# Discovery and Characterization of High-frequency Calls in North American Flying Squirrels (*Glaucomys sabrinus* and *G. volans*): Implications for Ecology, Behavior, and Conservation

by

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# Abstract

Studies of bioacoustics of vertebrates primarily have focused on amphibians and birds. Comparatively, the acoustic repertoire of mammals is poorly understood, and even less is known about the production of high-frequency sound. Thus far, the study of ultrasound in mammals has focused largely on echolocating abilities of bats and odontocete whales. Studies examining high-frequency sounds used for communication generally have been limited to laboratory experiments with mice and rats. Considerably less is known about high-frequency sound in other mammalian taxa. Similarly, most studies of bioacoustics focus on a single type of call emitted by a species. There are few studies describing the repertoire of calls of mammals. I describe the first known ultrasonic calls in North American flying squirrels (*Glaucomys sabrinus* and *G. volans*) and demonstrate a complex repertoire of calls within both species spanning both sonic and ultrasonic ranges. Passive recordings were collected from captive *G. sabrinus* and *G. volans* to generate a library of calls for each species. The acoustic model accurately classified calls correctly 92.2% of time. To test the library using natural populations, acoustic surveys were conducted in the Piedmont Plateau and Coastal Plains of Alabama where *G. volans* occur, and in the Appalachian Mountains of western North Carolina where southern flying squirrels and endangered Carolina northern flying squirrels (*G. s. coloratus*) occur. Recordings of flying squirrels from acoustic surveys conducted in the wild were quantified and discriminant-function analysis was used to compare recordings from the wild to calls of animals in captivity. Acoustic surveys in the wild detected squirrels at 86 of 180 sites sampled for 47.8% success of detection. The discovery of high-frequency calls in North American flying squirrels may be useful for behavioral studies addressing function of varying types of calls, evolution of ultrasound in gliding mammals, and for detecting, identifying, and monitoring species by using acoustical surveys.

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# DISCOVERY AND CHARACTERIZATION OF HIGH-FREQUENCY VOCALIZATIONS IN NORTH AMERICAN FLYING SQUIRRELS (*GLAUCOMYS SABRINUS* AND *G. VOLANS*)

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Studies of bioacoustics of vertebrates have focused on amphibians and birds. Comparatively, the acoustic repertoire of mammals is poorly understood, and even less is known about the production of high-frequency sounds. Thus far, the study of ultrasound in mammals has focused primarily on echolocating abilities of bats and odontocetid whales. Studies examining high-frequency sounds for communication generally have been limited to laboratory experiments with mice and rats. Considerably less is known about high-frequency sounds in other mammalian taxa. Similarly, most studies of bioacoustics focus on a single type of call emitted by a species. Studies describing the repertoire of calls of a species are rare in mammals. I describe the first known ultrasonic calls in North American flying squirrels (*Glaucomys sabrinus* and *G. volans*) and demonstrate a complex repertoire of calls within these species spanning both the sonic and ultrasonic ranges. Passive recordings were collected from captive *G. sabrinus* and *G. volans* to generate a library of calls for each species. Vocalizations were then quantified and analyzed using discriminant-function analysis to generate an acoustic model for distinguishing types of calls within and between species. I identified and characterized three unique types of high-frequency vocalizations for each species. The overall model correctly classified grouped calls 92.2% of the time. These discoveries may be useful for behavioral studies addressing function of varying types of calls, evolution of ultrasounds in gliding mammals, and for detecting and discriminating species by using acoustical surveys.

Key words: bioacoustics, flying squirrel, *Glaucomys sabrinus*, *Glaucomys volans*, ultrasonic vocalizations

The field of bioacoustics in vertebrates has focused primarily on anurans and avians. The wealth of acoustic knowledge for these two taxa can be attributed to how often both frogs and birds vocalize to defend territories or procure mates. High-frequency sounds above the range of hearing by humans (≥ 20 kHz) were first discovered in bats (Pierce and Griffen 1938). Since then, studies of ultrasonic signals emitted by mammals have been performed primarily on only two species (house mouse *Mus musculus* and brown rat *Rattus norvegicus*) in a laboratory setting. Outside of research published on these two species, high-frequency sounds are understood for only a few mammalian taxa including bats and cetaceans, which use ultrasonic vocalizations for echolocation.

Recent studies have identified and characterized multi-purpose uses of ultrasonic vocalizations in a variety of mammalian taxa. For example, Wilson and Hare (2004) discovered that Richardson’s ground squirrels (*Urocitellus richardsonii*) use ultrasonic vocalizations as a type of alarm to warn conspecifics of potential danger. Alarm calls with both sonic and ultrasonic components is species-specific in two species of golden-mantled ground squirrels (*Callospermophilus lateralis* and *C. saturates*; Eiler and Banack 2004). Complex ultrasonic songs that are similar to singing in some birds were first characterized quantitatively in male house mice (*Mus musculus*), and species-specific differences were detected in ultrasonic calls of baiomyine mice (Miller and Engstrom 2007). Briggs and Kalcounis-Rueppell (2011) reported evidence of male and female California deermice (*Peromyscus californicus*) producing ultrasonic vocalizations to defend territories and to maintain pair bonds. Other examples of ultrasonic vocalizations in Rodentia include neonatal voles (Blake 2002), rats (Brudzynski and Pniak 2002), and mice (Hahn and Schanz 2002) calling to mothers; male mice calling to females for reproductive purposes (Holy and Guo 2005; Pomerantz and Clemens 1981), and female mice increasing calls in the presence of same-sex conspecifics while foraging (Moles and D'Amato 2000). Fenton (1984) suggested that ultrasound probably was first used for communication and later became more specialized for navigation as the progenitor of bats evolved physiologically to leap, glide, and eventually fly. The discovery of high-frequency vocalizations in both species of North American flying squirrels (*Glaucomys*) provides a unique opportunity to study form and function of ultrasound in a gliding mammal.

Studies of bioacoustics of ultrasounds in mammals have thus far demonstrated that flying and aquatic taxa use ultrasounds for echolocation and communication, whereas terrestrial species more often use it for communication. As a nocturnal, gliding mammal, flying squirrels represent a functional intermediate to better understand production of ultrasound in mammalian systems. While it has been suggested that flying squirrels may emit sounds above the range of hearing in humans (Muul and Alley 1963), the only described calls include sonic tseets, bird-like chirps, chuck-chuck notes, and a soft, sneeze-like call (MacClintock 1970; Schwartz and Schwartz 2001; Sollberger 1940; and Perkins 1873). However, no one has described vocalizations of North American flying squirrels quantitatively. Thus, objectives of my study were to identify and quantitatively characterize vocalizations of *G. sabrinus* and *G. volans*, and to build an acoustic model to test intraspecific and interspecific differences.

## MATERIALS AND METHODS

To assess vocal repertoires of high-frequency sounds by northern and southern flying squirrels, passive recordings were made of captive squirrels. Recordings of 23 *G. sabrinus* were collected from a captive colony at Southeast Missouri State University and 26 *G. volans* were recorded from a captive colony at Wake Forest. The captive colony of northern flying squirrels was established with wild individuals from the northwestern United States. The colony at Wake Forest of southern flying squirrels was established with individuals from the southeastern United States. Squirrels were recorded passively from dusk until dawn for 3 nights at each facility during each spring  of 2006 and 2008.

Terminology used in bioacoustics is variable. Therefore, parameters of calls similar to those adopted by Holy and Guo (2005) and Kalcounis-Ruppell et al. (2006) were used to describe vocalizations in my study.  A syllable was defined as a single unit of sound emitted by a squirrel and separated from other such units of sound by silence. Syllables may contain multiple notes, which are changes in pitch. A type of syllable is one that is identifiable statistically and repeated regularly during vocalizations. A series of syllables (≥2) emitted in succession represents an acoustic phrase. Phrases comprised of a series of syllables that fall into one or more types of syllables are referred to as types of phrases. In general, a vocalization is a purposeful sound made by an animal, and a type of syllable is any call made by an animal that is distinct from other types of syllables and unique to that species. Thus, types of syllables and types of phrases are distinct from other such syllables and phrases. In my study, I describe general observations of types of phrases, but analyses were performed on individual types of syllables.

Vocalizations of *G. sabrinus* and *G. volans* were collected using iRiver® mp3 digital recorders (iRiver Limited, South Yarra, Victoria, Australia) attached to a Pettersson D240x ultrasound detector (Pettersson Elektronik AB, Uppsala, Sweden). The detector had a built-in microphone with a flat frequency response of 5-200 kHz. Digitized recordings were collected at a sampling rate of 44.1 kHz and 16-bit resolution. To better characterize ultrasonic calls, recordings were obtained in time-expansion mode (slowed down by a factor of 10). Time expansion allows for broadband recording and preserves all parameters of the original signal including harmonics. Thus, time expansion is better suited for studies describing and characterizing different types of vocalizations (Pettersson 2002). Calls of varying quality were recorded throughout the study, but those of low quality were excluded from analyses. High quality vocalizations were analyzed in Raven: Interactive Sound Analysis Software$^{©}$ (Cornell Lab of Ornithology, Ithaca, New York). Spectrograms were viewed in a Hann window with 50% overlap on a time grid and discrete Fourier transform (DFT) size of 256 samples on a frequency grid.

To describe types of syllables, 16 measurable characteristics were used including: duration, low frequency, high frequency, bandwidth, frequency at maximum amplitude, first-quartile frequency, third-quartile frequency, frequency at 5%, center frequency, frequency at 95%, bandwidth at 90% of call, aggregate entropy, average entropy, average power, energy, and maximum power (Table 1.1). Measurements of time, frequency, and amplitude were recorded in milliseconds (msec), kilohertz (kHz), and decibels (dB), respectively. Entropy was measured as a volume of energy (u). Means and standard deviations for each measured characteristic were calculated (Table 1.2). A forward stepwise-discriminant-function analysis (DFA) with cross validation (Table 1.3; Table 1.4) was used to classify vocalizations of each species and to create a predictive acoustic model for identifying types of syllables within species and between *G.*

*sabrinus* and *G. volans*. Statistical analyses were performed using SPSS 20.0 (SPSS, Inc., Chicago, Illinois).

## RESULTS

Across 6 nights of passively recording six adult females (3 nights) and 17 adult males and females of *G. sabrinus* (3 nights), I recorded 232 high-quality vocalizations. Vocalizations occurred in phrases 55.9% of the time. Three distinct types of syllables were detected in the total number of vocalizations and categorized as arc-whistles, trills, and upsweeps (Fig. 1). Arc-whistles were the most common type of syllable comprising 42.7% of vocalizations, followed by trills (38.8%) and upsweeps (18.5%). While all three types of syllables usually were emitted in phrases, 41.4% of arc-whistles were recorded as a single vocalization in one unit of time (1.7 sec). All trills and 90.7% of upsweeps occurred in phrases. Two consistent types of phrases were recorded. When trills were emitted, they always occurred in rapid succession and were the only type of syllable in the phrase with an average of 4.7 trills/sec. These were classified as trill phrases. Secondly, upsweep type of phrases of northern flying squirrels contained two types of syllables (single arc-whistle followed by multiple upsweeps) 88.9% of the time. Upsweeps occurred at an average of 2.6 per sec. These structured vocalizations were classified as bi-syllable phrases containing arc-whistles and upsweeps.

*Glaucomys volans* recorded during 6 nights at Wake Forest included 26 squirrels (14 males, 12 females). Of 215 individual high quality vocalizations recorded, 44.2% occurred in phrases. Multiple syllables were recorded; however, the most common vocalizations from all recordings of southern flying squirrels were of three distinct types of syllables: arc-whistles, trills, and crows (Fig. 2). The greatest number of types of syllables recorded was trills (45.1%), followed by arc-whistles (33.0%) and crows (21.9%). For southern flying squirrels, only crows were more likely to be emitted singly (85.1%) than repeated in phrases. Arc-whistles occurred in succession 67.6% of the time at a rate of 2.28 calls/sec, and similar to trills of northern flying squirrels, trills of *G. volans* always occurred in phrases with an average of 2.56 types of calls/sec. Of the three types of syllables identified, arc-whistles were typically the only vocalizations in arc phrases, and trills were the only type of syllable in trill phrases. Phrases that contained crows were not recorded often, but only contained that type of syllable (crow) when detected. The overall model in discriminant-functional analysis explained 90% of variation within calls in the first canonical discriminant function, and all five canonical discriminant functions used were highly significant $P < 0.001$ (Table 1.3). All six types of syllables for *G. sabrinus* and *G. volans* were classified correctly at 92.2% in the original model and 90.6% in cross validation (Table 1.4; Fig. 3).

## DISCUSSION

Trembock (1963) noted the lack of studies in mammalian acoustics. Some 30 years later, calls of only 5% of mammalian species had been archived (Gannon and Foster 1996). In 2004, Eiler and Banack reported that 21% of vocalizations of all species of mammals were archived and housed in three libraries (Macaulay Library of Natural Sounds, Ithaca, New York; British Library National Sound Archive, London, United Kingdom; and Borror Library of Bioacoustics, Columbus, Ohio). However, most of these recordings of mammals are of sonic calls or ultrasonic calls used for echolocation. Recently, there has been increased interest in mammalian bioacoustics. Several studies have demonstrated frequent and multi-purpose forms of communication using ultrasonic vocalizations in a variety of rodents (Briggs and Kalcounis-Rueppell et al. 2011; Holy and Guo 2005; Miller and Engstrom 2007; Panksepp and Burdorf 2000; Wilson and Hare 2004). The function of acoustic signals typically is assigned to general categories such as alarm, reproductive, or territorial calls. However, more quantitative research has demonstrated the ability of *Mus musculus* (Holy and Guo 2005), *Baiomys musculus*, and *B. taylori* (Miller and Engstrom 2007) to sing ultrasonically. Laughter-like chirps were discovered at 50 kHz in *Rattus norvegicus* (Panksepp and Burdorf 2000), as well as use of sonic syntax by rock hyraxes (*Procavia capensis*; Kershenbaum et al. 2012). With these discoveries and advancements in bioacoustic techniques, vocalizations in mammals have proven more complex and more common than previously believed. Yet, the wealth of knowledge for high-frequency vocalizations in mammals comes primarily from a few taxa including bats, whales, and myomorph rodents.

In my study, I describe the first quantifiable, high-frequency calls of *G. sabrinus* and *G. volans*. Both species frequently emitted these vocalizations throughout all recording sessions, suggesting that high-frequency sounds may play an important role in better understanding the communicative behavior of North American flying squirrels. Passive recordings demonstrated that each species most often emitted calls that were in one of three distinct types of syllables. While comparative studies of high-frequency repertoires in mammals are limited, Rehakova-Petru et al. (2012) discovered that a nocturnal primate (Philippine tarsier *Tarsius syrichta*) produced seven of eight types of calls with an upper frequency of 16-22 kHz. Within Rodentia, the brush deermouse *Peromyscus boylii* produced a minimum of three types of phrases and *P. californicus* a minimum of five types of phrases (Kalcounis-Rueppell et al. 2006). Syllables within types of phrases were similar in *Peromyscus*, but intraspecific differences in syllables were detected between laboratory and wild house mice (Kalcounis-Rueppell et al. 2010). These results are similar to my study. Two types of syllables for *G. sabrinus* (arc-whistles and trills) were acoustically and structurally similar to those of *G. volans* (arc-whistles and trills), although

DFA correctly classified all but trills of southern flying squirrels ≥89.6% of the time (Table 1.4). Nearly all of the incorrectly classified trills (16.5%) were categorized as arc-whistles of northern flying squirrels. Acoustically and spectrographically, these types of syllables were distinguished easily. Thus, in future studies examining interspecific differences, researchers should separate arc-whistles from trills based on how they sound and the spectrographic structure. Then, comparisons can be made on similar types of syllables between species (e.g., trills of *G. sabrinus* and trills of *G. volans*). The third type of syllable identified for each species was unique to each species. For example, upsweeps were recorded only from *G. sabrinus* and crows were collected only from *G. volans*. The overall model in DFA correctly classified 92.2% of the six types of syllables. Thus, interspecific differences in vocalizations of *Glaucomys* are distinct. Two other studies discovered species-specific differences in vocalizations of sciurids using quantitative analyses (Eiler and Banack 2004; Gannon and Lawlor 1989). Together with my study, these results show promise in using acoustical sampling as an additive measure of morphological and molecular techniques to distinguish species (Eiler and Banack 2004) and as a non-invasive surveying method to document presence of species.

I recorded samples of all types of syllables occurring in phrases, but crows for *G. volans* were the only syllable most likely to be emitted singly (85.1% of the time). Comparatively, crows were of longer duration (mean = 199.4 msec) than other types of syllables (Table 1). Usually, types of phrases consisted of the same types of syllables repeated in succession, regardless of species. However, arc-whistles usually preceded upsweeps in *G. sabrinus*. Other analyses of types of phrases have determined that male *M. musculus* use multiple syllables while exhibiting song-like calls (Holy and Guo 2005). My results are most similar to those reported by Kalcounis-Rueppell et al. (2006), where both *P. boylii* and *P. californicus* emitted multiple types of syllables in some types of phrases, while also repeating similar types of syllables in other phrases. It is likely that these differences were detected because researchers in the experiment on *Mus* were examining only vocalizations made during a specific behavioral context (male mice presented with sex-specific odors).

Frequencies of calls of northern flying squirrels were 4.8-26.7 kHz and those of southern flying squirrels were 5.3-30.1 kHz. While upper limits of their ranges are similar to other studies of high-frequency calls in rodents (Kalcounis-Rueppell et al. 2006; Wilson and Hare 2004), flying squirrels regularly make use of both sonic and ultrasonic ranges with frequency-modulated and tonal calls. Tonal calls like arc-whistles often contained both sonic and ultrasonic frequencies concurrently, while frequency modulated calls like trills, upsweeps, and crows typically oscillated between the two ranges.

In my study, quantifiable measurements of calls for both species of North American flying squirrels were used to build a predictive acoustic model allowing future studies to test species-specific differences. The variety of calls characterized demonstrated complexity within communicative behaviors of North American flying squirrels. Use of different types of syllables and phrases may convey different messages to conspecifics. Trill phrases in both species and upsweep phrases in *G. sabrinus* fit the definition of song adapted by Holy and Guo (2005). Prior to discovery of syntax in rock hyraxes, structured use of syllables had been studied in only three other mammalian taxa including primates, cetaceans, and bats (Kershenbaum et al. 2012). In my study, the number of syllables emitted and order of types of syllables suggests that *Glaucomys* also uses structured syntax. In addition, I present the first use of ultrasonic vocalizations in flying squirrels. Currently, the known functions of ultrasound in mammals include echolocation in bats, cetaceans, and insectivores, and various purposes of communication in bats, cetaceans, and rodents. Proposed advantages of ultrasonic communication include quick attenuation of the signal and occurring above the hearing range of most predators (Miller and Engstrom 2007). This allows nearby conspecifics to share information without revealing the location of the signaler. Overall, results of my study demonstrate that high-frequency vocalizations in flying squirrels are quantifiably distinct and emitted often by captive squirrels. Such information may be useful for behavioral studies addressing function of varying types of calls, evolution of ultrasound in gliding mammals, and for detecting and discriminating species by using acoustical surveys.

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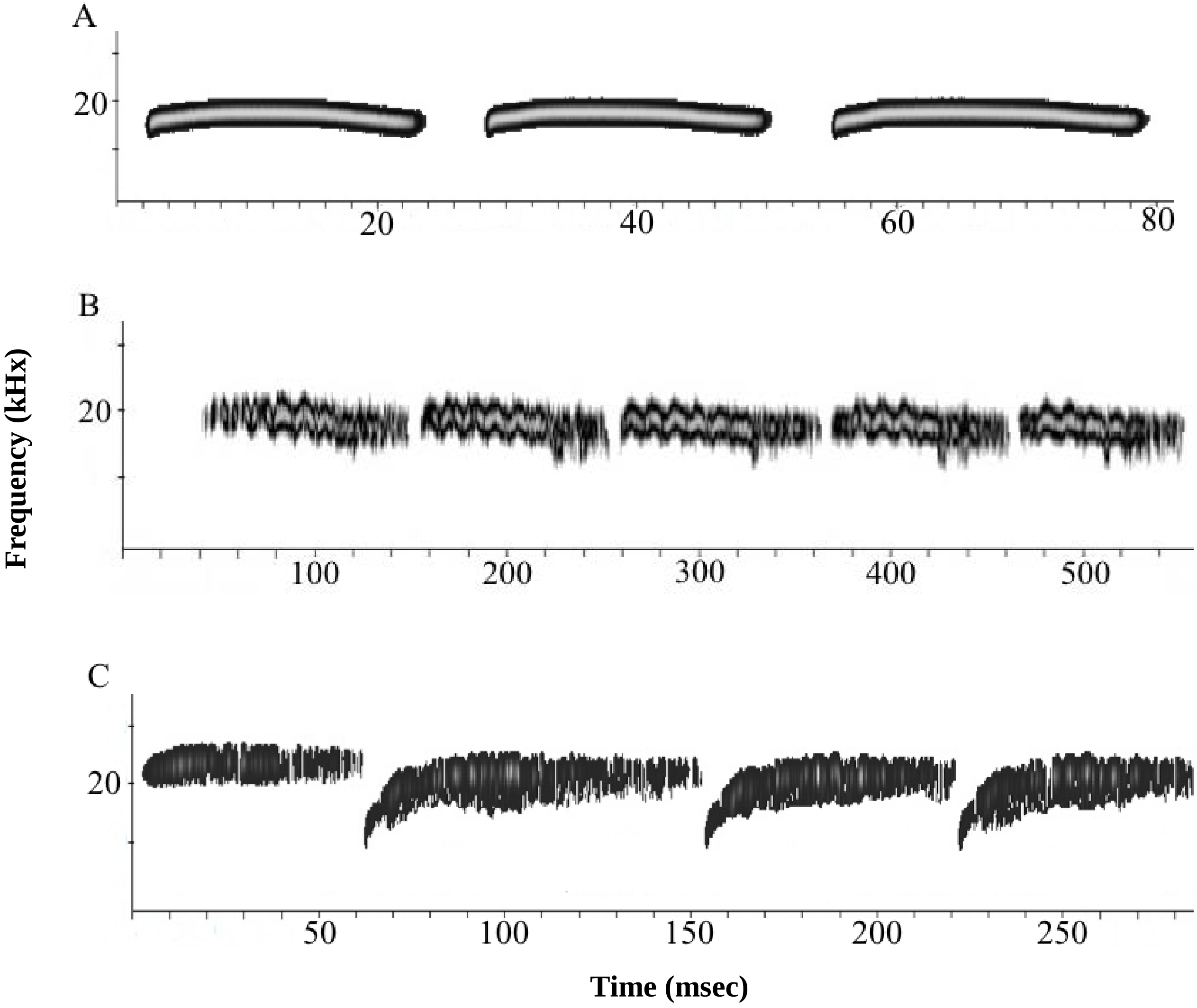

**FIG. 1.1.** – Spectrograms of three types of phrases emitted by *Glaucomys sabrinus*:

A) arc-whistle phrase with three arc-whistle syllables, B) trill-phrase with five trill syllables, and C) bi-syllable phrase with one arc-whistle syllable followed by three upsweep syllables.

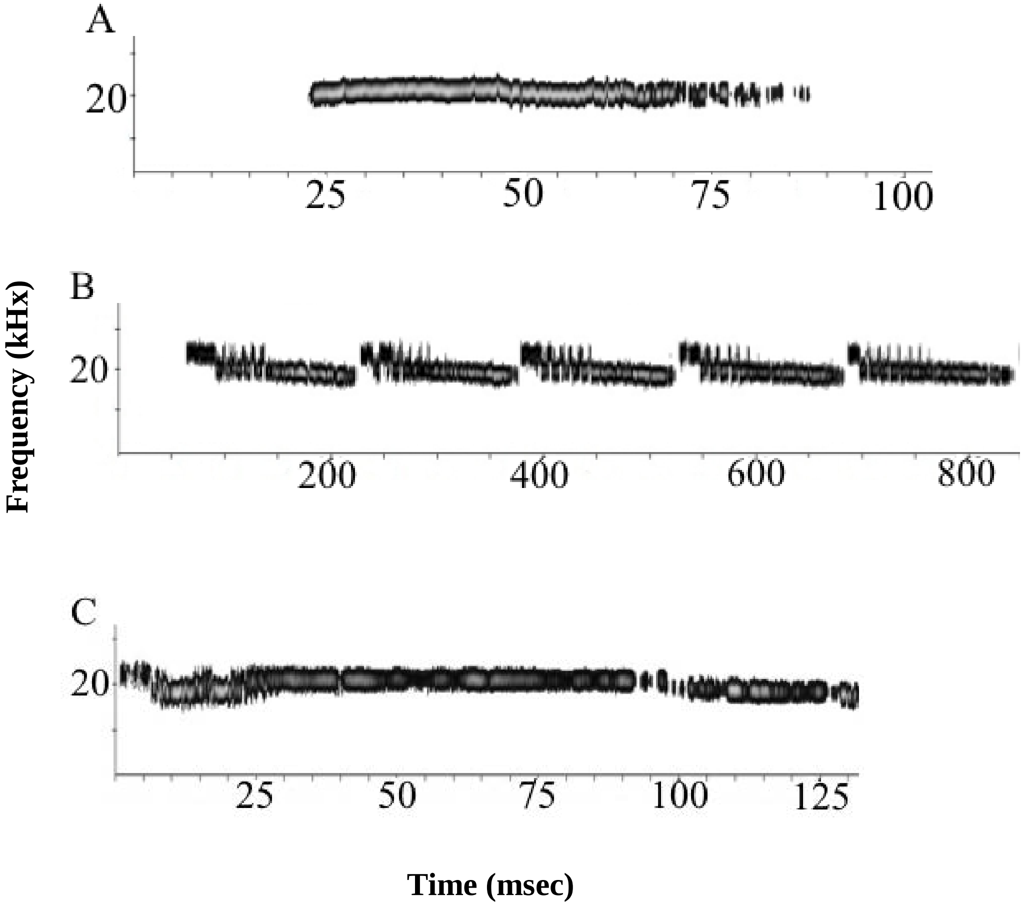

**FIG. 1.2.** – Spectrograms of three types of syllables emitted by *Glaucomys volans*: A) arc-whistle phrase with two arc-whistles syllables, B) trill-phrase with three trill syllables, and C) crow syllable.

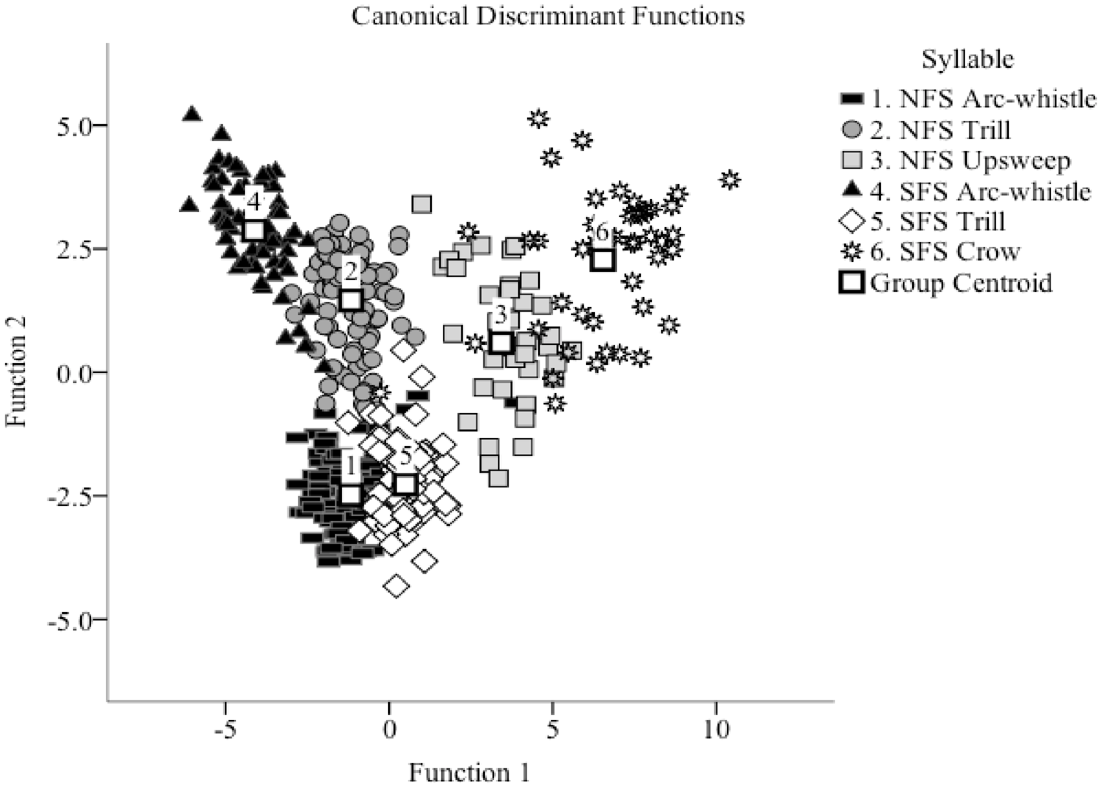

**FIG. 1.3.** – Canonical discriminant function of arrangement for six types of syllables emitted by *Glaucomys sabrinus* (NFS) and *G. volans* (SFS) during passive recordings. The overall model correctly identified 92.2% of syllables.

**TABLE 1.1. –** Descriptions of characteristics of calls used to quantify and classify vocalizations of captive *Glaucomys sabrinus* and *G. volans* during spring 2006 and 2008. Descriptions were modified from those in the Raven Pro 1.4 User's Manual (Cornell Lab of Ornithology, Ithaca, New York).

| Variable | Description |
|---|---|
| duration (msec) | length of call |
| low freq (kHz) | minimum frequency of call |
| high freq (kHz) | maximum frequency of call |
| bandwidth (kHz) | difference between upper and lower frequencies |
| max freq (kHz) | frequency at which call has the greatest amplitude |
| Q1 freq[a] (kHz) | divides the call into two frequency intervals containing 25 and 75% of the energy of the call and summed energy has to exceed 25% of total energy of call |
| Q3 freq[a] (kHz) | divides the call into two frequency intervals containing 25 and 75% of the energy of the call and summed energy has to exceed 75% of total energy of call |

| | |
|---|---|
| freq 5%[a] (kHz) | divides the call into two frequency intervals containing 5 and 95% of the energy of the call and summed energy has to exceed 5% of total energy of call |
| center freq[a] (kHz) | divides the call into two frequency intervals of equal energy and the left side of the call must have a summed energy that exceeds 50% of total energy of call |
| freq 95%[a] (kHz) | divides the call into two frequency intervals containing 5 and 95% of the energy of the call and summed energy has to exceed 95% of total energy of call |
| bandwidth 90%[a] (kHz) | difference between 5 and 95% frequencies |
| aggregate entropy (u) | measures the disorder in a sound by analyzing the energy distribution (higher entropy corresponds to greater disorder and pure tones would have zero entropy |
| average entropy (u) | measures entropy for each frame within a call and calculates the average |
| average power (dB) | value of spectrogram’s power spectral density, averaged over length of call |
| energy (dB) | total energy for entire length of call measured |
| max power (dB) | highest amplitude reached during call |

[a] Measurements considered robust because they were not dependent on method of selection by researched.

**TABLE 1.2.** – Means and standard deviations for 16 measures of parameters of calls used to quantify and classify high-frequency calls of northern and southern flying squirrels (*Glaucomys sabrinus* and *G. volans*).

| | *Glaucomys sabrinus* | | | | | | *Glaucomys volans* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Arc-whistles | | Trills | | Upsweeps | | Arc-whistles | | Trills | | Crows | |
| Variables | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Duration (msec.) | 171.23 | ± 3.89 | 128.98 | ± 8.65 | 166.07 | ± 30.83 | 75.72 | ± 20.69 | 177.51 | ± 23.73 | 199.42 | ± 48.48 |
| Low Freq (kHz) | 15.30 | ± 2.82 | 12.65 | ± 1.35 | 8.12 | ± 1.68 | 14.26 | ± 2.08 | 14.22 | ± 1.21 | 7.75 | ± 1.60 |
| High Freq (kHz) | 24.67 | ± 3.11 | 23.75 | ± 1.85 | 24.61 | ± 1.23 | 21.95 | ± 1.89 | 26.88 | ± 1.07 | 40.05 | ± 9.51 |
| Bandwidth (kHz) | 9.37 | ± 2.35 | 11.10 | ± 1.43 | 16.49 | ± 1.64 | 7.69 | ± 0.88 | 12.65 | ± 0.95 | 32.30 | ± 9.29 |
| Max Freq (kHz) | 20.03 | ± 2.84 | 17.99 | ± 0.97 | 19.23 | ± 2.23 | 17.81 | ± 1.87 | 19.64 | ± 1.95 | 15.54 | ± 3.36 |
| Q1 Freq (kHz) | 19.44 | ± 2.49 | 17.30 | ± 9.25 | 16.83 | ± 1.98 | 17.42 | ± 1.77 | 18.26 | ± 1.80 | 15.32 | ± 3.59 |
| Q3 Freq (kHz) | 20.67 | ± 2.66 | 19.14 | ± 9.25 | 20.51 | ± 1.45 | 18.92 | ± 1.91 | 20.74 | ± 2.00 | 18.05 | ± 3.45 |
| Agg Entropy (u) | 1.43 | ± 2.01 | 1.94 | ± 0.16 | 2.47 | ± 0.26 | 1.44 | ± 0.14 | 1.90 | ± 0.27 | 2.36 | ± 0.36 |
| Avg Entropy (u) | 1.50 | ± 1.22 | 1.66 | ± 0.09 | 2.23 | ± 0.15 | 1.39 | ± 0.08 | 1.63 | ± 0.10 | 1.97 | ± 0.22 |
| Avg Power (dB) | 79.45 | ± 6.22 | 93.85 | ± 5.03 | 82.23 | ± 5.75 | 94.03 | ± 6.24 | 79.38 | ± 6.06 | 84.83 | ± 7.70 |
| Freq 5% (kHz) | 18.20 | ± 2.41 | 15.50 | ± 1.03 | 13.38 | ± 1.59 | 16.30 | ± 1.73 | 17.71 | ± 1.33 | 12.45 | ± 2.91 |
| Center Freq (kHz) | 20.10 | ± 2.51 | 17.99 | ± 0.90 | 18.95 | ± 1.68 | 18.00 | ± 1.73 | 19.59 | ± 1.41 | 16.54 | ± 3.87 |
| Freq 95% (kHz) | 21.68 | ± 2.72 | 20.79 | ±1.22 | 21.83 | ± 1.17 | 19.63 | ± 1.69 | 23.16 | ± 1.49 | 21.25 | ± 3.02 |
| BW 90% (kHz) | 3.48 | ± 1.10 | 5.26 | ± 1.04 | 8.45 | ± 1.45 | 3.32 | ± 0.79 | 5.45 | ± 1.53 | 8.79 | ± 3.00 |
| Energy (dB) | 111.23 | ± 7.07 | 125.40 | ± 5.05 | 116.45 | ± 5.81 | 121.41 | ± 5.95 | 112.7 | ± 6.53 | 122.57 | ± 8.46 |
| Max Power (dB) | 95.95 | ± 6.33 | 105.7 | ± 2.90 | 99.75 | ± 5.17 | 102.82 | ± 5.17 | 95.31 | ± 5.64 | 100.60 | ± 6.59 |

**Table 1.3.** – Eigenvalues and test of significance for canonical discriminant functions used to classify types of calls emitted by *Glaucomys sabrinus* and *G. volans*.

Eigenvalues

| Function | Eigenvalue | % Variance | Cumulative % | Canonical Correlation |
|---|---|---|---|---|
| 1 | 9.134 | 48.9 | 48.9 | .949 |
| 2 | 4.866 | 26.0 | 74.9 | .911 |
| 3 | 2.830 | 15.1 | 90.0 | .860 |
| 4 | 1.576 | 8.4 | 98.4 | .782 |
| 5 | 0.291 | 1.6 | 100.0 | .475 |

Wilks' Lambda

| Test of Functions | Wilks' Lambda | Chi-square | df | Significance |
|---|---|---|---|---|
| 1 through 5 | 0.001 | 2890.485 | 70 | <.001 |
| 2 through 5 | 0.013 | 1880.755 | 52 | <.001 |
| 3 through 5 | 0.079 | 1109.422 | 36 | <.001 |
| 4 through 5 | 0.301 | 523.904 | 22 | <.001 |
| 5 | 0.774 | 111.418 | 10 | <.001 |

**Table 1.4.** – Results of classifications using discriminant-function analysis of six types of calls emitted by *Glaucomys sabrinus* and *G. volans.*

| | | | Predicted Group Membership | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Syllable | NFS Whistle | NFS Trill | NFS Upsweep | SFS Whistle | SFS Trill | SFS Crow | Total |
| Original | Count | NFS Whistle | 94 | 1 | 0 | 0 | 3 | 1 | 99 |
| | | NFS Trill | 2 | 87 | 0 | 1 | 0 | 0 | 90 |
| | | NFS Upsweep | 2 | 0 | 41 | 0 | 0 | 0 | 43 |
| | | SFS Whistle | 1 | 2 | 0 | 67 | 0 | 0 | 70 |
| | | SFS Trill | 16 | 1 | 0 | 0 | 80 | 0 | 97 |
| | | SFS Crow | 0 | 1 | 3 | 0 | 1 | 43 | 48 |
| | % | NFS Whistle | 94.9 | 1.0 | 0 | 0 | 3.0 | 1.0 | 100.0 |
| | | NFS Trill | 2.2 | 96.7 | 0 | 1.1 | 0 | 0 | 100.0 |
| | | NFS Upsweep | 4.7 | 0 | 95.3 | 0 | 0 | 0 | 100.0 |
| | | SFS Whistle | 1.4 | 2.9 | 0 | 95.7 | 0 | 0 | 100.0 |
| | | SFS Trill | 16.5 | 1.0 | 0 | 0 | 82.5 | 0 | 100.0 |
| | | SFS Crow | .0 | 2.1 | 6.3 | 0 | 2.1 | 89.6 | 100.0 |
| Cross-validated[a] | Count | NFS Whistle | 93 | 1 | 0 | 0 | 4 | 1 | 99 |
| | | NFS Trill | 2 | 87 | 0 | 1 | 0 | 0 | 90 |
| | | NFS Upsweep | 2 | 1 | 40 | 0 | 0 | 0 | 43 |
| | | SFS Whistle | 1 | 5 | 0 | 64 | 0 | 0 | 70 |
| | | SFS Trill | 18 | 1 | 0 | 0 | 78 | 0 | 97 |
| | | SFS Crow | 0 | 1 | 3 | 0 | 1 | 43 | 48 |
| | % | NFS Whistle | 93.9 | 1.0 | 0 | 0 | 4.0 | 1.0 | 100.0 |
| | | NFS Trill | 2.2 | 96.7 | 0 | 1.1 | 0 | 0 | 100.0 |
| | | NFS Upsweep | 4.7 | 2.3 | 93.0 | 0 | 0 | 0 | 100.0 |
| | | SFS Whistle | 1.4 | 7.1 | 0 | 91.4 | 0 | 0 | 100.0 |
| | | SFS Trill | 18.6 | 1.0 | 0 | 0 | 80.4 | 0 | 100.0 |
| | | SFS Crow | .0 | 2.1 | 6.3 | 0 | 2.1 | 89.6 | 100.0 |

# USE OF ACOUSTICAL SURVEYS IN DETECTING AND IDENTIFYING ENDANGERED CAROLINA NORTHERN FLYING SQUIRRELS (*GLAUCOMYS SABRINUS COLORATUS*) AND SOUTHERN FLYING SQUIRRELS (*G. VOLANS*)

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Acoustical surveys have long been a commonly used practice in documenting presence of species of birds and amphibians. More recently, with advancements in recording and analyzing high-frequency sounds, these same methods have been applied successfully to detecting and distinguishing individual species of bats and cetaceans. Because of the success seen in acoustic surveys for bats, this technique may be useful in detecting ecologically similar species such as flying squirrels. I assessed the efficacy of using acoustical surveys to document presence of both species of North American flying squirrels (*Glaucomys sabrinus* and *G. volans*). Acoustic surveys were conducted in the Piedmont Plateau and Coastal Plains of Alabama where *G. volans* occur, and in the Appalachian Mountains of western North Carolina where southern flying squirrels and endangered Carolina northern flying squirrels (*G. s. coloratus*) occur. Recordings of flying squirrels from acoustic surveys conducted in the wild were quantified and discriminant-function analysis was used to compare those calls to an existing library of calls recorded of animals in captivity. Acoustic detection of *G. volans* in Alabama occurred at 20 of 44 sites for a detection-success rate of 45.5%. Two new types of calls were discovered from field-surveys for *G. volans* including chirps and downsweeps. Discriminant-function analysis and cross validation correctly classified each group of calls 98.8% of the time. Acoustic detection of flying squirrels in western North Carolina occurred at 66 of 136 sites surveyed for a detection-success rate of

48.5%. Carolina northern flying squirrels were recorded at 44 sites and southern flying squirrels at 22 sites. Sympatry occurred at 15 sites. Results suggest that acoustical surveys may be significantly more effective in documenting presence of flying squirrels than techniques used previously. Such surveys may be useful in detecting and monitoring endangered populations of Carolina northern flying squirrels and disjunct or locally rare populations of southern flying squirrels in eastern Canada, Central America, and the mid-western United States.

Key words: acoustic surveys, discriminant-function analysis, Carolina northern flying squirrel, *Glaucomys sabrinus coloratus*, *Glaucomys volans*, southern flying squirrel.

Various techniques exist for surveying and documenting presence of mammals. Depending on objectives and species studied, methods may include live-traps, kill traps, scent-stations, bait stations, spot-lighting, aerial surveys, remote cameras, and use of scent-trained dogs (Dahlgren et al. 2012; Locke et al. 2012; Schemnitz et al. 2012). A relatively new technique used for detecting and identifying species of mammals is acoustic surveys (Britzke 2003; Gannon and Foster 1996). This method has been used for conducting presence-absence surveys of birds and amphibians, but more recently has been incorporated in surveys for a few selected mammalian groups. For example, acoustic surveys are used to distinguish among species of bats (Britzke 2003, O’Farrell and Gannon 1999) and cetaceans (Mellinger et al. 2007). The success of these surveys suggests that the method could be adapted to other taxa that frequently vocalize at higher frequencies, have species-specific calls, and emit calls with at least a moderate range of detection (≥ 2 m).

In a recent study, it was discovered that both species of North American flying squirrels (*Glaucomys*) regularly emit high-frequency vocalizations that were species-specific (Gilley 2013). While these recordings were made in a laboratory setting with captive squirrels, it is unknown if wild squirrels exhibit the same types of calls. A similar study of *Peromyscus* documented the first ultrasonic calls of two species in the wild (Kalcounis-Rueppell et al. 2006), although it was known previously that they exhibited this behavior in captivity (Vieira and Brown 2002; Wright and Brown 2004). While these results help to validate bioacoustical studies in a laboratory, Kalcounis-Rueppell et al. (2010) detected significant differences in calls of captive versus wild animals. Therefore, before acoustic surveys can be used as a viable sampling method, rigorous studies must be conducted in the field to validate observations made in the laboratory.

Sampling techniques that have been used to survey for flying squirrels include live-trapping, artificial nest-boxes (Althoff and Althoff 2001; Carey et al. 1991; C. A. Kelly in litt.; Risch and Brady 1996), and track-stations (Raphael et al. 1986). Efficacy of these techniques varies depending upon size of populations. Furthermore, trapping northern flying squirrels is considered labor intensive (Carey et al. 1991), and they have low susceptibility to entering live-traps (Menzel et al. 2006; Ford et al. 2010; Smith and Nichols 2003). Smith and Nichols (2003) reported success of capture per 100 trap nights to be 1.9-3.0% for northern flying squirrels. Trapping in trees significantly increased captures of southern flying squirrels (Loeb et al. 1999), but success of capture was 0.42-1.18% during >20,000 trap-nights (Laves and Loeb 2006). Checks of nest-boxes produced more detections for both species. Rate of occupancy for Carolina northern flying squirrels from 2007 to 2012 averaged 7.2% (C. A. Kelly in litt.). Weigl et al. (1992) found occupancy as high as 37.4% when nest-boxes were checked multiple times.

Average occupancy by southern flying squirrels was 17.8% in Ohio (Althoff and Althoff 2001) and 8.3% for boxes checked multiple times in Tennessee (Pitts 1992).

Distributions of northern and southern flying squirrels are mostly allopatric, but some sympatry does occur. One such example is in western North Carolina, eastern Tennessee, and southwestern Virginia where fragmented populations of *G. s. coloratus* and *G. volans* overlap. Weigl (2007) suggested that in these areas within the southern Appalachians, *G. sabrinus* poses a serious conservation problem because of loss of habitat. In the Appalachian Mountains, northern flying squirrels generally occur at high elevations in the spruce-fir and northern hardwood ecotone (Ford et al. 2004; Weigl 2007). Fragmented patches of suitable habitat for endangered Carolina flying squirrels (*G. sabrinus coloratus*) exist in eastern Tennessee, western North Carolina, and southwestern Virginia (USFWS 1990), but serious threats remain to current populations including encroachment by humans, clear-cutting, and climatic change (Weigl 2007). Such anthropogenic effects could alter composition of forests in favor of sympatric southern flying squirrels, leading to expansion into previously unoccupied habitats, increased competition, and spread of diseases (Weigl 1978).

In southern forests, *G. volans* is one of the dominant species (Loeb et al. 1999). However, southern flying squirrels can be rare locally in disjunct populations in eastern Canada, Central America (Diersing 1980; Lavers et al. 2006), and along the edge of their western distribution in the United States. They are listed as a species in need for varying degrees of conservation in Iowa, Kansas, Michigan, and Nebraska (Iowa Department of Natural Resources; Kansas Department of Wildlife and Parks; Michigan Department of Natural Resources, Nebraska Game and Parks Commission).

A non-invasive technique such as acoustic sampling that improves rate of detection and accurately distinguishes between species would prove valuable to the conservation of endangered northern flying squirrels. It could also serve as a more effective tool for monitoring locally rare and disjunct populations of southern flying squirrels. Furthermore, *Glaucomys sabrinus* is considered a management indicator species (Smith, et al. 2005), so better techniques that improve on efficiency of documenting its presence may provide greater measures for management and conservation of a variety of species and macrohabitats. I test the efficacy of using acoustic sampling in recording vocalizations of flying squirrels in the wild. Primary objectives were to test whether identity of species could be determined accurately from calls recorded in the wild when compared to an existing library of calls, and to establish a probability of detection for conducting acoustic sampling of both southern and northern flying squirrels.

## MATERIALS AND METHODS

Acoustic surveys for southern flying squirrels were conducted during spring and summer 2009 and 2010 in Lee and Washington counties, Alabama (Fig. 2.1). Point counts were performed at 12 sites by recording during two-minute intervals with an observer present. When squirrels were detected acoustically using a Pettersson bat detector spotlights were used to visually document presence. Passive recordings were conducted at 32 sites by attaching acoustical equipment to a tree 2-3 m above ground. All sites were sampled once. The equipment was then left recording from dusk until dawn, and collected the following morning. Sites surveyed by passive recording were sampled using a single detector set for one night. A total of 44 sites were surveyed for *G. volans* in Alabama.

Acoustic sampling for *G. s. coloratus* and *G. volans* in western North Carolina was conducted during autumn, spring, and summer 2010-2012 at 136 sites across 11 massifs in western North Carolina (Fig. 2.1). In addition, a few recordings were collected during winter 2009. Surveying efforts in North Carolina focused on Geographic Recovery Areas for *G. s. coloratus* outlined in the Appalachian Northern Flying Squirrel Recovery Plan (USFWS 1990) and non-recovery areas where suitable habitat existed for northern flying squirrels, but where presence had not been assessed or confirmed previously. Across four massifs in geographic recovery areas, 77 sites were surveyed, and across seven massifs in non-geographic recovery areas, 59 sites were surveyed. Because Carolina northern flying squirrels are endangered and considered rare, most sites were sampled for two to three nights and multiple (two to four) detectors were placed at most sites to increase chance of detection. Whenever calls were detected from a given area (regardless of detector), all quality calls were analyzed and counted as a single capture unless multiple individuals could be heard calling within a single recorded time frame (e.g., two squirrels calling at the same time). Because multiple syllables from a single squirrel were included in analyses, those phrases were checked to make sure all calls grouped as the same species. In 2012, three female and two male *G. s. coloratus* were radiocollared and telemetry data were collected concurrently with acoustical surveys. All recordings in North Carolina were conducted from dusk until dawn, and data were collected and downloaded daily to determine night of detection.

Calls of flying squirrels were collected using Pettersson D240x bat detectors (Pettersson Elektronik AB, Uppsala, Sweden), with a flat frequency response of 5-200 kHz. Digitized recordings of calls were collected at a sampling rate of 44.1 kHz and 16-bit resolution using iRiver® IFP, E100 mp3, (iRiver Limited, South Yarra, Victoria, Australia) and H2 zoom digital audio recorders (Samson Technologies, Hauppauge, New York). Detectors were set to time-expansion mode for recordings. Low-quality recordings and calls that overlapped with other calls were excluded from analyses. All other recordings of vocalizations of flying squirrels were analyzed using Raven: Interactive Sound Analysis Software© (Cornell Lab of Ornithology, Ithaca, New York). Spectrograms were viewed in a Hann window with 50% overlap on a time grid and discrete-Fourier-transform (DFT) size of 256 samples on a frequency grid.

In a previous study, three calls (arc-whistles, upsweeps, and trills) were described for captive northern flying squirrels and three calls (arc-whistles, crows, and trills) were described for southern flying squirrels (Gilley, 2013). In addition to these calls, three new types of calls (chirps, downsweeps, and tseets) were recorded from wild populations of *G. volans* in Alabama. These calls, along with those described by Gilley (2013), were used to build an acoustic library to test recordings from wild populations in western North Carolina. To determine species, 13 spectral characteristics of calls recorded during surveys were used in analyses including: duration, low frequency, high frequency, bandwidth, frequency at maximum amplitude, first-quartile (Q1) frequency, third-quartile (Q3) frequency, aggregate entropy, center frequency, energy, frequency at 5% call duration, frequency at 95% call duration, and maximum power. Measurements of time, frequency, and amplitude were recorded in milliseconds (msec), kilohertz (kHz), and decibels (dB), respectively. Entropy was measured as a volume of energy (u). To accurately discern between sweeps (upsweeps of *G. s. coloratus* and downsweeps of *G. volans*), two additional variables were added including starting frequency (kHz) and ending frequency (kHz).

Types of calls were sorted prior to analysis based on how they sounded and displays of spectrograms. For example, arc-whistles recorded during surveys were tested in a model with only known arc-whistles and no other type of call. Because of poor quality of recordings or lack of detection, crows (Gilley 2013), chirps, and tseets were not included in analyses. Forward stepwise-discriminant-function analysis (DFA) with cross validation was used to classify unknown arc-whistles, sweeps, and trills to species. Discriminant scores were calculated based on Mahalanobis distance from group centroids of known calls. Discriminant scores were then used to determine species by unknown calls recorded during surveys. All statistical analyses were performed using SPSS 20.0 (SPSS, Inc., Chicago, Illinois).

## RESULTS

During acoustical surveys in Alabama, southern flying squirrels were recorded at five of 12 sites by point counts and 15 of 32 sites by passive monitoring. Success of acoustical detection of *G. volans* in Alabama was 45.5%. Four types of calls were recorded including arc-whistles, trills (Gilley 2013), and two new types of calls: chirps and downsweeps (Fig. 2.3). Means and standard deviations for all variables of chirps and downsweeps recorded during the survey were calculated (Table 2.1). Forward, stepwise-discriminant-function analysis used three variables in the model to distinguish among types of calls: duration, low frequency, and high frequency (Table 2.2). Discriminant function explained 92.0% of the variation (Eigenvalue = 11.510, canonical correlation = 0.959; Table 2.3). Test of function (Table 2.3) was highly significant (function 1: $\lambda = .080$, $\chi^2 = 195.806$, $df = 3$, $P < .001$). The overall model and cross validation correctly classified 98.8% of the two new types of calls (Table 2.4; Fig. 2.4).

Passive recordings during winter 2009 and autumn, spring, and summer 2010-2012 acoustically detected flying squirrels in western North Carolina. Squirrels were detected at 66 of 136 sites surveyed for a 48.5% rate of detection. Collectively, surveying efforts in North Carolina recorded *G. s. coloratus* at 44 sites and *G. volans* at 22 sites. Sympatry occurred at 15 sites. Within geographic recovery areas, Carolina northern flying squirrels were detected at 39% of sites and southern flying squirrels at 15.6% of sites (Table 2.5). Overlap between the two species in geographic recovery areas occurred at seven sites within three massifs including Black Mountains, Great Balsams, and Roan Mountain (Table 2.5). In non-geographic recovery areas, Carolina northern flying squirrels were detected at 17 sites and southern flying squirrels at 15 sites (Table 2.5). Sympatry between the two species in non-geographic recovery areas occurred at eight sites across four massifs including Big Bald Mountain, Pond Mountain, Sugar Mountain, and Unaka (Table 2.5).

Types of calls recorded during acoustic surveys in North Carolina included arc-whistles and trills of both species, upsweeps of northern flying squirrels, and downsweeps of southern flying squirrels. Within geographic recovery areas, quality recordings of vocalizations included 64 arc-whistles, 8 sweeps, and 10 trills from *G. s. coloratus* and 34 arc-whistles and 16 downsweeps from *G. volans* (Table 2.5). In non-geographic recovery areas, quality recordings of Carolina northern flying squirrels included 92 arc-whistles and 20 upsweeps, and for southern flying squirrels, 32 arc-whistles, 12 downsweeps, and one trill (Table 2.5). Means and standard deviations were calculated for spectral variables of all calls recorded for both species during acoustic sampling (Table 2.6).

Forward, stepwise-discriminant-function analysis used four variables in the model to distinguish species by arc-whistles: duration, energy, frequency at 95% of call, and maximum power (Table 2.7). The model for upsweeps of *G. s. coloratus* and downsweeps of *G. volans* used three variables as predictors: frequency at 95% of call, starting frequency, and ending frequency (Table 2.7). Trills were assigned to species based on four variables: duration, bandwidth, frequency at 5% of call, and maximum power (Table 2.7). Because only two classifying groups (*G. s. coloratus* and *G. volans*) were used in each model, one discriminant function was produced. For arc-whistles, function 1 explained 93.5% of the variation

(Eigenvalue = 14.232, canonical correlation = 0.967; Table 2.8). Function 1 explained 97.6% of variation for sweeps (Eigenvalue = 39.516, canonical correlation = 0.988) and 88.9% for trills (Eigenvalue = 7.933, canonical correlation = .943; Table 2.8). Functions were statistically significant for arc-whistles ($\lambda = .066$, $\chi^2 = 452.08$, $df = 4$, $P < .001$), sweeps ($\lambda = .025$, $\chi^2 = 227.66$, $df = 3$, $P < .001$), and trills ($\lambda = .111$, $\chi^2 = 395.35$, $df = 4$ $P < .001$). The overall model classified 222 arc-whistles (156 as *G. s. coloratus* and 66 as *G. volans*; Fig. 2.5), 56 sweeps (28 as *G. s. coloratus* and 28 as *G. volans*; Fig. 2.6), and 11 trills (10 as *G. s. coloratus* and 1 as *G. volans*; Fig. 2.5) from individuals of 44 *G. s. coloratus* and 22 *G. volans*.

## DISCUSSION

Of the two species of flying squirrels that occur in North America, one subspecies of northern flying squirrel (*G. s. coloratus*) is federally listed as endangered, and disjunct populations of southern flying squirrels are considered rare locally in parts of their distribution in North and Central America (Lavers et al. 2006). Flying squirrels pose other difficulties for researchers in being arboreal, nocturnal, and trap-shy (Ford et al. 2010; Menzel et al. 2006; Smith and Nichols 2003). The success of documenting presence and discriminating among species of bats (Britzke 2003, O'Farrell and Gannon 1999) and cetaceans (Mellinger et al. 2007) with acoustical surveys provides support for adaptation of this technique to other taxa. Results of my study demonstrated rates of detection for both species of North American flying squirrels far greater (47% for all surveys combined) than published records of more traditional methods such as live-trapping and checking nest-boxes (Althoff and Althoff 2001; Carey et al. 1991; Ford et al. 2010; Laves and Loeb 2006; Menzel et al. 2006; Smith and Nichols 2003; Weigl et al. 1992). For example, I acoustically detected *G. s. coloratus* 39% of the time compared to a yearly average success of 7.2% checking nest-boxes in geographic recovery areas during 2009-2012.

For surveys in Alabama, point counts were first used in acoustic surveys to verify that calls were made by *G. volans* and not by bats, insectivores, or rodents that are known to also emit high-frequency calls. In a previous study, three types of calls from captive northern and three types from southern flying squirrels were quantified and described (Gilley 2013). Based on the library of calls generated by Gilley (2013), I detected arc-whistles, trills, and two new types of calls not described previously for flying squirrels during point counts and passive recording in Alabama. Calls recorded in North Carolina, however, matched all six types of calls described by Gilley (2013) and downsweeps recorded from wild *G. volans* in Alabama. The lack of recordings of arc-whistles, crows, and only one trill in Alabama may reflect sampling effort combined with situational behaviors associated with calls. Morton (1977) proposed the motivation-structural hypothesis that suggests calls of birds and mammals vary based on situations such as proximity or distance of other individuals and social context of calls (e.g., aggression and fear). Obvious differences associated with communicative behaviors may exist with squirrels in captivity compared to individuals in the wild. Interpretation of calls from captive settings should be validated when possible with calls from wild populations (Kalcounis-Rueppell 2010; Smith 1979).

Efforts in North Carolina were focused primarily on testing the efficacy of acoustic surveys in documenting presence of endangered *G. s. coloratus*. As such, surveys were conducted in geographic recovery areas of *G. s. coloratus* (USFWS 1990) and non-geographic recovery areas in an attempt to fill in gaps in distribution from previous records of trapping and nest-boxes (C. A. Kelly in litt.; Weigl et al. 1992). Southern flying squirrels also have been documented in several of these areas, although sustained sympatry is unlikely (Weigl 2007). Generally, elevation and composition of forest partition the two (Weigl 1969), but higher-elevation sites that contain beech trees (*Fagus grandifolia*) may support *G. volans*. Potential overlap in geographic and non-geographic recovery areas provided the chance to experimentally test an acoustic model of known calls to calls collected in the wild from both species. As much as possible, when calls were detected and assigned to species, these data were compared to an existing database maintained by the North Carolina Wildlife Resources Commission from trapping, nest-boxes, photographs, and telemetry.

Within geographic recovery areas, *G. s. coloratus* was detected at 30 sites and *G. volans* at 12 sites. All calls collected from known denning sites of *G. s. coloratus* were categorized as Carolina northern flying squirrels. Only five trills were recorded while simultaneously radio tracking five Carolina northern flying squirrels, but all grouped with 100% probability of detection in discriminant-function analysis as *G. s. coloratus*. The acoustic model predicted overlap at seven sites within geographic recovery areas. Trapping and surveys of nest-boxes at three of these sites within the Great Balsams and Black Mountains massifs confirmed overlap. However, data for the remaining four sites only support presence of *G. s. coloratus*. Reasons for this could be error in distinguishing between arc-whistles, as this type of call is the most similar for both species. Existing data of captures, photographs, and telemetry, however, greatly support most acoustic detections, even for arc-whistles. Another explanation could be the encroachment of *G. volans* at higher elevations where some hardwoods exist. Southern flying squirrels have been captured at 1,500 m elevation in the southern Appalachians (Weigl 1978). Acoustic monitoring should be repeated at these sites to gain a better understanding of occurrence of these species.

Acoustic sampling in non-geographic recovery areas detected *G. s. coloratus* at 17 sites and *G. volans* at 15 sites, with overlap occurring at eight sites within Big Bald, Pond Mountain, Sugar Mountain, and Unaka massifs. Captures or photographs supported all sites of overlap determined from acoustic surveys. Acoustic detection also produced four new sites (Big Bald, Pond Mountain, Nantahala, and Unaka Mountain) for Carolina northern flying squirrels were presence had not been documented previously. Photographs have subsequently confirmed presence of *G. s. coloratus* at Unaka and potentially at Pond Mountain.

When comparing calls of captive animals (Gilley 2013) and calls collected in Alabama to calls in North Carolina, general trends in differences of spectral features were observed. Specifically, in arc-whistles of both species and trills of *G. volans* (Figs. 2.5 and 2.7). Significant differences occurred between upsweeps of captive *G. sabrinus* and upsweeps of *G. s. coloratus* (Fig. 2.6). High-frequency calls of California deermice (*Peromyscus californicus*) recorded in a laboratory were significantly different in certain spectral characteristics from calls emitted by wild individuals but similar in overall motif and structure (Kalcounis-Rueppell et al. 2010). Results of my study may only reflect subtle differences in spectral measurements from calls collected in the laboratory compared to the wild. Another explanation could be geographical variation. Captive individuals of *G. sabrinus* used in the study by Gilley (2013) were collected from the northwestern United States, and although the geographical distance between populations of *G. volans* in Alabama and North Carolina is much less, regional dialects are a common occurrence in birds and mammals (Baker and Cunningham 1985; Kershenbaum et al. 2012). Even with observed differences, the acoustic library accurately discriminated between the two species based on calls. Future studies should incorporate known calls from the region being investigated.

Overall, acoustic sampling out-performed trapping and surveys of nest boxes in detecting both northern and southern flying squirrels. Based on existing data and analyses of habitats, almost all recordings collected during acoustic surveys in North Carolina accurately identified arc-whistles, sweeps, and trills to species. Other than the improved success of detection and identification of species achieved by acoustic sampling, this method has several advantages in surveying for flying squirrels. For example, it is minimally to non-invasive and can be conducted actively much like point-counts are done with birds, or remotely by leaving equipment in the field for periods of time. Modern acoustic equipment is portable and is cost effective when considering other commonly used practices of trapping or checking and maintaining nest-boxes. Furthermore, recent studies of vocalizations of mammals have shown geographical variation (Kershenbaum et al. 2012) and differences in calls between sexes, ages, and individuals (Blumstein and Munos 2005; Hooper et al. 2012). Previously, these sorts of demographical data were obtained only by capturing animals. Results presented herein suggest that success in detecting and monitoring flying squirrels can be greatly increased with the addition of acoustic surveys to traditional techniques.

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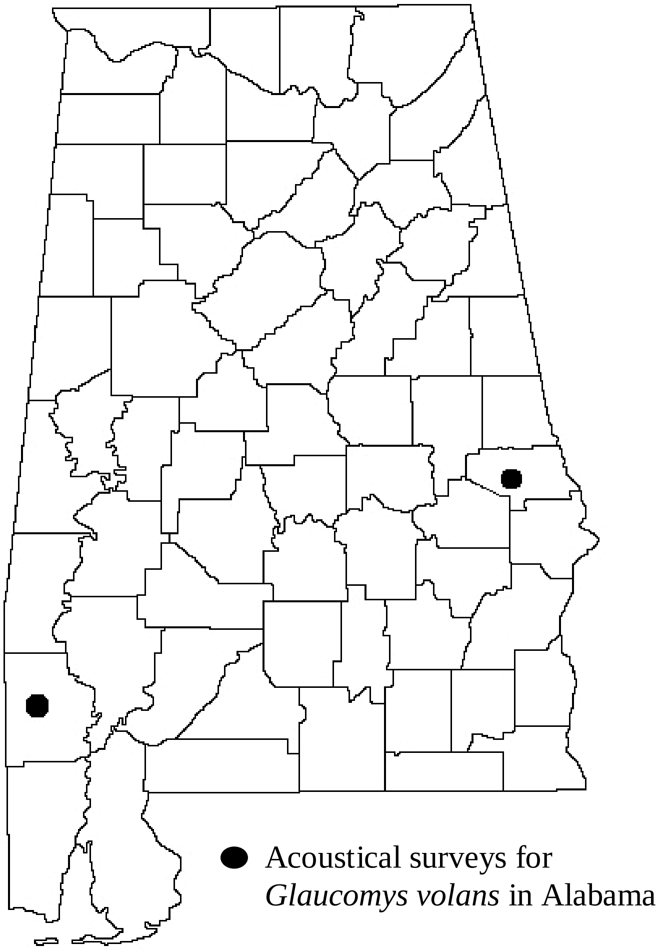

**Fig. 2.1.** – Locations of acoustical surveys for southern flying squirrels (*Glaucomys volans*) conducted in Lee and Washington counties, Alabama, during spring and summer 2009-2010.

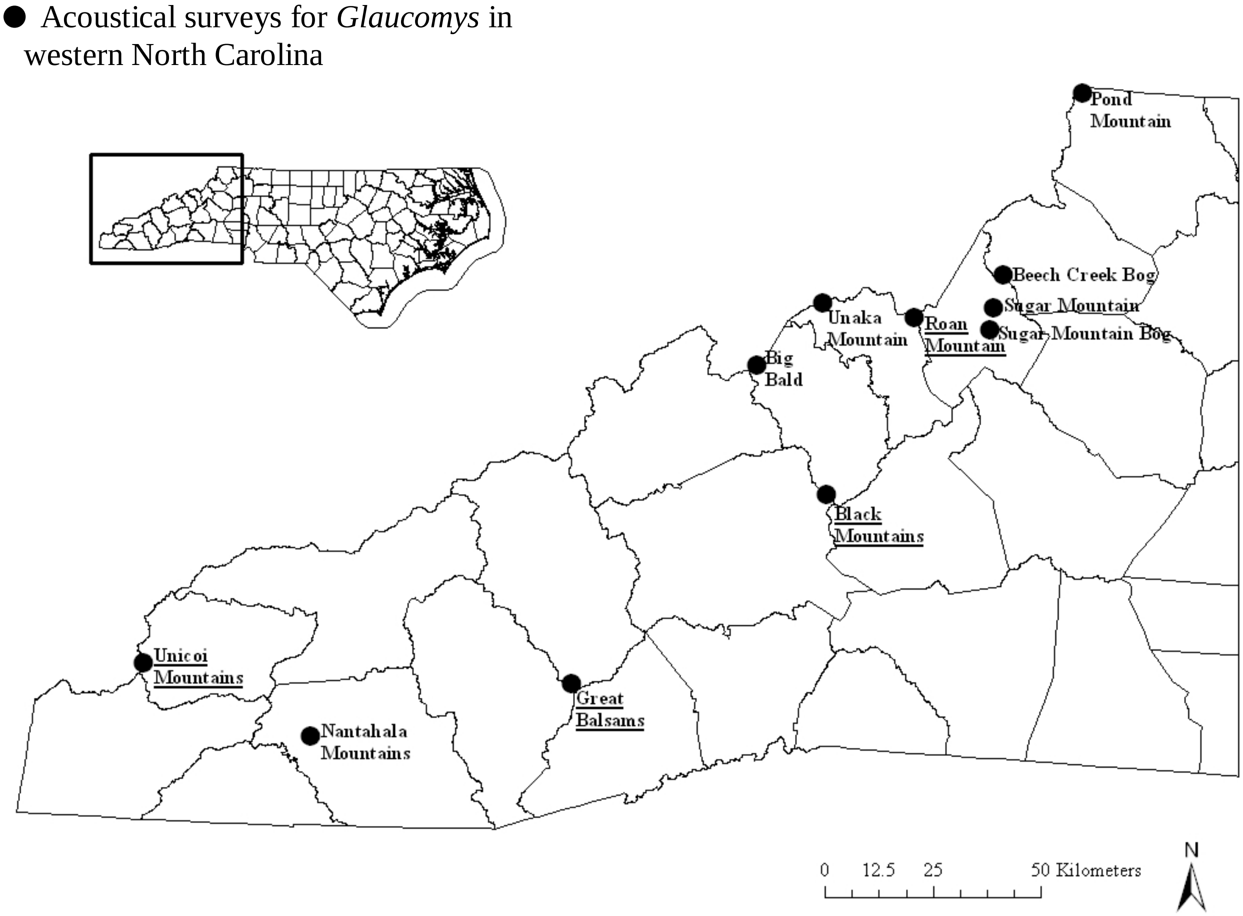

**Fig. 2.2.** – Locations of acoustical surveys for Carolina northern flying squirrels (*Glaucomys sabrinus coloratus*) and southern flying squirrels (*G. volans*) conducted in western North Carolina during 2009-2012. Surveys were conducted within geographic recovery areas for *G. s. coloratus* (underlined massifs), and within non-geographic recovery areas.

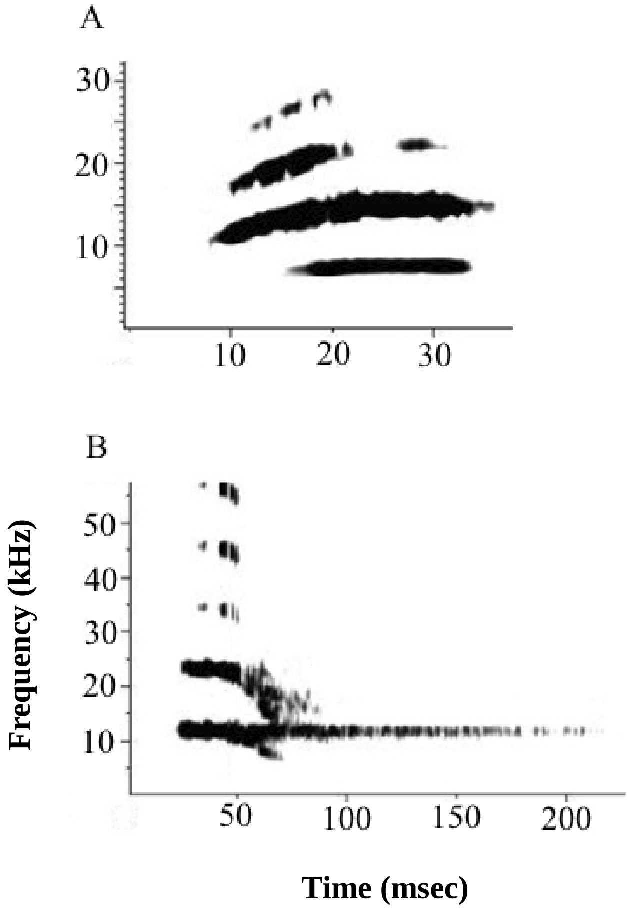

**Fig. 2.3.** – Two types of calls (A. chirp; B. downsweep) of wild, southern flying squirrels (*Glaucomys volans*) recorded in Alabama during 2009 and 2010.

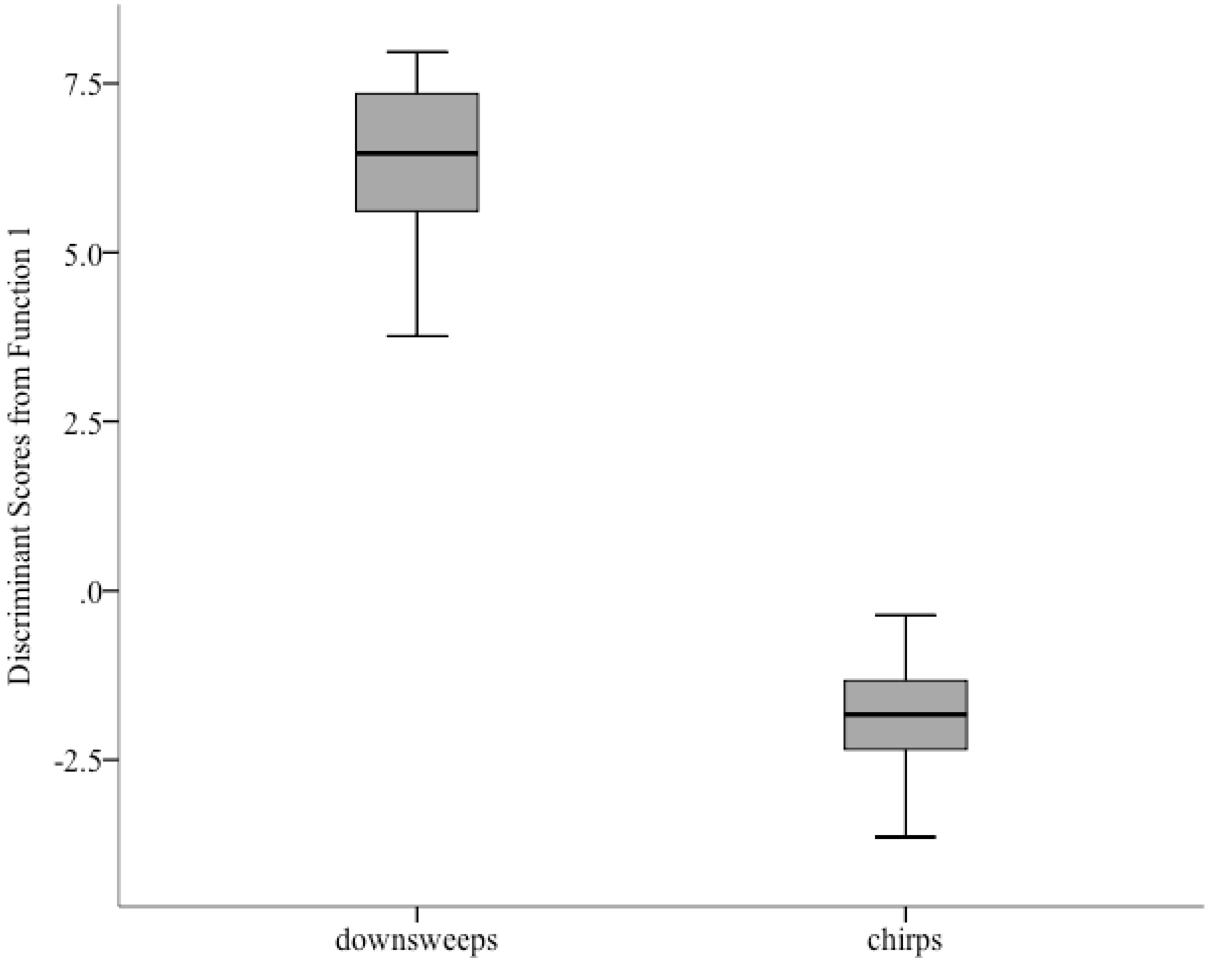

**Fig. 2.4.** – Boxplot of two calls (downsweeps and chirps) emitted by wild southern flying squirrels (*Glaucomys volans*) during spring and summer 2009-2010 in Lee and Washington counties, Alabama.

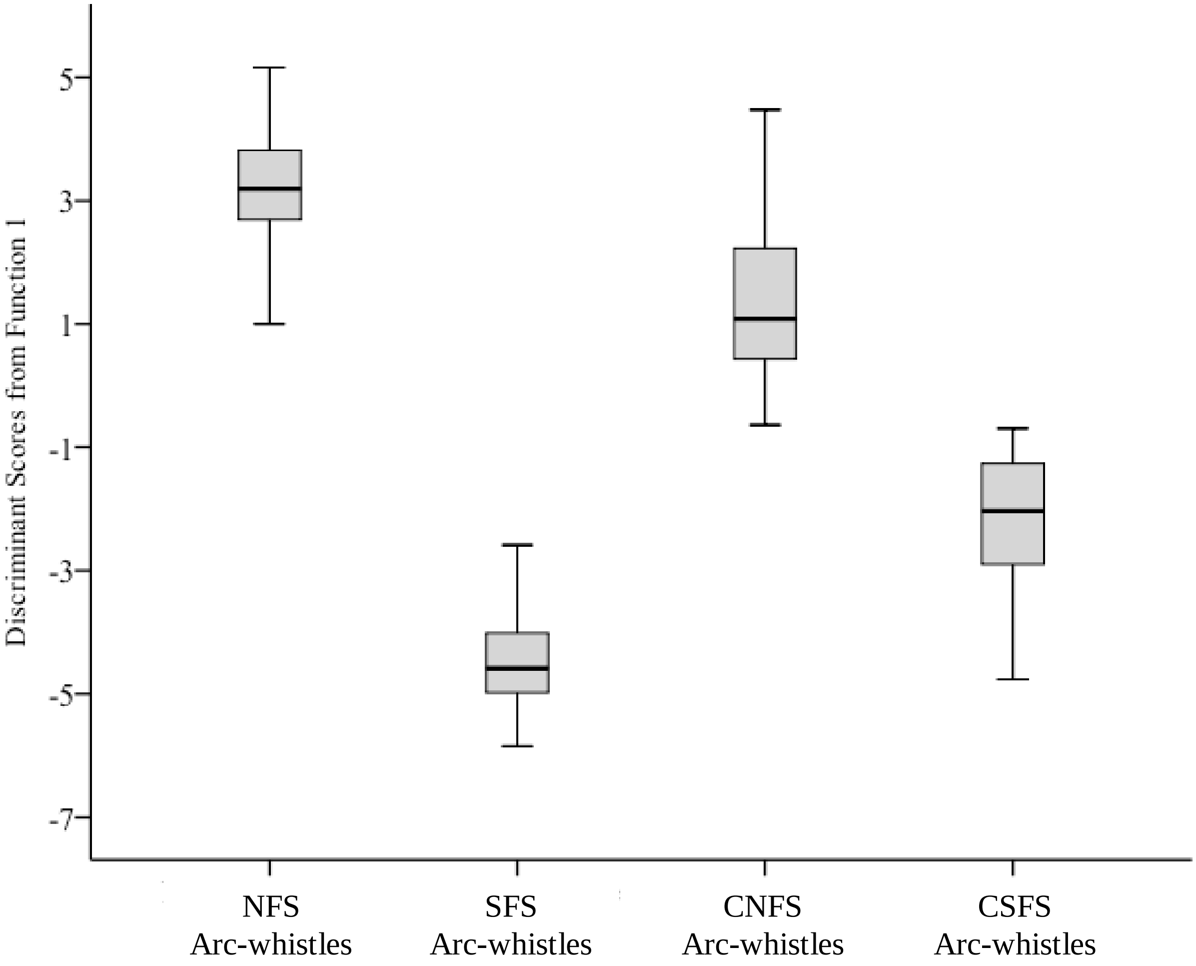

**Fig. 2.5.** – Boxplot showing arc-whistles of captive *Glaucomys sabrinus* and *G. volans* (Gilley 2012) compared to arc-whistles of wild flying squirrels (CNFS = Carolina northern flying squirrel; CSFS = Carolina southern flying squirrel) recorded in western North Carolina during 2009-2012. Classification of species (CNFS or CSFS) by arc-whistles was determined using discriminant scores from function 1 in discriminant-function analysis.

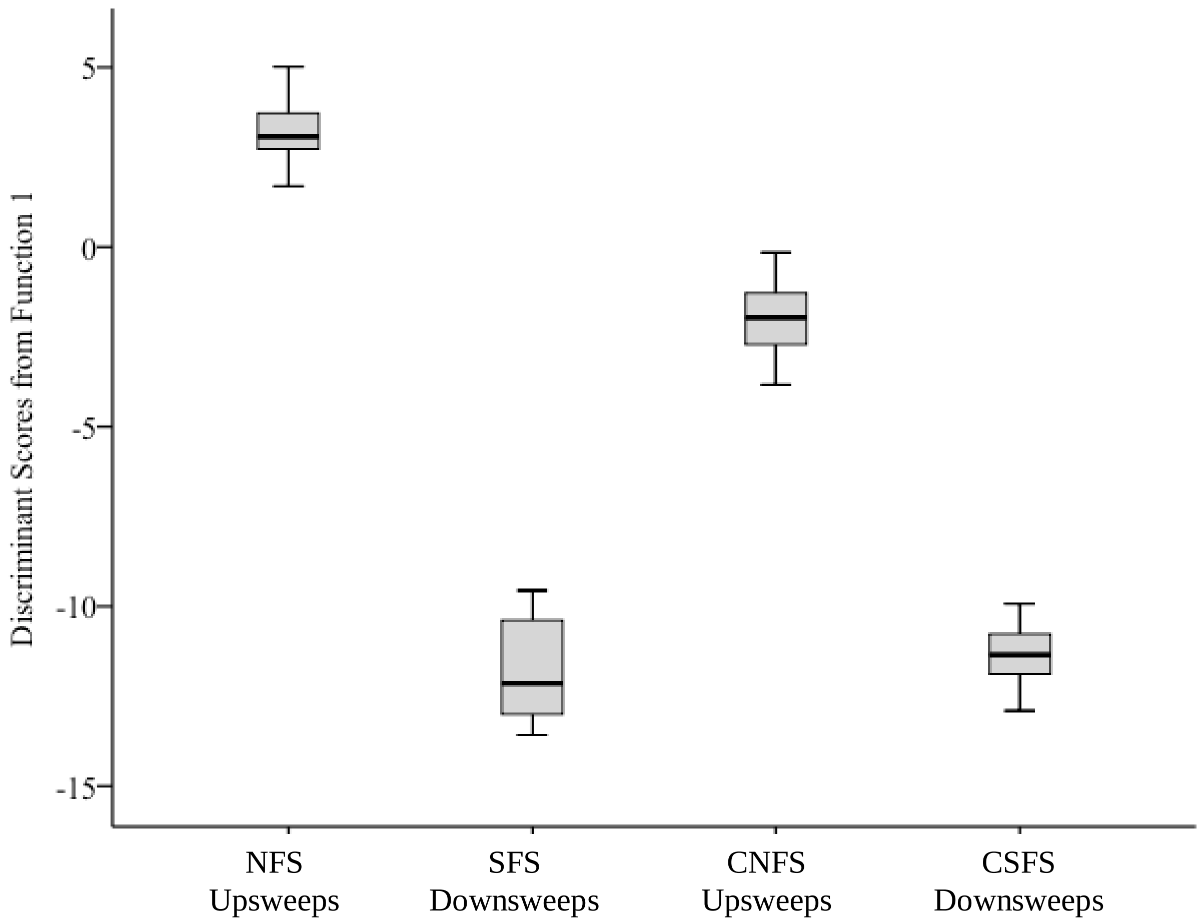

**Fig. 2.6.** – Boxplot showing sweeps of captive *Glaucomys sabrinus* (Gilley 2012) and *G. volans* compared to sweeps of wild flying squirrels (CNFS = Carolina northern flying squirrel; CSFS = Carolina southern flying squirrel) recorded in western North Carolina during 2009-2012. Classification of species (CNFS or CSFS) based on sweeps was determined using discriminant scores from function 1 in discriminant-function analysis.

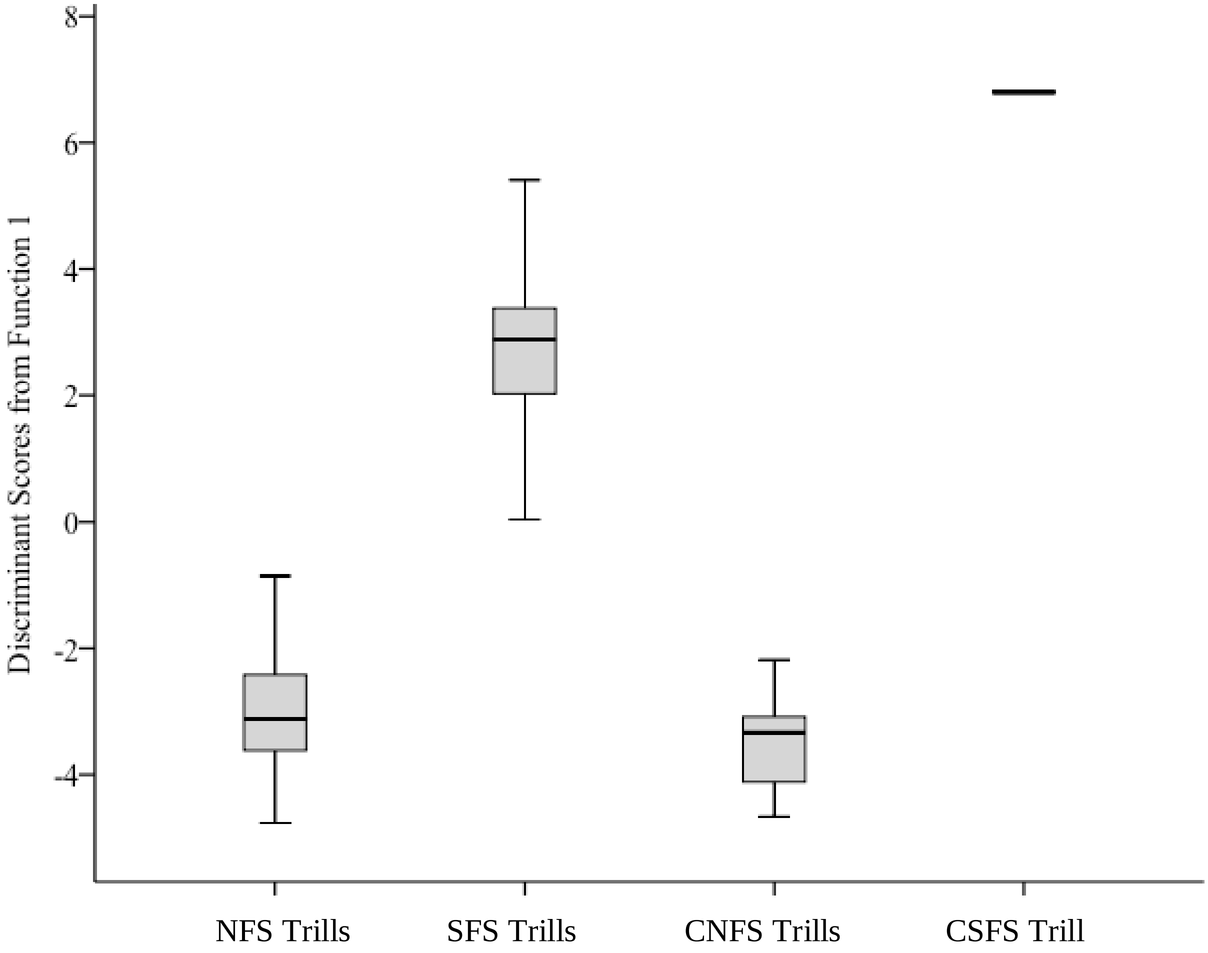

**Fig. 2.7. –** Boxplot showing trills of captive *Glaucomys sabrinus* and *G. volans* (Gilley 2012) compared to trills of wild flying squirrels (CNFS = Carolina northern flying squirrel; CSFS = Carolina southern flying squirrel) recorded in western North Carolina during 2009-2012. Classification of species (CNFS or CSFS) based on trills was determined using discriminant scores from function 1 in discriminant-function analysis.

**Table 2.1.** – Summary data for spectral measurements of two types of calls emitted by southern flying squirrels (*Glaucomys volans*) during acoustical surveys in Lee and Washington counties, Alabama, 2009 and 2010.

| | *Glaucomys volans* | | | |
|---|---|---|---|---|
| | Chirps | | Downsweeps | |
| Variables | Mean | S.D. | Mean | S.D. |
| Duration (ms) | 63.5 | 7.4 | 212.7 | 86.9 |
| Low Freq (Hz) | 5846.4 | 703.0 | 7220.7 | 1331.0 |
| High Freq (Hz) | 23950.1 | 1888.7 | 15959.0 | 1114.9 |
| Bandwidth (Hz) | 18103.7 | 1979.7 | 8738.3 | 1116.9 |
| Max Freq (Hz) | 15607.3 | 1346.3 | 12824.2 | 1249.5 |
| Q1 Freq (Hz) | 12782.1 | 592.6 | 12058.6 | 1023.4 |
| Q3 Freq (Hz) | 16985.4 | 843.9 | 13542.0 | 971.1 |
| Agg Entropy (u) | 3.7 | 0.3 | 2.3 | 0.3 |
| Avg Power (dB) | 112.4 | 1.3 | 105.7 | 6.0 |
| BW 90% (Hz) | 9302.3 | 2371.9 | 3732.4 | 934.2 |
| Center Freq (Hz) | 15228.3 | 410.1 | 12872.1 | 1084.3 |
| Energy (dB) | 140.7 | 1.0 | 135.8 | 6.1 |
| Freq 5% (Hz) | 10680.5 | 556.0 | 10623.1 | 934.3 |
| Freq 95% (Hz) | 19982.8 | 2080.3 | 14355.5 | 979.9 |
| IQR BW (Hz) | 4203.3 | 1251.2 | 1483.4 | 711.9 |
| Max Power (dB) | 127.0 | 0.2 | 121.9 | 5.3 |

**Table 2.2.** – Tests of equality of group means for three variables used in a forward, stepwise-discriminant-function analysis. Variables were spectral measures used to describe calls of southern flying squirrels (*Glaucomys volans*) during acoustic surveys in Alabama during 2009-2010.

| | Tests of Equality of Group Means | | | | |
|---|---|---|---|---|---|
| | $\lambda$ | F | df1 | df2 | *P* |
| Duration (s) | .192 | 331.862 | 1 | 79 | <.001 |
| Low Frequency (Hz) | .373 | 132.705 | 1 | 79 | <.001 |
| High Frequency (Hz) | .921 | 6.763 | 1 | 79 | .011 |

**Table 2.3.** – Eigenvalues and test of significance for canonical discriminant functions used to classify types of calls emitted by wild *Glaucomys volans* in Alabama during 2009-2010.

| Function | Eigenvalue | % Variance | Cumulative % | Canonical Correlation |
|---|---|---|---|---|
| 1 | 11.510 | 100.0 | 100.0 | .959 |

| Test of Functions | Λ | Chi-square | df | *P* |
|---|---|---|---|---|
| 1 | .080 | 195.806 | 3 | <.001 |

**Table 2.4.** – Classification results and cross validation of calls from wild southern flying squirrels (*Glaucomys volans*) in Alabama during 2009-2010.

| | | | Predicted Group Membership | | |
|---|---|---|---|---|---|
| | | Call | downsweep | chirp | Total |
| Original | Count | downsweep | 18 | 1 | 19 |
| | | chirp | 0 | 62 | 62 |
| | Percent[b] | downsweep | 94.7 | 5.3 | 100 |
| | | chirp | 0 | 100 | 100 |
| Cross-validated[a] | Count | downsweep | 18 | 1 | 19 |
| | | chirp | 0 | 62 | 62 |
| | Percent[c] | downsweep | 94.7 | 5.3 | 100 |
| | | chirp | 0 | 100 | 100 |

[a] Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

[b] 98.8% of original grouped calls were correctly classified.

[c] 98.8% of cross-validated grouped calls were correctly classified.

**Table 2.5.** – Acoustical surveys for *Glaucomys sabrinus coloratus* (CNFS) and *G. volans* (SFS) were conducted throughout 11 massifs in western North Carolina during 2009-2012. Squirrels were detected at 66 of 136 sites sampled, and calls recorded included arc-whistles (Arcs), sweeps, and trills of both species.

| | *Glaucomys sabrinus coloratus* | | | | | *Glaucomys volans* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| GRA Massif[a] | CNFS detected | Sites no CNFS | Arcs | Sweeps | Trills | SFS detected | Sites no SFS | Arcs | Sweeps | Trills |
| Black Mountains[b] | 13 | 9 | 39 | 3 | 0 | 4 | 18 | 11 | 14 | 0 |
| Great Balsams[b] | 14 | 22 | 19 | 2 | 10 | 4 | 32 | 12 | 0 | 0 |
| Roan[b] | 3 | 7 | 6 | 3 | 0 | 2 | 8 | 10 | 0 | 0 |
| Unicoi | 0 | 9 | 0 | 0 | 0 | 2 | 7 | 1 | 2 | 0 |
| Total GRAs | 30 | 47 | 64 | 8 | 10 | 12 | 65 | 34 | 16 | 0 |
| | | | | | | | | | | |
| nonGRA Massif | | | | | | | | | | |
| Big Bald[b] | 4 | 1 | 42 | 14 | 0 | 3 | 2 | 17 | 3 | 0 |
| Beech Creek Bog | 0 | 7 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 |
| Nantahalas | 0 | 6 | 0 | 0 | 0 | 7 | 1 | 3 | 9 | 0 |
| Pond Mountain[b] | 4 | 9 | 21 | 2 | 0 | 2 | 11 | 3 | 0 | 0 |
| Sugar Mountain[b] | 4 | 8 | 19 | 0 | 0 | 2 | 10 | 3 | 0 | 0 |
| Sugar Mnt. Bog | 0 | 2 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 |
| Unaka[b] | 5 | 9 | 10 | 4 | 0 | 2 | 12 | 6 | 0 | 0 |
| Total nonGRAs | 17 | 42 | 92 | 20 | 0 | 15 | 44 | 32 | 12 | 1 |
| **TOTAL** | **44** | **92** | **156** | **28** | **10** | **22** | **104** | **66** | **28** | **1** |

[a] Geographic recovery areas (GRA) represent areas defined by the Appalachian Northern Flying squirrel Recovery Plan (USFWS 1990). Non-geographic recovery areas (nonGRA) contain suitable habitat for *G. s. coloratus*, but little is known about presence.

[b] Massifs where both species were detected during acoustical surveys.

**Table 2.6.** – Means and standard deviations (SD) for calls of flying squirrels (CNFS = *Glaucomys sabrinus coloratus* and SFS = *G. volans*) recorded during acoustical surveys in western North Carolina during 2009-2012.

| | | Arc-whistles | | Sweeps | | Trills | |
|---|---|---|---|---|---|---|---|
| Variables | Species | Mean | SD | Mean | SD | Mean[a] | SD |
| Duration (s) | CNFS | 0.135 | 0.056 | 0.109 | 0.032 | 0.083 | 0.026 |
| | SFS | 0.089 | 0.035 | 0.189 | 0.055 | 0.175 | --- |
| LowFreq (kHz) | CNFS | 14.128 | 3.867 | 9.327 | 1.262 | 16.735 | 4.590 |
| | SFS | 13.247 | 3.334 | 5.836 | 1.053 | 18.488 | --- |
| HighFreq (kHz) | CNFS | 22.002 | 3.564 | 17.188 | 1.403 | 28.720 | 4.896 |
| | SFS | 19.972 | 3.174 | 15.661 | 1.843 | 25.884 | --- |
| Bandwidth (kHz) | CNFS | 7.875 | 2.124 | 7.861 | 1.599 | 11.985 | 2.196 |
| | SFS | 6.725 | 4.454 | 9.825 | 1.593 | 7.395 | --- |
| MaxFreq (kHz) | CNFS | 18.237 | 3.632 | 14.396 | 1.364 | 23.184 | 4.243 |
| | SFS | 16.809 | 2.252 | 12.397 | 1.312 | 18.949 | --- |
| Q1Freq (kHz) | CNFS | 17.630 | 3.642 | 13.966 | 1.292 | 21.749 | 3.675 |
| | SFS | 16.248 | 2.261 | 11.566 | 1.415 | 20.672 | --- |
| Q3Freq (kHz) | CNFS | 18.811 | 3.489 | 15.073 | 1.455 | 24.333 | 4.141 |
| | SFS | 17.318 | 2.237 | 12.797 | 1.496 | 22.395 | --- |
| CenterFreq (kHz) | CNFS | 18.226 | 3.633 | 14.458 | 1.270 | 22.969 | 3.978 |
| | SFS | 16.678 | 2.229 | 12.335 | 1.306 | 20.672 | --- |

**Table 2.6.** – continued.

| | | Arc-whistles | | Sweeps | | Trills | |
|---|---|---|---|---|---|---|---|
| Variables | Species | Mean | SD | Mean | SD | Mean[a] | SD[b] |
| Freq5 (kHz) | CNFS | 16.730 | 3.673 | 12.981 | 1.304 | 20.169 | 3.415 |
| | SFS | 15.217 | 2.543 | 10.705 | 1.182 | 18.949 | --- |
| Freq95 (kHz) | CNFS | 19.772 | 3.621 | 15.658 | 1.388 | 25.481 | 4.460 |
| | SFS | 17.892 | 2.330 | 13.720 | 1.463 | 24.117 | --- |
| StartFreq (kHz)[c] | CNFS | --- | --- | 10.253 | 1.198 | --- | --- |
| | SFS | --- | --- | 13.051 | 1.782 | --- | --- |
| EndFreq (kHz)[c] | CNFS | --- | --- | 15.763 | 1.213 | --- | --- |
| | SFS | --- | --- | 6.472 | 1.217 | --- | --- |
| AggEntropy (u) | CNFS | 1.348 | 0.156 | 1.795 | 0.338 | 2.605 | 0.545 |
| | SFS | 1.439 | 0.234 | 1.638 | 0.233 | 1.896 | --- |
| Energy (dB) | CNFS | 106.888 | 8.945 | 116.479 | 9.610 | 120.925 | 4.746 |
| | SFS | 116.364 | 10.966 | 120.375 | 13.644 | 93.700 | --- |
| MaxPower (dB) | CNFS | 93.177 | 8.517 | 103.146 | 9.842 | 109.192 | 4.829 |
| | SFS | 103.742 | 11.580 | 106.129 | 14.207 | 75.300 | --- |

[a.] Values for *G. volans* reflect actual measurements of variables as only one trill was recorded.

[b] Spectral measures used to distinguish between sweeps of *G. s. coloratus* and *G. volans.*

**Table 2.7.** – Tests of equality of group means for spectral measurements of three calls (arc-whistles, sweeps, and trills) emitted by wild flying squirrels (*Glaucomys sabrinus* and *G. volans*) in western North Carolina. Stepwise discriminant-function analysis classified species based on type of call using the following variables: duration (s), energy (dB), frequency at 95% of call (kHz), and maximum power (dB) for arc-whistles; frequency at 95% of call (kHz), starting frequency (kHz), and ending frequency (kHz) for sweeps; duration (s), bandwidth (kHz), frequency at 5% of call (kHz), and maximum power (dB) for trills.

| | Tests of Equality of Group Means | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Arc-whistles | | | | | Sweeps | | | | | Trills | | | | |
| Variables | λ | F | df1 | df2 | *P* | λ | F | df1 | df2 | *P* | λ | F | df1 | df2 | *P* |
| Duration (s) | 0.321 | 355.67 | 1 | 168 | <.001 | --- | --- | --- | --- | --- | 0.358 | 326.60 | 1 | 182 | <.001 |
| Bandwidth (kHz) | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | 0.716 | 72.09 | 1 | 182 | <.001 |
| Energy (dB) | 0.631 | 98.08 | 1 | 168 | <.001 | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Freq5 (kHz) | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | 0.537 | 156.97 | 1 | 182 | <.001 |
| Freq95 (kHz) | 0.841 | 31.71 | 1 | 168 | <.001 | 0.521 | 58.01 | 1 | 63 | <.001 | --- | --- | --- | --- | --- |
| MaxPower (dB) | 0.747 | 56.86 | 1 | 168 | <.001 | --- | --- | --- | --- | --- | 0.432 | 238.84 | 1 | 182 | <.001 |
| Start Freq (kHz) | --- | --- | --- | --- | --- | 0.611 | 40.07 | 1 | 63 | <.001 | --- | --- | --- | --- | --- |
| End Freq (kHz) | --- | --- | --- | --- | --- | 0.043 | 14.18 | 1 | 63 | <.001 | --- | --- | --- | --- | --- |

**Table 2.8.** – Eigenvalues and test of significance for canonical discriminant functions used to determine species based on types of calls. Calls were recorded from wild flying squirrels (*Glaucomys sabrinus coloratus* and *G. volans*) in western North Carolina during 2009-2012.

| Type of Call | Function | Eigenvalue | % of Variance | Cumulative % | Canonical Correlation | λ | Chi-square | df | *P* |
|---|---|---|---|---|---|---|---|---|---|
| Arc-whistles | 1 | 14.232[a] | 100 | 100 | 0.967 | 0.066 | 452.088 | 4 | <.001 |
| Sweeps | 1 | 39.516[a] | 100 | 100 | 0.988 | 0.025 | 227.655 | 3 | <.001 |
| Trills | 1 | 7.993[a] | 100 | 100 | 0.943 | 0.111 | 395.352 | 4 | <.001 |

[a]. First canonical discriminant function was used in each analysis.

# EFFICACY OF ULTRASONIC DETERRENT DEVICES IN DECREASING USE OF NEST-BOXES BY SOUTHERN FLYING SQUIRRELS (*GLAUCOMYS VOLANS*): IMPLICATIONS FOR THE ENDANGERED RED-COCKADED WOODPECKER (*PICOIDES BOREALIS*)

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Southern flying squirrels (*Glaucomys volans*) are opportunistic cavity nesters that often inhabit artificial roosting structures such as nest-boxes and natural cavities of various species of birds. Widespread occupancy of nest-boxes and natural cavities by these squirrels is believed to reduce fecundity in selected avian species such as the endangered red-cockaded woodpecker (*Picoides borealis*). Management practices have focused primarily on physically removing squirrels prior to breeding seasons, but the benefits of removal may be minimal. Because avian species, including woodpeckers, do not hear in the ultrasonic range (>20 kHz) and most rodents do, the use of ultrasonic deterrents could serve as an effective tool to repel kleptoparasitic species like *G. volans* from nesting cavities of selected birds. In my study, I tested efficacy of ultrasonic deterrents in repelling southern flying squirrels from nest-boxes over 6 consecutive nights. My study demonstrated that ultrasonic deterrents are 71% effective in deterring squirrels during the first night. Habituation to nest-boxes equipped with ultrasonic deterrents took on average 3.6 nights, with 24% of squirrels never nesting in an ultrasonic-equipped box. Because squirrels were tested in a laboratory with a limited area to escape the noise of ultrasonic deterrents, the rate of habituation could have been accelerated. In the field, where squirrels would have greater opportunity to escape, ultrasonic deterrents may significantly reduce kleptoparasitism of woodpecker nests. Additionally, over a 6-night period, squirrels chose quiet boxes significantly more often than ultrasonic boxes. For this reason, ultrasonic deterrents could be a more effective tool than physical removal of squirrels in reducing interspecific competition for nesting sites.

Key words: *Glaucomys volans*, nest-boxes, *Picoides borealis*, ultrasonic deterrent

Southern flying squirrels (*Glaucomys volans*) are one of the most common species of small mammals in southeastern forests (Loeb et al., 1999) and typically inhabit deciduous forests or mixed forests of pines and hardwoods (Dolan and Carter 1977; Weigl 1978). They are secondary cavity nesters that commonly den in existing cavities of snags or live trees (Dolan and Carter 1977; Holloway and Malcolm 2007; Muul 1974; Weigl 1978), but also use artificial structures such as nest-boxes (Borgo et al. 2010; Layne and Raymond 1994; Pitts 1992; Prange and Nelson 2006; Reynolds et al. 2009). Because of their abundance and propensity to aggregate in large numbers (Layne and Raymond 1994; Reynolds et al., 2009; Thorington et al. 2010), southern flying squirrels can pose potential problems for other cavity nesters such as bluebirds and woodpeckers. *G. volans* can reduce fecundity of endangered red-cockaded woodpeckers (*Picoides borealis*; Laves and Loeb 1999; Loeb and Hooper 1997). These woodpeckers are cooperative breeders and habitat-specialists that occupy old-growth pine forests and are the only woodpeckers in North America that excavate their nesting cavities in living pine trees (Costa 2001). Presently, only about 3% of the southern-pine ecosystem remains (Frost 1993). With the significant loss of habitat for red-cockaded woodpeckers, competition for suitable nesting sites could have detrimental effects on long-term survival of the species. Furthermore, even a single event of kleptoparasitism by southern flying squirrels within a breeding cluster of red-cockaded woodpeckers can negatively affect the fecundity of the colony (Loeb and Hooper 1997). Efforts to mitigate competition for nesting sites have included physical removal of squirrels (Borgo et al.

2010; Franzreb 1997; Laves and Loeb 1999; Mitchell et al. 1999; Richardson and Stockie 1995), supplementing clusters of red-cockaded woodpeckers with nest-boxes to lure southern flying squirrels out of cavities and into the boxes (Borgo et al. 2006; McComb and Noble 1991; Loeb and Hooper 1997), and use of predator-scent as a deterrent in captive (Borgo et al. 2006) and wild populations of southern flying squirrels (Stober and Conner 2007).

While Laves and Loeb (1999) reported that removal had a positive effect on red-cockaded woodpeckers by increasing rate of hatching and fledging, Mitchell et al. (1999) detected no difference in reproductive success after removal of southern flying squirrels. Furthermore, physically removing squirrels does not reduce future occupancy of cavities by *G. volans* (Borgo et al. 2010; Loeb and Ruth 1995). Similar conflicting results were reported with the use of predator-scents as a deterrent. In a study of captive southern flying squirrels, scents of several predators were highly effective in excluding southern flying squirrels from nest-boxes (Borgo et al. 2006), yet in wild populations, use of predator-scents had no significant effect on use of cavities (Stober and Conner 2007). Borgo et al. (2006) noted that adding external nest-boxes to existing clusters of red-cockaded woodpeckers significantly reduced usage of cavities by southern flying squirrels. However, this practice also could serve to attract a greater number of southern flying squirrels to the area.

Most management practices of removing squirrels focus primarily on sites with existing, active colonies of red-cockaded woodpeckers. However, due to large populations of southern flying squirrels, this approach is likely to overlook adjacent populations moving in and repopulating after removal of selected individuals (Borgo et al. 2010; Loeb 1993). Because time, effort, and equipment used to remove southern flying squirrels is costly with seemingly little return on minimizing effect, and the addition of nest-boxes could increase the population of southern flying squirrels locally, alternative methods warrant exploration.

Ultrasonic deterrents that emit high-frequency sounds above the range of hearing in humans (>20 kHz) have been marketed widely to the public as a cost-effective, non-toxic repellent to various nuisance or pest species. Studies testing these devices on various taxa including insects, birds, and mammals often have concluded that they are ineffective, but a few studies have yielded promising results. For example, Nelson et al. (2006) noted that ultrasonic deterrents producing 96 dB at 1 m had a moderate effect on reducing the number of domestic cats (*Felis catus*) entering gardens. Similarly, Phillipine rats (*Rattus rattus mindanensis*) were repelled successfully from bait-stations using a combination of three ultrasonic deterrents transmitting at 118, 116, and 103 dB at 30 cm (Shumake et al. 1982). Ultrasonic deterrents capable of broadcasting ultrasound >100 dB have proven effective in reducing foraging activity of various species of bats over ponds (Johnson et al. 2012) and around wind turbines (G. R. Spanjer in litt.; J. M. Szewczak and E. B. Arnett in litt.). While the effective range of reducing activity was limited (12–15 m), bats did not habituate to the broadcast over time (J. M. Szewczak and E. B. Arnett in litt.). Thus, ultrasonic deterrents may serve as an effective tool for repelling target species so long as sound-pressure level is high and the area targeted for exclusion is small. In my investigation, I used ultrasonic deterrents to test efficacy of excluding wild-caught southern flying squirrels from nest-boxes and the immediate area ≤45 cm surrounding the box. While studies addressing efficacy of these devices have focused primarily on their ability to reduce human-wildlife conflict, no study has assessed use of ultrasonic deterrents to mitigate interspecific competition. Non-mammalian vertebrates, including birds, do not hear in the ultrasonic range (Heffner and Heffner 2008), making ultrasonic deterrents ideal for targeting kleptoparasitic species like rodents. Thus, I test the hypotheses that nest-boxes equipped with ultrasonic deterrents will have reduced occupation by southern flying squirrels and consumption of food by southern flying squirrels will decrease in the immediate area surrounding the ultrasonic-equipped box. Additionally, I determine average rate of habituation of *G. volans* to ultrasonic deterrents over time.

## MATERIALS AND METHODS

The study was conducted at Auburn University during spring and summer 2011. Forty-four southern flying squirrels (30 ♂♂: 14 ♀♀) were captured by hand from nest-boxes of red-cockaded woodpeckers in Russell County, Alabama. After capture, demographic characters were recorded including sex, reproductive condition, weight, and age (adult or subadult). Subadults were classified based on weight (<65 g; Linzey and Linzey 1979). Squirrels where housed in wire-mesh cages measuring 60 x 60 x 60 cm and provided with two wooden nest-boxes per cage. No more than five squirrels were housed per cage and food and water were provided ad libitum.

For all experiments, I used 90 x 45 x 45-cm cages made of 1.9-cm wire-mesh that were partitioned with 1.9-cm hardware cloth creating two equal sides (A and B) of 45 x 45 x 45 cm. A small hole was cut into the partition to allow access to both sides of the cage. Two finch-sized nesting boxes (Ware Manufacturing, Inc., Phoenix, Arizona) were provided per cage (one on each side of the partition). One nest-box was equipped with a Black and Decker Ultrasonic Pest Repeller® (model EP321-2P; Black and Decker, Towson, Maryland). A hole was drilled through the back of the nest-box and the pest repeller was attached to the outside of cage with the speaker positioned over the hole drilled into box. This method prevented squirrels from having direct access to repellers. To contain the noise of the pest repeller on side A and reduce the effect of the noise on side B, I created two anechoic chambers by covering the interior floor, partition, and ceiling with 3.81-cm foam. I then insulated each nest-box with Foamular$^{®}$ acoustic sheeting (Owens Corning, Toledo, Ohio), and the remaining exterior walls of the cage with Quiet Brace$^{®}$ sound-deadening structural sheeting (Temple-Inland, Diboll, Texas). Frequency bandwidth and sound-pressure levels of the ultrasonic pest repellers were measured for all four dependent variables: quiet box, quiet area, ultrasonic box, and ultrasonic area (Table 3.1).

Squirrels were assigned randomly to control ($n$ = 20) or ultrasonic-experiment ($n$ = 24) groups. For the control-group, ultrasonic deterrents were not activated and squirrels were only tested for 1 night to determine if there was any difference between ultrasonic boxes and quiet boxes. Individual southern flying squirrels were placed into testing cages 1800–2000 h and given 15 minutes to explore and become familiar with the experimental cage. At the conclusion of this period, equal amounts of food and water were placed on sides A and B, and southern flying squirrels were left in the experimental cage all night. The following morning, boxes and consumption of food were checked to confirm activity. Categorical data were recorded for boxes (presence = 1; absence = 0) and consumption of food (all = 2; some = 1; none = 0). I then applied the same protocol to the ultrasonic experiment; except southern flying squirrels were tested over 6 consecutive nights, and ultrasonic deterrents were activated at the conclusion of the initial 15-minute period and just prior to placement of food and water. To determine if ultrasonic deterrents had a significant effect on reduction of nesting activity in ultrasonic boxes, I compared data obtained from observed frequencies of use of nest-boxes in control trials to frequencies of night 1 in experimental trials. I then proceeded with testing the experimental group over the remaining 5 nights, completing a total of 6 consecutive nights for the study. If a squirrel habituated to an ultrasonic box prior to night 6, average number of nights to habituate was recorded and they were returned to their containment cages. In addition to assessing probabilities of use of boxes for all *G. volans,* I evaluated how selected groups (adult males, adult females, and subadults) differed. The same data for all trials were collected on each of the following mornings until completion of the experiment.

For the control group, differences in usage of nest-boxes were tested using a binomial distribution. I next determined if ultrasonic-broadcast boxes could reduce nesting activity significantly by comparing control to experimental frequencies using a Chi-square test with Yates' correction (GraphPad Software, Inc., La Jolla, California). To assess whether consumption of food differed between sides A and B for both groups (control and ultrasonic experiment), I used a Chi-square test. Rate of habituation was calculated using mean number of nights it took for southern flying squirrels to start nesting in a box equipped with an ultrasonic deterrent. I used a one-way analysis of variance (ANOVA) to assess whether experimental groups (adult males, adult females, and subadults) differed in mean number of nights it took to habituate to ultrasonic-broadcast boxes. A logistic regression for repeated measures was used for the overall model to determine how probability of usage of boxes by southern flying squirrels differed over time. For tests examining probability of activity (between nest-boxes and consumption of food), I used a one-tailed hypothesis ($H_0$: quiet area ≤ultrasonic broadcast area; $H_A$: quiet area > ultrasonic broadcast area). Statistical significance was based on a rejection level of $\alpha = 0.05$. Statistical analyses were performed using SPSS 20.0 (SPSS, Inc., Chicago, Illinois).

## RESULTS

Sex ratios of southern flying squirrels collected from nest-boxes of red-cockaded woodpeckers were skewed toward males (2.4♂♂:1♀♀). In control trials with ultrasonic-broadcast boxes not activated, there was no significant difference in the probability that a squirrel would choose one box over the other (*n* = 20, *P* = 0.50, binomial test; Fig. 3.1), and all

20 squirrels consumed food from both sides A and B. However, during night 1 of activated testing of the ultrasonic-deterrent boxes, there were significant differences in observed usage of boxes, with a greater proportion of southern flying squirrels using quiet boxes ($\chi^2 = 3.06$, $df = 1$, $P = 0.04$ Fig. 3.1). Significantly less food was consumed in the ultrasonic-broadcast area than in the quiet area ($\chi^2 = 8.597$, $df = 2$, $P = 0.01$; Fig. 3.2). Results showed that food was consumed on both sides during most trials, but less consumption occurred in the broadcast-area.

Average sound-pressure levels were 53.8 dB in quiet boxes and 98.2 dB in ultrasonic-broadcast boxes. Frequency range of ultrasonic deterrents was 28.8–42.5 kHz (Table 3.1). Average number of days for southern flying squirrels to habituate to ultrasonic boxes in the experimental trials was 3.6, with 25% of *G. volans* never habituating. Mean rate of habituation for adult males = 2.8 days, adult females = 4 days, and subadults = 5.3 days. While this trend suggested subadults took longer to habituate to ultrasonic boxes, there was no statistical difference between groups ($F = 2.336$, $P = 0.12$, one-way ANOVA). The overall model using logistic regression for repeated measures demonstrated significant differences in the probability that southern flying squirrels were more likely to use quiet boxes (Wald $\chi^2 = 14.943$, $df = 5$, $P = 0.01$; Fig. 3.3).

## DISCUSSION

Studies that have tested efficacy of ultrasonic transmission in repelling certain species generally have shown ultrasonic deterrents to be ineffective. Reasons for lack of success likely are due to limitations of the physical properties of ultrasound. For example, higher-frequency sounds are highly directional, reflective, and attenuate quickly. Other factors contributing to limited success of ultrasonic deterrents include non-mammalian vertebrates, such as birds, having a hearing range that does not extend into the ultrasonic range (Dooling 2000), and animals habituating to the sound over time (Dooling 2002; Erickson et al. 1992). While the hearing range of red-cockaded woodpeckers is not known, Ramp (1964) reported that the upper hearing range of the hairy woodpecker (*P. villosus*) reaches 18.4 kHz at 90 dB, but other studies of hearing in birds have determined that upper limits usually do not exceed 12 kHz (Dooling 2000; Fay 1988; Heffner and Heffner 2007). For these reasons, ultrasonic deterrents repeatedly have been ineffective in repelling birds (Dooling 2002; Erickson et al. 1992), thereby providing a possible solution to alleviate kleptoparasitism of nests by small mammals. I used ultrasonic deterrents that emitted high-frequency sounds well above the highest known hearing ranges of birds (bandwidth 28.8–42.5 kHz; Table 1).

While some studies have had similar results to my findings for sex ratios of southern flying squirrels using boxes biased toward males (Heidt 1977; Layne and Raymond 1994), others have had nearly equal ratios. For example, Reynolds et al. (2009) noted that sex ratios of adults were biased toward males, but ratios were nearly equal when including all age-classes. Similarly, in a study of occupation of nesting cavities of red-cockaded woodpeckers by southern flying squirrels, overall sex ratios were not significantly different (Laves and Loeb 2006). It is likely that these sex ratios reflect sampling bias, where squirrels were taken from a single cluster and time (late spring). Other studies demonstrating equal or nearly equal sex ratios have included larger samples and multiple seasons.

Night 1 of activating the ultrasonic deterrents resulted in a significant decrease in usage of ultrasonic boxes as 71% of squirrels were observed in quiet boxes. Food consumption over the length of study was significantly greater on the quiet side ($\chi^2 = 8.597$, $df = 2$, $P = 0.01$; Fig. 3.2), but failed to deter squirrels from consuming food in ultrasonic areas. Only seven times out of 145 opportunities did a squirrel not consume any food from the ultrasonic area. Overall, squirrels consumed more food on the quiet side, but ultrasonic deterrents were unsuccessful in excluding squirrels from within 45 cm of ultrasonic-deterrent boxes. While sound-pressure level was relatively high in these areas (mean = 84.2 dB), my results suggest that for optimal results, a sound-pressure level >84 dB is needed and >100 dB probably is best.

The average sound-pressure levels of all ultrasonic boxes was 98.2 dB with a maximum of 107.1 dB. These levels are consistent with other studies that were successful in repelling selected taxa (Johnson et al. 2012; Nelson et al. 2006; Shumake et al. 1982; G. R. Spanjer in litt.; J. M. Szewczak and E. B. Arnett in litt.). Average rate of habituation to ultrasonic-deterrent boxes was 3.6 days with 25% of southern flying squirrels never habituating over a 6-day period. No significant difference was present for rate of habituation between groups (adult males, adult females, and subadults), although a general trend indicated subadults taking an average of 5.3 days. Furthermore, my results may reflect an accelerated rate of habituation due to the limited area that southern flying squirrels had to escape the noise and because food could have served as extra motivation for entering the ultrasonic area. Other studies using food to assess efficacy of ultrasonic deterrents have demonstrated that when thirsty or hungry, animals will ignore high-frequency sounds (Edgar et al. 2007; La Voie and Glahn 1977; Ward et al. 2008). In absence of food lures and limited space, southern flying squirrels in the wild that initially find a nesting site unsuitable (due to transmissions from ultrasonic deterrents) may be less likely to return if other sites are available. The breeding and nesting period for red-cockaded woodpeckers usually begins in late March, with fledging in late May or early June (Costa 2001). Thus, in an effort to reduce exposure and subsequent habituation of *G. volans* to ultrasonic deterrents, the devices could be activated just prior to breeding season and deactivated following fledging.

During the 6-day trial, the proportion of southern flying squirrels choosing quiet boxes each night was significantly greater than those nesting in ultrasonic-deterrent boxes (Fig. 3.3). When broadcasted ultrasound was >100 dB, studies assessing effectiveness of repelling bats have had considerable success with little-to-no habituation in areas <12 m (Johnson et al. 2012; J. M. Szewczak and E. B. Arnett in litt.). Future studies should test ultrasonic deterrents in the field to better determine efficacy of deterrence and rate of habituation. Overall, the general success of repelling southern flying squirrels from nest-boxes in the laboratory merits additional testing in the field.

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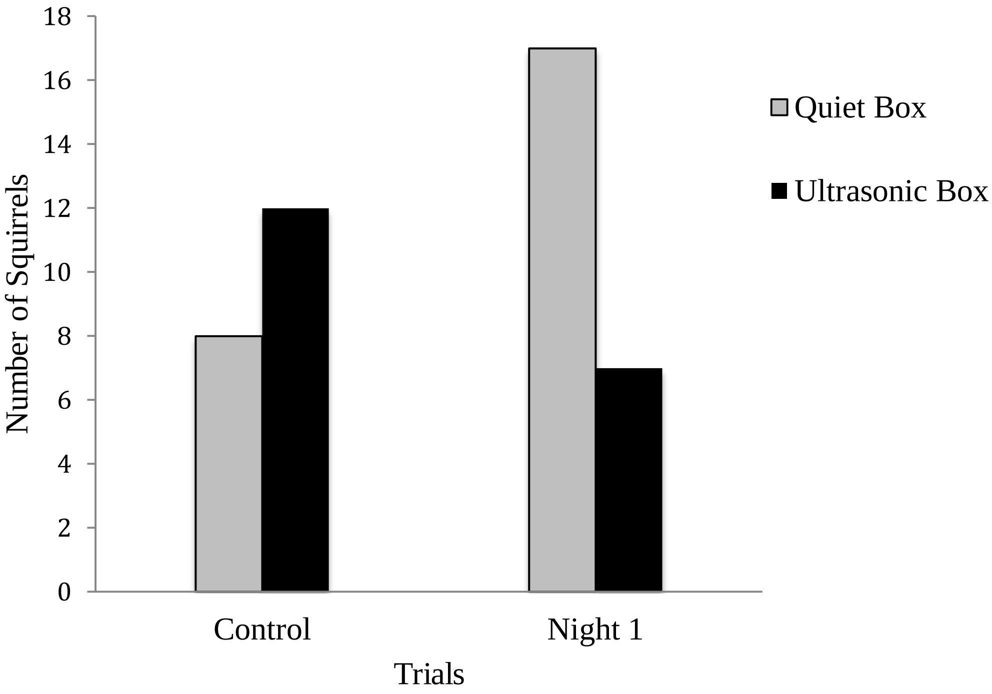

**FIG. 3.1.** – The number of southern flying squirrels (*Glaucomys volans*) nesting in boxes equipped with an ultrasonic deterrent was reduced significantly when compared to nesting activity in the experimental control ($\chi^2$ = 3.06, *df* = 1, *P* = 0.04).

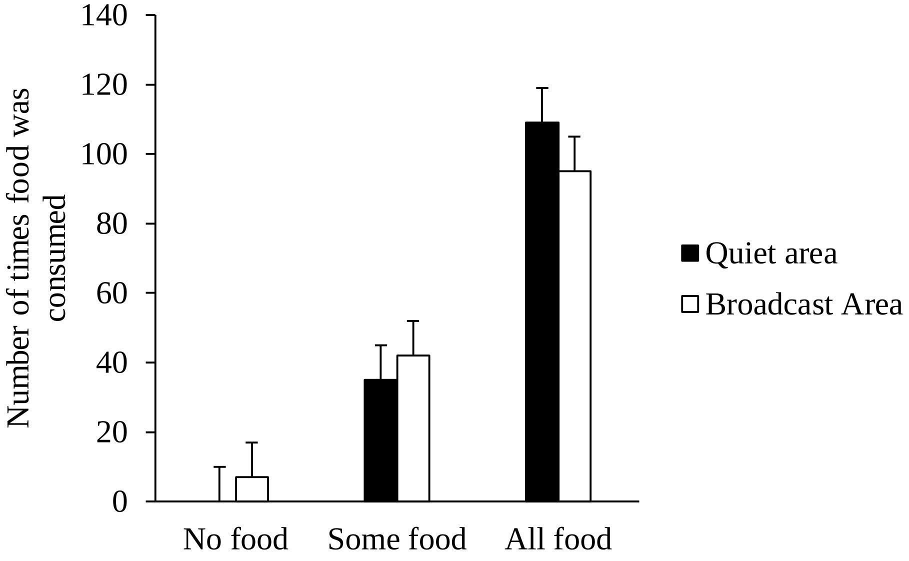

**Fig. 3.2.** – Number of times no food, some food, and all food was consumed by southern flying squirrels (*Glaucomys volans*) in quiet areas and broadcast areas. Overall, proportions consumed on the quiet side was greater than could be expected by chance ($\chi^2 = 8.597$, $df = 2$, $P = 0.01$).

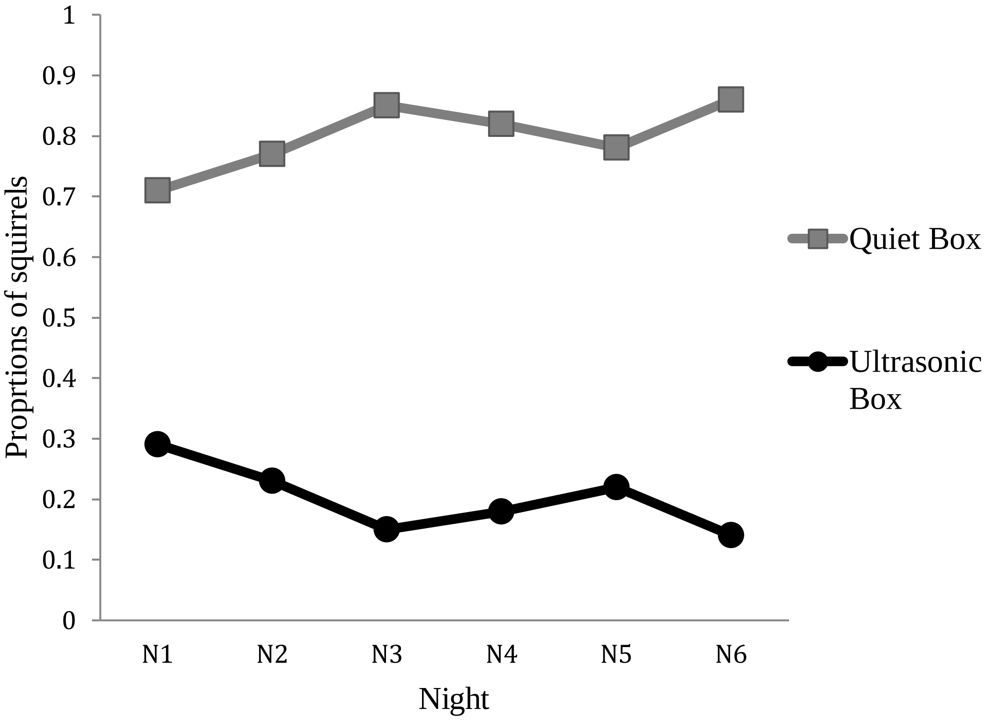

**FIG. 3.3. –** Probabilities of southern flying squirrels (*Glaucomys volans*) nesting in a box equipped with an ultrasonic deterrent over 6 consecutive nights (Wald $\chi^2 = 14.943$, $df = 5$, $P = 0.01$).

**TABLE 3.1.** – Measurements of Ultrasonic Pest Repeller® (Black and Decker, model EP321-2P; Towson, Maryland) used to test the efficacy of deterring southern flying squirrels (*Glaucomys volans*) from using nest-boxes and immediate area (≤45 cm) surrounding nest-boxes.

Quiet box

| Cage # | Ave Power (dB) | Max Power (dB) | Max Freq (kHz) | Low Freq (kHz) | High Freq (kHz) | Bandwidth (kHz) |
|---|---|---|---|---|---|---|
| 1 | 50.1 | 63.6 | 36.1 | 30.1 | 41.9 | 11.8 |
| 2 | 64.6 | 79 | 36.1 | 29.8 | 41.3 | 11.5 |
| 3 | 49.5 | 63.7 | 34.5 | 27.3 | 41.9 | 14.6 |
| 4 | 52.7 | 66.1 | 36.2 | 30.4 | 40.1 | 9.7 |
| 5 | 50.8 | 65.8 | 34.5 | 29.2 | 40.7 | 11.5 |
| 6 | 55.1 | 72.3 | 36.2 | 29.2 | 41.9 | 12.8 |
| Mean | 53.8 | 68.4 | 35.6 | 29.3 | 41.3 | 12 |

Ultrasonic box

| Cage # | Ave Power (dB) | Max Power (dB) | Max Freq (Hz) | Low Freq (kHz) | High Freq (kHz) | Bandwidth (kHz) |
|---|---|---|---|---|---|---|
| 1 | 99.1 | 107.2 | 34.5 | 29.0 | 43.0 | 14.0 |
| 2 | 99.4 | 107.1 | 34.5 | 29.0 | 41.9 | 12.9 |
| 3 | 96.7 | 107.0 | 29.3 | 26.7 | 45.0 | 18.2 |
| 4 | 97.9 | 107.0 | 31.0 | 28.5 | 43.1 | 14.6 |
| 5 | 97.0 | 107.0 | 31.0 | 27.9 | 42.5 | 14.6 |
| 6 | 99.1 | 107.1 | 34.5 | 28.5 | 43.7 | 15.2 |
| Mean | 98.2 | 107.1 | 32.5 | 28.3 | 43.2 | 14.9 |

**TABLE 3.1.** – continued.

Quiet area

| Cage # | Ave Power (dB) | Max Power (dB) | Max Freq (Hz) | Low Freq (kHz) | High Freq (kHz) | Bandwidth (kHz) |
|---|---|---|---|---|---|---|
| 1 | 62.0 | 76.7 | 36.2 | 30.1 | 43.0 | 12.9 |
| 2 | 71.7 | 86.4 | 36.2 | 29.2 | 41.9 | 12.8 |
| 3 | 59.8 | 74.6 | 36.2 | 27.9 | 43.1 | 15.2 |
| 4 | 64.4 | 79.6 | 32.7 | 29.2 | 41.9 | 12.8 |
| 5 | 64.1 | 79.9 | 34.5 | 27.9 | 41.3 | 13.4 |
| 6 | 69.8 | 86.4 | 36.2 | 29.2 | 43.1 | 14.0 |
| Mean | 65.3 | 80.6 | 35.3 | 28.9 | 42.4 | 13.5 |

Ultrasonic area

| Cage # | Ave Power (dB) | Max Power (dB) | Max Freq (Hz) | Low Freq (kHz) | High Freq (kHz) | Bandwidth (kHz) |
|---|---|---|---|---|---|---|
| 1 | 83.2 | 99.3 | 37.9 | 28.0 | 44.1 | 16.1 |
| 2 | 93.9 | 106.6 | 36.2 | 28.5 | 42.5 | 14.0 |
| 3 | 79.0 | 96.2 | 29.3 | 27.9 | 43.7 | 15.8 |
| 4 | 78.1 | 91.1 | 36.2 | 29.8 | 42.5 | 12.8 |
| 5 | 83.6 | 98.7 | 31.0 | 27.9 | 42.5 | 14.6 |
| 6 | 87.3 | 103.8 | 36.2 | 28.5 | 41.9 | 13.4 |
| Mean | 84.2 | 99.3 | 34.5 | 28.5 | 42.9 | 14.4 |