



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

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Abstract

Avian predators vary in their degree-of-threat to chickadees; for example, smaller owls and hawks are of higher threat to chickadees as they can easily maneuver through the trees, while larger predators cannot. We conducted an operant go/no-go discrimination task to investigate the effect of signal degradation on perceived threat. Chickadees were trained to respond to high-threat northern saw-whet owl (NSWO) or low-threat great horned owl (GHOW) calls that were recorded at short distances, and then tested with high- and low-threat owl calls that were rebroadcast and re-recorded across six distances (25 m, 50 m, 75 m, 100 m, 150 m, and 200 m). Subjects were further tested with high-threat and low-threat synthetic tones produced to mimic the natural calls across the six distances. We predicted that birds would perceive and respond to: (1) high-threat predator calls at longer distances compared to low-threat predator calls, and (2) synthetic tones similarly compared to the stimuli that they were designed to mimic. We believed chickadees would continue to perceive and respond to predators that pose a high threat at further distances; however, only responding to low-threat stimuli was consistent across distance recordings. Synthetic tones were treated similarly to natural stimuli but at lower response levels. Thus, the results of this study provide insights into how chickadees perceive threat.

Keywords Threat perception · Predator perception · Black-capped chickadee · Operant conditioning

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 behaviors should be selected

(Lima and 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 and Becker 2002; red-breasted nuthatches, *Sitta canadensis*, eavesdropping on black-capped chickadee mobbing calls, Templeton and 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

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high- and low-threat, respectively (Templeton et al. 2005). The stark contrast in chickadee responses between these species influenced stimuli selection for our experiment, and for the purposes of this paper, we will be referring to our NSWO and GHOW stimuli as high- and low-threat, respectively. 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 et al. (2011) conducted a study examining immediate early gene expression based on the work of Templeton et al. (2005) 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, that chickadees demonstrate the ability to perceive predator threat from both visual and auditory cues (Templeton et al. 2005; Avey et al. 2011), and the knowledge that calling makes an owl more susceptible to being detected and mobbed by nearby prey (Chandler and 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?

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, and then tested with additional high-threat NSWO and low-threat GHOW calls that were originally recorded across six distances (25 m, 50 m, 75 m, 100 m, 150 m, and 200 m). Subjects were further tested with high-threat and low-threat synthetic tones produced to mimic the respective and natural NSWO and GHOW calls across the six distances. We predicted that birds would perceive and respond to: (1) high-threat predator calls at farther distances compared to low-threat predator calls, and (2) synthetic tones similarly

compared to the stimuli that they were designed to mimic. Each of these predictions are described below.

First, we predicted that chickadees have the ability to perceive predators that pose a high threat from further distances as it would assist in survival in comparison to responding to low-threat predators. Although songbirds change their behaviors 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 to differentially to non-threat pigeon, *Columba livia*, nearby and distant dummies; Kryštofková et al. 2011), we believe that prey still perceive the threat posed and responding 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 high-threat NSWO or low-threat GHOW calls. 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 stimuli, 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) 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 1 year of age (determined by examining the color 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 during equipment shaping, eight failed pretraining (likely due to this initial exposure to the aversive, predator stimuli), 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–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, 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 ($30 \times 40 \times 40$ 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. A personal computer connected to a single-board computer (Palya and 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 vocalizations produced by small, high-threat northern saw-whet owls (NSWO) and large, low-threat great horned owls (GHOW) were obtained due to their high quality, originally recorded at short (i.e., close) distances; from here, the original recordings will be referred to as “short”-distance recordings. Four acoustic stimuli of both species were then rebroadcast and re-recorded (speakers and microphones were affixed at 1.5 m) 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 200 m (resulting in 48 stimuli that were used in the experiment).

All stimuli broadcast in the field were normalized using peak amplitude. Stereo mics from SM2 units were tested annually to ensure standardized gain on both channels and within 3 dB of manufacturer specifications. All field broadcasts were conducted in a remote field setting with no presence of anthropogenic sound, wind = 1 on the Beaufort scale. Recordings were also collected midafternoon to avoid overlap with avian vocalizations, and variation in vegetation sound was controlled using the same transect for all playbacks.

During the experiment, the short-distance stimuli were presented at approximately 80 dB as measured by a Brüel and Kjær Type 2239 (Brüel and Kjær Sound and 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. By maintaining the decibels according to the original, short-distance stimuli, the rebroadcast, further-distance stimuli maintained their natural attenuation.

To further investigate signal degradation across distance, we contrasted background and foreground noise from each recording to calculate signal-to-noise ratios (SNR) using Audacity 2.4.2. (The Audacity Team, Carnegie Mellon University, PA, USA). Using the SNRs, we compared the original stimuli to each set of rebroadcast stimuli across the six distances by conducting paired-samples *t* tests; see Table 1 for averages of the SNRs at each distance and Table 2 for the statistical results. From these analyses, it is evident that SNR did not change significantly over distance for GHOW stimuli; whereas, the SNR of NSWO stimuli was significantly different, which is an effect of signal degradation.

Synthetic stimuli

Four natural NSWO and GHOW stimuli were rebroadcast and re-recorded across six distances (25, 50, 75, 100, 150,

Table 1 Average signal-to-noise ratio of all great horned owl (GHOW) and northern saw-whet owl (NSWO) calls at each distance of the original and rebroadcast stimuli, calculated using Audacity 2.4.2

Distance	GHOW	NSWO
Short	35.920	31.460
25 m	26.075	13.835
50 m	20.930	13.288
75 m	19.943	13.135
100 m	18.920	12.555
150 m	15.988	12.030
200 m	13.703	4.800

The Audacity Team, Carnegie Mellon University, PA, USA

Table 2 Results of the paired-samples *t* tests comparing signal-to-noise ratios of the original, short-distance stimuli to the rebroadcast stimuli of the six additional distances

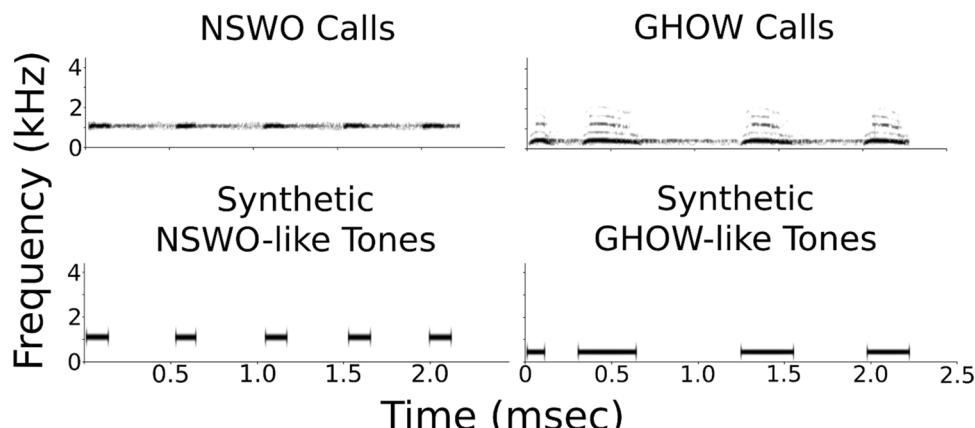
Paired samples <i>t</i> test comparison	GHOW <i>t</i> value (<i>df</i> =3) and <i>p</i> value	NSWO <i>t</i> value (<i>df</i> =3) and <i>p</i> value
Short v. 25 m	2.183, 0.117	8.063, 0.004
Short v. 50 m	2.408, 0.095	8.314, 0.004
Short v. 75 m	2.636, 0.078	7.104, 0.006
Short v. 100 m	2.775, 0.069	8.224, 0.004
Short v. 150 m	2.854, 0.065	6.432, 0.008
Short v. 200 m	2.885, 0.063	10.452, 0.002

Bonferroni corrections have been applied ($p=0.05/6$ tests=0.0083)

Bold font indicates significance

and 200 m; 48 stimuli total) and 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 high-threat NSWO and low-threat GHOW calls to produce high- and low-threat synthetic tones (see Fig. 1). See additional equipment and recording details above.

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



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). Pretraining is critical as the procedure is designed to remove any pre-existing biases and to ensure that birds approached the feeder for all stimuli. A trial began when the bird landed on the request perch and remained for between 900 and 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 $\geq 60\%$ responding on average to all stimuli, at least four 308-trial blocks $\leq 3\%$ difference in responding to future rewarded and unrewarded stimuli, and at least four 308-trial blocks in which the bird had $\leq 3\%$ 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 $\geq 60\%$ responding on average to all stimuli, completed one 308-trial block of $\leq 3\%$ difference in responding to future rewarded and unrewarded stimuli, and completed one 308-trial block of $\leq 3\%$ 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 (High-Threat S+ subgroup: three male and three female subjects), while the other subgroup discriminated 12 rewarded GHOW calls from 12 unrewarded NSWO calls (Low-Threat 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 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, to prepare birds for Transfer Testing in which stimuli were neither rewarded nor 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 (5 stimuli per species; 10 stimuli total); NSWO calls and GHOW calls recorded at six distances (i.e., 25 m, 50 m, 75 m, 100 m, 150 m, 200 m; four stimuli per type and distance; 72 stimuli total); and synthetic high- and low-threat 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., 5 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 and GHOW recorded at 25 m, 50 m, 75 m, 100 m, 150 m, 200 m (four stimuli per type and distance), and high- and low-threat synthetic stimuli replicating all six distances (four stimuli per type and distance). During transfer testing, the stimuli from Discrimination 85 training were presented thirteen 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 responding to stimuli during transfer testing, we split stimuli into: natural stimuli and synthetic stimuli. First, we conducted a three-way repeated-measures ANOVA for the true group with Condition (High Threat S+, Low Threat S+) \times Stimulus Species (NSWO, GHOW) \times Stimulus Distance (short distance, 25 m, 50 m, 75 m, 100 m, 150 m, 200 m) 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 (High Threat S+, Low Threat S+) \times Stimulus Species (NSWO, GHOW) \times Stimulus Distance (25 m, 50 m, 75 m, 100 m, 150 m, 200 m) as fixed factors and the scaled proportion of responding to synthetic 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 (High Threat S+: $X + SEM = 143.000 \pm 8.881$, $N=6$; Low Threat S+: $X + SEM = 132.167 \pm 9.141$, $N=6$). There was no significant difference, $t_{10} = 0.850$, $p = 0.415$, $d = 0.0538$, 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: $X + SEM = 502.667 \pm 167.185$, $N=3$; Pseudo 2 Group: $X + SEM = 513.500 \pm 133.878$, $N=4$). There was no significant difference, $t_5 = -0.039$, $p = 0.970$, $d = 0.025$, 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. Thus, this suggests that NSWO vs. GHOW calls are both perceptual and biologically relevant categories according to our subjects.

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 (High Threat S+, Low Threat S+) \times Stimulus Species (NSWO, GHOW) \times Stimulus Distance (short distance, 25 m, 50 m, 75 m, 100 m, 150 m, 200 m) 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 \times Stimulus Species \times Stimulus Distance ($F_{1,1,6} = 9.293$, $p < 0.001$, $\eta^2 = 0.650$), indicating that there was differential responding to stimulus species according to condition across all seven stimulus distances. The interaction of Condition \times Stimulus Species ($F_{1,6} = 36.109$, $p = 0.002$, $\eta^2 = 0.878$), and the two-way interaction of Stimulus Species \times Stimulus Distance were also significant ($F_{1,6} = 4.779$, $p = 0.002$, $\eta^2 = 0.489$), 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 ($F_1 = 62.038$, $p = 0.001$, $\eta^2 = 0.925$); however, the two-way interaction of Condition \times Species Distance and the main effects of Condition and Stimulus Distance were non-significant (all $p > 0.148$). To further investigate the three-way interaction, we conducted independent-samples and paired-samples *t* tests; see Tables 3 and 4 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 Low-Threat S+ subgroup responded significantly more to GHOW stimuli compared to the High-Threat S+ subgroup across all seven distances. In contrast, the High-Threat S+ group responded significantly more to stimuli recorded at short distances compared to stimuli recorded at 150 m; see Fig. 2. This suggests that there is a perceptual difference from the subjects' perspective, which is likely due to the physical difference between signals (i.e., a result of signal degradation).

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 (High Threat S+, Low Threat S+) \times Stimulus Type (synthetic high threat, synthetic low threat) \times Stimulus Distance (short distance, 25 m, 50 m, 75 m, 100 m, 150 m, 200 m) 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 \times Stimulus Type \times Stimulus Distance ($F_{1,1,5} = 4.420$, $p = 0.005$, $\eta^2 = 0.469$), indicating that there was differential responding to stimulus species according to condition across all seven stimulus distances.

Table 3 Results of the independent samples *t* test comparing subjects' responding in High-Threat S+ vs. Low-Threat S+ groups to each natural stimulus across distance during transfer testing, with Bonferroni corrections ($p = 0.05/14 = 0.0035$)

	<i>t</i> test	<i>p</i> value	Cohen's <i>d</i>	Confidence intervals
High-Threat S+ Group vs. Low-Threat S+ Group (<i>df</i> =10)				
NSWO stimuli				
Short	4.086	0.002	2.584	0.207, 0.704
25 m	0.585	0.571	0.370	-0.253, 0.434
50 m	1.065	0.312	0.674	-0.159, 0.451
75 m	1.343	0.209	0.849	-0.119, 0.480
100 m	-3.850	0.003	-2.435	-0.614, -0.164
150 m	-1.883	0.089	-1.191	-0.546, 0.046
200 m	-3.341	0.007	-2.113	-0.625, -0.125
GHOW stimuli				
Short	-8.910	<0.001	-5.635	-0.799, -0.479
25 m	-6.975	<0.001	-4.411	-0.852, -0.440
50 m	-11.859	<0.001	-7.500	-0.924, -0.632
75 m	-8.470	<0.001	-5.357	-0.833, -0.486
100 m	-5.057	<0.001	-3.198	-0.680, -0.264
150 m	-9.502	<0.001	-6.010	-0.832, -0.516
200 m	-6.750	<0.001	-4.269	-0.859, -0.433

Negative *t* values indicate that Low Threat S+ responded more than High Threat S+

Bold font indicates significance

The two-way interaction of Condition \times Stimulus Type ($F_{1,5} = 37.465$, $p = 0.002$, $\eta^2 = 0.882$), and the two-way interaction of Condition \times Stimulus Distance were also significant ($F_{1,5} = 2.751$, $p = 0.044$, $\eta^2 = 0.355$), 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 \times Stimulus Distance and the main effects of Condition, Stimulus Type, and Stimulus Distance were non-significant (all $p > 0.245$). To further investigate the three-way interaction, we conducted independent samples and paired-samples *t* tests; see Tables 5 and 6 for these statistical results. These results indicate that black-capped chickadees in the High-Threat S+ group were able to transfer training to stimuli recorded at 25 m and 50 m, and responded significantly more to stimuli recorded at 25 m compared to stimuli recorded at 100 m. However, unlike responding to the natural stimuli, the Low-Threat S+ subgroup did not respond significantly more to low-threat stimuli compared to the High-Threat S+ subgroup across distances; see Fig. 3. These results demonstrate that chickadees in the High-Threat S+ group performed similarly to natural and synthetic stimuli, suggesting that chickadees trained to recognize and respond to highly threatening stimuli generalized this training to other stimuli based on acoustic similarity.

Table 4 Results of the paired-samples *t* tests comparing subjects' responding in High-Threat S+ and Low-Threat 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 ($p=0.05/6=0.0083$)

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

Bold font indicates significance

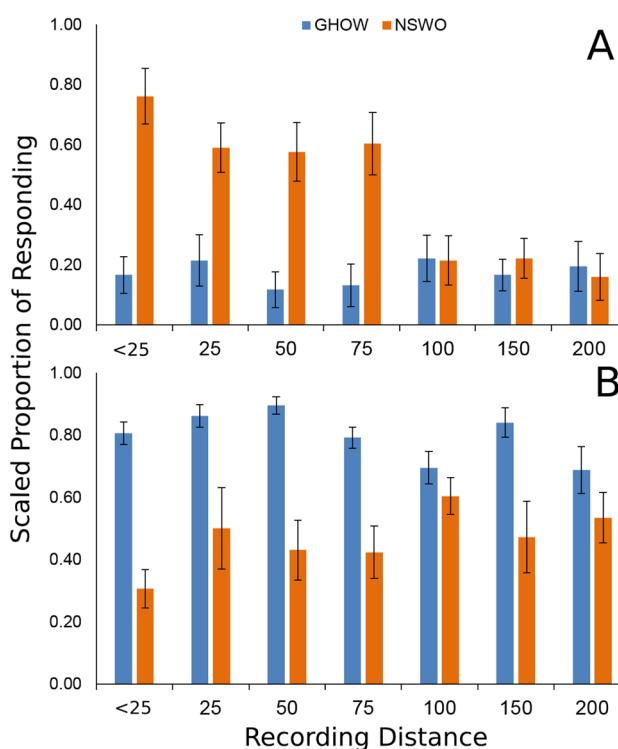


Fig. 2 Scaled proportion of responding to natural high-threat NSWO and low-threat GHOW calls by black-capped chickadees in the High-Threat S+ subgroup (a; $n=6$) and Low-Threat S+ subgroup (b; $n=6$) during the transfer testing phase \pm SEM across recording distances (short distances/<25, 25, 50, 75, 100, 150, 200 m)

Table 5 Results of the independent samples *t* test comparing subjects' responding in High-Threat S+ vs. Low-Threat S+ groups to synthetic stimuli across distance during transfer testing, with Bonferroni corrections ($p=0.05/10=0.005$)

	<i>t</i> test	<i>p</i> value	Cohen's <i>d</i>	Confidence intervals
High-Threat S+ Group vs. Low-Threat S+ Group (<i>df</i>=10)				
High-threat synthetic stimuli				
25 m	6.864	<0.001	4.341	0.352, 0.690
50 m	3.607	0.005	2.281	0.111, 0.472
75 m	2.573	0.038	1.627	0.036, 0.505
100 m	0.532	0.607	0.336	-0.177, 0.288
150 m	-0.425	0.680	-0.269	-0.347, 0.236
200 m	0.863	0.409	0.546	-0.088, 0.199
Low-threat synthetic stimuli				
25 m	-2.105	0.079	-1.331	-0.584, 0.043
50 m	-1.119	0.289	-0.708	-0.374, 0.124
75 m	-2.945	0.015	-1.863	-0.354, -0.049
100 m	-3.195	0.010	-2.021	-0.460, -0.082
150 m	-1.633	0.134	-1.033	-0.476, 0.073
200 m	-1.593	0.142	-1.008	-0.450, 0.075

Negative *t* values indicate that Low Threat S+ responded more than High Threat S+

Bold font indicates significance

Discussion

In this 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

Table 6 Results of the paired-samples *t* tests comparing subjects' responding in High-Threat S + and Low-Threat S + groups to each natural stimulus between synthetic high-threat and low-threat stimuli recorded at short distances vs. distant stimuli during transfer testing, with Bonferroni corrections ($p=0.05/5=0.01$)

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

Bold font indicates significance

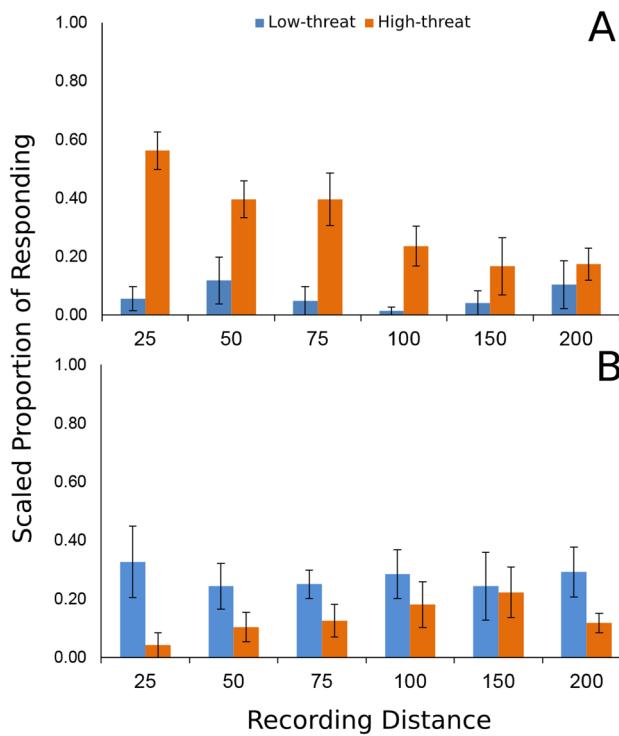


Fig. 3 Scaled proportion of responding to synthetic high-threat and low-threat synthetic tones by black-capped chickadees in the High-Threat S + subgroup (a; $n=6$) and Low-Threat S + subgroup (b; $n=6$) during the transfer testing phase \pm SEM across recording distances (25, 50, 75, 100, 150, 200 m)

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, and synthetic high- and low-threat tones, 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 as high- and low-threat, respectively (Templeton et al. 2005). In addition, 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 High-Threat S + and Low-Threat 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 rebroadcast and 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 naturally selected through evolution. However, we found that only the chickadees in the Low-Threat S + subgroup responded consistently to GHOW stimuli across all distances, whereas the High-Threat

S + subgroup appeared to decrease responding to high-threat NSWO stimuli as distance increased (i.e., stimuli recorded at short distances vs. 150 m).

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 high-threat NSWO signals over distance, as signal degradation may be a cause for the error in perception. It is likely that our higher-frequency, high-threat NSWO calls may be more affected by signal degradation than low-frequency, low-threat GHOW calls (see Fig. 4). 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). This is further supported by the analyses conducted on our stimuli set in which there were statistical differences between the original, short-distance NSWO stimuli and the stimuli at each distance; this was not true of GHOW stimuli (see Table 2). 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 is primarily

coniferous, this could explain our results, suggesting a strong influence of signal degradation on high-frequency, high-threat NSWO calls compared to low-frequency, low-threat GHOW calls. In general, call propagation rates can vary depending on the species due to acoustic traits like call frequency and structure (i.e., NSWO could attenuate more quickly than GHOW) and signals will degrade at differing rates depending on those acoustic traits over distance (i.e., due to reverberation, refraction, and absorption; Yip et al. 2017). Thus, we propose that the degradation impacts signal perception by impairing category perception.

Nonetheless, we recommend further investigation to examine this lack of responding to originally high-threat NSWO calls, recorded at further distances, perhaps by including stimuli that have similar acoustic characteristics to high threat calls, 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 and 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 high-threat NSWO and low-threat GHOW calls. We predicted chickadees would respond to synthetic high-threat and low-threat tones similarly to the natural predator calls. Chickadees in the High-Threat S + and Low-Threat S + subgroups did in fact respond appropriately to high-threat and low-threat 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. The most pivotal result is that the High-Threat S + group outperformed the Low-Threat S + group at responding to appropriate stimuli across distance, which

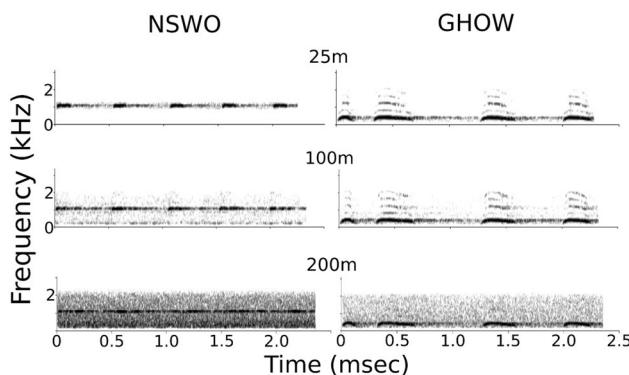


Fig. 4 Sample sound spectrograms of high-threat northern saw-whet owl (NSWO) and low-threat great horned owl (GHOW) calls across distances (25 m, 100 m, and 200 m), with time (ms) on the x-axis and frequency (kHz) on the y-axis, to demonstrate the effect of signal degradation on NSWO stimuli compared to GHOW stimuli

suggests a biologically relevant finding, critical for survival: Chickadees recognized and responded similarly to stimuli that mimicked a natural, high-threat predator's call.

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 High-Threat S + subgroup responded in this way as they were discriminating threat not species as at further distances small predators may no longer pose a high threat. This would explain the difference between the High Threat S + and Low Threat 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 tones similarly compared to natural predator calls. If chickadees had inappropriately responded to synthetic stimuli that were acoustically similar to high-threat predator calls, then the species may not survive. In general, an inability to discriminate between biologically relevant and biologically irrelevant acoustic stimuli may result in a loss of opportunities to feed or mate due to antipredator behaviors, or could instead result in habituation to the incorrect signals (i.e., not producing antipredator behaviors in the presence of a high-threat predator). Thus, this ability to perceive and respond to threat appropriately was likely selected. In total, the natural and synthetic stimuli used in the current experiment provide many insights into the threat perception of songbirds, including the effects of distance and signal degradation, and the perceptual similarities between natural and synthetic stimuli.

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