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EFFECTS OF ANTHROPOGENIC LIGHT AND NOISE ON ANURAN BREEDING
BEHAVIOR

By

ASHLEY KOBISK, Bachelor of Science

Presented to the Faculty of the Graduate School of

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ABSTRACT

The natural environment can be negatively impacted by a variety of human activities, including the production of artificial light at night and anthropogenic noise. Recent studies suggest that pollution from anthropogenic light and noise alters animal behavior. Despite being highly nocturnal and vocal animals, little attention has been given to anurans and the effects artificial light at night and anthropogenic noise have on their behavior. This study investigated the effects of artificial light at night and anthropogenic noise on anuran breeding systems in eastern Texas. Specifically, this study investigated whether (1) artificial light and anthropogenic noise altered calling behavior in male anurans, (2) artificial light influenced male call site selection, and (3) artificial light influenced female mate choice. Ambient light and sound levels were quantified at five sites that varied in urbanization and, therefore, artificial light and anthropogenic noise levels. At these sites, calling males were recorded and ambient light was then measured at the male's call site. Call parameters including call dominant frequency, call duration, pulse rate, and call rate were analyzed for differences among urban and rural populations. Call site light microhabitat measurements were compared to the general light environment as well as among populations. Additionally, females were tested in two phonotaxis experiments to determine their mate choice preferences under dark and elevated light conditions. Effects of artificial light and anthropogenic noise varied among

species. All species studied exhibited alterations in either call dominant frequency, call duration, or call rate. At urban sites, most anuran species called from sites almost significantly or significantly darker than the general light environment. While most anurans preferred call sites darker than the surrounding environment, urban anuran populations had brighter call sites than rural anuran populations. In female mate choice experiments, female Green Treefrogs (*Hyla cinerea*) from a rural population preferred lower frequency calls under elevated light conditions. These results suggest anuran species may vary in their sensitivity and response to artificial light at night and anthropogenic noise.

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INTRODUCTION

Many human activities come with a cost to the environment. Through transportation, urbanization, and industrialization, humans have drastically altered the natural environment via light and noise pollution (Swaddle et al. 2015). However, biologists have only recently given attention to the widespread negative impacts artificial light at night and anthropogenic noise have on natural systems. In response to these anthropogenic pollutants, some animals have developed changes in essential behaviors; foraging, predator-prey interactions, orientation, territory defense, and mate attraction are all documented to be affected by artificial light at night and anthropogenic noise (Longcore and Rich 2004, Francis and Barber 2013). Alterations in these behaviors can be maladaptive, impacting the survival and fitness of not only individuals, but also populations, creating conservation concerns (Francis and Barber 2013, Swaddle et al. 2015). Further, light and noise pollution are highly correlated with each other, yet few studies have investigated the effects of artificial light at night and anthropogenic noise together (Swaddle et al. 2015; Fuller et al. 2007, Nordt and Klenke 2013, Da Silva et al. 2014, Dominoni et al. 2020, Hennigar et al. 2019). Even fewer studies have explored the interaction of artificial light at night and anthropogenic noise on anuran breeding systems (McMahon et al. 2017, Halfwerk et al. 2019).

Artificial Light at Night

Light pollution, or artificial light at night, results from human reliance on street, building, and vehicular lighting for nighttime activities (Gaston et al. 2013). Artificial light is highly concentrated in human population centers, but can extend hundreds of kilometers from its source into rural areas as skyglow, created by Rayleigh scattering or the scattering of light by natural or pollutant aerosols suspended in the lower atmosphere (Kyba et al. 2011, Gaston et al. 2017). Pollution from artificial light increases in both extent and intensity on average by 2.2% per year (Kyba et al. 2017). As of 2016, 83% of the world's population, including 99% of the U.S. and Europe, is considered to be living under light polluted skies (Falchi et al. 2016). Artificial light is commonly quantified by its illuminance, the intensity of light that illuminates a surface as perceived by the human eye, in units of lux (Rich and Longcore 2006). Lunar light typically varies by three orders of magnitude with clear moonless nights having an illuminance of 0.001 lux and full moon nights having an illuminance of 0.1-0.3 lux (Rich and Longcore 2006, Gaston et al. 2017). In comparison, artificial light can increase natural nighttime levels by several orders of magnitude with skyglow having an illuminance of 0.2-0.5 lux and anthropogenic light sources having illuminances ranging from tens to thousands of lux (Rich and Longcore 2006, Bennie et al. 2016, Gaston et al. 2017).

Artificial light at night disrupts a variety of behaviors across taxa. Temporally dependent behaviors like foraging and reproduction are affected by the extension of photoperiod created by artificial light (Gaston et al. 2017). Diurnal species of birds and

diurnal reptiles, such as those in the genus *Anolis*, can exploit this extension of photoperiod by feeding under lights to which their prey are attracted (Henderson and Powell 2001, Lebbin et al. 2007, Maurer et al. 2019). Alternatively, nocturnal mammals like bats and rodents reduce foraging when artificial light is present due to an increase in perceived risk of predation (Kramer and Birney 2001, Bird et al. 2004, Kuijper et al. 2008, Lewanzik and Voight 2014). Changes in the times at which animals forage can affect temporal niche partitioning, creating unoccupied niches and increasing the potential for competition within species (Rotics et al. 2011).

Studies investigating the effects of artificial light at night on reproduction have largely focused on birds. Artificial light causes many species of songbirds to begin singing minutes to hours prior to the rising of the sun (Miller 2006, Kempenaers et al. 2010, Da Silva et al. 2014). In a laboratory setting, urban European Blackbirds (*Turdus merula*) exposed to low levels of artificial light (0.3 lux) reach reproductive maturity earlier and stay reproductively capable for longer than their forest dwelling counterparts (Dominoni et al. 2013). When nesting near artificial lighting, Great Tit (*Parus major*) females lay eggs earlier and feed nestlings at a higher rate while males are more successful in obtaining extra pair copulations (Kempenaers et al. 2010, Titulaer et al. 2012).

In other species, artificial light can influence mate attraction and choice. Female Winter Moths (*Operophtera brumata*) are less likely to mate as male moths are less attracted to female sex pheromones in lighted conditions (van Geffen et al. 2015).

Additionally, female Australian Black Field Crickets (*Teleogryllus commodus*) raised under high intensity light (100 lux) exhibit weaker mating preferences while male crickets raised under high light levels are more likely to be selected as mating partners (Botha et al. 2017).

Lastly, artificial light at night has detrimental effects on orientation and navigation. Some species rely on natural light cues for these behaviors, but these cues can be masked by artificial light. Insects, sea turtle hatchlings, and migrating birds are all attracted to and become disoriented by artificial light, increasing mortality in these species (Tuxbury and Salmon 2005, Eisenbeis 2006, Gauthreaux and Belser 2006).

Anthropogenic Noise

Noise pollution is created by urban developments, transportation networks, and resource extraction sites (Barber et al. 2009). Out of these sources, transportation networks are the largest contributor to noise pollution as they have tripled in the U.S. since the 1970s (Barber et al. 2009, Mennitt et al. 2013). Similar to pollution created by artificial light at night, noise pollution is positively correlated with population density (Mennitt et al. 2013). However, rural and even protected lands are not truly quiet as traffic noise can travel up to 10 kilometers from its source and noise from aircraft can travel up to 40 kilometers (Mennitt et al. 2013). As a result, in the U.S., 88% of the human population and 63% of protected areas are subjected to artificially increased sound levels (Swaddle et al. 2015, Buxton et al. 2017). Loudness of sound is measured by

amplitude, or the pressure difference between a measured sound and the softest sound audible to humans (Bradbury and Vehrencamp 2011). To simplify this measurement, amplitude is reported on a logarithmic scale in units of decibels (dB) (Bradbury and Vehrencamp 2011). Noise from natural sources typically ranges from 8-69dB (Mennitt and Fristrup 2016). Noise from anthropogenic sources can increase these natural levels by up to 32dB (Mennitt et al. 2013).

Like artificial light at night, anthropogenic noise changes animal behavior. Changes in foraging and predator-prey interactions vary among species. In the Greater Mouse Eared Bat (*Myotis myotis*), foraging success decreases and search time increases when hunting in close proximity to roads due to the auditory masking of cues from prey (Siemers and Schaub 2011). Common prey animals like rodents, ungulates, and small passerine birds reduce foraging time and exhibit increased vigilance for predators, translating into energetic and fitness costs (Stockwell et al. 1991, Quinn et al. 2006, Rabin et al. 2006, Shannon et al. 2016). Alternatively, animals can become distracted by anthropogenic noise. When Three-Spined Stickelbacks (*Gasterosteus aculeatus*) are exposed to artificial noise, their foraging efficiency decreases because they have trouble acquiring food items due to a shift in attention to the noise stimuli (Purser and Radford 2011). Distraction of attention also makes prey species, such as Ambon Damsel fish (*Pomacentrus amboinensis*) and Caribbean Hermit Crabs (*Coendoita clypeatus*), more susceptible to predation as they allow predators to approach at a closer distance before retreating (Chan et al. 2010, Simpson et al. 2016).

Anthropogenic noise can alter reproductive behavior. The presence of noise pollution causes some individuals to exhibit plasticity in vocalizations used in acoustic communication. Songbirds will sing at higher frequencies or at night in an effort to overcome auditory masking of their songs (Slabbekoorn and Peet 2003, Wood and Yezerinac 2006, Fuller et al. 2007, Dowling et al. 2011). In addition, male Humpback Whales (*Megaptera novaeangliae*) will extend the duration of their courtship songs when they encounter interference from sonar (Miller et al. 2000). Vocalizations used during courtship can help strengthen pair bonds between mates. In the Zebra Finch (*Taeniopygia guttata*), female preference for their pair bonded mate weakens when the pair bond maintaining call is masked by artificial noise (Swaddle and Page 2007). Anthropogenic noise can inhibit courtship behavior altogether. Male Greater Sage Grouse (*Centrocercus urophasianus*) will decrease their attendance at leks in response to chronic anthropogenic noise at lek locations (Blickley et al. 2012). Lastly, anthropogenic noise can influence a male's willingness to defend his territory from intruding males. Male House Wrens (*Troglodytes aedon*) defend their territories more aggressively from simulated intruders as they have to approach the intruder at a closer distance to better assess the threat (Grabarczyk and Gill 2019). In contrast, male African Cichlids (*Astatotilapia burtoni*) show an increased latency to fight intruding males as they may view noisy territories as poor quality and not worth the risks of defense (Butler and Maruska 2020).

Other effects of anthropogenic noise include changes in population densities of animals and alterations in community structure. Migrating and breeding bird species

richness is diminished in environments subjected to noise from roads and resource extraction sites (Reijen et al. 1995, Francis et al. 2009, McClure et al. 2013). Changes in population densities can alter community structure, creating a cascading effect through the environment. For example, in Rattlesnake Canyon Habitat Management Area in New Mexico, noise from natural gas wells may mask prey cues, reducing nest predation of Black-Chinned Hummingbirds (*Archilocus alexandri*) by Western Scrub-Jays (*Aphelocoma californica*; Francis et al. 2012). As a result, areas with elevated sound levels have denser populations of hummingbirds and less dense populations of scrub-jays (Francis et al. 2012). These population density changes increase pollination by hummingbirds while reducing seed dispersal by scrub-jays, thus altering ecological services and community structure (Francis et al. 2012).

Study System

Anuran Breeding Systems

Breeding in anurans is, for the most part, dependent upon temperature, rainfall, and photoperiod (Saenz et al. 2006, Schalk and Saenz 2016). Some species also regulate their breeding behavior based on the phases of the lunar cycle (Grant et al. 2012, Underhill and Höbel 2018a). When conditions are right, explosive or prolonged breeding events will occur during which males will gather and produce calls to attract females. These species-specific calls vary in different parameters such as frequency, duration, pulse rate, and call rate (Pough et al. 2016). Females will evaluate males based upon

these parameters and choose a male whose call fits her preferences. After a female has chosen a male, the pair will join in amplexus.

Amphibian Decline

Amphibian populations are declining worldwide. One-third of known amphibian species are threatened with extinction (Stuart et al. 2004). Habitat loss, road kills, chemical pollution, ultraviolet radiation, disease, parasites, invasive species, exploitation, climate changes, or some combination of these factors are all accepted causes of amphibian decline (Fahrig et al. 1995, Alford and Richards 1999, Hels and Buchwald 2001, Beebee and Griffiths 2005, Rowley et al. 2013). However, understudied types of pollution such as that from artificial light at night and anthropogenic noise may also be contributing factors as the consequences are just starting to be uncovered.

Effects of Artificial Light at Night on Anuran Behavior

Research on the effects of artificial light at night on anuran behavior is lacking. What research does exist suggests that anurans are not immune to its effects. When exposed to red, as well as low and high intensity white light, the foraging behavior of Cope's Gray Treefrog (*Hyla chrysoscelis*) becomes impaired as they require more time to find and capture prey (Buchanan 1993). Conversely, Fowler's Toads (*Anaxyrus fowleri*) are attracted to light from streetlights at which they feed on the insects who are also attracted (Ferguson 1960). However, streetlights can impede the migration of some species to breeding sites, such as the Common Toad (*Bufo bufo*), who are less likely to cross roads illuminated by green or white light (van Grunsven et al. 2016). When

crossing roads and caught in the headlights of a car, some anuran species become immobile, increasing their chance of mortality (Mazerolle et al. 2005). Anuran breeding choruses subjected to acute artificial light show reduced calling effort and increased movement, opposite behaviors than those exhibited under ambient light conditions (Baker and Richardson 2006, Hall 2016). For some species, breeding in continuously lit areas can shift the timing of the breeding season entirely, resulting in males calling earlier in the year and more continuously throughout the night, ultimately shortening the length of the breeding season (Dias et al. 2019). Exposure to artificial light can weaken physical breeding behavior. When male Common Toads (*Bufo bufo*) are exposed to elevated light levels, they take longer to achieve amplexus and are more likely to separate from a female before fertilizing her eggs (Touzot et al. 2020). Further, artificial light can reduce activity levels in tadpoles, making them more susceptible to biotic stressors such as parasites (May et al. 2019).

Effects of Anthropogenic Noise on Anuran Behavior

Exposure to anthropogenic noise changes anuran behavior. Many studies have focused on behavioral changes caused by traffic noise. Crawfish Frogs (*Lithobates areolatus*) will produce non-reproductive calls from their burrows in response to aircraft and traffic, possibly mistaking the noise for the calls of other males (Engbrecht et al. 2015). Marsh Frogs (*Pelophylax ridibundus*) exposed to traffic noise will walk instead of jump and transverse shorter distances than when in quiet conditions (Lukanov et al. 2014). In larval anurans, the presence of traffic noise decreases foraging efficiency and

increases activity level, potentially making them more vulnerable to predation (Castaneda et al. 2020).

Anthropogenic noise largely impacts anuran breeding behavior. Traffic noise can mask breeding choruses, making it harder for females to detect and travel to them (Bee and Swanson 2007, Tennessen et al. 2014, Senzaki et al. 2018). In response to this acoustic masking, many anuran species will modulate the parameters of their calls or reduce calling effort altogether (Sun and Narins 2005, Lengagne 2008, Kaiser and Hammers 2009, Parris et al. 2009, Cunnington and Fahrig 2010, Hanna et al. 2014). How anurans modulate their calls in response to anthropogenic noise varies by species. Green Frogs (*Lithobates clamitans*) and Northern Leopard Frogs (*Lithobates pipiens*) living in areas where traffic noise exceeds 60dB produce calls with higher dominant frequencies (Cunnington and Fahrig 2010). Spring Peepers (*Pseudacris crucifer*) exposed to low and high frequency noise will shorten the duration of their calls (Hanna et al. 2014). Alternatively, Oak Toads (*Anaxyrus quercicus*) increase the duration of their calls when road noise is present (Grace and Noss 2018). In Thailand, anuran species such as *Microhyla butleri*, *Rana nigrovittata*, and *Kaloula pulchra* decrease their call rate while the species *Rana taipehensis* increases its call rate during periods of elevated noise levels caused by aircraft and motorcycles (Sun and Narins 2005). Other anuran species are unable to modify their call parameters to compensate for acoustic masking. Pacific Chorus Frogs (*Pseudacris regilla*) lower the frequency of their calls in response to road noise, increasing the likelihood that their calls will be masked (Nelson et al. 2017). As an

alternative way to overcome the acoustic masking of their calls, some anuran species exhibit gap calling behavior, calling only when noise intensity is low and ceasing to call when noise intensity increases (Vargas-Salinas and Amézquita 2013, Vargas-Salinas et al. 2014). To further reduce interference from traffic noise during breeding, some anuran species may reduce their abundance near noisy roads or limit the number of days and amount of time spent at choruses (Eigenbrod et al. 2009, Kaiser et al. 2011, Grace and Noss 2018).

Objectives

The main purpose of this study is to investigate the effects of artificial light at night and anthropogenic noise pollution on anuran breeding systems in eastern Texas. Specific objectives include (1) determine if artificial light at night and anthropogenic noise pollution alter calling behavior in male anurans, (2) determine if artificial light at night influences male call site selection, and (3) determine if artificial light at night influences female mate choice.

Hypotheses and predictions:

Objective 1: Calling Behavior

H₀: Artificial light at night and anthropogenic noise do not alter calling behavior in male anurans.

H_a: Artificial light at night and anthropogenic noise do alter calling behavior in male anurans.

Prediction: Male anurans living at bright and noisy sites will exhibit alterations in call parameters such as dominant frequency, call duration, pulse rate, and call rate.

Objective 2: Male Call Site Selection

H₀: Artificial light at night does not influence male call site selection.

H_a: Artificial light at night does influence male call site selection.

Prediction: Male anurans living at sites with higher environmental light levels will call from sites darker than the general light environment.

Objective 3: Female Mate Choice

H₀: Female mate choice is not influenced by artificial light at night.

H_a: Female mate choice is influenced by artificial light at night.

Prediction: Artificial light at night may influence female mate choice in several ways.

Under higher levels of artificial light, female anurans may be less choosy, more choosy, exhibit no change in their natural mate choice behavior, or refuse to choose a mate.

MATERIALS AND METHODS

Environmental Data

Study Sites

Five study sites were selected in the Nacogdoches, TX, USA area based on their variation in exposure to sources of artificial light and anthropogenic noise: Pecan Park, the Clint Dempsey Soccer Complex, the former horse track, Alazan Bayou Wildlife Management Area, and the Stephen F. Austin Experimental Forest (Figure 1). Based on pilot observations, these sites were classified *a priori* as bright, intermediate, or dark and noisy, intermediate, or quiet. Bright and noisy sites included Pecan Park and the Clint Dempsey Soccer Complex. The horse track was considered intermediate in both light and noise pollution. Alazan Bayou Wildlife Management Area was considered intermediate in light pollution and quiet in noise pollution. The Stephen F. Austin Experimental Forest was considered a dark and quiet site.

Measuring Artificial Light at Night

To estimate the general light level (lux) of each site, 30 random locations (latitude and longitude) were generated using the Geomidpoint Random Point Generator (<http://www.geomidpoint.com/random/>). The starting point for generating the randomized points was selected by randomly selecting a location (latitude and longitude)

within the area occupied by previously observed frog choruses. The maximum distance was set to 0.1 km when generating the randomized points to keep the points within the intended study area while accounting for variation in habitat. Each randomized point was located using GPS and the light level was measured using a PCE-L 100 lux meter (PCE Americas Inc., Jupiter, FL, USA). Light level measurements were measured on multiple nights to account for variation in light levels from moonlight, cloud cover, and anthropogenic sources.

At all sites, light levels were also measured at specific call sites of recorded male frogs (see below) once frogs had been captured after recording. This provided an estimate of the light microhabitat the frogs were experiencing.

Measuring Anthropogenic Noise

Sound levels (dB) were measured using a PCE-428 sound level meter (PCE Americas Inc., Jupiter, FL, USA) at the same randomized points used for light level measurements. Due to the sensitivity of this instrument, decibel levels fluctuated during sampling, so sound was measured for 30 seconds and the most constant value during that time was recorded. Sound levels were recorded on multiple nights to account for sound level variation from anthropogenic sources. Sound levels were also measured during the time of night when frogs would normally call, but on nights when frogs were not calling to ensure that frog choruses did not contribute to the measurements.

Statistical Analysis

Light and sound level measurements obtained from the randomized points were averaged to provide a generalized light and sound level for each site. A permutation ANOVA was used to determine if generalized light levels and generalized sound levels differed between all sites. Permutation tests (10,000 iterations) with sequential Bonferroni adjustment (Rice 1988) were then used to conduct pairwise comparisons between individual sites. An outlier of 167 lux measured at the Clint Dempsey Soccer Complex was excluded from the light analysis as it largely skewed the data.

To determine whether male frogs were calling from sites that differed in light level from the general light environment, permutation tests (10,000 iterations) were used to compare the light levels of male call sites to light levels of random point locations. Permutation tests (10,000 iterations) with sequential Bonferroni adjustment (original $\alpha = 0.05$) were also used to determine if light levels of male call sites varied within species among different sites. For all statistical tests performed, p-values ranging from 0.05-0.1 were considered biologically significant (MacLeod et al. 2018). Environmental data comparisons and male call site data comparisons were conducted using the PopTools extension (Hood 2010) for Microsoft Excel (Microsoft Corporation, Redmond, WA, USA).

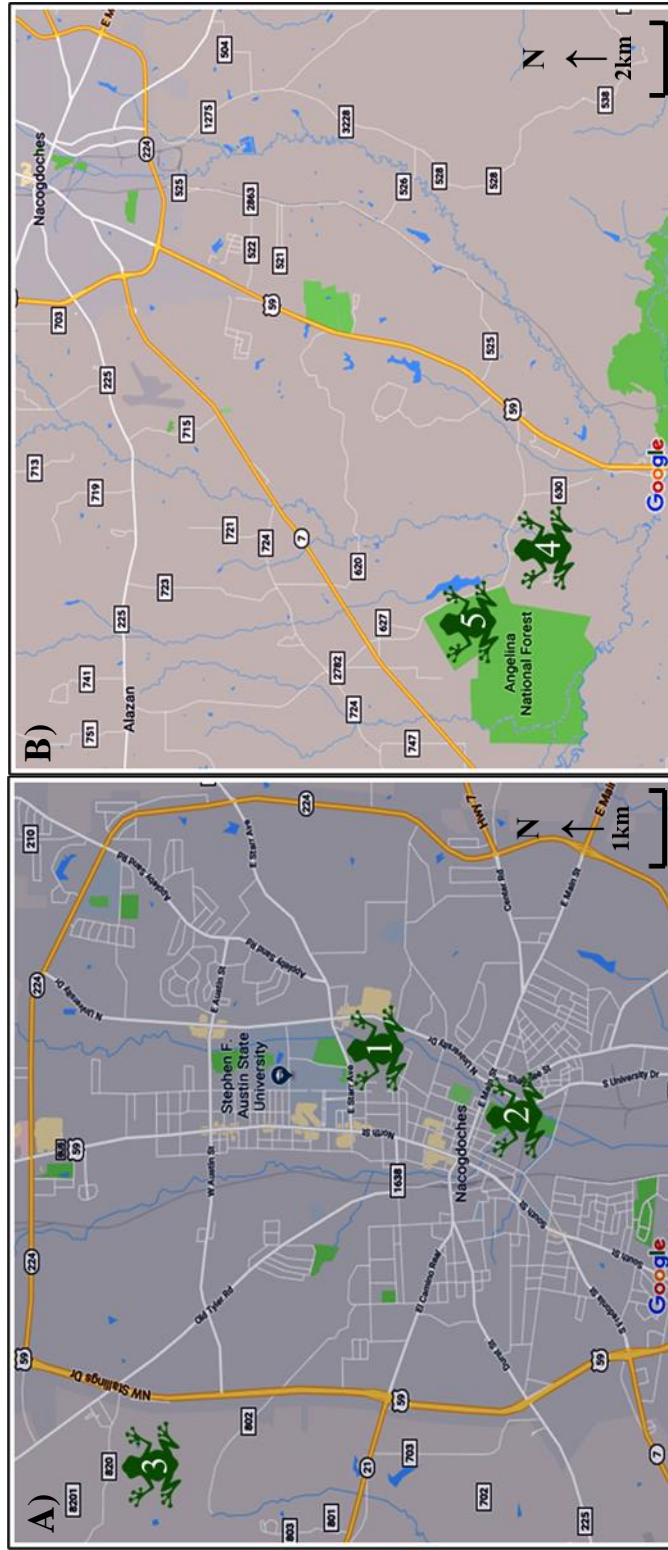


Figure 1. A) Map showing approximate locations of urban sites located within the city limits of Nacogdoches. Frog 1 is Pecan Park. Frog 2 is the Clint Dempsey Soccer Complex. Frog 3 is the former horse track. B) Map showing approximate locations of rural sites located 24 kilometers outside of the city of Nacogdoches. Frog 4 is Alazan Bayou Wildlife Management Area. Frog 5 is the Stephen F. Austin Experimental Forest.

Study Species

Data were collected for Spring Peepers (*Pseudacris crucifer*), Gray Treefrogs (*Hyla versicolor*), Green Treefrogs (*Hyla cinerea*), and Gulf Coast Toads (*Incilius nebulifer*). These species were selected based on their abundance at sites and published studies on male call parameters and female mate choice preferences. Data were recorded for Cope's Gray Treefrog (*Hyla chrysoscelis*) at one site, the Clint Dempsey Soccer Complex, after this species was mistaken for *Hyla versicolor*. Because data were available for only one population of this species, it was only included in the male call site vs general light environment analysis.

Call Recording and Analysis

Between 2018 and 2020, calling male frogs were recorded using a Sennheiser ME66 shotgun microphone (Sennheiser Electronic Corporation, Old Lyme, CT, USA) and a Marantz Professional PMD661 MKII solid state recorder (Marantz Professional, Cumberland, RI, USA) for a minimum of 10 calls. Since call parameters such as pulse rate and call rate are temperature dependent, body temperature was measured using a FLUKE 572 IR thermometer (Fluke Corporation, Everett, WA, USA) immediately after frogs were recorded. Substrate temperature of the frog's calling location was also recorded. Frogs were then caught by hand and placed in individual containers containing a small amount of water. Frogs were transported to the lab in the Department of Biology at Stephen F. Austin State University. In the lab, frogs were gently restrained so mass (g)

and snout-vent length (SVL) (mm) measurements could be taken. Frogs were recorded on multiple nights, so to avoid duplicating data, frogs were marked by injecting colored elastomer (Northwest Marine Technology, Shaw Island, WA, USA) between their toes, a common technique approved by the American Society of Ichthyologists and Herpetologists (ASIH) (Beaupre et al. 2004). Frogs were released at the site of capture within 72 hours. Recorded calls were later analyzed for the following call parameters: dominant frequency (Hz), call duration (s), pulse rate (pulses/s) (*Hyla versicolor* only), and call rate (calls/s) using Raven Pro 1.5 (Cornell Lab of Ornithology, Ithaca, NY, USA).

Statistical Analysis

Since call parameters can be dependent on temperature and body size (SVL), each call parameter for each species was tested for significance against these variables using linear regression (Microsoft Excel, Microsoft Corporation). For *Pseudacris crucifer*, call parameters were temperature adjusted to 15°C. For *Hyla versicolor*, *Hyla cinerea*, and *Incilius nebulifer*, call parameters were temperature adjusted to 25°C, with the exception of *H. cinerea* call duration (adjusted to 20°C). Temperature adjustments were based on the equation used by Platz and Forester (1988). In this equation, ambient temperature was replaced with body temperature so that $C_{25} = C_{\text{original}} - (T_{\text{body}} - 25.0)(\text{regression slope})$. “C” was the call parameter being adjusted, “C_{original}” was the original measured value of the call parameter, “T_{body}” was body temperature, and the regression slope was the slope of the linear regression testing the call parameter by temperature. If there was a

significant relationship between a call parameter and SVL, temperature adjusted values were then adjusted to population mean body size. This was done by substituting body size measurements for temperature in the temperature adjustment equation so that $C_{SVL} = C_{TempAdj} - (I_{SVL} - P_{SVL})(\text{regression slope})$. “ $C_{TempAdj}$ ” was the value of the temperature adjusted call parameter, “ I_{SVL} ” was an individual’s SVL, “ P_{SVL} ” was the population mean SVL, and the regression slope was the slope of the linear regression testing the call parameter by SVL. If a call parameter had a significant relationship with SVL in at least one population, then the parameter was body size adjusted for all populations.

Hyla cinerea calls are biphasic, exhibiting a low frequency phase (640-960 Hz) and a high frequency phase (2340-3450 Hz; Oldham and Gerhardt 1975). In all *H. cinerea* populations, for a select number of males (Pecan Park $n = 4$; Clint Dempsey Soccer Complex $n = 1$; Horse Track $n = 5$; Alazan Bayou Wildlife Management Area $n = 4$; Stephen F. Austin Experimental Forest $n = 1$), Raven Pro 1.5 classified the lower frequency phase to be the dominant frequency, resulting in abnormally low values compared to all other males. Hence, these males were considered outliers and excluded from call parameter analysis.

To determine if there was variation in male call parameters, a PERMANOVA was performed for each call parameter for each species using PAST v4.03 (Hammer 2001). Pairwise comparisons with sequential Bonferroni adjustment (original $\alpha = 0.05$) were used to determine differences in call parameters between populations. Call parameter

PERMANOVA results obtained from PAST v4.03 (Hammer 2001) were comparable to permutation test results obtained from PopTools (Hood 2010).

Female Mate Choice Experiments

Between 2019 and 2020, amplexant pairs were collected and placed in individual containers containing a small amount of water. Pairs were transported to Stephen F. Austin State University so that female mate choice experiments could be conducted in a sound chamber (2.7 m x 3.0 m; Industrial Acoustics Company Inc., Bronx, NY, USA) housed in the Department of Psychology. Female frogs were subjected to a dark treatment and a light treatment. All females were kept in the dark while being transported from the field until testing began (~1 hour later) and were tested under dark conditions before being tested under elevated light conditions. No artificial light was used during the dark treatment, although a small amount of light from the video camera (see below) was present. For the light treatment, females were given up to 30 minutes to acclimate to the elevated light conditions after the light was turned on. Females were kept in the sound chamber during testing to maintain adaptation of their eyes. Light pollution was simulated by a 16-watt LED shop light (Toggled, Troy, MI, USA) suspended over the center of the arena. The light bulb was wrapped in light filtering film (Rosco Roscolux Sheet 398 Neutral Gray, Stage Lighting Store, Jacksonville, FL, USA) and controlled by a dimmer switch in order to make the light level in the chamber approximately 2 lux, a light level based off of pilot data from an urban site (Pecan Park). To try to avoid side

bias, the light level in the chamber was measured at the female release point and speaker locations to make sure it was uniform.

During a trial, the female frog was separated from the male and placed under a clear plastic container located on one side of the chamber. She was played two male calls that varied in their call parameters from Micca MB42X speakers (Highland Technologies Ltd., Hong Kong, China) located on the opposite side of the chamber and angled towards the female release point. Different anuran species exhibit preferences for different call parameters, so the call parameter used varied by species. *Hyla versicolor* and *Incilius nebulifer* females were given a choice between short and long duration calls (*H. versicolor*: Klump and Gerhardt 1987, *I. nebulifer*: Wagner Jr. and Sullivan 1995). *Hyla cinerea* females were given a choice between low (750 Hz) and high (2718 Hz) frequency calls (Gerhardt 1987). Calls were natural, previously recorded calls that were modified for the extremes of the call parameter being used, while controlling for other call parameters. Calls were modified using Adobe Audition (Adobe Systems Inc., San Jose, CA, USA). Calls were played at 85 dB measured at the female release point to mimic a natural setting. To try to control for side bias, calls were randomly assigned to speakers between trials by flipping a coin.

Trial criteria were based on Laird et al. (2016). After a 2-5 minute acclimation period, the container was lifted and the female was given up to 10 minutes to choose a male call of her preference. A choice was considered if a female touched a speaker for at least 3 seconds. The trial was scored as “no choice” if the female climbed a wall of the

chamber, remained immobile for 2 minutes, or failed to choose a speaker within 10 minutes. All trials were recorded with a Sony Handycam FDR-AX100 4K Ultra HD camcorder (Sony Corporation, Tokyo, Japan) located inside the sound chamber. After trials were completed, the female was returned to the container with the male she was paired with. Pairs were weighed, measured for SVL, and marked with elastomer. Pairs were kept for no more than 72 hours and were released at the site at which they were captured.

All research activities were conducted with permission from Texas Parks and Wildlife, the U.S. Forest Service, and the Nacogdoches Police Department. Collection of frogs was permitted by Texas Parks and Wildlife under Scientific Research Permit No. SPR-0518-169. All methods were approved by Stephen F. Austin State University's Institutional Animal Care and Use Committee (Protocol #2018-002).

Statistical Analysis

Binomial tests were used to determine the probability of a female choosing one variation of a call parameter over the other for both the dark and light treatments. The probability of a female changing her preference between treatments was also determined. If a female did not respond in a trial, then the trial was not included in the analysis. Binomial tests were performed using an online binomial test calculator (<https://www.socscistatistics.com/tests/binomial/default2.aspx>).

RESULTS

Environmental Data

Artificial Light at Night

Mean (\pm SE) environmental light levels were 1.035 (\pm 0.499) lux at Pecan Park, 3.377 (\pm 1.431) lux at the Clint Dempsey Soccer Complex (soccer fields), 0.012 (\pm 0.002) lux at the horse track, 0.027 (\pm 0.037) lux at Alazan Bayou Wildlife Management Area (Alazan), and 0.003 (\pm 0.001) lux at the Stephen F. Austin Experimental Forest (SFAEF; Figure 2A, 2B). A permutation ANOVA confirmed that environmental light levels differed significantly among all five sites ($p = 0.001$). Light levels did not differ significantly between Pecan Park and the soccer fields, but were significantly brighter at Pecan Park than at the horse track, Alazan, or the SFAEF (Appendix Table A1). Light levels were also significantly brighter at the soccer fields than at the horse track, Alazan, and the SFAEF. Light levels at the horse track were significantly darker than those at Alazan and significantly brighter than light levels at the SFAEF. Light levels were also significantly brighter at Alazan than at the SFAEF.

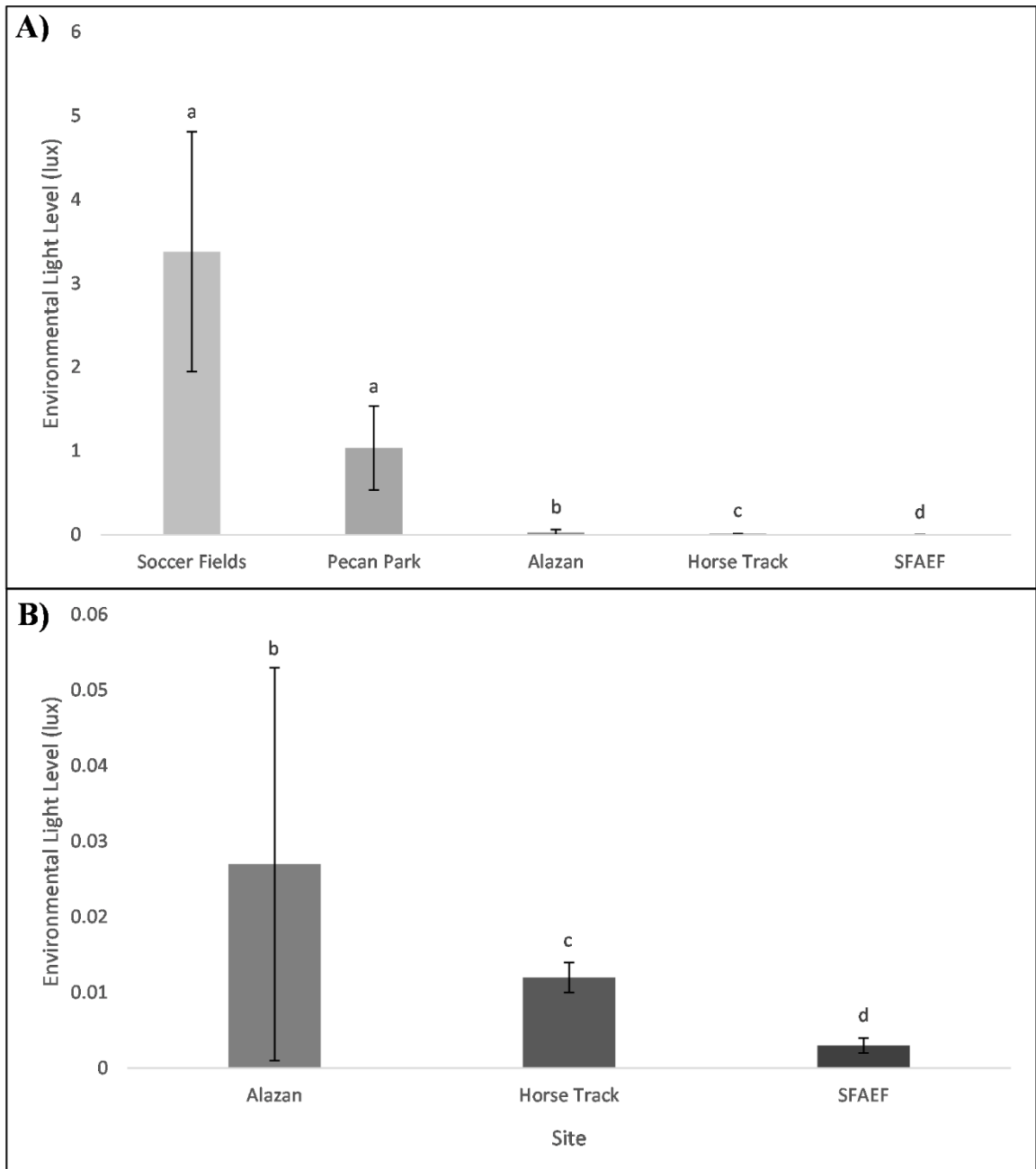


Figure 2. A) Mean (\pm SE) environmental light levels in lux by site. Sites are arranged from brightest to darkest light level. **B)** Close up of mean (\pm SE) environmental light levels for intermediate and dark sites. Sites are arranged from brightest to darkest light level. See Appendix Table A1 for p-values of pairwise comparisons.

Anthropogenic Noise

Mean (\pm SE) sound levels were 60.5 (\pm 0.3) dB at Pecan Park, 71.3 (\pm 0.4) dB at the soccer fields, 59.8 (\pm 0.8) dB at the horse track, 56.6 (\pm 0.4) dB at Alazan, and 57.2 (\pm 0.3) dB at the SFAEF (Figure 3). A permutation ANOVA determined that sound levels differed significantly among all five sites ($p < 0.0001$). Sound levels at Pecan Park were significantly quieter than sound levels at the soccer fields, did not differ from sound levels at the horse track, and were significantly louder than sound levels at Alazan and the SFAEF (Appendix Table A2). Sound levels at the soccer fields were significantly louder than those at the horse track, Alazan, and the SFAEF. The horse track was significantly louder than Alazan and the SFAEF. Sound levels did not differ between Alazan and the SFAEF.

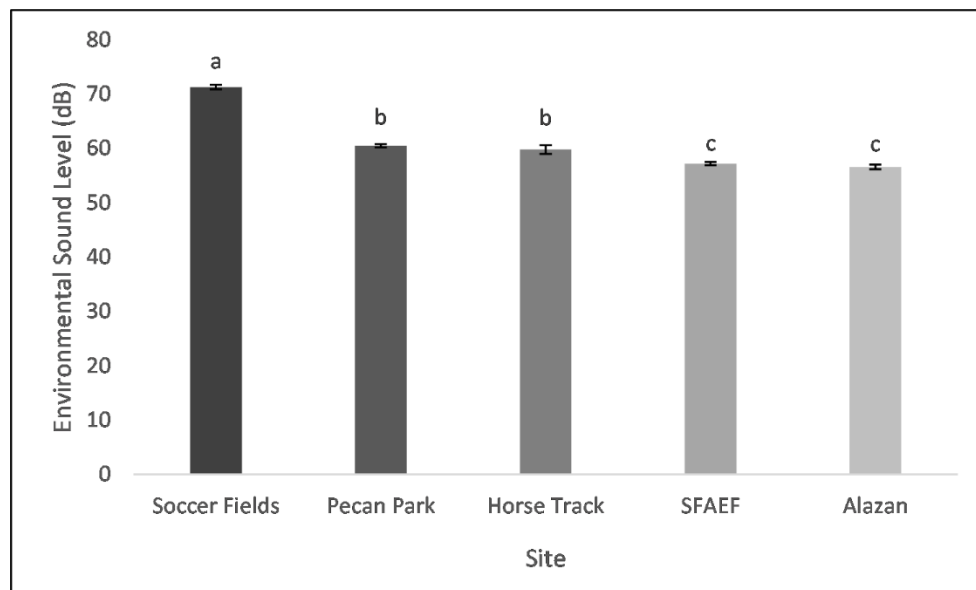


Figure 3. Mean (\pm SE) environmental sound levels in decibels (dB) by site. Sites are arranged from loudest to quietest site. See Appendix Table A2 for p-values of pairwise comparisons.

Male Call Sites

Male Call Sites vs General Light Environment

Permutation tests revealed that *Pseudacris crucifer* called from sites that were close to significantly darker than the general light environment at Pecan Park (Figure 4C, 4D) and the soccer fields (Figure 4A, 4B), and sites that were significantly brighter than the general light environment at the horse track (Figure 4F). In contrast, *P. crucifer* at the SFAEF did not call from sites with light levels that differed from those of the general light environment (Figure 4G). At the soccer fields, *Hyla chrysoscelis* called from sites that were almost significantly darker than the general light environment (Figure 4A, 4B). *Hyla versicolor* call site light levels were significantly darker than the general light environment at Alazan (Figure 4E) and close to significantly darker than the general light environment at the SFAEF (Figure 4G). *Hyla cinerea* from the Alazan population called from sites that were significantly darker than the general light environment (Figure 4E). However, *H. cinerea* from the Pecan Park (Figure 4C, 4D), soccer field (Figure 4A, 4B), horse track (Figure 4F), and SFAEF (Figure 4G) populations did not call from sites that differed in light level from the general light environment. *Incilius nebulifer* called from sites that were significantly darker than the general light environment at both Pecan Park (Figure 4C, 4D) and Alazan (Figure 4E).

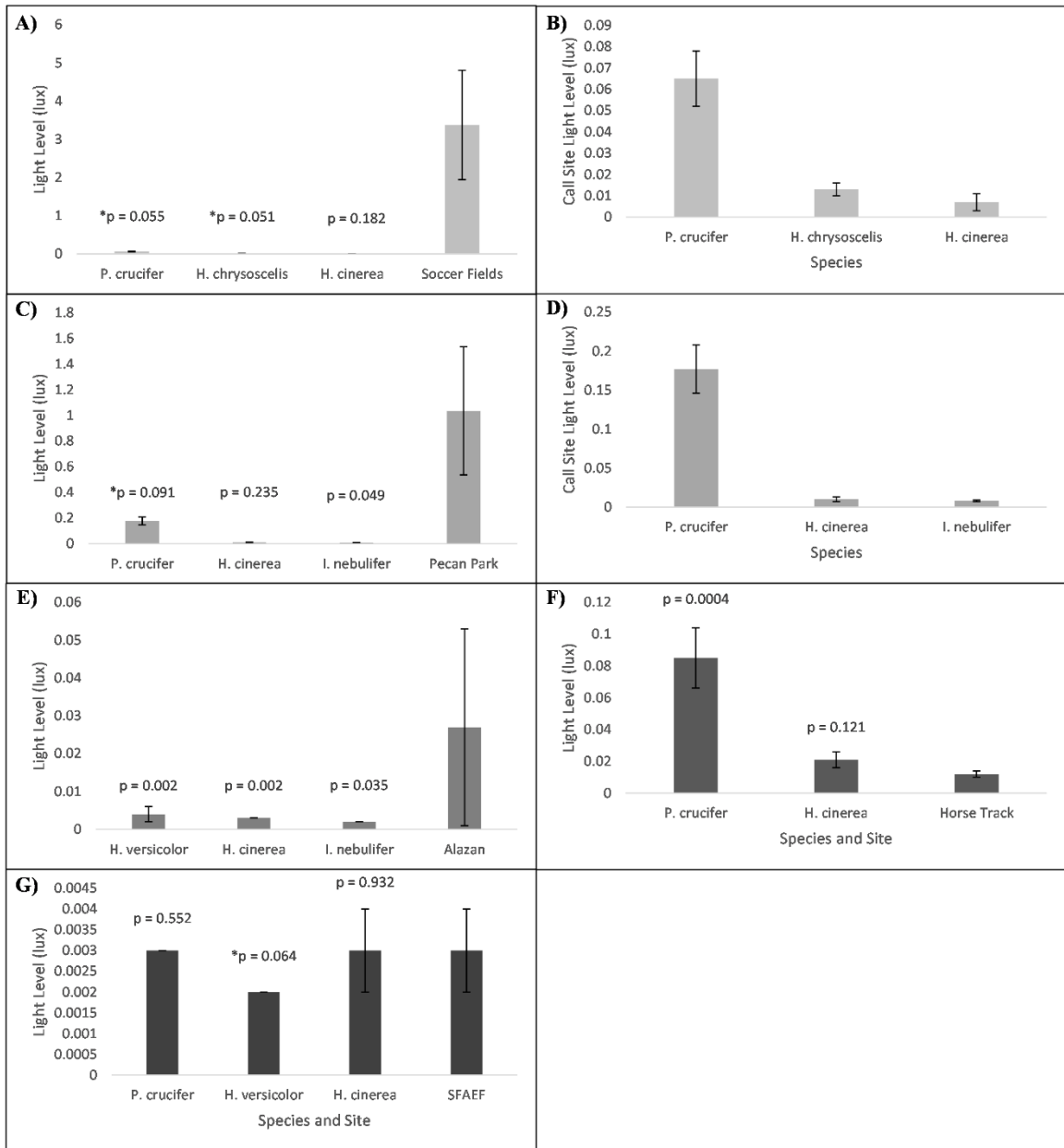


Figure 4. Mean (\pm SE) male call site levels in comparison to the general light environment in lux at **A)** the soccer fields with **B)** close up of male call site light levels, **C)** Pecan Park with **D)** close up of male call site light levels, **E)** Alazan, **F)** the horse track, and **G)** the SFAEF. Study sites are arranged from brightest to darkest site. P-values indicate the significance between the male call site light level of a species and the general light environment. P-values marked with an asterisk are significant at the biological significance level.

Comparison of Call Sites Among Populations

Pseudacris crucifer at Pecan Park called from sites that were significantly brighter than the call sites of the soccer field, horse track, and SFAEF populations. *Pseudacris crucifer* call site light levels did not differ between the soccer field and horse track populations. However, both the soccer field and horse track populations called from sites that were significantly brighter than the call sites of the SFAEF population (Figure 5A; Appendix Table A3). Light levels of *Hyla versicolor* call sites did not differ between the Alazan and SFAEF populations ($p = 0.229$; Figure 5B). Light levels of *Hyla cinerea* call sites did not differ between the Pecan Park, soccer field, or horse track populations, but the Pecan Park population called from sites that were significantly brighter than the call sites of the Alazan and SFAEF populations. Call site light levels also did not differ between the soccer field, horse track, or SFAEF *H. cinerea* populations, but the soccer field population called from significantly brighter sites than the Alazan population. The horse track population called from sites that were significantly brighter than the call sites of the Alazan and SFAEF populations. *Hyla cinerea* from the Alazan and SFAEF populations did not call from sites that differed in light level (Figure 5C, Appendix Table 4A). *Incilius nebulifer* at Pecan Park called from brighter sites than at Alazan ($p = 0.002$; Figure 5D).

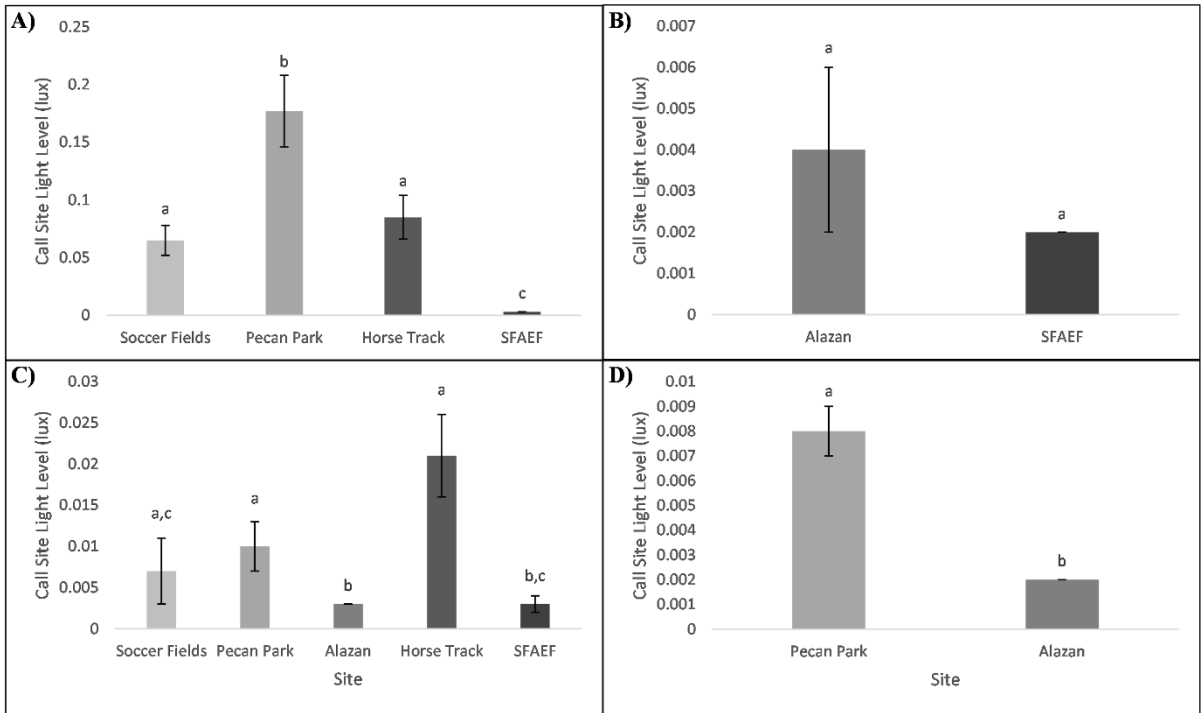


Figure 5. Mean (\pm SE) male call site light levels among **A)** *Pseudacris crucifer*, **B)** *Hyla versicolor*, **C)** *Hyla cinerea*, and **D)** *Incilius nebulifer* populations. Means with different letters are significantly different. Populations are arranged from brightest to darkest site. See Appendix Tables A3 and A4 for p-values of pairwise comparisons for *P. crucifer* and *H. cinerea* populations.

Male Call Variables

Pseudacris crucifer

Pseudacris crucifer were recorded at Pecan Park ($n = 30$), the soccer fields ($n = 21$), the horse track ($n = 18$), and the SFAEF ($n = 20$). Dominant frequency differed significantly among males from all four populations ($p = 0.0001$; Figure 6A). Males at Pecan Park called at significantly lower dominant frequencies than males at the SFAEF and at almost significantly lower dominant frequencies than males at the soccer fields

(Appendix Table A5). Dominant frequencies did not differ between the Pecan Park and horse track populations. Males from the soccer field population did not differ in dominant frequency from males of the horse track population, but called at significantly lower dominant frequencies than males from the SFAEF population. Males from the horse track population also called at significantly lower dominant frequencies than males from the SFAEF population.

Call duration did not differ among populations ($p = 0.220$; Figure 6B). However, pairwise comparisons showed that males from the horse track population had close to significantly longer calls than males from the SFAEF population (Appendix Table A6).

Call rate differed significantly among all populations ($p = 0.030$; Figure 6C). Pecan Park males called at significantly faster rates than males at the SFAEF, but not males at the soccer fields or horse track (Appendix Table A7). Similarly, males from the soccer fields called at significantly faster rates than males at the SFAEF, but not males at the horse track. Call rates of the horse track population did not differ from those of the SFAEF population.

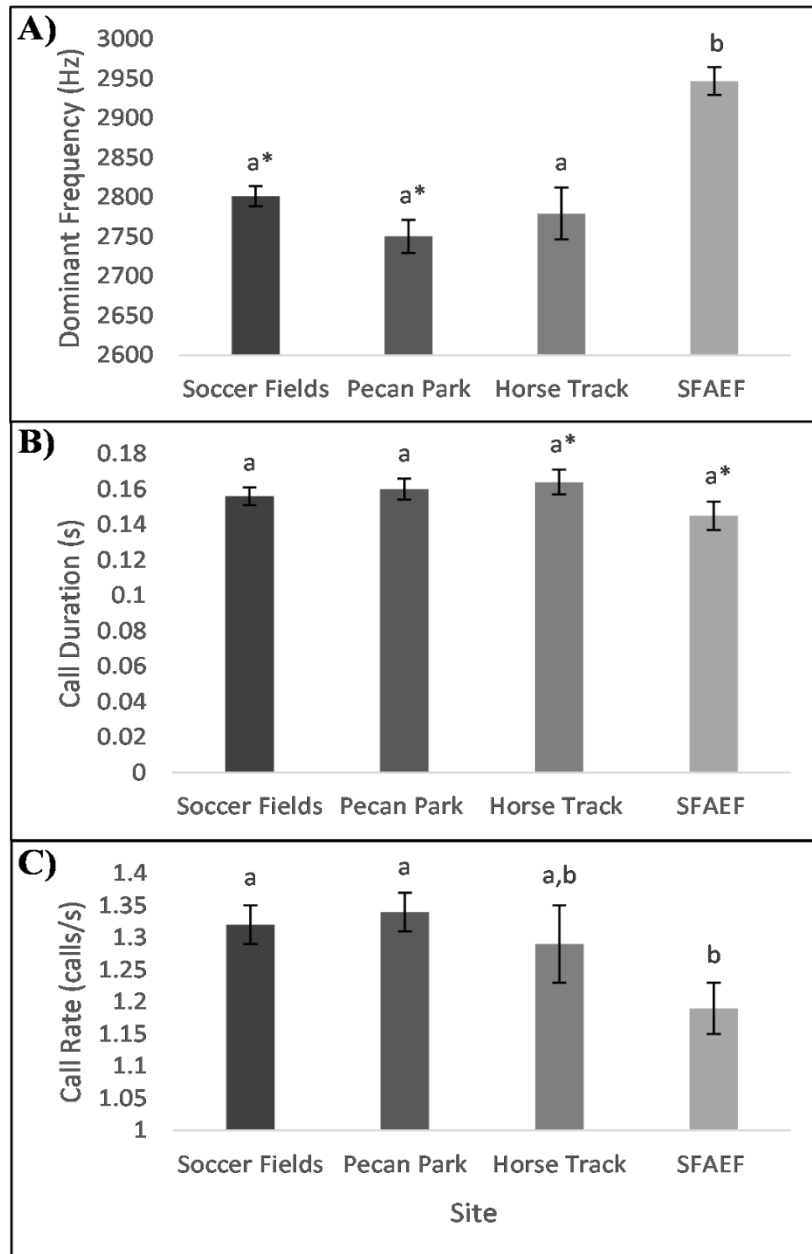


Figure 6. Mean (\pm SE) **A)** dominant frequency (Hz), **B)** call duration (s), and **C)** call rate (calls/s) for *Pseudacris crucifer* populations. Means with different letters are significantly different. Means with letters marked with an asterisk differ from each other at the biological significance level. Populations are arranged from loudest to quietest site. See Appendix Tables A5-A7 for p-values of pairwise comparisons.

Hyla versicolor

Hyla versicolor were recorded at Alazan ($n = 30$) and the SFAEF ($n = 30$).

Dominant frequency differed significantly between these populations with males from Alazan calling at higher dominant frequencies than males at the SFAEF ($p = 0.0001$; Figure 7A). There were no significant differences in call duration ($p = 0.894$; Figure 7B), pulse rate ($p = 0.339$; Figure 7C), or call rate ($p = 0.772$; Figure 7D) between these populations.

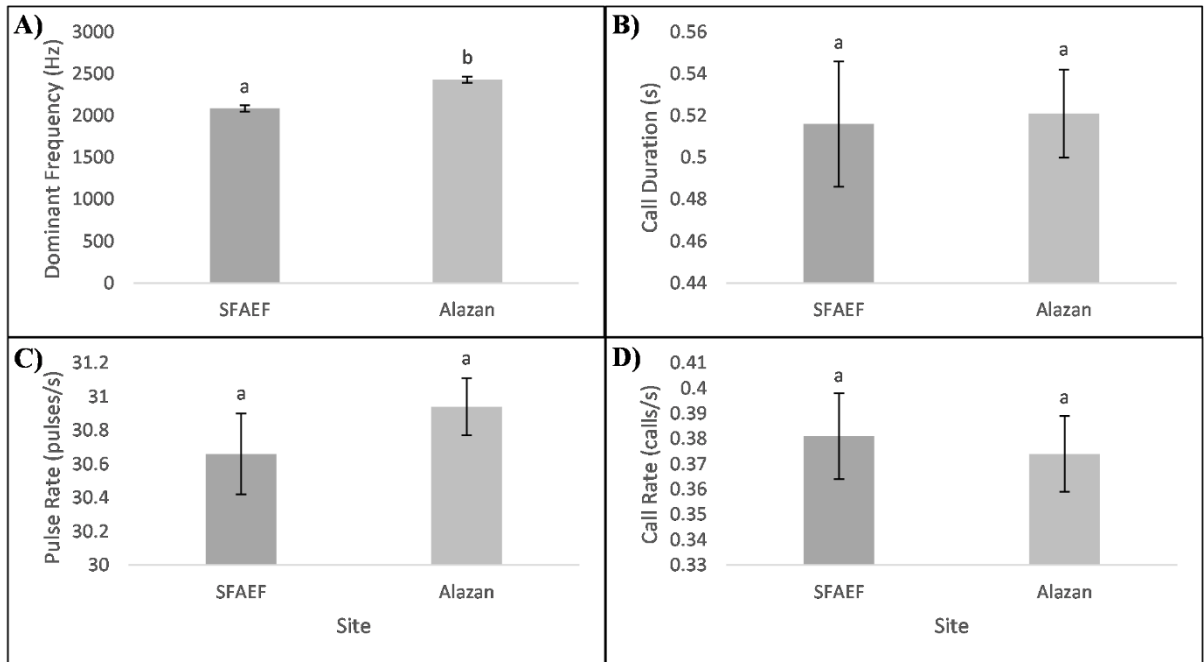


Figure 7. Mean (\pm SE) **A)** dominant frequency (Hz), **B)** call duration (s), **C)** pulse rate (pulses/s), and **D)** call rate (calls/s) for *Hyla versicolor* populations. Means with different letters are significantly different. Populations are arranged from loudest to quietest site.

Hyla cinerea

Hyla cinerea were recorded at Pecan Park ($n = 9$), the soccer fields ($n = 9$), horse track ($n = 29$), Alazan ($n = 26$), and the SFAEF ($n = 14$). Dominant frequency was not significantly different among all populations ($p = 0.147$; Figure 8A). However, males at Pecan Park and males at the horse track called at dominant frequencies almost significantly lower than males at Alazan (Appendix Table A8).

Call duration differed significantly among all populations ($p = 0.0001$; Figure 8B). Males from the Pecan Park population had significantly shorter calls than males from the soccer field, horse track, Alazan, and SFAEF populations (Appendix Table A9). Call duration did not differ between soccer field and horse track males, but soccer field males had significantly longer calls than males from the Alazan and SFAEF populations. Males from the horse track population and Alazan population did not have differences in call duration, but horse track males had significantly longer calls than males at the SFAEF. Call duration did not differ between Alazan and the SFAEF populations.

Call rate also differed significantly among all populations ($p = 0.0001$; Figure 8C). Males from the Pecan Park population called at significantly faster rates than males from the soccer field and Alazan populations, but there was no difference in call rate between Pecan Park males and males from the horse track or SFAEF populations (Appendix Table A10). The soccer field population had a significantly slower call rate than the horse track, Alazan, or SFAEF populations. There was no difference in call rate

between the horse track and Alazan or SFAEF populations. There was also no difference between the Alazan and SFAEF populations.

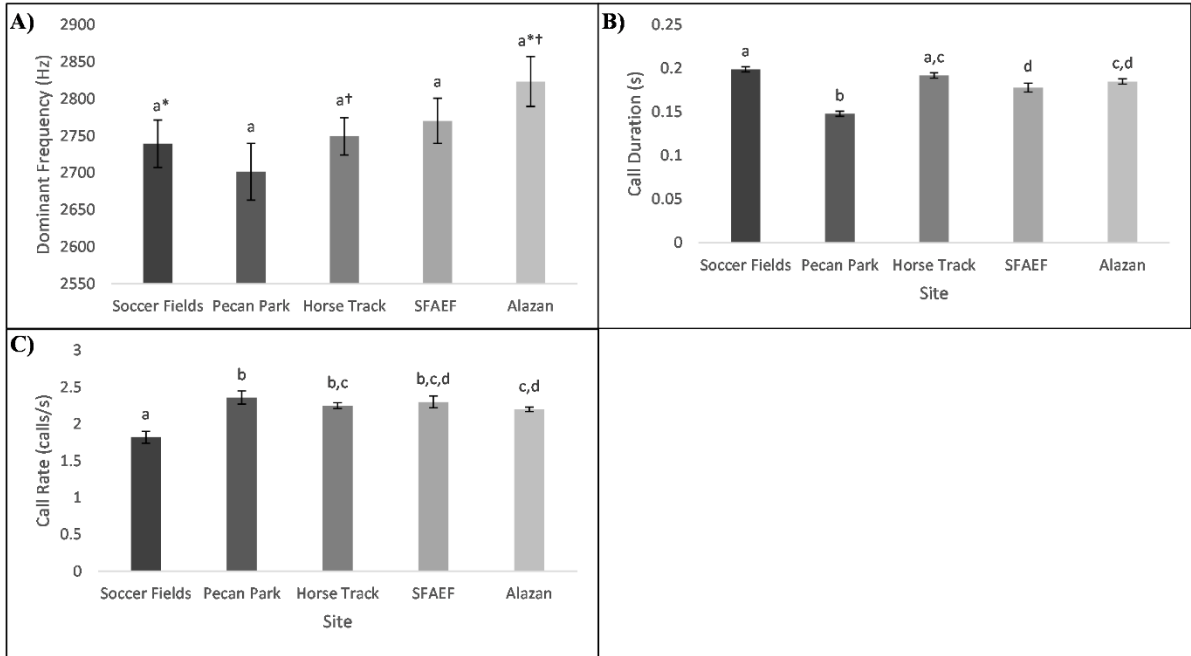


Figure 8. Mean (\pm SE) **A)** dominant frequency (Hz), **B)** call duration (s), and **C)** call rate (calls/s) for *Hyla cinerea* populations. Means with different letters are significantly different. The soccer field and Pecan Park populations had lower frequency calls than the Alazan population at the biological significance level, indicated by an asterisk and dagger symbol respectively. Populations are arranged from loudest to quietest site. See Appendix Tables A8-A10 for p-values of pairwise comparisons.

Incilius nebulifer

Incilius nebulifer were recorded at Pecan Park ($n = 30$) and Alazan ($n = 11$).

There was a significant difference in dominant frequency between these populations with males at Pecan Park calling at significantly higher dominant frequencies than males at Alazan ($p = 0.0001$; Figure 9A). Call rate also differed significantly, with males at Pecan Park calling at a significantly slower rate than males at Alazan ($p = 0.016$; Figure 9C). There was no difference in call duration between the two populations ($p = 0.246$; Figure 9B).

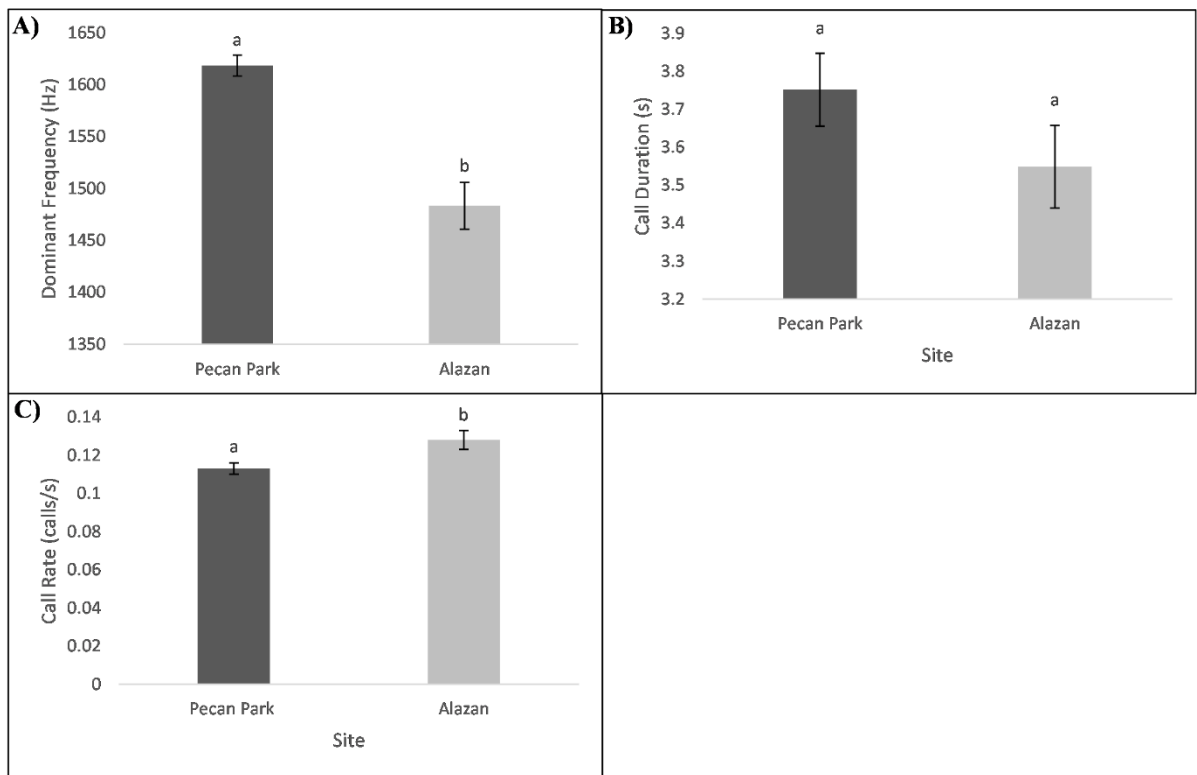


Figure 9. Mean (\pm SE) **A)** dominant frequency (Hz), **B)** call duration (s), and **C)** call rate (call/s) for *Incilius nebulifer* populations. Means with different letters are significantly different. Populations are arranged from loudest to quietest site.

Female Mate Choice

Hyla versicolor

Females were tested from the SFAEF population ($n = 3$). In the dark treatment, all three females chose a speaker, with one female preferring the short duration call and two females preferring the long duration call ($p = 0.375$). In the light treatment, two females chose a speaker, with both females preferring the long duration call ($p = 0.25$). One of these females switched her preference between the dark and light treatment ($p = 0.50$).

Gray Treefrog Species

Hyla chrysoscelis females were most likely tested from the soccer field population ($n = 6$). Out of these six females, only two chose a speaker. In the dark treatment, one female preferred the short duration call and one female preferred the long duration call ($p = 0.50$). In the light treatment, both females preferred the long duration call ($p = 0.25$), with the female preferring the short duration call in the dark treatment switching her preference ($p = 0.50$).

Hyla cinerea

Hyla cinerea females were tested from the soccer field ($n = 6$) and Alazan ($n = 18$) populations. From the soccer field population, four out of six females chose in both treatments. Under dark conditions, three females preferred the lower frequency call (750 Hz) and one female preferred the higher frequency call (2718 Hz) ($p = 0.25$). These preferences remained the same under elevated light conditions ($p = 0.25$), even though

two out of four females switched their preference between the dark and light treatments ($p = 0.375$).

From the Alazan population, 17 out of 18 females chose in both treatments. Under dark conditions, 13 out of 17 females chose a speaker with eight females preferring the low frequency call and five females preferring the high frequency call ($p = 0.157$). Under elevated light conditions, 14 females preferred the low frequency call and three females preferred the high frequency call ($p = 0.005$). Between the light and dark treatment, six out of 13 females switched their preference ($p = 0.209$).

Incilius nebulifer

One female was tested from the Alazan population. Under dark conditions, she did not display interest in either the short or long duration call played to her. Under elevated light conditions, this female did not leave the release point and displayed defensive behavior.

DISCUSSION

The purpose of this study was to expand our understanding of how artificial light at night and anthropogenic noise affect anuran breeding systems. The data reported here support the hypotheses that anthropogenic noise alters calling behavior in male anurans, male anurans living at sites with higher environmental light levels will call from darker sites, and that female mate choice is influenced by artificial light. First, anthropogenic noise altered the calling behavior of male anurans as all species studied exhibited alterations in their call parameters. Second, artificial light influenced male call site selection as most species called from sites almost significantly or significantly darker than the general light environment. Lastly, artificial light influenced female mate choice as female *Hyla cinerea* preferred lower frequency calls under brighter conditions.

Environmental Data

Artificial Light at Night

Light levels varied among study sites. While environmental light levels did not differ between Pecan Park and the soccer fields, light levels of these sites were significantly brighter than those of the horse track, Alazan, and SFAEF. Pecan Park receives artificial light from street lighting within the park, adjacent residences, Stephen

F. Austin State University, and skyglow from the surrounding city of Nacogdoches. Environmental light levels at the soccer fields may exceed those of Pecan Park as this site receives artificial light not only from downtown Nacogdoches, but also from stadium lighting at the soccer fields as well as the nearby softball field during practice and games. Light levels at the horse track were significantly darker than those of Alazan, but brighter than those of the SFAEF. While artificial light pollution at the horse track comes from nearby business, residential areas, and, occasionally, the Nacogdoches expo center, areas where frogs call at this site are somewhat sheltered by patches of trees and other vegetation. In comparison, ponds at Alazan are wide open with no canopy cover and this site receives skyglow from the neighboring cities of Nacogdoches and Lufkin. However, light level measurements may have been taken under more varied environmental conditions (e.g. moon phase; cloud cover) at Alazan than the horse track. Therefore, light levels at the horse track may be more similar to those of Alazan. Out of all five study sites, the SFAEF had the darkest environmental light levels. The focal pond at this site is an open grass-filled pond surrounded by tall trees and dense vegetation on all sides, blocking out most of the skyglow from Nacogdoches and Lufkin.

Anthropogenic Noise

Environmental sound levels were significantly louder at the soccer fields than at any other site as this location receives noise pollution not only from passing traffic and trains, but also constant noise from nearby industrial facilities. Sound levels did not differ between Pecan Park and the horse track, but were significantly louder than sound levels

at Alazan and the SFAEF. Pecan Park's elevated noise levels result from traffic noise, infrequent, attenuated train noise, and surrounding urban developments such as residences and Stephen F. Austin State University. While some individual sound levels at the horse track are similar to those at Pecan Park, this site receives intermittent noise from a nearby highway, resulting in sound level spikes. Events at the Nacogdoches expo center may also temporarily increase sound levels. Consequently, the overall environmental sound level of the horse track may vary in intensity based on traffic volume and other human activities. Environmental sound levels were quietest at Alazan and the SFAEF, which were not significantly different. These sites are located approximately 24 kilometers outside of the city of Nacogdoches and in-between US Highway 59 South and Texas State Highway 7. Traffic noise can infrequently be heard at these sites, but most of the noise is absorbed and attenuated by surrounding vegetation, decreasing its intensity.

Male Call Sites

Male Call Sites vs General Light Environment

With the exception of *Hyla cinerea*, males appeared to prefer call sites darker than the surrounding environment. Anurans whose call sites were slightly darker or brighter than ambient light levels may have been unable to avoid calling from sites with more ambient light due to the pervasiveness of artificial light in these environments. While ambient light levels at Alazan are relatively low, the ponds at this site are open and

anurans may call from darker sites to remain concealed from visually oriented predators. Environmental light levels were darkest at the SFAEF and as a result, *P. crucifer* and *H. cinerea* may not have had a need to call from more concealed sites. Meanwhile, *H. versicolor* call sites were likely slightly darker than the general light environment due to the fact that, unlike *P. crucifer* and *H. cinerea*, this species calls primarily from surrounding trees rather than from the open grass-filled area of the pond (A. Kobisk, personal observation).

Male call site selection can be influenced by a variety of factors. For example, male *H. versicolor* prefer warm call sites as higher temperatures allow them to produce energetically efficient, attractive calls (Höbel and Barta 2014). Calling from elevated perches, especially in forested habitat, or calling near water can limit the degradation of attractive calls as they are transmitted through the environment, minimizing the energetic expenditure of calling (Parris 2002, Schwartz et al. 2015). In closely related species such as *H. versicolor* and *H. chrysoscelis*, calling from species specific sites when in sympatry can reduce the chance of mating errors (Ralin 1968, Ptacek 1992). Lastly, some male anurans may call near suitable oviposition sites to further increase the likelihood they will be selected as mating partners (Mitchell and Miller 1991, McCallum et al. 2006). This study did not examine the influence of these factors on male call site selection.

Some anuran breeding systems benefit from multimodal communication, particularly the combination of acoustic and visual signals. For example, when presented with a choice between an attractive call alone (unimodal stimulus) or an attractive call

paired with an inflating vocal sac (multimodal stimulus), female Túngara Frogs (*Physalaemus pustulosus*) and Squirrel Treefrogs (*Hyla squirella*) prefer the multimodal stimulus (Rosenthal et al. 2004, Taylor et al. 2007, Taylor et al. 2008). This preference has also been demonstrated in *H. cinerea* (Laird et al. 2016). It is hypothesized that in noisy choruses, vocal sac movement can enhance a female's ability to detect and discriminate among calling males and therefore, males who call from visible positions may have a mating advantage (Rosenthal et al. 2004, Taylor et al. 2007, Taylor et al. 2008, Laird et al. 2016). More recent studies have suggested that brighter conditions may further improve detection and localization of male signals (Onorati and Vignoli 2017, Deng et al. 2019). As previously mentioned, light levels of urban *H. cinerea* call sites did not differ from those of the general light environment. Since female *H. cinerea* respond to visual cues (Laird et al. 2016), urban *H. cinerea* males may be utilizing the light to enhance the visibility of their vocal sac by calling from sites with elevated light levels. This may help attract females at urban sites, especially if acoustic signals are not always effectively transmitted because of anthropogenic noise. Additionally, *H. cinerea* at Pecan Park produced calls at faster rates while *H. cinerea* at the soccer fields and horse track produced calls of longer duration. Increasing the repetition of movement or length of time the vocal sac is visible may further improve the likelihood a male can be detected, evaluated, and located by a female. Contrarily, calling from brighter sites may weaken the effectiveness of multimodal communication. While female Túngara Frogs (*P. pustulosus*) typically prefer multimodal signals, under simulated full moonlight, they

choose unimodal signals more frequently as the multimodal signal becomes more conspicuous and increases predation risk (Cronin et al. 2019). Furthermore, in the Gray Treefrog complex, preference for multimodal signals is weak in *H. versicolor* (Reichert et al. 2014, Reichert and Höbel 2015) and absent in *H. chrysoscelis* (Li 2020). Lack of multimodal communication in these species may partially explain the use of darker call sites by *H. chrysoscelis* at the soccer fields and *H. versicolor* at Alazan and the SFAEF.

Comparison of Call Sites Among Populations

Although males appear to prefer call sites darker than the surrounding environment, urban light pollution may limit the availability of such sites. This is likely due to the nature of where *Pseudacris crucifer* call at each site. At Pecan Park, *P. crucifer* call from an open flooded field that is primarily illuminated by an adjacent apartment complex. In contrast, *P. crucifer* at the soccer fields call from within a flooded patch of forest and *P. crucifer* at the horse track call from the cover of vegetation or woody debris in flooded edge habitat. At the SFAEF, *P. crucifer* call from the grass-filled pond and tall trees and dense vegetation block out most light from outside sources. Consequently, ambient light levels were significantly brighter at urban sites than at the SFAEF. Thus, unlike *P. crucifer* at the SFAEF, urban *P. crucifer* populations may be unable to avoid calling from brighter microhabitats. In essence, with a lack of dark sites to call from, urban *P. crucifer* populations are experiencing a form of habitat loss. Female mate choice behavior under higher levels of ambient light can vary by species and mate choice preferences of female *P. crucifer* under these conditions so far remain unknown. One

possibility is that, like female Túngara Frogs (*Physalaemus pustulosus*), female *P. crucifer* may be more cautious and exhibit weakened preferences for attractive males with brighter call sites (Rand et al. 1997, Baugh and Ryan 2010, Bonachea and Ryan 2011a, Bonachea and Ryan 2011b, Cronin et al. 2019). Consequently, chronic exposure to artificial light in urban areas and consistent selection of less desirable males could reduce genetic quality and viability of offspring, lowering population fitness and, overtime, lead to population decline.

Individuals from both *Hyla versicolor* populations call from the refuge of trees or other vegetation. Due to the openness and brighter ambient light levels at Alazan, *H. versicolor* from this population may call from light microhabitats similar to those of the SFAEF population to reduce predation risk. Additionally, *H. versicolor* may have called from sites with optimal calling temperatures (Höbel and Barta 2014) or that minimized call degradation (Schwartz et al. 2015) and these sites may have coincidentally been darker than ambient light levels.

Hyla cinerea call from creek banks at Pecan Park, edge habitat at the soccer fields, and flooded edge habitat or aquatic vegetation in open swamp at the horse track; call sites which receive exposure to artificial light. Urban *H. cinerea* may purposefully call from brighter sites to enhance multimodal signaling, especially in the presence of anthropogenic noise (see above). In contrast, due to the open landscape and lower environmental sound levels, *H. cinerea* at Alazan may be less reliant on multimodal communication, preferring to remain concealed from predators. Further, the soccer field

and Alazan populations may be able to call from light microhabitats similar to those of the SFAEF population by calling from the cover of vegetation. Alternatively, the lack of difference in call site light levels between the soccer field and SFAEF populations may be an effect of sample size. Mean call site light level was not different between the Alazan and SFAEF population (0.003 lux), yet the soccer field population had significantly brighter call sites than the Alazan population which had more samples.

Incilius nebulifer at Pecan Park call on the banks of the creek running through the park or from the same flooded field where *P. crucifer* call, exposing this population to street lighting within the park or light from the neighboring apartment complex. In comparison, while *I. nebulifer* at Alazan call from open locations along pond edges, environmental light levels are significantly darker at Alazan than at Pecan Park, allowing for darker light microhabitats. Therefore, like *P. crucifer*, *I. nebulifer* at Pecan Park are experiencing loss of dark habitat with potentially similar consequences.

Male Call Variables

Pseudacris crucifer

Most anuran species that modulate frequency in response to anthropogenic noise shift their calls to higher frequencies (Parris et al. 2009, Cunnington and Fahrig 2010, Kruger and Du Preez 2016, Grenat et al. 2019, Leon et al. 2019). Decreasing frequency in response to anthropogenic noise is probably not a widely used strategy in anurans as most anthropogenic noise ranges in frequencies from 0-3000 Hz and is concentrated at

frequencies below 2000 Hz, the same frequency range of the calls of many anuran species (Roca et al. 2016, Simmons and Narins 2018). *Pseudacris crucifer* calls range in frequency from 2500-3500 Hz (Hanna et al. 2014), falling outside the concentration of most anthropogenic noise. As a result, lowering frequency may not be as detrimental in terms of acoustic masking for *P. crucifer* in comparison to other anuran species.

The downward shift in dominant frequency by *P. crucifer* is somewhat consistent with the findings of Hanna et al. (2014) who found that *P. crucifer* lowered the dominant frequency of their calls after exposure to high frequency (2850-3850 Hz) noise. They suggested that *P. crucifer* were mistaking the noise for the call of a rival male and lowering dominant frequency as an aggressive response, a behavior documented in other anuran species (Hanna et al. 2014). It is important to note that the study by Hanna et al. (2014) did not test the effects of anthropogenic noise, but rather the effects of white noise with low (1850-2850 Hz) and high (2850-3850 Hz) frequencies covering the frequency range of *P. crucifer* calls. While the general frequency range of anthropogenic noise is known (see above), this study did not look at the spectral range of anthropogenic noise at each site. Therefore, it is inconclusive if *P. crucifer* at Pecan Park, the soccer fields, and horse track are mistaking anthropogenic noise for rival male calls and lowering dominant frequency in response.

Two other species of anurans, Bischoff's Treefrog (*Boana bischoffi*) and the Pacific Chorus Frog (*Pseudacris regilla*) have been shown to lower their call frequencies in response to traffic noise (Caorsi et al. 2017, Nelson et al. 2017). For Bischoff's

Treefrog (*Boana bischoffi*), lowering dominant frequency may help transmit their call over longer distances when traffic noise is present (Caorsi et al. 2017). While lower frequencies are better for transmitting signals over longer distances, this transmission can be diminished by many factors including high amplitudes, a characteristic of anthropogenic noise (Forrest 1994, Francis et al. 2009, Nelson et al. 2017). Mean sound levels were significantly louder at the soccer fields than at Pecan Park. These high amplitudes could mask lower frequency calls and thus be the reason why *P. crucifer* at the soccer fields called at significantly higher frequencies than at Pecan Park. Interestingly, the Pacific Chorus Frog and the Spring Peeper not only both lower frequency in response to anthropogenic noise (Pacific Chorus Frog: Nelson et al. 2017; Spring Peeper: Hanna et al. 2014, this study), but are also both members of the genus *Pseudacris*. Lowering frequency in response to anthropogenic noise may be a characteristic response of this genus and should be further investigated.

In terms of other call parameters, call duration did not differ among populations. However, call rate differed significantly. These results contrast the findings of Hanna et al. (2014) who found that *P. crucifer* produced shorter duration calls but did not alter call rate after exposure to low and high frequency noise. As previously mentioned, Hanna et al. (2014) used white noise with a frequency range spanning that of a *P. crucifer* call played at 89-90 dB. Anthropogenic noise at the sites of this study varies in intensity and may vary spectrally, causing *P. crucifer* to exhibit different alterations in call parameters than previously observed. Additionally, the *P. crucifer* populations studied by Hanna et

al. (2014) were located in Ontario, Canada and may exhibit differences in call parameters due to genetic variation.

Advertisement calls are energetically costly for male anurans and males call for several hours across multiple nights (Pough et al. 2016). For *P. crucifer*, it may not be worth the energetic investment to alter both call duration and call rate when one call parameter is more important to female mate choice than the other. In this species, call rate has been documented to be important for female mate choice as males with faster call rates are more likely to be selected as mating partners (Forester and Czarnowsky 1985, Sullivan and Hinshaw 1990). By calling at faster rates at sites with higher levels of anthropogenic noise such as Pecan Park and the soccer fields, males may be further increasing their energy expenditure with a potentially negative impact on survival and fitness. Alternatively, these males may be making their calls more conspicuous to females. If males with faster call rates are more conspicuous to females against background noise, the increased energetic cost of a faster call rate may be offset by a benefit to selection (Kaiser and Hammers 2009). Another possible explanation is that *P. crucifer* may be exhibiting a type of gap calling behavior, increasing call rate when noise intensity is low (Vargas-Salinas and Amézquita 2013, Vargas-Salinas et al. 2014). There is anecdotal evidence for this as *P. crucifer* decreased calling effort when noise levels increased from passing trains at the soccer fields or passing cars in the apartment complex parking lot adjacent to Pecan Park (A. Kobisk, personal observation).

Hyla versicolor

The finding that males at Alazan called at significantly higher frequencies than males at the SFAEF is surprising as environmental sound levels did not differ between these sites. However, the contrast of open landscape at Alazan and dense vegetation at the SFAEF may impact the way noise is propagated at these sites. As a result, the Alazan population may experience stronger effects from anthropogenic noise spikes from surrounding roadways, causing this population to still exhibit an upshift in frequency. Alternatively, the observed difference in frequency may be an effect of habitat structure on call variation. For example, in closed habitat, anurans exhibit large frequency modulation ranges and birds call at lower frequencies to improve localization and reduce attenuation in the complex vegetated environment (Hunter and Krebs 1979, Bosch and De la Riva 2004, Boncoraglio and Saino 2007).

Conversely, Cunningham and Fahrig (2010) found that *Hyla versicolor* did not alter dominant frequency even when mean traffic noise reached 73.2 dB, a louder mean sound level than that of Alazan. They suggested that *H. versicolor* may not need to adjust frequency as this species calls at frequencies higher than the concentration of most anthropogenic noise (Cunningham and Fahrig 2010). Alternatively, they hypothesized that, due to the short breeding season of *H. versicolor* in Canada, actively searching for a mate may be more important than altering call parameters (Cunningham and Fahrig 2010). In Canada, *H. versicolor* call approximately from May to July (De Solla et al. 2006). In comparison, *H. versicolor* in eastern Texas call from March to September if temperature

and rainfall are sufficient (Saenz et al. 2006). This prolonged breeding season provides males with more opportunities to attend breeding choruses and attract a mate. Thus, for *H. versicolor* at Alazan, it may be a better strategy to raise frequency to be more conspicuous to females when anthropogenic noise is present than perform mate searching behavior. Further, variation in body size or genetics may also explain the difference in call parameter alteration among the Canadian and eastern Texas populations.

Cunnington and Fahrig (2010) also found that *H. versicolor* living at sites with higher levels of anthropogenic noise had faster call rates, but they did not examine call duration or pulse rate. Call duration, pulse rate, and call rate did not differ between the Alazan and SFAEF populations. Noise pollution at these sites may not have been loud or constant enough to cause *H. versicolor* to modify these call parameters. Due to the high energetic cost of maintaining call duration and call rate (Taigen and Wells 1985), *H. versicolor* may only alter these parameters when interference from anthropogenic noise is extreme.

If *H. versicolor* were not altering dominant frequency in response to anthropogenic noise, they may have been doing so in response to environmental light levels. Other anuran species have been shown to alter their calling behavior under higher levels of ambient light based on their perceived risk of predation. For example, *Smilisca silya* called more frequently and produced more complex calls under higher levels of ambient light as they could rely on visual cues to detect predatory bats (Tuttle and Ryan 1982). In contrast, Túngara Frogs (*Physalaemus pustulosus*) stopped calling during

predatory bat fly overs except when in almost complete darkness (Tuttle et al. 1982). Environmental light levels were significantly brighter at Alazan than at the SFAEF and *H. versicolor* at Alazan may have called at higher frequencies under these elevated light levels to compensate for predation risk from acoustically oriented predators. However, this suggestion requires further investigation as results of previous studies investigating *H. versicolor* calling behavior in the presence of predators are conflicting and did not examine the effects of varying light level (Schwartz et al. 2000, Höbel and Barta 2014).

Call data for an urban population of *H. versicolor* were not obtained as only its sister species, *Hyla chrysoscelis*, was recorded at the soccer fields. *Hyla chrysoscelis* were also observed calling in other urban areas such as Pecan Park and the Stephen F. Austin State University arboretum (A. Kobisk, personal observation). While little is known about competition between these two species, there is evidence of call site segregation when calling in sympatry (Ralin 1968, Ptacek 1992). *Hyla chrysoscelis* call at Alazan and the SFAEF, but in small numbers compared to *H. versicolor* (A. Kobisk, personal observation, Perez et al. 2021). In comparison, few, if any, *H. versicolor* were heard calling at urban sites (A. Kobisk, personal observation). Therefore, *H. chrysoscelis* may be better adapted to urban conditions. For example, its higher pulse rate (Johnson 1966) may allow this species to better compensate for the acoustic masking of anthropogenic noise. Alternatively, *H. versicolor* may outcompete *H. chrysoscelis* in rural areas, forcing this species to utilize suboptimal urban habitat.

Hyla cinerea

Unlike the other anuran species in this study, *Hyla cinerea* did not exhibit significant differences in dominant frequency among populations. These results partially contrast with Barrass (1985), who found that *H. cinerea* near Interstate 10 in Texas called at significantly higher frequencies than *H. cinerea* isolated within the Attwater Prairie Chicken National Wildlife Refuge (APCNWR). The mean environmental sound level of the soccer fields falls within the mean sound level range of Interstate 10 (64-78 dB) measured by Barrass (1985). However, *H. cinerea* at the soccer fields chorus further away from the factory at this site than other anuran species (A. Kobisk, personal observation), possibly reducing the intensity of noise this population experiences. Mean environmental sound levels of Pecan Park, the horse track, Alazan, and the SFAEF more closely resemble the mean sound level range of Texas FM 3013 (52-64 dB), an intermediate site used by Barrass (1985). Barrass (1985) found no significant difference in frequency between *H. cinerea* populations at Texas FM 3013 and Interstate 10 or between *H. cinerea* populations at Texas FM 3013 and the APCNWR. Thus, sound levels at sites in this study may not have been loud enough to force *H. cinerea* to adjust frequency. The need for *H. cinerea* to adjust frequency at lower sound levels may be reduced as the high frequency component of their call falls outside the concentration of most anthropogenic noise (Simmons and Narins 2018).

Call duration significantly differed among populations, however, the observed differences were inconsistent. Populations may have differentially adjusted call duration

based on the intensity of anthropogenic noise they were experiencing, a behavior documented in another anuran, the Fine-Lined Treefrog, *Boana leptolineata* (Caorsi et al. 2017). Sound levels at Pecan Park are not exceedingly loud and the occurrence of anthropogenic noise events at this site can vary. These conditions may have allowed *H. cinerea* at Pecan Park to minimize acoustic masking from noise by shortening their calls. When exposed to noise, Spring Peepers (*Pseudacris crucifer*) also reduced call duration to possibly avoid masking their calls with the noise stimulus (Hanna et al. 2014). In comparison to Pecan Park, the occurrence of anthropogenic noise events at the horse track can also vary. However, individual sound levels can spike approximately 10 dB above the mean environmental sound level (59.8 dB; A. Kobisk, personal observation). Additionally, the soccer fields are subject to loud and constant noise pollution. Unpredictable loud noise spikes at the horse track and constant noise at the soccer fields may prevent complete avoidance of acoustic masking. *Hyla cinerea* at these sites may have lengthened their calls to increase the likelihood that the calls would be detected against background noise. Similarly, Caorsi et al. (2017) hypothesized that when the Fine-Lined Treefrog increased call duration in response to noise, it was to maximize the amount of time its call was present in the environment.

Call rate also significantly differed among populations, but like call duration, the way *H. cinerea* altered this call parameter varied. Barrass (1985) found no difference in call rate among the Interstate 10, Texas FM 3013, and APCNWR *H. cinerea* populations. However, during female mate choice experiments, Barrass (1985) demonstrated that

female *H. cinerea* from the Interstate 10 and Texas FM 3013 populations decreased their latency to choose in masking noise conditions when a call with a faster rate was played. *Hyla cinerea* at Pecan Park may have not only shortened their calls to avoid acoustic masking, but also called at faster rates to maintain their detectability by females. Conversely, the soccer field population called at a significantly slower rate than the horse track, Alazan, and SFAEF populations. Other species of anurans decrease call rate during times of noise interference to reduce the chance that their calls will be masked (Sun and Narins 2005, Cunningham and Fahrig 2010, Caorsi et al. 2017). Additionally, there may be an energetic restraint on altering call duration and call rate, such that increasing call duration means calling at a slower rate (Wells and Taigen 1986). Due to the high anthropogenic noise levels at the soccer fields and because this population was producing longer calls, calling at a faster rate may not have been an efficient strategy when call transmission was likely to be disrupted.

Incilius nebulifer

The call frequency of *Incilius nebulifer* is below 2000 Hz, falling within the concentration of anthropogenic noise (Simmons and Narins 2018). Consequently, *I. nebulifer* at Pecan Park may have raised frequency to minimize the amount of overlap between their calls and anthropogenic noise. Calling at higher frequencies to escape the masking effects of anthropogenic noise is documented in many anuran species (Parris et al. 2009, Cunningham and Fahrig 2010, Kruger and Du Preez 2016, Grenat et al. 2019, Leon et al. 2019). However, few studies have investigated the effects of anthropogenic

noise in Bufonids and none have focused on *I. nebulifer* (Barrass 1985, Cunnington and Fahrig 2010, Kaiser et al. 2011, Vargas-Salinas et al. 2014, Grace and Noss 2018). Woodhouse's Toad (*Anaxyrus woodhousii*) living near an interstate with high anthropogenic noise levels called at higher frequencies (Barrass 1985). In contrast, anthropogenic noise had no effect on dominant frequency in American Toads, *Anaxyrus americanus* (Cunnington and Fahrig 2010).

Call duration did not differ between the Pecan Park and Alazan populations. However, call rate significantly differed between populations with *I. nebulifer* at Pecan Park calling at slower rates than *I. nebulifer* at Alazan. In contrast, the closely related species, *Incilius valliceps*, produced longer calls but did not alter call rate when exposed to anthropogenic noise (Kaiser et al. 2011). Other anuran species decrease call rate in response to anthropogenic noise to avoid calling when their call is less likely to be detected (Sun and Narins 2005, Cunnington and Fahrig 2010, Caorsi et al. 2017).

Conversely, the observed difference in call rate between the Pecan Park and Alazan populations may have been a response to con- or heterospecifics rather than anthropogenic noise. *Incilius nebulifer* decrease call rate when in close proximity to conspecifics, but increase call rate at intermediate distances (Sullivan and Wagner Jr. 1988). Males at Pecan Park call close enough to neighbors to sometimes elicit male combat, while males at Alazan are often evenly spaced around the pond's edge (A. Kobisk, personal observation). Furthermore, *I. nebulifer* at Alazan often call with large numbers of Green Treefrogs (*Hyla cinerea*) and occasionally, Gray Treefrogs (*Hyla*

versicolor) (A. Kobisk, personal observation). Accordingly, this population may have called at a faster rate not only based on proximity to neighbors, but also to maintain the conspicuousness of their call within the mixed chorus. Other anuran species naturally adjust call rate in the presence of heterospecifics (Schwartz and Wells 1984, Schwartz and Wells 1985). Additionally, while *Rana taipehensis* increased its call rate during anthropogenic noise events, it may have been a response to decreased call rate by heterospecifics rather than the noise stimulus (Sun and Narins 2005). At Alazan, environmental sound levels are low and chorus lulls caused by anthropogenic noise events were not observed during data collection (A. Kobisk, personal observation).

Female Mate Choice

Hyla versicolor

Three *Hyla versicolor* females were tested from the SFAEF population. In the dark treatment, one female chose the short duration call and two females chose the long duration call. In the light treatment, two females chose the long duration call, with one of these females switching her preference from the short duration call in the dark treatment. Female *H. versicolor* typically prefer longer duration calls (Klump and Gerhardt 1987). However, small sample size prevented adequate testing for this preference and whether it is affected by artificial light.

Female *H. versicolor* from a Wisconsin population retained their preference for longer duration calls when tested under simulated moonlight (0.2-2.0 lux; Underhill and

Höbel 2017) and bright artificial light (5-15 lux; Underhill and Höbel 2018b). For *H. versicolor* in Wisconsin, the cooler climate reduces predation pressure and shortens the breeding season, potentially limiting the influence of higher ambient light levels on female mate choice (Underhill and Höbel 2017). In contrast, predation pressure for *H. versicolor* in eastern Texas is likely higher as this species is preyed upon by at least five snake species (*Agkistrodon piscivorus*, *Nerodia erthyrogaster*, *N. rhombifer*, *N. fasciata*, *Thamnophis proximus*) as well as other nocturnal predators (A. Kobisk, personal observation). If further testing demonstrated that female preference for longer duration calls under elevated light levels is also maintained in the eastern Texas population, then female *H. versicolor* may use alternative behaviors for predator avoidance other than avoiding attractive mates. Additionally, the breeding season of *H. versicolor* in eastern Texas can span from March to September with adequate temperatures and rainfall (Saenz et al. 2006). However, temperatures can greatly fluctuate during the early part of the breeding season and the frequency of rainfall can diminish as the breeding season progresses (A. Kobisk, personal observation). Due to this environmental variability, like in the Wisconsin population, breeding with higher quality males when possible may take precedence over the consideration of other ambient conditions like light level (Underhill and Höbel 2017). One other possibility is that females from the darkest site, the SFAEF, may not exhibit altered mate choice behavior as they do not normally experience the bright conditions associated with artificial light at night.

Alternatively, higher ambient light levels may affect aspects of the *H. versicolor* breeding system other than a female's final mate choice. For example, Underhill and Höbel (2017, 2018b) hypothesized that elevated light conditions may alter how females travel to breeding ponds or alter male calling behavior such as call site selection. Artificial lighting impedes the migration of the Common Toad (*Bufo bufo*) to breeding ponds (van Grunsven et al. 2016). Thus, it is plausible that artificial light may have the same effect on other anuran species like *H. versicolor*. Furthermore, this study compared the light levels of male call sites to those of the surrounding environment. Male *H. versicolor* called from sites that were significantly darker than ambient light conditions at Alazan and slightly darker than ambient light conditions at the SFAEF. Perhaps if males can call from the refuge of dark call sites, then females may not need to change their mate choice behavior.

Gray Treefrog Species

All males recorded at the soccer fields were *Hyla chrysoscelis*. It is therefore likely that the six amplexed pairs collected at this site were of the same species. Out of six females tested, only two responded. Female response was likely low as it was not determined that this population was *H. chrysoscelis* until after mate choice trials had been conducted. As a result, females were tested with *H. versicolor* calls. However, mating errors do occur between *H. chrysoscelis* and *H. versicolor* (Ptacek 1992, Gerhardt et al. 1994), likely explaining why two females responded to the heterospecific call. In the dark treatment, one female chose the short duration call and one female chose the long

duration call. In the light treatment, both females chose the long duration call. These choices are comparable to those made by *H. versicolor* (see above). Therefore, the effects of artificial light on female mate choice may be similar in these sister species. However, more trials conducted with conspecific calls would be needed to provide solid evidence for this hypothesis.

Hyla cinerea

Hyla cinerea were tested from the soccer field and Alazan populations. Females from the soccer field population did not display a significant preference for the low or high frequency call in either the dark or light treatment. In comparison, females from the Alazan population also did not significantly prefer either call in the dark treatment, but did significantly prefer the low frequency call under elevated light conditions. However, it is worth noting that the low (750 Hz) and high (2718 Hz) frequency calls used in this experiment represent the low and high frequency phases of a *H. cinerea* call (Oldham and Gerhardt 1975), and using low and high frequency calls within the same frequency phase may yield different results.

The result that female *H. cinerea* from Alazan preferred the more attractive call under elevated light conditions is somewhat consistent with a previous study. When presented with two calls that are equally attractive, female Serrate-Legged Small Treefrogs (*Kurixalus odontotarsus*) prefer the call in brighter conditions as this call may be easier to locate (Deng et al. 2019). Additionally, female *H. cinerea* respond to multimodal signals and males who call from more visible perches may be more likely to

be chosen as mating partners (Laird et al. 2016). Thus, while a robotic frog was not present to deliver visual signals, the higher light level may have still helped *H. cinerea* females locate the speaker playing the more attractive call. Alternatively, the higher light level may have allowed females to see there were no predators present in the chamber. Or females may have still perceived a risk of predation, but were more selective to make their reproductive investment count.

The preference for more attractive calls under brighter conditions may also be present in the soccer field population, but non-significant due to small sample size. This may be likely as male *H. cinerea* at urban sites called from sites that did not differ in light level from the surrounding environment, possibly to aid multimodal communication. For some anuran species, producing attractive or conspicuous signals in urban environments may be beneficial. For example, in an urban population of Túngara Frogs (*Physalaemus pustulosus*), males produced more complex calls at faster rates and females significantly preferred these calls over the calls of rural males (Halfwerk et al. 2019). Additionally, in multimodal species, brighter light has the potential to strengthen selection on visual signals (Underhill and Höbel 2018b). Therefore, attractive calls or visual signals may be under stronger sexual selection in urban *H. cinerea* populations.

Incilius nebulifer

One female was tested from the Alazan population. Under dark conditions, she did not show interest in either the short or long duration call played to her. In the *Incilius nebulifer* breeding system, call duration and call rate are both important parameters for

female mate choice (Wagner Jr. and Sullivan 1995). However, it is possible that the call modified for duration in this experiment simply did not match this female's mate choice preferences. Additionally, the 10 minute trial criteria may not be suitable for *I. nebulifer* as one female observed in the field took up to 30 minutes to choose a mate (A. Kobisk and K. Wasley, personal observations). Alternatively, this female may not actually have been receptive. Most female anurans captured for female mate choice experiments in this study oviposited overnight after testing (A. Kobisk, personal observation). However, this female did not oviposit and the pair was unamplexed the next day (A. Kobisk, personal observation).

Under elevated light conditions, this female did not leave the release point and displayed defensive behavior by flattening her body to the floor of the chamber. It is likely that the higher light level increased this female's perceived risk of predation. Female Túngara Frogs (*P. pustulosus*) also exhibit more cautious mate choice behavior under brighter conditions due to a heightened perceived risk of predation by reducing their latency to choose and relaxing their preference for complex, attractive male calls (Rand et al. 1997, Baugh and Ryan 2010, Bonachea and Ryan 2011a, Bonachea and Ryan 2011b). Additionally, in one study, a majority of female Túngara Frogs (*P. pustulosus*) failed to make a choice by not moving from the release site (Rand et al. 1997). Female mate choice may be similarly altered by artificial light in *I. nebulifer*, but sample size would need to be increased to confirm this.

Conclusions

Artificial light at night and anthropogenic noise impact anuran breeding systems including those in eastern Texas. This study provides further support that anthropogenic noise alters male calling behavior. Call parameters such as call dominant frequency, call duration, and call rate were all affected by anthropogenic noise. While different species altered different parameters, in general, alterations in these call parameters are common in many other anuran species. Additionally, alteration in call frequency is documented in other animals that rely on acoustic communication such as birds. Furthermore, to our knowledge, this study is also the first to provide evidence that artificial light at night influences call site selection by male anurans. Males typically preferred to call from sites that were darker than the general light environment. Where male anurans call from may have implications on female mate choice and therefore, the impact of artificial light at night may weigh more heavily on how male anurans select call sites.

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APPENDIX

P-values of pairwise comparisons for environmental light and sound data, male call site light levels among *Pseudacris crucifer* and *Hyla cinerea* populations, and male call variables for *P. crucifer* and *H. cinerea* populations.

Table A1. P-values of pairwise comparisons comparing environmental light levels between sites. Bolded p-values are considered biologically or statistically significant. P-values significant after Bonferroni adjustment are marked with an asterisk.

	Soccer Fields	Horse Track	Alazan	SFAEF
Pecan Park	0.116	0.049	0.049	0.048
Soccer Fields	-----	0.023	0.023	0.024
Horse Track	-----	-----	0.044	0.0009*
Alazan	-----	-----	-----	0.002*

*Significant with Bonferroni adjustment

Table A2. P-values of pairwise comparisons comparing environmental sound levels between sites. Bolded p-values are considered biologically or statistically significant. P-values marked with an asterisk are significant after Bonferroni adjustment.

	Soccer Fields	Horse Track	Alazan	SFAEF
Pecan Park	<0.0001*	0.385	<0.0001*	<0.0001*
Soccer Fields	-----	<0.0001*	<0.0001*	<0.0001*
Horse Track	-----	-----	0.0005*	0.003*
Alazan	-----	-----	-----	0.215

*Significant with Bonferroni adjustment

Table A3. P-values of call site light level comparisons among populations for *Pseudacris crucifer*. Bolded p-values are considered biologically or statistically significant. P-values marked with an asterisk are significant after Bonferroni adjustment.

	Soccer Fields	Horse Track	SFAEF
Pecan Park	0.007*	0.033	0.0003*
Soccer Fields	-----	0.370	0.0006*
Horse Track	-----	-----	0.0002*

*Significant with Bonferroni adjustment

Table A4. P-values of call site light level comparisons among populations for *Hyla cinerea*. Bolded p-values are considered biologically or statistically significant. P-values marked with an asterisk are significant after Bonferroni adjustment.

	Soccer Fields	Horse Track	Alazan	SFAEF
Pecan Park	0.528	0.233	0.001*	0.010
Soccer Fields	-----	0.153	0.047	0.172
Horse Track	-----	-----	0.002*	0.024
Alazan	-----	-----	-----	0.617

*Significant with Bonferroni adjustment

Table A5. P-values of pairwise comparisons for dominant frequency (Hz) for *Pseudacris crucifer* populations from Pecan Park, the Clint Dempsey Soccer Complex, the horse track, and the Stephen F. Austin Experimental Forest. Bolded p-values are considered biologically or statistically significant. P-values marked with an asterisk are significant after Bonferroni adjustment.

	Soccer Fields	Horse Track	SFAEF
Pecan Park	0.068	0.438	0.0001*
Soccer Fields	-----	0.521	0.0001*
Horse Track	-----	-----	0.0001*

*Significant with Bonferroni adjustment

Table A6. P-values of pairwise comparisons for call duration (s) for *Pseudacris crucifer* populations from Pecan Park, the Clint Dempsey Soccer Complex, the horse track, and the Stephen F. Austin Experimental Forest. Bolded p-values are considered biologically significant. No p-values are significant after Bonferroni adjustment.

	Soccer Fields	Horse Track	SFAEF
Pecan Park	0.630	0.641	0.122
Soccer Fields	-----	0.347	0.226
Horse Track	-----	-----	0.077

Table A7. P-values of pairwise comparisons for call rate (calls/s) for *Pseudacris crucifer* populations from Pecan Park, the Clint Dempsey Soccer Complex, the horse track, and the Stephen F. Austin Experimental Forest. Bolded p-values are considered biologically or statistically significant. P-values marked with an asterisk are significant after Bonferroni adjustment.

	Soccer Fields	Horse Track	SFAEF
Pecan Park	0.665	0.348	0.004*
Soccer Fields	-----	0.552	0.009*
Horse Track	-----	-----	0.168

*Significant after Bonferroni adjustment.

Table A8. P-values of pairwise comparisons for dominant frequency (Hz) for *Hyla cinerea* populations from Pecan Park, the Clint Dempsey Soccer Complex, the horse track, Alazan Bayou Wildlife Management Area, and the Stephen F. Austin Experimental Forest. Bolded p-values are considered biologically significant.

	Soccer Fields	Horse Track	Alazan	SFAEF
Pecan Park	0.448	0.340	0.055	0.170
Soccer Fields	-----	0.836	0.176	0.498
Horse Track	-----	-----	0.078	0.621
Alazan	-----	-----	-----	0.300

Table A9. P-values of pairwise comparisons for call duration (s) for *Hyla cinerea* populations from Pecan Park, the Clint Dempsey Soccer Complex, the horse track, Alazan Bayou Wildlife Management Area, and the Stephen F. Austin Experimental Forest. Bolded p-values are considered biologically or statistically significant. P-values marked with an asterisk are significant after Bonferroni adjustment.

	Soccer Fields	Horse Track	Alazan	SFAEF
Pecan Park	0.0001*	0.0001*	0.0001*	0.0003*
Soccer Fields	-----	0.272	0.013	0.005*
Horse Track	-----	-----	0.119	0.021
Alazan	-----	-----	-----	0.212

*Significant with Bonferroni adjustment

Table A10. P-values of pairwise comparisons for call rate (calls/s) for *Hyla cinerea* populations from Pecan Park, the Clint Dempsey Soccer Complex, the horse track, Alazan Bayou Wildlife Management Area, and the Stephen F. Austin Experimental Forest. Bolded p-values are considered biologically or statistically significant. P-values marked with an asterisk are significant after Bonferroni adjustment.

	Soccer Fields	Horse Track	Alazan	SFAEF
Pecan Park	0.0003*	0.200	0.022	0.611
Soccer Fields	-----	0.0001*	0.0001*	0.0006*
Horse Track	-----	-----	0.242	0.544
Alazan	-----	-----	-----	0.147

*Significant with Bonferroni adjustment.

VITA

Ashley Kobisk graduated from Leonardtown High School in 2010 and enrolled at Salisbury University later that same year. As an undergraduate, she became involved in scientific research, assisting with a project investigating frog behavior and genetics under the guidance of Dr. Ryan Taylor and Dr. Kimberly Hunter. Ashley graduated with honors from Salisbury University in May 2014, receiving her Bachelor of Science degree. Before pursuing her master's degree, Ashley worked as a herpetology survey intern with the Environmental Division of the Patuxent River Naval Air Station and as a naturalist intern at Battle Creek Cypress Swamp Nature Center. She enrolled at Stephen F. Austin State University in the fall of 2017.

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Literature citations follow the format of the journal *Ecology*.

This thesis was typed by Ashley Kobisk.