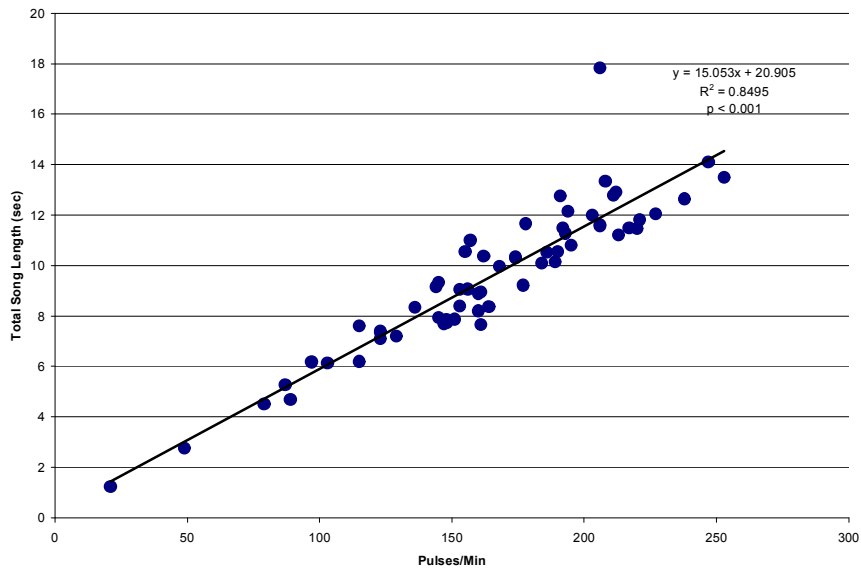


Figure 3. [A] Total song length increased across the breeding season and [B] pulses/min increased with increased in total song length.

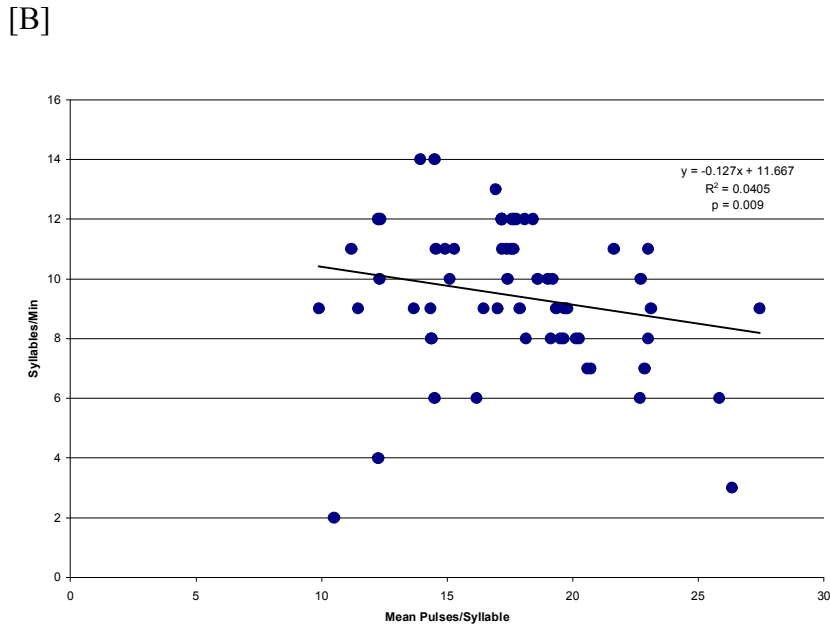
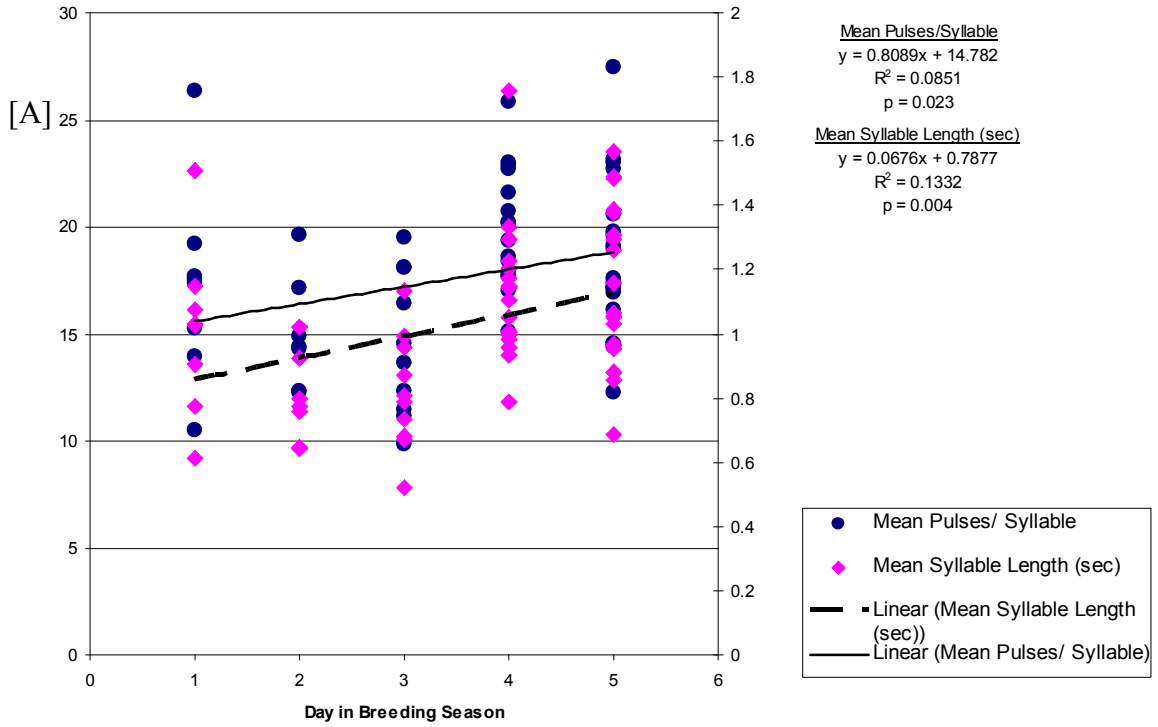


Figure 4. [A] Both the syllables/min and mean syllable length increased across the breeding season; but [B] as syllables/min increased, mean pulses/syllable increased.

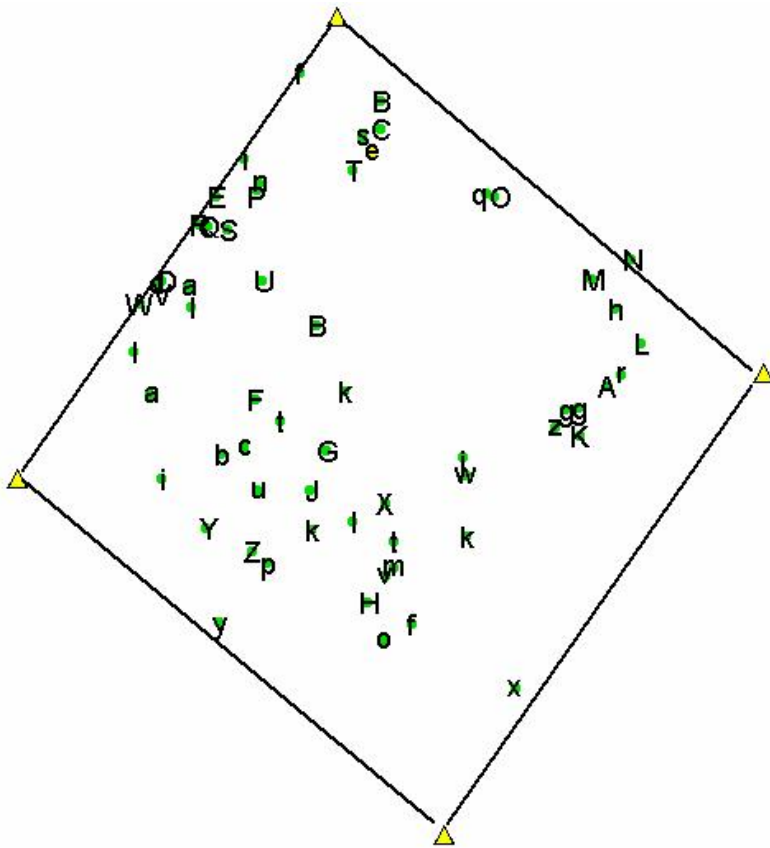


Figure 5. Spatial map of all male frogs over breeding season within perimeter of 225 m<sup>2</sup> grid.

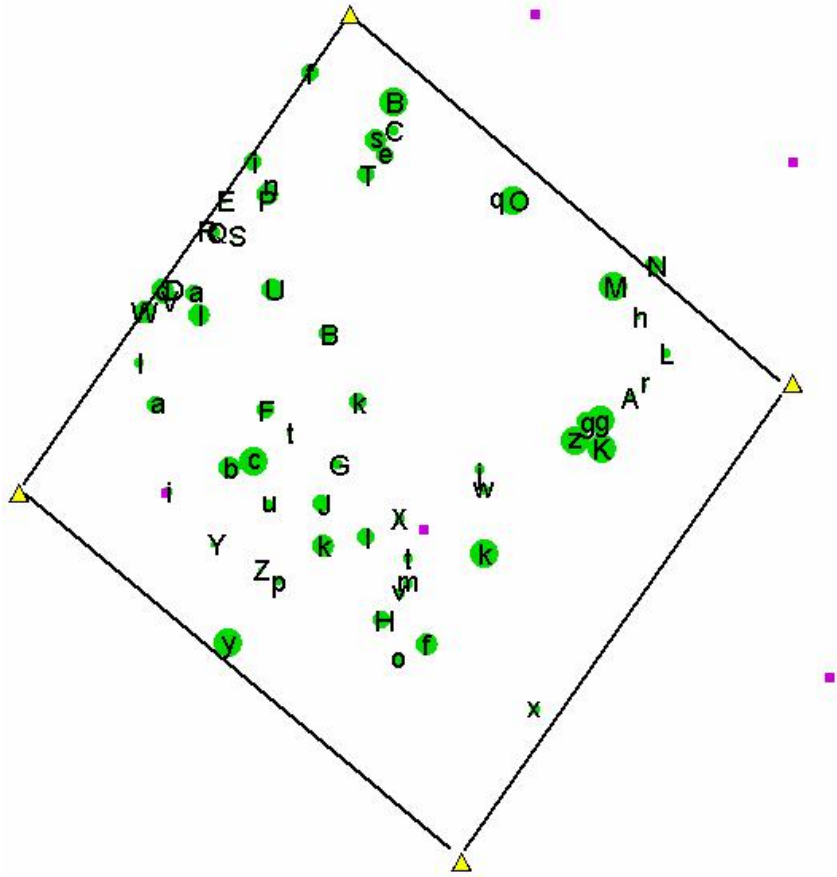


Figure 6. Spatial map of all male frogs over breeding season with “individual sexual quality” represented by area of circle encompassing individual identification.

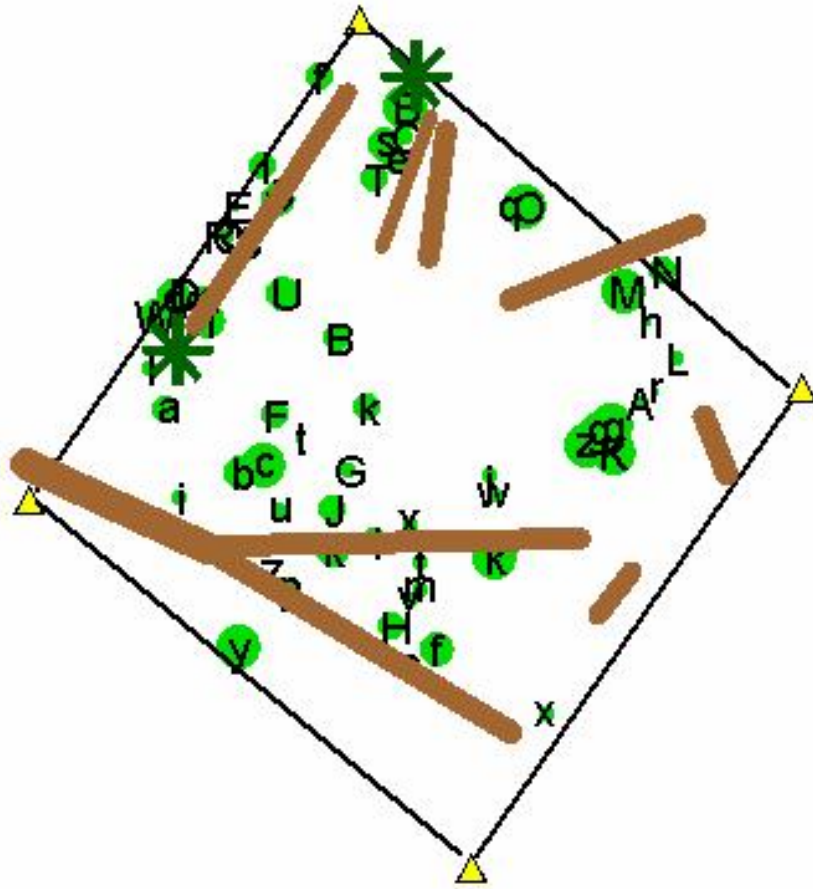
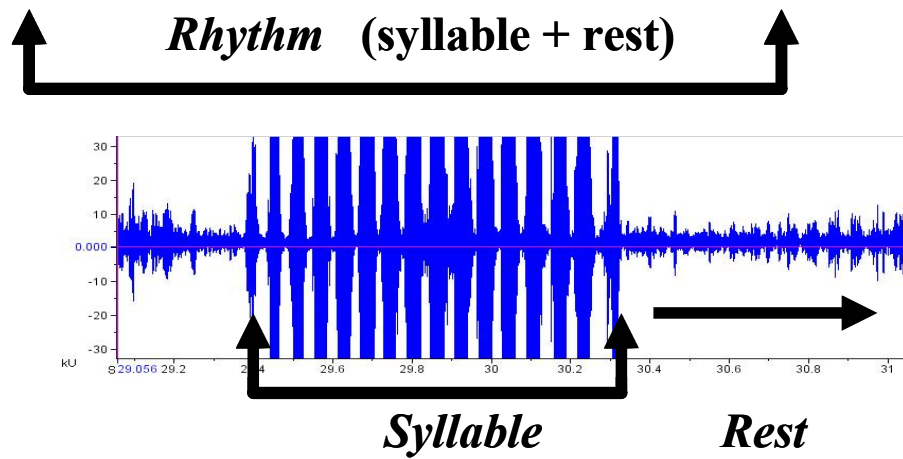


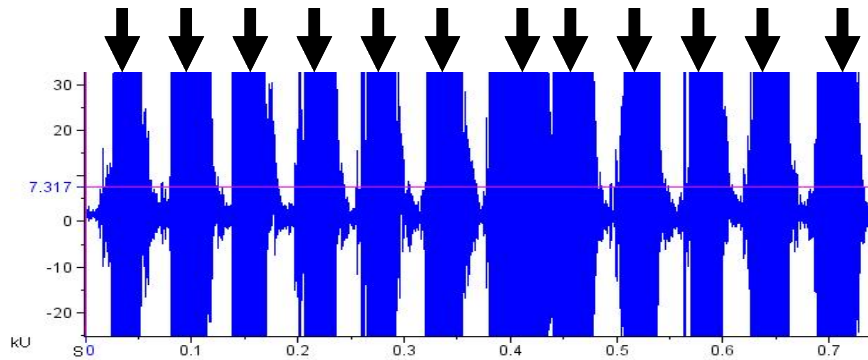
Figure 7. Spatial distribution of all calling males and their “sexual quality” in reference to physical environmental factors: clumps of grass are represented by dark green stars and logs are represented by brown lines. The fallen aspen tree is represented in the bottom left hand corner of the figure.

Appendix

**1-minute of frog vocalization: *Song* or *Call***



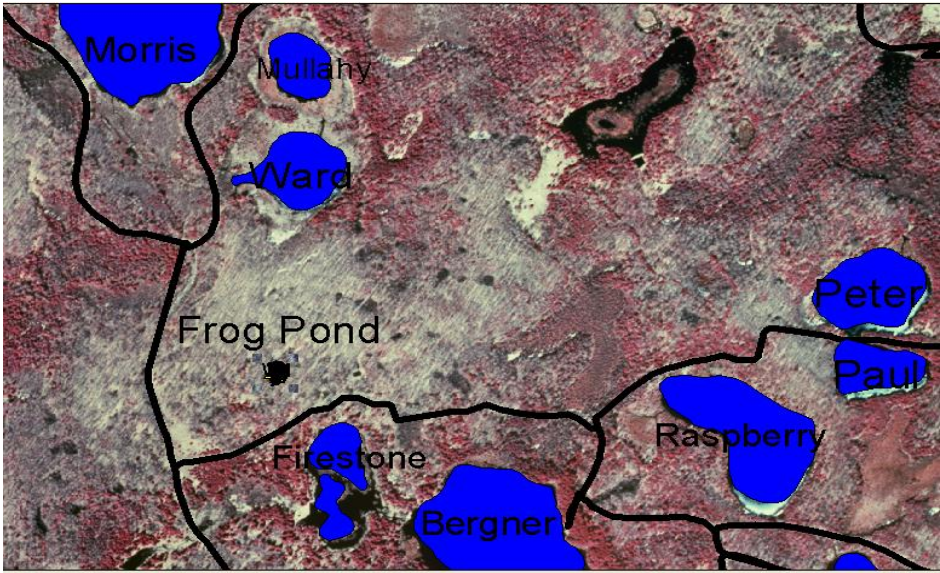
**12 individual *Pulses* (in this case) = 1 *Syllable***



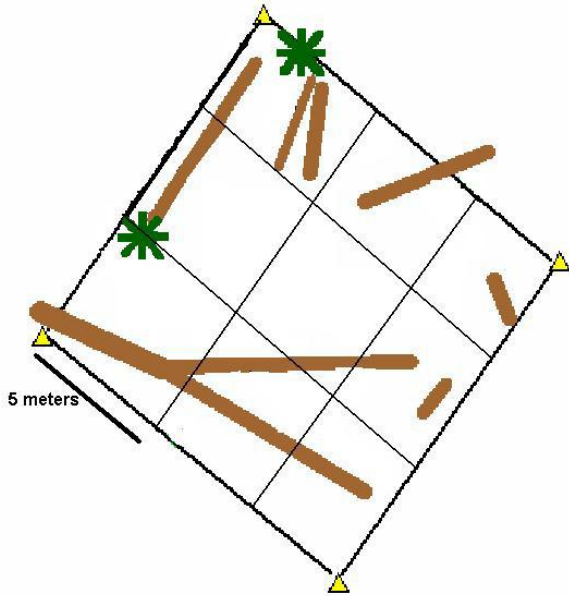
Appendix 1. Depiction of call breakdown that occurs during song analysis.

Breakdown occurs from largest component (song) to smallest component (pulses).





[A]



[B]

Appendix 2. [A] Map of gray tree frog pond site (vernal pond “V”) in Gogebic County, Michigan. [B] Grid set up around perimeter of gray tree frog pond with important environmental factors such as: a fallen aspen tree, logs and large clumps of grass.