

MALIN, REBECCA D., M.S. Response of *Eptesicus fuscus* and *Nycticeius humeralis* to Social Call Playback in North Carolina. (2020)
Directed by Dr. Matina C. Kalcounis-Rueppell. 41 pp.

Vocal communication is important for several bat behaviors, including navigation, foraging, and information transfer between individuals. This study sought to identify the responses of two temperate species of echolocating bats, big brown (*Eptesicus fuscus*) and evening bats (*Nycticeius humeralis*), to broadcasts of specific recorded bat social calls. Previous laboratory research on bat vocalizations suggest that certain social calls serve a group cohesion function, and other types of calls are associated with foraging. I hypothesized that broadcasts of recorded vocalizations containing upswEEP and downswEEP social calls would evoke increased levels of echolocation vocalizations and social calls. Further, broadcasts of vocalizations containing complex multi-pulse calls, would evoke increased production of foraging vocalizations. To test these hypotheses, trials were set up to monitor the responses of free-ranging bats to recorded playbacks of different types of bat vocalizations for three one-minute phases: pre-broadcast, during broadcast, and post-broadcast. Contrary to my hypothesis, the playback of evening bat social calls decreased responses with echolocation vocalizations, suggesting that these social calls might be used for group cohesion by repelling heterospecifics. Playback of big brown bat upswEEP social calls increased social call production responses, but not foraging or navigation vocalizations, supporting the hypothesis that upswEEP calls function in group cohesion. Big brown and evening bat echolocation broadcasts led to a decrease in echolocation responses. These

results indicate that the type of vocalization produced evokes different responses in bats, and that passive monitoring can be used to unveil group dynamics in a field setting.

RESPONSE OF *EPTESICUS FUSCUS* AND *NYCTICEIUS HUMERALIS* TO SOCIAL
CALL PLAYBACK IN NORTH CAROLINA

by

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A Thesis Submitted to
the Faculty of The Graduate School at
The University of North Carolina at Greensboro
in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Greensboro
2020

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ACKNOWLEDGEMENTS

I would like to thank my thesis committee chair Dr. Matina Kalcounis-Rueppell, and my committee members Dr. John J Lepri, Dr. Bryan McLean, and Dr. Han Li for all their support and guidance throughout this process.

I would also like to thank the other members of Dr. Matina Kalcounis-Rueppell's lab, including Dr. Rada Petric and Brian Springall for their suggestions, technological support, and feedback. I also thank my team of undergraduate research assistants: Beth Carswell, Joel Jacobs, Rukshan Fernando, Chase Carihfield, and Nicolas Mizero Sibomana for all for all of their help in the field and in the lab analyzing the files.

Additionally, I'd like to thank the UNCG Biology department for their support financially, academically, and mentally throughout this degree.

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CHAPTER I

INTRODUCTION

Vocal communication mediates social behaviors in a wide variety of animal taxa (Nomoto et al. 2018; Asaba et al. 2015; Musolf et al. 2015; Hammerschmidt et al. 2009; Bowers et al. 2018; Carter et al. 2015; Lewanzik et al. 2019; Jones et al. 2018). Vocal communication is an important component of bat (Chiroptera) behavioral repertoire. However, bat vocalizations in the context of social behaviors are understudied. In general, bats produce ultrasonic vocalizations (>20kHz) that are generally classified as echolocation and social calls. Echolocation and social calls differ in structure and function (Chaverri, Ancillotto, and Russo 2018).

Echolocation calls guide navigation and foraging at night (Griffin 1944; Warnecke, Falk, and Moss 2018; Pudlo and Kloepper 2019; Jones and Holderied 2007). In addition, echolocation calls potentially communicate species identity (Raw, Bastian, and Jacobs 2018, Culina and Garroway 2019, Lewanzik, Sundaramurthy, and Goerlitz 2019), age (Masters, Raver, and Kazial 1995), sex (Knörnschild et al. 2012), body size (Puechmaille et al. 2014), prey abundance (Culina and Garroway 2019, Lewanzik, Sundaramurthy, and Goerlitz 2019), and individuality (Yovel et al. 2009). Bats adjust their echolocation call structure in different environments. In cluttered environments, common sword-nosed bats (*Lonchorhina aurita*) shorten the duration of their echolocation calls, the shortened echoes return much faster to the sender, which allows

them to navigate more easily in the cluttered space (Gessinger et al. 2019). Big brown bats (*Eptesicus fuscus*) increase amplitude and duration of their echolocation calls when exposed to wideband random noise to increase the probability of signal transmission over the noise (Simmons 2017). In addition to adjusting echolocation call structure, Kuhl's pipistrelle (*Pipistrellus kuhlii*) bats alter their flight pattern to distinguish prey signals from background noise (due to cluttered flying environment such as foliage) (Taub and Yovel 2020).

Social calls are produced by bats both in the roost and while flying. Roost social calls include isolation calls emitted by pups that allow the mothers to identify them (Bohn, Wilkinson, and Moss 2007). Big brown bat echolocation and social calls develop from isolation calls in pups starting at 2 weeks of age (Monroy et al. 2011). Common noctule bats (*Nyctalus noctula*) were attracted to playbacks of roost social calls, supporting the proposition that these calls contain information about roost location (Furmankiewicz et al. 2011). Thomas's fruit-eating (*Artibeus watsoni*) and Honduran white bats (*Ectophylla alba*) produce social calls in the roost at dawn as a recruitment call (Gillam et al. 2013). The types of social calls produced varied between an autumn swarming site and a roost site in Natterer's bats (*Myotis nattereri*), with males producing one call primarily at the swarming site, and a different call produced primarily at the roost site, showing that different social calls serve different purposes (Schmidbauer and Denzinger 2019).

In-flight social calls are associated with foraging and species presence (Chaverri and Gillam 2015), though alarm calls are frequently used during attack or distressing events, and in the common pipistrelle (*Pipistrellus pipistrellus*) these calls induce mobbing behaviors by conspecifics (Russ, Racey, and Jones 1998). Type D (similar in structure to complex calls in Springall et al. 2019) social calls in *Pipistrellus pipistrellus* are primarily used in autumn and are associated with mating behaviors (Budenz, Heib, and Kusch 2009). Female greater spear-nosed bats (*Phyllostomus hastatus*) use screech calls to coordinate foraging, and playbacks of recordings attract conspecifics (Wilkinson and Wenrick Boughman 1998). Egyptian fruit bats (*Rousettus aegyptiacus*) make aggressive calls when competing for food, space, and mates (Prat, Taub, and Yovel 2016). These calls are specific to the emitter of the call, the intended receiver of the call, and the specific type of aggressive interaction taking place (Prat, Taub, and Yovel 2016). Common pipistrelle bats also increase social calling when insect density is low and exhibit a negative response to social call playbacks of conspecifics (Barlow and Jones 1997). Bechstein's bat (*Myotis bechsteinii*) social call broadcasts used as an acoustic lure attracted both conspecifics and heterospecifics at a very fine scale; 5 times as many bats were caught 0-1 meters from the speaker as from 2-3 meters from the speaker (Hill and Greenaway 2005). Florida bonneted bats (*Eumops floridanus*) were also attracted to playbacks of social calls used as a lure, however more males were caught than females (Torrez et al. 2017). Australian bats of multiple species were attracted to social call lures compared to no lure (Hill, Armstrong, and Barden 2015).

Previous research with social calls has most often focused on social calls in the roost, while in-flight social calls have remained difficult to study. The in-flight studies thus far have largely taken place in laboratory settings. However, Surlykke and Moss (2000) found that the duration between echolocation pulses as well as the bandwidth and frequency were different when conducted in the field compared to the laboratory. In the laboratory, the duration between echolocation pulses is longer, bandwidth of the pulses is larger, and frequency of the pulses was higher than in the field. Thus, in order to fully understand how temperate bats respond to social calls in nature, additional field studies are needed. Existing field studies have focused on alarm calls, but there is a knowledge gap for the functions of other in-flight social calls with free-living bats.

I hypothesized that bats will respond to in-flight social call playbacks in a call-specific manner. I tested my hypothesis with big brown (*Eptesicus fuscus*) bats and evening (*Nycticeius humeralis*) bats. Big brown and evening bats were chosen for this study due to their abundance in temperate bat communities, their seasonal behaviors, and known levels of social call production. Big brown and evening bats are both temperate and migratory species; however, evening bats produce more social calls than big brown bats despite their lower prevalence in the study area, Greensboro, NC. (Springall et al. 2019; 19.9 vs 2.4% respectively.). These species are common and there are seven different social call types identified from these species: complex, upsweeps, downsweeps, U-shaped, Inverted U-shaped, oscillating, and quasi-constant frequency (Springall et al. 2019).

Big brown bats live in colonies ranging from 2-500 individuals (Naughton and Canadian Museum of Nature 2012) and weigh 15-26 grams (Kurta and Baker 1990). They roost in old houses (Kalcounis-Rueppell, unpublished) and exhibit fission-fusion dynamics where maternity colonies disband (fission) and form new colonies (fusion) throughout the summer (Willis and Brigham 2004). Methany et al. (2008) suggest that these bats do not use kinship to choose their roost subgroups, finding that relatedness was not higher in subgroups than if they had been selected at random, so it's possible that the benefits of being in larger groups outweigh relatedness. Big brown bats typically hibernate less than 80km from their summer roosts (Naughton and Canadian Museum of Nature 2012), with some remaining at their roosts year-round (Kalcounis-Rueppell unpublished).

Evening bats travel between 100-200km to southern roosts when migrating (Watkins 1969) and live in colonies ranging in size from 2-275 individuals. They have an average lifespan of 6 years, usually producing twins each year (Humphrey and Cope 1970), and weigh 7-15 grams (Watkins 1972). Evening bats also participate in communal nursing, though they are more likely to share with female pups rather than male pups (Wilkinson 1992). These bats also forage in pairs, with an unsuccessful forager following a successful one (Wilkinson 1992). Evening bats do not roost in caves, instead roosting in trees and buildings (Watkins 1972).

In big brown bats, in-flight social calls have been classified and functionality tested in a laboratory (Wright et al. 2013, Wright et al. 2014). Frequency-modulated bouts (complex calls) of big brown bats repelled conspecifics, and upward frequency-

modulated calls (upsweeps), short frequency-modulated calls, long frequency-modulated (downsweeps), and quasi-constant-frequency calls attracted conspecifics (Wright et al. 2013, Wright et al. 2014). Big brown bat social calls are also linked to levels of aggression (Gadziola et al. 2012) and intraspecific distance while in flight (Wright et al. 2013). Complex social calls are most often produced concurrently with a high number of foraging buzzes, possibly indicating that the former calls have a foraging competition function, as noted in captive big brown bats (Wright et al. 2013, Springall et al. 2019). Upsweeps and downsweeps are most often produced when multiple species of bats are present, suggesting that these calls have a group cohesion function (Gillam and Chaverri 2012, Pfalzer and Kusch 2003). To date there has been no known investigation into the response of evening bats to social call playback, however, social call types for evening bats have been identified and classified (Springall et al. 2019).

Aim: Identify the social context of big brown bat and evening bat social calls in the field by evaluating response to playbacks.

Prediction 1: If downsweep and upsweep social calls attract bats, then there will be an increase in echolocation call production in the minute following the playback trial compared to the minute prior to the trial. This prediction follows from previous studies that show that downsweeps attract conspecifics in a laboratory setting (Wright et al. 2013, Wright et al. 2014) and have been suggested to be used for conspecific group cohesion (Gillam and Chaverri 2012, Pfalzer and Kusch 2003). Upsweeps are included in

this prediction because upsweeps and downsweeps frequently occur together (Springall et al. 2019).

Prediction 2: If upsweep and downsweep social calls attract bats, then evening bats and big brown bats will produce social calls in the minute following the playback trial compared to the minute prior to the trial. This prediction follows from previous studies that show that downsweeps attract conspecifics in a laboratory setting (Wright et al. 2013, Wright et al. 2014) and potentially being used for group cohesion (Gillam and Chaverri 2012, Pfalzer and Kusch 2003). Upsweeps are included in this prediction due to observations that upsweeps and downsweeps frequently occur together (Springall et al. 2019).

Prediction 3: If complex social calls are used for competition, then there will be fewer echolocation calls produced in the minute following the playback trial of complex social calls compared to the minute prior to the trial. This prediction follows from previous studies that have shown that complex calls repel conspecifics in a laboratory setting (Wright et al. 2013, Wright et al. 2014) and that complex social calls are associated with foraging buzzes (Wright et al. 2013, Springall et al. 2019).

CHAPTER II

METHODS

My research took place in Peabody Park at University of North Carolina at Greensboro (Parker et al. 2019). Permanent wetlands with standing water were constructed in Peabody Park in 2017, and an increase in bat activity and species richness occurred within a year of construction (Parker et al. 2019). This location is surrounded by a variety of canopy and subcanopy: Ash (*Fraxinus pennsylvanica*, *Fraxinus americana*), Black Cherry (*Prunus serotina*), Black Willow (*Salix nigra*), Buckeye (*Aesculus sylvatica*), Chinese Chestnut (*Castanea mollissima*), White Mulberry (*Morus alba*), Willow Oak (*Quercus phellos*), and Sycamore (*Planatus occidentalis*). The clearing of this site is a small (23m diameter) grassy opening, approximately 20 meters from a branch of North Buffalo Creek (Field Guide – Peabody Park | UNCG 2019). The opening is effectively a canopy gap and I will refer to this opening as a canopy cap throughout. Approximately 480m south of the site is the UNCG soccer stadium, and it is possible that the light from the stadium affected bat activity by increasing insect availability (Shimoda and Honda 2013). The bats foraging at this location appear to roost in older homes in downtown Greensboro (Kalcounis-Rueppell, unpublished data). There are seven known species of bats in the area: silver-haired bat (*Lasionycteris noctivagans*),

eastern red bat (*Lasiurus borealis*), tricolored bat (*Perimyotis subflavus*), hoary bat (*Lasiurus cinereus*), mexican free-tailed bat (*Tadarida brasiliensis*), evening bat, and big brown bat (Kalcounis-Rueppell et al. 2007). To test my hypothesis, I used playbacks of big brown and evening bat social calls previously recorded within Peabody Park at UNCG (Table 1). Data were collected in 2019 between June 4th and October 12th, for an extended summer. Previous research in this lab has identified summer (June 21-Sept 21) as having the highest bat activity (Springall et al. unpublished). All fieldwork followed the American Society of Mammalogists guidelines for use of wild animals in research and was approved by the North Carolina Wildlife Resources Commission (permit number 19-SC00162).

Recording Protocol

I identified bats to species and counted echolocation calls, foraging buzzes, and social calls using an ultrasonic microphone and recorder, an ultrasound speaker, and a thermal imaging camera to test my hypothesis (Figure 1). This allowed me to gain insight into the possible responses to the broadcasts of social calls. I attached an AT100 Ultrasonic Transmitter ((Binary Vocal Technology LLC. Tucson, Arizona, USA) to the top of a ~3m PVC pipe in the center of the canopy gap, and operated the broadcasting using G'Tools version 1.7 PLAY'R ultrasonic generation software (Binary Vocal Technology LLC). I also attached an Avisoft ultrasonic microphone (Emkay FG Series from Avisoft Bioacoustics, Berlin, Germany) ~0.3m below the ultrasonic speaker. The microphone was connected to an UltraSoundGate system 1216H (Avisoft Bioacoustics,

Berlin, Germany), which was connected to a laptop (DELL Latitude E6230) . Using Avisoft RECORDER Software, I recorded both sonic and ultrasonic sounds. I used a thermal imaging lens (Photon 320 14.25 mm; Flir/Core By Indigo) to assess possible interactions to the playbacks that could be difficult to assess from audio alone, however, I did not find any results that necessitated the thermal imaging footage. The thermal imaging lens was on a short tripod to capture the full view of the equipment and the canopy gap. The lens was connected to a JVC Everio HDD camcorder. All equipment was powered by an inverter to an external deep marine car battery. All data were regularly backed up to Seagate external hard-drives in triplicate.

Playback Stimuli

Consistency in terminology of social calls has been lacking. In 2003, common pipistrelle social calls were categorized by Type A/B/C/D (Pfalzer and Kusch 2003). Big brown bat calls have been classified with frequency modulated bouts, long frequency modulated calls, and short frequency modulated calls (Wright et al. 2013, Wright et al. 2014). I used the terminology used in Springall et al. (2019). I conducted different trials using silence, echolocation calls, and social calls. Playback files were created in Avisoft SASLab Pro (Avisoft Bioacoustics, Glienicke, Germany). Spectrograms had a 256 FFT (Fast Fourier Transform) and a 100-frame size with FlatTop window. Playbacks were determined based on a previous analysis of recordings collected at the same site 2 years prior to this experiment (Springall et al. 2019). I broadcasted the following calls: big brown bat echolocation, downsweeps, high frequency upsweeps, low frequency

upsweeps, upsweeps of both high and low frequency, upsweeps and downsweeps, and complex calls. I also broadcasted evening bat echolocation, downsweeps, and upsweeps with downsweeps. I broadcasted all calls that I had a sufficient number of recordings to create a playback file, and did not combine species within a playback file.

I used Avisoft to generate playback files for the broadcasting experiment. For a specific social call type, I clipped the social calls from recordings collected in Springall et al. (2019). I clipped the social calls and erased road noise [a repellent (Schaub, Ostwald, and Siemers 2008)], insect noise [an attractant (Hosken et al. 1994)], and echolocation calls between social calls, as echolocation calls are known to contain individual-specific signatures (Yovel et al. 2009). I then replaced poor quality (low amplitude, or overlapping) social calls with high quality (high amplitude, clear boundaries) social calls. A playback file was 1 minute long. To calculate natural calling rates, I identified files that contained 2 or more social calls of the same type, and measured the time between the calls. I repeated this until I had a minimum of 10 measurements for each call type. I clipped the social call files in Avisoft, preserving the calls and the time between them. I averaged the measurements and used the average time for spacing the clipped social calls of the same type from one another, and then repeated the sequence to make a 1-minute file. Neither white noise (equal intensity at all frequencies) or pink noise (decreasing intensity as frequency increases) were used as controls due to previous studies indicating that these are repellants to certain bat species (Schaub, Ostwald, and Siemers 2008, Horn et al. 2008).

Playback Trials

Each trial lasted 3 minutes and consisted of 1 minute each of pre-broadcast, broadcast, and post broadcast. Time between trials was 3 minutes to ensure any reaction to the previous stimuli ended. Calls were broadcast at 90-110 dB amplitude to replicate volumes of original recordings, in a random order each night. The first trial began after the first bat of any species was seen at sunset, as bat activity at this site is highest immediately after sunset and decreases thereafter (Springall et al. 2019). Trials ended once 5 minutes passed without any bats recorded on the Avisoft UltraSoundGate 1216H system. (Figure 2).

Data Analysis

The pre-broadcast minute was used as a control for every trial to account for differences in temperature, humidity, wind, and bat activity. Silence and echolocation call playbacks of both species were also used as controls, by comparing the difference between the post-broadcast minute to the pre-broadcast minute. Specifically, echolocation calls were used as a control to ensure that any response to social call playbacks was not due to a bat being present. Echolocation calls are often used as lures for bats (Lintott et al. 2013) so a comparison of how a social call playback could function as a lure versus echolocation is necessary. Results of the analysis that used echolocation as the control can be found in the supplemental materials. In response to each playback, I used software to count echolocation calls, foraging buzzes, social calls, and species presence (Table 2). Echolocation calls were counted with the pulse train analysis feature

in Avisoft SASLab Pro (Avisoft Bioacoustics, Berlin, Germany). Social calls and foraging buzzes were identified and counted manually in Kaleidoscope v.5 (Wildlife Acoustics Inc, Massachusetts, United States). Species identification of echolocation calls were completed in Sonobat version 4.0 (Szewczak, Arcata, California) using only those calls that were identified with 0.80 or greater confidence.

Data were checked for normality using qq plots and Shapiro-Wilks tests and were found to be not normal. For within trial comparison (post and during vs pre) I used paired Wilcoxon Rank-sum tests. For comparing broadcasts of social calls to broadcasts of silence, I subtracted the “pre” counts from the “during” and “post” phases of the trial to quantify the change in behaviors. A ratio was not suitable for the data due to the presence of zeros in the “post” or “during” minutes, as well as the high frequency of zeros in the data for foraging buzz and social call data. Variations of ratios were tested for the echolocation response data, which did not contain as many zeros, and results did not change compared to the results of subtracting “pre” from “post” or “during”. I used General Linear Models with the “Guassian” family for this analysis, using both silence and echolocation as the reference groups. Results of the analysis using broadcasts of echolocation as the control can be found in the supplemental materials (pg. 40). I used trial type, temperature, humidity, and wind speed data that were collected at the field site as predictors of bat activity for all three dependent variables. Beginning in September 2019, I added two additional trials of social calls that were collected specifically between September 2017 and November 2017 to assess potential seasonal differences in responses to the same calls, however, none were identified. I assessed species specific responses by

filtering the data set by species presence from Sonobat (Szewczak, Arcata, California). For big brown bats, the results did not change when filtering by species presence, and for evening bats the sample size was too small to calculate rank-sum tests or GLMs. Broadcasts were removed from the data set when equipment failed, or if there were no bats present during the “pre” minute of the trial (Table 3). I used the following R packages for my analysis: ggplot2 (Wickham, 2016) for visualization, tidyr 0.8.2 (Wickham and Henry, 2018) for organizing data, and MASS for glm calculations (Venables and Ripley, 2002). All data were analyzed in Rv3.2 (R Core Team, 2018).

CHAPTER III

RESULTS

Data were collected between June 11th and October 12th, 2019, for a total of 53 broadcasting nights. Successful broadcasts per call type were as follows: 58 trials of silence; 55 trials of big brown bat echolocation calls; 27 trials of big brown bat high-frequency upsweep social calls; 33 trials of big brown bat low-frequency upsweep social calls; 38 trials of big brown bat mixed-frequency upsweep social calls; 35 trials of big brown bat downsweep social calls; 27 trials of big brown bat mixed upsweep and downsweep social calls; 60 trials of big brown bat complex social calls; 51 trials of evening bat echolocation calls, 48 trials of evening bat downsweep social calls; 28 trials of evening bat mixed upsweep and downsweep social calls. In total, I successfully broadcasted for 460 trials (Table 1).

Response with “Pre” Minute as Control

Activity Response (Echolocation Counts)

Both species of interest are echolocating bats, therefore echolocation calls were used as a metric for bat activity. Big brown bat echolocation broadcasts decreased the number of echolocation pulses counted both in the “during” (35% decrease) and “post” (26% decrease) minutes when compared to the “pre” (n = 51, V= 1188.5, p < 0.0001;

n=51, V=988.5, $p < 0.001$, respectively). Likewise, evening bat echolocation broadcasts resulted in a 15% decrease in the number of echolocation pulses counted in the “during” phase only (n=45, V=917, $p < 0.001$). Evening bat downsweep social call broadcasts resulted in a 15% decrease in the number of echolocation pulses counted in the “during” minute only (n=43, V=809.5, $p < 0.01$). No other social calls showed any effect in the “during” or “post” minutes when comparing raw data (Table 2).

Foraging Response (Foraging Buzz Counts)

Big brown bat echolocation broadcasts resulted in a 45% decrease in foraging buzz production in the “post” minute compared to the “pre” minute (n=55, V=154, $p < 0.02$) (Table 2). Evening bat echolocation broadcasts did not have an effect on foraging buzz responses in either minute.

Social Call Response (Count)

Big brown bat low frequency upsweeps resulted in a 1,200% increase in social calling from bats in the “post” minute compared to the “pre” minute (n= 33, V=2, $p < 0.05$). Big brown bat high frequency upsweeps and mixed frequency upsweeps did not have an effect, however combining all big brown bat upsweep broadcasts revealed that upsweeps as a whole increased social calling by over 500% in the “post” minute when compared to the “pre” minute (n=98, V=17, $p < 0.05$). When combining all social call broadcasts, social calling increased by over 200% in the “post” minute when compared to the “pre” minute (n=296, V=397, $p < 0.03$) (Table 2).

Response with “Silence” Broadcast as Control

Activity Response (Echolocation Count)

Using silence as the reference group (control), big brown bat echolocation broadcasts resulted in a 460% decrease in activity in the “during” minute when compared to the “pre” minute (n= 305, df= 304, p= 0.001). In the “post” to “pre” minute comparisons, big brown bat echolocation showed a 250% decrease in echolocation call counts (n= 302, df= 301, p< 0.02). Big brown bat mixed frequency upsweeps showed a 222% decrease in echolocation call counts (n= 302, df= 301, p<0.04) (Figure 4), and evening bat downsweeps showed a 214% decrease in echolocation call counts (n=167, df= 166, p< 0.03) (Figure 5).

Social Call Response

Big brown bat low frequency upsweep broadcasts resulted in twice as many social calls produced in the “post” minute when compared to silence “post” minute (n= 346, df= 345, p<0.05) (Figure 6). Evening bat echolocation broadcasts decreased social call production by 127% in the “during” minute (n= 199, df= 198, p<0.05), and 160% in the “post” minute (n=199, df=198, p<0.03) (Figure 7) when compared to silence “during” and “post” minutes respectively.

Weather Results

Temperature and humidity positively influenced echolocation counts (n=1505 trials, df= 1504, p< 0.001, n= 1505, df= 1504, p< 0.001 respectively) but did not have an

effect on the differences between the “post” minute and “pre” minute (temperature: $n=464$, $df=463$, $p=0.378$; humidity: $n=464$, $df=463$, $p=0.996$), or on the differences between the “during” minute and the “pre” minute (temperature: $n=468$, $df=467$, $p=0.0767$; humidity: $n=468$, $df=467$, $p=0.639$).

CHAPTER IV

DISCUSSION

Under field conditions, these experiments revealed that social call playbacks from two temperate bat species (big brown and evening bats) impact bat community vocal responses. I broadcasted recordings of silence, echolocation calls, and a variety of social calls and combinations of social calls, previously reported in laboratory studies to test for effects on bats' behavioral responses. By examining behavioral responses of free ranging bats to broadcasts of recorded vocalizations, in a distinctively different field setting, I found that echolocation broadcasts from both species decreased echolocation calling. Big brown bat echolocation call broadcasts decreased foraging buzz responses, and big brown bat social call broadcasts increase social call responses. These changes in behavior indicate that in flight social calls have a communication function.

In this study, playback of echolocation vocalizations of both big brown and evening bats reduced echolocation call responses by 35% and 15% respectively for “post” minute to “pre” minute comparisons (Table 2). When big brown bats fly in pairs, one will sometimes stop echolocating and use the echolocation of the other bat for navigation, potentially reducing auditory signal interference for the other individual (Chiu, Xian, and Moss 2008). Thus, it is possible that bats that present in pairs might emit only one set of vocalizations, thus not representing to researchers the responses of both

members of the pair. This could have had an effect on the number of echolocation calls counted in this study. The present results are contradictory to previous research, which has suggested that heterospecifics were attracted to the echolocation calls of other bats (Barclay 1982, Lintott et al. 2013). A repulsive response has been seen in other mammals, including red squirrels (*Sciurus vulgaris*), which use calls to defend their territories against conspecifics (Siracusa et al. 2018). Echolocation calls, known to contain individual signatures (Yovel et al. 2009), could also communicate territoriality. In support of the latter, I found that big brown bat echolocation call broadcasts reduced the subsequent number of foraging buzzes.

I identified decreased echolocation call production in response to evening bat downsweeps. It is possible that downsweeps are used for resource or territory defense against heterospecifics, and this may be particularly important when heterospecifics have similar diets. In a similar manner, neotropical Chiriquí singing mice (*Scotinomys xerampelinus*) repel neighboring Alstons singing mice (*Scotinomys teguina*) from geographical areas using vocalizations (Pasch, Bolker, and Phelps 2013). Male ground finches on Galápagos Islands respond more aggressively to heterospecific songs than conspecific songs even though the songs are acoustically very similar (Ratcliffe and Grant 1985).

Big brown bats increased social calling in response to broadcasts of social calls, and specifically to broadcasts of upsweep social calls. Call and response are not limited to bats. Bigeyes (*Pempheris adspersa*), a nocturnal reef fish, increase their vocalizations and group cohesion behaviors during playback of conspecific vocalizations (van

Oosterom et al. 2016). Dugongs (*Dugong dugon*), a marine mammal, increase chirp calls in response to playbacks of dugong chirps and downsweeps compared to silence (Ichikawa et al. 2011). The upsweep social call used in this study is likely used for group cohesion in big brown bats, and social call broadcasts of upsweeps could be useful acoustic lures for increasing capture rates in big brown bats. It is also possible that the call is used for communicating a feeding patch or the quality of the feeding pack, however this is not as likely due to the lack of foraging buzz response. Low presence of evening bats did not allow for playback - response comparisons. Future researchers should focus on evening bats to examine possible associations between specific social calls and responses.

Calls used to create the broadcast files were collected from the same site that the trials took place, so it is possible that the bats recognized the individual signatures contained within the calls and this may have affected response. Trials took place beginning 5 minutes after the first bat and ended after 5 minutes without a bat recorded, typically 1-2 hours later. Overnight recording could potentially reveal calls used for returning to roosts, however it is known that most social calls are produced at the beginning of the night (Springall et al. 2019). Additionally, the individuality of the bats receiving the broadcasting stimuli may be important for response. I found that, despite similar shapes, social calls have immense plasticity between individuals, therefore it is very likely that there are individual signatures that other bats are able to recognize.

Overall, these results indicate that downsweep social calls do not increase bat echolocation counts as predicted. Instead, downsweep social calls, may assist with group

cohesion by keeping heterospecifics away rather than drawing conspecifics in. Broadcasts of upsweep social calls increased social call responses and therefore, these calls are likely used for communication. Additional research is needed to identify the specific information encoded within upsweep social calls. Group cohesion offers numerous benefits to animals across taxa, including predator avoidance and foraging success (Sogard and Olla 1997). How bats maintain groups while in flight has remained difficult to understand, however, the present study provides evidence that upsweep social calls may be involved. My study was conducted in a field setting where complex biotic and abiotic factors cannot be controlled, and despite this, I was able to identify two social calls that changed bat behaviors. These results can shed light on the coevolution of social calls and group cohesion in echolocating bats.

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APPENDIX A

TABLES AND FIGURES

Table 1. Total and Successful Broadcasts for Each Call Type. *Asterisks indicates control trial. E.f.* = *Eptesicus fuscus*, *N.h.* = *Nycticeius humeralis*. *Data were collected between June 11th and October 12th 2019 in Greensboro NC, USA. Data were analyzed in R v3.*

<u>Broadcasts</u>		
Broadcast	Total	Successful
Silence*	79	58
E.f. Echolocation	74	55
E.f. High Frequency Upsweep	38	27
E.f. Low Frequency Upsweep	45	33
E.f. Mixed Frequency Upsweep	49	38
E.f. Downsweep	52	35
E.f. Mixed Upsweep & Downsweep	37	27
E.f. Complex	77	60
N.h. Echolocation	61	51
N.h. Downsweep	61	48
N.h. Upsweep & Downsweep	35	28
Total	608	460

Table 2. Paired Broadcast Results by Call Type and Response Variable. *P*-values for Echolocation, foraging buzz, and social call responses from the bat community. “During” and “Post” were compared to the “Pre” minute for the corresponding response variable with Wilcoxon-Rank-Sum paired test in R v3.5. Arrows indicate the direction of the change, *n*=27-60 depending on the broadcast. Data were collected between June 11th and October 12th 2019 in Greensboro NC, USA.

Stimulus	Echolocation Response During	Echolocation Response Post	Buzz Response During	Buzz Response Post	Social Response During	Social Response Post
Silence	0.642	0.072	0.959	0.833	0.090	0.154
E.f. Echolocation	↓ <0.0001	↓ <0.001	0.161	↓ <0.02	0.531	0.396
E.f. Low Frequency Upsweep	0.331	0.645	0.659	0.526	0.281	↑ <0.05
E.f. High Frequency Upsweep	0.620	0.909	0.389	0.549	1	1
E.f. Mixed Frequency Upsweep	0.209	0.122	0.574	0.687	0.371	0.581
E.f. Downsweep	0.614	0.905	0.310	0.622	0.423	0.598
E.f. Upsweep & Downsweep	0.258	0.581	0.266	0.491	1	0.892
E.f. Complex	0.596	0.420	0.507	0.116	0.850	0.611
N.h. Echolocation	↓ <0.001	0.566	0.299	1	1	0.353
N.h. Downsweep	↓ <0.01	0.114	0.582	0.284	0.279	1
N.h. Upsweep & Downsweep	0.849	0.707	0.356	0.895	0.269	0.089

Figure 1. Spectrograms of Big Brown Bat and Evening Bat Social Calls. *Spectrograms were captured in Kaleidoscope v.5 (Wildlife Acoustics Inc, Massachusetts, United States). Social calls were collected from the UNCG Wetlands, Greensboro, NC, between March 15th, 2017 and June 30th, 2018.*

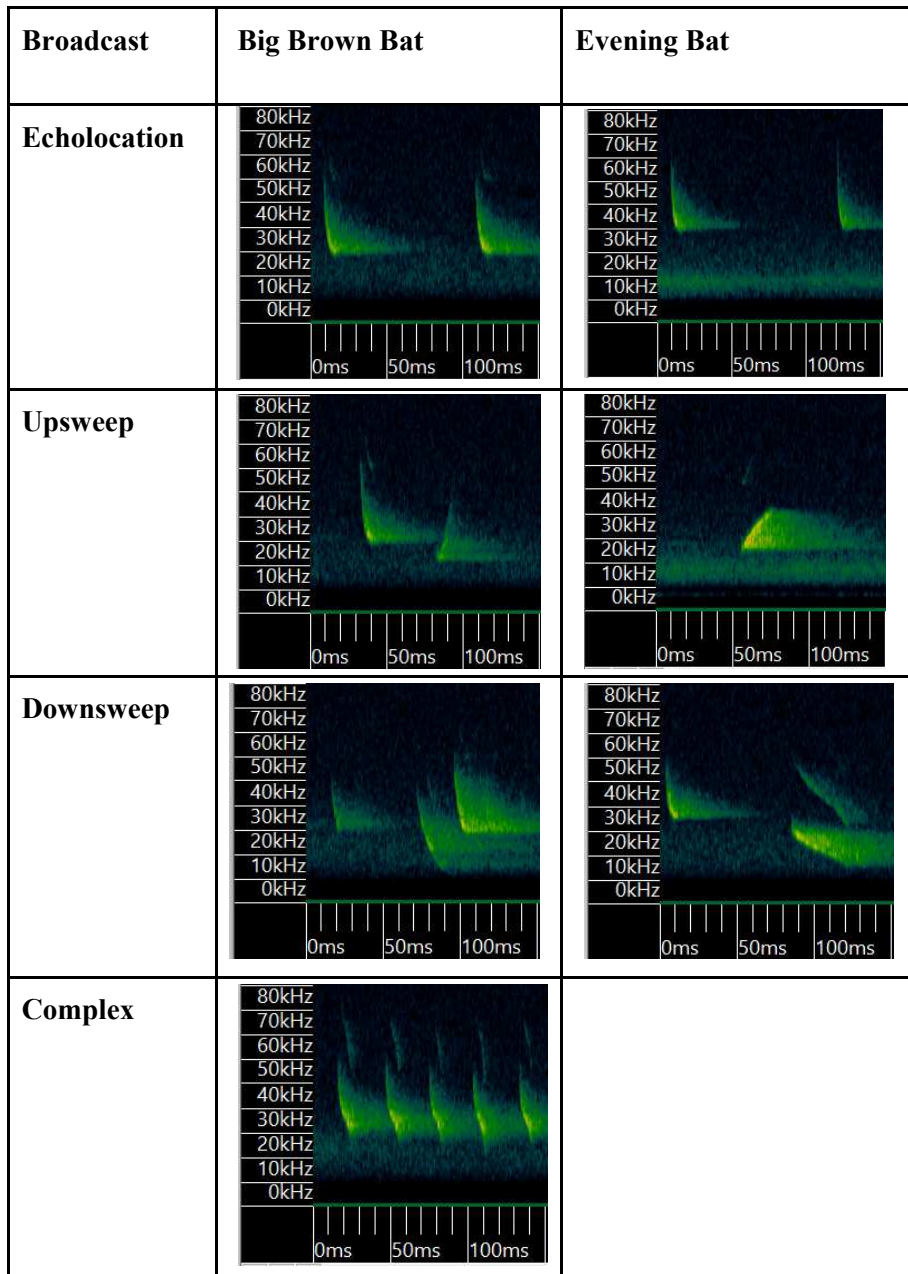


Figure 2. Schematic of Field Set-Up. *Not to scale. Actual size of pole is 3 meters. Research took place in Peabody park between June 11th and October 12th, 2019, in Greensboro NC, USA.*

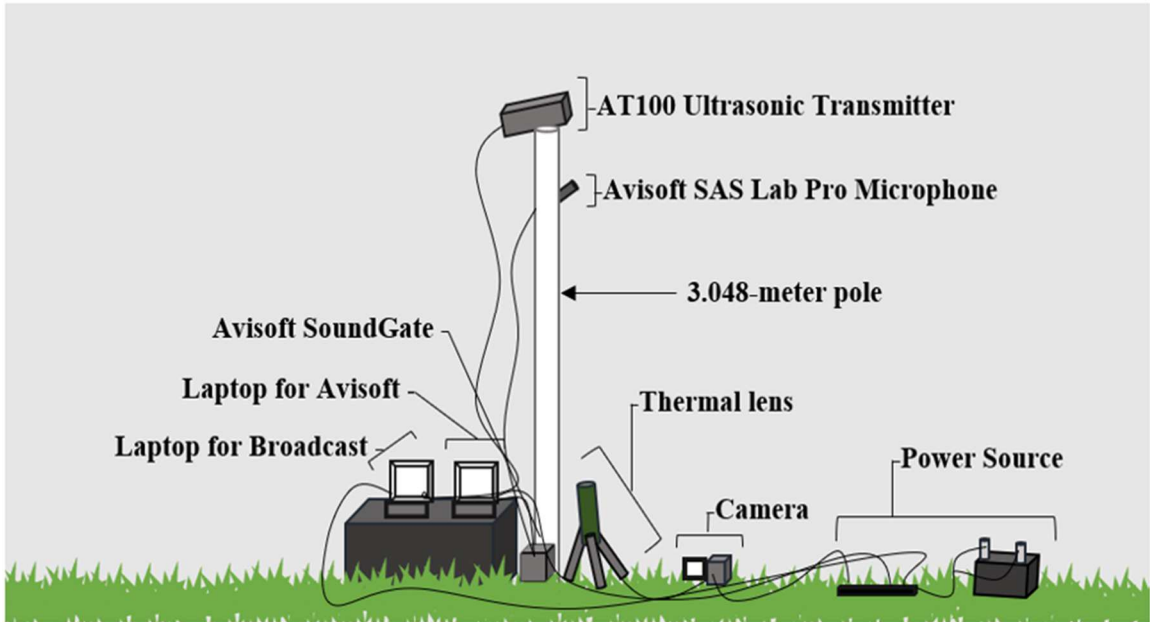


Figure 3. Visual Representation of Broadcast Design. *Timing of pre-observation, playback, post-observations, and recovery in a session within one night. Ranged from 3-22 trials per night depending on weather and level of bat activity.*

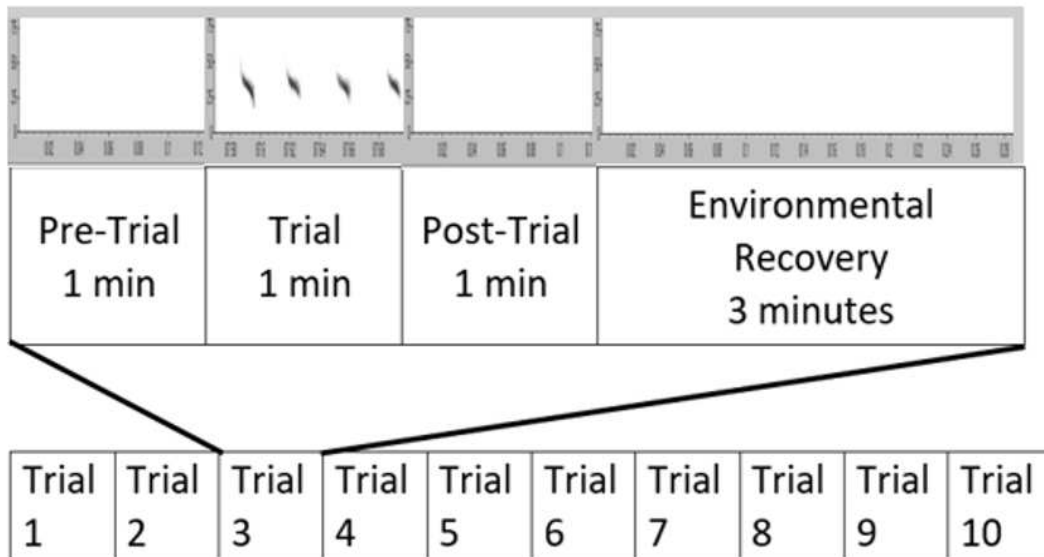


Figure 4. Echolocation Response to Big Brown Bat Broadcasts. Comparisons are of the “Post” minute to the “Pre” minute, using GLMs with Gaussian family distribution. One asterisks indicates a p -value <0.05 , two asterisks indicates a p -value of <0.001 , and three asterisks indicates a p -value <0.0001 . Data were analyzed in R v3.5, package: MASS (Venables and Ripley, 2002). Data were collected between June 11th and October 12th, 2019 in Greensboro NC, USA.

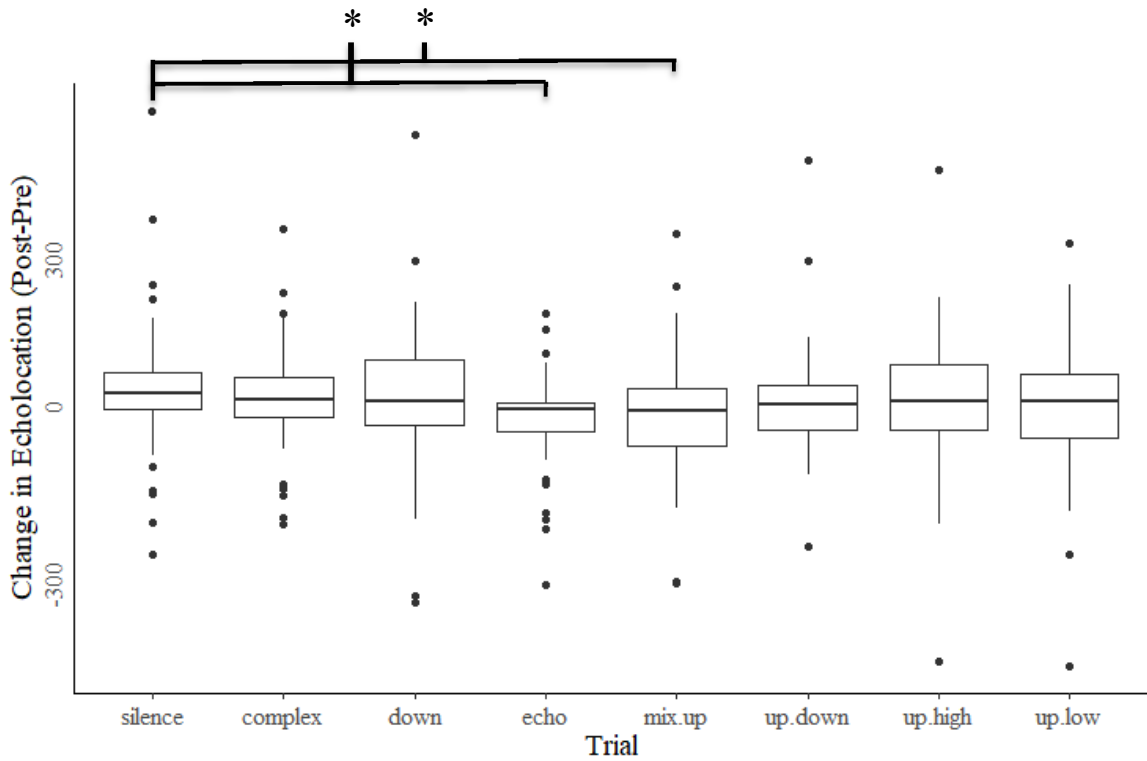


Figure 5. Echolocation Response to Evening Bat Broadcasts. Comparisons are of the “Post” minute to the “Pre” minute, using GLMs with Gaussian family distribution. Asterisks indicates a p -value < 0.05 . Data were analyzed in R v3.5, package: MASS (Venables and Ripley, 2002). Data were collected between June 11th and October 12th, 2019 in Greensboro NC, USA.

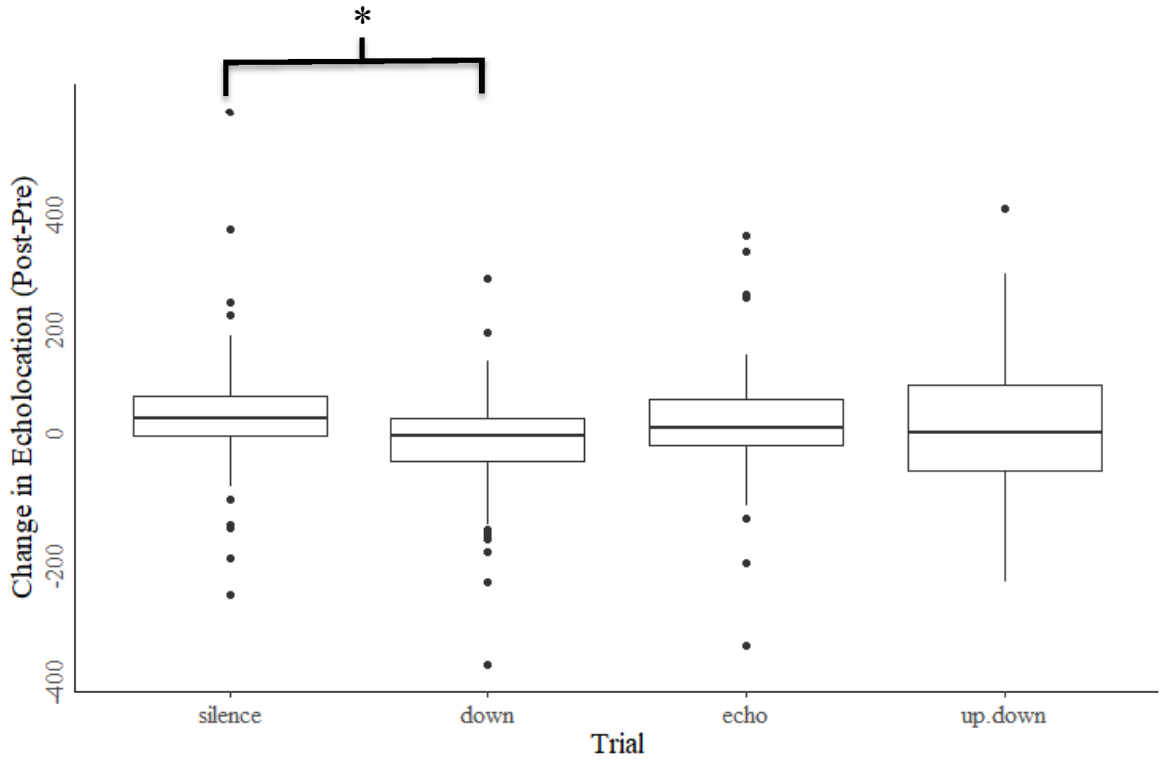


Figure 6. Social Call Response to Big Brown Bat Broadcasts. Comparisons are of the “Post” minute to the “Pre” minute, using GLMs with Gaussian family distribution. Asterisks indicates a p-value <0.05. Data were analyzed in R v3.5, package: MASS (Venables and Ripley, 2002). Data were collected between June 11th and October 12th, 2019 in Greensboro NC, USA.

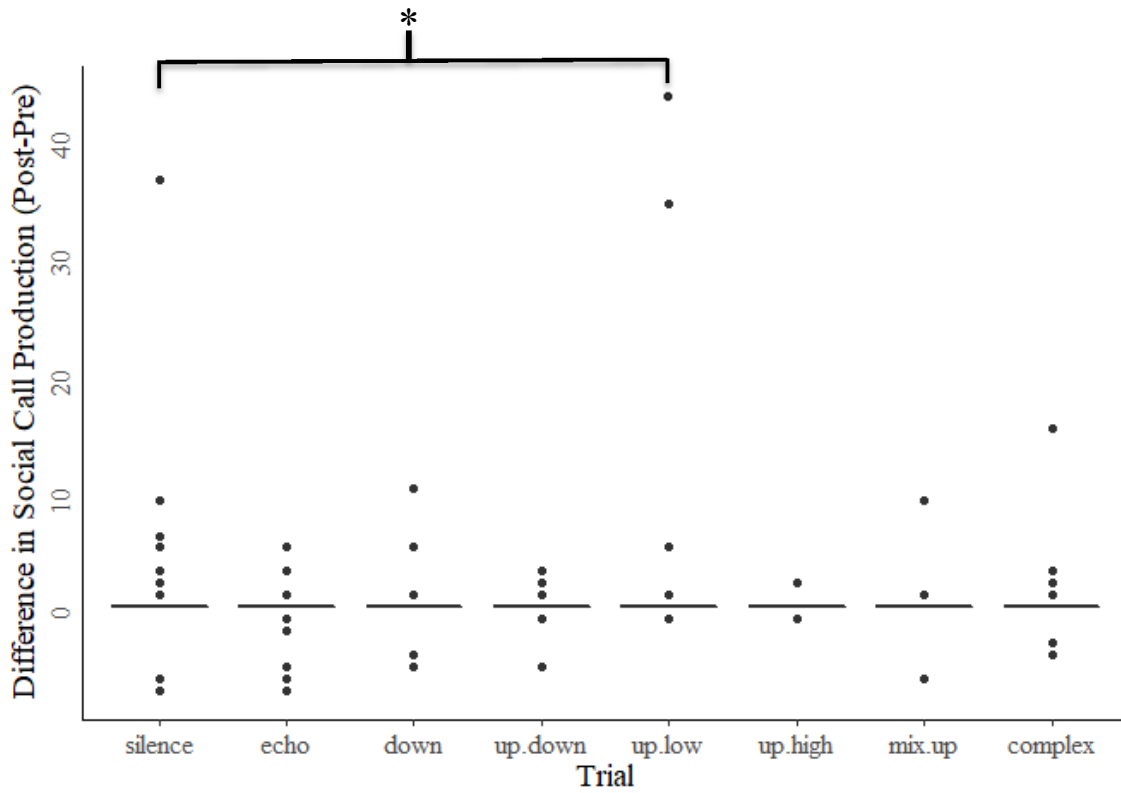
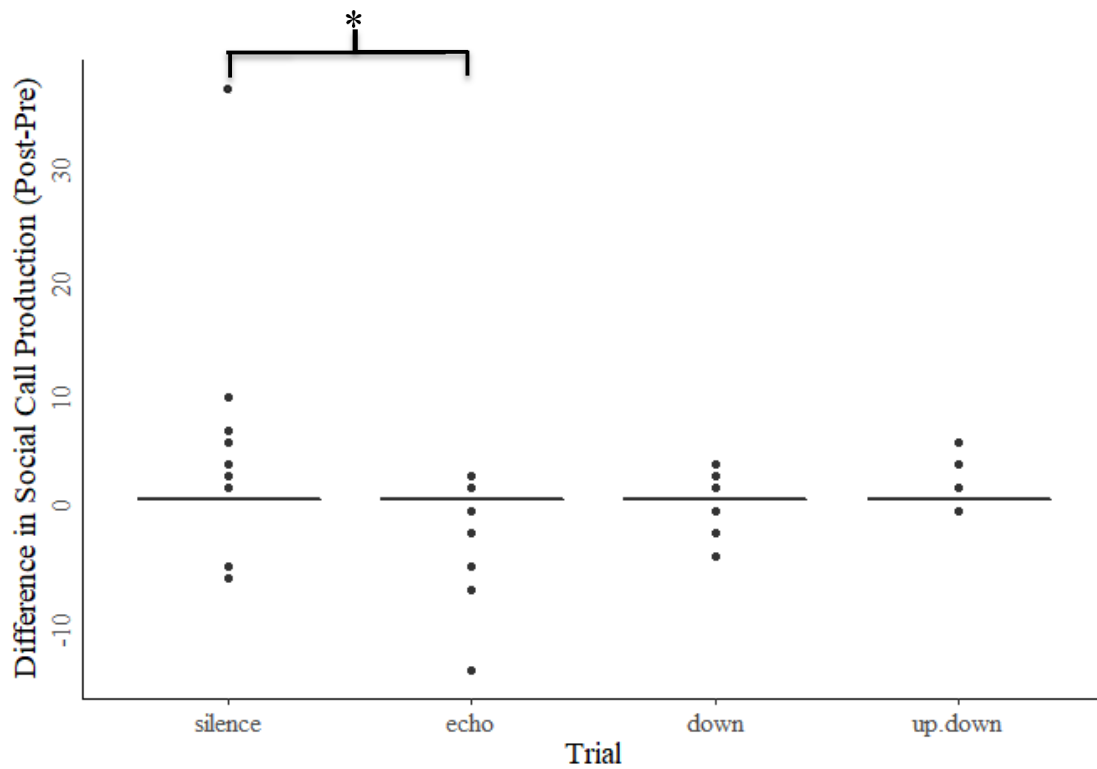


Figure 7. Social Call Response to Evening Bat Broadcasts. Comparisons are of the “Post” minute to the “Pre” minute, using GLMs with Gaussian family distribution. Asterisks indicates a p-value <0.05. Data were analyzed in R v3.5, package: MASS (Venables and Ripley, 2002). Data were collected between June 11th and October 12th, 2019 in Greensboro NC, US.



APPENDIX B

SUPPLEMENTAL MATERIAL

Echolocation as Control

Echolocation Response

Comparing the difference in activity “pre” minute echolocation counts subtracted from the “during” minute and “post” minute echolocation count I was able to compare responses across trials. Using echolocation as the reference group for each species, in the “during” minute, *Eptesicus fuscus* complex calls increased activity ($p < 0.01$), *Eptesicus fuscus* high frequency upsweeps increased activity ($p < 0.04$), and *Eptesicus fuscus* low frequency upsweeps increased activity ($p < 0.01$). No effects observed in the “post” comparisons.

Social Call Response

Using echolocation for each species as the reference group, *Eptesicus fuscus* low frequency upsweep broadcasts increased social calling when compared to *Eptesicus fuscus* echolocation broadcasts ($p < 0.001$)