

**Analysis of Call Quality and Lek Dynamics in *Hyla*
*versicolor***

BIOS 569: Practicum in Field Biology

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2005

ABSTRACT

Male attributes that are likely to be used in mate choice by females and function in sexual selection pressure was examined in a lek mating system of gray treefrogs, *Hyla versicolor*. Males attract females to the chorus with calls, which females presumably use in assessing the relative quality of males and in selecting a mate. This study focused on bioacoustically characterizing the songs of calling males from a chorus based on several call parameters (frequency, song length, pulses, and syllables), particular morphological features of the calling males (snout-vent length and cloacal temperature), and several abiotic features of the lek (ambient temperature and day in the breeding season). Both the pulses/minute and total song length increased significantly over course of the breeding season. Mean call frequency was affected significantly by cloacal temperature and snout-vent length (SVL). The positive relationship found between pulses/minute and total song length indicates that pulses may be relatively more important than syllables in controlling total song length. An inverse relationship was found between mean pulse/syllable and syllable/minute, suggesting that males may be able to modify the pulses and syllables of their calls to maintain a constant call effort. Preliminary data in spatial distribution suggests arrangement of male distribution across the breeding site may be nonrandom with reference to male quality and physical features of the breeding site.

INTRODUCTION

Sexual selection can be defined as “the difference in reproduction that arise from variation among individuals in traits that affect success in competition over mates and fertilization” (Andersson 1994). Female mate choice, in which females select a mate based on a particular characteristic or set of characteristics of the male rather than mating at random, is a fundamental component of intersexual selection. In many species, males use vocalizations to attract females, who consider multiple call characteristics, including call duration, frequency, pulses, and syllables in their selection of a mate.

Female mate choice is apparent in lek mating systems, which are characterized by a congregation of males in a breeding site, attempting to attract females to that location for the sole purpose of mating. There are no significant resources in the breeding site, indicating that females select mates in the lek based on nothing but the individual male and his displayed or implied characteristics. This suggests that leks are a suitable environment for the study of sexual selection. Current debates consider whether leks evolve as a result of males clustering around successful males or whether leks form where female densities are high or are likely to be high (Westcott 1994). Possible benefits of a lek mating system include decreased risk of predation, decreased mate search costs, and more efficient comparison of males (Westcott 1994).

Males of many anuran species use vocalizations within leks to communicate with both males and females. Calling males of the grey treefrog

species *Hyla versicolor* occupy their own individual territories within the breeding site, which they defend from other males with encounter calls when initially threatened and then with direct aggression if vocalizations do not determine the dominant male (Fellers 1979b). If a dominant male is established through these encounter calls, the other male may move to a different location in the breeding site or may remain silently in the territory – as a satellite male – of the calling male in hopes of gaining the perch or territory if the dominant male succeeds in attracting a female. Females, once they have selected a mate, enter the territory of the calling male and nudge him to initiate amplexus, which may last up to four hours, leaving the territory open to be used by satellite or subordinate males. Fellers (1979a) has shown that the perch used by calling *H. versicolor* males can have significant influence on the mating success of males and that preferred sites are horizontal and have relatively less vegetation, which allows for the best radiation and acoustics of vocalizations. This suggests that it may be to the satellite male's advantage to remain patiently in a calling male's territory in order to obtain his perch if he attracts a female. Several studies have suggested that the number of nights a male participates in a chorus greatly affects his mating success (Bertram et al. 1996; Ritke and Semlitsch 1989; Sullivan and Hinshaw 1992). As is the case with many anurans, it appears that sexual selection is operating through both male-male competition (intra-sexual selection) and female mate selection (inter-sexual selection) (Ritke and Semlitsch 1989).

Because females are attracted to the lek by the calling males, it seems that participation in a chorus is necessary for reproductive success (Burnmeister and Wilczynski 2000). The vocalizations of males help females recognize conspecifics and reject heterospecifics. Females use particular call characteristics to choose among males. Research has shown that female grey treefrog, *Hyla versicolor*, prefer long duration calls over shorter calls (Gerhardt et al. 1996; Sullivan and Hinshaw 1992), and that call duration is indicative of the energetic cost of calling (Wells and Taigen 1986). A call is composed of syllables, each of which is made up of a variable number of pulses and is broken up by periods of silence. Gerhardt et al. (1996) demonstrated that call effort (derived from call duration and call rate) is used by females to distinguish male callers. Males maintain a relatively stable call effort by increasing the number of pulses – and thus increasing call duration – while decreasing call rate, particularly in response to the vocalizations of other males (Wells and Taigen 1986). Calling males are sensitive to chorus size, especially when a chorus becomes large enough that chances of call overlap or interference are high (Gerhardt 2002). Females have been shown to select against overlapped calls (Schwartz 1987; Schwartz and Gerhardt 1995). Males respond to an increase in chorus size by lengthening their calls, while decreasing their call rate (Gerhardt 2002). Females respond to a higher number of total pulses particularly when there are differences in call

intensity, indicating that there is an advantage for males that produce more sound energy (Gerhardt 2001).

This study focused on characterizing the songs of *Hyla versicolor* bioacoustically based on several call parameters, particular morphological features of the calling males, and several abiotic features of the lek. Specifically, it was designed to find relationships by comparing frequency, pulses and syllables of calls to snout-vent length (SVL), cloacal temperature, ambient temperature, and day in the breeding season. It will also examine what relationships among song parameters. I hypothesize that call frequency would be correlated to SVL and that ambient temperature would directly affect cloacal temperature. I project that change in the total song length over a minute of calling would result from changes in the number of pulses/syllable, the number of pulses/minute, the mean length of syllables, and the number of syllables/minute. I hypothesize that SVL would be related to both the day in the breeding season and to the ambient temperature.

METHODS

Male grey treefrogs calling at a breeding site were observed over the five night mating season from 2 June to 6 June 2005. The breeding site was located at Vernal Pond “V,” north of Firestone Lake on UNDERC Property in Gogebic Co., MI. Situated in an aspen (*Populus tremuloides*) stand, the pond contained several fallen logs, including a large recently fallen quaking aspen, which was partially

submerged along the length of the pond (Figure 1). This site was selected based on previous observations of a chorus of calling *H. versicolor* at this location. Before beginning observations of calls, a 15m x 15m grid of colored stakes was constructed to encompass the pond in order to determine the exact locations of calling males. UTM coordinates were taken using a Garmin 12 Channel Global Positioning System (GPS) Unit at the corners of the grid and at particular physical features (trees, logs, etc.) of the pond, and ArcView GIS 3.0 was used to locate the pond, digitize features, and plot exact locations of calling males for use in future spatial analysis.

Each night of the breeding season, males were located by their calls during the chorus, which usually began at dusk and last several hours (typically 2100-2400 h). Once a male was located, his call was recorded for two minutes from a distance of 1 m and at a height level with the male. Recordings were made with a Dan Gibson model P350EPM Parabolic Microphone and an RCA model No. RP5012B Digital Voice Recorder were used. The direction in which the male was calling was recorded. A numbered flag or flagging tape was used to mark his location, and he was toe-clipped with the same number, using a toe-clipping method that incorporated numbering all toes on both front and back feet. His snout-vent length (SVL) (to the nearest mm) and cloacal temperature (to the nearest .2 °C) were measured and recorded before he was released from the same location where he was found.

The number and relative location of frog pairs found in amplexus each night was recorded. The air and water temperature were recorded three times during the chorus every evening – typically at the center of the pond and at the beginning, middle, and end of the chorus period. The next day, the exact location of the calling males was documented using the grid and the numbered flags from the previous night.

One-minute sections of each of call were analyzed using Raven 1.2 Bioacoustics software to assess several call characteristics: total song length per minute, mean frequency, mean syllable length, mean pulses per syllable, syllables per minute, and pulses per minute. These call parameters were compared to each other and to several physical factors, including snout-vent length, cloacal temperature, ambient temperature, and day of the breeding season, using regression analysis in Systat 11.0.

RESULTS

Over the five-night breeding season, 62 calling *H. versicolor* males were observed (Table 1). Their calls, location, and direction facing were recorded, and SVL and cloacal temperature were measured. Over the course of this study, there was as significant change in the ambient temperature ($r^2 = 0.2855$, $p < 0.001$; Figure 2a). Cloacal temperature was positively related to ambient temperature ($r^2 = 0.2003$, $p < 0.001$; Figure 2b).

Morphological features of the calling males and abiotic features of the lek had significant effects on some call parameters. Several of the call parameters significantly changed with day in the breeding season. There was an increase in the mean number of pulses/syllable ($r^2=0.0851$, $p=0.023$), as well as in the mean syllable length ($r^2=0.1332$, $p=0.030$) over the breeding period (Figure 3a). Total song length increased significantly with day in the breeding season ($r^2= p=0.004$; Figure 3b). Mean frequency of call appeared to be related to both SVL ($r^2=0.1583$, $p=0.001$) and cloacal temperature ($r^2= 0.0445$, $p=0.057$) (Figure 4).

Comparing call parameters to each other, several significant correlations were found. There was a strong positive correlation between the number of pulses in a minute and the total song length ($r^2=0.8495$; $p<0.001$; Figure 5). A negative relationship was found between mean pulses/syllable and the number of syllables/minute ($r^2=0.0405$, $p=0.009$; Figure 6).

The positions and relative quality of calling males in the grid are represented in Figure 7a. The relative quality of males was assessed by dividing each trait into three quality classes (low, medium, high) using assumed preferred characteristics: SVL (small, medium, large male classes with increasing quality), frequency (lower frequency = higher quality) and other call parameters (more pulses, longer syllables, increased syllables = higher quality). Though no statistical tests were run on the spatial distribution, certain trends of male

positioning based on relative location and physical features of the pond are visible (Figure 7b).

DISCUSSION

Comparisons among call parameters indicated that changes in total song length were reflected in changes in mean pulses/syllable, pulses/minute, mean syllable length, and syllables/minute. Fewer than expected correlations between call parameters and morphological and abiotic factors were found to be significant. The negative relationship between day in the breeding season and ambient temperature was unexpected, however cloacal temperature was positively correlated to ambient temperature as was expected with ectotherms, such as amphibians.

Day in the breeding season was significantly related to several aspects of males' calls, most significantly pulse/syllable and mean syllable length. With an increase in pulses/syllable and an increase in mean syllable length, it naturally follows that there would be an increase in total song length over the minute of analyzed call. That these call aspects increase over the course of the breeding season could suggest that males exhibit increased call effort as the breeding season progresses (when there is less time to mate before the end of the season), but a larger sample size in future experiments is needed to support this.

Mean call frequency (kHz) was significantly related to both SVL and cloacal temperature. As the SVL of the frog increases, it is expected that he will

have a larger buccal cavity capable of producing calls of lower frequency, which have been shown to be more appealing to females in some species, including the closely related treefrog, *Hyla chrysoscelis* (Morris and Yoon 1989). No significant relationship between SVL and cloacal temperature was found, however it is possible that more refined data is necessary in order to see the relationship between these two factors. This would require a finer resolution thermometer and a better method of assessing male size, such as mass or more morphological measurements in three dimensions in order to compare relative volumes.

The positive relationship between pulses/minute and total song length indicates that the pulses are a relatively more important factor in total song length than syllables. While pulses directly determine the length of the song, syllables are determined by where a song breaks and the periods of silence within the call. While the number of pulses/minute increased with total song length, the relationship between syllables/minute and total song length was not significant, suggesting that pulses are among the important aspects of a call in terms of stimulating and attracting females, a prediction supported by Wells and Taigen (1986), Klump and Gerhardt (1987), and Gerhardt (2001) (Table 1).

There was an inverse relationship between mean pulses/syllable and syllables/minute. Keeping total amount of call/minute constant, as a frog call decreases in the number of syllables/minute, it increases the number of

pulses/syllable and increases mean syllable length/minute. This suggests that there is some kind of trade-off between syllables and pulses and that the overall number of pulses in a minute would remain relatively constant. It is possible that the calling males are modifying their relative numbers of pulses and syllables while maintaining a constant call effort (call duration and call rate). Energetic costs may play a role in the relative number of pulses and syllables, especially if it is more costly to start calling. If so, it is reasonable to predict that males would conserve energy while lengthening their total song by increasing the number of pulses/syllable rather than increasing the number of syllables in order to limit the number of stops and starts in their call. More research is necessary to determine how energetic costs factor into male call effort.

Gerhardt (2001) found that males maintain call effort but adjust the number of syllables/minute in response to other calling males in the chorus to avoid overlapping calls or interference. Intuitively, this relationship can also be attributed to the fact that as syllables lengthen with greater number of pulses, the number of syllables in that minute must decrease. However, only two minutes of calls were recorded for each male, and only one minute was analyzed, which may not be representative of a male's performance over a whole night or the whole breeding season, suggesting that a larger sample size with greater chance of recapture is necessary in future experiments.

Future experiments attempting to characterize these call aspects might benefit from controlling for and separating the effects of ambient temperature, day in the breeding season, and levels of sexual excitement or receptiveness, because it was difficult to determine the nature of the relationship between all of these factors changing simultaneously and the call parameters. A larger sample size could give more conclusive results and more repeated catches and recordings of the same individual over time, which could be used to examine changes in male call characteristics and positions over a night or over the breeding season.

From the representation of relative spatial distribution (Figure 7), it appears that males of relatively higher quality are clumped together or near certain features of the pond, such as on logs, near grassy vegetation, or on the fallen aspen tree. In future experiments these trends can be assessed for significance. Spatial analysis of the locations and the direction calling males were facing when calling would be helpful in determining the distribution of males in the breeding site, especially in conjunction with bioacoustics analysis. Acquiring information on individual male mating success is essential to understanding leks and what male attributes a female is assessing in her mate choice. With this information, it would then be possible to examine the distribution of lekking males based on relative quality of calls, if females are using calls as a discriminating factor. Measuring male mating success is also needed to give added significance to the call analysis, morphological features, and physical

features within the lek, when using spatial analysis by verifying that particular call parameters or a particular spot with a lek are more important in attracting females.

ACKNOWLEDGEMENTS

I would like to thank Dr. Sunny Boyd, Dr. James English, Dr. Karen Francl, and Dr. Gary Belovsky for all their guidance, assistance, and patience in designing this experiment and analyzing the results. I thank Al Klein, Kathleen McAvoy, Tommy Hayes, Emmanuel Zervoudakis, Mara Irby, Keely Davidson, Rachel Wergin, Ashley Braun, and Matt Michel for their hard work, assistance, and good spirits in the field. My eternal gratitude goes to Lisa Himic, whose dedication, expertise, and willingness to take cloacal temperatures are a constant source of inspiration. I would like to acknowledge the funding for this course provided by The Bernard J. Hank Family Endowment.

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TABLES

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

	SVL	Cloacal Temperature	Ambient Temperature	Day in Breeding Season
Mean Pulses/Syllable	0	0.021	0.0149	0.0851
	(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	0.0828	0.1332
	(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	0.0775
	(p=0.825)	(p=0.232)	(p=0.143)	(p=0.03)
Mean Frequency	0.1583	0.0445	0.0122	0.031
	(p=0.001)	(p=0.057)	(p=0.395)	(p=0.176)
				x
Day in Breeding Season	0.049	0.001	0.2855	
	*(p=0.088)	(p=0.778)	(p<0.001)	
Ambient Temperature	0.028	0.2003	x	
	(p=0.198)	(p<0.001)		
Cloacal Temperature	0.002	x		
	(p=0.763)			
SVL	x			
*significant value not referred to in this study				

Table 1. continued

	Mean Frequency	Total Song Length	Syllable/Minute	Mean Syllable Length	Pulse/Minute	Mean Pulses/Syllable
Mean Pulses/Syllable	0.027	0.2266	0.0405	0.8161	0.2323	x
	(p=0.203)	*(p<0.001)	(p=0.009)	*(p<0.001)	*(p<0.001)	
Pulses/Minute	0.034	0.8495	0.0533	0.1487	x	
	(p=0.157)	(p<0.001)	*(p<0.001)	*(p<0.001)		
Mean Syllable Length	0.022	0.2972	0.053	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						

FIGURES

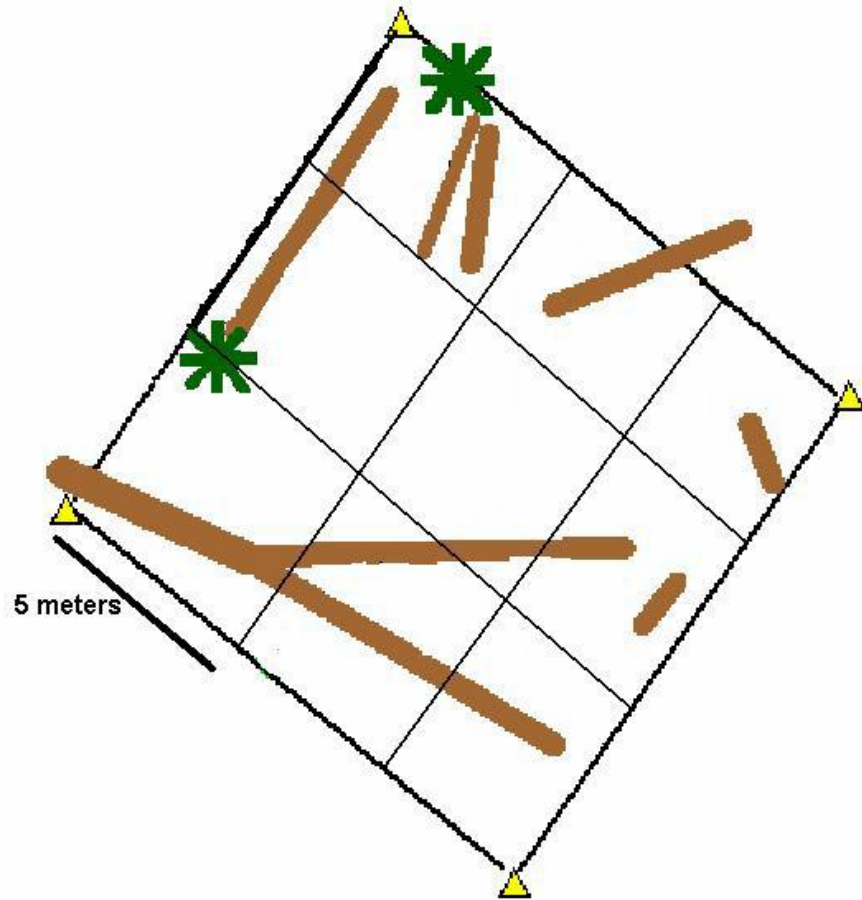


Figure 1. Representation of the Grid and Several Physical Features of the Vernal Pond.

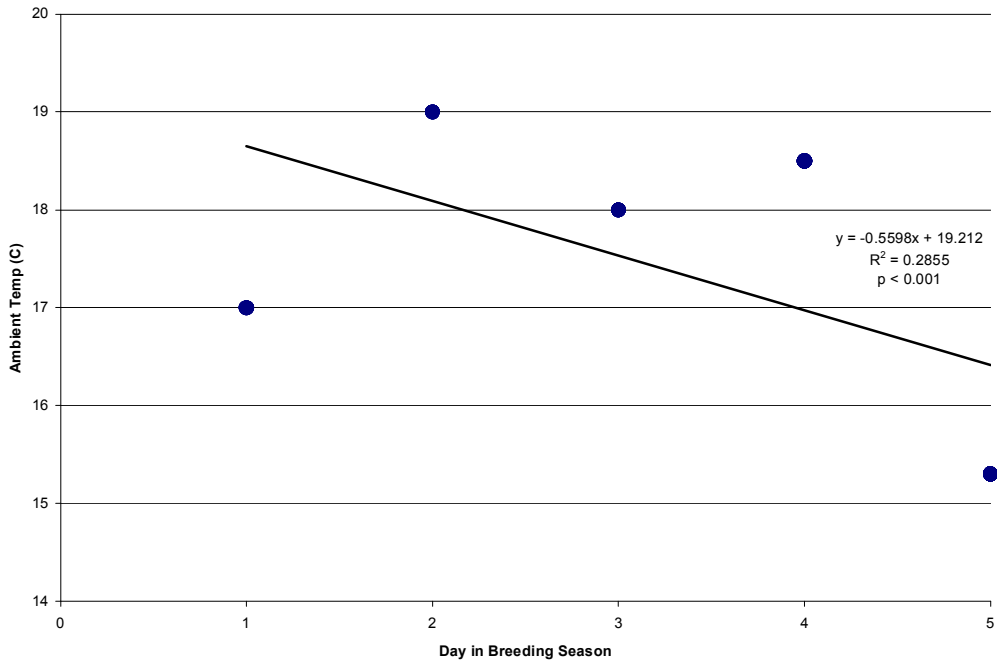
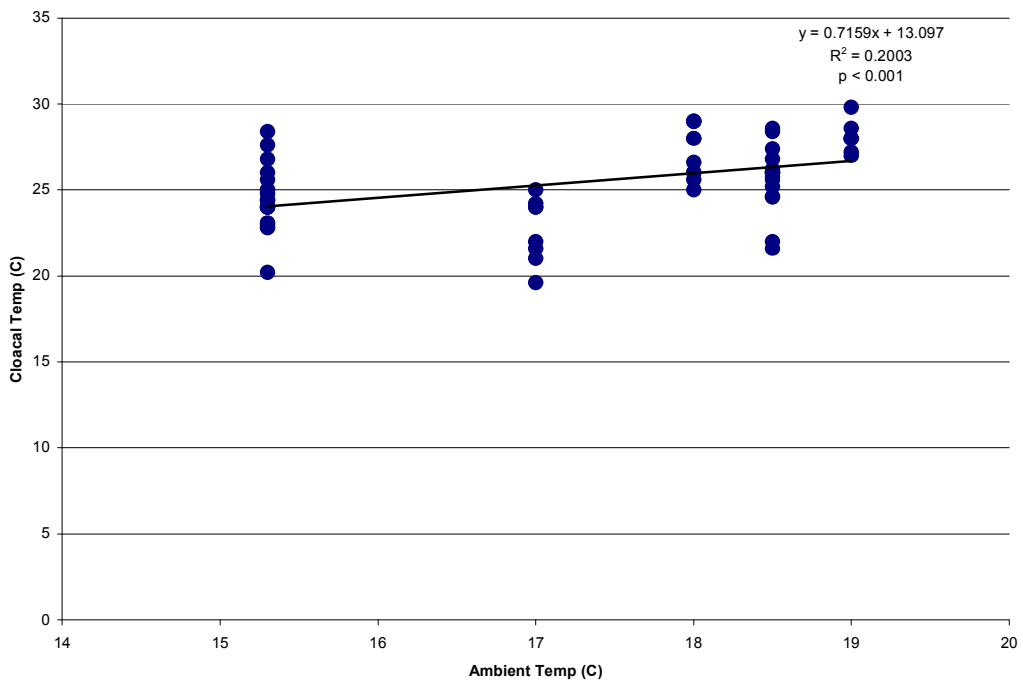


Figure 2. (a) Day in Breeding Season and Ambient Temperature. (b) Ambient Temperature and Cloacal Temperature.



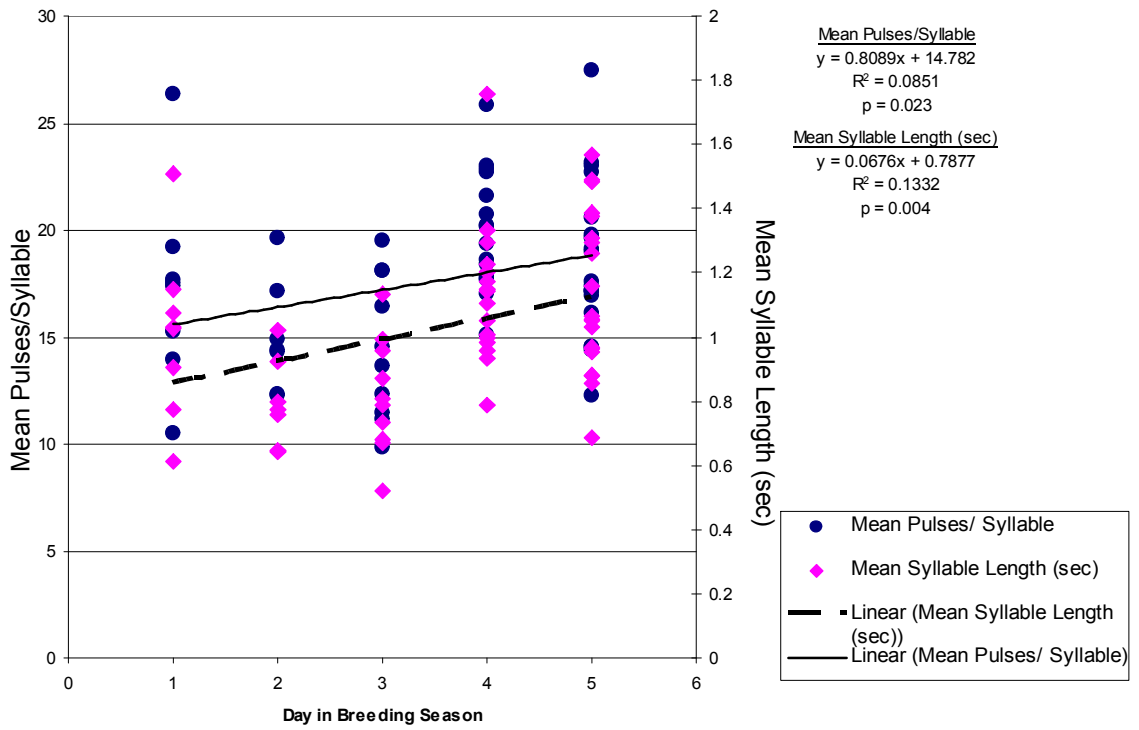
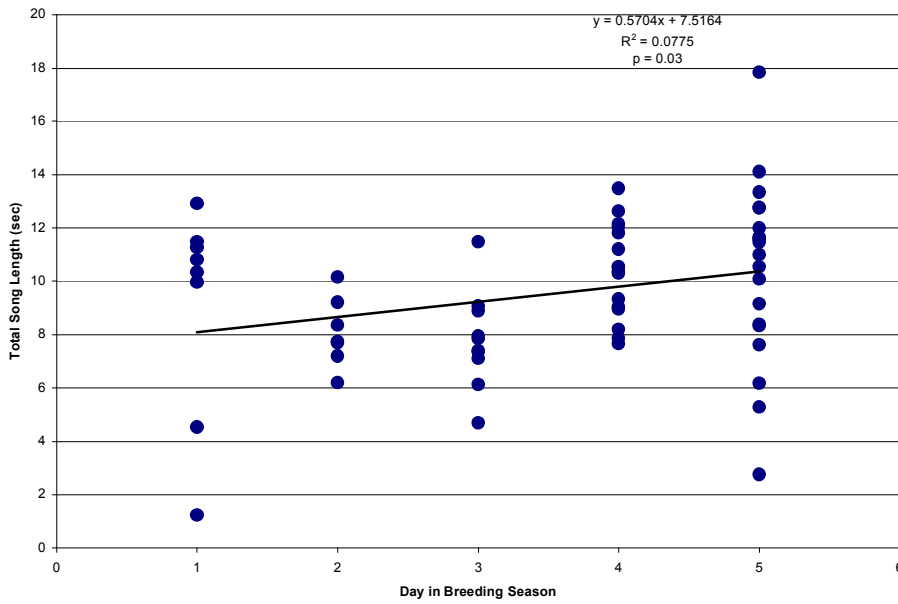


Figure 3. (a) Mean Pulses/Syllable and Mean Syllable Length as Functions of Day in Breeding Season. (b) Total Song Length and Day in Breeding Season.



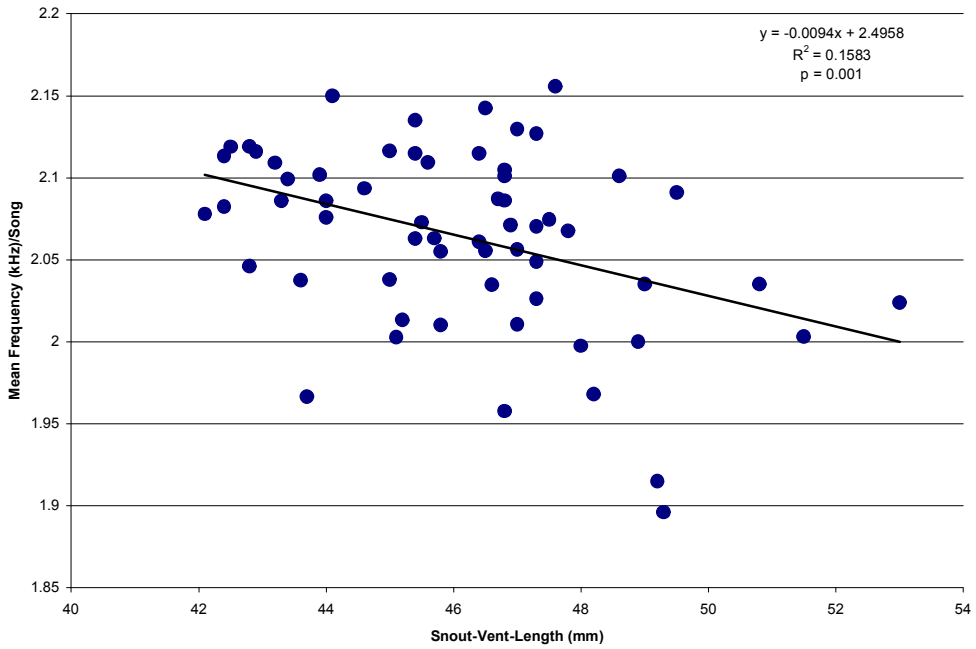
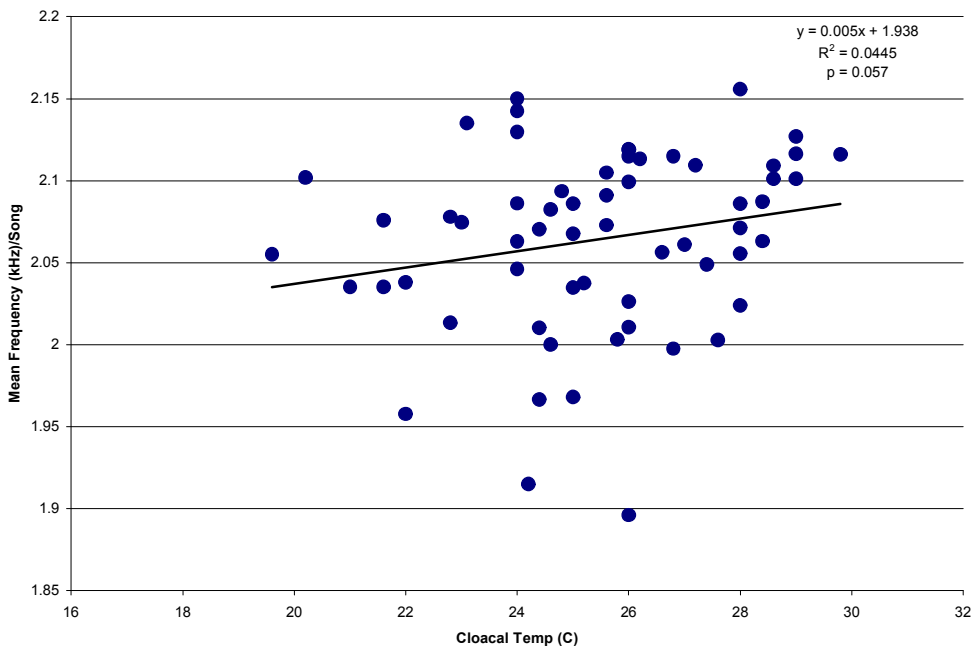


Figure 4. (a) SVL and Mean Frequency. (b) Cloacal Temperature and Mean Frequency.



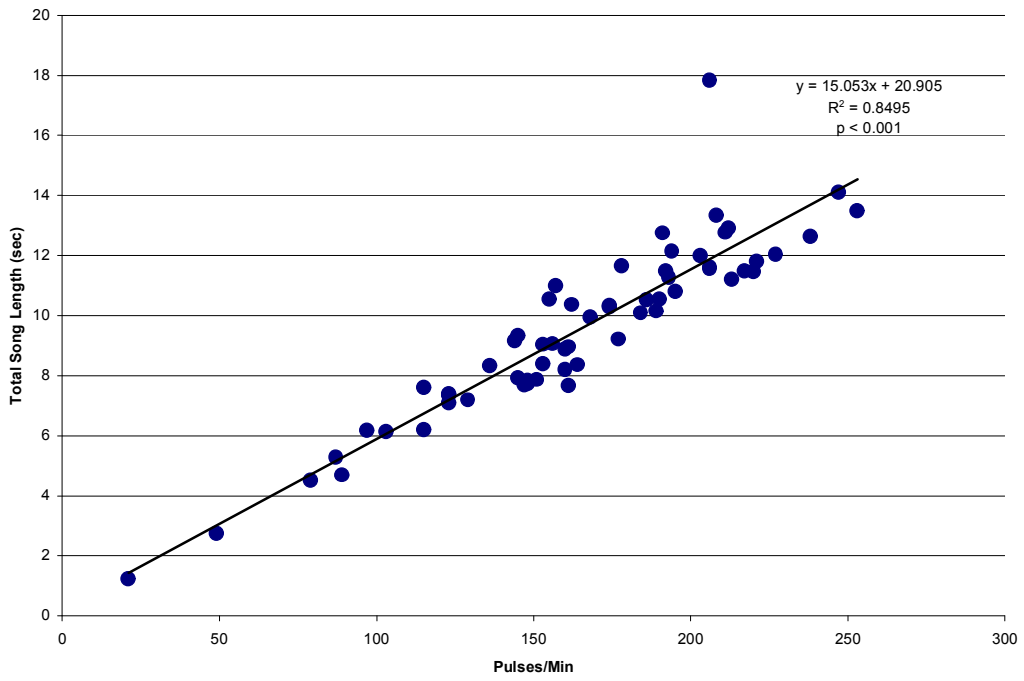


Figure 5. Pulses/Minute and Total Song Length.

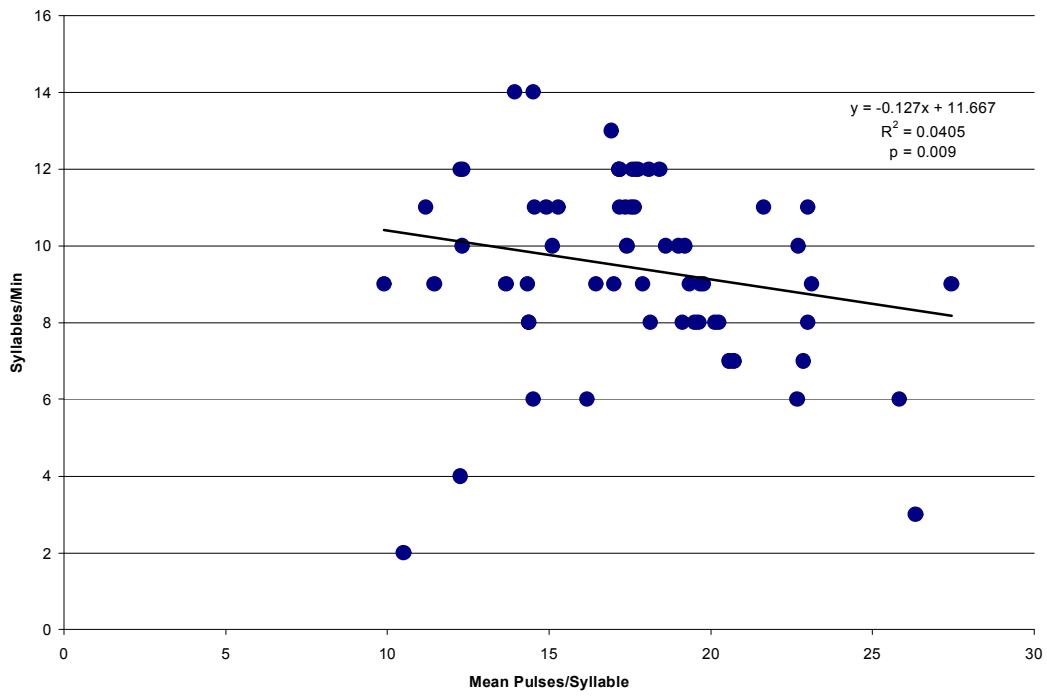


Figure 6. Mean Pulses/Syllable and Syllable/Minute.

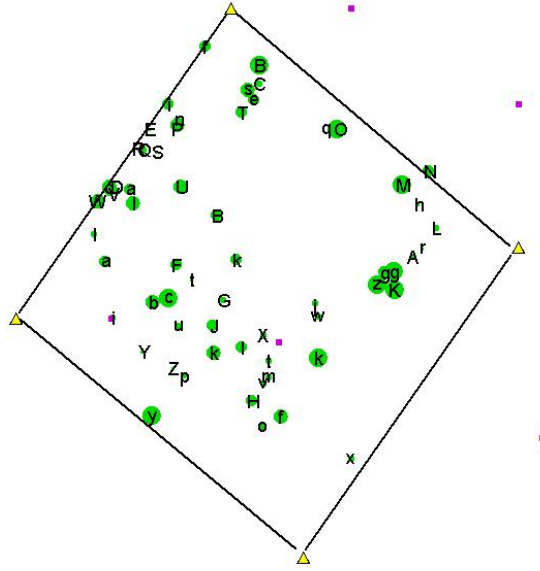


Figure 7. (a) The Distribution and Relative Quality of Calling Males in the Breeding Site. The larger points indicate a male of theoretically greater relative quality.
 (b) Location of Calling Male and Physical Features of the Breeding Site.

