BAT SPECIES RICHNESS AND EDGE HABITAT USE ON A COASTAL ISLAND IN SOUTH CAROLINA.

by

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DEDICATION

I would like to dedicate my thesis to my grandmothers, Francis Shelton Peterson and Martha Stewart Scott. Both of them have provided me with love, support, and guidance throughout my life. I wish they could read this. I also would like to dedicate my thesis to my wonderful parents, Gwin Peterson Scott and Ford Thackston Scott.

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ABSTRACT

Bats comprise approximately one quarter of all mammal species. The number of insectivorous, microchiropteran bats found along the Lower Coastal Plain (LCP) of South Carolina is declining. Little work has been conducted on coastal islands with regard to bat ecology and habitat use. The Lower Coastal Plain of South Carolina is home to possibly 12 species of bats. Bats in the Southeastern U.S. exhibit similarities in their call characteristics including but not limited to the visual representation of their calls (i.e. sonograms). Often, researchers base identification solely on the sonogram of a recorded call without considering other facets of call structure. Identification of bats inhabiting the coastal plain by acoustic monitoring is difficult and the results may be unreliable. Reference sonograms of known bat species were analyzed and characteristics including high and low frequencies, duration, and high to low frequency, slope, and bandwidth were compiled to set up a data library for each species. Ratios were developed for the

characteristics listed above. Ratios along with visual characteristics of species specific sonograms were used to identify bat calls recorded in the field. Species identified via the method also were the same species that were captured using mist nets and harp traps on Spring Island, SC. Captured bats were identified by visual and metric evaluation of high frequency call features.

Bat richness on Spring Island was assessed during the summer of 2007 using both capture and acoustic monitoring techniques. Bat calls along two general edge habitat types (pine and hardwood) were recorded to determine if bat activity differed significantly between edge habitats. Pine edge habitats on Spring Island are less vegetatively complex than hardwood edge habitats and bat activity was predicted to be greater along pine edges than hardwood edges. Larger bats may have greater difficulty foraging and flying in dense and cluttered vegetation than smaller bat species, and navigation through such areas may be more energetically costly than navigating through less dense and open-air areas. Insect dry mass along edge habitats was collected to determine if bat activity was dependent upon insect prey availability.

Six species of bats were identified on Spring Island through capture and acoustic monitoring. Bat activity (based on the number of recorded bat calls) did not differ significantly between the 2 edge types. Data did not support the prediction that the number of recorded bat calls would be greater along pine edges than recorded along hardwood edges. Bat activity along hardwood edge habitats was greater than expected suggesting that canopy-free areas like the fields on Spring Island may be important areas for bats. Unlike many other studies, bat activity was not dependent upon insect mass (g).

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CHAPTER 1: BAT CALL IDENTIFICATION METHODS

INTRODUCTION

Microchiropteran bats use echolocation for navigation and locating flying insect prey (Griffin et al. 1960). Bats often employ frequency modulated (FM) calls. Frequency modulated calls do not maintain a constant frequency, but sweep from high to low frequency and are typically made in short duration bursts. FM calls provide a much more detailed depiction of the environment that the bat is traveling than constant frequency (CF) calls (Altringham 1996). Constant frequency calls are often lower in frequency than FM calls. The lower frequency may prolong the intensity of the call, and allow it to cover greater distances. Some bats may use CF calls in more open environments to locate prey without expending as much energy as using more detailed (and typically higher energy) FM calls. Constant frequency calls also may allow a bat to detect changes in the amplitude of the sounds given off by the wing beats of flying insects (Bell and Fenton 1994). Few bats emit only FM or only CF calls (Altringham 1996); most emit both types. Bat calls can be monitored and recorded, using a bat detector, and their call characteristics visually displayed in the form of a sonogram. Because bat calls are often more similar in highest and lowest frequencies, fundamental frequency, duration, slope, bandwidth, and the number of individual harmonics within and between species, many investigators have used both real time monitoring and passive recording systems for species identification (O'Farrell et al. 1999, O'Shea et al. 2003). Yet, sonograms from bat species found along the lower coastal plain (LCP) of South Carolina, and the Southeast (as a whole), exhibit a great deal of similarity in their structure and morphology, therefore, identification of these species can be tedious and difficult. The six species of bat identified using this method are *Eptesicus fuscus* (Big Brown Bat), *Perimyotis* *subflavus* (Eastern Pipistrelle), *Nycticeius humeralis* (Evening Bat), *Tadarida brasiliensis* (Brazilian Free-tailed Bat), *Lasiurus seminolus* (Seminole Bat), and *Myotis lucifugus* (Little Brown Bat). All of these species listed above emit echolocation calls in ultrasound (above 20 kHz).

Although several studies have described bat activity using bat detectors in the LCP, most of these studies used heterodyne systems only for bat call detection (Kalcounis et al. 1999, Menzel et al. 2005). Many bat researchers use Anabat and SD1 detectors and Analook software (Titley Electronics, Ballina, New South Wales, Australia). Anabat detectors are heterodyne systems with zero-crossings analysis that can be used for both active and passive sampling (Titley Electronics website). Bat identification through the use of heterodyne systems may be qualitative and may require visual observations to positively identify each bat (O'Farrell et al. 1999, Duffy et al. 2000). Heterodyne systems monitor only a narrow range of frequencies at a given time (Parsons et al. 2000). Heterodyne detectors, like Anabat, are often used by researchers for their relatively low cost. Yet, heterodyning, zero-crossing and frequency division systems may track only the harmonic with the greatest energy (i.e. amplitude).

Bat research in the Southeastern United States may be problematic when using heterodyne detectors. Bat calls above or outside of the selected range will not be detected by heterodyne systems. The Pettersson D240x (Pettersson Elektronik, Uppsala, Sweden) is a time-expansion system. Time-expansion (like frequency division systems) is a broad-band system that can simultaneously detect a wide range of frequencies. Both heterodyning and time-expansion systems allow researchers to view frequency and spectral structure of bat calls. Time expansion systems (like the Pettersson D240x) lose no information for the incoming call, and when combined with the proper software, produce high-quality spectral call data. Unlike frequency division and heterodyning systems, time-expansion does not alter the spectral data of the incoming bat signal. This allows the SonoBat program to provide a highly detailed and accurate sonogram of the call along with the necessary pertinent data to view high/low frequencies and slopes etc.

The purpose of this paper is to provide both a qualitative and quantitative method of identifying 6 bat species of the LCP of South Carolina by evaluating sonograms recorded with a Pettersson D240x detector and digital recorder SonoBat Bat Call Analysis software (SonoBat, Arcata, CA.). This method can be used to identify bats of all types (FM and CF) that range in frequency from 14 kHz to 120 kHz.

METHODS

All bat calls recorded for identification were recorded on Spring Island, SC from May –August 2007, as described in chapter 2 (Scott 2008). Calls were detected and recorded using a passive sampling approach. The Pettersson D240x detectors were set as follows: The speaker was set to time expansion to avoid interference during recording, The volume was set to the lowest setting, high gain function was used to maximize the reception range for incoming bat calls, The trigger switch was set to auto with 1.7 second recording session time (this allows sufficient calls and call sequence data to be collected for later analysis). The high trigger function was used to minimize insect noise and interference. The high frequency setting was used. This triggered the detector to start recording anytime a high frequency sound was detected. The detector was set at 40 kHz for all sampling nights.

Calls were recorded using iRiver 800 digital recorders (iRiver America, Irvine, CA), and calls were later downloaded to a laptop computer. The number of files varied between and among sites for each night and for each edge habitat type. Since call files were recorded in MP3 format, it was necessary to convert them to a WAVE format for analysis by the SonoBat program. Acoustica MP3 to WAVE converter plus (Acoustica, Oakhurst, CA) was used to convert file formats. Bat calls were identified to species using both quantitative and qualitative analysis. SonoBat 2.5.8 was used to view and analyze bat calls and individual sonograms.

Bat calls were identified to species using both quantitative and qualitative analyses. Because some calls can be distorted (O'Farrell et al. 1999, Parsons et al. 2000), calls were first qualitatively determined to be classifiable. For qualitative assessment, sonograms for each bat species were examined for obvious similarities or differences in call structure. For each species, five sonograms were examined from the SonoBat eastern reference library. To ensure that species specific call structures that deviated from the norm were included, two reference library sonograms that appeared distinctive or exclusive in shape per species were selected. Next, three additional sonograms for each species were selected randomly using a number generator. The five sonograms were then saved as jpeg images, printed, and used for comparison with the sonograms collected from the Spring Island files, hereafter these sonograms are referred to as the Spring Island reference sonograms. Only *Myotis lucifugus* and *Tadarida brasiliensis* exhibited noticeable differences from the other four species. Most *T. brasiliensis* calls exhibited a relatively constant frequency at or below 25 kHz. However, some *T. brasiliensis* calls resembled the calls of *E. fuscus*, *P. subflavus*, *N. humeralis,* and *L. seminolus*. All of these species exhibit some overlap in their frequency ranges.

For qualitative classification, species specific call characteristics were described. Call characteristics for the six species netted on Spring Island were characterized for bat calls of known identity using the reference view collection. For each species, each call was characterized in the standard view setting and then the “enable analysis” function was selected. This function automatically locates and displays the locations on each sonogram of both the high and low frequencies and individual harmonics. The “enable analysis” function also allows the user to view pertinent data and characteristics of the call. Characteristics include bandwidth, duration, Fmax, and slope, in addition to the high and low frequencies and harmonics (Figure 1). The number of SonoBat reference view sonograms varied for each of the six species recorded on Spring Island (Table 1). For high frequency, low frequency, bandwidth, duration, and slope, ranges were determined and minimum, maximum and mean values were recorded. All species specific call characteristics were noted for each species. All characteristics overlapped between; *E. fuscus*, *T. brasiliensis*, *L.seminolus*, *P. subflavus*, and *M. lucifugus*. For these species, high frequency to slope, low frequency to slope, and low frequency to duration ratios were calculated for each sonogram (Table 2). When species specific minimum and maximum ratios did not overlap, ratios were used as a species specific characteristic for call assignment.

Unidentified calls were then assigned to species. First, the sonogram for an unidentified call was compared visually to the Spring Island reference sonograms. If the unidentified call resembled one of these reference view sonograms, the call was then evaluated using the “enable analysis” function of SonoBat, as described for the reference calls (i.e. high and low frequency, duration, slope, etc.). Calls were identified by excluding possible identities for each call characteristic. Unidentified call characteristics were compared to species specific ranges for all six bat species occurring on Spring Island. First, unidentified calls were compared to the low frequency reference ranges. Possible identities were excluded when the unidentified call’s low frequency fell outside of species specific ranges. Identities were assigned when the low frequency of the unidentified call fell within a species specific range that did not overlap with other species. Next, the following ratios were calculated for the unidentified calls: high frequency to slope, low frequency to slope, and low frequency to duration. The ratios were then used for comparison between the unidentified calls and the SonoBat reference library call mean values. The only close values for low frequency to slope ratio are between *E. fuscus* and *M. lucifugus* however *E. fuscus* and *M. lucifugus* calls may be distinguished from each other by viewing average slopes. *E. fuscus* has and average slope of 5.3, while *M. lucifugus* exhibited an average slope of 10.6 (Table 3). *M. lucifugus* exhibited the highest high frequencies of all six species reference view calls (highest high frequency 107.4 kHz), while the highest high frequency for *E. fuscus* was 81.9 kHz.

If an unidentifiable call’s sonogram qualitatively appeared similar to a species specific reference view sonogram and quantitatively fell within the ranges of 3 of the 5 characteristics unique to one species, the unidentified call was identified to the corresponding bat species. If the unidentified call did not fall within the range for the high and low frequency parameters (maximum and minimum) for a given species, the call data were then compared to other ratio parameter ranges to determine whether or not the call could be quantitatively identified as one of the six species listed above.

RESULTS AND DISCUSSION

The method was developed to provide a stronger means of acoustic identification for bat species that are difficult at best to identify strictly from visual sonogram characteristics. Of 9176 bat calls recorded on Spring Island, 7535 calls, or 82.1%, were identified to species.

Calls that could not be assigned to species appeared distorted. Some of the unidentifiable calls were too distorted by interference to be confidently identified to species using the SonoBat program (Figure 2). Distorted sonograms exhibited characteristic “sweep” shape and pulse in milliseconds. The images for distorted calls were often blurry; sometimes to the extent that the end of one call ran together with the beginning of another. The “enable analysis” function still indicated values for highest and lowest frequencies, however, these points did not match up with their sonograms and slope and duration ratios did not match the parameters set for identification (Figure 3), and therefore could not be identified with confidence using this method. It is possible that cryptic species that were not captured on Spring Island could have similar call characteristics as species captured and recorded on the island, and therefore, could have been recorded.

Several additional factors may contribute to error in call identification and thus should be considered when recording calls and in data analysis. Bat call identification is based on the recordings of known species, and compiled into a call library. These reference calls may have been recorded under ideal conditions or under less-than-ideal conditions. Less-than-ideal conditions may have been field related. Many bats exhibit plasticity in their calls and recorded call structure may differ from those in established call library calls, including but not limited to, many species displaying shorter calls in more cluttered environments and longer calls in more open environments. Bat monitoring programs using acoustic techniques may be improperly designed and the results improperly interpreted due to temporal variation in bat activity. Activity may vary seasonally or even on a daily basis in response to many factors including: temperature, rainfall, wind, relative humidity, energetic demands, interspecific competition, and insect availability (Anthony et al. 1981, Barclay 1991, Taylor and O'Neill 1998, Adam et al. 1994, Reith 1980, 1982, Audet 1990, Kunz 1973, Lacki 1984, Speakman and Racey 1989).

Although the method successfully identified a large proportion of bat calls, a test of efficacy is needed. To evaluate the proportion of successfully identified calls, a large number of calls of known identity must be blindly evaluated using the method described herein. Recorded bat calls should come from bats captured and released in the wild or in large flight cages, and from bats that are housed and recorded under indoor conditions. Differences in calls recorded from indoors and those recorded from the field can then be compared. Although untested, the bat call identification method described here shows considerable promise over Anabat identification and qualitative identification of calls with SonoBat.

CHAPTER 2: BAT SURVEY AND EDGE PREFERENCES

INTRODUCTION

Eleven species of bats are documented along the Lower Coastal Plain (LCP) of South Carolina (Menzel et al. 2003). Of the bat species occurring in the LCP, two are listed as species of special concern: *Myotis austroriparius* (Southeastern Myotis), *Corynorhinus rafinesquii* (Rafinesques's Big-eared Bat; SC Department of Natural Resources 2006), and *Lasiurus intermedius* (Northern Yellow Bat) which is considered "rare" (Menzel et al. 2003).

Based on the general tenants of island biogeography (MacArthur and Wilson 1963, MacArthur and Wilson 1967, Menzel et al. 2003) fewer species of bats are expected to occur on islands or insular habitats when compared to the neighboring mainland, yet no prior published work has described the occurrence of bats on islands along the southeastern coast of the United States.

Use of Habitat Edges by Bats

The junction between adjacent habitats or communities was first described by Clements (1905) as the ecotone, which can be defined as an area of ecological transition. Later, Leopold (1933) and Odum (1959) made similar statements. The concept of ecotone has been broadened to include abiotic and biotic variables and include different spatial and temporal scales (Laliberte et al. 2007). Edge systems have conditions that are defined by the interactions within and among the communities (Holland 1988, Risser 1993). Edge is the junction of different landscape elements (Yahner 1998). Examples may include intersections between different plant communities, successional stages, or regions that differ in land use. For this study Baker et al.'s (2002) definitions of ecotone and edge are adopted, with an ecotone defined as the two dimensional zone of transition between ecosystems and an edge defined as the line used to “demarcate” two adjacent ecosystems. Ecological edges and ecotones can have dramatic impacts on species composition, richness and diversity (often over short distances), differing significantly from the communities on either side of the edge. Edges have been shown to have either positive or negative effects on many species. Simply defined edge-effect is a measurable change in edaphic conditions that commonly results in an increase or decrease in the relative abundance of a species and/or community change in richness or diversity when compared to neighboring habitats (Alvarez et al. 1988; Harris, 1988; Yahner, 1988). For example, avian and ophidian nest predators are often more abundant along edges causing a negative effect on species richness and abundance of songbirds (Fleming and Giuliano. 2001, Chalfoun et al. 2002). Edge-effects may result in lesser or greater vegetative complexity along habitats. Land management practices in the LCP have produced numerous forest/grass edges adjacent to areas of human activity, including homes, shopping centers, and roads. Bat species found along the LCP are commonly hawking feeders (i.e., capture and consume flying insect prey while both the insect and bat are in flight). Canopy free edges and ecotones adjacent to forest stands provide an obstruction free environment for hawking bats to feed. Edges may be beneficial to larger species of insectivorous bats, as bats may have low wing loading and high aspect ratios that restrict maneuverability in cluttered thick canopy forests. At least two bats that occur in the LCP of South Carolina have low wing loading and high aspect ratios (i.e., Big Brown Bat (*Eptesicus fuscus*) and the Evening Bat (*Nycticeius humeralis*; Altringham 1996). Bats with high wing loading and high aspect ratio, like the Brazilian Free-tailed Bat (*Tadarida* *brasiliensis*) also common in the LCP need open-air environments over which to forage (Altringham. 1996). Bats not only exhibit strong habitat preferences (Fenton 1970, Estrada et al. 1993, Burford and Lacki 1995, Haymond 1998), but many species are more abundant along edges of water bodies, trails, and forests compared to forest interiors (Krusic et al. 1996). Bats appear to maximize dietary intake along forest edges where insect diversity and abundance are greater (Verboom and Huitema 1997, Voller 1998, Grindal and Brigham 1999). Edges and ecotones also are important to insectivorous bat species for commuting to and from roosts and may function as orientation points for navigation (Grindal 1998; Grindal and Brigham 1999).

Although the importance of edges to bats has been acknowledged (Grindal 1998; Grindal and Brigham 1999, Verboom et al. 1997, Menzel et al. 2005, Loeb and O'Keefe 2006), few studies have quantified edge/ecotone condition, potential edge effects (but see Ford et al. 2005), or examined biotic variables, such as insect abundance, that contribute to preferred habitat for bats. Microhabitat preferences of bat species vary with body size and echolocation call characteristics, with preferences varying from cluttered forest for small bats with high echolocation call frequencies to open spaces for heavier bats with lower frequency calls (Ford et al. 2005, Altringham 1996). Hogberg et al. (2002) and Kalcounis et al. (1999) found that both large bats that are canopy adapted and smaller more maneuverable species were significantly more active along forest edges and ecotones. Relative abundance of small bat species varied by study (x versus y respectively), whether or not differences were driven by regional variation in relative abundance or species specific habitat preferences is unknown. Insectivorous bat activity is generally positively related to insect abundance (Wickramasinghe et al. 2003, Fukui et al. 2006, Parks et al. 2006), although bat activity can be reduced in instances of high insect abundance when the structural complexity of adjacent forest reduces bat maneuverability (Fenton, 1990; Kalcounis and Brigham. 1995). An open forest ecotone likely provides an optimal combination of dense forest vegetation for insects and an open space for efficient foraging by bats. Energetic returns along edges for many bat species may be particularly great, and between-edge variation in insect activity may be as important in determining bat abundance as between-habitat differences in insect availability.

In the LCP, pine and hardwood forest fragments are common. Pine fragments or stands are largely associated with silviculture with a dominance of disturbance-adapted loblolly pine (*Pinus taeda*); whereas, hardwood fragments are common refugia for many native plants (Menzel et al. 2005). Many bats prefer the mixed strata and spatial complexity of hardwood/mixed forests over the openness of pine forests (Ball 2002). Bat activity on Spring Island was expected to be greater along pine edges. Vegetation was found to be less dense within pine forests and along pine ecotones than in hardwood fragments. Also it was predicted that bat activity would be positively related to insect prey abundance/biomass regardless of ecotone type.

METHODS

The study was conducted on Spring Island, Beaufort Co. SC (Lat/Lon: 32.3° N 80.9° W) between June 4 and August 4, 2007 and consisted of biweekly mist netting, harp trapping, acoustic monitoring of bats, quantification of available invertebrate prey and plant community description. The island is an approximately 2424 hectare private development located 0.5 km from the mainland between the Colleton and Chechessee rivers, north-west of Port Royal Sound (Figure 4). Although extensively developed, Spring Island utilizes applied wildlife and best management practices (BMP) to lessen and mitigate environmental impacts caused by development. The available foraging habitat for bats on Spring Island consists of riparian areas, pine forest, mixed forests (pine/hardwood or pine/shrub), live oak forests, salt marsh, planted fields of native and exotic grasses and an18-hole golf course.

Mist netting

Bat richness and species occurrence was characterized in a biweekly mist net study. Twenty-five potential sites along service roads and trails were identified based on visual characteristics suggestive of a good corridor both for commuting bats and effective netting distributed across the island (sensu Kunz 1988). Commuting corridors used by bats often have sufficient structure to restrict deviation from the corridor. Characteristics included low hanging tree canopy to reduce the number of bats flying over the net and moderate to dense vegetation on either side of the corridor to prevent bats from going around the net.

Netting sites were given a number and three locations randomly selected each trap night. Mist nets were erected between 8:00 PM and 10:00 PM. All 30 mm^2 mesh mist nets (Avinet, Dryden, NY) were 2.6m high (with 2 or 3 nets stacked atop one another) but varied in lengths of 2.6, 6, and 9 meters. Excluding severe weather, nets were monitored at twenty to thirty minute intervals for a minimum of three hours. Captured bats were removed from mist nets and placed in cotton “bat bags” for transport to a central location on the island for documentation. Data collected included species (W.R. Hood from Barbour and Davis 1969, Whitaker 1988, Webster et al 1985, Reid 2007), sex, relative age (adult or juvenile), body mass (± 0.01 grams), forearm length (± 0.1 mm), and the presence of any visible parasites and/or injuries. Bats were examined for the presence or absence of external genitalia to determine sex. Male bats with visibly descended testicles were recorded as reproductive. Each female's abdomen was palpated to determine whether or not she was pregnant and nipples palpated to determine whether or not she was lactating. Bats were identified as juveniles if epiphyseal gaps were visible at the proximal and distal ends of the metacarpels and phalanges when illuminated.

To survey bats roosting underneath the Spring Island to Callawassie Island Bridge, bats were netted on June 28 and July 25, 2008 using a harp trap suspended by ropes and placed near bridge expansion joints where *T. brasiliensis* were observed entering and leaving the roost. The capture of these animals was not including in the experimental design and thus, data from the animals trapped under bridge were not included in measurements of relative abundance. All protocols for capturing and data collection of bats were in compliance with the recommendations of the American Society of Mammalogists Animal Care and Use Committee guidelines (ASM 1998).

Acoustic Monitoring of Bats along Edges

Eleven pine and eleven hardwood ecotones were sampled for bat use (Figure 5). Edge habitats were selected based on the presence of grass fields (including native and/or introduced grasses) adjacent to either pine or hardwood habitat. Edges were linear, edge lengths and the dimensions of the fields adjoined are listed in Table 4. All edges selected for this study were a minimum of 500 m apart and at least130 m from the nearest body of water, reducing bias associated with bat activity (Stagliano et al. 1998, Seidman and Zabel, 2001, Ford et al. 2005, Menzel et al. 2005). To characterize edge types, tree species were surveyed along the length of the vegetation transect that extended 15 m into the forest side of the edge. The number of pine and hardwood trees were counted and a percentage of each type per site was calculated for trees greater than 3 m high with a diameter at breast height (dbh) of greater than 15 cm. Ecotones were designated as pine when >75% of the canopy trees belonged to pine species including Loblolly Pine (*P. taeda*), Longleaf Pine (*P. palustris*), and Slash Pine (*P. elliottii*). Edges were designated hardwood when >75% of the canopy trees were hardwoods (Carter et al. 2004). Hardwood edge tree species included Southern Live Oak (*Quercus virginiana*), Laurel Oak (*Q. laurifolia*), and Red Bay (*Persea borbonia*). Grasses along both edge types included; *Digitaria spp*., *Setaria spp*., *Cenchrus spp*., *Paspalum spp*., and *Andropogon spp*.

Bat activity was determined from the monitoring of echolocation along each edge type six nights per week. Each week was divided into two sampling bouts (Monday to Wednesday and Thursday to Saturday). For each sampling bout, two pine and two hardwood edges were randomly selected. Repeated samplings of individual sites occurred at least two weeks apart and were treated as independent of prior sampling events. Bat calls were recorded at each edge for 8-10 hours for 3 consecutive nights. Detectors with a digital recorder and insect traps were set up approximately 1 hr before sunset. Detectors, recorders and captured insects were collected the following morning. By collecting data over the full night, the post-dusk and pre-dawn peaks in activity common to many insectivorous bats were included (Hayes 1997, Erkert 1982, Kunz 1973, Kunz et al. 1995, Maier 1992, Taylor and O'Neill 1988). Bat calls were recorded with one of four Pettersson D-240X ultrasound detectors (Pettersson Elektronik AB, Uppsala, Sweden) connected to iRiver 800 MP3 digital recorders (Seoul, Korea). The detectors were set for passive listen and time expansion, and the MP3 recorder set for voice activation. Both the detector and recorder were enclosed within a plastic jar at each station. Stations were erected 15 m away from each edge at the midpoint, and raised to a height of approximately 3 m. All calls were downloaded daily to a laptop computer. Method for call identification and quantification are described in Chapter 1.

Insect Dry Biomass Analysis

Insect relative abundance and biomass was sampled along edges synchronous acoustic monitoring to determine if bat activity was associated with insect abundance. Sampling occurred for 3 consecutive nights per location. As with bat calls, insect mass was collected multiple times, however repeated collection of insects at sites occurred at least 2 weeks later than each previous collection. Because all species of bats found in the southeastern US are hawking species, insect traps were constructed to collect aerial insects. Black-light bucket traps were constructed from 5-gallon buckets, a PVC shaft extending vertically from the center of each bucket to ~30.5 cm above the rim, and two white, vinyl floor tiles that were attached to the PVC shaft using zip cable ties and epoxy. On each side of the tiles, a 4 watt black-light was attached to attract insect prey items (Bell 1981, Carter et al. 2004). Insects attracted to the light slide from the tiles into the bucket below. Approximately 3 quarts of 20% dish detergent solution was added to each bucket to prevent insects from climbing out. Buckets were suspended approximately 3 m above the ground positioned 15 m from the habitat edge and 10 m from the bat listening station. All insects were collected after dawn the following morning, removed from the traps, drained, and frozen in zip-lock bags for later analysis.

Insects collected on the second day of each sampling bout were identified to order, and the mass of all insects collected from each location determined for each sampling night. All insects were dried to constant mass at 60°C for 24 hours. Finally, the total insect mass was measured for each day and location collected and insect mass by order was determined by location for the second night of each three day sampling bout.

Statistical Analysis

Using SPSS 14.0 (2005) the lengths of the 11 pine edges were summed and compared with the summed lengths of the 11 hardwood edges to determine the percentage of length that each edge type composed of total edge length. Percentages also were calculated for the areas and perimeters of the grass fields of both pine and hardwood sites. Percentages were used to calculate values for the expected number of calls for both edge types. Expected values were compared with the number of calls recorded for both edge types using chi-square analysis to determine if the numbers of calls were related to site length, perimeter, and area metrics. Data were collapsed and all sampling events treated as independent, and compared across the sampling season. For analysis of differences in call numbers between pine and mixed edges an independent-samples t-test was used (*a priori* $p<0.05$). Independent-samples t-tests also were used to compare the bat calls of individual species between edge types.

Insect dry mass (g) data also were collapsed into total values per edge type for the entire sampling season. Total insect dry mass collected for each edge type was compared using an independent-samples t-test. A linear regression model was used to determine whether or not total bat calls were dependent upon total insect dry biomass. To determine if there was a relationship between *E. fuscus* calls and coleopteran dry mass by edge type, a linear regression model was used. A linear regression model also was used to determine if a relationship existed between the numbers of *T. brasiliensis* calls and lepidopteran dry mass by edge type.

RESULTS

Bat Capture Survey

A total of 50 bats belonging to 6 species were captured using mist nets (Table 5). The number of bat species found on Spring Island is less than the number of species that have been documented within Beaufort County (Table 6).Of the bats captured by mist-netting 34 were females and 16 were males. The majority of captured bats were adults, only 3 bats were juveniles. Almost half of female bats were either pregnant (n=3), lactating (n=7), or in post-lactating condition (n=7).

More than 170 bats were captured over the course of 2 nights June 28 and July 29, using a harp trap suspended from the Spring Island to Callawassie Island Bridge. These bats were from a maternity colony and consisted of mature females and immature/nursing males and females.

Acoustic monitoring along edge habitats

A total number of 7349 bat calls among both edge habitats were recorded on Spring Island. No distinct trends in the number of bat calls were observed between weeks of the sampling season. Pine edges comprised 62% of the total edge habitat length of the sites sampled, and hardwood edges comprised the remaining 38% of the total edge habitat length. The total number of recorded calls was multiplied by the percentage of total edge habitat length to determine the total number of calls that would be expected for each edge habitat type. Values for total perimeter and total area of the fields adjacent to each edge were summed for each edge habitat type. Pine sites comprised 66% of total edge perimeter (m) and 63% of total site area (m^2). Total bat calls were multiplied by percentages to determine the number of expected calls based on pine and hardwood perimeter and area. Actual recorded calls along hardwood edges were greater than the number expected by chance for: edge length ($X^2 = 1002.77$, d.f. = 1, $p < 0.0001$), field area ($X^2 = 568.33$, d.f. = 1, $p < 0.0001$), and field perimeter ($X^2 = 663.37$, d.f. = 1, $p < 0.0001$) (Figure 6).

The mean calls recorded along pine edges were 46.5 per night ($\pm$5.05 SE). Hardwood ecotones averaged 48.5 calls per night ($\pm$5.14 SE) (Figure 7). The number of calls recorded each night did not significantly differ by forest type ($t = -0.277$, d.f. = 1,159, $p > 0.05$); although the number of bat calls were highly variable by night between and among both edge types. Bat activity also did not differ between edge habitats for individual bat species (Table 7, Figure 8).

Insect Abundance

Captured insects were from the following taxonomic Orders; Coleoptera, Hemiptera, Diptera, Hymenoptera, Odonata, Lepidoptera, Homoptera, and Blattaria (Figure 9). A total of 561.37 grams of insect dry mass were collected among both edge types. Biomass data were collapsed for the entire field season and treated as independent of individual weeks. Insect dry mass (g) was not significantly different between the two edge types ($t = -0.119$, d.f. = 1,159, $p > 0.05$) (Figure 10). Total bat calls showed no significant relationship with total insect dry mass when evaluated by linear regression ($F = 1.790$, d.f. = 1,159, $p > 0.05$). Calls emitted by *E. fuscus* did not appear dependent upon coleopteran dry mass ($F = 0.249$, d.f. = 1, 66, $p > 0.05$). Calls of *T. brasiliensis* were not dependent upon lepidopteran dry mass ($F = 2.616$, d.f. = 1, 64, $p > 0.05$).

DISCUSSION

Bat Capture Survey

Six species of bats were captured through the use of mist nets and harp traps on the island. When *T. brasiliensis* is excluded due to targeted collecting from a roost, *E. fuscus* was both the most common species trapped along road corridors and recorded acoustically along pine and hardwood forest fragments. *T. brasiliensis* was never captured in mist nets but is known to roost in structures around the perimeter of the island, including the Spring Island-Callawassie Bridge, as described herein, as well as a purple martin house, and plastic owl decoys (W.R. Hood, personal communication). Multiple colonies of *T. brasiliensis* are found beneath the Spring Island-Callawassie Bridge. It was impossible to determine an accurate number of these bats inhabiting the expansion joints of this bridge however, it is probable that this number is in the thousands. *T. brasiliensis* is adapted to open spaces and was not expected to penetrate interior areas of forest. Nevertheless, the results of acoustic monitoring indicate that forest edges across the island are important for this species.

Relative to previous studies in the LCP, low number of bats species recorded on Spring Island could be associated with an island or insular habitat effect (MacArthur and Wilson. 1963). Six species were captured on Spring Island, whereas 11 species have been documented in Beaufort County (Menzel et al 2003). Interestingly, *Perimyotis subflavus* was captured on Spring Island but was not documented in Beaufort County by Menzel et al. (2003). To further examine the effect of islands on bat distributions, mist-netting and acoustic monitoring must be conducted on neighboring islands and the adjacent mainland.

Acoustic monitoring along edge habitats

Bat captures on Spring Island support the acoustic identification methods used for this project. The six species of bats identified acoustically were the same as those six species captured through mist netting and harp traps. The majority of bat calls were composed of *E. fuscus* and *T. brasiliensis*. Both species are canopy-adapted/open-air specialists. *E. fuscus* and *T. brasiliensis* are greater in mass and are less maneuverable than smaller, clutter adapted species, which may explain why they were recorded most commonly along the canopy-free edge habitats. The fewest numbers of recorded calls were by *M. lucifugus* and *P. subflavus* which are clutter-adapted bats and typically forage in the more structurally complex forest interior. Recording stations in this study were biased for canopy and open-air specialists. Actual relative abundance of clutter species is likely higher than reported herein. My results are similar to those of Menzel et al. (2002). In Menzel et al. (2002), mist-netting and acoustic monitoring were used to evaluate bat passes and feeding buzzes in forest interiors and along service and logging roads, forest gaps, edges and other open areas. Their results found that *E. fuscus*, *N. humeralis*, *L. seminolus*, and *T. brasiliensis* exhibited higher activity levels along and within more open areas than within the forest interior.

Edge preferences of bats

Based on the total number of bat calls detected along pine and hardwood edges, bats displayed no significant preference for either pine or hardwood edges. Data did not support the prediction that bat activity would be greater along pine edges due to less dense vegetation found along this edge habitat type than along hardwood edges. However, bat activity did exhibit great variability both among and between sites and the two edge habitat types. Comparisons of individual species also exhibited great variability, suggesting that none of the six species occurring on Spring Island utilize one type of edge preferentially over the other for foraging and commuting. The actual number of bat calls along hardwood edges was significantly greater than what was expected based on edge length and field area and perimeter. The great number of total calls recorded along edges suggests that open air sites are important areas for bat activity. Relative bat activity is driven by a variety of factors, many of which are expected to be species specific. These factors include body size and relative clutter which can influence maneuverability and predation risk in open spaces, habitat specialization, prey availability, and proximity to refugia (Brigham et al. 1997, DeJong and Ahlen. 1991, Ford et al. 2005). The extent to which bats penetrate forest edges while foraging and commuting is not known. Prior work suggests that larger bodied species are more likely to penetrate pine forests than hardwood edges. Larger species like *E. fuscus*, *L. borealis*, and *L. seminolus* have been found in greater abundance in thinned and less vegetatively complex stands than unthinned stands (Elmore et al. 2005, Loeb and Waldrop, 2008), and avoid densely cluttered areas (Brigham et al. 1997, Erickson and West, 2003, Sleep and Brigham 2003). These species are habitat generalists that forage within many habitats that lack clutter that restricts or makes maneuvering more energetically costly. Smaller bodied bats such as *P. subflavus* and *Myotis* spp. are able to more efficiently fly and forage through more vegetatively complex environments. Menzel et al. (2005) found that bat activity above and within pine forests was greater than the activity levels above and within hardwood forests when pine forests were near riparian areas. This study did not state the distance these pine forests were from riparian areas. Pine edges on Spring Island that are less than 130 m from riparian areas and water bodies were not monitored for the Spring Island study. Some bats may utilize one habitat type for foraging and another type for roosting (Altringham. 1996). Seminole bats (*L. seminolus*) roost during winter months in trees with Spanish moss (*Tillandsia usneoides*) and under rocks, but during warmer months, roost on pine trees (Menzel et a. 1998 and 1999, Perry. 2007). Smaller bats like *P. subflavus* and *Myotis lucifugus* produced very few of the recorded calls for this study. Recordings from these species were sporadic and infrequent among both edge habitats, and captures on the island were also sporadic and infrequent. The few captures and recorded calls of *P. subflavus* and *M.* lucifugus were expected since these bats are clutter-adapted species and avoid open air spaces for foraging.

Spring Island recorded calls may have been distorted or attenuated due to thermal convection currents that result from falling temperatures and relative humidity through the progression of day to night (Griffin et al. 1960. Lacki 1984). It also should be noted that bats, particularly those in the southeastern United States, exhibit plasticity in their calls, both between and among individuals and species.

Insect Abundance

The diversity of insect prey (as per taxonomic order) captured for this study was similar to that found in other locations where bat activity is great (Anthony and Kunz 1977, P.W. Freeman 1979, Carter et al. 2003). The insect orders that represented the greatest relative biomass along the edges were: Coleoptera, Lepidoptera, Homoptera, and Hemiptera. All of these insect orders were collected on each night at each site. Therefore, prey was available within each habitat edge. The results are similar to those of Carter et al. (2004), which found that the above orders exhibited the highest percentages of occurrence in the dietary composition of *L. borealis*, *L.seminolus*, and *N. humeralis*. The order Coleoptera (beetles) represented the taxonomic order with the highest collected biomass. Coleopterans are important prey items to *E. fuscus* (Keeler and Studier, 1992, Hamilton and Barclay, 1998, Agosta and Morton, 2003). The diets of *T. brasiliensis* and *L. seminolus* are also largely comprised of coleopteran and lepidopteran prey. Coleopteran biomass constituted 58 – 100% of *E. fuscus* stomach contents (Kunz et al. 1995, Carter et al. 2004). There was no difference in the relative flying insect biomass between edge types, although foraging activity of insectivorous bats has been related to the abundance and distribution of prey (Kunz, 1973). Bat activity was not dependent upon insect dry mass. Insect dry mass was highly variable throughout the sampling season, and between and among edge sites. Mean bat calls were not significantly different between edge types, but the number of bat calls was highly variable throughout the sampling season, and between and among sites also. Barring a few artifacts (pine edge 5 and hardwood edge F) insect biomass was relatively similar across edge types among sites; therefore, there may not have been significant variation between the sites to allow detection of a relationship between bat calls and insect biomass. Likewise, insect biomass may have been sufficiently high at all sites to support high bat activity, as observed in this study.

This project was conducted during a period of drought along the LCP, as well as much of the Southeast. Drought conditions may have affected the abundance of emergent insects and therefore, the availability and abundance of insect prey. Insects preserved by freezing and then oven-dried and weighed may provide biased estimates of dry mass due to loss, fragmentation, and other damage (Leuven et al 1985).

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Table 1. The total number of SonoBat reference view sonograms by individual species found on Spring Island, SC.

Species	SonoBat Reference Sonograms
Eptesicus fuscus	71
Tadarida brasiliensis	62
Lasiurus seminolus	6
Nycticeius humeralis	10
Perimyotis subflavus	13
Myotis lucifugus	34

Table 2. Bat call ratio parameter means by species.

Species	High Frequency/Slope	Low Frequency/Slope	Low Frequency/Duration
E. fuscus	12.9	6.4	4.7
T. brasiliensis	51.1	40.7	2.5
L. seminolus	29.6	22.5	6.0
N. humeralis	18.0	12.4	7.6
P. subflavus	38.7	31.4	6.0
M. lucifugus	10.3	4.8	8.6

Table 3. Mean high, low, and slope values by bat species.

Species	High Frequency (kHz)	Low Frequency (kHz)	Slope
E. fuscus	58.9	27.6	5.3
T. brasiliensis	42.7	25.5	1.7
L. seminolus	61.6	40.9	3.3
N. humeralis	66.1	38.2	5.7
P. subflavus	61.4	42.2	2.8
M. lucifugus	92.8	41.2	10.6

Table 4. Site specific data by edge habitat type. Dimensions of the edges examined in this study. Area is based on data collected using a handheld Trimble GPS.

Pine				
Site	**Length (m)**	**Area (m2)**	**Bat Calls**	**Insect Dry Mass (g)**
1	310	13732	490	15.73
2	227	7236	601	23.29
3	186	6271	223	6.35
4	175	6673	505	13.76
5	391	49043	276	83.42
6	598	12022	311	20.15
7	734	11035	156	26.75
8	289	9328	399	24.05
9	117	7393	377	13.59
10	319	12519	394	19.63
11	218	7039	130	38.25
Total	**3564**	**142291**	**3862**	**284.97**

Hardwood				
Site	**Length (m)**	**Area (m2)**	**Bat Calls**	**Insect Dry Mass (g)**
A	159	7981	393	18.58
B	142	10229	452	10.59
C	175	6673	582	15.70
D	97	6378	531	5.17
E	172	5741	416	20.78
F	171	7425	235	77.97
G	99	2228	235	8.47
H	345	20610	130	49.42
I	86	4999	112	11.81
J	194	6179	112	37.97
K	232	10302	587	19.94
Total	**1872**	**88745**	**3785**	**276.4**

Table 5. Bats captured on Spring Island, SC.

Bat Species	Number of individuals captured
Eptesicus fuscus	26
Tadarida brasiliensis	>100*
Lasiurus seminolus	14
Nyticeius humeralis	5
Perimyotis subflavus	2
Myotis lucifigus	3
Total	**50**

*Bats were captured using a harp trap suspended adjacent to a known roost.

Table 6. Bat species found in Beaufort County and on Spring Island, SC.

Species	Beaufort County	Spring Island
Corynorhinus rafinesqui	X	
Eptesicus fuscus	X	X
Lasiurus borealis	X	
Lasiurus cinereus	X	
Lasiurus intermedius	X	
Lasiurus seminolus	X	X
Myotis austroriparius	X	
Myotis lucifugus	X	X
Nycticeius humeralis	X	X
Perimyotis subflavus		X
Tadarida brasiliensis	X	X

Table 7. Independent t-test significance values of species specific bat calls between edge habitat types.

Species	T	d.f.	Significance
Eptesicus fuscus	-0.277	1, 159	0.976
Tadarida brasiliensis	0.849	1, 159	0.397
Lasiurus seminolus	-0.685	1, 159	0.494
Nycticeius humeralis	-0.775	1, 159	0.439
Perimyotis subflavus	-1.503	1, 159	0.135
Myotis lucifugus	-1.647	1, 159	0.102
Unidentifiable	-1.544	1, 159	0.125

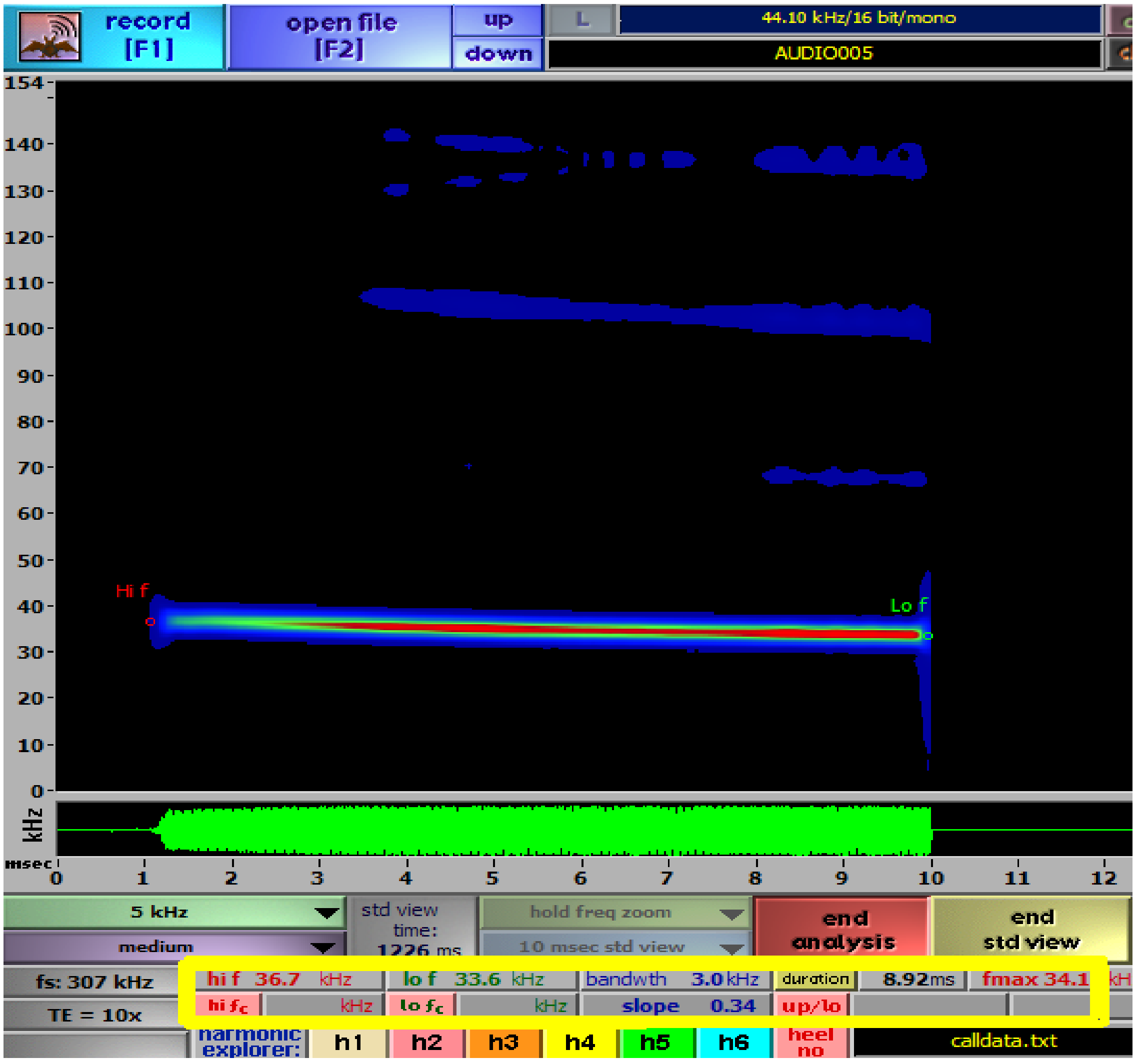

Figure 1. SonoBat sonogram with the "analysis" function turned on.
Call characteristic features are outlined by the yellow box.

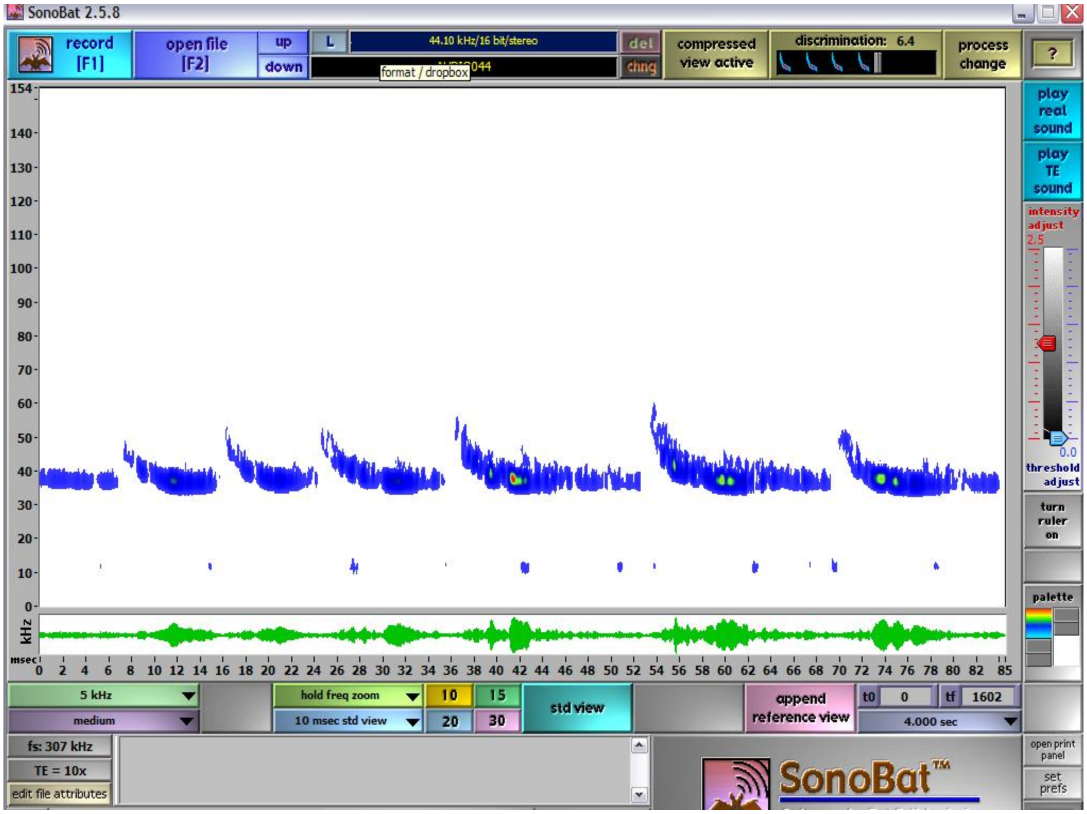

Figure 2. A sonogram of a bat call that has been distorted due to interference.

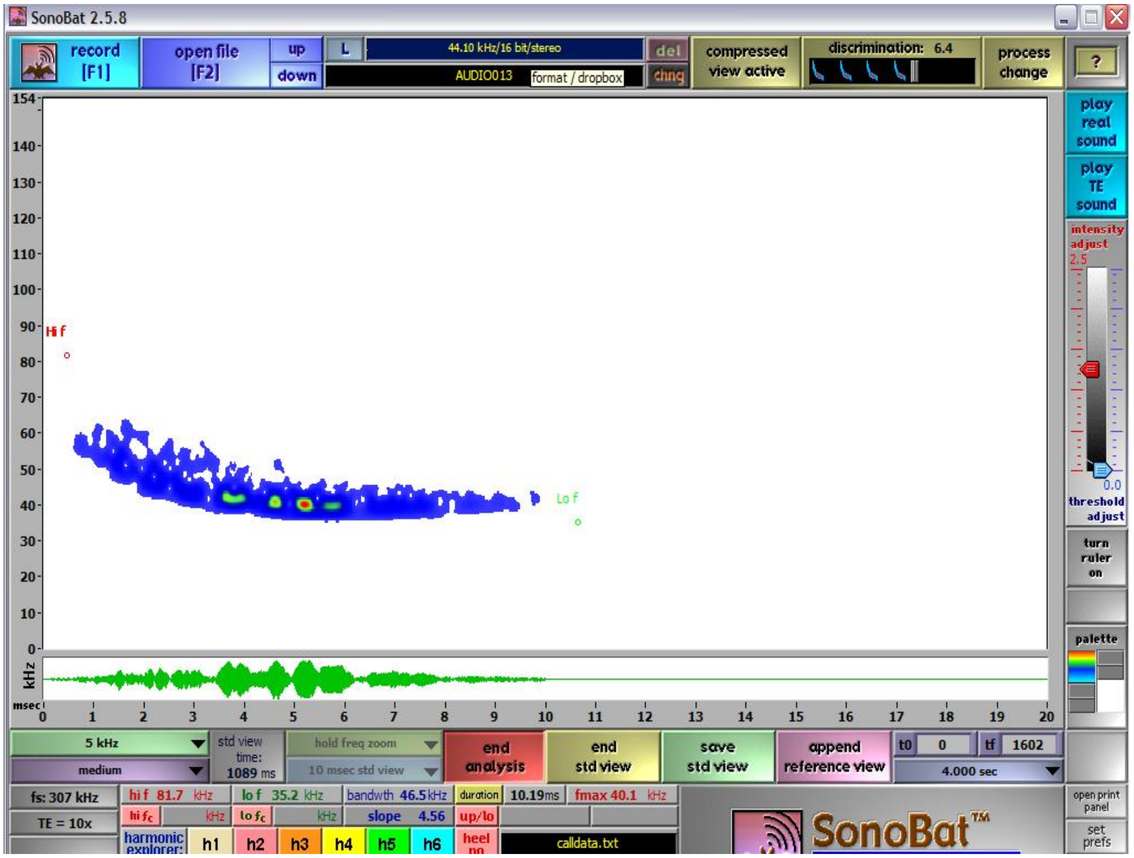

Figure 3. A sonogram with incorrect high and low frequency points.

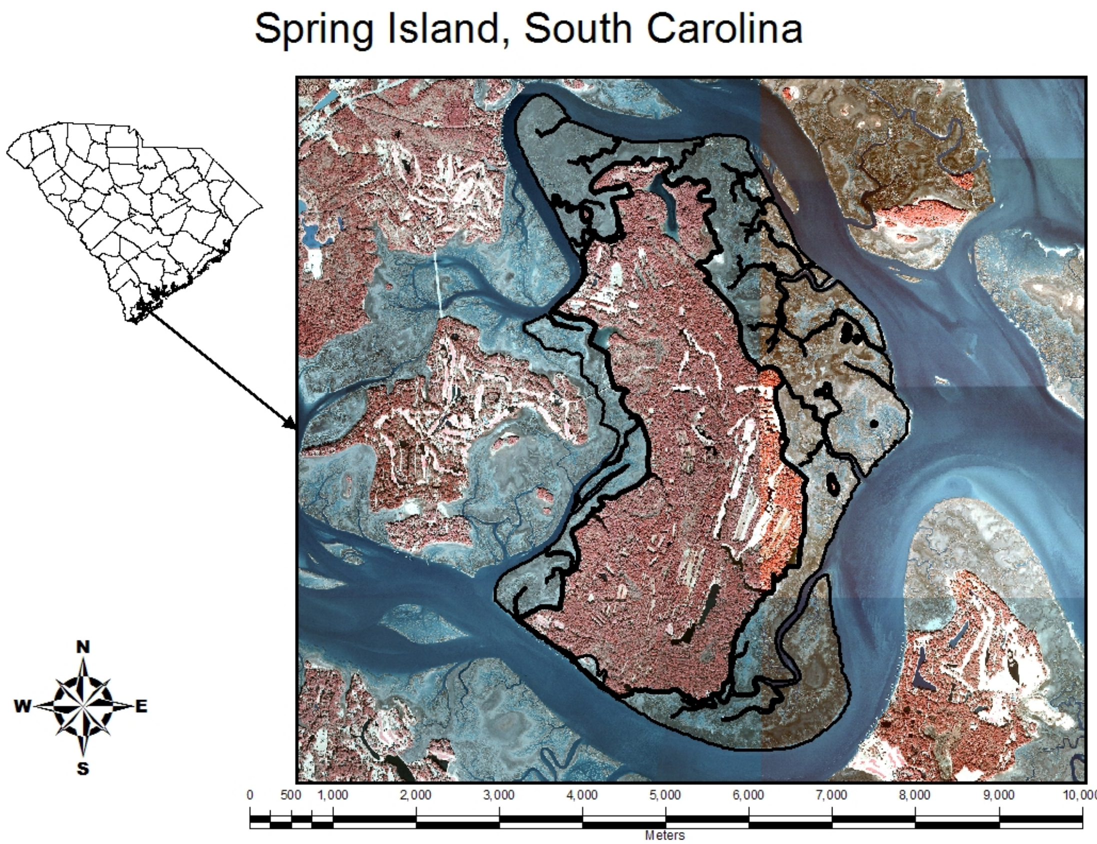

Figure 4. Digital aerial photograph (SCDNR 2006) ortho-rectified of Spring Island, Beaufort Co. SC (Lat/Lon: 32.3° N 80.9° W) bordered by the Chechessee and Colleton Rivers. Black and white map at left shows the position of Spring Island within the state of South Carolina. Image produced by G.A. Wood.

Figure 5. Digital aerial photograph of Spring Island, SC with marked locations of monitored edge sites. Pine edge sites are marked by Arabic numerals. Hardwood edge sites are marked by letters.

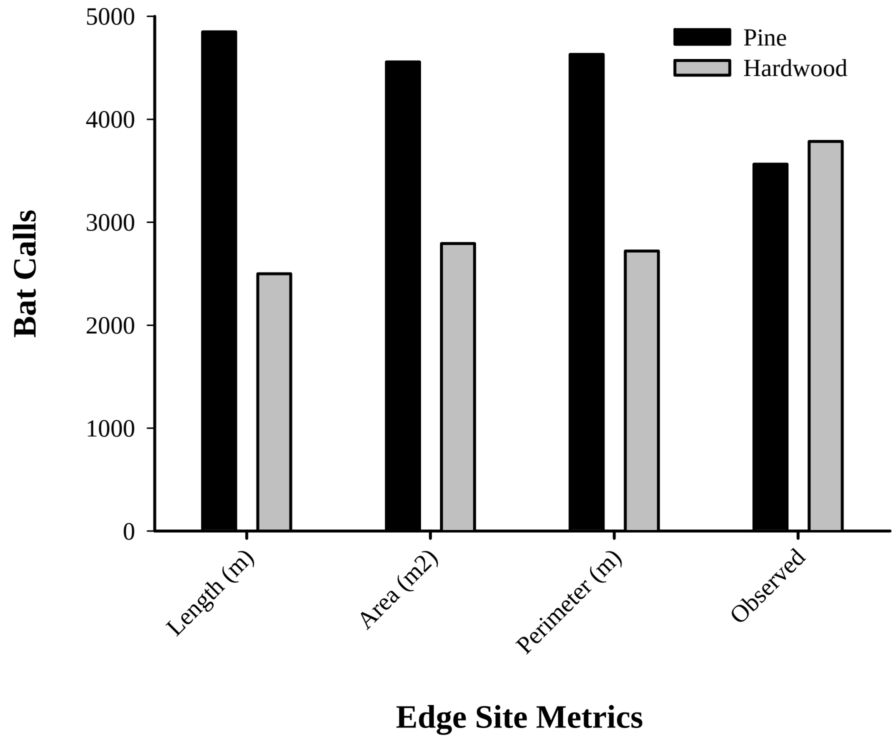

Figure 6. Expected calls based on total edge length (m), field area (m^2), field perimeter (m), and the total observed calls recorded per edge type.

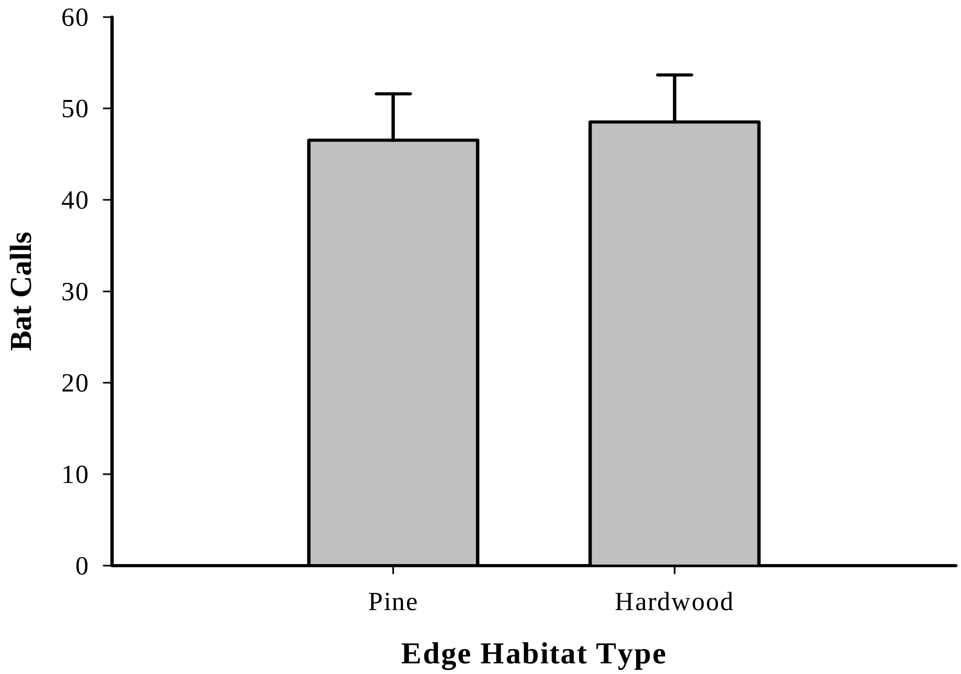

Figure 7. Mean (+ SE) bat calls per night by edge habitat type.

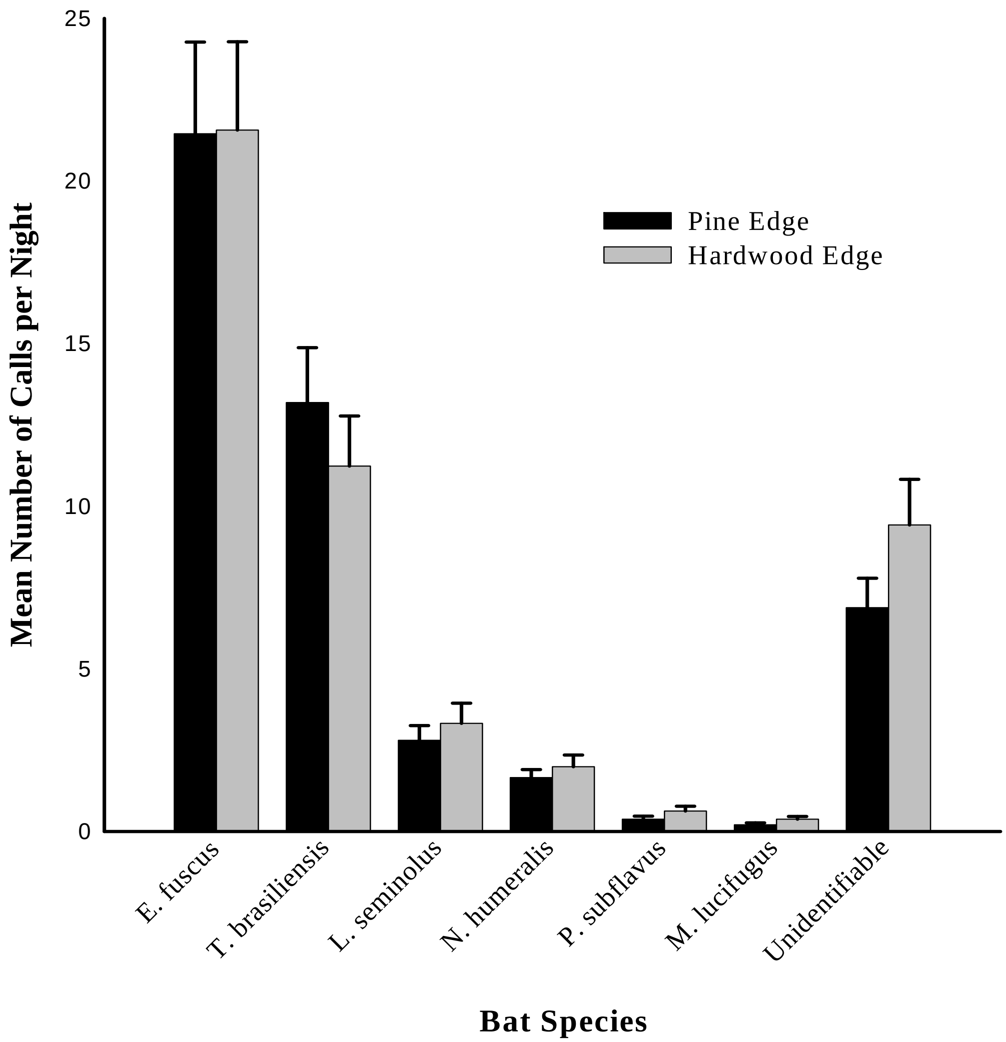

Figure 8. Mean (+ SE) calls per night by bat species.

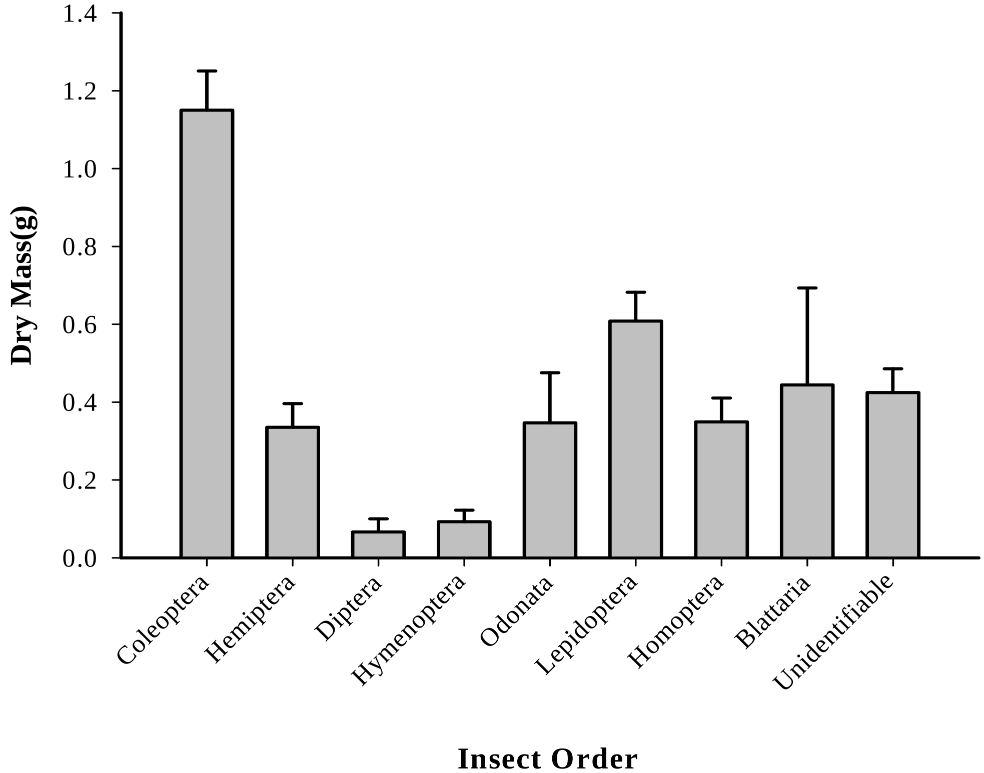

Figure 9. Mean (+ SE) insect dry mass (g) per night by taxonomic orders of captured, flying insects.

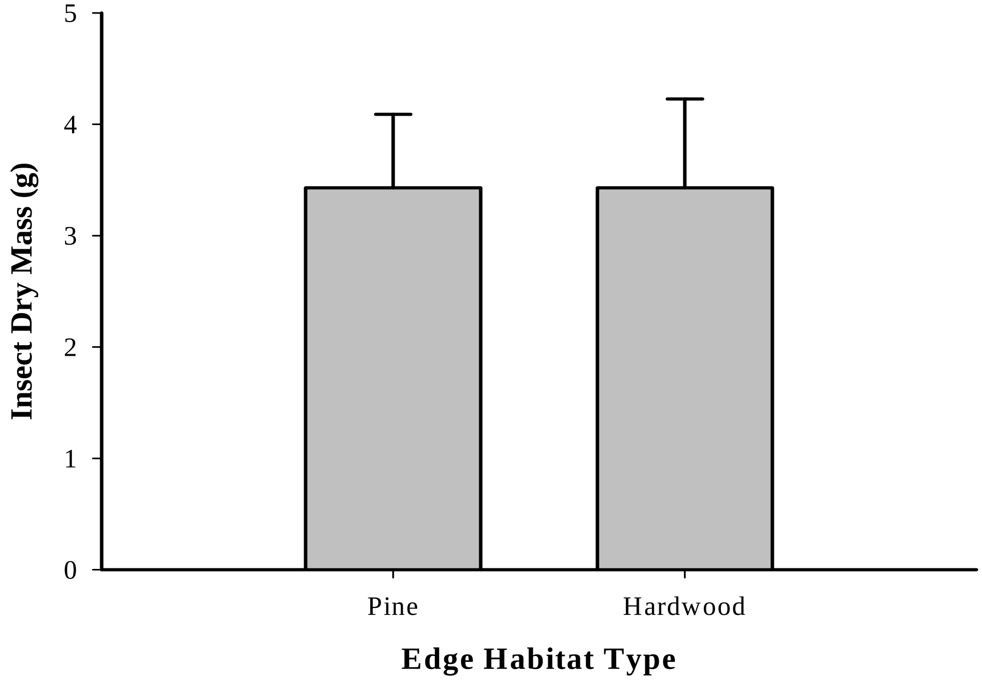

Figure 10. Mean (+ SE) insect dry mass (g) per night by edge habitat type.