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Acoustic Communication in the Temperate Treefrogs *Pseudacris* *Crucifer* and *Acris* *Crepitans*

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ACOUSTIC COMMUNICATION IN THE TEMPERATE TREEFROGS PSEUDACRIS
CRUCIFER AND ACRIS CREPITANS

A thesis

Presented to

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ABSTRACT

Spring peepers and cricket frogs produce advertisement calls to attract females. As ectotherms their body temperatures are greatly affected by ambient air temperature. Some characteristics of their advertisement calls are correlated with temperature. I analyzed advertisement calls of both species recorded in western-central Indiana. I compared call characteristic correlations with temperature found in our populations to those in populations in other geographic areas and found similar trends throughout the range of both species. Secondly, I examined aggressive calls in the spring peepers. Aggressive calls are used in male-male interactions, and in the spring peeper are a distinct call type different from advertisement calls in two characteristics. I used a habituation-discrimination protocol to test which of the two call characteristics, that differ between advertisement and aggressive calls, males use to distinguish advertisement and aggressive calls. I found that males responded with intermediate aggression to calls that only differ from advertisement in one of the two characteristics and responded with the most aggression to calls that differed from advertisement calls in both characteristics.

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CHAPTER 1

ADVERTISEMENT CALLS OF SPRING PEEPERS (*PSEUDACRIS CRUCIFER*) AND CRICKET FROGS (*ACRIS CREPITANS*) AND THEIR CORRELATION WITH TEMPERATURE

Introduction

Many animals such as anurans (Gerhardt 1994), insects (Greenfield 2002), birds (Searcy 1992), and mammals (Charlton et al. 2007) use acoustic communication to both find and choose mates. Males often use an advertisement signal to broadcast information about themselves to potential mates (Brown et al. 1996; Bee et al. 2001; Gerhardt & Huber 2002). Females from a wide array of species have been shown to choose mates based on signal characteristics (e.g., Eens et al. 1991; Shaw & Herlihy 2000; Craul et al. 2004; Baugh & Ryan 2010). For females to make these selections, there must be variation in signal characteristics between males. This variation can be caused by variation among individuals in age, size, and physical condition, as well as variation in abiotic factors such as temperature and time of year (Gerhardt & Huber 2002).

Male anuran amphibians use acoustic calls to both attract mates and to repel rival males (Gerhardt & Huber 2002; Wells 2007). The calls for a wide range of species have been recorded and measured for frequency, duration, call rate, amplitude, and structure, among other parameters (Gerhardt & Huber 2002). Many of these species have also been used in behavioral experiments examining the relationship between male calls and female mate choice (e.g.,

Forester & Czarnowsky 1985; Gerhardt 1991; Ryan et al. 1992; Burke & Murphy 2007).

Females have been shown to have preferences for call parameters such as amplitude, duration, call rate, and frequency (Gerhardt & Huber 2002).

Anurans are ectotherms, and therefore some of the call parameters that have been found to be important for female choice are affected by ambient temperature (Schneider 1977; Gayou 1984; Gerhardt & Doherty 1988), and some parameters exhibit variation across geographic ranges (Gerhardt & Davis 1988; Loftus-Hills & Littlejohn 1992; Gerhardt & Schul 1999). To understand fully how females make decisions based on male calls, we must understand these two sources of variation. Differences in male call parameters, due to temperature, have the potential to change which males are chosen by females, possibly allowing climate to have an impact on the selective pressure on males. Differences in how male call parameters are affected by temperature across geographic ranges can provide insight into the evolutionary history of a species.

This study examined the calls of two well-studied species, *Pseudacris crucifer* (spring peeper) and *Acris blanchardi* (cricket frog) recorded in west-central Indiana. I have recorded advertisement calls of both spring peepers and cricket frogs. Here I describe the calls from our recordings of each species' western-central Indiana population. I then examine in depth the effect of temperature on various call parameters. Finally, I make comparisons between the correlations between call parameters and temperature in my population and those published in populations from other geographic locations.

Methods

Study Site

This study was conducted in the spring of 2013 in west-central Indiana. Data for the cricket frog were collected at Indiana State University's Kiewig Wood's property, and data for the spring peeper were collected on private property near Brazil, Indiana.

Study Species

Spring Peeper

The spring peeper is a small, temperate-zone treefrog, approximately 29 mm in snout-vent length commonly found throughout the eastern United States (Forester & Czarnowsky 1985; Wells et al. 1996). They emerge early in the spring and males call from choruses for several weeks. Males call from emergent vegetation near the shoreline, on the ground, or in vegetation around small ponds (Rosen & Lemon 1974; Sullivan & Hinshaw 1990). Typical inter-male spacing is 20 – 120 cm (Fellers 1979; Gerhardt et al. 1989), and males will interact acoustically by entraining their calls with those of neighbors (Lemon & Struger 1980). Spring peepers will consistently call at temperatures lower and more variable (3.5 – 23 C) than many other anurans that use acoustic communication for breeding (Wells et al. 1996; Zimmitti 1999). Spring peepers have two distinct call types, an advertisement call for attracting females and an aggressive call for repelling rival males (Schwartz 1989). The advertisement call of the spring peeper is a simple, frequency-modulated tone with several harmonics (Fig. 1) (Forester & Czarnowsky 1985; Wells et al. 1996). The frequency modulation sweeps upwards from the beginning to the end of the call (Lemon & Struger 1980). The dominant frequency ranges from 2.3 – 3.5kHz (Lemon & Struger 1980). Call duration ranges 100 – 300ms (Lemon & Struger 1980). Call rates range from 30 – 110 calls per minute with an average call period of 1 call per

second (Lemon & Struger 1980). A second type of call is used in aggressive interactions; it shares the frequency structure of the advertisement call but is pulsed (Schwartz 1989).

Some call parameters are correlated with temperature. Call duration is negatively correlated with temperature, whereas call rate is positively correlated with temperature (Brown & Brown 1977; Lemon & Struger 1980; Sullivan & Hinshaw 1990; Wells et al. 1996; Zimmitti 1999). Frequency is also positively correlated with temperature (Sullivan & Hinshaw 1990).

Cricket Frog

The cricket frog is a common, small frog found throughout most of the eastern United States (Ryan et al. 1990). DNA sequencing and phylogenetic analysis have revealed that there are three species of cricket frogs (Gamble et al. 2008). The population we studied was formerly classified as *Acris crepitans blanchardi* (Lannoo 2005), but we follow the recommendation of Gamble et al. (2008) to classify this population as *Acris blanchardi*. Individuals range in size from 16-35 mm snout-to-vent length (Stebbins 2003). Males are heard calling from late April to July or early August (Lannoo 2005). They call from the shore or from floating vegetation near the edge of a pond with inter-male spacing ranging from 25 – 622 cm (Wagner 1989).

The call is a short click composed of a variable number of pulses (Fig. 2) (Perrill & Lower 1994; Ryan et al. 1995; Witte et al. 2001). This call is used to both attract females and repel males (Wagner 1989; Ryan et al. 1990). Calls consist of a single dominant frequency with multiple sidebands created by the amplitude modulation of pulses (Witte et al. 2001; Kime et al. 2004). Dominant frequency ranges from 2.7 – 4 kHz (Perrill & Lower 1994; Ryan et al. 1995; Witte et al. 2001), and males produce calls in a series or call groups of 25-90 calls in a single

calling bout (Perrill & Lower 1994; Ryan et al. 1995) (Fig. 3). Calls tend to increase in duration and number of pulses from the beginning to the end of a call group (Wagner 1989).

Call duration in the cricket frog is negatively correlated with temperature, whereas call rate, number of pulses per call, pulse rate, and frequency are positively correlated with temperature (Wagner 1989).

Call Analysis

I recorded 55 male spring peepers between April 4 and May 9 2013 and 42 male cricket frogs between April 30 and July 22 2013. All frogs were recorded using a Sennheiser K6 condenser shotgun microphone and a Roland R-09HR Studio WAVE/MPS3 recorder that used a sampling rate of 96kHz and 24 bit depth to create waveform audio files. The microphone was positioned 25-100cm away from the calling male. Air temperature at the calling site was measured immediately following the recording. Males were photographed after recording for individual identification to avoid later resampling. Calls were analyzed using Raven Pro 1.3. Five calls were randomly selected for analysis for each spring peeper. Each call was measured for duration, rise time, fall time using the waveform view. The power spectrum view with a Hann window type of size 1080 samples was used to measure the peak frequency (Hz) and relative amplitude (dB) for each harmonic at the beginning and end of each call. The difference in frequency from beginning to end was calculated for a measure of the frequency modulation. Call rates were calculated by counting each call produced by the male during the recording and dividing by the calling period.

The analysis was more complicated for the cricket frogs because many of call characteristics are correlated with a call's location in a call group (Wagner 1989). The power

spectrum view with a Hann window type of size 1080 samples was used to measure the dominant frequency (Hz). Other than dominant frequency, call characteristics were analyzed separately by call group location (Wagner 1989). Dominant frequency was not analyzed separately based on group location because it was found not to vary over the course of a call group (Wagner 1989). Five calls were randomly selected from the beginning, middle, or end of a call group. Each call was measured for duration, number of pulses, pulse rise time, pulse fall time, and interpulse interval in the waveform view. Call rates were calculated by counting the number of calls within calling groups using the only total period of call groups instead of the total time, as in Wagner (1989). Additionally, call group duration and intergroup intervals were measured. I present the variable “call bout” to describe multiple call groups produced within rapid succession, call group is defined as in Wagner (1989). Call bouts are separated by an interval more than double the interval between the call groups contained within the bout. Call bout durations and interbout intervals were measured.

Statistical Analysis

Pearson’s r correlations were calculated for each call characteristic and temperature. A sequential Bonferonni adjustment was performed on each group of call characteristics to maintain family-wide Type I error rates at 0.05 (Rice 1989). Correlations coefficients of call characteristics and temperature from other studies were compared to the correlations coefficients found in this study using Fisher’s Z tests. Correlation coefficients were compared because they were the statistic consistently reported in other studies. Sequential Bonferonni adjustments were also performed for all comparisons for each species. Only one other study has reported correlations between temperature and call characteristics in the cricket frog (Wagner 1989). The

data in his study were analyzed separately based on year collected (1986 and 1987). Therefore, I compared the correlations coefficients found in the present study to those found significant in each year separately.

Results

Call Description

Spring Peeper

Advertisement call characteristics for spring peepers in my study population are listed in Table 1. Calls were found to have roughly equal rise and fall times, although both parameters exhibited substantial variability. The calls of males in this population usually had twelve measurable harmonics (Table 2).

Cricket frog

The call rate characteristics for my study population of cricket frogs are listed in Table 3. Call rates reported are for calls produced within a call group.

Individual call characteristics are listed in Table 4. Call duration, number of pulses per call, and inter-pulse interval increased from the beginning of a call group to the end. Pulse rates decreased from the beginning to end of a call group, as did the amplitude of the last pulse relative to the first pulse of a call. Pulse duration, rise time, and fall time remained relatively constant throughout the call group.

Relationship between call characteristics and temperature

In spring peepers, call rate and fundamental frequency were significantly positively correlated with temperature and call duration was negatively correlated with temperature (Table 1). Rise times, fall times, and frequency shift were not correlated with temperature.

In cricket frogs, call rate was significantly positively correlated with temperature (Table 3).

Calls at the beginning of call groups had call durations and number of pulses per call significantly negatively correlated with temperature (Table 4). Calls in the middle and end of call groups had no call characteristics significantly correlated with temperature. Pulse rate, duration, rise time, fall time, and inter-pulse interval were not significantly correlated with temperature in any of the three bout locations.

Comparison of correlations with temperature to those of other studies

Spring peepers

I found no difference between this study and previous studies in the magnitude and direction of correlations between temperature and call characteristics. Four studies of spring peepers found call rates positively correlated with temperature (Table 5). None of these correlations were statistically significantly different from mine (Table 5). Further, there are no obvious geographic trends in correlation with temperature (Figure 4). Three studies have reported a correlation between call duration and temperature in the spring peeper (Table 5). The correlation reported here does not differ significantly from any of these three studies.

Correlation coefficients do increase from west to east, but show no trend north to south (Figure 4). Only one other study has looked for a correlation between temperature and fundamental

frequency in the spring peeper (Sullivan & Hinshaw 1990), and the correlation is not significantly different from that of the present study.

Cricket frogs

In most cases, the magnitude and direction of correlations between temperature and call characteristics for cricket frogs were not significantly different between the present study and Wagner (1989) (Table 6). Wagner (1989) found statistically significant correlations between temperature and dominant frequency, call rate, call duration, pulses per call, and pulse rate. Only the correlation between pulse rate at the beginning of call group was significantly different from the correlation in the present study; Wagner (1989) found a negative relationship, whereas there was no relationship detected in the present study.

Discussion

Spring peeper advertisement call harmonics

Most males in this population had twelve measureable harmonics in their advertisement calls. Previously the advertisement call has been described only by its dominant frequency (Schwartz 1989, Marshall et al. 2003). The second harmonic (dominant frequency) has higher relative amplitude than any of the other harmonics. The harmonic with the next highest relative amplitude is the third, which is 31.5 dB lower than the dominant frequency. A sound that is 30 dB less than another has only about 3% of the root mean squared pressure of the louder sound. Therefore, the other eleven harmonics may not contribute a significant enough amount of energy to the call to be of biological relevance. Playback experiments are needed to test if females distinguish between calls with only the dominant frequency and calls with additional harmonics.

Cricket frog call bouts

Inter-bout spacing measured here was found to have a wide range (range = 3.85 – 93.43 s). Intergroup spacing meanwhile, had a much narrower range (0.08 – 2.99 s). Call characteristics such as call duration, pulses per call, pulse rate, and interpulse interval vary predictably over the course of a call group, but appear to reset at the start of a subsequent group within the same calling bout. Therefore call group is still a valid variable within the larger unit of call bout.

Cricket frog call pulses

Cricket frog call pulses have been analyzed previously only as pulses per call and pulse rate (within a call)(Wagner 1989, Ryan & Wilczynski 1991, Burmeister et al. 2002). This study is the first to analyze the pulse duration, rise time, fall time, and interpulse interval. In other studies, call duration and pulses per call have been found to increase, and pulse rate to decrease, from the beginning to end of a call group (Wagner 1989, Ryan & Wilczynski 1991), a pattern found in the present study. More pulses per call should clearly make the total call duration longer, unless pulse duration or interpulse intervals were to shorten. Here, I found that there is no clear change in pulse duration over the course of a call group. Interpulse intervals increase from the beginning to the end of a call group. This increase in interpulse interval, combined with the increase in number of pulses, is the cause of longer duration calls at the end of a call group. Pulse rise times and fall times remain relatively constant throughout the call group, along with pulse duration. The pulses themselves do not appear to vary throughout the call group, only the number of pulses and the duration of the interval between them.

Relationship between call characteristics and temperature

The significant correlations between call rate, duration, and fundamental frequency found here do not differ significantly from those reported for spring peepers in other studies (Table 5). These relationships appear to be highly conserved throughout the range of spring peepers. Because these frogs are one of the first to start chorusing in the spring, they are likely encounter a wide range of temperatures (Wells et al. 1996; Zimmitti 1999). One hypothesis for the widespread similarity in relationship between temperature and call characteristic is that having plasticity in call characteristics based on temperature may allow these frogs to successfully attract mates at these wide ranging temperatures. Another hypothesis is that the differences in call characteristics represent physiological constraints on the ability of males to produce calls that selection cannot abolish.

Spring peeper call durations in my population exhibited a significant negative correlation with temperature, but did not exhibit temperature-dependent rise and fall times. These patterns mean that, as temperatures increase through a breeding season, males shorten the length of time their calls are at peak intensity. Since call rates increase with temperature, one hypothesis is that males are shortening their calls in order to produce more, shorter calls at a faster rate. There is also likely a physiological constraint on either or both the call rate and duration. In colder temperatures the muscles of ectotherms will contract less rapidly (Gayou 1984), lengthening the call. But we would also expect this effect of temperature on the muscles to lengthen the rise and fall times of the call. Slower contractions of muscles should taper the ends of the call along with lengthening it at its peak intensity. Hence, there is evidence supporting that males may have some behavioral plasticity in the lengths of their calls over different temperatures.

In my population of spring peepers, fundamental frequencies were significantly positively correlated with temperature. One hypothesis for this correlation is that males' muscles are contracting faster creating higher call frequencies. Fundamental frequencies are negatively correlated with body size (Sullivan et al. 1990), and females prefer lower fundamental frequencies (Forester & Czarnowsky 1985). Future experiments should be conducted to see if the correlation between fundamental frequency and temperature varies with male body size.

As with the spring peeper, call rate in cricket frogs was positively correlated with temperature (Table 3). Call duration and number of pulses per call were negatively correlated with temperature for calls located at the beginning of a call group. The significant correlations found in the present study are all similar to those found in Wagner (1989). Temperature appears to have a larger impact on calls at the beginning of a call group than at the middle or end of a call group. These calls are in general shorter and more widely spaced than those in the middle and at the end of a call group. At lower temperatures, the shorter duration calls at the beginning of a call group become longer and males produce more pulses in each call. Since middle and end calls are already longer and contain more pulses than those at the beginning of the call group, perhaps they are close to a limit on call duration and number of pulses.

Pulse duration, rise time, fall time, and interpulse interval did not appear to vary over the course of a call group or with temperature. The time intervals measured for these variables are quite small (thousandths to ten thousandths of a second), so there is a possibility that I did not measure them at precise enough values to discover any significant differences.

Summary

The results of this study confirm relationships between temperature and call characteristics found in other populations of our two study species. Temperature was found to have similar effects on the call characteristics of both species as found in other populations. In general, temperature has a predictable effect on call rates of both species. In both species, there is a negative effect of temperature on call duration to some degree. Both of these effects have been seen widely throughout anurans and appear to be the most important effects temperature has on calling characteristics.

CHAPTER 2

DISTINGUISHING TWO TYPES OF CALLS BY MALE SPRING PEEPERS (PSEUDACRIS CRUCIFER)

Introduction

Males of many species of animals gather in large groups called choruses during the breeding season (Bevier 1997; Ewart 2001; Drummer et al. 2011; Herman et al. 2013). At the chorus, males produce acoustic advertisement signals to attract females that choose a mate from among the group (Andersson 1994). Within a chorus there can be high densities of males. High densities can cause interference between overlapping signals, (Pollack 1986; Schwartz & Gerhardt 1995) making it more difficult for females to distinguish and locate the signals of individual males (Greenfield 1994; Tauber et al. 2001) and increasing the probability of interception by competitors (Lance & Wells 1993), thereby reducing the efficacy of female choice (Telford 1985; Pollack 1986; Dyson & Passmore 1992; Schwartz & Gerhardt 1995).

Physical combat is one strategy for reducing interference but can decrease a male's mating success because he risks injury and loses time for advertising. A second signal, the aggressive call, has evolved in some species to shorten the interaction and avoid physical combat (Enquist & Leimar 1983; Robertson 1986). Males will use the aggressive call if a neighboring male exceeds some threshold amplitude (Marshall et al. 2003). Males generally respond to aggressive calls with aggressive calls of their own (Humfeld et al. 2009). These interactions

rarely escalate to fighting and generally end with one male moving from the calling site (Wells 2007).

In anurans, aggressive calls can either be distinct or graded (Gerhardt & Huber 2002). In the distinct system, males have two separate calls, and a male can either produce an advertisement or an aggressive call. In the graded system, aggressive calls are modified advertisement calls that can be made more or less aggressive depending on the caller's aggressive intent (Gerhardt & Huber 2002). Graded calls have been hypothesized to allow a male to tradeoff between aggressive intent towards intruding males and attractiveness to potential female mates (Gerhardt & Huber 2002). On the other hand, distinct calls may allow males to more quickly convey aggressive intent and resume advertising to females. Acoustic differences between the two calls have been measured for many species, but how males identify and distinguish them has not been determined (Wells 2007).

The cricket frog (*Acris crepitans*) is an example of a species with graded aggressive calls, and one that has been examined in multiple studies (Wagner 1989; Burmeister et al. 1999). Cricket frogs both lower their dominant frequency and increase call duration when increasing the aggressiveness of their call (Wagner 1989). In one study males lowered the frequency of their calls but did not increase call duration in response to a male call that only had a lower frequency (Wagner 1989). Another study showed that males increased call duration but did not lower frequency in response to calls that only had longer duration (Burmeister et al. 1999). Therefore, cricket frogs can distinguish differences in both parameters separately and respond by changing their own parameters independently of each other (Burmeister et al. 1999).

This study examined a system with distinct advertisement and aggressive calls, the spring peeper (*Pseudacris crucifer*). The advertisement and aggressive calls of male spring peepers

differ in two parameters: duration and amplitude envelope shape (Schwartz 1989). Aggressive calls are both longer in duration than advertisement calls and have a pulsed structure that the advertisement call lacks (Figure 5). Males respond more aggressively to aggressive calls than to advertisement calls (Humfeld et al. 2009). However, it is not known whether the discrimination made by male spring peepers between the two types of calls is based on differences between the calls in duration or amplitude envelope or both.

My objective was to determine what acoustic characteristics male spring peepers use to distinguish the two types of signals: advertisement and aggressive calls. I used a habituation-discrimination protocol as in Humfeld et al. (2009), to test which call characteristics elicit an aggressive response from males. Males will be habituated to an intruder that produces advertisement calls, then in the discrimination phase will be exposed to different synthetic calls with varying characteristics. In Humfeld et al. (2009), male spring peepers responded to an intruder producing advertisement calls initially with aggression and then became habituated to the intruder over a four minute phase. Then, in the discrimination phase males responded more aggressively to intruders that switched to aggressive calls than to intruders that continued producing advertisement calls. This confirmed the hypothesis that males that have been habituated to an intruder will respond aggressively if the intruder switches to aggressive calls. I use this hypothesis to test which of the two call characteristics that differ between aggressive and advertisement calls elicit the aggressive response. If calls with longer duration elicit an aggressive response after habituation, then duration is used by males to distinguish the two call types. If calls with short duration but pulsed structure elicit an aggressive response after habituation, then pulsed structure is used by males to distinguish the two call types.

Methods

Study Site and Species

This study was conducted in the spring of 2014 on private property near Brazil, Indiana with a pond in a pasture with some trees on the margin. My study species, the spring peeper (*Pseudacris crucifer*), is a small, temperate-zone treefrog that responds readily in playback experiments (Schwartz 1989; Marshall et al. 2003; Humfeld et al. 2009). They emerge early in the spring and call from the ground or small shrubs around small ponds in dense choruses for up to two months. The spring peeper uses distinct advertisement and aggressive calls (Schwartz 1989). Both calls have the same frequency structure. Each call has several harmonics, with the second being the dominant (Figure 5). Calls produced by males in my study population have a mean fundamental frequency of 1487 Hz (N = 55). The two calls differ in their duration and amplitude envelope (Figure 5). The aggressive call is longer and has a pulsed structure, whereas the advertisement call is shorter and has a pure tonal structure. In my study population, the duration of an average aggressive call is 340 ms and that of an average advertisement call is 90 ms (N = 55).

Playback Protocol

Males in relative isolation were tested to reduce interference from other males calling. A speaker and a Sennheiser K6 condenser shotgun microphone were placed 50 cm from the subject male. This distance is both within the range of natural spacing for this species and far enough to allow placement without disturbing the male. The speaker played a call, representing an intruding male.

Calls produced by the subject male during the test were recorded using a Roland R-09HR Studio WAVE/MPS3 recorder that used a sampling rate of 96kHz and 24 bit depth to create waveform audio files. Each male was used in one, randomly assigned treatment. Males were photographed for individual identification after the test.

Experimental Design

A habituation-discrimination protocol (Humfeld et al. 2009)(Figure 6) was used to test male responses to four different treatments that differ in duration and temporal structure (Table 7). After placement of the speaker and microphone males were allowed to resume normal calling behavior. Then, a four-minute habituation phase of advertisement calls at 1 call/s was followed by a one-minute discrimination phase with call rates maintained at 1 call/s (Figure 6).

Treatments

Males were randomly assigned to one of four treatments: nonaggressive, aggressive, long-unpulsed, and short-pulsed (Table 7). My first two treatments were similar to the non-aggressive and aggressive treatments in Humfeld et al. (2009). The discrimination phase of the non-aggressive treatment consisted of advertisement calls. The aggressive treatment had a discrimination phase of aggressive calls. Calls in the discrimination of the long-unpulsed treatment had the duration of an aggressive call but was not pulsed. Calls in the discrimination phase of the short-pulsed treatment had the duration of an advertisement call but was pulsed.

Call Synthesis

Calls were synthesized using the open-source program Audacity. I used mean call parameters from male spring peepers recorded from this population in 2013 (Table 7). Each treatment had the same harmonic structure. The mean duration, rise time, and fall time of advertisement calls in 2013 were used for my synthesized advertisement calls and long-unpulsed calls. The mean duration, rise time, and fall time of aggressive call pulses in 2013 were used for my synthesized aggressive and short-pulsed calls. Since the duration of calls is correlated with temperature, I made five sets of each type of call set to 5 °C, 10°C, 15°C, 20°C, and 25°C. Insufficient data for the correlation between temperature and aggressive call duration was available from 2013, so aggressive calls were created for each of the five temperatures using the correlation between temperature and advertisement calls. Ambient air temperature was measured next to the focal male before each test and the call set within 2.5°C of the temperature was used.

Data Analyses

The response variables, the numbers of advertisement and aggressive calls produced, represent count data type and hence are not distributed normally. Therefore, generalized equations estimation (GEE) with the negative binomial distribution was used to analyze the repeated measures of advertisement and aggressive calls in the two phases. GEE produces two measurements of the goodness of fit of a model: quasi likelihood under the independence model criterion (QIC) and the corrected quasi likelihood under the independence model criterion (QICC). QIC was used to determine which correlation structure to use. Since all models had QIC less than 2 different from each other, AR(1) structure was used. This structure allows repeated measurements that are closer in time to each other to be more highly correlated. The

goodness of fit measure QICC was used to determine which effects to include in each model. I tested models including terms for temperature, treatment, phase, and the interaction of treatment and phase. The interaction term between treatment and phase is of the most importance for testing my hypothesis. If this interaction increases the fit of the model, males responded differently to the discrimination phase based on which treatment they were in.

Pairwise comparisons were also made using Tukey's tests. Comparisons were made between the mean aggressive calls produced by males in the discrimination phase between pair of treatments. Since I did not find the interaction between treatment and phase to be significant for advertisement calls, pairwise comparisons were not used for advertisement calls. I also made pairwise comparisons between the fourth minute of the habituation phase and the discrimination phase to see which treatments showed a significant change in aggressive call production.

Results

Playback experiments were conducted on 124 male spring peepers. A total of 101 were used in analysis. Two were removed because the temperature of the test was below the threshold of prepared playback calls (2.5 C). Eight males fled from the speaker at the onset of the playback, eight males did not respond with any calls during the playback, and five males stopped producing calls before the discriminant phase. None of these males produced calls in the discriminant phase and hence could not be included in analysis.

The model with the best fit for advertisement calls produced by males included only the temperature term. Inclusion of terms for phase, treatment, or the interaction of the two did not increase the fit (Table 8). The model with the best fit for aggressive calls included the terms

phase, treatment, and the phase-treatment interaction. Temperature did not increase the fit of this model (Table 8).

Pairwise comparisons of the mean aggressive calls produced in each treatment revealed that males in different treatments did not produce significantly different aggressive calls in the fourth minute of the habituation phase (Table 9). However, in the discrimination phase males in the nonaggressive treatment produced significantly less aggressive calls than those in the aggressive treatment. No other treatment pairs had significantly different aggressive calls.

Males in the nonaggressive treatment did not increase aggressive calls produced between the fourth minute of the habituation phase and the discrimination phase (Figure 7 and Table 10). Males in each of the other three treatments did increase aggressive calls in the discrimination phase compared to in the fourth minute of the habituation phase, although it was only statistically significant in the aggressive treatment and in the short-pulsed treatment (Table 10).

Discussion

The number of advertisement calls produced by a male was predicted best by a model that only included temperature regardless of which treatment or phase. By contrast, the number of aggressive calls produced by a male was best predicted by treatment, phase, and interaction of treatment and phase but not temperature. The interaction of treatment and phase was the most important term in testing my hypothesis. Since it was included in the best model for aggressive calls, this indicates that males have different aggressive call rates between treatments.

To test where the difference in treatments was we used the pairwise comparisons. The results suggest that males respond to the intermediate treatments with increased aggression, but not as strongly as to the aggressive treatment. I confirmed the result in Humfeld et al. (2009)

that males respond with a greater number of aggressive calls to the aggressive treatment than to the nonaggressive treatment. In addition I found that males did increase their aggressive calls in response to the long-unpulsed treatment and the short-pulsed treatment, although it was only statistically significant in the short-pulsed treatment. This suggests that males are responding to each of these parameters with increased aggression, but that the combination of both longer duration and pulsed structure produces the strongest aggressive response.

Schwartz (1989) found that the intensity of both advertisement and aggressive calls affect the number of aggressive calls produced by a male in a playback experiment. He also found that the duration of aggressive calls had an effect on the number of aggressive calls. Males produced more aggressive calls in response to the medium duration aggressive call than to the short duration aggressive call. There was not a significant difference in response to the medium and long duration aggressive calls. Intensity was held constant in this experiment for each treatment, but clearly based on the results from Schwartz (1989) it is an important factor in eliciting an aggressive response.

Duration is an important characteristic for males in distinguishing call types as suggested by my results and those of Schwartz (1989). I found that males produced more aggressive calls to long duration advertisement calls than to normal duration advertisement calls. Schwartz (1989) found that males produce more aggressive calls to longer duration aggressive stimuli. Pulsed structure is also clearly an important factor in eliciting an aggressive response based on the increased aggressive calls in that treatment between the fourth minute of the habituation phase and the discrimination phase. It is possible that the two parameters are redundant increasing the success in signaling aggressive intent to other males.

In the graded system, cricket frogs are able to respond to calls with either longer duration or lowered dominant frequency with calls that are also either longer in duration or lower in frequency. Since these calls are graded males may have more plasticity in their ability to respond to calls with different parameters. I found that male spring peepers respond with more aggressive calls to calls with either longer duration or a pulsed structure than to advertisement calls but not if the response calls vary in these parameters. In this discrete system, males may also be able to respond to calls with some plasticity. Schwartz (1989) found that males can vary the length of their aggressive calls. Future analysis of our data will examine the duration of both aggressive and advertisement calls given by males in the discrimination phase of each treatment.

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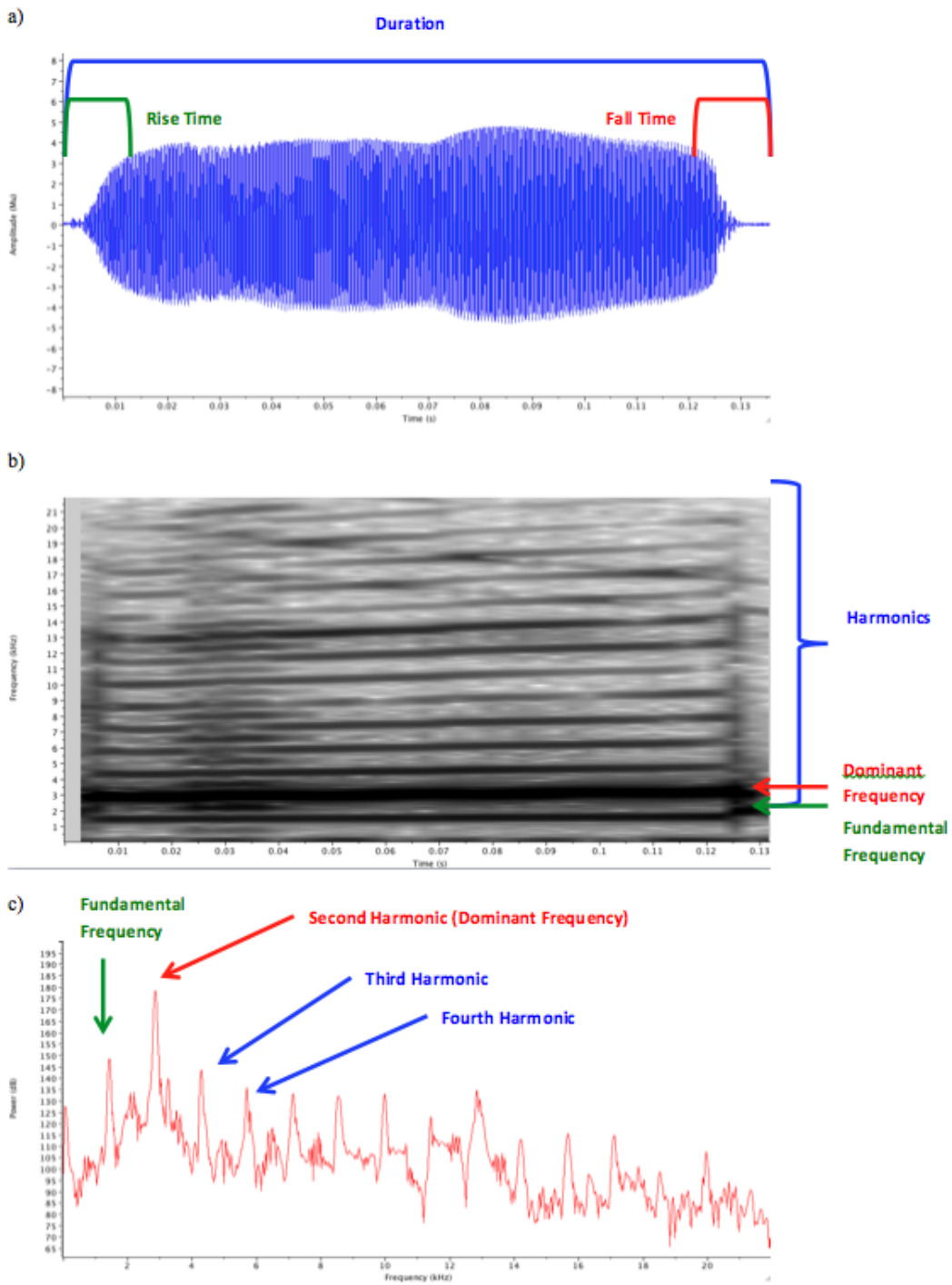


Figure 1. A spring peeper advertisement call shown in waveform (a), spectrogram (window size = 270) (b), and spectrogram slice (window size = 1080) (c).

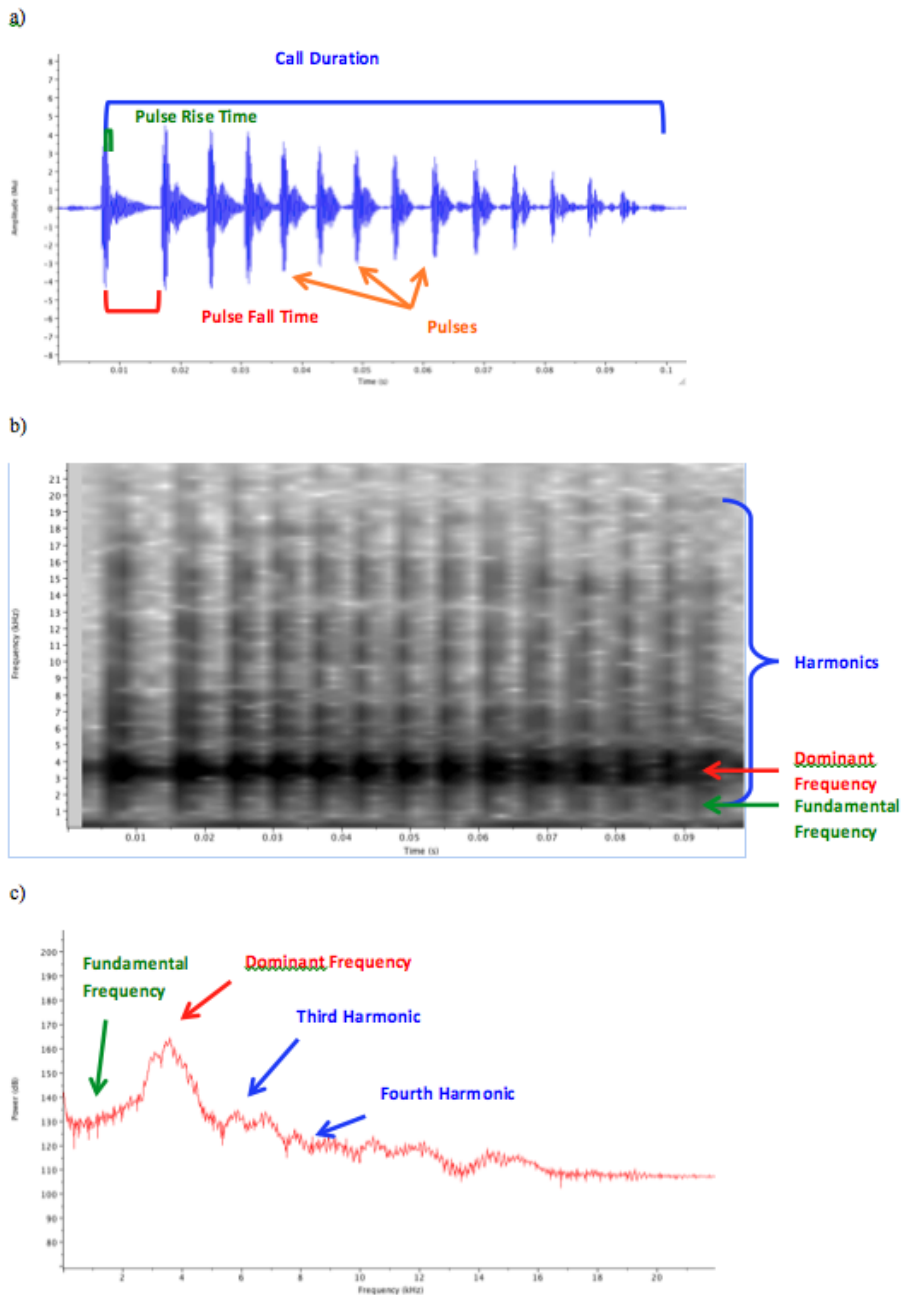


Figure 2. A cricket frog advertisement call shown in waveform (a), spectrogram (b) (spectrogram window size 180), and spectrogram cross section (c) (spectrogram window size 1080).

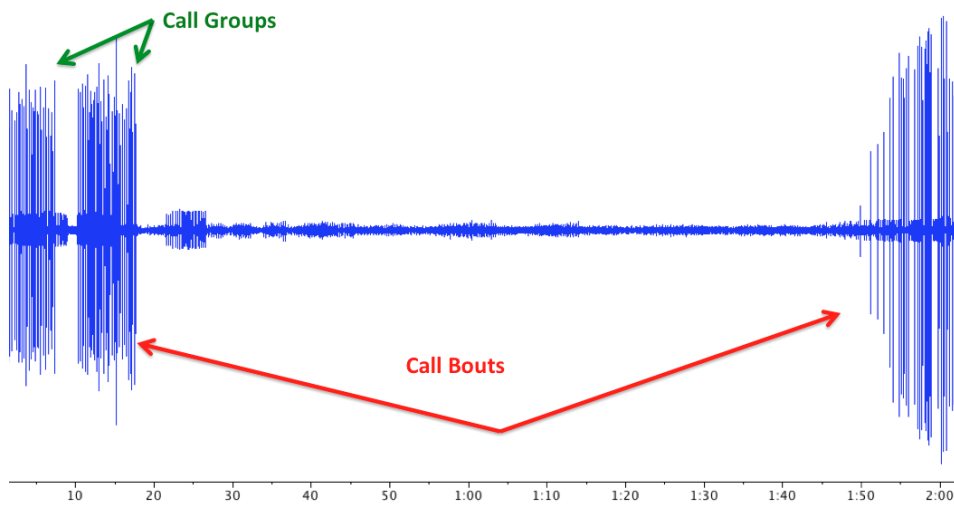


Figure 3. A cricket frog recording showing the call groups and call bouts.

Table 1. Call characteristics and their correlation with temperature for spring peepers in western central Indiana (N = 51)

Call Characteristic	Mean	Median	SD	Regression with temperature	r	p
Call rate (calls/s)	1.27	1.23	0.36	$y = 0.0541x + 0.3628$	0.76	<0.001*
Duration (s)	0.123	0.121	0.029	$y = -0.004x + 0.1894$	-0.68	<0.001*
Rise time (s)	0.014	0.012	0.010	$y = -0.0003x + 0.0201$	-0.17	0.223
Fall time (s)	0.013	0.009	0.011	$y = 0.0002x + 0.0107$	0.08	0.592
Fundamental Frequency (Hz)	1450.4	1438.4	75.2	$y = 7.4149x + 1326$	0.50	<0.001*
Frequency shift (%) from beginning to end of call	7.6	7.9	2.2	$y = -0.043x + 8.8073$	-0.70	0.037

* p values judged statistically significant at alpha = 0.05 after application of the sequential Bonferonni technique

Table 2. Call harmonics and their amplitude relative to the dominant frequency for spring peepers in western central Indiana (N= 51)

Harmonic	Average	Median	SD	Amplitude relative to the dominant frequency (dB)
Fundamental	1450.4	1438.4	75.2	-35.5
Dominant	2895.5	2876.8	158.0	0.0
3rd	4350.7	4315.2	229.7	-31.5
4th	5803.7	5753.7	308.9	-40.4
5th	7248.2	7196.4	379.8	-48.6
6th	8694.4	8647.7	458.2	-52.0
7th	10150.2	10090.5	535.4	-52.9
8th	11610.9	11520.3	609.2	-52.3
9th	13071.1	12980.2	670.9	-55.3
10th	14504.7	14410.0	744.1	-61.0
11th	15956.6	15848.5	824.0	-68.3
12th	17406.2	17278.2	900.6	-74.3

Table 3. Call rate characteristics of cricket frogs in western central Indiana (N = 42)

Call Characteristic	Mean	Median	SD	N	Regression with temperature	r	p
Call rate (calls/s)	3.55	3.25	1.15	42	$y = 0.1929x + 0.3337$	0.60	<0.001*
Call groups per call bout	2.26	1.80	1.77	42	$y = -0.0978x + 3.8908$	-0.20	0.209
Inter-bout spacing (s)	17.26	13.05	16.25	35	$y = -1.5897x + 42.85$	-0.33	0.056
Call group duration (s)	6.71	5.81	4.14	42	$y = 0.3709x + 0.5203$	0.32	0.039
Intergroup spacing	1.56	1.47	0.55	37	$y = -0.0551x + 2.4478$	-0.33	0.045

* p values judged statistically significant at alpha = 0.05 after application of the sequential Bonferonni technique

Table 4. Call characteristics of cricket frogs in western central Indiana (N = 42)

	Mean	Median	SD	N	Regression with temperature	r	p
Dominant Frequency (Hz)	3552.9	3509.9	192.3	42	$y = 22.757x + 3172.9$	0.42	0.005
Call duration (s)							
Beginning	0.0278	0.0293	0.0105	37	$y = -0.0016x + 0.055$	-0.54	0.001*
Middle	0.0420	0.0391	0.0203	40	$y = 4E-05x + 0.0413$	0.01	0.965
End	0.0622	0.0578	0.0219	39	$y = -0.0003x + 0.0671$	-0.05	0.774
Pulses per call							
Beginning	4.51	5.00	1.35	37	$y = -0.2479x + 8.655$	-0.64	<0.001*
Middle	5.73	6.00	1.66	40	$y = -0.181x + 8.7599$	-0.40	0.011
End	7.70	8.00	2.10	39	$y = -0.0376x + 8.3355$	-0.06	0.697
Pulse rate (pulses/s)							
Beginning	177.69	160.64	65.59	37	$y = 1.6786x + 149.65$	0.09	0.598
Middle	156.90	151.12	68.94	40	$y = 0.761x + 144.14$	0.04	0.805
End	132.28	133.93	38.32	39	$y = 1.4987x + 106.99$	0.14	0.392
Pulse duration (s)							
Beginning	0.00509	0.00513	0.00126	36	$y = 9E-05x + 0.0036$	0.25	0.146
Middle	0.00506	0.00510	0.00111	40	$y = 1E-05x + 0.0049$	0.04	0.807
End	0.00535	0.00511	0.00107	39	$y = 6E-05x + 0.0043$	0.21	0.192
Pulse rise time (s)							
Beginning	0.00089	0.00089	0.00013	36	$y = -8E-08x + 0.0009$	0.00	0.990
Middle	0.00090	0.00088	0.00017	40	$y = 6E-06x + 0.0008$	0.12	0.445
End	0.00091	0.00091	0.00014	39	$y = 4E-06x + 0.0008$	0.09	0.577
Pulse fall time (s)							
Beginning	0.00412	0.00412	0.00126	36	$y = 7E-05x + 0.0029$	0.21	0.218

Middle	0.00404	0.00417	0.00109	40	$y = 2E-05x + 0.0038$	0.05	0.752
End	0.00439	0.00418	0.00098	39	$y = 5E-05x + 0.0036$	0.18	0.269
Inter-pulse interval (s)							
Beginning	0.00189	0.00095	0.00419	35	$y = 0.0004x - 0.0042$	0.28	0.107
Middle	0.00272	0.00182	0.00302	40	$y = 0.0002x - 0.0006$	0.24	0.139
End	0.00449	0.00318	0.00558	39	$y = -8E-05x + 0.0058$	-0.05	0.768
Relative amplitude of last pulse to first pulse (%)							
Beginning	0.40	0.38	0.18	36	$y = 0.0142x + 0.1587$	0.28	0.100
Middle	0.38	0.34	0.18	40	$y = 0.0187x + 0.0707$	0.38	0.014
End	0.34	0.33	0.13	39	$y = 0.0112x + 0.1492$	0.30	0.061

* p values judged statistically significant at $\alpha = 0.05$ after application of the sequential Bonferonni technique

Duration*Dominant frequency**Call rate*

Figure 4. Locations of spring peeper and cricket frogs studies used for comparisons a) the present study: Terre Haute, IN, b) Brown & Brown 1977: Bath, IL, c) Lemon & Struger 1980: Mont St. Hilaire, Quebec, d) Sullivan & Hinshaw 1990: Penobscot County, ME, e) Wells 1996: Storrs, CT

Table 5. Comparisons of spring peeper call correlations with temperature

	Brown & Brown (1977)				Lemon & Struger (1980)				Sullivan & Hinshaw (1990)				Wells (1996)			
	r	p _r	z	p _z	r	p _r	z	p _z	r	p _r	z	p _z	r	p _r	z	p _z
Duration	-				-				-							
(s)	0.68	<0.001	1.51	0.131	0.73	<0.01	1.37	0.169	0.81	<0.05	2.56	0.011				
Dominant frequency																
(Hz)									0.56	<0.05	1.51	0.131				
Call rate							-									
(calls/min)	0.68	<0.001	0.69	0.490	0.87	<0.01	0.97	0.330	0.60	<0.05	1.88	0.060	0.79	<0.001	0.67	0.502
N	25				12				21 ^a ,	97 ^b			272			

r = the original correlation for each study

p_r = the p value for the original correlation

z = the value of Fisher's z comparing each study's r to the present study's r

p_z = the p value for the Fisher's z

^a N for duration and dominant frequency

^b N for call rate

* p values judged statistically significant at alpha = 0.05 after application of the sequential Bonferonni technique

Table 6. Comparisons of cricket frog call characteristic correlations with temperature

	Wagner 1986				Wagner 1987			
	r	p _r	Z	p _z	r	p _r	Z	p _z
Dominant Frequency (Hz)	0.23	<0.05	1.15	0.250	0.36	<0.001	0.41	0.681
Call rate (calls/s)	0.59	<0.001	0.09	0.925	0.77	<0.001	-1.80	0.072
Call duration (s)	-	<0.001	1.95	0.052	-0.77	<0.001	2.15	0.032
Beginning	0.76							
Middle	-	<0.001	2.12	0.034	-0.56	<0.001	3.48	0.001*
End	-	<0.001	1.64	0.102	-0.45	<0.001	2.35	0.019
Pulses per call	0.35							
Beginning	-	<0.001	-0.50	0.620	-0.55	<0.001	-0.75	0.456
Middle	0.58							
End	-	<0.001	0.33	0.743	-0.35	<0.001	-0.31	0.760
Pulse rate (pulses/s)	0.45							
Beginning	-	<0.001	1.55	0.121	-0.01	n.s	-0.29	0.770
Middle	0.35							
End	0.7	<0.001	-3.88	<0.001*	0.73	<0.001	-4.35	<0.001*
Pulse rate (pulses/s)								
Beginning	0.3	<0.01	-1.40	0.161	0.48	<0.001	-2.62	0.009
Middle	0.27	<0.01	-0.70	0.487	0.44	<0.001	-1.78	0.076
End								

r = the original correlation for each study

p_r = the p value for the original correlation

z = the value of Fisher's z comparing each study's r to the present study's r

p_z = the p value for the Fisher's z

* p values judged statistically significant at alpha = 0.05 after application of the sequential Bonferonni technique

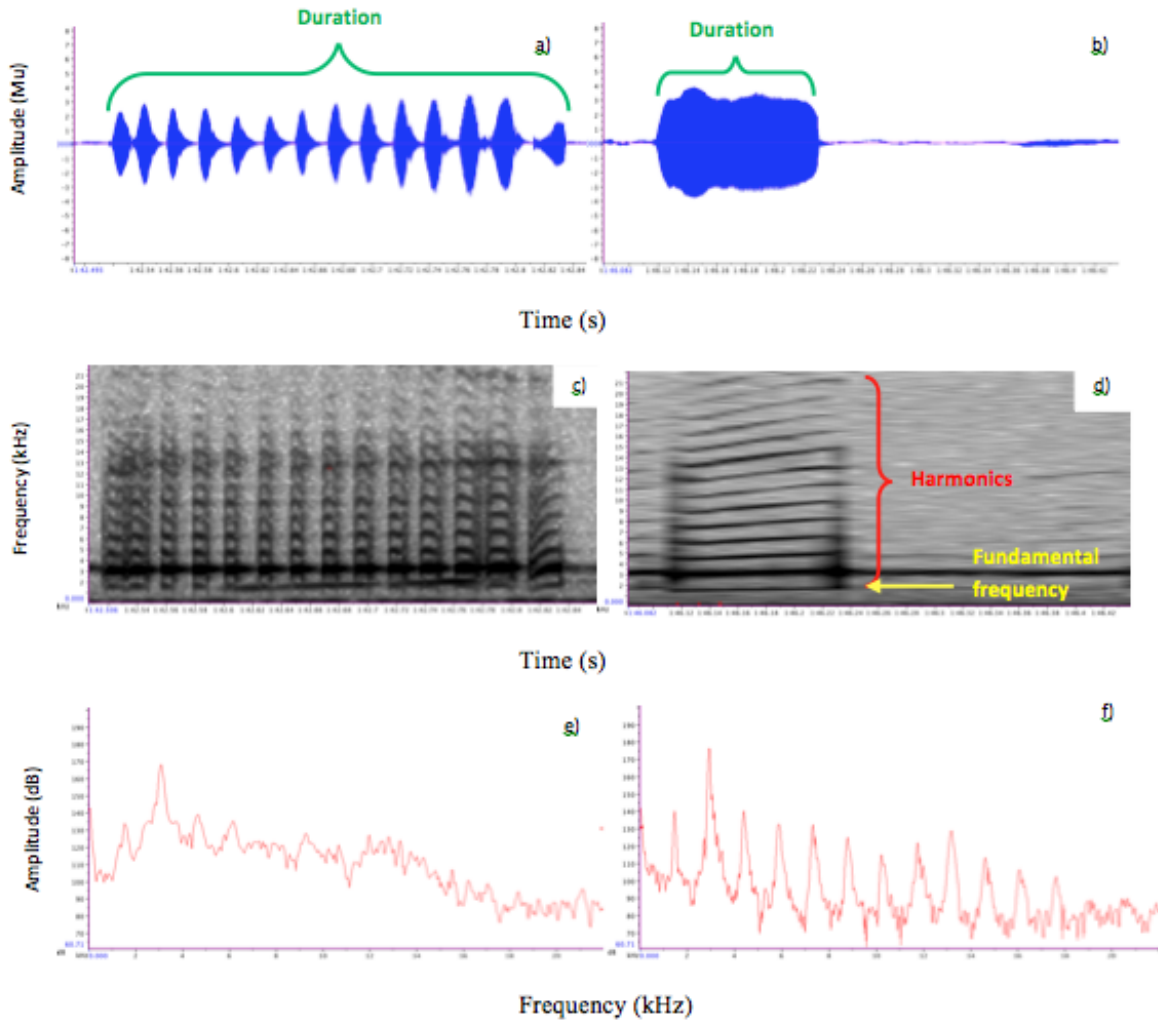


Figure 5. Spring peeper aggressive (a, c, e) and advertisement (b, d, f) calls from the same individual shown in waveform (a, b), spectrogram (c, d), and spectrogram cross section (e, f).

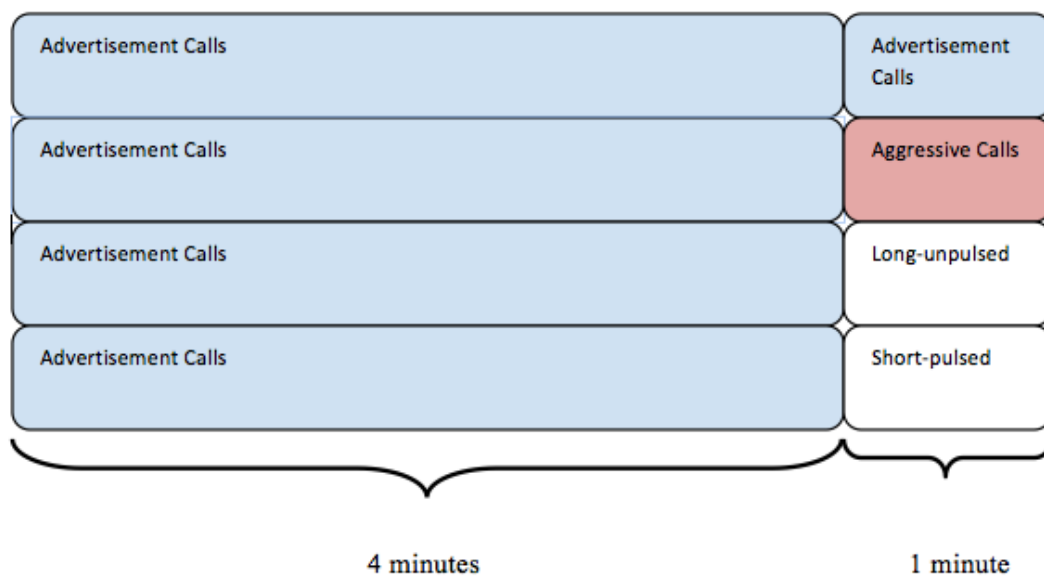
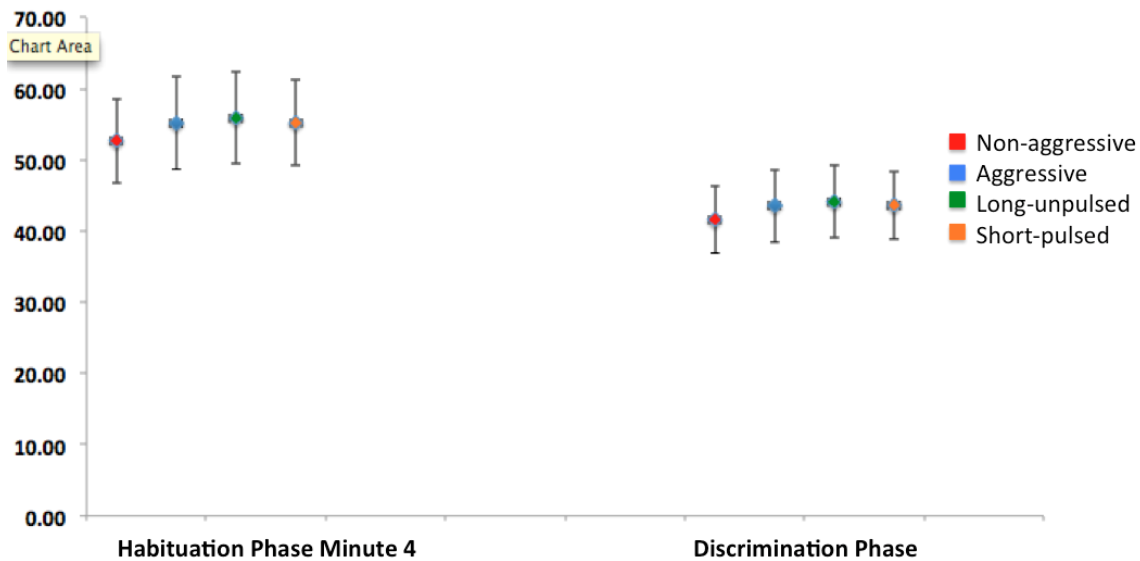


Figure 6. Habituation-discrimination protocol.

a)



b)

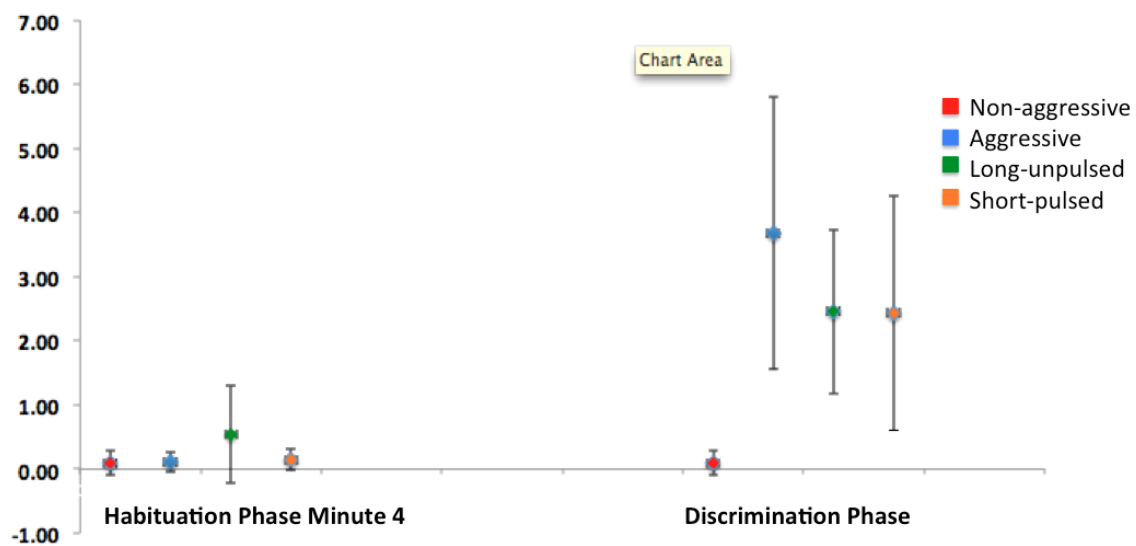


Figure 7. Estimated marginal means for a) advertisement calls (at the fixed covariate value of temperature = 12.002) and b) actual means for aggressive calls for each treatment in the fourth minute of the habituation phase and the discrimination phase.

Table 7. Call duration and pulsed structure for each of the four treatments

Treatment	Duration	Pulsed
Nonaggressive	90 ms	no
Aggressive	340 ms	yes
Long-unpulsed	340 ms	no
Short-pulsed	90 ms	yes

Table 8. GEE model effects for the general equations estimation for advertisement (a) and aggressive (b) calls

	Advertisement calls	Aggressive Calls
Term	Change in QICC	Change in QICC
Temp	-11.2	1.1
Treat	7.2	-40.3
Phase	2.7	-93.8
Treatment*Phase	3.9	-6.8

Table 9. Pairwise comparisons of the estimated marginal means aggressive calls for each treatment in the fourth minute of the habituation phase (a) and the discrimination phase (b) using Tukey's test

a)

Treatment		Mean	Q	p	95% Confidence interval	
		difference (I-J)			Lower	Upper
Non-aggressive	Aggressive	-0.01	-0.05	0.94	-0.83	0.81
	Long-unpulsed	-0.45	-1.99	0.50	-1.27	0.37
	Short-pulsed	-0.05	-0.21	1.00	-0.87	0.77
Aggressive	Long-unpulsed	-0.43	-2.07	0.46	-1.20	0.34
	Short-pulsed	-0.04	-0.18	1.00	-0.76	0.68
Long-unpulsed	Short-pulsed	0.40	1.90	0.54	-0.37	1.17

b)

Treatment		Mean	Q	p	95% Confidence interval	
		difference (I-J)			Lower	Upper
Non-aggressive	Aggressive	-3.58	-4.03	0.03	-6.84	-0.32
	Long-unpulsed	-2.36	-2.65	0.24	-5.62	0.90
	Short-pulsed	-2.33	-2.62	0.26	-5.59	4.48
Aggressive	Long-unpulsed	1.22	1.47	0.73	-1.83	4.27
	Short-pulsed	1.25	1.59	0.67	-1.62	4.12
Long-unpulsed	Short-pulsed	0.03	0.04	0.80	-3.02	3.08

Table 10. Pairwise comparisons between phases for each of the four treatments using Tukey's test

	Mean	Q	P	95% Confidence Intervals	
	difference (I-J)			Lower	Upper
Non-aggressive	0	0			
Aggressive	-3.57	-6.35	<0.001	-5.63	-1.51
Long-unpulsed	-1.92	-3.16	0.12	-4.14	0.30
Short-pulsed	-2.29	-3.99	0.03	-4.39	-0.19