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

Jenna V. Congdon[ad], Allison H. Hahn[e], Kimberley A. Campbell[ad], Erin N. Scully[ad], Daniel A. Yip[bd], Erin M. Bayne[bd], & Christopher B. Sturdy[acd]

Department of Psychology[a], Department of Biology[b], Neuroscience and Mental Health Institute[c],
University of Alberta[d];
Department of Psychology, St. Norbert College[e].

Corresponding author: Christopher B. Sturdy, csturdy@ualberta.ca, 1-780-492-7843
P-217 Biological Sciences Building, Edmonton, AB, Canada, T6G 2E9

Smaller owls and hawks are high-threat predators to small songbirds, like chickadees, in comparison to larger avian predators due to smaller raptors' agility (Templeton et al. 2005). The current literature focuses only on high- and low-threat predators. We propose that there may be a continuum in threat perception. In the current study, we conducted an operant go/no-go experiment investigating black-capped chickadees' acoustic discrimination of predator threat. After obtaining eight hawk and eight owl species' calls, we assigned each species as: (1) large, low-threat, (2) mid-sized, unknown-threat and (3) small, high-threat predators, according to wingspan and body size. Black-capped chickadees were either trained to respond ('go') to high-threat predator calls or respond to low-threat predator calls. When either low-threat predator calls were not reinforced or high-threat predator calls were not reinforced the birds were to withhold responding ('no-go') to those stimuli. We then tested transfer of training with additional small and large predator calls, as well as with the calls of several mid-sized predators. We confirmed that chickadees can discriminate between high- and low-threat predator calls. We further investigated how chickadees categorize mid-sized species' calls by assessing transfer of training to previously non-differentially reinforced (i.e., pretraining) calls. Specifically, transfer test results suggest that 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. However, mid-sized Cooper's hawks and northern hawk owls 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.

Keywords: threat perception, predator perception, black-capped chickadee, operant conditioning

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 and 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 Family (*Paridae*; Smith, 1991), and all three species demonstrate strong antipredator responses to relatively small predators (Templeton et al. 2005; Soard and Ritchison 2009; Courter and 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). 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. 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 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 and 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. 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 had higher levels of 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 with reference to, the same predator 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’ whether subjects treated stimuli as high and low threat categories, via transfer of training to novel stimuli, to determine how they perceived specific predator calls in an attempt to determine if threat perception occurs along a continuum or as more discrete high and low threat categories. 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 et al. 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 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 Weisman and Ratcliffe (2004) for a schematic of the apparatus, and 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 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 1; see Figure 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 across the call using SIGNAL 5.10.24 software (RMS sound = 1.0; 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).

Acoustic Analyses. We measured multiple acoustic features using SIGNAL software, including the start frequency (SF), peak frequency (PF), end frequency (EF), duration, and number of notes per acoustic stimulus. We then ran multiple one-way ANOVAs across predator size (Small, Mid-sized, Large) using the averages of these features per stimulus type and found that PF and EF were the only significant features ($p = 0.030$, and $p = 0.030$, respectively). Paired-samples *t*-tests revealed that the PF ($p < 0.001$) and EF ($p = 0.002$) features were only differences between Mid-sized and Large predators. However, upon running *post-hoc* one-way ANOVAs with predator stimuli classified according to birds' responding, there were no significant differences (*p*s > 0.078). This indicates that there are few or no individual acoustic features that were driving chickadees' performance when discriminating acoustic predator stimuli. See Table 2.

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 discriminated using the categories 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 categories 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.

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 v. Low Threat group; with Dunn–Šidák corrections) 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 Dunn–Šidák 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. Last, we conducted two linear regressions (Regression 1: High Threat group; Regression 2: Low Threat group) to determine if the average wingspan of the stimulus-producing species (in cm) predicted the proportion of responding during Transfer testing; these regressions needed to be conducted separately as the proportion of responding should be opposite due to the contingencies of transfer of training (i.e., High Threat group responding to “high-threat” species’ vocalizations and the Low Threat group responding to “low-threat” species’ vocalizations, respectively).

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 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 ($F_{3.202, 16.012} = 4712.493$, $p < 0.001$, $\eta^2 = .999$), 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 ≥ 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 ($F_{7,35} = 35.133$, $p < 0.001$, $\eta^2 = .875$), indicating that there was a significant difference in responding to Stimulus Species based on Condition. The main effect of Stimulus Species was significant ($F_{7,35} = 2.626$, $p = 0.027$, $\eta^2 = .334$, 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 Dunn–Šidák corrections ($p < 0.0064$). There were significant differences in responding to sharp-shinned hawk ($t_5 = 6.273$, $p = .001$, $d = -4.877$, 95% CI = 0.305, 0.682), great gray owl ($t_5 = -9.994$, $p < .001$, $d = -7.131$, 95% CI = -0.978, -0.610), broad-winged hawk ($t_5 = 16.039$, $p < .001$, $d = 10.144$, 95% CI = 0.722, 0.955), and short-eared owl ($t_5 = -4.656$, $p = .001$, $d = -2.945$, 95% CI = -0.734, -0.259); Note: 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. Responding to northern pygmy-owl, red-tailed hawk, Cooper's hawk, and northern hawk owl were non-significant ($ps \geq 0.093$). See Figure 3 for these statistical results.

We then conducted paired-samples *t*-tests (with Dunn–Šidák corrections; $p \leq 0.0018$) on the proportion of responding to each Transfer testing Stimulus Species, separately for the High Threat and Low Threat conditions, to further examine the significant Condition × Stimulus Species interaction; see Table 3 for these results.

Last, we conducted two linear regressions to determine if average wingspan of the stimulus-producing species (in cm; see Table 1) predicted the proportion of responding during Transfer testing. Two linear regressions were conducted in order to analyze proportion of responding according to wingspan for the High Threat group and the Low Threat group independently. There was a non-significant negative correlation between wingspan and proportion of responding for the High Threat group, with only 6.9% of the variance explained ($F_{1, 46} = 3.423$, $p = 0.071$, 95% CIs = -.005, $\beta = -.263$). There was a significant positive correlation between wingspan and proportion of responding for the Low Threat group, with 29.5% of the variance explained ($F_{1, 46} = 19.264$, $p < 0.001$, 95% CIs = .003, .007, $\beta = .543$). Therefore, the regression equation for the Low Threat group subjects' responding is equal to .114 + .005 (wingspan) proportion of responding when wingspan is measured in cm (see Figure 4). Taken together, the concept of threat appears to be more complex than wingspan alone (i.e., size) as prey may be taking into consideration many other factors, including each predator's diet.

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 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 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 et al. 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. It is important to note that the acoustic analyses demonstrate that the acoustic features that we measured (i.e., start frequency, peak frequency, end frequency, duration, and number of notes per stimulus) were not used by chickadees to classify stimuli.

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 and 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. The linear regressions that we conducted, using wingspan, were unable to account for the complexity of songbirds' predator perception, which suggests that there are multiple factors that determine where predators are perceived along a potential continuum of threat (e.g., predator diet). 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 that our classification of predators (i.e., according to wingspan) 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.

Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

Experiments conceived and designed: JVC, AHH, and CBS. Collected stimuli: DAY. Stimuli selection: JVC and KAC. Collected data: JVC, KAC, and ENS. Analyzed data: JVC, KAC, and AHH. Drafted paper: JVC and AHH. Revised paper: JVC, AHH, KAC, ENS, DAY, EMB, and CBS.

Acknowledgements

We would like to thank Alyshia M. M. Skurdal and Raphael Q. Gastrock, the research assistants who supervised our operant chambers throughout the length of the experiment. In addition, Alyshia M. M. Skurdal assisted with determining Edmonton-native species and the threat level according to wingspan of each species. We thank Isaac Lank and Al Denington for their technical assistance in the chickadee operant chambers.

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.

This research was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant and Discovery Accelerator Supplement, an Alberta Ingenuity Fund (AIF) New Faculty Grant, a Canada Foundation for Innovation (CFI) New Opportunities Fund (NOF) and Infrastructure Operating Fund (IOF) grants along with start-up funding and CFI partner funding from the University of Alberta (UofA) to CBS. JVC and KAC were each supported by an Alexander Graham Bell Canada Graduate Scholarship-Doctoral (NSERC CGS D).

Table 1. Information regarding species calls that were used as stimuli, including how the stimuli were sorted according to high, mid, or low threat level 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.

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	Training

					mammals and reptiles	
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 Occasionally small birds	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 2. Information regarding the acoustic stimuli produced by owls and hawks. The average ± SEM of acoustic features of the stimuli, including the Start Frequency (SF), Peak Frequency (PF), End Frequency (EF), Duration, and Number of Notes per vocalization.

Species	**Start Frequency (SF)**	**Peak Frequency (PF)**	**End Frequency (EF)**	**Duration**	**Number of Notes**
American kestrel	3876 ±118	5048 ±124	3820 ±87	1142 ±71	6.5 ±0.8
Boreal owl	636 ±141	815 ±36	659 ±16	1712 ±25	12.9 ±1.1
Merlin	3608 ±95	4275 ±157	3704 ±185	1520 ±175	10.6 ±1.6
Northern pygmy-owl	980 ±107	1094 ±44	952 ±47	1268 ±50	2.0 ±0.0

Northern saw whet owl	997 ±78	1139 ±21	1003 ±20	2009 ±18	4.7 ±0.2
Sharp-shinned hawk	3577 ±211	4101 ±409	3303 ±397	1241 ±335	8.0 ±1.4
Broad-winged hawk	4703 ±90	4820 ±72	4316 ±88	1004 ±62	2.0 ±0.0
Cooper's hawk	2985 ±149	3662 ±360	2939 ±332	1404 ±332	8.4 ±0.9
Northern hawk owl	2367 ±152	4608 ±310	3051 ±332	1027 ±116	1.0 ±0.0
Short-eared owl	2459 ±27	4101 ±409	3303 ±397	1241 ±335	8.0 ±1.4
Barred owl	301 ±98	596 ±34	275 ±12	1678 ±26	4.0 ±0.3
Great gray owl	118 ±80	299 ±17	192 ±8	2094 ±12	3.0 ±0.0
Great horned owl	241 ±115	408 ±7	256 ±10	2040 ±12	3.8 ±0.1
Long-eared owl	246 ±17	405 ±14	263 ±18	473 ±21	1.0 ±0.0
Peregrine falcon	2578 ±115	2899 ±159	2649 ±162	1561 ±171	6.8 ±0.6
Red-tailed hawk	2663 ±213	3461 ±66	2422 ±68	1238 ±122	1.0 ±0.0

Table 3. The results (*p*-values) of the paired-samples *t*-tests, with Dunn–Šidák corrections ($p \leq 0.0018$), 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**	0.0154	-	-	-	-	-	-	-
Broad-winged hawk	**BWHA**	**0.0014** **BWHA>NOPO**	0.0435	-	-	-	-	-	-
Cooper's hawk	**COHA**	0.8937	**0.0012**	**0.0013**	-	-	-	-	-

			SSHA>COHA	**BWHA>COHA**					
Northern hawk owl	**NHOW**	0.3408	0.0356	**0.0015** **BWHA>NHOW**	0.4774	-	-	-	-
Short-eared owl	**SEOW**	0.9945	0.0374	0.0022	0.8835	0.5776	-	-	-
Great gray owl	**GGOW**	0.0186	**0.0004** **SSHA>GGOW**	**<0.0001** **BWHA>GGOW**	0.0150	0.0098	0.0129	-	-
Red-tailed hawk	**RTHA**	0.1078	0.1567	0.0163	0.0701	0.3758	0.1181	0.0044	-
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**	0.0483	-	-	-	-	-	-	-
Broad-winged hawk	**BWHA**	0.0073	0.0044	-	-	-	-	-	-
Cooper’s hawk	**COHA**	0.2684	0.0142	0.0023	-	-	-	-	-
Northern hawk owl	**NHOW**	0.0395	0.0022	**0.0010** **NWHA>BWHA**	0.2373	-	-	-	-
Short-eared owl	**SEOW**	**0.0009** **SEOW>NOPO**	**0.0009** **SEOW>SSHA**	**<0.0001** **SEOW>BWHA**	0.0121	0.1927	-	-	-
Great gray owl	**GGOW**	0.0027	**0.0005** **GGOW>SSHA**	**<0.0001** **GGOW>BWHA**	0.0416	0.1383	0.8953	-	-
Red-tailed hawk	**RTHA**	0.0119	0.0033	0.0021	0.1978	0.7152	0.0878	0.1423	-

Note: **Bold** font indicates statistical significance ($p \leq 0.0018$).

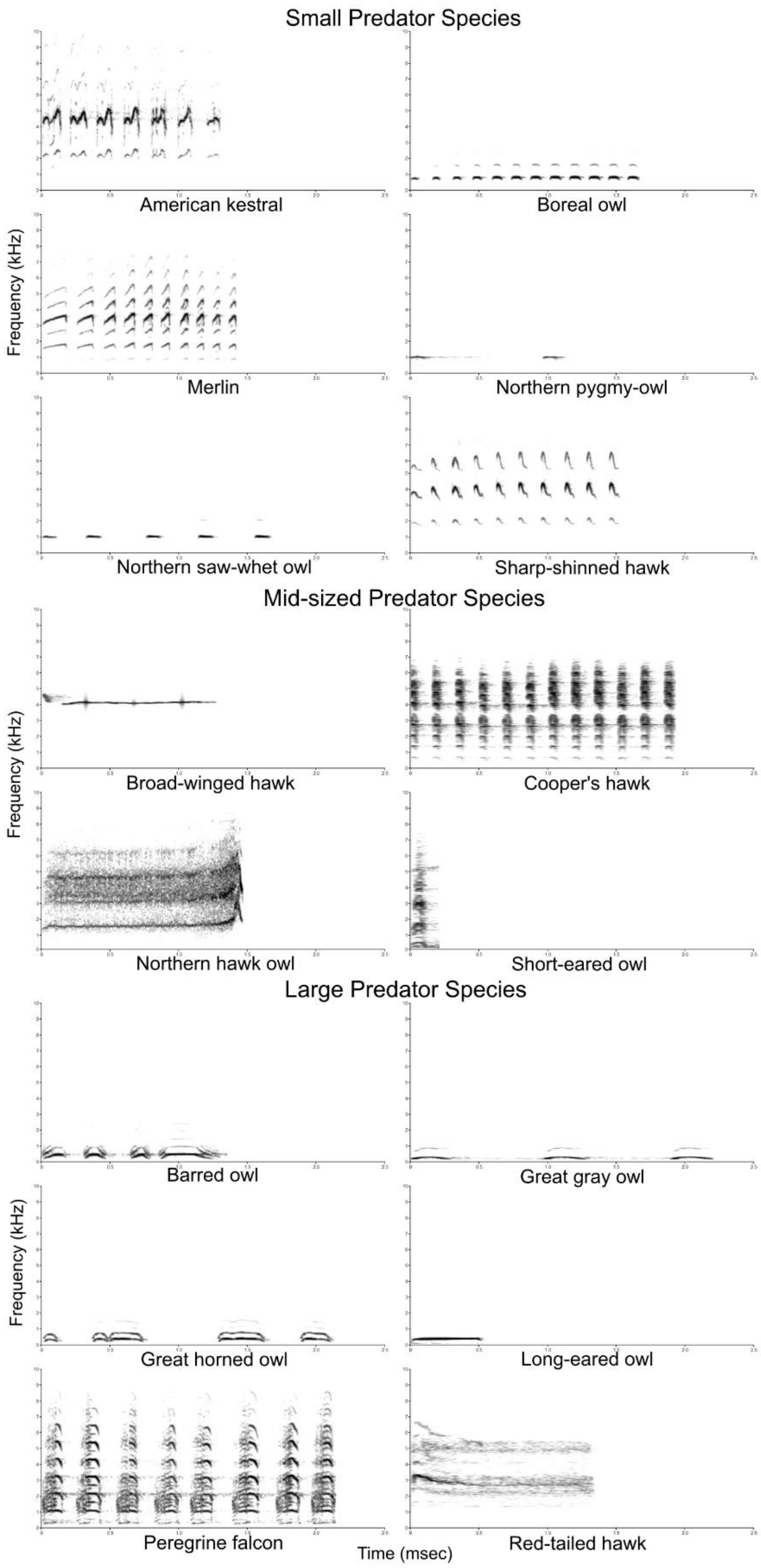

Figure 1. Sample sound spectrograms of the vocalizations produced by the 16 species of predators used as experimental stimuli, divided into small-sized, mid-sized, and large-sized predators with time (msec) on the *x*-axis and frequency (kHz) on the *y*-axis.

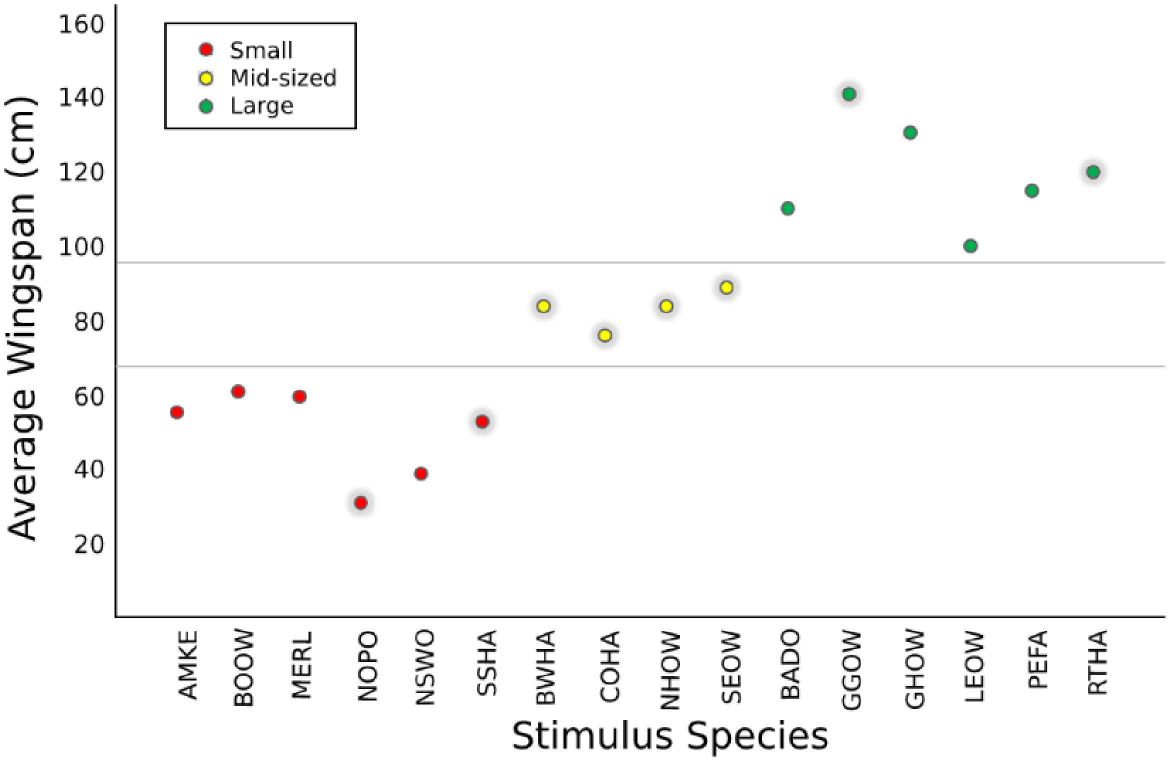

Figure 2. Owl and hawk species (*x*-axis) plotted according to average wingspan (*y*-axis), categorized by size: small, mid, and large (Sibley, 2000; Templeton et al., 2005; see Table 1). Small species were used as training and testing high-threat stimuli; large species were used as training and testing 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.

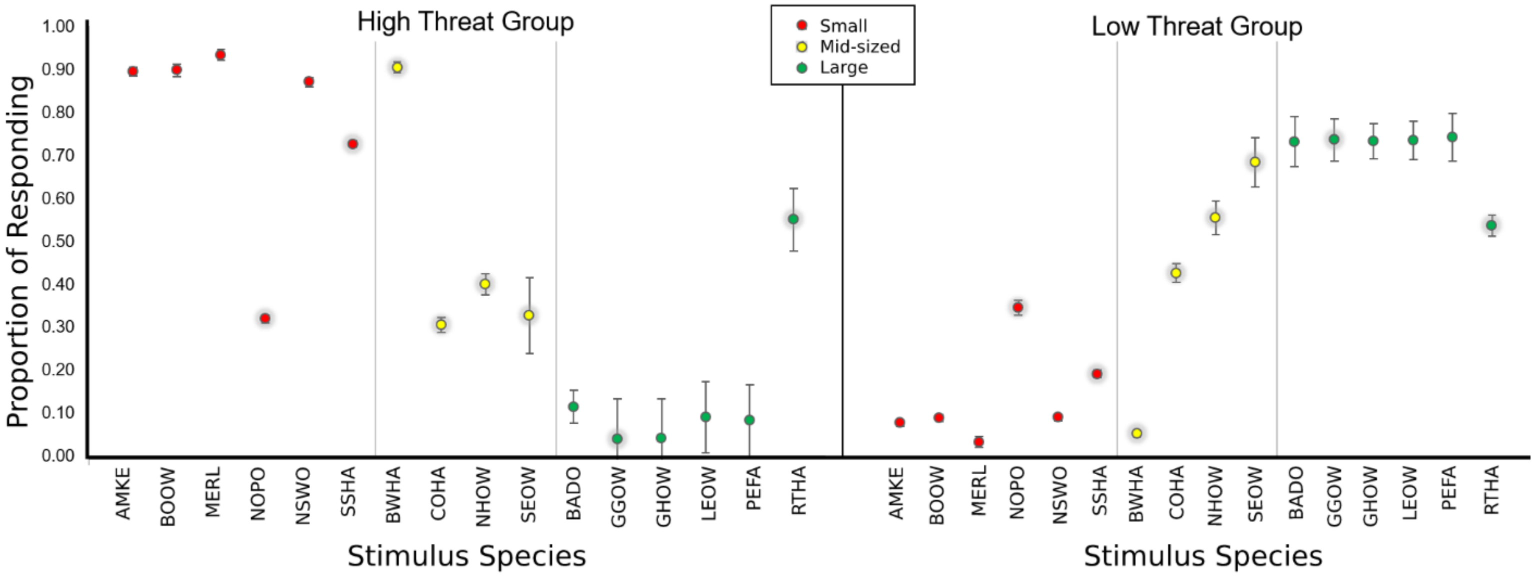

Figure 3. Average ± SEM proportion of responding to each stimulus species by black-capped chickadees in the High Threat (left) and Low Threat (right) groups during Transfer testing. AMKE: American kestrel; BOOW: boreal owl; MERL: merlin; NOPO: northern pygmy-owl; NSWO: northern saw-whet owl; SSHA: sharp-shinned 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.

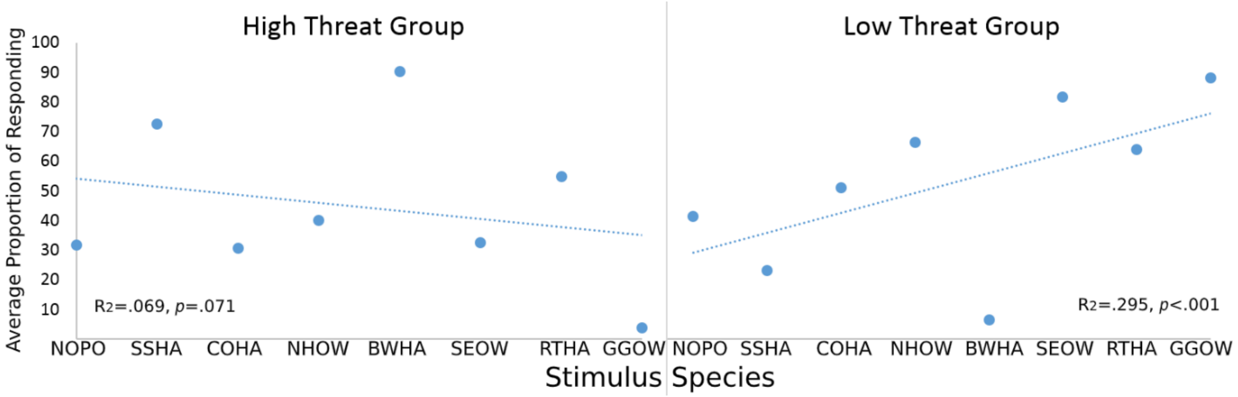

Figure 4. Average ± SEM proportion of responding to each stimulus species (ranging smallest to largest in cm) by black-capped chickadees in the High Threat (left: $R^2 = .069$) and Low Threat (right: $R^2 = 0.295$) groups during Transfer testing. The regression equation for the Low Threat group subjects' responding is equal to .144 + .005 (wingspan) proportion of responding when wingspan is measured in cm. NOPO: northern pygmy-owl/31cm; SSHA: sharp-shinned hawk/53cm; COHA: Cooper's hawk/76cm; NHOW: northern hawk owl/84cm; BWHA: broad-winged hawk/84cm; SEOW: short-eared owl/89cm; GGOW: great gray owl/120cm; RTHA: red-tailed hawk/141cm.

References

Avey MT, Hoeschele M, Moscicki MK, Bloomfield LL, Sturdy CB (2011) Neural correlates of threat perception: Neural equivalence of conspecific and heterospecific mobbing calls is learned. PLoS One 6:e23844.

Bayne EM, Hobson KA (2002) Effects of red squirrel (*Tamiasciurus hudsonicus*) removal on survival of artificial songbird nests in boreal forest fragments. The American Midland Naturalist 147:72-80.

Book DL, Freeberg TM (2015) Titmouse calling and foraging are affected by head and body orientation of cat predator models and possible experience with real cats. Animal Cognition 18:1155-1164.

Blancher P (2013) Estimated number of birds killed by house cats (*Felis catus*) in Canada. Avian Conservation and Ecology 8:3.

Charrier I, Bloomfield LL, Sturdy CB (2004) Note types and coding in parid vocalizations. I: The chick-a-dee call of the black-capped chickadee (*Poecile atricapillus*). Canadian Journal of Zoology 82:769-779.

Courter JR, Ritchison G (2010) Alarm calls of tufted titmice convey information about predator size and threat. Behavioral Ecology 21:936-942.

Griffiths R, Double MC, Orr K, Dawson RJ (1998) A DNA test to sex most birds. Molecular Ecology 7:1071-1075.

Kyle SC, Freeberg TM (2016) Do Carolina chickadees (*Poecile carolinensis*) and tufted titmice (*Baeolophus bicolor*) attend to the head or body orientation of a perched avian predator? Journal of Comparative Psychology 130:145.

Njegovan M, Hilhorst B, Ferguson S, Weisman R (1994) A motor-driven feeder for operant training in song birds. Behavior Research Methods, Instruments, & Computers 26:26-27.

Palya WL, Walter DE (2001) *Document set for the high-performance experiment controller*. Retrieved 25 October 2014 from http://www.jsu.edu/depart/psychology/sebac/Exp-Ctl.html

Pyle P (1997) Identification guide to North American birds. Slate Creek Press, Bolinas.
Semenchuk, G. P. (Ed.). (1992) *The atlas of breeding birds of Alberta*. Nature Alberta

Sieving KE, Hetrick SA, Avery ML (2010) The versatility of graded acoustic measures in classification of predation threats by the tufted titmouse *Baeolophus bicolor*: exploring a mixed framework for threat communication. Oikos 119:264-276.

Sibley DA (2000) The Sibley guide to birds. Alfred A.

Smith SM (1991) *The black-capped chickadee: Behavioral ecology and the natural history*. Cornell University Press: Ithaca, NY

Soard CM, Ritchison G (2009) ‘Chick-a-dee’ calls of Carolina chickadees convey information about degree of threat posed by avian predators. Animal Behaviour 78:1447-1453.

Sturdy CB, Weisman RG (2006) Rationale and methodology for testing auditory cognition in songbirds. Behavioural Processes 72:265-272.

Templeton CN, Greene E (2007) Nuthatches eavesdrop on variations in heterospecific chickadee mobbing alarm calls. Proceedings of the National Academy of Sciences 104:5479-5482.

Templeton CN, Greene E, Davis K (2005) Allometry of alarm calls: Black-capped chickadees encode information about predator size. Science 308:1934-1937.

Weisman RG, Ratcliffe L (2004) Relative pitch and the song of black-capped chickadees. American Scientist 92:532-539.