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Vocalizations, vocal behaviour, and geographic variation in the calls, duets, and duetting behaviour of a nonpasserine, the Barred Owl (Strix varia)

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VOCALIZATIONS, VOCAL BEHAVIOUR, AND GEOGRAPHIC VARIATION IN THE CALLS, DUETS, AND DUETTING BEHAVIOUR OF A NONPASSERINE, THE BARRED OWL (*STRIX VARIA*)

by

KARAN JESSICA ODOM

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2009

Vocalizations, vocal behaviour, and geographic variation in the calls, duets, and duetting behaviour of a nonpasserine, the barred owl (*Strix varia*)

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Declaration of Co-Authorship / Previous Publication

I. Co-Authorship Declaration

I hereby declare that this thesis incorporates material that is result of joint research, as follows:

I am the sole author of the Chapter 1, the introduction. I am the primary author for the research in all other chapters, Chapters 2, 3, and 4. All three data chapters are co-authored by my supervisor, Dr. Daniel J. Mennill. The ideas, field work, analysis, and writing were conducted primarily by me. Dr. Mennill contributed financial assistance and guidance through logistical support and feedback during research design, analysis, and writing.

Chapters 1 and 4 are written in the style of The Auk. Chapter 2 is written in the format of the journal The Condor is intended for publication by September 2009. Chapter 3 is written in the form of the journal Behaviour and was submitted for publication on July 26, 2009.

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This thesis includes one original paper that has been submitted for publication in peer reviewed journals, as follows:

Thesis Chapter	Publication title/full citation	Publication status
Chapter 2	Odom, K. J. & Mennill, D. J. In review. A quantitative description of Barred Owl vocalizations, sex differences, and vocal activity. Condor.	In review
Chapter 3	Odom, K. J. & Mennill, D. J. In review. Duet function in barred owls: territory defense, neighbour-stranger discrimination, and communication networks. Behaviour.	In review

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Abstract

This thesis provides the first quantitative analysis of Barred Owl (*Strix varia*) vocalizations and their function. Male and female Barred Owls produce thirteen distinct vocalizations, often combining their calls to perform duets. Female and male calls exhibit distinguishable vocal characteristics useful for sex discrimination. Owls have different diel patterns for different call types and they incorporate specific call types into duets. An experimental playback of neighbour versus stranger owl's duets indicate that Barred Owls used duets to confront territory intruders, but do not discriminate between duets of neighbours versus strangers. Recordings made with a 3.5km transect of automated recording devices demonstrate that nearby owls within the population do not vocalize differently during playback versus silent pre-playback periods. A comparison of Barred Owl calls, duets, and duet behaviour throughout the southeastern United States reveals much variation, but this variation does not follow a consistent geographic pattern.

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Chapter 1

Introduction

Introduction

Birds are one of only four taxa recognized to learn their vocalizations. Among birds, only three taxonomic groups are known to learn song: the songbirds (suborder oscine of the order Passeriformes) and two non-songbird orders, the hummingbirds (Apodiformes) and parrots (Psittaciformes; Nottebohm 1972). Approximately half of the remaining bird species are suboscine passerines and non-songbirds (nonpasserines) considered to not learn song. The majority of bird song studies have focused on the oscine passerines, presumably because of their vocal learning abilities and the intriguing question that follows: *why do some birds learn their songs?* Aspects of bird vocalizations that are considered unique to song-learning species include large repertoires and distinct patterns of geographic variation that arise in the vocalizations of song-learning species, known as dialects (Krebs and Kroodsma 1980, Mundinger 1982). Much research has successfully shown both aspects of learned bird song are linked to the complex ways birds use their song to attract mates and mediate social interactions (reviewed in Beecher and Brenowitz 2005). Nevertheless, recent research has revealed ways that songs of suboscines and non-songbirds are complex and some studies have suggested they use song in functionally similar ways to song-learning species (Lovell and Lein 2005b, Leger 2005, Bretagnolle and Genevois 1997). However, comparatively few studies have actually studied non-song-learning species with respect to the complexity of their vocalizations, the ways that they use their vocalizations, or their patterns of vocal geographic variation (exceptions include Leger and Mountjoy 2003, Seddon and Tobias 2005, Fitzsimmons et al. 2008a). This thesis examines the vocalizations, vocal behaviour, and vocal geographic variation of a duetting nonpasserine, the Barred Owl

(*Strix varia*; Figure 1). The following topics in this introduction provide the necessary background to connect this work to our current knowledge of bird song. I begin with a brief review of vocal learning, function, and geographic variation of oscine songbirds and then explain the complex and similar use of vocalizations of suboscine songbirds and non-songbirds. I conclude with an introduction to my study species and a description of the chapters that follow.

Vocal learning in oscines, suboscines, and non-songbirds

Extensive laboratory experiments provide the foundation for what we know about passerine song learning. In one of the earliest examples, male Chaffinches (*Fringilla coelebs*) were reared in isolation and some individuals were exposed to conspecific song, while others were not (Thorpe 1958, Nottebohm 1968). Those that heard conspecific song developed normal song; those deprived of hearing song did not (Nottebohm 1968). Similar studies in the White-crowned Sparrow (*Zonotrichia leucophrys*) indicated that the timing of exposure to song is also very important; most males required hearing adult conspecific song between 8-56 days of age, otherwise they never developed normal song (Marler 1970). Much additional research in a variety of species showed that many other factors influence song learning. Day length and timing within the breeding season can drastically change the length and timing of the song-learning period. Young Marsh Wrens (*Cistothorus palustris*) that hatched early in the breeding season were restricted to memorization the season they were born, whereas late season hatchlings memorized songs into the next spring (Kroodsma 1982). In some species, the influence of a tutor can also have profound effects. Some species were more likely to learn heterospecific song if tutored by a live bird of a different species, even if they were exposed to conspecific auditory stimuli (Baptista and Gaunt 1997). Among the many species that learn songs, the extent to which song can be learned after the first year of life is also very variable. Bird song researchers often refer to ‘open-ended’ and ‘close-ended’ learners, meaning species that can and cannot learn song after the first year of life, respectively (Catchpole and Slater 2008). However, recent research indicates vocal learning occurs in several species into the second year of life, demonstrating that song learning in passerine birds is a complex and diverse process (Brenowitz and Beecher 2005).

In contrast to the extensive research on oscine songbirds, only a few species of suboscine songbirds and non-songbirds have been examined for evidence of song learning. Kroodsma (1984, 1985) provided evidence that three species of flycatchers in the family Tyrannidae (suboscine songbirds) produced normal song when reared in isolation without conspecific song. One of those species, the Eastern Phoebe (*Sayornis phoebe*), did not produce an exact copy of conspecific song when it was provided (Kroodsma 1989). Other studies indicate an absence of learning in Domestic Chickens (*Gallus domesticus*; Konishi 1963) and doves (*Steptopelia spp.*; Lade and Thorpe 1964, Nottebohm and Nottebohm 1971). Several other suboscine songbirds and non-songbirds lack the necessary neuronal circuitry for song learning (Metzdorf et al. 1999, Silverin et al. 2000, Matsunaga et al. 2008). Aromatase and estrogen-receptors associated with the vocal control center of the songbird brain were absent across diverse non-songbirds, including Ring Doves (*Streptopelia risoria*), Common Swifts (*Apus apus*), Grey Partridges (*Perdix perdix*), Barn Owls (*Tyto alba*), and Japanese Quails (*Coturnix japonica*) (Metzdorf et al. 1999, Silverin et al. 2000). Japanese Quails and Emerald

Doves (*Chalcophaps indica*) also lacked gene expression present in the song control center of song-learning oscines and Psittaciformes (Matsunaga et al. 2008). Given the extreme simplicity of many of these species' vocalizations, often consisting of simple one- to two-note phrases, the lack of learning is not surprising. Nevertheless, recent evidence suggests learning in a suboscine, the Three-wattled Bellbird (*Procnias tricarunculata*), indicating vocal acquisition in suboscines and non-songbirds may be more complex than previously thought (Saranathan 2007). Many bird song researchers have called attention to the need for more studies on vocalizations and vocal learning in suboscines and non-songbirds (Marler and Peters 1982, Mundinger 1982, Kroodsma 1996).

Bird song function

Bird song has two main functions: mate attraction and territory defense (Catchpole and Slater 2008). Hypotheses concerning the evolution of song learning frequently focus on selection for song complexity, large repertoires, and vocal flexibility to meet these two ends (Beecher and Brenowitz 2005). In some species, individuals with more complex song and larger repertoires attract more females than conspecifics with less elaborate or fewer songs (Mountjoy and Lemon 1991, Searcy and Marler 1984, reviewed in Searcy and Yasukawa 1996). This type of mate choice directionally selects for increasing repertoire size in some species and is one explanation for the huge repertoires of some species. For example, the Nightingale (*Luscinia megarhynchos*) can combine up to 230 songs into its repertoire (Kipper et al. 2004).

Bird songs and repertoires also play an important role in interaction and recognition at territory boundaries (Stoddard 1996, Beecher and Brenowitz 2005). Many songbirds possess individually recognizable vocalizations that facilitate conspecific discrimination and recognition (reviewed in Stoddard 1996). Neighbour-stranger discrimination and recognition is an important component of song in many songbirds. A bird's ability to distinguish familiar from unfamiliar individuals can reduce the costs associated with confronting territory intruders, as predicted by the 'dear-enemy' hypothesis (*sensu* Fisher 1954, Temeles 1994). Thus, many songbirds have individually distinguishable vocalizations or share population-specific songs (Stoddard 1996). Birds also use shared songs in intricate ways to communicate with intruding individuals. In a study by Burt et al. (2001), Song Sparrows (*Melospiza melodia*) were shown to use shared song to selectively match rivals in order to escalate aggressive encounters or to avoid mathching a rival to de-escalate an encounter. Conspecific recognition and the ability to effectively share songs selects for fewer, simpler, easily recognized songs within a bird's repertoire (Beecher and Brenowitz 2005). Thus, optimal song complexity and repertoire size varies with selection for mate attraction or territory defense. It may be this balance between repertoire size and social interaction that selects for the diversity of song learning strategies seen in songbirds (Beecher and Brenowitz 2005).

Recent evidence suggests that in both mate attraction and territory defense contexts, bird song also imparts information to multiple individuals simultaneously, either intentionally or unintentionally, within a communication network (McGregor 2005). A communication network occurs when individual territories are sufficiently small that an animal's vocalization can be heard by multiple individuals (McGregor

2005). This allows signalers to communicate with multiple conspecifics with a single vocalization and allows extra-pair individuals to eavesdrop on intra-pair interactions (McGregor and Peake 2000, Mennill et al. 2002). The communication network model is a valuable system for evaluating bird song function.

Vocal geographic variation and dialects

The variety of song learning strategies in songbirds gives rise to a diversity of vocal geographic patterns. Research on several songbird species provides the basis for what we know about dialect formation and maintenance. Below I outline some of the major patterns in several key species as examples of the kinds of vocal geographic patterns seen in songbirds and how they correspond to song learning strategies and bird song function.

White-crowned Sparrow

White-crowned Sparrows were one of the first species documented with clear dialects (e.g. males share songs over large areas among nearby populations, but have abrupt changes in several distinct song characteristics over short geographic areas between adjacent populations; Marler and Tamura 1962). Interestingly, different White-crowned Sparrow subspecies have different dialect patterns (Nelson et al. 1995, Nelson 1999, Nelson et al. 2001). In subspecies with shorter breeding seasons, juvenile White-crowned Sparrows have little time to learn songs, so they learn an exact copy of one male’s song (Nelson 1999, Chilton et al. 2002). Southern migrants or sedentary subspecies selectively choose their song from a combination of males’ songs (Nelson et al. 1995). These adaptations lead to differences in dialect area and amount of song-sharing within populations (Nelson 1999, Nelson et al. 2001).

Also, White-crowned Sparrow dialects do not coincide with substantial genetic boundaries, but local dialects may facilitate mating decisions and reflect ecological adaptations. Two subspecies of White-crowned Sparrows have little to no reduced gene-flow between dialect boundaries (MacDougall-Shackleton and MacDougall-Shackleton, Soha et al. 2004). However, female White-crowned Sparrows do prefer local dialects. Plus, local males sire more young and have lower parasite loads (MacDougall-Shackleton et al. 2002).

Brown-headed Cowbird

Research on Brown-headed Cowbirds (*Molothrus ater*) provides support for a social function of dialects, as well as absence of genetic boundaries (Rothstein and Fleischer 1987, O'Loghlen and Rothstein 1995, Anderson et al. 2005). Young cowbirds learn their vocalizations in the first two years of life (O'Loghlen 1995). Their song is difficult to produce, so it takes this amount of time to learn an accurate copy of a local dialect (O'Loghlen 1995). Females prefer complete songs of the local dialect, indicating there may be an advantage to performing a local dialect (O'Loghlen and Rothstein 1995). However, Brown-headed Cowbirds within the eastern Sierra Nevadas show moderate to high movement between dialect boundaries (Anderson et al. 2005). This gene flow likely inhibits a genetic boundary between dialects, but could favor local ecological adaptations and habitat familiarity (Anderson et al. 2005).

Wood Warblers

Warblers offer a unique opportunity for understanding the evolution and adaptive significance of dialects because they sing two song types with two distinct functions: dawn song, typically associated with mate attraction, and day song, associated with territory defense. Byers' (1996) research on the Chestnut-sided Warbler (*Dendroica pensylvanica*) showed two distinctly different vocal geographic patterns between these two song types: dawn songs of Chestnut-sided Warblers changed little over space and time, but day songs varied substantially, both between populations and years. Day songs of also exhibited much greater individual variability which could be attributed to recognition and song matching (Byers 1996). At least two other warbler species, the Blue-winged Warbler (*Verminvora pinus*) and Golden-winged Warbler (*Verminvora chrysoptera*) exhibit this trend (Kroodsma 1981, Highsmith 1989).

Black-capped Chickadee

One of the most unique patterns of vocal geographic variation among songbirds occurs in the Black-capped Chickadee (*Poecile atricapillus*; Kroodsma et al. 1999). Over most of their geographic range, Black-capped Chickadees have one song that they sing over a wide frequency range throughout mainland North America (Kroodsma et al. 1999, Mennill and Ratcliffe 2004). They use frequency to match and counter sing with neighbours in a complex social network (Mennill and Ratcliffe 2004, Fitzsimmons et al. 2008b). However, Black-capped Chickadees off the coast of New England differ in their vocal patterns. Chickadees on Martha's Vineyard sing only high and low frequency songs (Kroodsma et al. 1999). On Chappaquiddick Island, they sing mostly on one middle frequency in some areas, but use high and low frequencies on other areas of the island (Kroodsma et al. 1999). Black-capped Chickadee use their vocalizations in complex interactions with conspecifics, so social influences appear to have largely shaped their vocal uniformity and dialect patterns (Mennill and Ratcliffe 2004, Fitzsimmons et al. 2008b).

Duets and duetting species

Many songbirds produce long, complex, variable vocalizations referred to as song and short, simple, stereotyped vocalizations referred to as calls (Catchpole and Slater 2008). In most temperate songbirds, males produce both songs and calls, whereas females produce only calls (Langmore 1998). However, in some temperate species and many tropical species, both males and females sing and call, and in these species mated pairs of birds often combine their vocalizations to produce duets (Hall 2004). Over 220 birds are known to duet, and of these, the majority occur in the tropics (Farabaugh 1982). Some tropical passerines produce complex, highly coordinated duets (Mann et al. 2009). For example, Plain Wren (*Thryothorus modestus*) duets are initiated by a male's introductory phrase and followed by several antiphonal, alternating female and male phrases combined so precisely they sound like a single song (Mann et al. 2003, Cuthbert and Mennill 2007). These duets and the duets of many other tropical wrens are also combined specifically based on call type, with mates selectively using songs from their repertoire to match songs within their mate's repertoire (Logue 2005, Marshall-ball and Slater 2008). Birds in other orders are also known to produce complex duets. Yellow-naped Parrots (*Amazona auropalliata*) perform duets with a specific syntax in which males and females combine their calls in a predictable order (Wright and Dahlin 2007).

The complexity of duets and why they evolved has received considerable attention in birds in recent years (Rogers et al. 2004, Logue 2005, Molles and Waas 2006, Mennill 2006, Seddon and Tobias 2007, Hall and Magrath 2007). Research has focused particularly on several hypotheses for the functions of duets, including joint territory defense, mate-guarding, and pair-bond formation (Hall 2004). Many studies provide support for many of these hypotheses, arguing that duets serve multiple functions (Marshall-ball et al. 2006, Hall and Magrath 2007, Mennill and Vehrencamp 2008). However, little research has examined how animals acquire duets or develop coordinated duetting behaviour (Levin 1996, Price 1998, Marshall-ball et al. 2006). A study on geographic variation of male and female components of duets of Eastern Whipbirds (*Psophodes olivaceus*) showed male Eastern Whipbirds' songs vary only slightly between locations, whereas females' duets vary substantially (Mennill and Rogers 2006). This indicates a difference in function and possibly ontogeny of sex-specific vocalizations and duet components.

Complex song in suboscine songbirds and non-songbirds

Until recently, suboscine and non-songbird vocalization research has been scarce (Kroodsma 1996). However, an increasing body of research points to the complexities of suboscine vocalizations (Leger and Mountjoy 2003, Leger 2005, Lovell and Lein 2004a, Lovell and Lein 2004b, Lein 2008, Seddon and Tobias 2005, Seddon and Tobias 2007, Fitzsimmons et al. 2008a). Alder Flycatchers (*Empidonax alnorum*), suboscine songbirds with a simple, innate vocalization, have individually distinct vocalizations (Lovell and Lein 2004a) that they use in individual discrimination (Lovell and Lein 2004b). A close relative, the Buff-breasted Flycatcher (*Empidonax fulvifrons*) also exhibits individual variation and vocal variation between populations (Lein 2008). Among the strongest arguments for complex suboscine vocalizations is the use of song in combinatorial syntax (Leger 2005) and complex, coordinated duets and choruses (Roper 2005). The Flammulated Attila (*Attila flammulatus*) combines elements within its dawn and day songs with a level of complexity previously seen only in oscine passerines (Leger 2005). The closely related Bright-rumped Attila (*Attila spadiceus*) has two different geographic patterns between its dawn and day song (Leger and Mountjoy 2003), shown previously in warblers (Byers 1996). Chestnut-tailed Antbirds (*Myrmeciza hemimelaena*) discriminate between the calls of conspecifics within their population and other populations based on dialects (Seddon and Tobias 2007). Nevertheless, Spotted Antbirds are not able to discriminate neighbours from strangers based on vocalizations (Bard et al. 2002).

A growing body of literature demonstrates that non-songbirds also have complex vocalizations that are used in complex ways (Bretagnolle and Genevois 1997, Bolton 2007, Klenova et al. 2008). Blue Petrels (*Halobaena caerulea*) are a nocturnal breeder in which males and females coordinate their vocalizations into duets. Male and female vocalizations vary differently geographically (Bretagnolle and Genevois 1997). Between several widely distributed archipelagos and seven closely spaced breeding islands, male call pitch varied drastically by archipelago and temporal characteristics varied between breeding islands (Bretagnolle and Genevois 1997). Female vocalizations did not vary on either level (Bretagnolle and Genevois 1997). Madeiran Storm-petrels (*Oceanodroma castro*) have two temporally separated breeding populations that are sympatric (Bolton 2007). Petrels in each population possess spectrographically distinct vocalizations and each population responds only to its own population's vocalizations (Bolton 2007). Differences between vocalizations of populations of both petrel species likely facilitate breeding decisions and reproductive isolation (Bretagnolle and Genevois 1997, Bolton 2007). Red-crowned Cranes (*Grus japonensis*) have not only individually distinct vocalizations, but individually distinct duets (Klenova et al. 2008). They use their vocal signatures in conspecific discrimination (Klenova et al. 2009).

Many owl species also have a variety of calls that they use in similar contexts to the songs of songbirds. Male and female Eurasian Eagle Owls (*Bubo bubo*) call the most during the pre-laying period, females preferentially respond to male calls, and males selectively choose call posts that are closer to their neighbour's territory (Penteriani 2002, Delgado and Penteriani 2006). Both mate attraction and territory defense appear to play important roles in owl calls. Owls also use their vocalizations within sex-specific contexts and to communicate with extra-pair individuals. Female Tawny Owls (*Strix aluco*), for example, respond more to female conspecific calls, whereas males respond to both sexes in playback experiments (Appleby et al. 1999). Additionally, several species have individually distinctive vocalizations that could be used for individual recognition (Cavanaugh and Ritchison 1987, Galeotti and Pavan 1991, Fitton 1991, Freeman 2000, Lengagne 2001, Holschuh and Otter 2005) and behavioural tests show that Tawny Owls and Little Owls (*Athene noctua*) use their vocalizations to discriminate between familiar neighbour and unfamiliar stranger owls (Galeotti and Pavan 1993, Hardouin et al. 2006). Owl calls can also be honest indicators of quality (Penteriani 2003, Appleby and Redpath 1997). In Tawny Owls, call rate and frequency is related to parasite load (Appleby and

Redpath 1997) and Eurasian Eagle Owl territory quality and breeding density is portrayed in their vocalizations (Penteriani 2003).

The Barred Owl

Owls provide an interesting model system for vocalization research because most owls are nocturnal, which may select for more complex vocal communication than in diurnal non-songbird species (Galeotti and Pavan 1991). Barred Owls are highly vocal and produce a wide variety of vocalization types (Eckert 1974). Males and females sometimes overlap their vocalizations in long duets, often referred to as caterwaul bouts (Mazur and James 2000). Both sexes produce most vocalization types and male and female vocalizations appear to be distinguishable by pitch (Mazur and James 2000). Recent anecdotal evidence suggests female Barred Owls might also have extended terminal notes with more vibrato than males, which would facilitate sex identification by vocalizations (Kroodsma 2005). Their home ranges are large in the northern United States and Pacific Northwest, averaging 1.18 km^2 to 2.82 km^2 (Elody and Sloan 1985). They can, however, maintain smaller, contiguous territories averaging 250-500 m in diameter in the southeastern United States (Odom pers. obs., pers. comm. R. Bierregaard, Jr.), distances that should allow between pair communication for social and territorial contexts within a communication network.

The Barred Owl's historic range extends from southeastern Texas throughout most portions of the eastern United States and Canada, with several smaller populations in the eastern mountain ranges of Mexico (Mazur and James 2000). Their range expanded in the mid 1900's to include central portions of southern Canada and northwestern Alberta and northeastern British Columbia (Mazur and James 2000). Very recently, Barred Owls have expanded their range into the northwest United States, including Washington (Roger 1966), Idaho (Stephens and Sturts 1997), Oregon (Taylor and Forsman 1976), and northern California (Evens and LeValley 1982). Current taxonomy argues that there are four, morphologically distinct subspecies of Barred Owl (Figure 2): the Northern Barred Owl (*S. v. varia*), the Florida Barred Owl (*S. v. georgica*), the Texas Barred Owl (*S. v. helveola*), and the Mexican Barred Owl (*S. v. sartorii*; Mazur and James 2000). Because of an association with mature and old-growth forest, the Barred Owl has been used as an indicator species in the eastern United States (U.S. Dept of Agriculture 1985, 1986, 1987). Its expansion into the Pacific Northwest also makes it a further species of interest for monitoring because of its potentially detrimental effects on threatened California and Northern Spotted Owls (*S. occidentalis occidentalis* and *S. o. caurina*; Buchanan et al. 2007). Because Barred Owls are nocturnal and difficult to detect visually, vocal detection and playback are particularly useful for monitoring (Mosher et al. 1999). Nevertheless, Barred Owl vocalizations and vocal behaviour are poorly described (Eckert 1974, McGarigal and Fraser 1985, Mazur and James 2000). For these reasons, it is important to better understand their vocalizations and vocal behaviour for studies of non-songbird vocalizations and duet function, as well as conservation monitoring.

Summary

Oscine songbirds exhibit impressive vocal complexity and diversity, often associated with song learning. Non-song-learning suboscine songbirds and non-songbirds also use their vocalizations in functionally similar and complex ways that warrant greater attention. In this thesis, I examine the vocalizations, vocal behaviour, and vocal geographic variation of Barred Owls in three data chapters. In Chapter 2, I provide a complete, quantitative description of the repertoire of the Barred Owl, as well as differences between the vocalizations of males and females, diel vocal variation, and use of vocalizations inside and outside of duets. Chapter 3 is an experimental study in which I test the use of Barred Owl duets in territory defense, neighbour-stranger discrimination, and communication networks via playback. Chapter 4 describes geographic variation of Barred Owl duets within the Florida Barred Owl across the southeastern portion of the Barred Owl's range.

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Figure 1. A pair of Barred Owls (*Strix varia*). Male (left) is slightly smaller than female (right). Photo from birdforum.net.

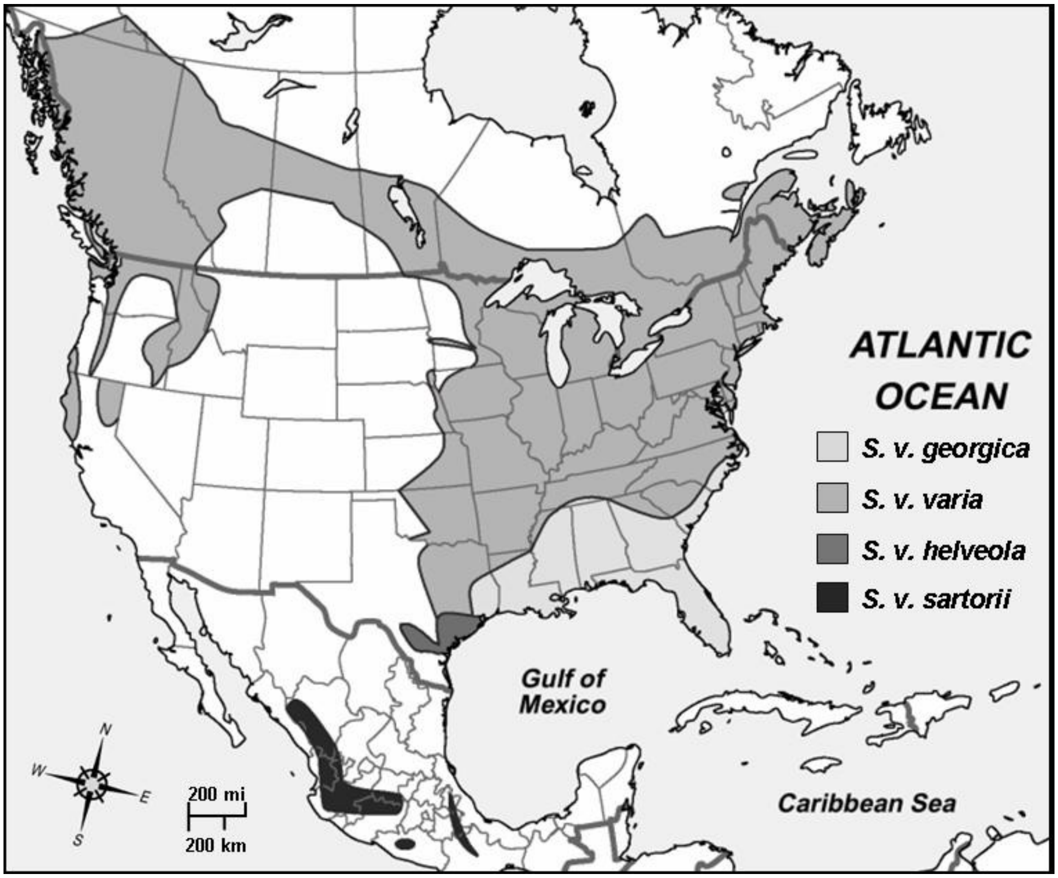

Figure 2. Map of the four Barred Owl subspecies' ranges. Entire species range boundary modified from Mazur and James (2000) and subspecies boundaries based on Bent (1938) and Eckert (1974).

Chapter 2

A quantitative description of barred owl vocalizations, sex differences, and vocal activity

Chapter summary

Barred Owls (*Strix varia*) are highly vocal and perform a diverse array of vocalizations. Barred Owls are often monitored by acoustic surveys, yet their vocalizations and vocal behavior are poorly described. In this study we present a detailed analysis of Barred Owl vocal behavior with four goals: (1) to provide a full, quantitative description of Barred Owl vocalizations, including vocalizations within duets; (2) to quantify differences between male and female vocalizations and evaluate their use for identifying owls of unknown sex; (3) to examine diel variation in vocal output for multiple vocalizations; (4) to explore the use of vocalizations inside and outside of duets. Adult Barred Owls produced 13 distinct vocalizations that could be assigned to call type based on fine-structural measurements. Female owls had higher pitched calls with longer terminal notes and more vibrato than males. We assigned sex with 91% accuracy using these differences. Barred Owls vocalized throughout the day, but were substantially more vocal at night with peaks in vocal activity from 02:00 to 05:00 and 18:00 to 20:00. Most calls were produced throughout the night, but some, particularly alarm calls, were produced at specific times. Two types of vocalizations were produced primarily within the context of duets, one type of vocalization occurred primarily outside duets, and the remaining types occurred as both solos and duets. We discuss possible functions of vocalization types and explain how understanding the vocal repertoire and sex differences of this species' acoustic signals is useful for behavioral studies and monitoring, including applications for Spotted Owl (*Strix occidentalis*) conservation.

Key words: *Barred Owl,* Strix varia*, vocalizations, duet, behavior*

Introduction

Barred Owls (*Strix varia*) are very vocal animals, with one of the most diverse vocal repertoires among North American owls (Eckert 1974). They are abundant throughout the eastern United States and Canada, with rapidly increasing numbers in the Pacific Northwest (Mazur and James 2000, Gutiérrez et al. 2007). Barred Owls have been used as an indicator species in several national forests (e.g. U.S. Department of agriculture 1985, 1986, 1987) and have recently became a species of interest to conservation biologists because they are expanding their range into the current range of the federally-threatened Spotted Owl (*Strix occidentalis*, Buchanan et al. 2007). As a nocturnal species, vocalizations play an important role in Barred Owl communication, behavior, and biology (Galeotti and Pavan 1991) and multiple studies have highlighted the usefulness of vocal surveys for detecting Barred Owls (McGarigal and Fraser 1985, Mosher et al. 1990, Crozier et al. 2006). Understanding the natural vocal activity of Barred Owls, the vocalizations they produce, and diel variation in vocal output has conservation implications for guiding monitoring efforts, including playback protocols and passive monitoring procedures.

Several accounts of the Barred Owl vocal repertoire exist, however they are anecdotal, they do not provide consistent nomenclature for the call types described, and they lack information on behavioral context and differences between the sexes (Brewster and Chapman 1891, Bent 1938, Eckert 1974, Johnsgard 1988, Mazur and James 2000). Bent (1938) provides the most thorough account, describing 11 types of vocalizations as well as duets, but the absence of nomenclature makes his descriptions difficult to use and inaccessible to anyone unfamiliar with this species' calls. All published accounts treat

Barred Owls' complex vocal duets (commonly referred to as "caterwaul bouts", Mazur and James 2000) as a single vocalization. However, Barred Owl duets are a complex combination of vocalizations, usually performed by a male and female breeding pair. Very few studies have looked at calling behavior and no studies to date have examined diel variation in calling patterns (McGarigal and Fraser 1985, Dunstan and Sample 1972, Mazur and James 2000). Two reports provide information on the context of vocalizations, but these focus on only a few call types (McGarigal and Fraser 1985, Bird and Wright 1991). Several accounts explain that female Barred Owls produce higher pitched calls than males (Bent 1938, Johnsgard 1988, Bird and Wright 1991). A recent observational account by Kroodsma (2005) adds to this by suggesting that male and female vocalizations differ in length and amount of vibrato in the terminal note of the call, with females performing elongated terminal notes with more rapid pitch undulations during the descent of the terminal note. However, consistency in these differences and use in assigning sex have not been evaluated.

In this study, we provide a detailed description of the vocalizations and vocal behavior of Barred Owls and present a standard nomenclature for future Barred Owl vocalization studies. We provide the first quantitative account of the vocalizations and vocal behavior of the Barred Owl including: (1) a full quantitative description of all vocalizations produced by Barred Owls, including vocalizations performed within duets, and a statistical comparison of fine-structural properties of similar vocalizations; (2) a quantification of the described differences between male and female vocalizations, with an evaluation of whether these differences can be used to accurately identify individuals of unknown sex; (3) a description of diel variation in call output for each type of vocalization; (4) an analysis of vocalizations that occur inside and outside of the context of duets.

Methods

We recorded Barred Owls of both known and unknown sex. Individuals of known-sex were captive birds recorded at wildlife rehabilitation facilities in central Florida in March 2004 and March and August 2005. Individuals of unknown sex were recorded from a wild population along the Choctawhatchee River Basin in Washington and Holmes counties in northwestern Florida from January to May 2007 and January to February 2008. Wild owls were recorded at 26 different locations within this site. Each recording location was separated by at least 500 m from all other locations. We considered a 500 m separation distance to be adequate for soliciting different pairs of owls based on our experience hearing two to three pairs of Barred Owls from a single location, as well as observations from radiotelemetry studies indicating that other southeastern populations maintain contiguous territories averaging 200 m in diameter (R. Bierregaard, Jr. pers. comm.).

We recorded calls of wild owls during spontaneous bouts of calling, or using standardized playback to elicit vocalizations. Standardized playback consisted of four tracks of common vocalizations: (i) a single note call given by each a male and female owl, (ii) eight two-phrased hoots (Mazur and James 2000) given by a male and female in alternation, (iii) ascending hoots (Mazur and James 2000) given by both males and females, and (iv) a 13 s duet. The first and fourth tracks were created from recordings of Barred Owls from central Florida provided by Cornell's Library of Natural Sounds and the second and third tracks were created from two mated pairs of wild owls we recorded from the north Florida population. Tracks were given in consecutive order with a 5 minute silent period between each playback. We ceased playback once owls responded vocally. Track four was seldom given.

Sound recording and analysis

Focal recordings of captive owls were made with a Sony TC-D5 Pro II tape recorder and an Audiotechnica AT835 microphone. Wild owls were recorded both focally and with automated recording devices. Focal recordings were made with a Marantz PMD- 670 solid-state digital recorder and a Sennhieser ME67 shotgun microphone with K6 power module. Automated recordings were made with a Marantz PMD-670 digital recorder powered by a sealed lead-acid battery and a Sennheiser ME-62 omni-directional microphone with K6 power module. Microphones for automated recording devices were mounted from shelf brackets attached to 3m wooden posts and were attached to small trees within the study site. Recordings were collected as MP3 files on a Hitachi 3GB microdrive at a sampling rate of 44.1 kHz, 16 bit, 160 kbps. Full details of the automated recording devices can be found in Hill et al. (2006). Focal recordings were collected as WAV files with the same parameters as above. Taped recordings from 2004 and 2005 recordings were digitized at a sampling frequency of 16 kHz using the program Cool Edit 2000 (Syntrillium Software Corp. 2002).

Using Syrinx-PC (Burt 2006) to visualize sounds as spectrograms, we isolated vocalizations from focal and automated recordings to create separate data sets for analyzing Barred Owl vocalizations, diel variation in vocal output, and vocal activity in and outside of duets. We used the same data set for providing quantitative descriptions of vocalizations and for evaluating differences between male and female vocalizations. Fine-scale measurements of vocalizations for quantitative vocal descriptions and sex differences were made using Audition 2.0 software (Adobe Systems, Inc. 2005) with settings as follows: Blackman-Harris window function; 512 Hz resolution; 100% window width. These settings provided an effective time resolution of 1 msec and effective frequency resolution of 22 Hz.

Quantitative description of vocalizations. Using recordings of captive, known-sex individuals, we separated vocalizations into a repertoire of vocalization types based on structural differences, including number of notes, differences in note length, inter-note interval, maximum frequency, and bandwidth. We compared this repertoire with observations in the field from 2007 and 2008. In the field, we observed several vocalizations that we did not record from the captive birds, and we added these vocalization types to the repertoire. Using this repertoire, we attempted to isolate one clear example of each vocalization of a male and female from each of the 26 recording locations from 2007 and 2008. We could not find good recordings of all vocalizations from every location, but we collected at least ten (and a maximum of 23) well-recorded examples of common vocalization types from males and females. For a few call types, it was difficult to obtain clear, non-overlapped recordings because they were typically produced as part of a duet, and usually overlapped by the mate's calls. We obtained as many good recordings of these less-common vocalizations as possible.

Ten variables were measured for each example of each call. Measurements were designed to provide information about overall characteristics of the call as well as parameters expected to distinguish males from females based on the description by

Kroodsma (2005). These variables were: (i) call duration, (ii) number of notes, (iii) duration of the terminal note, (iv) frequency of maximum amplitude (FMA) for the entire call, (v) duration from the point of FMA to the end of the call, (vi) FMA of the terminal note, (vii) duration from the point of FMA of the terminal note to the end of the call, (viii) maximum frequency (F_{max}) of the terminal note, (ix) duration from F_{max} of the terminal note to the end of the call, and (x) number of inflection points in the terminal note. Descriptive statistics with means (± SE) were calculated for each variable for each call type for both males and females. These values are presented as averages across males and females in the text, but the average value for each sex is presented separately in Table 1. Several vocalization types were previously described as distinct vocalizations and were noticeably different from all others, based solely on hearing without the aid of spectrographic analysis; these obvious vocalization types were considered distinct and not subject to further analysis. Several other vocalizations were quite similar to each other, so we performed canonical discriminant function analysis to determine if they could be separated into distinct vocalization types.

Male-female differences. To confirm Kroodsma's (2005) and our own observed differences in male and female vocalizations, we conducted two comparisons of male and female vocalizations based on the fine-structural measurements described previously, one using recordings of individuals of known sex and one using recordings of wild owls of unknown sex. For known-sex owls, we compared means ± SE for duration of terminal note, F_{max} of the terminal note, and number of inflection points in the terminal note. Because of small sample sizes of captive owls (two males, four females), we did not perform statistical analyses on these data. In our recordings of wild owls, we observed that duets were always composed of vocalizations by both an owl with lower calls and truncated terminal notes (the presumed male) and a second owl performing higher pitched calls with a longer terminal note and more vibrato (the presumed female; Kroodsma 2005). Females are known to produce higher pitched calls (Mazur and James 2000). Knowing this, we attempted to connect differences in pitch to length of the terminal note and the amount of vibrato in Barred Owls of known sex.

To test if these differences could be used to distinguish between wild owls of unknown sex, we used all ten variables measured for the quantitative description to examine differentiation between predicted males and females in our wild population of owls. First, we assigned a predicted sex to each vocalization in our data set based on Kroodsma’s (2005) and our own observed differences in the structure of male versus female calls (see above). We then used cluster analysis to determine if owls of unknown sex could be separated into two groups and if those groups corresponded to our predicted sex.

Diel variation and vocal behavior. To examine diel variation in Barred Owl vocalizations and to quantify use of vocalizations within and outside of duets, we scanned 24-hour recordings from automated recording devices. For the diel variation analysis, we used recordings from 12 different locations, each recorded on a different day from early January to mid-February 2007. This corresponds to the period just prior to the breeding season of Barred Owls in north Florida, and a time of year when Barred Owls are highly vocal. We determined the time and type of each vocalization produced within the entire 24 hours for each of the 12 recordings. Duets were treated as single vocalizations for this analysis, because the overlapping vocalizations of distant birds made it challenging to classify calls beyond this level. We calculated the average (± SE) output for each vocalization for each hour over a 24-hour period. We used a linear mixed-model ANOVA to compare diel variation in call rates for all vocalizations combined to determine if vocal activity varied significantly with time of day. We evaluated diel variation in call rates of seven common solo vocalizations and duets purely on a descriptive basis, to avoid reduced power from multiple statistical comparisons and because of low occurrence of many vocalization types during many hours of the day.

We also examined the prevalence of particular vocalizations inside and outside of Barred Owl duets. We counted the number of each vocalization type and the total number of calls that occurred in each duet from 26 duets, each recorded from a different pair of owls, isolated from automated and focal recordings at the 26 recording locations from 2007 or 2008. To create a comparison dataset for solo calls (calls produced outside the context of duets), we isolated the same number of solo calls from each recording to match the number of calls within the duet recorded at that location. Starting at midnight, we tallied calls until the necessary number of solo calls was reached and then calculated how often each of the vocalization types occurred.

Statistical analyses

We performed canonical discriminant function analysis on the fine structural measurements of the six common vocalizations using a cross-validation technique. We randomly selected 80% of the data, constructed the canonical discriminant analysis, and then evaluated the ability to correctly classify call type for the remaining 20% of the data. We report eigenvectors and canonical scores for the 80% of the data used to run the analysis, and we report accuracy as the percent of the 20% of the data tested by cross-validation. We considered canonical vectors with eigenvalues greater than 1.0 to contribute significantly to differentiation between vocalization types. We used variables with correlation coefficients greater than ± 0.5 to explain the variation described by canonical vectors.

To evaluate sex differences using fine-structural measurements of owl calls, we used hierarchical cluster analysis using Ward's method with two clusters specified to construct a phenogram based on all fine-structural measurements. We created the phenogram using the six common vocalization types (184 calls including 98 predicted males and 86 predicted females). We calculated correct assignment as the percentage of predicted males and females that were assigned to distinct, singly-rooted clusters. We chose this method based on the ability of cluster analysis to arbitrarily assign individuals to groups (clusters) based on fine-scale measurements without group association specified by the researcher.

To evaluate diel variation in call output for all vocalizations combined, we used a linear-mixed model ANOVA with a repeated measures design. We chose this method instead of a general linear model repeated measures ANOVA because repeated measures ANOVA eliminates cases (in our study, automated recording locations) with missing values. We had three recording locations that each had one of the 24 hours missing due to the recording finishing early. The model was constructed with call occurrence as the dependent variable, hour as a fixed factor, and each automated recording location as a repeated measure. We ran the model on the covariance matrix and an unstructured model was specified.

To compare call occurrence inside and outside of duets, we used log-linear regression, run as a three-way repeated measures ANOVA. We used an expanded data set of presence or absence, with each row representing a single vocalization within a duet. In our model, In our model, the within-subjects factor was vocalization type, and the between-subjects factor was whether the vocalization occurred within a duet or outside of a duet. We evaluated the results based on the interaction of vocalization type and in or outside of duets.

Canonical discriminant function analysis and cluster analysis were performed in JMP 5.0 (SAS Institute 2002). All other analyses were performed in SPSS 17.0 (SPSS Inc. 2008). All descriptive statistics are presented as mean ± SE.

Results

Quantitative description of vocalizations

Description of vocal repertoire. Adult Barred Owls performed 13 types of vocalizations and one non-vocal sound (Fig. 1, Table 1). Table 1 describes the fine-structural properties of each of these vocalization types.

Inspection call. This call is a single, loud, long note (0.97 ± 0.04 sec) with a slight rise in pitch (FMA: 586 ± 21 Hz; F_{max}: 613 ± 8 Hz) followed by a rapid descent: *Hooooahh.*

Two-phrased hoot. This call consists of two sets of four syncopated notes; three short and one long (3.19 ± 0.07 s total duration). The terminal note of the second phrase is the longest note (0.58 ± 0.03 s; nearly 20% of the call), and has an emphasized, downward finish. This call is known by the popular mnemonic, *Who cooks for you? Who cooks for you all?* and is sometimes referred to as the "cook call" (Bent 1938, Freeman 2000).

One-phrased hoot. This call is made up of 4.07 ± 0.07 notes, similar to either the first or second phrase of the two-phrased hoot (1.82 ± 0.10 s total duration). Females often give a drawn-out, evenly-accented version in duets or following alarm calling.

Ascending hoot. On average, this call has 8.62 ± 0.42 evenly-spaced notes, but can be longer. It is 3.27 ± 0.15 s and steadily increases in pitch and amplitude. The terminal and penultimate notes are in quick succession and are strongly accented. The terminal note is longer than other notes in the call (0.64 ± 0.06 s; 20% of the call) with an elaborate, rapid descent: *hoo-hoo-hoo-hoo-hoo-hoo-TO-WAH*. Also referred to as the "legato hoot" (Freeman 2000).

Short ascending hoot. This call is similar to the ascending hoot, with an even progression of ascending notes, ending with a final long, descending note, but is only 5.29 ± 0.19 syllables and shorter total duration (2.15 ± 0.10 sec) than the ascending hoot. We separated short ascending hoots from ascending hoots because short ascending hoots appeared to be more closely associated with duets.

Fast ascent. This series of evenly-spaced notes ascends rapidly with an emphasized, descending terminal note, also similar to the ascending hoot. However, it has a slightly shorter duration (2.84 ± .019 sec), but one to two more notes on average than the ascending hoot (Table 1). Its note and inter-note lengths are shorter, making the call sound hurried.

Gurgle. This call consists of 5.95 ± 0.04 hollow, throaty notes with substantial harmonic stacking and a higher fundamental frequency, sounding like maniacal laughter (Eckert 1974; total duration is 1.99 ± 0.36 sec). The gurgle call pattern is variable, but most similar to an ascending hoot. The terminal note of the call can be quite short (0.52 ± 0.16 sec), but still descending. In combination, the last two notes can create an abrupt up and down inflection, similar in tone and pace to the double-note *Uh-uh* call of a Fish Crow (*Corvas ossifragus*; McGowan 2001).

Two-note. Both notes in this two-note call are heavily accented. The total duration is short (0.87 ± 0.08 sec), but the first note is longer than most beginning notes of the other calls and is relatively consistent in pitch with a very subtle upward sweep. The second note is longer than the first (0.53 ± 0.07 s; 61% of the call) with an elaborate, downward trailing end: *Hooo-HOOOAAAH*.

Three-note. The three-note is three distinct, evenly spaced notes performed on approximately the same pitch. The terminal note (0.48 ± 0.04) makes up 40 - 54% of the total duration (1.01 ± 0.07 sec) caused by its extended, descending finish, as in the ascending hoot. The first two notes are similar in length to one another, and either or both may be heavily accented and equal in intensity to the terminal note: *HOO-HOO-HOOOO*.

Mumble. This is a short call (1.09 ± 0.12 sec) with three low, grumbled notes and a slight nasal quality. The middle note is higher pitched and can be longer than the first and third (Fig. 1K). The terminal note (0.55 ± 0.05 sec) comprises up to 50% of the total duration, but can be shorter, comprising only 36% of the call in males. All three notes are short, quick, slurred, and indistinct, as the name implies, sounding like *err-ERR-err*.

Twitter. This series of 7.25 ± 1.11 s variable-pitched, squeaky notes are high-pitched with a broad bandwidth (F_{min}: 2666 ± 337 Hz; F_{max}: 4120 ± 636 Hz). They are delivered in a cyclical up and down pattern, similar to the chipper calls of Chimney Swifts (*Chaetura pelagica*; Cink et al. 2002), but slower, sharper, and pulsed. We only observed males perform these calls, but females may also give them (Bird and Wright 1977).

Female begging. This a one-note long (1.32 ± 0.15 sec), thin, whistled call rising slightly in pitch (FMA: 802 ± 32 Hz; F_{max}: 828 ± 23 Hz). It is eerie (Eckert 1974) and slow: *errrrrrrrit*.

Scream (alarm call). One or two long (2.63 ± 0.48 sec), loud gradually ascending, and sharply climaxing notes, like a human scream. A sharp, high-pitched, and resonating: *IIIIIIEEET!* Two to three accented one-phrased hoots often follow this call and the call is performed mainly by females (identifiable by these one-phrased hoots), but may be produced by males (D. Wiens pers. comm.).

We also observed Barred Owls producing non-vocal sounds by snapping their bills (Fig. 1N). Most often this occurred as a series of solo bill snaps, but once occurred within a duet.

Our recordings and analyses focused on adult vocalizations, but juvenile and nestling begging calls were also observed. Juvenile and nestling begging was similar to female begging, but weaker, higher pitched, and with a raspier quality.

Vocalizations were sometimes preceded by one to three introductory notes, we called “pick-up notes”. Pick-up notes were often single, accented, elongated notes, slightly higher and rising in pitch than the following notes of the vocalization; a syncopated *Wooot* before the remainder of the call (e.g. beginning notes of Fig. 1E and F). Pick-up notes appeared to be most often performed by females in association with duets and most often preceded ascending hoots, short ascending hoots, one-phrased hoots, and sometimes gurgles.

Discriminant function analysis. Of the 13 quantitatively described vocalizations, gurgles, twitters, female begging, and screams exhibited unique peak frequencies (Table 1) and overall structure (Fig. 1), and were noticeably different to the ear. We considered these distinct call types and did not perform further analyses. We conducted canonical discriminant analysis on two-phrased hoots, one-phrased hoots, ascending hoots, fast ascents, short ascending hoots, and inspection calls (mumbles, three-notes, and two-notes were infrequent and usually performed within duets, so we were unable to obtain a sufficient sample size of non-overlapped recordings for inclusion in our canonical discriminant analysis). Canonical discriminant function analysis based on 10 fine-scale measurements capably discriminated between these six call types. Vocalizations were assigned to the correct call type with 69% accuracy, well above the 17% accuracy expected by chance (Chi-squared test: $\chi^2_{6,25} = 85.0$, $P < 0.0001$). There was some overlap in the 95% confidence intervals of one-phrased hoots and short ascending hoots, as well as between two-phrased hoots and ascending hoots and it was misclassification of these two groups that resulted in the majority of incorrect assignments. The first canonical vector explained 88% of the variation in the 80% of data used to conduct the discriminant analysis, and was correlated most strongly with call duration ($r = 0.91$), number of notes ($r = 0.98$), and duration of terminal note ($r = -0.53$).

Male-female differences

Male and female Barred Owls of known sex differed substantially in maximum frequency, duration of the terminal note, and number of inflection points in the terminal note (Fig. 2, Fig. 3). Females gave higher pitched calls than males (Fig. 2A), and had substantially longer terminal notes (Fig. 2B) with many more inflection points (Fig. 2C).

Wild Barred Owls of unknown sex were readily separated into two groups based on hierarchical cluster analysis. A phenogram formed from the six common calls produced by both males and females grouped owls into two distinct clusters that corresponded to predicted sex with an accuracy of 91%. Of 86 predicted female calls, 75 were assigned to a single cluster, suggesting that females could be predicted based on vocalizations with 87% accuracy. Of 98 predicted male calls, 92 were assigned to the second cluster, suggesting that males could be predicted based on vocalizations with 94% accuracy.

Diel vocalization rates and vocal behavior

Barred Owls exhibited significant variation in vocal output throughout the day and night (Linear mixed model: $F_{9,40} = 16.0$, $P < 0.0001$; Fig. 4). Although they were vocal at all hours, they were most vocal between 18:00 and 06:00, the hours corresponding to twilight or dark in January and early February in north Florida. Barred Owls showed peaks in vocal activity between 02:00 to 05:00 and 18:00 to 20:00. This first peak occurred in the early morning until just before dawn, and was associated mostly with an increase in inspection calls and two-phrased hoots. The second peak occurred shortly after dark and was driven by an increase in two-phrased hoots, ascending hoots, fast ascents, and duets. The most common vocalization type was inspection calls, which were given consistently throughout the night, followed by two-phrased hoots, which peaked in activity at 05:00 and 20:00. Screams were given substantially less often than other vocalizations and exhibited a unique pattern, peaking around 19:00, shortly after dark. Barred Owls were least vocal between 07:00 and 14:00.

Barred Owls used different vocalizations inside versus outside of duets (Log-linear regression: $F_{1,258} = 151.1$, $P < 0.0001$). Fast ascents, one-phrased hoots, gurgles, three-notes, mumbles, and two-notes occurred more often within duets, whereas two-phrased hoots and ascending hoots occurred more often outside of duets (Table 2). Short ascending hoots occurred almost equally inside and outside of duets (Table 2). Inspection calls, one of the most common vocalizations recorded from Barred Owls (Fig. 4), were used almost exclusively outside of duets (Table 2).

Discussion

Barred Owls have a diverse vocal repertoire of over 13 distinct vocalizations. The calls we described correspond to previous accounts (Brewster and Chapman 1891, Bent 1938, McGarigal and Fraser 1985), but we provided a standardized vocabulary, quantitatively verified differences between calls, and described new vocalizations used primarily within duets. We also found structurally similar vocalizations were used in separate contexts, shown primarily by certain vocalizations being duet-specific. Lastly, we found empirical support confirming that females perform a higher pitch call with a more elaborate terminal note than males (Kroodsma 2005) and these differences can be used to identify sex.

The vocalizations we describe match very closely with those described by Bent (1938), who described nine vocalizations included in our description in addition to duets and bill snapping. Bent's (1938) descriptions included two-phrased hoots, one-phrased hoots, gurgles, mumbles, inspection calls, screams, and female begging. Brewster and Chapman (1891) describe ascending hoots and explains that this vocalization can be long or short and is sometimes delivered quickly, possibly representing ascending hoots, short ascending hoots, and fast ascents. Bent's (1938) description differs slightly from ours, with two vocalizations described that we did not observe in our population: (1) a prolonged, tremulous call…[with a] whining quality", phoneticized "*wee-ow-o-w-ow-ow*", also noted by Eckert (1974), and (2) dog-like barking. We think the former might be a description of what we identify as a twitter, but Bent's description differs substantially from ours. Other explanations include that these vocalizations are rare, products of innovation, regionally specific, or originally misidentified. McGarigal and Fraser (1985) describe "an irregular and patternless assemblage of hoots." We observed Barred Owls in wildlife rehabilitation centers vocalize this way when woken and when falling to sleep. Lastly, our research focused on adult vocalizations, but accounts describing vocalizations of young describe hissing noises (Bent 1938, Eckert 1974) and a "ratlike squealing cry" (Eckert 1974) by nestlings and fledglings, in addition to the begging call we describe. Bird and Wright (1977) describe a distraction display of a female imitating vocalizations of young Barred Owls, including "chitters and squeals."

Our analyses also added to what is known about Barred Owl vocalizations. We described two vocalizations not previously mentioned in the literature: two-notes and three-notes. Previous researchers may have overlooked these vocalizations because both calls are relatively uncommon and are given primarily within duets, making them difficult to detect by auditory observation. Quantifying vocalizations within duets also allowed greater detection of vocalizations previously given little attention. Vocalizations such as gurgles, one-phrased hoots, and mumbles likely have an important function within duets, but each is only mentioned briefly in two published accounts (Brewster and Chapman 1891, Bent 1938). Additionally, our analysis of vocal activity showed inspection calls are one of the most abundant vocalizations given by Barred Owls (Fig. 4). This stands in contrast to early accounts, which give little or no recognition to this prominent vocalization (Brewster and Chapman 1891, Bent 1938). Furthermore, quantitative assessment allowed us to officially distinguish between similar calls, such as ascending hoots, short ascending hoots, and fast ascents. These three calls may show slight functional differences, perhaps driven by changes in motivation or intensity of the situation, such as territory intrusion or copulation solicitation. We noticed, specifically, that fast ascents were given most often by males at the onset of a duet while short ascending hoots are given by both sexes in the middle or toward the end of duets (Odom, unpublished data). Ascending hoots were less often associated with duets. Because of these apparent functional differences, it is worthwhile to consider these vocalizations separately for behavioral and bioacoustic studies.

In our discrimination of call types, we found a slight overlap of 95% confidence intervals of one-phrased hoots and short ascending hoots, and two-phrased hoots and ascending hoots. We did not predict that these vocalizations would show such similarity and we believe the overlap resulted from the contribution of the number of notes variable to canonical discrimination. These two groups of vocalizations have similar numbers of notes, but temporally distinct note spacing (two-phrased hoots have a distinct gap between the fourth and fifth notes absent in ascending hoots, and one-phrased hoots are syncopated, caused by a slightly longer internote after the first note; short ascending hoots have evenly spaced notes until the penultimate note; see Fig. 1A and B). Previous accounts also clearly define two-phrased hoots and ascending hoots as separate vocalization types (Mazur and James 2000) and our analyses show that these vocalizations also have different diel variation patterns and use inside and outside of duets.

Although our research did not focus explicitly on the behavioral context of individual vocalization types, our analyses of diel vocal patterns and vocal activity inside and outside of duets allow us to make some inferences about behavior, especially when viewed in the context of previously proposed functions and observations (Brewster and Chapman 1891, McGarigal and Fraser 1985, Johnsgard 1988). Johnsgard (1988) suggests two-phrased hoots are the territorial call of Barred Owls and Brewster and Chapman (1891) observed two-phrased hoots used as a contact call. Our findings that two-phrased hoots are used throughout the night and inside and outside of duets supports the idea of a mixed use call. Additionally, two-phrased hoots are given often during both spontaneous bouts of vocal activity and when confronting conspecific individuals (Odom unpublished data), indicating they are important in both aggressive and non-aggressive contexts. Inspection calls are believed to be a contact call (Johnsgard 1988). Their abundance and substantial use outside of duets agrees with this prediction. Also, we observed inspection calls given when two birds approached one another, or a when one member of a mated pair moved away from its partner. Also, inspection calling decreases during territorial interactions (Chapter 3). Conversely, ascending hoots, increase with territory intrusion, suggesting a territory defense function (Chapter 3). Playback studies indicate duets also serve a territorial function, increasing with simulated territory intrusion (Chapter 3). Therefore, vocalizations associated with duets may play a role in territory defense. However, without knowing what other functions Barred Owl duets serve, such as in paternity guarding or maintaining pair bonds, we cannot claim territory defense is the primary function of all vocalizations associated with duets. For example, mumbles are performed mainly within duets, but are very low amplitude, suggesting a within-pair function rather than a territorial signal to intruding individuals. We suggest the scream serves as an alarm call, supported by the unique peak in screams shortly after dark, the peak foraging time of mammalian nocturnal nest predators (Picman and Schriml 1994). Screams were also observed when researchers approached a nest site or while banding (D. Wiens pers. comm., R. Bierregaard, Jr., pers. comm.), further supporting an alarm call function.

We readily distinguished between male and female Barred Owls based on vocal characteristics. As proposed by Kroodsma (2005), the captive owls of known sex showed distinct differences in pitch, length of the terminal note, and amount of vibrato. Females are known to produce higher pitched vocalizations (Johnsgard 1988) and two other characteristics (longer terminal notes and the amount of vibrato in the end of the terminal note) were associated with higher pitched calls. Wild owls of unknown sex were assigned to two groups based on these features using cluster analysis, and their assignment matched with predicted sex based on the calls' terminal syllables and amount of vibrato. Not only did the two groups agree with our predicted sex, but the clusters were roughly even in size (with a slight skew towards males (56%), but more predicted males were also used in the analysis (53%)), the distribution expected for a sexually determined trait. Brewster and Chapman (1891) suggest that pace of vocalizations may also be a distinguishing feature between sexes. We noticed that females often give slower vocalizations, most noticeable in two-phrased, one-phrased, and ascending hoots, although we did not quantify this difference. Pace may also change with motivation or context, so we think pitch, length of the last note, and amount of vibrato are more reliable distinguishing characteristics of sex.

The ability to distinguish males from females and the ability to classify an animal's vocal repertoire is important for understanding the ecology and evolution of avian vocal duets. Barred Owls are one of few temperate duetting species (Farabaugh 1982, Benedict 2008). Determining duet structure, the contribution of each sex to a duet, and differential responses of males and females to duet playback provides a basis for evaluating the function and cooperative versus conflict-based motivations for duetting (e.g. Rogers et al. 2004, Hall 2006, Mennill 2006). Evaluating these duet components in a temperate species, where duets are less common, will help determine how and why such complex vocal behaviors evolved.

Distinguishing between males and females and understanding Barred Owl vocal behavior also provides a useful tool for surveying Barred Owl populations. Identifying males and females will allow surveyors to assess presence of multiple individuals at a location and potential mated pairs. As Barred Owls expand their range into the Pacific Northwest, improving assessment of Barred Owl presence and abundance is essential for Spotted Owl conservation (Buchanan et al. 2007). Identification of Barred Owls by their vocalizations is obviously important for detection. In addition, we suggest that understanding vocal activity patterns and context of vocalizations is important for designing effective survey methods that optimize Barred Owl detection. Broadcast of Barred Owl vocalizations may be disruptive to Spotted Owls (Crozier et al. 2006), so choosing peak calling periods for surveys (i.e. between 02:00 and 05:00 or 18:00 and 20:00) can increase Barred Owl detection while reducing the use of playback. Call context is also important in selecting playback stimuli that increase Barred Owl response, but reduce stress to Spotted Owls. We hope these improvements to the current literature on vocal descriptions, sex differences, and vocal behavior of the Barred Owl prove useful in designing Barred Owl survey protocols and facilitate future Barred Owl vocal behavior research.

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Figure 1. Spectrographs showing 13 vocalizations, one non-vocal sound, and a duet produced by Barred Owls: (A) ascending hoot, (B) two-phrased hoot, (C) fast ascent, (D) one-phrased hoot, (E) short ascending hoot, (F) gurgle, (G) inspection call, (H) scream (alarm call), (I) female begging, (J) two-note, (K) three-note, (L) mumble, (M) twitter, (N) bill snap, and (O) duet.

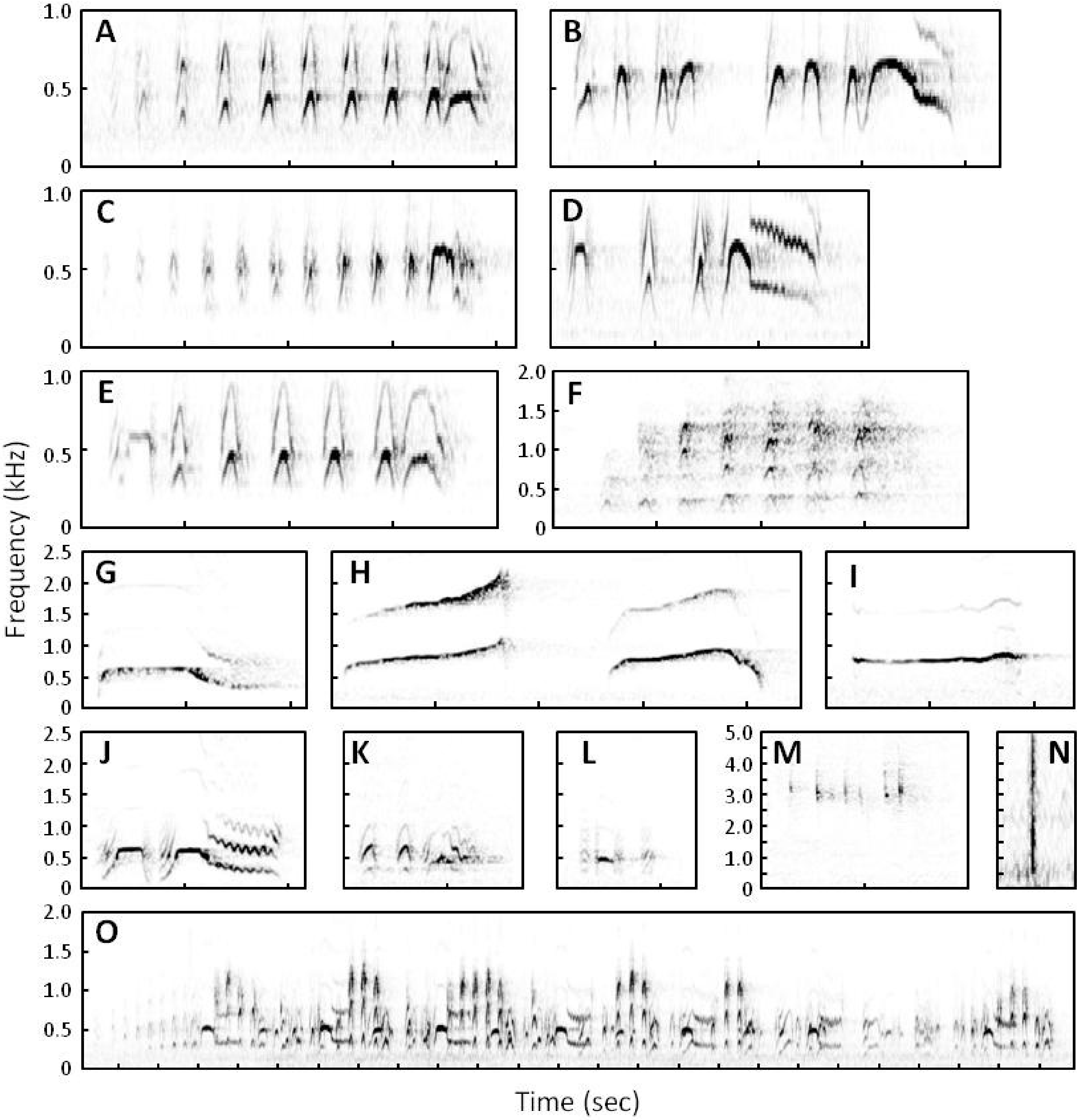

Figure 2. Fine-structural differences between captive owls of known sex (n = 4 females, 2 males), including (A) duration of the terminal note, (B) maximum frequency, and (C) number of inflection points in the terminal note.

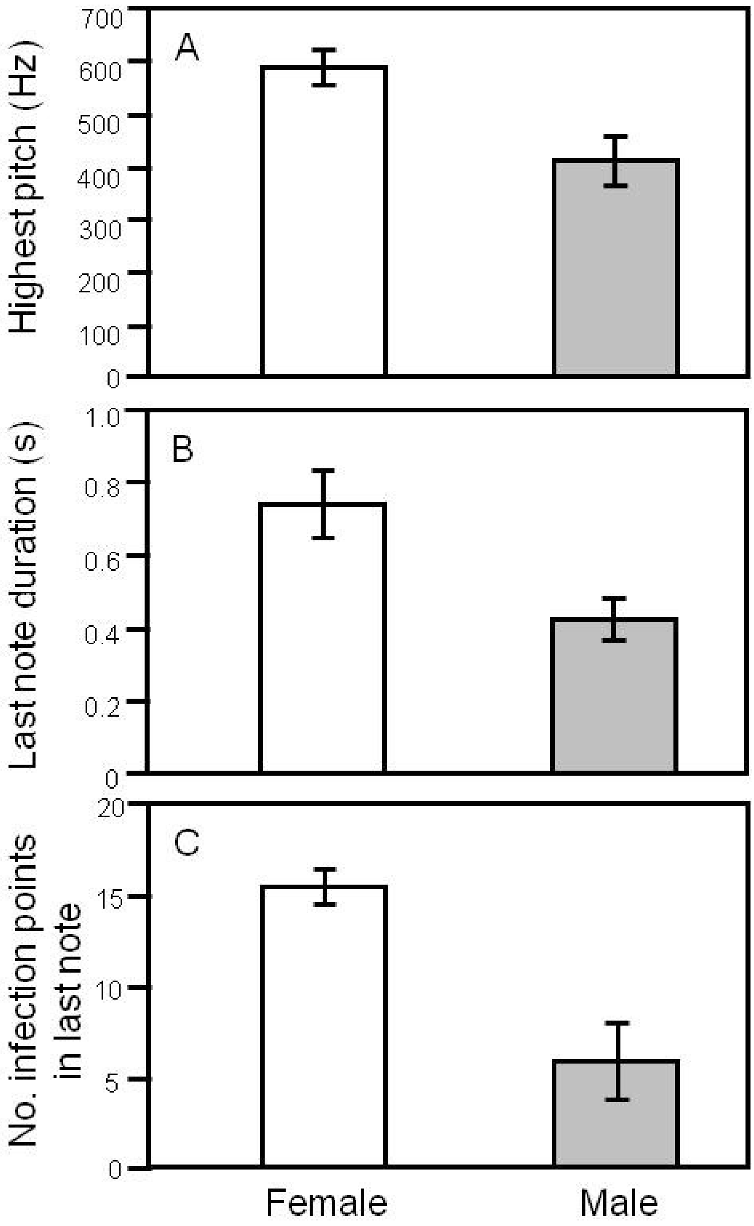

Figure 3. Spectrographic differences between the vocalizations of male (A and B) and female (C and D) one-phrased hoots (A and C) and inspection calls (B and D).

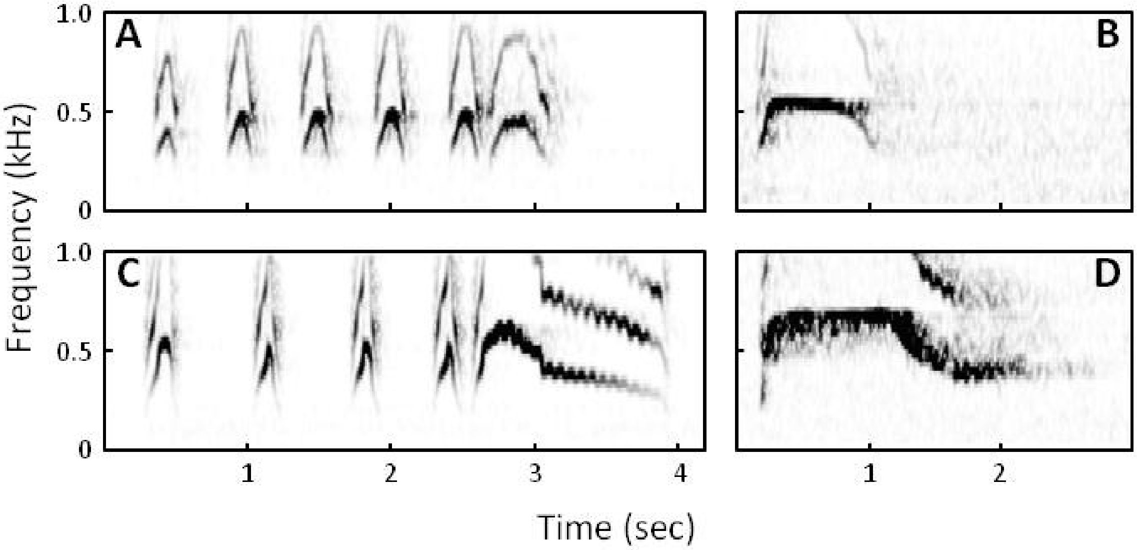

Figure 4. Diel variation in calling by Barred Owls indicating rates of (A) all vocalizations combined, (B-H) seven of the most common solo vocalizations, and (I) duets.

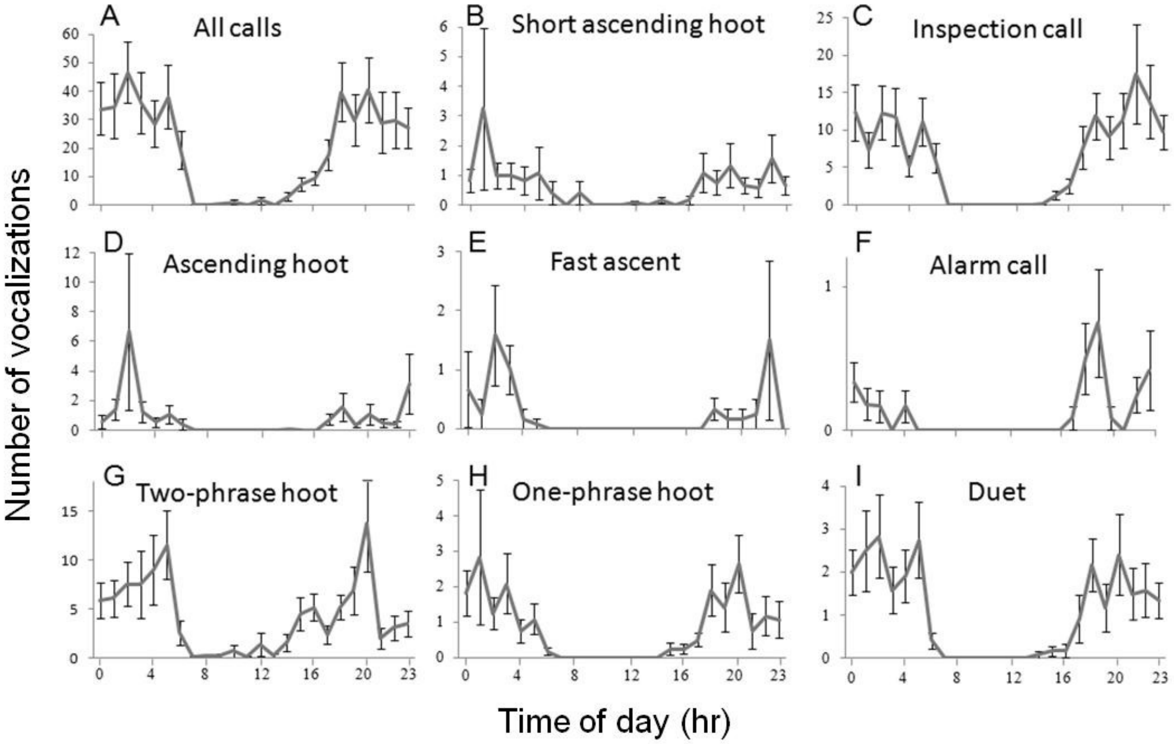

Table 1. Barred Owl vocalizations and average (± SE) fine-structural measurements for females and males.

Call type	Sex	n	Call duration (sec)	Mean no. notes	Duration terminal note (sec)	F_{max} (Hz)	FMA (Hz)	No. inflection points
Inspection Call	F	17	1.20 ± 0.05	1.00 ± 0.00	1.20 ± 0.05	639 ± 6	585 ± 19	11.1 ± 1.6
Inspection Call	M	20	0.73 ± 0.03	1.00 ± 0.00	0.72 ± 0.03	589 ± 10	511 ± 23	5.1 ± 1.1
Two-phrase hoot	F	22	3.40 ± 0.08	8.00 ± 0.00	0.79 ± 0.03	618 ± 7	573 ± 14	11.2 ± 1.1
Two-phrase hoot	M	23	2.98 ± 0.06	7.96 ± 0.08	0.38 ± 0.02	537 ± 10	481 ± 20	2.9 ± 0.7
One-phrase hoot	F	18	2.10 ± 0.10	4.00 ± 0.00	0.76 ± 0.05	591 ± 13	515 ± 24	11.7 ± 1.4
One-phrase hoot	M	13	1.53 ± 0.09	4.15 ± 0.15	0.43 ± 0.03	518 ± 22	423 ± 31	3.9 ± 0.9
Ascending hoot	F	12	3.61 ± 0.15	8.67 ± 0.43	0.83 ± 0.04	593 ± 13	519 ± 18	14.6 ± 1.3
Ascending hoot	M	18	2.92 ± 0.14	8.56 ± 0.41	0.44 ± 0.02	506 ± 16	491 ± 11	5.1 ± 0.8
Short ascending hoot	F	13	2.40 ± 0.11	5.08 ± 0.14	0.78 ± 0.05	607 ± 11	493 ± 34	15.1 ± 2.1
Short ascending hoot	M	12	1.89 ± 0.08	5.50 ± 0.23	0.38 ± 0.03	495 ± 21	401 ± 32	4.2 ± 1.2
Fast acent	F	4	2.91 ± 0.11	9.00 ± 0.71	0.53 ± 0.04	561 ± 32	442 ± 61	8.0 ± 1.1
Fast acent	M	12	2.78 ± 0.26	9.17 ± 0.84	0.40 ± 0.02	520 ± 17	465 ± 28	4.3 ± 0.9
Gurgle	F	2	2.04 ± 0.64	5.50 ± 0.50	0.70 ± 0.28	551 ± 14	325 ± 38	12.5 ± 10.5
Gurgle	M	18	1.94 ± 0.08	6.39 ± 0.30	0.33 ± 0.03	613 ± 55	518 ± 51	2.4 ± 0.8
Two-note	F	5	0.96 ± 0.11	2.00 ± 0.00	0.62 ± 0.09	627 ± 15	595 ± 22	6.6 ± 2.7
Two-note	M	3	0.79 ± 0.05	2.00 ± 0.00	0.44 ± 0.04	655 ± 164	470 ± 31	6.3 ± 2.3
Three-note	F	1	1.06	3.00	0.57	543	508	13.0
Three-note	M	5	0.92 ± 0.07	3.00 ± 0.00	0.39 ± 0.04	549 ± 30	436 ± 54	5.0 ± 1.7
Mumble	F	2	1.35 ± 0.17	3.00 ± 0.00	0.80 ± 0.06	606 ± 7	589 ± 1	12.0 ± 4.0
Mumble	M	6	0.83 ± 0.08	3.00 ± 0.00	0.30 ± 0.04	423 ± 61	417 ± 51	2.0 ± 1.0
Twitter	M	4	1.19 ± 0.15	7.25 ± 1.11	0.04 ± 0.01	4121 ± 637	3230 ± 116	0.0 ± 0.0
Female begging	F	5	1.32 ± 0.15	1.00 ± 0.00	1.32 ± 0.15	828 ± 23	802 ± 32	0.0 ± 0.0
Scream (alarm call)	F	13	2.63 ± 0.48	1.46 ± 0.24	1.91 ± 0.14	1056 ± 89	896 ± 25	0.0 ± 0.0

Table 2. Certain calls of ten distinct Barred Owl vocalizations were preferentially used inside or outside of duets. Totals based on an equal sample of 408 calls that occurred inside of duets and 408 calls that occurred outside of duets.

Location	Vocalization									
	Inspection call	Two-phrased hoot	One-phrased hoot	Ascending hoot	Short ascending hoot	Fast ascent	Gurgle	Two-note	Three-note	Mumble
Outside duet	142 (98%)	126 (80%)	14 (13%)	89 (67%)	29 (48%)	3 (13%)	0 (0%)	2 (20%)	3 (30%)	0 (0%)
Inside duet	3 (2%)	31 (20%)	96 (87%)	44 (33%)	31 (52%)	20 (87%)	161 (100%)	8 (80%)	7 (70%)	7 (100%)

Chapter 3

Duet function in barred owls: territory defense, neighbour-stranger discrimination, and communication networks

Chapter summary

Mated pairs of animals coordinate their vocalizations into duets in many different taxa, yet most research on duetting has focused on songbirds. Here we examine the duetting behaviour of barred owls (*Strix varia*) by addressing three questions: (1) Do owl duets function in territory defense? (2) Do owls discriminate between duets of strangers versus neighbours? (3) Do duets play a role in extended communication networks among a neighbourhood of owls? We used playback to simulate territory intrusions by an adjacent, territory-holding pair of owls (neighbours) and distant owls (strangers). We assessed responses using a 3.5 km transect of automated recording devices. We compared vocal activity during a pre-playback period and following both playback treatments for the focal pair, their neighbours, and more distant owls within the neighbourhood. After playback, focal owls gave significantly more duets, vocalized for a longer duration, and emphasized different call types compared to the pre-playback period, demonstrating that barred owls use duets in territory defense. Focal owls did not respond differently to neighbours versus strangers. At the neighbourhood level, owls did not behave differently during silent pre-playback periods or post-playback periods. Our results suggest barred owl duets function primarily in immediate confrontations with territory intruders.

Keywords: *Strix varia*, duet, communication, discrimination

Introduction

Coordinated vocalizations between male and female breeding partners, known as duets, have evolved independently in many different animals (e.g. Emerson & Boyd, 1999; Geissmann, 2002; Bailey, 2003; Hall 2004; Mann et al., 2009). Hypotheses for the primary functions of duets include pair bond maintenance, acoustic contact, mate guarding, joint territory defense, and breeding synchrony (reviewed in Hall, 2004). Proposed secondary functions of duets have received considerably less attention, and include sex recognition, individual recognition, maintaining reproductive isolation, and ritualized appeasement (Hall, 2004). Within both primary and secondary functions of duets, duetting is often more broadly explained as being cooperative or conflict-based (Logue, 2005; Mennill & Vehrencamp, 2008; Seddon & Tobias, 2009). Males and females may cooperatively combine their vocalizations to jointly defend a territory or maintain a pair-bond (Thorpe, 1972), or each could join its partner's song to guard their mate or their paternity (Sonnenschein & Reyer, 1983; Hall, 2004). Because duets signal cooperation or conflict between a pair, they likely serve the additional function of relaying information about rivalry or coalition between duetting animals to eavesdropping conspecific individuals in the neighbourhood around them (Hall, 2004; Hall & Magrath, 2007). We explored one primary and one secondary function of duets, as well as use of duets by nearby conspecifics through examining territory defense and neighbour-stranger discrimination of duets within a communication network.

Many duet studies have specifically focused on primary functions of duetting, particularly territory defense (e.g. Thorpe, 1972; e.g. Hall, 2006; Mennill, 2006). Many studies support a territory defense function of duetting by demonstrating aggressive responses to duet playback, including increased calling, duetting, or approach to the playback speaker (e.g. Rogers et al., 2004; Seddon & Tobias, 2005; Molles & Waas, 2006; Mennill, 2006). Connecting natural duet bouts with conspecific interactions with rivals or neighbours has also been used to assign a territorial function to duets (e.g. Mennill & Vehrencamp, 2008). Previous studies, however, involve a relatively small number of study species, all of them passerine birds. With the diversity of oscine passerine, suboscine passerine, and nonpasserine birds that are known to duet (Farabaugh, 1982), more extensive research on duet function across a broader taxonomic spectrum is needed.

Few studies have examined secondary functions of duetting, such as conspecific discrimination and individual recognition (however, see Wiley & Wiley, 1977; Mitani, 1985; Brown & Farabaugh, 1991; Hall, 2000; Grafe & Bitz, 2004; Klenova et al., 2008). Many studies support the idea that songbirds can distinguish between the voices of different individuals and much research supports the idea that individually distinctive vocalizations facilitate territorial interactions (reviewed in Stoddard, 1996). Recent research indicates that fairly stereotyped vocalizations of non-song-learning species, as well as complex signals such as duets, can also carry information on individual identity (Lovell & Lein, 2005; Lein, 2008; Roper, 2005). For example, two owl species have been shown to discriminate between neighbours and strangers based on playback experiments of solo calls (Galeotti & Pavan, 1993; Hardouin et al., 2006) and many other Strigiformes exhibit individually distinctive vocalizations (Cavanaugh & Ritchison, 1987; Galeotti & Pavan, 1991; Fitton, 1991; Freeman, 2000; Lengagne, 2001; Holschuh & Otter, 2005). Beyond owls, three studies have demonstrated neighbour stranger discrimination on the basis of duets, including two passerine species (*Campylorhynchus nuchalis*: Wiley & Wiley, 1977; *Laniarius aethiopicus*: Grafe & Bitz, 2004) and one gibbon species (*Hylobates muelleri*: Mitani, 1985). However, no experiments have tested a nonpasserine bird's ability to discriminate between conspecifics based on duets. Conspecific discrimination and recognition is classically studied in the form of a neighbour-stranger experiment (Brooks & Falls, 1975). The results are typically assessed in the context of the 'dear-enemy' hypothesis (*sensu* Fisher, 1954), which posits that an animal should respond more aggressively to an unfamiliar stranger than to a known neighbour (Brooks & Falls, 1975; reviewed in Temeles, 1994).

Another challenge in understanding duet function is determining which individuals within a population are listening to and responding to duets. The idea that duetting animals may signal to receivers other than their partner is fairly recent (Hall, 2004) and has developed alongside the communication network perspective of animal communication (reviewed in McGregor, 2005). The communication network model accounts for individuals exchanging signals with multiple receivers simultaneously, either intentionally or unintentionally (McGregor, 2005). Not only might animals communicate with individuals other than their mate when they contribute to a duet, but outside individuals may be able to gain information from listening to the duets of other nearby individuals. To date, no one has explored duet function within an extended communication network. Traditional communication network research has focused on the response of nearby individuals to song contests and territory intrusions (e.g. McGregor & Peake, 2000). Research on communication networks has demonstrated that interactions observed by eavesdropping individuals can influence the immediate vocal behaviour

(Fitzsimmons et al., 2008) or the future mating and territorial decisions of eavesdroppers (e.g. Peake et al., 2001; Mennill et al., 2002). Vocal duets are a prime candidate for imparting information to eavesdroppers within a communication network, because they may impart information about multiple individuals (i.e. the two duetting animals) to the neighbours around them.

In this study we evaluate the responses of barred owls (*Strix varia*) to playback of duets of familiar and unfamiliar individuals. We employed a neighbour-stranger experimental design and played back duets of familiar, adjacent territory-holding owls (neighbours) and unfamiliar, distant owls (strangers) to territorial, mated pairs of barred owls. We evaluate the vocal response of pairs of owls to playback within their territory and we also evaluate the responses of owls in neighbouring territories. Our investigation has three goals. (1) We test the hypothesis that barred owl duets play a role in territory defense. (2) We evaluate whether individuals can distinguish between the duets of neighbour versus stranger pairs. (3) We examine the influence of duets broadcast at one location on the vocal behaviour of the surrounding neighbourhood of birds. If barred owl duets are used in territory defense, we expected pairs would respond aggressively to duet playback, particularly by increasing their own duet output. If barred owl duets are used in neighbour-stranger discrimination, we expected an increased response to playback of stranger duets compared to neighbour duets. Lastly, based on the communication network model, we predicted that a simulated territory intrusion would lead to heightened vocal behaviour among neighbours and possibly other nearby owls in the population.

Methods

We studied barred owls at three sites within Holmes and Washington counties along the Choctawhatchee River in northwest Florida (Figure 1). Playback trials took place during the pre-breeding season of barred owls in January and February 2008. We simulated duets through a single loudspeaker positioned at the territory boundary of focal pairs of owls. We recorded the responses of playback subjects and adjacent owls using a 3.5 km transect of automated recording devices. Preliminary observations indicated that each automated recording device was capable of detecting a far-carrying signal up to 250 m, so we spaced devices 500 m apart for continuous recording across the 3.5 km transect. We used the same design at each of the three recording locations.

Study species

Vocal signalling plays a large role in the communication behaviour of owls, which are largely nocturnal (Galeotti & Pavan, 1991). Barred owls are highly vocal and perform long, far-carrying duets (Mazur & James, 2000). Surveys and observations in our study site and other southeastern populations suggest barred owls maintain contiguous territories averaging 250-500 m in diameter in bottomland forests (Odom, unpublished data; R. Bierregaard, Jr., pers. comm.). We often heard two to three pairs of barred owls from a single location, demonstrating that communication network effects are possible. Freeman (2000) showed that barred owls possess spectrographically individually-distinctive vocalizations, an important precursor for individual discrimination (Stoddard, 1996). Pair bonds are maintained across multiple years, mated pairs defend territories year-round, and territory boundaries are stable over several years (Mazur & James, 2000). Barred owl duets are continuous bouts of polyphonal calling between a mated male and female (Mazur and James, 2000; pers. obs.). Duets usually begin with a male call, and duets are easily distinguished from solo vocalizations by repeated boisterous gurgle vocalizations that do not typically occur outside of duets (Odom, unpublished data).

Recording and Playback Equipment

Each of the seven automated recording devices consisted of a Sennheiser ME-62 omni-directional microphone with K6 power module and Marantz PMD-670 solid-state digital recorder powered by a sealed lead-acid battery. Microphones were mounted on shelf brackets attached to 3m wooden posts and were attached to small trees within the study site. Recordings were collected as MP3 files on a Hitachi 3GB microdrive at a sampling rate of 44.1 kHz, 16 bit, 160 kbps. Full details of the automated recording devices can be found in Hill et al. (2006). Focal recordings were made with a Marantz PMD 670 and a Sennhieser ME67 shotgun microphone. Focal recordings were collected as WAV files with the same sampling rate as above. Playback was broadcast through an Anchor-Audio loudspeaker (model: PB-25 Minivox, 15 W).

Playback design

We used playback to simulate an intrusion at the boundary of a focal pair's territory by both a familiar, neighbouring pair of owls, and an unfamiliar, stranger pair of owls. Stranger vocalizations were recorded from territories at least 4 km from the focal pair, but usually 10-20 km from the focal pair. Neighbour vocalizations were recorded in a territory adjacent to the focal pair and played at the adjoining territory boundary. We assessed territory positions by evaluating positions of vocalizing pairs of owls in the vicinity of each playback location several nights in a row prior to playback. Duets used to create playback stimuli were elicited using a standardized playback of four tracks of a male and female owl performing two common solos: one track of eight two-phrased hoots and three identical tracks of three minutes of ascending hoots (Mazur & James, 2000). We ceased playback once the target pair began to vocalize and we recorded the pair until several clear duets were obtained at close range (10-30 m). All stimuli were recorded within thirty minutes from the broadcast of the first playback.

Playback stimuli were prepared using Audition (Adobe Systems Inc., San Jose, CA). All playback stimuli were created from one natural duet bout per pair lasting 35 ± 5 s. The duet stimulus was repeated four times with 30 s of silence between each repetition. Duets chosen for the stimuli consisted of several male-initiated two-phrased or ascending hoots joined by the female and escalating into the main caterwaul bout (Mazur & James, 2000). Duets finished with a few soft, short ascending hoots. This arrangement was typical of duets in our study population (Odom, unpublished data). Twelve stimuli were made and used in different combinations to create unique neighbour-stranger paired stimuli for each experimental trial. Eleven stimuli were used to simulate one neighbour and one stranger in different trials in different parts of the study population, and the remaining stimulus was used for a neighbour simulation. Stimulus amplitude was standardized to -1 dB using the normalize feature of Audition, and then burned onto CD as uncompressed WAV files for playback in the field.

Stimuli were broadcast at the same volume across all trials, based on an amplitude which we assessed to be a natural volume by comparing sounds from our playback device to owl duets in the field. Playback trials consisted of an initial 20 minutes of silent observation, the broadcast of the first playback stimulus (either neighbour or stranger stimulus), 20 minutes of observation to assess responses to playback, 20 minutes of silence, the broadcast of the second (opposite) playback stimulus, and a final 20 minutes of observation to assess responses to the second stimulus. We alternated the order of neighbour and stranger playback stimulus so that half the trials began with neighbour playback and half began with stranger playback. Playback response was recorded with a directional microphone near the site of playback and also with the 3.5 km transect of seven automated recording devices. We conducted eleven paired neighbour-stranger playback trials in total, in three to four territories in each of three transects (Figure 1). No owls responded to either stimulus in one trial. Several of the automated recorders failed in another trial, preventing analysis of neighbourhood-level responses for that trial. In total, we assessed responses of the focal pair across n=10 trials and responses of the surrounding neighbourhood across n=9 trials.

Sound analysis

We visualized recordings as spectrograms in Syrinx-PC (J. Burt, Seattle, WA). We located all owl vocalizations that occurred in each of three 20 minute periods: (I) a silent period 20 minutes prior to the first playback, (II) a post-neighbour playback period starting at the beginning of the neighbour stimulus, and (III) a post-stranger playback period starting at the beginning of the stranger stimulus. We calculated five parameters within each period: (i) duration of response, (ii) number of solo calls, (iii) number of duets, (iv) latency to first solo, and (v) latency to first duet. We defined duration of response as the period of time that owls vocalized after the start of the first playback until a five minute period of silence, based on our observation that owls were unlikely to resume vocalizing after five minutes of silence. We counted duets as bouts of overlapping male and female vocalizations. A gap in vocalizing greater than 1 sec was used to differentiate between duet bouts or distinct solo calls because owls usually transitioned to solo calls by one sex or ceased vocalizing after a gap of more than 1 sec (pers. obs.). Number of calls was the number of solo vocalizations that occurred outside of duet bouts. Both latency parameters were calculated from the start of each stimulus until the first call or duet, respectively. We also calculated the number of each of two common calls given by barred owls: inspection calls (contact calls) and ascending hoots (Chapter 2).

We compared these analyses at three levels: the response of the focal pair receiving the playback, the response of neighbours in immediately adjacent territories, and the neighbourhood response of all owls except the focal pair across the entire 3.5 km recording transect (approximately 8 territories of mated pairs). The focal pair's response was calculated from focal recordings taken at the playback location. The immediate neighbours' and the neighbourhood response were calculated from passive recordings taken by the automated recording devices. Immediate neighbours were defined as owls recorded at the two automated recording devices adjacent to the recording device at the location of the playback, and each parameter was calculated as the sum of vocal activity at both of these devices. The neighbourhood response was calculated as the sum of vocal activity of all owl vocalizations detected at all the automated recording devices minus the activity at the device nearest to the playback location.

Statistical analysis

We examined each of our three main questions using separate statistical comparisons. For parametric tests, the five parameters of response were transformed to achieve normal distributions, with log transformations applied to all continuous variables (both latency measures and duration of response) and square root transformations applied to counts (number of calls and duets).

To examine duet function in territory defense, we used data from the focal pair's response. We used linear mixed-models to conduct repeated measures ANOVA with restricted maximum likelihood method, type III sum of squares, and treatment as a fixed main effect. We examined all five main parameters for differences between the pre-playback period and neighbour and stranger stimuli. To understand how inspection calls and ascending hoots contributed to the overall number of calls and territory defense, we also compared the occurrence of each of these call types between the three treatments using Kruskal-Wallis tests on untransformed data. We used planned *post hoc* comparisons to evaluate differences between treatments for both sets of tests.

To examine neighbour-stranger discrimination based on duets, we used data from the focal pair's response. We used the results of the *post hoc* tests from the territory defense analysis for the five main parameters to evaluate differences in response between the neighbour and stranger treatments. This approach was equivalent to conducting paired t-tests between neighbour-stranger response on the five parameters.

To test the role of duets in a communication network, we used the linear mixed-model design used to examine territory defense to conduct repeated measures ANOVA. Comparisons were between the pre-playback period and neighbour and stranger treatments for both immediate neighbours and the wider neighbourhood (i.e. all birds recorded at all automated recording devices minus the device nearest the site of playback). In order to minimize the number of statistical tests performed, only those parameters for which the focal pair showed a significant response to playback were examined for the immediate neighbours and neighbourhood.

To determine if our design was sufficient to detect an effect for any nonsignificant results, we calculated effect sizes for our data using Cohen's d (Cohen, 1988; Nakagawa & Cuthill, 2007). We also calculated effect sizes for three published studies with similar designs to our three primary questions (question 1: Brooks & Falls, 1975; question 2: Hardouin et al., 2006; question 3: Fitzsimmons et al., 2008). As suggested by Thomas & Juanes (1996), we used these previous studies' effect sizes as standardized values for comparison to our effect sizes. We corrected for multiple comparisons within each of our main questions by accepting an alpha level of 0.007 for the territory defense analysis (seven comparisons) and 0.01 for communication network analysis (four comparisons). Linear mixed-models and *post hoc* analyses were conducted in SPSS (SPSS Inc., Chicago, IL). Kruskal-Wallis tests were conducted in JMP 5.0.1 (SAS Systems Inc., Cary, NC).

Results

Territory defense

Barred owls responded strongly to territory intrusion simulated through playback. Focal owls at the site of playback vocalized for a significantly longer duration (repeated measures ANOVA: $F_{2,27} = 6.3$, $p < 0.006$; Figure 2a) and performed significantly more duets ($F_{2,27} = 6.0$, $p = 0.007$, Figure 2b) in response to playback of neighbour or stranger stimuli than during the pre-playback period. The number of solo calls by focal owls did not differ between the playback and pre-playback periods when all call types were combined ($F_{2,27} = 1.3$, p = 0.30; Figure 2c). Neither latency to first solo ($F_{2,27} = 0.5$, p = 0.63) nor latency to first duet ($F_{2,27} = 0.1$, p = 0.94) were significantly different between the pre-playback period or the two playback treatments (Figure 2d and e). There were, however, significant differences in numbers of particular types of calls before and after playback. Inspection calls decreased significantly following playback (Kruskal-Wallis: $T = 11.5$, n = 10, p = 0.003; Figure 3a), while ascending hoots increased significantly between the pre-playback period and stranger playback ($T = 9.8$, n = 10, p = 0.007; Figure 3b).

Neighbour-stranger discrimination

There were no significant differences in response to neighbour and stranger stimuli by the focal pair (Table 1, Figure 2). Although differences were not significant, stranger stimuli elicited an increased response compared to neighbour stimuli from the focal pair: focal owls vocalized for a longer duration (Table 1, Figure 2a) and responded more quickly with solos (Figure 2d) in response to strangers than neighbours. The effect sizes from both these values were comparable to those calculated from the results of another similar study (Table 1).

Communication networks

Looking beyond the level of the focal pair, we found that neither immediate neighbours nor the entire recorded neighbourhood performed significantly different numbers of duets (repeated measures ANOVA: $F_{2,24} = 1.5$, p = 0.24 and $F_{2,24} = 1.8$, p = 0.18) or for significantly different durations ($F_{2,24} < 0.1$, p = 0.96 and $F_{2,24} = 1.0$, p = 0.40) across all treatments (Figure 4). We compared immediate neighbour and neighbourhood response based only on numbers of duets and duration because these were the only two of the five calculated parameters that showed a significant difference within the focal pair analysis. Although nonsignificant, all four comparisons (Figure 4) were in the predicted direction, with higher responses to neighbour and stranger playback than control periods. Effect sizes for communication network analyses were small compared to results of another similar study (Table 1).

Discussion

Barred owls used their duets in direct confrontations with simulated territory intruders. We found that only the barred owls within the territory experiencing the intrusion responded strongly to duets. We did not find compelling support for duets as a signal of individual discrimination or as a signal that transmits information within a communication network. Owls showed nonsignificant increases in their response to strangers versus neighbours and heightened levels of vocal activity to interactions taking place beyond their territory boundaries. Our findings are in agreement with previous studies of duet function in songbirds, showing that duets are important in territory defense. However our results stand in contrast to previous studies on both conspecific discrimination and communication networks, showing that owls do not respond differently to neighbour versus stranger playback and that playback does not induce communication network-level effects.

Territory defense

Barred owls demonstrated a territorial response to duets by increased calling and duetting, and by vocalizing for extended periods compared to the silent pre-playback period. The increased number of duets, in particular, points to the fact that barred owls use their duets to confront territory intruders. Our findings agree with the majority of studies that have examined the territory defense hypothesis of duetting (Hall, 2004; Rogers et al., 2004; Molles & Waas, 2006; Mennill & Vehrencamp, 2008). Previous studies show increased duetting and calling in response to duets in several passerines and one suboscine species (Rogers et al., 2004; Molles & Waas, 2006; Mennill, 2006; Seddon & Tobias, 2005). To our knowledge, this is the first support for the territory defense hypothesis of duetting in a nonpasserine bird.

Neighbour-stranger discrimination

Barred owls did not show strong differences in response to playback of neighbours' versus strangers' duets, although we found a consistent, nonsignificant heightened response toward strangers. There are several explanations for barred owls' similar responses to neighbours versus strangers. First, the heavy degree of overlap within the duets of barred owls may encumber individual recognition within their complex duets. Second, barred owls may not have been selected to discriminate neighbours from strangers. Third, our sample size may have been too small to allow us to detect neighbour-stranger discrimination. The first explanation seems unlikely considering the structure of barred owl duets. Although their complex duets have a high degree of overlap, the beginning and end of each duet typically features several calls given by the male or female independently, which would facilitate individual discrimination. Furthermore, Radford (2005) provides evidence for neighbour-stranger discrimination in green woodhoopoes (*Phoeniculus purpureus*) based on a group signature within their choruses ('rallies'), even though the individual contributions within these choruses show a very high degree of overlap. Even if barred owl duets do not possess such a signature, it seems likely that barred owls should be able to discriminate between individuals within a duet during sections of little overlap (Freeman 2000).

We consider the possibility that barred owls do not discriminate neighbours from strangers, especially recognizing that current literature on neighbour-stranger discrimination and recognition among suboscine and nonpasserine birds has found mixed results. Many studies show conspecific discrimination and recognition in songbirds (Stoddard, 1996; Blumenrath et al., 2007). However, the spotted antbird (*Hylophylax naevioides*: Bard et al., 2002), a suboscine passerine, has individually distinct vocalizations, but does not exhibit conspecific discrimination in playback experiments. Some song-learning species with complex vocalizations and large repertoires (e.g. red-eyed vireo, *Vireo olivaceous*) show a distinct lack of neighbour recognition (Godard, 1993), while other species without complex vocalizations or song-learning (e.g. alder flycatcher, *Empidonax alnorum*) do exhibit individually distinct vocalizations and neighbour-stranger discrimination (Lovell & Lien, 2004). Many owl species, including barred owls, possess the important prerequisite of individually distinctive vocalizations (Cavanaugh & Ritchison, 1987; Galeotti & Pavan, 1991; Fitton, 1991; Freeman, 2000; Lengagne, 2001; Holschuh & Otter, 2005). Furthermore, neighbour-stranger discrimination of solo vocalizations has been supported in two owl species (tawny owl *Strix aluco*: Galeotti & Pavan, 1993; little owl *Athene noctua*: Hardouin et al., 2006). If territorial interactions with neighbours are a substantial threat in barred owls, then duetting at territory boundaries by either neighbours or strangers would warrant an equal or increased aggressive response to neighbours (Temeles, 1994; Müller & Manser, 2007). However, barred owl pairs maintain year-round territories and territory boundaries are stable (Mazur & James, 2000). This suggests established neighbours are lower risk than non-neighbour intruders and are likely familiar, consistent with conditions of the 'dear-enemy' hypothesis (Fisher, 1954).

Within owls, evidence for neighbour-stranger discrimination is contradictory. Galeotti & Pavan (1993) and Hardouin et al. (2006) clearly showed neighbour-stranger discrimination and recognition on the basis of solo vocalizations in two owl species. However, Waldo (2002) did not find evidence for neighbour-stranger discrimination in the spotted owl (*Strix occidentalis*), the closest relative of the barred owl. Waldo's (2002) sample size was similar (n=13) to that used in this study, while Galeotti & Pavan (1993) and Hardouin et al. (2006) each reported nearly twice the sample size (n=20 and n=21, respectively). In addition, Waldo (2002) used unpaired treatments and suffered from some experimental design problems that may have contributed to the lack of definitive neighbour-stranger discrimination.

Although we failed to find neighbour-stranger discrimination in barred owls, trends and effect sizes of our results do not allow us to conclude that barred owls are incapable of discriminating between the duets of neighbours and strangers. For nearly all parameters, the reaction to the stranger stimulus was in the direction predicted by the 'dear-enemy' hypothesis (Temeles, 1994). Also, effect sizes for neighbours versus strangers were comparable to those from other published studies (Table 1), indicating the trends toward neighbour-stranger discrimination in barred owls were meaningful. These trends, in combination with comparable effect sizes to published studies, suggests that neighbour-stranger discrimination may occur in barred owls, but a larger sample size would be needed to detect an effect.

Communication networks

Our playback study with barred owls did not find evidence for communication taking place within a network that extends beyond the territory boundaries of the focal pair. This result was contrary to our expectations; given that barred owls produce far-carrying acoustic signals and closely-spaced territories, they seem to be an ideal candidate for information being transferred in a network-like fashion. Again, there are several possible explanations for the absence of a communication network among barred owls. First, our sample size may have been too small to detect significance. Second, our methods may not have allowed us to detect how communication networks function in this species. Third, extended communication networks may not exist among barred owls. In contrast to Fitzsimmons et al. (2008), we did not find immediate response to an observed territorial interaction through a communication network. However, sample sizes of both experiments were similar. Our sample size did allow us to detect a distinct response of focal owls to playback when compared to a pre-playback period. Thus, it is possible we would have detected a difference between at least the pre-playback period and playback trials within a communication network had such a relationship existed. Effect sizes for immediate neighbour and neighbourhood responses were small in comparison to standardized effect sizes (Table 1). These results do not allow us to conclude that communication networks do not exist among barred owls, however, the disparity with published research indicates a much greater sample size would be needed to detect an effect if one exists. Trends for the communication network analysis were in the predicted direction, but nonsignificant and weak in comparison to studies that did see a significant difference.

Another possibility is that communication networks exist within barred owl populations, but function in other ways. For example, Schmidt et al. (2007) found that song contests observed by eavesdropping nightingales (*Luscinia megarhynchos*) influenced how they responded to the observed nightingales the next day. Nightingales that lost song contests were more likely to be approached and challenged in future song contests (Schmidt et al., 2007). A barred owl's decisions and future interactions may be influenced by listening to duet bouts and territorial encounters in adjacent territories, but our experiment evaluated neighbourhood-level responses only in the twenty minutes following playback. Alternatively, communication network level responses may occur for variables that we did not measure in our study, such as non-vocal or sex-specific vocal responses of neighbours. Other owl species do use their vocalizations for between-pair communication (Appleby et al., 1999; Delgado & Penteriani, 2006), suggesting owl vocalizations could function in a communication network and the need for additional research.

Owls (Strigiformes) and songbirds (Passeriformes) show many differences in life histories, and these differences may help to explain why we did not find support for the communication network model. Most birds of prey are fairly solitary with large home ranges determined by their prey abundance (Mazur et al., 1998). Even in northern populations of barred owls, individuals maintain territories averaging 1.18 – 2.28 km^2 depending on the season (Elody & Sloan, 1985), two to four times the size of the territories observed among our birds. Such distances between pairs likely inhibits or decreases the use of a communication network. Complex communication networks may therefore be reduced among birds of prey, even though the potential for communication networks is present in the dense populations of barred owls that we studied in the southern United States.

Conclusions

In summary, barred owl duets played a role in territory defense, but we did not find support that barred owl duets function in neighbour-stranger discrimination or communication networks. Barred owls used duets to directly confront territory intruders. They also vocalized for extended periods of time, gave more ascending hoots, and gave fewer inspection calls in response to duet playback, indicating heightened aggression toward duet playback. Barred owls gave more calls and responded sooner to strangers than neighbours, but we were not able to detect a significant effect with our sample size. Other studies show conspecific discrimination in owls and barred owls maintain pair bonds and territories over multiple years that should favour recognition. We cannot conclude that barred owls do not discriminate familiar and unfamiliar owls. Distant barred owls did not vocalize more after neighbour or stranger playback than during a pre-playback silent period, indicating immediate neighbours and nearby owls do not strongly respond vocally to duets. Nevertheless, barred owl duets may function within a communication network in non-vocal or sex-specific ways.

An important direction for future research on barred owl duets is playback experiments of sex-specific response to male and female solos or duets in a dual-speaker design. Such research would further our knowledge of whether the territory defense function of barred owl duets is a cooperative or conflict-based behaviour. Additionally, we could gain insight into extra-pair response to duets for evaluating eavesdropping and secondary information transfer via duets. Investigating non-vocal and delayed responses in a communication network is also an important direction for future research in this system. We encourage continued research on multiple functions of duets, including conspecific discrimination and communication networks, in a diversity of oscine and suboscine passerines and nonpasserines with a variety of life histories.

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Figure 1. Map of study area within the Choctawhatchee River basin, northwest Florida, showing locations of three 3.5-km transects used to record Barred Owl responses to playback. Recording transects are outlined by boxes and microphone locations shown by filled circles.

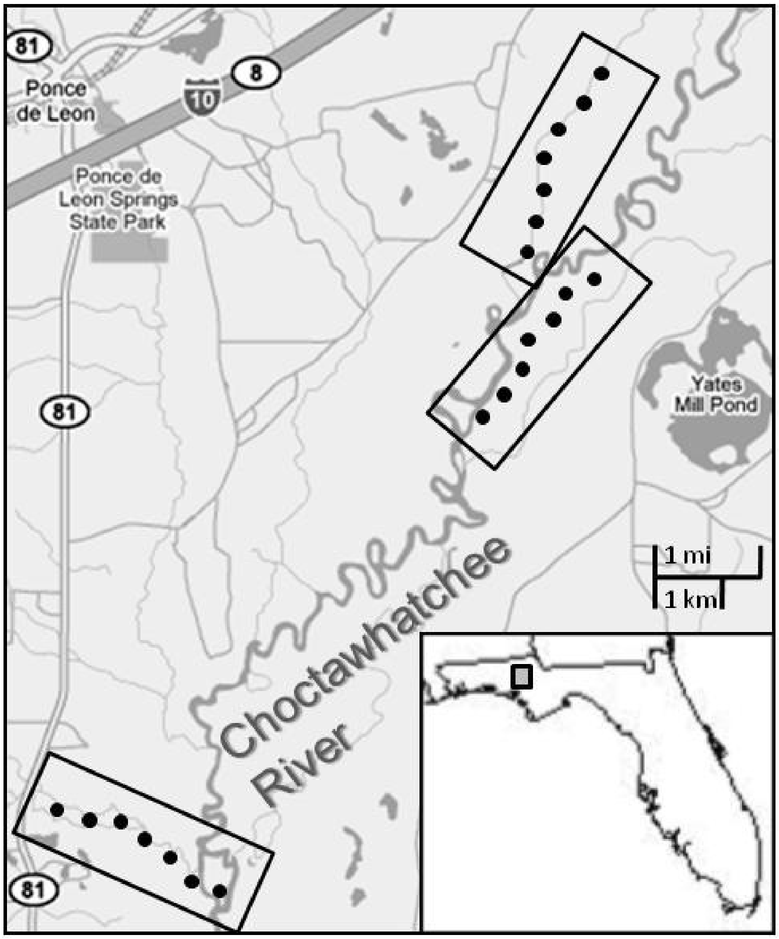


Figure 2. Singing behaviour of focal barred owls during a pre-playback silent period and in response to playback of duets of a neighbour and stranger pair of owls. Duration of vocal behaviour (A) and number of duets (B) increased significantly after playback when compared to a silent pre-playback period. Number of calls (C), latency to first solo (D), and latency to first duet (E) did not vary significantly across the three periods. Asterisk shows significant differences based on Tukey's test.

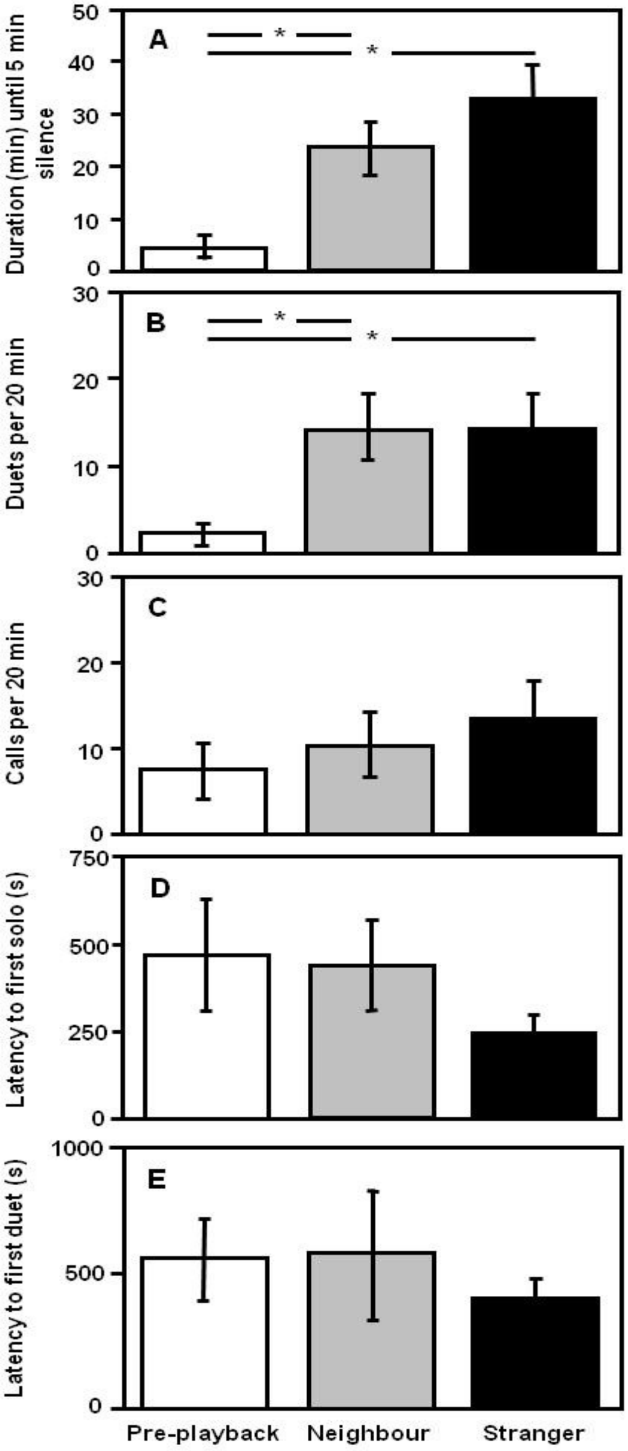


Figure 3. The number of inspection calls (A) decreased significantly after playback, whereas the number of ascending hoots (B) increased significantly after playback when compared to a silent pre-playback period for the focal pair. Asterisk shows significant differences based on Tukey's test.

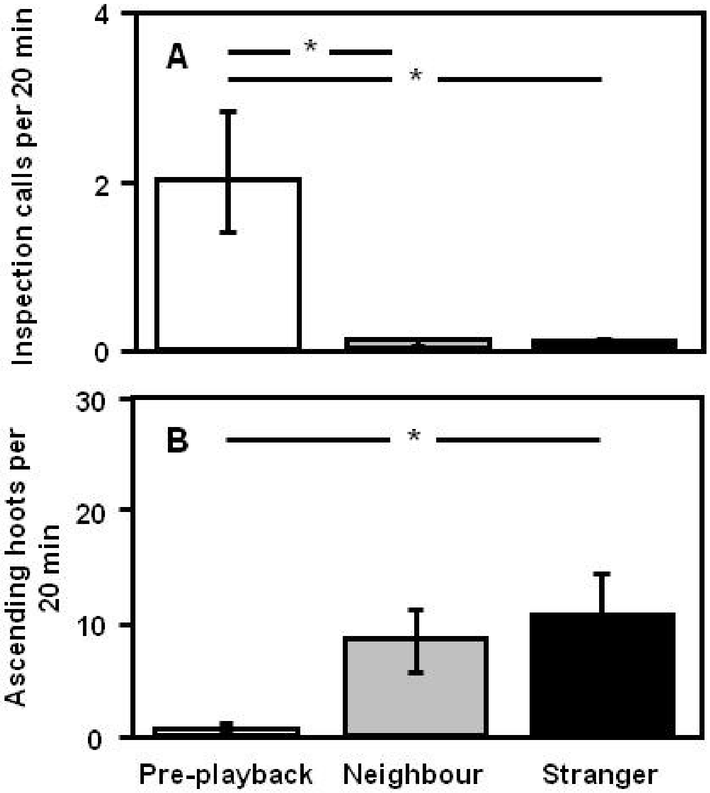
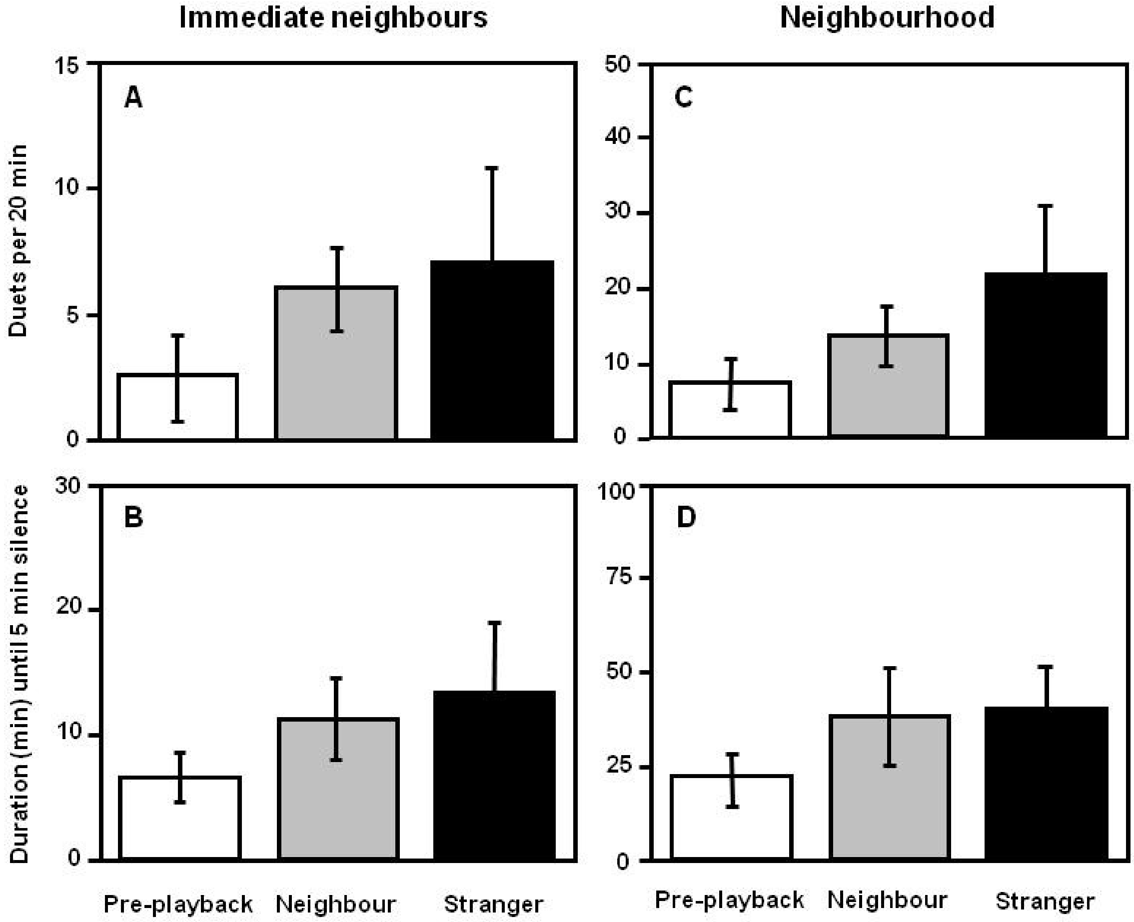


Figure 4. Vocal behaviour of territorial owl pairs adjacent to the site of playback (A and B) and of the entire neighbourhood of owls (C and D) recorded with a 3.5-km transect of microphones. No significant differences were detected for number of duets or duration outside of the focal pair's territory.



A. Territory defense

Group	Parameter	n	Mean Control	Mean Neighbour	Mean Stranger	Mean StdErr	P-value	d*
Focal pair	No. calls	10	7.3	10.5	13.3	3.3	0.30	0.31
Focal pair	**No. duets**	**10**	**2.3**	**14.5**	**14.7**	**3.4**	**< 0.01**	**1.13**
Focal pair	**Duration (min)**	**10**	**4.5**	**23.8**	**33.2**	**4.7**	**< 0.01**	**1.31**
Focal pair	Latency to first solo (min)	10	7.9	7.4	4.1	2.0	0.63	-0.08
Focal pair	Latency to first duet (min)	10	9.4	9.8	6.8	2.9	0.94	0.03
Comparison[1]	*No. Songs*	*18*	*0.8*	*2.0*	*4.1*	*0.1*	*< 0.01*	*2.71*

B. Neighbour-stranger discrimination

Group	Parameter	n	Mean Neighbour	Mean Stranger	Mean StdErr	P-value	d**
Focal pair	No. calls	10	10.5	13.3	3.3	0.37	0.26
Focal pair	No. duets	10	14.5	14.7	3.8	0.91	0.02
Focal pair	Duration (min)	10	23.8	33.2	5.1	0.41	0.54
Focal pair	Latency to first solo (min)	10	7.4	4.1	2.2	0.42	-0.62
Focal pair	Latency to first duet (min)	10	9.8	6.8	4.1	0.79	-0.30
Comparison[2]	*Hoots/min*	*21*	*10.0*	*13.6*	*1.0*	*0.0001*	*0.56*
Comparison[2]	*Duration of response*	*21*	*135.0*	*210.0*	*16.1*	*0.0001*	*0.72*
Comparison[2]	*Latency to first response*	*21*	*105.0*	*40.0*	*16.9*	*0.0001*	*-0.59*

C. Communication network

Group	Parameter	n	Mean Control	Mean Neighbour	Mean Stranger	Mean StdErr	P-value	d***
Immediate neighbour	No. duets	9	2.6	6.2	7.4	2.6	0.24	0.16
Immediate neighbour	Duration (min)	9	6.8	11.5	13.7	3.9	0.96	0.19
Neighbourhood	No. duets	9	8.4	15.2	24.8	6.2	0.18	0.54
Neighbourhood	Duration (min)	9	24.1	42.4	45.0	11.6	0.40	0.08
Comparison[3]	*Song output-Neighbourhood*	*10*	*12.5*	*9.0*	*17.5*	*2.2*	*0.04*	*1.16*
Comparison[3]	*Song output-Individuals*	*10*	*3.3*	*2.6*	*4.0*	*0.9*	*0.06*	*1.48*

Table 1. Effect sizes for the current study compared to standard effect sizes calculated from previous studies examining each of the hypotheses examined: (A) territory defense, (B) neighbour-stranger discrimination, and (C) communication networks.

* Calculated from means and mean square error with pre-playback as the comparison group.

**Calculated from means and standard deviations.

***Calculated from means and mean square error with stranger as the comparison group.

[1] From Brooks and Falls 1975.

[2] From Hardouin et al. 2006.

[3] From Fitzsimmons et al. 2008; treatments were 'submissive' and 'aggressive' rather than 'neighbour' and 'stranger'.

Chapter 4

No evidence for clinal variation or regional dialects in Barred Owl calls, duets, or duetting behaviour

Chapter summary

Research on vocal geographic variation in oscine passerine birds has provided insight into vocal learning and the function of bird song. Recent studies reveal more complexity in suboscine passerine and nonpasserine vocal behaviour than previously thought, yet we still have a very rudimentary understanding of geographic patterns in suboscine and nonpasserine species' vocalizations. Here we examine geographic variation in the vocalizations of Barred Owls (*Strix varia*). We explore variation in the structure of male calls, female calls, and duets, as well as two components of duetting behaviour: call occurrence and transition frequencies. We examine variation across ten locations that comprise a 1,350 km transect predominantly across the range of one subspecies of Barred Owls (*S. v. georgica*) in the southeastern United States. No components of vocalizations or vocal behaviour could be assigned to the correct location through discriminant analysis, nor did they vary with geographic distance. Some characteristics of male calls, female calls, call occurrence, and transition frequencies varied between locations, however, there was no discernible geographic pattern. Vocal geographic variation in Barred Owls appears to be random. We suggest random variation is one vocal geographic pattern expected for species that do not learn their vocalizations. We discuss the applications of our research for examining ontogeny of complex, coordinated vocal behaviour, such as duets.

Introduction

Patterns of geographic variation in bird vocalizations can provide insight into the ecology and evolution of animal sounds and vocal behaviour. Many oscine songbirds exhibit vocal dialects, where multiple characteristics or a major component of species-specific vocalizations change between populations over short geographic distances (Marler and Tamura 1962, reviewed in Mundinger 1982). Research on vocal variation in temperate, oscine songbirds has provided the foundation for what we know about the ontogeny of vocal learning in birds and has informed research on bird song function (reviewed in Beecher and Brenowitz 2005, Catchpole and Slater 2008). Studies of variation in avian dialect patterns and boundary size have connected ecological factors, such as dispersal distance and length of the breeding season, to timing and selectivity of vocal learning (e.g. Nelson 1999, Nelson et al. 2001). Vocal geographic patterns are also used to explore the function and evolution of song learning by linking dialects to song sharing among populations for mate attraction or social interactions (Mundinger 1982, Beecher and Brenowitz 2005).

Songs of non-song-learning suboscine and nonpasserine species can be complex and functionally similar to oscine species (e.g. Trainer et al. 2002, Lovell and Lein 2004b, Leger 2005) and also exhibit interesting vocal geographic patterns (Bretagnolle and Genevois 1997, Leger and Mountjoy 2003, Fitzsimmons et al. 2008). Alder Flycatchers (*Empidonax alnorum*) have a single, stereotyped song, but individuals have distinct vocalizations from one another and use those differences in neighbour-stranger discrimination (Lovell and Lein 2004a, b). Other suboscines also exhibit individually distinct vocalizations that vary between geographically separated groups (Chelén et al.

2005, Lein 2008, Fernández-Juricic et al. 2008, Fitzsimmons et al. 2008). For example, the Flammulated Attila (*Attila flammulatus*) selectively combines elements of its song in a way only previously known in song-learning species (Leger 2005), and the Bright-rumped Attila (*Attila spadiceus*) exhibits different geographic patterns between its dawn and day song, reminiscent of the functionally distinct songs of warblers (Byers 1996, Leger and Mountjoy 2003). However, few studies have documented large-scale vocal geographic patterns of suboscines (exceptions include Lindell 1998, Isler et al. 2005) and almost no studies have explored vocal geographic patterns in nonpasserines (exceptions are Goldstein 1978, Galeotti et al. 1996, Bretagnolle and Genevois 1997, Mager 2007).

Studies that have looked at large-scale patterns of geographic variation in suboscines with presumably innate vocalizations show two patterns: (1) steady, clinal patterns with increasing geographic distance (Isler et al. 2005), and (2) diffuse, random patterns that do not correlate to geographic distance (Lindell 1998). These two patterns should be expected for vocal geographic variation of non-song-learning suboscines and nonpasserines because vocal variation should be closely linked to genetic variation in these species (Soha et al. 2004, Isler et al. 2005). Variation in innate vocalizations is expected to correspond to genetic patterns, which may be clinal, but are also subject to local adaptation or drift, resulting in random variation (Mundinger 1982, Isler et al. 2005, Podos and Warren 2007). To our knowledge, only two studies have found substantial vocal variation in large-scale geographic studies in nonpasserines (Tawny Owl, *Strix aluco*: Galeotti et al. 1996; Blue Petrel, *Halobaena caerulea*: Bretagnolle and Genevois 1997). However, these studies examined differences between subspecies and separate habitat types (Galeotti et al. 1996) and among geographically separated island populations (Bretagnolle and Genevois 1997).

Many animals, including a diversity of oscines, suboscines, and nonpasserines, perform coordinated vocal duets between mated individuals (Farabaugh 1982). For example, mated male and female Yellow-naped Parrots (*Amazona auropalliata*) combine their vocalizations with a specific syntax (Wright and Dahlin 2007). Because of the precision and complexity of many avian duets, researchers have suggested they may require coordination or learning between duet partners (Harcus 1977, Levin 1996, Mann et al. 2009). However, scant attention has been given to vocal geographic patterns of duets (exceptions include Trainer and Parsons 2001, Mennill and Rogers 2006) and no studies have looked at geographic variation in syntax of such complex vocal behaviours. If duets do require learning, we suggest vocal geographic patterns of duets and duetting behaviour may vary similarly to dialects of learned bird song. It could be particularly informative to examine geographic variation in duets for species with otherwise innate vocalizations.

In this study we explore geographic patterns in the calls, duets, and duetting behaviour of a nonpasserine, the Barred Owl (*Strix varia*). Male and female Barred Owls combine their vocalizations into extended duets with a specific syntax and distinct male and female roles (Odom and Mennill in prep.). We tested the presence of geographic variation in male calls, female calls, duet structure, call occurrence, and transition frequencies. To examine how each of these vocalization types and behaviours vary geographically, we recorded owls from ten locations in a transect throughout the southeastern United States. We explored three questions: (1) Can owl vocalizations and vocal behaviour be assigned to specific locations? (2) Do vocalizations and vocal behaviour vary with geographic distance (i.e. are vocalizations and vocal behaviour more similar between closer locations)? (3) Are vocal characteristics shared consistently among groups of nearby locations with shifts in prominent vocal characteristics between more distant locations (i.e. do some populations show vocal similarity separated by dialect boundaries, as seen in oscine passerines; Mundinger 1982)? If Barred Owl calls or duets vary in a pattern similar to that seen for the vocalizations of many oscine passerines, we expected that calls and duets would show a strong signature of the recording location, or that multiple vocal characteristics would be shared between several locations with an abrupt change in characteristics between other locations. If Barred Owl vocalizations and duets lack meaningful vocal geographic variation, we expected that calls and duets would show a clinal pattern of variation where vocal divergence increases with geographic distance, or random variation with respect to geographic distance. We predicted that the simpler, stereotyped Barred Owl calls might lack meaningful geographic variation, whereas complex duets might exhibit patterns more similar to oscine passerine dialects.

Methods

We recorded Barred Owl calls and duets from 13 locations across five southeastern United States, focusing primarily within one subspecies range. Recording locations spanned from the western to the northeastern boundary of the Florida Barred Owl (*S. v. georgica*) subspecies range with the northern-most recording location in the range of the Northern Barred Owl (*S. v. varia*; Fig. 1). Barred Owls were uncommon at three locations (fewer than five pairs recorded at each location) resulting in ten locations for analysis: (i) Beaumont Unit and John's Lake of Big Thicket National Preserve, Koontz, TX (n= 9 pairs), (ii) Sherburne Complex Wildlife Management Area, Krotz Springs, LA (n= 7 pairs), (iii) Barataria Preserve, New Orleans, LA (n= 8 pairs), (iv) Pearl River Wildlife Management Area, Slidell, LA (n= 9 pairs), (v) Northwest Florida Water Management District of the Choctawhatchee River, Ponce de Leon, FL (n= 10 pairs), (vi) Florida and Stix Rivers within Apalachicola National Forest, Bristol, FL (n= 8 pairs), (vii) Suwannee River, Fargo, GA (n= 10 pairs), (viii) Harris Neck National Wildlife Refuge, MacIntosh Co., GA (n= 6 pairs), (ix) Congaree National Park, Hopkins, SC (n= 11 pairs), and (x) Charlotte, NC (n= 6 pairs). The majority of these locations were predominately bottomland hardwood forest, characterized by low to substantial levels of standing water year round and large numbers of Bald Cypress (*Taxodium distichum*). Harris Neck National Wildlife Refuge possessed only small portions of bottomland forest, and was otherwise predominated by Wax Myrtle (*Myrica spp.*), Juniper (*Juniperus spp.*), and Virginia Live Oak (*Quercus virginiana*). Owls recorded in Charlotte were recorded in the upland suburbs directly surrounding the city, also predominated by Virginia Live Oak.

We spent three to five days at each location, recording six to eleven pairs of owls at each site. Recordings were collected between 21 February and 08 April, 2008, excluding the Choctawhatchee River, where we recorded owls from 23 January to 15 February, 2008. February through April in the southeastern United States corresponds to the incubation period and early nestling period of Barred Owls. Barred Owls are nonmigratory and pairs maintain year-round territories at all these locations (Mazur and James 2000).

Equipment and recording protocol

All owls were recorded with a Marantz PMD- 670 solid-state digital recorder and a Sennhieser ME67 shotgun microphone with K6 power module. Recordings were collected as WAV files at a sampling rate of 44.1 kHz, 16 bit, 160 kbps. Most recordings were made between 0200 and 0800 h (in the dark or early twilight of dawn). Less than half of the pairs at Big Thicket, Choctawhatchee, Apalachicola, and Congaree were recorded between 1800 and 2400 h (twilight of late evening or in the dark). Focal recordings used for analysis were made from 10 to 40 m from the focal pair, but usually at a distance of approximately 20 m. Within a location, pairs of owls were recorded a distance of at least 500 m from each previous pair. This distance was sufficient to detect separate pairs of owls within the southeastern United States based on observations of multiple pairs of owls at single recording locations throughout a population in northwest Florida, and radiotelemetry studies indicating contiguous territories averaging 200 m in diameter (R. Bierregaard, Jr. pers. comm.). Vocalizations of owls are spectrographically distinct (Freeman 2000), so we visually inspected spectrographs of owls that approached from adjacent recording locations. If we doubted two recordings from adjacent locations were separate pairs, we eliminated one of the recordings from our analyses.

Calls and duets were solicited from all pairs using a standardized playback stimulus consisting of two tracks of common vocalizations: (1) 1 min 40 sec of eight two-phrased hoots alternating between male and female, and (2) 3 min of ascending hoots by both males and females (Chapter 2). We played track one once and track two up to three times at a location with five minutes of silence between each playback. We stopped playback as soon as owls responded vocally. If owls did not respond within five minutes of the end of the third playback of track two, we moved to a new location. Both playback stimuli were prepared from recordings of mated pairs of wild owls from the Choctawhatchee River Basin in northwest Florida.

Sound analysis

Sounds were visualized as spectrograms and measurements were made using SYRINX-PC (J. Burt, Seattle, WA) (settings: Blackman FFT, transformation size 1024 Hz, providing an effective time resolution of 3 msec and frequency resolution of 4 Hz). To assess vocal variation in call structure, duet structure, and duetting behaviour, we measured multiple variables for each of five vocalization types or vocal behaviours: male calls, female calls, duets, call occurrence, and transition frequencies (Table 1). To evaluate male and female call structure, we measured features of male gurgle calls and female one-phrased hoots (Figure 2). Both calls were isolated from consecutive gurgle to one-phrased hoot transitions within duets. We selected these vocalizations because gurgles and one-phrased hoots are the most common calls and the most common transition within Barred Owl duets (Odom and Mennill in prep.). We counted number of notes and measured call duration, maximum frequency (F_{max}), minimum frequency (F_{min}), and duration of the final note for each male gurgle and female one-phrased hoot (Table 1, Figure 2). For duet structure, we used the same gurgle to one-phrased hoot duet section. We counted number of calls within the duet and measured duration of the entire duet. We also measured time delays and frequency differences between the gurgle call, the one-phrased hoot, and the preceding female call, including: start of the first female call to the start of the male gurgle, start of the male gurgle to start of the female one-phrased hoot, end of the male gurgle to end of the female one-phrased hoot, and male gurgle F_{max} minus female one-phrased hoot F_{max} (Male F_{max} – Female F_{max}; Table 1, Figure 2). We selected gurgle to one-phrased hoot duet sections from the first duet in a recording where the entire duet was high enough quality to allow each call within the duet to be readily identified. We isolated only one gurgle to one-phrased hoot duet section from one duet from each pair recorded. We modified this procedure from Klenova et al. (2008) in order to obtain a comparable section of a duet from each pair. In several locations, gurgle to one-phrase hoot transitions were less common. If we could not find a gurgle to one-phrased hoot transition for a pair, we measured the same variables in the next most-similar call combinations, gurgle to short ascending hoot or gurgle to ascending hoot transitions.

We examined geographic variation in duetting behaviour by assessing two components of duets: call occurrence, as a measure of call use, and transition frequencies between consecutive pairs of vocalizations, as a representation of duet syntax. The same duet from each pair used for fine-scale measurements was used in these two analyses. For call occurrence, we counted how often each of ten common call types occurred within each duet (see Chapter 2 for call descriptions). We added all other calls that occurred to an additional category of ‘other’, for eleven call occurrence categories. For transition frequencies, we sequenced the order of each call within duets. We then counted the number of each transition within a duet. We selected the eleven most common transitions for analysis by canonical discriminant function analysis and multivariate analysis of variance. We used all possible transitions to create proximity matrices for comparisons to geographic distance.

Statistical analysis

We conducted three analyses to assess possible vocal geographic patterns: (1) canonical discriminant function analysis (DFA) to determine if calls, duets, and duetting behaviour could be assigned to geographic location, (2) mantel tests comparing geographic distance to variation in calls, duets, and duetting behaviour, (3) multivariate comparisons (multivariate analysis of variance and log-linear regression) to assess differences and similarities among multiple locations in calls, duets, and duetting behaviour. For each set of analyses, we tested all five vocalization types or behaviours (male calls, female calls, duet structure, call occurrence, and transition frequencies). We corrected for examining five vocalization types or behaviours within each analysis by accepting a significance value of $P = 0.01$.

Discriminant function analysis. We used a cross-validation technique to see if canonical scores calculated with 80% of the data could assign the remaining 20% of the data to the correct location. Correct discrimination was evaluated by a chi-square analysis of known location by the predicted location by DFA. We report correct classification as the 20% of the data that was correctly classified to location. DFA was carried out in JMP 5.0.1 (SAS Systems Inc., Cary, NC).

Geographic distance, dissimilarity matrices, and mantel tests. We used mantel tests to compare a similarity matrix of geographic distance to each of five dissimilarity matrices representing the five vocalization types or vocal behaviours. We calculated geographic distance using the central most GPS UTM coordinate for the recordings collected at each location and converted each UTM coordinate into longitude and latitude decimal degrees. We then calculated distance between each pair of locations in kilometers to create a similarity matrix of geographic distance. Dissimilarity matrices were constructed by between-group linkage using Euclidean distances for male call, female call, and duet fine-scale measurement data sets. For call occurrence and transition frequencies, we used chi-squared measures in place of Euclidean distance to compensate for count data. Fine-scale measurement data were standardized as z-scores. All matrices were rescaled to 0-1 range.

Mantel tests were used to compare the distance matrix to each dissimilarity matrix separately. Each model specified 10 locations. We interpreted significance based on an expected inverse relationship between geographic location and dissimilarity of the measurements within the matrix (i.e. geographic distance should increase with decreasing dissimilarity between locations). Mantel tests were performed in Isolation by Distance, v.3.16 (Jensen et al. 2005) and dissimilarity matrices were constructed in SPSS (SPSS Inc., Chicago, IL).

Multivariate comparisons. We used general linear models to compare locations in a multivariate analysis of variance (MANOVA) design for both continuous fine-scale measurements and count data. For male call, female call, and duet fine-scale measurements, we ran three separate multivariate general linear models with all variables specified as dependent variables and location as a fixed factor. We used repeated measures general linear models to compare count data of call occurrence and transition frequencies as log-linear regressions on expanded data sets. The data sets for each were structured as contingency tables of presence or absence of each vocalization type or transition for each call or pair of calls within a duet. We specified 11 factors, treating each of the eleven variables of call type or transitions as a within-subjects factor. Location was specified as a between-subjects factor. All models were run with a full factorial design and type III sum of squares. For tests with significant results, we ran planned *post hoc* comparisons to evaluate which variables and locations were significantly different. MANOVA and log-linear regression were conducted in SPSS.

Results

Male calls, female calls, and duetting behaviour varied between locations. However, only a few geographically separated locations differed for several variables and the locations exhibiting differences were not consistent across variables. Barred Owl calls or duets could not be assigned to recording location, and did not vary according to geographic distance.

Discriminant function analysis

No aspects of Barred Owl calls or duets could be assigned to location using Discriminant Function Analysis (DFA). DFA could not correctly classify male or female calls to location better than expected by chance; only 6.3% of male calls ($\chi^2 = 44.0$, N = 16, P = 0.88) and 20.0% of female calls ($\chi^2 = 47.6$, N = 15, P = 0.93) were correctly classified based on fine-scale measurements. Fine-scale measurements of duets also could not be predicted based on location, with only 25.0% of duets classified to the correct location ($\chi^2 = 45.6$, N = 16, P = 0.84). Call occurrence and transition frequencies could not be assigned to location, either, with only 23.5% ($\chi^2 = 52.3$, N = 17, P = 0.96) and 25.0% ($\chi^2 = 41.2$, N = 16, P = 0.93) of duets assigned to the correct location, respectively. There was substantial overlap between the 95% confidence intervals for nearly all locations for all comparisons, shown by the initial DFA using 80% of each data set.

Geographic distance

Variation in Barred Owl calls, duets, and duetting behaviour was not related to geographic distance. Fine-scale measurements of male calls showed no significant changes as geographic distance increased (r = -0.11, P = 0.26). Female calls also showed no significant changes with geographic distance (r = 0.13, P = 0.81). Duet structure became increasingly dissimilar with increasing geographic distance, but this relationship was not significant after correction for multiple comparisons (r = -0.27, P = 0.05). Similarly, duet behaviour based on occurrence was weakly correlated to geographic distance, but was non-significant after correction for multiple comparisons (r = -0.26, P = 0.04). Duet behaviour based on transition frequencies was not significantly related to geographic distance (r = -0.05, P = 0.46).

Multivariate comparisons

Barred Owl calls and duetting behaviour varied between locations, but there was no discernable geographic pattern to this variation (Figures 3 and 4). Male calls varied significantly between locations (MANOVA: $F_{45,307}$ = 1.8, P = 0.002), driven by significantly fewer notes in calls given in Barataria Preserve, LA than along the Suwannee River, GA and significantly higher minimum frequencies in the Pearl River Wildlife Management Area, LA than in Apalachicola National Forest, FL, the Suwannee River, GA, Harris Neck National Wildlife Refuge, GA, or Congaree National Park, SC

(Figure 3a and b). Females gave significantly shorter calls in Apalachicola National Forest, FL and the Suwannee River, GA compared to Harris Neck National Wildlife Refuge, GA ($F_{45,303} = 1.8$, $P = 0.002$; Figure 3c). Duet structure varied between locations, but not significantly after corrections for multiple comparisons ($F_{54,336} = 1.4$, $P = 0.05$).

Duetting behaviour varied between locations across several variables, again, with no discernable geographic pattern (Figure 4). Call use within duets differed significantly between locations (Log-linear regression: $F_9 = 2.6$, $P = 0.005$). In the Pearl River Wildlife Management Area, LA, owls gave more two-phrased hoots (Figure 4a), and in Apalachicola National Forest, FL, they gave more three-note calls (Figure 4b) than at most other locations. Barred Owls also gave fewer short ascending hoots at Pearl River Wildlife Management Area, LA, than in Charlotte, NC, more one-phrased hoots along the Suwannee River, GA than in Harris Neck National Wildlife Refuge, GA and Charlotte, NC, and more fast ascents in Sherburne Wildlife Management Area than most other locations. Transition frequencies of Barred Owl duets also varied significantly between locations ($F_9 = 2.8$, $P = 0.003$), with owls in Apalachicola National Forest, FL performing more ascending hoot to ascending hoot combinations compared to Congaree National Park, SC and owls in Charlotte, NC gave fewer one-phrased hoot to gurgle transitions than in the Pearl River Wildlife Management Area, LA and the Suwannee River, GA.

Discussion

Barred Owl calls, duets, and duetting behaviour showed inconsistent geographic variation across the southeastern United States. No aspects of vocalizations or vocal behaviour could be assigned to recording location, and duet structure showed no increase in variation with geographic distance. There was some variation between locations for calls and duetting behaviour, however, the locations showing variation were not consistent across variables. While clinal variation is an accepted vocal geographic pattern of non-song-learning species (Mundinger 1982, Isler et al. 2005), we suggest the random and inconsistent vocal variation shown in Barred Owl calls and duets should also be an expected pattern for non-song-learning suboscine and nonpasserine species.

Many studies provide evidence for distinct dialects in oscine species, linking multiple, observable changes in vocalizations between populations to the cultural transmission of bird song (Marler and Tamura 1962, Mundinger 1982). Recent studies confirm that these changes can occur in the absence of genetic variation (MacDougall-Shackleton and MacDougall-Shackleton 2001, Soha et al. 2004), making vocal geographic variation a useful tool for assessing cultural transmission of vocalizations and song learning (Saranthan et al. 2007). Nevertheless, vocal variation can also result from natural selective pressures and adaptations, including geographic barriers, changes in habitat, and morphological adaptations (reviewed in Podos and Warren 2007). These mechanisms are known to contribute to vocal variation in songbirds (Handford and Lougheed 1991, Chilton and Lein 1996, Podos 2001). Suboscine and nonpasserine vocalizations should also be influenced by direct selection for effective communication, but are also susceptible to be byproducts of genetic adaptation or changes in gene flow (Zink and Remsen 1986, Bretagnolle and Genevois 1997, Podos and Warren 2007).

Local adaptations can alter vocalizations of innate vocalizing species, such as the increase in frequency with decreasing body size of Common Loons (*Gavia immer*) in northwestern regions (Mager et al. 2007). If vocalizations are genetically controlled, we may expect overall similarity in vocalizations among genetically similar populations, with slight variation in vocal traits between populations that exhibit genetic variation due to local adaptations or drift (Podos and Warren 2007). Such random differences in vocal characteristics were seen in the vocalizations of a suboscine, the Pale-breasted Spinetail (*Synallaxis albescens*; family: Furnariidae; Lindell 1998), as well as among our Barred Owls.

We evaluate the overall consistency and random variation among Barred Owl vocalizations in a single species range in light of several factors, including high levels of individual variation, habitat differences, and local genetic adaptations. Barred Owls have individually distinct vocalizations that can be readily visually identified by spectrographs (Freeman 2000). Obvious spectrographic differences between individuals in all locations likely resulted in large amounts of variation within each location, making differentiation of vocalizations between locations difficult. Additionally, we noticed duet structure and syntax was highly variable even within a single pair of birds, particularly in timing of overlap between calls (Figure 5). This intra-individual variation inhibited us from visually or auditorially distinguishing calls and duets between locations and likely contributed to statistical inability to discriminate between locations. The effect of high individual variability was noticeable statistically by the large dispersion of points within each location by DFA and substantial overlap of 95% confidence intervals.

We consider that the random differences between locations may have arisen due to habitat differences or other environmental factors, particularly between the two upland sites of Harris Neck National Wildlife Refuge, GA and Charlotte, NC. Some of the geographic differences were associated with these sites, but not all, and habitat was similar between the remaining eight locations. Also, we conducted preliminary correlations between habitat and vocal variation, using habitat measurements taken at each site. We found no evidence for a relationship between vocalizations and habitat (Odom unpublished data). Therefore, habitat may have played a role in vocal variation, but we do not think it accounts for most of the vocal differences seen. Tawny Owls (*Strix aluco*) exhibit vocal variation between open farmland and forested woodland sites (Galeotti et al. 1996). However, they exhibited much greater differences in vocalizations between subspecies ranges (Galeotti et al. 1996), indicating habitat may play a role in vocal variation of *Strix* species, but genetics has a dominant influence. We also consider changes in vocalizing throughout the breeding season may have contributed to perceived differences in vocalizations between locations (Chelén et al. 2005). If breeding season was a major reason for variation, we would have expected to see the greatest variation in the Choctawhatchee River, which was sampled just prior to incubation and nestling stages when owls at all other locations were recorded. However, the Choctawhatchee River was intermediate for most vocalizations and vocal behaviour variables, suggesting other factors had a greater influence on vocal variation.

We intentionally focused primarily on a single subspecies range to target populations that are presumably genetically similar. Current Barred Owl subspecies boundaries are morphologically defined, with owls from east Texas to southeast North

Carolina sharing many physical traits, predominately plumage color, size, and amount of feathering on the toes (Mazur and James 2000). While phenotypic similarity implies genetic similarity, it does not guarantee genetic homogeneity (e.g. ‘genetic compensation’, Grether 2005) and some traits are more susceptible to vary with environmental change (Zink and Remsen 1986). While plumage color and size may be fairly constant across a subspecies range with potential local genetic adaptations, vocal characteristics and behaviour are likely more plastic, which would lead to disruptive vocal geographic patterns (Zink and Remsen 1986). If vocalizations vary strictly with genetic structure, we might have expected to see shifts in more vocal characteristics with the shift in subspecies boundaries between Charlotte, NC and the other recording locations. No call or duet structure variables and only three of seven significant duet behaviour characteristics varied between Charlotte and other locations. While this may indicate vocal variation is attributed to other environmental and ecological factors besides genetics, Barred Owls exhibit substantial morphological intergradations at subspecies boundaries, so may not be very genetically different (Pyle 1997). In Charlotte, Barred Owls have morphological traits of both *S. v. georgica* and *S. v. varia* (R. Bierregaard, Jr. pers. comm.). Therefore, environmental adaptations, but also local genetic adaptations, differences in gene flow, and drift could all contribute to the random vocal differences seen in Barred Owls (Podos and Warren 2007).

Our study is one of the first to examine vocal variation in a nonpasserine across a subspecies range. The inconsistent, random variation agrees with previous research on non-song-learning species (Lindell 1998), although varies from the traditionally expected clinal pattern (Mundinger 1982, Isler et al. 2005). More research on vocal variation in a diversity of suboscine and nonpasserine species within subspecies boundaries will help verify both patterns. Also, an analysis of genetic variation across the range of the Florida Barred Owl would help verify whether these vocal differences arise from genetic variation and adaptation.

Our research has interesting implications for acquisition of not only Barred Owl vocalizations, but vocal behaviour. Vocal geographic variation has proven a valuable tool for examining vocal learning in stereotyped songs of a suboscine (Saranathan et al. 2007). We suggest it may also be useful to explore learning of complex vocalizations and vocal behaviour, such as duets. We propose the inconsistent geographic variation in Barred Owl call structure, duet structure, and duetting behaviour may indicate all aspects of vocalizations and vocal behaviour are innate in Barred Owls. Nevertheless, evidence is surmounting that vocal learning is more widespread and plastic than originally thought (Brenowitz and Beecher 2005, Saranathan et al. 2007), so more attention should be paid to vocal ontogeny in a diversity of vocalization types and behaviours. We encourage continued research on vocal geographic variation of suboscines and nonpasserines, particularly within complex, coordinated vocalizations.

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Wildlife Management Area for assistance and access to land. We are especially grateful to R. Bierregaard, Jr. for access to his owls in Charlotte, North Carolina and his advice and discussion about Barred Owl behaviour and vocalizations. We also thank N. Barker, J. Ciborowski, T. Pitcher, and R. Sosa for help with analysis. We thank the Natural Sciences and Engineering Research Council of Canada (NSERC), the Canada Foundation for Innovation, the Government of Ontario, and the University of Windsor for grants to DJM, and the American Ornithologists' Union for a research award to KJO. We also thank NSERC, Pelee Island Winery, Nokuse Plantation Inc. and M. C. Davis, Northwest Florida Water Management District, Florida Fish and Wildlife Conservation Commission, and the U.S. Fish and Wildlife Service for support in parallel with a search for Ivory-billed Woodpeckers in north Florida.

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Figure 1. Map of the range of the Barred Owl (*Strix varia*), showing subspecies boundaries (inset). Recording locations through the geographic range of *S. v. georgica* are shown in the expanded section: (i) Big Thicket National Preserve, TX, (ii) Sherburne Complex Wildlife Management Area, LA, (iii) Barataria Preserve, LA, (iv) Pearl River Wildlife Management Area, LA, (v) Choctawhatchee River Basin, FL, (vi) Apalachicola National Forest, FL, (vii) Suwannee River Basin, GA, (viii) Harris Neck National Wildlife Refuge, GA, (ix) Congaree National Park, SC, and (x) Charlotte, NC. Entire species range boundary modified from Mazur and James (2000) and subspecies boundaries based on Bent (1938) and Eckert (1974).

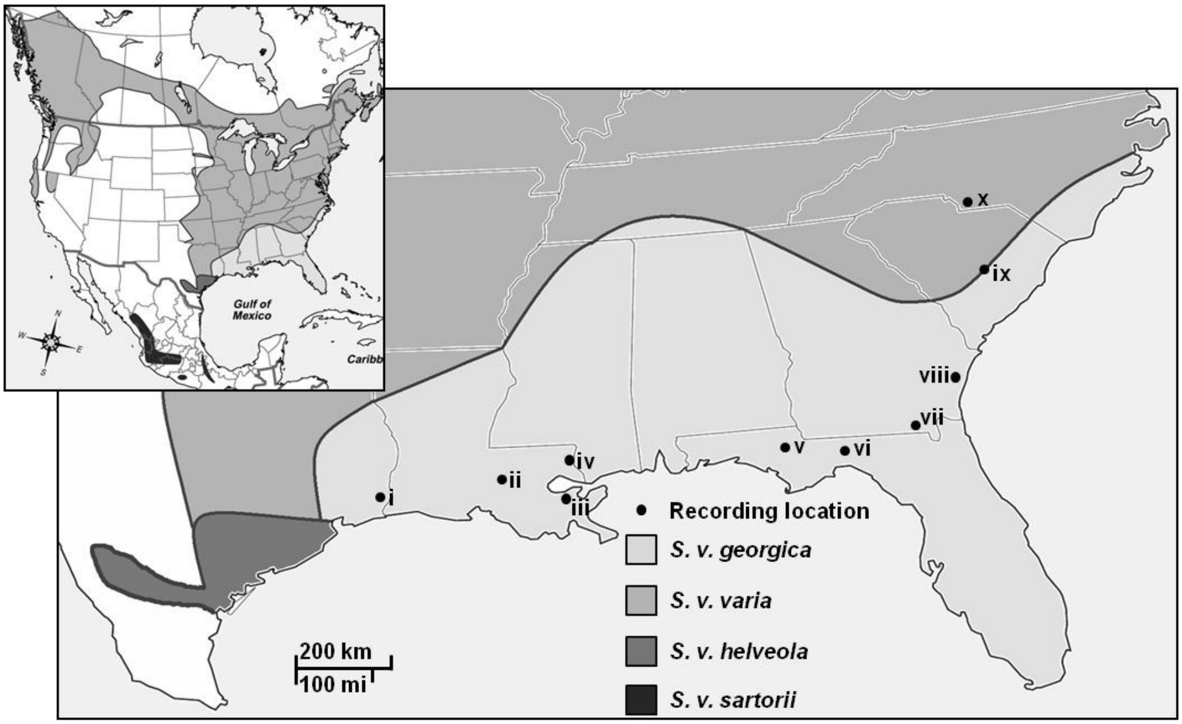

Figure 2. Top: Sound spectrograph of a Barred Owl duet. Bottom: Sound spectrograph of a portion of a duet, showing structural components measured for a female-male-female gurgle call to one-phrased hoot duet section. Female contributions are underlined in black, and male contributions are underlined in grey. Thirteen variables were measured: (i) male call duration, (ii) male call maximum frequency (F_{max}), (iii) male call minimum frequency (F_{min}), (iv) male call duration of the final note, (v) female call duration, (vi) female call F_{max}, (vii) female call F_{min}, (viii) female call duration of the final note, (ix) start of the first female call to the start of the male gurgle, (x) start of the male gurgle to start of the female one-phrased hoot, (xi) end of the male gurgle to end of the female one-phrased hoot, (xii) male gurgle F_{max} minus female one-phrased hoot F_{max} (M_{Fmax}-F_{Fmax}), and (xiii) duration of the entire duet.

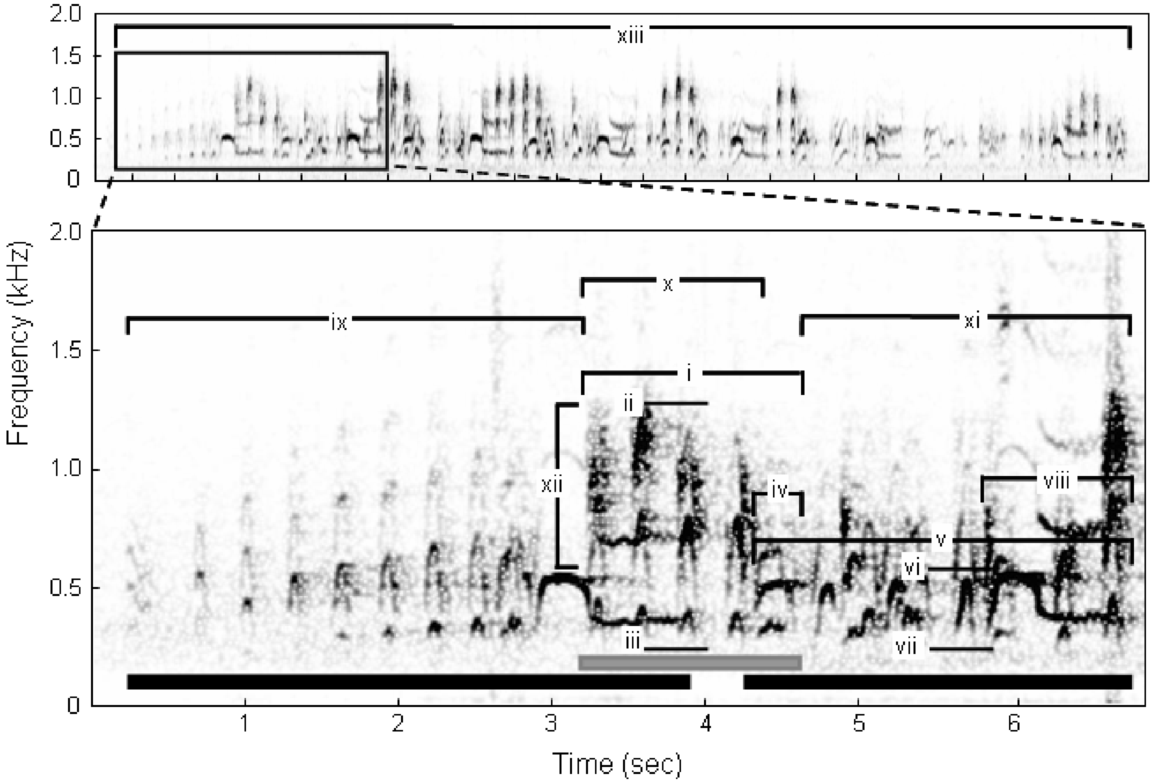

Figure 3. Comparisons of Barred Owl calls between ten locations based on fine-structural features of (i) male call number of notes, (ii) male call minimum frequency, and (iii) female call duration.

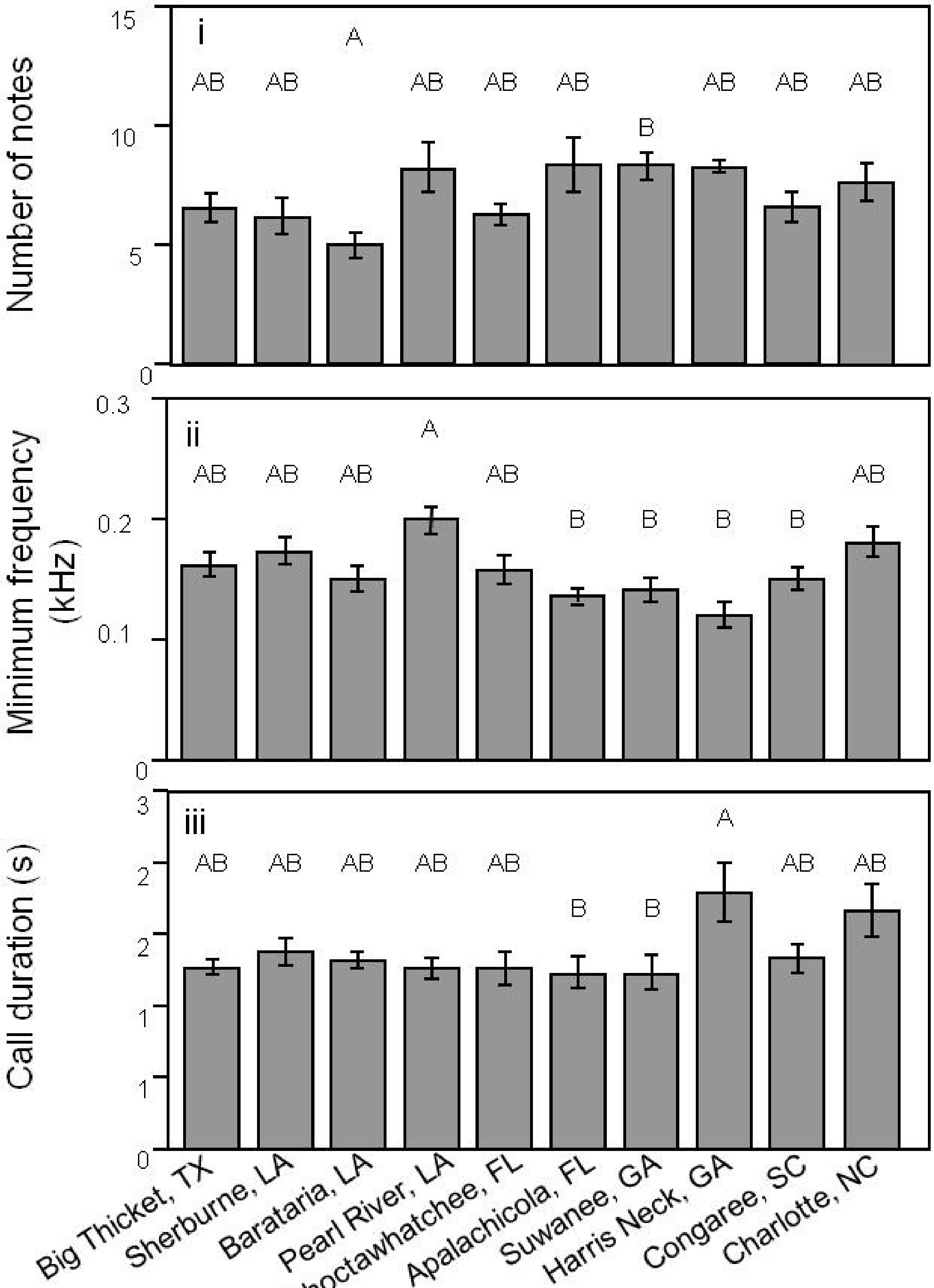

Figure 4. Comparisons of Barred Owl calls between ten locations based on call occurrence of (i) two-phrased hoots and (ii) three-note calls, and transition frequencies between (iii) ascending hoots to ascending hoots and (iv) one-phrased hoots to gurgles.

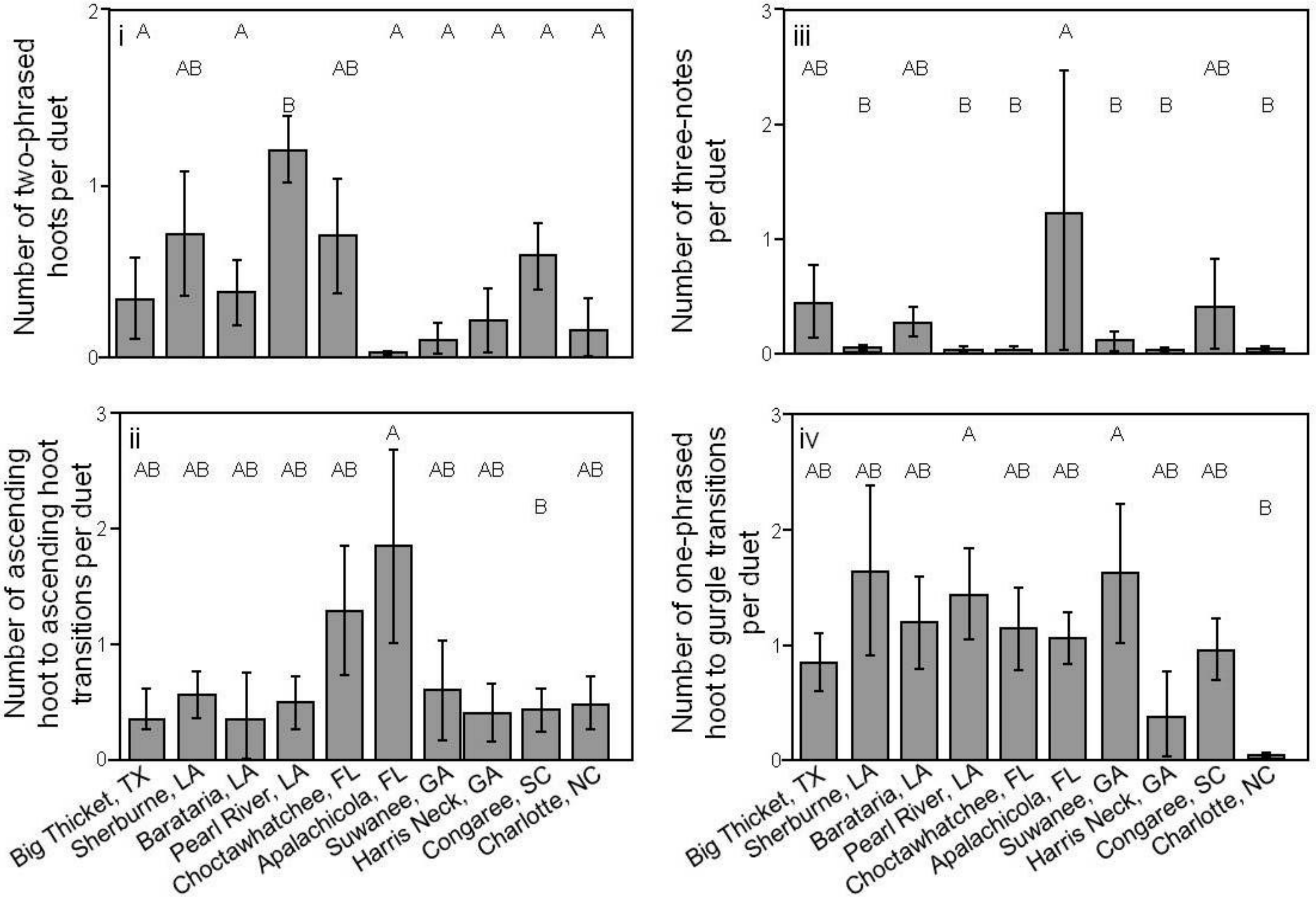

Figure 5. Spectrographs of Barred Owl female-male-female gurgle to one-phrased hoot duet sections from the Choctawhatchee River, FL (i-iii), Charlotte, NC (iv and vii), Suwannee River, GA (v and viii), and Big Thicket Preserve, TX (vi and ix). Time delays within duets (indicated by underlines: female = black; male = grey) at a single location can be similar (i versus ii) or different (i versus iii) and time delays between locations can be similar (i versus iv, or v versus vi) or different (iv, v, and vi versus vii, viii, and ix).

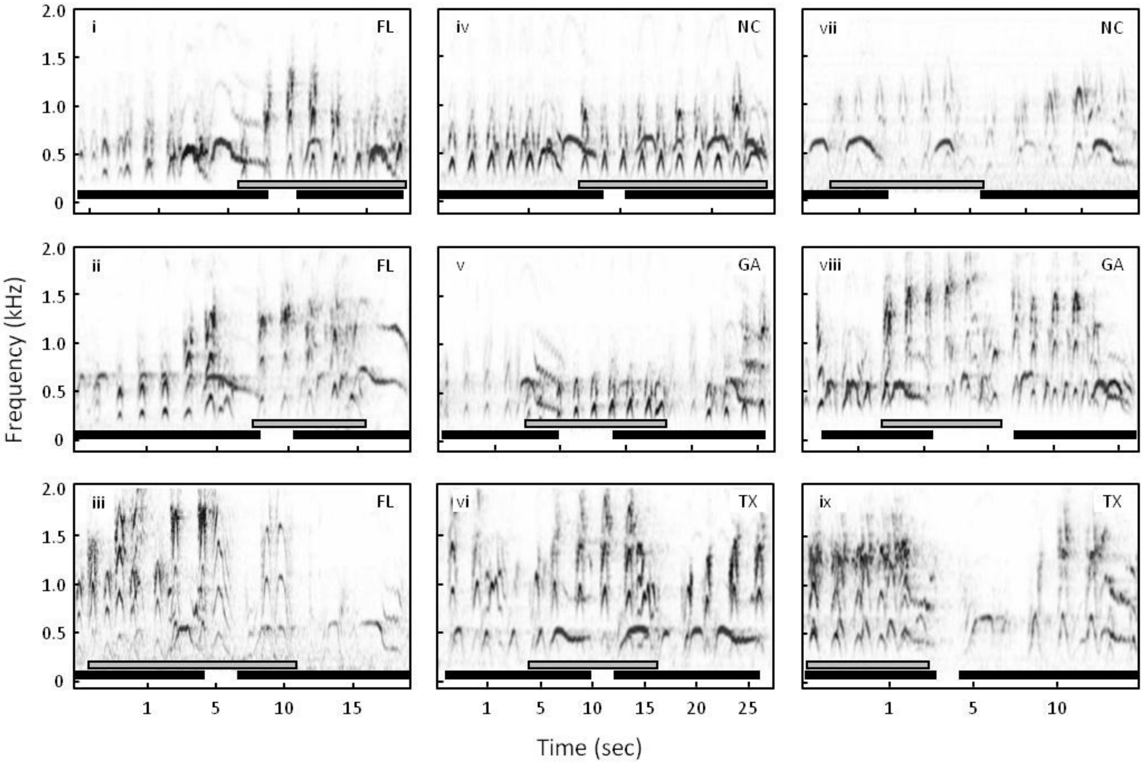

Table 1.

Vocal category	vocalization type or behaviour	No. variables	Variables
Call Structure	Male call (gurgle)	5	Number of notes, call duration (s), maximum frequency (F_{max}; kHz), minimum frequency (F_{min}; kHz), duration of the final note (s)
Call Structure	Female call (one-phrased hoot)	5	Number of notes, call duration (s), maximum frequency (F_{max}; kHz), minimum frequency (F_{min}; kHz), duration of the final note (s)
Duet structure	Duet section (female call + male gurgle + female one-phrased hoot)	6	Number of calls, duration of entire duet (s), start of the first female call to the start of the male gurgle (s), start of the male gurgle to start of the female one-phrased hoot (s), end of the male gurgle to end of the female one-phrased hoot (s), Male F_{max} - Female F_{max} (kHZ)
Duet behaviour	Call occurrence	11	Number of: two-phrase hoots, one-phrase hoots, ascending hoots, short ascending hoots, fast ascents, gurgles, mumbles, two-notes, three-notes, other
Duet behaviour	Transition frequencies*	11	Number of: ascending hoots to ascending hoots, ascending hoots to gurgles, gurgles to ascending hoots, gurgles to gurgles, gurgles to one-phrased hoots, gurgles to short ascending hoots, one-phrased hoots to gurgles, one-phrased hoots to one-phrased hoots, one-phrased hoots to short ascending hoots, short ascending hoots to gurgles, short ascending hoots to one-phrased hoots

Table 1. Summary of variables measured to assess vocal geographic variation in call structure, duet structure, and duetting behaviour of Barred Owls.
*Eleven most common transition frequencies used in multivariate comparisons, but all call transition combinations used to create dissimilarity matrix.

Thesis Summary

Vocalizations and vocal behaviour of oscine songbirds have been well studied. Many songbirds possess complex vocalizations, large repertoires, and exhibit distinctive patterns of geographic variation, all considered indicators of vocal learning. Vocalizations and vocal behaviour of suboscine songbirds and non-songbirds are less well studied, but these birds can also exhibit complex vocalizations with similar functions. Barred Owls (*Strix varia*) are non-songbirds that perform long, complex duets between males and females of mated pairs. I contribute to knowledge of birdsong by providing an account of the vocalizations, vocal behaviour, and vocal geographic variation in calls and duets of Barred Owls.

Chapter 2 revealed that adult Barred Owls have a large vocabulary of 13 different vocalizations. I examined sex-specific characteristics and found females performed higher-pitched calls with longer terminal notes and increased vibrato compared to males. I tested whether these vocal characteristics could be used to distinguish between owls of unknown sex and found sex could be predicted and correctly classified based on these vocal differences. I examined the diel variation in vocal activity of Barred Owls and found peaks in vocalizing between 02:00 to 05:00 and 18:00 to 20:00. Barred Owls also used different vocalizations in different diel patterns, indicating context-specific use of certain vocalizations. Additionally, Barred Owls used some vocalizations exclusively within duets, while other vocalizations were used both in and outside of duets or primarily outside of duets. This also indicated specific context of these vocalizations. This information will be useful for planning surveys and for continued research on the function of Barred Owl vocalizations and duetting behaviour.

In Chapter 3, I tested the use of duets in territory defense, neighbour-stranger discrimination, and communication networks in a playback study of familiar, neighbour and unfamiliar, stranger duets at territory boundaries. I recorded the response of focal owls as well as immediate neighbours and other nearby owls within a 3.5 km transect of automated recording devices. Focal owls responded strongly to playback of duets with increased duetting, they duetted for longer durations, and switched from contact calls to ascending hoots. Focal owls did not respond significantly more strongly to stranger than neighbour playback, although the trends were in the predicted direction and the effect was similar to other published neighbour-stranger studies. Immediate neighbour and other nearby owls did not show a significantly increased response to the playback stimuli that were broadcast outside their territory boundaries. Trends were also in the predicted direction, but effect sizes were not comparable to published studies, indicating a much larger sample size was needed to detect difference if they exist. I conclude Barred Owls primarily use duets to confront territory intruders for territory defense. I do not conclude that neighbour-stranger discrimination is absent or that communication networks do not exist in Barred Owls, but I suggest that additional research is needed with alternative stimuli, larger sample sizes, and additional response variables.

In Chapter 4, I show that Barred Owl calls, duets, and duetting behaviour do vary between locations, but not in a consistent pattern. I recorded Barred Owls in a 1,350 km transect across five southeastern United States and measured components of male calls, female calls, duet structure, call occurrence, and transition frequencies. I examined three ways these components could vary geographically: (1) location-specific variation where vocal characteristics can be assigned to location, (2) variation by distance where vocal characteristics become more different with increasing geographic distance, and (3) sharing of characteristics between multiple locations with changes in prominent vocal characteristics between other populations. In songbird dialects, the third pattern is often observed with abrupt vocal changes taking place between two adjacent groups of populations. Barred Owl calls, duets, and duetting behaviour could not be assigned to location and did not vary according to geographic distance. I observed differences between locations in male calls, female calls, call occurrence, and transition frequencies. However, the locations exhibiting variation were not consistent across the variables I measured. I conclude such variation is random and likely results from substantial individual variation, or genetic and environmental variation. I suggest random variation is one form of vocal geographic variation that should be expected in non-song-learning species. Barred Owls do not exhibit a consistent pattern in geographic variation in calls, duets, or duetting behaviour, which has implications for the presumably innate nature of calls as well as duets and duetting behaviour.

Most suboscine songbirds and non-songbirds examined for complex vocalizations, function, and vocal geographic variation show similar vocal complexity and function to oscine songbirds, but lack distinct patterns of geographic variation. This thesis reveals that Barred Owls possess an extensive vocabulary of calls that they use to different extents at different times. They use certain calls exclusively in the context of duets, indicating duets themselves have a unique function. Like many oscine songbirds, Barred Owls use their duets in territory defense. Unlike many oscine and suboscine songbirds and some owl species, Barred Owls do not show an obvious ability to distinguish neighbours from strangers, nor do they appear to exhibit extended communication networks. Lastly, Barred Owls do not show distinct dialects in their calls, duets, or duet behaviour, as seen in many songbirds. This suggests that while Barred Owls may have a diverse vocabulary that can be used to communicate a variety of motives, communication in Barred Owls appears rudimentary compared to many oscine songbirds.

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Association of Field Ornithologists Student Travel Award, Spring 2009

American Ornithologists' Union Student Travel Award, Spring 2008

American Ornithologists' Union Research Award, Spring 2008

University of Windsor International Graduate Excellence Scholarship, Summer 2007

University of Windsor International Graduate Student Scholarship, Spring 2007

University of Windsor President's Excellence Scholarship, Spring 2007

Ohio Wesleyan University Zoology Department awards for excellence in research and greatest contribution to the department, Spring 2006

Inductee into Phi Beta Kappa, Spring 2006

Alexander E. Bergstrom Grant, Association of Field Ornithologists, Spring 2005

Travel award from the Wilson Ornithological Society, Spring 2005

Three-year honorary student membership to the American Ornithologists' Union, Winter 2005

Scholarship to attend 8 weeks of summer coursework at the Rocky Mountain Biological Laboratory, Summer 2004

Ohio Wesleyan University Four-year Trustee Scholarship, Spring 2002