

Influence of Sound Pressure Level of Frog Advertisement Calls on Attraction of Frog-Biting Midges

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Abstract

Frog-biting midges (Diptera: Corethrellidae) have evolved the ability to eavesdrop on the distinctive acoustic signals emitted by male frogs from which they obtain a blood meal. Acoustic traps broadcasting the advertisement calls of Collett's treefrog, *Polypedates colletti*, at 80 dB versus 90 dB sound pressure level were used to test the influence of sound pressure level on midge attraction. An increase in call amplitude by 10 dB led to a 5-fold increased capture rate, suggesting that amplitude is a critical factor in determining frog-biting midge attraction and the incidence of parasitism.

Index Terms: acoustic communication, Anura, *Corethrella*, sound pressure level, phonotaxis

1. Introduction

Parasites are known to be profound drivers of the evolution of communication systems in their role as eavesdroppers.^{1,2} Using specialized host-seeking behaviors and sensory abilities, parasites can home in on acoustic communication signals of their host with great precision, and in turn hosts have evolved different ways of evasion, including changes in signal characteristics,²⁻⁴ reduced signaling activity,⁵⁻⁷ the adoption of alternative signaling modalities or mating strategies,^{8,9} and even the loss of acoustic signaling altogether.^{10,11}

Frog-biting midges (Diptera: Corethrellidae) are highly specialized ectoparasites that exclusively feed on the blood of frogs that they locate by eavesdropping on their advertisement calls. Frog-biting midges experience strong selective pressures to locate and suck blood from their frog hosts, while at the same time, frogs are expected to show avoidance strategies.^{12,13} Frog-biting midges have a pantropical distribution,¹⁴ but their behavior has mostly been studied in the Neotropics,¹⁵⁻¹⁷ Australia,^{18,19} tropical Japan,²⁰ and Borneo.^{7,12,21} These midges use sound as a primary cue to locate their host. Although call

rate, call amplitude, and call frequency have been identified as important components of host localization and choice,^{7,12,22} the role of source amplitude in attracting midges has not been investigated explicitly.

Within heath forests of Brunei, and after heavy rains, male Collett's treefrog *Polypedates colletti* loosely aggregate near pools of water where they call from elevated perches. In this study I measure the sound pressure level (SPL) of the biphasic advertisement call of *Polypedates colletti* and how sensitive frog-biting midges are to the SPL of calling males. I hypothesized that louder calls would attract more frog-biting midges and thus calling softly might be a behavioral strategy to reduce parasitism risk.

2. Materials and methods

2.1. Study site

The study was carried out in a heath forest located 12.5 km south of the town Sungai Liang (Paya Gambut, Mukim Liang; N04.584, E114.505). Previous studies on frog-biting midges (*Corethrella*) and their frog hosts have been carried out at this same location.^{7,12,21} The experiments were carried out from May to

October 2022 along a 300 m transect through the forest along an abandoned logging road.

2.2. Study species and experimental setup

Frog-biting midges were captured in modified mosquito traps (Biogents) that had been fitted with small loudspeakers (EasyAcc Model LX-839) placed on top of the trap above the funnel.^{7,23} Pre-recorded advertisement calls of *Polypedates colletti* were used as lures to attract the midges and capture them. Calls had previously been recorded from the study site and three files were created with a single file containing 10 different calls from one of three males normalized to a call rate of 0.4 calls/s for playback. Each file contained the two call types of the biphasic call. Files (.wav) were transferred to microSD cards and one chosen at random for each playback. Each file was 15 s long and the files were played back as a loop. **Figure 1** shows a representative 6 s of the playback loop.

To obtain information on playback levels to be used for the experiment, male *P. colletti* were located in the field and the peak sound pressure level (SPL) of their calls measured using the C-weighted and fast response setting of a SPL meter (Extech 407703A) at a distance of 50 cm. Males call from elevated perches on understory vegetation and produce a biphasic call that serves to both attract females and aid in male

spacing. The SPL of between 2-5 calls of five males were measured and averaged for each male. Average (\pm SD) peak sound pressure level was 79.6 ± 2.5 (dB SPL re 20 μ Pa; n=5) at a distance of 50 cm. The sound pressure level of the advertisement calls of the syntopically-calling rough-sided frog (*Hylarana glandulosa*), the more attractive host to frog-biting midges,¹² was used to set the upper value of SPL values to which the midges are likely to be exposed. With SPL in *H. glandulosa* averaging (88.6 ± 2.7 , n=5), the upper amplitude was set at 90 dB. This was convenient because if a sound is 10 dB louder, it has 10 times the sound intensity, which causes it to be perceived as roughly twice as loud by the human ear (the 10 dB rule).²⁴ A paired experimental design was used in which the two traps were set 50 m apart and the two stimuli (80 vs 90 dB) randomly assigned to one of the two traps. Amplitudes of loudspeakers were set using the C-weighted and fast response setting of a SPL meter (Extech 407703A) for each loudspeaker each evening. The acoustic traps were activated to run for 40 minutes from 1900 - 1940 hr in the evening when the midges are most active.²³ Midges were identified to the species level using the key provided in Borkent and Grafe.²¹

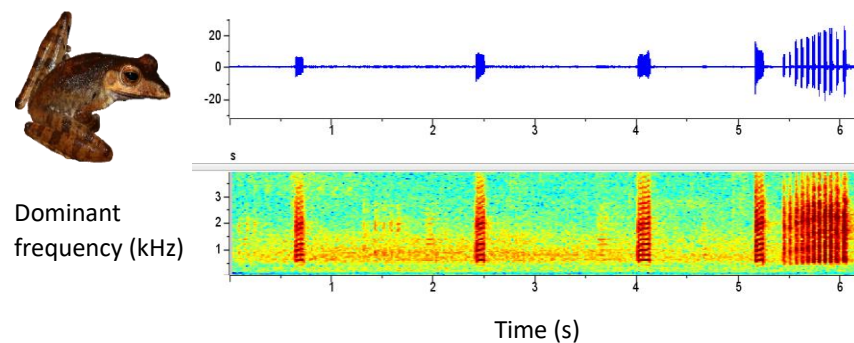


Figure 1. Waveform and spectrogram of the advertisement calls of *Polypedates colletti* used in this study showing the two call types, four honk notes followed by a trill.

To control for the effects of variation in microhabitat on the number of midges attracted, the traps were moved randomly along a 300 m forest transect with traps placed at least 50 m

from their previous locations. We temporarily removed any calling frog from within 50 m of the experiments each night to minimize the influence of other calling frogs on our results. A single

replicate was run approximately once a week throughout the season to reduce seasonal effects with a total of 14 replicates.

The data were analyzed and violin plots generated using the online tool Numiqo.com. Since data was not distributed normally, we used a Wilcoxon signed-rank test to determine the effect of amplitude on catch rate.

3. Results and Discussion

Over the course of the 5-month study period, a cumulative total of 180 frog-biting midges were captured, 30 in the traps set at 80 dB and 150 in the traps set at 90 dB. The most abundant frog-biting midge by far was *Corethrella lutea* with an overall total of 83.3% of individuals captured. The other midge species captured were *C. pauciseta*, *C. gilva*, and *C. tigrina*, with 9.4%, 6.1%, and 1.1% of the total, respectively.

Results of the Wilcoxon signed rank test indicated that there was a significantly higher number of frog-biting midges captured in the louder *P. colletti* traps at 90 dB ($Z=2.9811$, $p=0.003$, $df=13$, $N=14$; see **Figure 2**). The observed effect size r was large (0.83), indicating that the magnitude of the difference between the mean ranks was large.

Although frog-biting midges are known to be sensitive to the amplitude of frog advertisement

calls, the role of source amplitude in attracting midges has not been investigated explicitly. All previous experiments designed to understand the temporal and spectral parameters attractive to midges, tightly controlled amplitude.^{7,15-23} As expected, the present study shows that increased call amplitude increases the attraction of frogs to frog-biting midges. Furthermore, this study shows the magnitude of the sensitivity of amplitude in attracting midges. A 10 dB increase in amplitude led to a five-fold increase in the number of midges captured. This indicates that the relationship between sound intensity and fly attraction was not linear, likely a result of non-uniform midge density.

The cost to males of attracting midges goes beyond the loss of blood. *Corethrella* have been shown to transmit trypanosome blood parasites^{7,25,26} with the infection prevalence in midges, depending on country, region, and study site, ranging from 2.9% to 23.5%.²⁶ The prevalence of infected midges was 6.1% at my Liang study site.²⁶ Thus, frogs are expected to modulate amplitude to reduce parasite attraction, while at the same time call amplitude will increase their range in attracting females. For example, the West African running frog *Kassina fusca* shows high variation in call amplitude possibly to balance the costs associated with eavesdropping predators and/or parasites and attracting females.²⁷

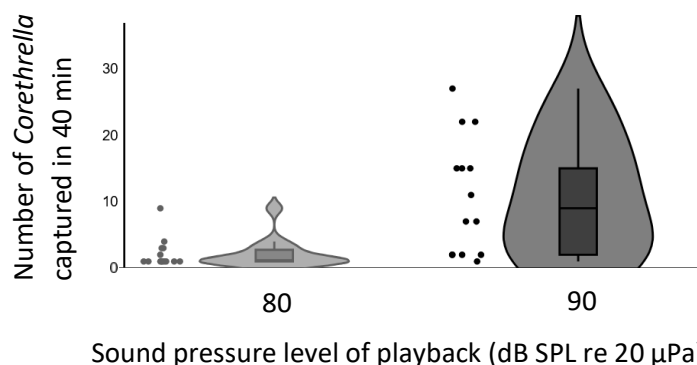


Figure 2. Number of frog-biting midges (*Corethrella*) captured in traps broadcasting the advertisement calls of Collett's treefrog (*Polypedates colletti*) at two different sound pressure levels. The width of each violin reflects the kernel density estimate with wider sections indicating higher data density. Plots were truncated at zero. Box plots show median and interquartile range. Raw data is shown left of each violin.

Several factors, apart from lowering call amplitude, have been identified as evasive strategies used by frogs to reduce or avoid being parasitized. In Borneo, frogs vary in attractiveness depending on the dominant frequency of their calls and call repetition rate.^{7,12} Perch height of a calling male also influences attractiveness, since some species of *Corethrella* (*C. gilva*) are less abundant at 4 m above ground.⁷ This suggests that some frogs, in particular *Hylarana baramica*, which call several meters above ground, are less likely to attract midges.¹² Moreover, the timing of calls can be another strategy used by frogs.⁸ *H. glandulosa* is known to call before sunset, before midges become active and thus substantially reducing its parasitism risk.

Recent evidence from studies of the antennal sensitivity and actionable acoustic space in the mosquito *Aedes aegypti* (Diptera: Culicidae), suggest they can hear in the far field up to 10 m.²⁸ With the Corethrellidae forming a monophyletic group (Culicomorpha) together with the Chaoboridae and Culicidae, it seems reasonable to assume that the *Corethrella* have similar antennal sensitivity as *A. aegypti*, allowing them to home in with great precision on frogs from several meters away. The actionable acoustic space of *Corethrella* can be as high as 2.5 m,²⁹ i.e. the range within which frog-biting midges detect and react to auditory cues. The results reported here and those of Eltz *et al.*,²⁹ support the observation that midges travel over distances of several meters. Determining the actionable acoustic space of frog-biting midges remains to be explored in future experiments since this has implications for the ecology and evolution of interactions with their frog hosts.

In conclusion, this study shows that a reduction in amplitude by 10 dB significantly reduced the attractiveness of *P. colletti* calls, suggesting that amplitude is a critical factor in determining the incidence of parasitism from frog-biting midges.

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