The perception, structure, and function of female song in the black-capped chickadee (*Poecile atricapillus*)

by

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Abstract

The black-capped chickadee (*Poecile atricapillus*) is a non-migratory songbird found throughout North America who communicates primarily through its numerous vocalizations, each with various functions. The vocalizations of the black-capped chickadee can be divided into calls and songs. Calls serve functions crucial for survival (e.g., altering to predator presents; keeping track of flockmates) while songs are produced specifically for mate attraction and territorial defense. The black-capped chickadee has a single song called the *fee-bee* song, a short (~1s) two-note vocalization. While songbird songs are traditionally thought to be produced primarily by males, there is an increasing number of studies of female song in songbirds. The studies contained in this dissertation further support the argument that females produce song and that female song serves a function, through investigating female song in the black-capped chickadee. A recent study has shown that female black-capped chickadees do sing, and that female song is distinct from male song and suggests that female-produced song may be used for mate recognition (Hahn et al., 2013). In Chapter 2, I conducted operant conditioning go/no-go discrimination tasks to test black-capped chickadees' perception of female song. The results indicated that female and male chickadees can learn to discriminate among individual females via their songs and generalize responding to novel songs from the same individual females after training. In addition, both sexes can generalize their responding using only the *bee*-note portion of the female song, meaning that when given only a portion of the *fee-bee* song, subjects could still identity individual females. Next, I conducted discriminant function analyses and used artificial neural networks to examine which acoustic measures are important for classifying individual female songs (Chapter 3). Analyses showed that both notes of the *fee-bee* song likely play a role in classification and thus discrimination, and that song can also be classified by the season it was produced in (i.e., spring vs. fall). These findings are in line with the differences that are evident in male chickadee song by season and in that males can be identified as individuals by their *fee-bee* song. In Chapter 4, I used an operant conditioned go/no-go discrimination task to investigate the impact of anthropogenic noise on the ability of female and male chickadees to discriminate among individual female chickadee songs. Findings suggested that even low-level noise (40dB) performance decreased compared to performance in silence, and high-level noise was increasingly detrimental to discrimination. We learned that perception of *fee-bee* songs does change in the presence of anthropogenic noise such that birds take significantly longer to learn to discriminate between females, but birds were able to generalize responding after learning the discrimination. Overall, the results of the above studies reveal that the female *fee-bee* song contains cues that allows both sexes of chickadees to identify individual females. While chickadees can discriminate between females when listening to the *bee*-note portion of a female's song, bioacoustic analysis identified that both notes (i.e., *fee* and *bee*) are important in classifying female song. In addition, differences in acoustic features differ by season, further suggesting that female song may have a similar function to male song. Lastly, in the presence of anthropogenic noise, the ability to discriminate between individual females decreases significantly. With the urbanization of the natural world increasing over time, it is important to recognize how anthropogenic noise impacts communication in species that communicate acoustically, such as the black-capped chickadee. Specifically, focus should be directed to investigating female-produced song; although the function of this type of song remains under investigation, current research suggests that female song may hold importance to both sexes.

Preface

All procedures followed the Animal Care (CCAC) Guidelines and Policies and were approved by the Animal Care and Use Committee for Biosciences at the University of Alberta (AUP 108). This dissertation follows the format prescribed by the APA Style Manual and the University of Alberta's Department of Psychology.

A version of Chapter 2 of this thesis has been published as C. Montenegro, W. D. Service, E. N. Scully, S. K. Mischler, K. A. Campbell, & C. B. Sturdy, "Black-capped chickadees (*Poecile atricapillus*) can identify individual females by their *fee-bee* songs", The Auk, 137. I was responsible for concept formation, experimental design, stimuli preparation, data collection, data analysis, and manuscript composition. W. D. Service, E. N. Scully, and S. K. Mischler assisted with data collection and contributed manuscript edits. K. A. Campbell contributed stimuli and manuscript edits. C. B. Sturdy was the supervisory author and was involved in concept formation and manuscript revision.

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<u>Chapter 1 General Introduction</u>

Animal communication

Traditionally, theories surrounding communication have taken a human-centric approach in differentiating forms of animal communication from human speech (Hockett & Hockett, 1960). Even by name, humans possess language whereas animals only possess communication. Language is defined by Hockett's Design Features as having several properties including but not limited to arbitrariness (i.e., language is mostly made-up), displacement (i.e., communicating about future or past events), productivity (i.e., rules of production; syntax/grammar), and semanticity (i.e., arbitrary signals have meaning; Hockett & Hockett, 1960). However, several studies have demonstrated that animal communication does contain some of these properties (Cheney & Seyfarth, 1990; Ficken et al., 1978; Nowicki, 1989). Animal communication specifically refers to the process by which a signal is transmitted between the signaler who produced the signal and the receiver who perceives the signal. A signal is defined by a wide range of characteristics (behavioral, physiological, morphological) that convey information to other organisms (Otte, 1974). Therefore, the information conveyed by a signal can vary in terms of form, for example through visual, chemical, electrical, tactile and thermal, vibrational, and auditory signals (Hauser, 1996). While not the only form of signal that possesses this ability, auditory signals can include information about the external environment (Cheney & Seyfarth, 1990; Rauber & Manser, 2017; Templeton et al., 2005; Templeton & Green, 2007) as well as information regarding the signaler (Becker, 1982; Parejo & Avilés, 2007; Suzuki, 2012), providing evidence for the rich content provided within the domain of animal communication.

Concerning the external environment, signals can include context-specific information regarding potential predators. Vervet monkeys (*Chlorocebus pygerythrus*) produce a specific type of alarm call in response to varying predators, for example they produce a different call for leopards (*Panthera pardus*) vs. aerial predators (e.g., eagle, *Polemaetus bellicosus*) vs. snakes (e.g., python, *Python sebae*; Cheney & Seyfarth, 1990). Since these different calls are produced in response to specific predators, and since their acoustic structures seem arbitrary, vervet monkey communication can be described as having semanticity and arbitrariness. Meerkat (*Suricata suricatta*) sentinels will vary their vocalizations to warn individuals feeding about predators, which is reflected in the response behavior of other meerkats. Short note calls from the signaler result in decreased vigilance and increased foraging behavior in the receiver while long calls result in the opposite response (Rauber & Manser, 2017). In regard to songbirds, black-capped chickadees (*Poecile atricapillus*) produce different mobbing calls in response to different predators. The greater the number of terminal D-notes in the chickadee mobbing calls the higher-threat the predator (e.g., high threat, northern saw-whet owl, *Aegolius acadicus*, vs. low threat, great horned owl, *Bubo virginianus*; Templeton et al., 2005). In addition, the D-note modification can serve as a cue, inadvertently communicating threat to other species (e. g., red-breasted nuthatches, *Sitta canadensis*; Templeton & Greene, 2007), demonstrating a form of learnability for other species vocalizations.

In addition to containing information regarding external events, a signal can just as importantly convey information concerning the signaler. When living in an environment where visual signals would be lost (e.g., dense vegetation) animals often rely on acoustic communication which can include information regarding species, sex, quality, and/or individual identity (Becker, 1982; Parejo & Avilés, 2007; Otter et al., 1997; Suzuki, 2012). For example, many songbird species rely on acoustic signals in discriminating between heterospecifics and conspecifics as vegetation can interfere with visual cues (Becker, 1982). In terms of heterospecifics, studies have shown that signals containing species information are used to form mixed-groups (Suzuki, 2012). In addition, avian brood parasites may evaluate host nests based on signals indicating higher or lower individual quality (e.g., host, Eurasian magpie, *Pica pica* and parasite, great-spotted cuckoo, *Clamator glandarius*; Parejo & Avilés, 2007). Just as important is the information regarding the individual signaler to conspecifics (Carlson et al., 2020). The information communicated can ensure the correct response; information on sex can ensure an individual is responding to a possible mate, while information regarding individual identity and quality can ensure an individual is choosing a high-quality mate or avoiding a high-quality conspecific (Catchpole & Slater, 2007; Carlson et al., 2020).

Considering the importance of vocal signals, anthropogenic (human-produced) noise can interfere with auditory signals and thus impact information in relation to the signaler and the external environment. Anthropogenic noise (e.g., traffic noise, sonar systems, oil drilling and production) can mask acoustic signals which may disrupt the ability to separate a signal's content from the noise. As a result, some songbirds have adjusted their vocalizations in response to noise, specifically their songs (Otter et al., 1997). And songs have been shown to contain important information specific to sex, and individual identity (Hahn et al., 2013; Phillmore et al., 2002). Some songbirds will sing earlier in the day to avoid high traffic noise (Fuller et al., 2007; Proppe et al., 2011), alter the frequency or amplitude of song (Brumm, 2004), or change frequency and duration of song as a consequence of human-produced noise (Gentry et al., 2017). With respect to external environments, when exposed to playbacks of tufted titmouse (*Baeolophus bicolor*) alarm calls in noisy areas, cardinals (*Cardinalis cardinalis*) are less likely to produce predator avoidance behaviors compared to behavior in quieter areas suggesting the ability to eavesdrop has decreased due to the presence of anthropogenic noise (Grade & Sieving, 2016).

Acoustic communication has proven a complex and interdisciplinary topic with most research focusing on the observation and description of vocalizations, the comparison of structure of vocalizations, the behavioral function of vocalizations, and the production and perception of vocalizations (Garcia & Favaro, 2017). One approach to scrutinizing questions on these topics includes using a model species, meaning studying a common and accessible animal species. Thus, results can be generalized to a wider array of species. For acoustic communication, a model species can be found within songbirds, specifically black-capped chickadees.

Songbirds

Nearly half of all 10,000 known bird species are songbirds, or oscines (Mischler et al., 2017; Sibley & Monroe, 1990). Oscines are vocal learners, meaning that these particular birds learn to produce their species-specific vocalizations, which are critical to survival, via a tutor (Ball & Hulse, 1998; Catchpole & Slater, 2008; Doupe & Kuhl, 1999). While there are other groups of animals who are vocal learners, the number is limited (i.e., humans, parrots, hummingbirds, cetaceans, bats, and elephants; Jarvis, 2006; Tyack, 2008). Songbirds are also characterized by their vocal organ, the syrinx, which allows songbirds to produce vocalizations (Catchpole & Slater, 2008; Larsen & Goller, 1999) and songbirds have specialized neural pathways for learning and producing their vocalizations (Catchpole & Slater, 2008; Mello et al., 2004). In addition, being a vocal learner allows comparison between humans and songbirds as the processes and brain areas involved in communication parallel language learning, which again is human-specific (Bolhuis et al., 2010; Doupe & Kuhl, 1999).

Based on their form and function, songbird vocalizations are divided into two categories, calls and songs (Catchpole & Slater, 2008; Mischler et al., 2017). Calls serve functions crucial for survival (e.g., flock cohesion, warning of predators, and locating individuals) and are produced year-round. Songs are produced specifically for mate attraction and territorial defense often with increased production in the spring during mating seasons. By convention, song is a sexually selected signal produced primarily by males and driven through selection pressures of intrasexual competition and intersexual mate choice, thus, song function can vary based on the sex of the receiver, female vs. male. Females may assess a male's song in terms of potential for a high-quality mate and males may assess a male's song in terms of the likelihood of attack and quality. Therefore, we should consider that song characteristics can serve both functions simultaneously (e.g., chaffinch, *Fringilla coelebs*; Leitáo & Riebel, 2003; Riebel & Slater, 1998) or independently (green reed warbler, *Acrocephalus arundinaceus*, Catchpole, 1983; dusky warbler, *Phylloscopus fuscatus*, Forstmeier & Balsby, 2002).

As a result of the historical male-only song convention, male song has been widely studied despite the reality that females also produce song (Langmore, 1998; Odom et al., 2004; Riebel, 2003). It has long been known that in tropical species female song exists as a function of duetting with male mates (Langmore 1998; Slater & Mann, 2004), however, an increasing number of studies support that in many temperate songbird species, females also sing, thus creating a geographic bias for reporting and investigating which female songbirds sing (Garamszegi et al., 2007; Odom et al., 2014). The idea that both sexes sing is rational as song can contain information regarding the sex of the signaler as a possible way to quickly discern a potential mate from a rival (Hahn et al., 2013). Accordingly, there should be acoustic differences in song that allow a receiver to discriminate the sex, the quality, or the identity of the signaler. Prior research has shown differences between female and male song based on acoustic features (e.g., white-crowned sparrows, *Zonotrichia leucophrys*, Baptista et al., 1993; bellbirds, *Anthornis* *melanura*, Brunton & Li, 2006), and these types of differences also seem to allow individuals to discriminate between female vs. male songs of the same species such as in the European robin (*Erithacus rubecula*; Hoelzel, 1986) or the black-capped chickadee (Hahn et al., 2013).

Black-capped chickadees

Black-capped chickadees are a small non-migratory songbird commonly found throughout North America (Smith, 1991). Black-capped chickadees are also vocal learners, meaning that their song (Shackleton & Ratcliffe, 1993) and calls (Ficken et al., 1985; Hughes et al., 1998) are learned from adult conspecific tutors. The black-capped chickadee uses its *fee-bee* song for territorial defense and mate attraction. The song contains two notes, the first note (*fee*) is produced at a higher frequency compared to the second note (*bee*),and the two notes are produced at a consistent pitch ratio independent of the starting frequency of the song. Prior studies have shown some geographic variation in the structure of the *fee-bee* song (Kroodsma et al., 1999) Calls are used for every other function, including but not limited to, the *gargle* call used for aggressive interactions, the *tseet* call used as contact calls, and the *chick-a-dee* call which is used as a mobbing and alarm call to conveying species information (Ficken et al., 1978). Bearing in mind Hockett's design features, the black capped-chickadee’s namesake *chick-a-dee* call demonstrates productivity (i.e., rules of production; syntax/grammar; Hockett & Hockett, 1960). The *chick-a-dee* call consists of four-notes, ABCD, while these notes can be omitted or repeated when producing the call, the order of the notes will always remain the same, displaying syntax (Ficken et al., 1978).

Given the numerous vocalizations of the black-capped chickadee and research potential, this dissertation will focus on the structure, perception, and function of the *fee-bee* song of the black-capped chickadee. While both female and male chickadees are able to sing (Hahn et al., 2013), the majority of research has focused on male song and the current function of female song is unknown. Recent research surrounding female song in other songbirds has suggested that the function of female song is most likely similar to the function of male (Langmore, 1998; Odom et al., 2004; Riebel, 2003), meaning territorial defense and mate attraction. And in black-capped chickadees the proposed function of female song is mate recognition (Hahn et al., 2013). In order to further explore the function of female song first we must consider studies surrounding male song first.

In respect to function, an increase in male song production can be noted in the spring (Avey et al., 2008) when high-ranking males will begin singing earlier during the dawn chorus, will sing for longer durations, and sing at higher rates compared to low-ranking individuals (Otter et al., 1997). We can also observe the function of song in males through frequency matching where during a singing bout a male will either increase or decrease the frequency of their *fee-bee* song in order to match the frequency of a competing male's song (Hill & Lien, 1987; Horn et al., 1992; Mennill & Ratcliffe, 2004; Ratcliffe & Weisman, 1985). Prior research has shown that the amplitude ratio of the *fee-* and *bee*-notes is more consistent when produced by dominant males versus subordinate males (Hoeschele et al., 2010). Dominant males are also able to maintain a more consistent internal ratio between notes (*fee* and *bee*) compared to subordinate males (Christie et al., 2004). Additionally, studies suggest that females attend to differences in dominant and subordinate male song, observed by differences in response to playback of male song based on dominance rank (Mennill et al., 2003). Considering that the above studies have found potential cues for dominance, and thus quality, within male *fee-bee* songs, we can extend this idea to songs also containing information surrounding individual identity. And a past operant conditioning study has indeed found that male chickadees can discriminate between individual males based on their song (Phillmore et al., 2002).

Female song is not a new phenomena. However, traditionally female temperate songbirds are considered to not produce song (Garamszegi et al., 2007; Langmore, 1998; Riebel, 2003), and this idea extends to black-capped chickadees. Despite field reports of female chickadees producing *fee-bee* songs (Dwight, 1897; Hill & Lein 1987), we have limited studies on female song. Recent research has shown that there are differences in acoustic structure between female and male *fee-bee* songs. One such difference is that female songs have a more pronounced *fee* glissando or the decrease in frequency in the *fee*-note of the song (Hahn et al., 2013). In addition, female and male chickadees can discriminate between male and female song, further suggesting that the sex of the bird producing the song signal matters (Hahn et al., 2015; See Figure 1-1 for spectrograms of female and male song). Artificial neural network analyses of female and male songs identified the *fee* glissando as important for classifying song by sex (Hahn et al., 2015), corroborating the differences observed in the *fee* glissando between sexes. Researchers have also shown sex differences in the timing of *fee-bee* songs, with females being more variable in terms of inter-song intervals (1.5-8s) compared to males (2.5-5s; Kobrina et al., 2019). Lastly, a bioacoustic analysis of female and male songs has found that male songs show less variation in acoustic measures (frequency and duration) than female songs, and this difference is detected in the fall and spring. And for both sexes, songs produced in the spring have less variation compared to songs produced in the fall (Campbell et al., 2019). These results may suggest that male song consistency is more important than female song consistency if female song serves a similar function to male song, even so, for both sexes song appears to be more vital in the spring vs. fall. These results could suggest that female song does indeed have a function similar to male song, considering that spring is the chickadee mating season when song production would be high.

Overall, many questions remain unanswered in order to elucidate the function of female song, questions surrounding structure, production, and perception of female song. My dissertation research focuses on examining perception of female song use through behavioral tests (i.e., operant conditioning), the acoustic features that exist within the female black-capped chickadee *fee-bee* song (i.e., bioacoustic analysis, statistical classification, artificial neural networks), and assessing the impact of anthropogenic (human produced) noise on female song perception. Research has shown that chickadees will shift the pitch (Proppe et al., 2012) and frequency (LaZerte et al., 2016) at which they sing in the presence of anthropogenic noise. And as previously reviewed, anthropogenic noise has the potential to mask signals and jeopardize accurate discrimination of fine acoustic details in songs (Nemeth & Brumm, 2010; Nemeth et al., 2013), and differing levels of anthropogenic noise can negatively impact discrimination of conspecific *fee-bee* songs by black-capped chickadees (Mischler et al., unpublished). In order to increase our understanding of the function of female-produced black-capped chickadee song, we must explore the proposed functions of female song. If the function is territorial defense and mate attraction, then there are most likely cues relating to sex as well as quality and individual identify. By doing so, we also increase our understanding of animal communication and demonstrate that the signals passed between animals are complex.

Current research

The first aim in extending current research on female-produced black-capped chickadee song is to investigate the acoustic cues contained within the female *fee-bee* song. Previous research suggests that female song does differ from male song (Hahn et al., 2013), and females and males can discriminate between female- and male-produced songs (Hahn et al., 2015). These results indicate that chickadee song does contain cues relating to sex. And other research specific to black-capped chickadees has shown that male song in particular provides cues relating to status and quality (Christie et al., 2004; Hill & Lien, 1987; Hoeschele et al., 2010; Horn et al., 1992; Mennill et al., 2003; Otter et al., 1997; Ratcliffe & Weisman, 1985), and individual identity (Phillmore et al., 2002). Therefore, I questioned whether female song contains cues beyond the sex of the signaler, in terms of individual identity (Chapter 2).

Chapter 2 focuses on investigating chickadees' ability to discriminate between individual females. I conducted an operant conditioning go/no-go task in which female and male birds were trained to respond differentially to two groups of stimuli, (a) songs produced by three individual female chickadees vs. (b) the songs by three other individual females. Therefore, some birds were rewarded for responding to songs of three female birds and then punished for responding to songs of the other three female birds. Results indicated that female and male chickadees can discriminate between individual females based on their *fee-bee* songs and both sexes can generalize this discrimination to novel songs produced by the same previously rewarded females. In addition, we manipulated a novel set of songs by concatenating either a *fee*- or *bee*-note from a previously rewarded female with either a *fee*- or *bee*-note from a female bird not included in the study, thus creating complete novel songs. Data showed that chickadees were able to generalize responding to *bee*-notes of previously rewarded females but not *fee*-notes. Overall, these results suggest that both sexes may be attending to a specific cue or acoustic features within song which allows them to identify individual females, and that the specific cue may be within the latter half of the *fee-bee* song. Additionally, we now know that more than just sex (Hahn et al., 2013; Hahn et al., 2015) can be extrapolated from female song in chickadees.

In order to further investigate what acoustic features are important in identifying individual females, I then conducted bioacoustic analyses of female-produced *fee-bee* songs in the form of discriminant function analyses (DFA) and artificial neural networks (ANN; Chapter 3). Previous research has found that the *fee* glissando is an important acoustic feature in discriminating between female and male song (Hahn et al., 2013), while data from Chapter 2 suggested that the *bee*-note is important in discriminating between individual female songs. The DFA revealed that both notes of the song, as well as the internote interval, are important in classifying songs to the individual that produced it. A follow-up DFA was conducted to observe any differences in female song by season, as the female song stimuli were recorded in two different seasons (fall vs. spring). Results showed that *bee*-note features are important in classifying songs to season, as well as the *fee* glissando. In addition, ANNs were able to confirm both sets of results. Altogether, because the difference observed by season in female song is mirrored by the seasonal biological functions of male song (i.e., mate attraction, territory defense, solicitation of extrapair copulations; Avey et al., 2008; Ficken et al., 1978; Vélez et al., 2015), then perhaps we are observing a similar acoustic difference in females. With respect to the original DFA which classified female song by individuals, several acoustic features were identified, perhaps evidence of overall voice recognition as the mechanism underlying individual identification in many vocalizations of the black-capped chickadee.

Next, Chapter 4 moves in a different direction of research by examining the impact of anthropogenic noise on the ability of chickadees to identify females via their *fee-bee* songs. Here, I again used an operant conditioning go/no-go task and trained male and female black-capped chickadees to respond differentially to different groups of female chickadees using their song. Past research has shown that black-capped chickadees will shift the frequency at which they sing when in the presence of anthropogenic (human-produced) noise (Proppe et al., 2012), and that noise can mask acoustic signals and impede the transmission of specific cues and impact the function of signals (Grade & Sieving, 2016). Results suggested that the ability to discriminate between female chickadees via their *fee-bee* is indeed disrupted with increasing levels of anthropogenic noise, but, once birds learned the discrimination, they were able to generalize responding to novel female song stimuli in the presence of noise. These findings provide insight into how signals are perceived in the presence of anthropogenic noise and if female song has a function similar to male song or another function altogether, then the messages contained in this song are also potentially being overlooked.

Chapter 5 presents a general discussion to summarize the findings from each preceding chapter. In Chapter 5 I consider my findings in respect to the existing literature on the structure, perception, and how the perceptual mechanism of female song informs the function of female song in black-capped chickadees and songbirds, as well as discuss future directions for research based on current results.

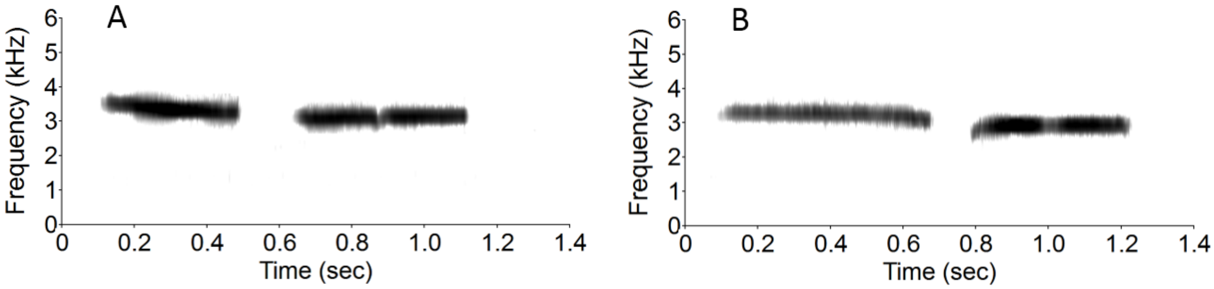

Figure 1-1. Spectrogram of the (A) female *fee-bee* song on the left, and spectrogram of the (B) male *fee*-*bee* song on the right.

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Chapter 2 Black-capped Chickadees (*Poecile atricapillus*) can identify individual females by their *fee-bee* songs

Introduction

In temperate bird species, song is considered a sexually selected signal used predominantly by males, and serving 2 main functions: (1) territorial defense and (2) mate attraction (Catchpole & Slater 2008). Most studies examining temperate songbird vocalizations have concentrated on males, leading to the misconception that females lack song (Langmore, 1998; Riebel, 2003). However, reports and studies of female song in temperate species are ever increasing, and in many species, it is now recognized that both males and females can and do produce song (Langmore, 1998; Odom & Benedict, 2018; Riebel, 2003; Riebel et al., 2019). In a review of 323 songbird species, it was reported that female song is present in 71% of species (Odom et al., 2004), including Yellow Warblers (*Dendroica petechia*; Hobson & Sealy, 1989), European Starlings (*Sturnus vulgaris*; Sandell & Smith, 1997), Red-winged Blackbirds *(Agelaius phoeniceus*; Yasukawa, 1989), and the focus of the current study, black-capped chickadees (*Poecile atricapillus*; Hahn et al., 2013). This is not to say that females of the remaining 29% of species do not sing, only that females have not been documented singing (Odom et al., 2014).

The black-capped chickadee *fee-bee* song is a simple 2-note vocalization that is used primarily by males for territorial defense and mate attraction (Ficken et al., 1978; Smith, 1991). There are several accounts of females singing songs that are acoustically similar to male *fee-bee* songs (i.e. songs are tonal and contain 2 notes) both in field (Dwight, 1897; Hill and Lein, 1987) and laboratory settings (Hahn et al., 2013). While observations and studies on female song in black-capped chickadees are very sparse, the song seems to serve a similar purpose. Early observations of breeding pairs indicate that while males produce the *fee-bee* song more often than females, both sexes produce the song to protect territories during the spring (Odum, 1942). The first note in the song (*fee*) is produced at a higher frequency compared to the second note (*bee*) and the frequency of the *fee*-note decreases over the duration of the note (referred to as the *fee* glissando; Hahn et al., 2013; See Figure 2-1). The *fee* glissando is less pronounced in males than in females. In male songs, the *fee* glissando is highly stereotyped (Christie et al., 2004b); however, the stereotypy of the female *fee* glissando has yet to be examined. Black-capped chickadees are able to identify the sex of an individual using the *fee* glissando within their *fee-bee* song (Hahn et al., 2015). In addition to being able to determine the sex of an individual via song, the ability to identify individuals via song is also beneficial in distinguishing between friend or foe, whether conspecific, heterospecific, or predator. In several species, discriminating between individuals via acoustic signals has been shown to facilitate identification of a familiar conspecific (e.g., Song Sparrow, *Melospiza melodia*, Stoddard et al., 1990) or a mate, (e.g., Zebra Finch, *Taeniopygia guttata*, Miller, 1979). While studies on individual identification on chickadee species outside of the black-capped chickadee are limited, a recent study has suggested that the *fee-bee* song in the black-capped chickadee is used for mate recognition (Hahn et al., 2013) and in order to be used for mate recognition the *fee-bee* song would need to contain information concerning individual identity.

Previous studies have indicated that male black-capped chickadee song contains information regarding individual identity (Christie et al., 2004b; Hahn et al., 2015; Hoeschele et al., 2010; Phillmore et al., 2002; Wilson & Mennill, 2010). A study examining *fee-bee* songs produced by black-capped chickadees in eastern Ontario suggested that the total duration of song is used by conspecifics to identify individual males and may encode male quality (Christie et al., 2004b). In addition, black-capped chickadee males and females eavesdrop on male singing contests, suggesting that both males and females attend to song bouts and use song to identify successful and unsuccessful conspecifics and their quality (Christie et al., 2004b; Mennill et al., 2002; Mennill & Ratcliffe, 2004). Past operant go/no-go discrimination tasks (Phillmore et al., 2002) and playback studies (Wilson & Mennill, 2010) have indicated that male black-capped chickadees can discriminate between individual males via *fee-bee* songs. Recently researchers have proposed that one of the functions of female song includes advertising individual quality (Langmore, 1998; Odom & Benedict, 2018; Riebel et al., 2019). The ability to differentiate between individual females could aid in assessing quality and identifying rank.

In the current study, we use an operant go/no-go paradigm to determine (1) if male and female black-capped chickadees can discriminate between individual female black-capped chickadee *fee-bee* songs, and (2) which song component(s) enable this discrimination. We trained black-capped chickadees in an operant discrimination task. We were interested in examining whether chickadees could identify individual female chickadees based on song and whether they use acoustic features in one or both notes within the song when discriminating between individuals. The results add to a growing body of literature on female song in temperate songbird species and provide valuable insights into sex-based discrimination of songs in this species and the function of the *fee-bee* song in females.

Methods

Subjects

Eighteen black-capped chickadees (9 males and 9 females) were tested between February and June, 2019. In total, 16 black-capped chickadees (8 males and 8 females) completed the experiment. One female failed to learn non-discrimination training (see description below) and was removed from the experiment, and one male died of natural causes (see Ethical Note). Sex was determined by deoxyribonucleic acid analysis of blood samples (Griffiths et al., 1998). Birds were captured in Edmonton (North Saskatchewan River Valley, 53.53°N, 113.53°W; Mill Creek

Ravine, 53.52°N, 113.47°W), Alberta, Canada, in January 2018. All birds were at least one year of age at capture, verified by examining outer tail rectrices (Pyle, 1997).

Prior to the experiment, birds were individually housed in parakeet cages (30 × 40 × 40 cm; Rolf C. Hagen, Montreal, Quebec, Canada) in colony rooms. Birds had visual and auditory, but not physical, contact with each other. Birds had ad libitum access to food (Mazuri Small Bird Maintenance Diet; Mazuri, St. Louis, Missouri, USA), water with vitamins supplemented on alternating days (Prime Vitamin Supplement; Rolf C. Hagen), grit, and a cuttlebone. Additional nutritional supplements included 3–5 sunflower seeds daily, one superworm (*Zophabas morio*) 3 times a week, and a mixture of hard-boiled eggs and greens (spinach or parsley) twice a week. The colony rooms were maintained at ~20°C and on a light:dark cycle that followed the natural light cycle for Edmonton, Alberta, Canada.

All birds had previous experience with at least one operant experiment involving chick-a-dee calls (7 birds with 1 unpublished experiment and 9 birds with 2 unpublished experiments) but none of the birds had previous experimental experience with black-capped chickadee–produced *fee-bee* songs in any experimental paradigm.

Apparatus

During the experiment, birds were housed individually in modified colony room cages (30 × 40 × 40 cm) placed inside a ventilated, sound-attenuating operant chamber. The chambers were lit with a full spectrum LED bulb (3W, 250 lm E26, Not-Dim, 5000 K; Lohas LED, Chicago, Illinois, USA) and the natural light:dark cycle for Edmonton, Alberta, was maintained throughout the experiment. Each cage contained 2 perches in addition to a perch fitted with an infrared sensor (i.e. request perch), a water bottle, grit cup, and cuttlebone. Birds had ad libitum access to water (with vitamins supplemented on alternating days), grit, and cuttlebone and were provided 2 superworms daily (one in the morning and one in the afternoon). An opening (11 × 16 cm) on the left side of the cage allowed the birds to access a motorized *fee*der, which was also equipped with an infrared sensor (Njegovan et al., 1994). Food was only available as a reward for correct responses to auditory stimuli during the operant discrimination task. A personal computer connected to a single-board computer (Palya & Walter, 1993) scheduled trials and recorded responses to stimuli. Stimuli were played from a personal computer hard drive through a Cambridge Integrated Amplifier (model A300 or Azur 640A; Cambridge Audio, London, England). Stimuli played in the chamber through a Fostex full-range speaker (model FE108 Σ or FE108E Σ; Fostex, Japan; frequency response range: 80–18,000 Hz) located beside the *fee*der. See Sturdy and Weisman (2006) for a detailed description of the apparatus; See Figure 2-2 for illustration of the operant conditioning chamber.

Recordings of Acoustic Stimuli

Six female black-capped chickadees' *fee-bee* song recordings were used for the current study; four birds were recorded in spring 2012 and two birds were recorded in fall 2014 (recording were collected from two separate years in order to increase total vocalizations used in the current study and decrease subjects' opportunity to memorize stimuli). Birds were captured in Edmonton (North Saskatchewan River Valley, 53.53°N, 113.53°W; Mill Creek Ravine, 53.52°N, 113.47°W), Alberta, Canada, in January 2010, 2011, 2012, and 2014. All birds were at least one year of age at capture, verified by examining outer tail rectrices (Pyle, 1997). A recording session for an individual bird lasted ~1 hr and all recordings took place at 0815 hours after colony lights turned on at 0800 hours. Birds were recorded individually in their colony room cages, which were placed in sound-attenuating chambers (1.7m × 0.84 m × 0.58 m; Industrial Acoustics, Bronx, New York, USA). Recordings were made using an AKG C 1000S

(AKG Acoustics, Vienna, Austria) microphone connected to a Marantz PMD670 (Marantz America, Mahwah, New Jersey, USA) digital recorder (16-bit, 44,100 Hz sampling rate). The microphone was positioned 0.1 m above and slightly behind the cage. Following a recording session, audio files were analyzed and cut into individual files using SIGNAL 5.03.11 software (Engineering Design, Berkley, California, USA).

Acoustic Stimuli

A total of 156 vocalizations were used as stimuli in the current experiment composed of 26 *fee-bee* songs produced by each of 6 female black-capped chickadees. Of the 26 songs produced by each bird, 24 songs were used without modification and 2 songs were edited to create spliced songs using SIGNAL. Spliced songs contained either a *fee*- note or a *bee*-note from the original song and, respectively, either a *bee*-note or a *fee*-note from another bird not included in the study. Songs were spliced in order to better determine what portion of the song is used to individual identification, given just the *fee*-note or *bee*-note from previously rewarded females will the subjects generalize responding? When creating the spliced songs, the internote interval was held constant at 100 ms, similar to the internote interval in natural songs (e.g., Xinternote = 135 ms; Ficken et al., 1978) and the internote interval used by other studies manipulating song features (e.g., Xinternote = 100 ms; Hahn et al., 2015; Hoeschele et al., 2010). Since songs are sung over a range of absolute frequencies (Horn et al., 1992; Mennill & Otter, 2007; Weisman et al., 1990), frequencies of replacement notes were manipulated using Audacity 2.2.2 software so that the start frequency of the *fee*-note used matched the start frequency of the *fee*-note that was being replaced in the song.

All vocalizations were of high quality (i.e. no audible interference) and were bandpass filtered (lower bandpass: 500 Hz, upper bandpass: 14,000 Hz) using GoldWave 6.31 (GoldWave, St. John's, Newfoundland, Canada) to reduce any background noise. For each stimulus, 5 ms of silence was added to the leading and trailing portion of the vocalization and tapered to remove transients (in order to reduce "popping" from transients during stimulus playback) and amplitude was equalized using SIGNAL 5.03.11 software. During the experiment, stimuli were presented at ~75 dB as measured by a Brüel and Kjær Type 2239 (Brüel and Kjær Sound and Vibration Measurement A/S, Nærum, Denmark) sound pressure meter (A-weighting, slow response).

Procedure

Non-discrimination training. Pretraining began once the bird learned to use the request perch and feeder to obtain food. During pretraining, birds were trained to respond to a tone (1,000 Hz) 1 s in length to receive access to food. Acclimatization to the chamber, feeder, and speaker occurred over an approximately 15-day period. The average time to complete non-discrimination training ranged from 10 days to 41 days (mean = 21.43, SD = 9). During non-discrimination training, birds received food for responding to all *fee-bee* song stimuli. Each trial began when a bird landed on the request perch and remained for 900–1,100 ms, at which point a randomly selected stimulus played. Songs were presented in random order from trial to trial until all 156 stimuli had been heard without replacement; once all 156 stimuli were used, a new random sequence was generated and initiated. If the bird left the request perch during a stimulus presentation, the trial was considered interrupted, resulting in a 30-s lights out period. If the bird entered the feeder within 1 s after the stimulus played, it was given 1 s access to food, followed by a 30-s intertrial interval. If a bird remained on the request perch during the stimulus presentation and the 1 s following the completion of the stimulus, it received a 60-s intertrial interval with the lights on, but this interval ended if the bird left the request perch. The above procedure engenders a high level of responding on all trials (Sturdy & Weisman, 2006). Birds continued on non-discrimination training until they completed six 468-trial blocks at ≥60% responding on average to all stimuli, at least four 468-trial blocks at ≤3% difference in responding to future rewarded vs. unrewarded discrimination stimuli, at least four 468-trial blocks at ≤3% difference in responding to future rewarded vs. unrewarded transfer stimuli, and at least four 468-trial blocks at ≤3% difference in responding to spliced stimulus types (*fee* replaced vs. *bee* replaced). Following a day of free feed, birds completed a second round of non-discrimination training in which they complete at least one 468-trial block that met each of the above requirements, in order to ensure the previous criteria were not completed by chance. A 468-trial block consisted of the bird experiencing each of the 156 stimuli 3 times. Non-discrimination training is necessary in order to expose the bird to all the stimuli that will be used in the experiment and to ensure the birds treat the stimuli equivalently (Sturdy & Weisman, 2006). See Figure 2-3 for flowchart of study stages.

Discrimination training. The discrimination training procedure was similar to previous training; however, of the original 156 stimuli, only 60 training stimuli were presented, and responses to these stimuli were differentially reinforced. In particular, responses to half of the stimuli (“rewarded stimuli”, S+) were positively reinforced with 1 s access to food, as before, and responses to the other half (“unrewarded stimuli”, S−) were instead punished with a 30-s intertrial interval with lights off (Sturdy & Weisman, 2006). Discrimination training continued until birds completed six 360-trial blocks with a discrimination ratio (DR) between S+ and S− of > 0.80 with the last 2 blocks being consecutive. For DR calculations see Response Measures below.

Subjects were randomly assigned to either a True category discrimination group (n = 10) or Pseudo category discrimination group (n = 6). Black-capped chickadees in the True category discrimination group were divided into 2 subgroups: (1) True 1 (n = 5) discriminated between 30 rewarded *fee-bee* songs produced by 3 individual chickadees (S+) and 30 unrewarded *fee-bee* songs produced by another 3 individual chickadees (S−); and (2) True 2 (n = 5) discriminated between the same songs with opposite rewards such that the 30 rewarded (S+) *fee-bee* songs were the S− from True 1 and the 30 unrewarded (S−) *fee-bee* songs were the S+ from True 1 (Sturdy & Weisman, 2006).

The Pseudo category discrimination group was also divided into 2 subgroups: (1) Pseudo 1 (n = 3) discriminated between 30 randomly selected rewarded (S+) *fee-bee* songs and 30 randomly selected unrewarded (S−) *fee-bee* songs; and (2) the second subgroup Pseudo 2 (n = 3) discriminated between the same songs with opposite reward structure such that the 30 rewarded (S+) *fee-bee* songs were the S− from Pseudo 1 and the 30 unrewarded (S−) *fee-bee* songs were the S+ from Pseudo 1 (S+) *fee-bee* songs and 30 randomly selected unrewarded (S−) *fee-bee* songs. The purpose of the Pseudo groups was to include a control in which subjects were not trained to categorize according to individual chickadee and would instead be required to memorize each vocalization independent of the producer.

Discrimination-85 training. This procedure was identical to discrimination training except that rewarded songs were reinforced with a reduced probability (i.e. P = 0.85). On 15% of trials when a rewarded stimulus was played and a bird entered the *fee*der, no access to food was granted. A 30-s intertrial interval ensued, during which the lights remained on. This was done to prepare birds for probe trials in which some stimuli were neither rewarded nor unrewarded. Discrimination-85 training continued until birds completed 2 consecutive 360-trial blocks with a DR of at least 0.80.

Probe I. During probe I the reinforcement contingencies from discrimination-85 training were maintained. In addition to the 60 stimuli from discrimination training, this stage included 12 novel *fee-bee* songs, 2 from each of the 6 individual females. For True groups, six of these novel songs were categorized as P+ and the other six as P−, based on whether they were produced by the same birds as the S+ or the S− training stimuli. For Pseudo groups, the novel songs were not assigned to categories or individual. For both groups, the 12 novel stimuli were neither rewarded nor unrewarded. The birds completed six 72-trial blocks in which the 60 familiar discrimination stimuli repeated once per block and the 12 probe sequences played once per block.

Transfer trainings. The transfer training procedures were generally the same as the discrimination trainings except as noted. Stimuli used were replaced by 60 new songs (recorded from the same 6 females). Responses to half of these stimuli (S−) were unrewarded with a 30-s intertrial interval with lights off. Transfer training continued until birds completed six 360-trial blocks with a discrimination ratio (DR) ≥ 0.80 with the last 2 blocks being consecutive. Subjects in the True 1 and True 2 groups discriminated between 30 new rewarded (S+) *fee-bee* songs produced by the same 3 individual chickadees heard in discrimination training (S+) and 30 new unrewarded *fee-bee* songs produced by the same 3 individual chickadees heard in discrimination training (S−). Subjects in the Pseudo 1 and Pseudo 2 groups discriminated between 30 new randomly selected, rewarded (S+) *fee-bee* songs and 30 new randomly selected unrewarded (S−) *fee-bee* songs. The transfer-85 training procedure was identical to discrimination-85 training but used songs from the first transfer training.

Probe II. During probe II the reinforcement contingencies from transfer-85 training were maintained. In addition to the 60 stimuli from transfer training, this stage included 12 novel *fee-bee* songs, 2 from each of the 6 individual females. As in probe I, the 12 novel stimuli were neither rewarded nor unrewarded, in order to assess how the birds responded to novel stimuli. P+ and P− were assigned in a similar manner to probe I. The birds completed six 72-trial blocks in which the 60 familiar transfer stimuli repeated once per block and the 12 probe sequences played once per block.

Probe III. Prior to probe III, subjects were given an additional stage of transfer-85 training in case their response rates had decreased during probe II. In probe III, reinforcement contingencies from transfer-85 training were maintained, and in addition to the 60 stimuli from transfer training, this stage included the 12 novel spliced *fee-bee* songs, including 2 songs derived from each individual chickadee: one using a *fee*-note from a previously rewarded female bird and a *bee*-note from another bird not included in the study, and one using a *fee*-note from another bird not included in the study and a *bee*-note from a previously rewarded female bird. Spliced songs were used in order to test which note is used in individual identification. For True groups, probe III stimuli were separated into 4 groups: (1) *fee*-note from a rewarded bird; 3 songs (Fee+); (2) *bee*-note from a rewarded bird; 3 songs (Bee+); (3) *fee*-note from an unrewarded bird; 3 songs (Fee−) and; (4) *bee*-note from an unrewarded bird; 3 songs (Bee−). The 12 novel stimuli were neither rewarded nor unrewarded. The birds completed six 72-trial blocks in which the 60 familiar transfer stimuli repeated once per block and the 12 probe sequences played once per block.

Response measures. For each 360-block trial during training, a percent response was calculated (R+/(N–I)): R+ is the number of trials in which the bird went to the feeder, N is the total number of trials, and I is the number of interrupted trials in which the bird left the perch before the entire stimulus played. For discrimination training, a DR was calculated by dividing the mean percent response to all S+ stimuli by the mean percent response to S+ stimuli plus the mean percent response to S− stimuli. A DR of 0.50 indicates equal response to rewarded (S+) and unrewarded (S−) stimuli; a DR of 1.00 indicates perfect discrimination between stimuli.

Statistical analyses. We conducted an analysis of variance (ANOVA) comparing the number of trials needed to reach criterion and the DRs between the True and Pseudo groups during discrimination training, an ANOVA comparing the number of trials needed to reach criterion and the DRs between True and Pseudo groups during transfer training, and an ANOVA comparing the number of trials needed to reach criterion and the DRs in True and Pseudo groups between discrimination training and transfer training. We also conducted a repeated measures ANOVA comparing responding to training stimuli and probe stimuli for probes I, II, and III. We also conducted post hoc tests to test for differences in the number of trials to reach criterion during discrimination training and responding to probe stimuli for probes I, II, and III. All statistical analyses were conducted using SPSS 20 (SPSS, Chicago, Illinois, USA).

Ethical note. Throughout the experiment, birds remained in the testing apparatus to minimize the transport and handling of each bird. One male subject died from natural causes during operant training. Following the experiment, birds were returned to the colony room for use in future experiments. All procedures were conducted in accordance with the Canadian Council on Animal Care (CCAC) Guidelines and Policies with approval from the Animal Care and Use Committee for Biosciences for the University of Alberta (AUP 1937), which is consistent with the Animal Care Committee Guidelines for the Use of Animals in Research. Birds were captured and research was conducted under an Environment Canada Canadian Wildlife Service Scientific permit (#13-AB-SC004), Alberta Fish and Wildlife Capture and Research permits (#56066 and #56065), and a City of Edmonton Parks permit.

Results

Discrimination and Transfer Training

In discrimination training, True group birds reached criterion (i.e. learned to discriminate) significantly faster than did Pseudo group birds based on DRs ($F_{1,14} = 12.022$, $p = 0.004$, partial $\eta^2 = 0.462$). There were no significant differences in trials to criterion by sex ($F_{1,8} = 0.870$, $p = 0.870$, partial $\eta^2 = 0.004$). Tukey's post hoc analysis showed no significant difference between True groups 1 and 2 ($p = 0.963$), and no significant difference between Pseudo 1 and Pseudo 2 ($p = 0.761$).

In transfer training, True group birds again learned to discriminate significantly faster than did Pseudo group birds based on DRs ($F_{1,14} = 15.981$, $p = 0.001$, partial $\eta^2 = 0.533$). Here too, there were no significant differences in trials to criterion by sex ($F_{1,8} = 0.621$, $p = 0.453$, partial $\eta^2 = 0.072$). Tukey's post hoc analysis showed no significant difference between True groups 1 and 2 ($p = 0.979$), and no significant difference between Pseudo 1 and Pseudo 2 ($p = 0.271$).

Comparing results from discrimination training and transfer training, True groups learned to discriminate transfer training stimuli to criterion in fewer blocks compared to discrimination training stimuli based on DRs ($F_{1,8} = 11.786$, $p = 0.009$, partial $\eta^2 = 0.596$). In contrast, Pseudo groups showed no difference in the rate of learning during discrimination training vs. transfer training ($F_{1,4} = 0.040$, $p = 0.851$, partial $\eta^2 = 0.010$). See Figure 2-4 for results of discrimination and transfer training.

Probe I

In probe I, percent response in True groups differed across the 4 stimulus types: rewarded discrimination stimuli, unrewarded discrimination stimuli, rewarded probe I stimuli, and unrewarded probe I stimuli ($F_{1,9} = 44.002$, $p < 0.001$, partial $\eta^2 = 0.830$). Tukey's post hoc analyses revealed a significant difference in responding between discrimination training S+ and S− ($p < 0.001$), with higher responding to S+ stimuli, and a significant difference in responding between probe I stimuli ($p < 0.001$), with higher responding to P+ stimuli, suggesting birds were able to generalize their responding (Figure 2-5). There were no significant differences within Pseudo groups by stimulus type ($F_{1,5} = 1.211$, $p = 0.340$, partial $\eta^2 = 0.195$). And no significant differences by sex across groups by stimulus type ($F_{3,36} = 0.602$, $p = 0.618$, partial $\eta^2 = 0.048$.

Probe II

In probe II, percent response in True groups differed across the 4 stimulus types: rewarded transfer stimuli, unrewarded transfer stimuli, rewarded probe II stimuli, and unrewarded probe II stimuli ($F_{1,9} = 63.487$, $p < 0.001$, partial $\eta^2 = 0.876$). Tukey's post hoc analyses showed a significant difference in responding between transfer training S+ and S− ($p < 0.001$), with higher responding to S+ stimuli, and a significant difference in responding between probe II stimuli ($p < 0.001$), with higher responding to P+ stimuli, suggesting birds were able to generalize their responding (Figure 2-6). There were no significant differences within Pseudo groups by stimulus type ($F_{1,5} = 0.027$, $p = 0.876$, partial $\eta^2 = 0.005$). And no significant differences by sex across groups by stimulus type ($F_{3,36} = 0.916$, $p = 0.443$, partial $\eta^2 = 0.071$.

Probe III: Spliced Songs

In probe III percent responding in the True groups differed across the 6 stimulus types: rewarded transfer stimuli, unrewarded transfer stimuli, and the Fee+, Bee+, Fee−, Bee− stimuli ($F_{1,9} = 47.878$, $p < 0.001$, partial $\eta^2 = 0.842$). There were also no significant differences by sex for True groups by stimulus type ($F_{3,24} = 0.009$, $p = 0.999$, partial $\eta^2 = 0.001$. Tukey's post hoc analyses showed a significant difference in responding between transfer training S+ and S− ($p < 0.001$), with higher responding to S+ stimuli. Analysis also showed a significant difference in responding between probe III stimuli ($p = 0.003$), with higher responding to Bee+ stimuli compared to Bee− stimuli, and no significant differences between Fee+ vs. Fee− stimuli ($p = 0.242$) or Fee+ vs. Bee+ ($p = 0.708$) (Figure 2-7). Results showed significant differences within Pseudo groups between stimulus types ($F_{1,5} = 17.531$, $p = 0.009$, partial $\eta^2 = 0.778$). However, Tukey's post hoc analyses showed no significant difference in responding between discrimination training S+ and S− ($p = 0.056$), and no significant differences in responding between all probe III stimuli ($p = 0.111$).

Discussion

Previous research has shown black-capped chickadees are capable of identifying individual chickadees by their *chick-a-dee* call (Charrier & Sturdy, 2005; Mammen & Nowicki, 1981) and possibly by *tseet* calls (Guillette et al., 2010). In addition, black-capped chickadees can identify individual males by their *fee-bee* songs (Christie et al., 2004a; Phillmore et al., 2002; Wilson & Mennill, 2010). Our study shows that female and male black-capped chickadees are able to discriminate between individual females based on *fee-bee* song. But the acoustic cues behind identification require further investigation.

Results showed that during discrimination training birds in the True groups (i.e. birds trained to respond to songs of particular individual females) were quicker to discriminate between rewarded female song and unrewarded female song when compared to Pseudo group birds (i.e. birds trained on random sets of female songs), suggesting True category discriminations were easier to learn vs. memorizing randomly selected rewarded songs. During transfer training, birds in the True groups were quicker to learn to respond correctly to rewarded female song stimuli compared to Pseudo group birds, again suggesting True category discriminations were easier to learn. Our data indicates that birds in the True groups used open-ended categorization while birds in the Pseudo groups used rote memorization in order to respond correctly in our tests (Sturdy et al., 2007). In addition, results from probe I and II trials showed that the categories learned by the True birds could be generalized to novel stimuli. Pseudo birds continued to show no difference in responding as they were not assigned stimuli to generalize to.

Probe III data showed that there was no significant difference between Fee+ and Bee+ stimuli in True groups, meaning birds did not respond differentially to either the *fee*-note or *bee*-note from their previously rewarded female. Birds showed no other differences in responding except for the Bee+ vs. the Bee− stimuli, with higher responding to the Bee+ stimuli (*bee*-notes from previously rewarded females). This suggests that the *bee*-note is sufficient for discriminating between individual females. However, research has shown that in males the *fee* glissando remains constant at different absolute pitches (Christie et al., 2004b) and is necessary for individual recognition (Shackleton et al., 1992). In females the *fee* glissando shows a greater change in frequency compared to males (Hahn et al., 2013) and may be used by chickadees to tell the difference between female and male conspecifics (Hahn et al., 2015) and perhaps for individual recognition. Past research has also shown that these two-note songs are rich in information about the producer including information such as individual identity (Christie et al., 2004a; Phillmore et al., 2002), sex (Hahn et al., 2013), rank (Christie et al., 2004b), and quality (Christie et al. 2004b; Otter & Ratcliffe, 1993). Further research is necessary to determine how chickadees are able to use the *fee-bee* song for individual identification.

We also observed no differences between sexes in speed of acquisition during discrimination training or transfer training. Recognition of individual females based on the *fee-bee* song might be equally important to both male and female chickadees. Individual recognition based on song is advantageous in that the listener can correctly identify an individual as a neighbor or an invading individual, especially when considering the typical uses of song in the current species, including mate attraction and territorial defense (Smith, 1991). While the purpose of song in female black-capped chickadees is less understood, studies have highlighted its potential use in aggressive female–female interaction, and future female–male mating interactions (Montenegro et al. personal observation). The current study showed no differences in responding between females and males, suggesting they are attending to the same features and both receive relevant information. The ability to quickly identify an individual female would be beneficial in saving time and energy if females are vocalizing to defend territories or to communicate with a mate or potential mate. However, testing in the wild is required.

Our results suggest that discrimination of individual females based on *fee-bee* song is easiest when both notes can be used but that the *bee*-note is sufficient for such discrimination (Figure 2-5). Because *fee-bee* songs are not the only vocalization used by black-capped chickadees to identify individuals, voice characteristics might be a mechanism that aids in identification. Perhaps all vocalizations from an individual share distinguishing features that allow for discrimination. If the categorization that we observed was due to voice recognition, such distinguishing features might be more prominent in *bee*-notes than in *fee*-notes as birds were able to generalize learning to *bee*-notes but not *fee*-notes. Given what we know about song in male and female Black-capped chickadees, future research should first explore both the *fee*- and *bee*-note portion of the *fee-bee* song. While past research has focused on the *fee*-note and shown that the *fee* glissando differs between males and females (Hahn et al., 2013), it appears that the *bee*-note is also important for identification. A more fine-grained comparison of acoustic differences in female song would also be worth exploring. Overall, further research with additional song manipulations is necessary to examine the acoustic mechanisms behind individual recognition via female-produced *fee-bee* songs as well as the function of female song.

Past studies have demonstrated that individual recognition is an ability seen in species other than the black-capped chickadee. For example, in birds, European starlings (*Sturnus vulgaris*) can discriminate between individuals using distinctive acoustic features of song and individual song repertoire (Gentner & Hulse, 1998), and great tits (*Parus major*) can extend this discrimination to novel vocalizations (Weary & Krebs, 1992). Black redstart (*Phoenicurus ochruros*) parents use vocal discrimination of fledglings to feed their offspring vs. another birds' offspring (Draganoiu et al., 2006). Similarly, male North American bullfrogs (*Rana catesbeiana*), whose vocalizations are significantly more variable between males compared to within males, use vocal discrimination to identify neighbors and strangers (Bee & Gerhardt, 2001). Giant otter (*Pteronura brasiliensis*) contact calls which are individually distinctive, are used to communicate and keep in contact with others when out of visual contact (Mumm et al., 2014). This ability is also observed in African elephants (*Loxodonta africana*), where females discriminate between the contact calls of elephants with familial bonds and members of other families with which they are associated (McComb et al., 2000) and can do so at a distance (2.5 km; McComb et al., 2003). Individual recognition has been seen in more species (See review by Carlson et al., 2020), and through the current study we have shown that female black-capped chickadee song can also be used for individual identification.

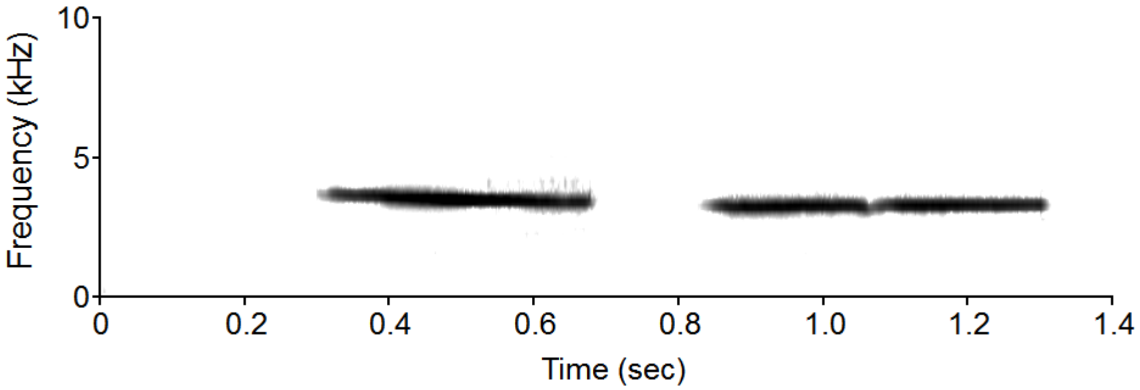

Figure 2-1. Spectrogram of the *fee-bee* song produced by the female black-capped chickadee.

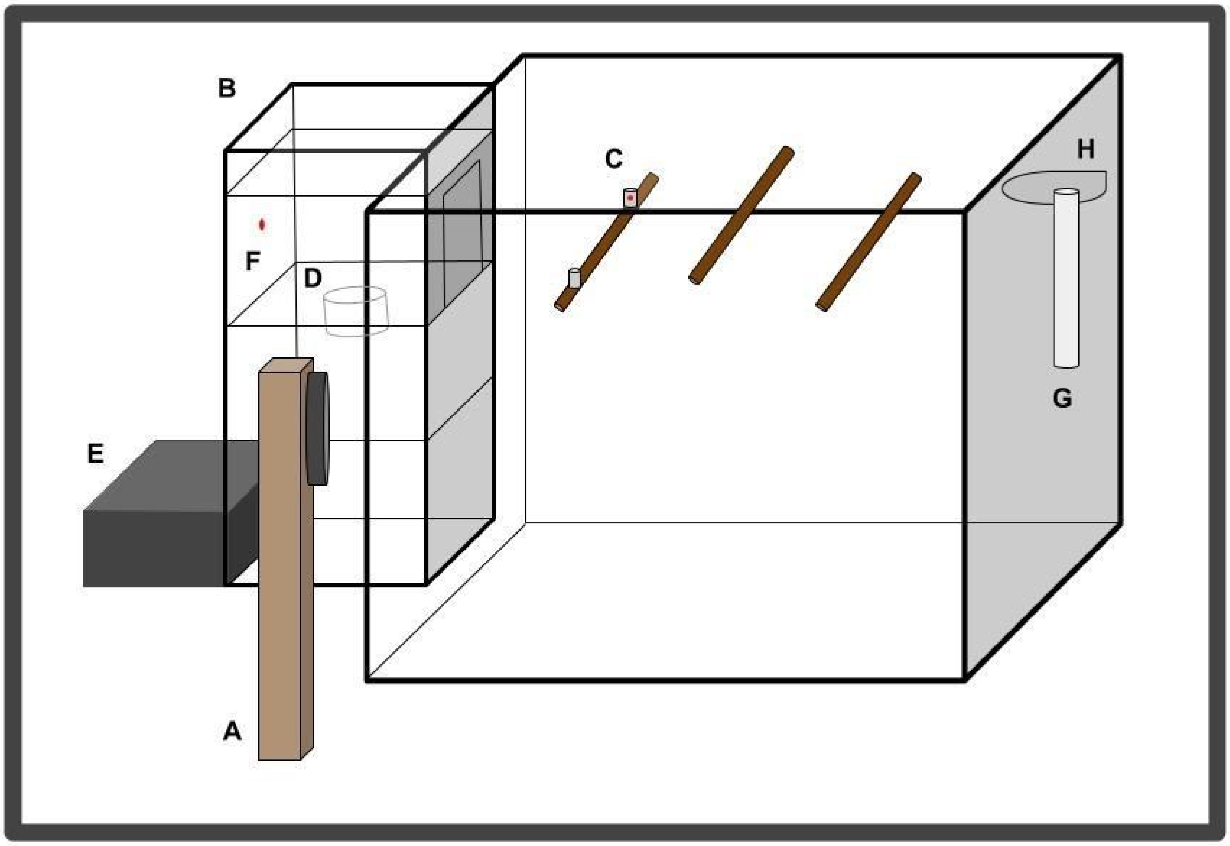

Figure 2-2. Illustration of the operant conditioning chamber, including: (A) speaker, (B) automated feeder, (C) request perch fitted with infrared photo-beam assembly, (D) feeder cup, (E) electrical inputs, (F) red LED, (G) water bottle, (H) and cuttlebone. Also shown is the feeder opening, and additional perches. To simplify the sketch the front and floor of the chamber, and the enclosure's acoustic lining are not included.

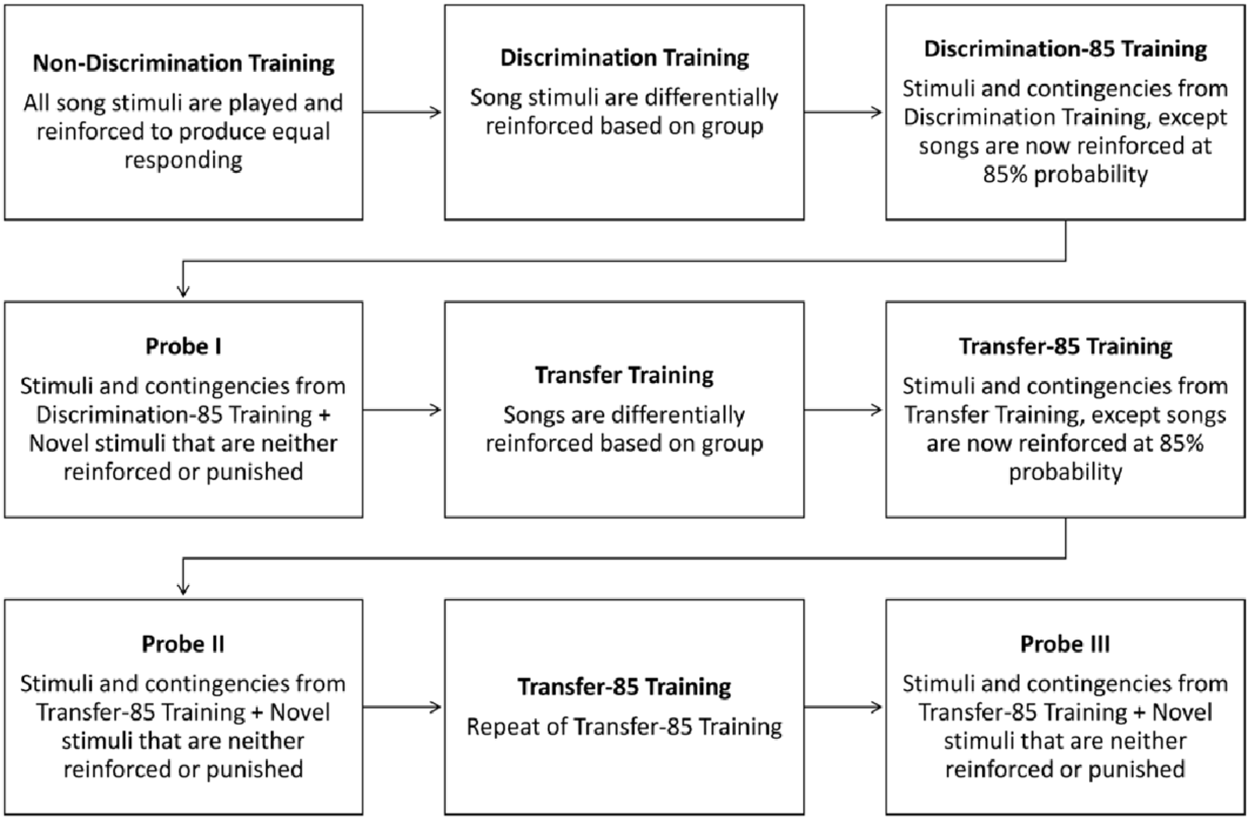

Figure 2-3. Flowchart depicting the stages of the experimental procedure including the name of each stage followed by a short description (see text for details). Non-Discrimination training includes all songs used in the study (156 stimuli). Following Non-Discrimination training, stimuli were divided into S+ (10 songs produced by three individual females each, 30 songs total) and S- (10 songs produced by three individual females each), 30 songs total. In addition, Probe stages introduced spliced stimuli divided into P+ (2 songs produced by three individual females each, six songs total) and P- (2 songs produced by three individual females each, six songs total).

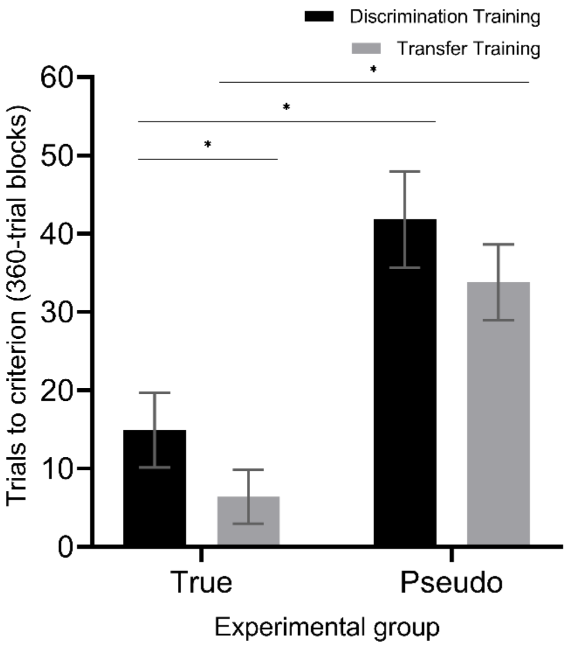

Figure 2-4. Trials to criterion by True groups and Pseudo groups in discrimination training and transfer training. The following differences were significant (indicated by asterisks): True groups vs. Pseudo groups in discrimination training (ANOVA, *p* = 0.004), True groups vs. Pseudo groups in transfer training (*p* = 0.001), and True groups in discrimination vs. transfer training (*p* = 0.009). Error bars represent standard error.

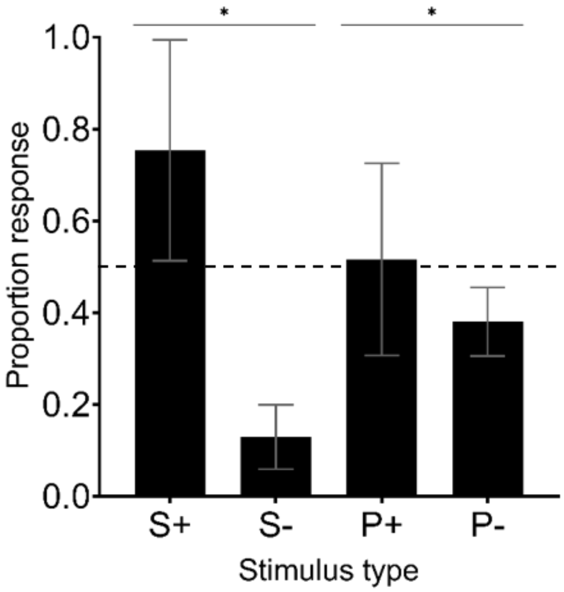

Figure 2-5. Percent response by True groups in probe I trials. The following differences were significant (indicated by asterisks): rewarded (S+) vs. unrewarded (S−) song stimuli (repeated measures ANOVA: $p < 0.001$), and rewarded (P+) vs. unrewarded (P−) probe stimuli ($p = 0.001$). Error bars represent standard error.

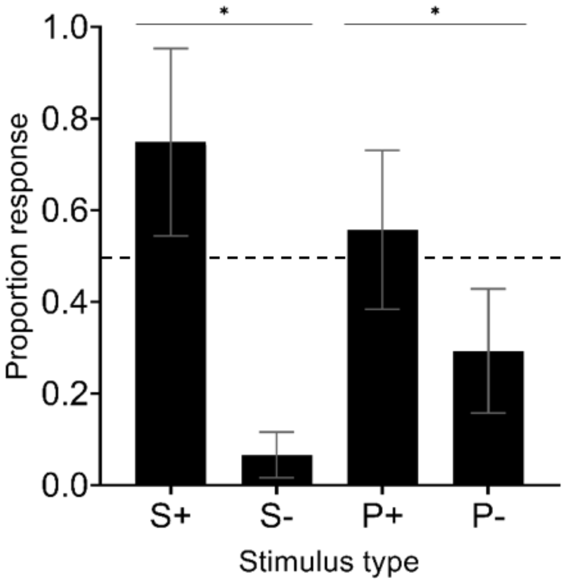

Figure 2-65 Percent response by True groups in probe II trials. The following differences were significant (indicated by asterisks): rewarded (S+) vs. unrewarded (S−) song stimuli (repeated measures ANOVA: $p < 0.001$), and rewarded (P+) vs. unrewarded (P−) probe stimuli ($p = 0.001$). Error bars represent standard error.

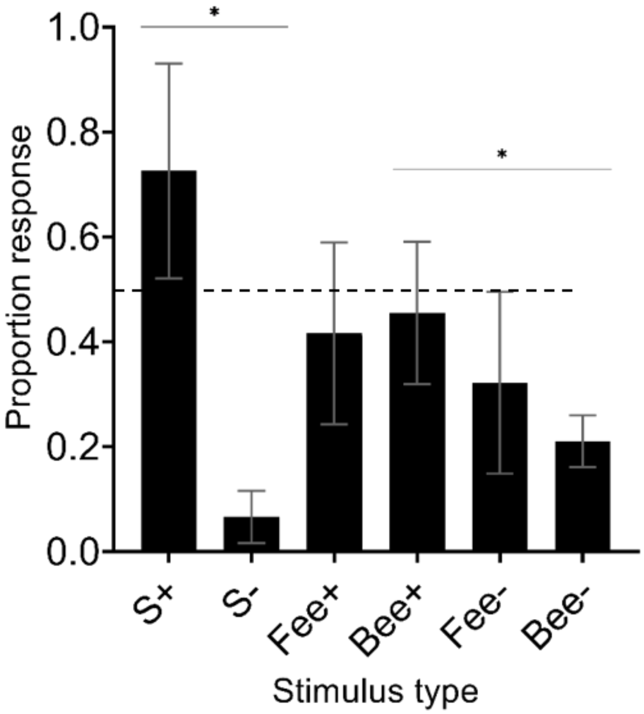

Figure 2-7. Percent response by True groups in probe III trials. The following differences were significant (indicated by asterisks): rewarded (S+) vs. unrewarded (S−) song stimuli (repeated measures ANOVA: $p < 0.001$), and rewarded (Bee+) vs. unrewarded (Bee−) probe stimuli ($p = 0.003$). Error bars represent standard error.

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Chapter 3 Individual acoustic differences in female black-capped chickadee (*Poecile atricapillus*) *fee-bee* songs

Introduction

Within oscines (the true songbirds), songs are traditionally considered a sexually selected signal, produced primarily by males, and serving two main functions, territorial defense and mate attraction (Catchpole & Slater, 2008). Prior studies examining songbird vocalizations suggest that females lack song (Langmore, 1998; Riebel, 2003). Nonetheless, there is an increasing number of studies of female song in songbirds, further supporting the argument that females do produce song and that their song serves a function (Langmore, 1998, Riebel, 2003). A review of songbird species (Odom et al., 2014), reported that female song is present in 71% of the reviewed 323 species and unknown in the remaining 29% songbird species. Recent studies have shown that female black-capped chickadees (*Poecile atricapillus*) also sing, however the function of female song in this species is currently unknown (Hahn et al., 2013; Montenegro et al., 2020).

Being able to determine the sex of an individual via song, and the ability to identify individuals via song, is advantageous in distinguishing among conspecifics to discriminate mate from non-mate, and among flockmates. In several species, discriminating between individuals via acoustic signals has been shown to facilitate identification of a familiar conspecific (e.g., Song Sparrow, *Melospiza melodia*, Stoddard et al., 1990) or a mate (e.g., great tits, *Parus major*, Lind et al., 1996). A recent study has suggested that the *fee-bee* song in the black-capped chickadee may be used for mate recognition (Hahn et al., 2013b), and in order to be used for mate recognition, the *fee-bee* song would need to contain information concerning individual identity. Previous studies have indicated that male black-capped chickadee song contains information that could be used for individual identity (Christie et al., 2004a; Hahn et al., 2015; Hoeschele et al., 2010; Phillmore et al., 2002; Wilson & Mennill 2010). A previous study examining *fee-bee* songs suggests that the total duration and the interval ratio is used to identify individual males (Christie et al., 2004a). In addition, males and female chickadees eavesdrop on male singing contests and use song to identify successful and unsuccessful conspecifics and their quality (Christie et al., 2004b; Mennill et al., 2002; Mennill & Ratcliffe, 2004).

The black-capped chickadee *fee-bee* song is a two-note vocalization that is primarily used for territorial defense and mate attraction and traditionally thought to only be used by males (Ficken et al., 1978; Smith 1991). However, there are several reports of females singing songs that are acoustically similar to male *fee-bee* songs (i.e. songs are tonal and contain two notes) in the laboratory (Hahn et al., 2013b) and field (Dwight, 1987; Hill & Lein, 1987). As in male black-capped chickadees, the first note in the female song (*fee*-note) is produced at a higher frequency than the second note (*bee*-note) and the frequency of the *fee*-note decreases over the duration of the note (referred to as the *fee* glissando; Hahn et al., 2013b; Weisman et al., 1990). A bioacoustic analysis of several acoustic features showed that the *fee* glissando is less pronounced in males than it is in females (Hahn et al., 2013b). A follow up operant go/no-go discrimination task suggested that black-capped chickadees are able to identify the sex of an individual using the *fee* glissando within their *fee-bee* song (Hahn et al., 2015). In addition, female song production is more variable acoustically, with inter-note intervals ranging from 1.5-8.0s, while male song is produced more regularly, with inter-note intervals running from 2.5-5.0s (Kobrina et al., 2019).

Prior operant go/no-go discrimination tasks (Phillmore et al., 2002) and playback studies (Wilson & Mennill, 2010) have also indicated that male black-capped chickadees can discriminate between individual males via their *fee-bee* songs. And a recent operant task showed that male and female chickadees can discriminate between females via their *fee-bee* songs

(Montenegro et al., 2020). A bioacoustic analysis of male *fee-bee* songs indicate that songs are more distinct and variable between individuals rather than within individuals, with song length, *fee*-note duration, and the *fee* glissando being the most variable features (Wilson & Mennill, 2010). Furthermore, during playback of the above analyzed song, wild chickadees remained within their testing area and sang significantly longer in response to *fee-bee* songs from different males compared songs from the same male, further suggesting the ability to discriminate between individuals based on song (Wilson & Mennill, 2010). To date, the particular acoustic differences between individual female *fee-bee* songs is unknown.

Here we measured 13 acoustic features in female black-capped chickadee *fee-bee* songs, including frequency and duration measurements, to investigate which acoustic features in song are used to identify individual females. We completed a bioacoustic analysis of 13 acoustic features using both discriminant function analyses and artificial neural networks to determine if the acoustic features measured could be used to identify the individual producing a specific song. Previous research has shown male and female black-capped chickadees can identify individual females via their *fee-bee* song even when presented with only the *bee* position of the song (Montenegro et al., 2020). Therefore, we predicted that the source of acoustic differences between female black-capped chickadee song would most likely be found in the *bee*-note portion of their *fee-bee* songs.

Methods

Subjects

We used *fee-bee* songs from six females (Female A-Female F) used in a previous study focused on individual identification of female chickadees (Montenegro et al., 2020). Sex was determined by deoxyribonucleic acid analysis of blood samples (Griffiths et al., 1998). Birds were captured in Edmonton (North Saskatchewan River Valley, 53.53°N, 113.53°W; Mill Creek Ravine, 53.52°N, 113.47°W), Alberta, Canada, in January 2010-2014. All birds were at least one year of age at capture, verified by examining outer tail rectrices (Pyle, 1997). All birds were individually housed in parakeet cages (30 × 40 × 40 cm; Rolf C. Hagen, Montreal, Quebec, Canada) in colony rooms. Birds had visual and auditory, but not physical, contact with each other. Birds had *ad libitum* access to food (Mazuri Small Bird Maintenance Diet; Mazuri, St. Louis, Missouri, USA), water with vitamins supplemented on alternating days (Prime Vitamin Supplement; Rolf C. Hagen), grit, and a cuttlebone. Additional nutritional supplements included 3–5 sunflower seeds daily, one superworm (*Zophabas morio*) 3 times a week, and a mixture of hard-boiled eggs and greens (spinach or parsley) twice a week. The colony rooms were maintained at ~20°C and on a light:dark cycle that followed the natural light cycle for Edmonton, Alberta, Canada.

Recordings of acoustic stimuli

Of the six birds, four were recorded in Spring 2012 and two birds were recorded in Fall 2014. A recording session for an individual bird lasted ~1 hr and all recordings took place at 0815 hours after colony lights turned on at 0800 hours. Birds were recorded individually in their colony room cages, which were placed in sound-attenuating chambers (1.7m × 0.84 m × 0.58 m; Industrial Acoustics, Bronx, New York, USA). Recordings were made using an AKG C 1000S (AKG Acoustics, Vienna, Austria) microphone connected to a Marantz PMD670 (Marantz America, Mahwah, New Jersey, USA) digital recorder (16-bit, 44,100 Hz sampling rate). The microphone was positioned 0.1 m above and slightly behind the cage. Following a recording session, audio files were analyzed and cut into individual files using SIGNAL 5.03.11 software (Engineering Design, Berkley, California, USA).

Acoustic measures

Each female provided 24 *fee-bee* songs, amounting to 144 *fee-bee* songs in total. Song composition was visually determined from spectrograms in SIGNAL (version 5.05.02, Engineering Design, Belmont, MA) by a single individual (CM) using Ficken et al. (1978) as a reference. All vocalizations were of high quality (i.e., no audible interference) and were bandpass filtered (lower bandpass: 500 Hz, upper bandpass: 14,000 Hz) using GoldWave 6.31 (GoldWave, St. John's, Newfoundland, Canada) to reduce any background noise. For each stimulus, 5 ms of silence was added to the leading and trailing portion of the vocalization to standardize duration. Individual songs were then saved as separate (.WAV) files.

For each song we measured 13 acoustic features examined previously in studies of identification in chickadee song (Christie et al., 2004a; Hahn et al., 2013a; Hahn et al., 2013b Hoeschele et al., 2010; Otter & Ratcliffe, 1993) and calls (Campbell et al., 2016; Guillette et al., 2010). Measurements included: (1) total duration of song, (2) *fee*-note duration, (3) the proportion of song duration occupied by the *fee*-note (*fee*-note duration divided by the total duration of the song) (4) *bee*-note duration, (5) the proportion of song duration occupied by the *bee*-note (*bee*-note duration divided by the total duration of the song), (6) *fee* glissando (decrease in frequency across the duration of the *fee*-note, calculated by dividing the start frequency of the *fee*-note by the end frequency of the *fee*-note), (7) *fee*-note start frequency (8) *fee*-note peak frequency, (9) *fee*-note end frequency, (10) *bee*-note start frequency, (11) *bee*-note peak frequency, (12) *bee*-note end frequency, (13) the internote interval between the notes (calculated by subtracting the *fee*- and *bee*-note duration from total song duration)

The above acoustic features were measured from sound spectrograms and power spectra using SIGNAL. Sound spectrograms of a *fee-bee* song were used for all duration (time resolution

5.8 ms) measurements and frequency (frequency resolution 172.3Hz) measurements. See Figure 1 for how the acoustic features were measured.

Statistical analysis

Discriminant function analysis (DFA) is commonly used in bioacoustic research to discriminate the vocalizations of groups or individuals based on specific acoustic features and can also suggest which features are used for identification (Mundry & Sommer, 2007). If the acoustic features previously measured in the *fee-bee* songs vary among individuals, then a DFA can use the features to accurately classify the songs to each individual (Tabachnick & Fidell, 2007). Thus, we used a stepwise DFA and the leave-one-out method of cross-validation where one case is withheld at a time and the discriminant function is derived from the remaining cases. Then using the discriminant function that was derived, the withheld case is then classified. These steps are repeated until all cases have been classified in this manner (Betz, 1987). We report the original and cross-validated percentage of correct classifications, the standardized coefficients, and eigenvalues for the discriminant functions derived from our analyses. Cross-validation can provide an estimate for how well the derived discriminant function can predict group membership with a new sample. The standardized coefficients express the relative importance of each variable to the discriminant score. A greater contribution is associated with a standardized coefficient with a larger magnitude. In addition, as the standardized coefficient's magnitude increases it represents a closer relationship between the variable and the discriminant function (Klecka, 1980). In addition, we report Cohen’s *Kappa*, this index was calculated in order to assess if the model’s performance differed from expectations based on chance (Titus et al., 1984). Following the DFA, we conducted a corresponding repeated measure multivariate analysis of variance (MANOVA) using the acoustic features to compare songs produced by each individual for significant differences. All statistical analyses were conducted using SPSS (Version 20, Chicago, SPSS Inc.).

Artificial neural networks (ANN) are widely used in bioacoustic research to identify species-specific signals and to identify specific individuals within a species by determining the distinct features within a vocalization (Hahn et al., 2013a; Parsons & Jones, 2000; Pozzi et al., 2009; Terry & McGregor, 2002). The networks used in the current study used similar settings as those described in Nickerson et al. (2006), Guillette et al. (2010), and Hahn et al. (2015). We trained the network using the Rosenblatt program (Dawson, 2004), and each network had an input unit for each acoustic feature which were connected to one of six output units. Each of the input units corresponded to one acoustic feature within the *fee-bee* song. The output units used a sigmoid-shaped logistic equation to transform the sum of the weighted signals from each input into an activity value that ranged between 0 and 1. The learning rate was set at 0.5, and we continued training until the output unit produced a ‘hit’ (defined as an activity level of 0.9 or higher when the correct response was to turn ‘on’ (i.e., correct bird), or an activity level of 0.1 or lower when the correct response was to turn ‘off’ (i.e., incorrect bird)). Prior to training, the connection weights for each network were set to a random weight between −0.1 and 0.1, so each network served as one ‘subject’.

Results

Acoustic analysis

A total of 144 songs were analyzed (24 songs from six female black-capped chickadees). Table I shows the mean, standard deviation, coefficients of variation between individuals (CV_b), coefficients of variation within an individual (CV_w), and potential for individual coding value

(PIC) for all acoustic features measured across each female. We calculated the coefficients of variation between individuals (CV_b) using the following formula:

$$CV_b = \left(\frac{SD}{MEAN}\right) \times 100$$

here the SD is the standard deviation and mean is the average for the total sample, and we calculated the coefficient of variation within an individual (CV_w) using the formula:

$$CV_w = \left(\frac{SD}{MEAN}\right) \times 100$$

here the SD and mean are calculated from each individual's songs (Sokal and Rohlf, 1995; Charrier et al., 2004; Hahn et al., 2013b; Campbell et al., 2016). For each acoustic feature, the PIC value is the ratio CV_b/mean CV_w, where mean CVw is the average CVw calculated for all individuals (Charrier et al., 2004; Hahn et al., 2013b; Campbell et al., 2016). If we observe a PIC value greater than 1, then that particular acoustic feature may be used for individual identification.

A correlation matrix showed that *fee* start frequency and *fee* peak frequency ($r(144) = 0.934$, $p < 0.001$), and *bee* start frequency and *bee* peak frequency ($r(144) = 0.897$, $p < 0.001$) are highly correlated. In addition, the *fee* proportion of the total song length was highly correlated to the *bee* proportion of the total song length ($r(144) = 0.875$, $p = < 0.001$). Thus, the acoustic features of *fee* start frequency, *bee* start frequency, *fee* proportion were removed from further DFA and MANOVA analyses.

DFA, MANOVA, & ANN (by individual)

The stepwise DFA used to classify songs based on the individual female producing the song used 10 measured acoustic features. In total, one stepwise analysis with six steps was performed. Stepwise analysis showed that *bee* duration, *fee*-note peak frequency, *bee*-note proportion *fee*-note end frequency, internote interval, and *bee*-note peak frequency can be used to classify 84.73% of songs by the individual female. The overall Cohen's *Kappa* coefficient was high (0.81), which indicates good model performance. See Table II for predicted group membership distributions by DFA and ANN. See Table III for Wilks' lambdas, *F* statistics, and *p* values for all acoustic features. See Table IV for standardized coefficients, eigenvalues, percentage of variance, and canonical correlations for the discriminant functions.

Results from the repeated measures MANOVA revealed significant differences between all six female chickadees based on the measured acoustic features, ($F_{(45, 584)} = 23.797$, $p < 0.001$, partial $\eta^2 = 0.606$). While the vocalizations of these females were significantly different, the repeated measures MANOVA cannot determine which acoustic features cause these differences. See Table V for significant differences between individual females. See Figure 2 for centroid plots for all females.

Pilot testing with female song stimuli indicated that the network never learned to classify the 144 songs to the six individual females based on the 10 measured acoustic stimuli with 100% accuracy, therefore we could not use perfect performance as the criterion to stop training. As a result, we stopped training the network after 30,000 training sweeps, which was approximately the number of sweeps that the artificial neural network reached its maximum number of hits ($\bar{x}$=783). Since each of the six female chickadees contributed 24 songs, there were 864 total measurements that could be used to identify one female. The 30,000 sweeps showed that 783 individual measurements were correctly classified (90% accuracy). See Table II for predicted group membership distributions.

DFA, MANOVA, & ANN (by season)

While our analysis of acoustic stimuli by the individual was highly accurate, results also showed a strong difference between the songs of the four individual females recorded in the

Spring and the two individual females recorded in the Fall. Thus, we performed a separate DFA, MANOVA, and complimentary ANN, for the vocalizations sorted by season (i.e., fall vs. spring based on the measured acoustic features).

The stepwise DFA used to classify songs based on season of female-produced song (fall vs. spring) used the identical 10 measured acoustic features as the above analysis by individual. In total, one stepwise analysis with three steps was performed. Stepwise analysis showed that *bee*-note *duration*, *bee*-note peak frequency, and *fee* glissando can be used to classify 98.45% of songs by the season they were produced. Our overall Cohen's *Kappa* showed high accuracy (0.96), indicating good model performance. See Table II for predicted group membership distributions by DFA and ANN. See Table III for Wilks' lambdas, *F* statistics, and *p* values for all acoustic features. See Table IV for standardized coefficients, eigenvalues, percentage of variance, and canonical correlations for the discriminant functions.

Results from the MANOVA revealed significant differences between Fall and Spring songs based on the measured acoustic features ($F_{(9,\,134)} = 133.595$, $p < 0.001$, partial $\eta^2 = 0.900$). While the songs of these females by season were significantly different, the repeated measures MANOVA cannot determine which acoustic features cause these differences. See Table V for significant differences between season.

For the ANN, we stopped training the network after 40,000 training sweeps, which was approximately the number of sweeps that the network reached its maximum number of hits ($\bar{x}$=268). As each of the six female chickadees contributed 24 songs by season, there were 288 total measurements that could be attributed to one season. The 40,000 sweeps showed that 268 individual measurements were correctly classified (93%). See Table II for predicted group membership distributions.

Discussion

Overall, using discriminant function analyses and artificial neural networks we were able to classify individual female-produced *fee-bee* songs to a high degree of accuracy; although some female birds showed slight overlap and we also observed an impact of season. The analyses identified many acoustic features which differed significantly between individuals. Several acoustic features including *bee*-note measurements and the *fee* glissando (for season only), were found to be in-line with previous research on individual identification in male and female black-capped chickadees (Montenegro et al., 2020).

The results of the discriminant function analysis (DFA) showed that of the acoustic features investigated, *fee*-note, *bee*-note, and internote interval were most accurate at classifying the individual female singer. Specifically, *bee*-note duration, *fee*-note peak frequency, *bee*-note proportion, *fee*-note end frequency, internote interval, and *bee*-note peak frequency could be used to distinguish individual females. The artificial neural networks (ANN) were used to confirm correct and incorrect classification of songs identified by the DFA. Both methods of classification, DFAs and ANNs, did find a degree of overlap between the songs of Female B and Female E, and showed the highest number of errors when classifying Female B and Female E.

The MANOVA results agreed with those of the DFA for all acoustic features including the *fee*- and *bee*-note acoustic features as best able to accurately classify individual identity. Tukey's *post-hoc* analysis and centroid plots revealed that not all the six identified acoustic features were significantly different between the females. Some females overlapped more with other females and some overlapped less with other females, and not all acoustic features were significantly different between individuals, suggesting individual differences in acoustic features between the female birds. For example, *bee*-note duration was significantly different between

Female C and all other birds, (p = 0.001), but *bee*-note duration for Female A was only significantly different from Female C and D. (p = 0.001). The centroid plot (Figure 2) shows the overlap between each song from each bird. Female C and Female D are shown as clusters separate from each other and from all other birds; comparatively, Female, A, B, E, F are closely clustered together. These two birds are distinct from the rest of the four birds, thus, the DFA and ANN were able to classify songs produced by Female C (DFA, 100%; ANN, 96%) and Female D (DFA, 95.8%; ANN, 97%) to highest degree of accuracy.

Female C and D were recorded in a different year and season (Fall 2014) than the rest of the females (Spring 2012). While, the previous operant study using these vocalizations showed no difference in response or ability to discriminate based on year of recording or season (Montenegro et al., 2020), we ran a separate DFA, MANOVA, and ANN in order to investigate identification via season of female-produced song. The DFA showed that *bee*-note duration, *bee*-note peak frequency, and the *fee* glissando were the most important features in classifying individuals by fall vs. spring and could be used to classify female song to a high degree (98.45%). However, the MANOVA showed significant differences between *bee*-note duration and *fee* glissando but not the *bee*-note peak frequency. The ANN was able to confirm that our female-produced *fee-bee* songs could be classified to a high degree (93%). Interestingly, the *fee* glissando in chickadee song has previously been associated with sex discrimination (Hahn et al., 2015) and all songs in this prior study were previously recorded in the spring. A prior acoustic analysis has also suggested that male- and female-produced songs do differ by season (Campbell et al., 2019). Perhaps the difference in the *fee* glissando in female song that we observe in the current study mirrors the biological functions of male song (i.e., mate attraction, territory defense, solicitation of extrapair copulations), which are more profound in the spring, the black-capped chickadee breeding season (Avey et al., 2008). Notably, a previous study on seasonal plasticity in chickadees and other songbirds used auditory evoked potentials (reflects the auditory ability level of an individual) to find that there are seasonal changes in the auditory processing systems of chickadees, and that these changes match the acoustic properties of songs during and outside of the breeding season (Vélez et al., 2015). These differences may also be what our results are indicating in terms of differences in individual song by season. That said, there appears to be no difference in the song system based on season and the *fee-bee* song (Smulders et al., 2006). Overall, while these possible functions compliment the current proposed function of female song (Langmore, 1998) and past literature on song and season, we must still consider that male and female songs do differ in form and function in this species, and recognize that the function of female song is still unknown, requiring behavioral testing.

The DFA results suggest that there are features within the latter half of the *fee-bee* song that signal the identity of the singer as well as features that match prior studies on male identification. A previous study found that female and male chickadees were able to identify individual females when listening to only the *bee*-note portion of their respective *fee-bee* songs. When discriminating between *fee*-note portions, the chickadees were no longer able to perform the discrimination (Montenegro et al., 2020), thus supporting the acoustic features identified by the DFA. In addition, it has been previously shown that internote interval is used by chickadees when discriminating between individual males via their song (Christie et al., 2004a). Internote interval was also identified by the current DFA and ANN as being an important feature in classifying female songs by individual. While the *fee* glissando was only significant when classifying female song by season, some *fee*-note acoustic features such as peak frequency, and end frequency were identified as significantly different amidst females. In addition, a past study has shown that female and male black-capped chickadees show no difference in frequency sensitivity, specifically that female and male chickadees exhibit the greatest sensitivity to frequencies between 2 and 4 kHz, as evidenced by auditory evoked potentials (Wong & Gall, 2015). These evoked potential results show that not only is the auditory system of both sexes sensitive in the frequency region of *fee-bee* song, but also suggest that song is important to both sexes. Considering we found parallels between female and male individual identification via song, perhaps the functions of song are similar in both sexes. Or perhaps the features that the current DFA selected for classification of individuals is evidence of overall voice recognition simply because many black-capped chickadee vocalizations lend themselves to individual identification. Prior research has shown that black-capped chickadees can identify individual chickadees by their *chick-a-dee* calls (Charrier & Sturdy, 2005; Mammen & Nowicki, 1981) and possibly by *tseet* calls (Guillette et al., 2010).

Collectively, our findings suggest the classification of female black-capped chickadees via female-produced *fee-bee* song is not note dependent or season dependent. While *bee*-note features were identified as significantly different between females and previously shown to be important to chickadees when discrimination between females, our analyses showed that some *fee*-note features were also important and these differences in acoustic features differ between seasons. Further studies manipulating acoustic features of female *fee-bee* songs can aid in further determining which features are most important for individual identification and how they may work together. In addition, further exploring female song use can also benefit identifying which acoustic features are used in discriminating individuals. Depending on how females use song, whether for territorial defense, mate attraction, or another function entirely, the way in which black-capped chickadees interpret the song may differ and thus the important acoustic features may differ. Female song studies in other species have found that there are differences in female song compared to male song. For example, female banded wren (*Thryophilus pleurostictus*) song is shorter compared to male song, females also have a smaller song repertoire (Hall et al., 2015). Female song use in this species also differs from male song in that is it primarily used for mate communication, while male song is used for territorial defense and mate attraction. The female Venezuelan Troupials (*Icterus icterus*) song also differs from male song in both frequency and duration but have similar syllable composition. In addition, female troupials sing all year-round (Odom et al., 2016). Thus, it is important to further study the female song as a whole and the way female song may differ in production and perception compared to male song in order to explore begin exploring function.

Table 3-1. Summary of acoustic features measured including means and SDs for all acoustic features measured across each female black-capped chickadee. SDs provided in parentheses.

Bird	**Value**	**Total duration (ms)**	***Fee*-note duration (ms)**	***Bee*-note duration (ms)**	***Bee*-note proportion (%)**	***Fee*-note peak (Hz)**	***Fee*-note end (Hz)**	***Fee* glissando (Hz)**	***Bee*-note peak (Hz)**	***Bee*-note end (Hz)**	**Internote interval (ms)**
All birds	**Mean**	895	399	392	43	4352	3432	1239	3813	3117	104
	SD	343	272	157	10	331	187	93	365	405	29
	$\text{CV}_{\text{between}}$	38.32	68.09	40.16	23.15	7.60	5.46	7.53	9.58	12.98	28.08
	PIC	1.91	2.05	2.54	1.61	1.57	1.38	1.26	1.55	1.35	1.28
Female A Spring	**Mean**	1004	432	469	47	4312	3571	1185	3946	3306	99
	SD	73	56	43	4	116	90	57	55	89	19
	$\text{CV}_{\text{within}}$	7.23	12.93	9.28	8.54	2.69	2.52	4.85	1.39	2.98	18.93
Female B Spring	**Mean**	1032	389	511	50	4347	3446	1231	3741	3089	132
	SD	103	84	24	4	272	201	71	123	114	18
	$\text{CV}_{\text{within}}$	9.96	21.60	4.69	8.31	6.27	5.84	5.79	3.29	3.69	13.61
Female C Fall	**Mean**	857	546	224	29	4594	3511	1270	3767	3050	90
	SD	613	604	54	9	229	98	82	113	679	27
	$\text{CV}_{\text{within}}$	71.47	111.05	24.13	31.28	4.98	2.79	6.48	21.57	22.25	29.72
Female D Fall	**Mean**	411	183	146	35	3985	3253	1164	3970	3262	82
	SD	68	56	47	7	99	113	69	76	110	34
	$\text{CV}_{\text{within}}$	16.49	30.62	32.42	19.91	2.49	3.46	5.95	1.91	3.37	41.02
Female E Spring	**Mean**	1082	462	495	46	4175	3292	1246	3646	2972	125
	SD	95	47	74	5	374	229	97	213	638	25
	$\text{CV}_{\text{within}}$	8.80	10.08	14.98	10.29	8.95	6.95	7.81	5.84	21.44	19.93
Female F Spring	**Mean**	986	382	507	51	4701	3516	1338	3808	3021	97
	SD	60	49	48	4	169	79	66	117	123	9
	$\text{CV}_{\text{within}}$	6.08	12.87	9.43	7.86	3.59	2.25	4.96	3.08	4.07	8.82

Table 3-2. Predicted group membership percentages by individual (A) and by season (B). First line includes original percentages and in parentheses are cross-validated percentages by DFA. Second line includes predicted group membership percentages by ANN results for comparison.

A

	Predicted group membership by individual					
Bird & Season	**Female A**	**Female B**	**Female C**	**Female D**	**Female E**	**Female F**
Female A **Spring**	91.7 (83.3) 92.0	0.0 (4.2) 3.0	4.2 (4.2) 1.0	0.0 (0.0) 0.0	0.0 (0.0) 0.0	4.2 (8.3) 3.0
Female B **Spring**	4.2 (4.2) 5.0	62.5 (54.2) 79.0	0.0 (0.0) 0.0	0.0 (0.0) 1.0	4.2 (4.2.0) 14.0	29.2 (37.5) 3.0
Female C **Fall**	0.0 (0.0) 2.0	0.0 (0.0) 0.0	100.0 (95.8) 97.0	0.0 (0.0) 1.0	0.0 (4.2) 0.0	0.0 (37.5) 0.0
Female D **Fall**	0.0 (0.0) 1.0	0.0 (0.0) 1.0	0.0 (0.0) 5.0	100.0 (100.0) 94.0	0.0 (0.0) 0.0	0.0 (0.0) 0.0
Female E **Spring**	8.3 (8.3) 3.0	0.0 (0.0) 1.0	4.2 (4.2) 5.0	0.0 (0.0) 0.0	87.5 (87.5) 90.0	0.0 (0.0) 1.0
Female F **Spring**	8.3 (8.3) 5.0	16.7 (20.8) 19.0	4.2 (4.2) 4.0	0.0 (0.0) 0.0	4.2 (4.2) 2.0	66.7 (62.5) 70.0

B

	Predicted group membership by season	
Bird	Fall	Spring
Fall	96.9 (96.6) 93.0	3.1 (3.1) 7.0

Spring	0.0 (2.1) 1.0	100.0 (97.9) 99.0

Table 3-3. Acoustic features that are used in the analysis at each step by DFA results showing relative importance of each feature in discriminating between individual female chickadees via their *fee-bee* song (A) and in discrimination between season via female *fee-bee* song (B).

A

Step	**Variable**	**Wilk's lambda**	***F* statistic**	**Significance**
1	*Bee*-note duration	0.100	248.854	< 0.001
2	*Fee*-note peak frequency	0.049	96.506	< 0.001
3	*Bee*-note (proportion)	0.27	67.642	< 0.001
4	*Fee*-note end frequency	0.018	52.465	< 0.001
5	Internote interval	0.015	42.117	< 0.001
6	*Bee*-note peak frequency	0.012	36.319	< 0.020

B

Step	**Variable**	**Wilk's lambda**	***F* statistic**	**Significance**
1	*Bee*-note duration	0.128	969.814	< 0.001
2	*Bee*-note peak frequency	0.121	512.093	< 0.001
3	*Fee* glissando	0.114	362.079	< 0.001

Table 3-4. Reported values for the five discriminant functions via individual female bird (A), including standardized coefficients, eigenvalues, percentage of variance, and canonical correlations, and for the one discriminant function via season (B).

A

	Function				
Standardized coefficients	**1**	**2**	**3**	**4**	**5**
Bee-note duration	1.31	0.24	-0.25	-0.37	-0.47
Bee-note (proportion)	-0.40	-0.72	0.84	0.68	0.57
Fee-note peak	0.04	0.75	-0.02	0.80	-0.02
Fee-note end	0.44	0.22	0.26	-0.78	0.60
Bee-note peak	-0.47	0.17	0.20	-0.44	-0.30
Internote interval	0.15	-0.35	-0.55	0.24	0.77
Eigenvalue	14.25	1.39	0.53	0.39	0.11
% of variance	85.5	8.3	3.1	2.4	0.6
Canonical correlation	0.969	0.762	0.587	0.531	0.309

B

	Function
Standardized coefficients	**1**
Bee-note duration	-0.27
Bee-note peak frequency	1.07
Fee glissando	-0.264
Eigenvalue	7.759
% of variance	100.0
Canonical correlation	0.941

Table 3-5. Repeated measures MANOVAs reported mean differences and significance by individual female chickadee (A) and by season (B) based on acoustic features.

A

Comparison		Mean difference	Significance
Female A	**Female B** **Female C** **Female D** **Female E** **Female F**	45.446 50.828 185.134 12.465 89.324	0.106 0.071 < 0.001* 0.656 0.002*
Female B	**Female C** **Female D** **Female E** **Female F**	5.382 139.688 -32.981 43.878	0.848 < 0.001* 0.240 0.119
Female C	**Female D** **Female E** **Female F**	134.306 -38.363 38.496	< 0.001* 0.172 0.171
Female D	**Female E** **Female F**	-172.669 -95.810	< 0.001* 0.001*
Female E	**Female F**	76.859	0.007*

B

Comparison		Mean difference	Significance
Fall	**Spring**	81.172	< 0.001

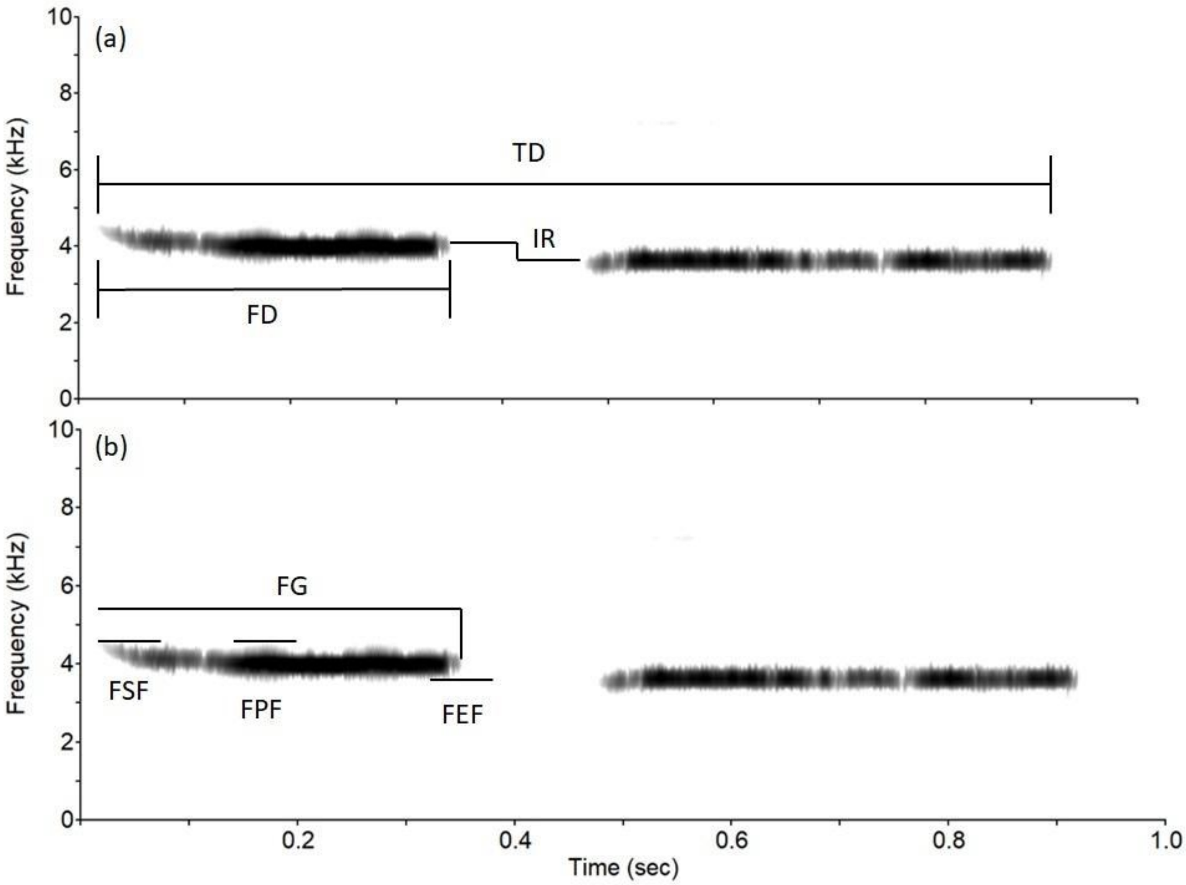

Figure 3-1. Sound spectrogram depicting acoustic measurements performed in *fee-bee* songs. All measurements depicted for *fee*-notes were measured similarly for *bee*-note measurements. (a) Sound spectrogram (time resolution 5.8 ms) of a *fee-bee* song. Measurements shown: total duration of song (TD) and *fee*-note duration (FD). (b) Sound spectrogram (frequency resolution 172.3Hz) of the same *fee-bee* song. Measurements shown: *fee* glissando (ratio of frequency decrease within *fee*-note) (FG), internote interval (II) (frequency ratio between the notes), *fee* start frequency (FSF), *fee* peak frequency (FPF), *fee* end frequency (FEF). All *bee* measurements not shown were measured using the identical procedure.

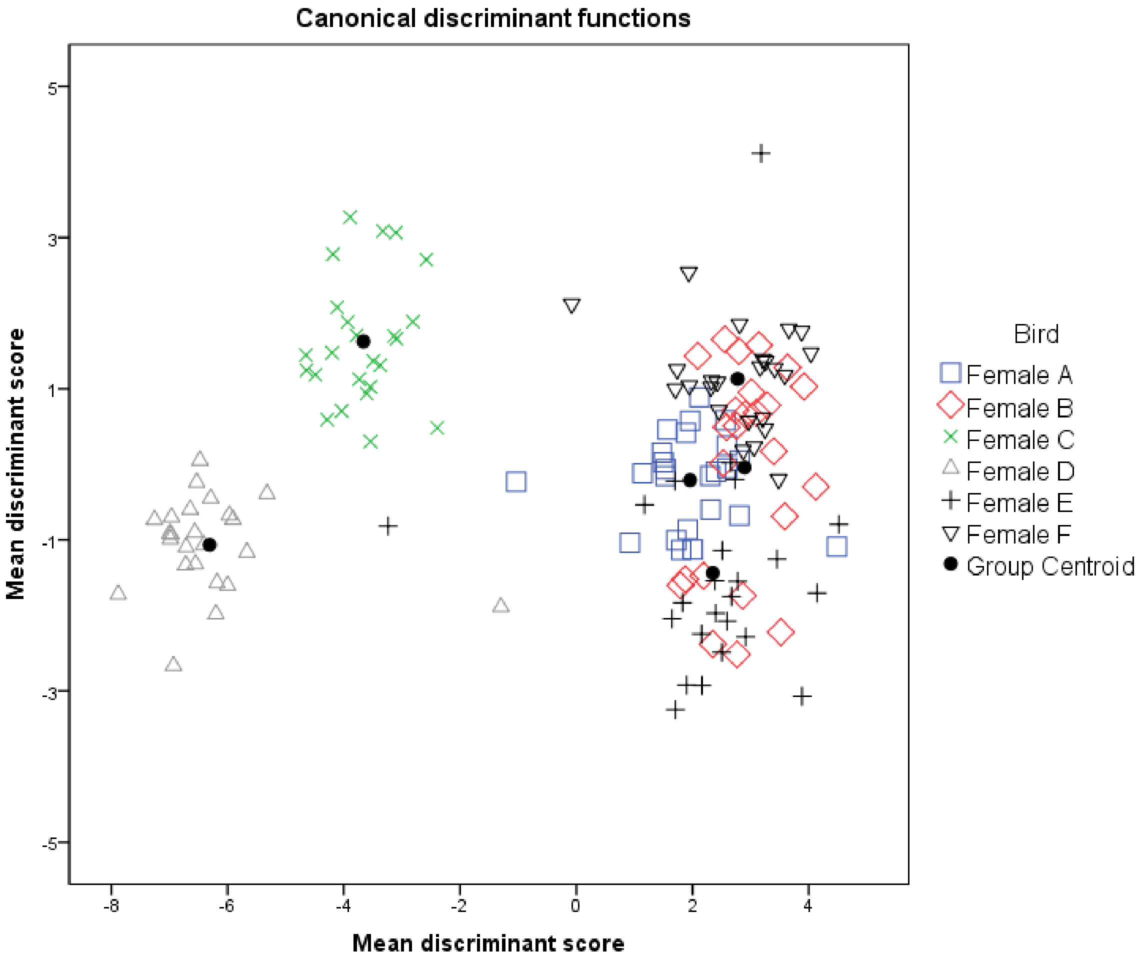

Figure 3-2. Centroid plot for all females showing the distribution of each song in relation to all songs. Each female, A-F has each of their classified songs plotted, remaining. Circles denote the group centroid for each bird.

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Chapter 4 The impact of anthropogenic noise on individual identification via female song in Black-capped chickadees (*Poecile atricapillus*)

Introduction

Throughout the world, the anthropogenic pressures of human activity, including anthropogenic noise, are increasing and have significant effects on animal behavior (Berger-Tal et al., 2019; Vitousek et al., 1997). Anthropogenic noise consists of a wide range of sounds including: road vehicles, airplanes, industrial machinery, and air movement machinery (Leventhall, 1988; Tempest, 1985). Anthropogenic noise levels have been shown to cause disturbances in human and non-human animals depending on the source and its proximity (Shannon et al., 2016). In non-human animals, the disturbances associated with exposure to long-term anthropogenic noise include physical and/or physiological damage, and masking of communication signals (Shannon et al., 2016; Slabbekoorn & Ripmeester, 2008).

The vocal adjustments made by songbirds as a consequence of anthropogenic noise are currently well studied. For example, songbirds can shift their temporal pattern of behaviour by singing earlier in the day to avoid noises associated with high traffic noise (Fuller et al., 2007; Proppe et al., 2011), alter the quality of their vocalizations by shifting frequency or amplitude (Brumm, 2004), and change frequency and duration as a consequence of anthropogenic noise exposure (Gentry et al., 2017). Killer whales (*Orcinus orca*) will increase call duration in the presence of anthropogenic noise (Foote et al., 2004), while bow-winged grasshoppers (*Chorthippus biguttulus*) will increase the frequency of courtship signals in response to increasing anthropogenic noise (Lampe et al., 2012).

The auditory masking of signals and its influences on perception of acoustic signals have been demonstrated in a variety of species. In the presence of traffic noise, great tit (*Parus major*) alarm calls are masked, and thus tits may face increased predation risk (Templeton et al., 2016). When exposed to playbacks of tufted titmouse (*Baeolophus bicolor)* alarm calls in noisy areas, cardinals (*Cardinalis cardinalis*) were less likely to produce predator avoidance behaviors compared to behavior in quieter areas suggesting the ability to eavesdrop had decreased (Grade & Sieving, 2016). A comparable impact has been demonstrated in hermit crabs (*Coenobita clypeatus*), where crabs were slower to hide in response to a visual stimulus in the presence of noise (Chan et al., 2010). In addition, at varying levels of traffic noise, female grey treefrogs (*Hyla chrysoscelis*) show an increase in response latency and decrease in orientation towards male advertisement calls (Bee & Swanson, 2007).

Similarly, there is ample research on vocal adjustment in black-capped chickadees *(Poecile atricapillus*), which includes several studies on masking of vocal signals, but few on the perception of masked signals. The black-capped chickadee *fee-bee* song is a two-note vocalization that is used primarily by males for territorial defense and mate attraction (Ficken et al., 1978; Smith, 1991). The function of songs in female songbirds is still unknown, but past research on female song suggests the function includes advertising individual quality (Langmore, 1989; Odom & Benedict, 2018; Riebel et al., 2019). Prior research has shown that black-capped chickadees will shift the pitch (Proppe et al., 2012) and frequency (LaZerte et al., 2016) at which they sing in the presence of anthropogenic noise. Furthermore, anthropogenic noise can mask acoustic signals and compromise discrimination of fine details in songs (Nemeth & Brumm, 2010; Nemeth et al., 2013) and differing levels of anthropogenic noise can negatively impact discrimination of conspecific *fee-bee* songs by black-capped chickadees (Mischler et al., unpublished).

In order to avoid wasting resources like time and energy when interacting and vocalizing, distinguishing between conspecifics and heterospecifics is a useful ability. For example, being able to tell your mate vs. the mate of your neighbor in the next-door territory. We can observe the ability to identify individuals by their vocalization in several species including o conspecifics in song sparrows (*Melospiza melodia;* Stoddard et al., 1990) and mates in zebra finches (*Taeniopygia guttata*: Miller, 1979). Most recently for black-capped chickadees, the *fee-bee* song has been shown to serve a function related to individual recognition in terms of mate recognition, in particular the *fee* glissando (Hahn et al., 2013). The *fee* glissando (decrease in frequency compared to the first *fee*-note and following *bee*-note; Hahn et al., 2013; Weisman et al., 1990) is perhaps an acoustic feature for identification as it is less prominent in male chickadees compared to female chickadees (Hahn et al., 2015). Furthermore, past song studies have suggested that features such as total song duration and the interval ratio are useful in discrimination between males (Christie et al., 2004). It is conceivable that due to the function of a chickadee's one song that these features may also signify male quality, especially when we clue into the other features such as relative amplitude of the two notes demonstrating significant differences between dominant and subordinate male chickadees (Hoeschele et al., 2010). Accordingly, song may be used to tell individuals apart based on sex, quality, and rank.

Recently, we have also shown that male and female black-capped chickadees can discriminate between individual females via their *fee-bee* songs (Montenegro et al., 2020). Therefore, based on our own findings and the findings of current anthropogenic noise literature, we questioned whether chickadees could discriminate among female songs in the presence of anthropogenic noise. We used an operant go/no-go paradigm to determine how anthropogenic noise impacts the ability of male and female black-capped chickadees to discriminate between individual female black-capped chickadee *fee-bee* songs. Male and female black-capped chickadees were trained and tested using unmanipulated *fee-bee* songs in addition to varying levels of anthropogenic noise. Our aim was to examine if the chickadees could identify individual female chickadees via their *fee-bee* song, as previously found, at differing levels of anthropogenic noise measured on how quickly the birds were able to learn to distinguish between individuals or if song would be masked by the noise.

Methods

Subjects

In total, twenty-two black-capped chickadees (nine males and 13 females) were tested between May and December 2019, and 16 black-capped chickadees (seven males and nine females) completed the experiment. To expand one male and one female failed to learn Pretraining, and one female failed to learn Non-differential training (see descriptions below for training information); as a result, all three were removed from the experiment. In addition, one male and two females died of natural causes during the course of the study (see Ethical Note). For all birds, sex was determined by deoxyribonucleic acid analysis of blood samples (Griffiths et al., 1998). All birds were captured in Edmonton (North Saskatchewan River Valley, 53.53°N, 113.53°W; Mill Creek Ravine, 53.52°N, 113.47°W), Alberta, Canada in January 2018 and January 2019 and were at least one year of age at capture, verified by examining outer tail rectrices (Pyle, 1997).

Prior to the current experiment, all chickadees were individually housed in Jupiter Parakeet cages (30 × 40 × 40 cm; Rolf C. Hagen, Inc., Montreal, QB, Canada) in a single colony room. Therefore, birds did not have physical contact with each other, but did have visual and auditory contact. Birds had *ad libitum* access to food (Mazuri Small Bird Maintenance Diet; Mazuri, St. Louis, MO, USA), water with vitamins supplemented on alternating days (Monday, Wednesday, Friday; Prime Vitamin Supplement; Hagen, Inc.), a cup containing grit, and a cuttlebone. Additional nutritional supplements included three to five sunflower seeds daily, one superworm (*Zophabas morio*) three times a week, and a mixture of hard-boiled eggs and greens (spinach or parsley) twice a week. The colony rooms were maintained at approximately 20°C and on a light:dark cycle that followed the natural light cycle for Edmonton, Alberta, Canada.

One bird had previous experience with one operant experiment involving *chick-a-dee* calls but showed no difference in responding compared to other subjects. The remaining 15 birds had no previous experimental experience with these particular black-capped chickadee-produced *fee-bee* songs or any experimental paradigm.

Apparatus

For the duration of the experiment, birds were housed individually in modified colony room cages (30 × 40 × 40 cm; described above) which were placed inside a ventilated, sound-attenuating operant chamber. All chambers were lit with a full spectrum LED bulb (3W, 250 lm E26, Not-Dim, 5000 K; Lohas LED, Chicago, IL, USA), and maintained the natural light:dark cycle for Edmonton, Alberta. Each cage within each operant chamber contained two perches and an additional perch fitted with an infrared sensor (i.e., the request perch). Each cage also contained a water bottle, grit cup, and cuttlebone. Birds had *ad libitum* access to water (with vitamins supplemented on alternating days; Monday, Wednesday, Friday), grit, and cuttlebone and were provided two superworms daily (a morning and afternoon worm). An opening (11 × 16 cm) located on the left side of the cage allowed the birds to access a motorized *fee*der, also equipped with an infrared sensor (Njegovan et al., 1994). The purpose of the sensor was so that food was only available as a reward for correct responses to auditory stimuli during the operant discrimination task. We should note that performance of the discrimination task is required for access to food and thus maintains motivation (See procedure for information on the discrimination task). For operation and data collection, a personal computer connected to a single-board computer (Palya & Walter, 2001) scheduled trials and recorded responses to stimuli. Stimuli were played from a personal computer hard drive through a Cambridge Integrated Amplifier (model A300 or Azur 640A; Cambridge Audio, London, England). Stimuli played in the chamber through a Fostex full-range speaker (model FE108 Σ or FE108E Σ; Fostex Corp., Japan; frequency response range 80-18,000 Hz) located beside the *fee*der. See Sturdy and Weisman (2006) for a detailed description of the apparatus.

Recordings of Acoustic Stimuli

The following acoustic stimuli were used in our previous published operant study which indicated that male and female chickadees can identify individual females via their song (Montenegro et al., 2020). Stimuli included the songs of six female black-capped chickadees. All females were captured in Edmonton (North Saskatchewan River Valley, 53.53°N, 113.53°W; Mill Creek Ravine, 53.52°N, 113.47°W), Alberta, Canada in January 2010, 2011, 2012, and 2014, and all females were at least one year of age at capture, verified by examining outer tail rectrices (Pyle, 1997). Four females were recorded in Spring 2012 and two females were recorded in Fall 2014. Each recording session lasted approximately 1 hr and all recordings took place after colony lights turned on at 08:00, specifically at 8:15. All females were recorded in silence, individually, within their respective colony room cages. Colony room cages were placed in sound-attenuating chambers for recording (1.7 m × 0.84 m × 0.58 m; Industrial Acoustics Company, Bronx, NY). An AKG C 1000S (AKG Acoustics, Vienna, Austria) microphone (positioned 0.1 m above and slightly behind the cage) was connected to a Marantz PMD670 (Marantz America, Mahwah, NJ) digital recorder (16 bit, 44,100 Hz sampling rate) and was used for all recordings. Audio recordings were analyzed and cut into individual files (songs) using SIGNAL 5.03.11 software (Engineering Design, Berkley, CA, USA).

Acoustic Stimuli

For the current study, a total of 150 vocalizations were used as stimuli, these vocalizations were comprised of 25 *fee-bee* songs produced by each of six recorded female chickadees. We ensured that all 150 were of high quality, meaning no audible interference, and all stimuli were bandpass filtered (lower bandpass 500 Hz, upper bandpass 14,000 Hz) using GoldWave version 6.31 (GoldWave, Inc., St. John's, NL, Canada) in order to reduce any background noise outside of the song stimuli spectrum. For each song stimulus, 5 ms of silence was added to the leading and trailing portion of the vocalization and each stimulus was tapered to remove transients, in addition amplitude was equalized peak to peak using SIGNAL 5.03.11 software. When triggered, stimuli were presented at approximately 75 dB SPL as measured by a calibrated Brüel & Kjær Type 2239 (Brüel & Kjær Sound & Vibration Measurement A/S, Nærum, Denmark) sound pressure meter (A-weighting, slow response), a level that corresponds with the natural chickadee vocalizations amplitudes (Nowicki, 1983; Proppe et al., 2010; Templeton & Greene, 2007). All dB measurements were made at the level of the request perch where birds trigger stimuli and where birds are required to remain for the length of the stimuli or house lights will extinguish.

Noise stimuli

Anthropogenic noise stimuli were originally created and used by Potvin and MacDougall-Shackleton (2015) and by Potvin, Curcio, Swaddle, and MacDougall-Shackleton (2016). The stimuli were recorded from an urban area in Melbourne, Victoria, Australia and other anthropogenic noise stimuli of various trains, cars, motorcycles, and lawnmowers downloaded from Soundbible.com were used. Within Victoria (Vic Roads, 2015) and Alberta (Government of Alberta, 2017; Patching Associates Acoustical Engineering, 2018) urban traffic noise averages 60-80 dB. The files used varied in length, with those recorded in Melbourne all being 10 minutes in length and those downloaded from Soundbible.com varying between 1-10 minutes (Potvin et al., 2016; Potvin & MacDougall-Shackleton, 2015). In total 10 tracks were used with 30 total minutes of noise stimuli. Three anthropogenic noise conditions were used in the study, including Silence (no noise), Low noise (anthropogenic noise stimuli played at ~40dB), and High noise (anthropogenic noise stimuli played at ~75dB) replicating the variation of traffic noise experienced in urban areas (Potvin et al., 2016; Potvin & MacDougall-Shackleton, 2015). For the Low and High noise conditions the 10 tracks repeated on a randomized and continuous loop during data collection (natural light of light:dark cycle) with, thus noise exemplars overlapped songs by chance, to further emulate urban areas. Noise stimuli had natural variations and modulations in frequency and amplitude over the course of the sound files. See Figure 4-1 for female song and traffic noise stimuli spectrograms and power spectrums.

Procedure

Non-differential training. Once a bird learned to use the request perch fitted with a sensor as well as learned to use the feeder to obtain food then pretraining began. During Pretraining, birds were trained to respond to a 1 second tone (1,000Hz) in order to receive access to food. Pretraining occurred over an approximately 15-day period in order to allow acclimatization to the chamber, feeder, and speaker. Following Pretraining was Non-differential training. During Non-differential training, birds received food for responding to all *fee-bee* song stimuli. All trials began when a bird landed on the request perch and remained on the perch for between 900-1100 ms, at which point a randomly-selected song stimulus played. Songs were presented in random order from trial to trial until all 150 stimuli had been triggered and played without replacement; once all 150 stimuli were played, a new random sequence initiated. In the event that the bird left the request perch during a stimulus presentation, the trial was deemed interrupted, and resulted in a 30 s lights out of the operant chamber and then received another randomly played song stimuli. If the bird entered the feeder within 1 s after the stimulus (any stimulus) was played, it was given 1 s access to food, followed by a 30 s intertrial interval. If a bird remained on the request perch during the stimulus presentation and the 1 s following the completion of the stimulus, then the bird received a 60 s intertrial interval with the lights on. The purpose of the above Non-differential training is to engender a high level of responding on all trials, across all stimuli (Sturdy & Weisman, 2006). Birds continued on Non-differential training until they completed six 450-trial blocks at ≥ 60% responding on average to all stimuli, at least four 450-trial blocks at ≤ 3% difference in responding to future rewarded versus future unrewarded Discrimination stimuli, at least four 450-trial blocks at ≤ 3% difference in responding to future rewarded versus unrewarded Discrimination stimuli. Then following a day of free *fee*d (where birds have *ad libitum* access to a food cup) birds completed a second round of Non-differential training in which they completed at least one 450-trial block that met each of the above requirements. To elaborate, a 450-trial block consisted of the bird experiencing each of the 150 stimuli three times. For the current study the average time to complete Non-differential training ranged from 10 days to 41 days (*M* = 21.43, *SD* = 9). See Figure 4-2 for flowchart of study stages.

Discrimination training. Discrimination training procedures included only 114 out of the 150 training stimuli that were presented, and responses to these stimuli were now differentially reinforced. Specifically, correct responses to half of the stimuli (“rewarded stimuli”, S+) were positively reinforced with 1 s access to food, and incorrect responses to the other half (“unrewarded stimuli”, S-) were instead punished with a 30-s intertrial interval of lights off within the operant chamber. In regard to criterion, Discrimination training continued until a bird completed six 342-trial blocks with a discrimination ratio (DR) between their respective S+ and S- of greater than 0.80 with the last two blocks being consecutive (Sturdy & Weisman, 2006). For DR calculations see Response Measures below.

The current subjects were randomly assigned to either a True category discrimination group (*n* = 10) or Pseudo category discrimination group (*n* = 6). Furthermore, chickadees in the True category discrimination group were divided into two subgroups: (a) True 1 (*n* = 5) discriminated between 57 rewarded *fee-bee* songs produced by three individual chickadees (S+) and 57 unrewarded *fee-bee* songs produced by another three individual chickadees (S-); and (b) True 2 (*n* = 5) discriminated between the same songs with opposite rewards, properly, the 57 rewarded (S+) *fee-bee* songs were the S- from True 1 and the 57 unrewarded (S-) *fee-bee* songs were the S+ from True 1.

In similitude, the Pseudo category discrimination group was divided into two subgroups: (a) Pseudo 1 (*n* = 3) discriminated between 57 randomly-selected rewarded (S+) *fee-bee* songs and 57 randomly-selected unrewarded (S-) *fee-bee* songs; and (b) the second subgroup Pseudo 2 (*n* = 3) discriminated between the same songs with opposite rewards, meaning, the 57 rewarded (S+) *fee-bee* songs were the S- from Pseudo 1 and the 57 unrewarded (S-) *fee-bee* songs were the S+ from Pseudo 1 (S+) *fee-bee* songs and 57 randomly-selected unrewarded (S-) *fee-bee* songs. To explicate, the purpose of the two Pseudo groups was to include a control in which subjects are required to memorize each vocalization independent of the producer rather than be trained to categorize songs according to individual chickadees as the True groups have *bee*n.

Discrimination-85 training. Discrimination-85 training was identical to the above Discrimination training except that rewarded songs were reinforced with a reduced probability, *P* = 0.85. Therefore, for 15% of trials when a rewarded stimulus was played and a bird correctly responded, no access to food was triggered. Instead, a 30 s lights on intertrial interval occurred. The change in reinforcement occurs in order to prepare birds for Probe trials in which novel song stimuli were neither rewarded with access to food nor unrewarded with a lights out, instead nothing occurs (Sturdy & Weisman, 2006). Discrimination-85 training continued until birds completed two consecutive 342-trial blocks with a DR of at least 0.80.

Discrimination-85 training with noise & Probes. Discrimination-85 training with noise was identical to Discrimination-85 training except one of the three noise stimuli conditions (Silence; Low noise, 40dB; High noise, 75dB) was played over the song stimuli. The noise stimuli condition was randomly-selected for each bird. Each bird went through three sessions of Discrimination-85 training with noise (Silence, Discrimination I; Low, Discrimination II; High, Discrimination III). Following each Discrimination-85 training with noise session was a Probe session. During Probe the reinforcement contingencies from Discrimination-85 training were maintained. In addition to the 114 stimuli from Discrimination training, this stage included 12 novel *fee-bee* songs, two from each of the six individual females. For True groups, six of these novel songs were categorized as P+ and the other six as P-, based on whether they were produced by the same birds as the S+ or the S- training stimuli. For Pseudo groups, the novel songs were not assigned to categories. For both groups, the 12 novel stimuli were neither rewarded (no food access) nor unrewarded (no lights out) (Sturdy & Weisman, 2006). The birds completed six 126-trial blocks in which the 114 familiar discrimination stimuli repeated once per block and the 12 probe sequences played once per block. In addition, one of the three noise stimuli conditions (Silence; Low noise, 40dB; High noise, 75dB) was played over the song stimuli, and each bird went through three sessions of Probe with noise (Silence, Discrimination I; Low, Discrimination II; High, Discrimination III). The noise stimuli condition was randomly-selected for each bird.

Response measures. For each 342-block trial during training, a percent response was calculated (R+/(N-I)): R+ represents the number of trials in which the bird went to the *fee*der, N represents the total number of trials, and I represents the number of interrupted trials in which the bird left the perch before the entire stimulus played. For Discrimination training and Discrimination-85 training, a DR was calculated by dividing the mean percent response to all S+ stimuli by the mean percent response to S+ stimuli plus the mean percent response to S- stimuli. A DR = 0.50 specifies equal response to rewarded (S+) and unrewarded (S-) stimuli, a DR = 1.00 specifies a perfect discrimination between S+ and S- stimuli.

Statistical analyses. All statistical analyses were conducted using SPSS (Version 20, Chicago, SPSS Inc.). In order to compare the number of trials needed to reach criterion and the DRs between True and Pseudo groups we conducted an analysis of variance (ANOVA). An ANOVA was also conduced to compare the number of trials needed to reach criterion and the DRs between True and Pseudo groups during Discrimination-85 I, II, and III (Silence, Low noise, High noise). A repeated measures ANOVA was conducted to compare responding to training stimuli and probe stimuli between True groups and Pseudo groups to Discrimination-85 I, II, and III and Probes I, II, and III. And lastly, we conducted *post-hoc* tests in order to reveal any differences in the number of trials to reach criterion during Discrimination training and to Discrimination-85 I, II, and III.

Ethical note. Throughout the experiment, birds remained in the testing apparatus to minimize the transport and handling of each bird. One male and two female subjects died from natural causes during operant training. Following the experiment, healthy birds were returned to the colony room for use in future experiments. All procedures were conducted in accordance with the Canadian Council on Animal Care (CCAC) Guidelines and Policies with approval from the Animal Care and Use Committee for Biosciences for the University of Alberta (AUP 1937), which is consistent with the Animal Care Committee Guidelines for the Use of Animals in Research. Birds were captured and research was conducted under an Environment Canada Canadian Wildlife Service Scientific permit (#13-AB-SC004), Alberta Fish and Wildlife Capture and Research permits (#56066 and #56065), and the City of Edmonton Parks permit.

Results

Discrimination training by Discrimination Ratios

Results showed that for Discrimination training, True group birds reached criterion (i.e., learned to discriminate in fewer sessions) significantly faster (22.985 ± 8.342) than did Pseudo group (69.167 ± 10.552) birds based on DRs ($F_{1,12} = 11.801$, $p = 0.005$, $\eta_p^2 = 0.496$). See Figure 4-3. And there was no significant difference in sessions to criterion by sex ($F_{1,8} = 0.294$, $p = 0.598$, $\eta_p^2 = 0.024$). Further Tukey's *post hoc* analysis showed no significant difference between True groups 1 and 2 ($p = 0.384$), and no significant difference between Pseudo 1 and 2 ($p = 0.125$).

Discrimination-85 with noise by Discrimination Ratios

There was a significant difference between the three noise conditions (Silence, 2.437 ± 0.388; Low, 9.708 ± 1.265; High, 43.896 ± 5.031) of Discriminatio-85 training based on DRs, ($F_{2,16} = 50.706$, $p < 0.001$, $\eta_p^2 = 0.864$). Specifically, there were significant differences in the rate of acquisition for the High noise condition compared to the Silence ($p < 0.001$) and Low noise conditions ($p < 0.001$), as well as between the Low noise and Silence conditions ($p = 0.002$). See Figure 4-4 for trials to criterion.

Results for Discrimination-85 with Silence showed no significant difference in trials to criterion between True and Pseudo group birds based on DRs ($F_{1,12} = 0.450$, $p = 0.835$, $\eta_p^2 =$

0.004) as well as no differences by sex ($F_{1,12} = 0.725$, $p = 0.411$, $\eta_p^2 = 0.060$). A follow-up Tukey's *post hoc* analysis also showed no significant difference between True groups 1 and 2 ($p = 1.000$), and no significant difference between Pseudo 1 and Pseudo 2 ($p = 0.504$).

For Discrimination-85 with Low noise, results showed that True group birds were able to reach criterion significantly faster than did Pseudo group birds based on DRs ($F_{1,12} = 15.501$, $p = 0.002$, $\eta_p^2 = 0.564$), meaning True birds learned to discriminate more quickly than Pseudo birds in the presence of Low noise. In addition, there was no significant difference in trials to criterion by sex ($F_{1,12} = 0.656$, $p = 0.222$, $\eta_p^2 = 0.121$). And Tukey's *post hoc* analysis also showed no significant difference between True groups 1 and 2 ($p = 0.220$), and no significant difference between Pseudo 1 and 2 ($p = 0.368$).

Lastly, results for Discrimination-85 with High noise, revealed that True group birds reached criterion significantly faster than did Pseudo group birds based on DRs ($F_{1,12} = 10.000$ $p = 0.008$, $\eta_p^2 = 0.455$), again meaning that True birds learned to discriminate between individuals faster than Pseudo birds when in the presence of High noise. There was a significant difference in trials to criterion by sex ($F_{1,12} = 9.173$, $p = 0.010$, $\eta_p^2 = 0.433$). Tukey's *post hoc* analysis showed no significant difference between True groups 1 and 2 ($p = 0.326$), but a significant difference between Pseudo 1 and 2 ($p = 0.008$). The Tukey's *post hoc* analysis also showed no significant difference by sex for True groups 1 and 2 ($p = 0.840$), but a significant difference between Pseudo 1 and Pseudo 2 ($p = 0.001$) with females learning the discrimination faster than males.

Discrimination-85 with noise by Percent Responding

In Discrimination-85 training with noise, percent response in True groups differed across the six stimulus types: rewarded stimuli during Silence, unrewarded stimuli during Silence rewarded stimuli during Low noise, unrewarded stimuli during Low noise, rewarded stimuli during High noise, and unrewarded stimuli during High noise ($F_{1,8} = 92.498$, $p < 0.001$, $\eta_p^2 = 0.920$). Tukey's *post-hoc* analysis revealed a significant difference in percent response between rewarded (S+) and unrewarded (S-) stimuli during Silence, Low, and High noise (*p*s < 0.001). We also found no difference in percent response between rewarded stimuli (S+) during Silence vs. Low noise ($p = 0.780$), or Low vs. High noise ($p = 0.164$) but there was a significant difference between rewarded stimuli (S+) during Silence vs. High noise, based on percent response ($p = 0.017$). Lastly, there were no significant differences in percent response between unrewarded stimuli (S-) during any noise condition (*p*s > 0.060). See Figure 4-5.

In Discrimintion-85 training with noise, percent response in Pseudo groups also differed across the six stimulus types: rewarded stimuli during Silence, unrewarded stimuli during Silence rewarded stimuli during Low noise, unrewarded stimuli during Low noise, rewarded stimuli during High noise, and unrewarded stimuli during High noise ($F_{1,4} = 30.904$, $p = 0.005$, $\eta_p^2 = 0.885$). For Pseudo birds, Tukey's *post-hoc* analyses revealed a significant difference in percent response between rewarded (S+) and unrewarded (S-) stimuli during Silence ($p = 0.022$), no difference between rewarded (S+) and unwarded (S-) stimuli during Low noise ($p = 0.091$), but a significant difference between rewarded (S+) and unrewarded (S-) stimuli during High noise ($p = 0.016$). Analyses also revealed no difference in percent response between rewarded stimuli (S+) during Silence and High noise ($p = 0.157$), and Low and High noise ($p = 0.609$) but there was a significant difference between rewarded stimuli (S+) during Silence and Low noise, based on percent response ($p = 0.036$). Lastly, there was no significant difference in percent response between unrewarded stimuli (S-) during any noise condition (*p*s > 0.182).

Probe with noise by Percent Responding

In Probe with noise, percent response in True groups differed significantly across the six stimulus types: rewarded stimuli during Silence, unrewarded stimuli during Silence rewarded stimuli during Low noise, unrewarded stimuli during Low noise, rewarded stimuli during High noise, and unrewarded stimuli during High noise ($F_{1,8} = 94.601$, $p < 0.001$, $\eta_p^2 = 0.922$). Tukey's *post-hoc* analysis showed a significant difference in percent response between rewarded (S+) and unrewarded (S-) stimuli during Silence, Low, and High noise (*p*s < 0.001). We also found no difference in percent response to rewarded stimuli (S+) between any noise condition (*p*s > 0.337), or any difference in percent response to unrewarded stimuli (S-) between any noise condition (*p*s > 0.211). See Figure 4-5.

Discussion

Based on discrimination ratios (DR) our results suggest that as noise level increased, discriminating between individual females via their song decreased. Low noise and High noise were detrimental to learning the discrimination (i.e., impaired discrimination performance), with High noise impairing discrimination more than Low noise. However, only in Silence did True group birds learn the discrimination significantly faster than Pseudo groups birds, suggesting True category discriminations were easier to learn versus memorizing randomly-selected rewarded songs. High or Low noise learning was disrupted in both True and Pseudo groups. Thus, even a low-level noise of 40dB impacted the bird's ability to discriminate between individuals.

At the Discrimination-85 with noise training stage, birds had already learned the discrimination and responded differentially to the six stimuli types: (1) rewarded stimuli during Silence, (2) unrewarded stimuli during Silence, (3) rewarded stimuli during Low noise, (4) unrewarded stimuli during Low noise, (5) rewarded stimuli during High noise, and (6) unrewarded stimuli during High noise. Percent response data for Discrimination-85 training with noise shows that both True and Pseudo group birds responded to rewarded and unrewarded *fee-bee* song stimuli consistently across noise types but differed in their responding by noise type. Meaning that for Silence, Low, and High noise, birds responded significantly more to rewarded compared to unrewarded, thus learning their discrimination which is also demonstrated by trials to criterion for all noise conditions. However, when looking at percent response to only rewarded stimuli across conditions for True groups, High noise had significantly less response compared to the Silence group, further indicating that noise was detrimental to discriminating between individual female songs. In addition, responding to unrewarded stimuli across noise conditions increased as noise increased, albeit not significantly. Lastly, Probe data suggest that True birds did learn to generalize responding in all noise conditions, demonstrating that birds did transfer their learning of specific female individual song to novel song stimuli. And birds in Silence, Low, or High noise conditions did not differ in responding across reward stimuli or across unrewarded stimuli, indicating that responding was maintained across noise conditions.

The songs used in the current study were produced and recorded in the relative silence of a sound attenuating chamber in a laboratory. These recorded songs were then presented to the subjects with the addition of anthropogenic noise. Previous research has shown that black-capped chickadees require prior experience with noise to adjust their vocalizations in response to noise (Slater & Mann, 2004). Conceivably, the same is true for accurately perceiving songs in anthropogenic noise and over time, or through multiple sessions over time, birds would improve their discrimination between individuals. In addition, different results may have been expected or observed if songs recorded in anthropogenic noise were used. Songs that have naturally been shifted in their amplitude or frequency to be heard over noise may no longer show masking effects. Although, a past study on great tits found that masking still impacted vocalizations produced in noise (Templeton et al., 2016). Tits were recorded in a lab setting with anthropogenic noise present, and recordings showed that the amplitude of calls increased. However, when the modified calls were used as stimuli in a playback field study, traffic noise masked the modified alarm calls.

In terms of noise stimuli, the current study used a combination of recorded anthropogenic noise stimuli as well as other anthropogenic noise stimuli (i.e., trains, cars, motorcycles, lawnmowers). We should note that the random combination of noise stimuli is not identical to what a bird would typically experience in an urban environment. A recent study has found that zebra finches do not increase their song frequencies as adults when exposed to natural anthropogenic noise during the sensorimotor learning period (Liu et al., 2012). A similar result has been found for synthetic noise with zebra finches (Potvin et al., 2016) and great tits (Moseley et al., 2018), however, another study has suggested that artificial noise mimicking the spectral shape of noise does impact the development of song in white-crowned sparrows (*Zonotrichia leucophrys*; Zollinger et al., 2017). It is possible that the generalization we observed in the current study was due to behavioral plasticity, but we should consider the combination of anthropogenic stimuli used in the current study.

While individual recognition was impaired in the current study, we may be also observing the result of impaired detection (Pohl et al., 2009); Langemann et al., 1998), suggested by the differences in responding based on noise condition. A past lab operant conditioning go/go-no study with great tits found that signal detection is impacted by anthropogenic noise (Moseley et al., 2018). The study used multiple independent masking effects (urban noise, woodland noise, dawn chorus) and found that auditory thresholds during noise, both urban and woodland, required louder signals compared to no-noise/no-masking effects. In addition, birds were better able to detect signals with a narrow frequency range vs. a wide frequency range in no-noise, urban, and woodland conditions. Perhaps the birds in the current study were experiencing a similar masking effect and impaired detection/recognition. Another operant conditioning go/no-go task using great tits unexpectedly found low critical masking ratios at high frequencies, suggesting that great tits can perceive high-frequency signals in order to communicate in the presence of white noise (Langemann et al., 1998), and that the songs in the current study could also be masked. In a similar vein, the reduced performance we are observing in the current study could be due also to other factors such as distraction (i.e., distracted prey hypothesis; See Introduction; Chan et al., 2010). Overall, these findings highlight the potential impacts of ever-increasing anthropogenic noise on wildlife. In particular, we highlight the impact on perception of auditory signals. As urbanization increases, birds either have to adapt to or avoid urbanized areas which can directly impact their success. Research has demonstrated that noise can mask communication between and within species, yet some species thrive in the city and show phenotypic differences in behavior, physiology, and morphology when compared to their rural conspecifics. Previous studies have shown that phenotypic and environmental variation are correlated (Stearns, 1989) and their relationship is reflected by distinct mechanisms such as vocal plasticity (Dingemanse et al., 2010). Studies of nightingales (*Luscinia megarhynchos*) and great tits demonstrate that birds adjust song amplitude (Brumm, 2004; Templeton et al., 2016) in response to background noise. Black-capped chickadees have been found to sing at a higher pitch with increases in anthropogenic noise (Proppe et al., 2012).

These and other findings suggest that birds are modifying their vocalizations as a result of noise in order to communicate with conspecifics (Fernandez-Juricic et al., 2005; Wood &

Yezerinac, 2012). However, how are the receivers, both conspecific and heterospecific, of these modified signals perceiving these modified vocalizations? And how does signal detection impact these perceptions?

For future studies, black-capped chickadees serve as an ideal subject given that they are both urban and rural birds, and further exploring the differences between these birds can aid in understanding how environmental pressures and evolutionary responses change vocalization function. For example, what differences do we see in urban vs. rural birds in terms of behavior, quality, and perceptual abilities. Behavioral traits such as aggressiveness and boldness have also been linked to urbanization and gradients of anthropogenic noise (Garamszegi et al., 2012; Hardman & Dalesman, 2018). A bolder individual may be drawn to urban areas as they are better equipped to survive there. The precise manner in which differences in behavioral traits can influence vocal characteristics is unknown, but it is possible that being more aggressive would be related to high plasticity in vocalizations between urban and rural environments. Urban male song sparrows (Melospiza melodia) are bolder and more territorial comparted to rural conspecifics (Evans et al., 2010). A study of dark-eyed juncos (*Junco hyemalis*) found an increase in boldness in those settling in an urban noisy environment (Atwell et al., 2012). A bolder individual may be drawn to urban areas as they better equipped to survive there. The way differences in behavioral traits can influence vocal characteristics is unknown, but it is possible that being more aggressive would mean high plasticity in vocalizations between urban and rural environments. Thus, it is also possible that this includes differences in perception. Overall, we find an abundance of research topics related to noise and animal communication left to explore.

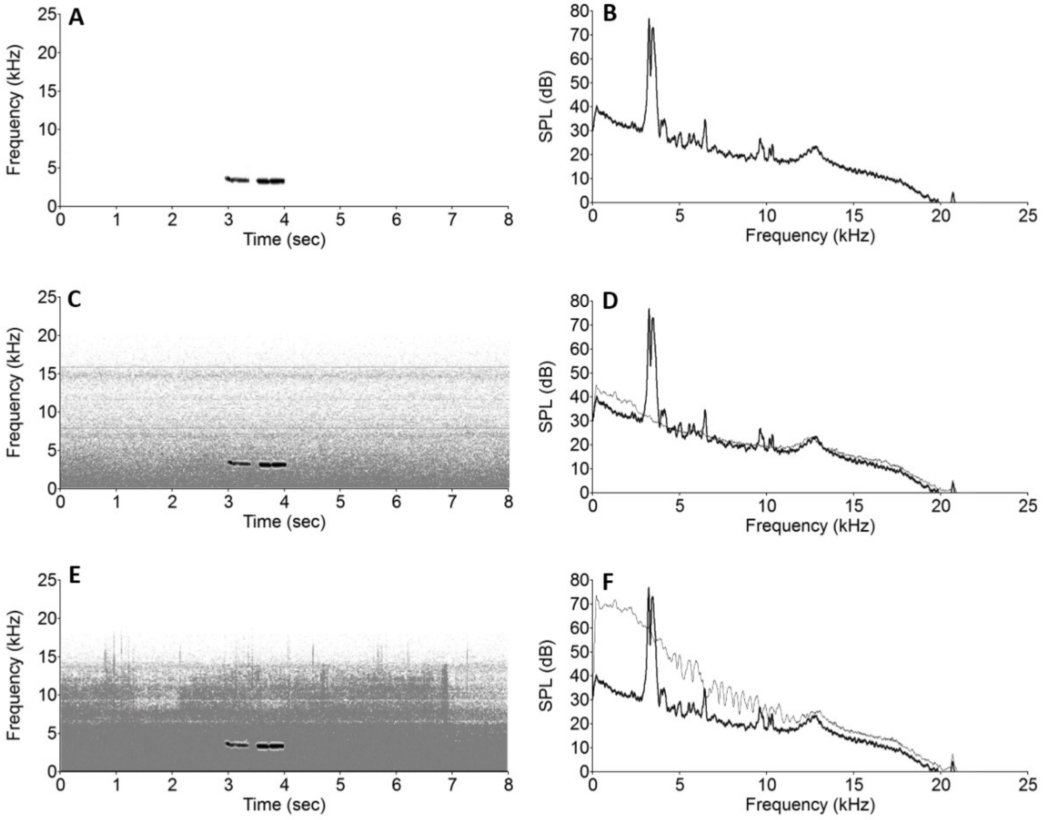

Figure 4-1. (A) Spectrogram of a female *fee-bee* song in silence. (B) Power spectrum of female *fee-bee* song in silence. (C) Spectrogram of female *fee-bee* song in low noise. (D) Power spectrum of female *fee-bee* song (black) in low noise (grey). (E) Spectrogram of female *fee-bee* song in high noise. (F) Power spectrum of female *fee-bee* song (black) in high noise (grey).

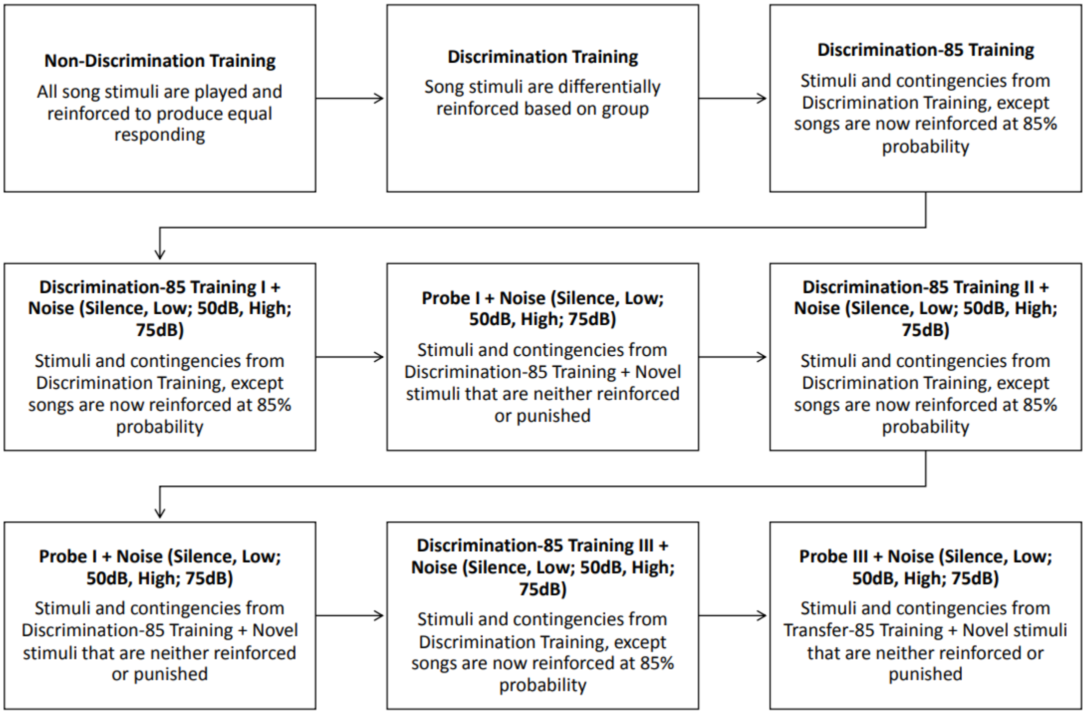

Figure 4-2. Flowchart depicting the stages of the experimental procedure including the name of each stage followed by a short description (see text for details).

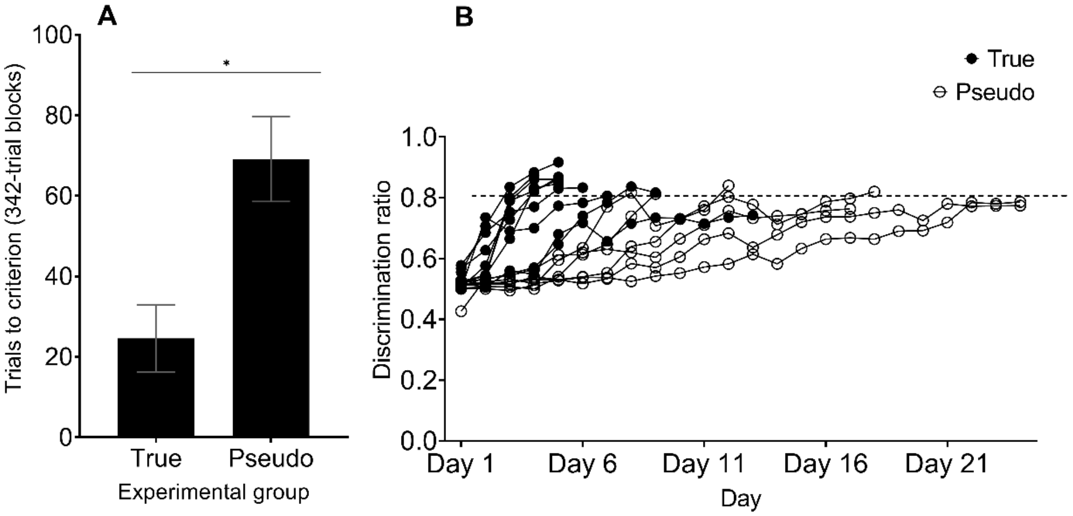

Figure 4-3. (A) Trials to criterion by True groups and Pseudo groups in Discrimination training. The following difference was significant (indicated by asterisk): True groups vs. Pseudo groups in Discrimination training (ANOVA, $F_{(1,12)} = 11.801$, $p = 0.005$). Error bars represent standard error. (B) Average discrimination ratio for all True ($n = 10$) and Pseudo ($n = 6$) birds by number of days during Discrimination training. Birds completed Discrimination training via six 342-trial blocks with a discrimination ratio greater than 0.80 (dashed line) with the last two blocks being consecutive.

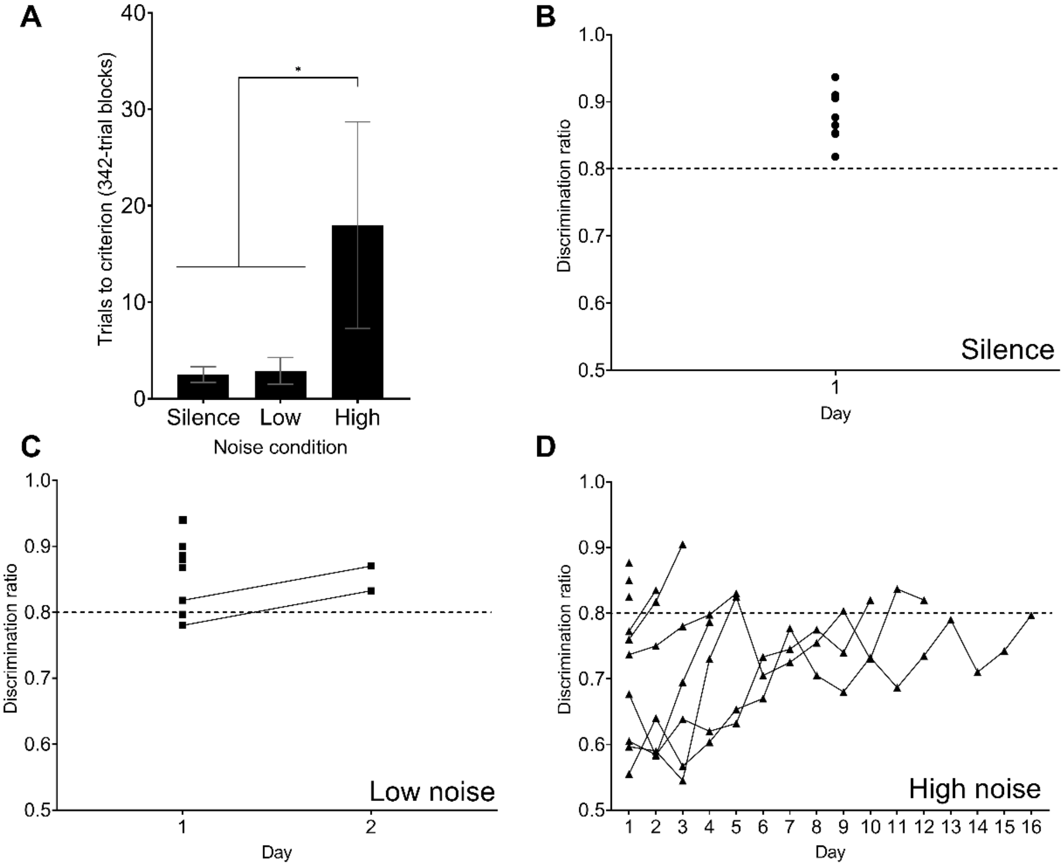

Figure 4-4. (A) Trials to criterion by True groups in Discrimination-85 with noise. The following differences were significant (indicated by asterisk): High noise condition vs. Silence ($p < 0.001$), High noise condition vs. Low noise condition ($p < 0.001$), and Low noise vs. Silence condition ($p = 0.002$). Error bars represent standard error. (B) Average discrimination ratio for all True ($n = 10$) birds by number of days during Discrimination-85 with silence. Birds completed Discrimination-85 with noise via two consecutive 342-trial blocks with a DR of at least 0.80 (dashed line). (C) Average discrimination ratio for all True ($n = 10$) birds by number of days during Discrimination-85 with low noise. (D) Average discrimination ratio for all True ($n = 10$) birds by number of days during Discrimination-85 with high noise.

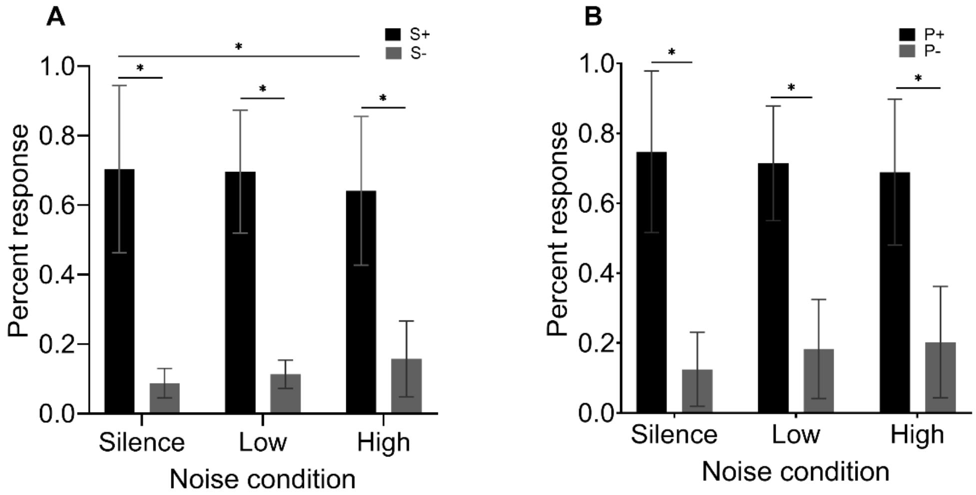

Figure 4-5. (A) Proportion response by True groups in Discrimination-85 with noise. S+ representing responses to rewarded stimuli and S- representing responses to unrewarded stimuli. The following differences were significant (indicated by asterisk): Silence (S+ vs. S-; $p < 0.001$, Low noise (S+ vs. S-; $p < 0.001$), High noise (S+ vs. S-; $p < 0.001$), and Silence vs. High (S+ vs. S+); $p < 0.017$). Error bars represent standard error. (B) Proportion response by True groups in Probe trials with noise. P+ and P- were based on whether stimuli was produced by the same birds as the S+ rewarded or the S- unrewarded stimuli. The following differences were significant (indicated by asterisk): Silence (S+ vs. S-; $p < 0.001$, Low noise (S+ vs. S-; $p < 0.001$), and High noise (S+ vs. S-; $p < 0.001$). Error bars represent standard error.

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Chapter 5 General Discussion

Summary of data chapters

In this dissertation, I investigated the perception, structure, and how the perceptual mechanism of female song informs function of female song in black-capped chickadees, as well as the impact that changes in our environment may have on chickadee communication. All data chapters (Chapters 2, 3, and 4) focused on female-produced song, a vocalization that is lacking in experimentation in both the laboratory and field, despite evidence of female chickadees singing in the laboratory (Campbell et al., 2019; Hahn et al., 2013; Hahn et al., 2015) and field (Dwight, 1897; Hill & Lein 1987). The studies conducted here included a variety of techniques including two operant conditioning go/no-go tasks; one of which examined individual identification of female chickadees via female song (Chapter 2) and the second examined the impact of noise on the ability to identify individual female chickadees (Chapter 3). Studies also included a bioacoustic analysis that used both discriminant function analyses and artificial neural networks in order to identify which acoustic features or cues are important in identifying individual female chickadees via their song (Chapter 4). I analyzed the song stimuli by individual but also by season in order to further explicate the function of female song in chickadees. When considering what is understood about the function of song in male chickadees, the proposed function of female song, and current results, we find that the two-note *fee-bee* song holds more information than just the sex of the bird producing the song. In addition, anthropogenic noise can negatively impact the transmission of information via chickadee song.

Perception: Categorization of female song

Chapter 2 was conceptualized in order to build on previous work that suggests there is a difference between female- and male-produced *fee-bee* songs. First, a previous bioacoustic analysis used potential for individual coding and discriminant function analyses (DFA) to find that female and male songs can be readily classified by sex (Hahn et al., 2013). A follow-up operant conditioning go/no-go task suggested that female and male chickadees can assess the sex of a singing conspecific (Hahn et al., 2015). Being able to identify the sex of the signaler would be extremely beneficial to a receiver in determining how to response. This is especially true in the black-capped chickadee which likely uses the same two-note song in both contexts of song use: mate attraction and territorial defense. Prior research has focused on male song in chickadees and suggests that more than just sex is encoded in song; song also includes information on quality/rank (Christie et al., 2004b; Otter & Ratcliffe, 1993) and individual identity (Christie et al., 2004a; Phillmore et al., 2002). However, we should note that these types of information may be related, meaning a receiver may be able to identify a signal individually by their high or low quality and/or rank.

In order to study these ideas further, I conducted an operant conditioning go/no-go task questioning if female and male chickadees can identify individual females based on hearing their *fee-bee* songs. Because our birds are housed individually in a laboratory setting, it is unlikely that rank based on a dominance hierarchy applies. Findings suggested that both sexes can discriminate between individual female chickadees via their song, and that True groups were able to learn this discrimination faster than Pseudo groups. True groups were also able to learn this discrimination faster when they were given a novel set of songs from the same previously reinforced females. In all Probe stages, True group birds were able generalize responding to a 12-song set of novel songs from the same previously reinforced and punished individual females. Probe III was particularly interesting as it introduced novel spliced *fee-bee* songs stimuli. Spliced songs contained either a *fee-* or *bee*-note from a previously rewarded female bird and, respectively, either a *bee-* or *fee*-note from another bird not included in the study, together creating a complete song with one note from a previously rewarded or unrewarded female. Data showed only one significant difference between songs containing a *bee*-note from a previously rewarded female vs. a *bee*-note from a previously unrewarded female, with higher responding to the previously rewarded stimuli. This finding suggests that the *bee*-note was sufficient for discriminating between individual females and to support generalized responding.

Further research is necessary to determine how chickadees are able to use the *fee-bee* song for individual identification. Chapter 2 found no differences between sexes at any stage of the study, leading to the conclusion that perhaps recognition of individual females based on song is equally important to both female and male chickadees. The idea of being able to identify a female being important to both sexes is appropriate if we consider the typical uses of song in black-capped chickadees, including mate attraction and territorial defense. The ability to quickly identify an individual female would be beneficial in saving time and energy if females are vocalizing to defend territories or to communicate with a mate or potential mate. While Chapter 2 provides support that both sexes attend to the same features and perceive relevant information, which features are responsible for this were unknown. Therefore, the next study in this dissertation focused on the acoustic features of female-produced *fee-bee* songs in chickadees.

Structure: Acoustic features of female song

Multiple studies have identified features within male and female song that may act as cues for individual differences and support the primary functions of song. Referring to studies conducted on male song in chickadees, the *fee* glissando is suggested as a necessary feature for individual recognition (Christie et al., 2004b; Shackleton et al., 1992). Other features such as total duration and the interval ratio (i.e., frequency at *fee*-note end divided by frequency at *bee*-note start) are used to identify individual males (Christie et al., 2004a). In addition, dominant males are able to maintain a more consistent interval ratio between notes (*fee* and *bee*) compared to subordinate males (Christie et al., 2004b). The *fee* glissando has also been identified as a feature used by female and male chickadees in identifying the sex of the chickadee producing the song (Hahn et al., 2013). Using the potential for individual coding and discriminant function analyses, a past study found that in females the *fee* glissando shows a greater change in frequency compared to the male *fee* glissando (Hahn et al., 2013). Chapter 2 found that both sexes can discriminate between females based on their song; Chapter 3 aimed to investigate if any of the features used to explore song in the past and more can be used to classify female song by the individual female who produces it (Christie et al., 2004a; Hahn et al., 2013; Hoeschele et al., 2010; Otter & Ratcliffe, 1993).

In order to investigate the acoustic features attended to for individual recognition via female song, I used discriminant function analyses (DFA) and artificial neural networks (ANN) to classify the set of female song stimuli previously used in Chapter 2. Two sets of analyses were run in order to observe differences in song by individual and by season (fall vs. spring). By individual, a DFA was able to classify a high percentage of songs to the correct female chickadee and identified six acoustic features as relevant for classification: (1) *bee*-note duration, (2) *fee*-note peak frequency, (3) *bee*-note proportion, (4) *fee*-note end frequency, (5) internote interval, and (6) *bee*-note peak frequency. A follow-up ANN was able to confirm classification by also correctly classifying a high percentage of songs, whereas a follow-up analysis found significant differences between the acoustic features identified by the DFA. By season, a DFA was also able to classify female song to the corresponding season to a high degree and identified three acoustic features: (1) *bee*-note length, (2) *bee*-note peak frequency, and (3) *fee* glissando. The ANN resulted in a similarly high performance, and a repeated measures MANOVA relieved significant differences between acoustic features as well.

Overall, results suggest that the ability to identify individuals is not note dependent, as both notes were identified as important in classifying songs, in terms of frequency and duration. In addition, the ability to classify songs is not season dependent. Further studies should focus on manipulating acoustic features of female *fee-bee* songs and measuring how specific manipulations impact the ability to discriminate between female chickadees based on song. A study of this nature would aid in further determining which features are most important for individual identification as well as how they may work cooperatively.

The perceptual mechanism informing the function of female song

Taken together (Chapter 2 and 3) found curious results in terms of individual identification as well as how the perceptional mechanism of female song may provide insight into the function of female song. Male song includes information regarding sex (Hahn et al., 2013; Hahn et al., 2015), quality and rank (Christie et al., 2004b; Mennill et al., 2002; Mennill & Ratcliffe 2004), and individual identity (Christie et al., 2004a; Hahn et al., 2015; Hoeschele et al., 2010; Phillmore et al., 2002; Wilson & Mennill, 2010), and all of these cues are beneficial to the function of male song, territorial defense and mate attraction. Female song contains information regarding sex (Hahn et al., 2013; Hahn et al., 2015) and from the current dissertation female song also contains information regarding individual identity (Chapter 2 and 3).

In support of the ability to identify individual females by their song, results show that both female and male chickadees are able to discriminate between females and also generalize responding to novel stimuli, suggesting that female song does matter. In addition, female song was able to be classified using many acoustic features within the two-note song. With so many features being significantly different and with females and males being able to perform this discrimination, an alternate explanation for this ability is that black-capped chickadees have an capacity for overall vocal recognition, similar to other animals (e.g., meerkats, dolphins, humans; Carlson et al., 2020). Prior research has also shown that chickadees are able to identify individuals by multiple vocalization including by *chick-a-dee* calls (Charrier & Sturdy, 2005; Mammen & Nowicki, 1981), possibly via *tseet* calls (Guillette et al., 2010), the aforementioned male *fee-bee* song (Phillmore et al., 2002), and now by female *fee-bee* songs. Even if true, the ability to discriminate between individual females by either voice or by vocalization suggests that female song is similar to male song.

We can find further support for the above reasoning in the *fee* glissando of female song. Chapter 3 found that the *fee* glissando in females is significantly different between the fall and spring. The *fee* glissando has also been identified as a sex-related cue, but all songs included in the study that found this were recorded in the spring (Hahn et al., 2013). Other bioacoustic analysis have also suggested that female and male songs differ by season (Campbell et al., 2019). If the function of female song is similar to male song, then perhaps we are observing a similar effect by season for females. Compared to the fall, the spring (breeding season) is when the biological functions of male song are most profound (Avey et al., 2008). Studies have also found seasonal changes in the auditory processing systems of chickadees, changes that match the acoustic properties of songs during and outside of the breeding season (Vélez et al., 2015). However, we must still consider that male and female songs do differ in form and thus, possibly function in this species.

Anthropogenic noise and female song

Chapter 4 of the current dissertation focused on introducing how environmental changes may be impacting animal communication, specifically increases in anthropogenic noise and auditory communication in black-capped chickadees. Increasing levels of anthropogenic noise have been connected to disturbances in human and non-human animals (Shannon et al., 2016). In non-human animals, disturbances via noise are associated with physical and/or physiological damage, and masking of communication signals (Shannon et al., 2016; Slabbekoorn & Ripmeester, 2008). However, several studies have shown that communication between animals is shifting in response to these challenges (Foote et al., 2004; Fuller et al., 2007; Gentry et al., 2017; Lampe et al., 2012; Proppe et al., 2012). Songbirds are particularly affected by anthropogenic noise as they are primarily vocal communicators, and we can examine this reflected in the adjustment's songbirds make to their vocalizations. European robins (*Erithacus rubecula*) are able to shift the temporal pattern of their vocalizations by singing earlier in the day to avoid high traffic noise (Fuller et al., 2007), and white-crowned sparrows (*Zonotrichia leucophrys nuttalli*) change the frequency and duration of vocalizations as a consequence of anthropogenic noise exposure (Gentry et al., 2017). Past research has also shown that black-capped chickadees will shift the frequency (Proppe et al., 2012) and timing (LaZerte et al., 2016) with which they sing when in the presence of anthropogenic noise. In addition, anthropogenic noise can mask acoustic signals, causing the fine acoustic details in songs to be obscured (Nemeth et al., 2013; Nemeth & Brumm, 2010).

In terms of anthropogenic noise, I was interested in how human-made noise pollution can impact the perception of signals. I was also interested in if female song has a function similar to male song or a completely different function then is it possible that the messages contained within the song could be masked. In order to answer this question, I used an operant conditioning go/no-go task to investigate how the presence of differing levels of noise impact the ability to discriminate between individual female chickadees via their *fee-bee* songs. Findings demonstrated that as noise level increased, learning to discriminate between individual females via their song decreased. Low-level (40dB) noise was detrimental to learning the discrimination, but high-level noise (75dB) was especially detrimental sometimes causing the birds to take almost three times as long to learn the discrimination, and this was true for both True and Pseudo groups. However, for the True groups, once the discrimination between individual females was learned, birds were able to generalize responding to novel stimuli at all levels of noise. Not only did this study replicate results from Chapter 2 but this study also suggests that the birds who did eventually learn the discrimination somehow extended their discrimination to novel songs even in the presence of the noise that delayed reaching criteria.

Conclusion

The aim of this dissertation was to increase our understanding of the perception, structure, and function of female song in black-capped chickadees, as well as explore the impact of our changing environments. The current studies demonstrate that female song in chickadees contains cues that receivers can use to identify individual females and that the *fee* glissando may serve a similar function to the male *fee* glissando, and thus supports that the function of female song may be territorial defense and mate attraction. However, these results were found through the exploration of the perceptual mechanisms of female song, further testing is required for finding the function of female song. I also found that anthropogenic noise could be masking information found in female song, yet over time that information can be heard. By focusing on one species of songbird and one type of song, produced by one sex, the studies in this dissertation have provided insights into female song use in songbirds, the overall cognitive abilities of chickadees, the impact of anthropogenic noise on wildlife, and a comprehensive knowledge and understanding of chickadee communication.

With that said, many questions remain unanswered. Beginning with female song, the development of female song is still largely unexplored. How do females learn their song and who do they learn it from? What differences might exist between male and female chickadees in the song control system, both in production and perception of female song? And ultimately, what is the function of female song? In order to further explore the function of female song, studies should focus on what other information female song in chickadees provides. It is possible that both sexes can also gather information about an individual female's rank and quality? Studies also need to focus on the context in which a female bird would sing. Lastly, anthropogenic noise-related studies should continue focusing on both the perception and production of song in noise. For example, the results seen in Chapter 4 may have differed if the song stimuli used was recorded in anthropogenic noise vs. silence. If the birds had naturally shifted in their amplitude or frequency to be heard over noise, then results might have not shown any masking effects or less of an effect. Addressing these many lines of research will allow us to have an even deeper understanding on vocal communication in chickadees and songbird in general.

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