GEOGRAPHIC AND SEASONAL VARIATION OF FLYING SQUIRREL VOCALIZATIONS IN CALIFORNIA

By

Travis A. Farwell

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Committee Membership
Dr. Barbara A. Clucas, Committee Chair
Dr. Micaela S. Gunther, Committee Member
Dr. Nicholas J. Kerhoulas, Committee Member
Dr. Joseph M. Szewczak, Committee Member
Dr. Erin Kelly, Graduate Coordinator

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ABSTRACT

GEOGRAPHIC AND SEASONAL VARIATION OF FLYING SQUIRREL VOCALIZATIONS IN CALIFORNIA

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Advancements in bioacoustics field studies have further elucidated spatial, temporal, and behavioral aspects of otherwise-cryptic species, as well as offering insights into species communication. The discovery of high-frequency vocalizations in North American flying squirrels in particular has allowed researchers to use ultrasonic acoustic recorders to detect these cryptic species in the wild. Investigations into vocalizations of northern flying squirrels (*Glaucomys sabrinus*) and southern flying squirrels (*G. volans*) have highlighted call type variation between species and the potential use of recorders as a tool to examine vocal activity patterns. However, high-frequency vocalizations had yet to be quantitatively analyzed for the recently discovered Humboldt's flying squirrel (*G. oregonensis*). Using ultrasonic acoustic recorders, I recorded flying squirrels in Humboldt and San Bernardino counties, California to: 1) measure and compare call properties and call types of Humboldt's flying squirrels and the San Bernardino subspecies (*G. o. californicus*) and test for geographic variation, 2) determine the extent of seasonal variation that exists between call type rates, and 3) test for seasonal differences in their nightly vocal activity patterns within and between counties. I hypothesized that variation in call properties may exist between Humboldt's flying squirrel and the San Bernardino subspecies, and that nightly call rates and vocal activity patterns would differ between summer and winter. I collected over 27,000 calls from 2018-2021 from different areas in Humboldt and San Bernardino counties and identified four previously described call types in flying squirrels: arcs, upsweeps, chirps, and trills. I found significant geographic variation in arc and trill call types when comparing call properties between counties, but these call properties were not significantly different across areas within each county. Additionally, I found that vocal activity patterns were similar between seasons within each county as well as between counties, but I found significant differences in the timing of call types across seasons within both counties. I also demonstrate the usefulness of ultrasonic recorders as a minimally-invasive tool for surveying for flying squirrels and their potential for assisting in future investigations into the behavior, ecology, and conservation of these enigmatic, forest-dwelling species.

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INTRODUCTION

The field of terrestrial bioacoustics has grown significantly in the past few decades. Technological advances have allowed researchers to utilize a variety of commercially available acoustic recorders as a minimally-invasive technique for investigating various ecological aspects of sound-producing taxa (Gaunt et al. 2005; Laiolo 2010; Aide et al. 2013). Acoustic studies have further elucidated spatial, temporal, and behavioral aspects of otherwise-cryptic species, offering insights into species communication that may be attained using passive, automated systems (Gilbert et al. 1994; Young 2003; Hartwig 2005; Laiolo 2010; Blumstein et al. 2011; Zwart et al. 2014; Buxton et al. 2016; Vallee 2018). Acoustic detectors may be deployed for weeks at a time, endure extreme climatic conditions, and have the ability to record ultrasonic frequencies (≥ 20 kHz) that exceed the threshold of human hearing (Sales and Pye 1974; Pye and Langbauer 1998; Fristrup and Mennitt 2012; Browning et al. 2017). The capacity to record ultrasonic-frequency vocalizations (USVs) enables detection of species utilizing this cryptic form of communication while implementing minimally-invasive survey techniques.

Mammalian USVs were first described in bats (*Myotis lucifugus* and *Eptesicus fuscus*) in the 1930s (Pierce and Griffin 1938) and a decade later in captive dolphins (*Tursiops truncatus*; McBride and Hebb 1948) and bank voles (*Myodes glareolus*; Schleidt 1948). While research into mammalian USVs was historically focused on bats and toothed cetaceans, relatively recent studies have explored ultrasonic calls of other mammals (see Kalcounis-Rueppell et al. 2010). USVs are well known to facilitate echolocation, however numerous studies have demonstrated that these calls may have additional functions. Technological advances in recent years have promoted further research and though contemporary studies have shed more light on mammalian USVs (e.g., Ter-Mikaelian et al. 2012; Kessler et al. 2012; Musolf et al. 2015; Panyutina et al. 2017; Rieger and Marler 2018), the extent of taxa exhibiting these types of vocalizations remains unknown. Moreover, unlike the audible vocalizations of birds (e.g., Mundinger 1982; Baker and Cunningham 1985) and mammals (Lameira et al. 2010), few studies have focused on the potential for geographic variation in USVs in terrestrial mammals with the exception of bats.

Rodents represent a significant proportion of known mammals that are capable of USVs and are mostly believed to use these calls for intraspecific communication. Anderson (1954) first described the ultrasonic vocalizations of Norway rats *(Rattus norvegicus*) in laboratory experiments, which prompted research focused on other rodents potentially utilizing USVs. Studies in the mid-20th century postulated the use of echolocation by certain rodents (Kahmann and Ostermann 1951; Anderson 1954; Rosenzweig et al. 1955), but contemporary research has since demonstrated several other behavioral applications of USVs (Kalcounis-Rueppell et al. 2010; Pultorak et al. 2017; Gilley et al. 2019). Current understanding of rodent USVs is mainly derived from studies focused on Muridae (Sales 1972; Maggio and Whitney 1985; Brudzynski and Ociepa 1992; Holy and Guo 2005; Portfors 2007; Kalcounis-Rueppell et al. 2010) and Cricetidae

(Rabon et al. 2001; Kalcounis-Rueppell et al. 2006; Miller and Engstrom 2007; Takahashi et al. 2010; Petric and Kalcounis-Rueppell 2010; Ferhat et al 2016), with relatively fewer studies focused on Sciuridae (Eiler and Banack 2004; Wilson and Hare 2006; Gilley et al. 2019). In general, known functions of rodent vocalizations include predatory alarm calls (Slobodchikoff et al. 1998; Matrosova et al. 2007), aggression displays (Kapusta et al. 2007), territorial defense (Rieger and Marler 2018), kin recognition (Rabon et al. 2001; Mandelli and Sales 2004; Blake and Hayes 2012), courtship behavior (Hoffmann et al. 2012; Pultorak et al. 2017), and recently-discovered arboreal echolocation in a species of Vietnamese dormouse (*Typhlomys chapensis*) (Panyutina et al. 2017).

Among the rodents known to produce USVs, sciurids have been found to use alarm calls extending into the ultrasonic range (Wilson and Hare 2006). Richardson’s ground squirrels (*Urocitellus richardsonii*) produce and react to USVs among conspecifics for predator avoidance and vigilance (Wilson and Hare 2006). Further, golden-mantled ground squirrels (*Callospermophilus lateralis* and *C. saturatus*) were discovered to use USVs for predator-specific alarm calls that vary based on spatial distribution, showing patterns consistent with dialects (Eiler and Banack 2004). These studies demonstrate the importance of cryptic communication in certain sciurids and highlight a deficit of studies focused on describing and interpreting their vocal behavior.

The production of USVs by North American flying squirrels (*Glaucomys* spp.) had been suspected since the mid-late 20th century (Muul and Alley 1963, Muul 1970, Wells-Gosling and Heaney 1984; Saunders 1988), but was not quantitatively investigated prior to 2013 (Gilley 2013; Murrant et al. 2013). While echolocation was hypothesized due to the arboreal, gliding, nocturnal behavior of these sciurids, evidence has not supported this premise and instead favors a communicative application (Chattin 1969). Gilley (2013) discovered that captive northern flying squirrels (*G. sabrinus*) and southern flying squirrels (*G. volans*) produce multiple types of USVs, classifying 5 distinct call types, defined as syllables, based on temporal and acoustic parameters. Additionally, 3 distinct call types produced by captive and wild northern and southern flying squirrels were described in Ontario, Canada (Murrant et al. 2013), with one call type found to be similar to Gilley (2013). Similar work was conducted on southern flying squirrels in Indiana to examine geographic variation, identifying 11 distinct call types and finding 7 to be unlike previously described vocalizations (Eisinger et al. 2016). Most recently, Gilley et al. (2019) described 10 distinct call types produced by northern flying squirrels and 27 call types by southern flying squirrels (28 call types in total), which represents the most robust study to date. These studies highlight the diverse ultrasonic vocal repertoire of flying squirrels in North America, further indicating potential communicative significance and suggesting geographic and species-specific variation in call types.

USVs in northern and southern flying squirrels allow researchers to use ultrasonic acoustic recorders that are designed for detecting echolocating bats to establish presence/absence of wild populations of flying squirrels in North America (Diggins et al. 2016; Gilley et al. 2019). In addition to being able to detect flying squirrels and other species that produce ultrasonic sounds, the timing of calls recorded by ultrasonic recorders can also be useful in understanding flying squirrel behavior, such as when they are active throughout the night. Camera traps are the traditional non-invasive method used to determine activity patterns of mammals (Bridges and Noss 2011; Buxton et al. 2016; Caravaggi et al. 2018); however, acoustic recorders may increase detection and thus accuracy of activity patterns given that they do not rely on the individual to pass directly in front of the recorder. Nevertheless, there are limited data on the efficacy of acoustic recorders to investigate activity patterns in flying squirrel species (see Diggins et al. 2016).

Mammalian circadian activity patterns, which are a facet of behavior and defined as the movements of an animal or its body parts (Bridges and Noss 2011), are generally categorized as diurnal, nocturnal, crepuscular, and cathemeral (Bennie et al. 2014). Variability in the circadian activity of Sciurids is influenced by evolutionary pressures of both biotic and abiotic conditions (Parker et al. 2014; Jastroch et al. 2016; Wassmer and Refinetti 2016; Schweiger and Frey 2021), and temperature and climatic conditions have been found to influence the nightly activity patterns of flying squirrels (DeCoursey 1961; Steinhoff et al. 2012; Suzuiki and Ando 2017). While North American flying squirrel species' activity is lower during colder months, they are not known to hibernate (Wells-Gossling and Heaney 1984). Both northern and southern flying squirrels have been found to spend a shorter amount of time away from the nest in colder, harsher climatic conditions than in warmer, milder conditions (Cotton and Parker 2000; Vernes 2004; Nelson and Sagot 2018).

As the thermoregulatory needs of smaller mammals are higher than of larger animals (Carbone et al. 2005; Swingle and Foreman 2009; Blake et al. 2012; Ikeda et al. 2016), shifts in temperature and climate have a significant influence on the activity patterns of flying squirrels. North American flying squirrel species occupy regions where weather patterns and temperatures can be vastly different, and we would expect to be able to detect plasticity across activity patterns in flying squirrels that occupy areas with these pronounced climatic differences. Establishing a baseline of the activity patterns of flying squirrels is fundamental for understanding the degree to which climate change (Schweiger and Frey 2021) and human disturbance (Clark et al. 2021) may affect their behavior. Furthermore, gaining insight into the activity patterns of different species and subspecies of flying squirrels is essential to understanding their behavior and natural history, and the timing of vocalizations, in both a nightly and seasonal context, may be used to elucidate the behavioral significance of this cryptic behavior.

Recently, northern flying squirrels ranging from southern British Columbia to central and southern California were found to be a distinct species based on molecular differences (Arbogast et al. 2017). Reclassified as Humboldt's flying squirrel (*G. oregonensis*), this taxonomic revision also affects the 5 subspecies within the range of this new species, which include the San Bernardino subspecies, previously known as *G. sabrinus californicus* and now determined *G. oregonensis californicus* (Wells-Gossling and Heaney 1984; Arbogast et al. 2017). USVs of Humboldt's flying squirrels and San Bernardino flying squirrels have not been analyzed or compared, and little information is available regarding their activity patterns. Seasonal variation in the activity patterns of

Humboldt's flying squirrels has been suggested by preliminary camera trap studies (Clucas and Atkins 2022); however, it has not been measured using ultrasonic recorders. Furthermore, the extent of activity pattern variation in San Bernardino flying squirrels remains unknown but may be more distinct than other populations, as they live in an area with greater temperature shifts than populations of Humboldt's flying squirrel living along the northern coast of California (potential subspecies *G. o. stephensi*, Wells-Gossling and Heaney 1984, hereinafter referred to as "Humboldt's flying squirrel"). These species are also potential candidates for geographic variation in the acoustic properties of call types and activity patterns. The limited extent of their range and the genetic uniqueness of San Bernardino flying squirrels (Yuan et al. 2022) offers an idiomatic insight into species-subspecies call type variation and differences in activity patterns in an area where little is known regarding the behavior and ecology of two distinctly separated populations.

To understand the extent of vocal variation between flying squirrels living in different regions of California, as well as the activity patterns of these flying squirrels, I used ultrasonic acoustic recordings to: 1) describe the acoustic properties of their vocalizations and test for geographic variation between the counties, 2) identify specific call types and determine if there are seasonal differences in the call type rates, and 3) test for seasonal differences in general vocal activity patterns within and between counties. I compared the degree of variation in call types, call properties, and timing of vocal activity between the flying squirrels living in northwestern California (Humboldt County) and Southern California (San Bernardino County) across multiple seasons. Based on the bird vocalization literature on geographic variation, I hypothesized that variation in call properties may exist between Humboldt's flying squirrel and the San Bernardino subspecies due to either the historic separation of San Bernardino populations or the different environmental conditions (or both), but that call types will be similar between regions. I predicted that a higher degree of variation exists between flying squirrels living in different regions of California than across flying squirrels living in the same region, and that I would find the same call types across all areas. Additionally, if call rates of different call types varied seasonally, this variation might provide insight into the functions of the different call types (e.g., mating calls may be more prominent in only particular times of the year). Finally, with regards to vocal activity patterns, I hypothesized that they would vary seasonally due to climatic conditions due to thermoregulatory needs while also fulfilling metabolic needs. I predicted that flying squirrels would be active for longer periods of time in warmer months, and that vocal activity patterns would differ between the more temperate Humboldt County and San Bernardino County, which has greater shifts in temperature (i.e., colder winters).

METHODS

Study Area

Humboldt County

I conducted a portion of this study in three areas of Humboldt County, situated in northwestern California, part of the Humboldt's flying squirrel range. The climate of Humboldt County's coast is defined by moderate temperatures that seldom go below freezing in the winter and rarely exceed 25° C during the summer months, averaging between 15-20° C and an average annual rainfall of approximately 115 cm, supporting ancient coast redwood (*Sequoia sempervirens*) forests in the remaining unlogged patches (McFarland et al. 2003; NOAA 2005).

The first area I surveyed was in Headwaters Forest Reserve (HFR) near Fortuna, California, USA (407909.24 m E, 4497336.26 m N 10T) (Figure 1), with 8 sites with squirrel detections (Table 1; Figure 1) surveyed between July 2018 and July 2020. HFR is managed by the Bureau of Land Management and the California Department of Fish and Wildlife, and contains patches of old-growth and second-growth redwood forest. I surveyed in old-growth patches that were situated ~200-500 m in elevation, with the sites closest together at ~200 m apart from each other and the sites furthest from each other at ~1,900 m apart. Sites were situated in the southwestern portion of the reserve, an area closed to the public with daytime logging operations conducted adjacent to the study area.

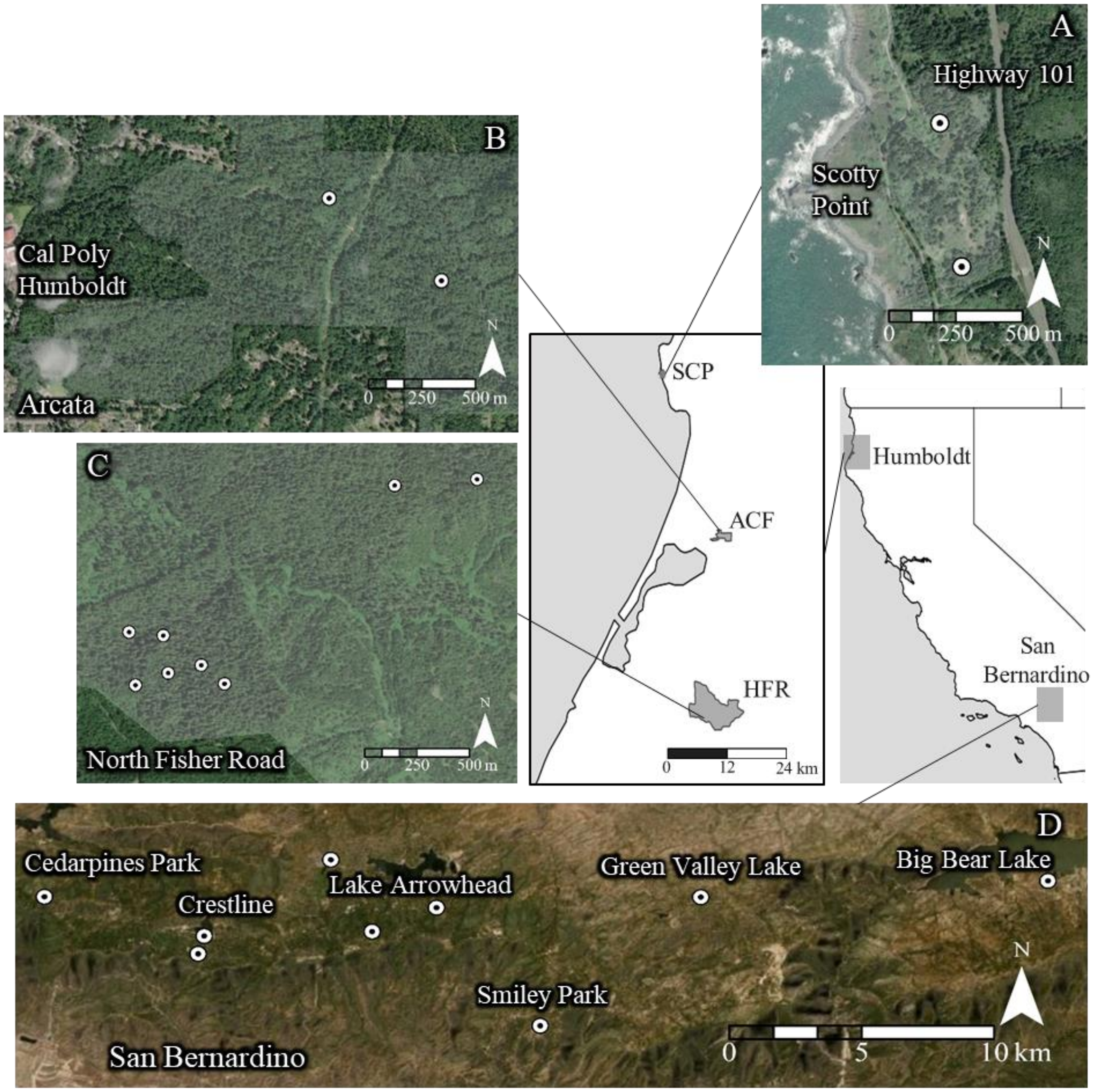

Figure 1. Sites (white circles) surveyed in two counties of California, USA from 2018-2021. Areas surveyed in Humboldt County: Seawood Cape Preserve (SCP; A), Arcata Community Forest (ACF; B), and Headwaters Forest Preserve (HFR; C). Shaded regions on the color map insets indicate areas of forest or preserve. Areas surveyed in San Bernardino County (Big Bear Lake [BBL], Green Valley Lake [GVL], Smiley Park [SP], Lake Arrowhead [LA], Crestline [CL], and Cedarpines Park [CP]; D). Source: Esri, Maxar, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community.

The second area I surveyed was 70 km north of HFR in the Arcata Community Forest (ACF), Arcata, California, USA (410922 m E, 4525527 m N 10T) (Figure 1). This forest contains a mix of second growth stands of coast redwoods and Douglas firs (*Pseudotsuga menziesii*) which were clear cut 20-30 years ago or thinned ≤ 10 years (COA 2016). I included 2 sites with flying squirrel detections in this area, one site in February 2019 and one site in March 2020. The sites were situated ~ 640 m apart from each other and situated at ~150-300 m in elevation and 20-100 m away from established trails but the forest was open to the public and therefore susceptible to a higher degree of human disturbance as compared to HFR and SCP.

The third area I surveyed was 25 km north of ACF at Seawood Cape Preserve (SCP), Trinidad, California, USA (402863 m E, 4550294 N 10T) (Figure 1), which consists of second growth stands. This preserve is managed by the Wildlands Conservancy, a nonprofit public benefit corporation. SCP lies on the coast of the Pacific Ocean, with a forest composition similar to ACF and HFR. Though the preserve stretches from Highway 101 to a rocky coastline, the 2 sites where I collected flying squirrel recordings were situated in a patch of forest west of the highway and east of Patrick's Point Drive, a main road that divides the preserve. The sites were located between 70-100 m above sea level and roughly 550 m from each other. These sites were closer to highway and road disturbance than sites in ACF and HFR, with site 5 situated within 100 m from Highway 101. Established trails are open to the public on the coastline west of Patrick's Point Drive but the sites were situated in the patch of forest east of this drive, which is closed to the public.

San Bernardino County

The San Bernardino flying squirrel subspecies is found in San Bernardino County, California. Sites were chosen using the iNaturalist (iNaturalist.org) database and Facebook community groups (Facebook.com) to identify posted observations of San Bernardino flying squirrels. I contacted landowners who had previously detected flying squirrels and set up recording stations on their properties. During the winter of 2018 and summer 2019, I surveyed one site in Lake Arrowhead (LAH) (484033.1m E, 3789402.9m N 11S) and one site in Crestline (CL) (474953.9m E, 3788053.3m N 11S) (Figure 1), which are situated ~9 km apart. I resurveyed these sites in the winter of 2019 and summer of 2020 and, in addition, surveyed two more sites in LAH, another site in CL, one site in Big Bear Lake (BBL) (507047.17 m E, 3789132.79 m N 11S), one site in Green Valley Lake (GVL) (493691.63 m E, 3789150.45 m N 11S), one site in Cedarpines Park (CP) (469451.14 m E, 3790757.24 m N 11S), and one site in Smiley Park (SP) (487595.92 m E, 3784308.98 m N 11S), for a total of 9 sites (Table 1; Figure 1).

Table 1. Sites surveyed in Humboldt (ACF, HFR, and SCP) and San Bernardino (BBL, CL, CP, GVL, LA, and SP) counties, California, USA from 2018-2021. Seasons surveyed for each location are specified as Winter (W) and Summer (S). Number of sites includes total number of sites where flying squirrel calls were collected (n = 22). See text for full site names.

Area	ACF	HFR	SCP	BBL	CL	CP	GVL	LA	SP
# of Sites	2	9	2	1	2	1	1	3	1
Season	W	WS	W	WS	WS	WS	WS	WS	WS

Site elevations in this region ranged from approximately 1,500 m above sea level (SP) to 2,500 m above sea level (BBL). Sites in San Bernardino were situated at significantly higher elevations than Humboldt County sites and experience moderately temperate climates with average annual rainfall of approximately 90 cm, annual snowfall averaging approximately 90 cm per year and temperatures ranging from -1° C in winter and 26° C in the summer (NOAA 2018). Though considered temperate, sites in this montane forest experiences more extreme shifts in temperature and climate than areas surveyed in Humboldt. The sites in San Bernardino were situated in forested residential neighborhoods and sites were often within 100 m of roads and neighboring houses, subjecting them to relatively higher levels of human disturbance. All sites in San Bernardino had bird feeders where flying squirrels had previously been observed feeding, so I considered them sites with "supplementary feeding".

Humboldt County sites were at significantly lower elevations as compared to the San Bernardino County sites and comprised of different floral and faunal species. While Humboldt County sites ACF and SCP were not dominantly comprised of old-growth redwoods, the floral makeup of these three areas is similar, characterized by coast redwoods and Douglas firs as the dominant tree species and huckleberry (*Vaccinium* spp.), salal (*Gaultheria shallon*), and sword ferns (*Polystichum munitum*) comprising the majority of understory vegetation (McFarland et al. 2003). Humboldt's flying squirrels had previously been detected in both ACF and HFR (Clucas and Atkins 2022; Clucas et al., unpublished), but had yet to be detected in SCP. In contrast, San Bernardino sites were largely characterized by mixed-conifer forest and dominated by Jeffery pines (*Pinus jeffreyi*) and white firs (*Abies concolor*), along with areas containing black oaks (*Quercus kelloggii*) and cultivated species at residences (Butler et al. 1991). In addition to the contrasting flora between Humboldt and San Bernardino, the above-mentioned differences in the degrees of human disturbance might influence the flying squirrel’s behavior (Carey 2000; Pyare and Longland 2001).

Data Collection

I recorded wild flying squirrel calls using Wildlife Acoustics SM4BAT-FS full-spectrum ultrasonic recorders (hereafter SM4; Wildlife Acoustics Inc., Maynard, MA, USA). SM4s are designed for acoustic bat surveys and are equally effective at detecting other USV-producing species. The SM4 recorders were set to a 500 kHz sample rate, at 12 decibels (dB) gain, using a 16 kHz analog high-pass filter to eliminate unwanted noise signals. Although lower sample rates conserve battery power, higher frequency calls require a higher sample rate to maximize quality (Abrahams 2018). Minimum trigger frequency and level were set to 12 kHz and 12 dB, respectively. Recordings were set for a minimum duration of 1.5 milliseconds (ms) and no maximum duration to record entirety of phrases (bouts of a single call type). Trigger sensitivity was set to high and the minimum time interval was set to 0 to capture a succession of calls in its entirety. I saved all call files as .wav files and did not compress them to preserve audio quality. Each recorder was scheduled to automatically turn on 1-2 hours before and after sunrise and sunset each night, respectively, and was deployed for a duration of 1-2 weeks per site. The recorder settings were adopted from the SM4 User Guide (Song Meter SM4 FS User Guide 2018, Wildlife Acoustics Inc., Maynard, MA, USA).

I baited all sites using peanut butter suet cakes (Peanut Delight, C&S Products, Iowa, USA) nailed to a tree approximately 2.5 m from the base of the trunk, with the SM4 and a Bushnell HD infrared camera (Model # 119836, Bushnell Corporation, Overland Park, Kansas, USA) placed on an adjacent tree roughly 3 m from the bait. I used camera trap data to supplement identification of flying squirrel calls by comparing the time of camera detection with the recording timestamp. Cameras were set to take 3 photos per trigger at 12M pixels with a 3 second interval at a normal sensor level. The recorder was locked to the trunks of trees and microphones were attached at approximately 2.5 m above the ground and approximately 1.5 m away from the trunk of the tree, facing upwards. To decrease the probability of recording the same individuals across study areas, sites were situated at least 500 m apart during each recording event (Lehmkuhl et al. 2006; Wilson et al 2008). SCP had only two sites that were ≥ 500m apart, so only these two sites were considered independent of each other. In San Bernardino, sites were situated at residences and bait was replenished by landowners when possible. Methods were in accordance with the Animal Welfare Act and were approved by the Cal Poly Humboldt Institutional Animal Care and Use Committee (No. 17/18.W.54-A).

Call Analysis

Measuring Call Properties and Identifying Call Types

I sorted call files using SonoBat 4.4.1 (SonoBat, Inc., Arcata, California, USA) to determine the species. This software was developed to identify bat species and uses a spectrogram and oscillogram for visual representation of each call expressed as frequency (kHz) and time (ms). SonoBat does not have an automated identification for flying squirrel vocalizations so I used manual identification using previous descriptions of call types (Gilley 2013; Murrant et al. 2013; Eisinger et al. 2016; Gilley et al. 2019). I also used the camera trap detections paired with recorder time stamps to verify that flying squirrels were present in the area around the time calls were recorded. I first used the "Batch File Scrubber" in SonoBat DataWizard 4.4.1, a program used for preparing acoustic data for analysis, and used the "high grade" and "5 kHz and above" settings to sort out the noise files, and then sorted out the flying squirrel calls from the other species. I used Raven Pro 1.4 (Cornell Lab of Ornithology, Ithaca, New York) to measure the acoustic properties of each call in the call file. A total of 6 variables were measured and adopted from Gilley et al. (2019) using measurements extracted from the Raven Pro 1.4 User's Manual (Cornell Lab of Ornithology, Ithaca, New York), which included: call duration (ms), delta frequency (bandwidth) (kHz), high and low frequencies (kHz), maximum frequency (kHz), and average entropy (u) (Table 2.).

Table 2. Measurements extracted using Raven Pro 1.4 (Cornell Lab of Ornithology, Ithaca, New York) and adopted from Gilley et al. (2019). Call descriptions are adopted from the Raven Pro 1.4 User's Manual (2010).

Measurement	Description
Duration	The time from the start of the fundamental harmonic to the end
ΔF	Bandwidth or the difference between the high and low frequencies
LF	Lowest frequency in the fundamental harmonic
HF	Highest frequency in the fundamental harmonic
MaxF	Frequency at the highest amplitude in fundamental harmonic
AvgE	Average of entropy values calculated within a single call

Extraneous noise in recordings may enshroud calls and make identification impossible when the extraneous noise is at a higher amplitude than the call. Due to the variability in recording quality, I created a quality grade for each call analyzed. Calls that were faint and the start or end frequencies could not be determined on the spectrogram were considered “low-incomplete”, calls that were complete but faint (compared to the ambient noise) were considered “low”, calls that were complete and had a stronger fundamental harmonic with less ambient noise were considered “high-low”, and calls that had little-to-no ambient noise and clearly defined fundamental harmonics were considered “high”. Low and low-incomplete calls were not included in the call property analyses but were included in the temporal overlap analysis as the fine-scale acoustic properties were not crucial for establishing presence of a vocalizing flying squirrel.

As there is no standardized bioacoustic terminology (Lameira et al. 2010), I adopted phrasing from Gilley et al. (2019), which was partially derived from Holy and Guo (2005) and Kalcounis-Ruppell et al. (2006). A call is defined as any intentional sound vocalized by an animal. Call types are described as any visually and audibly distinct and repeated call (as viewed/listened to in a time-compressed mode 10x). A call represents a single vocalization bordered by an interval of silence. A group of ≥ 2 calls that are bordered by an interval of silence represents a call bout. Call types were identified and categorized based on previous research describing *Glaucomys* spp. vocalizations (Gilley et al. 2019; C. A. Diggins, Virginia Polytechnic Institute and State University, personal communication). For preliminary call type identification, I viewed calls using a Hann window with a time expansion factor of 10 in SonoBat. Call-bouts were inferred as emanating from a single individual based on the inter-call durations and consistent amplitude of each call within a call bout.

Determining Seasonal Vocal Activity Patterns

I extracted the timing of calls using Raven Pro 1.4 and separated these calls times by a duration of 30 minutes to form independent detection events (Higdon et al. 2019), with any additional files within the 30-minute interval excluded from the temporal analysis. As the recorder does not record individual calls separately but records a bout of calls in a single file, individual time stamps for a single call within a call file were used for analysis. Due to the different sampling efforts and situational differences between Humboldt and San Bernardino sites, vocal activity patterns were not estimated and compared between counties but were compared within each county. Seasonal differences, defined as either summer (June-August) or winter (November-February), were compared between each call type and between all call types.

Data Analyses

Call Properties and Call Types: Discriminant Function Analyses

To determine the degree of geographic variation in flying squirrel call properties, I compared the call types that were all previously described call types for North American flying squirrel species (Gilley 2013; Murrant et al. 2013; Eisinger et al. 2016; Gilley et al. 2019). To account for non-independence of the call data (i.e., it was not known if the same individual was vocalizing at a site), I used a nested permuted discriminant function analysis (pDFA), which accounts for potential pseudoreplication (Mundry and Sommer 2007). Prior to running the pDFA, I tested for collinearity between call properties using all calls and found that bandwidth was positively related to maximum frequency ($r = 0.96$, $n = 882$, $p = < 0.001$; Appendix A.1) in trills, so I removed bandwidth from the parameter list. I also checked each call parameter for outliers and compared median call property values by creating box-plots (Appendix A.2). I ran a DFA to determine which call properties explained variation in call types between counties and across areas and created canonical discriminate functions plots to visualize the differences. Data used in the DFA were validated using Eigenvalues (E) and Wilks' Lambda (W) values to calculate statistical significance, with minimum tolerance tests set to 0.001 to maintain independence of variables (adopted from Gilley 2013). I used RStudio 1.2 (RStudio Inc., Boston, Massachusetts, USA) to conduct the pDFA using code written and provided by R. Mundry (R. Mundry, Institute for Biology/Behavioral Biology, Free University of Berlin, personal communication) and based on the "MASS" package “Ida”. I also used SPSS to run the DFA and generate the canonical discriminate function plots (SPSS version 28.0., SPSS Inc., Chicago, Illinois, USA).

Seasonal Difference in Call Type Rates

I determined call type rates for each call type for both summer and winter by quantifying the number of calls per survey night for each site (i.e., dividing the total number of call by the total number of survey nights; Appendix B.1, Appendix B.2). I then conducted a paired t-test to determine if there were significant differences in call type rates between seasons for each call type in San Bernardino. I was not able to conduct these tests for Humboldt due to the low sample sizes for each call type in the summer data and not all areas having both summer and winter data (Table 1; Appendix B.3).

Geographic and Seasonal Variation in Vocal Activity Patterns: Temporal Overlap Analyses

I used the “overlap” package in R to estimate seasonal vocal activity patterns of flying squirrels and compared these patterns within and between counties and between call types (Ridout and Linkie 2009). The temporal overlap of vocal activity is the overlap coefficient ($\widehat{\Delta}$ or D-hat), which ranges from 0 (no temporal overlap) to 1 (complete temporal overlap). For smaller sample sizes ($n < 50$) I used the $\widehat{\Delta}_1$ estimator (1) as described in Ridout and Linkie (2009):

$$\widehat{\Delta}_1 = \int_0^1 \min\left\{\hat{f}(t), \hat{g}(t)\right\} \mathrm{d}t \tag{1}$$

This estimator uses $\widehat{\Delta}_1$ as the coefficient of overlap for smaller sample sizes, where function $\hat{f}(t)$ describes the vocal activity patterns of one population of squirrels, $\hat{g}(t)$ describes the vocal activity patterns of another population, and $\widehat{\Delta}_1$ describes the integral of overlap between these two populations. The overlap coefficient for sites with larger sample sizes ($n \geq 50$) was described by equation (2),

$$\widehat{\Delta}_4 = \frac{1}{2}\left(\frac{1}{n}\sum_{i=1}^{n} \min\left\{1, \frac{\hat{g}(x_i)}{\hat{f}(x_i)}\right\} + \frac{1}{m}\sum_{j=1}^{m} \min\left\{1, \frac{\hat{f}(y_j)}{\hat{g}(y_j)}\right\}\right), \tag{2}$$

where $\widehat{\Delta}_4$ describes the coefficient of overlap for populations with sample sizes n and m, x_i is the timing of vocal activity for the first population over i detections, and y_j is the timing of vocal activity for the second population over j detections. These overlap coefficients were used to generate density curves for each comparison, though only $\widehat{\Delta}_4$ was used in the final analyses (Weitzman 1970; Ridout and Linkie 2009). To determine vocal activity timing relative to sunrise and sunset, I used the sunTime function in “overlap”, which accounts for the shifting sunrise and sunset times (Nouvellet et al. 2012). To determine if there were significant differences between overlap of variables, I conducted a 2-sample Anderson-Darling (AD) test using the R package “kSamples” with $\alpha = 0.05$ (Scholz and Zhu 2019).

I calculated the overlap of all calls within both counties to test for seasonal differences in timing of calls. In addition, I calculated the overlap of timing of each call type separately between the two seasons (summer and winter) within each county using call type rates. Call types included in the analysis were arcs, chirps, upsweeps, and trills; downsweeps were not included due to a low sample size. I also conducted an AD test to quantify seasonal changes in overlap of call type derived vocal activity patterns.

RESULTS

I surveyed for 230 nights in Humboldt County across 12 sites and recorded 12,628 Humboldt's flying squirrel calls, and 201 nights in San Bernardino County across 9 sites and recorded 15,247 San Bernardino flying squirrel calls (Appendix B.1, Appendix B.2). I identified low quality calls and ran all call property analyses using high-quality calls (n = 6,191; Appendix B.5, Appendix B.6).

Call Properties and Types

I identified 5 main call types: 4 were chirp-like calls which consisted of arcs, chirps, downsweeps, and upsweeps, and trills, a more complex call (Figure 2), all of which were detected in both counties and had previously been described for northern and southern flying squirrel species. The number of high quality calls per call type across sites and counties varied and were sufficient to run the analyses with the exception of downsweeps (Appendix B.3, Appendix B.4).

Main Call Types

The discriminant function analysis including all call types showed that trills were significantly different from all other call types and that this difference was driven by the call properties duration and max frequency, which explained 91.8% of the variance (E=9.54, W=0.05; Figure 2). I ran a separate DFA to test all calls without trills, which found upsweeps and chirps to the most different driven by low frequency and high frequency, which accounted for 86.9% of the variance (E = 0.97, W = 0.44; Appendix C).

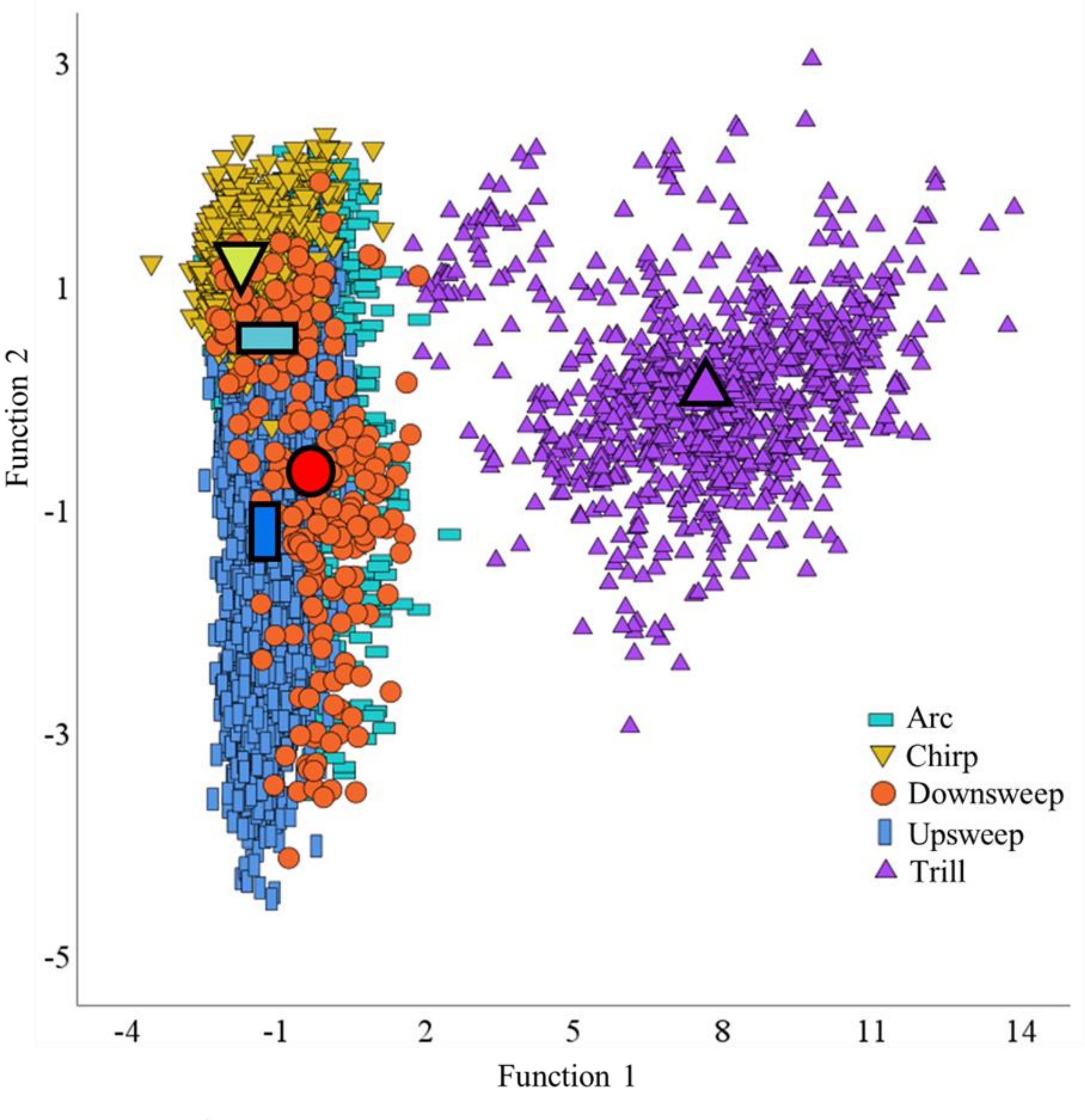

Figure 2. DFA of all main call types (n = 6,439) previously described (Gilley et al. 2019). Function 1 (x-axis) is defined by duration and max frequency and Function 2 is defined by low frequency and max frequency.

Arcs

Call properties for arcs (Figure 3) were significantly different between Humboldt and San Bernardino County (pDFA: $p = 0.008$). The pDFA found that 83% of the calls were correctly cross-classified. However, within both counties, arc call properties did not differ significantly across areas (pDFA: both $p > 0.05$). The DFA comparing counties showed that high frequency and duration explained the greatest amount of variance ($E= 0.969$, $W=0.508$; Figure 4, Appendix D.1). The DFA comparing areas across in both counties found 86.3% of the variance was explained by high frequency and duration ($E= 1.584$, $W=0.304$; Figure 5, Appendix D.2).

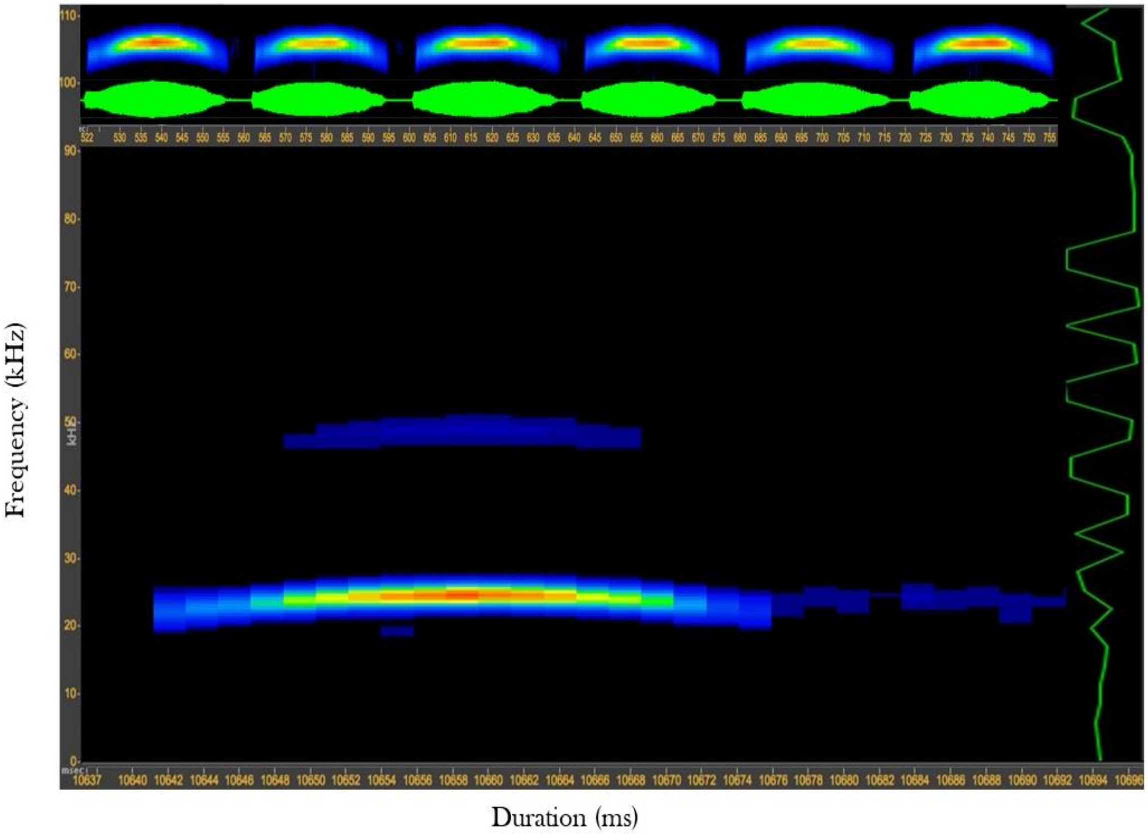

Figure 3. Spectrogram view of an arc as viewed in SonoBat. Spectrogram view is paired with waveform (right side of spectrogram) and call bouts (top of spectrogram) viewed in compressed mode to capture extent of bouts while maintaining the shape of the call.

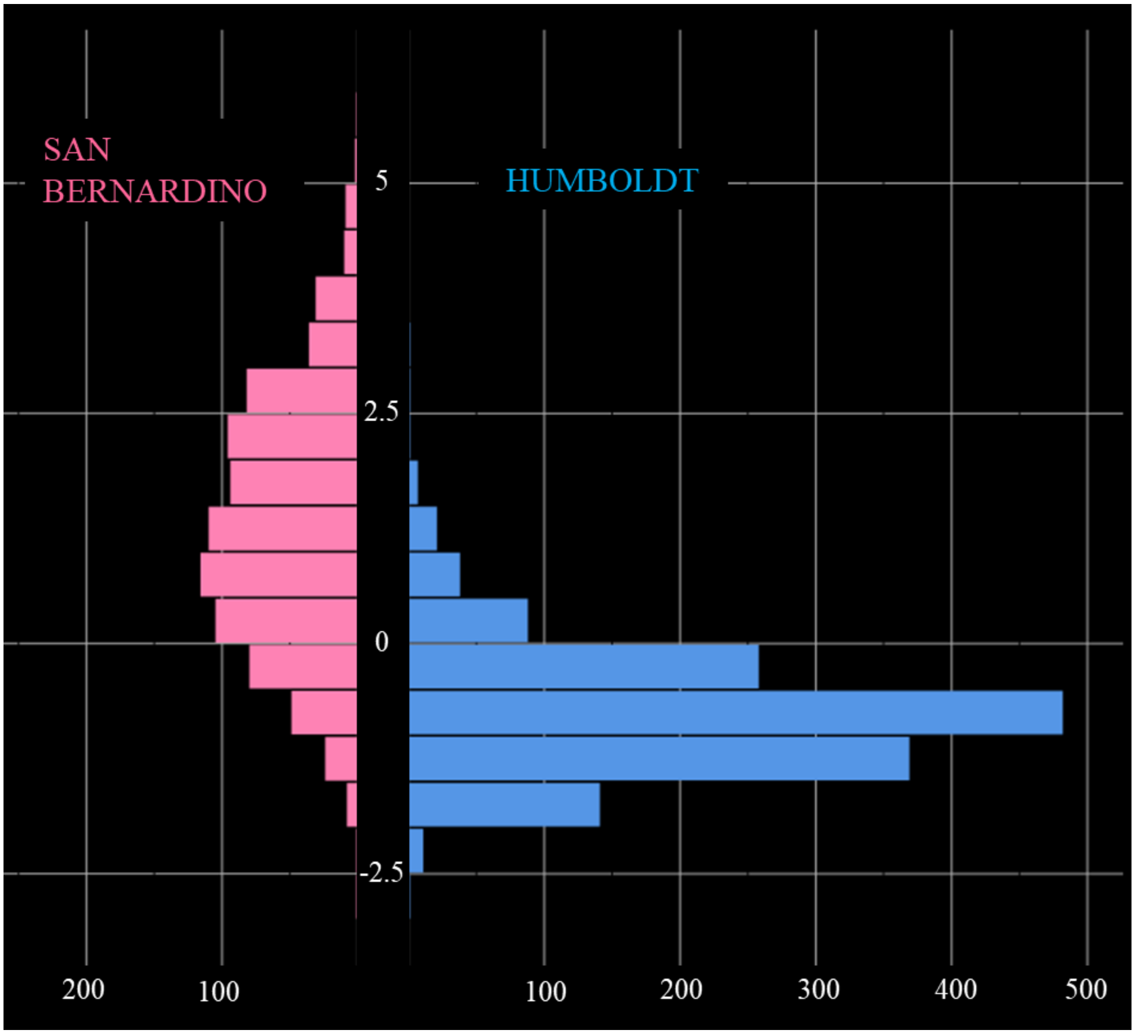

Figure 4. DFA function 1 histograms for arcs between counties. Function 1 is described by duration and high frequency. The vertical axis is described as the discriminant function score (a value generated from function 1) and the horizontal axis is number of calls.

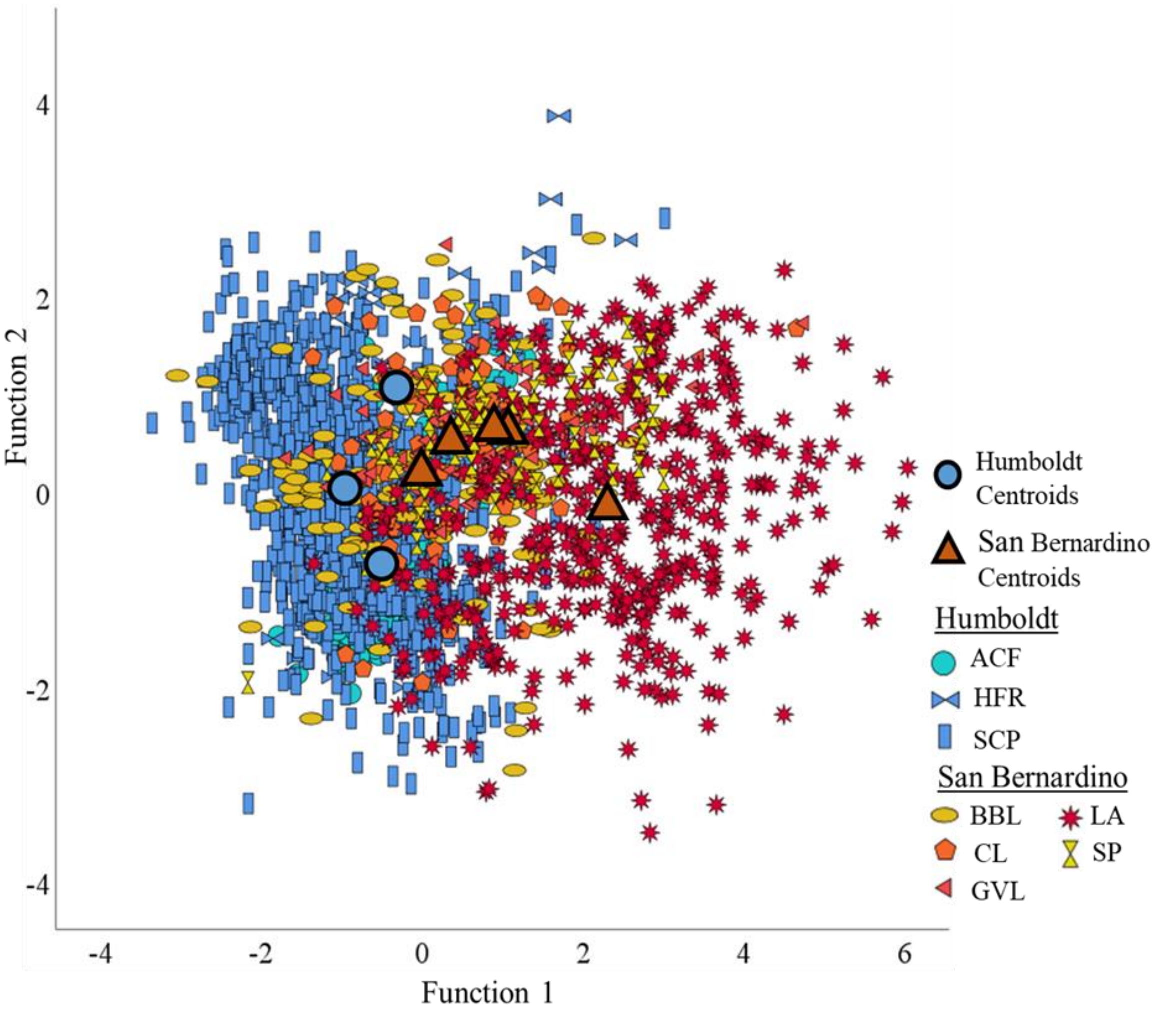

Figure 5. DFA for arcs across areas. All Humboldt and San Bernardino areas were included except for the CP area in San Bernardino, which was excluded due to no arcs detected. Function 1 (x-axis) is the discriminant function score generated from high frequency and duration while Function 2 (y-axis) is the discriminant function score generated from low frequency and high frequency.

Chirps

Call properties for chirps (Figure 6) were not significantly different between Humboldt and San Bernardino County (pDFA: p = 0.433), with 58% of chirps correctly cross-classified. The DFA comparing counties showed that high frequency and duration explained the greatest amount of the variance (E = 0.127, W = 0.887; Figure 7, Appendix

D.1). The DFA comparing areas across both counties found that only 49.9% of the variance was explained by high frequency and max frequency (E= 0.227, W=0.659; Figure 8, Appendix D.2).

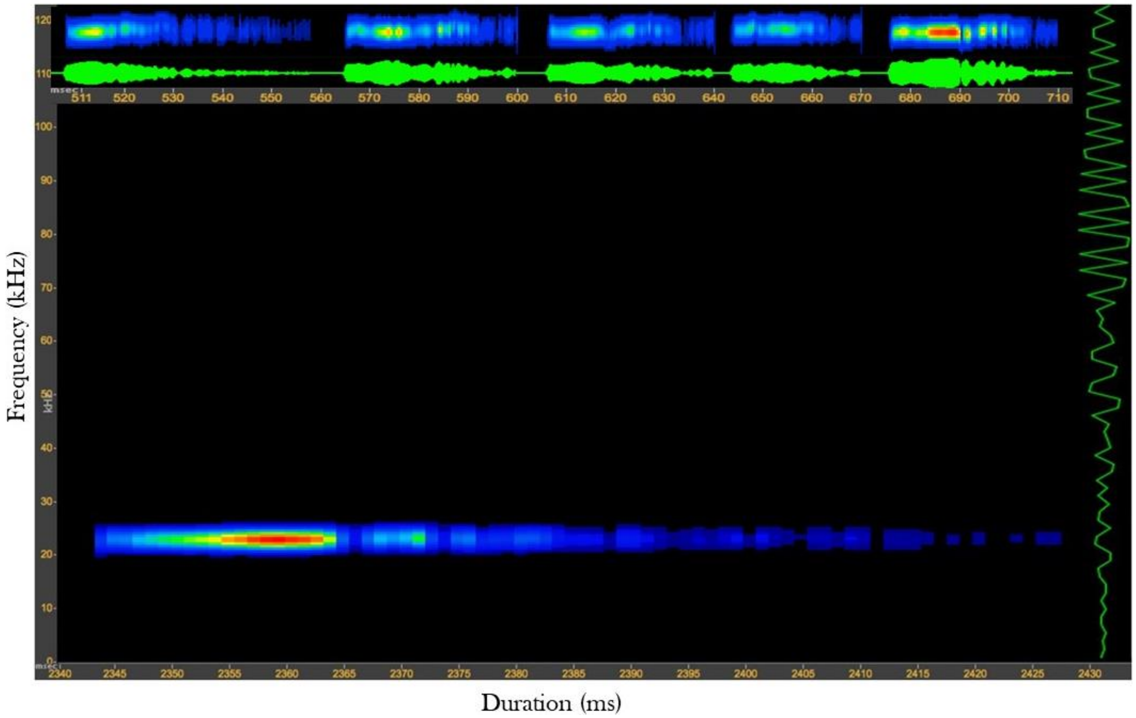

Figure 6. Spectrogram view of a chirp as viewed in SonoBat. Spectrogram view is paired with waveform (right side of spectrogram) and call bouts (top of spectrogram) viewed in compressed mode to capture extent of bouts while maintaining the shape of the call.

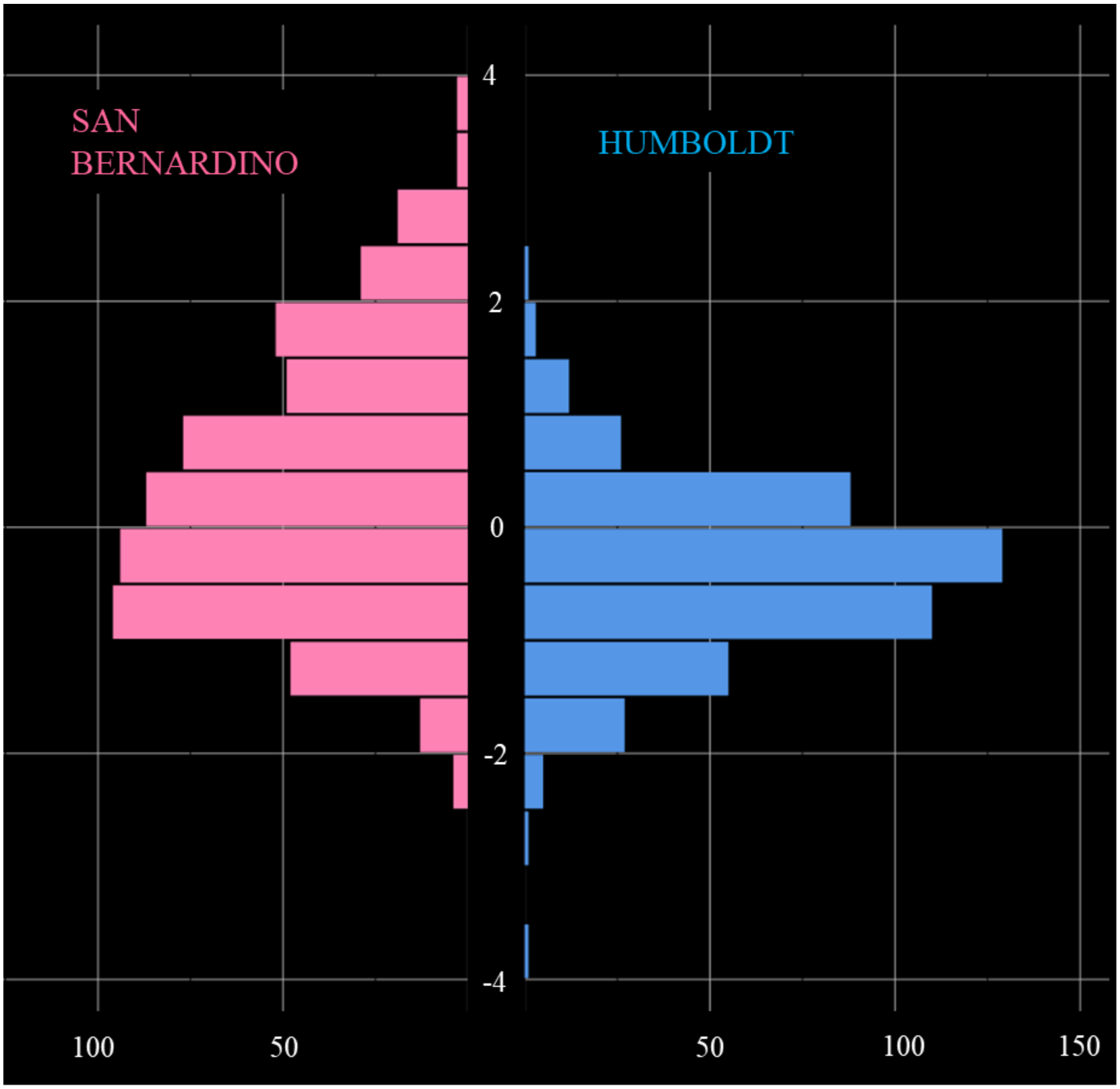

Figure 7. DFA function 1 histograms for chirps between counties. Function 1 is described by duration and high frequency. The vertical axis is described as the discriminant function score (a value generated from function 1) and the horizontal axis is the number of calls.

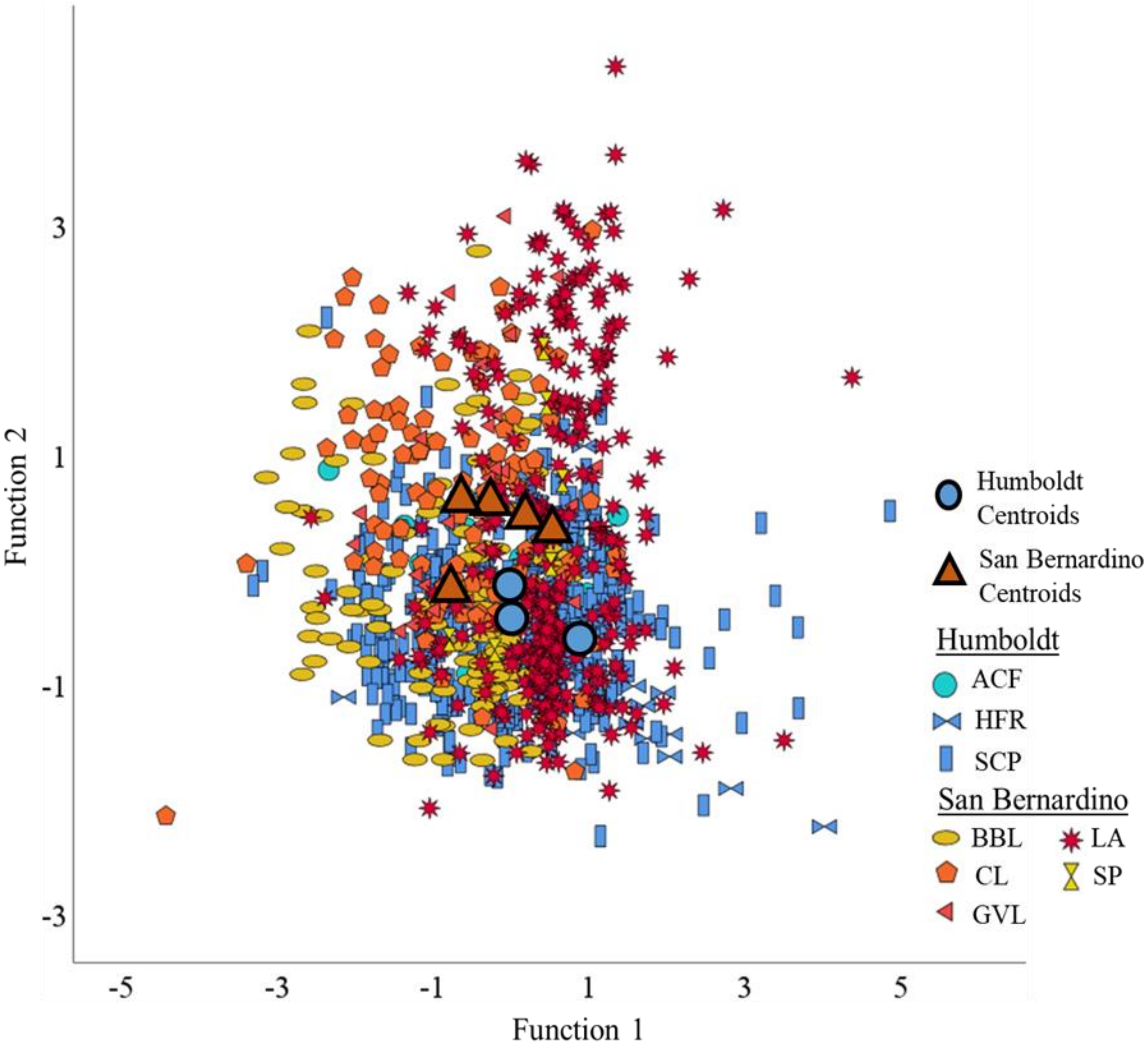

Figure 8. DFA for chirps across areas. All Humboldt areas were included and the CP area in San Bernardino was excluded due to no chirp detections. Function 1 (x-axis) is the discriminant function score generated from high frequency and max frequency while Function 2 (y-axis) is the discriminant function score generated from high frequency and duration.

Upsweeps

Call properties for upsweeps (Figure 9) were not significantly different between Humboldt and San Bernardino County (pDFA: p = 0.14), with 65.3% of upsweeps correctly cross-classified. The DFA comparing counties showed that high frequency and average entropy explained the greatest amount of the variance (E = 0.234, W = 0.810; Figure 10, Appendix D.1). The DFA comparing areas across both counties found that 70.4% of the variance was explained by high frequency and low frequency (E= 0.436, W=0.583; Figure 11, Appendix D.2).

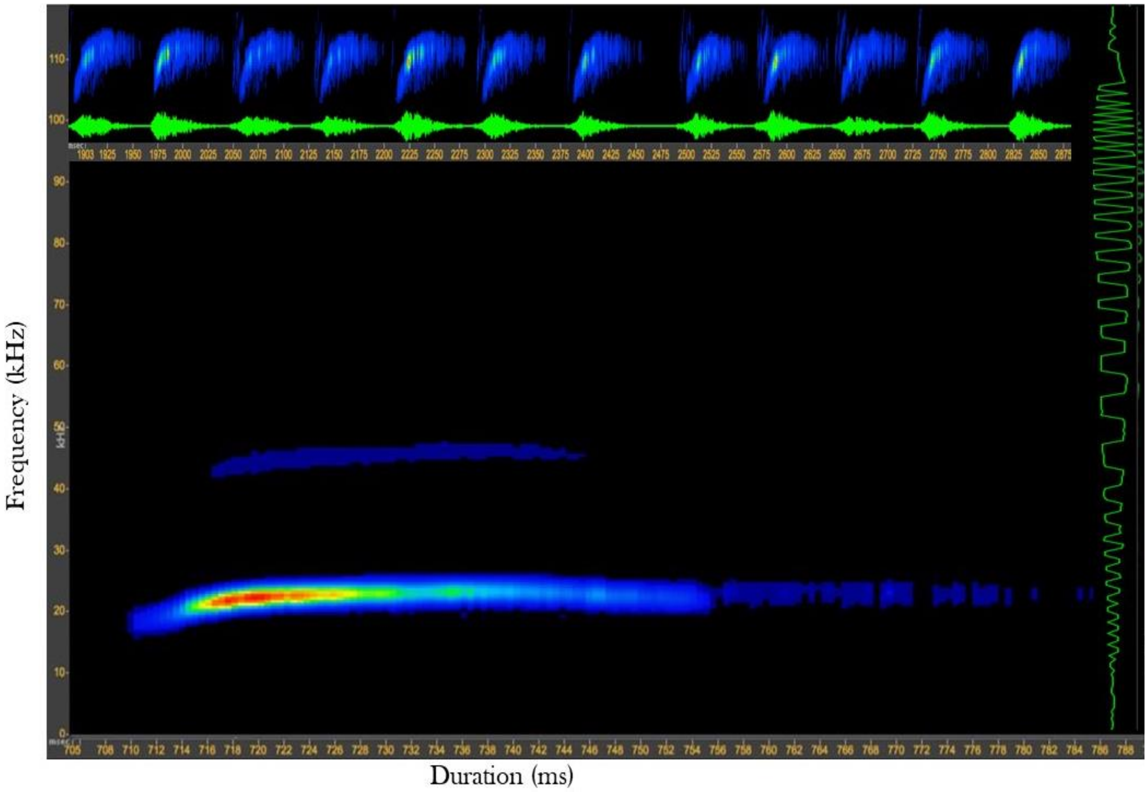

Figure 9. Spectrogram view of an upsweep as viewed in SonoBat. Spectrogram view is paired with waveform (right side of spectrogram) and call bouts (top of spectrogram) viewed in compressed mode to capture extent of bouts while maintaining the shape of the call.

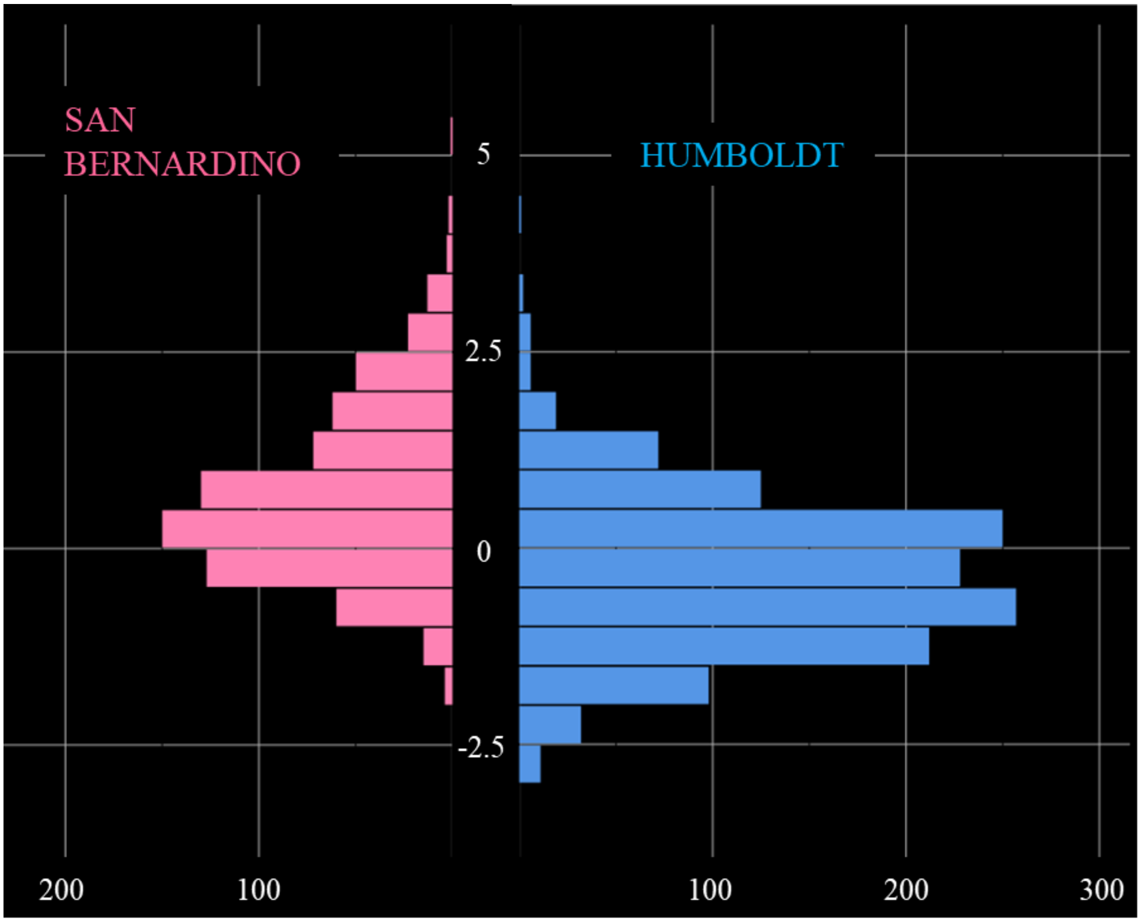

Figure 10. DFA function 1 histograms for upsweeps between counties. Function 1 is described by average entropy and high frequency. The vertical axis is described as the discriminant function score (a value generated from function 1) and the horizontal axis is the number of calls.

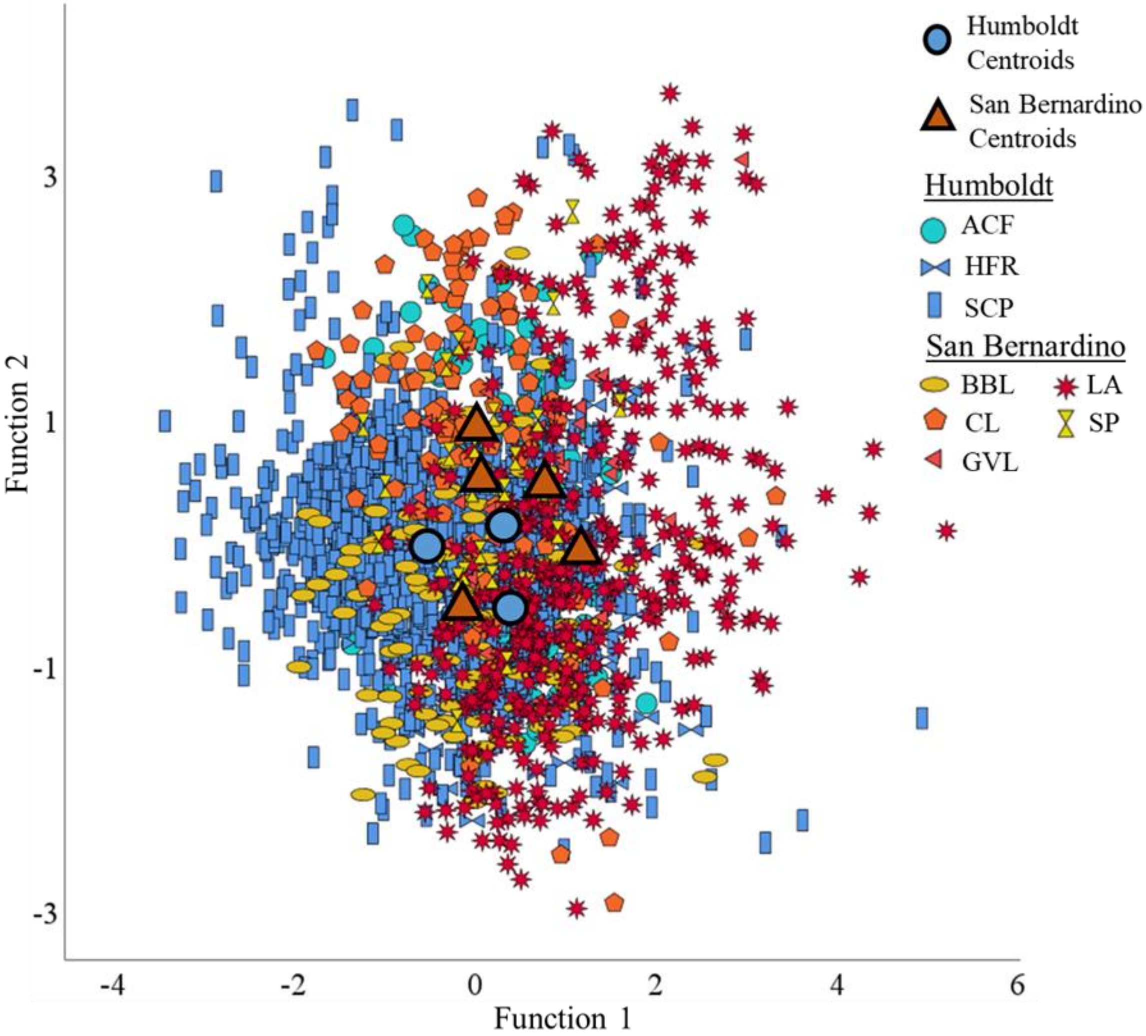

Figure 11. DFA for upsweeps across areas. All Humboldt areas were included and the CP area in San Bernardino was excluded due to no upsweep detections. Function 1 (x-axis) is the discriminant function score generated from low frequency and high frequency while Function 2 (y-axis) is the discriminant function score generated from high frequency and duration.

Trills

Call properties for trills (Figure 12) were significantly different between Humboldt and San Bernardino County (pDFA: p = 0.001), and was the strongest discrimination between counties. The pDFA found that 97% of the calls were correctly cross-classified. Trill call properties did not differ significantly across areas within each county (pDFA: $p > 0.05$). The DFA comparing counties showed that low frequency and duration explained the greatest amount of the variance (E = 2.652, W = 0.274; Figure 13, Appendix D.1). The DFA comparing areas across both counties found 88.3% of the variance was also explained by low frequency and duration (E = 2.825, W = 0.186; Figure 14, Appendix D.2).

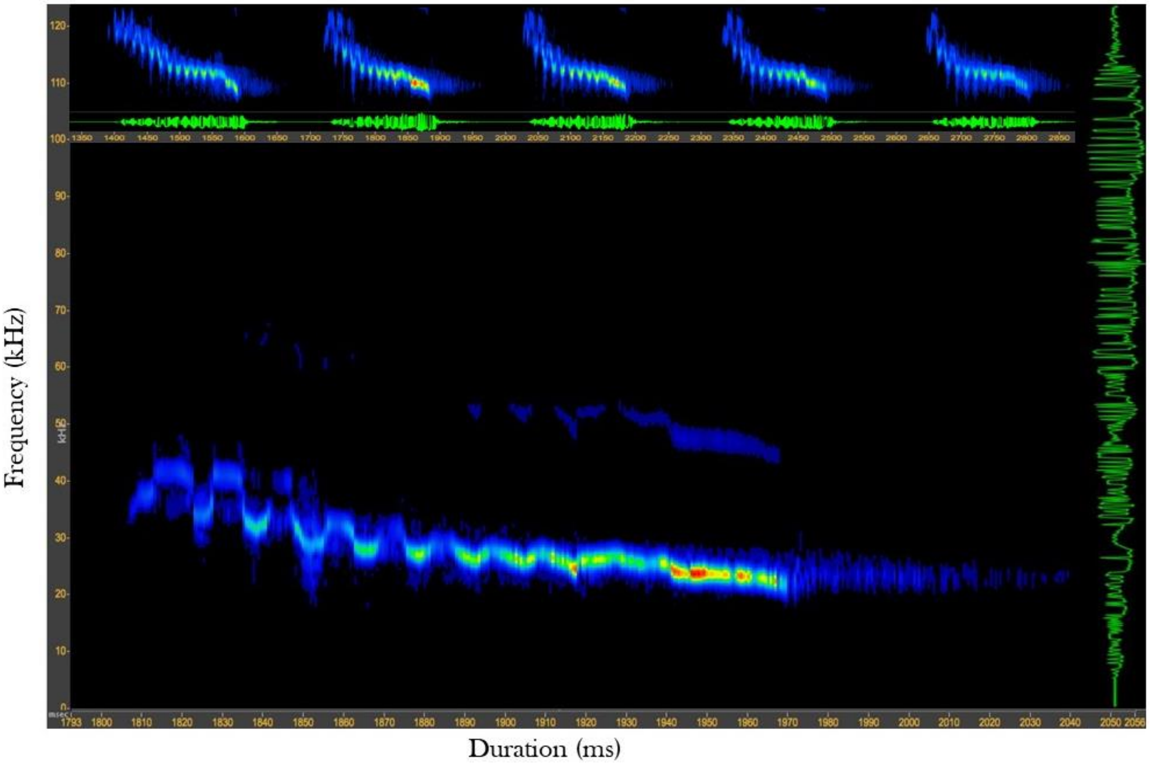

Figure 12. Spectrogram view of a trill as viewed in SonoBat. Spectrogram view is paired with waveform (right side of spectrogram) and call bouts (top of spectrogram) viewed in compressed mode to capture extent of bouts while maintaining the shape of the call.

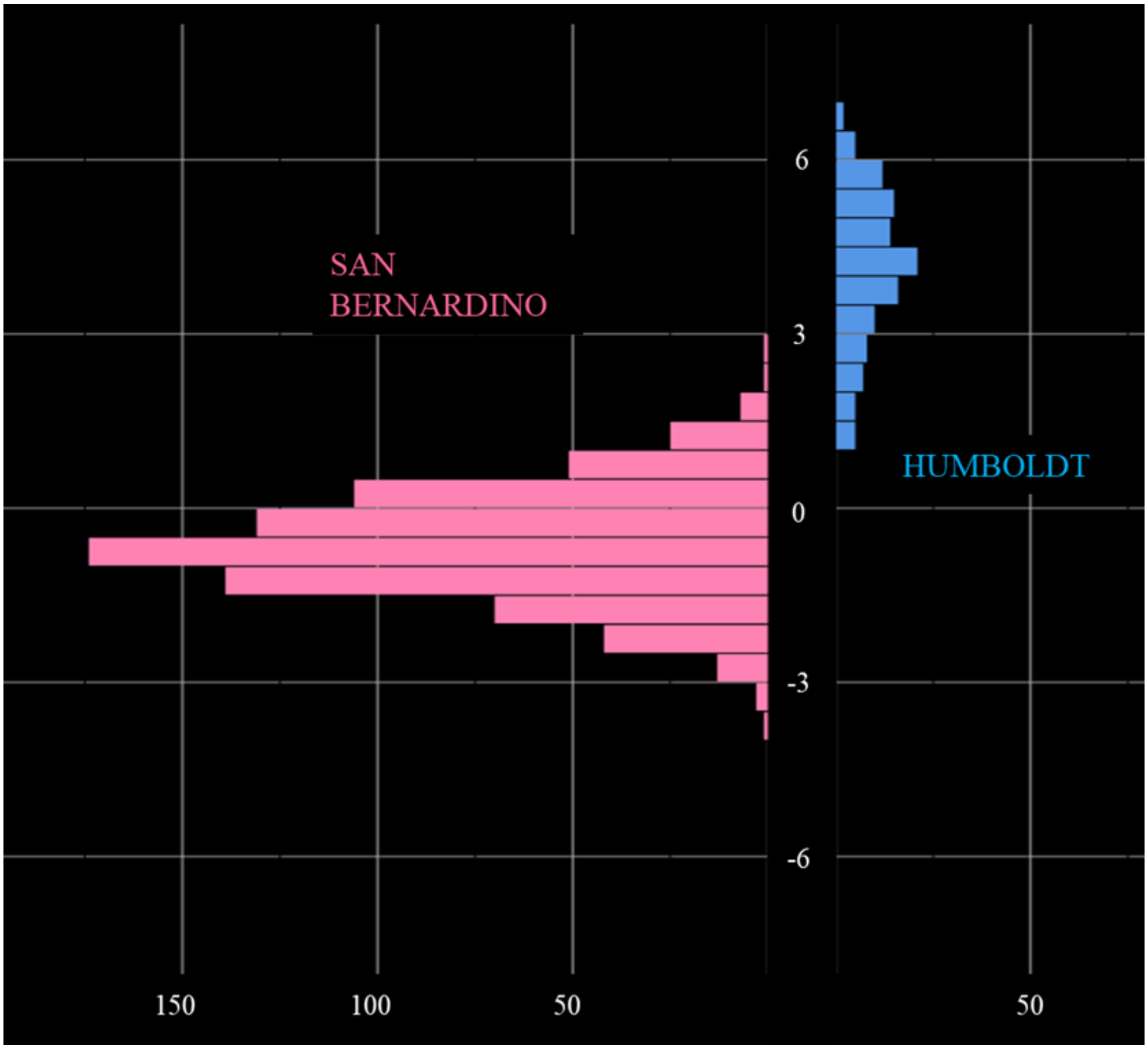

Figure 13. DFA function 1 histogram for trills between counties. Function 1 is described by average entropy and high frequency. The vertical axis is described as the discriminant function score (a value generated from function 1) and the horizontal axis is the number of calls.

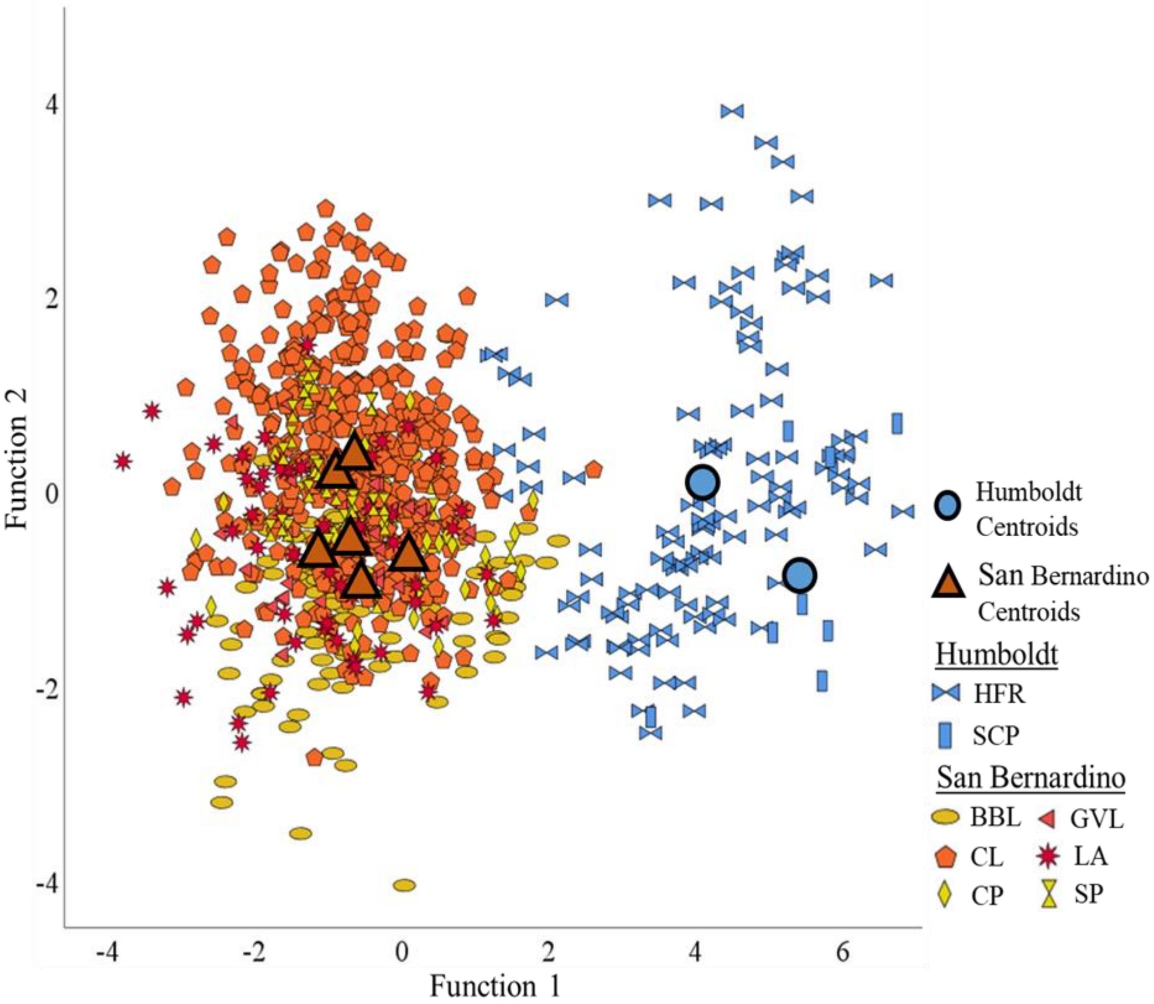

Figure 14. DFA for trills across areas. All San Bernardino areas were included and the ACF area in Humboldt was excluded due to no trill detections. Function 1 (x-axis) is the discriminant function score generated from low frequency and duration while Function 2 (y-axis) is the discriminant function score generated from average entropy and max frequency.

Seasonal and Geographic Variation

Seasonal Differences in Call Type Rates

In San Bernardino County, I recorded a total of 2,879 arcs (61.8% in the summer), 3,160 chirps (56.6% in the summer), 258 downsweeps (74.8% in the summer), 3,503 upsweeps (33.3% in the summer) and 4,454 trills (76.4% in the summer) across all sites (Appendix B.4). For each call type, call rates varied across sites within and between seasons (from 0 to 186.3 calls / survey night); however, I did not find any significant differences in call type rates between seasons for any of the call types (Table 3).

Table 3. Comparison of call type rates by season in San Bernardino County, California, USA using calls per night for each site and results of paired t-tests for each call type. Seasons are represented by "S" (summer) and "W" (winter).

	Arc		Chirp		Upsweep		Trill	
Sites	S	W	S	W	S	W	S	W
BB402	25.5	17.4	15.7	57.7	15.7	49.4	40.3	2.9
CL243	0.5	7	2.6	7.1	1.3	12.3	186.3	15.5
CL768	12.0	15.4	14	4	13.5	25.4	6	22.6
CP215	0.3	0	0	0	0	0	45.9	0
GVL338	38.8	16.8	7	4.3	16.8	13.8	13.4	17.6
LA427	38.5	11.4	28.1	7.7	16.1	28.9	4.9	2.8
LA828	4.1	1.1	3	0.6	21.8	2.8	9.6	0.7
LA850	13.8	2.5	8	3.7	20.3	17.6	72.7	7.8
SP005	47.6	1.9	2.6	0.6	29.9	0.3	7.8	6.9
Average	**20.1**	**8.2**	**9**	**9.5**	**15**	**16.7**	**43**	**8.5**
t-score	2.13		0.09		0.27		1.79	
p-value	0.07		0.93		0.79		0.11	

Geographic and Seasonal Variation in Vocal Activity Patterns

I found no significant difference between vocal activity patterns in Humboldt and San Bernardino counties (D-hat= 0.908, AD = 1.037, T.AD = 0.049, p = 0.338; Figure 15; Appendix G). There was a slightly higher number of calls in the early morning in Humboldt and a higher number of calls in the late evening in the San Bernardino.

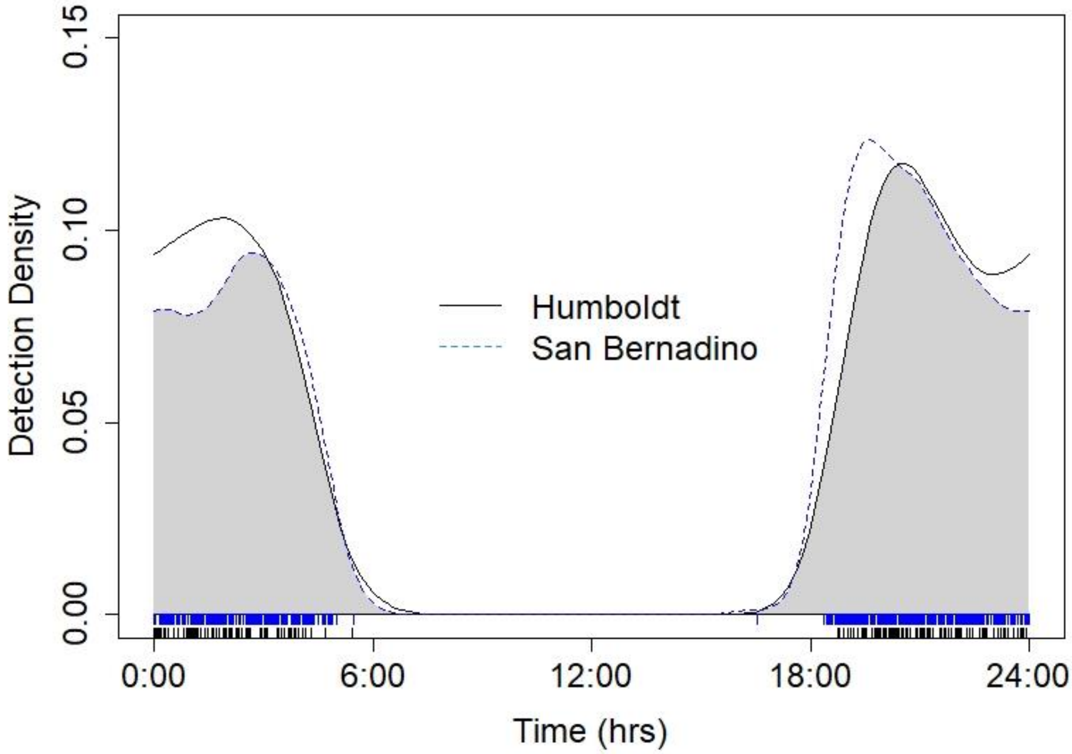

Figure 15. Overlap of vocal activity patterns in Humboldt (n = 13 sites) and San Bernardino counties (n = 9 sites), California, USA from 2018-2021.

I tested for seasonal differences in vocal activity patterns of all calls from both counties and within each county by comparing the overlap of timing of calls in summer (June – August; n = 10 Humboldt sites and n = 9 San Bernardino sites) and winter (November – February; n = 4 Humboldt sites and n = 8 San Bernardino sites). I found no significant difference between summer and winter vocal activity within Humboldt (D-hat= 0.831, AD = 1.073, T.AD = 0.097, p = 0.321; Appendix E). There was a slightly higher number of calls in the early morning during the summer than during winter, and a higher number of calls in the late evening in the winter in Humboldt. San Bernardino also had no significant difference in timing of calls between summer and winter (D-hat= 0.896, AD = 1.145, T.AD = 0.192, p = 0.289; Appendix E), though summer had a slight increase in calls in the early morning while winter had an increase in calls between in the late evening, and did not detect prolonged vocal activity during summer months.

Finally, I tested for differences in temporal overlap of each main call type within each county. I compared overlap in vocal activity patterns in summer and winter using all calls for each call type to compare the densities of total amounts of each call type during summer and winter. I tested arcs, chirps, upsweeps, and trills and found call types to be significantly different between seasons for both counties (all $p < 0.001$). In Humboldt County, I found that chirps had the highest degree of seasonal overlap (D-hat= 0.873, AD= 8.425, T. AD= 9.812; Appendix F, Appendix G) and trills had the least amount of seasonal overlap (D-hat= 0.577, AD= 28.66, T. AD= 36.45; Appendix F, Appendix G), with upsweeps (D= 0.643, AD= 149.9, T. AD= 195.6; Appendix F, Appendix G) and arcs (D-hat= 0.677, AD= 15.16, T. AD= 18.6; Appendix F, Appendix G) falling in the middle. In San Bernardino County, I found that trills had the highest degree of seasonal overlap (D-hat= 0.890, AD= 7.47, T. AD= 8.51; Appendix F, Appendix H) and chirps had the least amount of seasonal overlap (D-hat= 0.712, AD= 60.51, T. AD= 78.27; Appendix F, Appendix H), with upsweeps (D= 0.787, AD= 19.44, T. AD= 24.24; Appendix F,

Appendix H) and arcs (D-hat= 0.720, AD= 36.92, T. AD= 47.22; Appendix F, Appendix H) falling in the middle.

DISCUSSION

This is the first study to quantitatively describe the USVs from Humboldt’s flying squirrels and its San Bernardino subspecies, as well as to use acoustic recorders to describe vocal activity patterns of these species, offering preliminary evidence of geographic variation in call type properties and variation in the timing of call types across seasons. I found that significant ultrasonic vocal variation occurs between populations of flying squirrels in Humboldt and San Bernardino counties for two distinct call types, arcs and trills. I had hypothesized that species-subspecies variation in call properties may exist between flying squirrels from geographically separated populations and found this to be supported in certain cases. I also hypothesized that call types would be similar between regions and found that indeed they share call types. My prediction that a greater amount of variation in call properties exists between flying squirrels living in separate regions compared to flying squirrels in different areas within the same region was also supported. I did not find that call type rates varied between seasons; however, I did find that the temporal timing of call types within a nighttime period varied between seasons. Lastly, I predicted flying squirrels to be active for longer periods of time during summer months, but did not find that their vocal activity patterns were significantly different between summer and winter.

Seasonal vocal activity patterns, as inferred from patterns of vocalizations, were not significantly different, which contrasts with what is known of other North American flying squirrel vocal activity patterns, such as northern flying squirrels in British

Columbia, which were found to shift their activity patterns during harsh winters (Cotton and Parker 2000). Furthermore, Southern flying squirrels have been found to increase aspects of their activity such as increasing the distance that they travel from the nest and will expand their home ranges as temperature increases (Nelson and Sagot 2018). Though more distantly related, Japanese flying squirrels (*Pteromys momonga*) were found to reduce their activity the duration of activity bouts in the winter as opposed to summer (Suzuki and Ando 2017). These studies were conducted in regions that experience much harsher winter storms than the sites I surveyed in Humboldt or in San Bernardino, though the survey areas in San Bernardino can experience comparable low temperatures in the winter. It is possible there is less need for flying squirrels to limit activity during milder winter months or in areas that experience less extreme temperature variation.

The four distinct ultrasonic call types I described were based on previously-described call types and were identified using visual and auditory identification, though the analyses found that significant overlap exists between arcs, chirps, and upsweeps when compared to trills. Moreover, a continuum was observed across arcs, chirps, downsweeps, and upsweeps, which may be more-suitably described as chirp-like calls for the level of description in this study. As a result of this overlap in call structure, the probability of call type misidentification between chirp-like calls was higher as compared to trills. Downsweep calls, which are similar to upsweeps but terminate at the low frequency instead of the high frequency, have been described in previous studies and were also observed here (Gilley 2013; Murrant et al. 2013; Eisinger et al. 2016; Gilley et al. 2019), but, as mentioned previously, were not included in the analysis due to small sample sizes. I also observed two-toned chirps in few instances, and a more fine-scale study of calls within Humboldt and San Bernardino counties may uncover new call types that are specific to Humboldt's flying squirrels. It is also possible that call types produced by the San Bernardino subspecies could be more distinctly different from other North American flying squirrels due to their pronounced isolation and genetic uniqueness (Yuan et al. 2022). I found that trills were the most unique call type, often characterized by a large bandwidth, longer duration, and rapid modulation, features that may allow future researchers to identify individuals based on diagnostic traits of trills, but this would require knowing which individual is producing the trills to investigate this. There may also be potential for other trill types that were not previously described; for example I observed potential "arc trills" in a few instances. Trills were found to be the most species-specific call type in Gilley et al. (2019), which is in accord with the findings reported here. I also observed instances where two squirrels were vocalizing at the same time and using the same call type without overlapping frequencies (frequency alteration), which was also observed by Gilley et al. (2019).

The lack of seasonal differences in call type rates precludes gaining insight into the potential function of the call types. However, the analyses only compared summer versus winter, and call rates may change during other times of the year. Interestingly, the finer-scale temporal analyses of seasonal variation in the timing of call types across a night did result in significant differences and could inform future studies focused on the function of the different call types. The functions of flying squirrel USVs have not been investigated as extensively as those of ground squirrels and tree squirrels, likely due to their elusive and nocturnal behavior (Diggins 2021). A small number of studies have attempted to study the context and behavior associated with the vocalizations of flying squirrel species in Asia (e.g., Shen 2013, Terada et al. 2021), and they suggest the species potentially have specific alarm calls and mating calls; however, the functions of North American flying squirrel vocalizations remain relatively unknown (Gilley et al. 2019, Diggins 2021). Experimental studies, rather than passive recording surveys may be required to further elucidate these more complex behavioral questions of call function.

There are several potential limitations in this study. First, the smaller amount of Humboldt summer data may have influenced the seasonal vocal activity pattern results; more surveys in different areas of Humboldt during warmer months could provide a clearer picture of the vocal activity of flying squirrels in Northern California. Second, the smaller sample sizes of trills and arcs from Humboldt compared to San Bernardino may have influenced the analyses of geographic variation. Third, the incorporation of lower-quality calls (calls with broken fundamental harmonics, low amplitudes, background noise, etc.) in the temporal overlap analyses for call types may have led to the misidentification and/or skewed results of seasonal call type activity. Sound quality was an important factor in identifying call types as echoing can make the fundamental harmonic in a call appear longer (i.e., of greater duration), and low-quality calls increase probability of misidentifying calls (i.e., faint arcs/downsweeps/upsweeps appearing as chirps) due to the discriminating features of call types being lost as the recording quality degrades. In this study I oriented the microphone so that it was attached to the end of a stick which extended ~1 m from the recorder, which preliminary field tests found to be an effective method. Extending the microphone away from solid surfaces (in this case the tree the recording unit was attached to) limited the amount of echoing and interference. Finally, the previous conditions of the recording sites may also invite bias; San Bernardino recordings were collected from residential properties that had established feeders while the Humboldt recordings were collected from baited trees in more natural areas that were greater distances from human disturbance and without human-established food sources. This may have been pronounced during months where alternate food sources were scarce or metabolic needs were higher. These situational disparities between counties may have also contributed to the greater number of calls collected in San Bernardino than in Humboldt.

This study further supports that vocalizations predominantly range in the ultrasonic spectrum, though it is worth noting that many calls dropped into the sonic range, which has also been observed in previous studies (Gilley 2013; Murrant et al. 2013; Eisinger et al. 2016; Gilley et al. 2019). Discrepancies are apparent in frequency range between northern and southern flying squirrels, with southern flying squirrels found to produce specific call types at lower frequencies (Gilley 2013; Murrant et al. 2013; Gilley et al. 2019). Past research varies in the number of vocalizations described, which may be influenced by brand of monitoring equipment, microphone placement, circumstances of recording (i.e., wild versus captive individuals), observer bias, and the software used to view, measure, and analyze vocalizations (Gilley 2013; Murrant et al. 2013; Kaiser and O’Keefe 2015; Diggins et al. 2016; Eisinger et al. 2016; Ratcliffe and Jakobsen 2018; Gilley et al. 2019). Additionally, recording conditions and spatial distribution of vocalizing individuals may further influence the appearance of vocalizations in a spectrogram, increasing the potential for false identification (Ratcliffe and Jakobsen 2018). Recording wild populations also increases the potential for falsely identifying bats as squirrels, with *Lasionycteris noctivagans*, *Tadarida brasiliensis*, *Corynorhinus townsendii*, and *Lasiurus cinereus* producing structurally similar echolocation calls that may be confused with chirp-like calls produced by flying squirrels (Reichert et al. 2018; Gilley et al. 2019).

Future research into high-frequency vocalizations of North American flying squirrels should focus on elucidating the disparities in call types between Humboldt's flying squirrels and northern flying squirrels, which may improve monitoring efforts in areas of sympatry (Diggins et al. 2016; Arbogast et al. 2017). Gilley et al. (2019) produced fine-scale descriptions of vocalizations in northern and southern flying squirrels, and chirp-like call types observed in my study may be further broken into the call types described in this recently-published research, with others that may be unique to Humboldt's flying squirrels. The variation I detected between geographically-separated populations requires further investigation as this may be influenced by other factors. For instance, flying squirrels in dense forest patches with a greater potential for the sound signal to be broken by trees may produce lower-frequency calls as they attenuate less-rapidly than higher-frequency calls. The morphological constraints of the production of ultrasonic vocalizations in North American flying squirrels also warrants investigation as it could shed light on the plasticity of calls within individuals. Additionally, as this study only looked at two subspecies of Humboldt's flying squirrels (*G. o. stephensi* and *G. o.*

californicus), inclusion of the other subspecies throughout the entirety of their known range could provide further insights into the extent of geographic variation in this species and ultrasonic vocal repertoire of this species. Establishing a comparative index of vocalizations produced by North American flying squirrels may inform evolutionary lineages and support genetic distinctions between closely related taxa (Arbogast 2007; Kerhoulas and Arbogast 2010; Arbogast et al. 2017).

Study Implications

Understanding the distribution and ecology of North American flying squirrels in forests subject to human activities such as logging and recreation is essential for implementing appropriate conservation practices (Waters and Zabel 1995; Weigl 2007; Holloway and Smith 2011; Smith 2012). While live trapping can provide a wealth of data, mortality rates can be significant and may conflict with conservation efforts in areas with lower densities of at-risk flying squirrels, along with threatened subspecies (Payne et al. 1989; Rosenberg and Anthony 1992; Diggins et al. 2016). North American flying squirrels, in addition to being indicators of forest health, also constitute as prey for carnivores including those of conservation concern (e.g., Northern spotted owls [*Strix occidentalis caurina*] and fishers [*Pekania pennant*]), and further developing survey methods to establish habitat suitability will benefit an array of species in addition to flying squirrels (Zabel and Mckelvey 1995; Smith et al. 1999; Pyare and Longland 2002; Smith et al. 2005; Weigl 2007). Developing automated systems for detecting and measuring flying squirrel calls will be essential for long-term studies that collect large quantities of data, as sorting and measuring calls manually was highly time consuming. I recommend the inclusion of flying squirrel calls into bat-analysis programs to avoid potentially misclassifying bat species with flying squirrels, and this would also allow researchers to have an automated system for detecting flying squirrels in the wild. Additionally, assessing geographic variation in call types, as well as species-specific calls, may serve to bolster the efficacy of surveying for different species of flying squirrels in areas of geographic sympatry using minimally-invasive methods. The practicality and efficacy of bioacoustics as a minimally-invasive survey method has shown significant promise in recent studies (Blumstein et al. 2011; Diggins et al. 2016), and a benefit of this technique is that surveys are not species-specific and one can inspect an acoustic landscape to detect bats, insects, and other ultrasonic sound-producing species.

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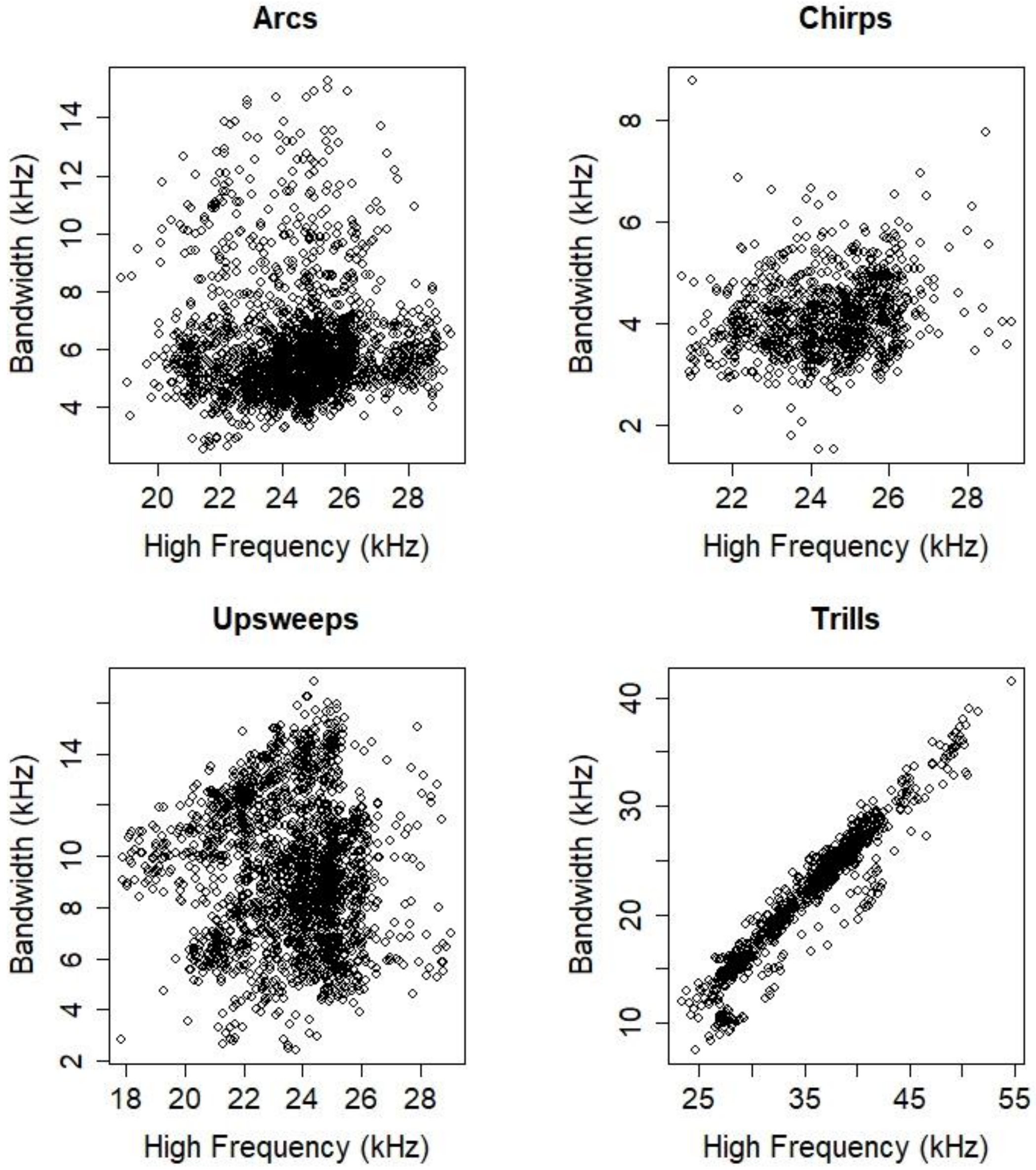
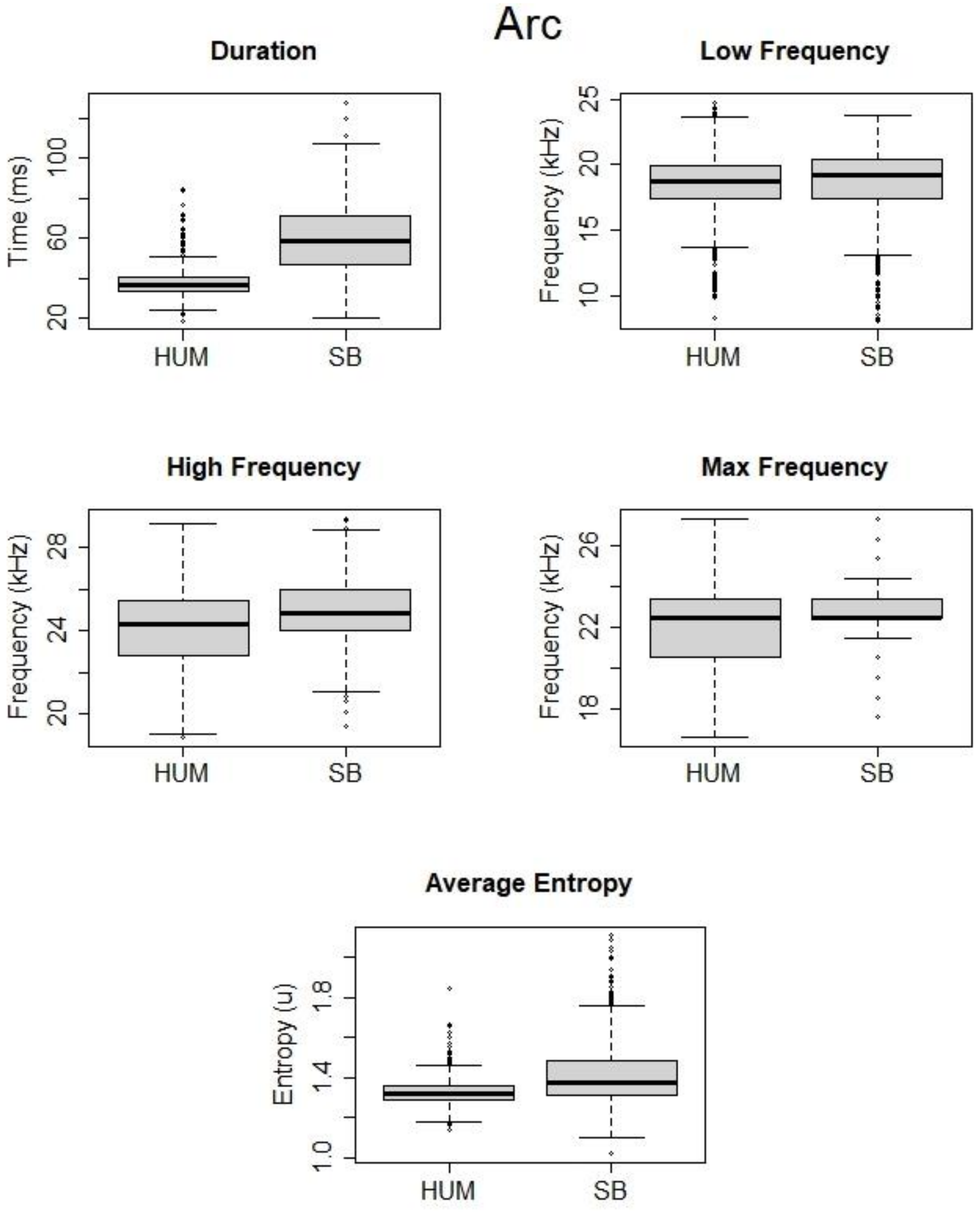
APPENDICES

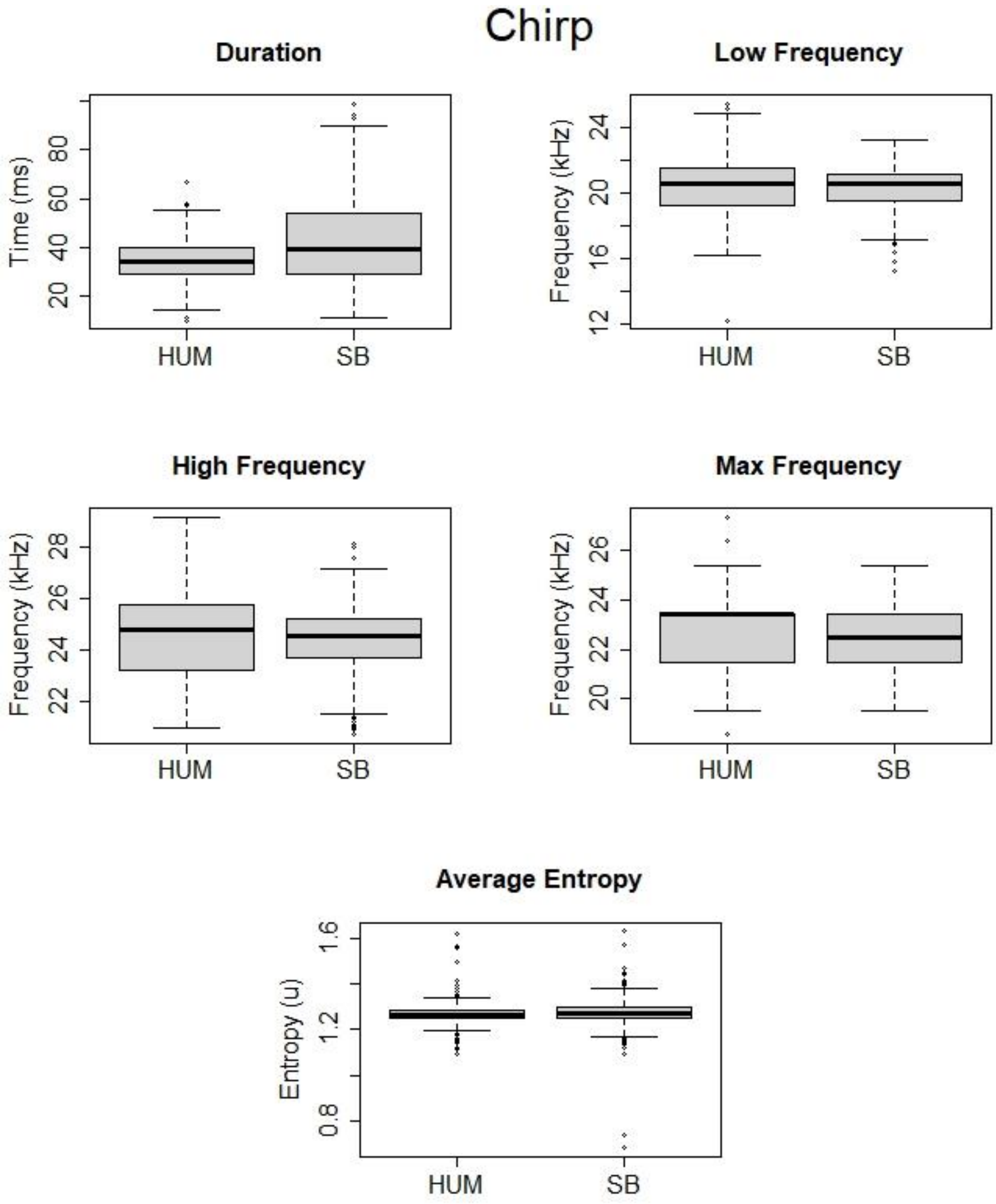
Appendix A

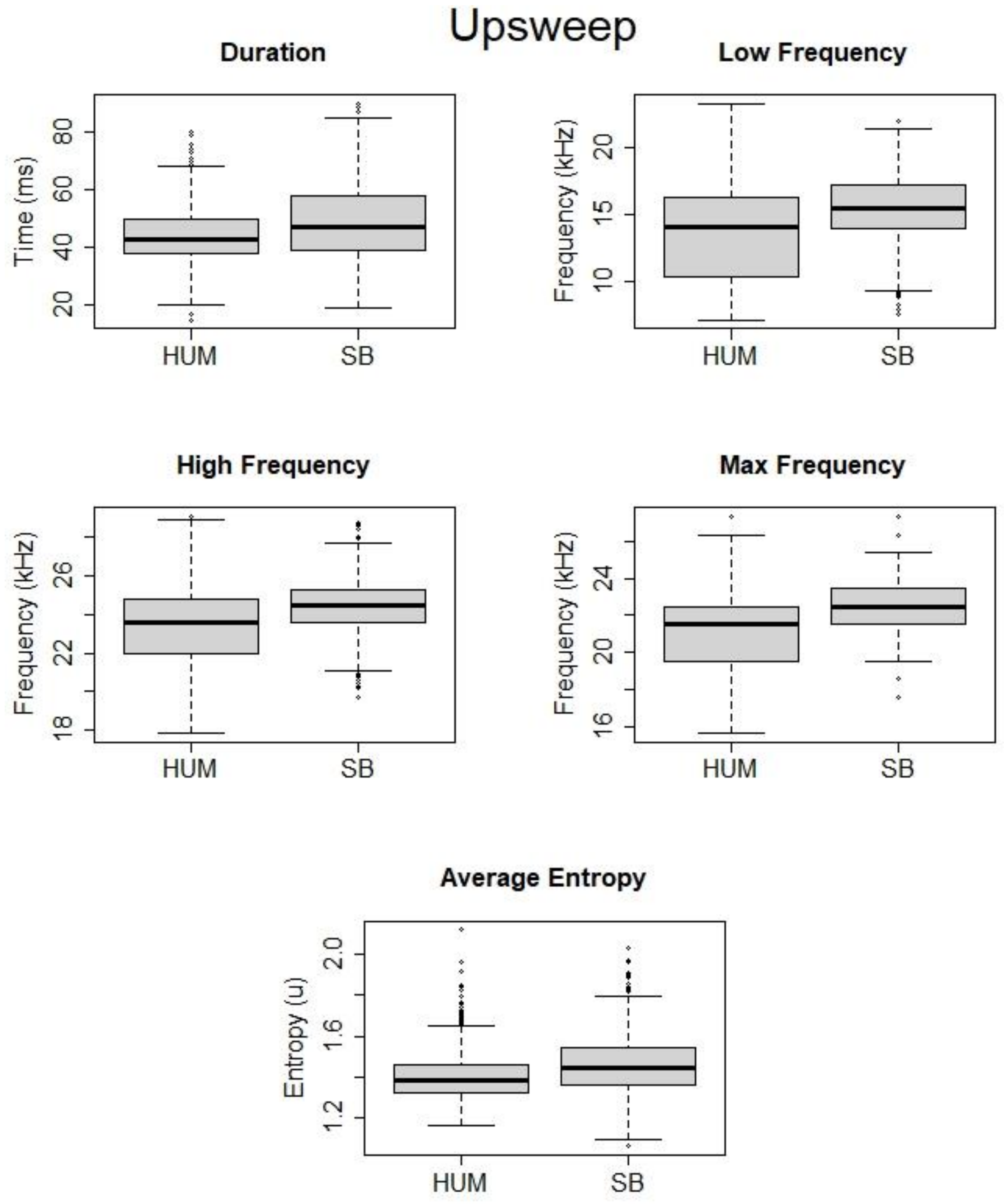
Appendix A.1: Correlation plots showing the parameters high frequency (x-axis) versus bandwidth (y-axis) for arcs (R = 0.019, p = 0.37), chirps (R = 0.22, p = < 0.001), upsweeps (R = 0.039, p = 0.077), and trills (R = 0.96, p = < 0.001).



Appendix A.2: Arc, chirp, upsweep, and trill measurement medians compared between Humboldt and San Bernardino counties for each parameter measured using Raven Pro 1.4.




Chirp
Duration
Time (ms)
20
40
60
80
HUM
SB
Low Frequency
Frequency (kHz)
12
16
20
24
HUM
SB
High Frequency
Frequency (kHz)
22
24
26
28
HUM
SB
Max Frequency
Frequency (kHz)
20
22
24
26
HUM
SB
Average Entropy
Entropy (u)
0.8
1.2
1.6
HUM
SB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Time (ms)
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40
60
80
HUM
SB
Low Frequency
Frequency (kHz)
10
15
20
HUM
SB
High Frequency
Frequency (kHz)
18
22
26
HUM
SB
Max Frequency
Frequency (kHz)
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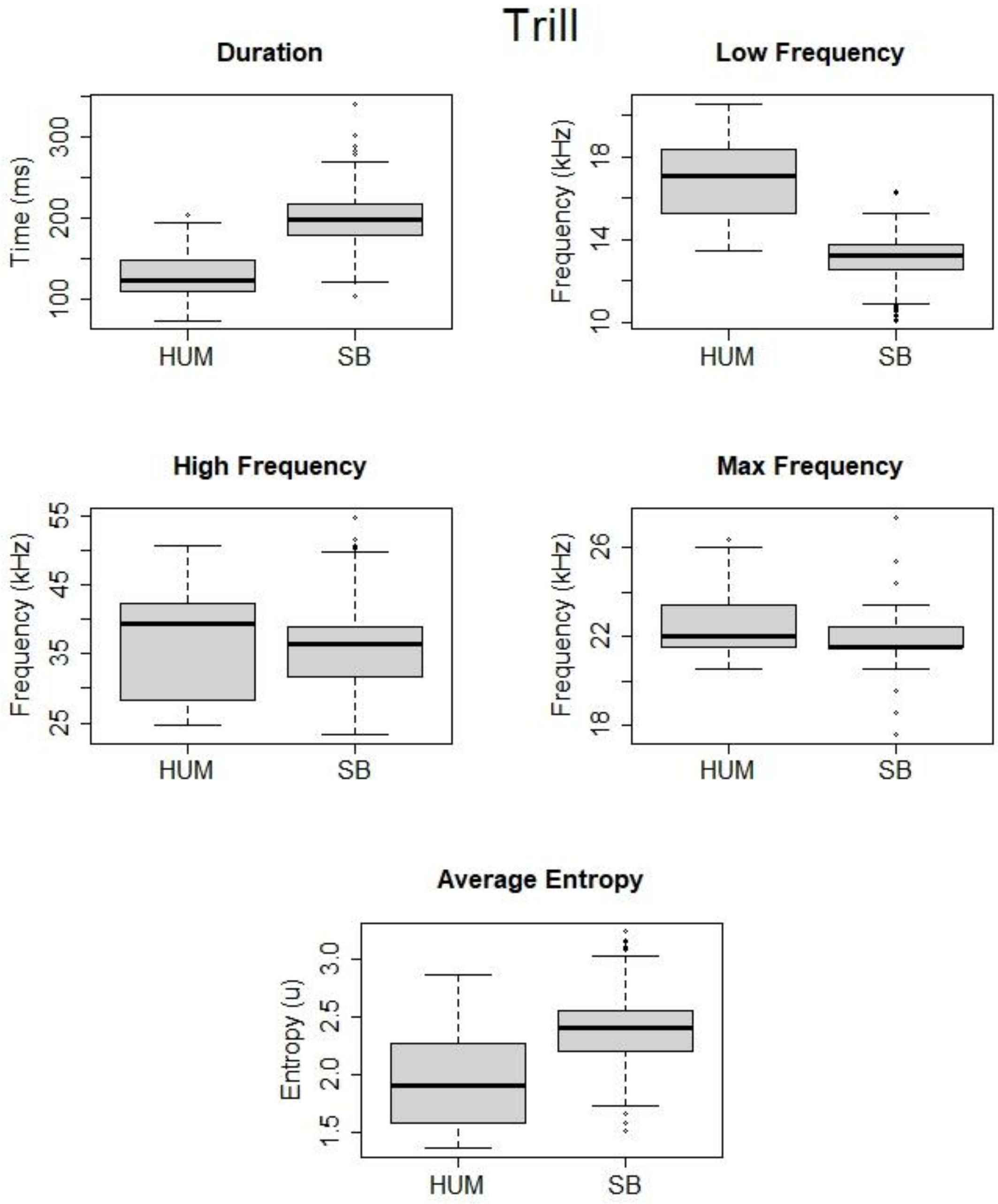
20
24
HUM
SB
Average Entropy
Entropy (u)
1.2
1.6
2.0
HUM
SB


Trill
Duration
Time (ms)
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200
300
HUM
SB
Low Frequency
Frequency (kHz)
10
14
18
HUM
SB
High Frequency
Frequency (kHz)
25
35
45
55
HUM
SB
Max Frequency
Frequency (kHz)
18
22
26
HUM
SB
Average Entropy
Entropy (u)
1.5
2.0
2.5
3.0
HUM
SB

Appendix B

Appendix B.1: Survey effort totals for surveys conducted in Humboldt County, California, USA. Seasons span from Winter 2018-Summer 2021 and recording nights were between the hours 1800 hrs and 0700 hrs. Sites included those that had recordings of flying squirrels (n= 13). Rate is calls per night and total call rate, along with seasonal percentages of call rates, is the average call rates for the county ("Total" row) and seasons ("Summer %" and "Winter %").

Season	Sites	Nights	Files	Calls	Rate
Summer	HFR1	3	3	14	4.7
	HFR4	14	2	15	1.1
	HFR18	15	61	307	20.5
	HFR21	31	34	240	7.7
	HFR23	31	49	431	13.9
Winter	ACF1	14	47	661	47.2
	ACF2	18	26	247	13.7
	HFR7	14	19	110	7.9
	HFR12	17	1	5	0.3
	HFR14	11	27	168	15.3
	HFR20	14	22	138	9.9
	SCP1	26	385	5012	198.8
	SCP5	22	321	5280	247.2
Total	**13**	**230**	**997**	**12628**	**54.9**
Summer %	28.6	40.9	14.9	8	10.7
Winter %	71.4	59.1	85.1	92	85.4

Appendix B.2: Survey effort totals for surveys conducted in San Bernardino, California, USA. Seasons span from Winter 2018-Summer 2020 and recording nights were between the hours 1800 hrs and 0700 hrs. Sites included those that had recordings of flying squirrels (n= 17). Rate is calls per night and total call rate, along with seasonal percentages of call rates, is the average call rates for the county ("Total" row) and seasons ("Summer %" and "Winter %").

Season	Sites	Nights	Files	Calls	Rate
Summer	BB402	11	656	1282	116.5
	CL243	10	1633	1867	186.7
	CL768	2	19	155	77.5
	CP215	7	77	327	46.7
	GVL338	8	204	816	102
	LA427	16	221	1621	101.3
	LA828	8	49	373	46.6
	LA850	6	186	821	136.8
	SP005	9	129	1002	111.3
Winter	BB402	15	200	1848	123.2
	CL243	24	242	1241	51.7
	CL768	5	86	458	91.6
	GVL338	14	189	1148	82
	LA427	25	307	1489	59.6
	LA828	15	26	147	9.8
	LA850	12	69	480	40
	SP005	14	36	172	12.3
Total	**17**	**201**	**4329**	**15247**	**75.9**
Summer %	47.1	38.3	73.3	54.2	107.3
Winter %	52.9	61.7	26.7	45.8	56.3

Appendix B.3: Call type totals for Humboldt County sites (n = 13), California, USA from 2018-2021. Call types listed are arcs (“A”), chirps (“C”), downsweeps (“D”), upsweeps (“U”), and trills (“T”). Total call rate, along with seasonal percentages of call rates, is the average call rates for the county (“Total” row) and seasons (“Summer %” and “Winter %”).

Season	Sites	A	A/night	C	C/night	D	D/night	U	U/night	T	T/night
Summer	HFR1	0	0	0	0	0	0	0	0	14	4.7
	HFR4	1	0.1	1	0.1	0	0	0	0	13	0.9
	HFR18	49	3.3	31	2.1	1	0.1	116	7.7	110	7.3
	HFR21	19	0.6	38	1.2	2	0.1	119	3.8	62	2
	HFR23	57	1.8	143	4.6	0	0	126	4.1	105	3.4
Winter	ACF1	283	20.2	139	9.9	1	0.1	238	17	0	0
	ACF2	160	8.9	54	3	4	0.2	29	1.6	0	0
	HFR7	2	0.1	31	2.2	0	0	0	0	77	5.5
	HFR12	0	0	0	0	0	0	0	0	5	0.3
	HFR14	7	0.6	9	0.8	0	0	16	1.5	136	12.4
	HFR20	10	0.7	52	3.7	0	0	76	5.4	0	0
	SCP1	1376	52.9	1832	70.5	246	9.5	1708	65.7	8	0.3
	SCP5	1496	68	1951	88.7	98	4.5	1872	85.1	21	1.0
	Total	**3460**	**15.0**	**4281**	**18.6**	**352**	**1.5**	**4300**	**18.7**	**551**	**2.4**
	Summer	126	1.3	213	2.3	3	0.0	361	3.8	304	3.2
	Winter	3334	24.5	4068	29.9	349	2.6	3939	29.0	247	1.8
	Total %	**26.7**		**33.1**		**2.7**		**33.2**		**4.3**	
	Summer %	12.5		21.2		0.3		35.8		30.2	
	Winter %	27.9		34.1		2.9		33.0		2.1	

Appendix B.4: Call type totals for San Bernardino County sites (n = 9), California, USA from 2018-2020. Call types listed are arcs (“A”), chirps (“C”), downsweeps (“D”), upsweeps (“U”), and trills (“T”). Site CP215 in San Bernardino had no detections during the winter and was not included in the call totals. Total call rate, along with seasonal percentages of call rates, is the average call rates for the county (“Total” row) and seasons (“Summer %” and “Winter %”).

Season	Sites	A	A/night	C	C/night	D	D/night	U	U/night	T	T/night
Summer	BB402	280	25.5	346	15.7	49	4.5	173	15.7	443	40.3
	CL243	5	0.5	51	2.6	0	0	13	1.3	1863	186.3
	CL768	24	12.0	89	14.0	3	1.5	27	13.5	12	6
	CP215	2	0.3	0	0	4	0.6	0	0	321	45.9
	GVL338	310	38.8	251	7.0	12	1.5	134	16.8	107	13.4
	LA427	616	38.5	650	28.1	62	3.9	257	16.1	78	4.9
	LA828	33	4.1	88	3	0	0	174	21.8	77	9.6
	LA850	83	13.8	167	8	12	2	122	20.3	436	72.7
	SP005	428	47.6	149	2.6	51	5.7	269	29.9	70	7.8
Winter	BB402	261	17.4	865	57.7	1	0.1	741	49.4	44	2.9
	CL243	168	7	170	7.1	10	0.4	294	12.3	372	15.5
	CL768	77	15.4	20	4	6	1.2	127	25.4	113	22.6
	GVL338	235	16.8	60	4.3	9	0.6	193	13.8	247	17.6
	LA427	284	11.4	193	7.7	38	1.5	722	28.9	69	2.8
	LA828	16	1.1	9	0.6	0	0	42	2.8	11	0.7
	LA850	30	2.5	44	3.7	1	0.1	211	17.6	94	7.8
	SP005	27	1.9	8	0.6	0	0	4	0.3	97	6.9
	Total	**2879**	**14.3**	**3160**	**15.7**	**258**	**1.3**	**3503**	**17.4**	**4454**	**22.2**
	Summer	1781	23.1	828	10.8	2210	28.7	1169	15.2	1573	20.4
	Winter	1098	8.9	621	5	66	0.5	2334	18.8	1047	8.4
	Total %	**20.2**		**22.2**		**1.8**		**24.6**		**31.2**	

Season	Sites	A	A/night	C	C/night	D	D/night	U	U/night	T	T/night
	Summer %	23.6		11		29.2		15.5		20.8	
	Winter %	21.3		12		1.3		45.2		20.3	

Appendix B.5: Call quality totals for calls recorded in Humboldt County, California, USA from 2018-2021 (“HQ” = high quality, high-low quality; “LQ” = low quality, low-incomplete quality).

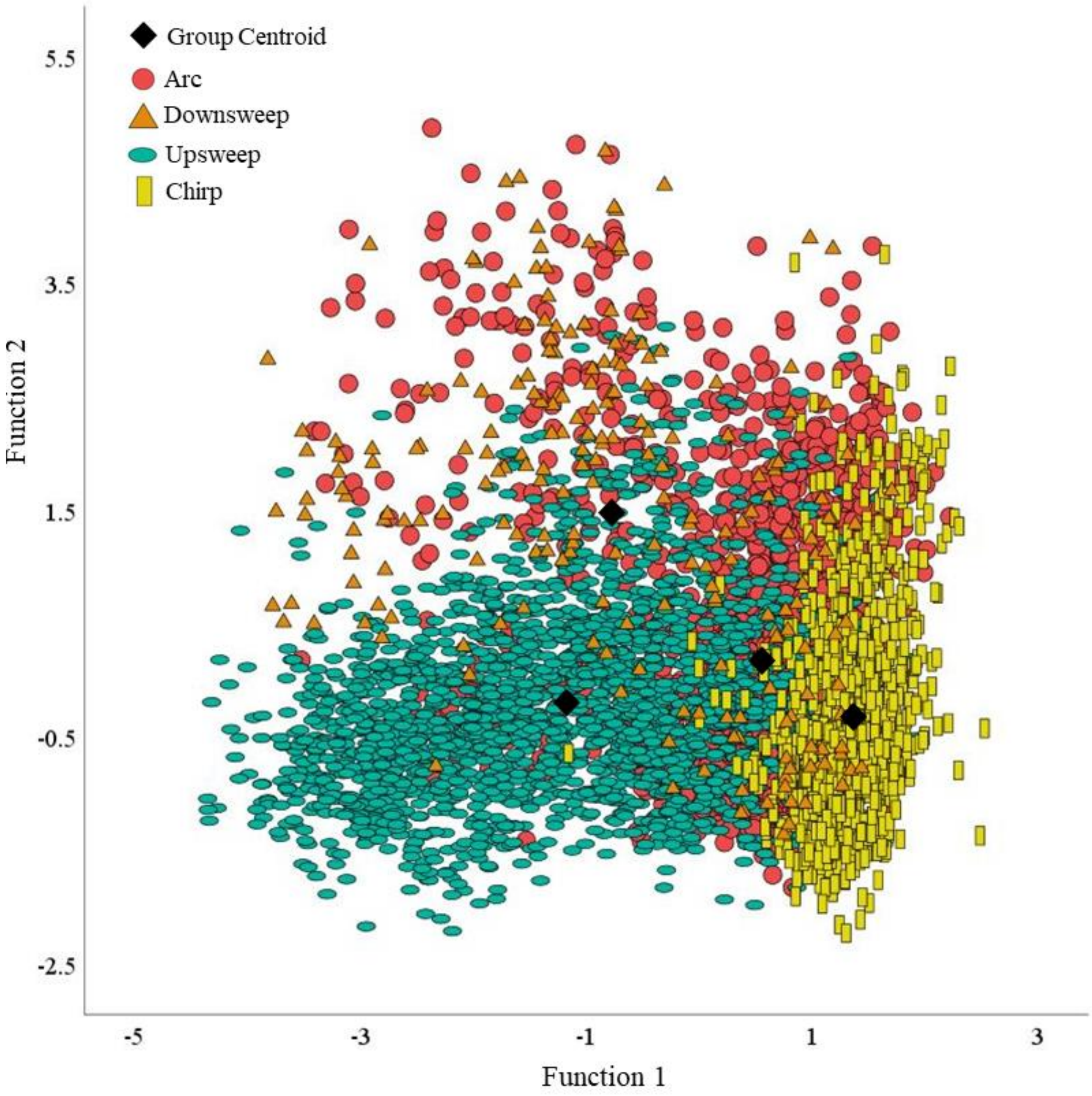
Call Type	HQ	LQ	**Total**
Arc	1420	2042	**3462**
Chirp	458	3828	**4286**
Upsweep	1322	2993	**4315**
Trill	125	440	**565**
Total	**3325**	**9303**	**12628**
%	26.3	73.7	45.3

Appendix B.6: Call quality totals for calls recorded in San Bernardino County, California, USA from 2018-2020 (“HQ” = high quality, high-low quality; “LQ” = low quality, low-incomplete quality).

Call Type	HQ	LQ	**Total**
Arc	818	2059	**2877**
Chirp	575	3848	**4423**
Upsweep	711	2790	**3501**
Trill	762	3684	**4446**
Total	**2866**	**12381**	**15247**
%	18.8	81.2	54.7

Appendix C

Appendix C: DFA results of call types excluding trills collected between 2018 and 2021 in both Humboldt and San Bernardino counties. Function 1 (x-axis) is defined by low frequency and high frequency and function 2 (y-axis) is defined by duration and high frequency.



Appendix D

Appendix D.1: DFA results for call type properties between Humboldt and San Bernardino counties, with all $p < 0.001$.

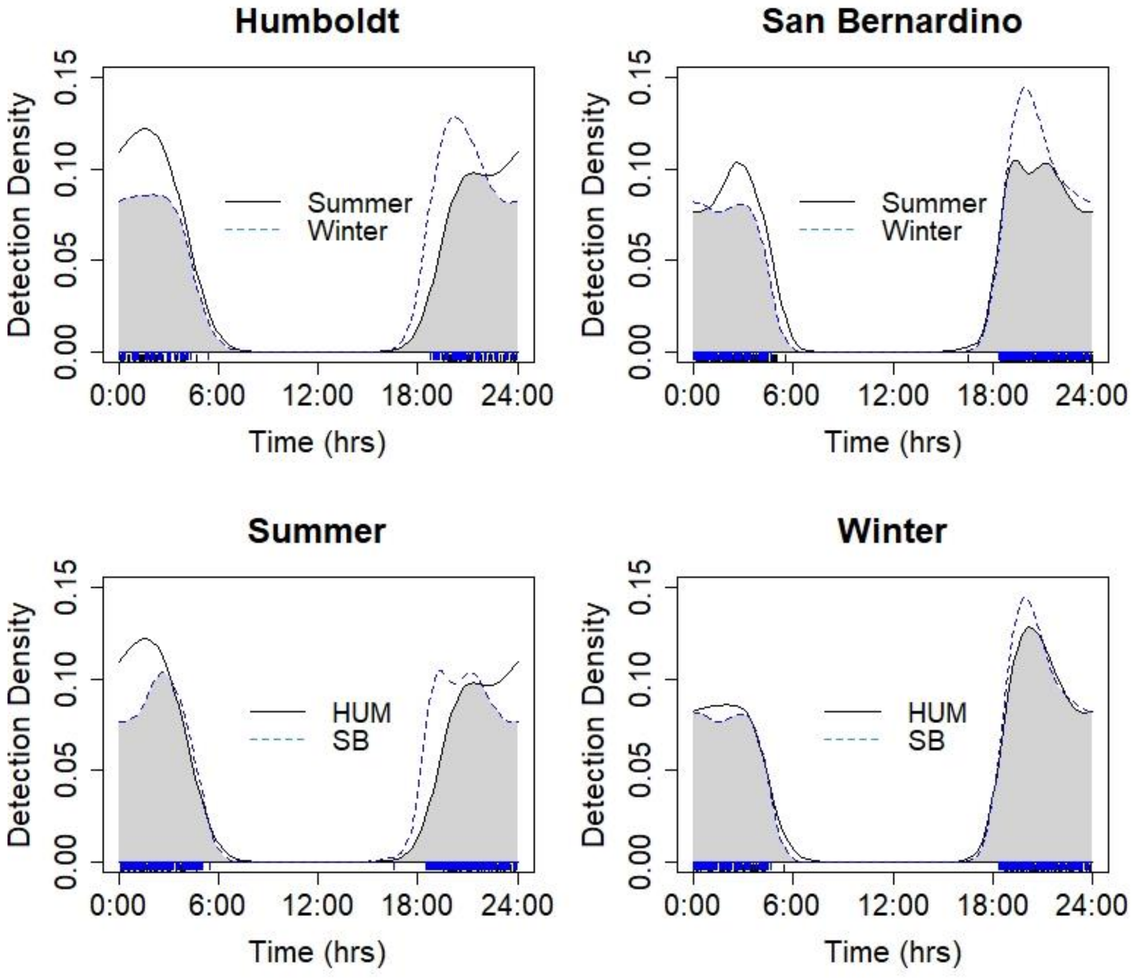
Call Type	Eigenvalue	Canonical Correlation	Wilk's Lambda	Chi-square
Arc	0.969	0.701	0.508	1537.140
Chirp	0.127	0.336	0.887	123.005
Upsweep	0.234	0.436	0.810	426.385
Trill	2.652	0.852	0.274	1139.224

Appendix D.2: DFA results for call type properties across areas within each county (Humboldt: n = 3, San Bernardino n = 6), with all $p < 0.001$.

Call Type	Eigenvalue	Canonical Correlation	Wilk's Lambda	Chi-square
Arc	1.584	0.783	0.304	2967.414
Chirp	0.227	0.430	0.659	427.641
Upsweep	0.436	0.551	0.583	1092.718
Trill	2.825	0.859	0.186	1474.881

Appendix E

Appendix E: Overlap of vocal activity patterns in summer (June – August) in Humboldt (n = 5 sites) and San Bernardino (n = 9 sites) counties and winter (November – February) in Humboldt (n = 8 sites) and San Bernardino (n = 8 sites) counties in California, USA from 2018-2021. “HUM” represents Humboldt County and “SB” represents San Bernardino County.



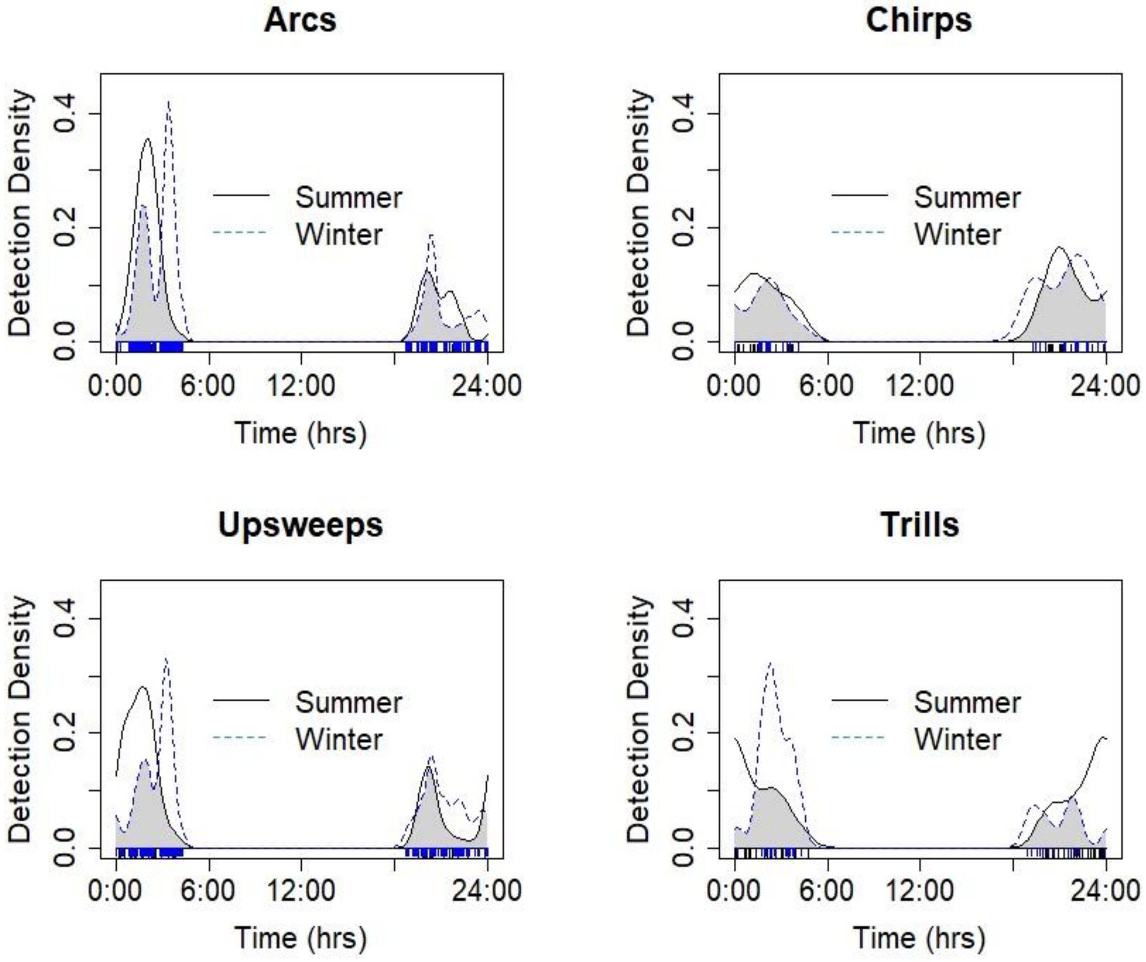
Appendix F

Appendix F: Bootstrapping results for Humboldt and San Bernardino seasonal overlap and the seasonal overlaps of four main call types recorded in each county during summer-winter seasons from 2018-2021 in California, USA. Lower and Upper Cs are 95% confidence intervals, “AD” is the Anderson-Darling test result and “T. AD” is (AD-mean/sigma).

County	Season/Call Type	Lower CI	Upper CI	D-hat	A-D	T. A-D	P-Value
Humboldt	All Seasons	0.728	0.935	0.831	1.073	0.097	0.321
	Arc	0.494	0.618	0.677	15.16	18.6	< 0.001
	Chirp	0.623	0.851	0.873	8.425	9.812	< 0.001
	Upsweep	0.501	0.599	0.643	149.9	195.6	< 0.001
	Trill	0.445	0.587	0.577	28.66	36.45	< 0.001
San Bernardino	All Seasons	0.853	0.956	0.896	1.145	0.192	0.288
	Arc	0.635	0.727	0.720	36.92	47.22	< 0.001
	Chirp	0.559	0.640	0.712	60.51	78.27	< 0.001
	Upsweep	0.690	0.762	0.787	19.44	24.24	< 0.001
	Trill	0.862	0.917	0.890	7.47	8.51	< 0.001
Both	All Seasons	0.883	0.966	0.908	1.037	0.049	0.338
Both	Summer	0.780	0.947	0.856	1.888	1.171	0.105
Both	Winter	0.936	0.984	0.908	0.200	1.056	0.991

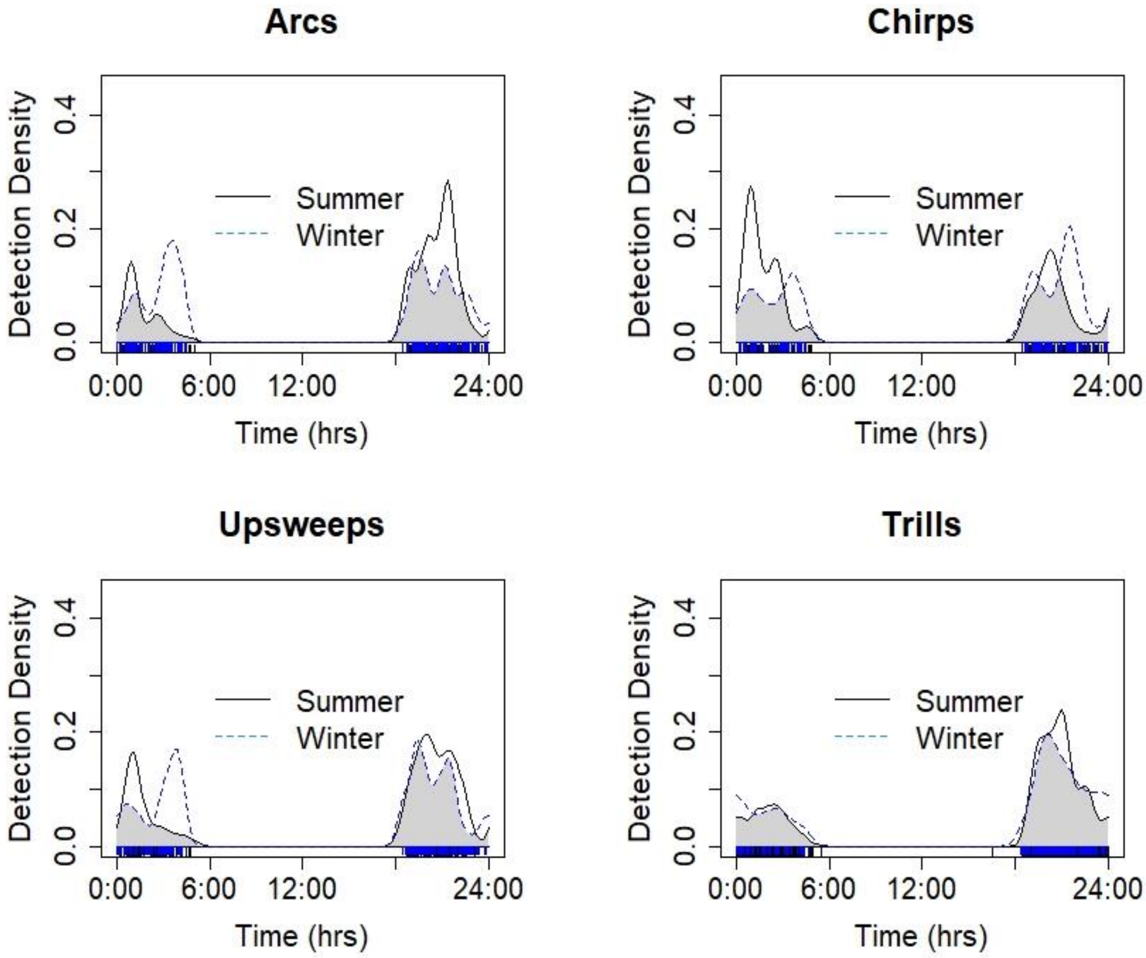
Appendix G

Appendix G: Overlap of temporal patterns of call types collected in Humboldt County, California, USA from 2018-2021. Call type data were not separated into events to show densities of total calls recorded for each call type during each season.



Appendix H

Appendix H: Overlap of temporal patterns of call types San Bernardino County, California, USA from 2018-2020. Call type data were not separated into events to show densities of all calls recorded for each call type during each season.



Arcs

Chirps

Upsweeps

Trills