Temporal Analysis of Chorus Frog Vocalizations

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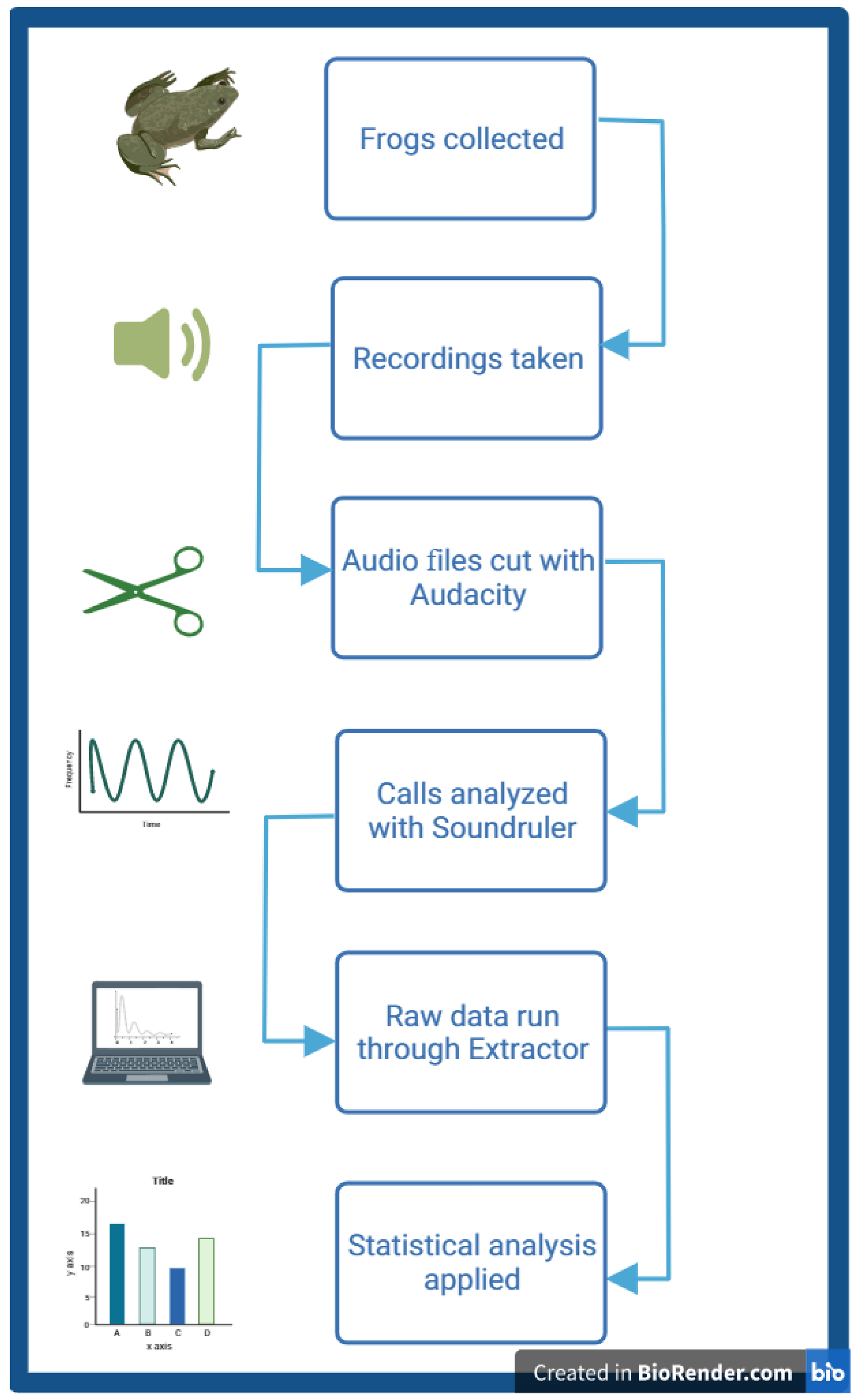
BACKGROUND

Acoustic signaling is vitally important in sexual selection, species recognition, and speciation across many taxa, including everything from reptiles to birds and mammals. Of these many taxa, anurans are an excellent choice for acoustic analysis, because acoustic signaling is known to be the strongest determining factor in their mate selection. Temporal variation across call sequences is known to be a factor in sexual selection (Benítez et. al 2016), however fine scale temporal variation, such as within a single calling sequence, has been under examined.

Pseudacris feriarum is an ideal system for this analysis because the spectral properties of their call sequences are known to show significant differences between allopatric and sympatric populations (*fig. 6*), resulting in dramatic effects on speciation.

In this study, I analyzed recordings of male *Pseudacris feriarum* vocalizations to determine how the acoustic characteristics of a call sequence changed from beginning to end.

OVERVIEW



METHODS

Data Collection

We collected the calls used in these preliminary analyses from a Florida population in January-March 2021. We collected males in amplexus, separated them from females, and placed them in a custom acoustically insulated "Frog Box". There, recordings of various frog calls were played, and the subject's response calls, if made, were recorded.

Acoustic Analysis

We isolated five sequential calls from the beginning and end of each sequence using Audacity. We then analyzed the selected calls using SoundRuler version 0.941. We derived the values of five target variables or took them directly from SoundRuler's raw data output. We measured a total of 140 calls from 14 subjects, and used 110 of those in our final analysis.

We examined a total of 5 call characteristics to explore patterns of temporal variation. We chose these characteristics, (call rate, call duration, pulse number, pulse rate, and frequency), because they are known to be important for mate choice and species recognition in other frogs (Köhler et al. 2017).

Statistical Analysis

To determine if each of the five call traits differ between the beginning and ending of a calling sequence, we used a Wilcoxon signed-rank test. We then used a sequential Bonferroni correction to correct for repeated tests on the same data set.

Fig. 1 Inside of a recording chamber, containing a round speaker to play chorus sounds to the male being recorded. Chorus frogs do not usually call in silence, so chorus sounds are necessary to stimulate calling.

Call Rate	1 / time from 10% maximum amplitude (call onset) to 10% maximum amplitude (onset) for next call
Call Duration	Duration of call from 10% maximum amplitude (call onset) to 10% maximum amplitude (offset)
Pulse number	Number of pulses in the call
Pulse Rate	1 / time from 10% maximum amplitude (pulse onset) to 10% maximum amplitude (onset) of next pulse within call
Frequency	Call dominant frequency at the call maximum amplitude

Fig. 2 Definitions of 5 call characteristics examined across P. feriarum. All pulse-related variables except pulse number were averaged across pulses within the call.

CALL PROPERTIES

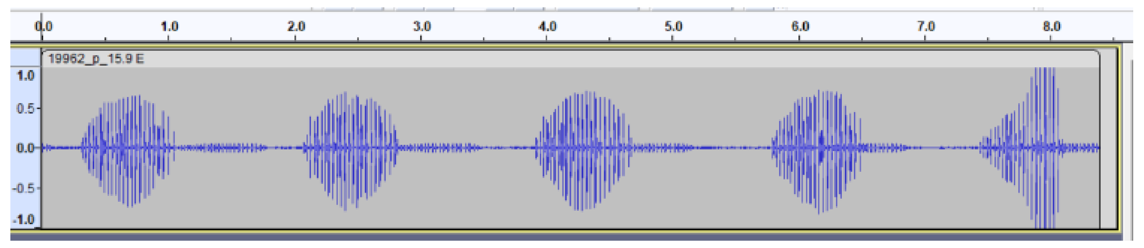


Fig. 3 Oscillogram of a sequence of five P. feriarum calls in Audacity

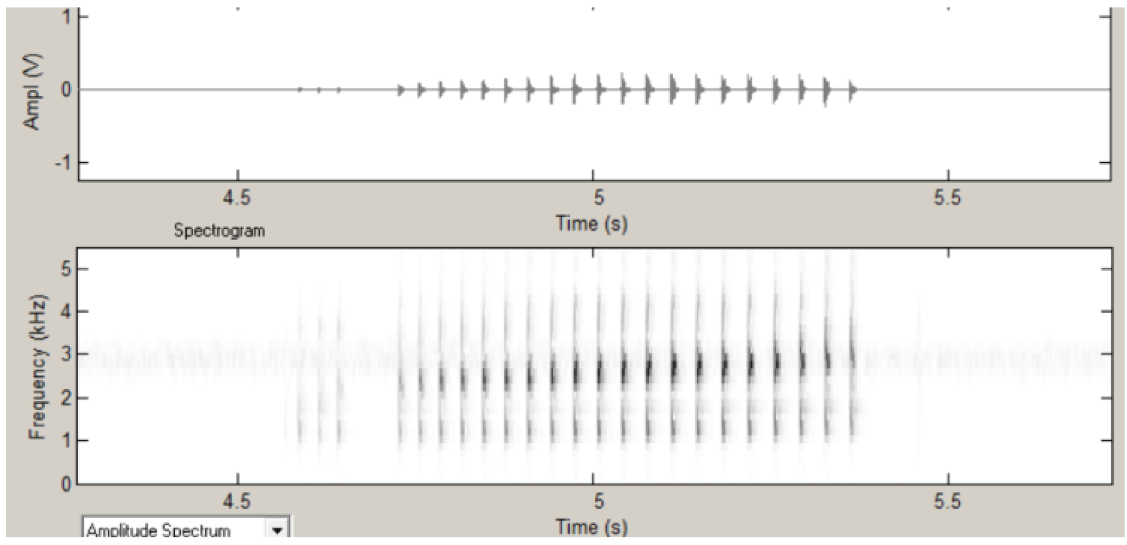


Fig. 4 Oscillogram (top) and spectrogram (bottom) of a single P. feriarum call in Soundruler

RESULTS

We found that peak dominant frequency, call duration, and pulse number significantly increased from the start of the calling sequence and the end of the calling sequence (P<0.05; *Fig. 5*). We did not find any change in pulse rate or call rate.

Variable Name	Beginning Average	End Average	P-Value
Frequency	**2974.969 ±232.850**	**3137.356 ±230.333**	0.004883
Call Duration	**0.584 ±.102**	**0.731 ±0.075**	0.002442
Call Rate	0.521±.084	0.550 ±0.034	1
Pulse Number	**20.875 ±3.867**	**25.121 ±2.606**	0.02669
Pulse Rate	34.444 ±2.426	33.414 ±2.182	1

Fig. 5 Pairwise comparison of beginning and ending calls. Variables that exhibited significance after a sequential Bonferroni correction are bolded.

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DISCUSSION

Our findings suggest that there may be fine-scale temporal variation that needs to be considered when conducting acoustic analyses.

Statistical vs. Biological Significance
While we found a statistically significant effect of time in call sequences, it is not clear whether these differences are likely to affect mating behaviors in these frogs.

Future Directions

Future studies could illuminate whether temporal variation has a significant effect on female mate preference. In particular, higher pulse number is more energetically costly than lower pulse number sequences (Lemmon 2009), and so directional selection for higher-cost calls invites further research.

Beyond intra-population research; cross-population comparison of sympatric and allopatric populations of *P. nigrita* and *P. feriarum* could determine whether temporal variation is a significant factor in speciation reinforcement.

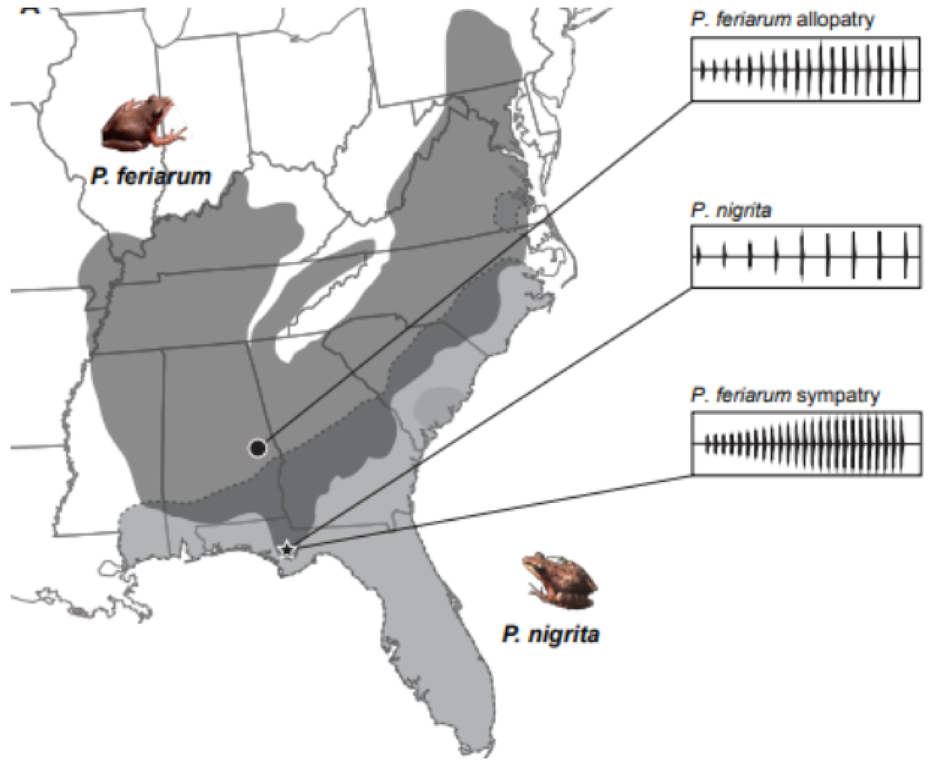

Fig. 6 Map of allopatric and sympatric distribution of P. nigrita (light grey) and P. feriarum (medium grey) in the Southern U.S, with sympatry indicated by the dark grey region.

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Figure 6. Dye, M., Lemmon, A.R., & Lemmon, E. (In Review), Female Chorus Frogs Delay Mate Choice Under Suboptimal Conditions.

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