2005

Quantification of Call Variations Between Sub-Regional Florida Scrub Jay (*Aphelocoma coerulescens*) Populations

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Quantification of call variations between sub-regional Florida Scrub Jay (Aphelocoma coerulescens) populations

A Thesis

Presented to

the Faculty of the Department of Environmental Science

Taylor University

In Partial Fulfillment

of the Requirements for the Degree

Master of Environmental Science

by

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June 2005

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Title: Quantifying call variations between sub-regional Florida Scrub Jay (Aphelocoma coerulescens) populations
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Degree Date: June, 2005

Abstract

The Florida Scrub Jay (*Aphelocoma coerulescens*) has seen significant and accelerating declines in its overall population numbers in the past 100 years. This is primarily the result of habitat loss and population fragmentation, which has isolated the extremely territorial Florida Scrub Jay into smaller sub-regional populations. In many ways this is a catalyst for social evolution and divergence of the species. An early indicator of divergent evolution can be witnessed in the development of distinct call dialects. By recording the female hiccup calls of the three largest distinct sub-regional populations (Atlantic Coast sub-region, Ocala sub-region and the Lake Wales Ridge sub-region), the divergence in dialects can be quantified. Using digital recording equipment and two popular sound analysis programs, 12 birds from each sub-region were compared for similarity. Principal Coordinate Analysis and ANOVA tests were then used to find the significance of the differentiation between the populations. The final analysis has revealed a significant difference between the female hiccup calls of the Lake Wales Ridge sub-region (Archbold Biological Station), the Ocala sub-region (Ocala National Forest) and the Atlantic Coast sub-region (Canaveral National Seashore/Merritt Island Wildlife Refuge).

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Introduction

The Florida Scrub Jay (*Aphelocoma coerulescens*) has been observed for centuries and intensely studied since 1969, predominantly at Archbold Biological Station (Woolfenden and Fitzpatrick 1984; Woolfenden and Fitzpatrick 1990; Woolfenden and Fitzpatrick 1996). This intense study of the Florida Scrub Jay has documented the population decline toward extinction, which in turn lead to its designation as a Threatened Species on June 3, 1987 (Cox 1987; USFWS 1999). However, the population numbers have continued to fall and by 1999, the scrub jay had lost 25%-50% of the total population over the past 12 years (USFWS 1999). The total number now has fallen below 10,000 birds despite protection, and is believed to be endangered (Woolfenden and Fitzpatrick 1996; USFWS 1999). The Florida Scrub Jay is highly sensitive to habitat and as a result, one of the largest causes of population decline is the loss and fragmentation of scrub habitat (Woolfenden and Fitzpatrick 1990; Bergen 1994; Woolfenden and Fitzpatrick 1996; USFWS 1999). Accelerated removal and fragmentation of scrub habitat is being driven primarily by urban and agricultural development (Myers 1990; Fitzpatrick et al. 1991, cited in USFWS 1999; Breininger et al. 1999). Historically, fragmentation of scrub habitat may have been a major factor in population isolation and divergent evolution (McDonald et al. 1999; Stenzler 2001). At present, the majority of remaining jays are found in five large, though disjointed, sub-regional populations (Fig. 1). Within these five sub-regions lie three "core populations:" the Lake Wales Ridge sub-region, the Atlantic Coast sub-region and the Ocala sub-region (Fitzpatrick et al. 1994, cited in USFWS 1999). The Florida Scrub Jay is strictly sedentary, unlike its close relative, the Western Scrub Jay (McDonald et al. 1999). The result is genetic variation, which may correlate to habitat fragmentation more than geographic distance (McDonald et al. 1999; Stenzler 2001).

Vocal dialects can be defined as temporal or spatial variations of the auditory communication system, which are limited to groups of individuals (Martens 1996). Distinct vocal dialects signaling early divergent populations are not a new idea; however, dialects driving divergent evolution remain controversial (Marler and Tamura 1962; Nottebohm 1969; Baker 1975; Baptista 1975; Baker and Mewaldt 1978; Baker and Cunningham 1985; Petrinovich 1985; Zink 1985; Slabbekoorn and Smith 2002). If the Florida Scrub Jay call has developed dialectic characteristics, it is likely due to learning, not from genetic transmission of the calls (Webber and Stefani 1990; Lynch 1996; Payne 1996; Midford 1999). Therefore, while dialect divergence may shadow genetic divergence, they are not directly connected (Baker and Thompson 1985). Any vocal dialects in the scrub jay population would most likely be a result of population fragmentation, which is propelling divergent evolution; however, in the future, vocal dialects could help to accelerate genetic isolation (McDonald et al. 1999; Slabbekoorn and Smith 2002).

Dialectic variations across songbird populations are a common occurrence and have been documented in many species (Lemon 1966; Thielcke 1969; Lemon 1971; Kroodsma 1974; Lemon and Harris 1974; Jenkins 1977; Bradley 1977; Goldstein 1978; Baker and Cunningham 1985; Slater 1986; Payne et al. 1988; Shackell et al. 1988; Lynch and Baker 1993; Martens 1996; Baker 2003; Baker et al. 2003; Baker and Logue 2003). In contrast, geographic variations in the most common calls of the Florida Scrub Jay have only been compared minimally. Specifically, the rapid multiple weep and female hiccup calls have been compared with short written descriptions and visual comparisons of spectrograms (Woolfenden and Fitzpatrick 1996). Visual and auditory inspections of the calls appear to indicate dialectic variations between sub-regions; in spite of this, there has previously been no statistical data to support this assumption (Woolfenden and Fitzpatrick 1996).

Digital acoustic analysis for the purpose of quantifying and characterizing similarities and differences in complex vocalizations has become increasingly popular (Nowicki and Nelson 1990; Wright 1996; Janik 1999; McCowan and Reiss 2001; Tchernichovski et al. 2000, 2001; Baker 2003; Baker et al. 2003; Baker and Logue 2003). Two of the more common techniques for digital sound comparison are spectrogram cross-correlation (SPCC) and multi-taper spectral analysis (MTSA) (Khanna et al. 1997; Cortopassi and Bradbury 2000; Tchernichovski et al. 2000, 2001; Baker 2003; Baker et al. 2003; Baker and Logue 2003). SPCC, the older method of sound comparison, is most commonly embodied in Cornell Lab of Ornithology's *Canary* software and is now being revamped for future release versions of its *Raven* software (Charif et al. 2003; Charif et al 1995). This technique slides two spectrograms over each other in small incremental steps to determine the percent similarity between the two sounds (Charif et al. 1995; Khanna et al. 1997; Cortopassi an Bradbury 2000; Baker and Logue 2003). SPCC has been debated in the past as an inferior method (Khanna et al. 1997); however, user error seems to be the cause of most problems, not a general failure of the method (Cortopassi and Bradbury 2000). MTSA is a newer technique which allows a greater level of accuracy in the analysis of complex bird songs. *Sound Analysis Pro* employs the most recent incarnation of the method (Tchernichovski et al. 2000, 2001; Baker 2003; Baker et al. 2003; Baker and Logue 2003; Tchernichovski and Mitra 2004).

Digital recording to hard drives, unlike digital acoustic analysis, is being adopted much more slowly. It is often shunned in favor of the more readily available and cheaper tape, R-DAT and MiniDisc recording setups (Grotke 2004). Digital recording to a hard drive appears much improved over conventional tape recordings because the final recordings are much cleaner and lack the traditional hiss and recording speed distortions found in tapes. Additionally, tapes, R-DAT and MiniDisc recording setups typically employ 3.5 mm mini-jack connectors, which degrade sound quality. Recording digitally to a mobile computer also allows immediate analysis in the field, and accommodates faster analysis of recorded sound. Both *Raven* and *Sound Analysis Pro* include built in recording features (Charif et al. 2003; Tchernichovski and Mitra 2004). Though mobile computers and hard drives may be more bulky, recording to this medium is more efficient to work with and produces cleaner sounds.

Modern digital recording techniques have not been applied in the majority of vocalization studies on the Florida Scrub Jay; however, one study did use *Canary* for call analysis (Barbour 1977; Elowson and Hailman 1991; Owen-Ashely et al. 2002). Combining the known benefits of modern digital computer recording and analysis into a study of the dialectic variations of the Florida Scrub Jay has the possibility to produce much more accurate results.

One of the more recognizable calls of the Florida Scrub Jay is the female hiccup (Barbour 1977; Woolfenden and Fitzpatrick 1984; Woolfenden and Fitzpatrick 1996). First officially classified in 1977, the female hiccup call is recognized by most field ornithologists to exhibit noticeable auditory differences between sub-regional populations. This characteristic, however, has only been noted briefly in publications and has never been fully explored (Barbour 1977; Woolfenden and Fitzpatrick 1996). Females issue the call most often in territorial disputes or while protecting the nest; it alerts males of an altercation and indicates the female's support for the male (Barbour 1977; Woolfenden and Fitzpatrick 1984, Woolfenden and Fitzpatrick 1996). The 1977 Barbour study focused exclusively on Florida Scrub Jay calls from the Lake Wales Ridge population at Archbold Biological Station (Barbour 1977). As a result, the classification ignores the variations found in other regions, which could be an early indicator of genetic isolation and divergent evolution.

The unique challenges of doing a similarity study on the female hiccup call is three fold. First, the call can be rather faint and difficult to capture from a distance. As a result, recordings must be taken within a close proximity to the female. Second, the call is a series of sharp "kloks" and hence recordings must have very little background noise (Barbour 1977). This makes noise reduction software difficult to use, as it tends to remove the hiccups along with background noise. Finally, because the call is not harmonically complex, there are not enough acoustic features for multi-taper signal analysis to examine for similarities in the traditionally accepted manor (Tchernichovski and Mitra 2004; Tchernichovski, 02-22-2005, personal communication, not referenced). The challenge is therefore to find a way to negotiate these difficulties and still find an appropriate statistical method to quantify call variations. Hence, it was necessary to design the study in such a way that it could answer the questions presented by the hypothesis but also avoid the pitfalls presented above.

The working hypothesis for this study is: digital hard drive recordings and digital analysis techniques will show a statistically significant differentiation in the female hiccup calls of the three largest sub-regional populations of the Florida Scrub Jay. The goal of this study was to examine the performance of two digital analysis programs as they classify several female hiccups from each of the three core populations within Florida. The similarity scores produced by *Canary* will be subjected to statistical analysis using Principal Coordinate Analysis (PCoA) and subsequent Linear Discriminate Analysis (LDA) for plotting samples in a two-dimensional acoustic space, (Legendre and Legendre 1998; Cortopassi and Bradbury 2000; Baker 2003; Baker and Logue 2003). Features calculated from *Sound Analysis Pro* will be tested using a one-way ANOVA and a (univariate) nested factorial. This will allow examination and classification of any dialectic variation of the female hiccup call that may be found to exist while evaluating two common sound analysis programs and their ability to handle the harsh, raspy and sharp sounds of the Florida Scrub Jay calls.

Methods and Materials

Population Samples and Recording

From June through August of 2004 and January of 2005, recordings were made in the three largest population sub-regions of the Florida Scrub Jay: Lake Wales Ridge (Archbold Biological Station), Ocala (Ocala National Forest), and Atlantic Coast sub-regions (Canaveral National Seashore/Merritt Island National Wildlife Refuge) (Fig. 2). Within each of these geographically distinct sub-regions, 12 individual female birds were analyzed. Local experts helped locate groups of banded birds for recording. All birds recorded in this study were banded with both US Fish and Wildlife metal bands and colored plastic band combinations specific to each recording location. This eliminated the possibility of repeat recordings of the same bird. The female calls were recorded digitally to an Apple iBook (700MHz PowerPC G3, 384 MB RAM, OS X Version 10.2.6, Apple Computer Inc., 2003) running *Felt Tip Sound Studio 2.1.1* (24-bit depth, 44 kHz rate, mono channel, WAV audio format, Kwok 2003) with a shotgun microphone (Audio-technica 897, line+gradient condenser microphone, frequency response 20-20,000Hz, Audio-technica U.S., Inc., Stow, Ohio) mounted on a 54 cm parabolic dish. The microphone was connected to the Apple iBook via an audio interface (Tascam US-122 USB, 24-bit depth, 44 kHz rate) (Fig. 3).

Calls were first run through a 1000Hz High Pass filter to remove background noises. They were then normalized, and re-sampled according to the specifications of the computer program used to analyze similarity. Calls were selected based on the greatest signal to noise ratio and relative freedom from other bird songs as per Baker and Logue (2003). The four clearest beginning sequential hiccups (syllables) were selected for each bird at each population for similarity analysis. Hiccups were taken from the beginning of each hiccup sequence thereby standardizing which hiccups were used. Call files were zero-padded before spectrogram correlation so that each sound file was exactly 500ms long. This was to eliminate problems associated with comparing spectrograms of different lengths (Cortopassi and Bradbury 2000; Baker and Logue 2003).

Analysis of Calls: Spectrogram Cross-Correlation

The first employed method of analysis was Cornell Laboratory of Ornithology's *Canary 1.2.4* (Charif et al. 1995). Spectrograms for each file were created in *Canary* with the following parameters: 128 point frame length (1398.80 Hz bandwidth), 0.0907ms time grid resolution with 96.88% overlap, 256 point Fourier transform size, Hamming window, boxy, and –80 dB default clipping level (Charif et al. 1995; Cortopassi and Bradbury 2000). *Canary* compared call files using Spectrogram Cross-Correlation (SPCC), which compares two sound spectrograms frame by frame in time-frequency domains (Charif et al. 1995; Cortopassi and Bradbury 2000). Visually, this means the four hiccups from two calls are slid over each other and a correlation coefficient is calculated at each incremental change (Baker and Logue 2003). The best match between the two files is given by a coefficient between 0 and 1. All 36 four-syllable calls were batch processed by *Canary*, which provided a triangular matrix of similarity values (Charif et al. 1995; Baker and Logue, 2003). Statistical analysis for SPCC was run using the similarity matrix in *R-package* (Version 4, Casgrain and Legendre 2001). The similarity matrix was imported into *R-package* and converted to a distance matrix using the function $D=(1-S)^{-0.5}$ (Legendre and Legendre 1998; Cortopassi and Bradbury 2000; Baker and Logue 2003). A Principal Coordinate Analysis (PCoA) was then performed on the distance matrix, which produced eigenvectors that described call positions in reduced space (Baker 2003; Baker and Logue 2003). The first five eigenvalues accounted for more than 50% of variance and therefore five eigenvectors were used in a Linear Discriminate Analysis (LDA) performed in *SPSS version 12* (Cortopassi and Bradbury 2000; Baker 2003; Baker and Logue 2003; SPSS Inc. 2003). The LDA provided canonical variate scores for plotting relative positions in two dimensions and Wilks' Lambda (λ) was used to determine significance (Baker 2003; Baker and Logue 2003) (Fig. 4). The post-hoc test, Fishers LSD, was used to determine statistical difference between the three samples (Baker 2003; Baker and Logue 2003).

Analysis of Calls: Multi-taper Spectral Analysis

The second method used *Sound Analysis Pro* (SA+ version 1.04a, Tchernichovski and Mitra 2004). *Sound Analysis Pro* measures similarity from five acoustic features extracted from the calls by multi-taper spectral analysis techniques. These five features: pitch, frequency modulation (FM), amplitude modulation (AM), Wiener entropy and goodness of pitch, are then compared using a batch similarity to produce a similarity matrix akin to the final matrix produced by the SPCC technique (SA+ version 1.04a, Tchernichovski and Mitra 2004). However, the female hiccup call is a very simplistic and feature poor call, for this reason *Sound Analysis Pro* could not perform a similarity batch which produced accurate and reliable results (Tchernichovski and Mitra 2004;

Tchernichovski, 02-22-2005, personal communication, not referenced). Therefore, on all 36 calls, *Sound Analysis Pro* was used to run feature batch procedures. This determined the inter-syllable space duration and the total time, starting at the beginning of the first syllable and ending after the last syllable (Fig. 5). Statistical tests run on this data were executed in *SPSS version 12* and *Minitab version 14* (SPSS Inc. 2003; Minitab Inc. 2003). All data was tested for normality and a one-way analysis of variance (ANOVA) test was performed on the 36 four-syllable total time durations. A (univariate) nested factorial model testing with an analysis of variance was used on the inter-syllable space duration call samples for all 36 jays, which resulted in the examination of 108 inter-syllable space durations.

Results

Canary results analyzed with Principal Coordinate Analysis

The first five eigenvalues accounted for 51.13% of the variance and there was no need for negative eigenvalue correction because 35 eigenvalues extracted by PCoA accounted for 100% of the variance. Comparisons of the first five eigenvalues indicated significant heterogeneity among sub-regions (Wilk's $\lambda = 0.006$, $p < 0.001$). Post hoc tests with Fisher's LDS showed that all three sub-regions differed from each other (LWR v. O, $p < 0.001$; LWR v. AC, $p < 0.001$; AC v. O, $p < 0.001$). The LDA correctly assigned all 36 calls to their respective sub-regions and a scatter plot of the degree of separation in coordinate space was created from canonical variates one and two (Fig. 4). The PCoA and LDA show that there are significantly distinct dialects at each sub-region location and that each sub-regional location is significantly different from each of the other sub-regional locations.

Sound Analysis Pro results analyzed with Analysis of Variance

The 36 four-syllable overall duration measurements were tested for normality. They were found to be normal using the Shapiro-Wilk test for normality $p > 0.05$. There was significant difference between the sub-regional call overall duration, $F(2, 33) = 174.8$, $p < 0.001$. The means were separated using Tukey's Honestly Significant Difference, results shown in Figure 6 (Ocala = 284.1; Atlantic Coast = 332.8; Lake Wales Ridge = 375.8). This shows that there are significant differences in the length of call delivery between each sub-regional population.

The 108 inter-syllable space duration measurements distilled from the 36 birds in three sub-regions were tested for normality and were found to be normal using the Shapiro-Wilk test for normality $p > 0.05$. Analysis of variance for interaction between sub-region and inter-syllable space duration showed a significant difference between populations, $F(4, 66) = 44.6$, $p < 0.001$. The means were separated out and the results of the inter-syllable space interaction with inter-syllable space mean duration and sub-region are displayed in Figure 7. This shows that the duration rhythm between syllables is not the same for all sub-regions and in fact it appears that Lake Wales Ridge sub-region was the reason. Hence, a second analysis of variance was run using just the Atlantic Coast and Ocala sub-regions and no significant difference was found in the interaction between sub-region and inter-syllable space duration, $F(2, 44) = 1.69$, $P = 0.197$. Therefore, the Lake Wales Ridge sub-region is significantly different than the other two in inter-syllable space duration delivery (slow-quick, slow-quick). The Atlantic Coast and Ocala sub-regions are not significantly different from each other in delivery of inter-syllable space duration, each duration delivery is of approximately the same length every time (quick-quick-quick-quick) (Fig. 7).

General descriptions of calls in their entirety

The Lake Wales Ridge sub-region call variation appears very similar to previous classifications (Barbour 1977). This is the overall slowest of the three variations, with a mean four-syllable length of 375.8 ms, and contains the unique two syllable, loud/soft staccato (Fig. 8). The sound, whut-TIK-whut-TIK-whut-TIK-whut-TIK, is the most commonly recorded and recognized representation of the hiccup call (Woolfenden and Fitzpatrick 1996). The rhythm is significantly different than the other two calls because the inter-syllable space duration is not similar between all syllables (Barbour 1977). Instead, there is a long 112 ms pause between the loud syllable and the soft syllable, then a shorter 88 ms pause between the soft syllable and the next loud syllable (Fig. 5). The entire length of the call varies but commonly is found in the 3-4 s range. The Lake Wales Ridge sub-regional call is significantly distinct from Ocala and Atlantic Coast sub-regions in regards to both rhythm and speed of call delivery.

The Ocala sub-region population in the Ocala National Forest produces the hiccup call very rapidly; the mean four-syllable duration is 284.1 ms (Fig. 9). The general sound, TK-TK-TK-TK-TK-TK-TK, has been described as analogous to the Atlantic Coast sub-region (Woolfenden and Fitzpatrick 1996) and this is correct to some degree. There is not a significant difference between the rhythms of the calls in the two sub-regions. However, the ANOVA of overall length does show a significant difference in speed of delivery. A second feature to note is the presence of a softer, preceding twin hiccup within each syllable. *Sound Analysis Pro* did not separate the twin hiccups into distinct syllables; instead, it grouped them as one larger syllable. Yet, examinations of the waveform and spectrogram created in *Canary* reveal its existence (Fig. 6).

It is worth noting that a few rare cases were recorded at the Ocala National Forest where the call speed started rapidly and slowed near the end of the call. Mean inter-syllable space duration in these calls was 54.2 ms during the first half of the call but slowed to an average of 89.3 ms in the second half. This call was recorded several times but always from the same bird and, as such, is likely an anomaly of that specific female's call. The Ocala sub-regional call is significantly distinct from Lake Wales Ridge and Atlantic Coast sub-regions in regards to speed of call delivery, but is not distinct from the Atlantic Coast sub-region in terms of rhythm.

The Atlantic Coast sub-region population at Canaveral National Seashore typically performs the hiccups in multiple short bursts (Fig. 10). These bursts are usually less then a second, decrease in length by one syllable with each repeated burst and are preceded by a soft conversational gluttural (Woolfenden and Fitzpatrick 1996). Even though it is more common to hear the short bursts, the population does occasionally perform the hiccup call for more extended periods of time. The guttural is only separated from the first syllable on average by 29.5 ms (Fig. 5). This is a very small distance when you consider that the mean inter-syllable space duration is 91.4 ms. The general sound is, TK-TK-TK-TK-TK-TK-TK, which is, as noted above, very similar to the Ocala sub-region (Woolfenden and Fitzpatrick 1996). However, the Canaveral population delivers the syllables at a significantly less rapid pace; the mean four-syllable duration is 332.8 ms in length. The Atlantic Coast sub-regional call is significantly distinct from Lake Wales Ridge and Ocala sub-regions in regards to speed of call delivery but is not distinct from the Ocala sub-region in terms of rhythm.

Discussion

For the first time, this study statistically demonstrates that significant dialectic variation in the female hiccup call exists between the three largest sub-regional populations of the Florida Scrub Jay. The PCoA followed by an LDA is a commonly practiced statistical method for determining the existence of dialectic variations in bird populations (Legendre and Legendre 1998; Cortopassi and Bradbury 2000; Baker 2003; Baker and Logue 2003). It is regularly used throughout the biological sciences as well as the social sciences and has been widely accepted as legitimate. However, the PCoA model and the canonical plot ignore some objectionable mathematics (Gower 1966). The problem lies in the model being based on perceived similarity tests instead of concrete, measurable characteristics. PCoA must be used with caution and recognized as a tool but not a perfect tool. That being said, the PCoA model produced discrete geographic separation and placed all the calls in their appropriate geographic sub-regions. This plot shows very clearly that there are in fact distinct variations of the female hiccup call in all three sub-regions.

The one-way ANOVA and (univariate) nested factorial ANOVA are important because they use sound, classic statistics to measure the variations in the rhythm of the calls. It was employed to bolster the findings of the PCoA and add some additional mathematical robustness to the findings. This is a unique situation because the nature of the hiccup call allowed inter-syllable space duration measurements, a luxury more complex call comparisons cannot examine. The one-way ANOVA and Tukey's HSD, which were run on the four-syllable total duration, showed significant differences between the three sub-regions. This is significant statistical evidence that all the sub-regions involved were distinct from one another in their speed of delivery. The (univariate) nested factorial looked more closely at the inter-syllable space duration lengths and how they varied over a four-syllable call section. This is noteworthy because it showed that not all the sequences of inter-syllable space are the same. Instead the Lake Wales Ridge sub-region delivers the call in a slow-quick, slow-quick time segment rhythm as opposed to the other two sub-regions, which deliver the syllables in approximately equal time segments. Therefore, not only is there significant variation between speeds of delivery, there is also significant variation in rhythm between the Lake Wales Ridge sub-region and the other two sub-regions. The Lake Wales Ridge sub-regional dialect is by far the most distinct of the three variations, displaying significant differences in syllable structure, duration and rhythm. The Ocala and Atlantic Coast variations are distinct from one another as a result of the significant differences in duration. However, they lack the significant differences in syllable structure and rhythm that make the Lake Wales Ridge sub-region so unique.

The female hiccup call has few acoustic features and presented a unique challenge to analyze. This resulted in an inability of the MTSA technique to perform similarity test in the traditionally accepted manor (Tchernichovski et al. 2000, 2001; Baker 2003; Baker et al. 2003; Baker and Logue 2003; Tchernichovski and Mitra 2004). The inability of MTSA to distill acoustic features from the female hiccup call is likely unique to this specific call and not all Florida Scrub Jay calls. Further dialect investigation of calls, such as the rapid multiple weep and screech scold, would likely be able to produce enough acoustic features to run MTSA similarity batch analysis. A focus shift to the rhythm of the call was therefore necessary in this study to produce call characteristic numbers for statistical analysis, which proved to be successful.

Conversely, SPCC did not struggle with the hiccup call primarily because of the simplicity of its comparative technique. The spectrograms created using Fast Fourier Transforms only use two characteristics to create an image, time and frequency (Charif et al. 1995). As a result, the lack of acoustic features did not affect the similarity comparisons. In fact, the SPCC was a good fit because it was comparing precisely the two elements that create the dialectic variation.

As noted before, the extent of background noise removal only entailed a 1000 Hz high pass filter. Further removal of background noise proved to be nearly impossible. Previous studies have used the shareware program *GoldWave* (v4.26; http://goldwave.com) to reduce the impact of background noise and improve the signal to noise ratio (Tchernichovski et al. 2001; Baker 2003; Baker et al. 2003; Baker and Logue 2003). However, in this study, attempting to remove background noise with the previously used technique resulted in the loss of the hiccup syllables. The software tended to view the hiccups as noise because of the lack of easily definable acoustic characteristics. For this reason, further noise reduction procedures were abandoned. In turn, this put added pressure on the recording process to produce the clearest, loudest and most noise free recordings possible. Hence, many of the calls we recorded were eliminated simply because they lacked the clarity and noise-free requirements. The ability to examine calls for recording quality in the field was a large improvement over taking a tape recording back to a lab and then having to convert it to digital files for examination.

The use of a laptop to record the sounds produced very good results primarily because it eliminated the use of a 3.5 mm mini-jack plug and tapes. Mini-jack plugs are the most common way to connect microphones to both tape and digital recorders. However, it causes a bottleneck in recording set-ups because it degrades sounds by adding significant amounts of background hiss before it even reaches the intended recording medium. Consequently, bypassing the use of a 3.5 mm mini-jack with the audio interface and USB cable produced considerably higher quality. Avoiding conventional tapes also improved recordings and sidestepped problems with background hiss and recording speed distortions. The end result was sound quality more akin to a studio then typical field recordings.

For the first time, modern digital techniques in recording and analysis were applied to Florida Scrub Jay calls. The results are more clearly defined and accurate because of the use of objective statistics instead of subjective descriptions. When comparing the two methods of statistical quantification, the combination of the two procedures strikes me as a much more robust and holistic process then singling out either PCoA or the analysis of variance specifically. It is worth noting that both methods need to be carefully monitored throughout the recording, filtering and statistical analysis phases. Without this constant vigilance, error can easily be introduced into both methods. However, I believe that the use of *Sound Analysis Pro* and the focus on rhythm provided the most repeatable, robust and simplest method for analyzing the female hiccup calls. This of course is only applicable in the case of the hiccup call because *Sound Analysis Pro* will likely be able to calculate the other Florida Scrub Jay calls acoustic features. This in turn will allow for both standard statistical analysis as well as PCoA calculated from similarity batches produced from acoustic features. Once again, provided *Sound Analysis Pro* can get a robust feature calculation, I believe that it will perform superior to *Canary*. This is in large part due to *Sound Analysis Pro's* ability to compare more features then *Canary*, and *Canary* is an older program which is being phased out. At the completion of this study, Cornell's Lab of Ornithology had not integrated a similarity comparison technique into the *Raven* software. It is therefore unclear how *Raven* will compare to *Sound Analysis Pro*. I am sure that there will remain debate concerning the best method, but both techniques can be used on the Florida Scrub Jay call, and each may be necessary to address the many unanswered questions remaining.

Conclusions and Future Study Recommendations

This is only a preliminary study with small call sample sizes of one of the most recognizable Florida Scrub Jay calls, from three specific locations within the three largest sub-regions. There are likely other dialectic variations in Florida Scrub Jay calls including the female hiccup, rapid multiple weep and the screech scold (Woolfenden and Fitzpatrick 1996). Because this study was limited in scope, one of the obvious first steps would be a more in depth research of the variations of hiccup calls.

There are a number of different issues for future studies to examine. Recordings from the smaller North Gulf Coast sub-region and Southern Gulf Coast sub-region should be compared to those of the other three sub-regions. Recordings and comparisons within sub-regional populations should be examined, particularly the Atlantic Coast sub-region, which spreads out over a far greater distance then Ocala and Lake Wales sub-region's combined. Additionally, there needs to be greater examination of the relationship between call variation, geographic distance and population fragmentation. One of the most interesting future options would include comparing genetic variation with call variation and examining the interaction, if any, of the two elements. Much like studies of genetic diversity, this research cannot be put off indefinitely. If the population numbers continue to plummet, many localized variations could be lost before further examination is completed.

Due to the distinctive and recognizable nature of the female hiccup call, it seems important that a general description and knowledge of dialects be presented to the general public. Typically, commercial audio birdcall collections only contain the Lake Wales Ridge variety of the female hiccup. Florida Scrub Jay literature intended for the general public also tends to lack detailed descriptions of sub-regional or geographic dialectic variations with the exception of Woolfenden and Fitzpatrick (1996). Hopefully, this re-examination of one of the most noticeable Florida Scrub Jay calls will lead to further investigation and a more detailed classification of the calls with specific consideration for dialects.

Acknowledgements

Thanks to Edwin Squiers. John Stiner and Mark Epstein for providing housing and advice. John Puschock, Glen Woolfenden, Reed Bowman and Becky Antworth for help locating banded birds to record. Ofer Tchernichovski for advice and logistics help, warm regards. Dr. Ken Constantine and Robert Reber for statistics help and advice. Alexis Cole, thanks for everything, you're indispensable. Research carried out under Canaveral National Seashore Scientific Research and Collecting Permit *CANA-2004-SCI-0007* and Merritt Island National Wildlife Refuge *2004 SUP 54*. Special thanks to Leland Boren for provided funding.

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Figure Legends

Figure 1. Locations of Florida Scrub Jay sub-regions and habitats connecting the sub-regions; over 50% live in the Ocala, Atlantic Coast and Lake Wales Ridge sub-regions. Adapted from Fitzpatrick et al. 1994; USFW 1999.

Figure 2. Three recording locations in relation to the distribution of Florida Scrub Jay populations with an 8.2 km dispersal buffer zone. Adapted from USFW 1999.

Figure 3. Diagram of sound recording setup. The elimination of 3.5 mm mini-jack plugs and tapes helped to produce superior recordings.

Figure 4. Scatterplot of canonical variate one and two describing the separation between sub-regions and site specific clustering of the 36 recorded Florida Scrub Jay female hiccup calls.

Figure 5. At top is a spectrogram and below is the wave form of an Atlantic Coast sub-region call short burst. The preceding glutteral was only found in the Atlantic Coast sub-region call. Additionally, inter-syllable space duration, syllable, and total time for four-syllables are all noted.

Figure 6. A comparison bar graph of means for overall four-syllable duration between sub-regions. *Means followed by same letter are not significant at the 0.05 alpha level.

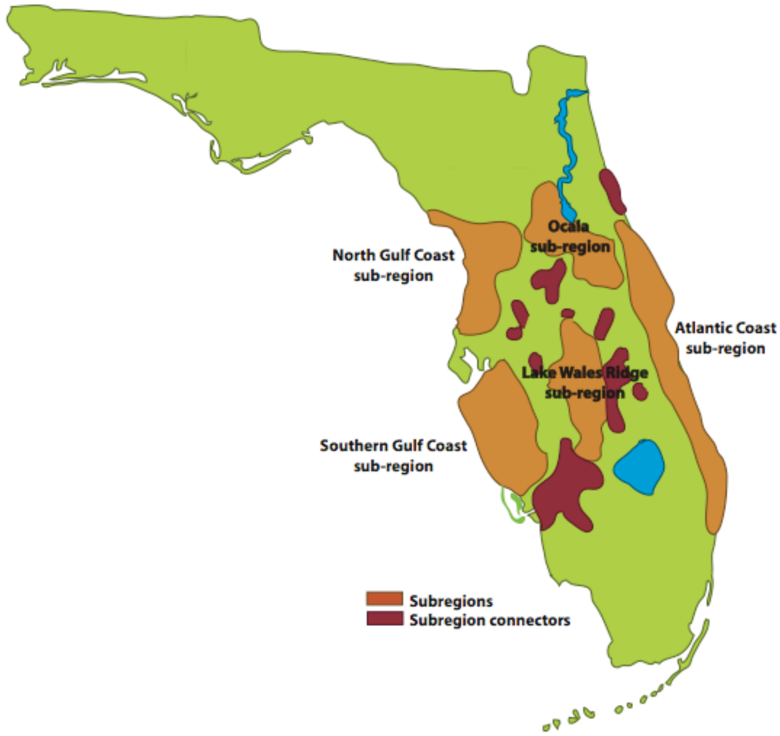
Figure 7. Inter-syllable space interaction with inter-syllable space mean duration and sub-region. The vertical axis shows the separation between each sub-region as it pertains to inter-syllable space duration for each inter-syllable space. The horizontal axis shows that the Lake Wales Ridge sub-region is significantly different in terms of rhythm than either the Ocala or Atlantic Coast sub-regions because it is not parallel to the others. Both Ocala and Atlantic Coast sub-regions are parallel and therefore are not significantly different from each other in terms of rhythm of inter-syllable space.

Figure 8. At top is a spectrogram and below is the wave form of the Lake Wales sub-region call. The loud-soft-loud-soft syllables and slow-quick-slow-quick rhythm are only found in the Lake Wales sub-region call.

Figure 9. At top is a spectrogram and below is the wave form of the Ocala sub-region call. The twin hiccup is not found in the Lake Wales or Atlantic Coast sub-regions.

Figure 10. Atlantic Coast sub-region short bursts preceded by guttural. Note the decreasing number of hiccups in the second series, this pattern of subtracting one syllable is common in the Atlantic Coast sub-region.

Figures



Ocala
sub-region
North Gulf Coast
sub-region
Atlantic Coast
sub-region
Lake Wales Ridge
sub-region
Southern Gulf Coast
sub-region
Subregions
Subregion connectors

Figure 1.

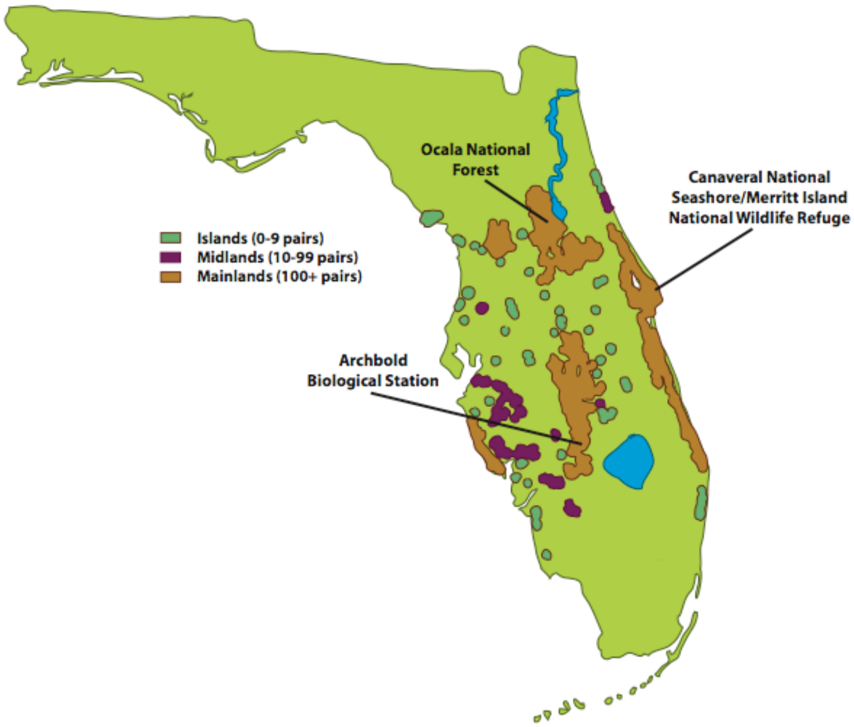

Ocala National Forest
Canaveral National Seashore/Merritt Island National Wildlife Refuge
Islands (0-9 pairs)
Midlands (10-99 pairs)
Mainlands (100+ pairs)
Archbold Biological Station

Figure 2.

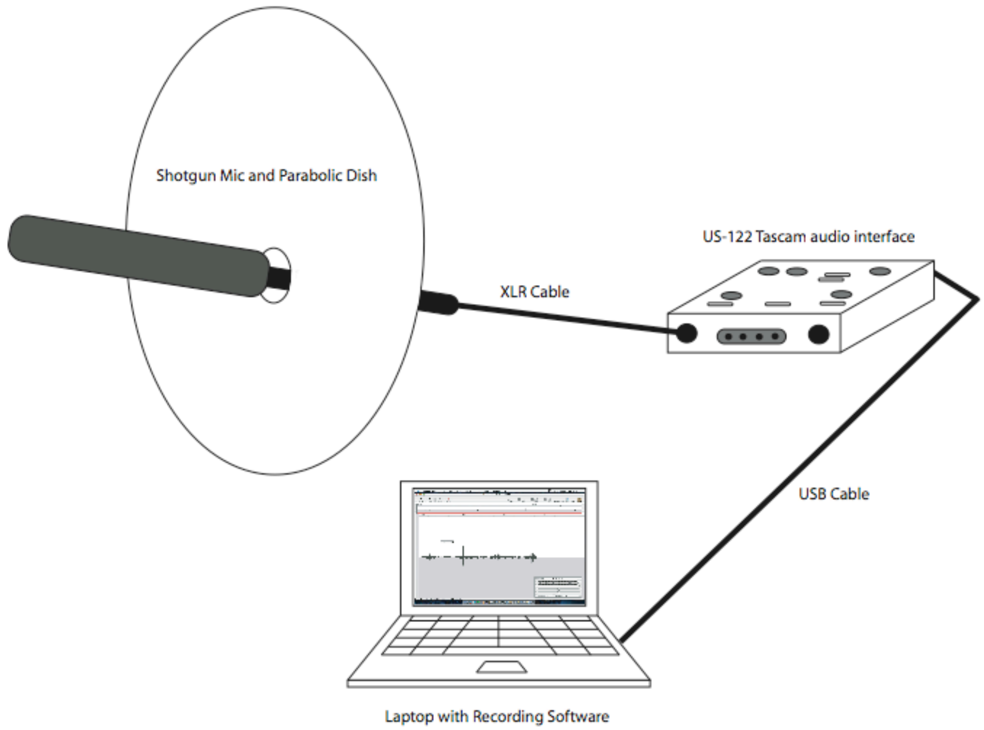
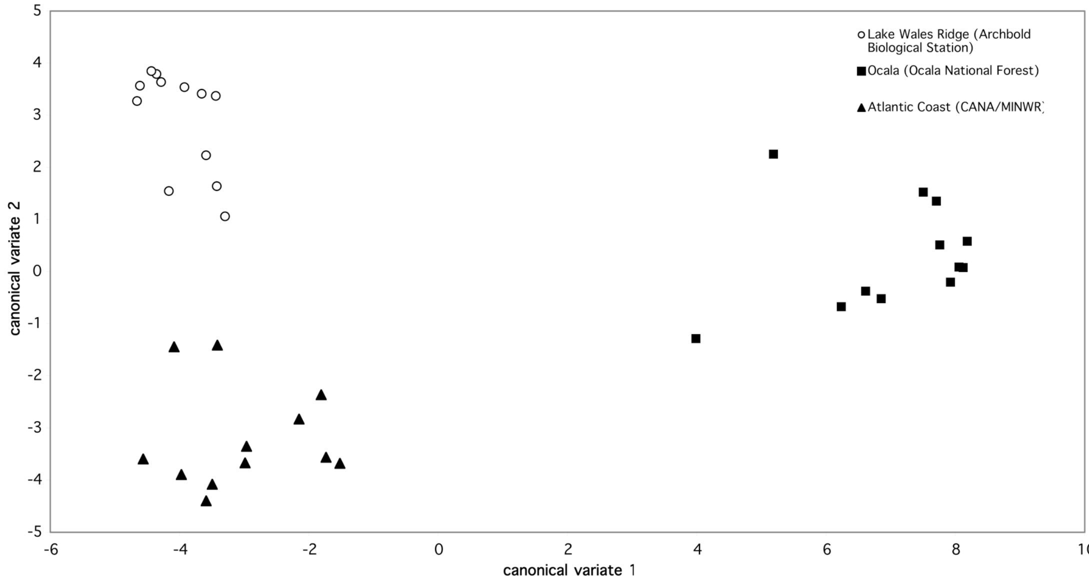

Shotgun Mic and Parabolic Dish
US-122 Tascam audio interface
XLR Cable
USB Cable
Laptop with Recording Software

Figure 3.



Lake Wales Ridge (Archbold Biological Station)
Ocala (Ocala National Forest)
Atlantic Coast (CANA/MINWR)
canonical variate 2
canonical variate 1
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
-4
-2
0
2
4
6
8
10

Figure 4.

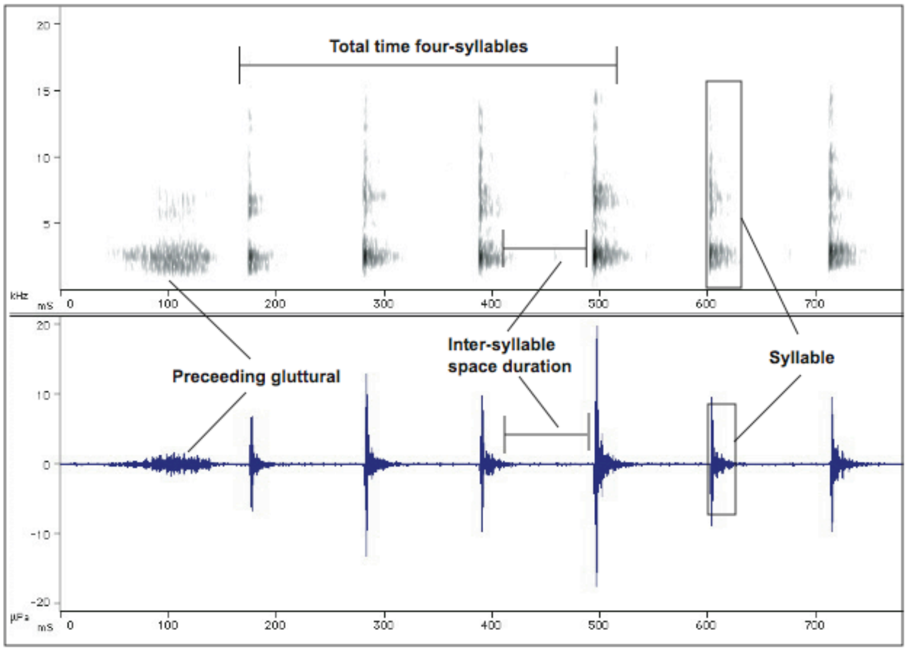

Total time four-syllables
Preceeding gluttural
Inter-syllable
space duration
Syllable
kHz
mS
μPa
mS

Figure 5.

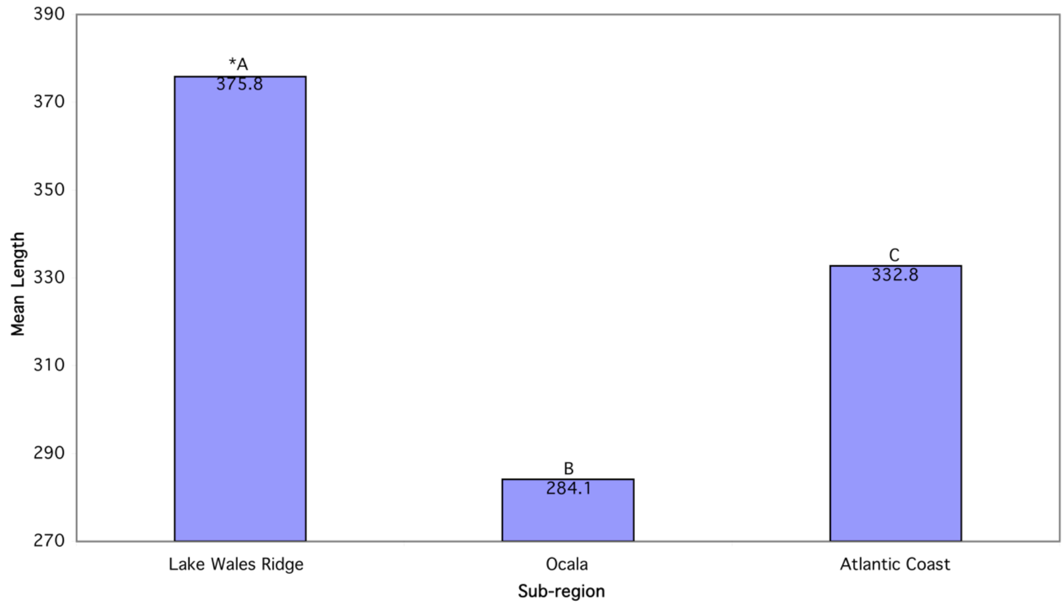

390
370
350
330
310
290
270
Mean Length
*A
375.8
B
284.1
C
332.8
Lake Wales Ridge
Ocala
Atlantic Coast
Sub-region

Figure 6.

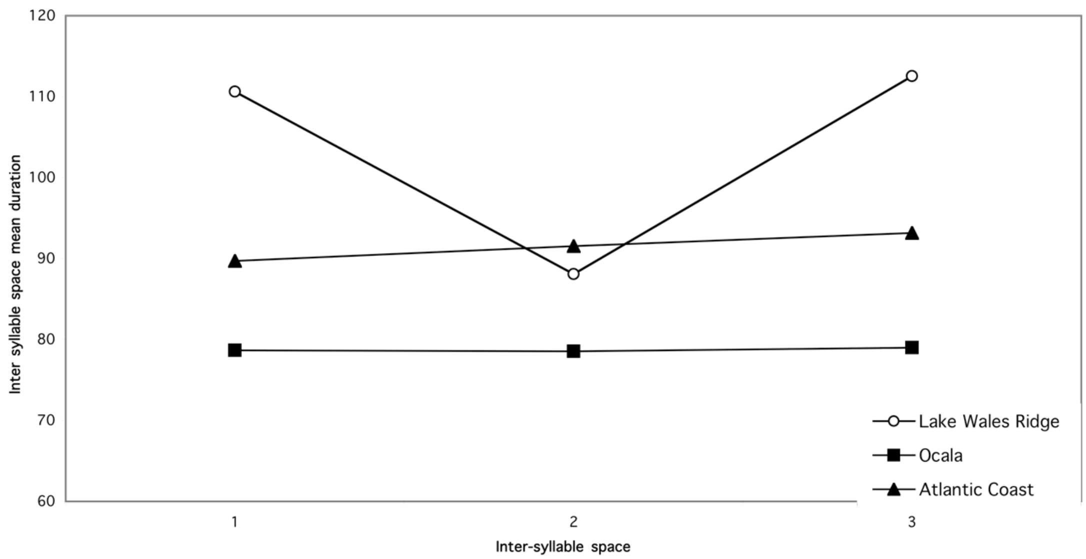
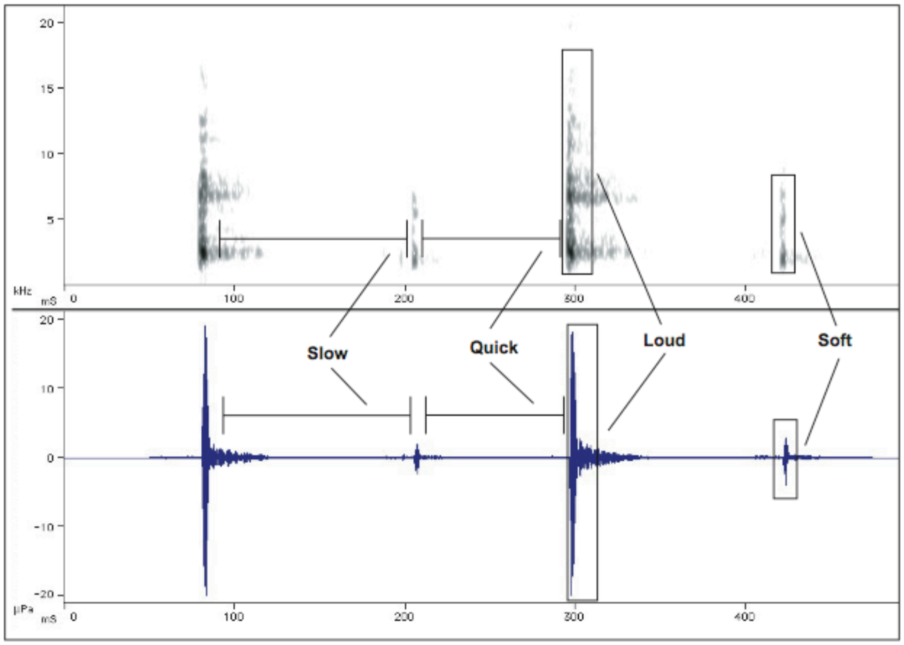

120
110
100
90
80
70
60
Inter syllable space mean duration
1
2
3
Inter-syllable space
Lake Wales Ridge
Ocala
Atlantic Coast

Figure 7.



20
15
10
5
kHz
mS
0
100
200
300
400
Slow
Quick
Loud
Soft
20
10
0
-10
-20
µPa
mS
0
100
200
300
400

Figure 8.

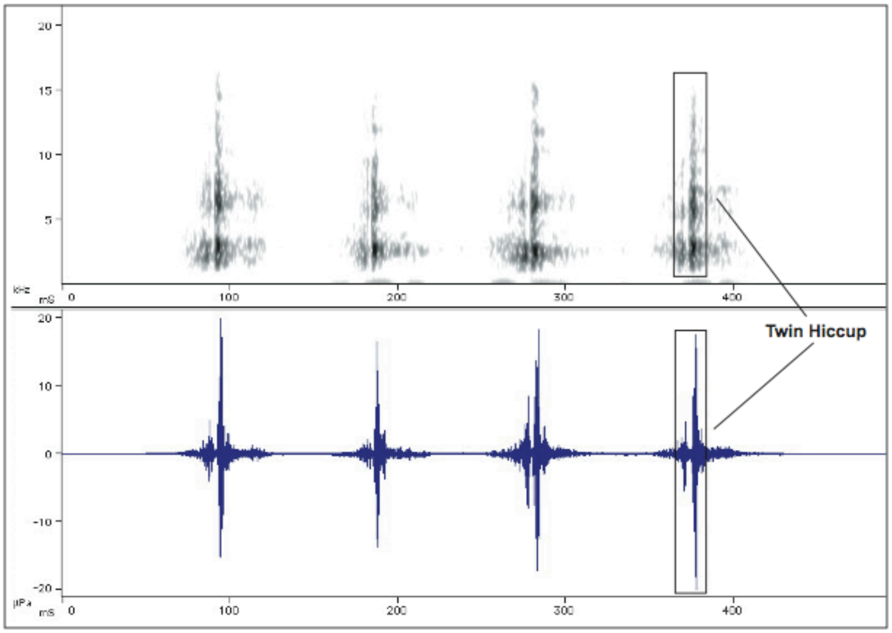


Figure 9.

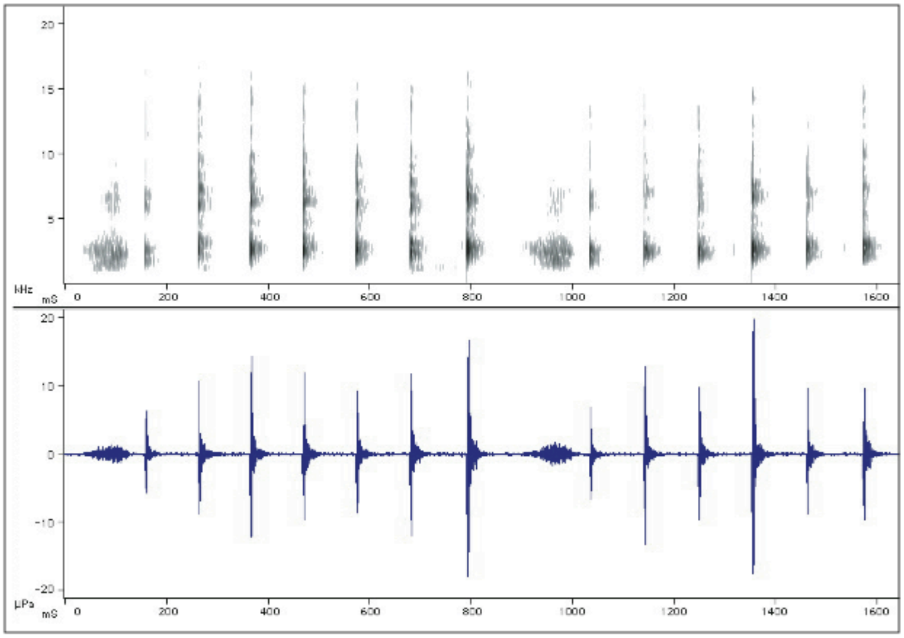


Figure 10.