Attending to the *chick-a-dee* call of the black-capped chickadee (*Poecile atricapillus*)

by

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Abstract

Black-capped chickadees (*Poecile atricapillus*) are a species of songbird commonly found across much of North America. Their namesake *chick-a-dee* call is an acoustically complex vocalization that is partially learned (Hughes, Nowicki, & Lohr, 1998) and whose syntax is comparatively similar to that of human language (Hailman & Ficken, 1986). This call serves a variety of functions including flock coordination and synchronization of mobbing behaviours, however there is much to learn about the production, perception, and meaning of this complex vocalization.

In Chapter 2, I conducted operant conditioning go/no-go discrimination tasks to test black-capped chickadees' perception of acoustic categories, specifically the sex of the signaller. I found that while chickadees can learn to discriminate male calls from female calls, they do so by memorizing training stimuli, rather than learning to discriminate between conceptual categories.

Chapter 3 investigated how the note order within calls is perceived using both categorization and individual preference as evaluated by a choice preference task. I found that chickadees not only learn to discriminate between natural-ordered and scramble-ordered *chick-a-dee* calls, but they do so using open-ended categories. Additionally, individual preference appears to be related to rate of learning on the discrimination task.

In Chapter 4, I used a behavioral task to investigate the impact of anthropogenic noise on feeding behavior, also asking if conspecific *chick-a-dee* calls might mitigate those changes. Though the playback of conspecific calls did not lessen the effect of anthropogenic noise, we learned that feeding behaviour does change when birds are exposed to anthropogenic noise such that birds feed less during playback than they do during silence.

Overall, the results of these experiments revealed the variety of acoustic features present in *chick-a-dee* calls, increasing our understanding of how chickadees do or do not perceive those features, and illuminating behaviors that occur in response to those features. This adds to our understanding of an acoustically and functionally complex vocalization of a small North American songbird.

Preface

All procedures outlined in this document followed the Canadian Council on 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 and 107).

Chapter 2 of this thesis is currently under revision to be submitted for publication. I was responsible for experimental design, data collection and analysis, and manuscript composition. A.H. Hahn contributed to experimental design and manuscript edits. J.V. Congdon and E.N. Scully contributed to data collection and manuscript edits. C.B. Sturdy was the supervisory author and was involved in experimental design and manuscript edits.

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Dedication

For Nannie and Papa. You encouraged me to learn, even if you didn’t know what about.

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Chapter 1: General Introduction

Vocal Communication

Communication is the process by which one organism, a signaler, produces a detectable signal that is perceived by another, a receiver; This signal is often described as containing information, which refers to the content of the signal (Stegmann, 2013). For example, meerkat (*Suricata suricatta*) sentinels use different types of vocalizations to warn of different types of predators, evoking behaviours appropriate for each type (Rauber & Manser, 2017). There are several forms of communication which each employ a different modality of signal (e.g., olfactory communication occurs via chemical signals, visual communication occurs via visual signals) however this research will focus on acoustic communication using auditory signals. Animals often rely on acoustic communication in environments where visual signals would be lost. For example, many avian species rely on acoustic rather than visual signals to tell con- and heterospecifics apart because vegetation and other obstructions can interfere with visual cues (Becker, 1982).

Obstructions may also interfere with auditory signals, such as physical barriers blocking transmission of sounds (e.g., trees, rocks, manmade sound barriers). Other sources of sound in the environment may also mask acoustic signals (e.g., acoustic signals from other signalers, wind moving through vegetation, traffic noise), leading to an inability to separate signal content from background noise. Many signalers incorporate redundant information into their acoustic signals, which aides in combating the potential for signal loss, masking, or degradation (Bradbury & Vehrencamp, 1998; Wiley, 1983). Redundancy may be enhanced by combining acoustic signals with other forms of signal (e.g., a visual display along with acoustic signaling), having the same information contained at different points within the same vocalization (e.g., at the start and end of the vocalization), and repetition of the same vocal signal are some methods of creating redundancy (Bateson, 1968; Endler, 2000; Hauser, 1996; Smith, 1977; Uetz & Roberts, 2002).

Researchers have long been interested in the nature of the information contained within acoustic signals as well as the ability of receivers to perceive and act upon those signals (e.g., Darwin, 1869); However, these factors are complex, interconnected, and therefore difficult to investigate. Most research into the content of vocal signals has focused on either the observation and categorical description of vocalizations produced in the natural habitat, the comparison of the acoustic structure of vocalizations and the apparent behavioural function (as determined by production context), or the investigation of biological mechanisms involved in the production and perception of vocalizations (for review, see Garcia & Favaro, 2017). Though individually these approaches have drawbacks (e.g., naturalistic observation is filtered through the restrictions of human perception, bioacoustic analyses are not guaranteed to identify biologically relevant differences in acoustic features, and neural assays reveal activation but not underlying processes), a more thorough understanding of communication systems may be obtained by combining methodologies (Sturdy & Weisman, 2006). One common practice is the use of model species, that is a common or easily accessible animal species that can be studied and the results applied more generally to a wider array of species, to investigate this topic. Songbird species have often served as models for understanding acoustic communication.

Songbirds

Songbirds, also called oscines, make up nearly half of the approximately 10 000 known species of birds that exist today (Sibley & Monroe, 1990). Compared to other bird species, songbirds possess a more complex vocal organ, the syrinx, allowing them to produce intricate and diverse vocalizations, particularly the songs after which they are named (Larsen & Goller, 1999). This subgroup of the order Passeriformes is unique from non-songbirds in both the production and perception of their vocalizations. Unlike other avian species, songbirds are vocal learners, which means that they learn to produce many of their species-typical vocalizations by listening to and imitating adult tutors of their own species (for review, see Ball & Hulse, 1998; Catchpole & Slater, 1995). This trait is shared by a limited number of other animal groups including humans, parrots, hummingbirds, cetaceans, bats, and elephants (Tyack, 2008) as well as pinnipeds (e.g., seals, sea lions, and walruses; Reichmuth & Casey, 2014). In the case of songbirds, it involves the use of both their morphologically complex syrinx and specialized neural pathways (Mello, Velho, & Pinaud, 2004). Vocal learning in songbird species is of particular interest because the stages of the process and brain areas involved parallel language learning in humans (Doupe & Kuhl, 1999; Bolhuis, Okanoya, & Scharff, 2010).

Songbird vocalizations are divided into two broad categories, songs and calls, based primarily on both their form and function. Songs are typically more acoustically complex than calls and are often used in the context of mating and territory defense, while calls are used more broadly and are produced year-round in contexts as varied as flock cohesion, warning of predators, and locating individuals (Catchpole & Slater, 2008). In most songbird species, songs are studied more extensively than are their calls (for review, see Slater, 2003). Research has focused primarily on the production and perception of song due to its complexity and the fact that many songs are learned. This has left calls comparatively ignored, due to the fact that their production is thought to be innate. However, both calls and songs can have learned and innate components, as seen in zebra finch (*Taeniopygia guttata*) long calls (Simpson & Vicario, 1990) and black-capped chickadee (*Poecile atricapillus*) *chick-a-dee* calls (Hughes, Nowicki, & Lohr, 1998).

Black-capped chickadees

Black-capped chickadees have been used as a model species to study vocal production and perception, particularly as it relates to learning to produce vocalizations. Unlike zebra finches, a common songbird model species in which only the males produce songs, black-capped chickadee males and females both have the ability to sing (Hahn, Krysler, & Sturdy, 2013). Black-capped chickadees are a small species of songbird that can be found across much of North America (Smith, 1991). As songbirds, black-capped chickadees are also vocal learners in that proper production of their song, as well as a call, is learned from adult conspecific tutors (Shackleton & Ratcliffe, 1993; Hughes, Nowicki, & Lohr, 1998). Unlike the pattern seen in the vocalizations of most songbird species, black-capped chickadees produce only a single, acoustically simple song (the *fee-bee* song) and produce multiple types of calls that range in acoustic complexity from a single note (e.g., *tseet* calls) to multiple notes arranged in a particular, syntax-governed order (e.g., *chick-a-dee* calls; Ficken, Ficken, & Witkin, 1978). For the purpose of this dissertation, I will focus on the information contained within *chick-a-dee* calls and how black-capped chickadees may attend to these signals.

***Chick-a-dee* calls**

The *chick-a-dee* call of the black-capped chickadee is composed of four main note types that may be repeated or omitted within a given call, however they almost always occur in the order A → B → C → D (Ficken, Ficken, & Witkin, 1978). This combinatorial nature yields a theoretically unlimited repertoire of producible call forms (Hailman, Ficken, & Ficken, 1985) and the computable syntax is not unlike that of human language (Hailman & Ficken, 1986). Produced year round by both sexes, utterance of this call occurs most often in the fall and winter (Avey, Quince, & Sturdy, 2008).

The *chick-a-dee* call is produced to coordinate flock movements and inform conspecifics of the presence of food sources (Freeberg and Lucas 2002; Gladdis 1985; Smith, 1991), maintaining flock cohesion (Nowicki, 1983), and the coordination of con- and heterospecific mobbing behaviour in response to predators (Hurd, 1996). In addition to these identified functions of the call, *chick-a-dee* calls are also known to differ acoustically depending on a number of qualities including species within the genus *Poecile* (Bloomfield et al., 2003; Bloomfield & Sturdy, 2008), flock membership (Mammen & Nowicki, 1981; Nowicki, 1989), and individual identity (Charrier et al., 2004).

With a variable form and myriad of known functions, there is still much to learn about the *chick-a-dee* call of black-capped chickadees. In this dissertation, I will describe studies designed to learn more about the types of information that may be contained within these calls, how individual chickadees perceive that information, and how their behaviour changes in response.

Current Research

My first goal was to investigate the ability of black-capped chickadees to perceive the difference between male- and female-produce *chick-a-dee* calls. Previous research has found some evidence of acoustic differences between male- and female-produced *chick-a-dee* calls in both black-capped chickadees (Campbell et al., 2016) as well as Carolina chickadees (*Poecile carolinensis*; Freeberg et al., 2003). However, there is no evidence that chickadees of either species perceive or attend to these identified acoustic features. In Chapter 2, I conducted two operant conditioning tasks in which birds were trained to respond differentially to two categories of stimuli, *chick-a-dee* calls produced by either male or female black-capped chickadees. Through the course of the first experiment, we determined that black-capped chickadees could learn to discriminate between male- and female-produce *chick-a-dee* calls but did not treat the two types of calls as distinct perceptual categories. Experiment 2 was a modification of the original experiment intended to determine if removing potentially distracting acoustic cues (here, removing the terminal "*-dee*" portion of the same call stimuli as used in experiment 1) would facilitate category learning based on the remaining portion of those calls. Again, we found that birds could learn the discrimination but were unlikely to use categorization as a mechanism to do so. The results suggest that, while sex may be a useful category in vocalizations related to mating, like *fee-bee* song, individual identity and recognition may provide sufficient information for other vocalizations (i.e., in calls). This finding is significant because it adds to what we know about the perception of information contained within these vocalizations. Specifically, we now know that the acoustic features identified by Campbell and colleagues (2016) are not necessarily features that chickadees attend to in order to discriminate between male and female signalers.

Since black-capped chickadee *chick-a-dee* calls are produced in a set order (Ficken, Ficken, & Witkin, 1978), I sought to better understand how individuals perceive vocalizations that follow and break this fixed syntax. Specifically, I wanted to determine if black-capped chickadees treat natural-ordered *chick-a-dee* calls as an open-ended perceptual category. In Chapter 3, I conducted two behavioral tasks: 1) a choice preference task designed to evaluate whether black-capped chickadees prefer to hear natural-ordered or scramble-ordered (i.e., unnatural) *chick-a-dee* calls based on how often and for how long they sat on perches associated with each type of call, and 2) a go/no-go operant discrimination task designed to evaluate whether or not birds could discriminate between natural-ordered or scramble-ordered calls and if they treated those types of calls as separate perceptual categories. I also examined the relationship between preference for a call type and performance on discriminating between the two call types. My results show that, not only do black-capped chickadees treat natural-ordered *chick-a-dee* calls as an open-ended category, their individual preference for natural-ordered or scramble-ordered calls was related to their performance in discriminating between the two call categories. This finding is significant because chickadees are treating “normal” calls (i.e., calls that follow standard syntax) as different from “abnormal” calls, indicating that order is important.

Research has shown that black-capped chickadees will shift the pitch at which they sing in the presence of anthropogenic, or human-produced, noise (Proppe et al., 2012). Another study by Schepers and Proppe (2017) found that playback of conspecific calls can increase population density of songbird species living adjacent to noisy roadways. The experiment described in Chapter 4 was designed to investigate how playback of black-capped chickadee *chick-a-dee* calls affect changes in feeding behavior that occur due to the presence of anthropogenic noise. We monitored visits to a feeder while birds heard silence, conspecific chickadee calls, brown noise, or a combination of calls and noise. Our results showed that birds fed less overall on days when they heard call playback than on days when they heard noise or call and noise playback, which may be due to an increased metabolism caused by the stress of hearing playback. We also found that birds fed less during all playback types then they did during silence, suggesting that they may decrease feeding behaviour to increase vigilance (as seen in Chaffinches [*Fringilla coelebs*]: Quinn et al., 2006). These results are important because they provide evidence that chickadees are capable of adjusting their behaviour in response to anthropogenic noise, but also because it highlights the importance of further research into how species adapt in the presence of anthropogenic noise.

Chapter 5 provides a general discussion, summarizing the results and conclusions from the earlier data chapters. Here, I connect my findings to the existing body of work and discuss possible future directions for research.

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Chapter 2: Perception of sex differences in black-capped chickadee (*Poecile atricapillus*) *chick-a-dee* calls

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Introduction

As a species that typically inhabits sparse to dense forest across much of North America, black-capped chickadees (*Poecile atricapillus*) often rely on vocalizations to communicate between a signaller, the individual producing the vocalization, and a receiver, the individual perceiving the vocalization (Smith, 1991). This is true of a variety of avian species which rely on acoustic, rather than visual, signals to discriminate between con- and heterospecifics because acoustic cues travel farther than visual cues, and vegetation, among other obstructions, can interfere with visual cues (Becker, 1982). In addition to containing species-specific cues, avian vocalizations also allow for individual recognition (Falls, 1982; Nelson & Poesel, 2007), identification of individual dominance rank (Massen, Pašukonis, Schmidt, & Bugnyar, 2014; Hahn, Guillette, Hoeschele, Otter, Ratcliffe, & Sturdy, 2017), as well as recognition of a signaller's sex (Hoelzel, 1986; Pavlova, Pinxten, & Eens, 2005).

Songbirds produce acoustically-complex learned vocalizations (i.e., songs) which function in territory defense and mate attraction, in addition to comparatively simple vocalizations (i.e., calls) which may or may not be learned and are produced in situations typically unrelated to mating behaviours (Catchpole & Slater, 2008). Unlike most songbirds, chickadees produce a single acoustically-simple song (i.e., *fee-bee* song), and numerous calls that vary in complexity from single notes (e.g., *tseet* call) to multiple notes with fixed, semantically-governed order (e.g., *chick-a-dee* call; Ficken, Ficken, & Witkin, 1978).

Here we focus on the *chick-a-dee* call, one of the most complex (in both acoustics and usage) and versatile vocalizations that black-capped chickadees produce. This call consists of four note types that occur in a relatively fixed order (A → B → C → D), though each note type may be repeated or omitted, allowing for numerous and varied combinations (see Figure 2-1;

Ficken, Ficken, & Witkin, 1978). This variation makes it possible for the call to serve multiple functions, including informing conspecifics regarding the presence of food (Smith, 1991), the coordination of con- and heterospecifics to assist in mobbing behaviour against predators (Hurd, 1996), and maintaining flock cohesion (Nowicki, 1983). Additionally, the *chick-a-dee* call has been found to differ acoustically based on a caller's individual identity (Charrier et al., 2004), flock membership (Mammen & Nowicki, 1981; Nowicki, 1989), and species (Bloomfield et al., 2003; Bloomfield & Sturdy, 2008).

Sex differences have been documented in black-capped chickadee vocalizations, including their *fee-bee* songs (Hahn et al., 2013) and *tseet* calls (Guillette et al., 2010), as well as in the *chick-a-dee* calls of the closely-related Carolina chickadee (*P. carolinensis*; Freeberg et al., 2003). Conceptually, determining the sex of an unknown signaler may be beneficial in informing a receiver's actions. For example, if an individual male hears another male in his territory, the correct response would be to defend the territory aggressively; however, this response would not be appropriate if the intruder is a female as there may be an opportunity to mate. Bioacoustic analyses of black-capped chickadee *chick-a-dee* calls found evidence of some acoustic differences between A notes produced by males and females (Campbell, Hahn, Congdon, & Sturdy, 2016). This featural analysis was conducted using a series of measurements related to call duration and frequency at various time points on a sound spectrogram. Chickadees may or may not attend to these human-selected acoustic features, or they may attend to other components within the call not captured by the bioacoustic analysis.

In the current study, we used an operant go/no-go discrimination task to evaluate if black-capped chickadees can discriminate between male- and female-produced *chick-a-dee* calls. We also manipulated calls to determine which portions of the call, if any, aid in this potential discrimination. Birds were, for example, trained to respond ('go') to male-produced *chick-a-dee* calls and withhold responding ('no-go') to female-produced calls; following training, birds were then tested with additional male- and female-produced calls, and manipulated calls. For the birds in the True groups, in which rewarded and unrewarded stimuli consisted of categories (i.e., male calls and female calls potentially forming perceptual categories), if the birds were using open-ended categorization to perform the discrimination, we would expect them to continue responding to these new calls in the Transfer stage using the same contingencies as they learned in Discrimination training (i.e., birds reinforced for responding to male calls would continue to respond to novel male calls). Conversely, if birds did not use categorization and instead relied on rote memorization to discriminate between rewarded and unrewarded stimuli in Discrimination training, we would expect to see non-differential responding to the novel calls presented in the Transfer stage as the learning would not transfer to new stimuli. Birds in the Pseudo groups, in which rewarded and unrewarded stimuli were randomly selected from between categories (i.e., random calls not forming perceptual categories), could only rely on rote memorization to discriminate between rewarded and unrewarded calls.

EXPERIMENT 1

Methods

Subjects

Twenty-one wild-caught black-capped chickadees (11 males, 10 females) captured between February 3, 2013 and January 20, 2015 were used in Experiment 1. Birds were captured in Edmonton (North Saskatchewan River Valley, 53.53°N, 113.53°W; Mill Creek Ravine, 53.52°N, 113.47°W) and Stony Plain (53.46°N, 114.01°W), Alberta, Canada and were aged as older than one year at time of capture using the shape and colour of outer tail retrices (Pyle, 1997). Sex was determined using DNA analysis (Griffiths, Double, Orr, & Dawson, 1998). All birds had previous experience with the operant discrimination paradigm, discriminating biological (owl calls, fee-bee songs; Congdon et al., 2019; Hahn, et al., 2015) and synthetic (tonal) stimuli (McMillan, et al., 2017). Fourteen birds had experience discriminating *chick-a-dee* calls, but none had experience with the calls used in the current experiment. Previous experience was balanced across groups.

Housing

Before and after the experiment, birds were housed in individual 30 × 40 × 40 cm cages (Rolf C. Hagen, Inc., Montreal, QC, Canada) in communal colony rooms which allowed for visual and auditory, but not physical contact between conspecifics. Birds had *ad libitum* access to food (Mazuri Small Bird Maintenance Diet; Mazuri, St. Louis, MO, U.S.A.), water, grit, and cuttlebone. Water was supplemented with vitamins three days a week (Prime Vitamin Supplement; Hagen, Inc.). Food supplements included three to five sunflower seeds daily, one superworm (*Zophobas morio*) three times a week, and a mixture of egg and either parsley or spinach twice a week. The light:dark cycle was maintained to match the local day:night cycle for Edmonton, AB, Canada.

Apparatus

During the experiment, birds were housed individually in an experimental apparatus that consisted of a modified 30 × 40 × 40 cm home cage within a sound attenuating chamber. The chamber was supplied with a constant flow of air and lit by a 9 W, full spectrum fluorescent bulb using an on-off schedule that matched the local day-night cycle for Edmonton, AB, Canada. An 11 × 16 cm opening on one side of the cage allowed the birds to access a motorized feeder. The cage contained three perches, a grit cup, a water bottle, and cuttlebone. Birds received one superworm twice daily. Mazuri food was accessible only after a correct response, as a reward. Infrared beams in the feeder and in the perch closest to the feeder tracked the position of the bird during testing. A single-board computer tracked responses and set up trials, sending and receiving data from a personal computer which also stored and played acoustic stimuli. Stimuli passed through an NAD310 Integrated Amplifier (NAD Electronics, London, U.K.) or a Cambridge A300 or 640A Integrated Amplifier (Cambridge Audio, London, U.K.) before coming through a Fostex FE108 Σ full range speaker beside the feeder (Fostex Corp., Tokyo, Japan; frequency response range: 80-18 000 Hz). See Sturdy and Weisman (2006) for a detailed description of the experimental setup.

Acoustic Stimuli

A total of 248 *chick-a-dee* calls were obtained from 44 black-capped chickadees (22 males, 22 females) and used to create 268 acoustic stimuli (summarized in Table 2-1). Individuals that provided vocal stimuli were unfamiliar to those that served as subjects. High-quality vocalizations were recorded from individuals isolated in sound-attenuating chambers (1.7 m × 0.84 m × 0.58 m; Industrial Acoustics Company, Bronx, NY). Individual *chick-a-dee* calls were cut from recordings and bandpass filtered outside of frequency range of *chick-a-dee* calls (2000 - 5000 Hz) using GoldWave 6.19 (GoldWave, Inc., St John's, NL, Canada). These calls were prepared as outlined below and played at a volume of ~75 dB as measured at the request perch (Type 2239, Brüel & Kjær Sound & Vibration Measurement A/S, Nærum, Denmark; A-weighting, slow response) during the experiment.

Natural call stimuli. One hundred and twenty unmanipulated calls from 24 individuals (12 males, 12 females) were used as stimuli for the discrimination stage and 38 unmanipulated calls from 20 individuals (10 males, 10 females) were used as stimuli for the transfer stage (see 'Procedure' below). Five ms of silence was added to the beginning and end of each stimulus using SIGNAL 5.06.07 (Engineering Design, Berkeley, CA, U.S.A.), then each file was tapered to remove transients and amplitude was equalized.

Manipulated call stimuli. Ninety additional calls from the same 20 individuals that provided stimuli for Transfer testing were modified as follows for Test trials.

Partial call stimuli. Ten male and 10 female calls (two calls each from 10 individuals) were cut into their initial "chick-a" portion (A, B, and/or C notes) and terminal "-*dee*" portion (D notes) such that each call yielded two stimuli (one "*chick-a*" and one "-*dee*" portion). These 40 portioned stimuli (10 male, 10 female "*chick-a*" stimuli; 10 male, 10 female "-*dee*" stimuli) were then prepared in the same manner as the natural call stimuli.

Spliced call stimuli. These stimuli were created by randomly splicing together the "*chick-a*" and "-*dee*" portions created as partial call stimuli to form *chick-a-dee* calls made of notes from two different individuals. Ten stimuli were created from male "*chick-a*" and female "-*dee*" portions (M-F calls), ten were created from female "*chick-a*" and male "-*dee*" portions (F-M calls), ten were created with portions from two males (M-M calls), and ten were created with portions from two females (F-F calls), yielding a total of 40 spliced stimuli which were then prepared in the same manner as the natural call stimuli.

Single note-type spliced call stimuli. To create these stimuli, a single note-type from one call was replaced with notes of the same type from another call. Care was taken to select calls that contained a similar number of the note type that was exchanged in order to account for potential redundancy on call composition contained within call notes (see Freeburg, Lucas, & Clucas, 2003). For example, replace the A notes from a call with five notes (e.g., AACDD) with

A notes from a call with four to six notes of similar note structure (e.g., AADD). As with spliced call stimuli, single note-type spliced call stimuli were created from calls with all possible sex combinations (i.e., M-F, F-M, M-M, and F-F). Additionally, these stimuli were created by splicing each of the three introductory note types (A, B, and C notes) between calls from different individuals (the spliced call stimuli function as D-note splices). Due to limitations of call composition where certain note types are produced less frequently than others (A notes are produced most often; C notes are produced least often; Campbell et al., 2016), fewer C-note splice stimuli were created than either A- or B-note splice stimuli. Overall, 12 A-note splice stimuli (5 M-F calls, 5 F-M calls, 1 M-M call, and 1 F-F call), 12 B-note splice stimuli (5 M-F calls, 5 F-M calls, 1 M-M call, and 1 F-F call), and six C-note splice stimuli were created (2 M-F calls, 2 F-M calls, 1 M-M call, and 1 F-F call). These 30 stimuli were then prepared in the same manner as the natural call stimuli.

Procedure

Pretraining. Following basic training (i.e., shaping) to familiarize birds with the process of obtaining food from the experimental apparatus, Pretraining began. This stage ensured that birds responded in sufficient quantity and non-differentially to stimuli that would be rewarded and unrewarded during later stages. During Pretraining, birds were exposed to all stimuli that would be presented throughout the experiment and reinforced for responding to all stimuli. Each trial began with the bird landing on the request perch, breaking the perch's infrared beam and triggering a stimulus to play. Stimuli were grouped into 536-trial bins and were presented in a random order, without replacement, until all stimuli had been heard. The bird was required to remain on the request perch for 1084-2825 ms after initiating a trial in order to ensure the whole stimulus was heard. If the bird left the perch before the stimulus finished playing, the trial was considered interrupted and the bird received a 30-s timeout in which the houselight was off and a new trial could not be initiated. If the bird flew to the feeder within 1 s of the completion of the stimulus, the bird was rewarded with 1 s access to food followed by a 30-s intertrial interval during which the houselight was on and a new trial could not be initiated. Remaining on the perch for 1 s longer than the stimulus triggered a 60-s intertrial interval during which the houselight was on and a new trial could not be initiated. However, this interval would end if the bird left the perch. Birds continued on Pretraining until they displayed 60% responding or higher for six 536-trial blocks, a less than 3% difference in responding to rewarded and unrewarded stimuli for four blocks, and a less than 3% difference in responding to categories of future testing stimuli for four blocks. During this stage, one male was removed from the study after failing to feed at a sufficiently high rate and one female was removed from the study due to equipment failure.

Discrimination training. In this stage, the method of stimulus presentation remained the same as in Pretraining, however only 120 of the stimuli played during the previous stage (unmanipulated natural call stimuli from 12 males, 12 females) were presented here. Additionally, these stimuli were differentially rewarded such that half remained rewarded (S+) and the other half were reassigned to be unrewarded (S-). Responding to these unrewarded stimuli now resulted in a 30-s intertrial interval in which the houselight was off and a new trial could not be initiated. Birds' experimental group determined which stimuli were rewarded and which were unrewarded.

Birds were randomly assigned to either a True category discrimination group or a Pseudo category discrimination group. The True category discrimination group consisted of two subgroups that were rewarded for either male calls (*n* = 6; 3 males, 3 females) or female calls (*n*

= 6; 3 males, 3 females). The Pseudo category discrimination group consisted of two subgroups (Pseudo 1: *n* = 2; 2 males, 1 female; Pseudo 2: *n* = 3; 1 male, 2 females) that were rewarded for responding to 60 randomly-selected calls (30 male, 30 female) and unrewarded for responding to the remaining 60 calls (30 male, 30 female).

Birds continued on Discrimination training until they completed six 120-trial blocks with a discrimination ratio (DR) of at least 80%. The last two of these blocks needed to be consecutive. DR was calculated by dividing the mean percentage of response to all rewarded stimuli by the mean percentage of response to rewarded stimuli plus the mean percentage of response to unrewarded stimuli, then multiplying by 100. With this calculation, a DR of 50% indicates equal responding to rewarded and unrewarded stimuli, while a DR of 100% indicates responding to only the rewarded stimuli (i.e., perfect discrimination). During this stage two males, one in the Female S+ group and the other in the Pseudo 1 group, were removed from the experiment for failing to learn the discrimination in more than double the number of trials required for the other subjects to complete this stage (91 and 110 calculations, respectively).

Discrimination 85 training. This training stage was identical to Discrimination training except that the rewarded stimuli were now only reinforced 85% of the time. This meant that on 15% of trials, when a stimulus from the rewarded category was played, entering the feeder resulted in a 30-s intertrial interval in which the houselight remained on without access to food. The purpose of this stage was to expose birds to trials in which responses to stimuli were neither rewarded or punished, as would be encountered in both Transfer and Probe trials. As with the Discrimination stage, birds continued on Discrimination training until they completed six 120-trial blocks with a discrimination ratio (DR) of at least 80%, where the last two of these blocks needed to be consecutive.

Transfer testing. The stimuli and associated reward-contingencies from Discrimination 85 training were maintained in Transfer testing. Additionally, birds were exposed to 38 calls (17 male, 17 female calls provided by different individuals than those that produced the calls used in Discrimination training) that had previously been non-differentially reinforced (i.e., had been played during Pretraining), but were not heard during Discrimination training. Responding to these novel Transfer stimuli resulted in a 30-s intertrial interval in which the houselight remained on without access to food being provided, meaning that the trials were neither rewarded nor punished. These stimuli were presented once during 278-trial blocks in which the stimuli presented during Discrimination training were presented twice each. Stimuli were randomly selected for playback from the total pool of available stimuli without replacement. Birds remained on Transfer until they completed three 278-trial blocks. Following this, they returned to Discrimination 85 training until they completed two 120-trial blocks with a discrimination ratio (DR) of at least 80%, where these blocks needed to be consecutive. Transfer calls were introduced to test for the method of learning that birds employed to discriminate between the rewarded- and unrewarded-contingencies presented in Discrimination training.

Test trials. Similar to Transfer testing, the stimuli and associated reward-contingencies from Discrimination 85 training were maintained in Test trials. Additionally, birds were exposed to 110 manipulated calls (40 partial call stimuli, 40 spliced call stimuli, and 30 single note-type spliced call stimuli provided by different individuals than those that produced the calls used in either Discrimination training or Transfer testing) that had previously been non-differentially reinforced (i.e., had been played during Pretraining) but were not heard during other stages. Responding to these novel Test stimuli resulted in a 30-s intertrial interval in which the houselight remained on without access to food being provided; these trials were neither rewarded nor punished. These Test stimuli were randomly divided into four unique 267- or 268-trial blocks. Within each block, the 27-28 Test stimuli were presented once and the Discrimination training stimuli were presented twice each. Stimuli were randomly selected for playback from the total pool of available stimuli without replacement. Birds remained on Test trials until they completed three 267- or 268-trial blocks. Between each Test trial session, birds returned to Discrimination 85 training until they completed two 120-trial block with a discrimination ratio (DR) of at least 80%, where these blocks needed to be consecutive.

Response Measures

In order to analyze responding to each type of stimulus, we calculated the proportion of responding by dividing the number of trials where the bird went into the feeder by the total number of trials in which the bird either went into the feeder or left the request perch after hearing the full call stimulus (i.e., all non-interrupted trials). To account for individual differences in responding to non-trained stimuli, we scaled the proportion of responding to each type of Test trial stimuli separately for each bird. The highest proportion of responding for each individual was rescaled to equal 1 and all other proportions of responding were proportionally rescaled. For example, if the highest proportion of responding was 0.5 and all others were 0.25, the values would be scaled to 1 and 0.5, respectively.

Statistical Analyses

We first evaluated whether the two True groups differed from one another in speed of acquisition. We conducted an independent-samples *t* test on the number of 120-trial blocks individuals took to reach criterion during Discrimination training. We also compared speed of acquisition between the two Pseudo groups. To compare rate of learning between True and

Pseudo groups, we conducted an independent-samples *t*-test on the number of 120-trial blocks individuals took to reach criterion during Discrimination training.

We then conducted a mixed ANOVA on the proportion of responding to each Stimulus Type (i.e., Discrimination male calls, Discrimination female calls, Transfer male calls, and Transfer female calls) during the three 278-trial blocks of Transfer testing. Similarly, we conducted six mixed ANOVAs, one for each type of manipulated call stimuli (e.g., partial call stimuli, spliced call stimuli, and single note-type spliced call stimuli) on the scaled proportion of responding to each Stimulus Type (i.e., Discrimination male calls, Discrimination female calls, Probe M-M calls, F-F calls, M-F calls, and F-M calls) during the three 267- or 268-trial blocks of Probe trials. All statistics were conducted in IBM SPSSStatistics v.22 (IBM Corp., Armonk, NY, U.S.A.).

Ethical note

Throughout the experiments, birds remained in the testing apparatus to minimize stress and discomfort caused by transport and handling. Following the experiments, birds were returned to the colony room for use in future experiments. Birds were closely monitored and remained healthy during the experiment. 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 107 and 108), 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 (#56076 and #56077), and City of Edmonton Parks Permit.

Results

Trials to criterion

The average ± SD number of trials required for each group to complete Discrimination training are as follows: Male S+ group = 29.17 ± 8.98; Female S+ group = 24.50 ± 5.68; Pseudo group 1 = 22.00 ± 7.07; and Pseudo group 2 = 39.00 ± 23.90. Independent-samples *t*-tests on the number of 120-trial blocks individuals took to reach criterion during Discrimination training revealed no significant difference between either the two true category groups (i.e., Male S+ and Female S+ groups; $t_{10} = 1.076$, $p = 0.307$, CI = -5.00 – 14.33) or the two Pseudo category groups ($t_3 = -0.934$, $p = 0.419$, 95% Confidence Intervals = -74.90 – 40.91). There was also no difference between the True and Pseudo category groups ($t_{15} = -0.839$, $p = 0.415$, CI = -19.01 – 8.28).

Transfer testing

To determine if individuals in the true category groups continued to respond to the reward-contingencies learned in Discrimination training when presented with novel stimuli in Transfer testing, we conducted a mixed ANOVA on the scaled proportion of responding during the first three 278-trial blocks of Transfer testing with Stimulus Type (e.g., Discrimination male calls, Discrimination female calls, Transfer male calls, Transfer female calls) as a within-subjects factor, category group (e.g., Male S+ group, Female S+ group) and individual sex as between-subjects factors. There was a significant main effect of Stimulus Type ($F_{3,24} = 4.330$, $p = 0.014$) and a significant Stimulus Type × Group interaction ($F_{3,24} = 32.070$, $p < 0.001$). All other main effects and interactions were not significant ($p > 0.296$).

We conducted post hoc comparisons using Bonferroni corrections to determine the nature of the significant interactions. For the main effect of Stimulus Type, there was no significant pairwise comparison ($p > 0.189$). We conducted pairwise comparisons on the proportion of responding to each stimulus type for each True group separately. For the Male S+ group, birds responded significantly more to rewarded male-produced calls than to unrewarded female-produced calls (adjusted $p < 0.001$, CI = -0.785 – -0.470). Birds in the Female S+ group also responded more to rewarded calls than unrewarded calls (adjusted $p = 0.004$, CI = 0.237 – 0.868), and also responded significantly more to Transfer female calls than to unrewarded Discrimination male calls (adjusted $p = 0.023$, CI = -0.909 – -0.086). All other pairwise comparisons were not significant (adjusted $p > 0.051$). Four independent-samples *t*-tests were conducted to compare the proportion of responding to each Stimulus Type (e.g., Discrimination male calls, Discrimination female calls, Transfer male calls, Transfer female calls) between the two True groups (e.g., Male S+ group, Female S+ group). The adjusted *p*-value for significance became $p = 0.05/4 = 0.013$ with Bonferroni corrections for four comparisons. There was a significant difference in responding between groups such that birds rewarded for responding to male stimuli (e.g., Male S+ group) responded more to male stimuli than did birds that were rewarded for responding to female stimuli ($t_{10} = -4.984$, $p = 0.001$, CI = -0.795 – -0.304) and birds rewarded for responding to female stimuli (e.g., Female S+ group) responded more to female stimuli than did birds that were rewarded for responding to male stimuli ($t_{10} = 10.071$, $p < 0.001$, CI = 0.491 – 0.770). All other between-group comparisons were not significant ($p > 0.690$).

Test trials

To determine if individuals in the true category groups responded to the novel stimuli presented in Probe trials, we conducted six mixed ANOVAs, one for each type of Test stimulus, on scaled proportion of responding during the first three 267- or 268-trial blocks of Transfer testing with Stimulus Type (e.g., Discrimination male calls, Discrimination female calls, Probe M-M calls, F-F calls, M-F calls, and F-M calls) as a within-subjects factor, category group (e.g., Male S+ group, Female S+ group) and individual sex as between-subjects factors.

Partial call stimuli. Two mixed ANOVAs were conducted, one on scaled proportion of responding to *chick-a* stimuli and one on scaled proportion of responding to *-dee* stimuli. In both cases, there was a significant Stimulus Type × Group interaction (*chicka*: $F_{3,24} = 27.519$, $p < 0.001$; *dee*: $F_{1.42,11.35} = 20.998$, $p < 0.001$). In addition to the same difference between rewarded and unrewarded Discrimination stimuli reported for the Transfer testing results (as the same proportion of responding to Discrimination stimuli is compared to all Transfer and Test trial responses), there was a significant difference in responding such that birds in the Female S+ group responded more to Discrimination female stimuli than to Partial call male "*chick-a*" stimuli (adjusted $p = 0.028$, CI = 0.060 – 0.878). All other pairwise comparisons were not significant (adjusted $p > 0.057$). Two independent-samples *t*-tests were conducted for each type of Partial call stimulus to compare the proportion of responding to male- and female-produced stimuli (e.g., "*chick-a*" male stimuli, "*chick-a*" female stimuli, "*-dee*" male stimuli, "*-dee*" female stimuli) between the two True groups (e.g., Male S+ group, Female S+ group). The adjusted *p*-value for significance became $p = 0.05/2 = 0.026$ with Bonferroni corrections for two comparisons. None of these tests were significant ($p > 0.131$).

Spliced call stimuli. A mixed ANOVA was conducted on scaled proportion of responding to spliced call stimuli. There was a significant Stimulus Type × Group interaction ($F_{1.87,14.94} = 20.550$, $p < 0.001$). For the Stimulus Type × Group interaction, there was a significant difference in responding such that birds in the Male S+ group responded more to Discrimination male stimuli than to Spliced call M-M stimuli (adjusted $p = 0.044$, CI = 0.010 –

0.681) and birds in the Female S+ group responded more to Discrimination female stimuli than to Call splice F-M stimuli (adjusted $p = 0.010$, CI = 0.138 – 0.786). All other pairwise comparisons were not significant (adjusted $p > 0.060$). Four independent-samples *t*-tests were conducted for each type of Spliced call stimulus to compare the proportion of responding to male- and female-produced stimuli (e.g., M-M stimuli, F-F stimuli, F-M male stimuli, M-F female stimuli) between the two True groups (e.g., Male S+ group, Female S+ group). The adjusted *p*-value for significance became $p = 0.05/4 = 0.013$ with Bonferroni corrections for four comparisons. None of these tests were significant ($p > 0.188$).

Single note-type spliced call stimuli. Three mixed ANOVAs were conducted, one on scaled proportion of responding to each type of single note-type spliced call stimuli (e.g., A note spliced calls, B note spliced calls, and C note spliced calls). There was a significant Stimulus Type × Group interaction (A note: $F_{2.35,18.83} = 8.280$, $p = 0.002$; B note: $F_{2.33,18.62} = 8.642$, $p = 0.002$; C note: $F_{2.07,16.55} = 6.764$, $p = 0.007$). For the pairwise comparisons for both A note and B note spliced call stimuli, there was a significant difference in responding where birds in the Female S+ group responded more to Discrimination female stimuli than to the single note-type call splice F-M stimuli (A note: adjusted $p = 0.013$, CI = 0.132 – 0.909; B note: adjusted $p = 0.020$, CI = 0.089 – 0.885). For the pairwise comparisons for C note spliced call stimuli, birds in the Male S+ group responded significantly more to single note-type call splice M-F stimuli than to the Discrimination female stimuli (adjusted $p = 0.017$, CI = -0.762 – -0.090; see Figure 2-2). All other pairwise comparisons were not significant (adjusted $p > 0.060$). Four independent-samples *t*-tests were conducted for each type of Single note-type spliced call stimulus (e.g., A note, B note, and C note spliced) to compare the proportion of responding to male- and female-produced stimuli (e.g., M-M stimuli, F-F stimuli, F-M male stimuli, M-F female stimuli) between the two True groups (e.g., Male S+ group, Female S+ group). The adjusted *p*-value for significance became $p = 0.05/4 = 0.013$ with Bonferroni corrections for four comparisons. None of these tests were significant ($p > 0.027$).

Rationale for Experiment 2

From the results of Experiment 1, we found that birds can discriminate between male- and female-produced *chick-a-dee* calls. However, they did not respond to new calls in the Transfer stage using the same contingencies as they learned in Discrimination training, suggesting that they simply memorized individual stimuli during training. Previous research on sex differences in *chick-a-dee* calls of black-capped chickadees (Campbell et al., 2016) and Carolina chickadees (Freeberg et al., 2003) found differences in the preliminary, "*chick-a*" portion of the call (e.g., the portion made of A, B, and C notes). The terminal "*-dee*" portion of the call (e.g., the D notes) is known to change based on individual flock identity (Mammen & Nowicki, 1981; Nowicki, 1989) and the presence of predators (Hurd, 1996). With this flexibility, it could be argued that the "*-dee*" portion of *chick-a-dee* calls may be unreliable for identifying a stable trait like individual sex, and this portion may serve as a distractor from any potential information contained within the "*chick-a*" portion. With this in mind, and knowing that "*chick-a*" only calls are commonly produced in nature (Smith, 1991), we designed Experiment 2 to investigate how removing the "*-dee*" portion of *chick-a-dee* calls affects the perception of sex differences by black-capped chickadees.

EXPERIMENT 2

Methods

The methods for Experiment 2 were identical to those of Experiment 1 except for the modifications noted below.

Subjects

Eighteen wild-caught black-capped chickadees (9 males, 9 females) captured between January 14, 2014 and January 30, 2016 were used in Experiment 2. 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 and were aged as older than one year at time of capture using the shape and colour of outer tail retrices (Pyle, 1997). Sex was determined using DNA analysis (Griffiths, Double, Orr, & Dawson, 1998). Eight birds had previous experience with the operant discrimination paradigm, discriminating biological stimuli (chick-a-dee calls: Scully, Campbell, Congdon, & Sturdy, 2019; chick-a-dee and owl calls: Congdon et al., 2019a, Congdon et al., 2019b), but none had experience with the calls used in the current experiment. Previous experience was balanced across groups.

Acoustic Stimuli

Experiment 2 used the same acoustic stimuli as Experiment 1, however the vocalizations were modified as follows: Natural call stimuli were truncated, removing the *-dee* portion of all calls. For the manipulated call stimuli, partial call stimuli were not modified, however, both spliced call and single note spliced call stimuli were also shortened to remove the *-dee* portion of the calls using SIGNAL 5.06.07 (Engineering Design, Berkeley, CA, U.S.A.) before being tapered and equalized as for Experiment 1.

Results

Trials to criterion

The average ± SD number of trials required for each group to complete Discrimination training are as follows: Male S+ group = 65.67 ± 26.99; Female S+ group = 62.00 ± 22.36; Pseudo group 1 = 75.00 ± 33.15; and Pseudo group 2 = 60.00 ± 26.51. Independent-samples *t*-tests on the number of 120-trial blocks individuals took to reach criterion during Discrimination training revealed no significant difference between either the two true category groups (i.e., Male S+ and Female S+ groups; $t_{10} = 0.256$, $p = 0.803$, CI = -28.21 – 35.55) or the two Pseudo category groups ($t_4 = 0.612$, $p = 0.574$, CI = -53.05 – 83.05). There was also no difference between the True and Pseudo category groups ($t_{16} = -0.292$, $p = 0.774$, CI = -30.33 – 22.99).

Transfer testing

We conducted a mixed ANOVA in the same manner as in Experiment 1. There was a significant Stimulus Type × Group interaction ($F_{3,24} = 23.362$, $p < 0.001$). All other main effects and interactions were not significant ($p > 0.103$).

As in Experiment 1, we conducted pairwise comparisons on the proportion of responding to each stimulus type for each True group separately. For the Male S+ group, birds responded significantly more to rewarded male-produced calls than to unrewarded female-produced calls (adjusted $p = 0.004$, CI = -0.744 – -0.210). Birds in the Female S+ group also responded more to rewarded calls than unrewarded calls (adjusted $p = 0.008$, CI = 0.164 – 0.761), and also responded significantly more to both Transfer male calls and Transfer female calls than to unrewarded Discrimination male calls (male: adjusted $p = 0.012$, CI = -0.626 – -0.106; female: adjusted $p = 0.011$, CI = -0.987 – -0.171). All other pairwise comparisons were not significant (adjusted $p > 0.059$). Four independent-samples *t*-tests were conducted to compare the proportion of responding to each Stimulus Type (e.g., Discrimination male calls, Discrimination female calls, Transfer male calls, Transfer female calls) between the two True groups (e.g., Male S+ group, Female S+ group). The adjusted *p*-value for significance became $p = 0.05/4 = 0.013$ with Bonferroni corrections for four comparisons. There was a significant difference in responding between groups such that birds in the Male S+ group responded more to Discrimination male stimuli than did birds in the Female S+ group ($t_{10} = -5.087$, $p < 0.001$, CI = 0.283 – 0.725), birds in the Female S+ group responded more to Discrimination female stimuli than did birds in the Male S+ group ($t_{10} = -4.438$, $p = 0.001$, CI = -0.653 – -0.217), and birds in the Female S+ group responded more to Transfer female stimuli than birds in the Male S+ group did ($t_{10} = -3.388$, $p = 0.007$, CI = -0.659 – -0.136). All other between-group comparisons were not significant ($p > 0.793$; see Figure 2-3).

Test trials

We conducted a mixed ANOVA in the same manner as in Experiment 1.

Partial call stimuli. Two mixed ANOVAs were conducted, one on scaled proportion of responding to *chick-a* stimuli and one on scaled proportion of responding to *-dee* stimuli. In both cases, there was a significant main effect of Stimulus type (*chicka*: $F_{3,24} = 5.723$, $p = 0.004$,; *dee*: $F_{1.52,12.19} = 5.167$, $p = 0.030$), however there was no significant pairwise comparison (*chicka*: $p > 0.056$; *dee*: $p > 0.146$). There was also a significant Stimulus Type × Group interaction for both "*chick-a*" and "*-dee*" Partial call stimuli (*chicka*: $F_{3,24} = 26.250$, $p < 0.001$; *dee*: $F_{1.42,11.35} = 20.998$, $p < 0.001$). There was a significant difference in responding such that birds in the Male S+ group responded more to Discrimination male stimuli than to either Partial call male or Partial call female "*chick-a*" stimuli (male: adjusted $p = 0.034$, CI = 0.042 – 0.865; female: adjusted $p = 0.032$, CI = 0.043 – 0.803). Birds in the Male S+ group also responded more to

Discrimination male stimuli than to either Partial call male or Partial call female "*-dee*" stimuli (male: adjusted $p = 0.013$, CI = 0.166 – 1.035; female: adjusted $p = 0.030$, CI = 0.064 – 1.076). All other pairwise comparisons were not significant (adjusted $p > 0.066$). Two independent-samples *t*-tests were conducted for each type of Partial call stimulus to compare the proportion of responding to male- and female-produced stimuli (e.g., "*chick-a*" male stimuli, "*chick-a*" female stimuli, "*-dee*" male stimuli, "*-dee*" female stimuli) between the two True groups (e.g., Male S+ group, Female S+ group). The adjusted *p*-value for significance became $p = 0.05/2 = 0.026$ with Bonferroni corrections for two comparisons. None of these tests were significant ($p > 0.037$).

Spliced call stimuli. A mixed ANOVA was conducted on scaled proportion of responding to spliced call stimuli. There was a significant Stimulus Type × Group interaction ($F_{1.87,14.94} = 20.550$, $p < 0.001$). For the Stimulus Type × Group interaction, there was a significant difference in responding such that birds in the Male S+ group responded more to Discrimination male stimuli than to Spliced call M-M stimuli (adjusted $p = 0.044$, CI = 0.012 – 0.856), Spliced call F-F stimuli (adjusted $p = 0.044$, CI = -0.966 – -0.013), and Spliced call M-F stimuli (adjusted $p = 0.039$, CI = 0.026 – 0.893). All other pairwise comparisons were not significant (adjusted $p > 0.184$). Four independent-samples *t*-tests were conducted for each type of Spliced call stimulus to compare the proportion of responding to male- and female-produced stimuli (e.g., M-M stimuli, F-F stimuli, F-M male stimuli, M-F female stimuli) between the two True groups (e.g., Male S+ group, Female S+ group). The adjusted *p*-value for significance became $p = 0.05/4 = 0.013$ with Bonferroni corrections for four comparisons. None of these tests were significant ($p > 0.191$).

Single note-type spliced call stimuli. Three mixed ANOVAs were conducted, one on scaled proportion of responding to each type of single note-type spliced call stimuli (e.g., A note spliced calls, B note spliced calls, and C note spliced calls). There was a significant Stimulus Type × Group interaction (A note: $F_{5,40} = 5.474$, $p = 0.001$; B note: $F_{1.49,11.93} = 8.642$, $p = 0.046$; C note: $F_{5,40} = 8.250$, $p < 0.001$). For the pairwise comparisons for B note spliced call stimuli, there was a significant difference in responding where birds in the Male S+ group responded more to Discrimination male stimuli than to the single note-type call splice M-F stimuli (adjusted $p = 0.003$, CI = 0.218 – 0.705). For the pairwise comparisons for C note spliced call stimuli, birds in the Male S+ group responded significantly more to Discrimination male stimuli than to single note-type call splice M-M stimuli (adjusted $p = 0.016$, CI = 0.142 – 1.126) and birds in the Female S+ group responded significantly more to Discrimination female stimuli than to single note-type call splice F-F stimuli (adjusted $p = 0.033$, CI = 0.050 – 1.023). All other pairwise comparisons were not significant (adjusted $p > 0.132$). Four independent-samples *t*-tests were conducted for each type of Single note-type spliced call stimulus (e.g., A note, B note, and C note spliced) to compare the proportion of responding to male- and female-produced stimuli (e.g., M-M stimuli, F-F stimuli, F-M male stimuli, M-F female stimuli) between the two True groups (e.g., Male S+ group, Female S+ group). The adjusted *p*-value for significance became $p = 0.05/4 = 0.013$ with Bonferroni corrections for four comparisons. None of these tests were significant ($p > 0.019$).

Discussion

In the current study, we evaluated the ability of black-capped chickadees to perceive sex differences in their *chick-a-dee* calls. We also compared responding to full *chick-a-dee* calls to truncated *chick-a* calls in order to determine if the *-dee* portion of the calls influences how birds perceive the sex of a caller. We found that for birds hearing either full calls or shortened calls, training with stimuli forming a True category provided no benefit in speed of acquisition over training using a Pseudo category. This suggests that there may not be an advantage in using categorization (i.e., True category group) instead of rote memorization (i.e., pseudo category group) in this particular task.

We tested responding to novel male- and female-produced *chick-a-dee* and *chick-a* calls to determine if birds had in fact learned a category, rather than simply memorizing the trained vocalizations. In Experiment 1, birds did not respond to new *chick-a-dee* calls following the reward contingencies introduced in Discrimination training. This suggests that birds did not learn to categorize calls based on the sex of the caller. In contrast, birds in experiment 2 that were rewarded for responding to female-produced *chick-a* calls in Discrimination training (e.g., female S+ group) responded significantly more to novel female-produced *chick-a* calls (e.g., Transfer stimuli) than birds rewarded for responding to male stimuli (e.g., male S+ group). However, birds in the female S+ group also responded more to all Transfer stimuli than they did to unrewarded male Discrimination *chick-a* calls, suggesting that birds may simply have been responding to Transfer stimuli nondifferentially (i.e., at chance levels).

Test trial stimuli were presented to evaluate whether exchanging certain portions of the *chick-a-dee* call would influence how birds perceived that call. We asked, for example whether birds in the female S+ group could be made to respond to a male-produced call by exchanging the male-produced A notes with female-produced A notes. In Experiment 1 we found that birds in the male S+ group responded significantly more to M-F C note spliced stimuli than to unrewarded female-produced Discrimination stimuli. However, since there was no evidence of category learning in this group, this result may simply be due to birds responding non-differentially to this type of test stimulus. Supporting this idea, the remainder of significant differences observed for Test trials in both experiments revealed that birds responded more to their rewarded Discrimination stimuli than to Test stimuli. This difference in responding indicated that they perceive the Test stimuli as different from the trained Discrimination stimuli. We conducted Experiment 2 after seeing no evidence of category learning with full *chick-a-dee* calls in Experiment 1. Previous bioacoustic analyses of sex differences in both black-capped chickadees and Carolina chickadees identified sex differences in acoustic features of A notes, which occur in the "*chick-a*" portion of calls (Campbell et al., 2016; Freeberg et al., 2003). As such, we expected the removal of the "*-dee*" portion of the call to facilitate category learning as the D notes might not contain information related to sex. The difference in Transfer testing results between Experiment 1 where there were no significant interactions involving Transfer stimuli and Experiment 2 where birds in the True groups responded differently to Transfer female calls support this prediction. However, given that the birds in the female S+ group responded more to both types of Transfer stimuli than to unrewarded stimuli, this evidence is not strong. When investigating perception of sex differences in black-capped chickadee *fee-bee* song, solid evidence of category learning was observed when employing a much smaller training stimulus set (30 male, 30 female song stimuli used by Hahn et al., 2015, versus 60 male, 60 female call stimuli employed here). As such, we suggest that knowing sex of a signaler may be more biologically relevant for reproductive-critical vocalizations like *fee-bee* song, but it may be less critical for *chick-a-dee* calls which are used for situations outside of reproduction (Smith, 1991). If this is the case, birds would be unlikely to learn "male-produced" or "female-produced" as perceptual categories in this operant discrimination paradigm.

Conclusions

Overall, our results provide evidence black-capped chickadees can learn to discriminate between male- and female-produced full *chick-a-dee* calls as well as shortened *chick-a* calls, but are unlikely to rely on category learning to do so. Considering that chickadees attend to sex differences in their *fee-bee* song but do not appear to do the same with their *chick-a-dee* calls, the biological relevance of the vocalization and the conditions within which it is employed may play a role in whether or not a vocalization differs between the sexes. *Chick-a-dee* calls differ acoustically between individuals, which would allow for a receiver to identify familiar signalers (Charrier et al., 2004). Since *chick-a-dee* calls function to indicate the presence of food (Smith, 1991), to coordinate mobbing behaviour against predators (Hurd, 1996), and to maintain flock cohesion (Nowicki, 1983), it is possible that knowing the identity of an individual is sufficient and knowing the sex of the signaler independently provides no benefit. Future studies should be conducted to investigate black-capped chickadees perception of sex differences in other chickadee vocalizations such as *tseet* or *gargle* calls which serve different biological functions to determine if function may be related to the presence or absence of sex differences in the vocalizations.

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Table 2-1. Summary of the 268 acoustic stimuli presented including stimulus type and quantity. Stimuli are listed under the experimental stage in which they were introduced, though stimuli introduced in Discrimination training were also presented during both Transfer training and Test trial stages.

Introduction Stage	**Natural Call Stimuli**	**Manipulated Call Stimuli**						**Total Call Stimuli**
		Partial call		**Spliced call**	**Single note-type spliced call**			
		“chick-a”	“-dee”		A Note	B Note	C Note	
Discrimination training	120	-	-	-	-	-	-	120
Transfer testing	38	-	-	-	-	-	-	38
Test trials	-	20	20	40	12	12	6	110
Total	**158**	**20**	**20**	**40**	**12**	**12**	**6**	**268**

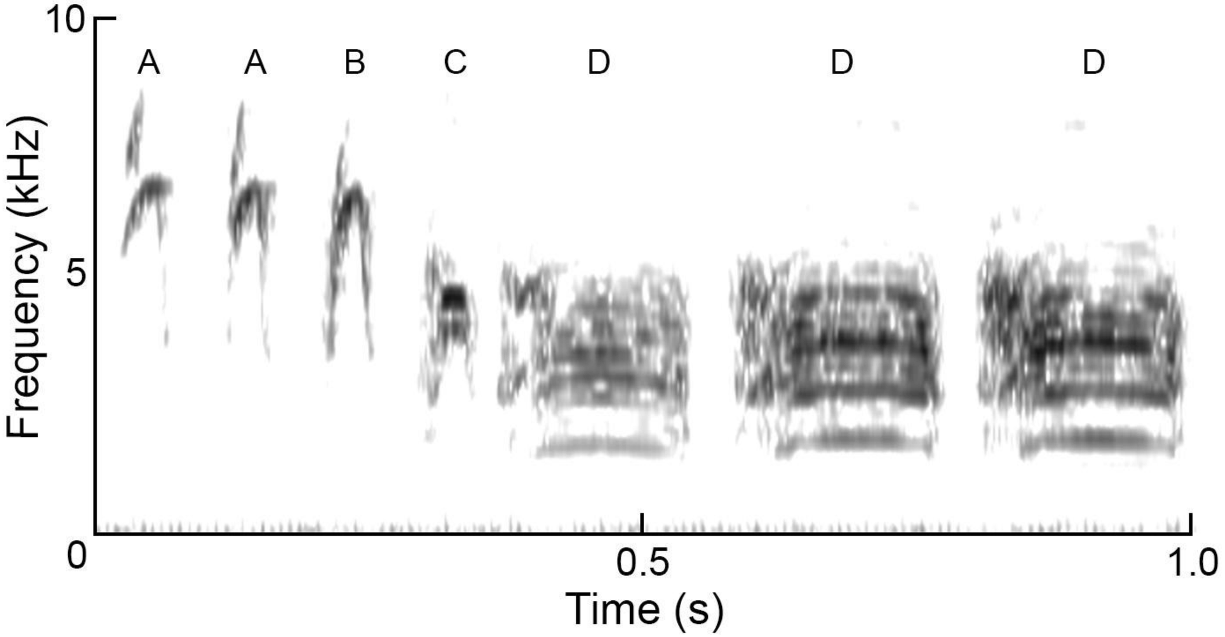

Figure 2-1. Sound spectrogram of a black-capped chickadee *chick-a-dee* call (window size = 256 points, time resolution = 5.8 ms). Call note types are indicated above each note.

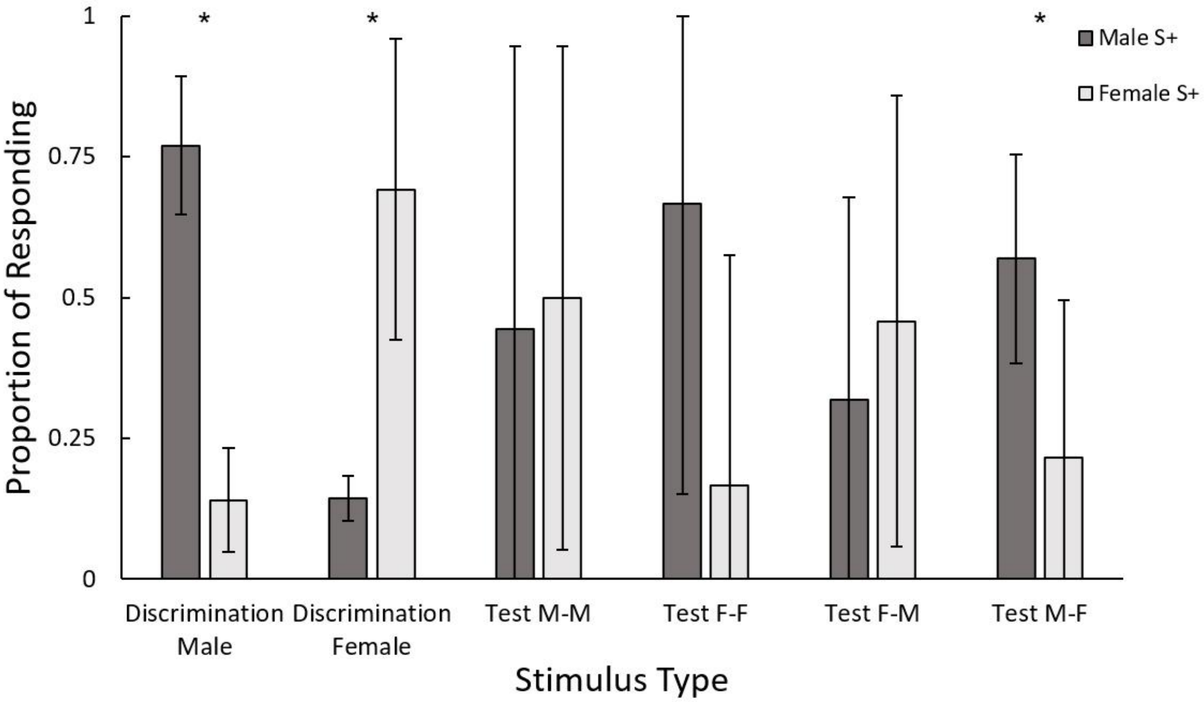

Figure 2-2. Mean ± SE proportion of responding to Discrimination Male, Discrimination Female, M-M, F-F, F-M, and M-F C note splice call stimuli for True groups (e.g., Male S+ and Female S+) in Experiment 1. * Indicates a significant difference in responding between the two groups ($p < 0.05$).

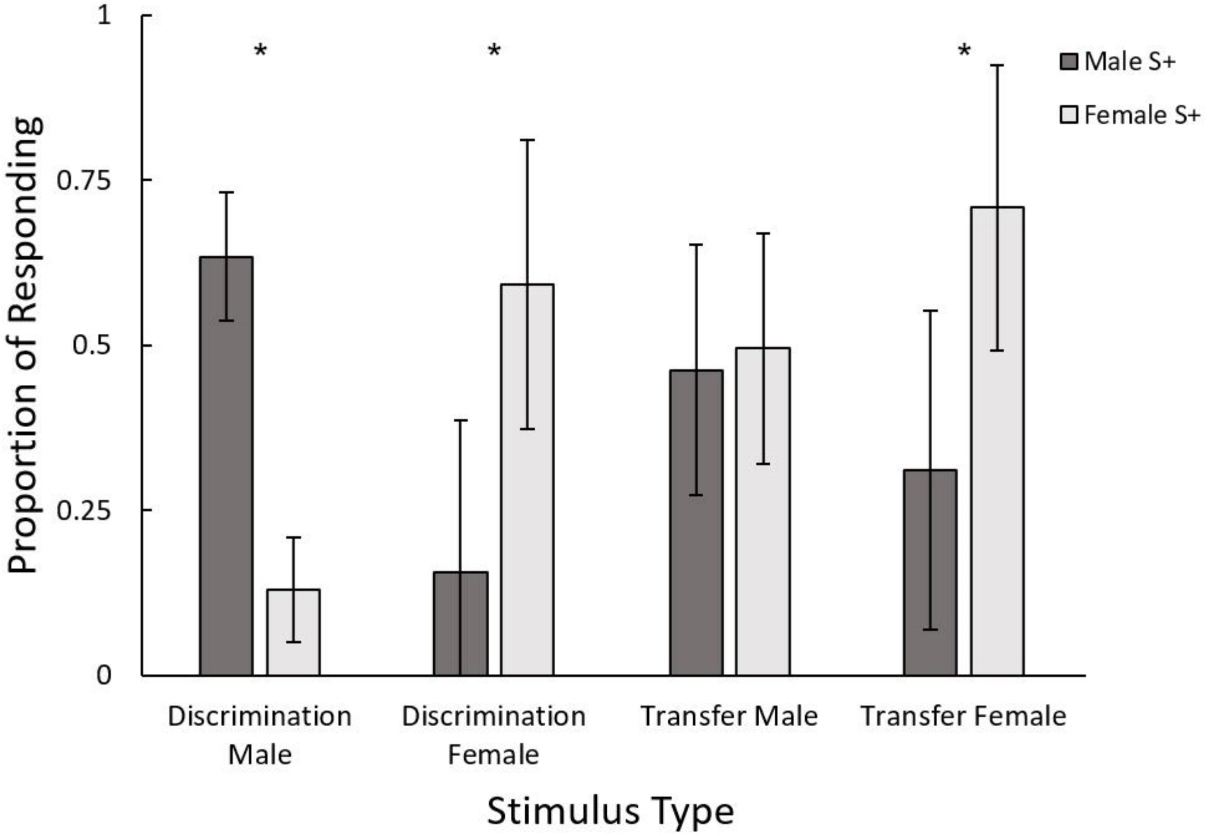

Figure 2-3. Mean ± SE proportion of responding to Discrimination Male, Discrimination Female, Transfer Male, and Transfer Female stimuli during the Transfer testing stage for True groups (e.g., Male S+ and Female S+) in Experiment 2. * Indicates a significant difference in responding between the two groups ($p < 0.05$).

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Chapter 3: Black-capped chickadees (*Poecile atricapillus*) discriminate between naturally-ordered and scramble-ordered *chick-a-dee* calls and individual preference is related to rate of learning

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Introduction

Most forms of animal communication are thought to be non-syntactic, meaning that unique signals are employed to serve different purposes in various situations (Nowak, Plotkin, & Jansen, 2000). For example, vervet monkeys (*Cercopithecus aethiops*) and Japanese tits (*Parus minor*) produce distinct alarm vocalizations in response to avian predators and terrestrial predators (Seyfarth, Cheney, & Marler, 1980; Suzuki & Ueda, 2013). In contrast, syntactic communication, as is seen in human speech, involves individual components of a signal having their own meaning and the combination of those components producing different and varied meanings (Nowak, Plotkin, & Jansen, 2000). A major hurdle of understanding the origins of human language is determining how human language could have evolved from animal communication through the process of natural selection (Suzuki, Wheatcroft, & Griesser, 2018). This endeavour is made more difficult by the fact that our closest evolutionary relatives, the great apes, do not possess syntactic communication systems (Fitch, 2010). Researchers have had to look more distantly to find species that share some of the traits involved in human language.

Hailman and Ficken (1986) have noted that the *chick-a-dee* call of black-capped chickadees (*Poecile atricapillus*), a small species of North American songbird, possesses a computable syntax not unlike that of human language. *Chick-a-dee* calls consist of four main note types that are amalgamated following a fixed order (A → B → C → D) though individual note types can be repeated or omitted, yielding a theoretically unlimited repertoire of producible call types (Hailman, Ficken, & Ficken, 1985). Chickadees can use their *chick-a-dee* call as a mobbing call, warning and recruiting both con- and heterospecifics of the presence of predators (Hurd, 1996). The composition of the call changes depending on how dangerous the predator is perceived to be (i.e., *chick-a-dee* calls with more D notes are produced to higher threat owls and hawks compared to low threat predators; Templeton, Green, & Davis, 2005). In this situation, the same vocalization can convey different meanings depending on the types of notes that make up that vocalization. The *chick-a-dee* call also contains information on species identity (Bloomfield & Sturdy, 2008; Bloomfield et al., 2005), flock membership (Mammen & Nowicki, 1981; Nowicki, 1989), and individual identity (Charrier, Bloomfield, & Sturdy, 2004). With so many essential functions and apparently strict syntactic rules governing the order of note production, it is evident that both the form and function of black-capped chickadee *chick-a-dee* calls require further investigation.

To that purpose, we endeavoured to understand how black-capped chickadees perceive and attend to the syntax of *chick-a-dee* calls (specifically, the order of notes in the call). In the first portion of the current study, we designed a behavioural choice preference task to investigate if chickadees demonstrated a preference for correctly-ordered (i.e., natural) *chick-a-dee* calls over incorrectly-ordered (i.e., scrambled) *chick-a-dee* calls. For the second stage of the experiment, we designed an operant conditioning discrimination task to determine if black-capped chickadees perceive natural ordered *chick-a-dee* calls as a separate perceptual category from scrambled order *chick-a-dee* calls. We also tested whether there was a relationship between preference in task one and performance in task two. The results of this study will strengthen our knowledge of the perception of structure and meaning of black-capped chickadee *chick-a-dee* calls and, more broadly, will add to our understanding of how syntactic communication systems may develop from non-syntactic systems.

Methods

Subjects

Twenty black-capped chickadees (9 male, 11 female) were tested between July 17, 2017 and March 9, 2018. Birds were captured in Edmonton, Alberta, Canada (North Saskatchewan River Valley, 53.53°N, 113.53°W; Mill Creek Ravine, 53.52°N, 113.47°W) between February 5, 2015 and February 6, 2017. Birds were determined to be at least one year of age at time of capture by examining the shape and colour of outer tail retrices (Pyle, 1997) and sex was determined by DNA analysis of blood samples (Griffiths, Double, Orr, & Dawson, 1998). No birds had experience with the experimental procedure.

Housing

Birds were individually housed in 30 × 40 × 40 cm cages (Rolf C. Hagen, Inc. Montreal, QB) in colony rooms where they had visual and auditory, but not physical, contact with conspecifics when not in experimental apparatus. Colony rooms were maintained on a light:dark cycle that matched the natural light cycle of Edmonton, Alberta, Canada. Birds had *ad libitum* access to food (Mazuri Small Bird Maintenance Diet; Mazuri, St Louis, MO, U.S.A.), water, grit, and cuttlebone. Birds received additional supplementation in the form of a superworm (*Zophobas morio*) and vitamins added to water (Prime vitamin supplement; Hagen, Inc.) three times a week, a mixture of eggs and spinach or parsley twice a week, and three to five sunflower seeds daily.

Apparatus

Choice preference task. Birds were individually tested in 117 × 120 × 200 cm sound-attenuating chamber (Industrial Acoustics Company, Bronx, NY) during the choice preference task. Within the chamber, a 67 × 116 × 116 cm testing space was constructed and contained three 1.75 cm diameter, 10 cm long perches monitored by an infrared beam. The back and side walls each had a single perch and each perch was mounted in front of a Fostex FE108E S full-range speaker (Fostex Corp., Tokyo, Japan; frequency response range 80e18000 Hz) 100 cm above the floor of the testing space (see Figure 3-1 for diagram). Landing on one of the two acoustic perches would break the infrared beam, signaling stimulus playback. Landing on the silent perch would still break an infrared beam, however no auditory playback occurred. Stimulus playback and response monitoring was controlled by a single-board computer (Palya & Walter, 2001) and a personal computer. Stimuli stored on a CD were played through a Cambridge Azur 640A Integrated Amplifier (Cambridge Audio, London, U.K.) to the speakers within the testing space. Birds had ad libitum access to food and water during testing sessions.

Operant conditioning task. Birds were individually housed in modified home cages (30 × 40 × 40 cm) located within ventilated sound-attenuating chambers during the instrumental learning task. Chambers were illuminated by 9W full-spectrum fluorescent bulbs on a light:dark cycle that matched the natural light cycle of Edmonton, Alberta, Canada. Cages contained three perches, a grit cup, a water bottle (water vitamin supplemented three days a week), and cuttlebone. Birds received one superworm twice daily. Mazuri food was accessible only after a correct response, as a reward. An 11 × 16 cm opening on one side of the cage allowed the birds to access a motorized feeder. Infrared beams in the perch closest to the feeder and in the feeder itself tracked the position of the bird during testing. A single-board computer tracked responses and set up trials in connection with a personal computer which stored and played the acoustic stimuli. Stimuli passed through an NAD310 Integrated Amplifier (NAD Electronics, London, U.K.) or a Cambridge A300 or 640A Integrated Amplifier (Cambridge Audio, London, U.K.) before playing through a Fostex FE108 Σ full range speaker (Fostex Corp., Tokyo, Japan; frequency response range: 80-18 000 Hz) located beside the feeder. See Sturdy and Weisman (2006) for a detailed description of the experimental setup.

Acoustic Stimuli

Four hundred and seventy-eight *chick-a-dee* calls were collected from four black-capped chickadees (two males: 290 calls, two females: 188 calls). Subjects had no experience with individuals that provided vocal stimuli. Note composition was determined by a single individual using visual inspection of spectrograms in SIGNAL (version 5.05.02, Engineering Design, 2013) using Ficken, Ficken, and Witkin (1978) as a reference. Identified notes were cut and saved individually using SIGNAL to create a pool of available notes.

One hundred seventy stimuli (85 natural order, 85 scrambled order) four to ten notes in length (X ± SD = 6.99 ± 2.04 notes) were constructed by randomly selecting notes from the pool without replacement (i.e., a given note was only used once) such that each created stimulus contained at least one A, B, C, and D note. After notes were selected for a stimulus, two paired stimuli were created: 1) a natural ordered stimulus in which notes were ordered A > B > C > D, and 2) a scrambled ordered stimulus where notes did not follow the natural order (e.g., D > B > A > C). Calls were assembled with 10 ms silence between adjacent notes and were bandpass filtered using GoldWave 6.19 (GoldWave, Inc., St John's, NL, Canada) outside of frequency range of *chick-a-dee* calls (2000-5000 Hz). Using SIGNAL, 5 ms of silence was added to the beginning and end of each call. Each file was also tapered to remove transients then amplitude was equalized using GoldWave.

Procedure

Choice preference task. The 170 call stimuli were pseudo-randomly divided into two stimulus sets (Set A: 84 stimuli, Set B: 86 stimuli) such that each set had the same number of calls of each note length (e.g., half of four-note-long calls assigned to Set A, half to Set B). Pairs of natural and scrambled calls that were constructed from the same set of notes were kept together such that if the natural call was assigned to Set A, so was the equivalent scrambled call. Within a test session, birds only heard stimuli from one set. The order in which stimulus sets were presented was randomized between birds such that half of subjects heard Set A first, and half heard Set B first. Once birds met criteria (see below) for the first stimulus set, they were then tested on the second stimulus set.

Within a test session, natural ordered calls were assigned to one perch, scrambled ordered calls to another, and no acoustic stimulus for the final perch. There were six unique ways that the three types of stimuli could be assigned to the left, back, and right perches. We randomly assigned perch configurations for the first stimulus set presented, then chose a configuration for the second set such that no stimulus category was associated with the same perch for both sets.

Test sessions lasted for two hours and occurred between 09:00 and 16:00 hours. Birds only received a single test session each day. For testing, birds were transported from the colony room in their home cage, removed from the cage, and released into the testing area. After the test session ended, the bird was caught, returned to their home cage, and transported back to the colony room.

During a test session, landing on a perch broke an infrared beam, signaling the computer. Number of visits to each perch as well as the time spent on each perch was recorded by the computer. If the perch was an acoustic perch (i.e., natural or scrambled order call), breaking the beam triggered playing a single call stimulus in its entirety. A second stimulus would not play unless the bird left and returned to the perch (i.e., calls did not play simultaneously). Stimuli were randomly selected without replacement from the pool of stimuli in the appropriate stimulus category for the set. Birds were tested on the same stimulus set until they had heard each acoustic stimulus at least five times or had completed a maximum of five test sessions (e.g., 10 h total).

After meeting these criteria, birds moved on to testing with the second stimulus set after at least one day or rest. After completing the choice preference task for both stimulus sets, birds were tested on the instrumental learning task.

Operant conditioning task.

Pretraining. First, birds underwent basic training (i.e., shaping) to ensure that they were able to obtain food from the experimental apparatus, then they began Pretraining. During basic training, a male bird was returned to the colony room after failing to feed successfully from the apparatus. Pretraining was included to ensure that birds responded non-differentially to stimuli that would be differentially rewarded during later stages and to ensure that they responded at a sufficiently high rate. To accomplish this, birds were presented with and non-differentially rewarded for responding to all of the stimuli that would be used throughout the experiment. Birds would initiate a trial by landing on the request perch, breaking the infrared beam and triggering the playback of a stimulus. During playback, a stimulus was randomly selected from the 170 stimuli in a bin without replaced until all stimuli had been heard. In order to ensure the entirety of a stimulus was heard, birds were required to remain on the perch for 1470-2377 ms after a trial was initiated. Trials were considered interrupted if the bird left the perch before playback was completed. This triggered a 30-s timeout in which the houselight was turned off and new trials could not be initiated. If the bird flew into the feeder within 1 s of the end of playback, they were rewarded with 1 s access to food followed by a 30-s intertrial interval in which the houselight remained on and new trials could not be initiated. If the bird remained on the perch for more than 1 s after the end of playback, a 60-s intertrial interval was initiated in which the houselight remained on and new trials could not be initiated. This interval ended if the bird subsequently left the perch. Birds remained on Pretraining until they responded to 60% or more of trials across six 170-trial bins, displayed a less than 3% difference in responding across four 170-trial bins to what would be rewarded and unrewarded stimuli in Discrimination training (see below), and displayed a less than 3% difference in responding across four 170-trial bins to categories of Transfer testing stimuli (i.e., transfer natural stimuli and transfer scrambled stimuli). During this stage, a female subject died due to human error in loading a program.

Discrimination training. After meeting criteria in pretraining, birds began Discrimination training. The method of stimulus presentation remained the same, however, only 80 of the total 170 stimuli were presented and half (40) of these calls were now unrewarded (i.e., responding to these stimuli now resulted in a 30-s intertrial interval in which the houselight was off and a new trial could not be initiated). Responding to the remaining 40 calls was rewarded as in pretraining with 1 s access to food.

Birds were randomly assigned to either a True category discrimination group (N = 12) or a Pseudo category discrimination group (N = 5). The True category group consisted of two subgroups that were rewarded for either responding to natural ordered calls (N = 6) or scrambled order calls (N = 6). The Pseudo category discrimination group also consisted of two subgroups (Pseudo 1: $n = 2$; 1 male, 1 female; Pseudo 2: $n = 3$; 1 male, 2 females) that were rewarded for responding to 40 randomly-selected calls (20 natural ordered, 20 scrambled ordered) and unrewarded for responding to the remaining 40 calls (20 natural ordered, 20 scrambled ordered).

Birds remained on Discrimination training until they completed six 80-trial blocks with a discrimination ratio (DR) of at least 80% with the last two of these blocks being consecutive. DR was calculated by dividing the mean percentage of response to all rewarded stimuli by the mean percentage of response to rewarded stimuli plus the mean percentage of response to unrewarded stimuli, then multiplying by 100. With this calculation, a DR of 50% indicates equal responding to rewarded and unrewarded stimuli, while a DR of 100% indicates responding to only the rewarded stimuli (i.e., perfect discrimination). During this stage a male in the Pseudo 2 group died of natural causes.

Discrimination 85 training. Nearly identical to Discrimination training, Discrimination 85 training differed only in that the rewarded stimuli were reinforced only 85% of the time. This meant that when a stimulus from the rewarded category was played, on 15% of trials entering the feeder resulted in a 30-s intertrial interval in which the houselight remained on without access to food. This stage served to expose birds to trials in which responses to stimuli were neither rewarded nor punished, as would be encountered in Transfer trials. As with the Discrimination stage, birds continued on Discrimination training until they completed six 80-trial blocks with a discrimination ratio (DR) of at least 80%, where the last two of these blocks needed to be consecutive.

Transfer testing. After meeting criteria in Discrimination 85 training, birds began Transfer testing. The stimuli and reward-contingencies introduced in Discrimination 85 training remained the same. Birds were also introduced to an additional 90 calls (45 natural order, 45 scrambled order) that had previously been non-differentially reinforced (i.e., had been played during pretraining), but were not heard during Discrimination training. Responding to these novel transfer stimuli was neither rewarded nor punished, in that a response resulted in a 30-s intertrial interval in which the houselight remained on without access to food being provided. Within a 650-trial bin, the 80 discrimination stimuli were played seven times each while the new transfer stimuli were only played once. Upon completion of three bins, the experiment was completed and birds were returned to the colony room.

Response measures

Choice preference task. During this task, the absolute number of visits to each perch and the amount of time spent on each perch was recorded. However, individual birds took varying numbers of test sessions to meet criteria. To account for this, we calculated the average number of visits to each perch and the average amount of time spent on each perch by dividing the total measure by the number of 2 h sessions it took to meet criteria. We tested whether these values differed between set A and set B using a paired-samples *t*-tests for each measure (e.g., average number of visits and average time) before combining the two data sets.

To quantify preference between the two types of acoustic stimuli, we calculated two preference scores, one using number of visits and the other using time on the perch, for each bird as follows:

$$preference\ score_{visits} = \frac{(visits\ to\ natural\ perch)}{(visits\ to\ natural\ perch)+(visits\ to\ scrambled\ perch)}$$

$$preference\ score_{time} = \frac{(time\ on\ natural\ perch)}{(time\ on\ natural\ perch)+(time\ on\ scrambled\ perch)}$$

Here, a preference score of 1 indicates a preference for natural ordered calls, a score of 0.5 indicates no preference, and a score of 0 indicates a preference for scrambled order calls.

Operant conditioning task. To analyze patterns of responding, we calculated the proportion of responding for each stimulus type by dividing the number of trials where the bird went into the feeder by the total number of trials in which the bird either went into the feeder or left the request perch after hearing the full call stimulus (i.e., all non-interrupted trials). A proportion of responding of 1 indicates responding to all stimuli in a category.

Statistical Analyses

For the choice preference task, we conducted a repeated measures ANOVA to evaluate differences in the average amount of time spent on each of the three perches. We conducted a similar repeated measures ANOVA on the average number of visits to each of the three perches.

To determine if the two True category groups differed from one another in speed of acquisition, we conducted an independent-samples *t*-test on the number of 80-trial blocks individuals took to reach criterion during Discrimination training. In the same fashion, we compared speed of acquisition between the two Pseudo category groups. In order to compare rate of learning between the True and Pseudo category groups, we conducted an independent-samples *t*-test on the number of 80-trial blocks individuals took to reach criterion during Discrimination training.

We then conducted a repeated measures ANOVA on the proportion of responding to each stimulus type (i.e., discrimination natural calls, discrimination scrambled calls, transfer natural calls, and transfer scrambled calls) during the three 650-trial blocks of Transfer testing. All statistics were conducted in IBM SPSSStatistics v.22 (IBM Corp., Armonk, NY, U.S.A.).

Ethical Note

During the choice preference task, birds remained in the testing apparatus for only two hours a day and had free access to food and water. During the instrumental learning task, birds remained in the testing apparatus to minimize stress and discomfort caused by transport and handling. Following the experiments, birds were returned to the colony room for use in future experiments. Birds were closely monitored throughout the experiments. One bird died during pretraining due to a program error and one bird died during Discrimination training due to natural causes. All other birds remained healthy during the experiment. These 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 108), 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 (#56076 and #56077), and City of Edmonton Parks Permit.

Results

Response to Perches

We conducted a repeated measures ANOVA where the average amount of time on each of the three perches (e.g., natural order, scrambled order, silence) was the within-subjects factor and sex was the between-subjects factor. There was a significant main effect of perch ($F_{2,30} = 10.095$, $p < 0.001$). A Bonferroni post hoc test revealed that birds spent significantly more time on the silent perch than on either acoustic perch (natural: $p < 0.001$; scrambled: $p < 0.001$). A similar repeated measures ANOVA on average number of visits to each of the three perches also revealed a significant main effect of perch ($F_{1.35,20.21} = 4.158$, $p = 0.044$). Here, birds were revealed to visit the natural order perch significantly more often than the scrambled order perch ($p = 0.019$). In both cases, there was no significant main effect of sex and no significant interaction ($p > 0.385$).

Trials to Criterion

The average ± SD number of trials required for each group to complete Discrimination training are as follows: Natural S+ group = 32.17 ± 14.05; Scrambled S+ group = 50.17 ± 35.43; Pseudo group 1 = 23.67 ± 23.03; and Pseudo group 2 = 77.33 ± 23.97. Independent-samples *t*-

tests on the number of 80-trial blocks individuals took to reach criterion during Discrimination training revealed no significant difference between either the two True category groups (i.e., Natural S+ and Scrambled S+ groups; $t_{10} = -1.157, p = 0.274$) or the two Pseudo category groups ($t_3 = -2.145, p = 0.121$). There was also no difference in speed of acquisition between the True and Pseudo category groups ($t_{15} = -1.307, p = 0.211$).

Transfer testing

True category groups. We conducted a repeated measures ANOVA on the proportion of responding during the three 650-trial bins of Transfer testing to evaluate if individuals in the True category groups continued to respond to the reward-contingencies learned in Discrimination training when presented with novel stimuli in Transfer testing. Stimulus type (e.g., discrimination natural order calls, discrimination scrambled order calls, transfer natural order calls, transfer scrambled order calls) was the within-subjects factor and both category group (e.g., natural S+ group, scrambled S+ group) and sex as between-subjects factors. There was a significant stimulus type × group interaction ($F_{3,24} = 129.70, p < 0.001$). All other main effects and interactions were not significant ($p > 0.196$).

We conducted post hoc pairwise comparisons using Bonferroni corrections on the proportion of responding to each stimulus type for each True group separately. For birds in the natural order S+ group, they responded significantly more to rewarded natural order calls than to both unrewarded scrambled order calls (adjusted $p < 0.001$, 95% Confidence Interval (CI) = -0.743 – -0.546) and transfer scrambled order calls (adjusted $p = 0.001$, CI = -0.857 – -0.352). They also responded significantly more to transfer natural order calls than to both unrewarded scrambled order calls (adjusted $p = 0.001$, CI = 0.330 – 0.818) and transfer scrambled order calls (adjusted $p = 0.001$, CI = -0.756 – -0.313). Similarly, birds in the scrambled order S+ group responded significantly more to rewarded scrambled order calls than to both unrewarded natural order calls (adjusted $p = 0.004$, CI = 0.268 – 0.990) and transfer natural order calls (adjusted $p = 0.002$, CI = -0.871 – -0.321). They also responded significantly more to transfer scrambled order calls than to both unrewarded natural order calls (adjusted $p = 0.007$, CI = 0.206 – 0.909) and transfer natural order calls (adjusted $p = 0.001$, CI = 0.296 – 0.752). All other pairwise comparisons were not significant (adjusted $p > 0.472$).

Four independent-samples *t*-tests were conducted to compare the proportion of responding to each stimulus type (e.g., discrimination natural order calls, discrimination scrambled order calls, transfer natural order calls, transfer scrambled order calls) between the two True groups (e.g., natural order S+ group, scrambled order S+ group). The adjusted *p*-value for significance became p = 0.05/4 = 0.013 with Bonferroni corrections for four comparisons. There was a significant difference in responding between groups such that birds rewarded for responding to natural ordered stimuli (e.g., natural order S+ group) responded more to natural ordered stimuli (both from discrimination and from transfer) than did birds that were rewarded for responding to scrambled order stimuli (discrimination: $t_{10} = -5.003$, $p = 0.001$, CI = -0.779 – -0.299; transfer: $t_{10} = -4.489$, $p = 0.001$, CI = -0.652 – -0.219) and birds rewarded for responding to scrambled order stimuli (e.g., scrambled order S+ group) responded more to scrambled order stimuli (both from discrimination and from transfer) than did birds that were rewarded for responding to natural ordered stimuli (discrimination: $t_{10} = 24.005$, $p < 0.001$, CI = 0.666 – 0.802; transfer: $t_{10} = 10.835$, $p < 0.001$, CI = 0.495 – 0.751; see Figure 3-2).

Pseudo category groups. In the same manner as was done for the True category groups, we also conducted a repeated measures ANOVA on the proportion of responding during the three 650-trial bins of Transfer testing to evaluate how individuals in the Pseudo category groups responded to the novel stimuli in Transfer testing. Again, there was a significant stimulus type × group interaction ($F_{3,3} = 95.486$, $p = 0.002$). There was also a significant main effect of both group and sex (group: $F_{1,1} = 697.173$, $p = 0.024$; sex: $F_{1,1} = 244.052$, $p = 0.041$). All other main effects and interactions were not significant ($p > 0.196$).

To examine the main effects, we conducted post hoc Bonferroni comparisons. Birds in the Pseudo 1 group responded more overall than did birds in the Pseudo 2 group ($p = 0.024$). Similarly, males responded more overall than females ($p = 0.041$). We conducted post hoc pairwise comparisons using Bonferroni corrections on the proportion of responding to each stimulus type for each Pseudo category group separately. Birds in the Pseudo 1 group responded significantly more to rewarded discrimination calls than to transfer scrambled order calls (adjusted $p = 0.036$, CI = 0.037 – 0.233) and more to transfer natural order calls than unrewarded discrimination calls (adjusted $p = 0.026$, CI = -0.908 – -0.289). All other pairwise comparisons were not significant (adjusted $p > 0.053$).

Four independent-samples *t*-tests were conducted to compare the proportion of responding to each stimulus type between the two Pseudo category groups. The adjusted *p*-value for significance became $p = 0.05/4 = 0.013$ with Bonferroni corrections for four comparisons. There was a significant difference in responding between groups such that birds in the Pseudo 1 group responded more to Pseudo 1 S+ stimuli (i.e., Pseudo 1 rewarded discrimination stimuli) than did birds in the Pseudo 2 group ($t_3 = 10.254$, $p = 0.002$, CI = 0.461 – 0.877). Similarly, birds in the Pseudo 2 group responded more Pseudo 2 S+ stimuli (i.e., Pseudo 2 rewarded discrimination stimuli) than did birds in the Pseudo 1 group ($t_3 = -18.409$, $p < 0.001$, CI = -0.812 – -0.573). There were no significant differences in responding between groups to transfer natural order stimuli ($p = 0.052$) or to transfer scrambled order stimuli ($p = 0.043$).

Acoustic preference and performance. We conducted multiple regression analyses to determine if a measure of learning speed, here bins to criteria, could be used to predict the strength of preference, as measured by preference score, for both time spent on and number of visits to each of the three perches. For the preference score calculated using the amount of time spent on the three perches, the regression equation was not significant ($R^2 = 0.075$, $R^2_{adj} = -0.131$, $F_{2,9} = 0.364$, $p = 0.704$). However, for the preference score calculated using the number of visits to each of the three perches, the regression was significant ($R^2 = 0.486$, $R^2_{adj} = 0.372$, $F_{2,9} = 4.258$, $p = 0.050$). The number of bins required to reach criterion significantly predicted the number of visits to the perch ($\beta = 0.711$, $p = 0.017$) such that birds that learned the discrimination in more trials demonstrated a stronger preference toward what would be the rewarded stimulus type (see Figure 3-3).

Discussion

In this experiment, we evaluated the relationship between individual preference and performance as it pertains to natural- and unnaturally-ordered conspecific calls. We first conducted a choice preference task to evaluate individual black-capped chickadees' responses to our two types of manufactured calls without the influence of differential reward. We then conducted an instrumental discrimination task in which responding to calls was differentially-rewarded. This allowed us to examine how individual variation in individual preference could influence performance.

Individual Preference

We found that black-capped chickadees spent more time on the perch that did not produce sound (i.e., silent perch) than on either of the acoustic perches (i.e., natural and scrambled order *chick-a-dee* call playback). This finding is consistent with observations by Hahn and colleagues (2017), who were investigating preferences between dominant and subordinate *fee-bee* song, and observed that birds spent more time on the silent perch compared to either of the acoustic perches. Chickadees in the current study also visited the perch associated with naturally-ordered calls more often than the perch associated with scrambled-order calls. Our results indicate that when birds landed on the silent perch, they remained on that perch, whereas for the natural-order perch, the birds would visit the perch, then leave and return again.

Perceptual Categorization

In this experiment, birds in the True category groups were differentially-reinforced for responding to one category of stimuli but not the other (e.g., rewarded for responding to natural-ordered stimuli but not scrambled-order stimuli). Whereas birds in the Pseudo category groups were differentially-reinforced for responding to groups of vocalizations that did not form categories (i.e., rewarded for responding to half natural- and half scrambled-order calls). This distinction allows us to evaluate whether black-capped chickadees make use of open-ended categorization when learning this discrimination. Black-capped chickadees have been shown to use open-ended categorization to discriminate between male- and female-produced *fee-bee* songs (Hahn et al., 2015), and con- and heterospecific *chick-a-dee* calls (Bloomfield, Farrell, & Sturdy, 2008; Bloomfield, Sturdy, Phillmore, & Weisman, 2003). By the nature of the training contingencies, only True category groups would be capable of learning the two categories (i.e., natural and scrambled) from Discrimination training. Evidence of True category groups using categorization can come from two sources. First, if we assume that learning a category (as could be seen with True category groups) is easier than memorizing each individual call (as required with Pseudo category groups), we would expect to see True category groups learning the discrimination faster than Pseudo category groups. In comparing the number of bins required to meet Discrimination training criteria between True and Pseudo category groups, we found no such difference in rates of acquisition. This could imply that both True and Pseudo category groups were relying on rote memorization to learn the discrimination. Second, determining category learning is involved requires testing whether birds can apply the categories that were learned in Discrimination training to testing stimuli. For example, birds in the natural-order call group would continue responding to natural-order calls that had not been associated with differential rewards. If birds had simply memorized the Discrimination training stimuli, they would respond non-differentially to the testing stimuli. Our results indicate that birds in both the True natural- and scrambled-order call groups continued to respond according to the contingencies that they learned in Discrimination training. Additionally, birds in the Pseudo category groups showed non-differential responding to the testing stimuli, as is expected since they did not learn True categories in Discrimination training. These results taken together indicate that black-capped chickadees are capable of learning and using the categories of natural- versus scrambled-ordered calls.

Acoustic Preference and Performance

In this study, we presented black-capped chickadees with two types of *chick-a-dee* calls that had been constructed by artificially assembling individual notes into either natural- or scrambled-order calls. In nature, *chick-a-dee* calls are almost exclusively produced with notes in a fixed order of A through D notes (A → B → C → D; Hailman, Ficken, & Ficken, 1985). Calls that deviate from this set syntax are rare (personal observation), suggesting that there may be information conveyed by the order itself, rather than simply within the individual notes (Hailman & Ficken, 1986). This quality can be seen in that some notes produced earlier in a call are thought to contain redundant information on notes that come later in the call (Freeberg, Lucas, &

Clucas, 2003). For example, the structure of an A note produced early in the call will change depending on how many other A notes will follow it. In our experiment, we observed a preference for natural-ordered calls over scrambled-ordered calls in that birds visited the perch associated with natural-ordered calls more compared to the perches associated with scrambled-order calls. This preference may be driven by an avoidance of the "unnatural" scrambled-order calls. Future research should investigate if our manufactured natural-ordered calls are perceived by black-capped chickadees as perceptually-similar to naturally-produced *chick-a-dee* calls. If both individual notes and the overall note structure both contain information, birds may perceive these manufactured calls as lacking information in comparison to naturally-produced calls, even though the note order is grammatically-correct.

Individual differences in order (natural vs. scrambled) and strength of preference may be related to how individuals perceive and respond to different vocalizations. For example, Riters and colleagues (2013) showed that some European starlings (*Sturnus vulgaris*) find hearing conspecific vocalizations to be rewarding whereas other individuals do not. This difference in perception may explain differences in motivation and, subsequently, learning. In this experiment, we found that birds with a strong preference for a stimulus type took longer to learn the discrimination task if the preferred stimulus type was the rewarded category. For example, birds in the Natural Order S+ group took longer to learn to respond to natural order stimuli if they had a strong preference for natural stimuli compared to if their preference was for scrambled order stimuli.

Conclusions

In the present study, we used two behavioural tasks to evaluate the relationship between preference for and ability to discriminate between natural- and scrambled-order *chick-a-dee* calls. Our results indicate that, not only do chickadees display individual preference for one type of call over the other, the magnitude and direction of their preference influences the rate at which they learn to discriminate between the two types of calls. Chickadees not only learned to discriminate between natural- and scrambled-order *chick-a-dee* calls, but they applied the learned contingencies to testing stimuli, suggesting that natural- and scrambled-order calls are distinct perceptual categories. To extend these findings, future research should evaluate the validity of using manufactured natural-ordered call stimuli as a substitute for naturally-produced calls.

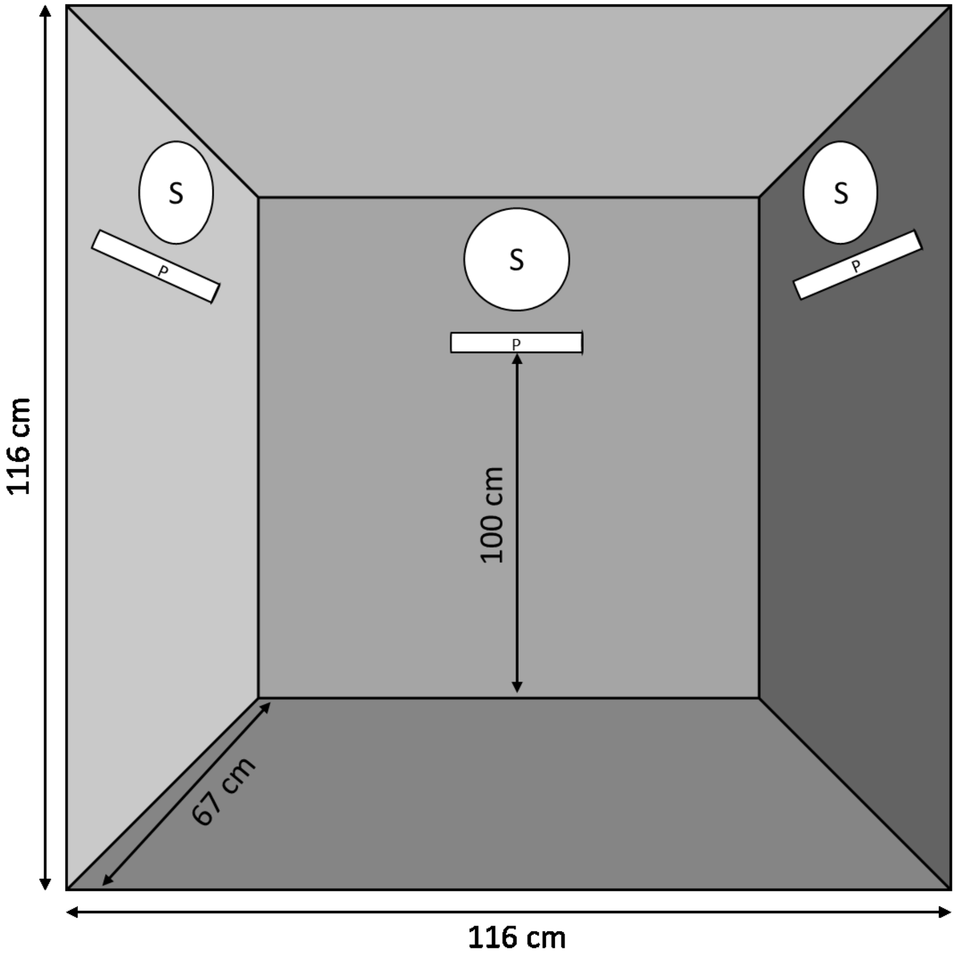

Figure 3-1. Diagram depicting the layout of the choice preference task testing chamber. "S" indicates a speaker. "P" indicates a perch with infrared sensors.

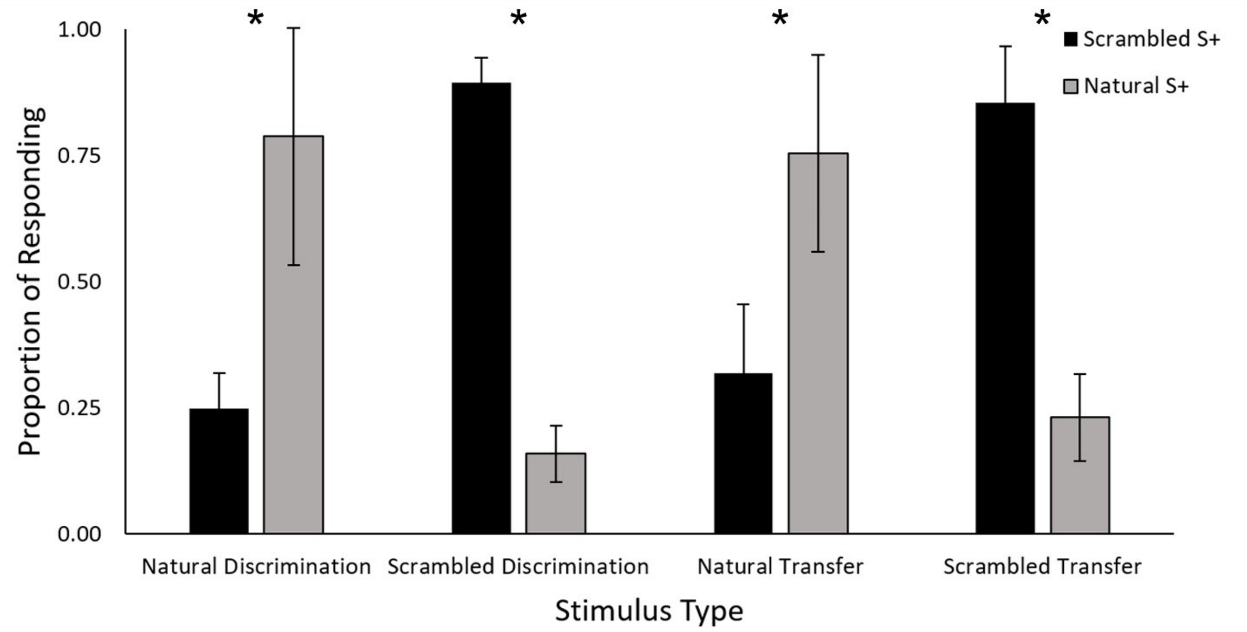

Figure 3-2. Average ± SE proportion of responding by birds in the True category groups (e.g., Natural order, Scrambled order) to four types of stimuli: natural order stimuli in Discrimination training, scrambled order stimuli in Discrimination training, natural ordered stimuli in Transfer testing, and scrambled order stimuli in Transfer testing. * indicates a significant difference ($p \leq 0.05$) between group means.

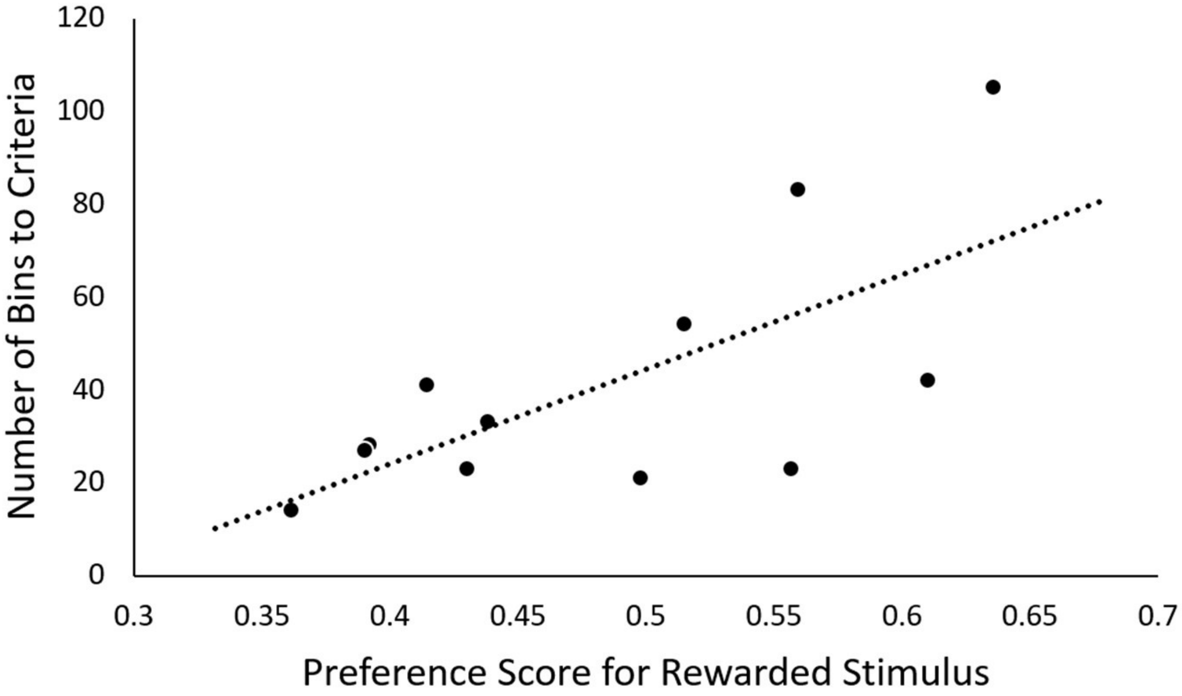

Figure 3-3. The relationship between the number of bins required to meet criteria in Discrimination training and the preference score calculated for the rewarded stimulus during Discrimination training using the number of visits to each perch.

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Chapter 4: The effects of anthropogenic noise on feeding behaviour in black-capped chickadees (*Poecile atricapillus*)

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Introduction

As the global population and level of urbanization increase, so too do the levels of noise associated with human activity. This human-produced noise, or ‘anthropogenic noise’, has extensively changed environmental soundscapes worldwide. Background noise exists in all habitats, but the sounds of urban environments and other areas of human activity exceed natural levels and include novel sounds not heard in a natural habitat. Anthropogenic noise tends to be composed of low frequency sounds from sources such as road vehicles, airplanes, industrial machinery, and air movement machinery (Tempest, 1985; Leventhall, 1988). Transportation networks (including rail, air, and road) are a widespread source of anthropogenic noise outside of urban areas. For example, 83% of the land in the continental U.S. is within 1 km of a road, highlighting the pervasiveness of noise pollution to our natural environments (Ritters & Wickham, 2003). High amplitude, low frequency noise is particularly damaging as it can be propagated at much longer distances, and therefore have farther reaching effects (Buxton et al., 2017).

Anthropogenic noise levels in the range of 40 to 100 dB have been shown to cause disturbances in both humans and non-human animals depending on the source and its proximity (Shannon et al., 2016). In humans, chronic noise exposure has been shown to cause a reduction in cognitive function (Szalma & Hancock, 2011) and sleep quality (Fyhri & Aasvang, 2010), as well as an increased risk of cardiovascular disease (Babisch et al., 2005; Hansell et al., 2013). In non-human animals, the impairments associated with chronic noise exposure include physical or physiological damage, masking of communication signals, and/or reduced available attention (Shannon et al., 2016; Slabbekoorn & Ripmeester, 2008).

While much is known about how noise impacts habitat selection (Slabbekoorn & Ripmeester, 2008), vocal behaviour (Francis & Barber, 2013), and population dynamics (Shannon et al., 2016), less is known about how it impacts daily foraging patterns in songbirds. Because songbirds often retain only enough fat to survive each night, reduced foraging - whether due to fear or to novelty - might have catastrophic impacts on individual survivorship. One recent study by Ware et al. (2015) found that migratory birds were less capable of gaining weight throughout the day when artificial noise was added to the habitat. However, Van Donselaar and colleagues (2018) found that urban black-chickadees were less likely than rural chickadees to avoid a feeder when noise was added. This suggests that some birds may become accustomed to noise, and resume normal foraging behaviour.

Making adjustments that reduce the impacts of noise has been well documented in other songbird behaviours. For example, individuals can shift their spatial patterns of behaviour (e.g., avoid areas with increased noise levels), shift their temporal pattern of behaviour (e.g., sing earlier in the day to avoid noises associated with rush hour traffic; Fuller et al., 2007), alter the quality of their vocalizations (e.g., change frequency or amplitude; Brumm, 2004; change frequency and duration; Gentry et al., 2017), or change their behaviour entirely (e.g., singing a different song type; Quinn et al., 2006; Fuller et al., 2007; Nemeth & Brumm, 2010; Rios-Chelen et al., 2015).

Although many bird species alter their behaviour in response to noise, some do not. The effectiveness of intentional mitigation strategies, other than noise reduction, has not been well studied. The most common means of reducing intensity of anthropogenic noise is the use of physical barriers and while there are other, more effective, means of reducing anthropogenic noise (such as restricting road traffic and reducing speeds) there is evidence that these barriers reduce noise in both urban and rural environments (Murphy & King, 2011; Slabbekoorn & Ripmeester, 2008). The downside of these barriers are that their benefits only extend short distances and their presence can cause habitat fragmentation, making them better used near especially sensitive roadside habitats or surrounding localized noise sources (Summers et al., 2011). Costs for constructing barriers can also be prohibitive. Recently, Proppe et al. (2017) suggested that perhaps behaviour could be manipulated to increase songbird productivity in moderately noisy areas where aversion might be due to novelty rather than fitness costs. They demonstrated that playing conspecific calls (a known acoustic attractant) near low-use roads increased the density of birds in noisy habitats (Scheppers & Proppe, 2017); however, density does not necessarily correlate with survivorship. For example, if foraging is less efficient in noisy habitats, birds drawn to these habitats may be less likely to survive and reproduce.

While a number of well-designed field experiments have tested the effects of noise on bird behaviour (e.g., Templeton et al., 2016; Bayne et al., 2008), it can be difficult to determine how particular behaviours are affected in isolation. For example, in a 20 year review of noise research Shannon and colleagues (2016) found that, while 88% of the 242 studies showed a significant biological response to noise, few studies took into account that the activities that cause anthropogenic noise are themselves a kind of disturbance (e.g., traffic, construction, agriculture, etc.). Although field studies are excellent at assessing behaviour within the myriad of signals and cues found in the natural environment, laboratory-based studies provide a more controlled environment, allowing the effects of anthropogenic noise on individuals to be studied without some of the confounding factors that must be accounted for in the field. For example, conspecific and heterospecific interactions, climactic events, and variations in natural and anthropogenic noise can all be tightly controlled in laboratory studies (e.g., Lohr, Wright, & Dooling, 2003; Potvin, Curcio, Swaddle, & MacDougall-Shackleton, 2016).

In the present study, we sought to investigate the effects of anthropogenic noise (as approximated by brown noise, a noise spectrum with more energy at lower frequencies) on the feeding behaviour of black-capped chickadees (*Poecile atricapillus*). Living primarily in forested areas, black-capped chickadees communicate mainly through acoustic signals (i.e., not visual or olfactory). Black-capped chickadees' ability to thrive in both urban and rural locations means that they inhabit environments with varying levels of anthropogenic noise. In addition to synthetic noise, we embedded *chick-a-dee* calls within noise tracks to determine if this potential mitigation strategy, as suggested by Proppe et al. (2016), altered foraging behaviour. We exposed 12 black-capped chickadees to either brown noise, *chick-a-dee* calls, or a combination of brown noise and *chick-a-dee* calls, and evaluated how this affected their feeding behaviour. We predicted that the presence of low-frequency noise would have a negative impact on feeding behaviour and that the presence of conspecific *chick-a-dee* calls may mitigate those negative effects.

Methods

Subjects

Twelve wild-caught black-capped chickadees (six males, six females) were used in this experiment. Subjects were caught in January 2016 in Edmonton, AB (North Saskatchewan River Valley, 53°53N, 113°53W) or Stony Plain, AB (53°46N, 114°01W) and determined to be at least one year of age at time of capture by referencing the shape and colour of their outer tail retrices (Pyle, 1997). Sex was determined using DNA analysis (Griffiths, Double, Orr, & Dawson, 1998). All subjects had experience with the experimental apparatus for auditory GO/NOGO operant conditioning tasks (e.g., Hahn et al. 2017), but had no experience with the acoustic stimuli used (i.e., *chick-a-dee* calls, brown noise). Testing occurred between August 1 and September 17, 2017.

Housing

Prior to the experiment, individual chickadees lived in Jupiter Parakeet cages (30 × 40 × 40 cm, Rolf C. Hagen, Inc., Montreal, QB, Canada) in a communal colony room having visual and auditory contact with other chickadees, but no physical contact. Birds had free access to food (Mazuri Small Bird Maintenance Diet; Mazuri, St Louis, MO, USA), water (supplemented with vitamins three days a week; Prime vitamin supplement; Hagen, Inc.), grit, and cuttlebone. They also received three to five sunflower seeds each day, one superworm (*Zophobas morio*) three days a week and a mixture of hard-boiled egg and spinach or parsley two days a week. Subjects were kept on a light/dark cycle that approximated the natural light cycle for Edmonton, AB.

During the experiment, chickadees were individually housed in the experimental apparatus (see Apparatus below). They had free access to water (vitamins added three days a week), grit, and cuttlebone and received a superworm twice a day. Mazuri food was provided following visits to the feeder. While in the experimental apparatus, subjects were kept on a 15:9 hour light:dark cycle to match the average natural summer day:night cycle for Edmonton, AB, Canada.

Apparatus

Chickadees were housed in modified Jupiter Parakeet cages (30 × 40 × 40 cm) that were singly enclosed in ventilated sound-attenuating chambers. Each chamber was lit with a full spectrum LED bulb (3W, 250lm E26, Not-Dim, 5000K; Lohas LED, Chicago, IL, USA). The modified cages had an 11 × 16 cm opening that allowed access to a motorized feeder (described in Njegovan et al., 1994). The feeder contained infrared beams that signalled feeder visits. A personal computer connected to a single-board computer (Palya & Walter, 2001) recorded feeder visits and triggered the food cup to rise upon each visit, allowing the bird access to food. Acoustic stimuli played from a full-range speaker (Fostex FE108 R or FE108E R; Fostex Corp., Japan; frequency response range 80–18,000 Hz) located next to the feeder after passing through an integrated amplifier (Cambridge A300, Azur 640A or Azur 351A; Cambridge Audio, London, England). See Sturdy and Weisman (2006) for a detailed description of the apparatus.

Procedure

On the first day of the experiment, birds were moved to the apparatus and provided with free access to food from both a food cup and from the raised feeder cup. This allowed birds to acclimatize to the apparatus. This acclimatization period was followed by three days of silence, which served as the baseline for establishing normal feeding behaviour in each bird. Following baseline, birds received three days of each of the three conditions (Call Stimulus, Noise Stimulus, Call and Noise Stimulus) separated by one day of silence, which allowed the bird's feeding behaviour to return to baseline (see Figure 4-1). Order of stimulus presentation was randomly assigned such that two individuals were assigned to each of the six possible stimulus presentation orders. Three subjects had an extra day of silence added between treatments to account for the loss of the data from the planned day of silence due to an error. This extra silence did not affect their performance.

Chamber houselights turned on each morning at 09:00. Stimulus playback started at 09:30 (average 09:29 ± 00:12) once the previous days' data was saved and the recording program was restarted. Seven hours of stimulus presentation was followed by silence until lights out at 24:00. Birds could access food by visiting the feeder at any time between lights on and lights out.

Stimuli

This experiment used three types of stimuli: (1) black-capped chickadee *chick-a-dee* calls, (2) brown noise, and (3) a combination of *chick-a-dee* calls and brown noise (see Figure 4-2). All stimuli consisted of 30 minutes of sound followed by 30 minutes of silence repeated for seven hours. After this seven-hour stimulus, no sounds played for the rest of the day. Background sound levels in experimental chambers were approximately 60 dB and the stimuli were presented at approximately 75 dB (measured approximately 20 cm from the speaker by a Brüel & Kjær Type 2239 decibel meter; Brüel & Kjær Sound and Vibration Measurement A/S, Nærum, Denmark; A weighting, slow response).

Chick-a-dee calls. Twenty adult black-capped chickadees (10 males, 10 females) captured in Edmonton, Alberta (North Saskatchewan River Valley, 53°53N, 113°53W; Mill Creek Ravine, 53°52N, 113°47W) and at Barrier Lake Field Station (51°02N, 115°03W) provided a total of 60 *chick-a-dee* calls (three calls per bird) for stimuli. Calls were randomly selected using a random number generator (i.e., random.org) from available recordings of those birds, however only calls that fell within the average number of notes per call plus or minus two standard deviations were used (4-9 notes; avg = 6.93, s.d. = 1.23; from previous acoustic study, Campbell et al., 2016). Birds that provided calls for stimuli were housed in a separate colony room from birds that were subjects so that subjects were not familiar with the stimuli. The calls were recorded in 2014 in individual sound-attenuating chambers (Industrial Acoustics Corporation, Bronx, NY, USA) using Marantz PMD670 digital recorders (Marantz America, Mahwah, NJ, USA). Calls were then bandpass filtered in GoldWave (version 6.30; GoldWave, Inc., St. John's, NL, Canada) to remove frequencies outside the range of the calls (500 – 13,000 Hz) and both ends of the stimulus were tapered at 5 ms to remove transients. Five ms of silence was added before and after each call in SIGNAL software (version 5.05.02, Engineering Design, Belmont, MA, USA).

Prepared calls from different individuals were randomly paired. Each pair was combined in SIGNAL software with three seconds of silence between the first and second calls, then silence was added following the second call to bring the total file length to 60 seconds. Two calls per minute is comparable to rates observed in the wild (15 calls/hour during summer days; Avey et al., 2008) and used in other playback experiments (2 calls/minute: Scully et al., 2018; 4 partial calls/minute: Avey et al., 2014). The 30 files created from the paired calls were concatenated in a randomly determined order to create a 30-minute file; silence was added at the end to bring the total file length to 60 minutes. This hour-long stimulus played seven times to make up the Call Stimulus.

Brown noise. Patange and colleagues (2013) conducted a spectral analysis of samples of traffic noise, concluding that it tends to behave as low frequency pink noise (1/f noise) with random fluctuations. Brown noise (also called red noise or Brownian noise) refers to a broadband spectrum of noise that has more energy at longer wavelengths than at shorter wavelengths and whose sounds randomly change from one moment to the next. Audacity (version 2.1.3; Audacity Team) was used to create a 30-minute noise file using the generate function to make amplitude 0.8 Brownian noise. Silence was added to the end of the stimulus in SIGNAL to bring the total length to 30 minutes. As with the Call Stimulus, this file was played seven times to make up the seven-hour Noise Stimulus.

Chick-a-dee calls and brown noise. To make a seven-hour Call and Noise Stimulus, the Call Stimulus and Noise Stimulus were combined in GoldWave by moving each stimulus into one of two channels in a stereo file.

Statistical Analyses

The time of each visit to the feeder was recorded and matched with the time of stimulus playback start. Visits were summed per 30-minute period for each bird for each day; lined up with 30 minutes playback and 30 minutes silence between. For analysis, the feeds from the seven 30-minute playback periods were summed and treated as a single time period called Stimulus Playback. Similarly, the feeds from the seven 30-minute periods of silence immediately following playback were summed and the time period was called Silence between Playback. The feeds from the remaining silence at the end of the day were also summed, with the time period called Late Silence.

To allow for comparison of feeding between different individuals with varying feeding patterns (total feeds ranged from 413 to 1359 per day with average of 1026), the first three days of silence were used to establish baseline feeding behaviour in the absence of playback. The feeds for silent baseline days were summed in the same manner as the treatment days, with the Stimulus Playback time period starting at the beginning of the day, even though no stimulus was used in the Silence condition. Using the same pattern of 30 minutes "Playback" followed by 30 minutes of Silence between Playback as during the stimulus playback conditions, the average feeds for each of the three time periods (Stimulus Playback, Silence between Playback, and Late Silence) were calculated. These baseline measures were subtracted from the number of feeds for the corresponding time period in each treatment day to scale each individual's performance.

Differences in feeding behaviour, as measured by baseline-scaled feeds, were analyzed using a repeated measures analysis of variance (rmANOVA) with Time (Stimulus Playback, Silence between Playback, and Late Silence) and Condition (Silence, Calls, Noise, Calls and Noise) as the between-subject factors. Sex of the individual and Order of stimulus presentation were included as within-subject factors. Tukey's post hoc tests were conducted to evaluate the nature of interactions between variables.

Ethical Note

Studies were conducted with approval from the Animal Care and Use Committee for Biosciences for the University of Alberta (AUP 108) and all procedures were in accordance with the Canadian Council on Animal Care (CCAC) Guidelines and Policies and the ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines. Chickadees were captured under an Environment Canada Canadian Wildlife Service Scientific permit, Alberta Fish and Wildlife Capture and Research permits, and City of Edmonton Parks Permit.

Results

We conducted a rmANOVA on the baseline-scaled feeds to determine the relationship between the type of playback heard (Silence, Calls, Noise, or Calls and Noise) and the time relative to the playback (during Playback, Silence between Playback, or Late Silence) while accounting for both the sex of the subject and the order in which the subject heard the types of playback. There was a significant effect of Condition on feeding behaviour ($F_{2, 24} = 11.62$, $p < 0.001$) such that birds visited the feeder fewer times on days when Calls were played than they did on days when Noise or Calls and Noise were played ($p = 0.025$; $p < 0.001$; see Figure 3).

Time also had a significant effect on feeding behaviour ($F_{3, 23} = 22.59$, $p < 0.001$), where birds fed less during Playback than they did during Silence between Playback ($p < 0.001$; see Figure 4-4).

There was a significant Time × Condition interaction ($F_{6, 20} = 11.89$, $p < 0.001$). When looking at the Silence between Playback, birds fed less on days when Calls were played than on days when Noise, or Calls and Noise, were played ($p = 0.001$; $p = 0.001$). There was no significant difference in feeding between Noise, and Calls and Noise, conditions ($p \geq 0.454$). Birds also fed less during Late Silence than would be expected if they fed at a constant rate (expected: 53.33%; observed: 46.78%).

There was no overall significant main effect of Sex ($F_{1, 25} = 0.28$, $p = 0.599$), meaning that the proportion of feeds was similar between males and females. Similarly, there was no significant main effect of presentation Order ($F_{5, 25} = 0.74$, $p = 0.599$). However, the three-way effects Condition × Time × Order ($F_{30, 120} = 1.71$, $p < 0.001$), Condition × Sex × Order ($F_{8, 50} = 2.47$, $p = 0.024$), and Time × Sex × Order ($F_{12, 75} = 4.40$, $p < 0.001$) were significant. Neither the three-way Condition × Time × Sex interaction ($F_{6, 20} = 2.66$, $p = 0.133046$) nor the four-way Condition × Time × Sex × Order interaction were significant ($F_{24, 92} = 1.68$, $p = 0.091$).

Discussion

In this experiment, we looked at the effects of brown noise on the feeding behaviour of captive black-capped chickadees in the absence of other factors associated with the production of anthropogenic noise. We predicted that the presence of anthropogenic noise, approximated by brown noise, would have a negative impact on feeding behaviour in black-capped chickadees and that the presence of conspecific *chick-a-dee* calls may mitigate those negative effects. Instead, we observed that individuals fed significantly less on days when *chick-a-dee* calls were played than on days when either brown noise or the combination of *chick-a-dee* calls and brown noise were played. Though we did not predict higher feeding during playback of noise than during call playback, there is some evidence that songbirds experiencing stress and variable feeding (both of which may here be caused by the pattern of playback and silence) increase feeding and associated activity to compensate for increased metabolism (Fokidis, Burin des Roziers, Sparr, Rogowski, Sweazea, & Deviche, 2012). The increase observed in this experiment, therefore, could indicate that noise is elevating stress hormones or other internal processes that are not immediately observable.

We also observed that birds fed significantly less during stimulus playback than during the periods of silence between the playback. When considering the type of stimulus being played, we found that birds who had heard playback of conspecific *chick-a-dee* calls fed less during the subsequent period of silence than did birds who heard either brown noise or a combination of calls and brown noise. Quinn and colleagues (2006) found that chaffinches (*Fringilla coelebs*) decrease feeding behaviour in favour of vigilance behaviours in the presence of anthropogenic noise. While we did not monitor what behaviours chickadees were engaging in when not feeding, this may provide evidence that black-capped chickadees prefer to feed when anthropogenic and other sources of noise are not present to mask important auditory cues from their surroundings (e.g., sound of an approaching predator).

Our results show that black-capped chickadees are capable of altering their foraging behaviour in response to noise cues, preferring to feed when noise is not present. This ability could be part of what allows these and other species to thrive in environments with variable noise levels, like cities. This feeding behaviour could be adaptive, akin to birds shifting singing behaviour to avoid rush hour noise. Bergen and Abs (1997) found that urban songbird species sing earlier in the day than those in the forest. Similarly, Brumm (2004) found that nightingales (*Luscinia megarhynchos*) sing louder when traffic noise is present, increasing song amplitude during the week but not on weekends. Proppe and colleagues also found that black-capped chickadees sing at higher frequencies (2012) and sing shorter songs as levels of anthropogenic noise increase (2011). Overall, Barber and colleagues (2010) suggest that the ability to adapt may determine which species thrive in urban environments and which are forced to find new habitats.

We had predicted that the presence of conspecific calls would mitigate the effects of anthropogenic noise on feeding behaviour, however, our results did not support this. Schepers and Proppe (2017) found that playback of conspecific vocalizations in a wooded roadside environment increased the population density of a number of bird species in that habitat, suggesting that song playback could provide a means of fortifying species density and diversity near roads. In looking at feeding behaviour, we found that black-capped chickadees behaved similarly to both noise and noise with calls, altering their behaviour after the playback rather than during. Damsky and Gall (2016) found that both black-capped chickadees and the tufted titmouse (*Baeolophus bicolor*), another small North American songbird, did not attend to tufted titmouse mobbing calls (calls used to recruit both con- and heterospecifics to ward off predators) when the calls were played in combination with anthropogenic noise. They suggest that anthropogenic noise may mask vocalizations, limiting their effectiveness. These results, taken together with our own, suggest that playback of conspecific calls embedded within noise may not enhance feeding behaviour by simulating safety through the presence of conspecifics.

We observed that birds fed significantly less during the silent period at the end of the day (i.e., Late Silence) than during either stimulus playback or the silence between stimulus playback, regardless of playback condition, order of playback exposure, or sex. We believe this to be due to natural feeding tendencies in black-capped chickadees as during baseline birds were observed to feed primarily between 9:00 and 16:00 with feeder visits steadily declining until lights out. In nature, midday hours would be optimal for feeding to ensure appropriate caloric intake before nightfall.

Here, we have demonstrated the importance of conducting research in both the field and in a controlled laboratory. Having conducted research with species as diverse as pigeons (e.g., *Columbia livia*), chickadees (e.g., *Poecile atricapillus* and *P. gambeli*; Batty et al., 2009), ants (e.g., *Melophorous bagoti* and *Veromessor pergandei*), and humans (i.e., *Homo sapiens*), Dr. Marcia Spetch seeks the best model for each research question. This results in a spectrum of research methods, ranging from field studies investigating natural behaviours with controlled manipulations (e.g., navigational abilities in both humans and desert ants; Spetch et al., 1997; Legge et al., 2014, respectively), to lab studies investigating specific biological capacity for a behaviour (e.g., risky decision making in pigeons and humans, or timing in pigeons and chickadees; Ludvig et al., 2014; McMillan et al., 2016, 2017, respectively), and anywhere in between. Marcia Spetch's extensive body of work clearly demonstrates the value of studying human and non-human animals in both the wild and the laboratory in an attempt to investigate perception and cognition. For example, Spetch and colleagues (1997) conducted several experiments comparing pigeons and humans on a searching task. When searching for a hidden goal, as the square array increased, pigeons searched according to the distance and direction from a landmark. However, humans instead searched the middle of the table top as the array increased (i.e., a location that was similar to the goal in comparison to the original landmark array). To determine how human participants would respond on a larger scale, a comparative task was designed in an outdoor grassy field. Responding in the different settings was similar even though the participants had to physically move through space to find the goal (i.e., unlike the laboratory setting). This provided further evidence of the differences between pigeon and human search behaviour, confirming that humans' search strategy was not confined by size or a lack of physical movement. In this case, a laboratory research question was taken from the laboratory to the field, but there are many reasons to bring field research questions into the laboratory.

In conclusion, we found that black-capped chickadees fed significantly less when presented with conspecific calls than when presented with brown noise (with or without calls). Chickadees also fed significantly more during the periods of silence between stimulus playback than during the playback itself, regardless of playback type. Our experiment helps demonstrate the value of conducting research under the correct conditions for the research question. Our results provide evidence supporting research (e.g., in chaffinches Quinn et al., 2016) that has demonstrated songbirds are capable of adjusting their behaviour in response to anthropogenic noise and that some of these observed behaviours may be due to stress-driven changes in metabolism. The ability to adapt both feeding and communication behaviours allows species to exist in the presence of anthropogenic noise and thrive in the ever-growing urban landscapes.

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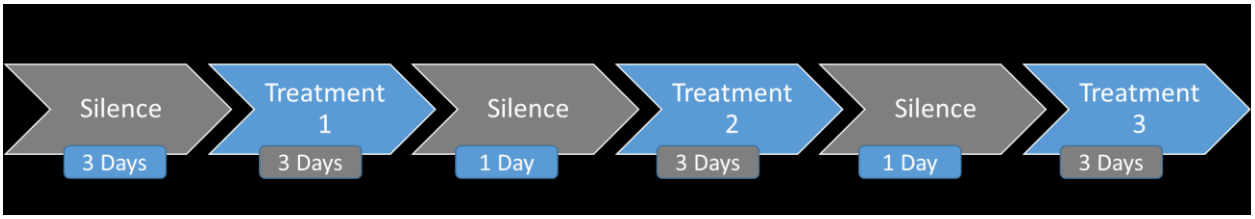

Figure 4-1. A flowchart showing order and number of days for each stage of treatment.

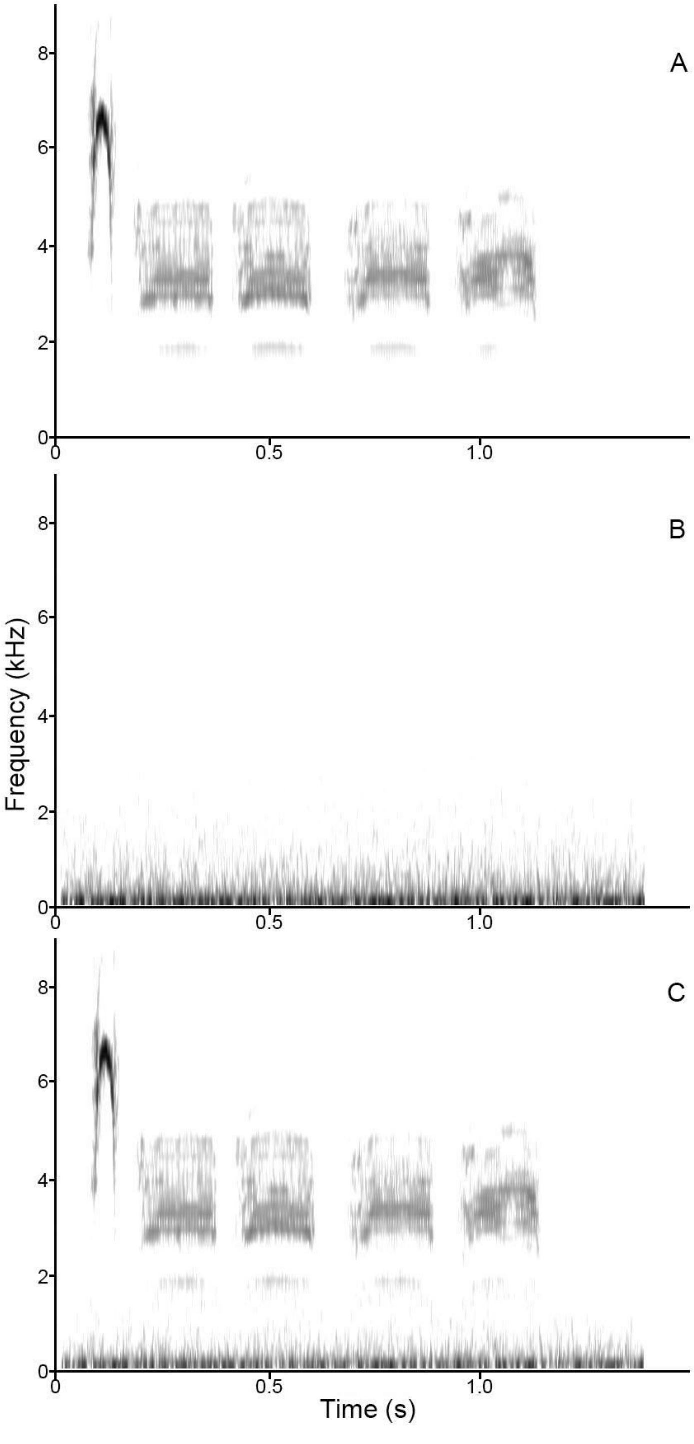

Figure 4-2. A sound spectrogram (window size = 256 points, time resolution = 5.8 ms) showing the three stimulus types used for playback: (A) black-capped chickadee *chick-a-dee* calls, (B) brown noise, and (C) a combination of *chick-a-dee* calls and noise.

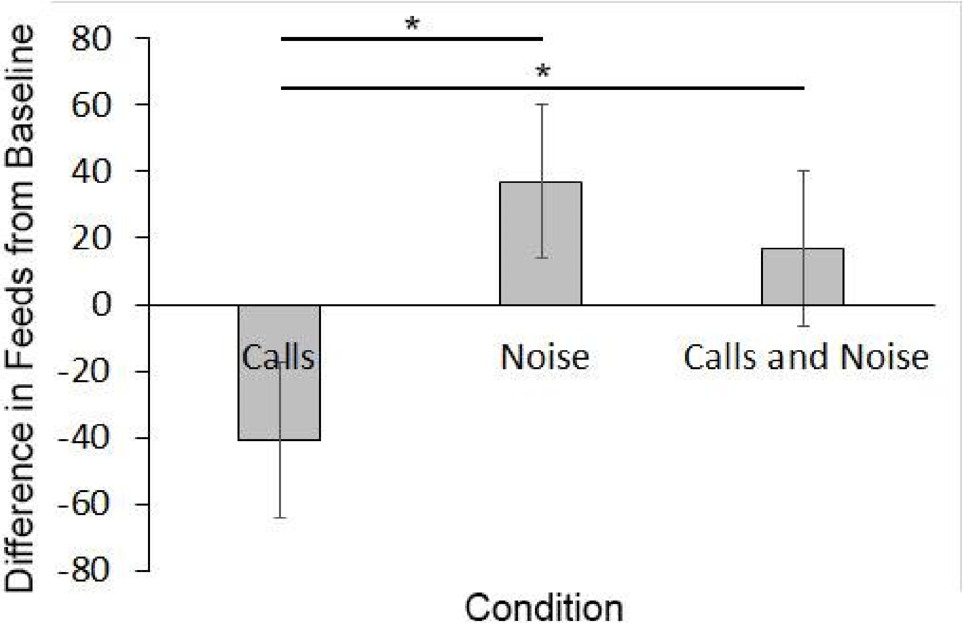

Figure 4-3. Mean ± SE of difference in total feeding across all subjects between each playback Condition (Calls, Noise, Calls and Noise) and Baseline. Here, a negative number means birds fed less during a treatment condition than during the baseline (silent) condition. * indicates a significant difference ($p \leq 0.05$) between group means.

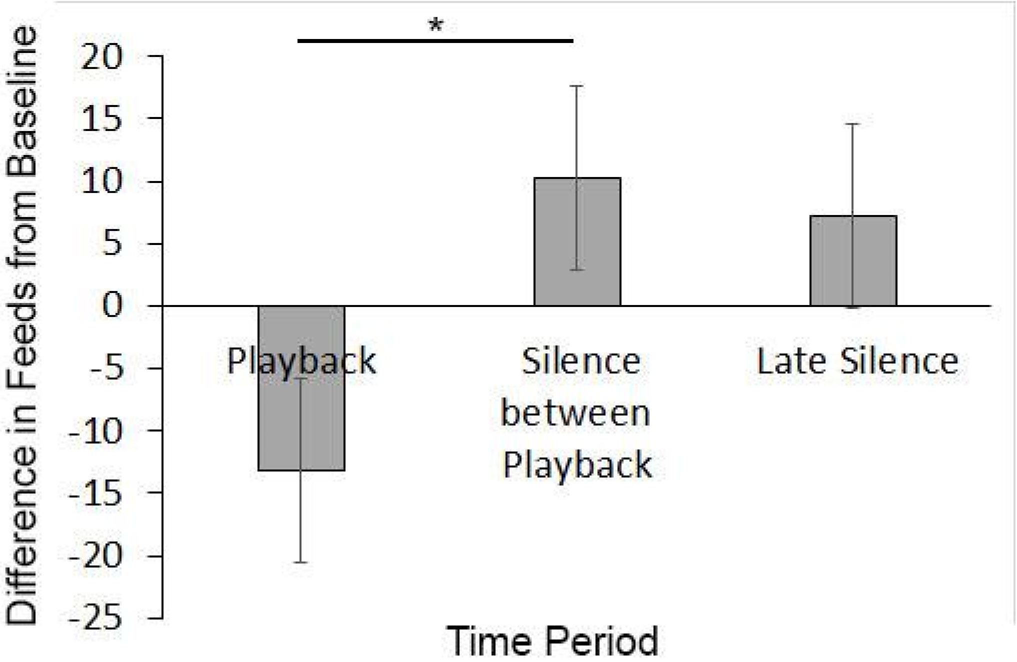

Figure 4-4. Mean ± SE of difference in total feeding across all subjects between each time period (Playback, Silence between Playback, Late Silence) and Baseline. Here, a negative number means birds fed less during a time period during treatment condition than at the equivalent time period during the baseline (silent) condition. * indicates a significant difference ($p \leq 0.05$) between group means.

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Chapter 5: General Discussion

Summary

This thesis examined the perception of information contained within the *chick-a-dee* calls of black-capped chickadees. Chapters 2 and 3 addressed potential natural categories that black-capped chickadees may attend to within conspecific *chick-a-dee* calls. Both chapters involved operant conditioning go/no-go discrimination tasks (two in Chapter 2, one in Chapter 3) to test for perception of acoustic categories, here sex of the signaller and note order within calls. Chapter 3 connected categorization with individual preference using a choice preference task. In Chapter 4, I conducted a behavioral task to determine how anthropogenic noise impacted feeding behavior and to see if conspecific *chick-a-dee* calls mitigated those changes. Overall, the results of these experiments revealed the variety of acoustic features present in *chick-a-dee* calls, increasing our understanding of how chickadees do or do not perceive those features, and illuminating behaviors that occur in response to those features. This adds to our understanding of an acoustically and functionally complex vocalization of a small North American songbird.

Sex differences in vocalizations and biological relevance

We conceptualized this experiment based on the idea that being able to identify the sex of an unknown caller simply from the acoustic features within a call appears to be biologically beneficial. For a male chickadee, if an unknown bird is also a male, he could be a potential rival and should be confronted. If a new bird is a female, they are a potential mate and should not be chased off. Sex differences have been identified in the acoustic structures of other black-capped chickadee vocalizations (*tseet* call: Guillette et al., 2010; *fee-bee* song: Hahn et al., 2013). Additionally, birds have been found to attend to these sex differences in *fee-bee* song, discriminating male- and female-produced songs in two separate open-ended categories (Hahn et al., 2017). Bioacoustic analyses have identified potential acoustic differences between male- and female-produced *chick-a-dee* calls in black-capped chickadees (Campbell, et al., 2016) as well as Carolina chickadees (Freeberg et al., 2003). Avey and colleagues (2008) also found differential neural expression in the brains of black-capped chickadees when exposed to male- and female-produced chickadee calls, suggesting differences in perception of those calls. The experiments detailed in Chapter 2 allowed us to evaluate if black-capped chickadees attend to any acoustic features that differ between the calls of male and female chickadees to discriminate between the calls.

The results of Chapter 2 provide evidence the black-capped chickadees do not treat male- and female-produced *chick-a-dee* calls as separate open-ended categories. In both experiments described in the chapter, chickadees in the true-category discrimination groups did not learn any faster than chickadees in the pseudo-category discrimination groups, suggesting that there is no advantage for speed of acquisition to discriminate true categories using categorization over rote memorization. This conclusion is further supported by the results of the transfer tests where birds in the true-category discrimination groups did not respond to novel calls using the same contingencies that they learned in discrimination training.

Here we propose that black-capped chickadees only attend to the sex of a signaler when it is biologically relevant to categorize those signals based on sex rather than relying on individual identity. For example, we see black-capped chickadees discriminate between male- and female-produced *fee-bee* songs using open-ended categorization (Hahn et al., 2017) because the *fee-bee* song is used to advertise their territory and attract mates as well as deter competitors (Mennill & Otter, 2007) where correctly identifying the sex of a signaler is crucial in such critical situations affecting fitness. In contrast, chickadee calls are employed in a variety of situations unrelated to mating such as flock coordination (Ficken, Ficken, & Witkin, 1978), notification of food sources

(Ficken, 1981), and mobbing of predators (Hurd 1996). Since these calls are not used in situations related to mating, it is less essential to be able to quickly identify the sex of the individual producing a call. In particular, chickadees tend to occupy set territories during the spring and summer months where they will know the individuals in surrounding territories (Smith, 1991). In the fall and winter, black-capped chickadees form relatively stable flocks that do not travel great distances (Smith, 1991). Since chickadee calls allow for individual (Charrier et al., 2004) and flock identification (Mammen & Nowicki, 1981; Nowicki, 1983) and since birds will be familiar with local individuals and flocks, it could be sufficient to rely on individual identification rather than identifying an individual's sex in situations where *chick-a-dee* calls are employed.

To evaluate this hypothesis, future research should determine whether or not chickadees treat other black-capped chickadee vocalizations, such as *tseet* and *gargle* calls, produced by male and female chickadees as separate open-ended categories. *Tseet* calls are contact calls used almost continually to keep in contact with nearby conspecifics (Smith, 1991), while *gargle* calls are aggressive signals often produced between members of winter flocks (Ficken et al., 1978). If I am correct and chickadees only attend to the sex of a signaler in situations where sex is critical, then we would expect that chickadees would treat both *tseet* and *gargle* calls in the same manner as they did *chick-a-dee* calls: learning to discriminate between male- and female-produced calls by relying on rote memorization rather than employing open-ended categorization.

Open-ended categorization of calls

Experiments in both Chapters 2 and 3 employed operant conditioning go/no-go discrimination tasks. These tasks are designed to allow for identification of the methods used by individuals to discriminate between two categories of stimuli. One method that can be used for discrimination is rote memorization in which individuals memorize individual vocalizations based on their reward contingencies. For example, an individual would learn that vocalization one is rewarded while vocalization two is unrewarded. Black-capped chickadees are particularly good at employing rote memorization, as was shown in both experiments of Chapter 2 where birds used rote memorization to discriminate between 120 male- and female-produced *chick-a-dee* calls. Another method of discrimination is open-ended categorization, in which individuals learn to classify stimuli into a category based on acoustic similarity. In this case, an individual would learn that vocalization one belongs to category A and is therefore rewarded while vocalization two belongs to category B and is therefore unrewarded. For small stimulus sets, the benefit of using open-ended categorization is minimal as it does not require large amounts of processing to memorize small numbers of stimuli. As stimulus sets get larger, it becomes beneficial to use open-ended categorization as individuals can learn the general rule that category A is rewarded and category B is unrewarded, then respond to each new vocalization as it appears based on the category to which it belongs. In nature, this is beneficial as it allows individuals to respond quickly to novel stimuli based on past experience.

There are two aspects to consider when determining whether an individual is using rote memorization or open-ended categorization within an operant go/no-go discrimination task. The first is investigating the speed of acquisition, or how long it takes an individual to learn to discriminate between two categories. As explained above, once a bird has learned the category rule of “respond to A, but not B,” it does not require increased cognitive load to apply that rule to new vocalizations, whereas if an individual is learning each vocalization separately, each new vocalization requires more cognitive resources. As such, making use of open-ended categorization should result in faster discrimination of stimuli than would rote memorization.

Individuals can be forced to rely on rote memorization by enforcing a discrimination that does not correspond to a perceptual category (i.e., if there is no "rule" for responding). This is seen in the ability of black-capped chickadees to discriminate between *fee-bee* songs produced by individual chickadees (Phillmore, Sturdy, Turyk, & Weisman, 2002; Wilson & Mennill, 2010) where the true-category discrimination groups (i.e., groups rewarded for responding to songs produced by one individual, but not another) leaned the discrimination significantly faster than individuals in the pseudo-category discrimination groups (i.e., groups rewarded for responding to some songs produced by one individual and some produced by another).

In both Chapters 2 and 3, we found no difference in rate of learning between true and pseudo category groups. This could mean that a) the two categories being investigated (e.g., male vs. female in Chapter 2; natural vs. scrambled in Chapter 3) did not belong to distinct perceptual categories and as such, both true and pseudo category groups relied on rote memorization, or b) open-ended categorization did not provide a speed advantage over rote memorization in acquiring the discrimination. To differentiate between these two options, we tested how birds responded to untrained calls (i.e., calls that had not been differentially rewarded). The results of transfer testing stages in Chapter 2 showed that birds did not use open-ended categorization to discriminate between male- and female-produced *chick-a-dee* calls and instead relied on rote memorization. In contrast, the transfer stage of Chapter 3 showed that birds did continue to respond based on the learned contingencies from discrimination training (i.e., the category "rules") when presented with new stimuli, revealing that chickadees do perceive natural and scrambled order *chick-a-dee* calls as separate perceptual categories.

Individual variation in preference and performance

Individual differences exist across many aspects of behavior. Black-capped chickadees exhibit individual differences in vocal production of their *tseet* call (Guillette et al., 2010), *chick-a-dee* call (Charrier et al., 2004), and *fee-bee* song (Christie, Mennill, & Ratcliffe, 2004). A large portion of recent research investigating individual variation focuses on how personality influences an individual’s behaviour (for breadth of research, see Carere & Maestripieri, 2013). In black-capped chickadees, there is evidence that individual personality is related to vocal-production in stressful conditions, such that individuals that more readily explore novel environments also more readily vocalize in response to conspecific mobbing calls (Guillette & Sturdy, 2011). Similarly, fast exploring black-capped chickadees also showed faster learning in an acoustic discrimination task than slow exploring individuals (Guillette, Reddon, Hurd, & Sturdy, 2009).

Recently, there has been a push to connect the study of individual cognitive ability and personality allowing for the identification of neural and biological mechanisms that may underlie both types of variation (reviewed by Griffin, Guillette, & Healy, 2015). Griffin and colleagues (2015) describe the difficulty of integrating studies of individual variation in cognitive ability with the existing field of personality, pointing out that in practice, most cognitive studies seek to minimize individual differences to get at species level cognitive ability. Matzel and colleagues (2003) describe a methodology in which they evaluated general learning ability in individual mice and found a positive correlation between scores on tasks associated with cognitive abilities such as rate of learning, spatial navigation, and fear conditioning, providing evidence for a common source of variation underlying general cognitive abilities.

In the experiments outlined in Chapter 3, we were interested in the relationship between individual differences in both preference and performance. Here, performance on an acoustic discrimination task, as quantified by rate of acquisition, provided a measure of individual cognitive ability while proportion of time on or proportion of visits to acoustic perches provided a measure of individual preference. Our results showed a significant correlation between individual preference and performance such that birds that displayed a strong preference for the rewarded stimulus group of calls had taken longer to learn the acoustic discrimination than did birds that preferred the unrewarded stimulus group of calls. Hahn and colleagues (2017) investigated individual preference for and performance in discriminating between *fee-bee* songs produced by dominant and subordinate black-capped chickadees, and found similar evidence for individual differences in acoustic preference but no correlation between preference and speed of acquisition of discrimination.

The relationship between individual preference and individual cognitive ability is not yet well understood. Additional research comparing these two areas is necessary to allow for a better understanding of what biological mechanisms may be involved in individual variation of behavior.

The importance of preliminary studies

In the introduction of this dissertation, I discussed the importance of combining different methodologies to create a more thorough understanding of communication systems (see Sturdy & Weisman, 2006). The studies contained within this thesis were designed to approach the scientific question in a manner that would supplement existing research. For example, the behavioral studies described in Chapter 2 were a continuation of an earlier bioacoustic analysis that investigated acoustic features that differed between the *chick-a-dee* calls of male and female black-capped chickadees (Campbell, et al., 2016). Additionally, within that same chapter, Experiment 2 was a follow-up to the original experiment intended to clarify and expand upon the results we obtained. In each case, the earlier research informed the studies that followed.

In Chapter 4, I described a behavioral experiment designed to investigate the effects of anthropogenic noise on feeding behavior in black-capped chickadees in controlled laboratory settings. This study was based, in part, on field research that showed black-capped chickadees will shift the acoustic structure of their songs in the presence of anthropogenic noise (Proppe et al., 2012) and that playback of conspecific calls may mitigate the effects of anthropogenic noise in songbird species living near roadways (Schepers & Proppe, 2017). Though our results did not show playback of *chick-a-dee* calls mitigating the changes in behaviour caused by playback of anthropogenic noise, they did raise a series of questions that future research should be designed to answer. For example, observations of increased feeding behaviour on days when anthropogenic noise was heard have been suggested to be due to increased metabolism resulting from stress caused by noise playback. This result may also be due to limitations of the experimental apparatus, in that any entrance into the feeder was registered as a feeding visit, even if a bird did not feed. In this case, increased motor behaviour could also be interpreted as increased feeding behaviour. To differentiate between these two options and to identify any additional behaviours that might be occurring, future studies should involve hormone assays to evaluate individual stress levels as well as video scoring of other behaviours to better understand what individuals are doing in response to playbacks.

Additionally, findings from the laboratory should also be considered with field studies employing comparative methodologies. For example, the two field studies this experiment was based on showed a shift in singing behaviour and of population density. To determine how our observations translate into nature, a field study should be conducted using playback of anthropogenic noise and *chick-a-dee* calls to investigate changes to feeding behaviour in nature.

Conclusions

My goal for the studies outlined in this thesis was to understand the information contained within black-capped chickadee *chick-a-dee* calls, how chickadees perceive that information, and how they respond to it. The results of these varied studies further illustrate the complexity and variability of these calls. Specifically, we now know that while *chick-a-dee* calls vary acoustically between the sexes, chickadees do not attend to these differences to categorize those calls. However, chickadees do successfully categorize vocalizations that differ in note composition, treating natural-ordered and scramble-ordered *chick-a-dee* calls as separate open-ended categories. Additionally, *chick-a-dee* calls do not appear to mitigate the effects of anthropogenic noise. Additional studies are needed to expand on the knowledge gained through this research by better understanding the role and importance of note order in *chick-a-dee* calls and increasing our understanding of the effects of anthropogenic noise on feeding behaviour. Future research should continue to consider individual variation when investigating cognitive ability, as well as the biological significance of events and stimuli being investigated.

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