Perception and communication of predator threat in the black-capped chickadee (*Poecile atricapillus*)

by

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Abstract

The black-capped chickadee (*Poecile atricapillus*) is a type of North American songbird that produces multiple vocalizations, including their namesake *chick-a-dee* call. This call is used as a mobbing call to recruit and coordinate conspecifics in the presence of a predator. These parids perceive and respond to avian predators of varying size, as well as elicited conspecific mobbing calls. Specifically, smaller hawks and owls are of higher threat to chickadees as they easily maneuver through the trees, while larger hawks and owls cannot. The *chick-a-dee* call consists of four note types that can be repeated or omitted (A, B, C, and D), and chickadees produce more D notes per call in response to small, high-threat predators compared to large, low-threat predators. In Chapter 2, I extended work investigating the perception of arousal in vocalizations produced by three biological classes by testing whether two vocal learning species, humans and chickadees, can discriminate vocalizations of high- and low-arousal using operant discrimination go/no-go tasks. Chickadees (Experiment 1) and humans (Experiment 2) learned to discriminate between high- and low-arousal stimuli and significantly transferred the discrimination to additional giant panda, human, and black-capped chickadee vocalizations. I also conducted discriminant function analyses using four acoustic measures, and found that fundamental frequency likely played a role in responding during the task; however, these analyses also suggest roles for other acoustic factors as well as familiarity. Next, in Chapter 3, I conducted an operant go/no-go discrimination task to investigate the effect of signal degradation and anthropogenic noise on perceived threat. The results indicated that chickadees responded to natural low-threat owl stimuli more consistently across distance than high-threat owl stimuli, synthetic tones were responded to similarly compared to natural stimuli but at lower levels, and truck alarms were not responded to as though they were of high-threat suggesting that although acoustically-similar, chickadees can discriminate between high-threat owl calls and anthropogenic stimuli. In Chapter 4, I used operant conditioning techniques to investigate referential communication, which has been defined as the exchange of information giving reference to objects or events. Few studies have examined referential alarm calls in songbirds, yet we found that high- and low-threat mobbing calls were not treated similarly to the corresponding predator's calls. Since owl stimuli are acoustically distinct in comparison to the conspecific stimuli, chickadees may have found predator stimuli easier to discriminate; this factor may have lead to the asymmetrical results. In Chapter 5, I conducted an operant go/no-go experiment investigating black-capped chickadees' acoustic discrimination of predator threat. I confirmed that chickadees can discriminate between high- and low-threat predator calls, and further investigated how chickadees categorize mid-sized species' calls via transfer of training according to perceived threat. Specifically, mid-sized broad-winged hawks were perceived to be of high threat whereas mid-sized short-eared owls were perceived to be of low threat. Mid-sized Cooper's hawks and northern hawk owls, however, were not significantly differentially responded to, suggesting that they are of medium threat which supports the notion that perception of threat is along a continuum rather than distinct categories of high or low threat. Last, in Chapter 6, I exposed black-capped chickadees to stationary avian and mammalian mounts of various threat levels in the field. This study revealed that only *chick-a-dee* call production in response to avian mounts significantly differed, chickadees produced more *chick-a-dee* calls with 4-8 D notes in response to the high-threat avian mount compared to the high-threat mammalian mount, chickadees produced significantly more 4 D note calls in response to the small, sharp-shinned hawk in comparison to the large, red-tailed hawk, and that feeding was most reduced to high- and low-threat avian predator mounts compared to the foam control.

Taken together, these studies reveal insights into chickadee perception and communication of threat. Specifically, these studies indicate that: chickadees are capable of discriminating between high and low arousal in vocalizations produced by multiple species (Chapter 2); signal degradation affects threat perception (Chapter 3); operant conditioning may not be an ideal paradigm for examining the referential elements of mobbing calls (Chapter 4); there is likely a continuum of threat according to predator size (Chapter 5); and antipredator responses of black-capped chickadees vary in response to avian and mammalian predators that pose a high, low, or no threat (Chapter 6).

Preface

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

Chapter 2 of this thesis has been published as J. V. Congdon, A. H. Hahn, P. Filippi, K. A. Campbell, J. Hoang, E. N. Scully, D. L. Bowling, S. A. Reber, & C. B. Sturdy, "Hear them roar: A comparison of black-capped chickadee (*Poecile atricapillus*) and human (*Homo sapiens*) perception of arousal in vocalizations across all classes of terrestrial vertebrates", Journal of Comparative Psychology, *in press*. I was responsible for concept formation, experimental design, stimuli preparation, data collection, data analysis, and manuscript composition. A.H. Hahn contributed advice for the experimental design, data analysis, and manuscript edits. P. Filippi contributed to provided collected acoustic stimuli and manuscript edits. K.A. Campbell assisted with collecting chickadee subject data, data analysis, and manuscript edits. J. Hoang contributed to stimuli preparation and collecting human participant data. E.N. Scully contributed to collecting bird subject data and manuscript revisions. D.L. Bowling and S.A. Reber provided recordings used as stimuli and manuscript advice. C.B. Sturdy was the supervisory author and was involved in concept formation and manuscript revision.

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Dedication

My dissertation is dedicated to my incredible family, friends, and colleagues, both past and present. Thank you. My successes, especially this dissertation, would not have been possible without each of you and your love, support, and guidance.

Clear eyes, full hearts, can't lose. – Coach Taylor

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Table of Contents

List of Tables

List of Figures

Chapter 1. General Introduction

Communication

Communication can be defined in many ways, typically involving the transmission of a ‘signal’. Signals have been defined as “behavioral, physiological, or morphological characteristics fashioned or maintained by natural selection because they convey information to other organisms” (Otte, 1974). Thus, a signal provides information, and when transmitted from a sender to a receiver, the receiver should then respond appropriately (Pearce, 2008). Conversely, Maynard Smith and Harper’s (2003) Signalling Theory suggests that a signal will only be selected for if both the signaller and the receiver benefit from the production of it. The latter definition is not universally accepted, however, as there are several examples in which the receiver does not also benefit from the production of the signal, such as mimicry or dishonest signalling (Pearce, 2008). Animals can communicate regarding their identity, mood, intentions, and information about the environment (e.g., food, mates, predators) through auditory, visual, chemical, electrical, tactile and thermal, and vibrational signals (Hauser, 1996). In addition, animals can also create unintentional signals (i.e., cues). For example, animals leave behind footprints, feces, scents or pheromones, and more that provide information about that individual. This type of passive communication can also occur including examples such as the colouring of bees and wasps, poison dart frogs (*Dendrobatidae*), and Eastern coral snake (*Micrurus fulvius*), or plumage (e.g., bright peacock feathers to attract mates, dull plumage when sick; Pearce, 2008).

Although there are many different types of animal communication, I am primarily interested in intentional (i.e., active) acoustic and visual behaviours. Acoustic signals are capable of travelling long distances, can be received from many angles, and allow for communication when conspecifics or heterospecifics are not visible (e.g., during thick summer foliage). Many animals produce alarm calls, vocalizations produced by a species in the presence of a predator (Struhsaker, 1967). It has been demonstrated that certain species' alarm calls convey specific meaning about a nearby predator (i.e., semantic information). A well-known example is the vervet monkey (*Cercopithecus aethiops*), that produces distinct vocalizations that refer to different types of threat. Vervet monkeys live in South Africa in social troops and require the ability to communicate with conspecifics in order to survive. Research has demonstrated that vervet monkeys are prey to several different predators, and have specific antipredator responses to each: (1) In the presence of a leopard (*Panthera pardus*), vervet monkeys will produce a loud bark; (2) in the presence of an aerial predator (e.g., eagle; *Polemaetus bellicosus*), vervet monkeys will produce a chuckle; and (3) in the presence of a snake (e.g., python; *Python sebae*), vervet monkeys will produce high-pitched chuttering (Cheney & Seyfarth, 1990). Through playback experiments, it is evident that conspecific receivers respond differentially to alarm calls produced in response to leopards, eagles, and snakes. Vervet monkeys either: (1) fled up a tree, (2) looked toward the sky and dove into a nearby bush for cover, or (3) searched the ground to potentially mob the predator, respectively (Seyfarth & Cheney, 1993). Seyfarth and Cheney (1993) found that vervet monkeys also produce a distinct alarm call in response to baboons, predators that attack their young.

Social animals require the ability to communicate in order to survive, but historically, human speech has been differentiated from other forms of acoustic animal communication according to the properties of language (Hockett, 1960). Language has been defined according to a number of properties including an arbitrariness of units (i.e., discrete units), displacement (i.e., communicating about events in another time or space), productivity (i.e., grammar/syntax, rules of production), and semanticity (i.e., specific meaning; Pearce, 2008). For example, focusing on the property of semanticity, for an auditory animal communication system to be considered as language, vocalizations must be capable of containing specific meaning (i.e., an external referent). The notion of semantics is connected to referential communication, defined as the use of signals to provide information about objects or events in the environment (Marler, Evans, & Hauser, 1992). As summarized, Seyfarth, Cheney, and Marler (1980) focused on semantic signals and demonstrated that vervet monkeys' alarm calls contain specific meaning and external referent for the receivers of these signals. Many studies have demonstrated that non-human animal communication satisfies certain properties of language, however, few studies have examined referential properties in songbird acoustic communication.

Passerines

Songbirds, or Oscine Passerines (i.e., "perching birds" that include more than half of all bird species; Mischler et al., 2017), are classified as 'vocal learners', groups of animals that require a model (e.g., parent) in order to learn species-specific vocalizations that are critical for survival (Doupe & Kuhl, 1999). Songbirds are among a small vocal learning groups of animals, including parrots, hummingbirds, bats, elephants, cetaceans (i.e., dolphins and whales), elephants, and humans (Jarvis, 2006).

My dissertation proposal is more specifically focused on my main model species, the black-capped chickadee (*Poecile atricapillus*). Black-capped chickadees are a species of non-migratory North American songbird that produce a number of vocalizations, classified based mainly on function as either songs or calls (Smith, 1991). Chickadees produce a song that is learned for mating and territoriality (Shackleton & Ratcliffe, 1993), but also learn parts of their calls (e.g., their namesake *chick-a-dee* call). Calls are used for every other function, and include the *gargle* call (used in aggressive situations), *tseet* call (a contact call), and *chick-a-dee* call. The *chick-a-dee* call can be used in a variety of contexts: conveying species information (Charrier & Sturdy, 2005), for recruitment to food sources (Mahurin & Freeberg, 2008), and for recruitment of conspecifics and heterospecifics to attack and harass (i.e., mob) perched predators (Ficken & Witkin, 1977).

Perception of Threat

A main focus of my dissertation is the investigation of predator-prey relationships, particularly how chickadees perceive and communicate about varying predator threat. According to Templeton et al. (2005), chickadees will produce *tseet* calls for flying predators, yet produce *chick-a-dee* mobbing calls for perched predators. The *chick-a-dee* call can contain four note types, produced in a strict order (A→B→C→D), that can be excluded or repeated for an infinite number of combinations (Ficken, Ficken, & Witkin, 1978). Templeton et al. (2005) investigated how chickadees respond to live predators of different sizes (i.e., wingspan and body length), and demonstrated that chickadees produce more D notes per call to smaller, high-threat hawks and owls compared to larger, low-threat predators. In addition, when mobbing call vocalizations were played back through a speaker, chickadees approached high-threat vocalizations more often and much closer, suggesting that the vocalizations include information about the nearby predator threat and initiate mobbing behaviour (Templeton et al., 2005). Avey et al. (2011) later designed an experiment to investigate the immediate early gene (IEG) expression of ZENK in black-capped chickadees' auditory brain areas in response to vocalizations of predators of varying threat levels. Northern-saw whet owls (NSWO; *Aegolius acadicus*) pose a high threat to chickadees as they can easily maneuver through the trees when chasing small prey; however, great horned owls (GHOW; *Bubo virginianus*) are much larger, slower, and do not typically consume songbirds. Avey et al. (2011) used playback stimuli of NSWO and GHOW vocalizations, and the control calls of the red-breasted nuthatch (RBNU; *Sitta canadensis*). In addition, chickadee mobbing calls in response to NSWO (i.e., MOB NSWO) and GHOW (i.e., MOB GHOW) mounts were used. In summary, Avey et al. (2011) found that higher levels of ZENK expression occur in response to small, high-threat owl predator calls (NSWO) and the corresponding high-threat *chick-a-dee* mobbing calls (MOB NSWO) in comparison to low-threat owl and *chick-a-dee* mobbing calls.

Avey et al.'s (2011) experiment provided strong evidence showing a relationship between chickadee IEG expression and varying levels and types (i.e., owl vs. mobbing calls) of predator threat, but did not provide information on how chickadee vocal and motor behaviour was altered by playback. Thus, my Master's thesis focused on conducting a playback experiment investigating the vocal and movement responses of chickadees to the same stimuli utilized by Avey et al. (2011). In line with Templeton et al.'s (2005) findings, I reported trends of 1-3 D notes per call to low threat mobbing calls (MOB GHOW) and 3-6 notes per call to high threat mobbing calls (MOB NSWO). I also found that chickadees produce more *tseet* calls following playback of heterospecific (including owl calls) in comparison to conspecific playback (i.e., mobbing calls), and that chickadees perch hop (a measure of general activity) more in response to heterospecifics, potentially as a way to prepare for fight-or-flight scenarios (Congdon et al., 2016).

Current Studies

Although vocal communication is well-studied in many species, there are many gaps in the literature regarding the information contained within these signals and how they are perceived. How do animals perceive the communication of threat? Specifically, can black-capped chickadees, a species of vocal-learning songbird, perceive arousal in heterospecific vocalizations (Chapter 2), perceive relative risk in the context of signal degradation (Chapter 3), and do the resulting vocalizations contain referential information about the predators they are perceiving (Chapter 4)? Also, how strong is the negative correlation between wingspan/body size and threat when discriminating the acoustic vocalizations of predators (Chapter 5) and visual predator and non-predator mounts in black-capped chickadees (Chapter 6)? Through the current studies, I try to address gaps in the literature regarding arousal in heterospecific vocalizations, the perception of threat following signal degradation, referential communication, and the negative correlation between predator size and perceived threat using both acoustic and visual stimuli. Through a combination of operant go/no-go discrimination tasks and mount exposure experiments, the objective of the research in this PhD dissertation explores the perception and communication of threat in the black-capped chickadee, and applies these responses on a larger comparative scale; specifically, I sought to investigate: (Chapter 2) how chickadees and humans perceive arousal in the vocalizations of multiple animal species (a comparative study of high- and low-arousal vocalizations); (Chapter 3) the effect of signal degradation on perceiving threat; (Chapter 4) the perception and categorization of predator and referential mobbing call signals; (Chapter 5) the categorization of the vocalizations of avian predators with respect to wingspan and perceived threat; and (Chapter 6) responses, in terms of vocalization and feeding, to mammalian predators in comparison to avian predators in the field.

In Chapter 2, I investigated if black-capped chickadees and humans can perceive arousal in heterospecific vocalizations produced by nine different species: giant panda (*Ailuropoda melanoleuca*), American alligator (*Alligator mississippiensi*s), common raven (*Corvus corax*), hourglass treefrog (*Dendropsophus ebraccatus*), human (*Homo sapiens*), African elephant (*Loxodonta africana*), Barbary macaque (*Macaca sylvanus*), black-capped chickadee, and domestic pig (*Sus scrofa*). I designed a comparative go/no-go discrimination task in which both subjects and participants were trained to respond to either high- or low-arousal stimuli then were tested with novel stimuli from each species to determine the ability for transfer of training. In addition, I conducted bioacoustic analyses, using multiple discriminant function analyses (DFA), to determine which acoustic features (i.e., duration, initial fundamental frequency, spectral centre of gravity, and/or harmonic-to-noise ratio) could explain the observed results.

Chickadees have previously been shown to respond differentially to owls of varying sizes/threat levels (live predators, Templeton et al., 2005; hetero- and conspecific vocalizations, Avey et al., 2011), but how are those signals affected by distance? In Chapter 3, I investigated the effect of signal degradation on perceived predator threat (high-threat NSWO and low-threat GHOW), and the resulting behaviours, of black-capped chickadees. In addition, I further investigated the perception of synthetic NSWO-like and GHOW-like stimuli and truck alarms, as these alarms are acoustically-similar to NSWO calls.

Many species produce alarm calls in the presence of predators, and it has been demonstrated that vocalizations change according to the predators that those species are communicating about (e.g., red squirrels', *Tamiasciurus hudsonicus*, *seet* and *bark* vocalizations in response to aerial and ground predators, respectively; Greene & Meagher, 1998). In Chapter 4, I aimed to further determine if chickadees' mobbing calls in response to NSWO and GHOW are perceived as equal to NSWO and GHOW predator calls, suggesting evidence for referential communication (i.e., semantic properties). I investigated this using an operant go/no-go discrimination task in which, for example, black-capped chickadees were initially trained to respond to either high- or low-threat owl vocalizations, then trained to respond to either high- or low-threat mobbing calls. I predicted that if chickadees completed the second round of discrimination, that included stimuli of the same contingency as the first round of discrimination (e.g., both vocalization types of the same threat level), in fewer trials than chickadees that stimuli included the opposite contingency compared to the first round of discrimination, then it would support the notion of referential communication in the mobbing calls of a species' of songbird.

In Chapter 5, I aimed to determine how strong the negative correlation in black-capped chickadees' perception of predator threat and actual predator wingspan is by training chickadees to respond to small, "high-threat" or large, "low- threat" owl and hawk calls, then tested responding to mid-sized owl and hawk calls (16 species in total). Previously, Templeton et al. (2005) demonstrated a strong negative correlation between wingspan/body length and the number of D notes produced per *chick-a-dee* call, yet how subjects respond in a go/no-go discrimination task will provide evidence on how the predators are perceived, to ascertain whether there is a continuum of threat perception rather than only categorical high- vs. low-threat.

In Chapter 6, expanding from operant go/no-go experiments, I designed a laboratory mount-exposure experiment with three species of both avian and mammalian predators: (1) a high-threat predator, (2) a low-threat predator, and (3) a non-threat species. This laboratory experiment resulted in few chickadee vocal or movement responses, and thus I designed a field extension to attempt to address the gap in the literature on songbird perception of mammalian predators. I conducted a field experiment with wild black-capped chickadees in which they were exposed to: (1) sharp-shinned hawk (*Accipiter striatus*), (2) red-tailed hawk (*Buteo jamaicensis*), and (3) northern flicker avian mounts (*Colaptes auratus*), and (1) cat (*Felis catus*), (2) American red squirrel, and (3) woodchuck mammalian (*Marmota monax*) mounts. In addition, I tested a control foam cylinder and a supplementary mount of a predator species, short-tailed weasel (i.e., a low-threat species; *Mustela erminea*). Overall *chick-a-dee* call production, the number of D notes produced per call, and feeding behaviour were analysed.

Chapter 7 provides a general discussion to summarize the findings of each experimental chapter. Here, I compare the findings of this dissertation to the literature on the perception of threat, focused on songbirds. The remaining gaps in the literature are discussed in terms of future directions for research.

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Chapter 2. Hear them roar: A comparison of black-capped chickadee (*Poecile atricapillus*) and human (*Homo sapiens*) perception of arousal in vocalizations across all classes of terrestrial vertebrates[1]

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Introduction

In *The Descent of Man*, Darwin proposed that our earliest terrestrial ancestors expressed emotion through their vocalizations (Darwin, 1871). This century-old hypothesis still underlies many theories of emotional communication today, highlighting the importance of understanding the specific information contained within vocalizations and the evolutionary origins of a trait critical to our survival (i.e., shared mechanisms for vocal emotional expression). Organisms produce acoustic signals in response to specific stimuli to convey information (Aubin & Jouventin, 2002). For example, signals may include the production of aggressive calls by hourglass treefrogs (*Dendrosophys ebraccatus*) during competitive signaling interactions with other males (Reichert, 2011), mobbing calls produced by black-capped chickadees (*Poecile atricapillus*) when they encounter predators (Templeton, Greene, & Davis, 2005), or distress calls produced by domestic pigs (*Sus scrofa domesticus*), all of which comprise information about an individual's emotional state and potential intentions (e.g., mating intentions, predator threat, distress; Linhart, Ratcliffe, Reby, & Spinka, 2015). Acoustic signals can vary based on different environmental contexts and adopt a wide range of functions in mate choice, potential threat perception, and species recognition in a broad range of taxa (Gerhardt, Humfeld, & Marshall, 2007). In diverse species, including insects, amphibians, birds, and mammals, acoustic signals convey a variety of cues: caller identity, location, social context, emotional state, current condition, and developmental state (Maynard Smith & Harper, 2004). Since the vocalizations of many taxa share similar functions, vocal characteristics are especially well-suited to investigate both within and between species similarities (i.e., do animals produce, and perceive signals that share overall meaning within and across species?). Moreover, some phylogenetic comparative studies support the idea that acoustic traits can predict patterns of diversification across genera by indicating lineage-specific acoustic differences (Seddon, Merrill, & Tobias, 2008). These studies suggest that organisms can identify the vocal signals not only within their own species, but also among others due to distinct characteristics. Overall, the capacity to perceive the information in acoustic signals is evolutionarily important because it allows individuals to respond appropriately in the face of specific environmental challenges (e.g., the presence of a predator), ultimately increasing their fitness.

Acoustic signals are produced in many contexts of intraspecies (i.e., conspecific) and interspecies (i.e., heterospecific) communication, which can be beneficial for the receiver as well as the signaler. For example, groups consisting of individuals of different species can benefit from alarm calls that warn of predators (Caro, 2005), as well as contact calls that maintain group cohesion by notifying nearby individuals of their location via the production of brief, soft short-range calls (Marler, 2004; e.g., *tseet* call of the black-capped chickadee: Smith, 1991). Black-capped chickadee mobbing calls have been observed to recruit conspecifics and other avian species (i.e., heterospecifics) to attack and harass nearby predators with mobbing behaviour (Templeton, Greene, & Davis, 2005), thus providing an example of both intra- and interspecies communication. There are many cases of species eavesdropping on heterospecifics' vocalizations; grey squirrels (*Sciurus carolinensis*) attend to the calls of blue jays (*Cyanocitta cristata*) in order to assess the risk of food caches being pilfered (Schmidt & Ostefeld, 2008), and migrating passerines assess the acoustic sounds of local species as an indicator of habitat quality (Mukhin et al., 2008).

Acoustic signals can reflect the signalers' physiological states, which result from the context of vocal production (Morton, 1977; Rendall et al., 2009). In particular, acoustic signals can contain information about an organism's state of arousal, which is the degree of physiological alertness or attentiveness (Russell, 1980); arousal plays a critical role in regulating attention, information processing, motivation, and emotional reactivity (Nesse, 1990). The ability for the receiver to discriminate arousal in vocalizations is especially important. While acoustic signals can provide details about the caller's species, sex, age, motivational state, dominance status, etc., cues expressing high arousal level convey immediately relevant information, such as the presence of a threat to the life of an organism (Stoeger, Baotic, Li, & Charlton, 2012). Arousal-based changes in physiology influence acoustic features of the voice such as duration and harmonic-to-noise ratio (HNR; i.e., harshness of a sound). For instance, infant African elephant roars primarily function to signal arousal levels, with duration and HNR being of particular importance (Stoeger, Charlton, Kratochvil, & Fitch, 2011). Detecting level of threat, or arousal, from another organism's vocalizations (conspecific or heterospecific) is perhaps one of the most important evolutionary functions for survival (e.g., red-breasted nuthatches, *Sitta canadensis*, eavesdrop on black-capped chickadees mobbing calls indicating a nearby predator; Templeton & Greene, 2007).

Recently, Filippi and colleagues (2017) found that humans (*Homo sapiens*) can identify levels of arousal in vocalizations produced by multiple species across three taxonomic classes: Amphibia, Mammalia, and Reptilia (including Aves). Thus, humans appear to have the ability to identify the emotional content contained in both conspecific and heterospecific vocalizations. So far, tests of this ability across such a wide range of species have only been made in humans. In order to better understand both the presence of and the ability to perceive these acoustic universals, we need to look beyond humans. Here, we seek to evaluate this ability in songbirds because their method of vocal acquisition parallels that of humans, suggesting that they may perceive or process vocalizations in a comparable manner produced through convergent evolution.

In Experiment 1, we tested black-capped chickadees, a non-migratory, North American songbird. Chickadees have been used as a comparative model as both songbirds and humans are vocal learners, meaning that the young of these animal groups learn their species-specific vocalizations, critical to their survival, from an adult model. These species belong to a relatively small group (i.e., humans, songbirds, hummingbirds, parrots, bats, elephants, and cetaceans; Jarvis, 2006) that are known to possess this ability. Therefore, we believe that there could be similarities in how these two evolutionarily-distinct species perceive and respond to auditory stimuli, specifically having similar abilities in perceiving arousal-based vocal cues communicated by the signaler. Thus, we investigated if black-capped chickadees and humans would respond similarly on a comparative task discriminating between high and low arousal vocalizations. Filippi and colleagues (2017) demonstrated that humans were capable of detecting arousal in vocalizations by directly asking participants to simultaneously differentiate between high and low arousal vocalizations (pairwise presentations), and their results provided evidence for acoustic universals. In the wild, animals would typically not hear two vocalizations (one of high arousal and one of low arousal) and then need to decide how to respond. As it is not natural to have to decide, from two vocalizations, which is higher arousal, it is more likely that animals would hear vocalizations and then have to judge if they are of high or low arousal to determine how they should respond to their surroundings (e.g., nearby predator). Considering how vocalizations would typically be heard in the wild, in the current study we presented vocalizations consecutively, such that subjects had to assess a single vocalization before hearing the next.

In summary, the current study used an operant conditioning go/no-go discrimination task, in which we could test chickadees (Experiment 1) and humans (Experiment 2) in an analogous manner (i.e., consecutive presentations with no instructions about the nature of the task) in order to determine the extent to which both species can discriminate vocalizations based on arousal levels. Considering that we tested humans again with this new paradigm, direct comparison to Filippi et al. (2017) is less critical. Overall, using a different task to ask a similar question also informs us about how robust the results are. In addition to testing, in order to investigate the mechanisms underpinning arousal perception in animal vocalizations, we explored which acoustic parameters predict category of vocalization (high or low arousal) across and within the vocalizing species included in this study.

Experiment 1: Black-capped Chickadees

Methods

Subjects

Twelve black-capped chickadees (eight males and four females, identified by DNA analysis; Griffiths, Double, Orr, & Dawson, 1998) were tested between September 2015 and April 2016. Birds at least one year of age (determined by examining the colour and shape of their outer tail retrices; Pyle, 1997) were captured in Edmonton (North Saskatchewan River Valley, 53.53˚N, 113.53˚W, Mill Creek Ravine, 53.52˚N, 113.47˚W), or Stony Plain (53.46˚N, 114.01˚W), Alberta, Canada between December 2010 and January 2015.

Prior to the experiment, birds were individually housed in Jupiter Parakeet cages (30 × 40 × 40 cm; Rolf C. Hagen, Inc., Montreal, QB, Canada) in colony rooms containing several other black-capped chickadees. Birds had visual and auditory, but not physical, contact with one another. Birds had *ad libitum* access to food (Mazuri Small Bird Maintenance Diet; Mazuri, St

Louis, MO, USA), water (vitamin supplemented on alternating days; Prime vitamin supplement; Hagen, Inc.), grit, and cuttlebone. Birds were given three to five sunflower seeds daily, one superworm (*Zophobas morio*) three times a week, and a mixture of greens (spinach or parsley) and eggs twice a week. Birds were maintained on a light:dark cycle that mimicked the natural light cycle for Edmonton, Alberta, Canada.

Throughout the experiment, birds were housed individually in operant chambers (see apparatus below), maintained on the natural light cycle for Edmonton, Alberta, and had *ad libitum* access to water (vitamin supplemented on alternate days), grit, and cuttlebone. Birds were given two superworms daily (one in the morning and one in the afternoon). Food (i.e., Mazuri) was only available as a reward for correct responding during the operant discrimination task. Birds had previous experience discriminating synthetic tones, musical chords, *fee-bee* songs, and/or black-capped chickadee call notes (McMillan et al., 2015; Hahn et al., 2016; Hoang, 2015; Guillette, Hahn, Hoeschele, Przyslupski, & Sturdy, 2015; respectively), but no operant experience with the stimuli used in this experiment.

Ethical Note

Throughout Experiment 1, birds remained in the testing apparatus to minimize the transport and handling of each bird. Following the experiment, birds were returned to the colony room for use in future experiments. Birds remained healthy during the experiment. All animal studies were conducted in accordance with the Canadian Council on Animal Care Guidelines and Policies and with approval from the Animal Care and Use Committee for Biosciences for the University of Alberta (“Neuroethology of Songbird Acoustic Communication: Laboratory Studies”, AUP00000107), and the University of Calgary Life and Environmental Sciences Animal Care Committee. 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.

Apparatus

During the experiment, birds were housed individually in modified colony room cages (30 × 40 × 40 cm) placed inside a ventilated, sound-attenuating chamber. The chambers were illuminated by a 9-W, full spectrum fluorescent bulb. Each cage contained three perches, a water bottle, and a grit cup. An opening on the side of the cage (11 × 16 cm) provided each bird access to a motor-driven feeder (see Njegovan, Hilhorst, Ferguson, & Weisman, 1994). Infrared cells in the feeder and the request perch (perch closest to the feeder) monitored the position of the bird. A personal computer connected to a single-board computer (Palya & Walter, 2001) scheduled trials and recorded responses to stimuli. Stimuli were played from the personal computer hard drive, through either a Cambridge A300 Integrated Amplifier, Cambridge Azur 640A Integrated Amplifier (Cambridge Audio, London, England), or an NAD310 Integrated Amplifier (NAD Electronics, London, England) and through a Fostex FE108 Σ or Fostex FE108E Σ full-range speaker (Fostex Corp., Japan; frequency response range 80-18,000 Hz) located beside the feeder. See Sturdy and Weisman (2006) for a detailed description of the apparatus.

Acoustic Stimuli

A total of 180 vocalizations produced by nine species were used as stimuli in the current experiment (originally collected and utilized in Filippi et al., 2017): infant giant panda (*Ailuropoda melanoleuca*), infant American alligator (*Alligator mississippiensis*), adult common raven (*Corvus corax*), adult hourglass treefrog, adult human (*Homo sapiens*; language: Tamil), infant African elephant (*Loxodonta africana*), adult Barbary macaque (*Macaca sylvanus*), adult black-capped chickadee, and infant domestic pig. We obtained 20 vocalizations per species, consisting of ten that were of high arousal and ten that were of low-arousal, in which any given individual produced both high and low arousal vocalizations (for spectrograms of each species' vocalizations, see Figure 2-1). These vocalizations were produced by male and female signalers, the same individual within a species, recorded and validated for arousal level in previous studies, and unpublished stimuli arousal levels were classified as high or low arousal according to the criteria presented in the original studies from which they were taken (giant panda, Stoeger et al., 2012; hourglass treefrog, Reichert, 2011, 2013; human, Bowling et al., 2013; African elephant, Stoeger et al., 2011; Barbary macaque, Fischer, 1995; domestic pig, Linhart et al., 2015; and unpublished works: American alligator recorded by S.A.R.; common raven recorded by A. Pašukonis; black-capped chickadee recorded by M. Avey and edited by J.V.C. and J.H.; see Table 2-1 for a summary of the nature of each species' vocalizations and published references). These same vocalizations were used in Filippi et al. (2017).

Below are descriptions of the conditions under which high- and low-arousal vocalizations are produced for each species. Infant giant pandas produce distress calls under multiple contexts (e.g., spontaneous agitation, feeding) which results in differing levels of motor activity (e.g., abrupt head movements) where higher levels of motor activity are associated with higher levels of arousal (i.e., >10 movements per 30s; Stoeger et al., 2012). Infant American alligators produce distress calls when pursued by a predator, and convey increases or decreases in the level of threat posed with the same call type; if they are grabbed, the peak frequency and overall energy of the high arousal calls (uttered with the palatal valve open) shift to significantly higher levels compared to low arousal calls (the same call uttered with the palatal valve closed; Britton, 2001). Common ravens produce defensive calls when physically confronted by a dominant conspecific;

the calls vary in arousal depending on the degree of threat posed (e.g., attack or not; Massen et al., 2014; Stocker et al., 2016). Hourglass treefrogs demonstrate an escalating level of competition during sexual advertisement by producing both aggressive calls (in the context of close-range inter-male agonistic interactions; i.e., high arousal) and advertisement calls (in the context of mate attraction; i.e., low arousal); for use as acoustic stimuli in the current study, these two call types were produced in close temporal proximity to ensure that they were recorded in a similar behavioural context (Reichart, 2011; Reichart, 2013). In a laboratory setting, human speakers were instructed to express emotions of anger (high-arousal) or sadness (low-arousal) through sentences spoken in Tamil (Bowling et al., 2012). Infant African elephants produce distress calls during disturbances in social context which result in greater physiological responses (e.g., larger quantities of secretions from the temporal glands, ears, head, and tail movements) in conditions of high arousal compared to low arousal (Stoeger et al., 2011). Barbary macaques produce disturbance calls when there are disturbances in the surroundings (e.g., a nearby predator), resulting in looking behaviour (low arousal) or increased physical distance from the source of the disturbance (i.e., playback speaker) to a point in which there is an assessed lack of danger (i.e., escape; high arousal; Fischer et al., 1995). Black-capped chickadees produce mobbing calls in response to nearby predators according to the threat level posed (Templeton et al., 2005), and neural activity in some brain regions has been shown to be greater in response to high-threat predator and conspecific calls compared to low-threat calls (arousal level dependent on the degree of threat posed; Avey et al., 2011). Infant domestic pigs produce distress calls when mobility is restricted, increasing motor activity with immobility, where higher levels of motor activity (attempted escape) are associated with higher levels of arousal compared to lower levels of motor activity (relaxed; Linhart et al., 2015). In summary, high arousal vocalizations were obtained from individuals under stressful conditions (e.g., threatening environments, agonistic interactions), while low arousal vocalizations were obtained from less adverse conditions, and despite the differences in indicators all stimuli were correlates of high- or low-arousal and negatively valenced.

All vocalizations were of high quality (i.e., no audible interference and low background noise when viewed on a spectrogram with amplitude cutoffs of -35 to 0 dB relative to vocalization peak amplitude) and were bandpass filtered (outside the frequency range of each vocalization type) using GoldWave version 5.58 (GoldWave, Inc., St. John's, NL, Canada) to reduce any background noise. For each stimulus, 5 ms of silence was added to the leading and trailing portion of the vocalization and tapered to remove transients, and amplitude was equalized using SIGNAL 5.10.24 software (Engineering Design, Berkeley, CA, USA).

During the experiment, stimuli were presented at approximately 75 dB as measured by a Brüel & Kjær Type 2239 (Brüel & Kjær Sound & Vibration Measurement A/S, Nærum, Denmark) decibel meter (A-weighting, slow response) at the approximate height and position of a bird's head when on the request perch.

Procedure

Pretraining. Pretraining began once the bird learned to use the request perch and feeder to obtain food. During Pretraining, birds received food for responding to all stimuli (future S+, S-, and transfer stimuli). A trial began when the bird landed on the request perch and remained for between 900-1100 ms. A randomly-selected stimulus played without replacement until all 180 stimuli had been heard. If the bird left the request perch before a stimulus finished playing, the trial was considered interrupted, resulting in a 30-s time out with the houselight turned off. If the bird entered the feeder within 1 s after the entire stimulus played, it was given 1 s access to food, followed by a 30-s intertrial interval, during which the houselight remained on. If a bird remained on the request perch during the stimulus presentation and the 1 s following the completion of the stimulus it received a 60-s intertrial interval with the houselight on, but this intertrial interval was terminated if the bird left the request perch. This was to encourage a high level of responding on all trials. Birds continued on Pretraining until they completed six 540-trial blocks of ≥ 60% responding on average to all stimuli, at least four 540-trial blocks ≤ 3% difference in responding to future S+ and S- stimuli, at least four 540-trial blocks in which the bird had ≤ 3% difference in responding to future high and low arousal transfer stimuli, and at least four 540-trial blocks in which the bird had ≤ 3% difference in responding to short and long stimuli to ensure that birds did not display stimuli preferences. Following a day of free feed, birds completed a second round of Pretraining in which they completed one 540-trial block of ≥ 60% responding on average to all stimuli, ≤ 3% difference in responding to future S+ and S- stimuli, ≤ 3% difference in responding to future high and low arousal transfer stimuli, and ≤ 3% difference in responding to short and long stimuli to confirm that each bird continued to not display preferences following the break.

Due to a low feeding percentage, one bird was put on a modified Pretraining criteria of twelve 540-trial blocks of ≥ 30% responding on average to all stimuli; all other criteria remained the same. The second round of Pretraining included two 540-trial blocks of ≥ 30% responding on average to all stimuli. The bird remained healthy throughout the experiment. There was a negligible statistical difference in whether or not this bird was included when analyzing overall performance on Discrimination Training. *See 'Trials to Criterion'*.

One other bird met Pretraining criteria, but was moved on to Discrimination Training before completing the second round. However, there was no statistical difference in whether or not this bird was included when analyzing overall performance on Discrimination Training. See ‘Trials to Criterion’.

Discrimination Training. The procedure was the same as during Pretraining; however, only the 108 training vocalizations were presented (with the remaining 72 withheld for use during Transfer testing), and responding to half of these vocalizations were then punished with a 30-s intertrial interval with the houselight off. As during Pretraining, responses to rewarded (S+) vocalizations resulted in 1 s access to food. Discrimination training continued until birds completed six 540-trial blocks with a discrimination ratio (DR) ≥ 0.80 with the last two blocks being consecutive. For DR calculations see Response Measures, below.

Birds were randomly assigned to either a True category discrimination group ($n = 6$) or Pseudo category discrimination group ($n = 6$). Black-capped chickadees in the True category discrimination group were divided into two subgroups (High S+ Group; two males and one female subject; Low S+ Group; two males and one female subject). The Pseudo category discrimination group was also divided into two subgroups (Subgroup 1: two males and one female subject; Subgroup 2: two males and one female subject). The purpose of the Pseudo group was to include a control in which subjects were not trained to categorize according to arousal level, investigating if True group acquisition is due to category learning (significantly fewer trials than the Pseudo groups) or simply rote memorization (similar number of trials compared to the Pseudo groups). See Figure 2-2 for how stimuli were divided into True and Pseudo subgroups.

Discrimination 85. This phase was identical to Discrimination training, except that the S+ vocalizations were rewarded with a reduced probability (i.e., $P = 0.85$). On unrewarded S+ trials, entering the feeder after the stimulus finished playing resulted in a 30-s intertrial interval, during which the houselight remained on, but there was no access to food. Discrimination 85 training was employed to introduce birds to trials in which there was no access to food, but the houselight remained illuminated, in order to prepare birds for transfer trials in which stimuli were neither rewarded nor punished. Discrimination 85 training continued until birds completed two 540-trial blocks with a DR ≥ 0.80.

Transfer Trials. During Transfer testing, the stimuli and reinforcement contingencies from Discrimination 85 were maintained. In addition, 72 stimuli (eight high- and eight low-arousal vocalizations from nine species heard during non-differential training but not discrimination training) were introduced. These new (i.e., transfer) stimuli were each presented once during a 612-trial block (S+ and S- stimuli from Discrimination 85 training were presented five times each; randomly-selected without replacement). Responses to transfer stimuli resulted in a 30-s intertrial interval with the houselight on, but no access to food; we did not differentially reinforce or punish transfer stimuli, and only presented each transfer stimulus once each per bin, so subjects did not learn specific contingencies associated with responding to these transfer stimuli. All birds completed a minimum of three blocks of Transfer trials and these were included for analysis. Following Transfer, birds were returned to their colony rooms.

Response Measures. For each stimulus exemplar, a proportion response was calculated by the following formula: R+/(N-I), where R+ is the number of trials in which the bird went to the feeder, N is the total number of trials, and I is the number of interrupted trials in which the bird left the perch before the entire stimulus played.

For Discrimination and Discrimination 85 training, we calculated a discrimination ratio (DR), by dividing the mean proportion response to all S+ stimuli by the mean proportion response of S+ stimuli plus the mean proportion response of S- stimuli. A DR of 0.50 indicates equal responding to rewarded (S+) and unrewarded (S-) stimuli, whereas a DR of 1.00 indicates perfect discrimination.

In order to analyze responding to each of the 18 stimulus types (nine high arousal, nine low arousal) during Transfer Trials, we calculated the proportion of responding for each stimulus type by averaging the percent response from the birds within each condition.

Statistical Analyses. We conducted independent-samples *t*-tests on the number of trials to criterion and DRs for the True and Pseudo category groups. Analyses of variance (ANOVAs) were conducted on DRs to determine if the birds had similar DRs to all species' vocalizations during Discrimination training. To determine if the True group continued to respond during Transfer to the contingencies they learned during Discrimination training, we ran a repeated measures ANOVA on the proportion of responding, and several paired-samples *t*-tests with Bonferroni corrections ($p < 0.0125$); similar tests were conducted with the Pseudo group. Huynh-Feldt correction was used on all repeated measures tests to correct for any possible violations in sphericity. We also conducted a three-way ANOVA (Condition × Arousal Level × Stimulus Species) on the proportion of responding during Transfer, followed by paired-samples *t*-tests to determine which of the nine species' vocalizations birds were transferring their responding to.

Results

Trials to Criterion

To determine whether birds in the two True category groups differed in their speed of acquisition, we conducted an independent-samples *t*-test on the number of 108-trial blocks to reach criterion for the two True category conditions (S+ High Group, S+ Low Group). There was no significant difference, $t_4 = -0.192$, $p = .857$, $d = .192$, 95% Confidence Interval [CIs] = -10.3290, 8.9957.

To determine whether birds in the two Pseudo category groups differed in their speed of acquisition, we conducted an independent-samples *t*-test on the number of 108-trial blocks to reach criterion for the two Pseudo category conditions (Pseudo Group 1, Pseudo Group 2). There was no significant difference, $t_4 = 0.761$, $p = .489$, $d = .761$, 95% CIs = -7.905, 13.9504. Since there were no differences in the speed of acquisition between the two Pseudo category groups, we combined the two groups in the remaining analyses.

To compare the acquisition performance of the True and Pseudo category groups and to determine if the True group learned to categorize in fewer trials than the Pseudo group, we conducted an independent-samples *t*-test on the number of 108-trial blocks to reach criterion for the True category and Pseudo category groups. There was a significant difference between the groups ($t_{10} = -2.244$, $p = .049$, $d = 1.419$, 95% CIs = -10.9603, -0.0397) in that True birds learned to discriminate significantly faster than Pseudo birds. If we removed the True group bird that was on modified Pretraining criteria, due to low feeding, the difference between group still approached significance, $t_9 = -2.173$, $p = .058$, $d = 1.449$, 95% CIs = -11.9052, 0.2385, meaning that acquisition during Discrimination was slightly slower for this particular bird. If we remove the True group bird that met Pretraining criteria, but was moved on to Discrimination Training before completing the second round, there is still a significant difference between the groups, $t_9 = -2.282$, $p = .048$, $d = 1.521$, 95% CIs = -12.0152, -0.0515.

DR Analysis

To examine if birds learned to discriminate all species' vocalizations equally we conducted a three-way ANOVA for the True group with Condition (S+ High and S+ Low), Arousal Level (High and Low Arousal), and Stimulus Species (Giant Panda, American Alligator, Common Raven, Hourglass Treefrog, Human, African Elephant, Barbary Macaque, Black-capped Chickadee, Domestic Pig) as fixed factors and DR as the dependent variable. There was a significant three-way interaction ($F_{1, 1, 8} = 2.635$, $p = .014$, $\eta^2 = .226$). There were significant main effects of Condition ($F_1 = 6.126$, $p = .016$, $\eta^2 = .078$, 95% CIs = 0.2304, 0.4192) and Arousal Level ($F_1 = 193.788$, $p = .029$, $\eta^2 = .064$, 95% CIs = 0.2356, 0.4242), and a significant interaction of Condition × Arousal Level ($F_{1, 1} = 193.788$, $p < .001$, $\eta^2 = .729$). All other interactions and main effects were non-significant (*p*s ≥ 0.277).

We conducted a three-way ANOVA for the Pseudo group with Condition (Pseudo 1 and Pseudo 2), Stimulus Type (Rewarded and Unrewarded stimuli), and Stimulus Species (Giant Panda, American Alligator, Common Raven, Hourglass Treefrog, Human, African Elephant, Barbary Macaque, Black-capped Chickadee, Domestic Pig) as fixed factors and DR as the dependent variable. There were significant main effects of Stimulus Species ($F_8 = 4.813$, $p < .001$, $\eta^2 = .348$, 95% CIs = 0.2528, 0.7259), Condition ($F_1 = 4.056$, $p = .048$, $\eta^2 = .053$, 95% CIs = 0.3307, 0.5277), and Stimulus Type ($F_1 = 336.523$, $p < .001$, $\eta^2 = .824$, 95% CIs = 0.5996, 0.7419), as well as a significant interaction of Stimulus Species × Stimulus Type ($F_{8, 1} = 4.548$, $p < .001$, $\eta^2 = .336$). All other interactions and main effects were non-significant (*p*s ≥ 0.085).

Category Learning

During Transfer trials, birds were intermittently presented with high- and low-arousal stimuli not presented during Discrimination training. A repeated measures ANOVA on the proportion of responding to the four stimulus types [Discrimination (DIS) S+ stimuli, Discrimination S- stimuli, Transfer (TRS) S+ associated stimuli, Transfer S- associated stimuli] by birds in the True Group was significant ($F_{1, 5} = 65.820$, $p = .001$, $\eta^2 = .943$, 95% CIs = 0.2757-0.5069). We then conducted four paired-samples *t*-tests with Bonferroni corrections ($p < .0125$). There were significant differences between responding to rewarded and unrewarded category stimuli during both Discrimination training and Transfer testing (DIS S+ vs. DIS S-, $t_5 = 7.264$, $p = .001$, $d = 3.3383$, 95% CIs = 0.3627, 0.7600; TRS S+ associated stimuli vs. TRS S- associated stimuli, $t_5 = 9.085$, $p < .001$, d =1.4662, 95% CIs = 0.1811, 0.32414), demonstrating that during Transfer testing birds continued to respond to the contingencies learned during discrimination training. There were no significant differences between responding to rewarded stimuli during training versus testing (DIS S+ vs. TRS S+ associated stimuli, $t_5 = 2.014$, $p = .100$, $d = 0.5846$, 95% CIs = -0.0335, 0.2758) or to unrewarded category stimuli during training versus testing (DIS S- vs. TRS S- associated stimuli, $t_5 = -0.3494$, $p = .031$, $d = -1.5329$, 95% CIs = -0.3494, -0.0258).

A repeated measures ANOVA on the proportion of responding to the four stimulus types [Discrimination (DIS) S+ stimuli, Discrimination S- stimuli, Transfer (TRS) S+ associated stimuli, Transfer S- stimuli] by birds in the Pseudo Group was significant ($F_{1,5} = 52.258$, $p = .001$, $\eta^2 = .913$, 95% CIs = 0.2870, 0.4958). We conducted four paired-samples *t*-tests with Bonferroni corrections ($p < .0125$). There were significant differences between responding to rewarded and unrewarded discrimination stimuli during transfer (DIS S+ vs. DIS S-, $t_5 = 16.333$, $p < .001$, $d = 5.5181$, 95% CIs = 0.4572, 0.6280), and transfer stimuli (TRS S+ associated stimuli vs. TRS S- associated stimuli, $t_5 = 4.433$, $p = .007$, $d = 0.5054$, 95% CIs = 0.0422, 0.1589), demonstrating that during transfer testing birds responded to the non-differentially rewarded stimuli according to the contingencies learned during discrimination training. There were no significant differences between responding to rewarded stimuli during training versus testing (DIS S+ vs. TRS S+ associated stimuli, $t_5 = 3.777$, $p = .013$, $d = 1.4528$, 95% CIs = 0.0757, 0.3985) or between responding to unrewarded stimuli during training versus testing (DIS S- vs. TRS S- associated stimuli, $t_5 = -3.009$, $p = .030$, $d = -1.3637$, 95% CIs = -0.3799, -0.0299).

Transfer Trials

To determine if the pattern of learning was the same across all species' vocalizations in transfer, we conducted a Condition (S+ High, S+ Low) × Arousal Level (high, low) × Stimulus Species (Giant Panda, American Alligator, Common Raven, Hourglass Treefrog, Human, African Elephant, Barbary Macaque, Black-capped Chickadee, Domestic Pig) three-way ANOVA on the proportion of responding of the True group during testing. There was a significant three-way interaction of Condition × Arousal Level × Stimulus Species ($F_{1,\,1,\,8}$ = 3.386, p = .002, η^2 = .273). There were also significant interactions of Condition × Stimulus Species ($F_{1,\,8}$ = 3.651, p = .001, η^2 = .289), and Arousal Level × Condition ($F_{1,\,1}$ = 27.836, p < .001, η^2 = .279). All other main effects and interactions were non-significant (*p*s ≥ 0.164). The main effect of Arousal Level was not significant as the True group was rewarded for either high or low arousal (S+ High, S+ Low), so half of the subjects would be expected to respond more to high arousal stimuli than low arousal stimuli and vice versa. However, the interaction of Condition and Arousal Level was significant, indicating that birds were transferring their learning to appropriately respond to rewarded-contingency high or low arousal novel stimuli, as demonstrated previously (see 'Category Learning'; Figure 2-3). To further investigate responding across Stimulus Species for each of the true category groups, we conducted paired-samples *t*-tests; see Table 2-2 for these statistical results. See Figure 2-4 for S+ High Group and S+ Low Group subjects' responding.

Discussion: Experiment 1

We demonstrated that black-capped chickadees are capable of discriminating vocalizations based on the arousal context, even though the vocalizations were produced by multiple species that our subjects likely had no prior experience with (subjects had experience with conspecific, human, and possibly raven vocalizations). The two True-category groups did not differ in speed of acquisition, nor did the two Pseudo-category groups; however, the True and Pseudo groups differed in speed of acquisition in that the True group learned to discriminate the high arousal stimuli from the low arousal stimuli in fewer trials compared to the Pseudo group. Due to the difference in speed of acquisition (i.e., the True group learned in fewer trials than the Pseudo group in training), it appears that chickadees might be using arousal-based category information over rote memorization. True group birds then transferred their training to novel stimuli based on the 'rules' they learned from discrimination. This is supported by the fact that there were no significant differences between responding by the True group to rewarded category stimuli during training versus testing (i.e., subjects continued to respond to the transfer stimuli based on the contingency learned during discrimination training); however, there was a significant difference between responding by the True group to unrewarded category stimuli during training versus testing.

Overall, we demonstrated that chickadees are capable of discriminating between vocalizations and transferring prior training to several species' vocalizations based on vocal components related to arousal. Specifically, chickadees demonstrated transfer of training to giant panda, human, and black-capped chickadee vocalizations, one group trended toward transfer of training to domestic pig vocalizations (See 'General Discussion' for further explanation of S+ Low group's transfer of training to low arousal domestic pig vocalizations), but neither group (S+ High or S+ Low) transferred their training to American alligator, common raven, hourglass treefrog, African elephant, or Barbary macaque vocalizations. After demonstrating successful categorical training based on arousal in a non-human animal species, we wanted to test human learning and transfer of training on a comparative task to provide a direct comparison with our chickadee results.

Experiment 2: Humans

In previous work, Filippi et al. (2017) demonstrated that humans can discriminate between high and low arousal vocalizations produced by multiple species. However, it is unclear how they would perform on a task that is directly analogous to that used to test chickadees. Thus, we designed a go/no-go discrimination task for humans to train then test their ability to discriminate based on arousal without explicit instructions on the categorical information, replicating our chickadee experiment with humans.

Methods

Participants

University of Alberta undergraduate students participated in this study between July 13, 2015 to August 10, 2015 in exchange for partial research credit towards their introductory psychology class. Students were required to have normal hearing and were not allowed to have previously participated in similar acoustic experiments.

Twenty-one participants were excluded from the study; three had incomplete surveys, 16 exhibited high responding during training (i.e., >95%), one exhibited low responding during training (i.e., <10%), and one participant was removed due to an incorrect file being used in testing. Thus, there was a total of 16 S+ High Arousal participants (seven males, nine females), 14 S+ Low Arousal (five males, nine females), 12 Pseudo 1 (four males, eight females), and 13 Pseudo 2 (six males, seven females). We processed the data of the remaining 55 participants (M_{age} = 21 years; SD_{age} = 3.121 years; 22 males and 33 females). Eighteen subjects were native English speakers, whereas 37 were non-native English speakers. Sixteen of our participants had one or more pets (dog, cat, hamster, and/or fish), and the average reported experience with animals was a rated three out of seven ($M_{\text{experience}} = 2.936$).

Ethical Note

All human studies were conducted under Songbird Neuroethology Laboratory's Auditory Perception and Cognition protocols with approval from the University of Alberta's Research Ethics & Management Online (REMO; "Auditory perception and cognition", Pro00016997). Participants were recruited via the University of Alberta, Department of Psychology's Research Pool in exchange for partial course credit toward the introductory psychology course in which they were actively enrolled.

Apparatus

Each participant was seated in front of an LG FLATRON W2442PA computer, equipped with an Intel Core i7 CPU and Windows 7 Professional. A pair of SENNHEISER HD 280 Pro headphones with nominal impedance of 64 Ohms was connected to each computer. The volume on each computer was set to a standardized level (i.e., headphone jack set to 20% of maximum volume), but participants were allowed to adjust the volume to the level they felt was most comfortable. A program from a previous experiment conducted in our laboratory (Hoeschele, Weisman, & Sturdy, 2012), utilizing a go/no-go task, was installed and placed on the desktop of each computer. Participants heard auditory stimuli through the headphones and used the computer mouse to make responses.

Stimuli

Experiment 2 used the same 180 stimuli from Experiment 1. One hundred and eight stimuli were used (six high- and six low-arousal vocalizations from each of the nine species) in the discrimination training phase, and 180 stimuli (108 discrimination stimuli plus an additional four high- and four low-arousal vocalizations from each of the nine species) during the transfer testing phase.

Procedure

Up to four participants completed the experiment at a time, randomly assigned to one of four conditions (High S+, Low S+, Pseudo 1, or Pseudo 2 group; Figure 2-2); they first read the information letter and signed a consent form. Then they filled out a survey in regards to their age, sex, native language, duration of musical training, experiences in sharing common area with pets at home, and level of animal experience (Appendix B).

Discrimination Practice Phase. A short Practice phase was completed before the Training phase to allow participants to understand the basics of using the program. The Practice phase included six stimuli, presented in a fixed order. Human infant cries were used as stimuli during this phase, but were not used as stimuli during the remainder of the experiment. The procedure for this phase was the similar to the other experimental phases, but utilized only these six stimuli, three high- and three low-arousal vocalizations.

Discrimination Training Phase. One hundred and eight stimuli were presented in the Training phase, consisting of six high-arousal and six low-arousal vocalizations from each of the nine species. During this training phase, participants were to categorize sounds that they heard into an “S+” category or a second, unnamed category. They received feedback from the program following their categorization, informing them of whether their response was “CORRECT” or “INCORRECT”. The goal of this stage was for the participants to successfully discriminate the sounds into the arbitrary category that had been chosen by the experimenters (i.e., “S+”). Experimental groups, and respective stimuli, were the same as for the birds (see Experiment 1: Discrimination Training; Appendix A, Figure 2-A1). Specifically, if an individual had been randomly assigned to S+ High Arousal Group, they would view a rewarded phrase of “CORRECT” if they clicked the S+ button when a high-arousal sound was heard (i.e., a correct “go” response; Appendix A, Figure 2-A2). Contrary, they would view an unrewarded response of “INCORRECT” if they clicked the S+ button when a low-arousal sound was heard (i.e., an incorrect “go” response; Appendix A, 2-A3). The individual would not receive any feedback if they choose to not press the S+ button whenever they heard a stimulus. This was viewed as a correct response when clicking S+ would have been incorrect (i.e., a correct “no-go” response). This concept was also applied when low arousal vocalizations were rewarded. For both of the Pseudo subgroups, responding (i.e., pressing the S+ button) to half of the high- and half of the low-arousal stimuli resulted in “CORRECT”. All participants moved onto Transfer Testing after completing 108 trials.

Transfer Testing Phase. A set of 180 stimuli were utilized in the Transfer Testing stage in which four additional high- and four additional low-arousal vocalizations of each of the nine species were added, for a total of 10 high- and 10 low-arousal stimuli for each of the nine species. These new stimuli provided the opportunity for us to test participants’ ability to generalize their knowledge of the categories based on what they learned from the training phase. Feedback was not provided in this stage (the feedback window was no longer depicted on the computer screen; Appendix A, Figure 2-A4). We expected that participants in the Pseudo group would respond non-differentially to the high and low arousal stimuli during this stage.

Statistical Analyses. All analyses conducted in Experiment 2 were the same as Experiment 1 (see Experiment 1’s ‘Statistical Analyses’), except that we could not conduct independent-samples *t*-tests on the number of trials to criterion as every participant moved on to testing following a certain number of trials.

Results

Discrimination Responding

To examine if humans in the True group learned to discriminate all species' vocalizations equally well we conducted a three-way ANOVA with Condition (S+ High and S+ Low Groups), Arousal Level (High and Low Arousal), and Stimulus Species (Giant Panda, American Alligator, Common Raven, Hourglass Treefrog, Human, African Elephant, Barbary Macaque, Black-capped Chickadee, Domestic Pig) as fixed factors and DR as the dependent variable. There was a significant three-way interaction ($F_{1,1,8} = 5.252$, $p < .001$, $\eta^2 = .077$). There were significant interactions of Stimulus Species × Condition ($F_{8,1} = 10.009$, $p < .001$, $\eta^2 = .137$) and Arousal Level × Condition ($F_{1,1} = 45.826$, $p < .001$, $\eta^2 = .083$). All other interactions and main effects were non-significant (ps ≥ 0.188).

We also conducted a three-way ANOVA for the Pseudo Group with Condition (Pseudo 1 and Pseudo 2), Stimulus Type (Rewarded and Unrewarded stimuli), and Stimulus Species (Giant Panda, American Alligator, Common Raven, Hourglass Treefrog, Human, African Elephant, Barbary Macaque, Black-capped Chickadee, Domestic Pig) as fixed factors and DR as the dependent variable. There was no significant three-way interaction, $F_{1,1,8} = 0.120$, $p = .998$, $\eta^2 = .002$. There was a significant main effect of Condition ($F_1 = 17.200$, $p < .001$, $\eta^2 = .040$, 95% CIs = 0.6328, 0.7048), but all other main effects and interactions were non-significant (ps ≥ 0.486).

Category Learning

To determine if the True group continued to respond to the contingencies learned in training, we conducted a repeated measures ANOVA on the proportion of responding to the four stimulus types (Discrimination (DIS) S+ stimuli, Discrimination S- stimuli, Transfer (TRS) S+ associated stimuli, Transfer S- associated stimuli) for the True group, $F_{1,29} = 283.007$, $p < .001$,

η^2 = .907, 95% CIs = 0.4126, 0.4821. We then conducted four independent-samples *t*-tests with Bonferroni corrections (p > .0125). There were significant differences between responding to rewarded and unrewarded stimuli during both Discrimination training and Transfer testing (DIS S+ vs. DIS S-, t_{29} = 6.814, p < .001, d = 1.2562, 95% CIs = 0.1464, 0.2720; TRS S+ associated stimuli vs. TRS S- associated stimuli, t_{29} = 5.688, p < .001, d = 0.9877, 95% CIs = 0.1081, 0.2296), with participants responding significantly more to rewarded category stimuli than unrewarded. There were also significant differences in responding to rewarded category stimuli during training versus testing (DIS S+ vs. TRS rewarded-contingency stimuli, t_{29} = 3.660, p = .001, d = 0.2289, 95% CIs = 0.0147, 0.0520), but no significant difference between responding to unrewarded category stimuli during training versus testing (DIS S- vs. TRS S-, t_{29} = -0.6577, p = .516, d = -0.0375, 95% CIs = -0.0288, 0.0148), as humans responded more to rewarded category stimuli from training than from testing but showed no difference in responding to unrewarded training and testing stimuli.

A repeated measures ANOVA on the proportion of responding to the four stimulus types (Discrimination (DIS) S+ stimuli, Discrimination S- stimuli, Transfer (TRS) S+ associated stimuli, Transfer S- associated stimuli) by humans in the Pseudo group was not significant ($F_{1, 24}$ = 188.993, p < .001, η^2 = 0.887, 95% CI = 0.4961, 0.5752). There was a significant difference between unrewarded training versus testing (DIS S- vs. TRS S-, t_{24} = 4.279, p < .001, d = 0.4862, 95% CIs = 0.0256, 0.0733). All other paired-samples *t*-tests were non-significant, *p*s > .057.

Transfer Trials

We conducted a Condition (S+ High, S+ Low) × Arousal Level (high, low) × Stimulus Species (Giant Panda, American Alligator, Common Raven, Hourglass Treefrog, Human, African Elephant, Barbary Macaque, Black-capped Chickadee, Domestic Pig) three-way

ANOVA on the proportion of responding during testing to determine if the pattern of learning was the same across all species' vocalizations. There was a significant three-way interaction of Condition × Arousal Level × Stimulus Species ($F_{1,\,1,\,8} = 4.955$, $p < .001$, $\eta^2 = .073$). There was a main effect of Condition ($F_1 = 4.439$, $p = .036$, $\eta^2 = .009$, 95% CIs = 0.3789, 0.4533). There was also a significant interaction of Arousal Level × Condition ($F_{8,\,1} = 44.070$, $p < .001$, $\eta^2 = .080$), and Stimulus Species × Condition ($F_{8,\,1} = 8.326$, $p < .001$, $\eta^2 = .117$). All other interactions and main effects were non-significant (*p*s ≥ 0.118). As in Experiment 1, Arousal Level was not significant as a main effect, but was significant as an interaction of Condition by Arousal Level; this indicates that the True group were responding appropriately by transferring their training to high and low arousal novel stimuli (See Figure 2-5 for group responding to categories by human participants during transfer testing). To further investigate the effect of Stimulus Species, we conducted paired-samples *t*-tests; see Table 2-3 for these statistical results. See Figure 2-6 for S+ High Group and S+ Low Group human participants' responding.

We also conducted a Pseudo Group (Pseudo 1, Pseudo 2) × Arousal Level (high, low) × Stimulus Species (nine species' vocalizations) three-way ANOVA on the proportion of responding during testing to determine if the pattern of responding was the same across all species' vocalizations. There were no main effects or interactions (*p*s ≥ .075). Thus, responding did not change with Arousal Level according to Condition as the Pseudo group did not respond to novel stimuli based on arousal, as expected for a Pseudo (control) group.

Human Experience

In order to determine if 'experience', a term used here to describe participants' current interaction level with animals, affected the ability to transfer discrimination abilities between high and low arousal stimuli, we ran four one-way ANOVAs. Participants' experience (1-7;

Appendix B) did not influence their responding (S+ High: $F_{143} = 0.633$, $p = .728$, $\eta^2 = .031$; S+ Low: $F_{125} = 0.984$, $p = .512$, $\eta^2 = .270$; Pseudo 1: $F_{107} = 0.686$, $p = .894$, $\eta^2 = .200$; Pseudo 2: $F_{116} = 1.131$, $p = .317$, $\eta^2 = .191$; CIs for listed experiences reported in Table 2-4).

Discussion: Experiment 2

In keeping with the results of Filippi et al. (2017), we demonstrated that humans are capable of discriminating vocalizations produced by multiple species based on the arousal context. Additionally, we predicted that human participants with more animal experience would be able to categorize at a higher level than those without such experience. However, we found that participants' experience, according to our survey, did not affect their responding. The fact that experience did not affect performance could be interpreted as support for the existence of perceivable acoustic differences in vocalizations that differ in levels of arousal, regardless of familiarity with animals; however, there were limitations on our survey as the wording used on the survey did not directly ask about prior experience with animals such as owning a pet when young.

Filippi and colleagues (2017) showed that humans are capable of discriminating across all species' vocalizations when a high and low arousal stimulus were paired; it may have been difficult for the human participants in the current study to learn the categorization task since they were presented with one stimulus at a time and were not given the specific instructions to categorize based on arousal. In contrast, we found that humans could discriminate giant panda, human, and black-capped chickadee vocalizations when presented consecutively (using the same stimuli as the previous experiment utilizing concurrent presentation; Filippi et al., 2017). The difference in the results from the current study and Filippi et al. (2017) is likely methodological, with the go/no-go design used here being more conceptually difficult than a choice task. In addition, vocalizations produced by some species were quite short, potentially increasing the difficulty for birds and humans to obtain arousal-based information in this design.

For both rewarded (S+) and unrewarded (S-) stimuli, True group participants continued to respond during Transfer testing based on the contingencies from Discrimination training, however, there was a significant difference in responding to rewarded stimuli during training versus testing such that they responded less to testing stimuli than to training stimuli. There was a significant difference in responding by the True group to high and low arousal transfer stimuli, demonstrating transfer of training to untrained stimuli. This indicates that humans can not only perceive and learn categories of arousal, but also transfer that learned categorization to some species' vocalizations. The human participants in the True group successfully transferred their training to the same three species that the chickadees transferred to (i.e., giant panda, human, and black-capped chickadee vocalizations; S+ Low Arousal group approached significance for responding to black-capped chickadee vocalizations, *p* = .053). In addition, participants in the S+ High arousal group transferred their training to vocalizations produced by African elephants. This indicates that acoustic features may vary significantly between vocalizations of high and low arousal level, as most humans do not have extensive experience with the majority of species whose vocalizations they were trained with, especially the giant panda vocalizations to which they demonstrated transfer of training. This also provides direct comparative findings of arousal perception as chickadees and humans demonstrated similar responding on analogous go/no-go discrimination tasks without instructions about the categories.

Bioacoustic Analysis

To examine the acoustic variation that black-capped chickadees and humans may have attended to when discriminating vocalizations that differed in arousal, we conducted bioacoustic analyses and discriminant function analyses on the discrimination and transfer stimuli presented during the go/no-go discrimination task.

Methods

For the bioacoustic analysis, we measured four acoustic features that had been previously measured to access variation in acoustic arousal (Filippi et al., 2017; Maruščáková et al., 2015): (1) duration of the vocalization, (2) initial fundamental frequency (F0), (3) spectral centre of gravity (SCG; a measure of the average frequency height), and (4) harmonic-to-noise ratio (HNR; harshness of a sound, a measure of relative pure and noisy signal components). We then conducted two categories of stepwise DFA using these acoustic features from the high and low arousal stimuli from each of the nine species. The first was a single DFA where vocalizations from all species and all arousal levels were categorized concurrently (i.e., 18 ‘vocal categories’ total) to determine which stimulus groups were most likely to be misclassified. The second was a series of nine DFAs in which the vocalizations for each species was categorized as high or low arousal separate from the other species. The purpose of these two types of DFAs was to differentiate between overall classification and within species classification. By comparing the misclassifications in the DFA to the black-capped chickadee and human performance errors, we hoped to determine if these acoustic features could explain the observed results. The four acoustic measures (duration, F0, SCG, HNR) were included as independent variables. The Discrimination training stimuli were used to create the discriminant function, a model to predict which vocal category a stimulus belongs to based on its four acoustic measures. In addition, based on the prediction errors from the DFA, we evaluated into which vocal categories the stimuli were incorrectly classified. Next, we used the discriminant function to classify the Transfer testing stimuli; again, we evaluated which stimuli were incorrectly classified by examining the prediction errors from the DFA. Some acoustic measures were not completed as the structure of certain vocalizations did not allow for the measurement of some features (i.e., ‘noisy’ vocalizations; one common raven, two African elephant, two Barbary macaque, and one domestic pig vocalization were not measured on F0 and HNR); the missing values were replaced with the mean of the remaining vocalizations for the species within the same arousal level for the statistical analysis. For each of the nine stepwise DFA, our criterion for a variable to be entered in the analysis was a minimum partial F = 3.84, and to be removed was a maximum partial F = 2.71, the same criterion as the first DFA.

While the behavioural task was to categorize high- versus low-arousal vocalizations, since stimuli included vocalizations produced by nine distinct species, it is possible that subjects were treating the task as nine separate categorization tasks. In order to determine which acoustic measures were driving the discrimination between high and low arousal vocalizations for each species, we conducted nine stepwise DFAs that classified the high- and low-arousal stimuli of each species separately (e.g., high arousal giant panda vs. low arousal giant panda vocalizations). Again, the Discrimination training stimuli were used to create a discriminant function and, based on the prediction errors from the DFA, we evaluated the percentage of stimuli that were incorrectly classified and which species’ vocalizations were most likely to be incorrectly classified. We then used the discriminant function to classify the Transfer testing stimuli.

Results

For the discriminant function we constructed to classify the stimuli based on the category of vocalization (high or low arousal level for each species), the overall Wilks’ λ was significant [Wilks’ $\lambda = 0.003$, $\mathcal{X}^2$ (68, $N = 108$) = 521.076, $p < 0.001$]. In addition, each residual Wilks’ λ was significant [test of functions 2 through 4: Wilks’ $\lambda = 0.043$, $\mathcal{X}^2 = 288.493$, $p < 0.001$; test of functions 3 through 4: Wilks' $\lambda = 0.250$, $\mathcal{X}^2 = 127.518$, $p < 0.001$; and test of function 4: Wilks' $\lambda = 0.610$, $\mathcal{X}^2 = 45.516$, $p < 0.001$].

The four discriminant functions assigned 88.0% of the original grouped cases to correct category of vocalizations (chance = 1/18 = 5.6%). After creating the discriminant functions with Discrimination training stimuli measures, we used the discriminant functions to predict the category membership for the Transfer training stimuli. The four discriminant functions assigned 52.8% of Transfer training stimuli grouped cases to correct category of vocalizations (chance = 1/18 = 5.6%; see Table 2-5).

For each of our nine stimulus species, we conducted a stepwise DFA to determine whether our four acoustic measures (duration, F0, SCG, HNR) could predict arousal level (high or low); see Table 2-6 for the statistical output. For the Discrimination stimuli, the DFA was able to correctly classify 100% of the giant panda vocalizations as high vs. low arousal, 91.7% of the American alligator vocalizations as high vs. low arousal, 83.3% of the common raven vocalizations as high vs. low arousal, 100% of the hourglass treefrog vocalizations as high vs. low arousal, 100% of the human vocalizations as high vs. low arousal, 100% of the African elephant vocalizations as high vs. low arousal, 91.7% of the black-capped chickadee vocalizations as high vs. low arousal, and 83.3% of the domestic pig vocalizations as high vs. low arousal (chance for each = 50%).

Next, we assessed how well the discriminant functions created with the Discrimination stimuli would classify the Transfer stimuli. We found that 87.5% of the giant panda vocalizations, 100% of the American alligator vocalizations, 62.5% of the common raven vocalizations, 100% of the hourglass treefrog vocalizations, 87.5% of the human vocalizations, 87.5% of the African elephant vocalizations, 62.5% of the black-capped chickadee vocalizations, and 75% of the domestic pig vocalizations were correctly classified according to arousal level. Although organisms may not consistently pay attention to all available acoustic features, these findings suggest that there was sufficient acoustic variation for discrimination between the high and low arousal transfer stimuli for each of these species.

General Discussion

Here we have demonstrated that black-capped chickadees and humans, can learn to discriminate between high and low arousal vocalizations. Across Experiment 1 and 2, the majority of True groups showed transfer of training to giant panda, human, and black-capped chickadee vocalizations. Human and giant panda vocalizations correspond to the species Filippi et al. (2017) found human participants were best capable of identifying when asked which was the high arousal vocalization in paired evaluations, with participants identifying black-capped chickadee vocalizations at 85% correct. Black-capped chickadee subjects' responding to black-capped chickadee and human vocalizations could be explained by familiarity; the subjects in this experiment were wild-caught black-capped chickadees housed in captivity with daily exposure to humans (i.e., lab staff and researchers). Familiarity has been demonstrated to be a factor in identifying information contained within vocalizations (e.g., content, context, etc.). For example, cat vocalizations (i.e., meow sounds) that had been recorded from different cats in multiple behavioural contexts were best classified by human participants with more exposure to cats (Nicastro & Owren, 2003). In the current study, human participants' significant transfer of training to human and black-capped chickadee vocalizations may have been due to familiarity, although it is possible that some participants did not have experience with black-capped chickadees. In addition, it is not likely that black-capped chickadee subjects or human participants had much (if any) exposure to giant panda vocalizations, which they significantly transferred their responding to during the task. Scheumann and colleagues (2014) found that human participants recognized the emotional content of human vocalizations, but had mixed results for animal vocalizations depending on individual experience with each species; they concluded that human recognition of the emotional content of animal vocalizations is determined by familiarity rather than acoustic universal-like coding. We found that our participants' overall experience with animals did not improve their responding when discriminating between vocalizations of high and low arousal; however, we did not collect information on the specific species that participants had experience with, so it is possible that all of our human participants had limited (if any) experience with all of the species whose vocalizations we used. Therefore, while familiarity may play a role in differentiating arousal level in vocalizations, this alone does not explain the responding by black-capped chickadees and humans during this task, especially to the giant panda stimuli.

We conducted a DFA to better understand which acoustic features could be contributing to the discrimination of high and low arousal vocalizations across all of the species whose vocalizations we used as stimuli. The DFA correctly classified more than half of the giant panda and black-capped chickadee vocalizations (chance = 5.6%), which is consistent with the responding by black-capped chickadees and humans, as both chickadees and humans significantly transferred their responding to vocalizations produced by these two species. However, there was a clear discrepancy between the classification performance of the DFA and that of the chickadees and humans; the DFA misclassified high arousal human vocalizations entirely (0% accuracy, with 75% accuracy in classifying low arousal human vocalizations), while the chickadee and human subjects significantly transferred their responding to human vocalizations. From the additional DFAs, we determined which acoustic features may be utilized to correctly classify between high and low arousal vocalizations for each species. Both birds and humans transferred to giant panda and black-capped chickadee vocalizations, stimuli in which the DFAs utilized the initial fundamental frequency (F0). Black-capped chickadees also transferred to domestic pig vocalizations. Maruščáková and colleagues (2015) found that the acoustic properties of piglet vocalizations were effective in human participants' judging of emotional content; specifically, that simple acoustic parameters (e.g., F0) were more effective than complex acoustic properties (e.g., harmonic-to-noise ratio: HNR). Consistent with this, the results from our DFA suggest that F0 is a useful acoustic feature to differentiate between high and low arousal domestic pig vocalizations. Taken together, these results suggest that F0 may be one fundamental acoustic feature that is useful when differentiating arousal level in various vocalizations. Although research (for instance Bowling et al., 2017) has found that F0 correlates negatively with signaler's body size within species, the link between the expression of emotional arousal and body size through modulation of F0 remains open to investigation. For black-capped chickadee vocalizations, in addition to using F0, the DFA also used duration to classify based on arousal. The only other DFA that used duration to classify based on arousal was the DFA classifying African elephant vocalizations. In our behavioural experiments, in addition to significant transfer of training to black-capped chickadee vocalizations, there was some transfer of training to African elephant vocalizations (i.e., in Experiment 2). These results suggest that duration may also be a useful feature when discriminating between vocalizations of arousal. Consistent with this, previous studies have demonstrated that animals discriminate among acoustic stimuli using duration. For example, Briefer's (2012) review concluded that physiological arousal (e.g., a change in respiration) is typically reflected in vocalization changes, including longer durations and higher F0.

When classifying American alligator, common raven, and human vocalizations, the DFA used the spectral centre of gravity (SCG). SCG has previously been utilized as a reliable acoustic feature as Sauter et al. (2010) and Faragó et al. (2014) found that higher intensity ratings consistently corresponded with higher SCG for human and dog vocalizations, respectively. Filippi et al. (2017) also demonstrated that F0 and SCG predicted accuracy in humans' ability to identify the high-arousal vocalizations. However, due to a lack of transfer to alligator and raven vocalizations, SCG is unlikely to be a useful acoustic feature for this type of discrimination. Belin and colleagues (2008) demonstrated that humans could discriminate between human vocalizations of positive and negative valence (affectivity; different from the calming/excitement of arousal), but not vocalizations produced by other species (i.e., cats and rhesus monkeys); however, fMRI imaging indicated that there was appropriate discrimination at a neurobiological, albeit unconscious, level based on cerebral blood flow. Thus, while behavioural responses failed to demonstrate discrimination of heterospecific vocalizations, accurate discrimination was revealed from the fMRI result. It is possible that chickadees and humans in the current experiment were capable of transfer to all nine species' vocalizations (negatively valenced), but did not demonstrate the discrimination at a behavioural level, similar to the results of Belin et al. (2008).

In conclusion, we demonstrated that birds and humans (without being given instruction about the nature of the task) that were previously rewarded for responding to vocalizations based on arousal level responded significantly more to untrained vocalizations associated with the same arousal level that was previously rewarded. Both species transferred their training to novel stimuli produced by a third of the species that we included in our task based on their previous training; however, consistent transfer of training was not demonstrated to the vocalizations of the remaining species by either birds or humans. Similar in that it is adaptive for red-breasted nuthatches to eavesdrop on black-capped chickadee mobbing calls (Templeton & Greene, 2007) or non-vocal iguanas to understand the emotional content of mockingbirds' alarm calls regarding a nearby shared predator (the Galapagos hawk; Vitousek et al., 2007), it would be advantageous to have the ability to perceive arousal in vocalizations produced by a variety of species, including unfamiliar species. We propose that future studies utilize non-vocal learning study species to further investigate the perception of arousal, and potentially incorporate fMRI techniques to provide a more thorough investigation, especially of unconscious perception. Our findings demonstrate that a species of songbird and humans, both vocal learners, perceive variations of arousal in vocalizations produced by multiple species (Mammalia and Aves), suggesting that acoustic features may exist that vary sufficiently between high- and low-arousal vocalizations to allow these two exemplars, and perhaps other species, to discriminate based on level of arousal.

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Table 2-1. The nature (i.e., vocalizations type, behavioural context under which vocalizations were recorded, and non-acoustic correlate(s) of arousal level) of the high- and low-arousal vocalizations collected from nine species that were used as stimuli for all three experiments explained with references (modified from Filippi et al., 2017's Table 2-S1).

Species (Latin)	Species (Common)	Vocalization Type	Level of Arousal	Behavioural context	Non-acoustic correlate(s) of arousal level	Original study reporting on stimuli set
Ailuropoda melanoleuca	Giant panda	Distress call	High	After stroking, placement in the incubator; spontaneous agitation	High occurrence of motor activity	Stoeger et al. (2012)
			Low	Anogenital stroking and feeding	Low occurrence of motor activity	
Alligator mississippiensis	American alligator	Distress call	High	Human handling	Open palatal valve (correlates with high arousal; Britton, 2001)	Recordings provided by S.A.R. (unpublished data)
			Low		Closed palatal valve (correlates with low arousal; Britton, 2001)	
Corvus corax	Common raven	Defensive call	High	Confrontation by a dominant	Physical attack or chase (Massen et al., 2014; Stocker et al., 2016)	Recordings provided by Pašukonis, A. (unpublished data)
			Low		No physical attack or chase (Massen et al., 2014; Stocker et al., 2016)	
Dendropsophus ebraccatus	Hourglass treefrog	Aggressive and advertisement calls	High	Male-male mating competition	Escalating competitive level in call timing interactions	Reichart (2011; 2013)
			Low	Sexual advertisement		
Homo sapiens	Human	Prosodically emotional sentences (language: Tamil)	High	Laboratory setting for audio recordings	Native Tamil speakers expressing anger	Bowling et al. (2012)
			Low		Native Tamil speaker expressing sadness	
Loxodonta africana	African elephant	Distress call	High	Disturbance in social context	Assistance context: The calf has fallen over or has been separated from the group	Stoeger et al. (2011)
			Low		Suckling context: The calf is begging unsuccessfully for access to the bottle or breast; calf is disturbed while suckling or bottle feeding	
Macaca sylvanus	Barbary macaque	Disturbance call	High	Disturbance in surroundings	Increase in physical distance (i.e., escape) from the source of the disturbance to where lack of danger is assessed	Fischer et al. (1995)
			Low		Assessment of a disturbance in the surroundings (i.e., looking)	
Poecile atricapillus	Black-capped chickadee	Mobbing call	High	Response to the visual model of a high-threat predator (Northern saw-whet owl)	Increase of neural activity in the telencephalic auditory areas in response to high threat (Avey et al., 2011)	Recordings provided by J.V.C. and J.H. (unpublished data)
			Low	Response to the visual model of a low-threat predator (Great horned owl)		
Sus scrofa	Domestic pig	Distress call	High	Immobility test	The piglet attempts to escape from human handlers during immobility test	Linhart et al. (2015)
			Low		The piglet lies relaxed on a scale during immobility test	

Table 2-2. Results of the paired-samples *t*-tests conducted on black-capped chickadee subjects' responding between high vs. low arousal vocalizations for each species during Transfer of Training.

Species	*t-test*	*p-value*	*Cohen's d*	*Confidence Intervals*
S+ High Group (*df* = 2)				
Giant panda	**4.491**	**0.046**	**6.351**	**.0252, 1.1769**
American alligator	0.948	0.443	1.341	-.2483, .3887
Common raven	-1.540	0.263	-2.178	-.2203, .1042
Hourglass treefrog	3.273	0.082	4.629	-.1310, .9644
Human	**6.062**	**0.026**	**8.573**	**.1693, .9974**
African elephant	0.378	0.742	0.535	-.1049, .1251
Barbary macaque	1.801	0.213	2.547	-.2139, .5220
Black-capped chickadee	**5.450**	**0.032**	**7.707**	**.1054, .8957**
Domestic pig	3.024	0.097	4.277	-.0944, .5409
S+ Low Group (*df* = 2)				
Giant panda	**-25.000**	**0.002**	**-35.355**	**-.8140, -.5749**
American alligator	-1.696	0.232	-2.398	-.7042, .3060
Common raven	1.214	0.349	1.719	-.4831, .8627
Hourglass treefrog	-1.211	0.349	-1.713	-1.4866, .8335
Human	**-10.771**	**0.009**	**-15.233**	**-.5728, -.2458**
African elephant	3.308	0.081	4.678	-.0533, .4079
Barbary macaque	-1.053	0.403	-1.489	-.4932, .2993
Black-capped chickadee	**-10.583**	**0.009**	**-14.967**	**-.3978, -.1678**
Domestic pig	**-6.804**	**0.021**	**-9.622**	**-.6596, -.1485**

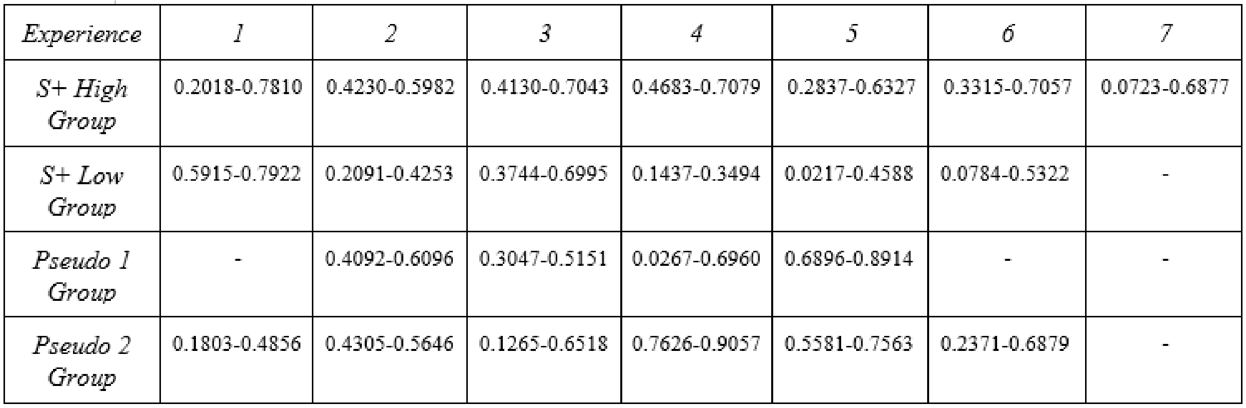
Bold font indicates significance.

Table 2-3. Results of the paired-samples *t*-test results of human participants' responding to each species' vocalizations during Transfer of Training.

Species	*t-test*	*p-value*	*Cohen's d*	*Confidence Intervals*
S+ High Group (*df* = 15)				
Giant panda	**4.901**	**<0.001**	**2.531**	**.2150, .5460**
American alligator	-0.490	0.631	-0.253	-.0836, .0523
Common raven	-0.382	0.708	-0.197	-.1389, .0967
Hourglass treefrog	0.544	0.595	0.281	-.1059, .1784
Human	**6.079**	**<0.001**	**3.139**	**.3790, .7882**
African elephant	**4.276**	**0.001**	**2.208**	**.1099, .3285**
Barbary macaque	0.746	0.467	0.385	-.0673, .1398
Black-capped chickadee	**6.199**	**<0.001**	**3.201**	**.3051, .6249**
Domestic pig	2.005	0.063	1.035	-.0082, .2701
S+ Low Group (*df* = 14)				
Giant panda	**-2.160**	**0.049**	**-1.155**	**-.5998, -.0022**
American alligator	-1.179	0.258	-0.630	-.1720, .0500
Common raven	-1.175	0.260	-0.628	-.1738, .0501
Hourglass treefrog	-2.062	0.058	-1.102	-1.935, .0038
Human	**-2.328**	**0.035**	**-1.244**	**-.07054, -.0289**
African elephant	-1.141	0.273	-0.610	-.2088, .0638
Barbary macaque	-0.322	0.753	-0.172	-.1278, .0945
Black-capped chickadee	-2.113	0.053	-1.128	-.4598, .0035
Domestic pig	0.127	0.901	0.068	-.0845, .0952

Bold font indicates significance, and underlined font indicates levels approaching significance.

Table 2-4. Confidence intervals (95% CIs) for listed participant experience (1-7).



Experience	*1*	*2*	*3*	*4*	*5*	*6*	*7*
S+ High Group	0.2018-0.7810	0.4230-0.5982	0.4130-0.7043	0.4683-0.7079	0.2837-0.6327	0.3315-0.7057	0.0723-0.6877
S+ Low Group	0.5915-0.7922	0.2091-0.4253	0.3744-0.6995	0.1437-0.3494	0.0217-0.4588	0.0784-0.5322	-
Pseudo 1 Group	-	0.4092-0.6096	0.3047-0.5151	0.0267-0.6960	0.6896-0.8914	-	-
Pseudo 2 Group	0.1803-0.4856	0.4305-0.5646	0.1265-0.6518	0.7626-0.9057	0.5581-0.7563	0.2371-0.6879	-

Table 2-5. Matrix of classification of Transfer testing stimuli by vocalization type using cross-validation in a Discriminant Function Analysis (DFA).

		Giant Panda		American Alligator		Common Raven		Hourglass Treefrog		Human		African Elephant		Barbary Macaque		Black-capped Chickadee		Domestic Pig	
		High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low
Giant Panda	High	**50**											25					25	
	Low		**75**	25															
American Alligator	High		25	**75**															
	Low				**75**								25						
Common Raven	High			50		**0**	50												
	Low			75			**25**												
Hourglass Treefrog	High		25					**50**						25					
	Low		25						**50**					25					
Human	High		50							**0**			25						25
	Low		25								**75**								
African Elephant	High											**100**							
	Low		50										**50**						
Barbary Macaque	High													**75**	25				
	Low													25	**50**			25	
Black-capped Chickadee	High															**50**	50		
	Low															25	**75**		
Domestic Pig	High															25	25	**0**	50
	Low																		**75**

The percentage of correct classifications is provided in bold along the diagonal. The percentage of misclassifications is given in corresponding rows and columns. Overall, 52.8% of the cross-validated cases were correctly classified. Empty cells indicate a percentage of zero.

Table 2-6. The acoustic measures used by the stepwise DFA to determine which four acoustic measures (duration, F0 SCG, HNR) could predict arousal level (high or low), and respective statistical output. Using the inclusion/exclusion criterion, only one or two acoustic measures were included in the discriminant function classifying each species:

Species	*Acoustic measure(s)*	*Wilks' λ*	$\mathcal{X}^2$	*p-value*
Giant panda	F0	0.138	18.826	<0.001
American alligator	SCG	0.198	15.389	<0.001
Common raven	SCG	0.494	6.698	0.010
Hourglass treefrog	F0/HNR	0.016	37.004	<0.001
Human	SCG	0.156	17.665	<0.001
African elephant	HNR/Duration	0.123	14.663	0.001
*Barbary macaque**	-	-	-	-
Black-capped chickadee	Duration/F0	0.307	10.617	0.005
Domestic pig	F0	0.386	9.035	0.003

*Note: Barbary macaque vocalization measures did not produce a discriminant function as none of the features met our criterion for inclusion in the model.

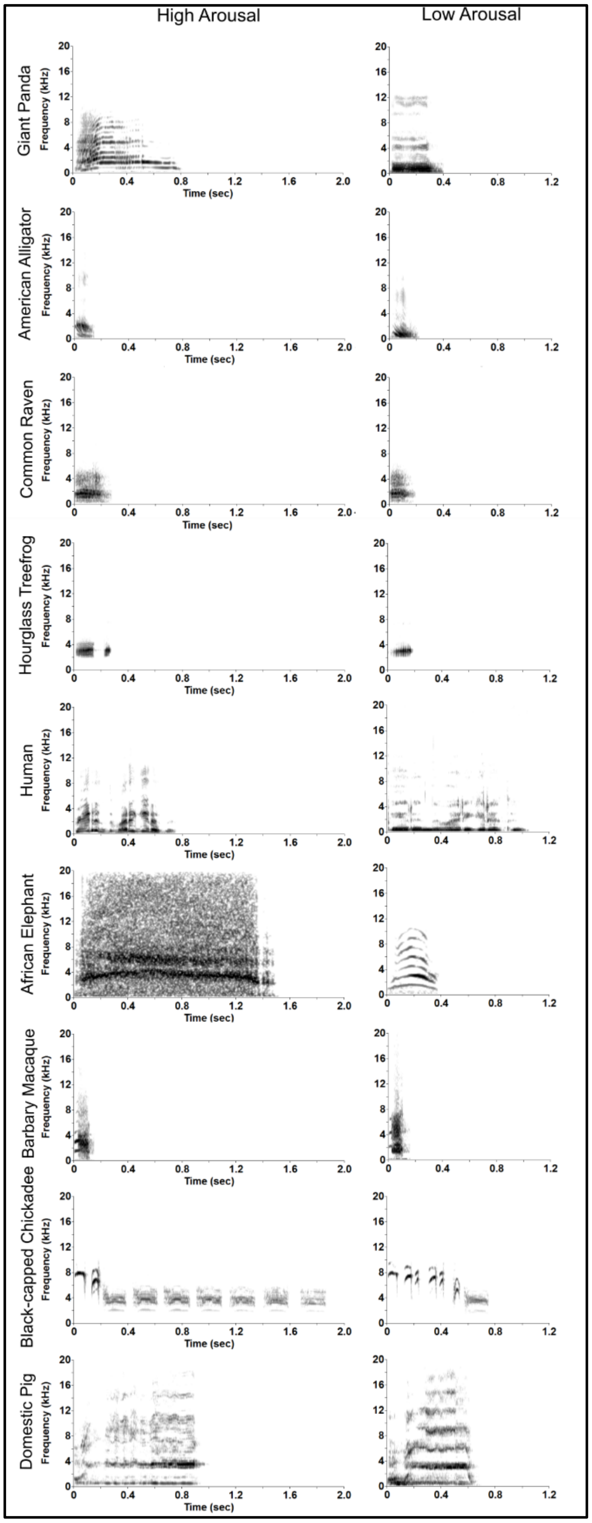


Figure 2-1. Spectrograms of high and low arousal vocalizations produced by each of the nine species (Giant Panda, American Alligator, Common Raven, Hourglass Treefrog, Human, African Elephant, Barbary Macaque, Black-capped Chickadee, and Domestic Pig), with Time on the x-axis (sec) and Frequency (kHz) on the y-axis.

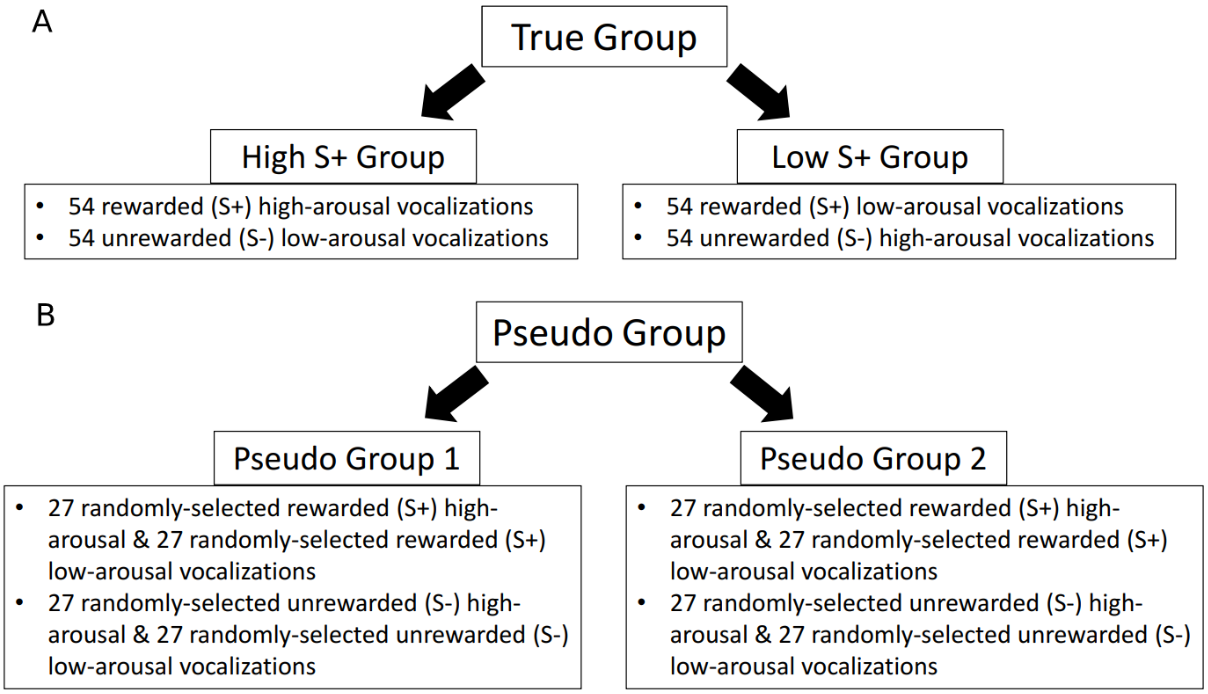


Figure 2-2. Black-capped chickadee subjects (Experiment 1) and human participants (Experiment 2) were randomly assigned to the (**A**) True or (**B**) Pseudo group and rewarded (S+) and punished (S-) for responding to different acoustic stimuli.

Experiment 1

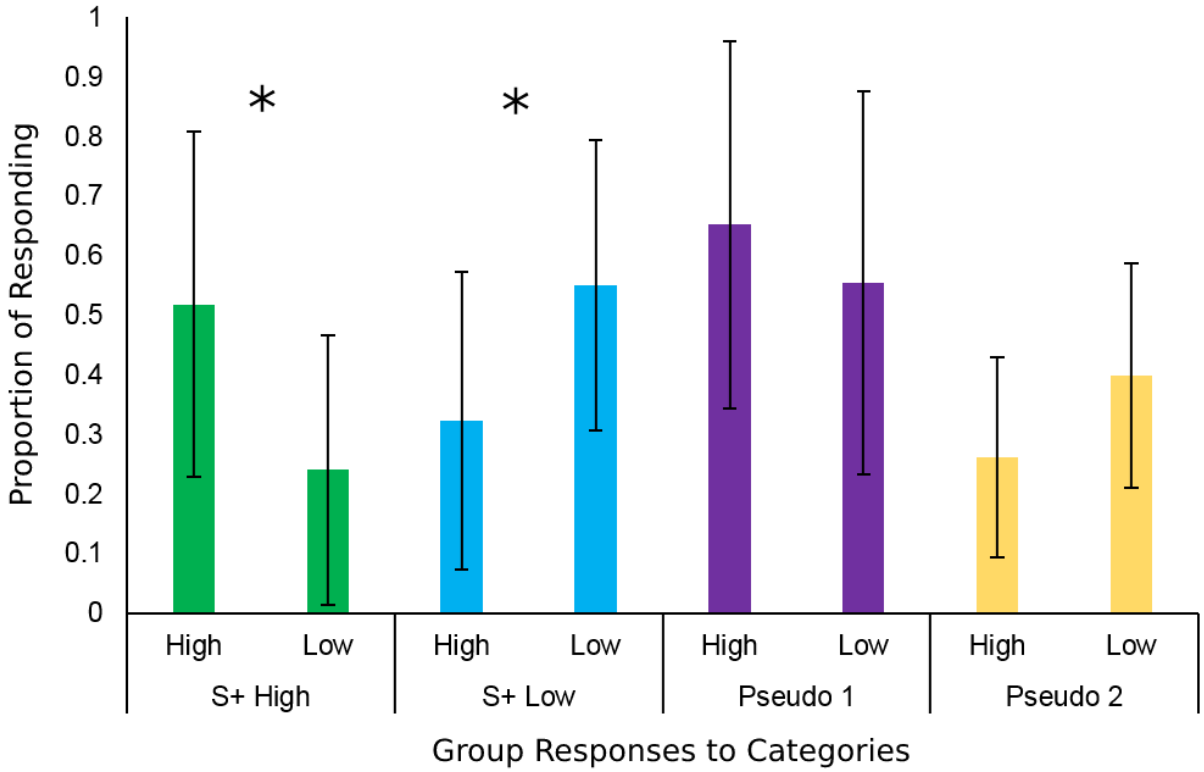


Figure 2-3. Proportion of responding to high vs. low arousal stimuli for each condition by black-capped chickadees (*n* = 12) during the transfer testing phase in Experiment 1 ± CI.
* indicates a significant difference between two adjacent bars.

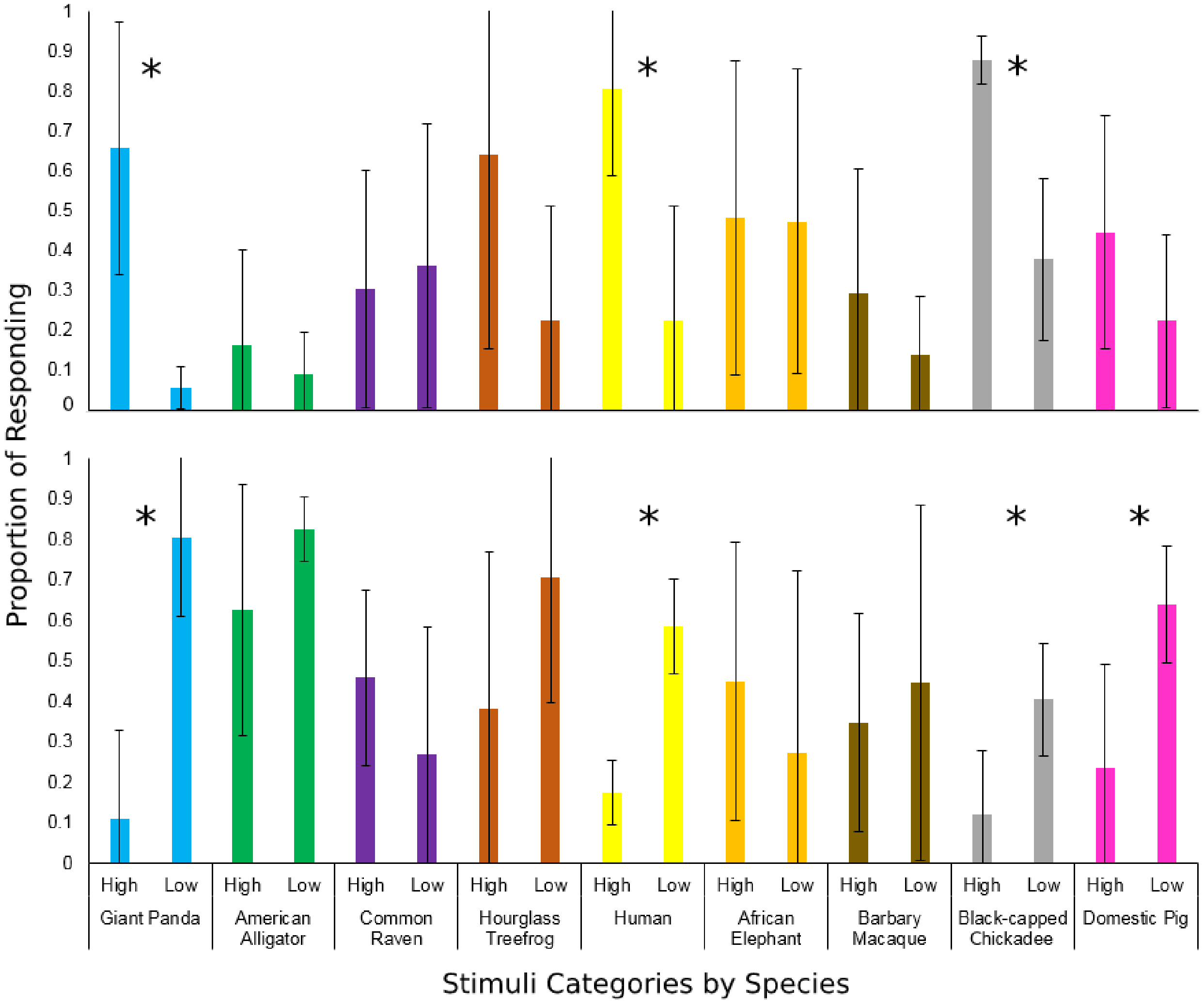


Figure 2-4. Proportion of responding to high arousal (previously rewarded) vs. low arousal (previously unrewarded) stimuli to each stimulus category (i.e., species) by black-capped chickadees in the S+ High group during the Transfer testing phase in Experiment 1 ± CI [top], and proportion of responding to low arousal (previously rewarded) vs. high arousal (previously unrewarded) stimuli to each stimulus category (i.e., species) by black-capped chickadees in the S+ Low group during the transfer testing phase in Experiment 1 ± CI [bottom].
* indicates a significant difference between two adjacent bars.

Experiment 2

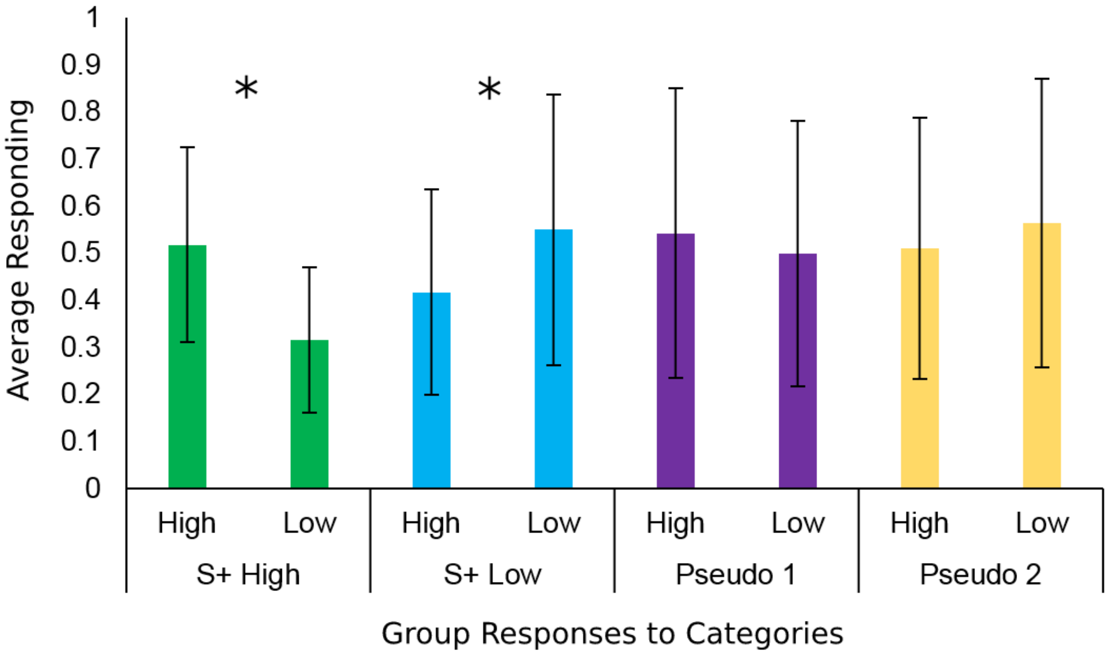


Figure 2-5. Average responding to high vs. low arousal stimuli for each condition by human participants (*n* = 76) during the transfer testing phase in Experiment 2 ± CI.
* indicates a significant difference between two adjacent bars.

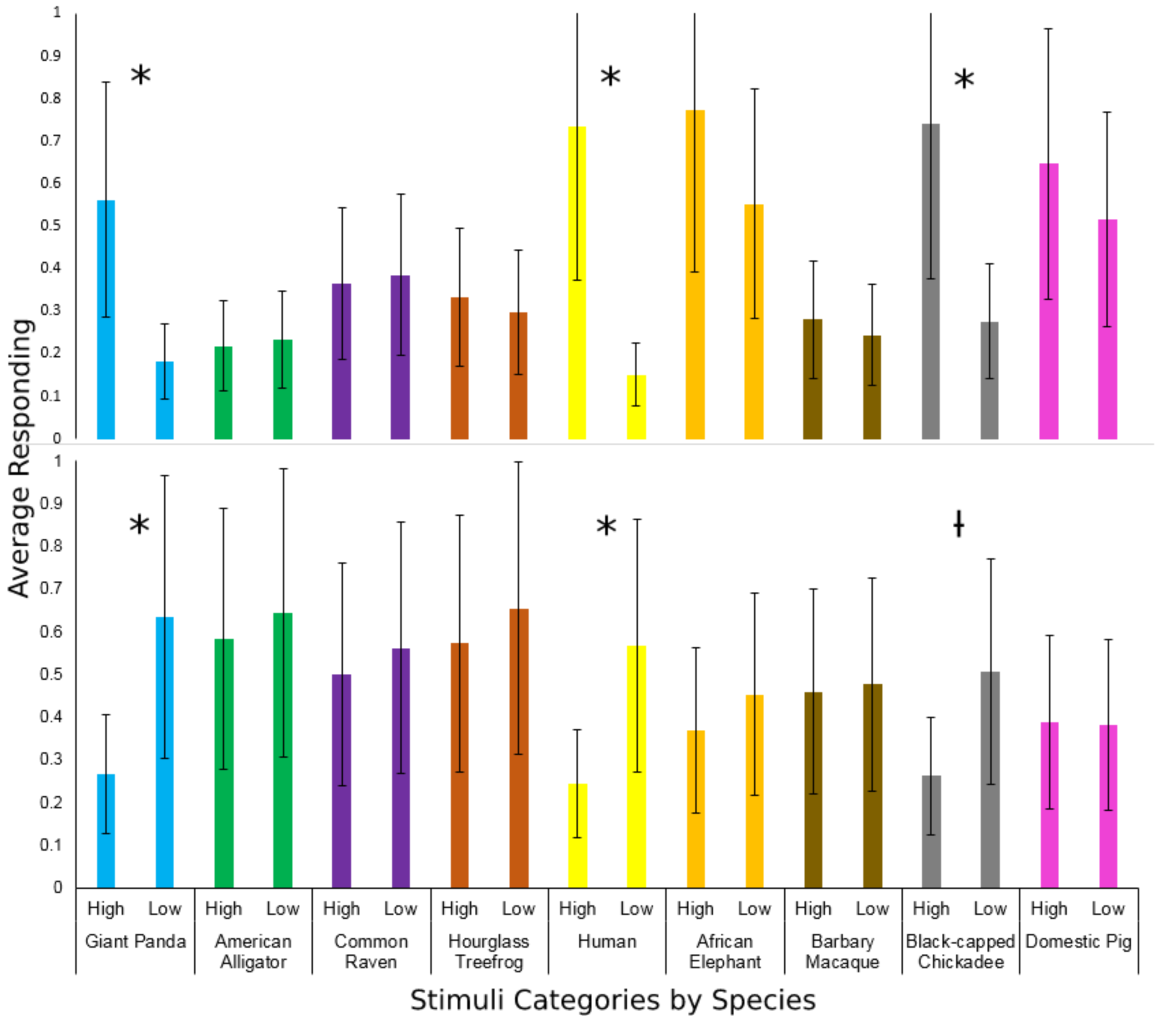


Figure 2-6. Average responding to high arousal (previously rewarded) vs. low arousal (previously unrewarded) stimuli to each stimulus category (i.e., species) by human participants in the S+ High group during the transfer testing phase in Experiment 2 ± CI [top], and average responding to low arousal (previously rewarded) vs. high arousal (previously unrewarded) stimuli to each stimulus category (i.e., species) by participants in the S+ Low group during the transfer testing phase in Experiment 2 ± CI [bottom].

* indicates a significant difference between two adjacent bars.

ł indicates a difference approaching significance between two adjacent bars.

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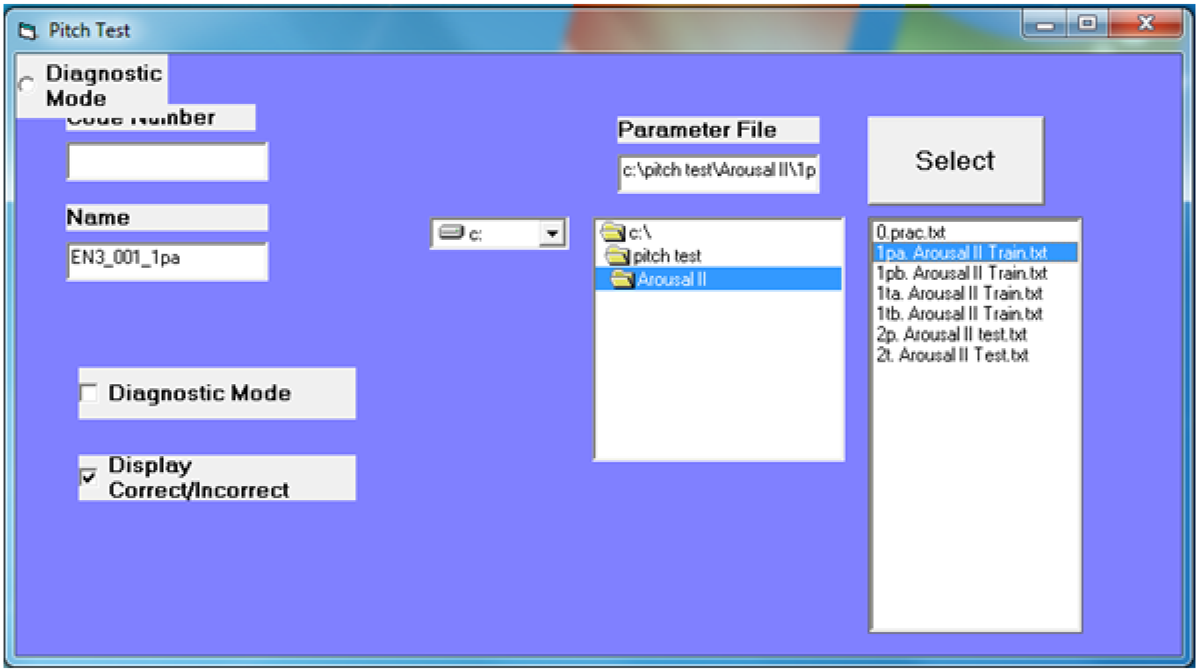
Appendix A: Supplemental figures for running the go/no-go human program



○ Diagnostic Mode

Code Number

Name: EN3_001_1pa

☐ Diagnostic Mode

☑ Display Correct/Incorrect

c:

c:\
pitch test
Arousal II

Parameter File: c:\pitch test\Arousal II\1p

0.prac.txt
1pa. Arousal II Train.txt
1pb. Arousal II Train.txt
1ta. Arousal II Train.txt
1tb. Arousal II Train.txt
2p. Arousal II test.txt
2t. Arousal II Test.txt

Figure 2-A1. Setting up the Arousal Discrimination Training Phase, Step #5 – Go/no-go training phase, in “1pa” pseudo condition.

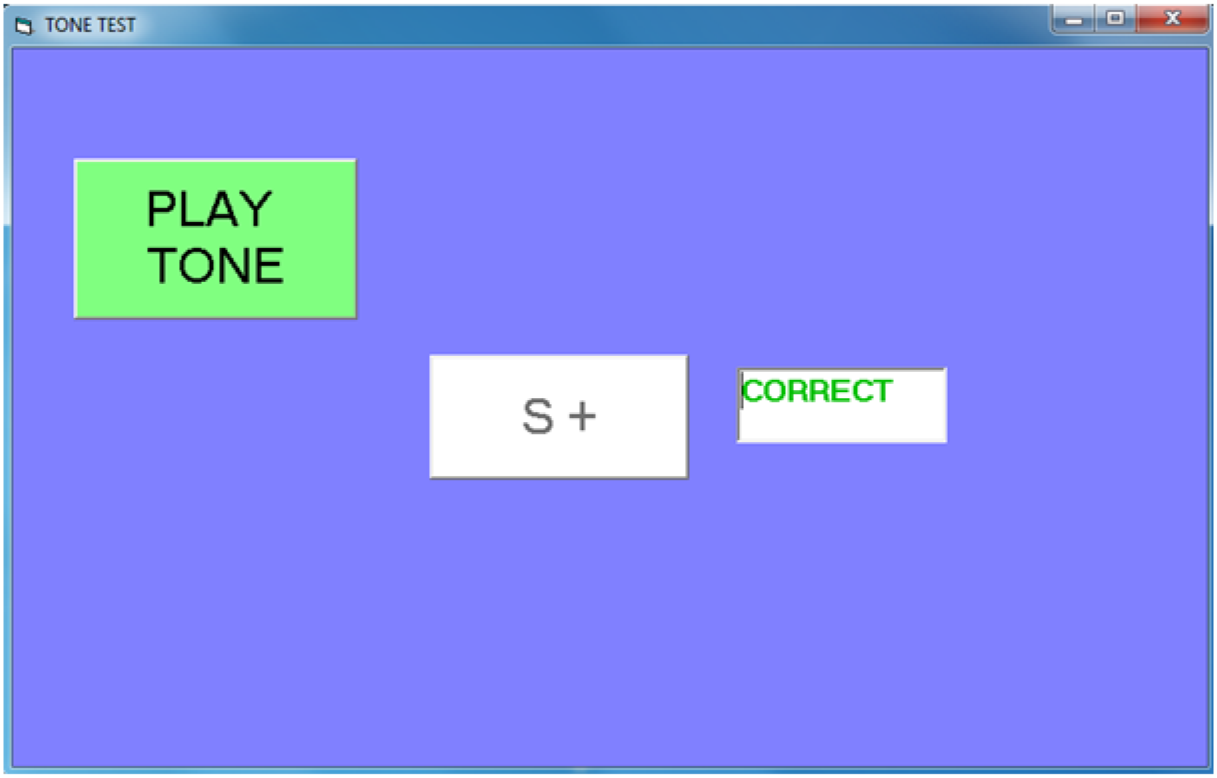


Figure 2-A2. Rewarded feedback received in the Training Phase.

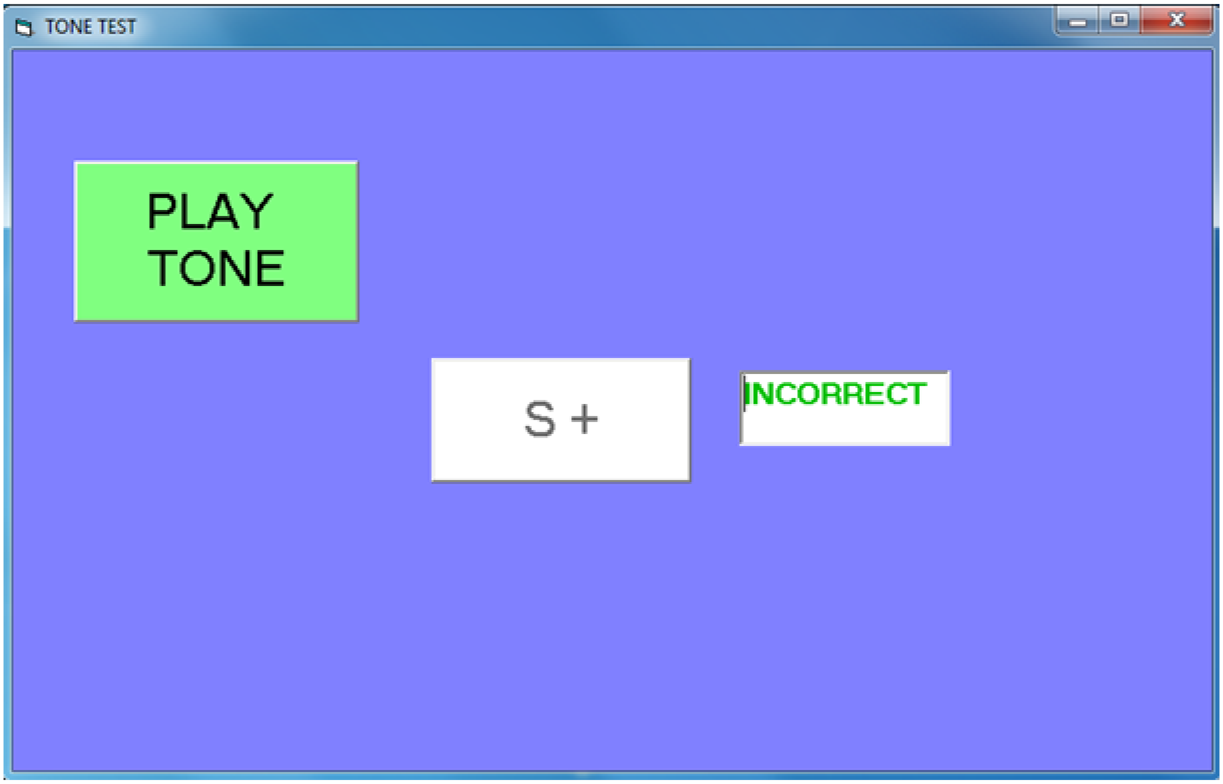


Figure 2-A3. Non-rewarded feedback received in the Training Phase.

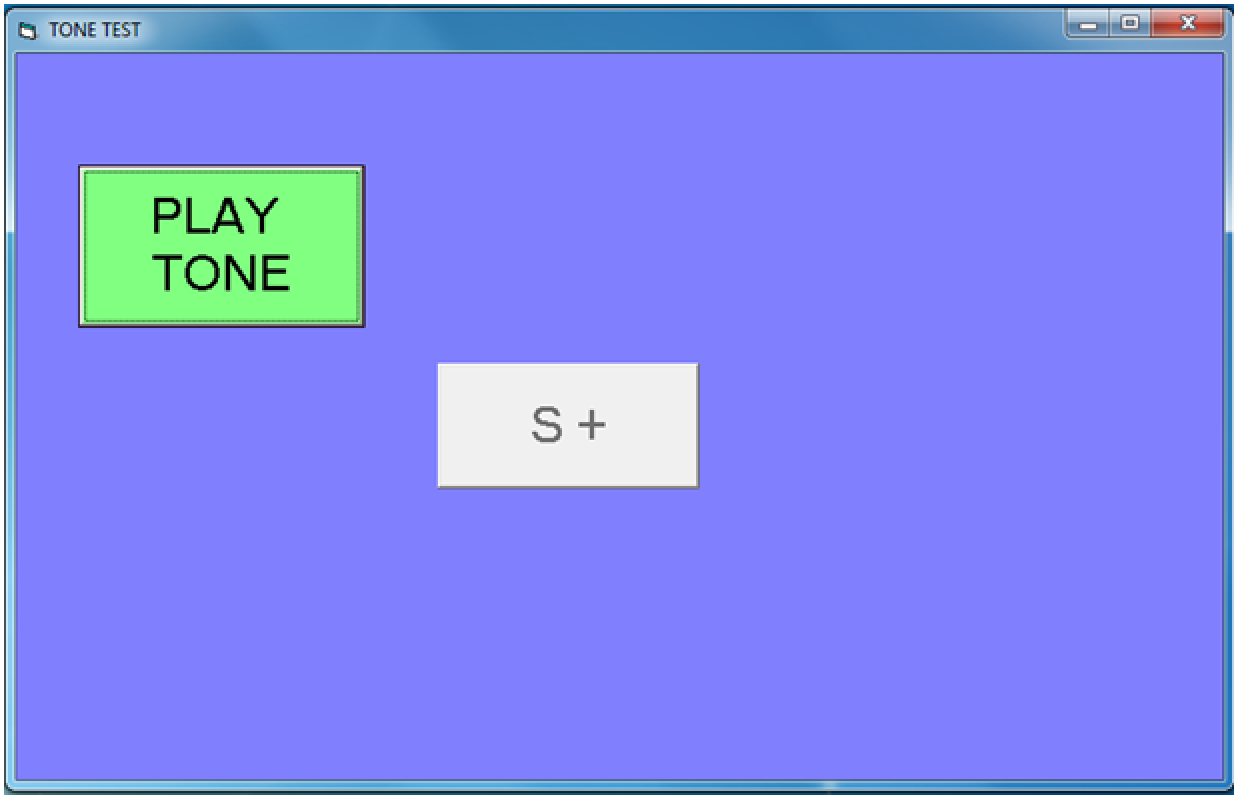


Figure 2-A4. Arousal Generalization Testing Phase (Note: No feedback box present).

Appendix B: Participant survey

Participant Survey (Experimenter use ONLY - Participant ID: ____________)

1) Age: ________

2) Sex: M or F

3) Are you a native English speaker? Y or N

4) Can you speak or understand Tamil? Y or N

5) Do you have 3 or more years of musical training? Y or N

6) Do you share your home space with a pet? If so please list the species.

7) Which of the following options best describes your experience with animals? Please circle your response.

1 2 3 4 5 6 7

1 - I have minimal interaction with animals (i.e., no pets, no friends or family with pets).

2 - I have some interaction with animals.

3 - I spend several hours a week with animals (e.g., friends have a pet).

4 - I have a pet.

5 - I have several species of pets.

6 - I work with animals 2-3 times a week.

7 - I work with animals 4 or more times a week (e.g., at a zoo or pet shop).

Chapter 3. Can you hear me now? The effect of signal degradation on perceived predator threat in black-capped chickadees (*Poecile atricapillus*)

Introduction

Almost every species on earth is or has been the prey of another species, since the time of the Cambrian explosion or longer (541-485 million years ago; Briggs, 2015). Predation is a major evolutionary force due to its impact on fitness, and consequently, the ability to perceive threat and possess adaptive antipredator behaviours should be selected for (Lima & Dill, 1990; Briggs, 2015). For example, the ability to communicate and interpret mobbing calls (i.e., vocalizations used to recruit conspecifics and heterospecifics) regarding nearby threat has been demonstrated to be beneficial to many species (e.g., black-capped chickadee mobbing calls, *Poecile atricapillus*, Baker & Becker, 2002; red-breasted nuthatches, *Sitta canadensis*, eavesdropping on black-capped chickadee mobbing calls, Templeton & Greene, 2007).

Black-capped chickadees are a non-migratory North American songbird that are typically predated by avian predators, including both hawks and owls (Smith, 1991). In the presence of a predator, black-capped chickadees produce mobbing calls to recruit and coordinate conspecifics and heterospecifics to attack and harass the nearby predator (Smith, 1991). Templeton et al. (2005) demonstrated that black-capped chickadee mobbing call production varies according to predator wingspan and body length, indicating that small-sized predators, including the northern saw-whet owl (NSWO; *Aegolius acadicus*), and large-sized predators, including the great horned owl (GHOW; *Bubo virginianus*) are on opposite ends of the threat spectrum as high- and low-threat, respectively (Templeton et al., 2005). Small songbird prey provide relatively little energetic benefit and are quite maneuverable, making them costly to pursue in forested areas (Pyke et al., 1977; Dudley, 2002); due to the large difference in wingspan and body length of these two species of owls, small NSWOs are more maneuverable in comparison to large GHOWs, and small songbirds are more likely to meet their energetic requirements (Templeton et al., 2005; Pyke et al., 1977). Avey and colleagues (2011) conducted a study examining immediate early gene expression based on the work of Templeton et al. (2005) in order to compare levels of ZENK expression in chickadees following exposure to high- and low-threat predator calls; upon hearing NSWO calls and GHOW calls, black-capped chickadees produced more gene expression in response to NSWO calls compared to GHOW calls. These results suggest that NSWO and GHOW predators, and the calls that they produce, are perceived to be different, potentially of high- and low-threat, respectively.

Despite knowledge of which predators are on opposite ends of the threat spectrum for black-capped chickadees, and that chickadees demonstrate the ability to perceive predator threat from both visual and auditory cues (Templeton et al., 2005; Avey et al., 2011), we do not know how anthropogenic noise (i.e., human-created noise) impacts the transmission of acoustic signals (i.e., signal degradation) in regards to threat perception. Unfortunately, due to the increase in human activity and urbanization across the globe, anthropogenic noise has been shown to have a detrimental impact on wild animals (Slabbekoorn & Ripmeester, 2008). For example, many species of songbirds are less abundant near highways (e.g., Forman & Alexander, 1998) and birds in those areas have lower breeding densities (e.g., Reijnen & Foppen, 1991). It has been hypothesized that female songbirds may prefer low-frequency male song, yet there have been many reported cases of songbirds increasing the frequency of their song to overcome low-frequency traffic noise; this suggests that male songbirds may have to choose between being an attractive mate or communicating clearly (Slabbekoorn & Ripmeester, 2008). These examples are just the tip of the proverbial iceberg regarding the impacts of anthropogenic noise on songbird communication. However, other than the knowledge that calling makes an owl more susceptible to being detected and mobbed by nearby prey (Chandler & Rose, 1988), few studies are focused on how acoustic signals produced by avian predators are perceived by songbirds, and the extent to which signal degradation affects perceived threat levels. Particularly, how do chickadees perceive the level of threat posed by potentially degraded predator acoustic signals (i.e., owl calls transmitted through forests)? For example, are high-threat predators perceived and responded to at further distances than low-threat predators? In addition, how do chickadees perceive acoustic stimuli that are classified as anthropogenic noise, such as the sound of truck alarms?

We sought to answer the above questions by collecting high- and low-threat owl calls and conducting an operant go/no-go discrimination task to investigate the effect of signal degradation on perceived threat. Specifically, chickadees were trained to respond to high-threat NSWO or low-threat GHOW calls that were recorded at short distances, then tested with additional NSWO and GHOW calls, NSWO-like and GHOW-like synthetic tones, and NSWO-like truck alarms, each across six distances (25m, 50m, 75m, 100m, 150m, and 200m). We predicted that birds would perceive and respond to: 1) high-threat predator calls at farther distances compared to low-threat predator calls, 2) synthetic tones similarly compared to the stimuli that they were designed to mimic, and 3) truck alarms as high-threat stimuli.

First, we predicted that chickadees have the ability to perceive predators that pose a high threat from further distances as this would assist in survival in comparison to responding to low-threat predators. Songbirds have been shown to change their behaviours depending on the distance of a predator (i.e., blackbirds, *Turdus merula*, mobbing intensity is higher to nearby magpie, *Pica pica*, predator dummies compared to distant magpies, whereas mobbing did not occur differentially to nearby versus distant dummies to non-threat rock dove, *Columbia livia*; Kryštofková et al., 2011). Thus, we anticipate that prey still perceive the threat posed and responses would be consistent in this type of task. Second, we were interested in investigating if chickadees perceive owl calls and synthetic stimuli as similar, generalizing the perception of threat, by creating synthetic tones that match the duration and frequency of NSWO or GHOW calls. Third, the calls produced by northern saw-whet owls (NSWO) are acoustically similar to the alarms that are produced when commercial vehicles reverse (i.e., backing up alarm; both acoustic stimuli have consistent frequencies throughout, and the two stimulus types have a similar frequency and duration of the individual segments); thus, we were interested in testing if black-capped chickadees perceive and respond to NSWO calls and truck alarms similarly, which would be detrimental to daily life in the wild (e.g., increased unnecessary vigilance, decreases in time spent feeding and mating) which affect survival and fitness. We know, for example, that black-capped chickadees have difficulty discriminating between acoustically-similar D notes produced by two different parid species (chestnut-backed chickadees, *Poecile rufescens*, and tufted titmice, *Baeolophus bicolor*; Hahn et al., 2017), but we do not know how chickadees will respond to acoustically-similar synthetic and truck alarm stimuli, both connected with anthropogenic noise. Overall, the results of this experiment will inform us about the perception of threat across distance, as well as inform us of whether or not our synthetic stimuli (i.e., tones and truck alarms) are perceived similarly to predator calls.

Methods

Subjects

Thirty-eight black-capped chickadees (19 males and 19 females; identified by DNA analysis; Griffiths et al., 1998) were originally used in this experiment, tested between September 2015 and April 2016. Birds at least one year of age (determined by examining the colour and shape of their outer tail rectrices; Pyle, 1997) were captured in Edmonton (North

Saskatchewan River Valley, 53.53˚N, 113.53˚W, Mill Creek Ravine, 53.52˚N, 113.47˚W), or Stony Plain (53.46˚N, 114.01˚W), Alberta, Canada between December 2010 and February 2015. However, two subjects failed early stages of the experiment, eight failed pretraining, one failed discrimination training, one failed due to low responding, and five birds died of natural causes. Thus, the data from only 19 birds (nine males, ten females) were used.

Prior to the experiment, birds were individually housed in Jupiter Parakeet cages (30 × 40 × 40 cm; Rolf C. Hagen, Inc., Montreal, QB, Canada) in colony rooms containing other black-capped chickadees. Birds had visual and auditory, but not physical, contact with one another. Birds had *ad libitum* access to food (Mazuri Small Bird Maintenance Diet; Mazuri, St Louis, MO, USA), water (vitamin supplemented on alternating days; Prime vitamin supplement; Hagen, Inc.), grit, and cuttlebone. Birds were given three to five sunflower seeds daily, one superworm (*Zophobas morio*) three times a week, and a mixture of greens (spinach or parsley) and eggs twice a week. Birds were maintained on a light:dark cycle that mimicked the natural light cycle for Edmonton, Alberta, Canada.

Throughout the experiment, birds were housed individually in operant chambers (see apparatus below), maintained on the natural light cycle for Edmonton, Alberta, and had *ad libitum* access to water (vitamin supplemented on alternate days), grit, and cuttlebone. Birds were given two superworms daily (one in the morning and one in the afternoon). Food (i.e., Mazuri) was only available as a reward for correct responding during the operant discrimination task. Sixteen birds had previous experience discriminating musical chords, black-capped chickadee *fee-bee* songs, parid and finch vocalizations, and/or *chick-a-dee* mobbing calls (Hoang, 2015; Hahn et al., 2016; Hahn et al., 2017; Congdon et al., 2019; respectively), but no operant experience with the stimulus types used in this experiment (owl vocalizations).

Apparatus

During the experiment, birds were housed individually in modified colony room cages (30 × 40 × 40 cm) placed inside a ventilated, sound-attenuating chamber. The chambers were illuminated by a 9W, full spectrum fluorescent bulb. Each cage contained three perches, a water bottle, and a grit cup. An opening on the side of the cage (11 × 16 cm) provided each bird access to a motor-driven feeder (see Njegovan et al., 1994). Infrared cells in the feeder and the request perch (perch closest to the feeder) monitored the position of the bird. A personal computer connected to a single-board computer (Palya & Walter, 2001) scheduled trials and recorded responses to stimuli. Stimuli were played from the personal computer hard drive, through either a Cambridge A300 Integrated Amplifier, Cambridge Azur 640A Integrated Amplifier (Cambridge Audio, London, England), or an NAD310 Integrated Amplifier (NAD Electronics, London, England) and through a Fostex FE108 Σ or Fostex FE108E Σ full-range speaker (Fostex Corp., Japan; frequency response range 80-18,000 Hz) located beside the feeder. See Sturdy and Weisman (2006) for a detailed description of the apparatus.

Acoustic Stimuli

Natural stimuli. Acoustic stimuli were obtained from the Bayne Laboratory (Department of Biological Sciences, University of Alberta, AB, Canada), Borror Laboratory of Bioacoustics (The Ohio State University, OH, USA), and the Macaulay Library (Cornell Lab of Ornithology, NY, USA; originally recorded between the years of 1954-2015 throughout Canada and USA). A total of 34 high quality vocalizations produced by small, high-threat northern saw-whet owls (NSWO) and large, low-threat great horned owls (GHOW) were obtained. Four acoustic stimuli of both species were then re-recorded using a Song Meter SM2+ automated audio recorder (Wildlife Acoustics, Maynard, Massachusetts, USA) in the boreal forest north of Fort

McMurray, AB (57.4998˚N, -111.4490˚W) on July 10, 2015, across six distances: 25, 50, 75, 100, 150, and 200m (resulting in 48 stimuli that were used in the experiment).

Synthetic stimuli. Four natural NSWO and GHOW stimuli across six distances (25, 50, 75, 100, 150, and 200m; 48 stimuli total) were reproduced as synthetic tones using Audacity 2.2.2. (The Audacity Team, Carnegie Mellon University, PA, USA). K.A.C. matched the frequency and amplitude (measured in the middle of the band using SIGNAL 5.10.24 software; Engineering Design, Berkeley, CA, USA) of natural NSWO and GHOW calls to produce NSWO- and GHOW-like tones (see Figure 3-1).

Truck alarm stimuli. Truck alarm stimuli (i.e., the back-up alarms produced when commercial trucks reverse) were originally recorded then re-recorded across six distances (25, 50, 75, 100, 150, and 200m; 24 stimuli) using a Song Meter SM2+ automated audio recorder (Wildlife Acoustics, Maynard, Massachusetts, USA) in the boreal forest north of Fort McMurray, AB (57.4998˚N, -111.4490˚W) on July 10, 2015.

All vocalizations used as acoustic stimuli were of high quality (i.e., no audible interference and low background-noise levels when viewed on a spectrogram with amplitude cutoffs of -35 to 0 dB relative to vocalization peak amplitude) and original (i.e., short distance) natural and truck alarm stimuli were bandpass filtered (outside the frequency range of each vocalization type) using GoldWave version 5.58 (GoldWave Inc., St. John's, NL, Canada) to reduce any background noise (Note: distant natural and truck stimuli were not possible to normalize without losing distant sound, and synthetic stimuli did not require normalizing). For all stimuli, 5 ms of silence was added to the leading and trailing portion of the vocalization and tapered to remove transients using SIGNAL 5.10.24 software. The amplitude of the original (i.e., "close") stimuli was equalized using SIGNAL 5.10.24 software.

During the experiment, stimuli were presented at approximately 75 dB as measured by a Brüel & Kjær Type 2239 decibel meter (A-weighting, slow response; Brüel & Kjær Sound & Vibration Measurement A/S, Nærum, Denmark) at the approximate height and position of a bird's head when on the request perch.

Procedure

Pretraining. Pretraining began once the bird learned to use the request perch and feeder to obtain food. During Pretraining, birds received food for responding to all stimuli (future rewarded stimuli, unrewarded stimuli, and testing stimuli). A trial began when the bird landed on the request perch and remained for between 900-1100 ms. A randomly-selected stimulus played without replacement until all 154 stimuli had been heard. If the bird left the request perch before a stimulus finished playing, the trial was considered interrupted, resulting in a 30-s time out with the houselight turned off. If the bird entered the feeder within 1 s after the entire stimulus played, it was given 1 s access to food, followed by a 30-s intertrial interval, during which the houselight remained on. If a bird remained on the request perch during the stimulus presentation and the 1 s following the completion of the stimulus it received a 60-s intertrial interval with the houselight on, but this intertrial interval was terminated if the bird left the request perch. This was to encourage a high level of responding on all trials. Birds continued on Pretraining until they completed six 308-trial blocks of ≥ 60% responding on average to all stimuli, at least four 308-trial blocks ≤ 3% difference in responding to future rewarded and unrewarded stimuli, at least four 308-trial blocks in which the bird had ≤ 3% difference in responding to future testing stimuli to ensure that birds did not display a bias for stimuli. Following a day of free feed, birds completed a second round in which they completed one 308-trial block of ≥ 60% responding on average to all stimuli, completed one 308-trial block of ≤ 3% difference in responding to future rewarded and unrewarded stimuli, completed one 308-trial block of ≤ 3% difference in responding to future testing stimuli to confirm that each bird continued to not display preferences following the break.

Discrimination Training. The procedure was the same as during Pretraining, except, only 24 training stimuli were presented (with the remaining 130 withheld for use during Transfer Testing), and responding to half of these stimuli were now punished with a 30-s intertrial interval with the houselight off and no access to food. As during Pretraining, responses to rewarded stimuli resulted in 1 s access to food. Discrimination training continued until birds completed six 312-trial blocks with a discrimination ratio (DR) ≥ 0.80 with the last two blocks being consecutive. For DR calculations see Response Measures, below.

Birds were randomly assigned to either a True category discrimination group (*n* = 12) or Pseudo category discrimination group (*n* = 7). Black-capped chickadees in the True category discrimination group were divided into two subgroups: one subgroup discriminated 12 rewarded NSWO calls from 12 unrewarded GHOW calls (NSWO S+ subgroup: three male and three female subjects), while the other subgroup discriminated 12 rewarded GHOW calls from 12 unrewarded NSWO calls (GHOW S+ subgroup: three male and three female subjects).

The Pseudo category discrimination group was also divided into two subgroups. Each subgroup discriminated six randomly-selected rewarded NSWO calls and six randomly-selected rewarded GHOW calls from six unrewarded NSWO and six unrewarded GHOW calls (Pseudo subgroup 1: two male and two female subjects; Pseudo subgroup 2: two male and two female subjects). The purpose of the Pseudo group was to include a control in which subjects were not trained to categorize according to threat level, investigating if True group acquisition is due to category learning (significantly fewer trials than the Pseudo groups) or simply rote memorization (similar number of trials compared to the Pseudo group); fewer trials to criterion would provide evidence of category learning, and transfer of training provides further support.

Discrimination 85. This phase was identical to Discrimination training, except that the rewarded stimuli were rewarded with a reduced probability of getting a reward (i.e., $P = 0.85$). On unrewarded rewarded S+ trials, entering the feeder after the stimulus finished playing resulted in a 30-s intertrial interval, during which the houselight remained on, but there was no access to food. Discrimination 85 training was employed to introduce birds to trials in which there was no access to food, but the houselight remained illuminated, in order to prepare birds for Transfer Testing in which stimuli were neither rewarded or punished. Discrimination 85 training continued until birds completed two 312-trial blocks with a DR ≥ 0.80.

Transfer Testing. During Transfer Testing, the stimuli and reinforcement contingencies from Discrimination 85 were maintained and 130 additional stimuli were included as stimuli. These stimuli were heard during Pretraining, but not Discrimination training. Testing stimuli consisted of additional NSWO and GHOW calls recorded at short distances (five stimuli per species; 10 stimuli total); NSWO calls, GHOW calls, and truck alarms recorded at six distances (i.e., 25m, 50m, 75m, 100m, 150m, 200m; four stimuli per type and distance; 72 stimuli total); and synthetic NSWO- and GHOW-like stimuli replicating all six distances (four stimuli per type and distance; 48 stimuli total).

Due to the number of testing stimuli, we created four rounds of Transfer Testing. First, birds completed at least three 322-trial blocks that included 10 additional NSWO and GHOW recorded at short distances testing stimuli (i.e., five additional stimuli recorded at short distances per species). Next, birds completed at least three 342-blocks of Transfer testing rounds 2, 3, 4, and 5, in a random order; these testing rounds included 30 additional testing stimuli comprised of a random assortment of NSWO, GHOW, and truck alarms recorded at 25m, 50m, 75m, 100m, 150m, 200m (four stimuli per type and distance), and NSWO- and GHOW-like synthetic stimuli replicating all six distances (four stimuli per type and distance). During Transfer Testing, the stimuli from Discrimination 85 training were presented 13 times each, randomly-selected without replacement and the testing stimuli were each presented once during the 322- or 342-trial block (round 1 and 2-5, respectively).

Birds completed a minimum of three blocks for each round of Transfer Testing and these were included in the analysis. Between each round of Transfer, birds completed two 312-trial blocks of Discrimination 85 with a DR ≥ 0.80. Following the final round of Transfer Testing, birds were returned to the colony room.

Responses to testing stimuli resulted in a 30-s intertrial interval with the houselight on, but no access to food; we did not differentially reinforce or punish testing stimuli, and only presented each testing stimulus once each per trial block, so subjects did not learn specific contingencies associated with responding to these testing stimuli.

Response Measures. For each stimulus exemplar, a proportion response was calculated by the following formula: R+/(N-I), where R+ is the number of trials in which the bird went to the feeder after the stimulus, N is the total number of trials during which that stimulus was presented, and I is the number of interrupted trials in which the bird left the perch before the entire stimulus played. For Discrimination and Discrimination 85 training, we calculated a discrimination ratio (DR), by dividing the mean proportion response to all rewarded stimuli by the mean proportion response to rewarded stimuli plus the mean proportion response to unrewarded stimuli. A DR of 0.50 indicates equal responding to rewarded and unrewarded stimuli, whereas a DR of 1.00 indicates perfect discrimination. For Transfer Testing, we scaled the proportion of response for each subject by rescaling the highest proportion of the response to a test stimulus to 1.0 and rescaling the proportion of response to all other stimuli as a ratio of the highest proportion of response.

Statistical Analyses. We conducted independent-samples *t*-tests on the number of trials to criterion for the True and Pseudo category groups during Discrimination training. To investigate responses to stimuli during Transfer Testing, we split stimuli into: natural stimuli, truck alarms, and synthetic stimuli. First, we conducted a three-way repeated measures ANOVA for the True group with Condition (NSWO S+, GHOW S+) × Stimulus Species (NSWO, GHOW) × Stimulus Distance (short distance, 25m, 50m, 75m, 100m, 150m, 200m) as fixed factors and the scaled proportion of responding to natural stimuli during Transfer Testing as the dependent variable; additional stimuli recorded at short distances were included with the distant stimuli to directly compare all natural stimuli. Second, we conducted a three-way repeated measures ANOVA for the True group with Condition (NSWO S+, GHOW S+) × Stimulus Species (NSWO, GHOW) × Stimulus Distance (25m, 50m, 75m, 100m, 150m, 200m) as fixed factors and the scaled proportion of responding to synthetic stimuli during Transfer Testing as the dependent variable. Last, we conducted a two-way repeated measures ANOVA for the True group with Condition (NSWO S+, GHOW S+) × Stimulus Distance (25m, 50m, 75m, 100m, 150m, 200m) as fixed factors and the scaled proportion of responding to truck alarm stimuli during Transfer Testing as the dependent variable. Where applicable, significant analyses were followed by independent samples *t*-tests on responding to stimulus type across distance to determine which stimuli birds demonstrated transfer of training (i.e., to which stimuli birds responded).

Ethical Note. Throughout the experiment, birds remained in the testing apparatus to minimize the transport and handling of each bird and reduce stress. Following the experiment, birds were returned to the colony room for use in future experiments. With the exception of five birds that died of natural causes, birds 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 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

To determine whether birds in the two True category groups differed in their speed of acquisition, we conducted an independent-samples *t*-test on the number of 312-trial blocks to reach criterion for the two True category conditions (NSWO S+: X+SEM = 143.000+8.881, N = 6; GHOW S+: X+SEM = 132.167+9.141, N = 6). There was no significant difference, t_{10} = 0.850, p = 0.415, d = .0538, 95% Confidence Interval [CIs] = -17.564, 39.230.

To determine whether birds in the two Pseudo category groups differed in their speed of acquisition, we conducted an independent-samples *t*-test on the number of 312-trial blocks to reach criterion for the two Pseudo category conditions (Pseudo 1 Group: X+SEM = 502.667+167.185, N = 3; Pseudo 2 Group: X+SEM = 513.500+133.878, N = 4). There was no significant difference, t_5 = -0.039, p = 0.970, d = .025, 95% CIs = -723.075, 701.409.

To compare the acquisition performance of the True and Pseudo category groups and to determine if the True group learned to categorize in fewer trials than the Pseudo group, we conducted an independent-samples *t*-test on the number of 312-trial blocks to reach criterion for the True category and Pseudo category groups. Due to a violation of Levene's test, we used the *p*-value that did not assume homogeneity of variance; there was a significant difference between the groups ($t_{6.030} = -2.962$, $p = 0.025$, $d = -2.412$, 95% CIs = -677.612, -64.935) in that True birds learned to discriminate significantly faster than Pseudo birds.

Analysis of Transfer Stimuli

Natural stimuli. To determine if the pattern of learning was the same across calls from testing species in Transfer Testing, we conducted a three-way repeated measures ANOVA for the True group with Condition (NSWO S+, GHOW S+) × Stimulus Species (NSWO, GHOW) × Stimulus Distance (short distance, 25m, 50m, 75m, 100m, 150m, 200m) as fixed factors and the proportion of responding during Transfer Testing as the dependent variable. Using a Huynh-Feldt correction, there was a significant three-way interaction of Condition × Stimulus Species × Stimulus Distance ($F_{1,1,6} = 9.293$, $p < 0.001$, $\eta^2 = .650$), indicating that there was differential responding to stimulus species according to condition across all seven stimulus distances. The interaction of Condition × Stimulus Species ($F_{1,6} = 36.109$, $p = 0.002$, $\eta^2 = .878$), and the two-way interaction of Stimulus Species × Stimulus Distance were also significant ($F_{1,6} = 4.779$, $p = 0.002$, $\eta^2 = .489$), indicating that there was a significant difference in responding to Stimulus Species based on the Condition, and Stimulus Distance based on Stimulus Species. The Stimulus Species main effect was significant ($F_1 = 62.038$, $p = 0.001$, $\eta^2 = .925$); however, the two-way interaction of Condition × Species Distance and the main effects of Condition and Stimulus Species were non-significant (all *ps* > 0.148). To further investigate the three-way interaction, we conducted independent samples and paired-samples *t*-tests; see Table 3-1 and 3-2 for these statistical results, respectively. These results indicate that black-capped chickadees in both groups were able to transfer training to reward-contingency stimuli recorded at short distances, and that the GHOW S+ subgroup responded significantly more to GHOW stimuli compared to the NSWO S+ subgroup across all seven distances. In contrast, the NSWO S+ group responded significantly differently to stimuli recorded at short distances compared to stimuli recorded at 150m; see Figure 3-2.

Synthetic stimuli. To determine if the pattern of learning was the same across calls from testing species in Transfer Testing, we conducted a three-way repeated measures ANOVA for the True group with Condition (NSWO S+, GHOW S+) × Stimulus Type (synthetic NSWO-like, synthetic GHOW-like) × Stimulus Distance (short distance, 25m, 50m, 75m, 100m, 150m, 200m) as fixed factors and the proportion of responding during Transfer Testing as the dependent variable. Using a Huynh-Feldt correction, there was a significant three-way interaction of Condition × Stimulus Type × Stimulus Distance ($F_{1,1,5} = 4.420$, $p = 0.005$, $\eta^2 = .469$), indicating that there was differential responding to stimulus species according to condition across all seven stimulus distances. The two-way interaction of Condition × Stimulus Type ($F_{1,5} = 37.465$, $p = 0.002$, $\eta^2 = .882$), and the two-way interaction of Condition × Stimulus Distance were also significant ($F_{1,5} = 2.751$, $p = 0.044$, $\eta^2 = .355$), indicating that there was a significant difference in responding to Stimulus Type on the Condition and Stimulus Distance based on Condition. However, the two-way interaction of Stimulus Type × Stimulus Distance and the main effects of Condition, Stimulus Type, and Stimulus Distance were not significant (all *ps* > 0.245). To further investigate the three-way interaction, we conducted independent samples and paired-samples *t*-tests; see Table 3-3 and 3-4 for these statistical results. These results indicate that black-capped chickadees in the NSWO S+ group were able to transfer training to stimuli recorded at 25m and 50m, and responded significantly more to stimuli recorded at 25m compared to stimuli recorded at 100m. However, unlike responding to the natural stimuli, GHOW S+ subgroup did not respond significantly more to GHOW-like stimuli compared to the NSWO S+ subgroup across distances; see Figure 3-3.

Truck alarm stimuli. To determine if the pattern of learning was the same across calls from testing species in Transfer Testing, we conducted a two-way repeated measures ANOVA for the True group with Condition (NSWO S+, GHOW S+) × Stimulus Distance (truck alarms: 25m, 50m, 75m, 100m, 150m, 200m) as fixed factors and the proportion of responding during Transfer Testing as the dependent variable. Using a Huynh-Feldt correction, the interaction of Condition × Stimulus Distance was not significant ($F_{1, 3.070} = 1.596$, $p = 0.231$, $\eta^2 = .242$), indicating that there was no significant difference in responding to truck alarm stimuli over distance based on Condition. The main effects of Condition and Stimulus Distance were not significant either (ps > 0.091); see Figure 3-4.

Discussion

In the current study, we confirmed that black-capped chickadees treat acoustically-distinct high-threat northern saw-whet owl (NSWO) and low-threat great horned owl (GHOW) calls as belonging to two separate perceptual categories. True group birds learned to discriminate stimuli in fewer trials compared to Pseudo group birds, suggesting that birds in the True group treated stimuli produced by two different species across multiple individuals as belonging to two perceptual categories leading to significantly faster task acquisition in the True group compared to the Pseudo group. Following training, chickadees were tested with NSWO and GHOW calls that were re-recorded across six distances, synthetic NSWO- and GHOW-like tones, and NSWO-like truck alarms to investigate the perception of threat and the effect of signal degradation on these two stimulus categories.

Natural Stimuli: Short Distance

Northern saw whet and great horned owls have been reported to be on opposite ends of the threat spectrum for black-capped chickadees (Templeton et al., 2005), and their calls have previously been used as acoustic stimuli to produce significantly different levels of immediate early gene expression in the black-capped chickadee auditory system (Avey et al., 2011). To ensure that the original stimuli were in fact treated as distinct perceptual categories, we tested True groups with additional NSWO and GHOW stimuli recorded at short distances. We found that both the NSWO S+ and GHOW S+ subgroups responded appropriately to testing stimuli (i.e., NSWO and GHOW calls, respectively). Thus, this provides further support for True group category learning compared to the Pseudo group, and demonstrates that both True subgroups demonstrated transfer of training to stimuli recorded at short distances that were previously non-differentially reinforced but of the rewarded category.

Natural Stimuli: Distant

We then tested birds with NSWO and GHOW calls that were re-recorded at multiple distances. We predicted that chickadees would perceive and respond to high-threat NSWO calls at further distances compared to low-threat GHOW calls as the ability to perceive predators that pose a higher threat from farther distances would assist in survival and thus, is likely to be selected for through evolution. However, we found that only the chickadees in the GHOW S+ subgroup responded consistently to GHOW stimuli across all distances, whereas the NSWO S+ subgroup appeared to decrease responding to NSWO stimuli as distance increased (i.e., stimuli recorded at short distances vs. 150m).

In light of these results, we propose that high-threat owls may be of lower salience when heard from further distances as chickadees do not perceive predators at such distances to continue to be of high-threat. Perhaps, if a small, high-threat predator is at a far enough distance, these predators are no longer considered to be an imminent danger, and if the signal is not perceived as high-threat, possibly explaining why chickadees ceased responding to high-threat testing stimuli recorded at far distances; in contrast, perhaps low-threat predators are considered low-threat, regardless of distance.

A second hypothesis is that there may be an issue with transmission of NSWO signals over distance, as signal degradation may be a cause for the error in perception. It is possible that higher-frequency NSWO calls may be more affected by signal degradation than low-frequency GHOW calls (see Figure 3-5). Upon transmission, the high-frequency notes contained in the black-capped chickadee *chick-a-dee* calls (i.e., A, B, C notes) attenuated most in dense coniferous forests compared to deciduous and mixed forests (Proppe et al., 2010). Yip et al. (2017) also demonstrated that sound attenuation appears to be frequency-dependent in that high-frequency songbird vocalizations had a lower effective detection radius compared to lower-frequency owl vocalizations, and that high-frequency vocalizations attenuated more when played back in the forest compared to roadside playback. Considering that our stimuli were re-recorded at multiple distances throughout the boreal forest which, although mixed throughout, was primarily coniferous in the area of recordings, this could explain our results. This suggests a strong influence of signal degradation on high-frequency NSWO calls compared to low-frequency GHOW calls.

Nonetheless, we recommend further investigation to examine this lack of responding to NSWO calls at further distances, perhaps by including stimuli that have similar acoustic characteristics yet are of low-threat, we can tease apart if responding is based on the threat posed by the vocalizations or the effect of signal degradation across distance. However, large animals typically produce vocalizations with lower frequencies compared to small predators (e.g., Martin et al., 2011) so finding a stimulus that would satisfy both higher-frequency and low-threat characteristics might be difficult. Conversely, NSWOs and GHOWs pose varying threat levels to chickadees, but not to humans (*Homo sapiens*). Yip et al. (2017) had human observers determine at what distances high-frequency songbird and lower-frequency owl vocalizations were detectable yet found that the results for NSWO and GHOW calls to be comparable in that both stimuli were detectable or not detectable, dependent on the observer. We propose that an extension of the current experiment could be completed as a comparative go/no-go task with human participants to further investigate if lack of responding to NSWO stimuli is based on threat perception or signal degradation.

Synthetic Stimuli

In the current study, synthetic stimuli were created to sound like NSWO and GHOW calls. We predicted chickadees would respond to synthetic NSWO-like and GHOW-like tones similarly to the natural predator calls. Chickadees in the NSWO S+ and GHOW S+ subgroups did in fact respond appropriately to NSWO-like and GHOW-like synthetic tones, respectively, but did so at lower and typically non-significant levels. The finding that our subjects responded to synthetic stimuli similarly, although at a lower level, to the original calls is important as it suggests that our synthetic signals contain some acoustic features of these owl calls that are related to the concept of high- and low-threat, but that the birds can still perceive them as different from owl calls.

Truck Alarm Stimuli

Truck alarms (i.e., the sound that commercial trucks produce when in reverse) are acoustically-similar to high-threat NSWO calls. To ensure that these truck alarms, commonly heard in the oil fields, were not an issue for chickadees living in areas where these alarms are heard, we used these as testing stimuli. However, we found that chickadees did not respond to truck alarms as if they were acoustically-similar to NSWO calls. These findings provide assurance that chickadees are capable of discriminating between NSWO calls and NSWO-like truck alarms and do not respond to the two as similar. Although anthropogenic noise is an extensive issue in regards to songbird communication, perception, and survival (e.g., Slabbekoorn & Ripmeester, 2008; Proppe et al., 2013; Bayne et al., 2008), if wild-caught laboratory chickadees had perceived and responded to synthetic commercial truck alarms in this experiment, it would have suggested that songbirds in the wild may regularly and inappropriately disrupt normal behaviour in response to acoustically-similar non-predators that do not pose a direct threat to survival.

Conclusions

Overall, this experiment provides insights into songbird perception of predator threat, and how that perception is affected by distance and signal degradation. Our findings do not support our prediction that black-capped chickadees would continue to discriminate high-threat signals at further distances compared to low-threat signals. Again, we propose that chickadees in the NSWO S+ subgroup responded in this way as they were discriminating threat not species as at further distances small predators may no longer pose higher threat. This would explain the difference between the NSWO S+ and GHOW S+ results. Future studies are necessary to parse threat perception from the effects of signal degradation, including stimuli of high-threat and low-frequency and/or comparative trials with humans. Synthetic tones that were created to match frequency and duration of NSWO and GHOW stimuli have demonstrated that chickadees will respond to constructed tones similarly to natural predator calls. In comparison to the findings that chickadees do not respond to truck alarms as similar to NSWO calls, it demonstrated that this species of songbird can perceive the similarities between natural calls and synthetic tones and generalize responding, but can also discriminate between natural NSWO calls and synthetic truck alarms. If chickadees were inappropriately responding to non-threatening objects as predators then populations may decline, as an inability to discriminate acoustic stimuli may result in a loss of opportunities for individuals to feed or mate due to antipredator behaviours, or could instead result in habituation to the incorrect signals (i.e., not producing antipredator behaviours in the presence of a high-threat predator). Thus, this ability to perceive and respond to threat appropriately is critical and was likely selected for. In total, the multiple stimuli used in the current experiment provides many insights into the threat perception of songbirds, including the effects of distance and signal degradation, perceptual similarities between natural and synthetic stimuli, and perceptual dissimilarities between high-threat owl calls and a type of anthropogenic noise.

Acknowledgements

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Table 3-1. Results of the independent samples *t*-test comparing subjects' responding in NSWO S+ vs. GHOW S+ groups to each natural stimulus across distance during Transfer Testing, with Bonferroni corrections (*p* = 0.05/14 = 0.0035).
Note: Negative *t*-values indicate that GHOW S+ responded more than NSWO S+.

	t-test	*p-value*	*Cohen's d*	*Confidence Intervals*
NSWO S+ Group vs. GHOW S+ Group (*df* = 10)				
NSWO stimuli				
Short	**4.086**	**0.002**	**2.584**	**0.207, 0.704**
25m	0.585	0.571	0.370	-0.253, 0.434
50m	1.065	0.312	0.674	-0.159, 0.451
75m	1.343	0.209	0.849	-0.119, 0.480
100m	**-3.850**	**0.003**	**-2.435**	**-0.614, -0.164**
150m	-1.883	0.089	-1.191	-0.546, 0.046
200m	-3.341	0.007	-2.113	-0.625, -0.125
GHOW stimuli				
Short	**-8.910**	**<.001**	**-5.635**	**-0.799, -0.479**
25m	**-6.975**	**<.001**	**-4.411**	**-0.852, -0.440**
50m	**-11.859**	**<.001**	**-7.500**	**-0.924, -0.632**
75m	**-8.470**	**<.001**	**-5.357**	**-0.833, -0.486**
100m	**-5.057**	**<.001**	**-3.198**	**-0.680, -0.264**
150m	**-9.502**	**<.001**	**-6.010**	**-0.832, -0.516**
200m	**-6.750**	**<.001**	**-4.269**	**-0.859, -0.433**

Bold font indicates significance.

Table 3-2. Results of the paired-samples *t*-tests comparing subjects' responding in NSWO S+ and GHOW S+ groups to each natural stimulus between natural NSWO and GHOW stimuli recorded at short distances vs. distant stimuli during Transfer Testing, with Bonferroni corrections ($p = 0.05/6 = 0.0083$).

	t-test	*p-value*	*Cohen's d*	*Confidence Intervals*
NSWO S+ (*df* = 5)				
NSWO Short v. 25m	4.101	0.009	0.698	0.064, 0.278
NSWO Short v. 50m	1.481	0.199	0.794	-0.136, 0.505
NSWO Short v. 75m	1.370	0.229	0.649	-0.138, 0.452
NSWO Short v. 100m	3.751	0.013	2.550	0.172, 0.920
NSWO Short v. 150m	**4.735**	**0.005**	**2.731**	**0.246, 0.831**
NSWO Short v. 200m	3.954	0.011	2.865	0.210, 0.992
GHOW S+ (*df* = 5)				
GHOW Short v. 25m	-1.220	0.277	-0.776	-0.604, 0.215
GHOW Short v. 50m	-1.085	0.327	-0.629	-0.421, 0.171
GHOW Short v. 75m	-1.765	0.138	-0.649	-0.290, 0.054
GHOW Short v. 100m	-3.630	0.015	-2.008	-0.510, -0.087
GHOW Short v. 150m	-1.520	0.189	-0.736	-0.449, 0.115
GHOW Short v. 200m	-3.287	0.022	-2.143	-0.408, -0.050

Bold font indicates significance.

Table 3-3. Results of the independent samples *t*-test comparing subjects' responding in NSWO S+ vs. GHOW S+ groups to synthetic stimuli across distance during Transfer Testing, with Bonferroni corrections ($p = 0.05/10 = 0.005$).
Note: Negative *t*-values indicate that GHOW S+ responded more than NSWO S+.

	t-test	***p-value***	***Cohen's d***	***Confidence Intervals***
NSWO S+ Group vs. GHOW S+ Group (*df* = 10)				
NSWO-like synthetic stimuli				
25m	**6.864**	**<.001**	**4.341**	**0.352, 0.690**
50m	**3.607**	**0.005**	**2.281**	**0.111, 0.472**
75m	2.573	0.038	1.627	0.036, 0.505
100m	0.532	0.607	0.336	-0.177, 0.288
150m	-0.425	0.680	-0.269	-0.347, 0.236
200m	0.863	0.409	0.546	-0.088, 0.199
GHOW-like synthetic stimuli				
25m	-2.105	0.079	-1.331	-0.584, 0.043
50m	-1.119	0.289	-0.708	-0.374, 0.124
75m	-2.945	0.015	-1.863	-0.354, -0.049
100m	-3.195	0.010	-2.021	-0.460, -0.082
150m	-1.633	0.134	-1.033	-0.476, 0.073
200m	-1.593	0.142	-1.008	-0.450, 0.075

Bold font indicates significance.

Table 3-4. Results of the paired-samples *t*-tests comparing subjects' responding in NSWO S+ and GHOW S+ groups to each natural stimulus between synthetic NSWO-like and GHOW-like stimuli recorded at short distances vs. distant stimuli during Transfer Testing, with Bonferroni corrections ($p = 0.05/5 = 0.01$).

	t-test	*p-value*	*Cohen's d*	*Confidence Intervals*
NSWO S+ (*df* = 5)				
NSWO-like 25 v. 50m	3.038	0.029	1.073	0.026, 0.308
NSWO-like 25 v. 75m	2.236	0.076	0.879	-0.025, 0.358
NSWO-like 25 v. 100m	**9.400**	**<0.001**	**2.017**	**0.237, 0.416**
NSWO-like 25 v. 150m	3.528	0.017	1.949	0.107, 0.684
NSWO-like 25 v. 200m	3.883	0.012	2.669	0.131, 0.646
GHOW S+ (*df* = 5)				
GHOW-like 25 v. 50m	-1.000	0.363	-0.553	-0.223, 0.098
GHOW-like 25 v. 75m	-2.236	0.076	-0.690	-0.179, 0.012
GHOW-like 25 v. 100m	-1.685	0.153	-0.900	-0.351, 0.073
GHOW-like 25 v. 150m	-2.229	0.076	-1.092	-0.389, -0.028
GHOW-like 25 v. 200m	-3.051	0.028	-0.830	-0.141, -0.012

Bold font indicates significance.

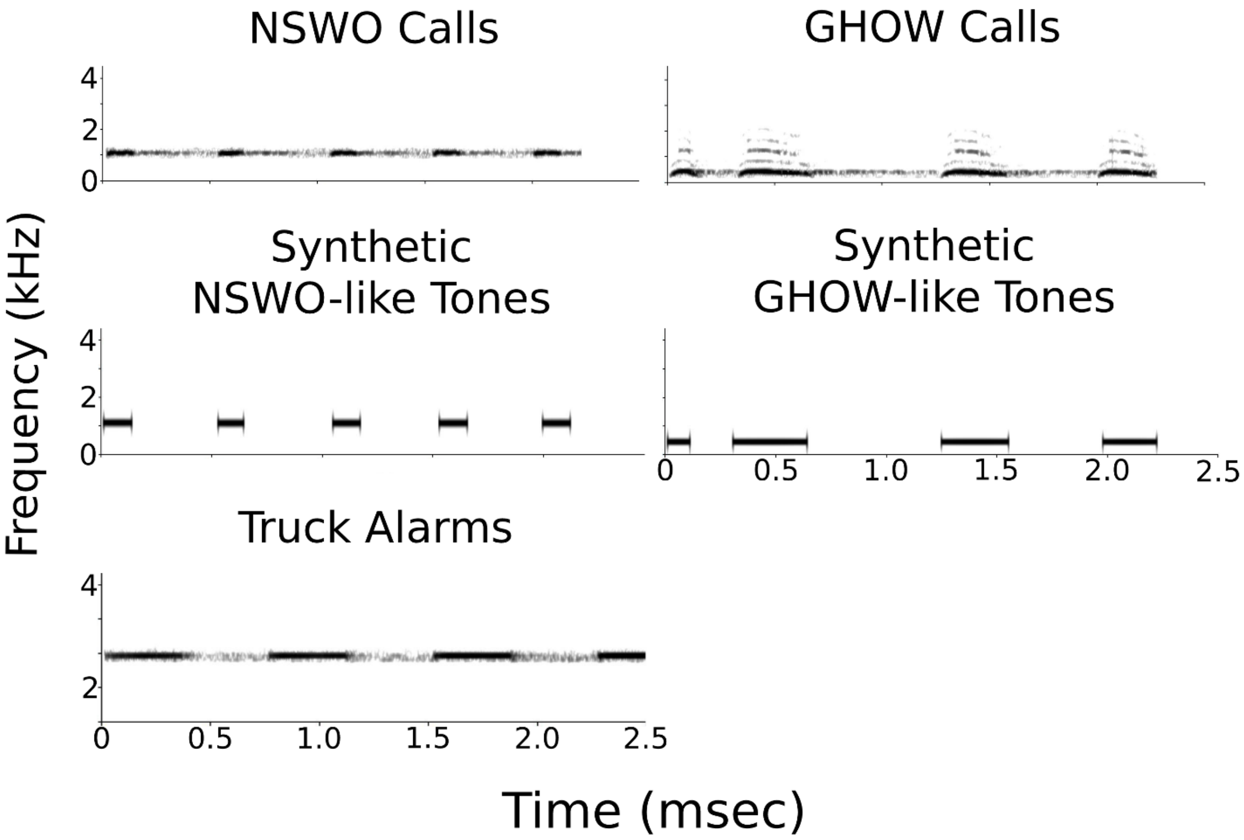

Figure 3-1. Sample sound spectrograms of high-threat northern saw-whet owl (NSWO) and low-threat great horned owl (GHOW) calls, synthetic NSWO-like and GHOW-like tones, and NSWO-like truck alarms used as acoustic stimuli with time (msec) on the *x*-axis and frequency (kHz) on the *y*-axis.

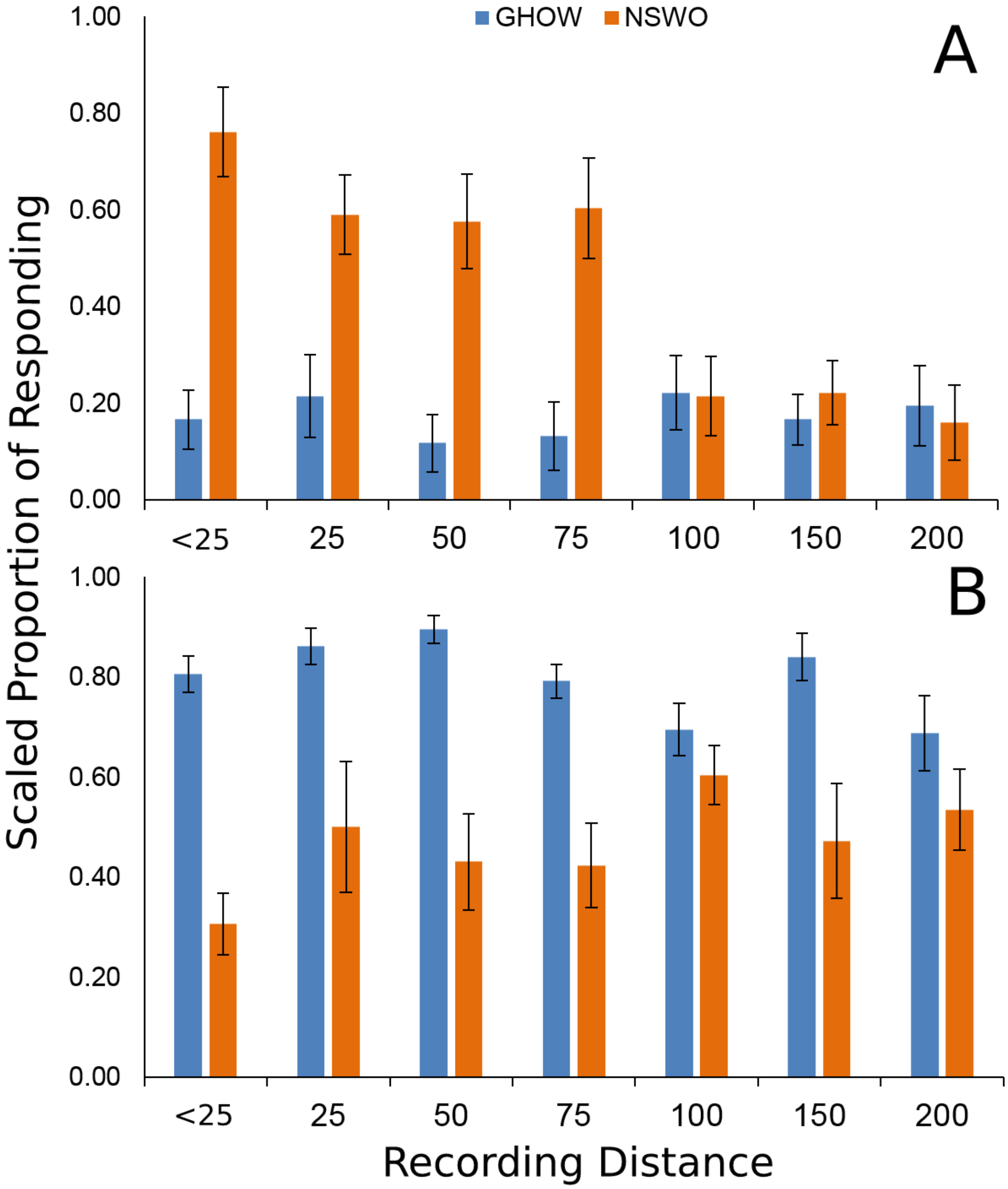

Figure 3-2. Scaled proportion of responding to natural NSWO and GHOW calls by black-capped chickadees in the NSWO S+ subgroup (**A**; *n* = 6) and GHOW S+ subgroup (**B**; *n* = 6) during the Transfer Testing phase ± SEM across recording distances (short distances/<25, 25, 50, 75, 100, 150, 200m).

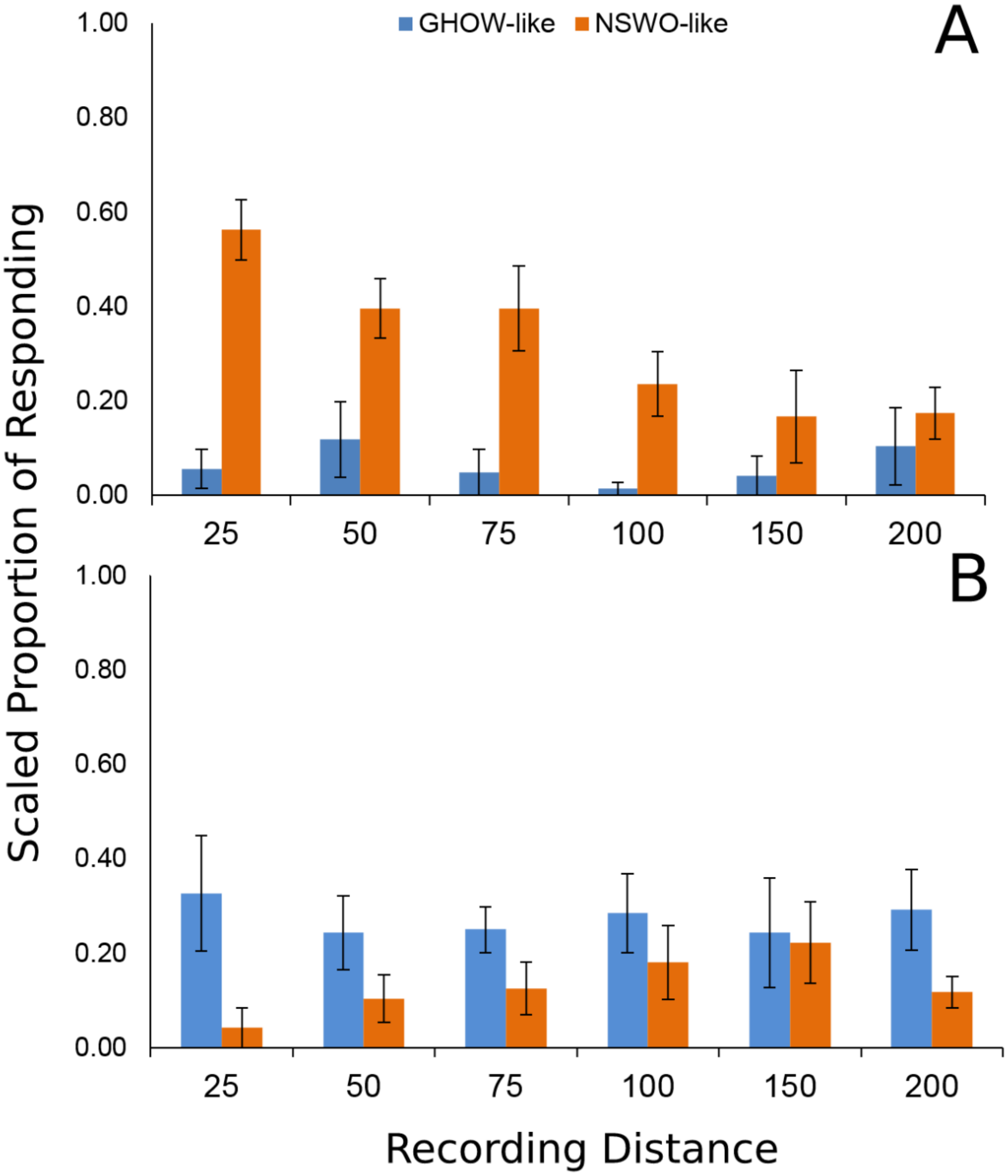

Figure 3-3. Scaled proportion of responding to synthetic NSWO-like and GHOW-like synthetic tones by black-capped chickadees in the NSWO S+ subgroup (**A**; *n* = 6) and GHOW S+ subgroup (**B**; *n* = 6) during the Transfer Testing phase ± SEM across recording distances (25, 50, 75, 100, 150, 200m).

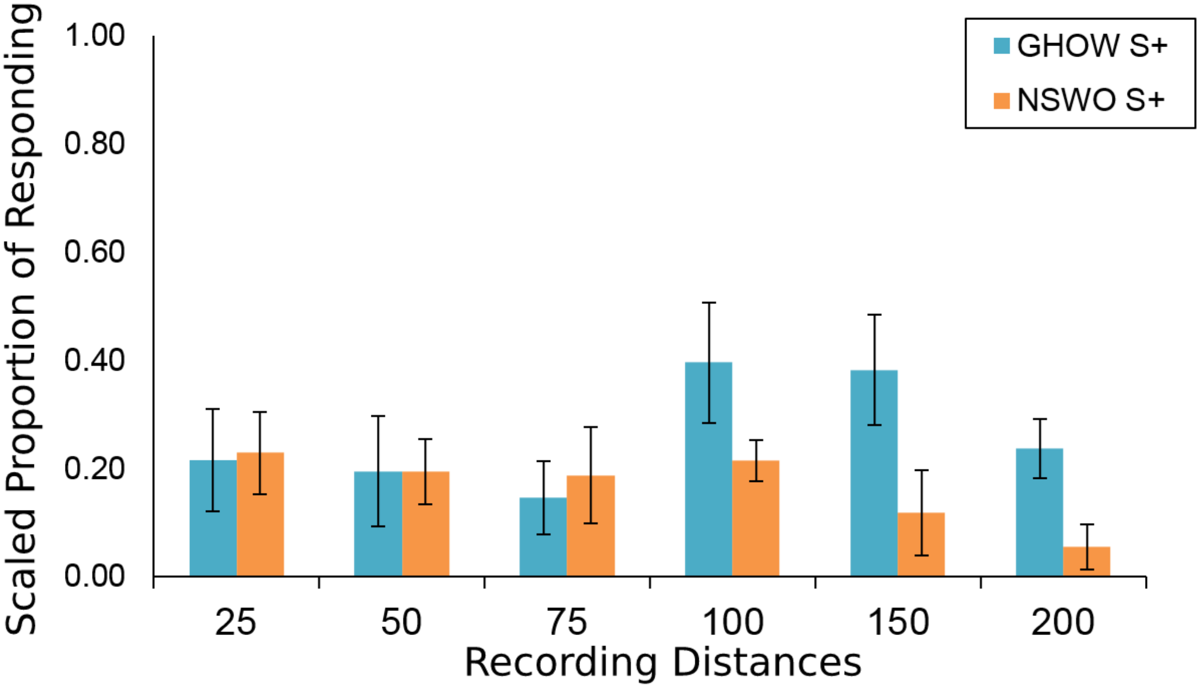

Figure 3-4. Scaled proportion of responding to NSWO-like truck alarm stimuli for each condition by True group black-capped chickadees (*n* = 12) during the Transfer Testing phase ± SEM across recording distances (25, 50, 75, 100, 150, 200m).

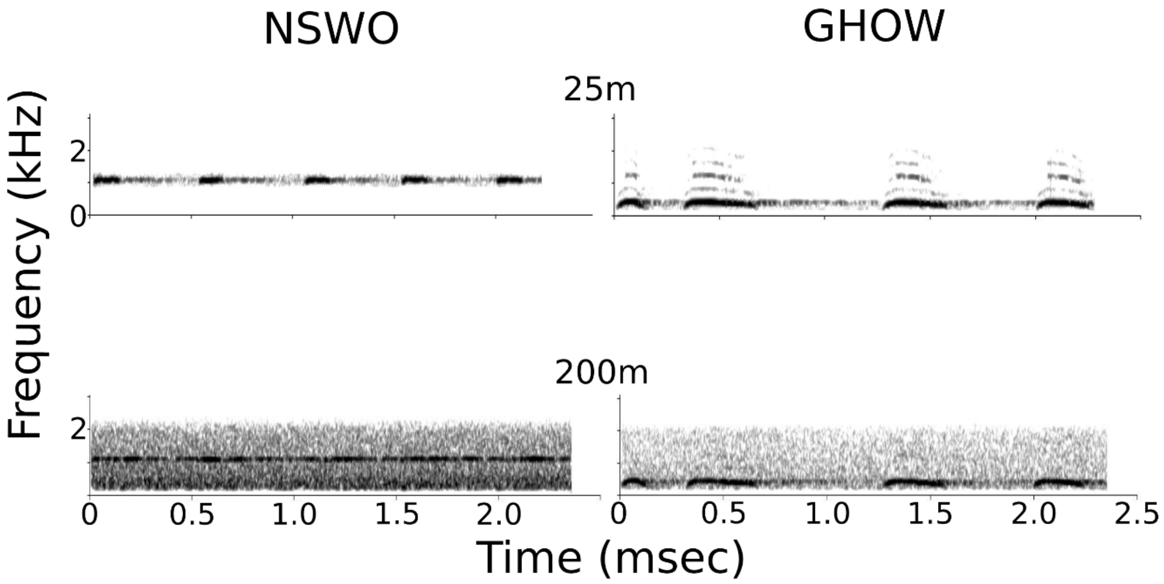

Figure 3-5. Sample sound spectrograms of high-threat northern saw-whet owl (NSWO) and low-threat great horned owl (GHOW) calls across distances (25m and 200m), with time (msec) on the *x*-axis and frequency (kHz) on the *y*-axis, to demonstrate the effect of signal degradation on NSWO stimuli compared to GHOW stimuli.

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Chapter 4. Discrimination of high- and low-threat vocalizations: An examination of referential communication in black-capped chickadee (*Poecile atricapillus*) mobbing calls

Introduction

Referential communication has been defined as the exchange of information giving reference to objects or events in the environment (Seyfarth & Cheney, 1990), is connected to semantics (Marler, Evans, & Hauser, 1992), and is used by a wide range of species. Evans (1997) clarified that in order for signals to be referential there must be production specificity for the signaler and perceptual specificity for the receiver. More generally, animals that have the ability for referential communication must: 1) be able to produce signals, 2) produce these signals under the correct circumstances, and 3) have receivers respond correctly (Smith & Harper, 2003). For example, referential communication is evident in honeybees (*Apis mellifera carnica*) that dance to communicate the location of a food source (i.e., direction and distance, including the language properties of displacement and semanticity; Riley et al., 2005), chickens (*Gallus gallus*) that produce 'food' calls that are distinct from their alarm calls (Evans & Evans, 1999; Evans et al., 1993), and arctic ground squirrels (*Citellus undulatus*) that produce acoustically-distinct *whistle* and *chatter* alarm calls in response to aerial danger and ground danger (Melchior, 1971).

Threat of predation is a major evolutionary force due to its impact on survival and fitness, and consequently, adaptive antipredator behaviours (such as informative signals) should be selected for over time (Lima & Dill, 1990). Vocal antipredator responses are a well-studied class of antipredator behaviours (Smith & Harper, 2003). In addition to signalling the presence of a predator, vocal antipredator responses can provide additional information about the predator type. Vervet monkeys (*Chlorocebus pygerythrus*) produce different alarm calls that signal either the presence of a leopard, eagle, or snake, and each of these calls results in an adaptive antipredator, species-specific behavioural response from nearby receiver vervet monkeys

(Seyfarth et al., 1980). In addition to predator type, vocal antipredator responses can also communicate the threat level posed by a predator (Note: Additional work is needed that includes more replicates in rank classes). Both Carolina chickadees (*Poecile carolinensis*) and black-capped chickadees (*P. atricapillus*) produce *chick-a-dee* calls containing four note types that can be included, omitted, or repeated (A→B→C→D; Bloomfield et al., 2005; Charrier et al., 2004). Both species produce the *chick-a-dee* call as a mobbing call in the presence of a predator, used to recruit and coordinate conspecifics and heterospecifics to attack (Smith, 1991). Typically, these mobbing calls are produced with a greater number of D notes per call in response to a small, high-threat predator relative to a large, low-threat predator (Templeton et al., 2005; Soard & Ritchison, 2009). This threat-specific modification in note composition allows for the communication of threat to other individuals and consequently adaptive responses from conspecifics and heterospecifics in the area (Templeton et al., 2005; Soard & Ritchison, 2009).

Furthermore, upon investigating neural expression in the auditory system of black-capped chickadees, Avey et al. (2011) found that more immediate early gene (IEG) expression occurs in response to high-threat predator and mobbing calls compared to low-threat predator and mobbing calls. This similarity in IEG expression within threat level occurred despite acoustic differences between the calls. Based on these findings of concordance between IEG expression across stimuli of the same threat level, we hypothesized that a similar pattern in perception of such calls would occur in a behavioural task. Specifically, if the neural response to predator and mobbing calls of the same threat level are similar, is it possible that chickadee-produced mobbing calls provide referential information about which predator is nearby?

Referential communication has most commonly been studied in humans and non-human primates (Seyfarth & Cheney, 1993); however, little evidence has been provided to support these abilities in songbirds. Thus, here we asked whether black-capped chickadees perceive predator calls and matched mobbing calls as similar. We designed a controlled laboratory experiment to train and test birds in an operant go/no-go discrimination task. Predator and mobbing calls have both been used in operant go/no-go discrimination tasks (Congdon et al., unpublished a; Congdon et al., unpublished b; Congdon et al., 2019), but responding to both predator and mobbing calls have never been compared. We trained one group of chickadees to respond (‘go’) to high-threat predator calls and withhold responding (‘no-go’) to low-threat predator calls (Discrimination Training I) and then trained those subjects with conspecific mobbing calls (i.e., high- or low-threat; Discrimination Training II; Owl S+ subgroups). Another group of chickadees were first trained with conspecific mobbing calls then trained with predator calls (MOB S+ subgroups). These were the True Transfer category subgroups in which there were true categories (i.e., high- vs. low-threat categories compared to pseudorandomized stimuli) to learn and the rewarded contingency was consistent between training rounds. Considering that the contingencies (e.g., high threat) were the same between the first and second round of training (e.g., mobbing calls then predator calls), if mobbing calls contain information about predators, transfer of training would be possible between the two types of stimuli and the subsequent round of training should be completed in fewer trials.

For a control, we included a group in which birds were rewarded for pseudorandomized stimuli with no category of threat level (Pseudo category subgroups). In addition, we included a reversal group in which the threat-contingencies were reversed between Discrimination Training I and Discrimination Training II; e.g., first trained with high-threat owl calls, then trained with low-threat conspecific mobbing calls; True Reversal category subgroups). This final group provided another type of control in an attempt to ensure that birds in the True Transfer category group were completing the task based on consistent contingencies between rounds of training. Specifically, the True Reversal category group should take a similar number of trials during to complete both rounds of training as the contingencies would differ and transfer of training would not be possible.

First, we predicted that True Transfer category groups and True Reversal category groups would complete Discrimination Training I in fewer trials compared to Pseudo category subgroups due to categories of perceptually similar stimuli being learned in fewer trials than groups of pseudorandomized stimuli. We also predicted that True Transfer category groups and True Reversal category groups would complete Discrimination Training II in fewer trials compared to Pseudo category groups. Third, we predicted that within True Transfer, True Reversal, and Pseudo category groups, the subgroups would not differ between the first and second round of training as each of the subgroups have similar transfer, reversal, or lack of contingencies. Finally, we predicted that birds that received the same threat-contingencies in Discrimination Training I and Discrimination Training II would show transfer of training. For example, birds first trained to respond to high-threat predator calls were predicted to demonstrate transfer of training in that they would then discriminate high-threat mobbing calls (i.e., different stimulus type, but same contingency of high-threat) in fewer trials. If transfer of training was demonstrated by all True Transfer category subgroups completing Discrimination Training II in fewer trials than Discrimination Training I, it would suggest that chickadees perceive classes of mobbing calls and predator calls as similar. In summary, these results would suggest that mobbing calls provide referential information about specific predators, and thus provide evidence of referential communication in a songbird.

Methods

Subjects

Thirty-seven black-capped chickadees (19 male, 18 female, identified by DNA analysis; Griffiths et al., 1998) were tested between June and September 2018; in total, 33 black-capped chickadees (15 males, 18 females) completed the experiment (see Ethical Note). Chickadees at least one year of age (determined by examining the colour and shape of their outer tail retrices; Pyle, 1997) 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 between January 9-26, 2018.

Prior to the experiment, birds were individually housed in Jupiter Parakeet cages (30 × 40 × 40 cm; Rolf C. Hagen, Inc., Montreal, QB, Canada) in colony rooms that were maintained on a light:dark cycle that mimicked the natural light cycle for Edmonton, Alberta, Canada. In the colony rooms, birds had visual and auditory, but not physical, contact with one another. Birds had *ad libitum* access to food (Mazuri Small Bird Maintenance Diet; Mazuri, St Louis, MO, USA), water (vitamin supplemented on alternating days; Prime vitamin supplement; Hagen, Inc.), grit, and cuttlebone. Birds were given three to five sunflower seeds daily, one superworm (*Zophobas morio*) three times a week, and a mixture of greens (spinach or parsley) and eggs twice a week.

Throughout the experiment, birds were housed individually in operant chambers (see apparatus below), maintained on the natural light cycle for Edmonton, Alberta, and had *ad libitum* access to water (vitamin supplemented on alternate days), grit, and cuttlebone. Birds were given two superworms daily (one in the morning and one in the afternoon). Food (i.e., Mazuri) was only available as a reward for correctly responding during the operant discrimination task. Only three birds had previous experimental experience hearing black-capped chickadee-produced *fee-bee* songs during a playback experiment in March 2018 (Montenegro et al., unpublished), but were naïve to operant experiments and the stimuli used in the current experiment.

Apparatus

During the experiment, birds were housed individually in modified colony room cages (30 × 40 × 40 cm) placed inside a ventilated, sound-attenuating chamber. The chambers were illuminated by a 9-W, full spectrum fluorescent bulb. Each cage contained three perches, a water bottle, and a grit cup. An opening on the side of the cage (11 × 16 cm) provided each bird access to a motor-driven feeder (see Njegovan et al., 1994). Infrared cells in the feeder and the request perch (perch closest to the feeder) monitored the position of the bird (i.e., perching and feeder entry). A personal computer connected to a single-board computer (Palya & Walter, 2001) scheduled trials and recorded responses to stimuli. Stimuli were played from the personal computer hard drive, through either a Cambridge A300 Integrated Amplifier, Cambridge Azur 640A Integrated Amplifier (Cambridge Audio, London, England), or an NAD310 Integrated Amplifier (NAD Electronics, London, England) and through a Fostex FE108 Σ or Fostex FE108E Σ full-range speaker (Fostex Corp., Japan; frequency response range 80-18,000 Hz) located beside the feeder. See Sturdy and Weisman (2006) for a detailed description of the apparatus.

Acoustic Stimuli

A total of 68 vocalizations was used as stimuli in the current experiment: 17 predator calls produced by high-threat northern saw-whet owls (NSWO; *Aegolius acadicus*), 17 predator calls produced by low-threat great horned owls (GHOW; *Bubo virginianus*), 17 mobbing calls produced by black-capped chickadees made in response to mounts of NSWO, and 17 mobbing calls produced by black-capped chickadees made in response to mounts of GHOW. According to Templeton et al. (2005), NSWO and GHOW are on opposite ends of the spectrum in regards to threat level, and henceforth we refer to NSWO as ‘high-threat’ and GHOW as ‘low-threat’ for the procedures of our study.

All owl calls were obtained through the Borror Laboratory of Bioacoustics (The Ohio State University) and field recordings contributed by the Bayne Laboratory (Department of Biological Sciences, University of Alberta). All black-capped chickadee-produced mobbing calls were recorded in the laboratory by Avey et al. (2011) and used in Congdon et al. (2019). The average +/- standard deviation for the duration of NSWO call stimuli was 2037.6 +/- 423.8 ms (range = 1558.2-2440.2 ms); the duration of GHOW call stimuli was 2095.6 +/- 325.8 ms (range = 708.6-2618.2 ms); the duration of mobbing call stimuli produced by black-capped chickadees made in response to mounts of NSWO was 1536.1 +/- 351.7 ms (range = 1050.9-2389.6 ms); and the duration of mobbing call stimuli produced by black-capped chickadees made in response to mounts of GHOW 765.3 +/- 200.7 ms (range = 516.5-1293.1 ms). There was an average of 4.8 D notes per call for stimuli in response to NSWO and 2.2 D notes per call for stimuli in response to GHOW. There is a significant difference in the duration of mobbing calls produced to NSWO versus GHOW ($t_{16} = -7.584$, $p < .001$, $d = 2.691$, 95% CIs = -986.283, -555.340).

All vocalizations were of high quality (i.e., no audible interference and low background noise when viewed on a spectrogram with amplitude cutoffs of -35 to 0 dB relative to vocalization peak amplitude) and were bandpass filtered (outside the frequency range of each vocalization type) using GoldWave version 5.58 (GoldWave, Inc., St. John’s, NL, Canada) to reduce any background noise. For each stimulus, 5 ms of silence was added to the leading and trailing portion of the vocalization and tapered to remove transients, and amplitude was equalized using SIGNAL 5.10.24 software (Engineering Design, Berkeley, CA, USA).

During the experiment, stimuli were presented at approximately 75 dB as measured by a Brüel & Kjær Type 2239 (Brüel & Kjær Sound & Vibration Measurement A/S, Nærum, Denmark) decibel meter (A-weighting, slow response) at the approximate height and position of a bird's head while on the request perch.

Procedure

Pretraining. Pretraining began once the bird learned to use the request perch and feeder to obtain food. During Pretraining, birds received food for responding to all stimuli (future S+, S-, and transfer stimuli). A trial began when the bird landed on the request perch and remained for between 900-1100 ms. A randomly-selected stimulus played without replacement until all 68 stimuli had been heard. If the bird left the request perch before a stimulus finished playing, the trial was considered interrupted, resulting in a 30-s time out with the houselight turned off. If the bird entered the feeder within 1 s after the entire stimulus played, it was given 1 s access to food, followed by a 30-s intertrial interval, during which the houselight remained on. If a bird remained on the request perch during the stimulus presentation and 1 s following the completion of the stimulus it received a 60-s intertrial interval with the houselight on, but this intertrial interval was terminated if the bird left the request perch. This was to encourage a high level of responding on all trials. Birds continued on Pretraining until they completed six 340-trial bins with ≥ 60% responding on average to all stimuli, at least four 340-trial bins with ≤ 3% difference in responding to future S+ and S- stimuli, at least four 340-trial bins in which the bird had ≤ 3% difference in responding to future high- and low-threat transfer stimuli, and at least four 340-trial bins in which the bird had ≤ 3% difference in responding to short and long stimuli to ensure that birds did not display biases. Once birds met the above criteria, they were given a day with unlimited access to food without auditory stimuli, then birds completed a second round in which they completed one 340-trial block with ≥ 60% responding on average to all stimuli, completed one 340-trial block of ≤ 3% difference in responding to future S+ and S- stimuli, completed one 340-trial block of ≤ 3% difference in responding to future high- and low-threat transfer stimuli, and completed one 340-trial block of ≤ 3% difference in responding to short and long stimuli, to confirm that each bird continued to not display biases following the break (the criterion has been used by similar operant go/no-go experiments; e.g., Hahn et al., 2015; Hahn et al., 2016; McMillan et al., 2017).

Discrimination Training I. The procedure was the same as during Pretraining; however, of the original 68 stimuli only the 34 training stimuli were presented (with the remaining 34 withheld for use during Transfer testing), and responding to half of these stimuli were punished with a 30-s intertrial interval with the houselight off. As during Pretraining, responses to rewarded (S+) stimuli resulted in 1 s access to food and responses to unrewarded (S-) stimuli resulted in a 30-s time out with the houselight turned off. Discrimination Training I continued until birds completed six 340-trial bins with a discrimination ratio (DR) ≥ 0.80 with the last two bins being consecutive. For DR calculations see Response Measures, below.

Birds were randomly assigned to either a True Transfer category discrimination group (*n* = 15), Pseudo category discrimination group (*n* = 6), or True Reversal category discrimination group (*n* = 12). Black-capped chickadees in the True Transfer category discrimination group were divided into four subgroups: 1) one subgroup discriminated 17 rewarded (S+) high-threat owl calls from 17 unrewarded (S-) low-threat owl calls (High Owl S+ Group: two male and two female subjects), 2) while the other subgroup discriminated 17 rewarded (S+) low-threat owl calls from 17 unrewarded (S-) high-threat owl calls (Low Owl S+ Group: two male and two female subjects); 3) another subgroup discriminated 17 rewarded (S+) high-threat mobbing calls from 17 unrewarded (S-) low-threat mobbing calls (High MOB S+ Group: one male and two female subjects), 4) while the other subgroup discriminated 17 rewarded (S+) low-threat mobbing calls from 17 unrewarded (S-) high-threat mobbing calls (Low MOB S+ Group: two male and two female subjects); see Figure 1.

The Pseudo category discrimination group was also divided into four subgroups. Two of the subgroups discriminated owl stimuli: eight randomly-selected rewarded (S+) high-threat owl and nine randomly-selected rewarded (S+) low-threat owl calls from nine unrewarded (S-) high-threat owl and eight unrewarded (S-) low-threat owl calls (Total of 34 stimuli; Pseudo 1 Owl Group: one female subject; Pseudo 2 Owl Group: one male and one female subject). The other two subgroups discriminated mobbing stimuli: eight randomly-selected rewarded (S+) high-threat mobbing and nine randomly-selected rewarded (S+) low-threat mobbing calls from nine unrewarded (S-) high-threat mobbing and eight unrewarded (S-) low-threat mobbing calls (Total of 34 stimuli; Pseudo 1 MOB Group: one female subject; Pseudo 2 MOB Group: one male and one female subject); see Figure 2. The purpose of the Pseudo category group was to include a control in which subjects were not trained to categorize according to threat level.

Black-capped chickadees in the True Reversal category discrimination group were divided into four subgroups (REV High Owl S+: one male and two female subjects, REV Low Owl S+: two males and one female subject, REV High MOB S+: two males and one female subject, REV Low MOB S+: one male and two female subjects) in which Discrimination Training I was the same as the True Transfer subgroups, but Discrimination Training II differed (see Discrimination Training II for this differentiation); see Figure 3.

Discrimination Training II. The procedure was the same as during Discrimination Training I; however, the 34 stimuli from Pretraining that were withheld from Discrimination Training I were presented. As during Pretraining and Discrimination Training I, responses to rewarded (S+) stimuli resulted in 1 s access to food and responses to unrewarded (S-) stimuli resulted in a 30-s time out with the houselight turned off.

True Transfer Owl S+ (High Owl S+, Low Owl S+) and Pseudo Owl S+ groups (Pseudo 1 Owl, Pseudo 2 Owl) were presented with mobbing stimuli during Discrimination Training II, whereas True Transfer MOB S+ (High MOB S+, Low MOB S+) and Pseudo MOB S+ groups (Pseudo 1 MOB, Pseudo 2 MOB groups) were presented with owl stimuli during Discrimination Training II (i.e., the opposite type of stimuli as presented with during Discrimination I). For example, High Owl S+ Group birds that were rewarded for responding to high-threat NSWO stimuli in Discrimination Training I were then rewarded for responding to high-threat mobbing stimuli in Discrimination Training II (i.e., the same contingency of ‘high-threat’; see Figure 1).

True Reversal groups also received the opposite type of stimulus during Discrimination Training II (owl stimuli during Discrimination Training I then mobbing stimuli during Discrimination Training II, or vice versa), but received the opposite contingencies during Discrimination Training II (high-threat stimuli were rewarded during Discrimination Training I then low-threat stimuli were rewarded during Discrimination Training II, or vice versa). For example, REV High Owl S+ Group birds that were rewarded for responding to high-threat NSWO stimuli in Discrimination Training I were then rewarded for responding to low-threat mobbing stimuli in Discrimination Training II; see Figure 3. The purpose of the True Reversal group was to determine if birds in this group would take longer in Discrimination Training II to complete a reversal of training compared to the True Transfer groups transferring training. This was expected as learning a reversal of contingencies (e.g., rewarded for responding to high-threat owl stimuli then low-threat mobbing stimuli) should take more trials than learning a transfer of contingencies (e.g., rewarded for responding to high-threat owl stimuli then high-threat mobbing stimuli; referential information of high-threat/NSWO). Discrimination Training II continued until birds completed six 340-trial bins with a discrimination ratio (DR) ≥ 0.80 with the last two bins being consecutive. For DR calculations see Response Measures, below.

Response Measures. For each stimulus exemplar, a percent response was calculated by the following formula: R+/(N-I), where R+ is the number of trials in which the bird went to the feeder, N is the total number of trials, and I is the number of interrupted trials in which the bird left the perch before the entire stimulus played. For Discrimination Training I, we calculated a discrimination ratio (DR), by dividing the mean percent response to all S+ stimuli by the mean percent response of S+ stimuli plus the mean percent response of S- stimuli. A DR of 0.50 indicates equal responding to rewarded (S+) and unrewarded (S-) stimuli, whereas a DR of 1.00 indicates perfect discrimination.

To determine whether groups differed in speeds of acquisition during Discrimination Training II compared to Discrimination Training I, the number of trial bins to criterion during Discrimination Training I was subtracted from the number of trial bins to criterion during Discrimination Training II (DIS2-DIS1).

Statistical Analyses. We conducted multiple one-way analyses of variance (ANOVAs) on the number of bins to discrimination criterion to compare between the subgroups. We conducted a separate ANOVA for the True Transfer, Pseudo, and True Reversal category groups. We also conducted between-groups independent-samples *t*-tests on the number of bins to discrimination criterion to compare True Transfer v. Pseudo, True Reversal v. Pseudo, and True Transfer v. True Reversal.

We conducted one-way ANOVAs on the number of bins to Discrimination Training II criterion between the subgroups of the True Transfer, Pseudo, and True Reversal category groups. We also conducted between-groups independent samples *t*-tests on the number of bins to Discrimination Training II criterion to compare True Transfer vs. Pseudo, True Reversal vs. Pseudo, and True Transfer vs. True Reversal.

We conducted one-way ANOVAs and independent samples *t*-tests, with Bonferroni corrections ($p = .008$), on the difference between the number of bins in Discrimination Training II and Discrimination Training I (Discrimination Training II-Discrimination Training) to reach criterion for the True Transfer, Pseudo, and True Reversal category groups.

We conducted independent samples *t*-tests on the difference between the number of bins in Discrimination Training II and Discrimination Training I (Discrimination Training II-Discrimination Training I = DIS2-DIS1) to reach criterion for the True Transfer, Pseudo, and True Reversal category groups according to MOB S+ compared to Owl S+.

Ethical Note. Throughout the experiment, birds remained in the testing apparatus to minimize the transport and handling of each bird. Three male subjects died from natural causes during operant training. One male subject became ill during operant training and was consequently humanely euthanized. At the completion of the experiment, birds were returned to the colony room for use in future experiments. All procedures were conducted in accordance with the Canadian Council on Animal Care (CCAC) Guidelines and Policies with approval from the Animal Care and Use Committee for Biosciences for the University of Alberta (AUP 1937), which is consistent with the Animal Care Committee Guidelines for the Use of Animals in

Research. Birds were captured and research was conducted under an Environment Canada Canadian Wildlife Service Scientific permit (#13-AB-SC004), Alberta Fish and Wildlife Capture and Research permits (#56066 and #56065), and City of Edmonton Parks permit.

Results

Bins to Criterion: Between subgroups

To determine whether chickadees in the four True Transfer category subgroups (High Owl S+, Low Owl S+, High MOB S+, Low MOB S+) differed in their speed of acquisition during Discrimination Training I, we conducted a one-way ANOVA on the number of 340-trial bins to reach criterion. There were no significant differences, $F_{3,11} = 1.898$, $p = .188$, $\eta^2 = 0.341$, 95% CIs = 9.994, 12.406.

To determine whether birds in the four Pseudo category subgroups (Pseudo 1 & 2 Owl, Pseudo 1 & 2 MOB) differed in their speed of acquisition during Discrimination Training I, we conducted a one-way ANOVA on the number of 340-trial bins to reach criterion. There were no significant differences, $F_{3,2} = 0.583$, $p = .681$, $\eta^2 = 0.466$, 95% CIs = 16.688, 49.312.

To determine whether birds in the four True Reversal category subgroups (REV High Owl S+, REV Low Owl S+, REV High MOB S+, REV Low MOB S+) differed in their speed of acquisition during Discrimination Training I, we conducted a one-way ANOVA on the number of 340-trial bins to reach criterion. There were no significant differences, $F_{3,8} = 2.278$, $p = .157$, $\eta^2 = 0.461$, 95% CIs = 9.809, 14.357. See Table 4-1 for the average number of bins to criterion ± SEM for each subgroup.

Bins to Criterion: Between groups

To compare the acquisition performance during Discrimination Training I of the True Transfer and Pseudo category groups and to determine if the True Transfer group learned to discriminate in fewer bins than the Pseudo category group, we conducted an independent-samples *t*-test on the number of 340-trial bins to reach criterion for the True Transfer category and Pseudo category groups. There was a significant difference between the groups ($t_{5.079}$ = -3.422, *p* = .018, *d* = -3.037, 95% CIs = -38.010, -5.500) in that chickadees in the True Transfer group learned to discriminate significantly faster than chickadees in the Pseudo category group.

To compare the acquisition performance during Discrimination Training I of the True Reversal and Pseudo category groups and to determine if the True Reversal group learned to categorize in fewer bins than the Pseudo category group, we conducted an independent-samples *t*-test on the number of 340-trial bins to reach criterion for the True Reversal category and Pseudo category groups. There was a significant difference between the groups ($t_{5.267}$ = -3.253, *p* = .021, *d* = -2.835, 95% CIs = -37.649, -4.639) in that chickadees in the True Reversal group learned to discriminate significantly faster than chickadees in the Pseudo category group.

Last, to compare the acquisition performance during Discrimination Training of the True Transfer and True Reversal category groups, we conducted independent-samples *t*-test on the number of 340-trial bins to reach criterion for the True Transfer category and True Reversal category groups. There was no significant difference between the groups (t_{25} = -0.792, *p* = .436, *d* = -0.319, 95% CIs = -30.080, -13.520). See Table 1.

Discrimination Training II: Between subgroups

To determine whether birds in the four True Transfer category subgroups differed in their speed of acquisition during Discrimination Training II, we conducted a one-way ANOVA on the number of 340-trial bins to reach criterion. There were no significant differences, $F_{3,11}$ = 1.834, *p* = .199, η^2 = 0.333, 95% CIs = 9.361, 13.039.

To determine whether birds in the four Pseudo category subgroups differed in their speed of acquisition during Discrimination Training II, we conducted a one-way ANOVA on the number of 340-trial bins to reach criterion. There were no significant differences, $F_{3,2} = 1.294$, $p = .464$, $\eta^2 = 0.660$, 95% CIs = 16.724, 58.610.

To determine whether birds in the four True Reversal category subgroups differed in their speed of acquisition during Discrimination Training II, we conducted a one-way ANOVA on the number of 340-trial bins to reach criterion for the True Reversal category conditions. There were no significant differences, $F_{3,8} = 3.510$, $p = .069$, $\eta^2 = 0.568$, 95% CIs = 9.537, 13.463.

Discrimination Training II: Between groups

To compare the acquisition performance during Discrimination Training II of the True Transfer and Pseudo category groups and to determine if the True Transfer group learned to transfer training in fewer bins than the Pseudo category group discriminated, we conducted an independent-samples *t*-test on the number of 340-trial bins to reach criterion for the True Transfer category and Pseudo category groups. There was a significant difference between the groups ($t_{5.111} = -3.231$, $p = .022$, $d = -2.858$, 95% CIs = -47.388, -5.544) in that True Transfer birds did learn to transfer discrimination significantly faster than Pseudo birds.

To compare the acquisition performance during Discrimination Training II of the True Reversal and Pseudo category groups and to determine if the True Reversal group learned to reverse discrimination in fewer bins than the Pseudo category group discriminated, we conducted an independent-samples *t*-test on the number of 340-trial bins to reach criterion for the True Reversal category and Pseudo category groups. There was a significant difference between the groups ($t_{5.120} = -3.193$, $p = .023$, $d = -2.822$, 95% CIs = -47.087, -5.246) in that True Reversal birds did learn to discriminate faster than Pseudo birds.

Last, to compare the acquisition performance during Discrimination Training II of the True Transfer and True Reversal category groups and to determine if the True Transfer group learned to transfer training in fewer bins than the True Reversal group learned the reverse contingencies, we conducted an independent-samples *t*-test on the number of 340-trial bins to reach criterion for the True Transfer category and True Reversal category groups. There was no significant difference between the groups ($t_{25} = -0.240$, $p = .812$, $d = -0.096$, 95% CIs = -2.869, 2.269) in that True Transfer birds did not learn to transfer discrimination significantly faster than True Reversal birds learned to reverse discrimination. See Table 4-1.

Discrimination Training II vs. Discrimination Training I

To determine whether birds in the four True Transfer category groups differed in their speed of acquisition between Discrimination Training I and Discrimination Training II, we conducted a one-way ANOVA on the difference between the number of 340-trial bins in Discrimination Training II and Discrimination Training to reach criterion (Discrimination Training II-Discrimination Training I; DIS2-DIS1) for the True Transfer category. There was a significant difference, $F_{3,11} = 4.187$, $p = .033$, $\eta^2 = 0.533$, 95% CIs = -1.964, 1.964. We conducted independent sample *t*-tests and applied Bonferroni corrections ($p = .05/6$ tests = .008). There were no significant differences between subgroups (*p*s $\geq$ .032); see Table 4-1 and Figure 4-4.

To determine whether birds in the four Pseudo category subgroups differed in their speed of acquisition, we conducted a one-way ANOVA on the difference between the number of 340-trial bins in Discrimination Training II and Discrimination Training I to reach criterion for the Pseudo category conditions. There was no significant difference, $F_{3,2} = 2.694$, $p = .282$, $\eta^2 = 0.802$, 95% CIs = -22.454, 31.787; see Table 4-1 and Figure 4-5.

To determine whether birds in the four True Reversal category subgroups differed in their speed of acquisition, we conducted a one-way ANOVA on the difference between the number of 340-trial bins in Discrimination Training II and Discrimination Training I to reach criterion for the True Reversal category conditions. There was a significant difference, $F_{3,8} = 19.703$, $p < .001$, $\eta^2 = 0.881$, 95% CIs = -3.339, 2.172. In order to further examine the significant ANOVA, we conducted independent samples *t*-tests and applied Bonferroni corrections ($p = .05/6$ tests = .008). REV Low Owl S+ took significantly more bins to reverse their discrimination learning compared to all three of the other subgroups: REV Low MOB S+ ($t_4 = 7.348$, $p = .002$, $d = 7.378$, 95% CIs = 3.733, 8.267); REV High Owl S+ ($t_4 = -6.047$, $p = .004$, $d = -6.047$, 95% CIs = -7.782, -2.885); and REV High MOB S+ ($t_4 = -6.025$, $p = .004$, $d = -6.025$, 95% CIs = -16.070, -5.931); all other *p*s ≥ .038. See Table 4-1 and Figure 4-4.

MOB S+ vs. Owl S+

Due to finding significant differences between the True Reversal subgroups that indicated owl stimuli may have been easier to learn to discriminate, we conducted an independent samples *t*-test on the difference between the number of 340-trial bins in Discrimination Training II and Discrimination Training I (DIS2-DIS1) for the True Transfer groups (MOB S+, Owl S+). There was a significant difference between DIS2-DIS1 for MOB S+ subgroups compared to Owl S+ subgroups ($t_{13} = -3.195$, $p = .007$, $d = -1.772$, 95% CIs = -7.633, -1.475). Specifically, both the MOB S+ and Owl S+ subgroups discriminated the owl stimuli in fewer trials than the mobbing stimuli. This result is not in line with our prediction as we predicted that Discrimination Training II would be completed in fewer trials than Discrimination Training I, regardless of whether stimuli were produced by heterospecifics or conspecifics.

To further investigate if this difference occurred between the Pseudo category subgroups, we conducted an independent sample *t*-test on the difference between the number of 340-trial bins in Discrimination Training II and Discrimination Training I for the Pseudo category groups (Pseudo MOB, Pseudo Owl). We found no significant difference between Pseudo MOB subgroups compared to Pseudo Owl subgroups ($t_4 = 1.054$, $p = .351$, $d = 1.054$, 95% CIs = -35.944, 79.944) in that both pseudorandomized owl and mobbing stimuli were discriminated in approximately the same number of trials.

To further confirm this difference between the True Reversal subgroups, we conducted an independent samples *t*-test on the difference between the number of 340-trial bins in Discrimination Training II and Discrimination Training I for the True Reversal groups (REV MOB S+, REV Owl S+). There was a significant difference between REV MOB S+ subgroups compared to REV Owl S+ subgroups ($t_{10} = -3.121$, $p = .011$, $d = -1.974$, 95% CIs = -9.999, -1.668) in that both the REV MOB S+ subgroups and the REV Owl S+ subgroup discriminated the owl stimuli in fewer trials than the mobbing stimuli.

Discussion

In the current study, we tested whether black-capped chickadees perceived two types of predator calls (high-threat NSWO and low-threat GHOW) as similar to conspecific mobbing calls produced in response to each of these predators (i.e., MOB NSWO and MOB GHOW, respectively) in order to investigate the referential information contained within chickadee mobbing calls. During Discrimination Training I, chickadees in the True Transfer and True Reversal groups learned to discriminate between rewarded and unrewarded stimuli significantly faster than chickadees in the Pseudo category group, suggesting that true categories were easier to learn than pseudo categories. This result was expected as there is ample evidence that songbirds learn acoustic categories faster than they memorize similar acoustic stimuli not arranged into categories (Sturdy et al., 2000). During Discrimination Training I, the discrimination task was the same for chickadees in the True Transfer and True Reversal groups and, as expected, there was no significant difference in the number of trials to reach criteria between the two groups. If mobbing calls contain referential information about the threat level posed by predators, chickadees in the True Transfer group would learn to transfer responding in fewer trials compared to chickadees in the True Reversal group during Discrimination Training II. However, during Discrimination Training II, the True Transfer group did not learn to transfer significantly faster than the True Reversal group.

Seyfarth and colleagues (1980) initiated the discussion of functionally referential alarm calls by suggesting that vervet monkeys produce specific alarm calls in response to multiple predators and respond distinctly to playback of each alarm call. Three decades later, Avey et al. (2011) found similar neural expression in chickadee auditory areas in response to hearing conspecific mobbing calls compared to hearing the predator's calls that elicited these mobbing calls, suggesting that these two acoustically-distinct vocalizations are perceived to be similar. The notion that information regarding predator threat could be contained in the varied mobbing calls produced by chickadees would be evidence of referentiality in a songbird species, however, the current study was unable to demonstrate that owl calls and mobbing calls produced in response to the same species of owls were perceptually-similar.

Additional tests compared the difference in bins to criteria between Discrimination Training I and Discrimination Training II, and indicated that there were significant differences in that both REV MOB S+ groups reversed to owl stimuli of opposite reward contingencies in fewer trials than REV Low Owl S+ reversed discrimination to high-threat mobbing stimuli.

Thus, it appears as though categorizing the owl species' calls according to threat level may be easier than categorizing mobbing calls. It is critical to survival to learn about the variations in production of mobbing calls produced by conspecifics as chickadees live in flocks and need to recognize a nearby threat and assist in antipredator mobbing behaviour (Charrier et al., 2004; Templeton et al., 2005); however, chickadees generally took longer to properly discriminate mobbing calls according to threat level. Perhaps, due to the biological relevance of conspecific mobbing calls it would be more appropriate to respond initially with mobbing behaviour rather than feeding; however, the required method of responding was approaching the feeder in the current task, which is consistent with mobbing behaviour. A previous study has shown that chickadees are capable of learning to approach operant feeders in response to high-arousal stimuli, including *chick-a-dee* mobbing calls produced in response to predators (Congdon et al., 2019). Congdon et al. (2019) investigated the perception of arousal by training chickadees to respond to high- or low-arousal stimuli, then testing with novel high- and low-arousal vocalizations, and found strong transfer of training to black-capped chickadee stimuli. Conversely, the design of the current experiment was quite different in that we directly compared the rates of acquisition in discrimination between calls produced by conspecifics and calls produced by two heterospecific species. During conception, this experimental design appeared most appropriate to determine if mobbing calls include referential information about predators by testing transfer of training. Alternatively, it is also possible that because the owl calls were produced by two different species (high-threat NSWO vs. low-threat GHOW), it is perceptually easier to categorize acoustically-distinct vocalizations rather than *chick-a-dee* mobbing calls produced by the same species but which vary in D-note composition and average duration (see Figure 6).

Not all animals, solitary or social, require the ability of referential communication as long as their communication system can allow the individuals to forage, reproduce, and survive. Originally it was thought that North American red squirrels (*Tamiasciurus hudsonicus*) a relatively solitary species, produced functionally referential alarm calls (i.e., *seet* and *bark* vocalizations in response to aerial and ground predators, respectively; Greene & Meagher, 1998), but more recent research has indicated that squirrels produce *seet-bark* vocalizations to all predators, regardless of type (Digweed & Rendall, 2009). In contrast to Templeton et al.'s (2005) conclusions that more D notes per call are produced to high-threat predators compared to low-threat predators, Baker and Becker (2002) and Wilson and Mennill (2011) argued that the rate of calling and duty cycle (i.e., proportion of time filled by vocalizations) of the *chick-a-dee* mobbing calls is the element that indicates urgency tied to the level of posed threat. The mobbing calls produced to NSWO and GHOW (i.e., MOB NSWO and MOB GHOW), used as stimuli in the current study, were individual calls with varying D notes; due to the constraints of the design (i.e., chickadees remaining on the request perch for the entirety of the call), we were only able to use individual calls rather than strings of calls. It is possible that if we were able to train birds to mobbing call stimuli that varied in both note repetition and calling rate, there would have been transfer of contingencies from the True Transfer MOB S+ subgroups in Discrimination Training II.

The purpose of this study was to investigate referential communication in a species of songbird as semantics are primarily studied in human and non-human primates (Seyfarth & Cheney, 1993). It has been suggested that a signal is referential if that vocalization contains variations that inform the receivers about environmental events, such as nearby predators (Evans, 1997). Maynard Smith and Harper (2003) suggested that animals that have the ability for referential communication must: 1) be able to produce signals, 2) produce these signals under the correct circumstances, and 3) have receivers respond correctly. The findings provided by Baker and Becker (2002; call rate variation), Templeton and colleagues (2005; D note variation), and Wilson and Mennill (2011; duty cycle variation) indicate that *chick-a-dee* mobbing calls contain variations that inform the receiver about nearby predators and that receivers respond appropriately. The findings from the current study suggest that chickadee mobbing calls may not be perceived to be signaling about a specific owl species or the resulting threat posed, and thus not parallel with the owl call stimuli in the way that we anticipated (i.e., indicating specific predator or threat level); however, chickadee mobbing calls may still be referential according to the criteria suggested by Maynard Smith and Harper (2003).

Conclusions

To our knowledge, no other studies have examined referential communication using a go/no-go procedure, as in the current experiment. We found that chickadees were able to discriminate the true categories of threat in fewer trials than pseudo categories, and True groups completed discriminations of acoustically-distinct owl stimuli in the fewest number of trials. However, chickadees in the True Transfer group did not learn the second discrimination faster in Discrimination Training II compared to the True Reversal group in the way that we predicted. Thus, we propose that the current task (go/no-go discrimination) may not be suitable to find a result that would support the proposal that mobbing calls are referential. For example, perhaps both True Transfer and True Reversal groups' 'true' categories are easy to discriminate, whether a transfer or reversal of threat contingencies is necessary (e.g., similar to mid-sessional reversal paradigms; i.e., a paradigm that includes switching the reward-contingencies mid-way through a session; Rayburn-Reeves & Cook, 2016). Future studies should further investigate the potential referential elements of chickadees’ mobbing calls by considering stimuli with varying duty cycles and/or alternative experimental designs.

Acknowledgements

All animal studies were conducted in accordance with the Canadian Council on Animal Care Guidelines and Policies and with approval from the Animal Care and Use Committee for Biosciences for the University of Alberta, and the University of Calgary Life and Environmental Sciences Animal Care Committee. Chickadees were captured and research was carried out under an Environment Canada Canadian Wildlife Service Scientific permit, Alberta Fish and Wildlife Capture and Research permits, and City of Edmonton Parks Permit.

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Table 4-1. The average number of bins to criterion ± SEM during Discrimination Training I and Discrimination Training II, and the difference scores, for each subgroup of the True Transfer, Pseudo, and True Reversal groups. The stimuli discriminated for each subgroup in Discrimination Training I and Discrimination Training II is indicated below values in parentheses.

Subgroup Names	Average Bins to Criterion in Discrimination Training I	Average Bins to Criterion in Discrimination Training II	Difference Score in Average Bins to Criterion (DIS2-DIS1)
True Transfer Group (*n* = 15)			
High Owl S+	105 ± 6.5 (High-threat owl)	137.5 ± 20.9 (High-threat mobbing)	32.5
Low Owl S+	97.5 ± 4.8 (Low-threat owl)	107.5 ± 11.1 (Low-threat mobbing)	10
High MOB S+	130 ± 15.3 (High-threat mobbing)	116.7 ± 20.3 (High-threat owl)	-13.3
Low MOB S+	120 ± 13.5 (Low-threat mobbing)	87.5 ± 9.5 (Low-threat owl)	-32.5
True Reversal Group (*n* = 12)			
REV High Owl S+	103.3 ± 14.5 (High-threat owl)	100 ± 10 (Low-threat mobbing)	-3.3
REV Low Owl S+	103.3 ± 8.8 (Low-threat owl)	153.3 ± 12 (High-threat mobbing)	50
REV High MOB S+	160 ± 30.6 (High-threat mobbing)	100 ± 20.8 (Low-threat owl)	-60
REV Low MOB S+	116.7 ± 6.7 (Low-threat mobbing)	106.7 ± 8.8 (High-threat owl)	-10
Pseudo Category Group (*n* = 6)			
Pseudo 1 Owl	220 (High- and low-threat owl)	210 (High- and low-threat mobbing)	-10
Pseudo 2 Owl	425 ± 175 (High- and low-threat owl)	335 ± 12.5 (High- and low-threat mobbing)	-90
Pseudo 1 MOB	430 (High- and low-threat mobbing)	230 (High- and low-threat owl)	-200
Pseudo 2 MOB	240 ± 40 (High- and low-threat mobbing)	575 ± 135 (High- and low-threat owl)	335

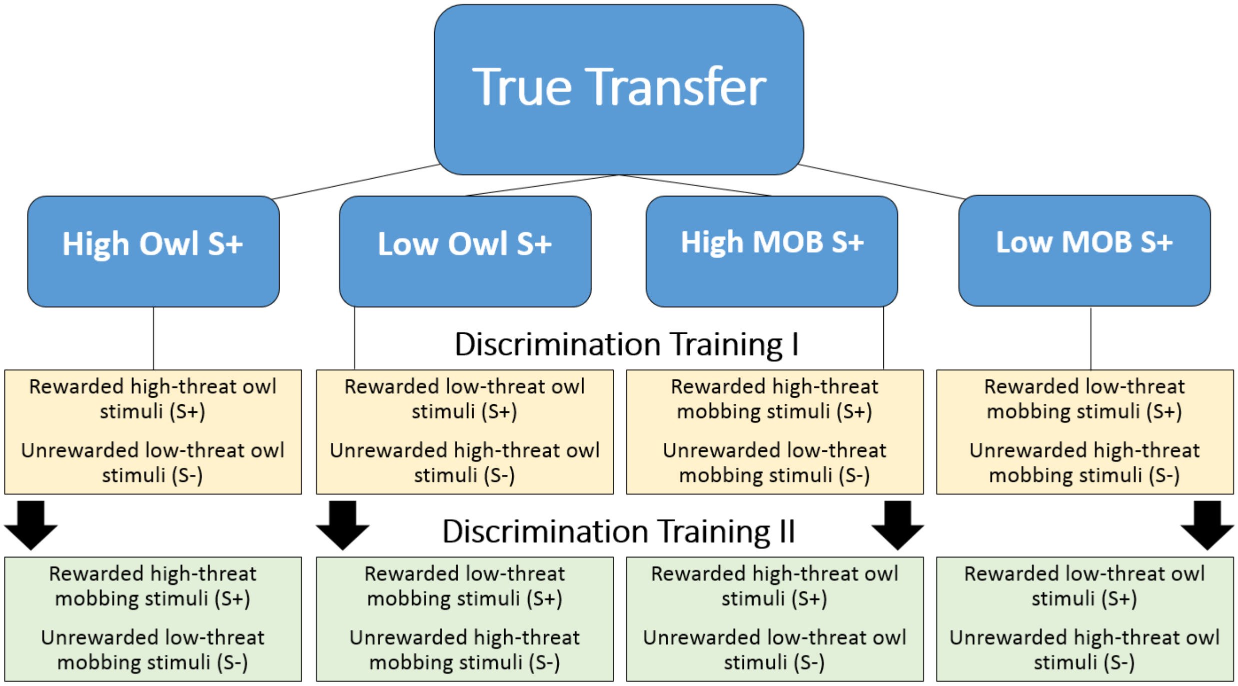

Figure 4-1. Schematic of the stimulus types and reward contingencies for Discrimination Training I and II for the four (4) subgroups of the True Transfer group.

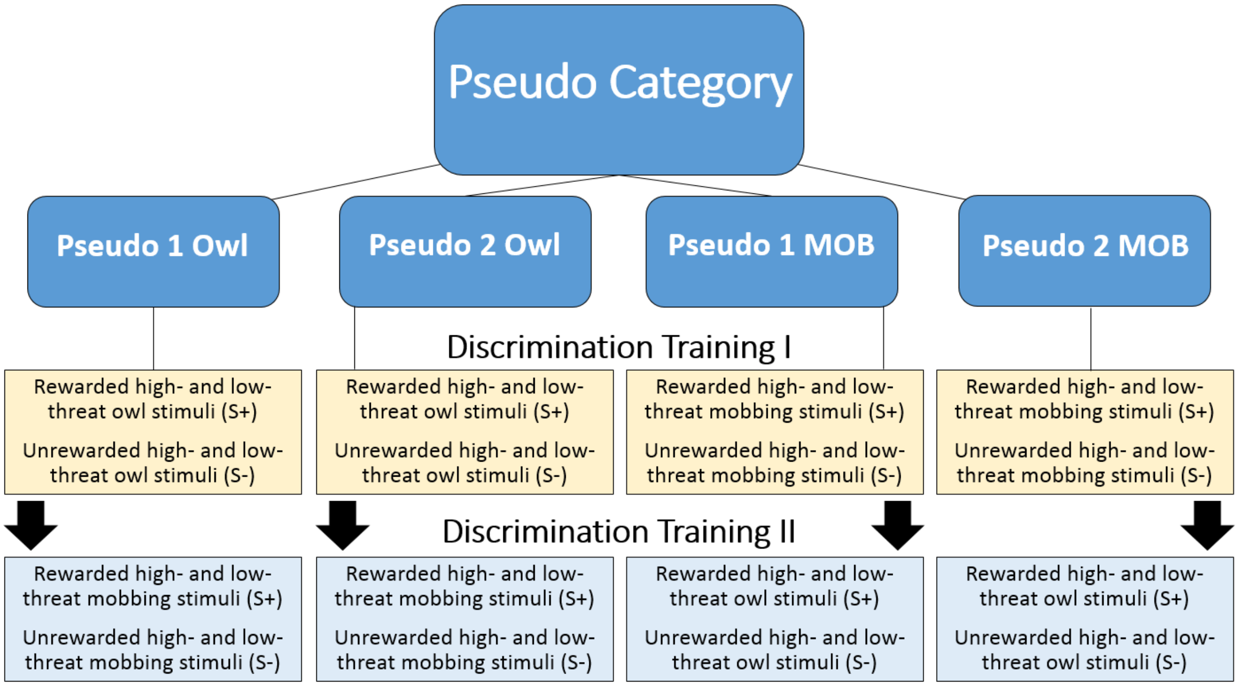

Figure 4-2. Schematic of the stimulus types and reward contingencies for Discrimination Training I and Discrimination Training II for the four (4) subgroups of the Pseudo Category group.

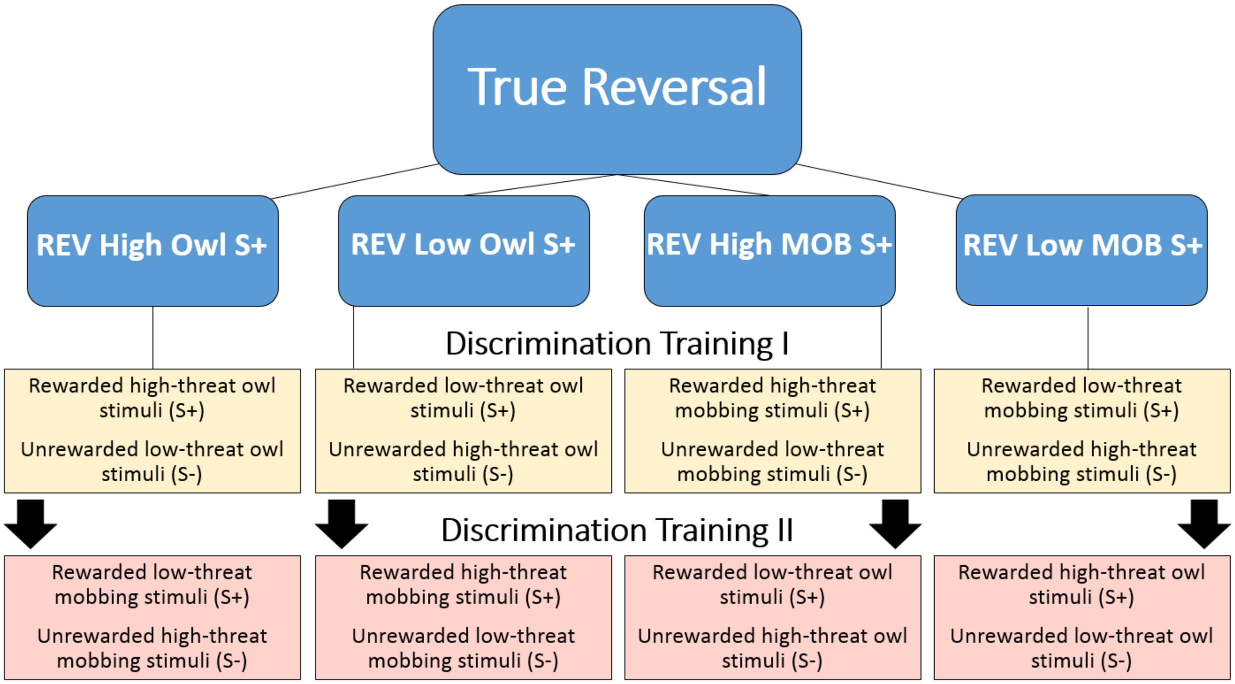

Figure 4-3. Schematic of the stimulus types and reward contingencies for Discrimination Training I and Discrimination Training II for the four (4) subgroups of the True Reversal group.

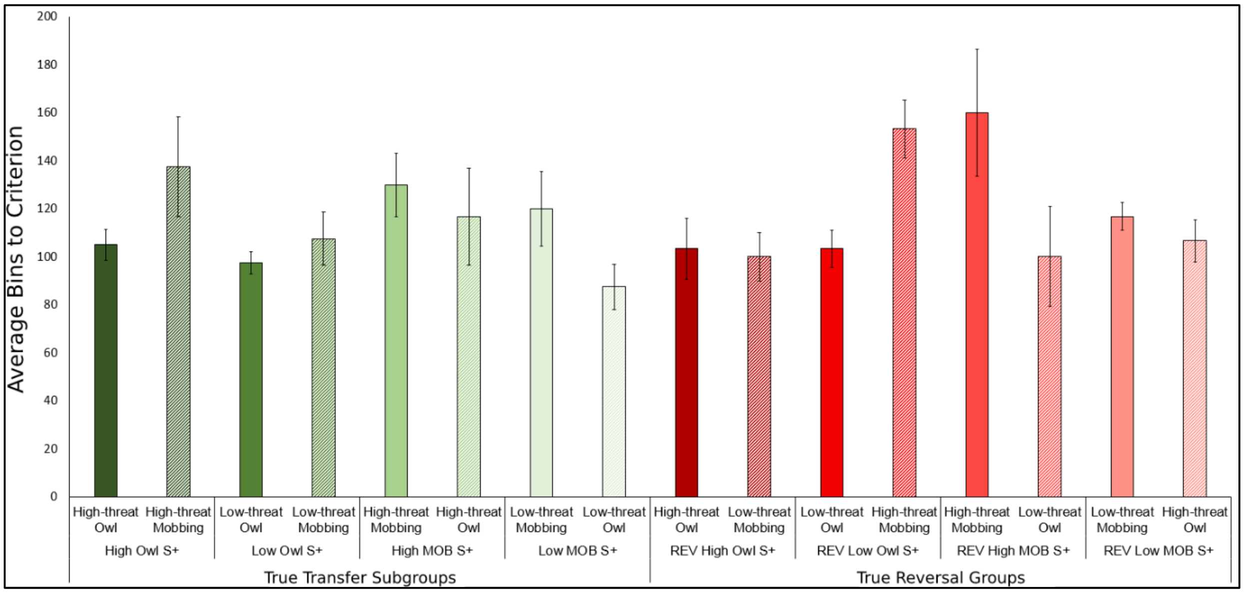

Figure 4-4. The average number of 340-trial bins to criterion ± SEM during Discrimination Training I (solid) and Discrimination Training II (thatched) for each subgroup in True Transfer and True Reversal. The stimuli discriminated for each subgroup in Discrimination Training I and Discrimination Training II is indicated below each bar.

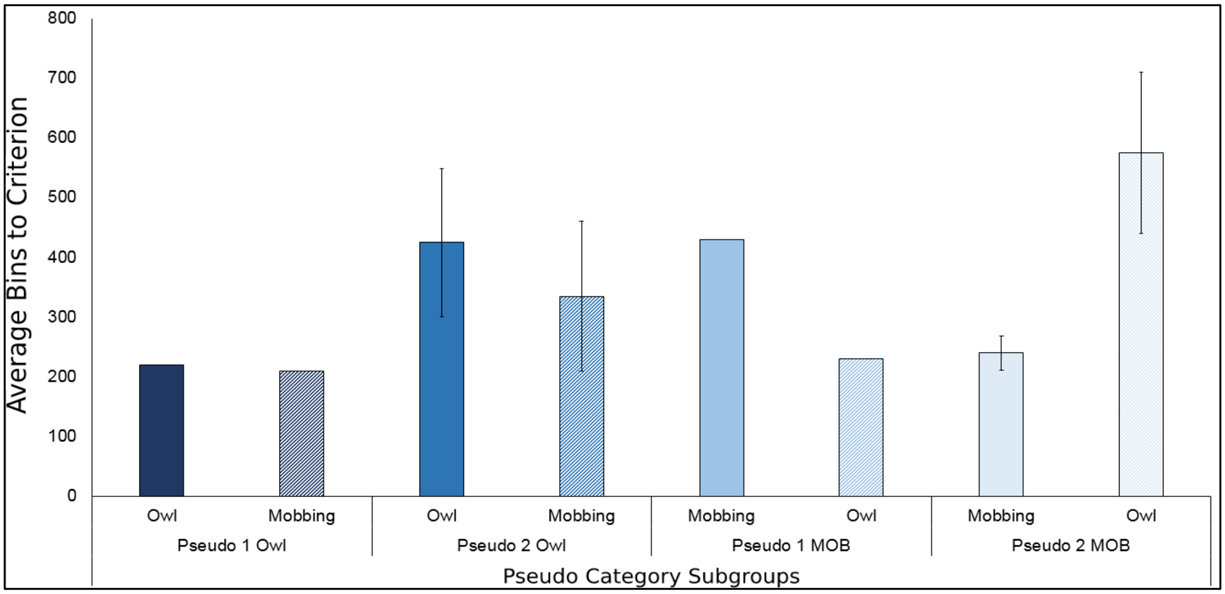

Figure 4-5. The average number of 340-trial bins to criterion ± SEM during Discrimination Training I (solid) and Discrimination Training II (thatched) for each subgroup in the Pseudo Category. The stimuli discriminated for each subgroup in Discrimination Training I and Discrimination Training II is indicated below each bar.

Note: Missing error bars indicate no calculated SEM as *n* = 1 in two of the Pseudo subgroups. *y*-axis is larger than that of Figure 4.

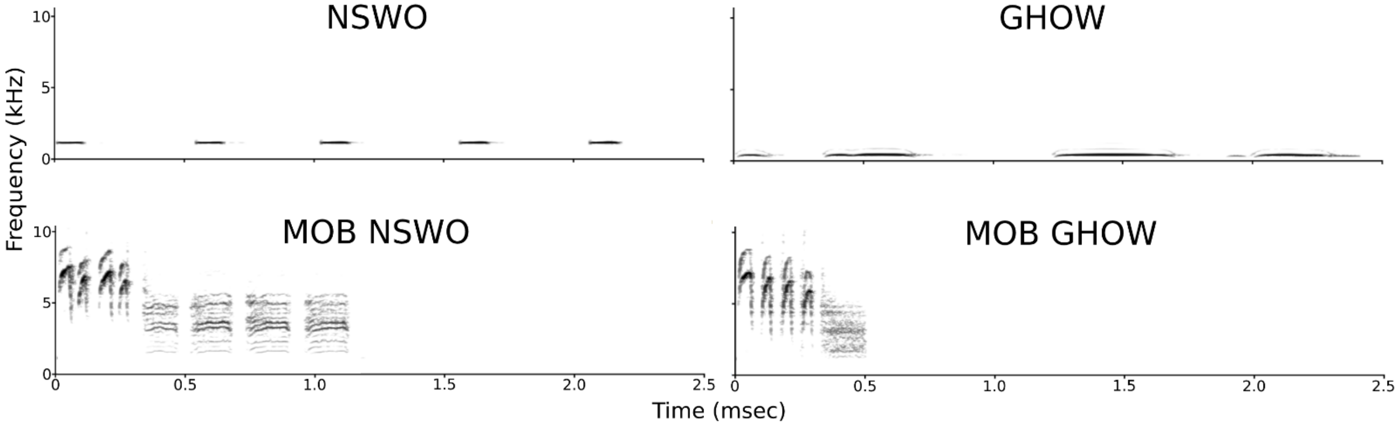

Figure 4-6. Sample sound spectrograms of the vocalizations produced by northern saw-whet owls (NSWO), great horned owls (GHOW), and black-capped chickadees' mobbing in response to both owl predators (MOB NSWO and MOB GHOW), used as experimental stimuli with time (msec) on the *x*-axis and frequency (kHz) on the *y*-axis.

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Chapter 5. Acoustic discrimination of predators by black-capped chickadees (*Poecile atricapillus*)

Introduction

Songbirds face predation by multiple species, including nest predators (e.g., squirrels, snakes), domestic and feral cats (*Felis catus*), and birds of prey (e.g., Bayne & Hobson, 2002; Suzuki, 2012; Blancher, 2013; Smith, 1991). Black-capped chickadees (*Poecile atricapillus*), Carolina chickadees (*P. carolinensis*), and tufted titmice (*Baeolophus bicolor*) are all relatively small songbirds in the same genus (*Paridae*; Smith, 1991), and all three species demonstrate strong antipredator responses to relatively small predators (Templeton, Greene, & Davis, 2005; Soard & Ritchison, 2009; Courter & Ritchison, 2010). This suggests that these three species of parids perceive smaller avian predators to be of higher threat than larger ones. This relationship is thought to be due to greater maneuverability of small avian predators (compared to large predators) and consequently, a better ability to capture a parid in a forested environment (Templeton et al., 2005).

In the presence of a predator, black-capped chickadees produce and use their *chick-a-dee* call as a mobbing signal to recruit and coordinate conspecifics and heterospecifics to attack and harass the predator (Smith, 1991). The *chick-a-dee* call is composed of four note types that occur in a fixed order (A→B→C→D) in which notes can be repeated or omitted (Charrier, Bloomfield, & Sturdy, 2004). Templeton et al. (2005) demonstrated a strong negative correlation between wingspan and body length of predators relative to the number of D notes produced in a *chick-a-dee* mobbing call when birds observed a predator. In other words, the smaller the predator, the more D notes produced per call by chickadees. Plotting the number of D notes in *chick-a-dee* mobbing calls by predator wingspan suggests that northern pygmy-owls (*Glaucidium gnoma*) and northern saw-whet owls (*Aegolius acadicus*), both small-sized predators, are viewed a higher threat compared to large-sized predators, such as great horned owls (*Bubo virginianus*) and great gray owls (*Strix nebulosa*; Templeton et al., 2005). Templeton et al. (2005) also demonstrated longer and more intense mobbing behaviour by chickadees in response to the playback of mobbing calls produced in the presence of a pygmy-owl compared to the playback of mobbing calls produced in the presence of a great horned owl or non-predator control (pine siskin, *Carduelis pinus*). In addition, Baker and Becker (2002) demonstrated that urgency is conveyed through the *chick-a-dee* call as predator mounts at 1m distance resulted in increased calling rate compared to predator mounts at a 6m distance. Tufted titmice also produce *chick-a-dee* mobbing calls that convey information about predator size and threat. When presented with various-sized raptors, titmice engaged in longer bouts of mobbing calls with more D notes given in response to smaller, high-threat predators (e.g., eastern screech-owl, *Megascops asio*) compared to larger, low-threat predators (e.g., red-tailed hawk, *Buteo jamaicensis*; great horned owl; Courter & Ritchison, 2010).

In terms of visual predator perception, prey species also seem to recognize visual features of predators, which is an ability that helps evaluate the threat of a potential predator. For example, when the head and body orientation of predator models faced toward a feeder, tufted titmice demonstrated greater avoidance compared to when predator models faced away (Book & Freeberg, 2015). Kyle and Freeberg (2016) also found Carolina chickadees and tufted titmice respond to head orientation of predators in that both species avoided feeding and produced more *chick-a-dee* calls when the predator head was oriented toward the feeder. These studies demonstrate the importance of predator recognition and how prey species react to differences in predator orientation based on visual cues.

Based on how different species alter their alarm calls in response to visual cues from different predators, many researchers have investigated if species perceive threat from vocalizations produced by conspecifics and heterospecifics in response to predators. For example, red-breasted nuthatches (*Sitta canadensis*) live in mixed flocks with chickadees during winter, and are typically attacked by the same species of predator. Nuthatches are known to eavesdrop on variations in heterospecific chickadee mobbing calls and approach a speaker (i.e., engage in mobbing behaviour) more during playback of black-capped chickadee *chick-a-dee* calls in response to high-threat (small-sized) predators compared to calls given in response to low-threat (large-sized) and non-threat house sparrow calls (*Passer domesticus*; Templeton & Greene, 2006). This ability to eavesdrop provides an advantage as the information gained can be used to determine what is present in the shared surroundings. Mammals also eavesdrop on avian alarm calling, as demonstrated by eastern chipmunks (*Tamias striatus*) responding more to the playback of titmouse mobbing calls in response to perched avian predators (high-threat), than "seet" calls in response to low-flying hawks (low-threat; Schmidt, Lee, Ostfeld, & Sieving, 2008). These results suggest that predator calls also vary in perceived threat level and can influence whether predator or alarm calls are perceived to indicate a great threat.

Avey and colleagues (2011) compared levels of neural expression in chickadees following exposure to predator and conspecific calls of high-threat (northern saw-whet owl calls or mobbing calls in response to northern saw-whet owl model) and low-threat (great horned owl calls or mobbing calls in response to great horned owl model). They found that chickadees produce more immediate early gene (IEG) expression in response to high-threat predator calls and the corresponding mobbing calls compared to low-threat predator calls and corresponding mobbing calls or the controls (heterospecific red-breasted nuthatch or reversed conspecific *chick-a-dee* calls). The results of Avey et al. (2005) suggest that chickadees perceive heterospecific and conspecific vocalizations produced by and regarding the same predator are perceived to be of similar threat levels, despite distinct acoustic differences.

Taken together, these previous findings indicate that parids perceive and attend to the threat posed by nearby visual predators, adjusting their alarm vocalizations accordingly, and vary their response to the vocalizations produced by both conspecifics and heterospecifics. The aims of the current study were to evaluate the perception of predator species in the middle of the body size spectrum that have not been evaluated, using an operant go/no-go discrimination task using predator-produced acoustic stimuli. Black-capped chickadee subjects were trained to respond to high or low threat hawk and owl stimuli. We then 'asked' subjects to 'categorize', via transfer of training to novel stimuli, how they perceived specific predator calls in an attempt to determine if threat perception is along a continuum rather than categorical high vs. low threat. This task is novel in its approach to studying songbird perception of predators in that it allows for the ability to investigate the threat level posed by predators according to chickadee perception. If threat perception is along a continuum as anticipated, certain testing species (e.g., particular mid-sized predators) will not be strongly responded to by either the high- or low-threat rewarded groups.

Methods

Subjects

Twenty-six black-capped chickadees (11 males and 15 females, identified by DNA analysis; Griffiths, Double, Orr, & Dawson, 1998) were tested between September 2016 and January 2017. Birds at least one year of age (determined by examining the colour and shape of their outer tail retrices; Pyle, 1997) were captured in Edmonton (North Saskatchewan River Valley, 53.53˚N, 113.53˚W, Mill Creek Ravine, 53.52˚N, 113.47˚W), or Stony Plain (53.46˚N, 114.01˚W), Alberta, Canada between January 2013 and February 2016. One male and one female subject died in operant training from natural causes. One female subject did not meet criterion while learning to use the operant equipment, and another female subject did not meet criterion during Pretraining; both were replaced. Three subjects (one male and two females) later failed Discrimination training due to reduced feeding and were returned to the colony room to preserve individuals' health. Thus, 19 black-capped chickadees (nine males and ten females) completed the experiment and their performance data were included in the statistical analyses.

Prior to the experiment, birds were individually housed in Jupiter Parakeet cages (30 × 40 × 40 cm; Rolf C. Hagen, Inc., Montreal, QB, Canada) in colony rooms containing several other black-capped chickadees. Birds had visual and auditory, but not physical, contact with one another. Birds had *ad libitum* access to food (Mazuri Small Bird Maintenance Diet; Mazuri, St Louis, MO, USA), water (vitamin supplemented on alternating days; Prime vitamin supplement; Hagen, Inc.), grit, and cuttlebone. Birds were given three to five sunflower seeds daily, one superworm (*Zophobas morio*) three times a week, and a mixture of greens (spinach or parsley) and eggs twice a week. Birds were maintained on a light:dark cycle that mimicked the natural light cycle for Edmonton, Alberta, Canada.

Throughout the experiment, birds were housed individually in operant chambers (see apparatus below), maintained on the natural light cycle for Edmonton, Alberta, and had *ad libitum* access to water (vitamin supplemented on alternate days), grit, and cuttlebone. Birds were given two superworms daily (one in the morning and one in the afternoon). Food (i.e., Mazuri) was only available as a reward for correct responding during the operant discrimination task. None of the 19 birds had previous operant experience or exposure to the stimuli used in this experiment.

Apparatus

During the experiment, birds were housed individually in modified colony room cages (30 × 40 × 40 cm) placed inside a ventilated, sound-attenuating chamber. The chambers were illuminated by a 9-W, full spectrum fluorescent bulb. Each cage contained three perches, a water bottle, and a grit cup. An opening on the side of the cage (11 × 16 cm) provided each bird access to a motor-driven feeder (see Njegovan, Hilhorst, Ferguson, & Weisman, 1994). Infrared cells in the feeder and the request perch (perch closest to the feeder) monitored the position of the bird. A personal computer connected to a single-board computer (Palya & Walter, 2001) scheduled trials and recorded responses to stimuli. Stimuli were played from the personal computer hard drive, through either a Cambridge A300 Integrated Amplifier, Cambridge Azur 640A Integrated Amplifier (Cambridge Audio, London, England), or an NAD310 Integrated Amplifier (NAD Electronics, London, England) and through a Fostex FE108 Σ or Fostex FE108E Σ full-range speaker (Fostex Corp., Japan; frequency response range 80-18,000 Hz) located beside the feeder. See Sturdy and Weisman (2006) for a detailed description of the apparatus.

Acoustic Stimuli

Acoustic stimuli were obtained from the Bayne Laboratory (Department of Biological Sciences, University of Alberta, AB, Canada), Borror Laboratory of Bioacoustics (The Ohio State University, OH, USA), and the Macaulay Library (Cornell Lab of Ornithology, NY, USA; originally recorded between the years of 1954-2015 throughout Canada and USA). Stimuli included 120 vocalizations produced by 16 predator species (eight owls and eight hawks): boreal owl (BOOW), northern pygmy-owl (NOPO), northern saw whet owl (NSWO), barred owl (BADO), great gray owl (GGOW), great horned owl (GHOW), long-eared owl (LEOW), and short-eared owl (SEOW), American kestrel (AMKE), merlin (MERL), sharp-shinned hawk (SSHA), peregrine falcon (PEFA), red-tailed hawk (RTHA), broad-winged hawk (BWHA), Cooper's hawk (COHA), and northern hawk owl (NHOW; Figure 5-1). Stimuli were recorded at a sampling rate of 44.1Khz, 16-bit, and in WAV format. All species were determined to be observed in Edmonton according to The Atlas of Breeding Birds of Alberta (Semenchuk, 1992). We determined average wingspan of the predators based on Sibley (2000) and Templeton et al. (2005). We then plotted stimuli according to their wing spans to determine perceived threat level (see Templeton, 2005; see Table 5-1; see Figure 5-2).

All vocalizations used as acoustic stimuli were of high quality (i.e., no audible interference and low background noise when viewed on a spectrogram with amplitude cutoffs of -35 to 0 dB relative to vocalization peak amplitude) and were bandpass filtered (outside the frequency range of each vocalization type) using GoldWave version 5.58 (GoldWave, Inc., St. John's, NL, Canada) to reduce any background noise. For each stimulus, 5 ms of silence was added to the leading and trailing portion of the vocalization and tapered to remove transients, and amplitude was equalized using SIGNAL 5.10.24 software (Engineering Design, Berkeley, CA, USA).

During the experiment, stimuli were presented at approximately 75 dB as measured by a Brüel & Kjær Type 2239 (Brüel & Kjær Sound & Vibration Measurement A/S, Nærum, Denmark) decibel meter (A-weighting, slow response) at the approximate height and position of a bird's head when on the request perch.

Small, "high threat" predators. A total of 50 auditory stimuli produced by six high-threat predator species were included: American kestrel (10 stimuli), boreal owl (10), merlin (10), northern pygmy-owl (5), northern saw-whet owl (10), and sharp-shinned hawk (5). A total of 40 calls produced by four species (i.e., American kestrel, boreal owl, merlin, and northern saw-whet owl) were used in training (Discrimination), whereas the remaining 10 calls produced by two high-threat species (i.e., northern pygmy-owl and sharp-shinned hawk) were excluded to test during generalization (Transfer).

Large, "low threat" predators. A total of 50 auditory stimuli produced by six high-threat predator species were included: barred owl (10 stimuli), great gray owl (5), great horned owl (10), long-eared owl (10), peregrine falcon (10), and red-tailed hawk (5). A total of 40 calls produced by four species (barred owl, great horned owl, long-eared owl, and peregrine falcon) were used in training (Discrimination), whereas the remaining 10 calls produced by two low-threat species (i.e., great gray owl and red-tailed hawk) were excluded to test during generalization (Transfer).

Mid-sized predators. A total of 20 auditory stimuli produced by four mid-threat predator species were included: broad-winged hawk (5 stimuli), Cooper's hawk (5), northern hawk owl (5), and short-eared owl (5). All 20 calls were used for testing during generalization (Transfer).

Procedure

Pretraining. Pretraining began once the bird learned to use the request perch and feeder to obtain food. During Pretraining, birds received food for responding to all stimuli (future rewarded stimuli, unrewarded stimuli, and transfer stimuli). A trial began when the bird landed on the request perch and remained for between 900-1100 ms. A randomly-selected stimulus played without replacement until all 120 stimuli had been heard. If the bird left the request perch before a stimulus finished playing, the trial was considered interrupted, resulting in a 30-s time out with the houselight turned off. If the bird entered the feeder within 1 s after the entire stimulus played, it was given 1 s access to food, followed by a 30-s intertrial interval, during which the houselight remained on. If a bird remained on the request perch during the stimulus presentation and the 1 s following the completion of the stimulus it received a 60-s intertrial interval with the houselight on, but this intertrial interval was terminated if the bird left the request perch. This was to encourage a high level of responding on all trials. Birds continued on Pretraining until they completed six 360-trial blocks of ≥ 60% responding on average to all stimuli, at least four 360-trial blocks ≤ 3% difference in responding to future rewarded and unrewarded stimuli, at least four 360-trial blocks in which the bird had ≤ 3% feeding on future transfer stimuli, and at least four 360-trial blocks in which the bird had ≤ 3% feeding on short and long stimuli to ensure that birds did not have a preference for the length of the stimuli. Following a day of free feed, birds completed a second round in which they completed one 360-trial block of ≥ 60% responding on average to all stimuli, completed one 360-trial block of ≤ 3% difference in responding to future rewarded and unrewarded stimuli, completed one 360-trial block of ≤ 3% feeding on future transfer stimuli, and completed one 360-trial block of ≤ 3% feeding on short and long stimuli to ensure that birds did not have a preference for the length of the stimuli, respectively, to confirm that each bird continued to not have preferences following the break.

Discrimination Training. The procedure was the same as during Pretraining, except, only 80 training stimuli were presented (with the remaining 40 withheld for use during Transfer testing), and responding to half of these stimuli were then punished with a 30-s intertrial interval with the houselight off. As during Pretraining, responses to rewarded stimuli resulted in 1 s access to food. Discrimination training continued until birds completed six 320-trial blocks with a discrimination ratio (DR) ≥ 0.80 with the last two blocks being consecutive. For DR calculations see Response Measures, below.

Birds were randomly assigned to either a True category discrimination group (*n* = 12) or Pseudo category discrimination group (*n* = 7). Black-capped chickadees in the True category discrimination group were divided into two subgroups: one subgroup discriminated 40 rewarded high-threat calls from 40 unrewarded low-threat calls (High Threat Group: three male and three female subjects), while the other subgroup discriminated 40 rewarded low-threat calls from 40 unrewarded high-threat calls (Low Threat Group: three male and three female subjects).

The Pseudo category discrimination group was also divided into two subgroups. Each subgroup discriminated 20 randomly-selected rewarded high-threat and 20 randomly-selected rewarded low-threat calls from 20 unrewarded high-threat and 20 unrewarded low-threat calls (Subgroup 1: two male and two female subjects; Subgroup 2: one male and two female subjects). The purpose of the Pseudo group was to include a control in which subjects were not trained to categorize according to arousal level, investigating if True group acquisition is due to category learning or simply rote memorization. For example, if the True group is discriminating using the classifications of ‘high’ and ‘low’ threat, these birds would complete training in significantly fewer trials compared to the Pseudo group that would have to rely on rote memorization. However, if birds did not perceive and respond to the classifications as expected, we would anticipate that both True and Pseudo groups would require a similar number of trials to complete acquisition based on rote memorization.

Discrimination 85. This phase was identical to Discrimination training, except that the rewarded stimuli were rewarded with a reduced probability of getting a reward (i.e., $P = 0.85$). On unrewarded rewarded trials, entering the feeder after the stimulus finished playing resulted in a 30-s intertrial interval, during which the houselight remained on, but there was no access to food. Discrimination 85 training was employed to introduce birds to trials in which there was no access to food, but the houselight remained illuminated, in order to prepare birds for Transfer testing in which stimuli were neither rewarded nor punished. Discrimination 85 training continued until birds completed two 320-trial blocks with a DR ≥ 0.80.

Transfer Testing. During Transfer testing, the stimuli and reinforcement contingencies from Discrimination 85 were maintained and 40 additional stimuli were also presented. Stimuli from Discrimination 85 training were presented four times each, randomly-selected without replacement and 13 or 14 new (i.e., transfer) stimuli were each presented once during a 333- or 334-trial block. Responses to transfer stimuli resulted in a 30-s intertrial interval with the houselight on, but no access to food; we did not differentially reinforce or punish transfer stimuli, and only presented each transfer stimulus once each per trial block, so subjects did not learn specific contingencies associated with responding to these transfer stimuli. See Figure 5-3.

These additional testing calls were produced by small (northern pygmy-owl, sharp-shinned hawk) and large predators (great gray owl, red-tailed hawk), as well as several mid-sized predators (broad-winged hawk, Cooper's hawk, northern hawk owl, short-eared owl). Eight high- and eight low-arousal calls from each species heard during Discrimination training, but not discrimination training, were introduced. Due to the number of testing stimuli, we created three rounds of Transfer testing that each included one or two stimuli per testing species. All birds completed a minimum of three blocks of Transfer testing and these were included for analysis. Between each round of Transfer, birds completed two 320-trial blocks of Discrimination 85 with a DR ≥ 0.80. Following final Transfer, birds were returned to their colony rooms.

Only True group Transfer testing data was analyzed. In order to analyze responding to each of the 16 stimulus species (six high threat, six low threat, four mid-sized predators), we calculated the proportion of responding for each stimulus type by averaging the percent correct response from the birds within each condition (described below).

Response Measures. For each stimulus exemplar, a proportion response was calculated by the following formula: R+/(N-I), where R+ is the number of trials in which the bird went to the feeder, N is the total number of trials, and I is the number of interrupted trials in which the bird left the perch before the entire stimulus played. For Discrimination and Discrimination 85 training, we calculated a discrimination ratio (DR), by dividing the mean proportion response to all rewarded stimuli by the mean proportion response to rewarded stimuli plus the mean proportion response to unrewarded stimuli. A DR of 0.50 indicates equal responding to rewarded and unrewarded stimuli, whereas a DR of 1.00 indicates perfect discrimination.

For Transfer testing, in order to analyze responding to each of the 16 predator stimuli (six high threat, six low threat, four mid-sized predators), we calculated the proportion of responding for each of the stimuli for all subjects.

Statistical Analyses. We conducted independent-samples *t*-tests on the number of trials to criterion for the True and Pseudo category groups during Discrimination training. A repeated measures Analysis of Variance (ANOVA) was conducted on the proportion of responding to determine if the birds differentially responded to Discrimination training stimuli during Transfer testing (Condition × Stimulus Species). To determine if differential responding was according to Threat Level, we conducted an independent *t*-test on the responding during Transfer testing of two True groups (High Threat vs. Low Threat). We also conducted a repeated measures ANOVA (Fixed factors: Condition, Stimulus Species) on the proportion of responding to determine if birds differentially responded to testing stimuli during Transfer. This analysis was followed by independent-samples *t*-tests (High Threat vs. Low Threat group) on responding to the Stimulus Species to determine which of the predator species' calls birds demonstrated transfer of training (i.e., to which stimuli birds responded). We then used paired-samples *t*-tests (with Bonferroni corrections) for each Condition (High Threat, Low Threat) separately, on the proportion of responding to the Stimulus Species in order to determine which predator species were responded to significantly more (or less) compared to other species. To further examine responding differences between rewarded species and each testing species, we conducted repeated-samples ANOVAs for each High Threat and Low Threat groups, and completed follow-up paired-samples *t*-tests (with Bonferroni corrections) on the proportion of responding during Transfer testing.

Ethical Note. Throughout the experiment, birds remained in the testing apparatus to minimize the transport and handling of each bird and reduce stress. Following the experiment, birds were returned to the colony room for use in future experiments. With the exception of one male and one female subject that died from natural causes, birds 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 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

To determine whether birds in the two True category groups differed in their speed of acquisition, we conducted an independent-samples *t*-test on the number of 320-trial blocks to reach criterion for the two True category conditions (High Threat Group: X+SEM =

21.333+6.427, *N* = 6; Low Threat Group: *X*+SEM = 12.333+1.022, *N* = 6). There was no significant difference, $t_{10} = 1.383$, $p = 0.197$, $d = .874$, 95% Confidence Interval [CIs] = -5.5001, 23.5001.

To determine whether birds in the two Pseudo category groups differed in their speed of acquisition, we conducted an independent-samples *t*-test on the number of 320-trial blocks to reach criterion for the two Pseudo category conditions (Pseudo 1 Group: *X*+SEM = 28.250+1.548, *N* = 4; Pseudo 2 Group: *X*+SEM = 91.000+18.610, *N* = 3). Due to a violation of Levene's test, we used the *p*-value that did not assume homogeneity of variance; there was no significant difference, $t_{2.028} = -3.360$, $p = 0.077$, $d = -4.719$, 95% CIs = -142.056, 16.556.

To compare the acquisition performance of the True and Pseudo category groups and to determine if the True group learned to categorize in fewer trials than the Pseudo group, we conducted an independent-samples *t*-test on the number of 320-trial blocks to reach criterion for the True category and Pseudo category groups. Due to a violation of Levene's test, we used the *p*-value that did not assume homogeneity of variance; there was a significant difference between the groups ($t_{6.660} = -2.569$, $p = 0.039$, $d = -1.991$, 95% CIs = -73.938, -2.681) in that True birds learned to discriminate significantly faster than Pseudo birds.

Analysis of Discrimination Stimuli during Transfer

To examine if birds learned to discriminate among calls from all species, we conducted a repeated measures ANOVA for the True group with Condition (High Threat, Low Threat) and Stimulus Species (American kestrel, boreal owl, merlin, northern saw-whet owl; barred owl, great horned owl, long-eared owl, peregrine falcon) as fixed factors and proportion of responding as the dependent variable. Using a Huynh-Feldt correction, there was a significant two-way interaction of Condition × Stimulus Species ($F_{1,7} = 4712.493$, $p < 0.001$, $\eta^2 = .999$), indicating that there was differential responding to stimulus species according to condition. The main effects of Condition and Stimulus Species were non-significant (*p*s $\geq$ 0.262).

Analysis of Transfer Stimuli

To determine if the pattern of learning was the same across calls from testing species in Transfer testing, we conducted a repeated measures ANOVA for the True group with Condition (High Threat, Low Threat) × Stimulus Species (northern pygmy-owl, sharp-shinned hawk; great gray owl, red-tailed hawk; broad-winged hawk, Cooper's hawk, northern hawk owl, short-eared owl) as fixed factors and the proportion of responding during Transfer testing as the dependent variable. Using a Huynh-Feldt correction, there was a significant interaction of Condition × Stimulus Species ($F_{1,7} = 35.133$, $p < 0.001$, $\eta^2 = .875$), indicating that there was a significant difference in responding to Stimulus Species based on Condition. The main effect of Stimulus Species was significant ($F_7 = 2.626$, $p = 0.027$, $\eta^2 = .334$, 95% CIs northern pygmy-owl = 0.253, 0.482; sharp-shinned hawk = 0.296, 0.663; great gray owl = 0.171, 0.756; red-tailed hawk = 0.489, 0.705; broad-winged hawk = 0.201, 0.768; Cooper's hawk = 0.274, 0.545; northern hawk owl = 0.387, 0.680; SEOW = 0.375, 0.773), indicating that there was a significant difference in responding based on the Stimulus Species. However, the main effect of Condition was non-significant ($p = 0.090$).

To further investigate responding across Stimulus Species between the true category groups, we conducted independent-samples *t*-tests of High Threat vs. Low Threat conditions responding to each Stimulus Species with Bonferroni corrections ($p < 0.006$). There were significant differences in responding to sharp-shinned hawk, great gray owl, broad-winged hawk, and short-eared owl. Responding to northern pygmy-owl, red-tailed hawk, Cooper's hawk, and northern hawk owl were non-significant (*p*s $\geq$ 0.093). See Table 5-2 and Figure 5-4 for these statistical results. We conducted paired-samples *t*-tests (with Bonferroni corrections; $p \leq 0.001$) on the proportion of responding to each Transfer testing species, separately for the High Threat and Low Threat conditions, to further examine the significant Condition × Stimulus Species interaction; see Table 5-3 for these results.

To then examine how responding to rewarded species compared to responding to each testing species, we conducted repeated measures ANOVAs for each Condition (High Threat, Low Threat), followed by paired-samples *t*-tests (with Bonferroni corrections; $p < 0.006$) on the proportion of responding during Transfer testing. Using Huynh-Feldt corrections, there were significant differences between rewarded and testing species for both True groups (High Threat: $F_8 = 18.228$, $p < 0.001$, $\eta^2 = .785$; Low Threat: $F_8 = 25.989$, $p < .001$, $\eta^2 = .839$). See Table 5-4 for the results of the paired-samples *t*-tests.

Discussion

Here we showed that chickadees perceive owl and raptor vocalizations as coherent groups (i.e., perceptual categories) based on the degree of threat. True group birds learned to discriminate acoustic stimuli produced by predators in fewer trials compared to Pseudo group birds, suggesting that birds in the True group perceived stimuli on the basis of threat level, grouping (classifying) species together based on their perceived level of threat, thereby leading to significantly faster task acquisition compared to the Pseudo group. This difference in acquisition during Discrimination training, along with significant transfer of training to sharp-shinned hawk (high threat) and great gray owl (low threat) vocalizations, supports the notion that predators that we classified as high or low threat (according to wingspan) for Discrimination training were of distinct threat levels and that birds are capable of discriminating between the calls produced by these species. By training chickadees to classify species at the polar ends of the continuum (i.e., high- and low-threat species), and testing with calls produced by additional predators, including mid-sized predators, we were able to determine the extent to which chickadees perceive the threat posed by predator species along a continuum rather than categorizing all predator species as high vs. low threat.

Transfer: Small vs. Large Predators

During Transfer testing we presented chickadees that were previously rewarded for responding to high or low threat stimuli with calls produced by multiple other predator species, including two additional small species (northern pygmy-owl and sharp-shinned hawk) and two additional large species (great gray owl and red-tailed hawk). Our results suggest that chickadees perceive sharp-shinned hawks as a high-threat predator as the High Threat group responded significantly more to sharp-shinned hawk calls during Transfer compared to Low Threat group. Similar to this result, presentation of sharp-shinned hawk mounts resulted in the production of more D notes per call by tufted titmice compared to the vocalizations produced in response to a live red rat snake (*Elphe guttata*; Sieving et al., 2010). In addition, Courter and Ritchison (2010) found that sharp-shinned hawk mounts resulted in more D notes per call within the first two minutes of presentation, and longer mobbing bouts, compared to responding to red-tailed hawk and great horned owl mounts, suggesting that sharp-shinned hawks are a high-threat predator to parids. According to Templeton et al. (2005), black-capped chickadees produce *chick-a-dee* mobbing calls containing approximately four D notes per call to both northern saw-whet owls and northern pygmy-owls, suggesting that these species are perceived as high threat. In Discrimination training birds were rewarded for responding to northern saw-whet owl calls as a high-threat predator, but did not demonstrate transfer of training to northern pygmy-owl calls in Transfer testing, despite the fact that these species of predator have equivalently small wingspans

(39 cm and 31 cm, respectively) and produce calls of similar frequency (see Figure 5-1). As the northern pygmy-owl is the smallest predator that we included, we would have expected responding to suggest it be of the highest threat. Because subjects were trained to respond (High Threat group) or withhold responding (Low Threat group) to northern saw-whet owl calls, it is possible that in the absence of this training (i.e., during a Transfer testing procedure) chickadees would not respond to northern saw-whet owl calls as a high-threat predator; this notion could be tested in a future experiment with similar methodology to the current study. It is also possible that black-capped chickadees perceive the vocalizations of northern pygmy-owls to not pose a high threat since northern pygmy-owls are fairly rare in Edmonton (observed, but not abundant), and although one of the only diurnal owl species, primarily produce vocalizations at night when black-capped chickadees are sleeping (Sibley, 2000). In contrast, due to the diurnal activity and diets of sharp-shinned hawks, this species is likely to be of greater risk than northern pygmy-owls (Sibley, 2000).

Similarly, we found that the High Threat group responded as though broad-winged hawks (mid-sized) are higher threat than northern pygmy-owls (small), and responded similarly to high-threat rewarded stimuli compared to sharp-shinned hawks, but not compared to northern pygmy-owl calls; this suggests that small northern pygmy-owls were not perceived to be of high-threat but instead lower on the proposed threat continuum (toward medium or low threat). In contrast, the Low Threat group responded as though northern pygmy-owls are higher threat than great gray owls (large), and did not respond similarly to low-threat rewarded stimuli compared to either northern pygmy-owls or sharp-shinned hawks; this suggests that small northern pygmy-owls were also not perceived to be of low-threat but instead higher on the threat continuum.

Thus, taken together, these results suggest that although northern pygmy-owls have a relatively short wingspan, they are perceived as a medium-threat predator along a threat continuum.

During Transfer testing, we presented chickadees with calls produced by great gray owls and red-tailed hawks, both species with relatively long wingspans (see Table 5-1), and therefore considered to be low-threat species (e.g., Templeton et al., 2005). We found that the Low Threat group responded significantly more to the great gray owl calls during Transfer than the High Threat group, confirming the perception of great gray owls as low threat; however, responding to red-tailed hawk calls was not significantly different between the two groups, suggesting that red-tailed hawks are not considered to be a high-threat or low-threat predator. Templeton et al. (2005) demonstrated that black-capped chickadees produce *chick-a-dee* mobbing calls containing approximately two D notes per call in response to great gray owls, but approximately 2.5 D notes per call in response to red-tailed hawks; thus, we suggest that great gray owls could be perceived by black-capped chickadees to be of lower threat than red-tailed hawks, along the continuum, although there was not a significant difference between these two species. Moreover, red-tailed hawks' wingspan of 120 cm is shorter than the great gray owls' wingspan of approximately 132-150 cm; this 10-30 cm difference in wingspan may be enough to increase maneuverability in red-tailed hawks in comparison to great gray owls that have a large body size and corresponding diet (i.e., they consume larger prey as rodent specialists). Red-tailed hawks have been classified as low-threat as chickadees are rarely preyed upon by this species (Houston, Smith, & Rohner, 1998). Soard and Ritchison (2009) found that responding to red-tailed hawks by Carolina chickadees resulted in *chick-a-dee* calls with more '*chick*' and fewer '*dee*' notes per call in comparison to smaller, high-threat predators. In the current study, during Transfer testing, both the High Threat and Low Threat groups responded to red-tailed hawk vocalizations at an intermediate level compared to the other testing species, and both groups responded to red-tailed hawk vocalizations in a manner similar to (i.e., no significant difference from) the High Threat rewarded and Low Threat rewarded stimuli, respectively. This suggests that red-tailed hawks are perceived as medium threat and provides support that there is a continuum in the perception of predator threat, rather than a high-threat versus low-threat dichotomy.

Transfer: Mid-sized Predators

During Transfer testing we included stimuli produced by mid-sized predators (broad-winged hawk, Cooper's hawk, northern hawk owl, short-eared owl) to investigate how chickadees perceived these predators compared to high- and low-threat predator species, and if the overall threat perception of songbirds is categorical in nature or occurs along a continuum. First, the High Threat group responded significantly more to broad-winged hawk calls compared to the Low Threat group, and as though broad-winged hawks are of higher threat than Cooper's hawks (mid-sized) and northern pygmy-owls (small).

Second, the Low Threat group responded significantly more to short-eared owl calls compared to the High Threat group, suggesting that chickadees perceived short-eared owls as a low-threat predator. In addition, the Low Threat group responded similarly to low-threat rewarded stimuli compared to short-eared owl calls, and as though short-eared owls are of lower threat compared to sharp-shinned hawks (small), northern pygmy-owls (small), and broad-winged hawks (mid-sized).

Last, High Threat and Low Threat groups did not respond significantly differently to Cooper's hawk or northern hawk owl calls, indicating that chickadees do not categorize these species of predator as either high or low threat. The High Threat group responded as though Cooper's hawks were of lower threat than sharp-shinned hawks (small) and broad-winged hawks (mid-sized), and the Low Threat group responded as though northern hawk owls were of lower threat than broad-winged hawks (mid-sized). Neither the High or Low Threat group responded similarly to high- or low-threat rewarded stimuli, respectively, compared to the Cooper's hawk calls further suggesting that this species is of medium-threat. Only the Low Threat group responded similarly to low-threat rewarded stimuli compared to the northern hawk owl calls, which taken together with the non-significant responding between High and Low Threat groups, suggests that this species may be perceived as medium-low threat. Overall, these results suggest a graded continuum of predator threat, as chickadees responded to these mid-sized predators (Cooper's hawk and northern hawk owl) at an intermediate level, rather than in a manner suggesting chickadees categorized these species as either high or low threat.

Similarly, Templeton and colleagues (2005) demonstrated that black-capped chickadees produced *chick-a-dee* mobbing calls containing approximately 3.25 D notes per call in response to Cooper's hawks, a species that falls between high-threat northern saw-whet owls (~4 Ds) and low-threat great horned owls (~2.5Ds). Templeton et al.'s study did not examine chickadees' response to broad-winged hawks or northern hawk owls, but concluded that short-eared owls are likely perceived as low threat considering that black-capped chickadees produced approximately 2.25 D notes per call in response to short-eared owls. In contrast, Courter and Ritchison (2010) found that tufted titmice likely perceive Cooper's hawks as a high threat predator given that titmice responded to Cooper's hawk mounts with more D notes per call compared to controls, which was comparable to titmice responding to sharp-shinned hawk mounts (a species that is comparable in body size). However, tufted titmice are approximately twice the size of chickadees (Pyle, 1997), and Cooper's hawks typically predate larger species of songbirds (e.g., house sparrow, *Passer domesticus*; Roth & Lima, 2006). Thus, Cooper's hawks may be a high-threat predator to tufted titmice, while the relatively smaller black-capped chickadees may perceive Cooper's hawks to be medium-threat.

Conclusions

Overall, this experiment provides insights into songbird perception of predator threat. By training chickadees to respond to either high- or low-threat predator vocalizations, and obtaining subsequent responses to additional calls produced by small, large, and mid-sized avian predators in Transfer testing, we were able to investigate perception of threat to determine whether threat occurs along a continuum. Black-capped chickadees demonstrated transfer of training and appear to consider sharp-shinned hawks and broad-winged hawks as high-threat predators, and great gray owls and short-eared owls as low-threat predators. Surprisingly, the calls of northern pygmy-owls (small predator) and red-tailed hawks (large predator) were not responded to differentially by the two groups; these responses do not indicate that chickadees were making incorrect responses, but rather than our classification of predators was not in line with chickadees' perception of threat. We predict that the nocturnal calling patterns of the northern pygmy-owl in comparison to diurnal chickadee activity, as well as the diurnal activity and slightly smaller wingspan of the red-tailed hawk compared to great gray owls, explains this responding. In a future study, subjects could be trained with northern pygmy-owl calls then tested with northern saw-whet owl calls; this would assist in clarifying how these two similar-sized owls, that produce a similar call, are perceived. Calls produced by mid-sized Cooper's hawks and northern hawk owls were also not responded to differentially, suggesting that black-capped chickadees do not perceive these species as the extremes of high or low threats. In summary, the lack of categorization by black-capped chickadees for a small predator (northern pygmy-owl), large predator (red-tailed hawk), and two mid-sized predators (Cooper's hawk and northern hawk owl) as high or low threat in the current study provides evidence that the perception of predator threat, according to wingspan, in songbirds may not be categorical, but rather along a graded continuum in which some species are considered to be of neither high nor low threat.

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All animal studies were conducted in accordance with the Canadian Council on Animal Care Guidelines and Policies and with approval from the Animal Care and Use Committee for Biosciences for the University of Alberta, and the University of Calgary Life and Environmental Sciences Animal Care Committee. Chickadees were captured and research was carried out under an Environment Canada Canadian Wildlife Service Scientific permit, Alberta Fish and Wildlife Capture and Research permits, and City of Edmonton Parks Permit.

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Table 5-1. Information regarding species calls that were used as stimuli, including how the stimuli were sorted according to small-, mid-, or large-sized predators and if they were initially presented during training (Discrimination) or testing (Transfer). The table is colour-coded: red for small-sized/high-threat predator species, green for large-sized/low-threat predator species, and yellow for mid-sized/unknown threat predator species during Transfer of Training. American Ornithology Union (AOU) Codes provided. Information regarding avian species' diets is from Sibley (2003), Fitch et al. (1946), and White & Stiles (1990). For birds, body length was measured from bill tip to tail tip, and reported body length and wingspan values are averages for each species (Sibley, 2000 and/or Templeton et al., 2005).

Species	***AOU Code***	***Threat Level***	***Wingspan***	***Resource***	***Diet***	***Stimulus Type***
American kestrel	**AMKE**	High	53 cm/58 cm	Sibley (2000)/Templeton et al. (2005)	Primarily insects, small rodents and birds	Training
Boreal owl	**BOOW**	High	61 cm	Sibley (2000)	Primarily insects, small mammals and birds	Training
Merlin	**MERL**	High	58 cm/61 cm	Sibley (2000)/Templeton et al. (2005)	Primarily small birds Occasionally small mammals and reptiles	Training
Northern pygmy-owl	**NOPO**	High	31 cm	Templeton et al. (2005)	Primarily rodents and small birds	*Testing*
Northern saw whet owl	**NSWO**	High	39 cm	Templeton et al. (2005)	Primarily small mammals; songbirds during migration	Training
Sharp-shinned hawk	**SSHA**	High	53 cm	Sibley (2000)	Primarily songbirds Occasionally insects	*Testing*
Broad-winged hawk	**BWHA**	Mid	84 cm	Sibley (2000)	Primarily small mammals, reptiles, and birds	*Testing*
Cooper's hawk	**COHA**	Mid	71 cm/81 cm	Sibley (2000)/Templeton et. al (2005)	Primarily mid-sized birds Occasionally small mammals	*Testing*
Northern hawk owl	**NHOW**	Mid	84 cm	Sibley (2000)	Primarily small mammals Occasionally small birds	*Testing*
Short-eared owl	**SEOW**	Mid	89 cm	Templeton et al. (2005)	Primarily small mammals Occasionally small birds	*Testing*

Barred owl	**BADO**	Low	110 cm	Sibley (2000)	Primarily small mammals Occasionally birds	Training
Great gray owl	**GGOW**	Low	150 cm/132 cm	Sibley (2000)/Templeton et al. (2005)	Primarily small mammals Occasionally birds	*Testing*
Great horned owl	**GHOW**	Low	140 cm/121 cm	Sibley (2000)/Templeton et al. (2005)	Primarily small mammals Occasionally birds	Training
Long-eared owl	**LEOW**	Low	100 cm	Sibley (2000)	Primarily small mammals Occasionally small birds	Training
Peregrine falcon	**PEFA**	Low	110 cm/120 cm	Sibley (2000)/Templeton et al. (2005)	Primarily birds	Training
Red-tailed hawk	**RTHA**	Low	120 cm/120 cm	Sibley (2000)/Templeton et al. (2005)	Primarily small mammals Occasionally birds	*Testing*

Table 5-2. The results of the independent-samples *t*-tests conducted on High Threat vs. Low Threat groups' responding to each testing stimulus species' calls with Bonferroni corrections ($p < 0.006$). The table is colour-coded: red for small predator species, yellow for mid-sized predator species, and green for large predator species during Transfer testing.
Positive *t*-values indicate more responding by chickadees in the High Threat group; negative *t*-values indicate more responding by chickadees in the Low Threat group.

Stimulus Species	***t* value**	***p* value**	**Cohen's *d***	**Confidence Intervals**
Northern pygmy-owl	-0.941	0.369	-0.595	-0.331, 0.134
Sharp-shinned hawk	6.273	**0.001**	-4.877	0.305, 0.682
Broad-winged hawk	16.039	**< 0.001**	10.144	0.722, 0.955
Cooper's hawk	-1.857	0.093	-1.174	-0.456, 0.042
Northern hawk owl	-2.399	0.037	-1.517	-0.514, -0.019
Short-eared owl	-4.656	**0.001**	-2.945	-0.734, -0.259
Great gray owl	-9.994	**< 0.001**	-7.131	-0.9778, -0.6102
Red-tailed hawk	-0.960	0.360	-0.607	-0.314, 0.125

Note: **Bold** font indicates statistical significance ($p < 0.006$).

Table 5-3. The results (*p*-values) of the paired-samples *t*-tests, with Bonferroni corrections ($p \leq 0.001$), conducted on the proportion of responding to each Transfer testing species, separately for the High Threat group and Low Threat group. The table is colour-coded: red for small predator species, yellow for mid-sized predator species, and green for large predator species during Transfer testing.

High Threat Group	**Species**	**Northern pygmy-owl**	**Sharp-shinned hawk**	**Broad-winged hawk**	**Cooper's hawk**	**Northern hawk owl**	**Short-eared owl**	**Great gray owl**	**Red-tailed hawk**
Species	**AOU Code**	**NOPO**	**SSHA**	**BWHA**	**COHA**	**NHOW**	**SEOW**	**GGOW**	**RTHA**
Northern pygmy-owl	**NOPO**	-	-	-	-	-	-	-	-
Sharp-shinned hawk	**SSHA**	0.015	-	-	-	-	-	-	-
Broad-winged hawk	**BWHA**	**0.001** **BWHA>NOPO**	0.044	-	-	-	-	-	-
Cooper's hawk	**COHA**	0.894	**0.001** **SSHA>COHA**	**0.001** **BWHA>COHA**	-	-	-	-	-
Northern hawk owl	**NHOW**	0.341	0.036	**0.001** **BWHA>NHOW**	0.477	-	-	-	-
Short-eared owl	**SEOW**	0.995	0.037	0.002	0.883	0.578	-	-	-
Great gray owl	**GGOW**	0.019	**<0.001** **SSHA>GGOW**	**<0.001** **BWHA>GGOW**	0.015	0.010	0.013	-	-
Red-tailed hawk	**RTHA**	0.108	0.157	0.016	0.070	0.376	0.118	0.004	-

Low Threat Group	**Species**	**Northern pygmy-owl**	**Sharp-shinned hawk**	**Broad-winged hawk**	**Cooper's hawk**	**Northern hawk owl**	**Short-eared owl**	**Great gray owl**	**Red-tailed hawk**
Species	**AOU Code**	**NOPO**	**SSHA**	**BWHA**	**COHA**	**NHOW**	**SEOW**	**GGOW**	**RTHA**
Northern pygmy-owl	**NOPO**	-	-	-	-	-	-	-	-
Sharp-shinned hawk	**SSHA**	0.048	-	-	-	-	-	-	-
Broad-winged hawk	**BWHA**	0.007	0.004	-	-	-	-	-	-
Cooper's hawk	**COHA**	0.268	0.014	0.002	-	-	-	-	-
Northern hawk owl	**NHOW**	0.040	0.002	**0.001** **NWHA>BWHA**	0.237	-	-	-	-
Short-eared owl	**SEOW**	**0.001** **SEOW>NOPO**	**0.001** **SEOW>SSHA**	**<0.001** **SEOW>BWHA**	0.012	0.193	-	-	-
Great gray owl	**GGOW**	0.003	**0.001** **GGOW>SSHA**	**<0.001** **GGOW>BWHA**	0.042	0.138	0.895	-	-
Red-tailed hawk	**RTHA**	0.012	0.003	0.002	0.198	0.715	0.088	0.142	-

Note: **Bold** font indicates statistical significance ($p \leq 0.001$).

Table 5-4. The results of the paired-samples *t*-tests, with Bonferroni corrections ($p < 0.006$), conducted on the proportion of responding to rewarded species compared responding to each testing species during Transfer testing, separately for the High Threat group and Low Threat group. The table is colour-coded: red for small predator species, yellow for mid-sized predator species, and green for large predator species during Transfer testing.

Stimulus Species	*t* value	*p* value	Cohen's *d*	Confidence Intervals
High Threat Group (*df* = 5)				
Northern pygmy-owl	**-5.895**	**0.002**	**-5.273**	**-0.827, -0.325**
Sharp-shinned hawk	-2.378	0.063	-2.127	-0.351, 0.014
Broad-winged hawk	0.367	0.729	0.328	-0.054, 0.072
Cooper's hawk	**-6.637**	**<0.001**	**-5.936**	**-0.817, -0.361**
Northern hawk owl	**-5.282**	**0.003**	**-4.724**	**-0.735, -0.254**
Short-eared owl	**-7.017**	**<0.001**	**-6.276**	**-0.777, -0.360**
Great gray owl	**-22.053**	**<0.001**	**-19.725**	**-0.956, -0.756**
Red-tailed hawk	-3.938	0.011	-3.522	-0.570, -0.120
Low Threat Group (*df* = 5)				
Northern pygmy-owl	**-8.378**	**<0.001**	**-7.494**	**-0.604, -0.320**
Sharp-shinned hawk	**-20.372**	**<0.001**	**-18.221**	**-0.728, -0.565**
Broad-winged hawk	**-18.961**	**<0.001**	**-16.959**	**-0.924, -0.703**
Cooper's hawk	-5.159	0.004	-4.614	-0.548, -0.183
Northern hawk owl	-3.893	0.011	-3.482	-0.352, -0.072
Short-eared owl	-0.814	0.453	-0.728	-0.235, 0.122
Great gray owl	-0.681	0.526	-0.609	-0.219, 0.127
Red-tailed hawk	-4.082	0.010	-3.651	-0.382, -0.087

Note: **Bold** font indicates statistical significance ($p < 0.006$).

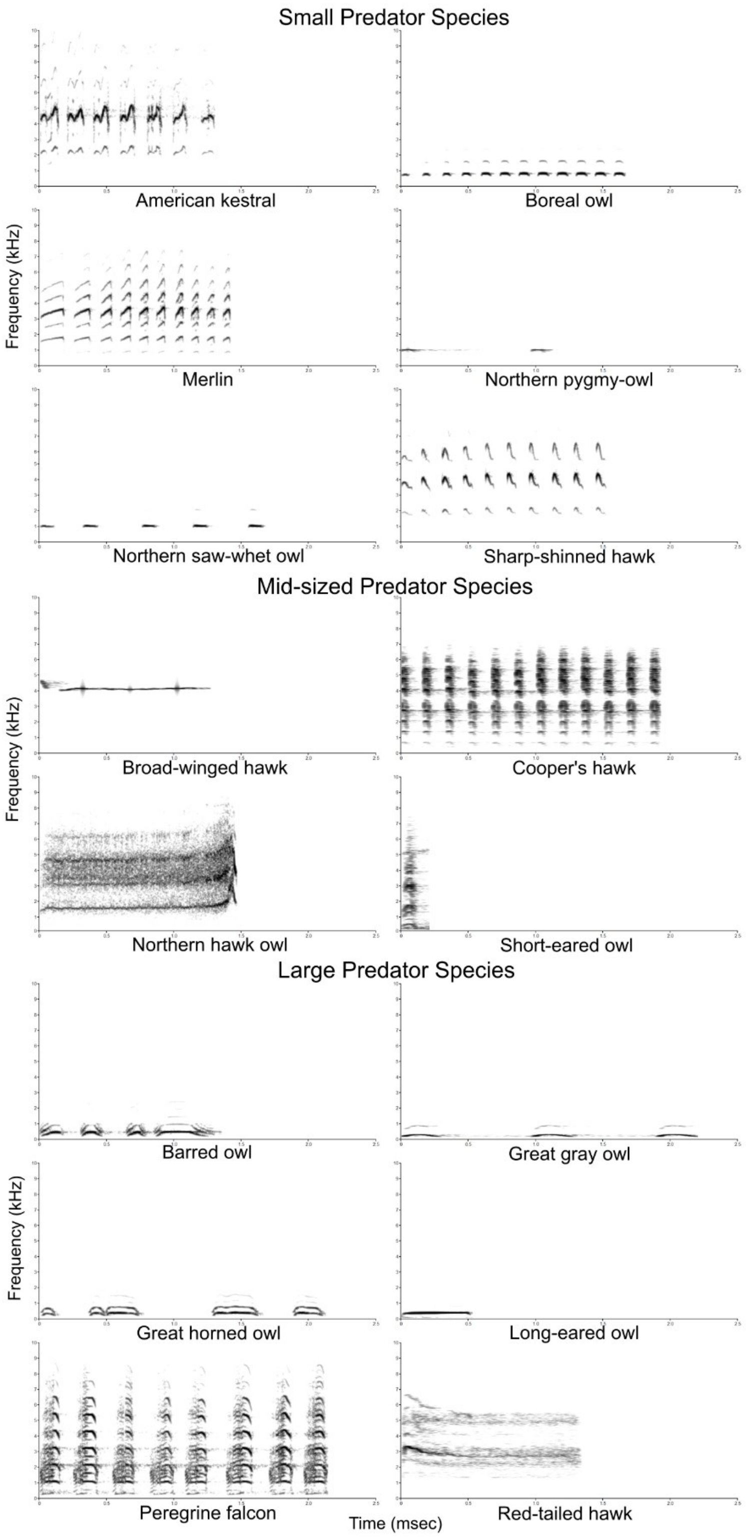

Figure 5-1. Sample sound spectrograms of the vocalizations produced by the 16 species of predators used as experimental stimuli, divided into small, mid-sized, and large predators with time (msec) on the *x*-axis and frequency (kHz) on the *y*-axis.

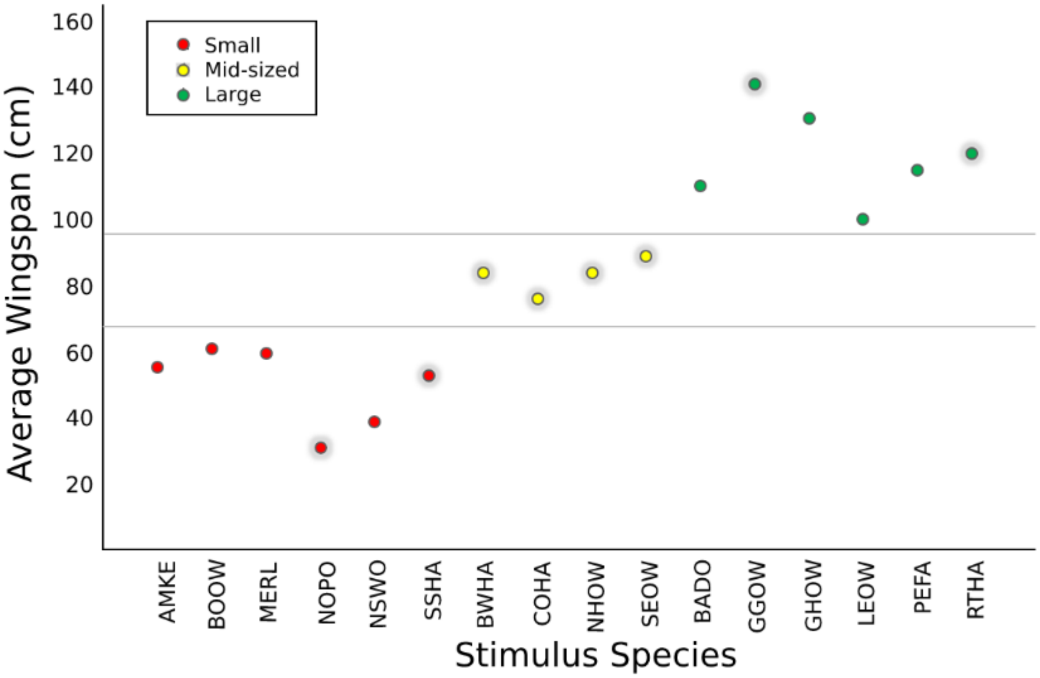

Figure 5-2. Owl and hawk species (*x*-axis) plotted according to average wingspan (*y*-axis), categorized by size: small, mid, and large (Sibley, 2000; Templeton et al., 2005; see Table 5-1). Small species were used as training and testing high-threat stimuli; large species were used as training and testing low-threat stimuli; and mid-sized species were used as testing stimuli to determine songbirds' perception of threat. AMKE: American kestrel; BOOW: boreal owl; MERL: merlin; NOPO: northern pygmy-owl; NSWO: northern saw-whet owl; SSHA: sharp-shinned hawk; BWHA: broad-winged hawk; COHA: Cooper's hawk; NHOW: northern hawk owl; SEOW: short-eared owl; BADO: barred owl; GGOW: great gray owl; GHOW: great horned owl; LEOW: long-eared owl; PEFA: peregrine falcon; RTHA: red-tailed hawk.
Note: Species with shadowed points indicate calls used as testing stimuli in Transfer.

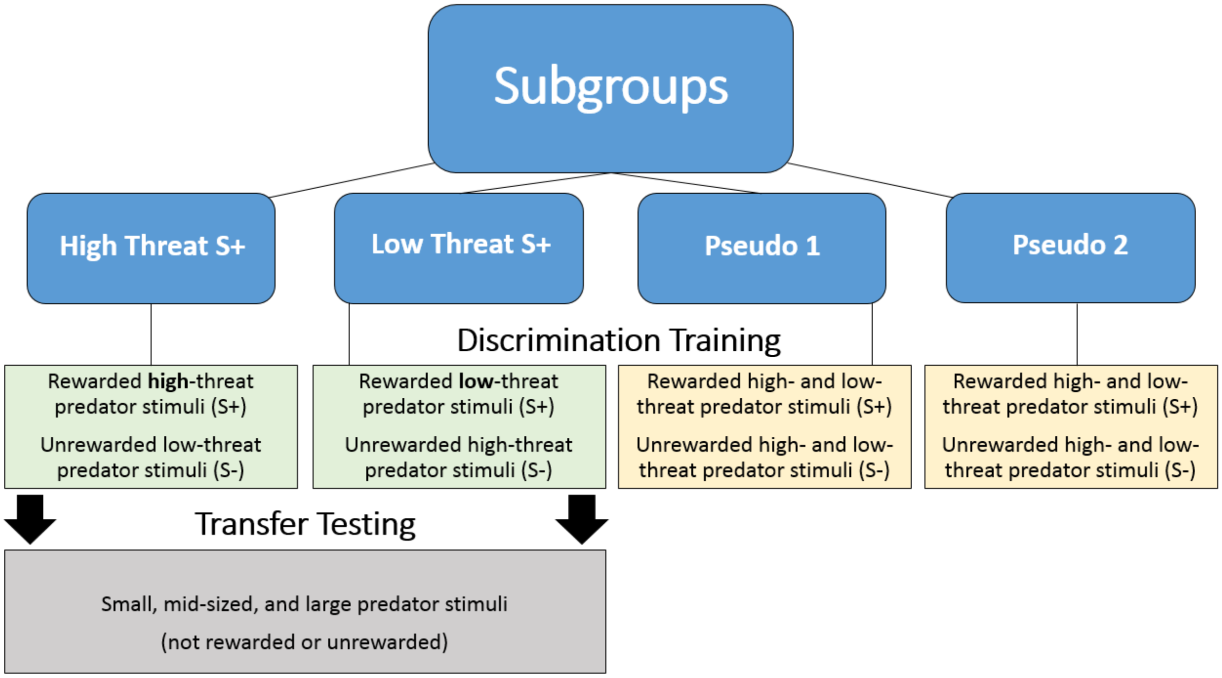

Figure 5-3. Schematic of the stimulus types and reward contingencies for Discrimination Training and Transfer testing for the four (4) subgroups. Only True groups (High Threat S+ and Low Threat S+ subgroups) moved on to Transfer testing in which testing stimuli were not rewarded or unrewarded.

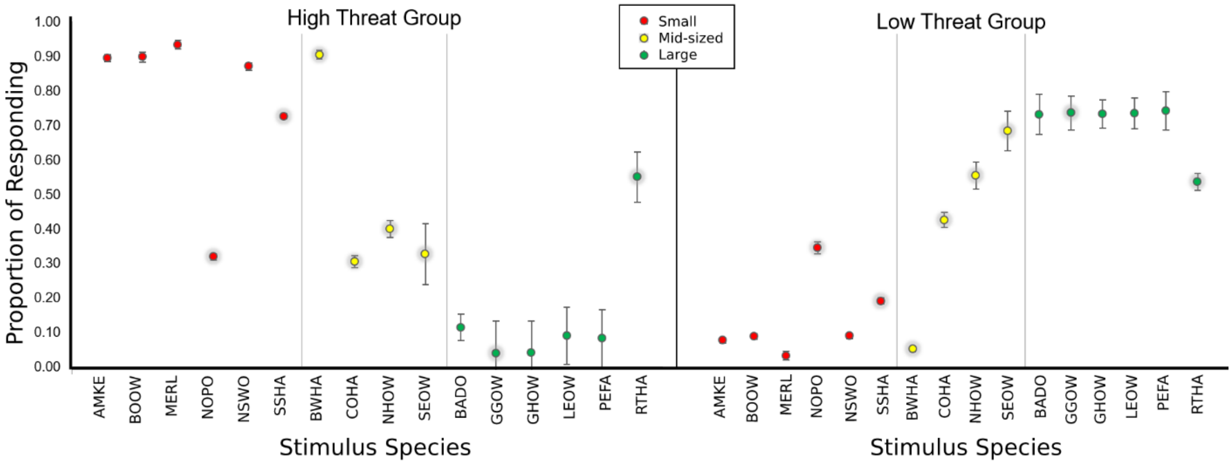

Figure 5-4. Average ± SEM proportion of responding to each stimulus species by black-capped chickadees in the High Threat (left) and Low Threat (right) groups during Transfer testing. AMKE: American kestrel; BOOW: boreal owl; MERL: merlin; NOPO: northern pygmy-owl; NSWO: northern saw-whet owl; SSHA: sharp-shinned hawk; BWHA: broad-winged hawk; COHA: Cooper's hawk; NHOW: northern hawk owl; SEOW: short-eared owl; BADO: barred owl; GGOW: great gray owl; GHOW: great horned owl; LEOW: long-eared owl; PEFA: peregrine falcon; RTHA: red-tailed hawk.
Note: Species with shadowed points indicate calls used as Transfer testing stimuli.

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Chapter 6. Black-capped chickadee (*Poecile atricapillus*) behavioural responses to avian and mammalian predator mounts of varying threat levels

Introduction

Predation is a major evolutionary force as it directly impacts fitness, and adaptive antipredator behaviours (Lima & Dill, 1990). In the presence of a predator, a common antipredator response is the production of alarm calls. In addition to signalling the presence of a predator, alarm calls can also provide information about the predator type (e.g., avian vs. mammalian). Seyfarth et al. (1980) demonstrated that vervet monkeys (*Cercopithecus aethiops*) produce different alarm calls to signal the presence of either a leopard (*Panthera pardus*), eagle (*Polemaetus bellicosus*), or snake (*Python sebae*), and each of these calls elicits a specific response from conspecifics. Vocal antipredator responses can also communicate the threat level of a predator. For example, black-capped chickadees (*Poecile atricapillus*) and Carolina chickadees (*P. carolinensis*) produce *chick-a-dee* calls, used as mobbing calls in the presence of predators (Smith, 1991; Templeton et al., 2005; Soard & Ritchison, 2009); this vocalization is composed of four note types that can be repeated or omitted: A, B, C, and D notes (Charrier et al., 2004; Bloomfield et al., 2005). Both species produce *chick-a-dee* mobbing calls with more D notes in response to high-threat predators relative to low-threat predators or non-threat species. This allows for the communication of threat level to be conveyed to conspecifics and elicits adaptive behavioural responses such as approaching the source of the alarm calls to assist with mobbing (black-capped chickadees to avian predators: Templeton et al., 2005; Carolina chickadees to avian predators: Soard & Ritchison, 2009).

Animals can also use a predator's size to evaluate potential threat and the relationship between predator size and perceived threat will vary for different species. For example, when male chickens (*Gallus gallus domesticus*) were exposed to three raptors of various sizes, they responded most evasively to the largest accipiter (Palleroni et al., 2005). This suggests that male chickens, a larger avian species, perceive larger avian predators as more of a threat than smaller avian predators. However, for relatively small songbirds, such as black-capped chickadees, Carolina chickadees, and tufted titmice (*Baeolophus bicolor*), the strongest antipredator response is displayed towards small avian predators compared to larger ones (Templeton et al., 2005; Soard & Ritchison, 2009; Courter & Ritchison, 2010). These studies suggest that parids (chickadees and titmice) perceive a predator to be of higher threat if it is smaller rather than larger. This relationship is thought to be due to the fact that smaller avian predators are more maneuverable and better able to capture a parid in a forested environment (Templeton et al., 2005). Size-threat relationships appear to be adaptive, with only a few exceptions (e.g., blue whales predating krill). Both chickens and parids display adaptive size-threat relationships, which matches the optimal foraging choices of predators. For example, large raptors would be unlikely to pursue small songbirds as they would receive little energetic benefit and because songbirds would be difficult to capture (Steenhof & Kochert, 1988; Pyke et al., 1977). Instead, small raptors are more likely to target parids as these raptors are maneuverable enough to efficiently capture small songbirds in forested areas and meet their energetic needs (Templeton et al., 2005; Pyke et al., 1977). Therefore, size should be an important factor that prey use when determining the threat of a predator.

Certain animals also have the ability to recognize key features of predators and this ability can be used to evaluate the degree of threat of a potential predator within a predator type. When presented with sparrowhawk (*Accipiter nisus*) and pigeon models that had either their beaks or eyes interchanged with the other species, Great tits (*Parus major*) exhibited no fear of pigeon models with hawk eyes and a reduction in fear responses to hawk models with pigeon eyes (e.g., as assessed by measuring the total occurrence of raising feathers on head, production of warning calls, etc.; Beránková et al., 2014). This suggests that prey use general features of raptors, such as body type, to determine if a bird is harmless or a potential predator (e.g., pigeon with hawk eyes is harmless). Meanwhile, specific features, such as eye colour (e.g., hawk with pigeon eyes), allow for a more refined assessment of the threat level of a predator. This would allow varying responses to different groups and species of raptors and may allow recognition of exceptions to size-threat relationships. While this ability has not been evaluated in responses by songbirds to mammals, it is likely that a similar ability exists due to the adaptive value of predator discrimination.

Although animals demonstrate the ability to evaluate predator threat and categorize predators based on visual stimuli, antipredator responses vary greatly among species as some species will have predator-type specific responses. For example, European rabbits (*Oryctolagus cuniculus*) produce a freezing response to avian predators, but flee in response to mammalian predators (Pongácz & Altbäcker, 2000). In contrast, other species have the same response to predators of different types, but modify this response based on threat. For example, California ground squirrels (*Spermophilus beecheyi*) responded similarly to a dog (*Canis lupus familiaris*) and a red-tailed hawk (*Buteo jamaicensis*) but fled more quickly from the hawk, likely due to the more immediate threat posed (Hanson and Coss; 1997). Currently, the factors that predict whether a species will have a predator type-specific response are not well-understood.

To further explore antipredator behaviour to visual cues, we investigated the responses of wild black-capped chickadees to avian and mammalian predator mounts of varying threat levels. Parids have been known to respond to acoustic stimuli with predator type-specific responses (Suzuki, 2012), and to visual stimuli according to the level of threat (Soard & Ritchison, 2009; Courter & Ritchison, 2010; Suzuki, 2011). In general, small avian predators pose a higher threat to chickadees than mammalian predators (Templeton et al., 2005), and therefore, we expected to find more intense antipredator behaviours in response to avian predators compared to mammalian predators (vocal: *chick-a-dee* call production; behavioural: reduced feeding activity).

In summary, although there has been much research investigating chickadee responses to avian predators, there is little on how chickadees respond to mammalian predators. Thus, the purpose of the current experiment is to address the gap in the literature regarding how black-capped chickadees respond to the mammalian equivalents of avian threats. We predicted that chickadees would react similarly when confronted with high-threat mammalian predators compared to high-threat avian predators by producing more *chick-a-dee* vocalizations to both avian and mammalian predators of high-threat, in comparison to low-threat and non-threat and control conditions. Furthermore, we predicted that more D notes would be produced per call in response to avian predators compared to mammalian predators as avian predators have easier access to chickadees located in trees. Last, we predicted that feeding behaviour would decrease most in response to high-threat avian and mammalian predators. These findings provide further insight into the antipredator responses of black-capped chickadees to both avian and mammalian predators.

Methods

Predator, Non-predator, and Control Mounts

We chose avian and mammalian species of three threat levels: (1) high-threat (sharp-shinned hawk, *Accipiter striatus*; domestic cat, *Felis catus*), (2) low-threat (red-tailed hawk; American red squirrel, *Tamiasciurus hudsonicus*), and (3) non-threat (northern flicker, *Colaptes auratus*; woodchuck, *Marmota monax*). Avian predators were chosen based on their degree of specialization as songbird predators, as well as their wingspan and size, based on the negative relationship between perceived threat and both wingspan and size (Templeton et al., 2005; Soard & Ritchison, 2009). Additionally, avian and mammalian non-threat species were chosen based on how closely they matched the size of the high-threat predator, which allows any size-threat effects to be controlled (Templeton et al., 2005; Soard & Ritchison, 2009). We chose the sharp-shinned hawk (*Accipiter striatus*) as our high-threat avian predator because it is a songbird specialist with a small wingspan (Sibley, 2003). The red-tailed hawk (*Buteo jamaicensis*) was chosen as the low-threat avian predator as they are large in size, have a relatively large wingspan, and only occasionally prey on songbirds (Sibley, 2003; Fitch et al., 1946). The northern flicker (*Colaptes auratus*) was selected as the avian non-threat species as they are approximately the same size as the sharp-shinned hawk with a similar wingspan, and do not prey on songbirds (Sibley, 2003).

Mammalian predator selection was based primarily on their degree of specialization on songbirds as size-threat relationships have not been thoroughly tested in mammals. The domestic cat (*Felis catus*) was chosen as a high-threat predator as they are estimated to prey upon 100 to 350 million birds per year in Canada (Blancher, 2013). Tufted titmice and Carolina chickadees are known to reduce feeding activity and alter call production in response to cat models being orientated toward a feeder (Book & Freeberg, 2015; Freeberg, Book, & Weiner, 2015). Fear of cats also appears to increase with urbanization (Van Donselaar et al., 2018). The American red squirrel (*Tamiasciurus hudsonicus*) was chosen as the low-threat mammalian predator as they are a nest predator of songbirds that only prey upon adult chickadees opportunistically (Fontaine & Martin, 2006; Steele, 1998). The woodchuck (*Marmota monax*) was chosen as the mammalian non-threat species as they approximate the size of a cat, but pose no threat to chickadees as they are herbivorous (Kwiecinski, 1998). The short-tailed weasel (*Mustela erminea*) was added as an additional low-threat testing species as they primarily consume mammals that are rabbit-sized or smaller, but when those food sources are scarce will occasionally consume birds and eggs (Erlinge, 1981). All predator and non-predator species can be found within our study location (Edmonton, AB, Canada); more details on these species can be found in Table 1.

All avian species were mounted in a perched position; head orientation varied, but during the exposures the mounts were orientated so that both eyes faced the feeder. Mammalian species were mounted in a seated position (domestic cat and American red squirrel) or in a standing positions on all fours (woodchuck and short-tailed weasel); head orientation varied, but during the exposures the mounts were orientated so that both eyes faced the feeder. In addition to the non-threat species (northern flicker and woodchuck), we included an additional inanimate control of a foam cylinder (Table 6-2; Figure 6-1).

The red-tailed hawk, northern flicker, and red squirrel taxidermy mounts were obtained from the University of Calgary (Calgary, AB, Canada). The sharp-shinned hawk was obtained from the Royal Alberta Museum then taxidermied (Edmonton, AB, Canada). Woodchuck and weasel pelts were obtained online and taxidermied in the Songbird Neuroethology Laboratory by A.M.M.S. (University of Alberta, Edmonton, AB, Canada). The cat mount was a toy white persian cat (FurReal Friends Lulu white persian cat 66520 Interactive Toy Hasbro Inc., Pawtucket, Rhode Island, USA). The foam cylinder (height: 13 cm; diameter: 9.9 cm; bottom circumference: 33.2 cm; top circumference: 27.5 cm; this mount was larger than the American red squirrel mount but smaller than the cat mount) was created by rolling and gluing a sheet of foam (Fabricland, Edmonton, AB, Canada). Each condition mount was affixed to a platform and surrounded by wire (i.e, cage-like) to reduce potential damage to our equipment by any mobbing activity displayed by chickadees.

Procedure

Mount exposures were conducted in the field to populations of wild black-capped chickadees between July 17 and August 23, 2017, after nestling chickadees had fledged (Dr. Kimberley Mathot & A.M.M.S.'s observations). In the month prior to the experiment, five feeder locations (containing black oil sunflower seeds) were established in Edmonton River Valley between 53.534770, -113.527915 and 53.530746, -113.525285 coordinates in Edmonton, AB, Canada. Feeders were a minimum of approximately 200 m apart in order to reduce the likelihood that birds were feeding at multiple feeders. Breeding territories have been reported as approximately 1.07 hectares (2.64 acres; Brewer, 1963). Thus, overlap in birds at our feeders is possible, but all measures were taken to reduce the likelihood that birds would have encountered the same mount twice (e.g., one mount per site per day).

Sites were visited between 09:00 to 13:00. Mount conditions were pseudo-randomly assigned in that each condition was successfully conducted at each feeder site over the course of the experiment, but no mount was used more than once per day. During the experiment, the mount was affixed to a platform and surrounded by a wire cage. The platform was placed on a stand (either "tall" or "short", 4m or 2m, respectively, to ensure the mounts' eyes were each at feeder height and attached to a 20lb Weider Barbell base plate; see Figure 6-2) approximately 1m from the feeder on a flat surface to hold the base of the stand in position. During baseline, the mount was covered by a black plastic bag. Baseline audio and video recordings began when black-capped chickadees were audible and visible in the area of the feeder. Audio recordings were conducted with Marantz Professional PMD561 handheld solid-state recorder (Marantz America Inc., Kanagawa, Japan) placed on the base of the mount prior to baseline. Video recordings were conducted with Canon HD VIXIA HF R500 camcorder (Canon Inc., Tokyo, Japan) by an experimenter sitting out of view of the mount and zooming in to obtain an image of the feeder and surrounding area. Additional notes about the number of birds in the area were dictated and recorded by the camera and also recorded in a notebook. Mounts were exposed by quietly removing the black plastic bag following five minutes of baseline observations. Mount Exposure started when the birds appeared to see and react to the mount exposure (e.g., looking toward the mount then initial freezing or call production). If baseline was conducted and chickadees left the area before mount exposure, the mounts were not revealed. If the chickadees did not appear to see the mount exposure, the condition was attempted again a subsequent day at the respective site.

Behavioural Analysis

Black-capped chickadee vocalizations were recorded at the location of the feeder during baseline and mount exposures. We examined behaviour for five minutes immediately prior to mount exposure (denoted as “Baseline”) and the first five minutes following exposure of the mount (denoted as “Mount Exposure”). Vocal analysis on the audio recordings was conducted using SIGNAL 5.10.24 software (Engineering Design, Berkeley, CA, USA). Non-vocal behaviour analysis was conducted using VLC media player (VideoLAN, Paris, France). The number of visible chickadees surrounding the feeder and feeding behaviour (defined as removing a seed from the feeder) was documented for five minutes of baseline prior to mount exposure and the first five minutes following exposure of the mount.

The number of chickadees audible and visible in the area, and number of chickadees in the immediate area (i.e., close to the feeder at approximately 5m or less), were recorded at the time of the experiment in a notebook and later confirmed using video footage. For *chick-a-dee* calling, we divided the number of calls during both baseline and mount exposure by the average number of black-capped chickadees audible and visible (from video recordings and field notes, not audio recordings). For feeding behaviour, we divided the number of feeder visits by black-capped chickadees during baseline by the average number of chickadees in the immediate area during baseline. We divided the number of feeder visits by black-capped chickadees during mount exposure by the average number of chickadees in the immediate area during mount exposure. Difference scores were then calculated by subtracting behaviour during baseline from behaviour during mount exposure.

Statistical Analyses

To determine if black-capped chickadees produced more *chick-a-dee* calls to both avian and mammalian predators of high threat, in comparison to low-threat, non-threat, and control conditions, we conducted a one-way ANOVA on the difference scores (Mount Exposure - Baseline). Significant differences were further investigated with independent samples *t*-tests, with Bonferroni corrections ($p < .05/6$ tests $< .008$; $p < .05/10$ tests $< .005$).

To determine if black-capped chickadees produced more *chick-a-dee* calls with a high number of D notes per call in response to high-threat avian predators compared to high-threat mammalian predators, we conducted independent samples *t*-tests using calls produced with 4-8 D notes as Templeton et al. (2005) indicated approximately four D notes per call were produced to high-threat predators. *Chick-a-dee* calls with zero to three D notes per call were not included in this analysis; recorded chickadees produced more than eight D notes per call on occasion, but only rarely so these calls were also not included in the statistical analysis of D notes.

To further compare our findings with that of Templeton et al.'s (2005), we investigated vocal response to small/high-threat vs. large/low-threat avian predators, specifically to determine if black-capped chickadees produced more calls with 4 D notes to the sharp-shinned vs. red-tailed hawk, and 2 D note calls to the red-tailed vs. sharp-shinned hawk; we conducted a one-way ANOVA of 2-4 D notes, then two independent samples *t*-tests using calls produced with 4 D notes and 2 D notes between the sharp-shinned and red-tailed hawk conditions to further investigate the significant differences.

To investigate feeding behaviour, we conducted two one-way ANOVA on the difference scores (Mount Exposure - Baseline) in feeding behaviour in avian (sharp-shinned hawk, red-tailed hawk, northern flicker, control) and mammalian conditions (domestic cat, American red squirrel, woodchuck, short-tailed weasel, control). Significant differences were further investigated with independent samples *t*-tests, with Bonferroni corrections ($p < .05/6$ tests $< .0083$).

Results

Audio Analyses: *Chick-a-dee* calls

Overall call production. To determine if black-capped chickadees produced more *chick-a-dee* calls predators of high-threat, in comparison to low-threat, non-threat, and control conditions, we conducted one-way ANOVAs on the difference scores (Mount Exposure - Baseline) for avian and mammalian conditions. There were significant differences in *chick-a-dee* call production across conditions for avian species and mammalian species (Avian: $F_{3,16} = 37.206$, $p < .001$, $\eta^2 = .406$; Mammalian: $F_{4,20} = 5.694$, $p = .003$, $\eta^2 = .388$); see Figure 3. We then conducted independent samples *t*-tests on *chick-a-dee* call production during avian conditions, with Bonferroni corrections ($p < .008$); Avian High- vs. Low-threat: $t_8 = 5.902$, $p < .001$, $d = 4.173$, 95% CIs = 17.653, 40.297; Avian High- vs. Non-threat: $t_8 = 9.157$, $p < .001$, $d = 6.475$, 95% CIs = 26.734, 44.733; Avian High-threat vs. Control: $t_8 = 19.318$, $p < .001$, $d = 13.660$, 95% CIs = 36.895, 46.897; all other *p*s > .024. We then conducted independent samples *t*-tests on *chick-a-dee* call production during mammalian conditions, with Bonferroni corrections ($p < .005$); short-tailed weasel vs. Control: $t_8 = 5.028$, $p = .001$, $d = 3.555$, 95% CIs = 7.057, 19.014; all other *p*s > .006. These results indicate that chickadees were only producing significantly more *chick-a-dee* calls in response to our “low-threat” short-tailed weasel in comparison to the control foam cylinder. See Table 6-2 for the remaining comparisons.

D note production. To determine if black-capped chickadees produced *chick-a-dee* calls with a higher number of D notes in response to high-threat avian mount exposure compared to high-threat mammalian mount exposure, we conducted independent samples *t*-tests using calls produced with 4-8 D notes: $t_{32.970} = 4.022$, $p < .001$, $d = 2.844$, 95% CIs = 1.628, 4.883. Thus, chickadees exposed to avian conditions produced more *chick-a-dee* calls contained 4-8 D notes per call in comparison to chickadees exposed to mammalian conditions. See Figure 4 and 5.

To further investigate vocal response to small/high-threat vs. large/low-threat avian predators, specifically to determine if black-capped chickadees produced more calls with 4 D notes to the sharp-shinned vs. red-tailed hawk, and 2 D note calls to the red-tailed vs. sharp-shinned hawk, we conducted a one-way ANOVA of 2-4 D notes, $F_{1,28} = 19.955$, $p < .001$, $\eta^2 = .632$. To investigate significant differences, we conducted three independent samples *t*-tests using calls produced with 4 D and 2 D notes in response to these two conditions, with Bonferroni corrections ($p < .025$). Calls with 4 D notes were produced significantly more often in response to the sharp-shinned hawk compared to the red-tailed hawk condition ($t_8 = 2.939$, $p = .019$, $d = 2.078$, 95% CIs = 1.123, 9.311), but calls produced with 2 D notes was not significantly different between the two conditions ($t_8 = 1.979$, $p = .083$, $d = 1.399$, 95% CIs = -0.502, 6.583).

Video Analyses: Feeding behaviour

To investigate feeding behaviour, we conducted two one-way ANOVAs on the difference scores (Mount Exposure - Baseline) in feeding behaviour to avian (sharp-shinned hawk, red-tailed hawk, northern flicker, control) and mammalian conditions (domestic cat, American red squirrel, woodchuck, short-tailed weasel, control). There were significant differences in feeding behaviour between avian conditions (high/low/non-threat/control; $F_{3,16} = 9.542$, $p = .001$, $\eta^2 = .177$, CIs = -3.4986, 0.0597), but there were no significant differences in feeding behaviour between the three mammalian conditions (high/low/non-threat/control; $F_{4,20} = 2.233$, $p = .102$, $\eta^2 = .028$, CIs = -2.064, 0.885). We then conducted independent samples *t*-tests, with Bonferroni corrections ($p < .0083$), to determine exactly where feeding behaviour differed across avian conditions. Feeding behaviour was significantly lower during sharp-shinned hawk (high-threat) mount exposure compared to the foam cylinder control ($t_8 = -5.319$, $p = .001$, $d = -1.075$, 95% CIs = -10.805, -4.270), and lower during red-tailed hawk (low-threat) mount exposure compared to the foam cylinder control ($t_8 = -3.822$, $p = .005$, $d = -2.703$, 95% CIs = -10.749, -2.659). There were no significant differences in feeding behaviour during sharp-shinned hawk (high-threat) mount exposure compared to northern flicker (non-threat; $t_8 = -2.935$, $p = .019$, $d = -1.075$, 95% CIs = -7.121, -0.855), or in any other comparisons ($ps \geq .053$); see Figure 6.

Discussion

In the current study, we predicted that wild black-capped chickadees would produce more *chick-a-dee* mobbing vocalizations to both avian and mammalian predators of high-threat (sharp-shinned hawk, domestic cat) in comparison to low-threat (red-tailed hawk, American red squirrel), non-threat (northern flicker, woodchuck), and control conditions (foam cylinder), conducted as separate avian and mammalian analyses. Only *chick-a-dee* call production in response to avian mounts, and between the short-tailed weasel and control, differed significantly.

Furthermore, we predicted that D notes would be produced at a higher rate in response to avian predators directly compared to mammalian predators as avian predators have easier access to chickadees located in trees. We found that chickadees produced more *chick-a-dee* calls with four to eight D notes in response to avian mounts compared to mammalian mounts. In addition, comparing between avian threats, we found that chickadees produced significantly more four D note calls in response to the sharp-shinned hawk in comparison to the red-tailed hawk, in parallel with Templeton et al.'s (2005) results. Last, we predicted that feeding behaviour would decrease most in response to high-threat avian and mammalian predators, conducted as separate avian and mammalian analyses. Instead, we found that feeding was decreased in response to both high-threat and low-threat avian conditions compared to the control, and did not alter across mammalian conditions.

Wilson and Mennill (2011) argue that the duty cycle (i.e., note and call frequency) of the *chick-a-dee* call determines the varying responses instead of the fine structure of the call (i.e., how many notes per call) as proposed by Templeton et al. (2005). Compared to change in fine structure, Wilson and Menill (2011) found that changes in duty cycle of *chick-a-dee* calls resulted in more conspecific and heterospecific receivers approaching the speaker, quicker speaker approach, and receivers remaining within 10m of the speaker for longer. Thus, we predicted that chickadees would produce more *chick-a-dee* calls to avian and mammalian species that we had classified as high-threat compared to low-threat, non-threat, or control based on perceived threat level and irrespective of body size. Instead, we found that *chick-a-dee* call production only significantly differed across avian mounts, specifically, with more calls being produced in response to sharp-shinned hawk (high-threat) compared to northern flicker (non-threat), and sharp-shinned hawk compared to the foam cylinder (control), but not the sharp-shinned hawk compared to red-tailed hawk (low-threat). These results parallel those of Wilson and Mennill (2011) in that more *chick-a-dee* calls were produced (i.e., higher duty cycle) to high-threat avian predators. These results also demonstrate that avian predators of high- and low-threat level are not perceived differently. This suggests that chickadees may perceive avian predators on a continuum of varying threat. Additionally, chickadees may not perceive mammalian predators in the same way that we categorized them. Mammalian diet may not be the best predictor of threat perception by parids.

Next, we predicted that chickadees would react differentially when confronted with avian predators compared to mammalian predators, in both *chick-a-dee* call D note production and feeding behaviour. We confirmed that high-threat avian mounts resulted in the production of more *chick-a-dee* calls containing four to eight D notes compared to the high-threat mammalian mounts, but that feeding was reduced only in response to sharp-shinned and red-tailed hawk mounts compared to the control mount and non-significant across mammalian conditions. Templeton et al. (2005) reported that chickadees produced mobbing calls with varying note production for predators of varying sizes predators. Specifically, chickadees produced an average of approximately four D notes in response to small predators (e.g., northern pygmy and saw-whet owls; *Glaucidium californicum*, *Aegolius acadicus*), but only an average of approximately 2.5 D notes per call to large, red-tailed hawks (Templeton et al., 2005). In addition, we confirmed that chickadees produced 4 D notes per call significantly more to the sharp-shinned hawk in comparison to the red-tailed hawk. Therefore, we had also predicted that chickadees' feeding behaviour would differ to the sharp-shinned hawk mount, a smaller, higher threat predator, compared to the larger red-tailed hawk. However, this was not confirmed. Similar to *chick-a-dee* call production, feeding behaviour was similar to both the high- and low-threat avian predators. This suggests that the sharp-shinned hawk and red-tailed hawk were not perceived and responded to be of significantly different threat levels, further suggesting a continuum of threat. However, these finding that these two species are not perceived as significantly different threat levels is in support of other studies that found no significant differences in mobbing behaviour between what they classified as intermediate-sized avian predators, such as the sharp-shinned hawk, and large predators, such as the red-tailed hawk (Soard & Ritchison, 2009; Courter & Ritchison, 2010).

As suggested above, chickadees may not perceive mammalian predators in the way that we categorized them according to posed threat. Due to a lack of research on how chickadee behaviour varies in response to mammalian predators, there is little evidence for how black-capped chickadees respond to avian and mammalian predators of similar threat levels. The majority of the avian research equated higher posed threat to a smaller body size (e.g., Templeton et al., 2005; Wilson & Mennill, 2011), yet we predicted that mammalian body size would not be a determining factor of threat perception. According to avian predator diets, we determined what level of threat predators would pose to chickadees based on their diet rather that the size of their body (i.e., the smaller avian predator being high-threat). Diet appeared to be a more appropriate indicator of threat than body size as, for example, cats are of more danger to chickadees compared to smaller American red squirrels or short-tailed weasels that sometimes consume chickadee eggs (Blancher, 2013).

Previous studies on Carolina chickadees and tufted titmice have shown threat-based responses by these species, significant differences in mobbing intensity typically only occur between small and consequently high-threat owls compared to large, low-threat avian predators, but not between intermediate-sized and large predators (Soard & Ritchison, 2009; Courter &

Ritchison, 2010). Therefore, these results suggest that perhaps large differences in perceived threat are required to produce significantly different threat-based responses, thus having important implications concerning studies using mammalian predators. It is impossible to confirm this currently since little is known about how chickadees perceive mammalian threat, however, it is possible that a mammalian equivalent to a small, high-threat avian predator may not exist. Instead, high-threat mammalian predators, such as the domestic cat, may be perceived similar to intermediate-sized avian predators. Evidence of this can be seen in the vocal responses of black-capped chickadees to domestic cats and to intermediate-sized raptors as they produced approximately the same number of D notes in response to both the domestic cat and intermediate-sized raptors (e.g., Cooper's hawk; Templeton et al., 2005). While the perceived level of threat posed by the American red squirrel mount is currently unknown, it is likely that since the red squirrel is not typically a predator of adult chickadees that it would be perceived to be at the lower bounds of mammalian threat (Fontaine & Martin, 2006; Steele, 1998). If accurate, this would make the threat level posed by the American red squirrel approximately the same as a large raptor. Therefore, it is possible that a lack of significant differences in responding between the red squirrel (comparable to low-threat raptors) and domestic cat (comparable to mid-threat raptors) mount may simply be due to there not being enough of a difference in perceived threat. If this is the case, it suggests that it may be difficult to find significant threat-based responses to mammalian predators in future experiments. Despite that domestic cats are a more recent pervasive issue in Canada, we continue to support our choice of the cat mount as the high-threat mammalian condition as evolution of antipredator responses can develop rapidly (e.g., evolution of the peppered moth, *Biston betularia*; Kettlewell, 1973). However, since we know that fear of cats increases with urbanization and antipredator responses are stronger in areas with cats (Van Donselaar et al., 2018; Book & Freeberg, 2015), future experiments using additional mammalian predators, and experiments conducted nearer to residential homes to provide more conclusive results regarding cat predators, will be required to confirm this.

While there may not be enough variation in perceived threat among mammalian predators to have quantitative differences within a particular response (i.e., call production or feeding behaviour), it is possible that chickadees respond to mammalian predators of varying threat levels with qualitatively different antipredator behaviours. This idea is supported in that some species produce different antipredator responses to predators of adults compared to nest predators. For example, lapwings (*Vanellus vanellus*) have been found to engage in diving and striking behaviours when they are presented with a nest predator, a crow (*Corvus corone*), while they engage in various other types of behaviours, such as leading and distraction behaviours, when they encountered a red fox (*Vulpes vulpes*; Elliot, 1985). These qualitatively different responses are thought to be a product of the threat the predator poses to an adult lapwing; a crow is low-threat to an adult lapwing and consequently they engaged in contact behaviours. Black-capped chickadees are known to engage in a variety of antipredator behaviours when a predator is presented close to their nest, such as wing waving and body waving (Clemmons & Lambrechts, 1992); therefore, perhaps the location of our mount exposure (i.e., near a feeder) was not enough to elicit notable qualitatively different antipredator responses in wild black-capped chickadees. It is possible that chickadees only engage in these diverse behaviours near their nest if they have offspring, and instead may ignore nest predators that pose no harm to adults if they do not have a nest to defend. Ignoring nest predators prior to laying their eggs has been observed in other species, such as black-billed magpies (*Pica hudsonia*; Buitron, 1983). If this is also the typical response of chickadees without a nest, it would explain the general lack of behavioural differences observed to the American red squirrel and short-tailed weasel in comparison to the domestic cat mount.

Future studies are required to determine if other species or locations with more cat exposure elicit quantitatively different responding, if qualitatively different antipredator behaviours occur in response to predators of adults compared to nest predators, and if the presence or absence of offspring is required to elicit nest predator specific behaviours. Despite our efforts in contacting museums, conservation sites, stores, and in online shopping, we were only able to obtain one mount per condition. Thus, we also recommend that future studies obtain more predator mounts and the recordings are conducted at more feeder sites. Due to these potential drawbacks, we suggest that our findings are interpreted with caution. Taken together, we propose that these findings provide further insight into the antipredator responses of black-capped chickadees to the visual exposure of avian and mammalian predators of varying sizes and diets. Although we found little evidence for varying responses to mammalian predators, these findings provide further insight into the antipredator responses of black-capped chickadees to both avian predators and advocate for future experiments investigating the perception of mammalian predators.

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Table 6-1. Summary of predator species presented to wild black-capped chickadees. Information regarding avian species is from Sibley (2003), Fitch et al. (1946), and White & Stiles (1990); for birds, body length was measured from bill tip to tail tip, and reported body length and wingspan values are averages for each species. Information regarding mammalian species is from Turner & Bateson (2000), Jones (1977), Steele (1998), Kwiencinski (1998), Merrit (1987), and Erlinge (1981); for mammals, body length does not include tail length.

Predator Type	Predator Species	Length (cm)	Wingspan (cm)	Diet	Threat Level
Avian	Sharp-shinned hawk (*Accipiter striatus*)	28	57	Primarily songbirds Occasionally insects	High-threat
	Red-tailed hawk (*Buteo jamaicensis*)	48	124	Primarily small mammals Occasionally birds	Low-threat
	Northern flicker (*Colaptes auratus*)	32	51	Primarily insectivorous Occasionally frugivorous	Non-threat
Mammalian	Domestic cat (*Felis catus*)	50	N/A	Commercial cat food (indoor), small mammals, songbirds	High-threat
	American red squirrel (*Tamiasciurus hudsonicus*)	17	N/A	Primarily seeds from spruce cones Opportunistically eggs and young birds	Low-threat
	Woodchuck (*Marmota monax*)	45	N/A	Primarily herbivorous Occasionally insectivorous	Non-threat
	Short-tailed weasel (*Mustela erminea*)	25	N/A	Primarily mammals (rabbit-sized or smaller) Occasionally birds, eggs, and insects	Low-threat (additional)
Control	Foam cylinder	height: 13 cm; diameter: 9.9 cm; top/bottom circumference: 27.5/33.2 cm			Control

Table 6-2. The results of the independent-samples *t*-tests conducted on *chick-a-dee* call production during avian and mammalian conditions, with Bonferroni corrections (Avian: $p < 0.008$; Mammalian: $p < 0.005$).
Positive *t*-values indicate more responding by chickadees to the first condition in the comparison; negative *t*-values indicate more responding by chickadees in to the second condition in the comparison.

Comparisons (*df* = 8)	***t*-value**	***p*-value**	**Cohen's *d***	**Confidence Intervals**
Avian Conditions (*p* < .008)				
High vs. Low	5.902	**<.001**	4.173	17.653, 40.297
High vs. Non-	9.157	**<.001**	6.475	26.734, 44.733
High vs. Control	19.318	**<.001**	13.660	36.895, 46.897
Low vs. Non-	1.191	.268	0.842	-6.332, 19.850
Low vs. Control	2.773	.024	1.961	2.178, 23.664
Non- vs. Control	1.721	.124	1.217	-2.097, 14.421
Mammalian Conditions (*p* < .005)				
High vs. Low	1.039	.329	0.735	-3.638, 9.609
High vs. Woodchuck	-0.650	.534	-0.460	-10.730, 6.009
High vs. Short-tailed weasel	-2.133	.065	-1.508	-15.160, 0.590
High vs. Control	2.137	.065	1.511	-0.456, 11.956
Low vs. Short-tailed weasel	-3.694	.006	-2.612	-16.681, -3.860
Low vs. Woodchuck	-1.759	.117	-1.244	-12.355, 1.663
Low vs. Control	1.520	.167	1.075	-1.430, 6.960
Short-tailed weasel vs. Woodchuck	1.385	.204	0.979	-13.126, 3.277
Short-tailed weasel vs. Control	5.028	**.001**	3.555	7.057, 19.014
Woodchuck vs. Control	2.827	.022	1.999	1.495, 14.727

Note: **Bold** font indicates statistical significance.

Figure 6-1. Photographs of all eight mounts (**A**: sharp-shinned hawk; **B**: domestic cat; **C**: red-tailed hawk; **D**: American red squirrel; **E**: northern flicker; **F**: woodchuck; **G**: control foam cylinder; **H**: short-tailed weasel).
Note: Size is not comparative between photos; photos have been provided for visual details of the experimental mounts only.

Figure 6-2. A photograph of the northern flicker (non-threat avian species) mount on the "tall" stand at feeder height, approximately 1m from the feeder.

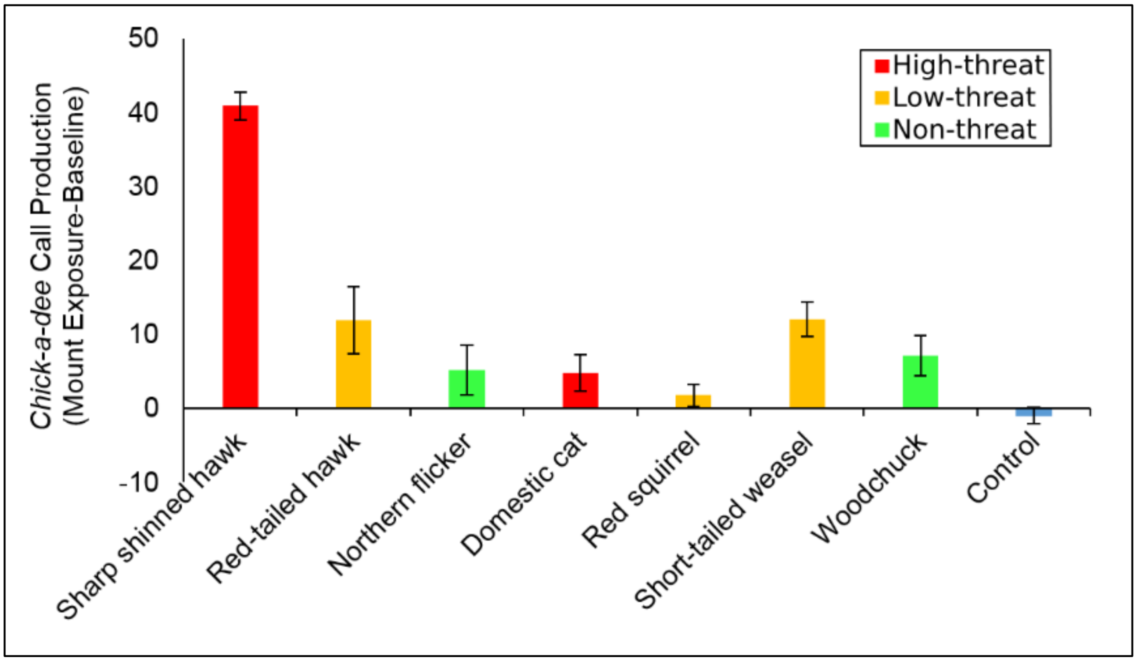

Figure 6-3. *Chick-a-dee* call production as a difference score (Mount Exposure - Baseline) ± SEM in response to each experimental condition.

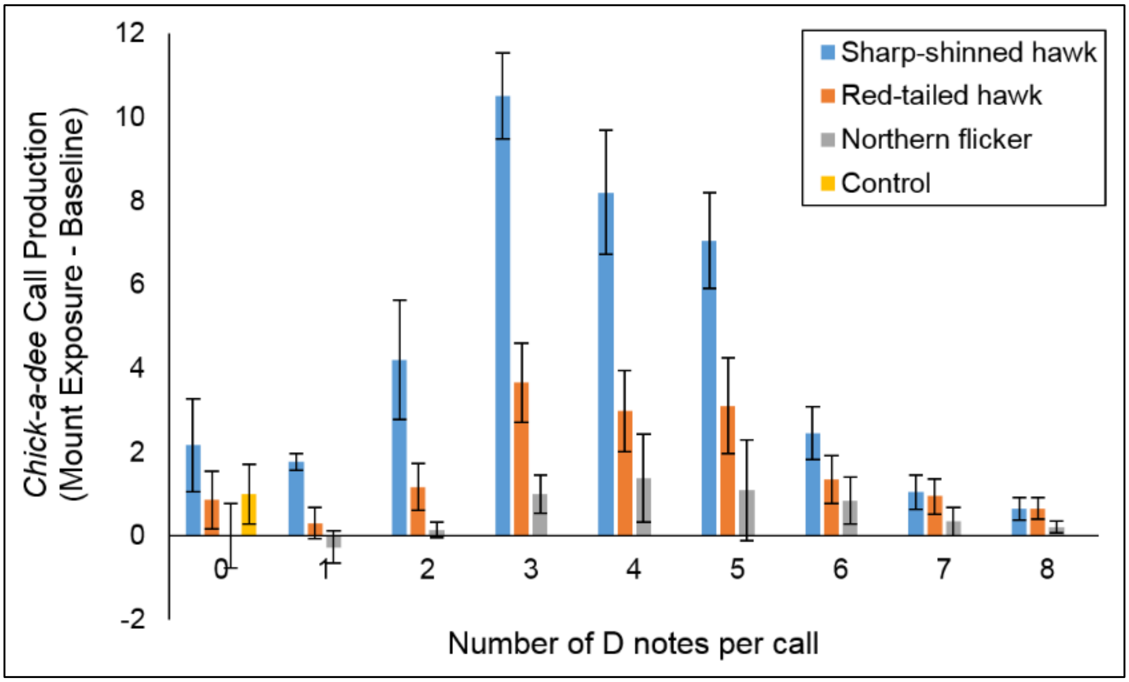

Figure 6-4. *Chick-a-dee* call production as a difference score (Mount Exposure - Baseline) ± SEM in response to avian mounts by the number of D notes produced. Negative values indicate a decrease in production of *chick-a-dee* call type from baseline.

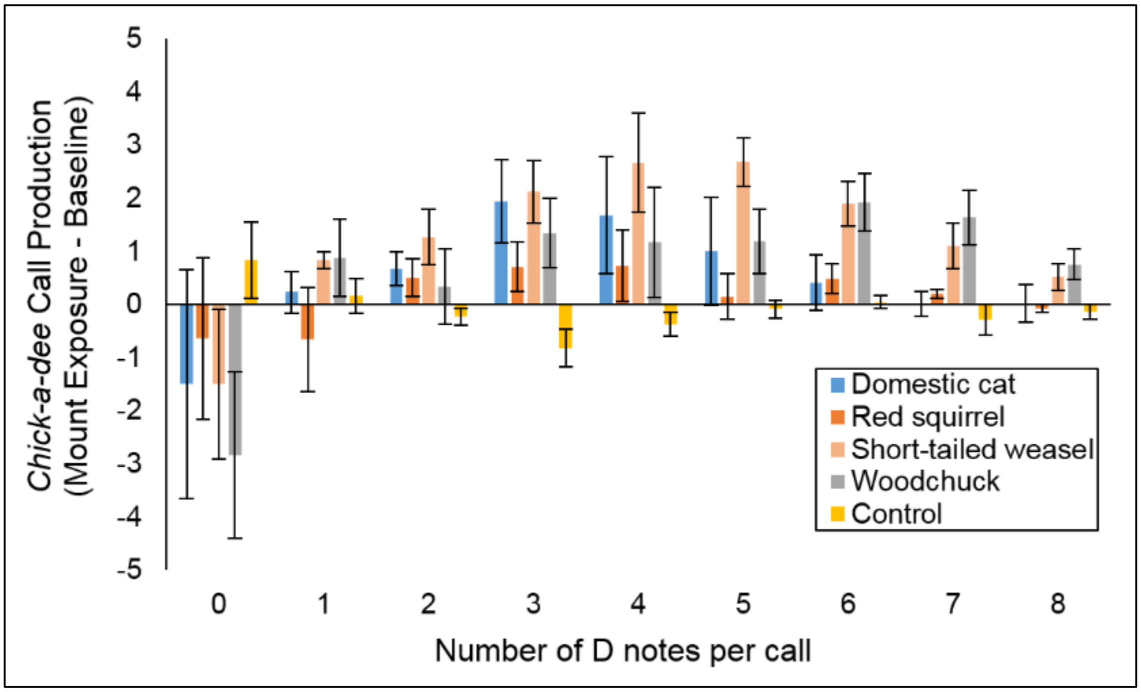

Figure 6-5. *Chick-a-dee* call production as a difference score (Mount Exposure - Baseline) ± SEM in response to mammalian mounts by the number of D notes produced. Negative values indicate a decrease in production of *chick-a-dee* call type after baseline.

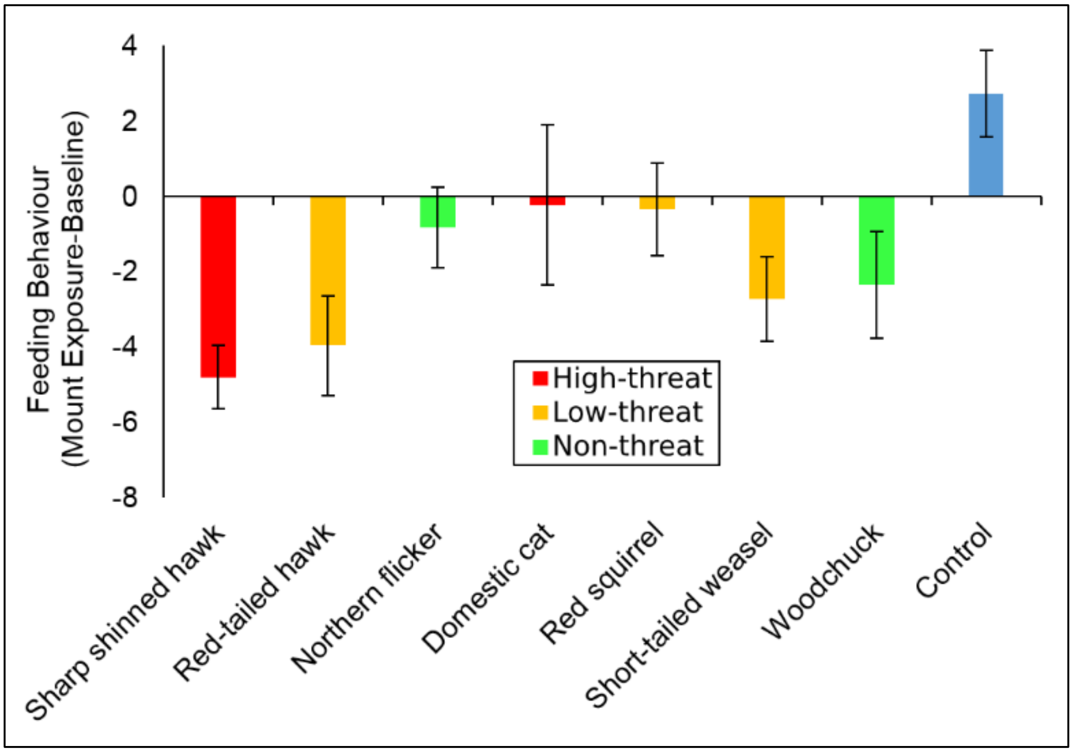

Figure 6-6. Feeding behaviour as a difference score (Mount Exposure - Baseline) ± SEM in response to each experimental condition.

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Chapter 7. General Discussion

Summary of Data Chapters

The research in this dissertation was novel in its use of both conspecific and heterospecific vocalizations to investigate songbird perception and communication of threat. This dissertation included a variety of techniques including a comparative go/no-go discrimination task investigating both chickadee and human perception of arousal (Chapter 2), several chickadee-focused operant go/no-go discrimination tasks investigating threat conveyed by predator and/or mobbing calls (Chapter 3, 4, and 5), and a predator mount exposure experiment conducted in the field (Chapter 6). The experiments in Chapter 2 were developed due to an interest in answering broader questions about the perception of arousal, whereas my other studies were focused on more specific questions, including chickadee's perception of varying predator threat (i.e., the effect of distance and signal degradation on predator threat, predator size and a threat perception continuum, and the threat posed by avian vs. mammalian predators; Chapter 3, 5, and 6, respectively), and an attempt to provide evidence of referential communication in the *chick-a-dee* mobbing call (Chapter 4).

First, in Chapter 2, I focused on the level of arousal conveyed through conspecific and heterospecific vocalizations as perceived by humans and chickadees on a comparative go/no-go task; this was an extension of a collaboration conducted with humans (see Filippi et al., 2017). Discriminant function analyses were also conducted to examine which acoustic features were likely attributed to the results. Next, Chapter 3 included an operant go/no-go discrimination task with chickadees, conducted to test the effect of signal degradation on the perception of threat in chickadees to high-threat northern saw-whet (NSWO; high-threat) and low-threat great horned owl (GHOW; low-threat) calls across multiple distances, and in comparison to synthetic and anthropogenic noise. In Chapter 4, I then conducted another operant go/no-go discrimination task to test the perception of threat posed through mobbing calls (i.e., referential communication); in addition to NSWO and GHOW calls, birds were predicted to transfer training to mobbing calls produced in response to both predators (i.e., MOB NSWO and MOB GHOW). In Chapter 5, I used vocalizations produced by sixteen predator species of hawks and owls of varying size; chickadees were trained to respond to high- or low-threat in an attempt to determine if threat perception is categorical or along a continuum. Lastly, in Chapter 6, I conducted a field experiment using visual stimuli in an attempt to replicate the findings of Templeton et al. (2005) and extend the literature on antipredator responses to investigate mammalian predators. Taken together, the results of this research demonstrate that chickadees perception of threat is complex; although this species of songbird is capable of discriminating between many acoustic stimuli, threat perception is not as black-and-white as the literature suggests, but rather is along a graded continuum. The studies within this thesis expand on previous research examining perceptual abilities in chickadees using acoustic stimuli produced by both heterospecifics and conspecifics, as well as visual stimuli of both avian and mammalian predators.

Operant Conditioning: Go/no-go Discrimination Tasks

In four of the five data chapters that compose this dissertation, go/no-go discrimination tasks were a major part of the experimental design. This technique allows the experimenter to “ask” non-human species perceptual questions, by training subjects to respond to a particular category then providing previously non-differentially rewarded stimuli to test perceptual similarity, known as open-ended categorization (e.g., Bloomfield et al., 2003). Many species of birds have demonstrated their use of open-ended categories, ranging from pigeons to zebra finches (*Columbia livia* and *Taeniopygia guttata*, respectively; e.g., Herrnstein et al., 1976; Sturdy et al., 1999; respectively). In chapters 2, 3, 4, and 5, operant conditioning techniques were used to investigate the use of categories based on arousal in heterospecific vocalizations (Chapter 2), then expanded to investigate the extent of threat perception following signal degradation (Chapter 3), referential elements of mobbing calls compared to predator calls (Chapter 4), and then beyond the perceptual categories altogether as a threat continuum (Chapter 5). Using a True/Pseudo category paradigm, "True category" birds learned to discriminate between high- and low-arousal/threat whereas "Pseudo category" birds received the same stimuli but randomly divided, without a category. The True category birds can use the concepts of 'arousal' and 'threat' by transfer of training to categorize testing stimuli whereas the Pseudo category birds would have to rely on rote memorization from training which requires experience with individual acoustic stimuli. Black-capped chickadees are capable of discriminating between *chick-a-dee* calls (used as stimuli in Chapter 2 and Chapter 4) produced by black-capped, Carolina, and mountain chickadees (*P. carolinensis*, *P. gambeli*; Bloomfield et al., 2003; Bloomfield & Sturdy, 2008); through the studies in this dissertation, I sought to determine if chickadees were capable of discriminating threat across multiple species of heterospecifics, in addition to conspecific vocalizations, and using open-ended categorization would reduce the cognitive load. Each of the four chapters that used go/no-go, true/pseudo paradigms found a difference in the trials to criterion for the true category groups compared to the pseudo category groups as the true category groups met criterion in significantly fewer trials, suggesting that high- and low-arousal/threat are categories but the pseudo category groups relied on rote memorization. This allowed for further testing on the perception of threat to determine the lack of boundaries (i.e., perceptual threat continuum) yet limitations (e.g., signal degradation, synthetic tones, anthropogenic noise).

The operant go/no-go discrimination task in Chapter 4, however, was designed to first train chickadees to discriminate between high-threat NSWO calls or low-threat GHOW calls then transfer training (either to high- or low-threat) on a second round of discrimination to high-threat mobbing calls produced in response to NSWOs (i.e., MOB NSWO) or low-threat mobbing calls produced in response to GHOWs (i.e., MOB GHOW); some subjects received mobbing calls in the first round of discrimination and predator calls in the second round, and other subjects were rewarded for opposite contingencies during the second round of discrimination (i.e., first rewarded for high-threat, then rewarded for low-threat). If predator and mobbing calls were perceived as similar, providing referential information, the second round of discrimination would require fewer trials based on transferring the concept of 'threat'. Although True category birds met criteria in fewer trials compared to Pseudo category birds, birds that discriminated predator and mobbing calls of the same threat did not complete criteria in fewer trials than birds that discriminated opposite contingencies. In fact, it appears that chickadees best discriminated owl stimuli, regardless of the threat-contingencies. This asymmetrical result suggests that the acoustic distinctions between vocalizations produced by different owl species may be perceptually easier to discriminate compared to conspecific mobbing calls. For Chapter 4 in particular, I propose that the operant go/no-go discrimination task used here was not suitable to demonstrate findings that would properly conclude that mobbing calls are referential.

Acoustic Features

Originally, I sought to extend Filippi et al.'s (2017) experiment investigating acoustic universals in vocalizations of arousal produced by multiple species, across all three biological classes of terrestrial vertebrates (Amphibia, Mammalia, and Reptilia, including Aves). In Chapter 2, I designed two comparative experiments that could be conducted with chickadees and humans, both vocal learners. Acoustic universals, a notion originally proposed by Darwin (1871), suggest that all species diverged from the earliest terrestrial ancestor with an ability to express emotion vocally. The ability to detect arousal through another species' vocalizations could help notify the listener of a nearby potential threat, including a shared predator. To extend this study, black-capped chickadees subjects and human participants were trained to discriminate between high- and low-arousal vocalizations when hearing them consecutively, compared to previous research that demonstrated that humans were capable of discriminating between paired stimuli (i.e., responding to choosing which stimulus is of high arousal). Both chickadees and humans were capable of discriminating across vocalizations produced by all nine species, but demonstrated transfer of training to vocalizations produced by black-capped chickadees, humans, and giant pandas. Both subjects and participants would have had experience with both black-capped chickadees and humans; birds would have been familiar with humans as they were wild-caught within Edmonton then housed in the laboratory, whereas University of Alberta students would have at least heard chickadees throughout campus. Transfer of training to the giant panda vocalizations, however, could not be explained by familiarity as subjects could not have been exposed to pandas and many students would have not heard juvenile panda vocalizations. Third, I ran a discriminant function analysis to investigate which acoustic features may have allowed subjects and participants to discriminate between high- and low-arousal vocalizations and found that initial fundamental frequency (i.e., F0) was most useful for classifying giant panda and black-capped chickadee vocalizations. Although research (for instance, Bowling et al., 2017) has found that F0 correlates negatively with signaler's body size within species, the link between the expression of emotional arousal through adjustments of F0 requires further investigation.

In Chapter 3's experiment, I was focused on investigating how signal degradation affects the transmission of acoustic signals and influences the perception of predator threat. There is importance in understanding how signals are perceived, directly connected to the issues of how anthropogenic noise may be affecting the perception of threat (i.e., predator calls) by both a potential signaller (i.e., a chickadee that initiates mobbing calls) and multiple receivers (i.e., nearby hetero- and conspecifics to aid in mobbing the nearby threat). From the results of this experiment, it appears that high-frequency NSWO calls may be more affected by signal degradation than low-frequency GHOW calls, resulting in chickadees ceasing to respond to distant high-threat NSWO calls. Further experiments are required to tease this notion apart from the notion that chickadees were instead discriminating threat and NSWOs may no longer be "high-threat" when further away. However, if the former is accurate, it would be consistent with the literature. For example, Proppe et al. (2010) demonstrated that in dense coniferous forests, the high-frequency notes contained in black-capped chickadee *chick-a-dee* calls (i.e., A, B, and C notes) degrade most, whereas Bloomfield et al. (2008) demonstrated that low-frequency D notes are likely to be resistant to degradation.

In addition to the natural owl stimuli used in Chapter 3, synthetic tones created to mimic the frequency and duration of the NSWO and GHOW calls, and NSWO-like truck backing-up alarm were used to investigate the perception of anthropogenic noise. Chickadees did demonstrate transfer of training to NSWO-like and GHOW-like synthetic tones, but did not respond to truck alarms as high-threat stimuli; this suggests that although the frequency and duration of NSWO-like and GHOW-like synthetic tones are critical for generalization, the frequency and duration of truck alarms are distinct enough from NSWO calls that chickadees are capable of appropriately discriminating between these stimuli as to not be deterred by non-threatening anthropogenic noise.

As described above, chickadees appear to be able to discriminate between high- and low-threat owl stimuli in fewer trials than conspecific high- and low-threat mobbing stimuli (Chapter 4). This suggests that, because high- and low-threat owl stimuli are produced by different species, it is conceptually easier to discriminate between acoustically-distinct calls even when compared to mobbing calls produced by members of their own species. Therefore, between Chapter 2, 3 and 4 we gained many insights into which acoustic features (i.e., F0) may be fundamental to discriminating between vocalizations of high- and low-arousal, and how signal degradation may affect threat perception, and that certain anthropogenic noises and owl calls remain perceptually-distinct, likely based on frequency and duration.

Perception of Threat

Categorization. Overall, much of this dissertation has been focused on the concept of categorizing threat, starting with high- and low-arousal/threat categories in Chapter 2, 3, 4, and 5. First, chickadees were trained to discriminate between high- and low-arousal across vocalizations produced by nine species to transfer training in an attempt to provide further evidence of acoustic universals. Next, chickadees were to discriminate between high- and low-threat owl calls to transfer training across vocalizations re-recorded at farther distances to investigate the effect of signal degradation and perceptual differences in comparison to anthropogenic noise. In an attempt to investigate referential communication in a species of songbird's alarm calls, chickadees were to discriminate between high- and low-threat owl calls then high- and low-threat mobbing calls. Last of the go/no-go discriminations, chickadees were to discriminate between small, high-threat hawk and owl calls compared to large, low-threat hawk and owl calls to investigate how mid-sized predators were perceived and categorized according to threat. In summary, black-capped chickadees were able to complete all of these discriminations, although transfer of training did not necessarily occur as predicted.

A threat continuum. Chapter 5 was originally focused on chickadees' perceptual categorization of vocalizations produced by small and large hawks and owls into high- or low-threat based on predator wingspan and body size, originally demonstrated with live predators by Templeton et al. (2005; i.e., smaller predators pose a higher threat to chickadees compared to larger predators). Following training, responding to additional small and large predators, as well as mid-sized predators, was investigated. However, responding was not black-and-white as either high- or low-threat. Instead, the findings revealed that chickadees did not respond to northern pygmy-owls (small predator) and red-tailed hawks (large predator) as of high- or low-threat. Specifically, both the High Threat and Low Threat groups responded to red-tailed hawk vocalizations at an intermediate level compared to the other testing species, and both groups responded to red-tailed hawk vocalizations in a manner similar to the High Threat rewarded and Low Threat rewarded stimuli, respectively. This suggests that red-tailed hawks are perceived as "medium threat" and provides support that there is a continuum in the perception of predator threat, rather than a high-threat versus low-threat dichotomy. These results are likely due to the nocturnal activity patterns of the northern pygmy-owl in comparison to diurnal chickadees, as well as the diurnal activity and slightly smaller wingspan of the red-tailed hawk compared to great gray owls (low-threat). In addition, the calls produced by mid-sized Cooper's hawks and northern hawk owls were also not responded to differentially; this suggests that black-capped chickadees do not perceive these species as the extremes of high or low threats, but rather along a continuum. Templeton et al.'s (2005) results could have also suggested that the perception of threat is along a continuum (i.e., a linear negative relationship between notes per call relative to body size), yet only the extremes of small and large predators were ever discussed. Thus, this is the first study to my knowledge that focuses on mid-sized predators and proposes a continuum of threat. Recent research, however, has demonstrated that there are situations in which perceivers can discriminate between otherwise continuous signals; for example, Caves et al. (2018) supported that female zebra finches are capable of visually discriminating along the orange to red spectrum of male beak colour. The results of Caves et al. (2018) also demonstrates that zebra finches had more difficulty discriminating between stimuli that were close on the spectrum in comparison to those at the boundaries, which supports my findings of chickadees' intermediate responding to particular species.

In Chapter 6, I investigated overall *chick-a-dee* mobbing call production, *chick-a-dee* call D note variation, and feeding behaviour in response to multiple avian and mammalian predators, using a field experiment. Vocal responding to avian predators varied similarly as reported in Templeton et al. (2005; i.e., approximately four D notes per call to the high-threat sharp-shinned hawk compared to lower-threat red-tailed hawk mount), but feeding behaviour was reduced most in response to both the sharp-shinned hawk (small predator) and red-tailed hawk (large predator) mounts. Chickadees also did not elicit quantitatively and qualitatively different responding across mammalian predators despite the fact that cats are estimated to prey upon 100 to 350 million birds in Canada per year (Blancher, 2013), these "high-threat" predators are unlikely to be perceived as high threat as small avian predators. Thus, due to non-differential feeding behaviour between the avian predator species and antipredator responding across mammalian species, chickadees appear to perceive and respond to visual predators as less distinct than originally predicted. Overall, these two studies (Chapter 5 and Chapter 6) provided insights and have initiated a conversation regarding the extent to which chickadees perceive the threat posed by predator species along a continuum rather than categorizing as a high/low dichotomy.

Conclusions

Through the five studies in this thesis, the aim was to increase the understanding of the perception and communication of threat in black-capped chickadees. Taken together, these studies demonstrate that chickadees can perceive threat (or arousal) in vocalizations produced by both conspecific and heterospecific signallers. By focusing on one species of songbird, the studies in this thesis have provided insights for threat perception in small parid species. Specifically, I sought to determine how signalling properties vary in response to differing levels of arousal and threat, degraded by distance, altered according to predator size, and in response to auditory and visual stimuli (i.e., auditory discrimination vs. mounts). With this knowledge, we have gained a further understanding of the complex perceptual abilities and communication system of the black-capped chickadee.

Although this dissertation provides many insights into threat perception and communication, future studies are required to explain certain results. First, as chickadees and humans did not consistently demonstrate transfer of training to arousal vocalizations produced by six of nine species, I propose an experiment incorporating fMRI techniques to provide a more thorough investigation of the perception of arousal, specifically unconscious perception (Chapter 2). For example, despite that humans could not discriminate between vocalizations of positive and negative valence (affectivity; although different from the calming/excitement of arousal) produced by other species (i.e., cats and rhesus monkeys), fMRI imaging indicated that there was appropriate discrimination at an unconscious, neurobiological level based on cerebral blood flow (Belin et al., 2008). Next, I propose an experiment in which to parse apart threat perception from the effects of signal degradation, including stimuli of high-threat/low-frequency or a comparative go/no-go task with humans (Chapter 3). Unfortunately, Chapter 4 did not find definitive evidence for referential communication in mobbing calls. Instead, it appears as though categorizing the owl species' calls according to threat level may be easier than categorizing mobbing calls, or, due to the biological relevance of mobbing calls, chickadees were in conflict between feeding and mobbing behaviour. Future studies should further investigate the potential referential elements of chickadees' mobbing calls by considering stimuli with varying duty cycles or alternative experimental designs as the go/no-go discrimination task did not provide compelling results. In Chapter 5, chickadees did not respond to northern pygmy-owl (small predator) calls as if they were of high-threat, despite the fact that responding continued to the training calls of the great horned owl (large predator). Since northern pygmy-owls and saw-whet owls are two similar-sized owl species that produce acoustically-similar calls, a future study could train subjects with northern pygmy-owl calls then test with northern saw-whet owl calls to clarify how these species are perceived. Last, future studies are required to investigate if other mammalian species, or urban areas that have higher cat populations, elicit quantitatively different responding. In addition, it is of interest to determine if qualitatively different antipredator behaviours occur in response to predators of adults compared to nest predators, and if the presence or absence of offspring is required to elicit nest predator specific behaviours. Although Chapter 6 provided little evidence for varying responses to mammalian predators, the findings provided further insight into the antipredator responses of black-capped chickadees to predators and advocates for future experiments investigating the perception of mammalian predators.

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