Variation in Four-note Location Calls
of Male Spotted Owls (*Strix occidentalis*)

by

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ABSTRACT

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Male Spotted Owls (*Strix occidentalis*) use Four-note Location Calls for territorial defense and communication with females; yet little is known about Spotted Owl vocal behavior. There are three subspecies of Spotted Owls that are genetically distinct, occupy different forests in North America, and vary in body size. I measured three temporal and four frequency vocalization variables to assess call variation among the three subspecies. I used MANOVA, under an information-theoretic model selection framework, to rank 17 *a priori* models, which represented hypotheses explaining variation in the structure of Spotted Owl calls. I also experimentally tested the Acoustic Adaptation Hypothesis by broadcasting calls of all three subspecies and measuring their attenuation and degradation when transmitted through forests used by Northern Spotted Owls (*S. o. caurina*). A genetics (i.e., subspecies) hypothesis, where all three subspecies were distinct, best explained variation in temporal structure of calls among Spotted Owl subspecies. The statistical model based on this hypothesis described 49% of the variation in temporal structure sampled. A habitat hypothesis which considered mean basal area of conifers and broadleaf trees at owl nest sites best explained variation in the frequency structure among the three subspecies. This model described 52% of the variation in frequency structure of owl calls. Frequencies of Spotted Owl calls decreased in relation to conifer basal area and increased in relation to broadleaf basal area. My results provided limited support for the Acoustic Adaptation Hypothesis; owls from more open forests produced shorter calls composed of higher frequencies. In support of the Acoustic Adaptation Hypothesis, calls from owls that inhabited more closed forests attenuated 2 dB less per 25 m. However, contrary to the Acoustic Adaptation Hypothesis, these same calls also degraded by 58 msec more per 25 m. Ultimately, habitat may be a strong selective force behind the vocal frequencies used in Spotted Owl calls. Spotted Owl morphology did not explain variation in owl call frequencies.

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TABLE OF CONTENTS

Page

ABSTRACT iii

ACKNOWLEDGEMENTS v

TABLE OF CONTENTS viii

LIST OF TABLES x

LIST OF FIGURES xii

INTRODUCTION 1

STUDY SITES 5

  Northern Spotted Owl: Northwestern California 5

  California Spotted Owl: Sierra Nevada, California 6

  Mexican Spotted Owl: Arizona and New Mexico 6

MATERIALS AND METHODS 8

  Collection of Spotted Owl Vocalizations 8

  Call Spectrograms and Waveforms 9

  FLC Response Variables 10

  Explanatory Variables 13

TABLE OF CONTENTS (CONTINUED)

Page

Development of *A Priori* Hypotheses 16

Genetics and vocalizations 16

Habitat, vocalizations, and the acoustic adaptation hypothesis 17

Morphology and vocalizations 21

Analyses 22

AAH Call Transmission Experiment 27

RESULTS 30

Variation in Temporal Structure of Spotted Owl Four-note Location Calls ... 30

Variation in Frequency Structure of Spotted Owl Four-note Location Calls .. 37

AAH Call Transmission Experiment 40

DISCUSSION 45

Variation in Temporal Structure in Spotted Owl Four-note Location Calls 45

Variation in Frequency Structure of Spotted Owl Four-note Location Calls .. 48

Limitations and Future Research 51

LITERATURE CITED 55

PERSONAL COMMUNICATIONS 62

APPENDIX A 63

LIST OF TABLES

Table Page

1 Mean basal area (m^2/ha) of conifers and broadleaf trees around nest sites within each study area where Spotted Owl vocalizations were collected. The numbers of Spotted Owl nest sites used in each study area are indicated in parentheses. 14

2 *A priori* hypotheses developed to explain variation in male four-note location call in Northern, California, and Mexican Spotted Owls....................15

3 MANOVA models based on *a priori* hypotheses addressing temporal variables in male four-note location calls in Northern, California, and Mexican Spotted Owls (*Strix occidentalis*). Response variables (V) in all models were the note length of note 2 plus note 3 (NL2+3), the internote length between notes 3 and 4 (INL3-4), and the length of note 4 (NL4). 23

4 MANOVA models based on *a priori* hypotheses addressing frequency variable in male four-note location calls in Northern, California, and Mexican Spotted Owls (*Strix occidentalis*). Response variables (V) in all models were the maximum frequency of note 2 and 4 (MFN2 and MFN4), the note width of note 3 (NWN3), and the frequency slope of note 4 (FSN4)....................24

5 Ranking and weighting, based on AIC_c, of 8 *a priori* models describing variation in temporal structure of Four-note Location Calls among the three subspecies of Spotted Owls. 31

6 Parameter estimates and 95% confidence intervals from a MANOVA model distinguishing the three subspecies of Spotted Owls based on temporal call characteristics of Four-note Location Calls. Response variables were the note length of note 2 plus note 3 (NL2+3), the internote length between notes 3 and 4 (INL3-4), and the length of note 4 (NL4). 32

7 Least-square means and 95% confidence intervals from a MANOVA model distinguishing the three subspecies of Spotted Owls based on temporal call characteristics of Four-note Location Calls. Response variables were the note length of note 2 plus note 3 (NL2+3), the internote length between notes 3 and 4 (INL3-4), and the length of note 4 (NL4). 34

LIST OF TABLES (CONTINUED)

Table Page

8 Effect sizes and 95% confidence interval results for temporal call characteristics across Spotted Owl subspecies. The effect size values represent the differences in treatment effect between two subspecies. The subspecies are represented as follows: NSO – Northern Spotted Owl, MSO – Mexican Spotted Owl, and CSO – California Spotted Owl..........................35

9 Mean values ± standard errors for frequency response variables (*n* = 40) and temporal response variables (*n* = 39) across all three subspecies of Spotted Owls. Frequency measures are: maximum frequency of note 2 and 4 (MFN2,4), width of note 3 (NWN3), and frequency slope of note 4 (FSN4). Temporal measures are: note length of notes 2 and 3 (NL2+3), internote length between notes 3 and 4 (INL3-4), and note length of note 4 (NLN4). Temporal values were summed to create the total mean call length..36

10 Ranking and weighting, based on AIC_c, of 9 *a priori* models describing variation in frequency characteristics in Four-note Location Calls among the three subspecies of Spotted Owls...38

11 Parameter estimates from a MANOVA model examining the relationship of mean basal area (m^2/ha) of conifers and broadleaf trees at nest sites to frequency characteristics of Spotted Owls Four-note Location Calls. Response variables were the maximum frequency of note 2 and 4 (MFN2 and MFN4), the note width of note 3 (NWN3), and the frequency slope of note 4 (FSN4)..39

12 Results from repeated measure ANOVA's testing the Acoustic Adaptation Hypothesis. Spotted Owl calls of all three subspecies were broadcast at 10 Northern Spotted Owl sites. Recordings at 3 sites were slightly distorted. However, results indicate negligible effects with their inclusion. Alpha level of 0.05 was used to determine significant *P*-values................................41

LIST OF FIGURES

Figure Page

1 Spectrogram depicting the 7 response variables measured for each call across Spotted Owl subspecies. Temporal characters measured were the total distance from beginning of note 2 to the end of note 3 (INL2+3), the internote distance between the end of note 3 and beginning of note 4 (INL3-4), and the length of note 4 (NL4). Frequency variables sampled were the maximum frequency of note 2 (MFN2) and note 4 (MFN4), the width of note 3 (NWN3), and the frequency slope of note 4 (FSN4)..............11

2 Distribution of tree diameters from sample plots around Spotted Owl nest sites: (A) 425 trees at 29 California Spotted Owl nests (mean dbh = 82.6 cm), (B) 1654 trees at 142 Northern Spotted Owl nests (mean dbh = 81.9 cm), (C) 1334 trees at 152 Mexican Spotted Owl nests (mean dbh = 44.1 cm) (Gutiérrez and Franklin unpublished data). 20

3 Mean change over 25 m in Peak Intensity per Hertz per call (PIH) of Spotted Owl vocalizations after being transmitted through the acoustic environment of the Northern Spotted Owl. Vertical bars represent 95% confidence intervals. 43

4 Mean change over 25 m in the internote length between notes three and four (INL3-4) of Spotted Owl vocalizations after being transmitted through the acoustic environment of the Northern Spotted Owl. Vertical bars represent 95% confidence intervals..44

INTRODUCTION

The Spotted Owl (*Strix occidentalis*) is one of the world's most intensely studied birds (Gutiérrez et al. 1995) because of its association with old growth forests and conservation status (Forsman et al. 1984, United States Department of the Interior 1990, 1993, Perry et al. 1999, Seamans et al. 1999). Despite extensive research concerning Spotted Owl natural history (Forsman et al. 1984, Gutiérrez et al. 1995), genetics (Barrowclough et al. 1999), population dynamics (Seamans et al. 1999), and habitat use (Moen and Gutiérrez 1997, Gutiérrez et al. 1998, Ganey et al. 1999, Peery et al. 1999), little is known about Spotted Owl behavior (Gutiérrez et al. 1995). In particular, the factors influencing structure and variation in Spotted Owl vocalizations have not been investigated.

"Bioacoustics and conservation intercept where animals are affected by and use sound," (Baptista and Gaunt 1997: 232). However, if sound communication research is to be a helpful conservation tool, we must first understand animal behavior and communication in its' natural setting (Baptista and Gaunt 1997). No constants concerning avian call rhythms and genetic heritability are known, except that different patterns of heritability do occur, whereas genotype appears to control the range of call frequencies produced (Baptista 1996). However, differences within species frequency ranges exist in terms of what frequencies are employed. The Acoustic Adaptation Hypothesis (AAH) (Morton 1975) attributes frequency and rhythm of calls to the sound environment within which a species evolved. In particular, selective forces produce vocal adaptations that are best suited to transmit information correctly and for optimal distances in relation to habitat structure (Morton 1975). Numerous field studies support the AAH (Nottenbohm 1975, Hunter and Krebs 1979, Wasserman 1979, Anderson and Conner 1985, Sorjonen 1986a, Waas 1988, Wiley 1991, Tubaro and Segura 1994). Conversely, research has also shown call frequencies to be inversely related to syrinx size (Baptista 1996), with morphology constraining what vocal frequencies are physiologically cost-effective to produce (Bradbury and Vehrencamp 1998). The goal of my study was to inspect potential factors affecting Spotted Owl calls through statistical examination of call characteristics in order to provide a foundation from which future research could explore Spotted Owl vocal ontogeny.

Vocal repertoires have been described for the Northern (*S. o. caurina*) and Mexican (*S. o. lucida*) Spotted Owl (e.g., Forsman et al. 1984, Ganey 1990), but not for the California Spotted Owl (*S. o. occidentalis*) (Miller 1934, Bent 1938, Forsman et al. 1984, Ganey 1990, Fitton 1991, and Gutiérrez et al. 1995). Forsman et al. (1984) described 12 distinct call types used by adult Northern Spotted Owls. At least nine of these calls were documented in adult Mexican Spotted Owls and used in similar behavioral contexts (Ganey 1990). Hoots, whistles, cooing sounds, and barks characterize vocalizations (Forsman et al. 1984, Gutiérrez et al. 1995) and both sexes produced most call types (Forsman et al. 1984). Of all known Spotted Owl calls, the Four-note Location Call (FLC) was the most frequently heard vocalization, especially from males (Forsman et al. 1984, Gutiérrez et al. 1995). Descriptive and quantitative analyses of frequency and temporal characteristics of Spotted Owl calls have only been conducted on the FLC and Series Location Call of the Northern subspecies (Fitton 1991). In this study, I examined male FLC's because they were the primary vocalization of all Spotted Owl subspecies.

The latitudinal distribution of the Spotted Owl extends from southern British Columbia to central Mexico (Gutiérrez et al. 1995). The American Ornithologists' Union (1957) recognizes three Spotted Owl subspecies based on morphological characteristics. Subsequent genetic research supports this delineation (Barrowclough et al. 1999). To sufficiently examine geographic variation in vocalizations, samples from multiple populations, both within and across geographic regions, are required (Mundinger 1982). Although I was unable to meet this prerequisite, I did examine Spotted Owl FLC's from four populations representing the three extant subspecies.

For this study, I measured three temporal and four frequency variables in vocalizations and assessed whether observed differences in owl genetics (Barrowclough et al. 1999), owl nesting habitat (Hunter et al. 1995, Moen and Gutiérrez 1997, Peery et al. 1999, May 2000), or owl morphology (Miller 1934, Gutiérrez et al. 1995) best explained variation in call structure among the three subspecies. I used MANOVA, under an information-theoretic model selection framework (Burnham and Anderson 1998), to rank 17 *a priori* models that represented hypotheses explaining variation in temporal and frequency call structure. I also experimentally tested the Acoustic Adaptation Hypothesis (Morton 1975) by broadcasting calls of all three subspecies in forests occupied by Northern Spotted Owls. I used ANOVA to test the change in call characteristics in relation to territory and subspecies.

STUDY SITES

Northern Spotted Owl: Northwestern California

Northern Spotted Owl vocalizations were recorded in two areas of northwestern California. The Willow Creek Study Area (40° 45′ N latitude), a 292.4 km^2 site located in Humboldt County, California, is centrally located within the 1,784 km^2 Regional Study Area. Spotted Owl research has been conducted in the Willow Creek Study Area since 1985 and the Regional Study Area since 1987 (Franklin et al. 2000).

Mixed Evergreen Forests (Küchler 1977) constituted 92% of forests on the Willow Creek Study Area (Solis 1983, Hunter et al. 1995) and were characterized by a multi-layered Douglas-fir (*Pseudotsuga menziesii*) canopy with conifer trees reaching 79 m tall, and a hardwood mid-story reaching 38 m. These mixed hardwood-conifer forests were dominated by Douglas-fir, sugar pine (*Pinus lambertiana*), tanoak (*Lithocarpus densiflora*), and pacific madrone (*Arbutus menziesii*), with a subdominant strata of canyon live oak (*Quercus chrysolepis*), big-leaf maple, (*Acer macrophyllum*), and chinquapin (*Chrysolepis chrysophylla*) (Solis 1983, Hunter et al. 1995). Forest stands used by Northern Spotted Owls for nesting contained conifers greater than 90 cm dbh, low amounts of herbaceous land cover, and high structural diversity due to a hardwood mid- and understory and a wide range of tree ages (Hunter et al. 1995, LaHaye and Gutiérrez 1999).

California Spotted Owl: Sierra Nevada, California

California Spotted Owl vocalizations were recorded on the 355 km^2 Eldorado Study Area (39° 00′ N latitude) located 16 km northeast of Georgetown, California (Moen and Gutiérrez 1997). Mid-elevation mixed-conifer forests common to the Sierra Nevada characterized this site (Moen and Gutiérrez 1997). Red fir (*Abies magnifica*), ponderosa pine (*Pinus ponderosa*) and white fir (*A. concolor*) dominated at higher elevations, whereas elevations below 1,500 m were dominated by Douglas-fir, sugar pine, incense cedar (*Calocedrus decurrens*), canyon live oak, and black oak (*Quercus kelloggii*) (Bias and Gutiérrez 1992). The majority of California Spotted Owl nest sites occurred in forests with greater than 70% canopy cover, residual trees of greater than 100 cm dbh, and a high density and basal area of live trees (Bias and Gutiérrez 1992, Moen and Gutiérrez 1997). Forest stands at sampled nest sites contained high structural diversity, resulting in multi-layered canopies, dominated by a mixed-conifer overstory (Bias and Gutiérrez 1992).

Mexican Spotted Owl: Arizona and New Mexico

Mexican Spotted Owl vocalizations were recorded in central Arizona (the Coconino Study Area) and in west-central New Mexico (the Gila Study Area). The Coconino Study Area encompassed 585 km^2 (34°50′ N latitude) on the Coconino Plateau. Three forest types occurred on the Coconino Study Area, pine-oak (ponderosa pine-Gambel oak, *Q. gambelii*), mixed-conifer/fir-aspen (dominated by both Douglas-fir and white fir, and characterized by a ponderosa pine, Gambel oak, and quaking aspen, *Populus tremuloides* understory), and piñon-juniper woodland (*P. edulis-Juniperus spp.*) (May 2000).

The majority of Spotted Owl nests on the Coconino Study area were found in forest stands with high canopy closure and mature and old growth trees (greater than 45.7 cm dbh) (May 2000). Most owls nested in pine-oak stands and this forest type was used in proportion to its availability on the landscape (May 2000). Gambel oaks were a critical part of this landscape; 41% of nests were in oak cavities and a high prevalence of large oaks was noted at most nest sites (May 2000). While mixed-conifer forest comprised only 4.3% of the Coconino Study Area, these stands were used in greater proportion than their availability (May 2000). Although owls used both young and mature conifer forest stands, all contained mature forest components and owls did not nest in open canopy, mature forests (May 2000).

The Gila Study Area was located in west-central New Mexico (33° 40´ N latitude) in the Tularosa Mountains. Owls nested in upper montane forests characterized by pure mixed-conifer stands or stands having a mixed-conifer overstory with a dominant or codominant Gambel oak understory (Seamans and Gutiérrez 1995). Nesting and roosting owls also used mature, mid-elevation pine forests defined by a ponderosa pine overstory and Gambel oak understory (Peery et al. 1999). Owl nest stands were characterized by high structural diversity, late seral stage characteristics, and mature trees greater than 45 cm dbh (Seamans and Gutiérrez 1995, Peery et al. 1999).

MATERIALS AND METHODS

Collection of Spotted Owl Vocalizations

Field crews working on owl demography research collected male Spotted Owl vocalizations from April through August during the 2000 breeding season. Owl calling was stimulated by vocal imitation (Forsman 1983). Recording was conducted in conjunction with demographic studies where owls were individually identified by unique color bands and numbered U. S. Fish and Wildlife Service bands (Franklin et al. 1996). The Institutional Animal Care and Use Committee approved this study on April 12, 2000 (# 99/00.W.94.0).

I collected recordings of Northern Spotted Owls using a Sennheiser ME66 shotgun microphone, K6 power module, WZM66 windscreen, and Marantz PMD-222 mono cassette deck. Recordings of California and Mexican Spotted Owls were collected using an identical Sennheiser microphone system, but using a Sony WM-D3 Professional Walkman. Sixty minute, Type I (Normal Bias) TDK tapes were used in all recorders. I trained project personnel on equipment use and provided a detailed instruction manual.

All recordings were collected while in sight of the bird (approximately 5–25 m), and with the least possible amount of vegetation between the owl and microphone. Vocalizations were recorded on calm days with no precipitation. Field data included: study site, date, temperature, wind speed, location, approximate distance to owl(s), owl identification (unique color band combination and U.S. Fish and Wildlife Service number), sex of owl(s), start and stop time of recording, and type of call(s) recorded. All owls sampled from the study populations were initially included and all recordings were statistically independent from each other (i.e., represented different owls).

Call Spectrograms and Waveforms

I digitized and edited taped calls in mono format, at 16 bits, with a sampling rate of 44,100 Hz, on a Dell PC, with Sound Blaster Live! Wave Studio (Creative Technology Limited 1997). I saved edited Four-note Location Calls as both wave files and Audio Interchange Format files by using SoundApp PPC (Franke 1998) for conversions. I extracted frequency variables from spectrograms created from Audio Interchange Format files using program Canary (Chariff et al. 1995). I extracted temporal measurements from spectrograms and waveforms created from wave files using program Syrinx (Burt 1999).

I created spectrograms for the frequency analysis using 4096-point Fast Fourier Transform with a filter bandwidth of 174.85 Hz, within a Hamming window, with 50% overlap, which produced a 10.77 Hz frequency resolution (Charif et al 1995). I created spectrograms and waveforms for the temporal analysis using 1024-point Fast Fourier Transform, within a Hamming window, which produced a 43.07 Hz frequency resolution (Charif et al 1995). I defined the beginning (or onset) and ending (or offset) of a note in a waveform as the point where decibel level changed from 0.00 dB to 0.05 dB. I defined the beginning and ending of a note in a spectrogram as the outside point where the fundamental frequency and the first harmonic converged.

FLC Response Variables

FLC's typically consist of 3 or 4 pure tones (Fitton 1991) that can be phonetically described as *hoo--hoo hoo---hooo*. The second and third notes are temporally closer and the fourth note ends with a downward frequency modulation (Forsman et al. 1984). Males primarily use the FLC during territorial disputes, to proclaim territory occupancy, while delivering prey to a nesting female, and to maintain pair contact (Forsman et al. 1984, Gutiérrez et al. 1995).

I selected variables describing call structure based on my *a priori* hypotheses, previous vocalization research (Richards and Wiley 1980, Wiley 1991, Daniel and Blumstein 1998, Slobodchikoff et al. 1998), and other Spotted Owl studies (Fitton, 1991, Baptista 1993, Kuntz and Stacey 1997). For instance, Baptista (1993) documented a variety of unique shapes for note four on Northern Spotted Owl FLC spectrograms and hypothesized that individual owls may be identified based on these note structures. Kuntz and Stacey (1997) reported distinctive motifs in the third note morphology of male FLC's from different subpopulations of Mexican Spotted Owls (single-peak, double-peak, and an intermediate note shape).

I measured only the second, third, and fourth note of calls because owls often omit a FLC's introductory note (Forsman et al. 1984, Gutiérrez et al. 1995). Mexican Spotted Owls often produce only three of the notes in the FLC regardless of aggressive state (Eakle et al. 1989, Gutiérrez et al. 1995). Thus, I measured structural components of notes typically produced by all subspecies.

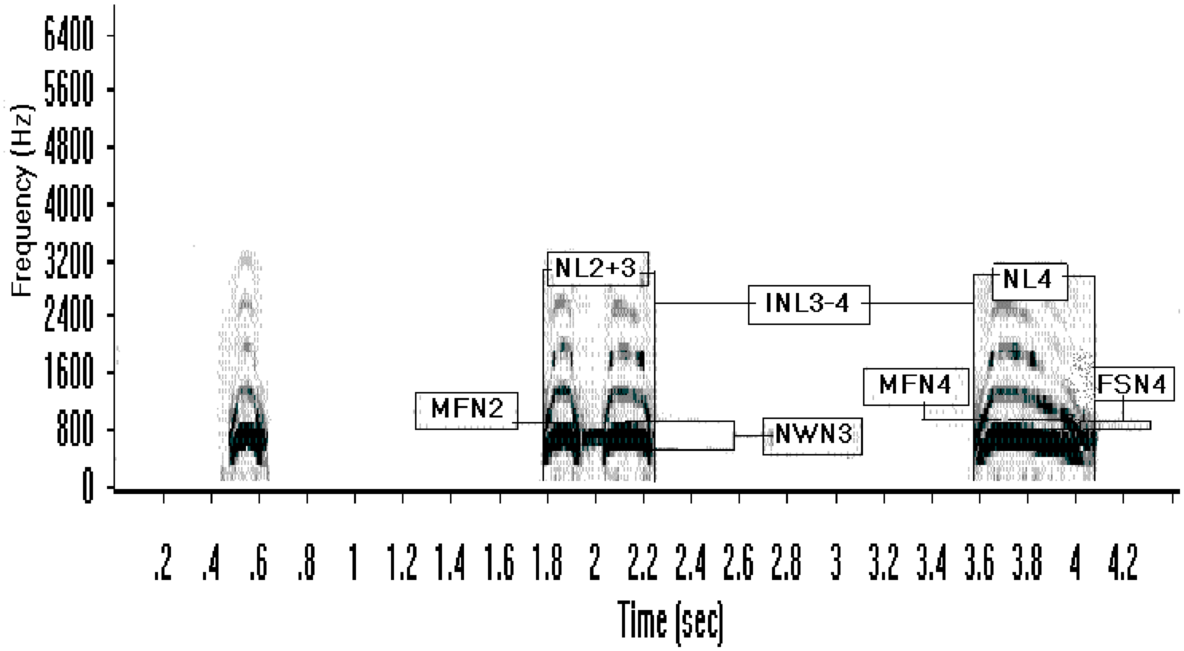


Figure 1. Spectrogram depicting the 7 response variables measured for each call across Spotted Owl subspecies. Temporal characters measured were the total distance from beginning of note 2 to the end of note 3 (INL2+3), the internote distance between the end of note 3 and beginning of note 4 (INL3-4), and the length of note 4 (NL4). Frequency variables sampled were the maximum frequency of note 2 (MFN2) and note 4 (MFN4), the width of note 3 (NWN3), and the frequency slope of note 4 (FSN4).

I only included owls for which at least two FLC's were recorded. The number of calls recorded per individual owl ranged from 2 to 24. I collected 7 measurements from each vocalization, four frequency and three temporal response variables (Figure 1). If I could not obtain either all frequency variables or all temporal variables from an individual owl, the bird was removed from the sample. I collected all measurements from the fundamental frequency of each note. A Spotted Owl call note was defined as "a continuous registration of similar intensity longer than 50 msec" (Fitton 1991, page 7).

I measured four frequency variables (Figure 1) from a total of 343 spectrograms taken from 40 individual owls. The maximum frequency of note 2 (MFN2) and maximum frequency of note 4 (MFN4) were both measured at the greatest hertz output for each fundamental frequency. The measure of note width [frequency range] from note 3 (NWN3) was the width of the fundamental frequency at the point of maximum frequency. The frequency slope [change of frequency/time] of note 4 (FSN4) was the change in hertz from the point of maximum frequency through the next 0.20 seconds or end of note 4, whichever came first.

I also measured three temporal variables (Figure 1) from a total of 330 spectrograms taken from 39 individual owls. The length of note 2 plus note 3 (NL2+3) was the total time from the beginning of note 2 to the end of note 3, and included the internote length between these two notes. The length of note 4 (NL4) was the total duration of note 4. The internote length between note 3 and note 4 (INL3-4) was the time between the offset of note 3 and the onset of note 4. I measured all 3 temporal variables within an FLC sequentially by repeating the use of each ending time as the beginning point for the next variable, e.g. ending time for INL3-4 also equaled beginning time for NL4.

Explanatory Variables

Vegetation sampling around Spotted Owl nests had been conducted in all study areas (Bias and Gutiérrez 1992, Seamans and Gutiérrez 1995, LaHaye and Gutiérrez 1999, May 2000). From all available nest plot data collected between 1983 and 2000, I extracted the dbh (diameter at breast height) and species of all sampled trees. I calculated mean basal area (m^2/ha) of conifers and broadleaf trees within each study area, as well as total basal area for all trees sampled (Bell et al. 1993) (Table 1). Since tree diameter is related to tree height (Mueller-Dombois and Ellenberg 1974), and Spotted Owls typically broadcast from mid to upper tree perches (Gutiérrez et al. 1995, personal observation), I chose conservative measures and only included conifer trees $\geq$ 25 cm dbh and broadleaf trees $\geq$ 50 cm dbh. My goal was to incorporate structural features that were characteristic of both mid and overstory strata in the forests through which owls transmit their calls. These continuous variables were used in hypotheses concerning habitat effects on vocalizations (Hypotheses 4-7, Table 2) to represent the general landscape features in the forests of each of the four study areas where vocalizations were collected. In using these data, I assumed that the forest structure around nests represented the forest structure through which spotted owls transmitted their vocalizations.

I used the mass of every adult male Spotted Owl captured from 1984 to 1994 to calculate mean subspecies mass (Wiley 1991) per study area (Willow Creek Study Area:

Table 1. Mean basal area (m^2/ha) of conifers and broadleaf trees around nest sites within each study area where Spotted Owl vocalizations were collected. The numbers of Spotted Owl nest sites used in each study area are indicated in parentheses.

	Basal Area (m^2/ha)	
Study Site	Conifer Trees	Broadleaf Trees
Willow Creek Study Area (n = 142)	18.85	1.18
Eldorado Study Area (n = 29)	23.91	0.75
Coconino Study Area (n = 97)	3.50	0.71
Gila Study Area (n = 55)	2.78	0.04

Table 2. *A priori* hypotheses developed to explain variation in male four-note location call in Northern, California, and Mexican Spotted Owls.

No.	Hypothesis
Genetic Factors	
1	Each Spotted Owl subspecies has a distinctly structured FLC (American Ornithological Union 1957 and Barrowclough et al. 1999).
2	FLC for the Mexican and California Spotted Owl are similar in structure yet distinct from the FLC of the Northern Spotted Owl (Barrowclough et al. 1999).
3	FLC for the California and Northern Spotted Owl are similar in structure yet distinct from the FLC of the Mexican Spotted Owl (Haig et al. 2001).
Habitat Factors	
4	The structure of Spotted Owl FLC's is a function of the mean basal area of coniferous trees at nest sites (Morton 1975, Martin and Marler 1977, Brown and Handford 1996).
5	The structure of Spotted Owl FLC's is a function of the mean basal area of broadleaf trees at nest sites (Morton 1975, Martin and Marler 1977, Brown and Handford 1996).
6	The structure of Spotted Owl FLC's is a function of the mean basal area of coniferous and broadleaf trees at nest sites (Morton 1975, Martin and Marler 1977, Brown and Handford 1996).
7	The structure of Spotted Owl FLC's is a function of the total mean basal area of all trees at nest sites (Morton 1975, Martin and Marler 1977, Brown and Handford 1996).
Morphological Factor	
8	The structure of Spotted Owl FLC's is a function of body mass due to variation in syrinx size. (Miller 1934).
N*o Factors*	
9	The structure of Spotted Owl FLC's is similar among the three Spotted Owl subspecies (genetic, habitat or morphological factors do not explain variation in FLC structure).

574.3 g (*n* = 88); Eldorado Study Area: 573.4 g (*n* = 41); Gila Study Area: 510.7 g (*n* = 41); and Coconino Study Area: 509.1 g (*n* = 43), Franklin and Gutiérrez unpublished data). These continuous variables were used to test my hypothesis that frequency of Spotted Owl FLC's would change with mean mass across subspecies (Hypothesis 8, Table 2).

Development of *A Priori* Hypotheses

To investigate subspecific vocal variation, I developed three sets of *a priori* hypotheses from the published literature. Hypotheses were based on genetic, habitat, and morphological differences previously observed among Spotted Owl subspecies.

Genetics and vocalizations

Although mtDNA genes do not control behavior, and are neutral markers, mtDNA analyses provide phylogeographic templates that may serve as the foundation for genetic differences among the three subspecies. Barrowclough et al. (1999) found that the previous taxonomic designation of the three subspecies by the American Ornithologists' Union (1957) corresponded with mtDNA genetic differences. Chronologically, Northern Spotted Owls branched off first from the Mexican and California Spotted Owls; a secondary biogeographic event likely led to further division between the Mexican and California subspecies (Barrowclough et al. 1999). Calculation of isolation time between these three subspecies resulted in the likelihood that all lineages have been distinct for greater than 10,000 years (Barrowclough et al. 1999).

In contrast, Haig et al. (2001) used random amplified polymorphic DNA (RAPD) and inferred no difference between the Northern and California Spotted Owl subspecies. The resulting lineage suggested that only the Mexican Spotted Owl represented a monophyletic group whereas the Northern and California subspecies represented a paraphyletic group (Haig et al. 2001). Haig et al. (2001) concluded that Spotted Owl genetics exhibited clinal variation, where geographic proximity and natural dispersal have rendered the California and Northern Spotted Owls no longer distinct subspecies. It is important to note that Haig et al. (2001) used, by default, the Mexican Spotted Owl to root their phylogenetic analysis. Despite this flaw, I used their grouping of Northern and California owls as an alternate phylogeographic template to hypothesize patterns of vocal variation among Spotted Owl subspecies. Reflecting these genetic patterns, I constructed three hypotheses modeling possible owl phylogenies and their plausible relation to owl vocalizations (Hypotheses 1-3, Table 2).

Habitat, vocalizations, and the acoustic adaptation hypothesis

I also investigated the relationship of vegetation structure to call variation, since it was possible that subspecific variation in Spotted Owl calls would not mirror genetic differences. The Acoustic Adaptation Hypothesis (AAH) suggested that animal vocalizations were a function of the acoustic environment in which a species evolved (Morton 1975). In particular, selective forces produced vocal adaptations that were best suited to transmit information correctly and for optimal distances in relation to habitat structure (Morton 1975). The AAH directly predicted variation in the frequencies and temporal patterns used in vocalizations. That is forest dwelling species should produce lower frequency calls, with fewer amplitude or frequency modulations, and avoid trills while stressing tonal or whistled elements (Morton 1975, Brown and Handford 1996). Numerous field studies support the AAH (Nottenbohm 1975, Hunter and Krebs 1979, Wasserman 1979, Anderson and Conner 1985, Sorjonen 1986a, Waas 1988, Wiley 1991, Tubaro and Segura 1994), as well as laboratory experiments (Brown and Handford 1996, 2000).

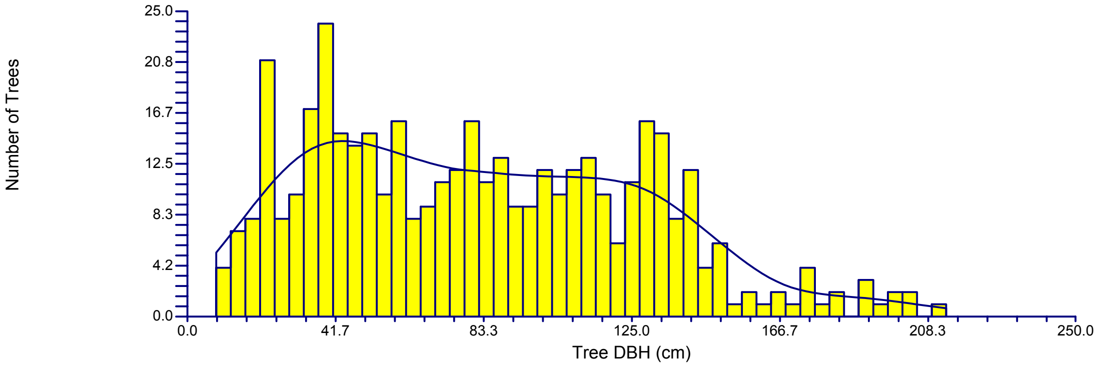
Two main factors affect sound transmission, attenuation and degradation (Catchpole and Slater 1995). Attenuation is the reduction of sound intensity that results from sound waves propagating through natural environments (Wiley and Richards 1982). Spherical spread is the constant reduction of amplitude expected for signals in a homogenous environment, and occurs at a rate of 6dB per doubling of distance (Catchpole and Slater 1995). Excessive attenuation is the loss of sound intensity that occurs in addition to spherical spreading and is influenced by many factors including vegetation structure (e.g., tree trunks and foliage) and call frequencies (Wiley and Richards 1982).

Degradation is the distortion of a signal during its broadcast and is a product of irregular amplitude fluctuations and reverberations (Wiley and Richards 1982). Sound transmission within forested habitats is primarily distorted due to reverberations or the multiple reflection and scattering of signals produced during propagation (Wiley and Richards 1982). Call frequency and complexity of scattering surfaces affects the degree by which sound reverberates, which in turn influences temporal characteristics within and between notes due to bounce and accumulation of echoes (Wiley and Richards 1982, Tubaro and Segura 1994, Catchpole and Slater 1995). The degree by which a soundwave is both attenuated and degraded is influenced by signal frequency and the complexity of habitat through which the signal must travel.

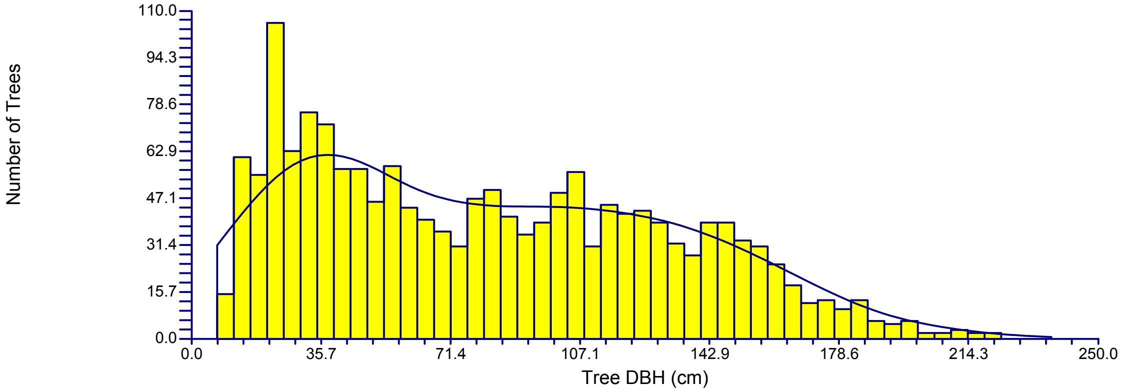
Spotted Owls inhabit mature and old-growth forests throughout their range (Gutiérrez et al. 1995), yet these forests vary by species composition and structure (Moen and Gutiérrez 1997, LaHaye and Gutiérrez 1999, Peery et al. 1999). This variation alone may have placed selective pressures on the evolution of Spotted Owl call structure. For instance, conifer and broadleaf trees present different scattering surfaces because conifer needles offer less resistance than broadleaf leaves to frequencies below 10 kHz (Martin and Marler 1977). For frequencies between 1-10 KHz, broadleaf forests generally attenuate sound by 2-35 dB/100 m versus 2-20 dB/100 m in conifer forests (Martin and Marler 1977, Bradbury and Vehrencamp 1998). In preliminary investigations of Northern Spotted Owl FLC's, I found average male fundamental frequencies were <1 KHz, with harmonics spanning higher frequencies. Low frequency calls, which are characterized by long wavelengths, are not reflected by small objects (Catchpole and Slater 1995), yet tree type (conifer or deciduous) and basal area (m^2/ha) likely influence scattering of both the fundamental and harmonic frequencies.

Most studies that examined the AAH have based comparisons between open and closed habitats. I believe structural variation between forested habitats could also impose selective pressures that influence subspecific vocal variation. I first assessed the acoustic environment occupied by Spotted Owls by inspecting the distribution of tree sizes

(A) California Spotted Owl



(B) Northern Spotted Owl



(C) Mexican Spotted Owl

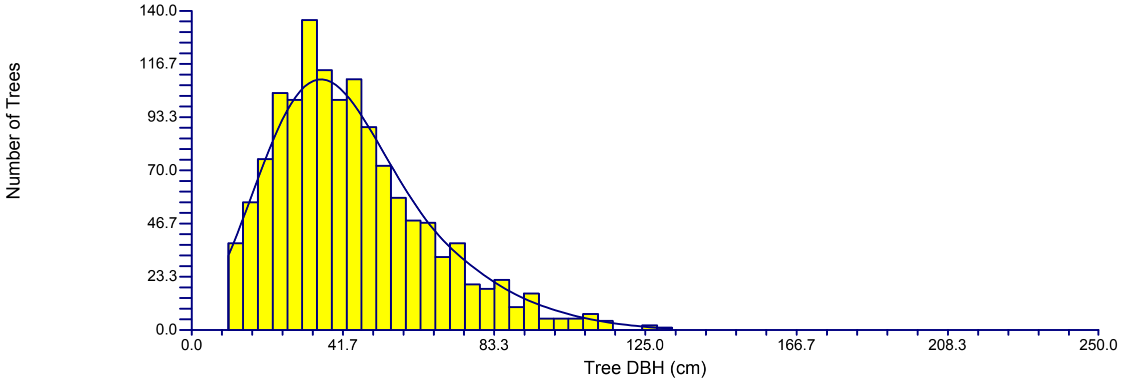


Figure 2. Distribution of tree diameters from sample plots around Spotted Owl nest sites: (A) 425 trees at 29 California Spotted Owl nests (mean dbh = 82.6 cm), (B) 1654 trees at 142 Northern Spotted Owl nests (mean dbh = 81.9 cm), (C) 1334 trees at 152 Mexican Spotted Owl nests (mean dbh = 44.1 cm) (Gutiérrez and Franklin unpublished data).

sampled around Spotted Owl nest sites (Figure 2). Two major groups were apparent; California and Northern Spotted Owls occupied more structurally closed nesting habitats, defined by a higher diversity of tree sizes (maximum dbh of 237.0 cm), while Mexican Spotted Owls nested in sites with predominantly smaller trees (maximum dbh of 132.4 cm) and less size variability (Figure 2). Therefore, based on the AAH, I predicted male California and Northern Spotted owls would produce lower frequency calls with limited modulations, while Mexican Spotted Owls would produce calls composed of higher frequencies and more frequency modulations.

Also based on the AAH, I predicted variation in the temporal structure within FLC call patterns among subspecies because alternating components within calls are expected to vary as a function of vegetation (Morton 1975). Signals that are slowly modulated are more favored in structurally closed habitats because they limit the deleterious effect of echoes from surrounding reflective and scattering surfaces (Morton 1975, Turbano and Segura 1994). Therefore, I predicted that California and Northern Spotted Owls would produce longer announcement vocalizations, defined by longer note and internote durations, than the Mexican Spotted Owls (Hypotheses 4-7, Table 2).

Morphology and vocalizations

The structure of Spotted Owl vocalizations could also be a function of morphological differences. The vocal apparatus of eight owl species, including both the Northern and California Spotted Owl, differed based on bronchial enlargement (Miller 1934). Both syrinx size and call frequency were a function of body size with larger syringes producing lower frequencies (Miller 1934). This inverse relationship between body size and vocal frequency has also been observed in other birds (Baptista 1996). Thus, as body size changes in male Spotted Owls, syrinx size should also change proportionately, which would influence call frequency.

Spotted Owl body characteristics vary across subspecies. Owl size appears to vary by latitude, with Northern Spotted Owls largest, California Spotted Owls slightly smaller than the Northern subspecies, and Mexican Spotted Owls the smallest (Gutiérrez et al. 1995). Therefore, I expected average frequencies emitted by male Spotted Owls would increase from north to south following the gradient in body sizes (Hypothesis 8; Table 2). In particular, the larger Northern and California Spotted Owls would emit similar, low frequency calls, while the smaller Mexican subspecies would produce the highest average call frequencies.

Analyses

I used an information-theoretic model selection approach (Burnham and Anderson 1998) to assess which of my proposed hypotheses best explained variation in male Spotted Owl FLC's. In the information-theoretic approach, Akaikes Information Criterion (AIC) was used as the basis for model selection; statistical models representing my *a priori* hypotheses were ranked according to their AIC values (Burnham and Anderson 1998). In contrast to classical null hypothesis testing, this approach allowed multiple hypotheses to be examined simultaneously (Burnham and Anderson 1998). I used a bias-corrected version of AIC, AIC_c, as an objective model selection criterion where models were ranked based on minimum AIC_c values. Akaike weights were used to address strength of evidence for each model in the set of models I examined

Table 3. MANOVA models based on *a priori* hypotheses addressing temporal variables in male four-note location calls in Northern, California, and Mexican Spotted Owls (*Strix occidentalis*). Response variables (V) in all models were the note length of note 2 plus note 3 (NL2+3), the internote length between notes 3 and 4 (INL3-4), and the length of note 4 (NL4).

Hypothesis Number	Model Statement	Explanatory Variables
Genetics Hypotheses		
1	V = ß$_0$ + ß$_1$ (NSO) + ß$_2$ (CSO)	Subspecies: (Northern versus Mexican versus California Spotted Owl)
2	V = ß$_0$ + ß$_1$ (CSO & MSO)	Subspecies: (Northern versus Mexican and California Spotted Owl)
3	V = ß$_0$ + ß$_1$ (NSO & CSO)	Subspecies: (Northern and California versus Mexican Spotted Owl)
AAH Hypotheses		
4	V = ß$_0$ + ß$_1$ (Conifer)	Habitat: Conifer Basal Area (m^2/hectare)
5	V = ß$_0$ + ß$_1$ (Broadleaf)	Habitat: Broadleaf Basal Area (m^2/hectare)
6	V = ß$_0$ + ß$_1$ (Conifer) + ß$_2$ (Broadleaves)	Habitat: Conifer Basal Area (m^2/hectare) + Broadleaf Basal Area (m^2/hectare)
7	V = ß$_0$ + ß$_1$ (Conifer & Broadleaf)	Habitat: Total (Conifer + Broadleaf) Basal Area (m^2/hectare)
No Variation		
8	V = ß$_0$	Intercept Only

Table 4. MANOVA models based on *a priori* hypotheses addressing frequency variable in male four-note location calls in Northern, California, and Mexican Spotted Owls (*Strix occidentalis*). Response variables (V) in all models were the maximum frequency of note 2 and 4 (MFN2 and MFN4), the note width of note 3 (NWN3), and the frequency slope of note 4 (FSN4).

Hypothesis Number	Model Statement	Explanatory Variables
Genetics Hypotheses		
1	$V = ß_0 + ß_1$ (NSO) + $ß_2$ (CSO)	Subspecies: (Northern versus Mexican versus California Spotted Owl)
2	$V = ß_0 + ß_1$ (CSO & MSO)	Subspecies: (Northern versus Mexican and California Spotted Owl)
3	$V = ß_0 + ß_1$ (NSO & CSO)	Subspecies: (Northern and California versus Mexican Spotted Owl)
AAH Hypotheses		
4	$V = ß_0 + ß_1$ (Conifer)	Habitat: Conifer Basal Area (m^2/hectare)
5	$V = ß_0 + ß_1$ (Broadleaf)	Habitat: Broadleaf Basal Area (m^2/hectare)
6	$V = ß_0 + ß_1$ (Conifer) + $ß_2$ (Broadleaf)	Habitat: Conifer Basal Area (m^2/hectare) + Broadleaf Basal Area (m^2/hectare)
7	$V = ß_0 + ß_1$ (Conifer & Broadleaf)	Habitat: Total (Conifer + Broadleaf) Basal Area (m^2/hectare)
Morphology Hypothesis		
8	$V = ß_0 + ß_1$ (Weight)	Morphology: Mean Owl Weight (gms)
No Variation		
9	$V = ß_0$	Intercept Only

(Tables 3, 4) (Burnham and Anderson 1998). This information-theoretic approach acknowledged that full reality was unknown (a “true model” did not exist), and, therefore, the “best model” was one that permitted the strongest inference from my set of candidate models (Anderson et al. 2000,Burnham and Anderson 1998). I examined variable contributions to the model of interest by examining the magnitude of parameter estimates and their 95% confidence intervals (Anderson et al. 2001).

I developed MANOVA models (Tables 3, 4) to represent each verbal hypothesis in Table 2 and used PROC GLM in Program SAS (SAS Institute Inc. 2000) to analyze these 17 statistical models. Individual owls were considered the experimental unit. To determine the extent of sampling variation within individuals, I first estimated the percentage coefficient of sampling variation (%CV) of each response variable for each individual (Appendix A). Eighty-six percent of the %CV’s for all response variables, for all owls, were ≤ 10.00%, with values ranging from 0.3% to 28.0% (Appendix A). Since little sampling variation existed for individuals, I used the mean value of each response variable per individual in the MANOVA models, rather than using a repeated measures design. I used categorical dummy variables to distinguish among subspecies in Hypotheses 1 - 3 (Tables 3, 4) and continuous variables for basal area and owl mass for hypotheses 4 - 7 (Tables 3, 4) and hypothesis 8 (Table 4), respectively. I chose MANOVA because there was a high degree of interrelatedness among my FLC response variables and it was therefore more appropriate to use multiple explanatory variables (Bray and Maxwell 1985). In addition, I did not believe subspecific call variation could be identified by examining only one frequency or temporal variable; I wanted to examine owl calls in terms of the relationship among variables (Bray and Maxwell 1985).

I conducted two separate analyses, one for frequency response variables and one for temporal response variables, to reduce the number of response variables in the MANOVA and because the morphology hypothesis relied purely on frequency characteristics. Due to small sample sizes and a multivariate application, a second order Akaike's information criteria formula specific to multivariate analysis was used:

$$\mathrm{AIC}_c = \mathrm{AIC} + 2\,\frac{K(K+v)}{np-K-v}$$

where AIC = $-2\log_e$ (likelihood) + $2K$, v was the number of distinct parameters, n was the number of independent multivariate observations, p was the number of non-independent components of n, and K was the total number of estimable parameters of the model (Burnham and Anderson 1998, page 303). Using program SAS (SAS Institute Inc. 2000), I transformed the least square error terms for each model to a maximum likelihood estimate by: 1) dividing each element of the error sums of squares and cross-product matrix by the sample size ($n = 40$ for frequency models and $n = 39$ for temporal models), 2) calculating the determinant of the resulting matrix, and 3) computing the natural log of the determinant and multiplying it by n to estimate the log likelihood (K. P. Burnham, personal communication). I next calculated Akaike weights to estimate the relative strength of inference for each *a priori* hypothesis, and to assess how well models described the information in the data (Anderson et al. 2000). The models selected for inference were based on minimum AIC_c values and large Akaike weights.

Once the best model for each analysis was selected, I examined the proportion of variance in my data set that this model actually explained. Since the determinant of the covariance matrix represents a scalar measure of the generalized variance, the determinant of the error sums of squares matrix represented an estimate of the variation remaining beyond that described by the model (A. B. Franklin and K. P. Burnham, personal communications). Therefore, the error sums of squares matrix from the intercept-only model represented the total amount of variation to be explained whereas the error sums of squares matrix from the best model represented the residual variation after the model has explained some proportion of variation (A. B. Franklin and K. P. Burnham, personal communications). Hence, I estimated the amount of variation explained by my best MANOVA models by calculating:

$$\begin{array}{c}\text{Proportion Variation}\\ \text{Explained}\end{array} = 1- \frac{\text{Determinant \{best model\}}}{\text{Determinant \{intercept only model\}}}$$

Because some temporal models used categorical dummy variables, I used the Newman-Keuls multiple range test to examine treatment effects in these models (Zar 1999). I used an alpha level of 0.05 to determine my *q* statistics (Rohlf and Sokal 1995).

AAH Call Transmission Experiment

If structural characteristics predicted by the AAH were present in Spotted Owl vocalizations, I predicted that vocalizations of Mexican Spotted Owls should attenuate and degrade more than Northern or California Spotted Owl calls when transmitted through denser forests occupied by Northern Spotted Owls. I tested this prediction by conducting a call transmission experiment.

I broadcast calls of all three subspecies in 10 forest stands occupied by Northern Spotted Owls on the Willow Creek Study Area. I randomly selected 10 independent owl stands from among 304 total owl roost and nest stands detected between 1985-1994 using GIS (ArcView 3.2; Environmental Systems Research Institute, Inc. 1997). These 10 stands were validated as having mature forest habitat using air photos.

I randomly selected three male owls from the sample of individuals recorded within each subspecies ($n = 9$), and then randomly selected a single, well recorded FLC for each owl, to be used in all broadcasts. I then created a compact disc of the selected digitized calls in order to limit analog tape wear and ensure equal transmission quality.

I conducted experimental call transmissions from 0900 to 1200 PST on calm days, 10-18 August 2000. I randomly assigned the order of tests and broadcast owl calls a minimum of 125 m from roads or clear cuts to avoid edge effects (Chen et al. 1995). By conducting all broadcasts within a short time frame, I avoided the need to control for environmental and time effects (Brown and Handford 2000). I used a SME-AFS amplified playback speaker on a tripod 2 m high and broadcast digitized owl calls from a Sony Discman. I randomly assigned speaker orientation to face either 90° or 180° across slope. Using a decibel meter, I set a minimum standard volume of 60 dB at 3 m from the speaker. I recorded all broadcasts with Marantz and Sennheiser equipment (see Collection of Spotted Owl Vocalizations section) at both 5 m (the reference distance) and 30 m from the speaker to control for any irregularities caused by signal transmission (Daniel and Blumstein 1998). A 25 m distance was used to control for topographical effects across owl stands (Fotheringham et al. 1997, Brown and Handford 2000).

Digitizing, editing, and variable extraction processes were identical to the observational study.

I analyzed a total of 180 recordings (10 sites x 9 owls x 2 distances). I measured five response variables per call: 1) peak intensity per hertz per call (note 2 through note 4) (PIH; in dB), 2) internote length between notes 3 and 4 (INL3-4; in seconds) 3) peak frequency of note 2 (PFN2; Hz), 4) peak frequency of note 4 (PFN4; Hz), and 5) length of note 4 (NL4; in seconds). All measurements were taken on the fundamental frequency. Because decibel levels were constant across samples, I also included waveforms in the AAH analyses. I used the change in each response variable from 5m (the reference distance) to 30 m (the transmission distance) to create a total of 90 data points. I conducted five one-way repeated measures ANOVA's, one for each response variable. Since this was a true experiment, I used a hypothesis testing approach with α = 0.05 to determine when results were statistically significant.

RESULTS

Variation in Temporal Structure of Spotted Owl Four-note Location Calls

I analyzed 17 *a priori* models (Tables 3, 4). Of 8 temporal models, the model representing the genetics hypothesis inferring all three subspecies distinct [$V = \beta_0 + \beta_1 (NSO) + \beta_2 (CSO)$] (Hypothesis 1, Table 2) (AOU 1957, Barrowclough et al. 1999) was the top ranked model for describing variation in Four-note Location Calls among Spotted Owls (Table 5). This model had the lowest AIC_c value and accounted for 83.6% of the Akaike weight, which indicated strong evidence that this model best explained the data from this set of models (Table 5, Burnham and Anderson 1998). The strength of evidence for the genetics model was 10 times that of the next best model, a habitat model [$V = \beta_0 + \beta_1 (Conifer) + \beta_2 (Broadleaf)$] (Hypothesis 6, Table 2) which suggested that temporal characters within owl calls were also related to the sum basal area of conifers and broadleaf trees.

In the selected genetics model, the length of note 2+3 (NL2+3) and the internote length between note 3 and 4 (INL3-4) explained most of the variation in temporal structure, $R^2 = 0.26$ and 0.17, respectively. Length of note 4 (NL4) explained less variation ($R^2 = 0.13$). The best model explained 49% of the variation in temporal structure sampled.

Parameter estimates were most precise for NL 2+3; 95% confidence intervals did not overlap zero for all three subspecies (Table 6). Estimates for NL4 and INL3-4 were

Table 5. Ranking and weighting, based on AIC_c, of 8 *a priori* models describing variation in temporal structure of Four-note Location Calls among the three subspecies of Spotted Owls.

Model	K	AIC_c	Rank	ΔAIC_c[a]	Akaike Weights
$V = ß_0 + ß_1 (NSO) + ß_2 (CSO)$	9	-566.47	1	0.000	0.836
$V = ß_0 + ß_1 (Conifer) + ß_2 (Broadleaf)$	9	-561.91	2	4.567	0.085
$V = ß_0 + ß_1 (Broadleaf)$	8	-559.88	3	6.591	0.031
$V = ß_0 + ß_1 (MSO + CSO)$	8	-559.57	4	6.905	0.026
$V = ß_0 + ß_1 (NSO + CSO)$	8	-558.39	5	8.089	0.015
$V = ß_0 + ß_1 (Total\ Conifer + Broadleaf)$	8	-555.53	6	10.948	0.004
$V = ß_0 + ß_1 (Conifer)$	8	-555.13	7	11.346	0.003
$V = ß_0$	7	-544.95	8	21.526	0.000

a. $\Delta AIC_c = AIC_{ci} -$ minimum AIC_c

Table 6. Parameter estimates and 95% confidence intervals from a MANOVA model distinguishing the three subspecies of Spotted Owls based on temporal call characteristics of Four-note Location Calls. Response variables were the note length of note 2 plus note 3 (NL2+3), the internote length between notes 3 and 4 (INL3-4), and the length of note 4 (NL4).

	Parameter Estimates (95% Confidence Intervals)		
Response Variable	Mexican Spotted Owl	California Spotted Owl	Northern Spotted Owl
NL2+3 (sec)	0.0573 (0.0246, 0.0900)	0.0483 (0.0142, 0.0824)	0.4690 (0.4433, 0.4947)
INL3-4 (sec)	-0.1389 (-0.3020, 0.0242)	0.0678 (-0.1023, 0.2379)	1.3250 (1.1970, 1.4530)
NL4 (sec)	0.0596 (-0.0037, 0.1229)	0.0736 (0.0074, 0.1398)	0.3296 (0.2798, 0.3794)

less precise with California Spotted Owls only having an effect in NL4, whereas Northern Spotted Owls did not overlap zero for either parameter (Table 6). California Spotted Owls had longer NL2+3 and NL4 relative to both the Northern and Mexican Spotted Owls, whereas Mexican Spotted Owls had the shortest INL 3-4 (Table 7). For NL2+3 and NL4, California and Mexican owls only differed by 9 and 14 msec, respectively (Table 8). Overall, the greatest temporal difference in FLC call structures occurred at the internote duration between notes 3 and 4 (Table 8) and mean time ranged from 1.1861 sec to 1.3928 sec (Table 7). Newman-Kuells results indicate that the Northern Spotted Owl can be distinguished from both the California and the Mexican Spotted Owl by the total duration of notes 2 and 3 and the Mexican Spotted Owl distinguished from the California Spotted Owl by the internote length between notes 3 and 4 (Table 8).

Interestingly, even though habitat was not selected as an explanatory factor for temporal differences, all subspecific, total call lengths agreed with AAH predictions; owls from more closed forest conditions produced longer calls (Table 9). However, individual components that comprised those calls did not uniformly conform to AAH predictions. In agreement with AAH predictions, Mexican Spotted Owls produced the overall shortest calls (2.1017 sec, Table 9), despite production of the longest NL2+3 duration (Table 7). Northern Spotted Owls produced the shortest NL2+3 and NL4 durations (Table 7); hence, these owls produced FLC's of intermediate length (2.1225 sec, Table 9). And California Spotted Owls emitted the longest Four-Note Location Calls

Table 7. Least-square means and 95% confidence intervals from a MANOVA model distinguishing the three subspecies of Spotted Owls based on temporal call characteristics of Four-note Location Calls. Response variables were the note length of note 2 plus note 3 (NL2+3), the internote length between notes 3 and 4 (INL3-4), and the length of note 4 (NL4).

	Least Square Means (95% Confidence Intervals)		
Response Variable	Mexican Spotted Owl	California Spotted Owl	Northern Spotted Owl
NL2+3 (sec)	0.5263 (0.5054, 0.5473)	0.5173 (0.4940, 0.5405)	0.4690 (0.4425, 0.4955)
INL3-4 (sec)	1.1861 (1.0815, 1.2908)	1.3928 (1.2767, 1.5089)	1.3250 (1.1926, 1.4574)
NL4 (sec)	0.3892 (0.3485, 0.4299)	0.4032 (0.3581, 0.4484)	0.3296 (0.2782, 0.3811)

Table 8. Effect sizes and 95% confidence interval results for temporal call characteristics across Spotted Owl subspecies. The effect size values represent the differences in treatment effect between two subspecies. The subspecies are represented as follows: NSO – Northern Spotted Owl, MSO – Mexican Spotted Owl, and CSO – California Spotted Owl.

	Effect Sizes (95% Confidence Intervals)		
Response Variable	NSO versus MSO	NSO versus CSO	MSO versus CSO
NL2+3 (sec)	0.0573 (.0165, .0981)	0.0483 (.0130, .0836)	0.0090 (-.0223, .0403)
INL3-4 (sec)	0.1389 (-.0299, .3077)	0.0678 (-.1445, .2801)	0.2067 (.0503, .3631)
NL4 (sec)	0.0596 (-.0058, .1250)	0.0736 (-.0087, .1559)	0.0140 (-.0466, .0746)

Table 9. Mean values ± standard errors for frequency response variables (*n* = 40) and temporal response variables (*n* = 39) across all three subspecies of Spotted Owls. Frequency measures are: maximum frequency of note 2 and 4 (MFN2,4), width of note 3 (NWN3), and frequency slope of note 4 (FSN4). Temporal measures are: note length of notes 2 and 3 (NL2+3), internote length between notes 3 and 4 (INL3-4), and note length of note 4 (NLN4). Temporal values were summed to create the total mean call length.

	Mean ± Standard Error		
Response Variables	Mexican Spotted Owl[a]	California Spotted Owl[b]	Northern Spotted Owl[c]
MFN2 (Hz)	657.2290 ± 7.44	691.4427 ± 7.51	636.4318 ± 16.37
MFN4 (Hz)	649.0776 ± 7.53	677.2264 ± 8.12	644.6266 ± 9.42
NWN3 (Hz)	124.5591 ± 2.39	123.7938 ± 3.28	122.7928 ± 4.40
FSN4 (Hz)	180.7828 ± 10.37	254.1262 ± 19.60	237.8297 ± 25.05
NL2+3 (sec)	0.5174 ± 0.01	0.5140 ± 0.02	0.4689 ± 0.02
INL3-4 (sec)	1.1990 ± 0.04	1.3924 ± 0.08	1.3241 ± 0.05
NL4 (sec)	0.3853 ± 0.13	0.4032 ± 0.02	0.3295 ± 0.03
Total Call Length (N2-N4) (sec)	2.1017 ± 0.18	2.3096 ± 0.12	2.1225 ± 0.10

a. *n* = 16
b. *n* = 14 (Hz); *n* = 13 (sec)
c. *n* = 10

(2.3096 sec, Table 9), a result of producing, on average, the longest INL3-4 and NL4 duration (Table 7).

Variation in Frequency Structure of Spotted Owl Four-note Location Calls

Of the 9 *a priori* models for the frequency analysis (Table 10), the AAH model that included both conifer and broadleaf tree mean basal area [$V = \beta_0 + \beta_1 (Conifer) + \beta_2 (Broadleaf)$] (Hypothesis 6, Table 2) best explained variation in owl call frequencies. This model had the lowest AIC_c value and accounted for 90.8% of the Akaike weight, which indicated strong evidence that this model best explained the data (Table 10, Burnham and Anderson 1998). The strength of evidence for this top habitat model was 10 times greater than the next best model [$V = \beta_0 + \beta_1 (NSO) + \beta_2 (CSO)$] (Hypothesis 1, Table 2) (Table 10), the genetics model that separated Spotted Owls into three separate subspecies (Barrowclough et al. 1999). These two models were also the same top ranked models in the temporal analysis, yet in reverse order (Tables 5, 10). The best frequency model explained 52% of the variation sampled.

Basal area of conifer and broadleaf trees explained variation in the mean frequency of note 2 (MFN2) and note 4 (MFN4), ($R^2 = 0.3182$ and 0.2943), whereas only conifer basal area best explained the frequency slope of note 4 (FSN4) ($R^2 = 0.2041$). Note width of note 3 (NWN3) did not contribute much to the model ($R^2 = 0.0050$). An increase in basal area of conifers was correlated with an increase in frequency of MFN2, MFN4, and FSN4 (Table 11). Conversely, MFN2, and MFN4 were negatively associated with the amount of broadleaf basal area (Table 11). Although there was a negative association between FSN4 and mean basal area of broadleaf trees, the parameter estimate

Table 10. Ranking and weighting, based on AIC_c, of 9 *a priori* models describing variation in frequency characteristics in Four-note Location Calls among the three subspecies of Spotted Owls.

Model	K	AIC_c	Rank	ΔAIC_c[a]	Akaike Weights
$V = \beta_0 + \beta_1$ (Conifer) + β_2 (Broadleaf)	13	1068.07	1	0.000	0.908
$V = \beta_0 + \beta_1$ (NSO) + β_2 (CSO)	13	1072.68	2	4.611	0.091
$V = \beta_0 + \beta_1$ (Conifer)	12	1083.88	3	15.812	0.000
$V = \beta_0 + \beta_1$ (Total Conifer + Broadleaf)	12	1084.17	4	16.104	0.000
$V = \beta_0 + \beta_1$ (MSO + CSO)	12	1084.50	5	16.432	0.000
$V = \beta_0 + \beta_1$ (NSO + CSO)	12	1085.50	6	17.432	0.000
$V = \beta_0 + \beta_1$ (Weight)	12	1085.56	7	17.489	0.000
$V = \beta_0 + \beta_1$ (Broadleaf)	12	1085.95	8	17.885	0.000
$V = \beta_0$	11	1092.43	9	24.362	0.000

a. $\Delta AIC_c = AIC_{ci} -$ minimum AIC_c

Table 11. Parameter estimates from a MANOVA model examining the relationship of mean basal area (m^2/ha) of conifers and broadleaf trees at nest sites to frequency characteristics of Spotted Owls Four-note Location Calls. Response variables were the maximum frequency of note 2 and 4 (MFN2 and MFN4), the note width of note 3 (NWN3), and the frequency slope of note 4 (FSN4).

	Parameter Estimates (95% Confidence Intervals)	
Response Variable	Conifer Basal Area	Broadleaf Basal Area
MFN2 (Hz)	3.386 (1.6122, 5.1598)	-79.075 (-118.1417, -40.0083)
MFN4 (Hz)	2.691 (1.2622, 4.1198)	-57.903 (-89.3532, -26.4528)
NWN3 (Hz)	-0.061 (-0.6372, 0.5152)	- 0.686 (-13.3574, 11.9854)
FSN4 (Hz)	3.586 (0.4931, 6.6789)	- 8.181 (-72.2773, 59.9153)

was not different from zero based on the 95% confidence intervals (Table 11). In general, Spotted Owl call frequencies were positively correlated with mean conifer basal area and negatively correlated with mean broadleaf basal area. Overall, parameter estimates from the frequency MANOVA were less precise than estimates from the temporal MANOVA analyses (Tables 7, 11).

As predicted by the Acoustic Adaptation Hypothesis, Northern Spotted Owls produced calls with the lowest mean frequency (Table 9). However, contrary to AAH predictions, Mexican Spotted Owls produced lower frequencies for both note 2 (657.23 Hz) and note 4 (649.08 Hz) than did California Spotted Owls (691.44 and 677.23 Hz respectively). Also contradictory to the AAH, California Spotted Owls produced the greatest frequency modulation within note 4 (FSN4; Table 9). California Owls averaged a pitch change of 254 Hz while vocalizing note 4, this was 74 Hz greater than the smallest change averaged by Mexican Spotted Owls (Table 9). However, the frequency slope of note 4 also was the least precise of all response variables (Appendix A).

AAH Call Transmission Experiment

I conducted five one-way repeated measures ANOVA's, one for each response variable (Table 12). Sources of variation included both subspecies and territory. I included broadcasts from all 10 territories in each ANOVA. During sonogram processing, I discovered 3 Mexican Spotted Owl recordings that were broadcast too loud and therefore distorted. I performed ANOVA's with and without the three distorted samples. The separate analyses resulted in similar conclusions (Table 12).

Table 12. Results from repeated measure ANOVA's testing the Acoustic Adaptation Hypothesis. Spotted Owl calls of all three subspecies were broadcast at 10 Northern Spotted Owl sites. Recordings at 3 sites were slightly distorted. However, results indicate negligible effects with their inclusion. Alpha level of 0.05 was used to determine significant *P*-values.

	All Samples Included		Three Samples Removed	
Source Of Variance	df	*P*	df	*P*
Peak Intensity Per Hertz Per Call:				
Territory	9	0.0000	6	0.0000
Subspecies	2	0.0002	2	0.0138
Internote 3-4 Distance:				
Territory	9	0.5227	6	0.3369
Subspecies	2	0.0000	2	0.0002
Length of Note 4:				
Territory	9	0.5401	6	0.4849
Subspecies	2	0.9425	2	0.7869
Peak Frequency Note 2:				
Territory	9	0.9826	6	0.9525
Subspecies	2	0.9107	2	0.7847
Peak Frequency Note 4:				
Territory	9	0.8851	6	0.7984
Subspecies	2	0.3445	2	0.3330

Of the five response variables measured, one temporal measurement and one frequency measurement were significant among the three subspecies (Table 12). As predicted, Peak Intensity per Hertz of Mexican Spotted Owl calls decreased more over 25 m, in comparison to the Northern and California subspecies (Figure 3). Mexican owl calls lost 11.423 dB (SE = 0.285) in comparison to 9.377 dB (SE = 0.285) and 9.901 (SE = 0.285) for the California and Northern owl, respectively. Contrary to my prediction, the duration between note 3 and note 4 changed the least over 25 m in Mexican owl calls (0.037 sec, SE = 0.007) compared with the other two subspecies (0.095 sec, SE = 0.0067 for California and 0.067 sec, SE = 0.0067 for Northern subspecies, Figure 4). In addition, there was a significant territory (stand) effect in the Peak Intensity per Hertz analysis (Table 12). Different owl stands influenced owl broadcast energy by as much as 17.840 dB to as little as 5.778 dB (SE = 0.7547). I found no significant subspecies or stand effects for the length of note 4 or the peak frequency of note 2 or 4 (Table 12).

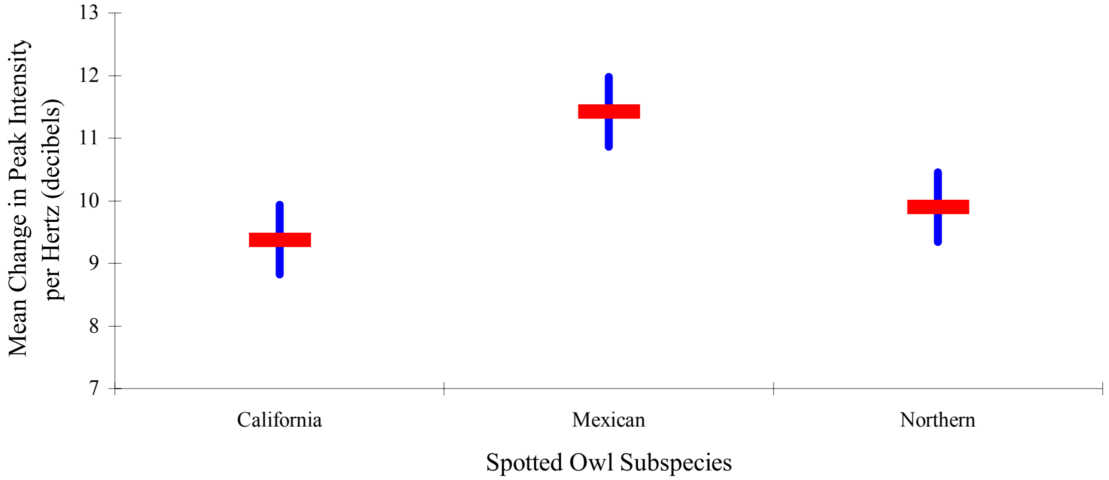


Figure 3. Mean change over 25 m in Peak Intensity per Hertz per call (PIH) of Spotted Owl vocalizations after being transmitted through the acoustic environment of the Northern Spotted Owl. Vertical bars represent 95% confidence intervals.

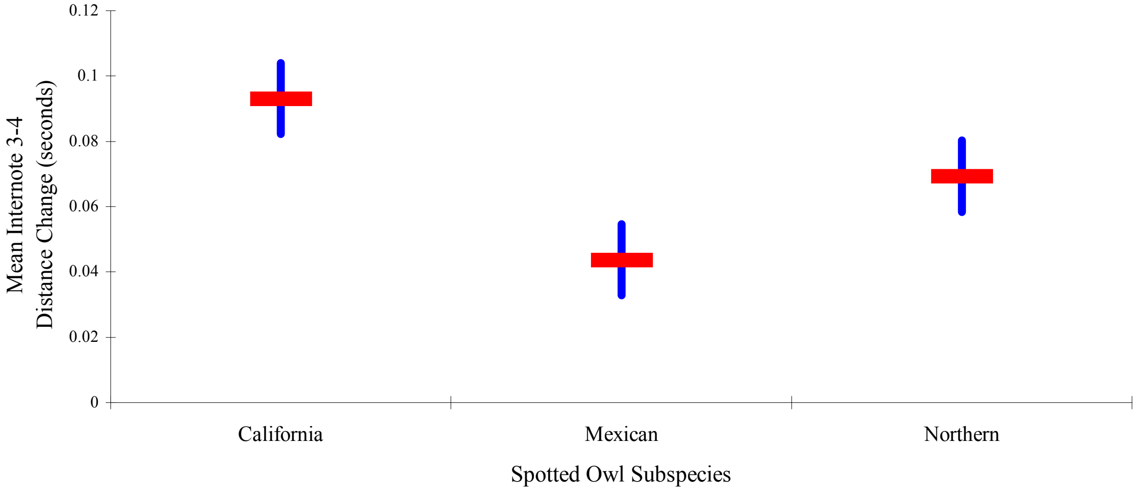


Figure 4. Mean change over 25 m in the internote length between notes three and four (INL3-4) of Spotted Owl vocalizations after being transmitted through the acoustic environment of the Northern Spotted Owl. Vertical bars represent 95% confidence intervals.

DISCUSSION

Variation in Temporal Structure in Spotted Owl Four-note Location Calls

Identifying each Spotted Owl subspecies as a distinct group provided the strongest evidence for differences among their temporal call structures. The remaining two genetic models, which proposed alternate groupings, did not explain variation in temporal structure. My results for temporal call characteristics were consistent with previous delineations of the Spotted Owl into three subspecies (American Ornithological Union 1957, Barrowclough et al. 1999). However, my results do not imply a cause and effect relationship because my genetics hypothesis was based on mtDNA results. Rather they represent a model for differentiation among the recognized three species. Additionally, although my habitat models were unable to explain temporal characteristics in Spotted Owl calls, my AAH experimental results suggested different rates of degradation for subspecific transmissions of internote 3-4 segments in Four-note Location calls. The ranging hypothesis (Morton 1982) provided an explanation for temporal call qualities that limit transmission degradation in native forests.

Subspecific mtDNA results indicated high rates of dispersal between individuals of the same subspecies and a scarcity of historical gene flow among the three subspecies of Spotted Owls (Barrowclough et al 1999). My model selection results also reflected this pattern; temporal qualities of Spotted Owl calls were more similar within individuals of the same subspecies. Mexican Spotted Owls produced the shortest calls despite producing the longest NL2+3 duration (Table 9). Northern Spotted Owls produced the shortest NL2+3 and NL4 durations, resulting in these owls producing FLC's of intermediate length (Table 9). The California subspecies produced the longest Four-note Location Calls even though the California and Northern Spotted Owl produced similar call characteristics for the INL3-4 variable (Table 9). Overall, although best explained by a subspecific model, some temporal properties of owl calls were also in agreement with bioacoustic predictions for limiting acoustic degradation (Morton 1975). However, only subspecific total call lengths (Table 9) agreed with AAH predictions for long distance transmission of owl calls in their native habitat, and these values were *ad hoc* summations.

In the ranging hypothesis, Morton (1982) proposes that selection would favor acoustic properties that limit degradation because use of sound cues that disguise the proximity of a rival could disrupt interactions between territory owners. The ranging hypothesis relies on birds using degradation to determine the location of probable contenders because sound in a homogeneous environment degrades predictably over propagation distance (Morton 1982, Wiley and Richards 1982, Fotheringham et al. 1997, Brown and Handford 2000). Research that supports the ranging hypothesis (Naguib 1995, 1996, Morton and Derrickson 1996) demonstrated that not all species use the same degradation cues to determine their responses. For example, Carolina Wrens (*Thryothorus ludovicianus*) use overall amplitude, reverberation, and frequency-dependent attenuation as unique stimuli for ranging (Naguib 1995). If territory owners use the estimated distance of a potential rival to determine their response, this could translate into active selection of energetic expenditures, e.g. forage, roost, or defend (Morton 1982). Since their auditory systems are specialized for sound localization, it is highly possible owls use these acoustic cues (Gill 1995). The ranging hypothesis also requires the receiver to be familiar with call types or call features (Morton 1982, Naguib 1996), and the limited repertoire size and high site fidelity of Spotted Owls (Forsman et al 1984, Gutiérrez et al. 1995) meets this requirement.

In my transmission experiment, internote duration change between notes 3 and 4 (INL3-4) measured the degradation effect of scatter and bounce (reverberation) incurred by a call during transmission (Fotheringham et al. 1997). Although subspecific call degradation did not conform to Acoustic Adaptation Hypothesis predictions, expectedly all INL3-4 durations decreased in length due to accumulation of echoes (Figure 4). Unexpectedly, however, the rate of degradation was opposite to that predicted. Owl calls from closed habitats (Northern and California Spotted Owls) degraded more over 25 m in comparison to calls from open habitats (Mexican Spotted Owls) (Figure 4) when broadcast through Northern Spotted Owl habitat. Although this result was counterintuitive, it was important to note that calls from owls that evolved in more closed breeding habitats--that is calls with longer internote spaces (Table 9)--transmitted and degraded similarly (Figure 4). Transmission experiments have been used to test this theory (Fotheringham et al. 1997), but behavioral observations during playback experiments are typically required for stronger evidence supporting the ranging hypothesis (Naguib 1995, 1996, Morton and Derrickson 1996). Ultimately, if these subspecific temporal characters were adaptive, and Spotted Owls used call degradation for ranging, it could influence management of their energetic expenditures while maintaining territory spacing.

Variation in Frequency Structure of Spotted Owl Four-note Location Calls

Mean basal area of both conifers and broadleaf trees in nesting habitat occupied by Spotted Owls provided the strongest evidence for observed differences in vocal frequencies among the subspecies. Thus, it was not only the physical area (total mean basal area) occupied by the trees themselves (Table 2, Hypothesis 7), but also the unique forest stands created by the distinctive presence of each tree type (Table 2, Hypothesis 6). These results, which support the Acoustic Adaptation Hypothesis (Morton 1975), lend further credibility to the importance of the broadleaf component previously documented between Mexican Spotted Owls and Gambel oaks (Seamans and Gutiérrez 1995, Ganey et al. 1999, May 2000). Accordingly, if the acoustic environment within which a species evolved plays a role in call formation (Morton 1975), then my findings also present new information for examining current views (see page 50) of the pre-settlement conditions of Sierra Nevada forests (Skinner and Chang 1996).

My results concurred with overall expected physical properties of soundwaves for effective transmission of Spotted Owl vocalizations. Since forests composed primarily of broadleaf trees are known to attenuate sound up to 15 dB/100 m more than conifer forests (Martin and Marler 1977, Bradbury and Vehrencamp 1998), it was logical that owl call frequencies were negatively correlated with broadleaf basal area. The degree of hertz change found for conifers was very slight in comparison to the strong relationship with broadleaf trees (Table 11). For example, an increase in 1 m^2/ha basal area of conifers resulted in an increase in maximum frequency of note 2 (MFN2) Hz by 3.4 Hz, whereas an increase in 1 m^2/ha basal area of broadleaf trees resulted in a 79.1 Hz decrease in

MFN2 (Table 11). In other words, if owl calls were to successfully broadcast in broadleaf forests, use of lower frequencies would be needed for calls to attenuate less during transmission.

Viewed as a gross measure of attenuation, my results on Peak Intensity per Hertz per call (PIH) exemplifies subspecific differences in the transmission qualities of owl vocalizations. PIH measures the greatest volume given per hertz or the frequency an owl emphasizes most while vocalizing (Charif et al. 1995). Ideally, lower frequencies, which degrade and attenuate less, would receive the greatest energy because they are more likely to transmit intact vocalizations to intended receivers (Wiley and Richards 1982). Despite this assumption, California Spotted Owls produced the highest frequency calls (Table 9), which attenuated less than the lower pitched calls of Mexican Spotted Owls (Figure 3). Thus although my results agree with AAH predictions, Mexican Spotted Owl calls lost 2 dB more over 25 m than either the Northern or California subspecies (both of which occupy denser forests). It is apparent that these differences are not just a product of frequency-dependent attenuation (Table 9, Figure 3).

Mexican Spotted Owls occupied smaller, more open territories than Northern or California owls (Gutiérrez et al. 1995). Thus, average broadcast energies required to propagate lower pitches in native habitats were perhaps less than that required for advertising in larger, more closed territories. Because Mexican Spotted Owl calls had lower frequencies and their habitat contained a strong oak component, I hypothesize that Mexican Spotted Owl calls would not attenuate at an increased level when broadcast through their native, less dense habitat. An experiment where the calls of all three subspecies were broadcast in all three habitats would be required to further test this assumption.

Although lower frequency calls attenuate less regardless of habitat (Wiley and Richards 1982), morphology constrains what vocal frequencies are physiologically cost-effective to produce (Bradbury and Vehrencamp 1998). Energetic costs are minimized when animals produce sounds with wavelengths equal to or smaller than their body size; use of lower frequencies results in increased production costs (Bradbury and Vehrencamp 1998). Since avian sound production efficiency ranges from 2-15%, use of lower frequency calls results in energetic tradeoffs (Bradbury and Vehrencamp 1998). Thus, the reason why the larger California Spotted Owl produced higher frequency calls than the smaller, Mexican Spotted Owl is still unclear. Although the three subspecies were delineated based on morphological characteristics (American Ornithological Union 1957), this did not translate into a model that separated the three Spotted Owl subspecies based on average call frequencies. As previously stated, numerous examples exist of an inverse relationship between bird mass and call frequency; larger birds produced lower pitched calls (Hoste and De Smet 2000) because of a direct relation to syrinx size (Miller 1934). Other research provided no evidence for relation of body size to vocal frequency (Handford and Lougheed 1991, Baptista 1996) and no direct evidence for genetic control of vocal frequency has been shown to exist (Baptista 1996). My results support these latter findings.

I believe the Spotted Owl Four-note Location calls characteristics I sampled are a product of pre-European settlement forest conditions. Assuming this is correct, and that the high structural diversity found at existing nest stands is similar to historic nest stands, it appears that both Northern and California Spotted Owls evolved in more closed forest conditions than Mexican Spotted Owls. My conclusions concerning the Acoustic Adaptation Hypothesis, and owl call frequencies and nesting habitat, contradict deductions about primitive forest conditions in the Sierra Nevada (Skinner and Chang 1996). Skinner and Chang (1996) predicted the historical presence of high structural diversity in Sierra Nevada mixed-conifer forests, specifically created by the presence of large, older trees, and a hardwood understory. However, the belief is "that many [mixed-conifer] forest areas were generally more open than they are today" because of a frequent fire interval (Skinner and Chang 1996, page 1054). Yet, due to poor historical records, these deductions were inferred from fire regime information extrapolated from southern Cascades and Klamath Mountain forests and incorporated with knowledge of fire history, behavior, and consequences (Skinner and Chang 1996). The effects of 100 years of fire suppression are not being challenged here. However, my results do not entirely match their conclusions; Spotted Owl call frequencies were directly correlated to the conifer and broadleaf components sampled at modern day nest sites. When viewed as a reflection of historical nesting habitat, California Spotted Owl call frequencies predict that historical nest sites were composed of dense forest stands, likely with a broadleaf understory, and similar to those undisturbed sites where owls nest today.

Limitations and Future Research

My results represent the first iteration of research directed at understanding the evolution of Spotted Owl Four-note Location Call structure. Since my best models only explain 50% of the variation in vocalization structure, it is likely that more parsimonious models exist. For example, better explanatory models may include a combination of frequency and temporal response variables and interactions between subspecies and habitat. Relating landscape-scale habitat values to represent all owls within a study area dictated it inappropriate to extrapolate these findings beyond Spotted Owl nesting habitat. If I could have related each owl to its own nest site, perhaps more variation would have been explained, yet this data was not available for all recorded males. Ultimately, I would have preferred to model all forests used by owls (nesting, roosting, and foraging) within their territories. Additionally, my morphology hypothesis would have been stronger if I could have attributed a unique, physical weight to each individual owl. Since all recorded males were not captured and weighed, this also was not possible.

Future analyses should consider controlling for, or including as covariates, other factors that can affect the structure of vocalizations, such as the length of time an owl was in a sampled population (emigrant versus native, Sorjonen 1986b), number of neighbors (Fitton 1991), subpopulation genetic templates (Ryan and Rand 1999), topography (Fotheringham et al. 1997, Brown and Handford 2000), altitude (Handford and Lougheed 1991), and roosting and foraging habitat (unique stand characteristics per owl). All of these potential sources of variation could affect either individual call production or sound propagation.

Further evaluation of my results could also include examination of recordings from California Spotted Owls in the insular San Bernardino population (LaHaye et al. 1994). The physical proximity of this population to Mexican Spotted Owl sites, as well as the structure of southern California forests (LaHaye et al. 1994), makes this study area an excellent test site for both my genetic and habitat models. Additionally, I would include this area in further testing of the Acoustic Adaptation Hypothesis. A more rigorous broadcast experiment, in which calls of all three subspecies were transmitted through habitats occupied by all Spotted Owl subspecies (Daniel and Blumstein 1998), might provide stronger evidence for adaptation of owl calls to habitat. Incorporation of additional broadcast distances (up to 100 m) and broadcast heights, as well as more individuals per subspecies, would undoubtedly assist future research.

My results provide a foundation for examining individual identification of Spotted Owls using vocalization structure. Baptista (1993) hypothesized that individual Northern Spotted Owls could be identified by the unique shape of their fourth note. The 28% coefficient of variation for my frequency slope of note 4 variable (Appendix A) suggests there is a moderate degree of variation among individuals. In addition, high variability across individuals was also present for the length of note 4 (Appendix A). Moreover, Kuntz and Stacey (1997) speculated that Mexican Spotted Owl subpopulations could be discriminated based on unique note 3 shapes. I propose that analytical tools used in geographic information systems (e.g., McGarigal and Marks 1995) could be useful to further investigate note morphology. Common response variables measure only a portion of a note’s character, e.g. a change over time or a maximum frequency. Yet, if the entire area defined by a fundamental frequency were measured as a shape, analogous to a landscape polygon, then unique attributes per individual owl could be more closely examined, creating a vocal “fingerprint" or voice print (Baptista and Gaunt 1997).

Ultimately, it will likely require a combination of studies that examine population, subpopulation, and individual vocal differences, to explain more of the variation in vocalization structure within and among Spotted Owl subspecies.

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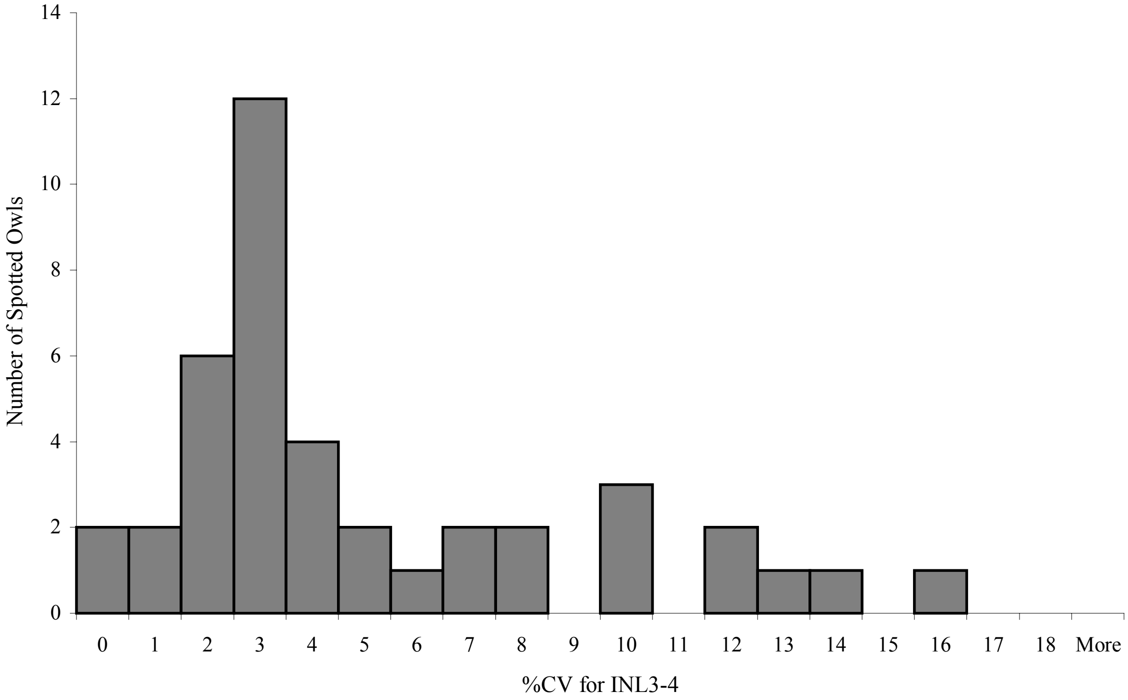
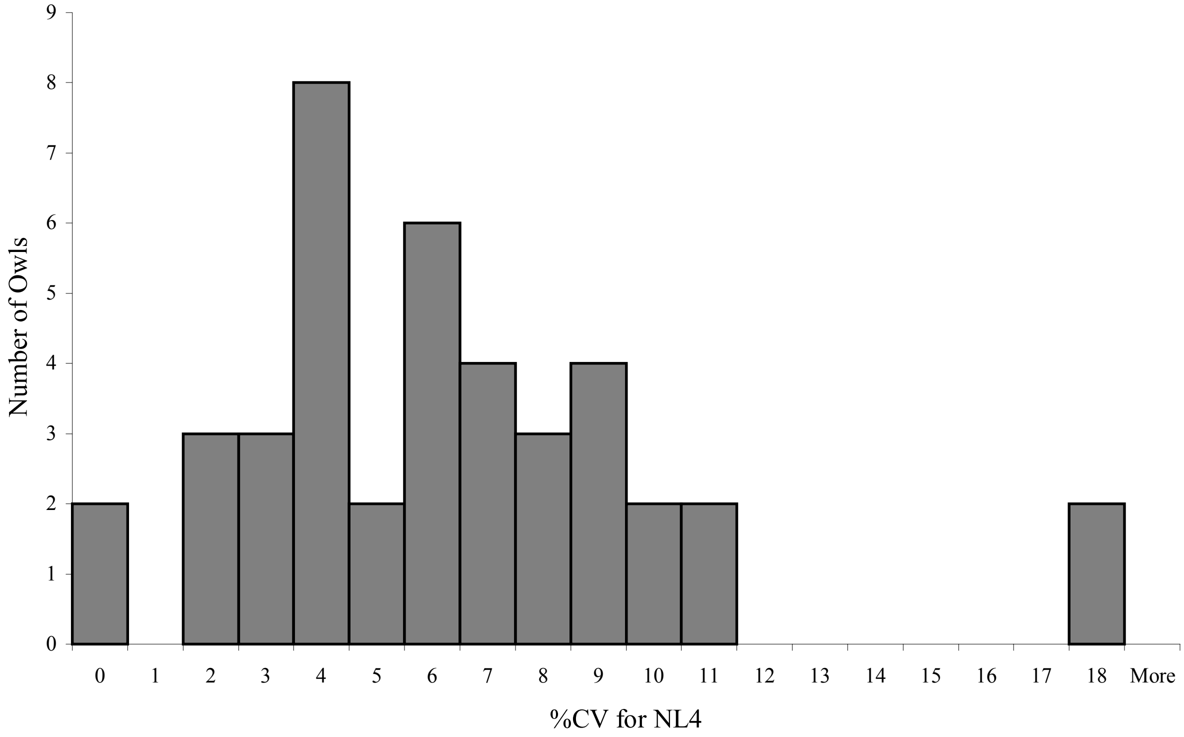
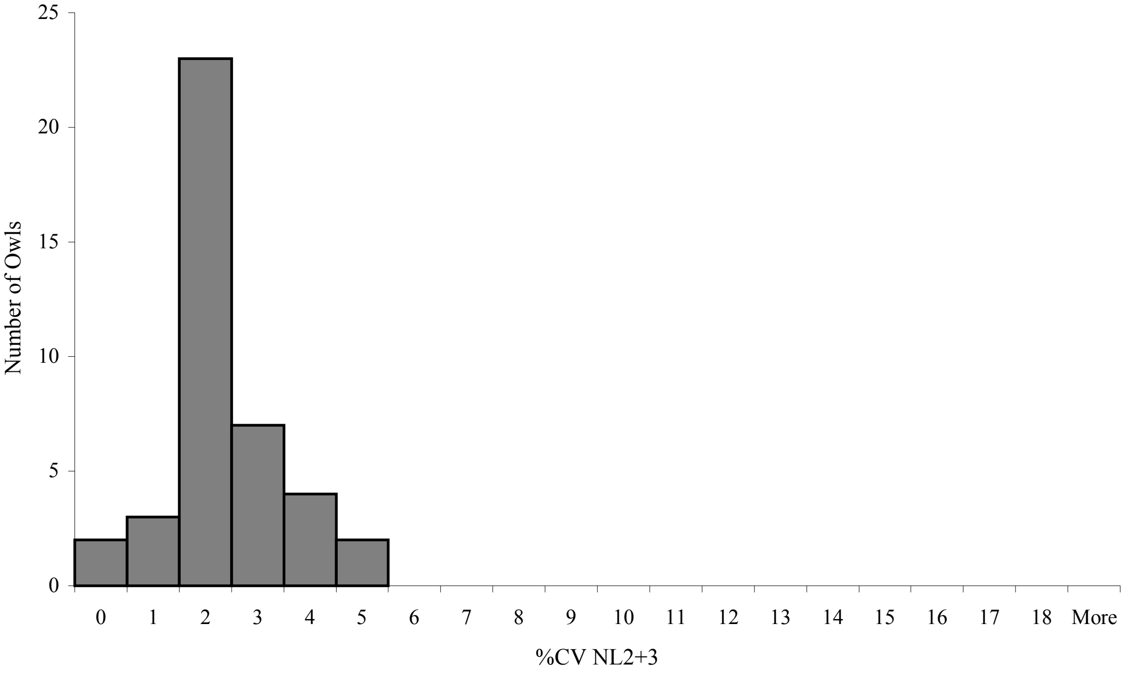
Appendix A. Histograms depicting the distribution of percent coefficient of variation for seven response variables used in MANOVA analyses testing variation in the Four-note Location Calls in Spotted Owls. Percent coefficient of sampling variation for temporal response variable internote length between notes 3 and 4 (INL3-4) from individual spotted owls representing the three subspecies.



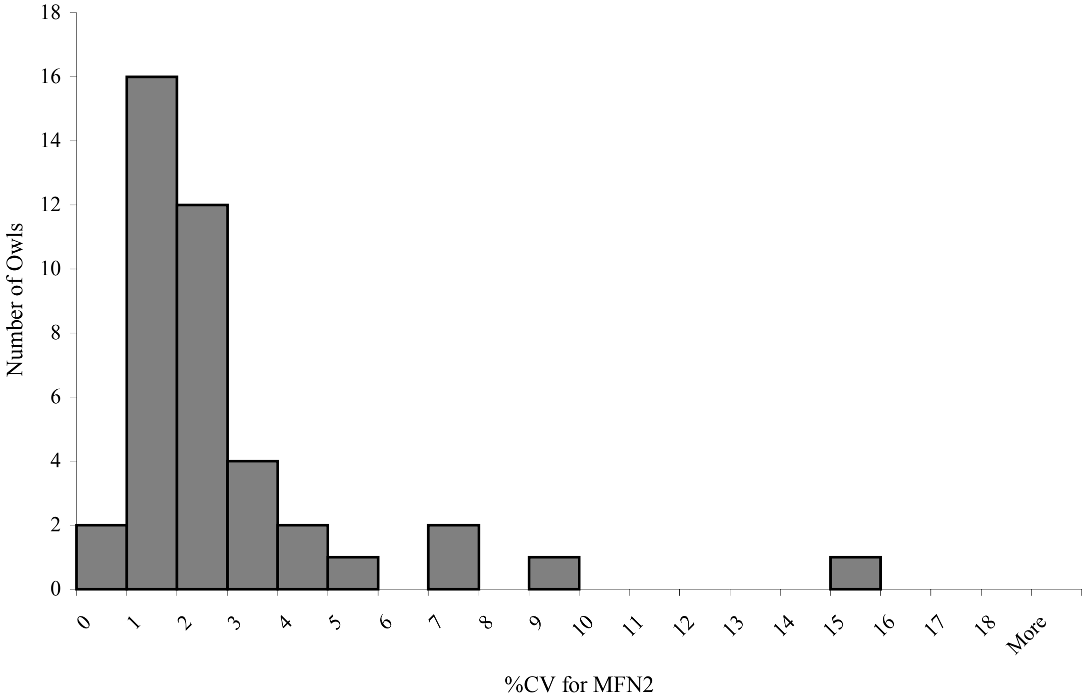
Appendix A. Histograms depicting the distribution of percentage coefficient of variation for seven response variables used in MANOVA analyses testing variation in the Four-note Location Calls in Spotted Owls (continued). Percentage coefficient of sampling variation for temporal response variable note length of note 4 (NL4) from individual spotted owls representing the three subspecies.



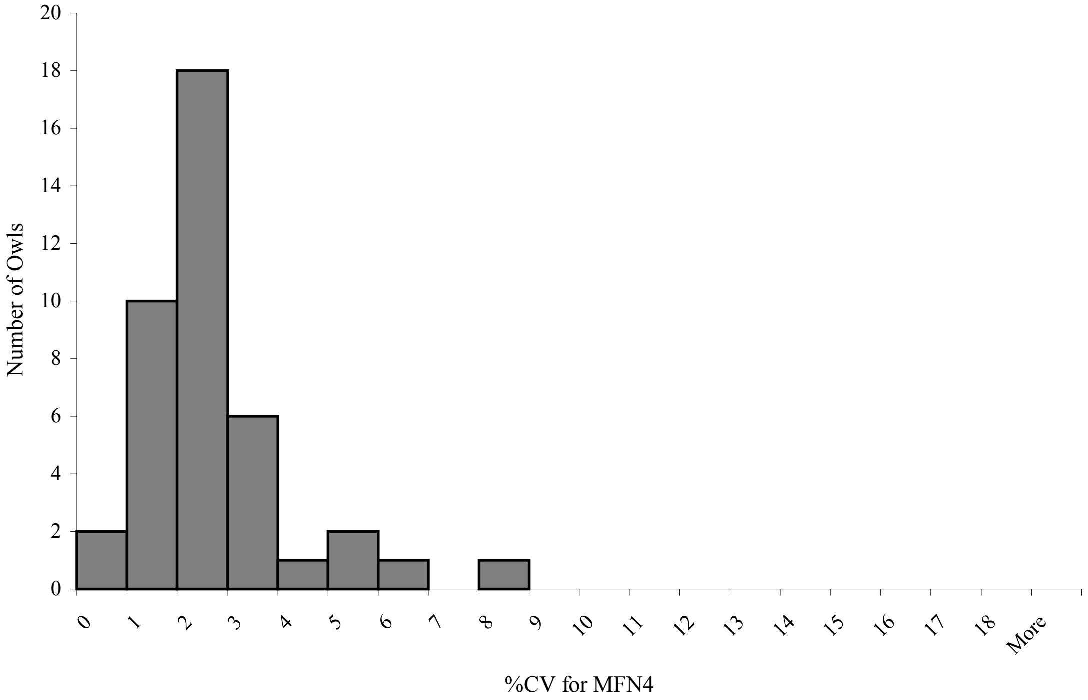
Appendix A. Histograms depicting the distribution of percentage coefficient of variation for seven response variables used in MANOVA analyses testing variation in the Four-note Location Calls in Spotted Owls (continued). Percentage coefficient of sampling variation for temporal response variable total length from beginning of note 2 to end of note 3 (NL2+3) from individual spotted owls representing the three subspecies.



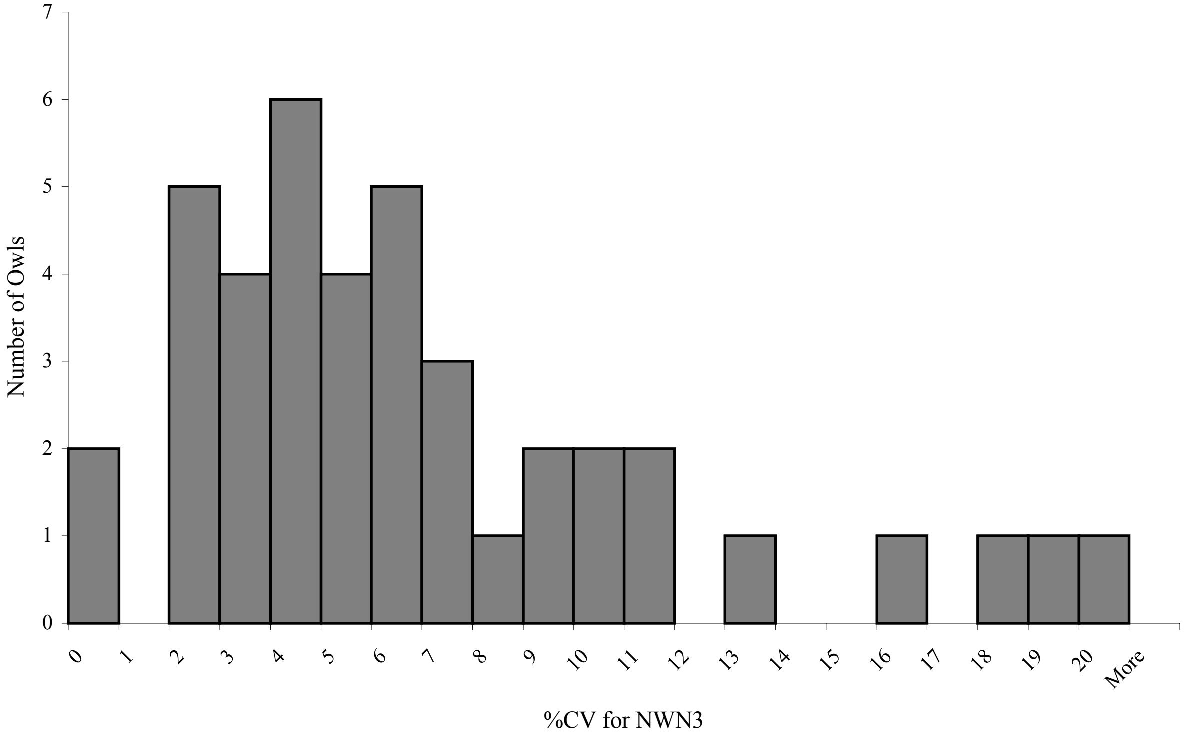
Appendix A. Histograms depicting the distribution of percentage coefficient of variation for seven response variables used in MANOVA analyses testing variation in the Four-note Location Calls in Spotted Owls (continued). Percentage coefficient of sampling variation for frequency response variable maximum frequency of note 2 (MFN2) from individual spotted owls representing the three subspecies.



Appendix A. Histograms depicting the distribution of percentage coefficient of variation for seven response variables used in MANOVA analyses testing variation in the Four-note Location Calls in Spotted Owls (continued). Percentage coefficient of sampling variation for frequency response variable maximum frequency of note 4 (MFN4) from individual spotted owls representing the three subspecies.



Appendix A. Histograms depicting the distribution of percentage coefficient of variation for seven response variables used in MANOVA analyses testing variation in the Four-note Location Calls in Spotted Owls (continued). Percentage coefficient of sampling variation for frequency response variable note width of note 3 (NWN3) from individual spotted owls representing the three subspecies.



Appendix A. Histograms depicting the distribution of percentage coefficient of variation for seven response variables used in MANOVA analyses testing variation in the Four-note Location Calls in Spotted Owls (continued). Percentage coefficient of sampling variation for frequency response variable frequency slope of note f (FSN4) from individual spotted owls representing the three subspecies.