



# Assessing Acoustic Signal Variability and the Potential for Sexual Selection and Social Recognition in Boreal Chorus Frogs (*Pseudacris maculata*)

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## Abstract

An important and necessary step in the analysis of any communication system involves quantitative descriptions of how signals vary at multiple levels of organization (e.g., species, populations, individuals). Such descriptions provide a basis for generating testable predictions about the functions of signals and their specific physical properties in different behavioral contexts. Here, we report results from acoustical and statistical analyses of the advertisement calls of the boreal chorus frog, *Pseudacris maculata*. In addition to characterizing measures of central tendency and dispersion for our study population, we assess how calls encode potentially relevant information in the contexts of sexual selection and social recognition. Specifically, we describe the relationships between call properties and both body size and condition, and relate these findings to predictions about female mate choice and male-male competition. We also make predictions about the shapes of female preference functions based on analyses of the patterns of within-individual variation in call properties. Findings from multivariate analyses of advertisement calls are used to generate testable predictions about the potential for these signals to function in social recognition. We also discuss our results in relation to wildlife conservation and relevant studies of geographic variation.

## Introduction

Animal signals vary at multiple scales (e.g., among species, among populations within species, among individuals within populations, and within individuals). Characterizing such variation is a critical first step toward understanding the mechanisms of signal production and perception, the adaptive functions of signals, and the broad-scale patterns of signal evolution (Bradbury & Vehrencamp 1998; Gerhardt & Huber 2002). Consequently, assessing variation in communication signals remains a central focus in the study of evolution, behavior, and neuroethology.

The primary objective of the present study was to characterize the sources and patterns of individual variation in the acoustic signals of the boreal chorus frog, *Pseudacris maculata* (Hylidae), in order to derive testable predictions about the potential function of these signals in the contexts of sexual selection and social recognition.

The most common anuran vocalization is the loud and conspicuous advertisement call that males produce to attract mates and to announce their occupancy of calling sites or territories to rival males (Wells 1977). Previous sexual selection studies of male-male competition have shown that the males

of some frog species can assess the size and fighting ability of rivals based on size-related variation in spectral call properties (reviewed in Gerhardt & Bee 2007). Other studies of sexual selection and female choice have shown that female frogs commonly prefer the low-frequency calls of larger males or traits that potentially depend on a male's condition, such as call duration and calling rate (reviewed in Gerhardt & Huber 2002). Therefore, one aim of this study was to assess the extent to which individual differences in body size and condition were predicted by the acoustic properties of advertisement calls.

In a review of the literature on signal variability and female preferences in anurans and insects, Gerhardt (1991) and Gerhardt & Huber (2002) pointed out that acoustic properties of signals can often be placed along a continuum ranging between 'static' and 'dynamic' based on patterns of within-individual variability. Moreover, call properties at the static and dynamic ends of the continuum are often under different selection regimes. In terms of female preferences, for example, static properties tend to be under stabilizing or weakly directional selection for values that are close to the population mean. Preferences for dynamic properties, on the other hand, tend to be directional in favor of values that equal or exceed the most extreme values in natural populations (Gerhardt 1991). Following Gerhardt (1991), we classified call properties along a continuum from 'static' to 'dynamic' based on patterns of within-individual variation to establish testable predictions about the shapes of female preference functions.

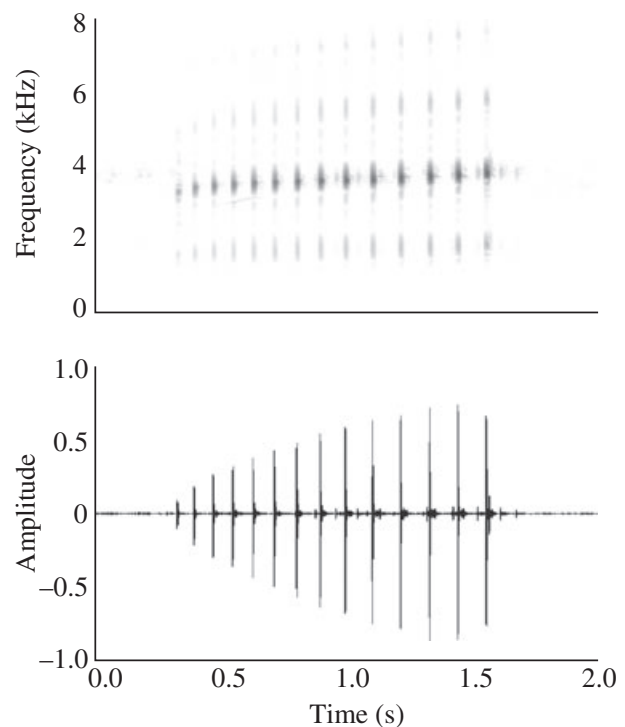
Previous studies of social recognition in anurans have sought to identify possible 'individual signatures' in advertisement calls (Bee & Gerhardt 2001a; Bee et al. 2001; Bee 2004; Feng et al. 2009; Gasser et al. 2009). Such individually distinctive signatures may be important for 'dear enemy' recognition in some frog species in which males acoustically and behaviorally discriminate between strangers and nearby territorial neighbors, such as in North American bullfrogs, *Rana catesbeiana* (Davis 1987; Bee & Gerhardt 2001a,b; Bee 2004; but see also Bee 2003). A good social recognition cue has two important qualities: (i) it is individually distinctive (i.e., varies more among individuals than within individuals), and thus contributes to statistically discriminating among individuals, and (ii) the differences among individuals can be resolved by the receiver's sensory systems. Here, we investigated the first of these qualities by quantifying the ability of advertisement calls to statistically discriminate among males.

## Methods

### Study System

We recorded 720 advertisement calls (Fig. 1) from 36 male boreal chorus frogs during their breeding season between 14 Apr. and 5 May, 2009. The boreal chorus frog belongs to a 'trilling frog clade' in the genus *Pseudacris* (Moriarty & Cannatella 2004), so named because all of its members produce advertisement calls comprising series of pulses (Fig. 1). Formerly considered one of four subspecies of the western chorus frog (*P. triseriata maculata*), Platz & Forester (1988) and Platz (1989) suggested species status for *P. maculata* based on morphology and advertisement call structure. Recent molecular studies have confirmed species status for *P. maculata* (Moriarty & Cannatella 2004; Lemmon et al. 2007).

Our recordings were made during active choruses (2200–0100 h) at two field sites in east-central Minnesota (USA) that are located 70 km apart (Carlos Avery Wildlife Management Area, 45°18'21.87"N, 93° 4'31.92"W, Anoka Co., n = 19 individuals recorded; Carver Park Reserve, 44°52'49.29"N, 93°43'3.10"W; Carver Co., n = 17 individuals



**Fig. 1:** The pulsed advertisement call of the boreal chorus frog (*Pseudacris maculata*). The top trace shows the spectrogram of a single call (FFT = 1024 points, Hanning window, 75% overlap) and the bottom trace shows the corresponding waveform.

recorded). Both field sites are large (approx. 9300 and 1335 ha, respectively) and comprise numerous ponds and marshes in which boreal chorus frogs were abundant. At both study sites, males called while in contact with the water, for example while sitting partially submerged on floating mats of filamentous algae or on dead or newly emerged vegetation. A preliminary investigation revealed no significant between-site differences in any call properties (see Supporting information); therefore, we did not include site as a between-subjects factor in the statistical analyses described below.

### Acoustic Recordings

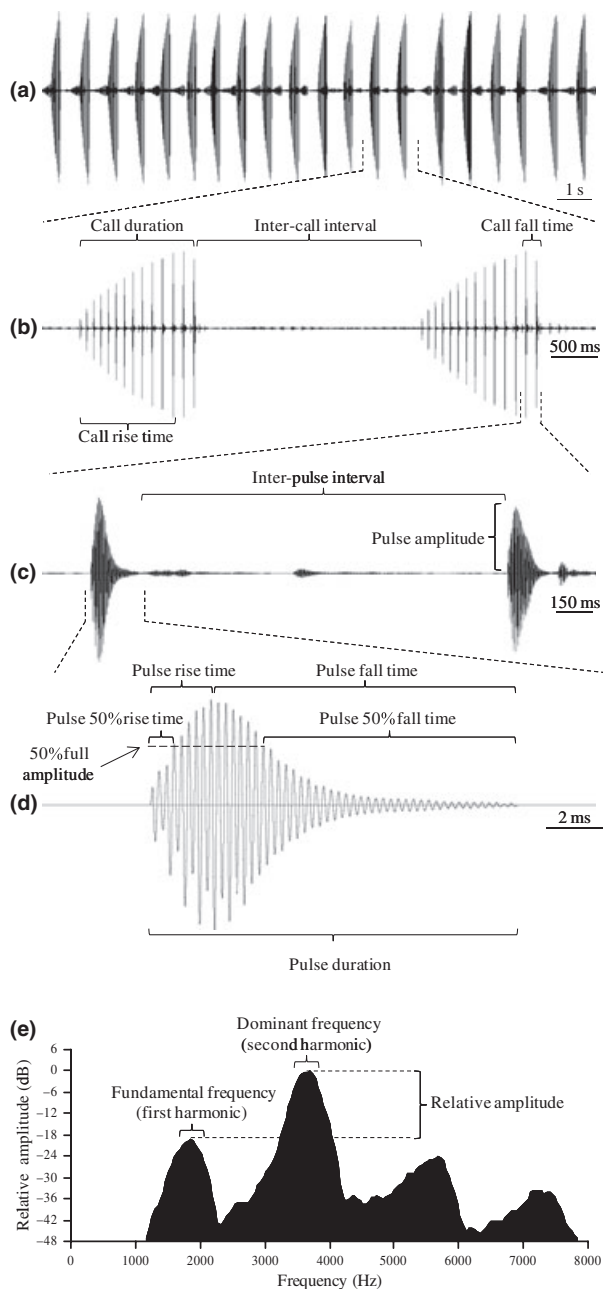
We preferentially recorded males that did not have calling neighbors within 1 m to minimize interference from neighbors and to maximize the signal-to-noise ratios of our recordings. We recorded calls using a handheld Sennheiser ME66 microphone (Sennheiser USA, Old Lyme CT, USA) with Sennheiser MZW 60-1 blimp windscreen and MZS 20-1 suspension grip. The tip of the microphone was positioned approx. 50 cm from the focal animal. We recorded 20 consecutive calls (44.1 kHz, 16-bit resolution) from each male using a Marantz PMD 670 solid-state digital recorder (D&M Professional, Itasca, IL, USA). We used a quick-reading cloacal thermometer (Avinet, Inc., Dryden NY, USA) to record the water temperature to the nearest 0.1°C at the position from which the focal animal called. Water temperatures ranged between 7.9°C and 18.4°C ( $\bar{X} \pm SD = 12.7 \pm 3.0^\circ\text{C}$ ). Immediately after most recordings, we also captured the focal animal and measured its snout-vent-length (SVL,  $n = 29$ ; approx. 80% of recorded individuals) to the nearest 0.1 mm using dial calipers and its body mass ( $n = 28$ ; approx. 78% of recorded individuals) to the nearest 0.1 g using Pesola spring scales. The SVLs and masses of our subjects varied between 21.5 and 28.1 mm ( $\bar{X} \pm SD = 24.7 \pm 1.6$  mm) and 0.5 g and 1.0 g ( $\bar{X} \pm SD = 0.7 \pm 0.1$  g), respectively. We used these data on SVL and mass to compute an index of body condition (i.e., size-independent body mass) as the residuals from a linear regression of the cube root of mass on SVL divided by SVL (Baker 1992). Subjects were released at their calling site immediately following measurements of temperature, SVL, and mass. In lieu of marking animals individually (e.g., by toe-clipping), we assured that individuals were not recorded more than once by making our recordings in different, widely separated areas both within and between nights.

We also measured the sound pressure levels (dB SPL re 20  $\mu\text{Pa}$ , fast RMS, C-weighted) of 10 calls from each of 11 additional males using a CEL-430.A1 Type 1 sound level meter (Casella USA, Amherst, NH, USA). For these measurements, we positioned the tip of the recording microphone just above the surface of the water at a distance of 1 m from the calling male. We calculated individual medians and inter-quartile ranges of SPL based on the 10 calls recorded from each individual. We determined the overall median and inter-quartile range based on these 11 individual median values. Data on SVL, mass, and temperature are not available for these recordings of sound pressure levels.

### Acoustical Analyses

We used Raven v1.2.1 (Cornell Lab of Ornithology, Ithaca, NY, USA) to measure or calculate 26 different call properties from each of the 720 recorded calls (20 calls/individual,  $n = 36$ ). We measured 18 properties in the time domain using Raven's waveform display (see Fig. 2 for schematic details). We counted the number of pulses per call, and measured call duration as the time between the onset of the first pulse and the offset of the last pulse. We measured inter-call interval as the time between the offset of the last pulse of one call and the onset of the first pulse of the next consecutive call. We calculated a male's call rate (calls per minute) by taking the reciprocal of the average call period for each call, which was the sum of the call's duration and the subsequent inter-call interval. We characterized the amplitude envelope of the call in two ways. First, we measured the call rise time (from the onset of the first pulse to the onset of the first full-amplitude pulse) and the call fall time (from the onset of the last full-amplitude pulse to the offset of the last pulse). Second, we measured the amplitude (in dimensionless units) of the first, middle, and last pulses of a call and converted values for the middle and last pulses to ratios relative to that of the first pulse. (For calls with an even number of pulses, we averaged values over the two middle pulses.)

We determined the overall pulse rate of a call with  $N$  pulses by taking the reciprocal of the overall pulse period averaged over the first  $N-1$  pulses in the call. An initial inspection, however, revealed that pulse rate also varied somewhat within a call. Therefore, we determined the instantaneous pulse rate at the beginning, middle, and end of the call by separately measuring and taking the reciprocal of the pulse period for the first, middle, and next-to-last pulses in



**Fig. 2:** Analysis of temporal and spectral call properties. Plots (a–d) depict waveforms of (a) 20 consecutive calls from one male, (b) a close-up showing the two indicated calls from the same male, (c) a close-up showing two pulses from the indicated call in (b), and (d) a close-up of the indicated pulse shown in (c). (e) Power spectrum (FFT = 1024 points, Hanning window, 75% overlap) showing the relative amplitudes (in dB) of frequencies below 8 kHz after averaging over the duration of a single call. Text and brackets indicate the ranges of time, frequency, or amplitude over which the indicated call properties were measured. Scale bars in (a–d) indicate time along the x-axis of each waveform. All times were measured to the nearest 1.0 ms. The two calls depicted in (b) are available in Audio S1 in the Supporting information.

a call. (Again, we averaged values for the two middle pulses of calls with an even number of pulses.) For the single pulse having the greatest relative amplitude in each call, we also measured pulse duration, pulse rise time (onset to maximum amplitude), 50% pulse rise time (onset to 50% maximum amplitude), pulse fall time (maximum amplitude to offset), 50% pulse fall time (50% maximum amplitude to offset), and inter-pulse interval (pulse offset to onset of the next pulse).

We made six measurements in the spectral domain using Raven's spectrogram and power spectrum displays (Fig. 2). We measured the overall dominant frequency (i.e., spectral peak of greatest relative amplitude) and the spectral peak having the second highest amplitude (in Hz), and their relative amplitudes (in dB), after averaging the spectrum over the entire call. In all cases, the dominant frequency was the second harmonic, and the spectral peak with the second highest amplitude was the fundamental frequency. For the purpose of all statistical analyses described below, we converted our logarithmic measures of relative amplitude (in dB) to a linear pressure ratio (i.e., 0 dB = 1.0, –6 dB = 0.50, –12 dB = 0.25, etc). We also measured the dominant frequency by taking separate slices through the first, middle, and last pulses of each call. From these spectral measurements of separate pulses, we calculated two additional properties. We determined the total range of upward frequency modulation (FM range) from the beginning to the end of the call by subtracting the dominant frequency of the first pulse from that of the last pulse. We then determined the rate of upward frequency modulation (FM rate) by dividing the FM range by the call duration.

### Statistical Analyses

All statistical analyses were computed using Statistica 7.1 (StatSoft 2006). We used a mixture of parametric and non-parametric tests. Decisions to use one or the other type of test were based on considerations of (i) the underlying distributions of the data, (ii) the assumptions of the tests, (iii) the ability (or inability) of transformations to bring the data in line with those assumptions, and (iv) the robustness of various analyses to violations of those assumptions.

#### *Assessing individual variation in relation to sexual selection*

In our dataset, water temperature was significantly positively correlated with SVL ( $r = 0.66$ ,  $p = 0.0001$ ,  $n = 29$ ) but not with the condition index ( $r = -0.03$ ,



$p = 0.8714$ ,  $n = 28$ ). To examine the independent influences of water temperature and SVL on call properties, we used multiple linear regression and examined separately the partial correlations between the unadjusted individual mean values of each call property (i.e., before standardizing to a common temperature) and both water temperature and SVL (see Supporting information). We subsequently standardized each measured value to a common water temperature following Platz & Forester (1988). We chose a standard water temperature of 14°C because it is close to the average water temperature at which our recordings were made (12.7°C), and because it is the same standard temperature used in the previous studies of *P. maculata* by Platz & Forester (1988) and Platz (1989). We investigated relationships between call properties and the condition index both before and after standardizing to a common water temperature of 14°C. All other statistical analyses were performed using temperature-corrected values of call properties. Because our analyses of relationships between call properties and SVL and condition were exploratory in nature, we did not correct for multiple comparisons and used a significance criterion of  $\alpha = 0.05$ .

For each focal male, we also determined the individual mean ( $\bar{X}_w$ ) and standard deviation ( $SD_w$ ) of each call property ( $n = 20$  calls/male), and we used these values to calculate a within-individual coefficient of variation ( $CV_w = 100\% \times SD_w / \bar{X}_w$ ). Following Gerhardt (1991), call properties with a  $CV_w$  less than about 5% were considered static properties; those with a  $CV_w$  greater than about 12% were considered dynamic properties. Properties with a  $CV_w$  between 5% and 12% were classified as 'intermediate'.

#### *Assessing individual variation in relation to social recognition*

We investigated the individual distinctiveness of advertisement calls using procedures fully described elsewhere (Bee & Gerhardt 2001a; Bee et al. 2001; Bee 2004). Briefly, we determined a grand mean and standard deviation based on averaging the 36 individual means from our sample, and we used these values to calculate the among-individual coefficient of variation ( $CV_a = 100\% \times \text{grand } SD / \text{grand } \bar{X}$ ). We determined the ratio of among-individual and within-individual variation as  $CV_a / CV_w$ . As a second measure of among-male variability, we expressed the difference between the largest and smallest individual means as a percentage of the grand mean for each call property. We also used

model II ANOVAs to describe the extent to which each call property varied significantly more among individuals than within individuals. We report the effect size for each ANOVA (partial  $\eta^2$ ) as an additional indication of the extent to which among-individual variation in call properties exceeded that within individuals. We also used principal components analysis (PCA) to reduce our 26 spectro-temporal properties to a smaller set of orthogonal principal components. Next, we used a discriminant function analysis (DFA) of the principal component scores to examine the extent to which calls could be assigned to the correct individual. Our measures of classification success were based on a cross-validation procedure, in which each call was classified using discriminant functions that were calculated after excluding the to-be-classified call. We assessed the statistical significance of correct classifications following Titus et al. (1984).

## Results

### Acoustic Properties of Advertisement Calls

We report specific values for temperature-corrected means, standard deviations, and ranges for all 26 call properties in the Supporting information. The typical call was approx. 750–950 ms in duration and comprised a series of approx. 14 short pulses delivered at a rate of approx. 16 pulses/s (Fig. 1). The pulse rate was slightly faster at the beginning of the call compared with the middle and end of the call. The amplitude envelope of the call comprised a gradual rise of approx. 500–750 ms over the first 7–10 pulses, followed by a short period of relatively steady amplitude and then a rapid decrease in amplitude that typically involved only the last 1–3 pulses. Each pulse in a call comprised several harmonically-related spectral peaks. The average frequencies (and relative amplitudes) of the first harmonic (i.e., the fundamental frequency) and the second harmonic (i.e., the dominant frequency) were approx. 1900 Hz (–14 dB) and 3800 Hz (0 dB), respectively. Over the duration of the call, the dominant frequency swept upward from approx. 3500 to 4100 Hz (Fig. 1). The typical pulse was approx. 14 ms in duration and had an explosive onset (<1–2 ms rise time) and an offset that lasted 10–12 ms. **Males usually repeated the call about every 2–3 s, yielding typical call rates of approx. 20–30 calls per minute.**

The overall median SPL was 89.1 dB at a distance of 1 m. There was little variation in recorded SPL values within and among individuals, and much of

the variation was within the recognized measurement error of type I sound level meters (e.g.,  $\pm 1.0$ – $1.5$  dB). The overall 25th and 75th percentiles of SPL based on the individual medians from all 11 subjects were 88.5 and 90.1 dB, respectively (inter-quartile range = 1.6 dB). Within individuals, the magnitude of the inter-quartile range based on the 10 calls recorded from each male ranged between 0.4 and 4.2 dB, with values for 9 of 11 subjects being less than or equal to 2.0 dB. Overall, these values of SPL for *P. maculata* are generally in line with those previously reported for other hylid tree-frogs (Gerhardt 1975).

### Sources and Patterns of Individual Variation

None of the temporal properties that we measured were significantly correlated with SVL after using multiple linear regression to partial out the effects due to variation in water temperature ( $0.02 \leq |r| \leq 0.28$ ,  $0.14 < p < 0.94$ ,  $n = 29$ ; see Supporting information). The only spectral property significantly correlated with SVL was the dominant frequency of the first pulse of a call ( $r = -0.44$ ,  $p = 0.0199$ ,  $n = 29$ ). The relationships between SVL and other measures of fundamental frequency and dominant frequency were negative but not significantly so ( $-0.30 \leq r \leq -0.17$ ,  $0.12 < p < 0.39$ ,  $n = 29$ ; see Supporting information). Neither the range nor rate of FM were related to SVL ( $0.17 \leq r \leq 0.28$ ,  $p > 0.14$ ,  $n = 29$ ).

There was a significant correlation between the mean, temperature-corrected values of call rise time and our condition index ( $r = 0.39$ ,  $p = 0.0421$ ,  $n = 28$ ). No other correlations between the condition index and temperature-corrected mean values for other call properties were significant ( $0.04 \leq |r| \leq 0.36$ ;  $0.0620 < p < 0.8088$ ;  $n = 28$ ). There were also no significant correlations between the condition index and any call property prior to standardizing values to 14°C ( $0.03 < |r| < 0.36$ ;  $0.0624 < p < 0.8487$ ;  $n = 28$ ).

We categorized 11 acoustic properties as 'static' properties (Table 1). Most of these 11 properties could be grouped together into measures of three more general call features that included (i) the spectral characteristics of the call (e.g., fundamental and dominant frequencies; Table 1), (ii) the length of the call (e.g., both call duration and pulse number; Table 1), and (iii) the rate of pulses in the call (e.g., overall and instantaneous pulse rates; Table 1). We categorized seven call properties as 'dynamic' (Table 1). Many of these properties could be grouped together to describe call features related to (i) the overall rate of calling (e.g., call rate and inter-call

interval; Table 1) and (ii) the amplitude envelope of the call (e.g., call fall time, middle:first pulse and last:first pulse amplitude ratios; Table 1). Most of the intermediately variable properties ( $5\% < CV_w < 12\%$ ) were related either to the duration and shapes of individual pulses (e.g., pulse duration, pulse rise time, pulse fall time, and pulse 50% fall time; Table 1), or to the magnitude and rate of frequency modulation over the duration of the call (e.g., FM range and FM rate; Table 1).

All of the call properties we examined varied significantly more among individuals than within individuals (Table 1). In general, properties that were less variable within individuals were also less variable among individuals (Table 1). This is indicated, for example, by significant positive correlations between the magnitude of  $CV_w$  and both that of  $CV_a$  (Spearman  $r = 0.93$ ,  $p < 0.0001$ ,  $n = 26$ ) and the maximum difference between individuals (Spearman  $r = 0.92$ ,  $p < 0.0001$ ,  $n = 26$ ). Nevertheless, the extent to which among-individual variation exceeded within-individual variation was greater for static properties compared with dynamic properties. For example, the  $CV_a/CV_w$  ratios of static properties (range = 2.1–4.8; Table 1) were generally higher than those of dynamic properties (range = 0.6–2.5; Table 1). This same trend is apparent in the effect sizes associated with the model II ANOVAs (Table 1). Call properties categorized as 'static' tended to be associated with larger effect sizes than call properties categorized as 'intermediate', which in turn had larger effect sizes than call properties categorized as 'dynamic'.

The initial PCA extracted eight factors with eigenvalues greater than 1.0, which together explained 79% of the variance in the measured call properties (Table 2). When the raw factor scores from the PCA were used as inputs in a DFA, the overall discriminant model was significant (Wilks'  $\lambda < 0.0001$ ,  $F_{280,5307} = 59.7$ ,  $p < 0.0001$ ), and all eight factors contributed significantly to discrimination ( $11.2 < F_{35,677} < 396.3$ , all  $p < 0.01$ ). The first six canonical roots from the DFA had eigenvalues greater than 1.0 and together they explained 98.3% of the variation in the PCA factor scores. The DFA classified, on average, 91.1% of calls to the correct individual (range = 55–100%), which is significantly greater than expected by chance (2.8% or 1/36; Cohen's Kappa = 0.909; 95% confidence interval = 0.887–0.930;  $Z = 144.2$ ,  $p < 0.01$ ; Titus et al. 1984). Table 3 summarizes the factor structure from the DFA depicting the correlations between the input variables (in this case factor scores from the PCA) and the resulting discriminant functions.

**Table 1:** Measures of within-individual and among-individual variability for 26 acoustic properties standardized to a common water temperature of 14°C (n = 36 individuals)

Categorization	Call property	CV <sub>w</sub> (mean)	CV <sub>w</sub> (range)	CV <sub>a</sub>	Maximum between- individual difference	CV <sub>a</sub> /CV <sub>w</sub>	F <sub>35, 684</sub>	Partial η <sup>2</sup>
Static	Fundamental frequency (overall)	1.9	0.0–9.9	4.1	17.5	2.1	42.7	0.69
	Dominant frequency (overall)	1.1	0.0–2.6	4.1	18.4	3.7	215.6	0.92
	Dominant frequency (first pulse)	1.3	0.6–3.5	4.1	17.9	3.2	159.1	0.89
	Dominant frequency (middle pulse)	0.9	0.3–2.2	4.3	17.7	4.8	394.8	0.95
	Dominant frequency (last pulse)	1.2	0.5–2.2	4.2	17.9	3.6	224.7	0.92
	Call duration	4.6	0.9–13.8	11.2	47.8	2.4	81.7	0.81
	Pulse number	4.1	0.0–8.9	9.3	36.1	2.3	88.3	0.82
	Pulse rate	1.7	0.8–3.2	7.5	30.3	4.5	370.4	0.95
	Instantaneous pulse rate (first pulse)	3.7	1.2–14.3	9.0	41.1	2.4	72.8	0.79
	Instantaneous pulse rate (middle pulse)	2.2	1.1–5.0	8.1	33.2	3.7	245.9	0.93
	Pulse 50% rise time	0.8	0.0–28.0	1.7	10.2	2.1	2.1	0.10
Intermediate	Pulse duration	6.0	3.2–15.5	9.4	35.3	1.6	46.7	0.71
	Inter-pulse interval	7.1	2.3–27.4	13.5	60.0	1.9	64.3	0.77
	Pulse rise time	6.0	0.0–21.0	7.6	31.4	1.3	8.9	0.31
	Pulse fall time	8.0	4.6–19.9	10.6	39.9	1.3	33.7	0.63
	Pulse 50% fall time	10.1	4.9–24.4	12.5	47.7	1.2	29.8	0.60
	Call rise time	10.0	3.9–23.9	17.7	90.1	1.8	58.8	0.75
	FM range	11.0	5.2–20.5	21.5	81.4	2.0	64.8	0.77
	FM rate	9.7	4.7–23.6	22.7	91.9	2.3	85.3	0.81
Dynamic	Call rate	12.3	3.8–40.9	11.1	53.8	0.9	14.3	0.42
	Inter-call interval	39.1	3.2–215.6	38.7	242.1	1.0	2.2	0.10
	Call fall time	34.4	14.2–80.2	46.1	242.8	1.3	40.8	0.68
	Middle:first pulse amplitude ratio	16.7	5.2–59.3	30.5	122.2	1.8	51.6	0.73
	Last:first pulse amplitude ratio	29.6	12.8–58.6	40.3	160.2	1.4	29.5	0.60
	Instantaneous pulse rate (pulse N–1)	18.1	2.1–47.0	11.6	51.2	0.6	5.5	0.22
	Relative amplitude	18.7	1.9–117.8	46.0	187.5	2.5	25.4	0.56

Results from model II ANOVAs of each call property are also shown (all p < 0.001).  
FM, frequency modulation.

A joint analysis of the PCA factor loadings in Table 2 and the DFA factor structure in Table 3 reveals how individual call properties contributed to individual distinctiveness. For the sake of brevity and clarity, we focus here on the first four canonical roots, which together explained 93% of the variance in PCA factor scores, and we limit our discussion to correlations in the DFA factor structure greater than 0.40. The first and second canonical roots, which explained 51% and 25% of the variance, respectively, were both correlated with PCA factor 2 (Table 3), which loaded heavily on fundamental frequency and dominant frequency (Table 2). Hence, spectral properties contributed most toward statistically discriminating among individuals. The third canonical root explained 10% of the variance (Table 3) and was correlated with PCA factors 4 and 6, which loaded most heavily on temporal properties related to call rate, call duration, and the shape of the call envelope (e.g., call rise time, call fall time,

and the amplitude ratio of the first and last pulses; Table 2). Finally, the fourth canonical root, which explained an additional 7% of the variance, was strongly correlated with PCA factor three, which loaded most heavily on properties related to frequency modulation (FM range and FM rate) and the amplitude ratio of the middle and first pulses (Tables 2 and 3). The fourth canonical root was also correlated with PCA factor 4 (Table 3), which loaded most heavily on call duration, call rise time, and call rate (Table 2). The remaining roots each explained less than 4% of the variance in PCA factor scores.

## Discussion

### Individual Variation and Sexual Selection

In the present study, the only significant negative correlation between a call property and body size was that between the dominant frequency of the

**Table 2:** Results from a principal components analysis showing the factor loadings for the eight factors with eigenvectors greater than 1.0

Call property	Factor							
	1	2	3	4	5	6	7	8
Call rate (calls/min)	0.45	0.14	0.28	<b>0.77</b>	0.09	-0.01	0.02	-0.06
Call duration (ms)	-0.20	0.04	-0.01	<b>0.92</b>	-0.13	-0.12	-0.07	0.00
Pulse number	0.00	-0.04	-0.04	0.01	-0.06	0.09	<b>-0.81</b>	0.04
Inter-call interval (s)	0.29	0.07	-0.02	-0.09	0.00	0.09	<b>0.78</b>	0.00
Call rise time (ms)	-0.13	-0.24	-0.22	<b>0.63</b>	-0.06	0.48	-0.12	-0.08
Call fall time (ms)	-0.20	0.09	0.24	0.11	-0.05	<b>-0.81</b>	0.04	0.04
Middle:first pulse amplitude ratio	-0.17	-0.04	<b>0.75</b>	0.31	-0.11	0.08	0.15	0.06
Last:first pulse amplitude ratio	-0.03	-0.11	0.52	0.23	0.11	<b>0.68</b>	0.09	0.12
Pulse rate (pulses/s)	<b>0.82</b>	0.12	0.31	-0.14	0.24	0.15	0.21	0.00
Instantaneous pulse rate (first pulse; pulses/s)	0.52	0.18	0.54	0.00	0.04	0.17	0.20	0.08
Instantaneous pulse rate (middle pulse; pulses/s)	<b>0.73</b>	0.12	0.39	-0.07	0.27	0.21	0.20	0.03
Instantaneous pulse rate ( <i>N</i> -1 pulse; pulses/s)	<b>0.65</b>	0.06	-0.12	0.14	0.05	-0.19	0.03	-0.16
Pulse duration (ms)	0.15	-0.20	-0.02	-0.03	<b>0.93</b>	0.04	0.03	-0.10
Inter-pulse Interval	<b>-0.71</b>	0.01	-0.07	0.24	-0.36	-0.19	0.00	0.10
Pulse rise time (ms)	-0.04	-0.19	-0.03	0.08	0.00	-0.11	0.21	<b>-0.67</b>
Pulse 50% rise time (ms)	0.19	-0.03	-0.03	0.00	0.00	0.06	-0.13	<b>-0.71</b>
Pulse fall time (ms)	0.14	-0.09	0.00	-0.04	<b>0.96</b>	0.06	0.01	0.03
Pulse 50% fall time (ms)	0.16	-0.01	0.03	-0.03	<b>0.94</b>	0.00	0.05	0.04
Fundamental frequency (overall; Hz)	0.08	<b>0.78</b>	0.14	-0.02	0.03	-0.05	-0.03	0.06
Dominant frequency (overall; Hz)	0.04	<b>0.91</b>	0.14	0.04	-0.11	0.08	0.05	-0.04
Relative amplitude (%)	0.44	-0.13	-0.08	0.11	-0.12	-0.34	-0.01	0.42
Dominant frequency (first pulse; Hz)	-0.07	<b>0.83</b>	-0.40	0.01	-0.08	-0.02	0.12	0.12
Dominant frequency (middle pulse; Hz)	0.03	<b>0.93</b>	0.02	0.00	-0.14	-0.06	0.06	0.06
Dominant frequency (last pulse; Hz)	0.18	<b>0.88</b>	0.30	-0.02	-0.04	-0.21	-0.02	0.07
FM range (Hz)	0.30	0.23	<b>0.79</b>	-0.04	0.03	-0.24	-0.15	-0.04
FM rate (Hz/s)	0.29	0.17	<b>0.75</b>	-0.38	0.06	-0.17	-0.09	-0.01

Loadings greater than 0.60 are highlighted in boldface type.

FM, frequency modulation.

**Table 3:** Factor structure from a DFA showing the correlations between resulting canonical roots and factor scores from a PCA on the original 26 call properties (see Table 2)

PCA factor	DFA canonical root							
	1	2	3	4	5	6	7	8
Factor 1	-0.15	0.40	-0.15	0.09	-0.18	<b>-0.79</b>	-0.02	0.36
Factor 2	<b>-0.75</b>	<b>-0.47</b>	-0.02	0.15	0.27	0.25	-0.02	0.25
Factor 3	-0.08	0.20	0.15	<b>-0.80</b>	-0.11	<b>0.52</b>	0.13	-0.05
Factor 4	0.04	-0.16	<b>-0.68</b>	<b>-0.46</b>	0.36	-0.40	-0.08	0.04
Factor 5	-0.01	0.18	-0.04	0.16	<b>0.89</b>	0.32	0.10	-0.18
Factor 6	-0.02	0.09	<b>-0.43</b>	0.23	<b>-0.43</b>	<b>0.67</b>	0.31	-0.12
Factor 7	-0.02	0.03	-0.03	0.01	-0.07	-0.01	<b>-0.69</b>	<b>-0.72</b>
Factor 8	-0.02	-0.02	0.03	-0.02	-0.03	-0.24	<b>0.66</b>	<b>-0.71</b>
Eigenvalue	23.02	11.16	4.64	3.30	1.47	1.04	0.45	0.30
Unique variance explained	0.51	0.25	0.10	0.07	0.03	0.02	0.01	0.01
Cumulative variance explained	0.51	0.76	0.86	0.93	0.96	0.98	0.99	1.00

Correlations greater than 0.40 are highlighted in boldface type. Also shown are the eigenvalues and estimates of the unique and cumulative proportions of variance explained by each canonical root.

DFA, discriminant function analysis; PCA, principal components analysis.

first pulse and SVL. Because the signal is upward frequency-modulated (Fig. 1), this result suggests that the lowest frequency a male can produce might be

constrained by, and thus predict, his body size. Importantly, however, SVL never explained more than 20% of the variation in fundamental or



dominant frequency. Similarly, we found little evidence to suggest that male condition (size-independent mass) was able to predict the spectral and temporal properties of advertisement calls. Thus, in the context of sexual selection, we would predict that among-individual differences in acoustic signals alone may be insufficient to allow accurate and reliable assessments of the body size and condition of competitive rivals by males and of potential mates by females, at least in our study population.

We categorized as static call properties related to pulse rate, spectral content, and call duration. Based on these categorizations, we would predict patterns of stabilizing or weakly directional female preferences generally favoring values near the population means of these properties. That pulse rate and spectral content represent static properties is consistent with previous studies in other anurans and insects (reviewed in Gerhardt & Huber 2002). Platz (1989) identified pulse rate as an important diagnostic trait for distinguishing between populations he designated as *P. maculata* and *P. triseriata* (but see Lemmon et al. 2007). Thus, preferences for values of pulse rate near the population mean might be important for correct species recognition in mixed species assemblages of *P. maculata* and *P. triseriata*, as it is for some members of other closely related species pairs of treefrogs (e.g., Bush et al. 2002).

Our characterization of call duration ( $\bar{X} = 859$  ms) as a static property deserves additional comment because it departs from the general patterns seen in other anurans and insects. Typically, relatively long signals (e.g., >500 ms) exhibit greater within-individual variability (e.g.,  $CV_w = 5\text{--}30\%$ ) compared with shorter signals (e.g., <500 ms). In addition, the females of many other frogs, including other hylid treefrogs, commonly exhibit strongly directional preferences for longer calls (Ryan & Keddy-Hector 1992; Gerhardt & Huber 2002). Hence, our prediction for stabilizing selection for call durations near the population mean is generally at odds with what would be expected based on studies of other frogs. Platz (1989) suggested that call duration might serve as a potentially important diagnostic trait for correct species identification, which is consistent with our prediction of stabilizing selection for this trait. It will be particularly important to test predictions about female preference functions for call duration in light of the patterns of among-species variation in this call property within the trilling frog clade.

Among the properties categorized as dynamic were those related to overall calling activity (call rate and inter-call interval). Numerous studies have shown

that female frogs almost invariably exhibit strong directional preferences for signals produced at higher rates (reviewed in Ryan & Keddy-Hector 1992; Gerhardt & Huber 2002). Therefore, we would predict strong directional selection favoring higher rates of calling in *P. maculata*, similar to those demonstrated in other anurans. We also categorized as dynamic several call properties related to the amplitude envelope of the call. While features related to the amplitude envelope of individual sound elements (e.g., pulses) may be important for call recognition in some species (Gerhardt & Doherty 1988; Gerhardt & Schul 1999), how females assess variation in temporal characteristics related to the overall call envelope have not been intensively studied in frogs.

### Individual Variation and Social Recognition

The DFA assigned 91% of advertisement calls to the correct individual, indicating that the advertisement calls of *P. maculata* are individually distinctive. This result is in line with several recent studies that have also used multivariate statistical approaches to characterize the individual distinctiveness of anuran advertisement calls (Bee & Gerhardt 2001a; Bee et al. 2001; Bee 2004; Feng et al. 2009; Gasser et al. 2009). These studies have reported that discriminant function models typically assign calls to the correct individual at rates (e.g., 50–100%) that are significantly higher than expected by chance.

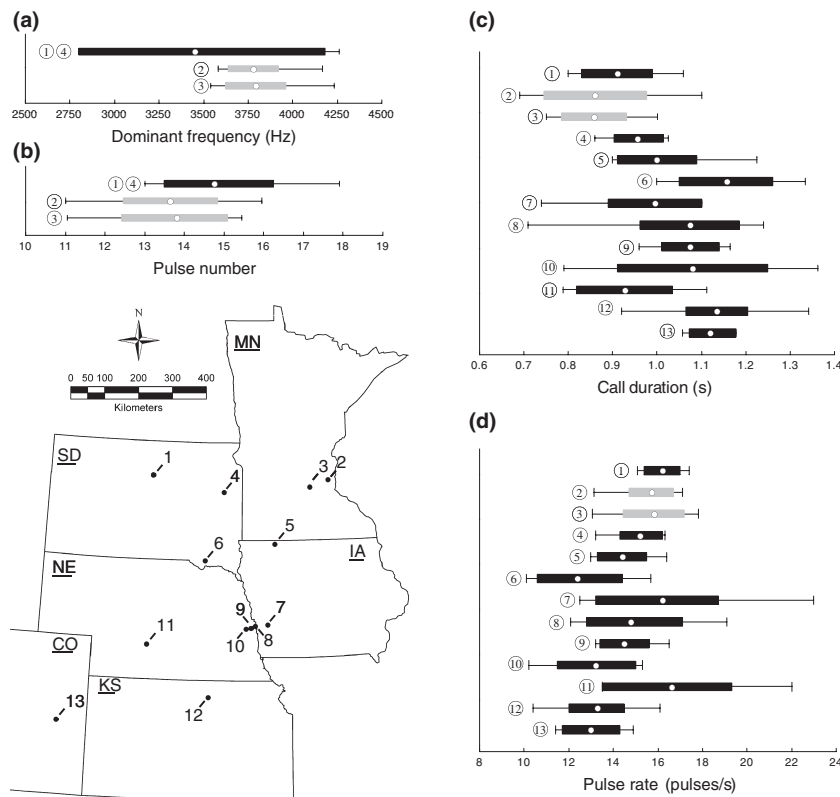
Results from purely descriptive studies of individual vocal distinctiveness should always be regarded as a first step in investigating various forms of social recognition (Bee et al. 2001). The ability of statistical models to discriminate among individuals based on the acoustic properties of their calls has no bearing on whether or not the animals in question can perceptually discriminate among individuals in the same way or actually do behaviorally discriminate among biologically relevant classes of individuals (Bee 2003). For example, we would actually hypothesize that in spite of a high degree of individual distinctiveness in *P. maculata* advertisement calls, vocally mediated dear enemy recognition between calling neighbors probably does not occur in this species. This hypothesis is based on two observations. First, the defense of long-term breeding or multi-purpose territories may be a pre-condition for the evolution or expression of dear enemy recognition (Temeles 1994; but see Bee 2003). However, there is no evidence to suggest that males of *P. maculata* defend long-term territories with resources or oviposition sites. Instead, the mating system is probably best

described as a lek (Höglund & Alatalo 1995). Second, the auditory system of *P. maculata* may not be able to discriminate unambiguously the most individually distinctive attributes of the call. Recall that the acoustic properties that contributed most toward the individual distinctiveness of the calls were those related to fundamental and dominant frequency. Given that the former is produced with a relative amplitude that is 14 dB less than that of the latter, and is thus more likely to be masked in mixed-species choruses (Bee MA, unpublished data), among-male differences in dominant frequency would be expected to be the more salient acoustic cue for individual discrimination. With an average frequency of about 3800 Hz, however, the dominant frequency of the call is almost certainly encoded by the basilar papilla, a sensory organ in the anuran

inner ear that probably cannot accurately resolve typical among-individual differences in frequency independently of differences in level (reviewed in Gerhardt & Huber 2002). Future field studies and playback experiments will be necessary to test these predictions about vocally mediated recognition.

**Individual Variation and Conservation**

Beyond studies of sexual selection and social recognition, our results and those of others on the individual distinctiveness of frog advertisement calls highlight their potential for use as a bioacoustics tool for anuran conservation. Recent studies have shown that the individual distinctiveness of animal vocalizations can be used as a reliable and non-invasive tool to census threatened wildlife populations



**Fig. 3:** Geographic variation in *Pseudacris maculata* advertisement calls. Shown here is a comparison of calls recorded during the present study in east-central Minnesota (gray bars) to previously published values from the literature (black bars). Points, boxes, and whiskers depict  $\bar{X}$ ,  $\pm 1$  SD, and range, respectively. (a) Dominant frequency (in Hz) and (b) pulse number compared to values reported in figures published in Platz & Forester (1988). (c) Call duration (in s) and (d) pulse rate (in pulses/s) compared to values reported in figures published in Platz (1989). Encircled numbers correspond to the following populations (and numbers of individuals recorded) in this study and the studies by Platz & Forester (1988) and Platz (1989): (1) Potter Co., South Dakota (n = 11), (2) Anoka Co., Minnesota (n = 19), (3) Carver Co., Minnesota (n = 17), (4) Hamlin Co., South Dakota (n = 12), (5) Dickinson Co., Iowa (n = 12), (6) Bon Homme Co., South Dakota (n = 15), (7) Pottawattamie Co., Iowa (n = 19), (8) Douglas Co., Nebraska (n = 19), (9) Douglas Co., Nebraska (n = 10), (10) Douglas Co., Nebraska (n = 9), (11) Dawson Co., Nebraska (n = 8), (12) Cloud Co., Kansas (n = 27), (13) Lincoln Co., Colorado (n = 8). In (c) and (d), each of the above populations is rank ordered in latitude from north to south. (Platz (1989) did not separate populations 1 and 4 in their figures and hence these are not separated in (a) and (b)).

(e.g., Terry & McGregor 2002; Terry et al. 2005). The occurrence and causes of global declines in amphibian populations are now well documented (Semlitsch 2003). However, bioacoustics tools are probably underutilized in research on the conservation of anuran species compared to their use with other taxa, especially birds (e.g., Peake et al. 1998; Bretagnolle & McGregor 2006; Grava et al. 2008). Given recent advances in multi-microphone arrays that can record a chorus of calling male frogs and recover individual sources (Jones & Ratnam 2009), future studies could examine the potential for deploying such arrays to identify all of the calling males in a chorus for purposes of population censuses and monitoring.

### Geographic Variation

Boreal chorus frogs are one of the most widely distributed frogs in North America. We would be remiss if we did not also compare our results to those from two previous studies of *P. maculata* (Platz & Forester 1988; Platz 1989) that reported mean values across 11 populations for four of the 26 acoustic properties that we also analyzed (overall dominant frequency, pulse number, call duration, and overall pulse rate). As illustrated in Figure 3, there was extensive overlap between the values of these call properties in Minnesota and those across other parts of the species' geographic range. Platz (1989) reported significant clinal variation in dominant frequency, with mean values of about 2910–3110 Hz in Kansas ranging upward to 3280–3580 Hz in South Dakota. The overall dominant frequency of 3785 Hz for boreal chorus frogs in Minnesota (Table S1 in Supplementary Material) is consistent with the trend for higher dominant frequencies to occur at higher latitudes. The extent to which this trend may be directly attributable to geographic variation in body size in presently unknown, although such a relationship seems plausible given that Platz & Forester (1988) reported a significant negative correlation between dominant frequency and body size across 20 populations. Platz (1989) reported no significant clinal or geographic variation in pulse number, and the mean number of pulses in calls recorded in Minnesota was within one SD of that in calls recorded in South Dakota (Fig. 3; Platz & Forester 1988).

Both call duration and pulse rate exhibited significant geographic variation between populations that Platz (1989) designated as *P. maculata* (formerly *P. triseriata maculata*) and *P. t. triseriata*. Although current molecular evidence suggests that these populations may all belong to *P. maculata* (Lemmon et al.

2007), call duration and pulse rate were the two most important acoustic properties leading Platz (1989) to suggest separate species status for *P. maculata* and *P. triseriata*. Call durations were longer, and pulse rates slower, in populations designated as *P. maculata* compared with *P. triseriata*. Our data on call duration (Fig. 3c) and pulse rate (Fig. 3d) for *P. maculata* in Minnesota are similar to those reported previously for this species by Platz (1989). A re-evaluation of geographic variation in the calls of boreal and western chorus frogs in light of more recent molecular evidence would be welcomed.

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## Supporting information

Additional Supporting Information may be found in the online version of this article:

**Audio S1.** Two boreal chorus frog advertisement calls.

**Figure S1.** Scatterplots of six temporal properties versus water temperature. Shown here are the mean values ( $n = 36$  individuals) for (a) call rate (calls/min), (b) call duration (ms), (c) inter-call interval (ms), (d) overall pulse rate (pulses/s), (e) pulse duration (ms) and (f) inter-pulse interval (ms). Data are shown for all individuals for which water

temperature data were available. Also shown in each plot is the output from a linear regression of each call property on water temperature along with the best-fit regression line.

**Table S1.** Means, standard deviations (SD), and ranges for 26 acoustic properties standardized to a common temperature of 14 °C (20 calls per individual;  $n = 36$  individuals).

**Table S2.** Partial correlations between call properties and both water temperature and SVL ( $n = 29$ ).

Because these analyses were exploratory, and did not test any particular hypothesis, we did not adjust our significance criterion ( $\alpha = 0.05$ ) for multiple comparisons. Significant correlations ( $p < 0.05$ ) are highlighted in boldface type.

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## Supplementary Material

Values for the means, standard deviations, and ranges of all 26 call properties, corrected to a standard temperature of 14 °C, are reported in Supplementary Material Table 1. These values represent data averaged over males recorded in the Carlos Avery Wildlife Management Area (n = 19) and the Carver Park Reserve (n = 17). A multivariate analysis of variance (MANOVA) failed to reveal a between-site difference when all 26 call properties were included as dependent variables (Wilk's  $\lambda = 0.84$ ,  $F_{25,10} = 0.07$ ,  $p = 1.00$ , partial  $\eta^2 = 0.15$ ). There were also no between-site differences in separate univariate ANOVAs for each dependent variable ( $0.004 < F_{1,34} < 1.3$ ,  $0.27 < p < 0.94$ ).

Temperature-dependent patterns of variation in several acoustic properties were fairly typical for anurans (reviewed in Gerhardt and Huber 2002). Call rate increased significantly with water temperature (Supplementary Material, Table 2, Fig. 1a). The positive relationship between call rate and temperature resulted because males produced shorter calls with shorter inter-call intervals as water temperature increased (Supplementary Material, Table 2, Figs 1b and 1c). There were also significant negative relationships between water temperature and both the rise time and the fall time of the overall call envelope, which can largely be explained by the tendency for males to produce shorter calls as temperature increased. We also found a significant positive correlation between water temperature and pulse rate (Supplementary Material, Table 2, Fig. 1d), indicating that males produced calls with faster pulse rates at warmer temperatures. The effect of water temperature on pulse rate was largely mediated by the negative relationships between water temperature and both pulse duration and inter-pulse interval (Supplementary Material, Table 2; Figs 1e and 1f). There were also significant negative relationships between water temperature and both measures of pulse fall time (Supplementary Material, Table 2). With the exception of the FM rate, which was positively related with temperature (Supplementary

Material, Table 2), there were no other significant relationships between water temperature and spectral call properties. However, the relationship between temperature and FM rate is probably best explained by the negative relationship between water temperature and the temporal property of call duration, which served as the denominator for converting the overall range of FM to the rate of FM during a call.

**Supplementary Material - Table 1.** Means, standard deviations (SD), and ranges for 26 acoustic properties standardized to a common temperature of 14 °C (20 calls per individual; n = 36 individuals).

Call property	Mean	SD	Range
<b>Call rate (calls/min)</b>	<b>23.4</b>	<b>2.6</b>	<b>18 - 31</b>
Call duration (ms)	859	96	691 - 1102
Pulse number	14	1	11 - 16
Inter-call interval (s)	2.1	0.8	1.0 - 6.1
Call rise time (ms)	639	113	378 - 954
Call fall time (ms)	179	82	34 - 467
Middle:first pulse amplitude ratio	5.2	1.6	2.5 - 8.9
Last:first pulse amplitude ratio	4.7	1.9	1.7 - 9.1
Pulse rate (pulses/s)	15.8	1.2	13.0 - 17.8
Inst. pulse rate (first pulse; pulses/s)	22.8	2.1	18.1 - 27.5
Inst. pulse rate (middle pulse; pulses/s)	15.1	1.2	12.4 - 17.5
Inst. pulse rate (N-1 pulse; pulses/s)	15.8	1.8	11.0 - 19.1
Pulse duration (ms)	14	1	11.5 - 16.3
Inter-pulse Interval	67	9	43.4 - 83.6
Pulse rise time (ms)	2	0	2.0 - 2.6
Pulse 50% rise time (ms)	1	0	1.0 - 1.1
Pulse fall time (ms)	11	1	9.0 - 13.5
Pulse 50% fall time (ms)	9	1	7.4 - 11.9
Fundamental frequency (overall; Hz)	1906	78	1783 - 2116
Dominant frequency (overall; Hz)	3785	153	3538 - 4233
Relative amplitude (%)	20.8	9.6	3.0 - 42.1
Dominant frequency (first pulse; Hz)	3365	137	3128 - 3730
Dominant frequency (middle pulse; Hz)	3643	155	3391 - 4036
Dominant frequency (last pulse; Hz)	3937	166	3679 - 4384
FM range (Hz)	572	123	343 - 808
FM rate (Hz/s)	708	160	447 - 1098

**Supplementary Material - Table 2.** Partial correlations between call properties and both water temperature and SVL (n = 29). Because these analyses were exploratory, and did not test any particular hypothesis, we did not adjust our significance criterion ( $\alpha = 0.05$ ) for multiple comparisons. Significant correlations ( $p < 0.05$ ) are highlighted in boldface type.

Call property	Water temperature		SVL	
	r	p	r	p
Call rate	<b>0.86</b>	<b>&lt; 0.0001</b>	-0.04	0.8456
Call duration	<b>-0.86</b>	<b>&lt; 0.0001</b>	0.13	0.4967
Pulse number	-0.10	0.6091	0.11	0.5678
Inter-call interval	<b>-0.51</b>	<b>0.0059</b>	-0.06	0.7743
Call rise time	<b>-0.66</b>	<b>0.0001</b>	-0.13	0.5251
Call fall time	<b>-0.39</b>	<b>0.0377</b>	0.28	0.1464
Middle:first pulse amplitude ratio	-0.03	0.8616	0.03	0.8835
Last:first pulse amplitude ratio	0.03	0.8694	-0.09	0.6581
Pulse rate	<b>0.91</b>	<b>&lt; 0.0001</b>	-0.11	0.5699
Instantaneous pulse rate (first pulse)	<b>0.90</b>	<b>&lt; 0.0001</b>	-0.10	0.6022
Instantaneous pulse rate (middle pulse)	<b>0.89</b>	<b>&lt; 0.0001</b>	-0.06	0.7771
Instantaneous pulse rate (N-1 pulse)	<b>0.80</b>	<b>&lt; 0.0001</b>	-0.18	0.3497
Pulse duration	<b>-0.59</b>	<b>0.0011</b>	0.05	0.7942
Inter-pulse interval	<b>-0.76</b>	<b>&lt; 0.0001</b>	0.03	0.8947
Pulse rise time	-0.18	0.3679	0.13	0.5074
Pulse 50% rise time	-0.13	0.4967	0.27	0.1665
Pulse fall time	<b>-0.53</b>	<b>0.0039</b>	0.02	0.9337
Pulse 50% fall time	<b>-0.51</b>	<b>0.0056</b>	0.06	0.7781
Fundamental frequency (overall)	0.35	0.0686	-0.27	0.1608
Dominant frequency (overall)	0.31	0.1163	-0.30	0.1253
Relative amplitude	0.37	0.0546	0.02	0.9122
Dominant frequency (first pulse)	0.33	0.0904	<b>-0.44</b>	<b>0.0199</b>
Dominant frequency (middle pulse)	0.26	0.1734	-0.30	0.1263
Dominant frequency (last pulse)	0.27	0.1571	-0.17	0.3802
FM range	0.04	0.8385	0.28	0.1422
FM rate	<b>0.59</b>	<b>0.0009</b>	0.17	0.3959

**Supplementary Material - Figure 1:** Scatterplots of six temporal properties versus water temperature. Shown here are the mean values ( $n = 36$  individuals) for (a) call rate (calls/min), (b) call duration (ms), (c) inter-call interval (ms), (d) overall pulse rate (pulses/s), (e) pulse duration (ms) and (f) inter-pulse interval (ms). Data are shown for all individuals for which water temperature data were available. Also shown in each plot is the output from a linear regression of each call property on water temperature along with the best-fit regression line.

