Original Article

Silence is sexy: soundscape complexity alters mate choice in túngara frogs

Derek A. Coss,[a,●] Kimberly L. Hunter,[a] and Ryan C. Taylor[a,b,●]

[a]Department of Biology, Salisbury University, 1101 Camden Avenue, Salisbury, MD 21801, USA
[b]Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancón, Republic of Panama

Many animals acoustically communicate in large aggregations, producing biotic soundscapes. In turn, these natural soundscapes can influence the efficacy of animal communication, yet little is known about how variation in soundscape interferes with animals that communicate acoustically. We quantified this variation by analyzing natural soundscapes with the mid-frequency cover index and by measuring the frequency ranges and call rates of the most common acoustically communicating species. We then tested female mate choice in the túngara frog (*Physalaemus pustulosus*) in varying types of background chorus noise. We broadcast two natural túngara frog calls as a stimulus and altered the densities (duty cycles) of natural calls from conspecifics and heterospecifics to form the different types of chorus noise. During both conspecific and heterospecific chorus noise treatments, females demonstrated similar preferences for advertisement calls at low and mid noise densities but failed to express a preference in the presence of high noise density. Our data also suggest that nights with high densities of chorus noise from conspecifics and heterospecifics are common in some breeding ponds, and on nights with high noise density, the soundscape plays an important role diminishing the accuracy of female decision-making.

Key words: **animal communication, chorus noise, mate choice, sexual selection, soundscape, túngara frog.**

INTRODUCTION

Every environment has a unique acoustic signature based on biological, geophysical, and anthropogenic parameters, called the soundscape (Krause 2008; Mullet et al. 2017). The soundscape experienced by animals can play an important role influencing animal communication (e.g., Mathevon et al. 2008; Luther and Gentry 2013), habitat selection (e.g., Hahn and Silverman 2006; Mullet et al. 2017), orientation (e.g., Vélez et al. 2017; Chang et al. 2018), and mate choice (e.g., Wollerman and Wiley 2002a; Vélez and Bee 2013; Huet des Aunay et al. 2014). In the context of mate choice, soundscapes can simply be viewed as combinations of biotic and abiotic noise that have the potential to interfere with the production, reception, and perception of mating signals.

An environment's soundscape changes frequently because of the dynamics of sound inputs across diel cycles and seasons, so animals may experience drastic shifts in noise over the course of months, days, or even hours (Pijanowski et al. 2011; Towsey et al. 2014b; Gottesman et al. 2018). For example, the onset of a sudden rainfall can lead to increased attenuation and harder discrimination tasks of acoustic signals in tawny owls (e.g., Lengange and Slater

2002). Additionally, acoustic communication signals produced by a wide range of taxa from birds to fish occupies a large portion of the biologically relevant frequency spectrum and has frequent temporal fluctuations. Some species are vocally active at night, while others are only active during the day, altering the frequency distribution of the soundscape over short time spans (Ruppé et al. 2015; Gottesman et al. 2018). This nocturnal and diurnal partitioning is common among different taxa like birds, insects, amphibians, mammals, and even fish (e.g., Ruppé et al. 2015; Ferreira et al. 2018; Gottesman et al. 2018).

According to the Acoustic Niche Hypothesis (ANH), each animal in a habitat occupies a different frequency bandwidth or exhibits temporal or spatial partitioning to avoid overlap in acoustic signals (Krause 1993; Farina et al. 2011; Villanueva-Rivera 2014). For example, avian communities demonstrate spatiotemporal partitioning for species with similar songs to minimize acoustic interference (Nelson and Marler 1990; Klump 1996; Luther 2009). Likewise, insects such as cicadas have also demonstrated partitioning in space, time, and acoustic signaling components (Sueur 2002). Chek et al. (2003) found that some of the examined anuran communities exhibited acoustic partitioning, but most did not. The acoustic niches of different species are of ecological interest for understanding how sympatric species share an acoustic space and potentially mask signals across species (Planqué and Slabbekoorn 2008; Ruppé et al.

2015). Even with partitioning, the ubiquitous and variable nature of noise can present unique challenges to signalers and receivers, potentially inducing errors in communication and mating decisions (Brumm and Slabbekoorn 2005; Lee et al. 2017; Vélez et al. 2017).

Noise generated by multiple signaling individuals, both conspecific and heterospecific, can lead to noise-induced errors in mate choice, particularly when there is spectral and temporal overlap of high-energy signals from multiple nearby sources (Wollerman and Wiley 2002a; de Groot et al. 2010; Römer 2013). Vocal activity in mixed-species aggregations can create spectrally and temporally complex soundscapes (Putland et al. 2017; Gottesman et al. 2018), where species composition and calling density may be important factors influencing call masking (Schwartz et al. 2001, 2002; Balakrishnan et al. 2014). Studying how receivers process mating signals within natural soundscapes can provide information on how receivers respond to natural fluctuations in the acoustic environment.

Anurans are excellent organisms for studying how noise densities affect communication between signalers and receivers (Gerhardt and Huber 2002; Wells and Schwartz 2007; Bee 2015). Male frogs commonly form mixed-species aggregations where they produce advertisement calls to attract females (Wollerman and Wiley 2002a; Wells and Schwartz 2007). These aggregations vary in density and, thus, intensity of chorus noise (Gerhardt and Huber 2002). Consequently, individuals are subjected to chorus noise ranging from a few, to hundreds of other frogs (Murphy 2003). In general, female frogs select specific properties of male advertisement calls, exhibiting mate preferences in their choices (Ryan 2001; Gerhardt and Huber 2002). Thus, mating outcomes are typically dependent on a female's ability to detect, localize, and discriminate conspecific calls from the rest of the chorus noise. In behavioral experiments, "choice" is typically demonstrated by phonotaxis, defined as movement toward one of two or more advertisement calls broadcast from different speakers, while "preference" is determined by the choice of multiple females, statistically favoring one call compared to others (Rosenthal 2017). Mate choice experiments establish preferences for particular calls within controlled conditions, and then deviations from preferences due to variance in experimental conditions can be investigated in follow-up trials (Rosenthal 2017).

We tested the effects of various chorus noise densities (duty cycles) on mate preferences in female túngara frogs (*Physalaemus pustulosus*), a common Neotropical frog found throughout Central America and northern South America. During the rainy season (May–November), males aggregate at night in ephemeral ponds/puddles and produce advertisement calls to attract females (Ryan 1985). The advertisement call consists of two components, a frequency-modulated whine followed by 0–7 harmonically rich chucks (Rand et al. 2006). Females will approach choruses and then evaluate males based on their call characteristics. Based on 19 years of data, females are known to demonstrate a consistent preference for males that produce complex calls (Ryan 1980; Ryan et al. 2019). In denser choruses with higher intraspecific competition, males produce more complex calls by appending additional chucks (Ryan et al. 2019).

Choruses of túngara frogs can become very crowded, reaching up to hundreds of frogs on any given night (Ryan et al. 1981), and are often confined to small puddles with loud and temporally dense noise. Additionally, other species of frogs are commonly found in close proximity to calling male túngara frogs, adding to the overall chorus noise that females must navigate to acquire mates. Considerable variation in chorus densities exists across nights, however. Due to the dynamic interaction between the environment and calling frog species, on some nights there are only a small number of males calling.

In this study, our primary aim was to characterize variation in natural chorus noise structure and subsequently test the ability of female túngara frogs to express preferences relative to these natural variances in chorus noise structure. We currently lack a comprehensive understanding of how variation in natural noise densities influences auditory processing and mate choice in frogs (but see Velez and Bee 2013; Lee et al. 2017; Christie et al. 2019). Therefore, our goal was to estimate the density of natural chorus noise over multiple nights at breeding locations in order to examine variation in chorus noise structure and identify potential maskers for túngara frog communication. We predicted that high density of either conspecific or heterospecific noise would mask the stimuli from overlap of calls, whereas low and mid noise densities would not mask calls due to gaps present in chorus structure. We also tested if females maintain call preferences in the presence of varying chorus noise densities based on natural soundscapes. Furthermore, these data provide information useful for understanding the threshold at which noise induces mating errors in this species, allowing for predictions of which nights will generate stronger selection from female choice.

METHODS

Soundscape

Study sites/soundscape sampling

All sound recordings and experimental procedures were conducted at the Smithsonian Tropical Research Institute (STRI) in Gamboa, Republic of Panama. We set up two passive Song Meter digital audio recorders (SM3; Wildlife Acoustics 2015; Maynard, MA) equipped with two omnidirectional microphones to record soundscapes at different breeding sites, Santa Cruz and Ocelot ponds. Santa Cruz is a "semi-urban" environment with a small, artificial breeding pond established near a natural stream and forest. A paved road, residential houses, and the Smithsonian lab are also located at Santa Cruz. This area experiences artificial nighttime lighting and occasional anthropogenic noise. Ocelot is a larger, natural pond and is located within a forest less affected by anthropogenic activity. Ocelot is located near a moderately quiet road that leads to Gamboa. The Song Meter recorders were strapped ~1.5 m above the ground to trees centered along the perimeter of the breeding ponds. These passive acoustic recorders allowed us to record and monitor changes in the soundscape over long periods of time with minimal intrusion. We set them to record from 2000 to 0030 h each night throughout July 2018 and June–July 2019 (sampling at 24 kHz; frequency spectrum from 0 to 11 kHz), though not every night was recorded during these time periods due to necessary maintenance and battery changes. Frog choruses were most active during this time frame, and June and July are part of the rainy season (May–November) when túngara frogs breed. Files were saved onto a secure digital (SD) card in stereo WAV format (16 bit).

Soundscape analyses

To quantify noise densities and identify target species potentially affecting the acoustic communication of túngara frogs, audio files were input into R (R Core Team 2018) and subsequently analyzed using QUT Ecoacoustics Analysis Programs (Towsey et al. 2014b,

2018). This program produces long-duration, false-color (LDFC) spectrograms and calculates acoustic indices (Figure 1; see Towsey et al. 2014b). Acoustic indices are summary statistics of the energy distribution across frequency, time, and space. These indices can be used to estimate spectral and temporal density, species richness, background noise, and differences among soundscapes (Sueur et al. 2014; Towsey et al. 2014b) and are especially useful for analysis of complex, long-duration sound files. For the purpose of this study, we prioritized one index, the mid-frequency cover (MFC). The MFC index is the fraction of mid-frequency (482–3500 Hz) noise-reduced spectrogram cells that exceed 3 dB above the acoustic energy that remains constant (Towsey et al. 2014a). For example, while the distant stridulations from crickets are registered as constant acoustic energy, nearby cricket chirps are picked up as acoustic events contributing to relevant background noise. This mid-frequency spectrum is a good match for ecologically relevant sounds for túngara frog hearing and communication (Ryan et al. 1990). Higher MFC index values correlate with more energy (from greater intensity and temporal and spectral activity) distributed across the mid-frequency spectrum, indicating higher noise densities that have the potential to mask túngara frog mating signals.

We divided recordings into 1-min, nonoverlapping segments and resampled at 22.05 kHz (sensu Towsey et al. 2014a). We converted each segment to a spectrogram with 512 frequency bins (21.48 Hz per bin) and a fast-Fourier transform (FFT) with Hamming window. Acoustic indices were calculated from these 1-min segments as scalar and vector values, where the scalar represented the entire 1-min segment and the vector represented the index values for each frequency bin (512 values) (sensu Towsey et al. 2014b). See Towsey et al. (2014a) for additional details on how acoustic indices were calculated.

We averaged the MFC index scalar values for each night from 2000 to 0030 h and categorized nights as having a low, medium, or high density of chorus noise based on the interquartile ranges (IQRs) of the MFC index (Figure 1). These ranges served as the general basis for selecting 2018 recordings to measure call rate of túngara frogs, gladiator tree frogs (*Hypsiboas rosenbergi*), and hourglass tree frogs (*Dendropsophus ebraccatus*), which are prevalent species
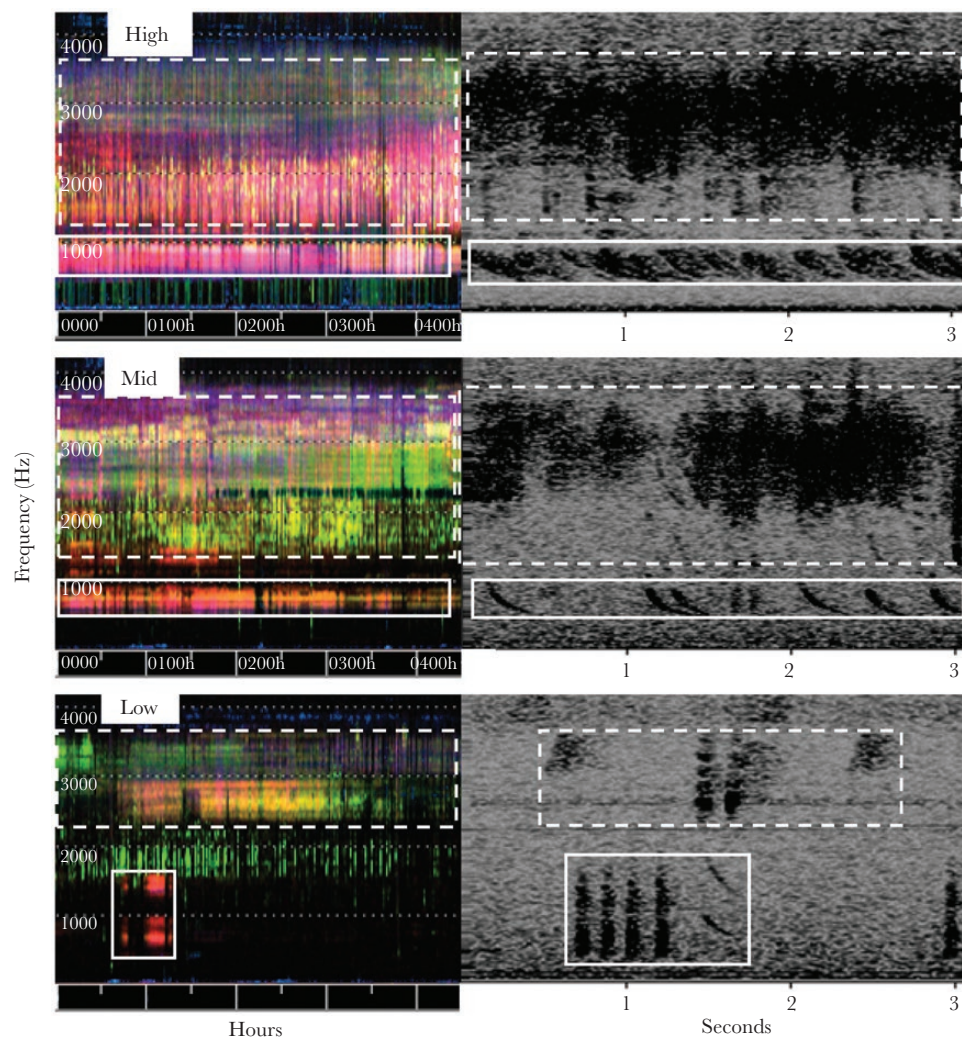
Figure 1
LDFC spectrograms of nights (from 2030 to 0030 h) with variable chorus noise densities: low, mid, and high. LDFC spectrograms were produced using QUT Ecoacoustics Analysis Programs (Towsey et al. 2014b, 2018) with red, green, and blue colors corresponding to the Acoustic Complexity Index, Temporal Entropy, and Event Count acoustic indices, respectively. Dashed and solid lines highlight frog calls depicted in LDFC spectrograms compared to 3-s, pseudo-color (grayscale) spectrograms of the same night (for color figure refer to online version).

at our study sites and are most likely to mask túngara frog calls. We used Raven Lite 2.0 (Cornell Laboratory of Ornithology) to measure call rates of these species from soundscape recordings. Unlike acoustic indices, call rate and frequency measurements were made from spectrograms with an FFT size of 1024 bins (frequency resolution 10.74 Hz, temporal resolution 15.2 s, Hamming window). We defined call rate as the number of calls per minute and visually measured it every 10 min from 2030 to 2130 h, as MFC index values were highest during this time period and thus suggested the greatest amount of calling activity on a given night. We then averaged these measurements for each night. In cases where there were too many calls to distinguish in the 1-min period, the max distinguishable call rate value for that species was used.

By quantitatively and visually comparing recordings across nights, sites, and years using MFC index values, LDFC spectrograms, and grayscale spectrograms, we also estimated the percentage of nights that contained high noise densities of the relevant species examined in this study. Nights that had high densities of túngara frog calls, high densities of hourglass and gladiator tree frog calls, or moderately high densities (between the mean values of mid and high densities) of all three species were considered to have high noise densities.

We measured other parameters including frequency ranges of the vocally dominant species and power spectra (amplitude distribution across frequencies) of each soundscape. We calculated the frequency ranges of these species by measuring the minimum and maximum frequencies in Raven. Dominant frequency peaks were computed every 30 min in R using the *meanspec()* and *fpeaks()* functions in the seewave package (Sueur et al. 2008). We then calculated power spectral density (PSD) by setting the value *PSD* in the function *meanspec()* to true and graphed the averages across frequency from 0 to 8 kHz.

Behavioral trials

Túngara frog pairs were collected in amplexus between 1930 and 2100 h from June to August 2019 in Gamboa, Republic of Panama. We subsequently brought the pairs to our laboratory at STRI facilities and placed them in total darkness in a cooler for at least 1 h before testing. This provided time for their eyes to dark-adapt after collection with flashlights (Cornell and Hailman 1984; Fain et al. 2001; Taylor et al. 2008). We tested all females the same night as collection from 2200 to 0330 h and at temperature of 27 °C. Following testing, we toe-clipped females that made a choice to ensure that females were not retested in the same experiment after any potential recaptures. The toe clips were placed in ethanol and saved for genetic analysis. At this time, we also measured their mass and snout-vent length (SVL) before releasing them with their male partners at their respective collection sites.

For each trial, we positioned a female under an acoustically and visually transparent plastic funnel (ca. 10 cm diameter) in the center (hereafter referred to as the "funnel zone") of a sound attenuation chamber (2.7 × 1.8 × 2 m; Acoustic System, ETS-Lindgren, Austin, TX). For all experiments, a nightlight (GE 55507; Fairfield, CT) illuminated the sound chamber with ambient light adjusted to ca. 5.8×10^{-10} W/cm² to mimic a nocturnal light level within the natural range experienced by túngara frogs (Taylor et al. 2008). We placed two speakers (Nanosat Black; Mirage) equidistant from the funnel zone at 80 cm and separated them by 60°. These speakers broadcast the amplified (NAD C-316BEE; Pickering, ON, Canada) acoustic stimuli antiphonally. Using Adobe Audition 2.0, we played

a recorded pair of natural calls with a known preference function (75% of females prefer "Od" call over the "Sc" call, from Ryan and Rand 2003). We chose to use a single pair of calls across experiments and trials so as not to confound differences in stimulus call properties with detection in noise. Specifically, we were testing the ability of females to express a known mating preference in chorus noise, rather than testing general female preference functions. Each stimulus consisted of a whine and single chuck. We adjusted stimuli so that the peak amplitude measured 82 dB SPL (fast, C weighting, re 20 µPa) from the funnel zone using a Larson Davis 831 Class 1 SPL meter (Larson Davis, Depew, NY). For all sound files, the acoustic stimuli were played on a 3-s loop separated by 1.1 s. We alternated which speaker broadcast which stimulus to avoid potential side bias.

Trials consisted of placing females under the funnel, playing the sound files, stimuli and noise for 2 min to acclimate the frogs, and then releasing the female remotely (outside the chamber) via raising the funnel with a rope and pulley system. We scored a choice if the female entered a 5 cm radius around a speaker and remained there for at least 3 s. Alternatively, if the female did not leave the initial funnel zone within 2 min or wandered around the chamber for 10 min without making a choice, we removed her from the chamber and retested her once more later that evening. If a female failed to make a choice on her second attempt, we recorded her as a "foul out" and did not include her in the data set. Additionally, we recorded the time that the female took to choose one of the stimuli (latency). All trials were video-recorded using an infrared video camera, which was connected to EthoVision (Noldus) software and was mounted on the ceiling of the chamber directly above the funnel zone. We tested a different set of 32 randomly sampled females for each experiment.

We first tested female preferences for the natural call pair in quiet conditions to confirm the 75% preference for the "Od" call. We then tested if female túngara frog preference for the natural call pair persisted in a variety of background noise treatments, consisting of conspecific or heterospecific frog calls (eight trials: six with 82-dB noise and two with 76- and 70-dB noise). We also varied the temporal density of chorus noise (the number of calls in a period of time) and categorized these variations as a low, medium, or high amount of background chorus noise (Table 1; Figure 2). These treatments were designed after analyzing the 2018 soundscape recordings to replicate natural variation in chorus noise experienced by túngara frogs. For the conspecific noise, we used a natural túngara frog call known to be less attractive when compared with the attractive stimulus (Call ID: Sb; from Ryan and Rand 2003). For the heterospecific noise, we recorded natural calls from gladiator and hourglass tree frogs, because both species often call in dense aggregations intermixed with túngara frogs. Additionally, both species have call characteristics (e.g., spectral overlap, relatively long call duration, high amplitude, and high call rate) that suggest they are potential maskers of túngara frog calls (hourglass: Wells and Schwartz 1984; gladiator: Höbel 2000). For the gladiator tree frog, we used a natural advertisement call consisting of four, 70-ms pulses (30- to 50-ms interpulse interval, 400-ms call duration) and having a dominant frequency of 945 Hz. For the hourglass tree frog, we used a natural advertisement call consisting of 16, 9-ms pulses (~3-ms interpulse interval) and a click note 90 ms after the introductory note (334-ms call duration, 189-ms introductory note duration, 55-ms click note duration) and having a dominant frequency of 2906 Hz.

Table 1

Treatment summary and corresponding number of calls that make up chorus noise treatments

Treatment	# Túngara frog calls (noise)	# Hourglass tree frog calls	# Gladiator tree frog calls
Control	—	—	—
Heterospecific Low	—	2	2
Heterospecific Mid	—	4	4
Heterospecific High	—	6	4
Conspecific Low	2	—	—
Conspecific Mid	6	—	—
Conspecific High	10	—	—

In all treatments, the same túngara frog stimuli were used: Od and Sc (Ryan and Rand 2003). Low, mid, and high chorus noise treatments reflect the number of calls present in each playback file for every 3-s loop, not including the stimuli.
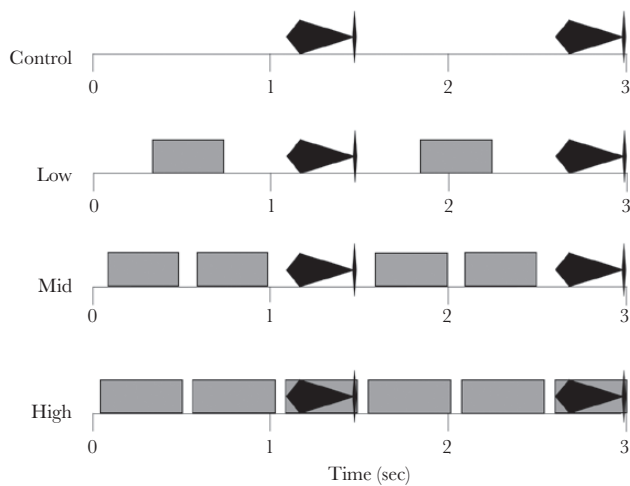
Figure 2

Graphical representation of noise densities (low, mid, and high) in 3-s looped sound files for either conspecific or heterospecific noise treatments. Gray boxes represent either conspecific or heterospecific noise, and black waveforms represent túngara call pair stimuli: Od and Sc (Ryan and Rand 2003). In high density chorus noise, there is acoustic interference of chorus noise with the stimuli.

For both conspecific and heterospecific noise treatments, we mounted two noise speakers on the back wall (behind the speakers playing the stimuli) in each of the corners, 125 cm from the ground and 210 cm from the funnel zone. The positions of these speakers were kept constant across all noise treatments, as were the speakers broadcasting the stimuli. Mounting two noise-broadcasting speakers above the floor of the chamber and on the corners generated an even, chorus-like distribution of noise within the chamber. Peak amplitudes of the noise (both conspecific and heterospecific) were calibrated to 82 dB SPL from the funnel zone. For high conspecific noise density, we also tested female túngara responses at two additional noise amplitudes: 76 and 70 dB.

Statistical analyses

Soundscape comparison

We tested differences in MFC index per hour starting at 2030 h using an analysis of variance (Anova) followed by Tukey honestly significant difference (HSD) post hoc tests. This analysis was performed on 2018 recordings to estimate the time period with the most calling activity. We also compared the soundscapes of Santa Cruz and Ocelot in 2018 and 2019 using MFC index and call rate to infer whether these sites differed from each other in the amount of chorus noise. Comparisons were performed on dates with recordings from both sites (e.g., 1–30 June at Santa Cruz vs. Ocelot). At our Santa Cruz site, we were able to compare mid-band activity across years as well, since we had recordings of the same calendar days for both years. Differences in MFC index and call rate were tested using independent sample t and two-sample Wilcoxon tests, respectively. Both assumptions of normality and homogeneity of variance were met for MFC index, but call rate data were not normally distributed.

Behavioral analyses

We analyzed female phonotactic preferences using a binomial distribution (SISA binomial calculator; Uitenbroek 1997), and we reported significance levels for preferences as mid-P-values, which have been advised for use in categorical data for smoothing radical changes in P-value generated by discrete data (Agresti 2001; Hwang and Yang 2001). A preference rate of 75% for the attractive call has been documented in the control of this study and in the original study (see Ryan and Rand 2003). Thus, we set the binomial expected value to 0.75 for all experimental treatments. We analyzed latency differences with an Anova, after transforming to satisfy assumptions of normality and homogeneity of variance. Following results from the Anova, we performed multiple comparisons using Dunnett's test to examine if chorus noise treatments had higher latencies than the control. We also report effect sizes using Cohen's d (Cohen 1992). We conducted all statistical analyses, except phonotactic preferences, using R (R Core Team 2018) with $\alpha = 0.05$ for all analyses.

Ethical approval

All procedures performed involving animals followed ABS ethical guidelines for treatment of animals (Buchanan et al. 2012). The guidelines discourage toe-clipping, if other options are available. We preserved the toes, however, for use in ongoing genetic analyses of the population, and thus get additional information. We only removed the distal tip of a maximum of one toe per foot. Our long-term experience with this species suggests that this procedure does not cause excessive stress or mortality. We suggest this because bleeding rarely occurs, males will resume calling behavior shortly after toe-clipping, and we routinely recapture marked individuals of both sexes exhibiting breeding behaviors. The toe tips also show some level of regeneration after a few weeks. The experiments were approved by IACUC protocols issued by Salisbury University and the Smithsonian Tropical Research Institute (IACUC: SU-0052 and STRI 2018-0411-2021). No animals were sacrificed for this research. Collecting permits were approved by Panama's MiAmbiente (ANAM: SE/A-44-18 and SE/A40-19).

RESULTS

Soundscape structure

Overall, we recorded 482 h of soundscape audio. We first wanted to examine which hour(s) of a typical night would have the most calling activity. We found statistically significant differences among the 4 h recorded each night, from the 160 recorded hours in 2018

($F_{3,156}$ = 4.37, P = 0.006). The results from the Tukey HSD post hoc tests showed that, on a given night, the MFC index means for the first 2 h (2030–2230 h, $\bar{x}_1$ = 0.142, $\bar{x}_2$ = 0.153) were both significantly higher than the last hour (2330–0030 h, $\bar{x}_4$ = 0.107) (P = 0.049 and P = 0.004, respectively) but did not significantly differ from the third (2230–2330 h, $\bar{x}_3$ = 0.139) (P = 0.997 and P = 0.725, respectively) or each other (P = 0.835), suggesting that noise densities in the mid-frequency band increased early on most nights and slowly diminished after 2230 h. This is consistent with years of observations of frogs at these breeding ponds.

In 2018, the MFC index was significantly greater at Ocelot (natural site) than Santa Cruz (semi-urban site) ($\bar{x}_{Ocelot}$ = 0.151, $\bar{x}_{Santa\ Cruz}$ = 0.083, t = 2.94, degrees of freedom [df] = 10, P = 0.015). However, in 2019, we did not find significant differences in MFC index between Santa Cruz and Ocelot ($\bar{x}_{Santa\ Cruz}$ = 0.173, $\bar{x}_{Ocelot}$ = 0.153, t = 1.76, df = 32, P = 0.087). Additionally, Santa Cruz had significantly greater MFC index values in 2019 compared to 2018 ($\bar{x}_{2019}$ = 0.126, $\bar{x}_{2018}$ = 0.097, t = 2.46, df = 32, P = 0.02). Out of 69 recorded nights at Santa Cruz, 42% were estimated to have high chorus noise densities. Out of 23 recorded nights at Ocelot, 78.3% were estimated to have high chorus noise densities.

When comparing call rates of túngara, hourglass, and gladiator frogs (Table 2) by site on the same days, Santa Cruz was significantly lower than Ocelot for both túngara frogs (W = 0, P = 0.004) and hourglass tree frogs (W = 4.5, P = 0.034). Gladiator tree frogs were absent from Ocelot and therefore were not tested for differences.

Across all the commonly occurring species at Santa Cruz and Ocelot, we found consistent overlap in their frequency ranges (Figure 3). Notably, dominant frequencies of the túngara frog's whine (466–906 Hz) and gladiator tree frog's call (610–1442 Hz) overlapped. Additionally, the dominant frequency of the túngara frog's chuck (2163–2989 Hz) overlapped with the frequency range of the hourglass tree frog's call (1919–4298 Hz). We observed no temporal or spatial partitioning across species; both gladiator and hourglass tree frogs were commonly situated near calling male túngara frogs.

When comparing the distribution of dominant frequencies across sites and years, we found a clear overlap in peaks from 450 to 850 Hz and 3000 to 3100 Hz (Figure 4). There was minor activity from 1.2 to 1.8 kHz. We also observed a high amount of activity from 3.8 to 7.7 kHz. Santa Cruz (semi-urban site) followed a similar distribution in dominant frequencies across years, while Ocelot (natural site) had slight variation between years.

Table 2
Call rate averages (sum of multiple individual callers) and standard deviations of túngara, hourglass, and gladiator frogs on nights with variable chorus noise densities: low, mid, and high

	Frog species		
Call rates (calls × min^{-1})	Túngara	Hourglass	Gladiator
Low	25 ± 16	25 ± 16	27 ± 13
Mid	61 ± 15	59 ± 15	54 ± 5
High	156 ± 6	88 ± 4	79 ± 14

Note that on nights with high chorus noise, call rate values for túngara and hourglass frogs were not precise above the averages due to a high amount of overlap from calling males.

Behavioral experiments

Both heterospecific and conspecific chorus noise had similar effects on the females' ability to distinguish between call stimuli (Figure 5). In the presence of low- and mid-density chorus noise, females maintained preference for the attractive call. At high densities of chorus noise, females chose randomly between the call stimuli.

Ryan and Rand (2003) demonstrated that females exhibit a 75% preference for the Od over the Sc call (15:5). We retested female preferences for this natural call pair in quiet background conditions and confirmed the 75% preference in favor of the Od call (24:8 preference for Od). In background noise experiments, with conspecific noise played at 82 dB, mate preference was not significantly different than the expected response rate of 0.75 for both low (Figure 5; 27:5, two-tailed binomial test: P = 0.265) and mid (Figure 5; 20:12, two-tailed binomial test: P = 0.084) densities of chorus noise. Preference was significantly reduced at the high noise density (Figure 5; 16:16, two-tailed binomial test: P = 0.002), with females choosing at random. For high conspecific noise density played at lower amplitudes, preference was not significantly different from expected at either 70 dB (25:7, two-tailed binomial test: P = 0.762) or 76 dB (19:13, two-tailed binomial test: P = 0.052). In the presence of heterospecific noise played at 82 dB, preference was not significantly different from expected for both low (Figure 5; 24:8, two-tailed binomial test: P = 0.919) and mid (Figure 5; 22:10, two-tailed binomial test: P = 0.361) densities of chorus noise, but preference was significantly lower during the high noise density treatment (Figure 5; 16:16, two-tailed binomial test: P = 0.002).

Latencies were significantly different among the nine treatments (32 samples each) ($F_{8,279}$ = 2.14, P = 0.033). However, when chorus noise treatments were compared to the control, we did not find any significant differences among pairwise comparisons (Figure 6; heterospecific: low t = −0.41, P = 0.958; mid t = 0.14, P = 0.854; high t = 1.80, P = 0.171; 82 dB conspecific: low t = 2.37, P = 0.052; mid t = 0.43, P = 0.754; high t = 1.38, P = 0.324; 76 dB conspecific: high t = 2.35, P = 0.056; 70 dB conspecific: high t = 0.89, P = 0.553). Conspecific low and 76 dB conspecific high noise treatments both had medium effect sizes (conspecific low: Cohen's d = 0.679; 76 dB conspecific high: Cohen's d = 0.582; Cohen 1992). Additionally, high heterospecific and 82 dB conspecific noise treatments had low effect sizes (heterospecific Cohen's d = 0.47; conspecific Cohen's d = 0.35; Cohen 1992).

DISCUSSION

The acoustic niches and signal characteristics of different species are of ecological interest for understanding how sympatric species share an acoustic space and potentially mask signals across species (Planqué and Slabbekoorn 2008; Ruppé et al. 2015). We did not find support for the ANH for the species examined in this study at either site (Figure 3). At Santa Cruz, gladiator and hourglass tree frogs overlapped with the túngara frog whine and chuck, respectively. Both gladiator and hourglass tree frogs have relatively high call rates in groups compared with calling alone (Table 2), and our behavioral results suggest these frogs interfere with mating decisions of female túngara frogs. We found no prominent spatial partitioning (species grouped on separate ends of the pond) among the species observed at either site, aside from clusters of male túngara frogs in ephemeral pools around Santa Cruz. This is likely a constraint imposed by the frog's reproductive ecology and the size of these breeding ponds.
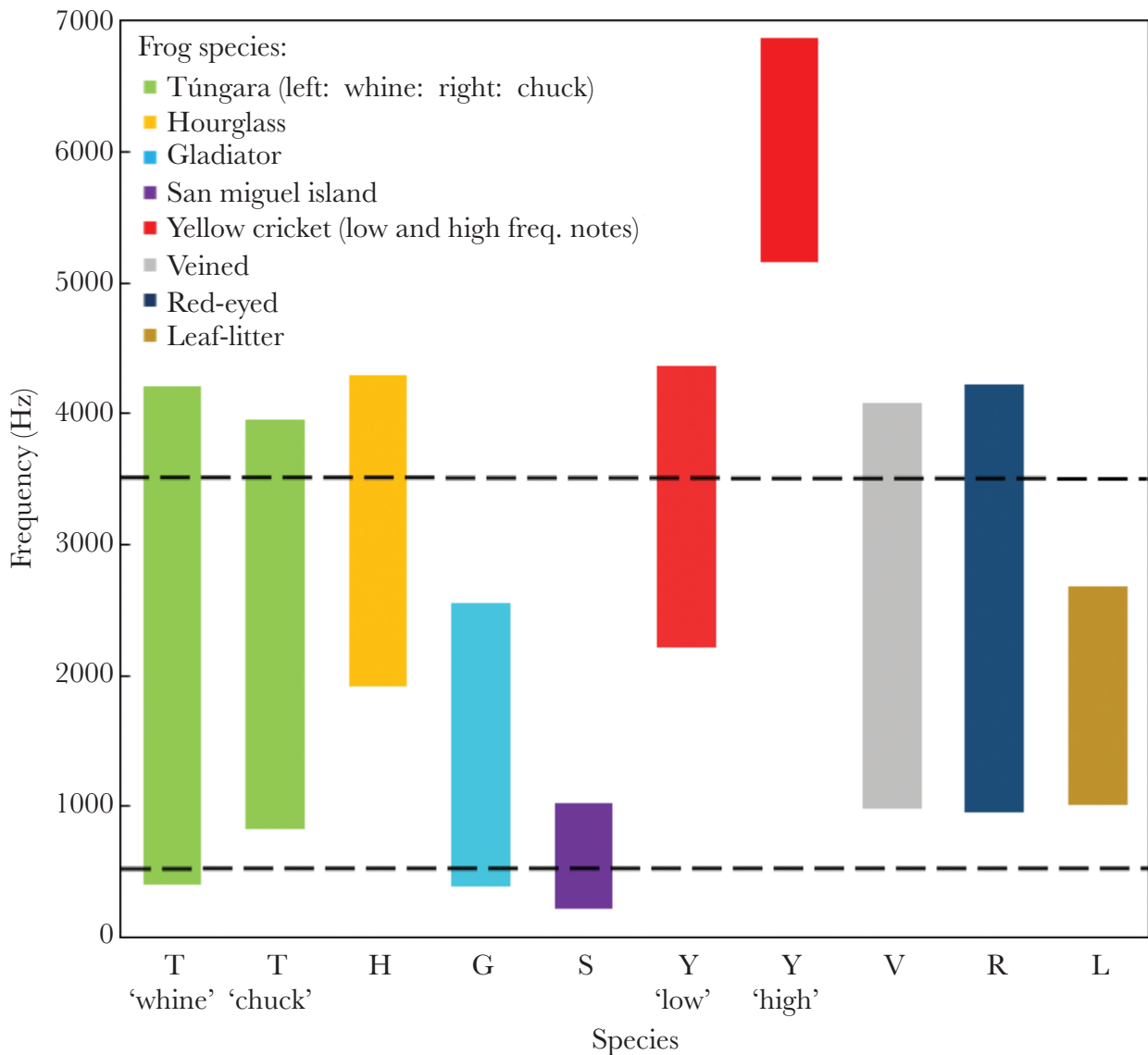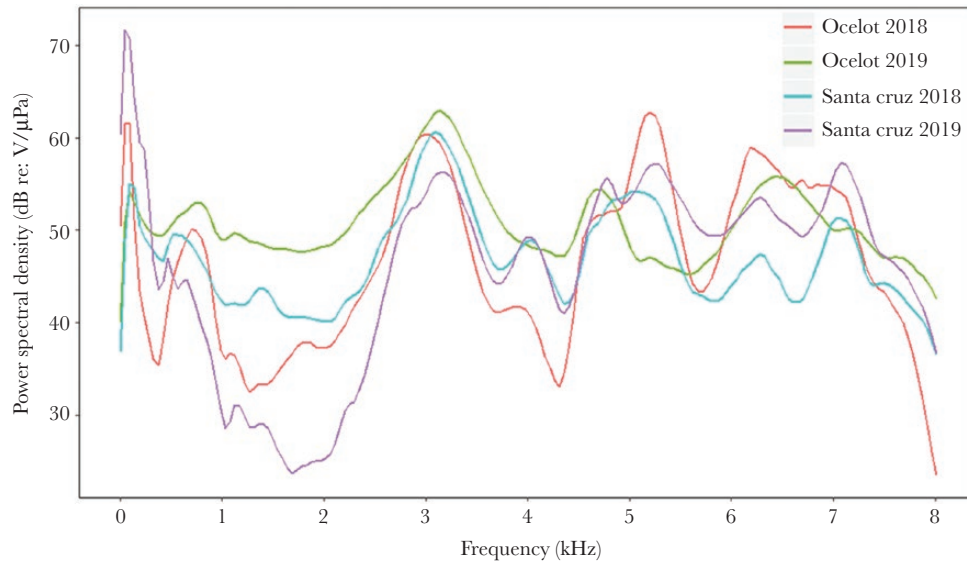
Figure 3

Frequency ranges of the prominent species calls recorded. Frog species from left to right: túngara frogs (*Physalaemus pustulosus*), hourglass tree frogs (*Dendropsophus ebraccatus*), red-eyed tree frogs (*Agalychnis callidryas*), San Miguel Island frog (*Leptodactylus insularum*), yellow cricket tree frog (*Dendropsophus microcephalus*), veined tree frog (*Trachycephalus venulosus*), gladiator tree frogs (*Hypsiboas rosenbergi*), leaf-litter toads (*Rhinella alata*). The whine and chuck of túngara frogs and low and high notes of yellow cricket tree frogs are measured separately (left to right, respectively). Both gladiator tree frogs and leaf-litter toads were absent from Ocelot soundscapes. The dashed line indicates range of frequencies most sensitive to túngara frog hearing (see Ryan et al. 1990).

During the part of the rainy season that we tested (June–August), we estimated that 42% of nights at Santa Cruz and 78.3% of nights at Ocelot consisted of high densities of chorus noise that significantly increased mating decision errors. This suggests that a relatively high proportion of nights have noise densities high enough to reduce the skewed male mating success that results from female mate choice. This was especially true at the less urbanized Ocelot. In our study, we found that a semi-urbanized area can potentially decrease noise-induced mating errors associated with high calling activity from other frogs. Furthermore, as areas become more urbanized, túngara frogs experience increased pressures from sexual selection and decreased pressures from natural selection (Halfwerk et al. 2019). Given the reduced risk of predation and parasitism in more urbanized areas (e.g., McMahon et al. 2017; Halfwerk et al. 2019) in combination with fewer nights with high chorus noise,

urbanized areas are potentially more beneficial for female expression of mating preferences. In other words, females can reduce their probability of making an error during mate choice. However, other factors that alter female expression of mating preferences like anthropogenic noise and light pollution should also be considered in these more urbanized areas.

Chorus noise and its effects on anuran communication are traditionally studied using artificially synthesized sounds like band-filtered and chorus-shaped white noise (e.g., Vélez and Bee 2010, 2013). White noise is a useful tool in acoustic studies, because the characteristics of the noise playbacks can be precisely controlled. Although white noise can be band-filtered to cover the spectrum typical of conspecific or heterospecific choruses, it commonly lacks the frequency modulation, temporal features (rise time, fall time, interpulse interval), and amplitude modulation typical of natural
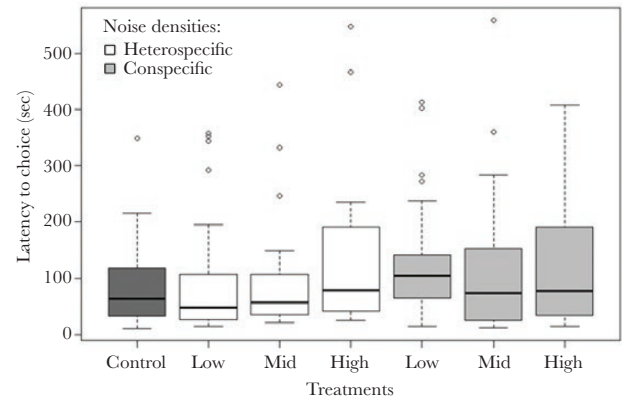
Figure 4

Average dominant frequencies compared by sites and years using PSD curves. PSD values were calculated in R using the *meanspec()* function and averaged for each frequency by site and year.
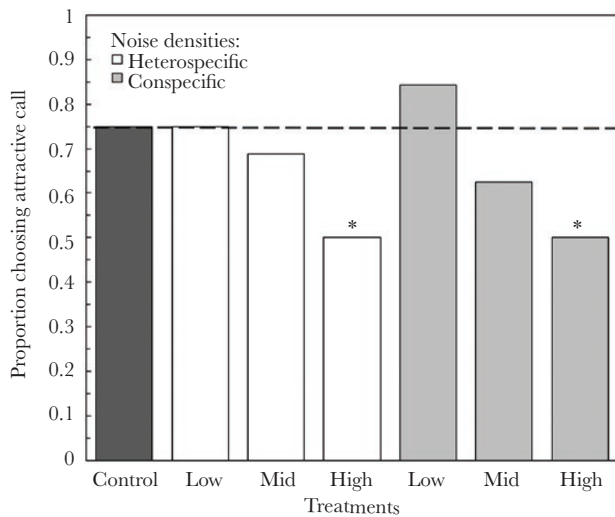
Figure 5

Female túngara frog responses to stimuli in quiet and 82 dB chorus noise treatments. Low, mid, and high treatments represent noise densities for heterospecific (white bars) and conspecific (gray bars) noise. Values indicate the proportion of females in each treatment that chose the attractive stimulus. The dashed line denotes the expected proportion of choices based on the quiet control treatment. The asterisk indicates significant differences from the control when $P < 0.05$.

Figure 6

Latency distributions of response times in seconds for female túngara frogs to make a choice during 82 dB chorus noise treatment trials. Low, mid, and high treatments represent noise densities for heterospecific (white bars) and conspecific (gray bars) noise. Abbreviations are the same as in Figure 3. Boxplots represent median (dark line) and interquartile ranges (IQRs) of latency values. Whiskers extend to the most extreme data points within ± 1.5 IQR. Outliers are depicted as open circles.

noise (but see Vélez and Bee 2013; Lee et al. 2017). Assessing how this natural variation influences signal detection is critical for understanding signal evolution, since noise-induced errors affect female mate choice and thus which male's signal traits are passed to the next generation (Lee et al. 2017). We suggest more studies employ both white noise and natural chorus noise for a broader understanding of the effects of noise on anuran communication.

Animal perception is often controlled by neurons selective in their responses for specific spectrotemporal patterns of natural sounds (Rose et al. 2011; Theunissen and Elie 2014). For example, certain neurons in the higher auditory areas of primates

(Rauschecker et al. 1995) and birds (Margoliash and Fortune 1992) fail to exhibit significant responses to either a white noise or tone burst stimulus but appear to be most responsive to specific spectrotemporal properties of natural animal vocalizations. In the Emei music frog (*Babina daunchina*), advertisement calls with spectral features characteristic of conspecific calls induced greater neural responses than only temporally matching white noise (Fan et al. 2019). Likewise, for many frogs, neurons in the torus semicircularis (homologous to the inferior colliculus in birds and mammals) respond either preferentially or only to specific temporal characteristics (e.g., Penna et al. 2001; Edwards et al. 2002). How differences in processing may affect a female's ability to make choices in natural versus synthetic noise remain unclear, but comparisons of both types of noise are important for future studies on mate choice and signal evolution.

In our study, both heterospecific and conspecific noise broadcast at 82 dB had similar results when compared to the control on the expression of female mate preferences (Figure 5). For both noise types, low and mid densities did not significantly reduce preference for the attractive call, but high densities significantly reduced preference from 75% to 50%, effectively making female choice random. These results suggest that female túngara frogs experience comparable cognitive challenges, similar to the "cocktail party problem" in humans (Cherry 1953; Bee 2008), under high densities of either heterospecific or conspecific choruses. This finding was surprising. Heterospecific calls exhibit some frequency separation and differ from conspecific calls dramatically in temporal structure. In conspecific noise, females must also process the biologically salient background noise and make decisions about whether to attend to this noise or ignore it. Thus, auditory discrimination of relevant signals should be easier for female túngara frogs in the presence of heterospecific noise (Nityananda and Bee 2011). In other words, it should be easier for females to detect conspecific calls in heterospecific noise. Although we predicted that conspecific noise would have a stronger influence degrading female mating decisions, it did not. Additionally, when high noise densities were broadcast at lower amplitudes (higher signal-to-noise ratios), females were able to distinguish between the call pair stimuli and express their preferences. Thus, increased spatial separation among males may allow females to discriminate among males more easily due to lower chorus noise intensities (Bee 2007).

When combining the behavioral results with the call densities, species compositions, and MFC at Ocelot and Santa Cruz, our data suggest that female túngara frogs experience different chorus noise structure at each site yet encounter similar challenges when deciphering signals. While females at Ocelot may need to contend with high densities of conspecific noise, females at Santa Cruz likely struggle more with high densities of heterospecific noise. Previous research has shown that the relative abundance of each species is an important factor for consideration, as some species have a higher potential for acoustic interference than others (e.g., Balakrishnan et al. 2014). In our study, however, high densities of both conspecific and heterospecific noise have the potential to mask túngara frog calls in a natural setting.

While changes in preference can reveal how effectively females discriminate in the presence of noise, the speed at which they choose is also an important consideration. In decision-making, there is often a trade-off between decision speed and accuracy associated with the decision-making process (Chittka et al. 2009). These speed-accuracy trade-offs (SATs) can be observed from frog phonotaxis in: 1) latency to choose a mate and 2) accuracy to choose the attractive mate. If the female takes longer to choose but can eventually locate the attractive mate, she experiences a trade-off where speed decreases, but accuracy is maintained. For this female, slow decision-making may result in choosing a preferred mate but at the cost of increasing her exposure to predators. Chorus noise may lead to either SATs or, under high noise densities, an overall decrease in both speed and accuracy.

Interestingly, our findings do not show a clear latency pattern (Figure 6). The low conspecific noise treatment had a moderate effect on female latency to choose, but this effect was not statistically significant. Likewise, while not statistically significant, we also reported a small effect size for both high conspecific and heterospecific noise at 82 dB. These results suggest that higher noise densities may generally increase latencies and that low conspecific noise may

result in a trade-off in speed for greater accuracy, but additional data are needed to verify these trends. Regardless, females were unable to discriminate between the attractive and unattractive males in the presence of high densities of chorus noise (Figure 5). While our study did not specifically test for the threshold at which noise density impairs discrimination, it does reveal the density at which females are no longer able to discriminate mating signals (Figure 5).

Implications

In this study, we estimated that 42–78.3% of the nights will result in a condition in which higher than expected errors in mating decisions occur. This range provides additional insight as to why the intensity of sexual selection can vary in different populations (reviewed in Miller and Svensson 2014). How much this range varies across locations and seasons is relatively unexplored and warrants further investigation. It is important to note that we used a single stimulus pair to examine how noise influences mate choice decisions for calls. We specifically chose this design to incorporate the known preference function for these calls. Further, mate preferences have been extremely well characterized in this species (Ryan 1985; Ryan et al. 2019) and we therefore were not testing general mate preferences. It is possible that calls with different properties (e.g., frequencies or a chuck versus no chuck) may generate different outcomes than demonstrated in our study. This is something that could be addressed in future studies. We already know, however, that a call with a chuck will be preferred to one without, irrespective of noise conditions (Ryan 1985). Compared with some frog species, the range in variation of túngara frog call properties is relatively small (James LS et al., unpublished data). The natural variation in calls, such as duration and rate, is therefore unlikely to have strong influences on signal detection in noise. Finally, our stimulus calls were within the centroid of properties for túngara frog calls (Ryan and Rand 2003), and thus representative of typical calls experienced by females in nature.

To our knowledge, this study is the first attempt using soundscape analysis and behavioral experiments to quantify the probability of females making errors in mating decisions. Wollerman and Wiley (2002a) performed playback experiments with natural chorus noise at multiple signal-to-noise ratios and found that female discrimination of calls differed among experiments (see also Wollerman 1999). Furthermore, the relative abundances of species in a chorus could affect female mate choice, where detection of a conspecific signal is more likely for common than uncommon species (Wollerman and Wiley 2002b). Tanner and Bee (2020) demonstrated that inconsistency in male gray tree frog (*Hyla versicolor*) calls can also have profound effects on female mating decisions. An important next step in understanding signal evolution is to examine how variation in signal properties (e.g., frequencies or presence of additional chucks) influence female discrimination in naturally fluctuating soundscapes.

We have demonstrated how natural variation in soundscape structure plays an important role in anuran communication and female mate choice. Noise shapes the evolution of animal communication, whether by driving novel signaling adaptations or limiting the evolution of social behavior (Wiley 2015). Therefore, studying soundscapes and their influence on signal discrimination is integral to understanding auditory processing, mate choice, and signal evolution.

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