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

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

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64 *Key words: threat perception, predator perception, black-capped chickadee, operant*  
65 *conditioning*

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## Introduction

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Almost every species on earth is or has been the prey of another species, since the time of the Cambrian explosion or longer (541-485 million years ago; Briggs 2015). Predation is a major evolutionary force due to its impact on fitness, and consequently, the ability to perceive threat and possess adaptive antipredator behaviours should be selected for (Lima 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).

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Black-capped chickadees are a non-migratory North American songbird that are typically preyed upon by avian predators, including both hawks and owls (Smith 1991). In the presence of a predator, black-capped chickadees produce mobbing calls to recruit and coordinate conspecifics and heterospecifics to attack and harass the nearby predator (Smith 1991). Templeton et al. (2005) demonstrated that black-capped chickadee mobbing call production varies according to predator wingspan and body length, indicating that small-sized predators, including the northern saw-whet owl (NSWO; *Aegolius acadicus*), and large-sized predators, including the great horned owl (GHOW; *Bubo virginianus*) are on opposite ends of the threat spectrum as high- and low-threat, respectively (Templeton et al. 2005). 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

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115 difference in wingspan and body length of these two species of owls, small NSWOs are more  
116 maneuverable in comparison to large GHOWs, and small songbirds are more likely to meet their  
117 energetic requirements (Templeton et al. 2005; Pyke et al. 1977). Avey and colleagues (2011)  
118 conducted a study examining immediate early gene expression based on the work of Templeton  
119 et al. (2005) in order to compare levels of ZENK expression in chickadees following exposure to  
120 high- and low-threat predator calls; upon hearing NSWO calls and GHOW calls, black-capped  
121 chickadees produced more gene expression in response to NSWO calls compared to GHOW  
122 calls. These results suggest that NSWO and GHOW predators, and the calls that they produce,  
123 are perceived to be different, potentially of high- and low-threat, respectively.

124         Despite knowledge of which predators are on opposite ends of the threat spectrum for  
125 black-capped chickadees, that chickadees demonstrate the ability to perceive predator threat from  
126 both visual and auditory cues (Templeton et al. 2005; Avey et al. 2011), and the knowledge that  
127 calling makes an owl more susceptible to being detected and mobbed by nearby prey (Chandler  
128 and Rose 1988), few studies are focused on how acoustic signals produced by avian predators are  
129 perceived by songbirds, and the extent to which signal degradation affects perceived threat  
130 levels. Particularly, how do chickadees perceive the level of threat posed by potentially degraded  
131 predator acoustic signals (i.e., owl calls transmitted through forests)? For example, are high-  
132 threat predators perceived and responded to at further distances than low-threat predators?

133         We sought to answer the above questions by collecting high- and low-threat owl calls and  
134 conducting an operant go/no-go discrimination task to investigate the effect of signal degradation  
135 on perceived threat. Specifically, chickadees were trained to respond to high-threat NSWO or  
136 low-threat GHOW calls that were recorded at short distances, then tested with additional high-  
137 threat NSWO and low-threat GHOW calls that were originally recorded across six distances

138 (25m, 50m, 75m, 100m, 150m, and 200m). Subjects were further tested with high-threat and  
139 low-threat synthetic tones produced to mimic the respective and natural NSW0 and GHOW calls  
140 across the six distances. We predicted that birds would perceive and respond to: 1) high-threat  
141 predator calls at farther distances compared to low-threat predator calls, and 2) synthetic tones  
142 similarly compared to the stimuli that they were designed to mimic. Each of these predictions are  
143 described below.

144 First, we predicted that chickadees have the ability to perceive predators that pose a high  
145 threat from further distances as it would assist in survival in comparison to responding to low-  
146 threat predators. Although songbirds change their behaviours depending on the distance of a  
147 predator (i.e., blackbirds, *Turdus merula*, mobbing intensity is higher to nearby magpie, *Pica*  
148 *pica*, predator dummies compared to distant magpies, whereas mobbing did not occur to  
149 differentially to non-threat pigeon, *Columbia livia*, nearby and distant dummies; Kryštofková et  
150 al. 2011), we believe that prey still perceive the threat posed and responding would be consistent  
151 in this type of task. Second, we were interested in investigating if chickadees perceive owl calls  
152 and synthetic stimuli as similar, generalizing the perception of threat, by creating synthetic tones  
153 that match the duration and frequency of high-threat NSW0 or low-threat GHOW calls. We  
154 know, for example, that black-capped chickadees have difficulty discriminating between  
155 acoustically-similar D notes produced by two different parid species (chestnut-backed  
156 chickadees, *Poecile rufescens*, and tufted titmice, *Baeolophus bicolor*; Hahn et al. 2017), but we  
157 do not know how chickadees will respond to acoustically-similar synthetic stimuli, connected  
158 with anthropogenic noise. Overall, the results of this experiment will inform us about the  
159 perception of threat across distance, as well as inform us of whether or not our synthetic stimuli  
160 (i.e., tones) are perceived similarly to predator calls.

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## Methods

### Subjects

163           Thirty-eight black-capped chickadees (19 males and 19 females; identified by DNA  
164 analysis; Griffiths et al. 1998) were originally used in this experiment, tested between September  
165 2015 and April 2016. Birds at least one year of age (determined by examining the colour and  
166 shape of their outer tail rectrices; Pyle 1997) were captured in Edmonton (North Saskatchewan  
167 River Valley, 53.53°N, 113.53°W, Mill Creek Ravine, 53.52°N, 113.47°W), or Stony Plain  
168 (53.46°N, 114.01°W), Alberta, Canada between December 2010 and February 2015. However,  
169 two subjects failed during equipment shaping, eight failed pretraining (likely due to this initial  
170 exposure to the aversive, predator stimuli), one failed discrimination training, one failed due to  
171 low responding, and five birds died of natural causes. Thus, the data from only 19 birds (nine  
172 males, ten females) were used.

173           Prior to the experiment, birds were individually housed in Jupiter Parakeet cages (30 × 40  
174 × 40 cm; Rolf C. Hagen, Inc., Montreal, QB, Canada) in colony rooms containing other black-  
175 capped chickadees. Birds had visual and auditory, but not physical, contact with one another.  
176 Birds had *ad libitum* access to food (Mazuri Small Bird Maintenance Diet; Mazuri, St Louis,  
177 MO, USA), water (vitamin supplemented on alternating days; Prime vitamin supplement; Hagen,  
178 Inc.), grit, and cuttlebone. Birds were given three to five sunflower seeds daily, one superworm  
179 (*Zophobas morio*) three times a week, and a mixture of greens (spinach or parsley) and eggs  
180 twice a week. Birds were maintained on a light:dark cycle that mimicked the natural light cycle  
181 for Edmonton, Alberta, Canada.

182           Throughout the experiment, birds were housed individually in operant chambers (see  
183 apparatus below), maintained on the natural light cycle for Edmonton, Alberta, and had *ad*

184 *libitum* access to water (vitamin supplemented on alternate days), grit, and cuttlebone. Birds  
185 were given two superworms daily (one in the morning and one in the afternoon). Food (i.e.,  
186 Mazuri) was only available as a reward for correct responding during the operant discrimination  
187 task. Sixteen birds had previous experience discriminating musical chords, black-capped  
188 chickadee *fee-bee* songs, Parid and finch vocalizations, and/or *chick-a-dee* mobbing calls (Hoang  
189 2015; Hahn et al. 2016; Hahn et al. 2017; Congdon et al. 2019; respectively), but no operant  
190 experience with the stimulus types used in this experiment (owl vocalizations).

### 191 **Apparatus**

192         During the experiment, birds were housed individually in modified colony room cages  
193 (30 × 40 × 40 cm) placed inside a ventilated, sound-attenuating chamber. The chambers were  
194 illuminated by a 9W, full spectrum fluorescent bulb. Each cage contained three perches, a water  
195 bottle, and a grit cup. An opening on the side of the cage (11 × 16 cm) provided each bird access  
196 to a motor-driven feeder (see Njegovan et al. 1994). Infrared cells in the feeder and the request  
197 perch (perch closest to the feeder) monitored the position of the bird. A personal computer  
198 connected to a single-board computer (Palya and Walter 2001) scheduled trials and recorded  
199 responses to stimuli. Stimuli were played from the personal computer hard drive, through either a  
200 Cambridge A300 Integrated Amplifier, Cambridge Azur 640A Integrated Amplifier (Cambridge  
201 Audio, London, England), or an NAD310 Integrated Amplifier (NAD Electronics, London,  
202 England) and through a Fostex FE108 Σ or Fostex FE108E Σ full-range speaker (Fostex Corp.,  
203 Japan; frequency response range 80-18,000 Hz) located beside the feeder. See Sturdy and  
204 Weisman (2006) for a detailed description of the apparatus.

### 205 **Acoustic Stimuli**

206           **Natural stimuli.** Acoustic stimuli were obtained from the Bayne Laboratory (Department  
207 of Biological Sciences, University of Alberta, AB, Canada), Borror Laboratory of Bioacoustics  
208 (The Ohio State University, OH, USA), and the Macaulay Library (Cornell Lab of Ornithology,  
209 NY, USA; originally recorded between the years of 1954-2015 throughout Canada and USA). A  
210 total of 34 vocalizations produced by small, high-threat northern saw-whet owls (NSWO) and  
211 large, low-threat great horned owls (GHOW) were obtained due to their high quality, originally  
212 recorded at short (i.e., close) distances; from here, the original recordings will be referred to as  
213 “short” distance recordings. Four acoustic stimuli of both species were then rebroadcast and re-  
214 recorded (speakers and microphones were affixed at 1.5m) using a Song Meter SM2+ automated  
215 audio recorder (Wildlife Acoustics, Maynard, Massachusetts, USA) in the boreal forest north of  
216 Fort McMurray, AB (57.4998°N, -111.4490°W) on July 10, 2015, across six distances: 25, 50,  
217 75, 100, 150, and 200m (resulting in 48 stimuli that were used in the experiment).

218           All stimuli broadcast in the field were normalized using peak amplitude. Stereo mics  
219 from SM2 units were tested annually to ensure standardized gain on both channels and within  
220 3dB of manufacturer specifications. All field broadcasts were conducted in a remote field setting  
221 with no presence of anthropogenic sound, wind = 1 on the beaufort scale. Recordings were also  
222 collected midafternoon to avoid overlap with avian vocalizations, and variation in vegetation  
223 sound was controlled for by using the same transect for all playbacks.

224           During the experiment, the short distance stimuli were presented at approximately 80 dB  
225 as measured by a Brüel & Kjær Type 2239 (Brüel & Kjær Sound & Vibration Measurement A/S,  
226 Nærum, Denmark) decibel meter (A-weighting, slow response) at the approximate height and  
227 position of a bird’s head when on the request perch. By maintaining the decibels according to the

228 original, short distance stimuli, the rebroadcast, further distance stimuli maintained their natural  
229 attenuation.

230 To further investigate signal degradation across distance, we contrasted background and  
231 foreground noise from each recording to calculate signal-to-noise ratios (SNR) using Audacity  
232 2.4.2. (The Audacity Team, Carnegie Mellon University, PA, USA). Using the SNRs, we  
233 compared the original stimuli to each set of rebroadcast stimuli across the six distances by  
234 conducting paired-samples t-tests; see Table 1 for averages of the SNRs at each distance and  
235 Table 2 for the statistical results. From these analyses, it is evident that SNR did not change  
236 significantly over distance for GHOW stimuli whereas the SNR of NSWOW stimuli were  
237 significantly different, an effect of signal degradation.

238 **Synthetic stimuli.** The four natural NSWOW and GHOW stimuli were rebroadcast and re-  
239 recorded across six distances (25, 50, 75, 100, 150, and 200m; 48 stimuli total) were reproduced  
240 as synthetic tones using Audacity 2.2.2. (The Audacity Team, Carnegie Mellon University, PA,  
241 USA). K.A.C. matched the frequency and amplitude (measured in the middle of the band using  
242 SIGNAL 5.10.24 software; Engineering Design, Berkeley, CA, USA) of natural high-threat  
243 NSWOW and low-threat GHOW calls to produce high-and low-threat synthetic tones (see Figure  
244 1). See additional equipment and recording details above.

## 245 **Procedure**

246 **Pretraining.** Pretraining began once the bird learned to use the request perch and feeder  
247 to obtain food. During Pretraining, birds received food for responding to all stimuli (future  
248 rewarded stimuli, unrewarded stimuli, and testing stimuli). Pretraining is critical as the procedure  
249 is designed to remove any pre-existing biases and to ensure that birds approached the feeder for  
250 all stimuli. A trial began when the bird landed on the request perch and remained for between

251 900-1100 ms. A randomly-selected stimulus played without replacement until all 154 stimuli had  
252 been heard. If the bird left the request perch before a stimulus finished playing, the trial was  
253 considered interrupted, resulting in a 30-s time out with the houselight turned off. If the bird  
254 entered the feeder within 1 s after the entire stimulus played, it was given 1 s access to food,  
255 followed by a 30-s intertrial interval, during which the houselight remained on. If a bird  
256 remained on the request perch during the stimulus presentation and the 1 s following the  
257 completion of the stimulus it received a 60-s intertrial interval with the houselight on, but this  
258 intertrial interval was terminated if the bird left the request perch. This was to encourage a high  
259 level of responding on all trials. Birds continued on Pretraining until they completed six 308-trial  
260 blocks of  $\geq 60\%$  responding on average to all stimuli, at least four 308-trial blocks  $\leq 3\%$   
261 difference in responding to future rewarded and unrewarded stimuli, at least four 308-trial blocks  
262 in which the bird had  $\leq 3\%$  difference in responding to future testing stimuli to ensure that birds  
263 did not display a bias for stimuli. Following a day of free feed, birds completed a second round  
264 in which they completed one 308-trial block of  $\geq 60\%$  responding on average to all stimuli,  
265 completed one 308-trial block of  $\leq 3\%$  difference in responding to future rewarded and  
266 unrewarded stimuli, completed one 308-trial block of  $\leq 3\%$  difference in responding to future  
267 testing stimuli to confirm that each bird continued to not display preferences following the break.

268 **Discrimination Training.** The procedure was the same as during Pretraining, except,  
269 only 24 training stimuli were presented (with the remaining 130 withheld for use during Transfer  
270 Testing), and responding to half of these stimuli were now punished with a 30-s intertrial interval  
271 with the houselight off and no access to food. As during Pretraining, responses to rewarded  
272 stimuli resulted in 1 s access to food. Discrimination training continued until birds completed six

273 312-trial blocks with a discrimination ratio (DR)  $\geq 0.80$  with the last two blocks being  
274 consecutive. For DR calculations see Response Measures, below.

275 Birds were randomly assigned to either a True category discrimination group ( $n = 12$ ) or  
276 Pseudo category discrimination group ( $n = 7$ ). Black-capped chickadees in the True category  
277 discrimination group were divided into two subgroups: one subgroup discriminated 12 rewarded  
278 NSW0 calls from 12 unrewarded GHOW calls (High Threat S+ subgroup: three male and three  
279 female subjects), while the other subgroup discriminated 12 rewarded GHOW calls from 12  
280 unrewarded NSW0 calls (Low Threat S+ subgroup: three male and three female subjects).

281 The Pseudo category discrimination group was also divided into two subgroups. Each  
282 subgroup discriminated six randomly-selected rewarded NSW0 calls and six randomly-selected  
283 rewarded GHOW calls from six unrewarded NSW0 and six unrewarded GHOW calls (Pseudo  
284 subgroup 1: two male and two female subjects; Pseudo subgroup 2: two male and two female  
285 subjects). The purpose of the Pseudo group was to include a control in which subjects were not  
286 trained to categorize according to threat level, investigating if True group acquisition is due to  
287 category learning (significantly fewer trials than the Pseudo groups) or simply rote memorization  
288 (similar number of trials compared to the Pseudo group); fewer trials to criterion would provide  
289 evidence of category learning, and transfer of training provides further support.

290 **Discrimination 85.** This phase was identical to Discrimination training, except that the  
291 rewarded stimuli were rewarded with a reduced probability of getting a reward (i.e.,  $P = 0.85$ ).  
292 On unrewarded rewarded S+ trials, entering the feeder after the stimulus finished playing  
293 resulted in a 30-s intertrial interval, during which the houselight remained on, but there was no  
294 access to food. Discrimination 85 training was employed to introduce birds to trials in which  
295 there was no access to food, but the houselight remained illuminated, in order to prepare birds for

296 Transfer Testing in which stimuli were neither rewarded or punished. Discrimination 85 training  
297 continued until birds completed two 312-trial blocks with a  $DR \geq 0.80$ .

298       **Transfer Testing.** During Transfer Testing, the stimuli and reinforcement contingencies  
299 from Discrimination 85 were maintained and 130 additional stimuli were included as stimuli.  
300 These stimuli were heard during Pretraining, but not Discrimination training. Testing stimuli  
301 consisted of additional NSW0 and GH0W calls recorded at short distances (5 stimuli per  
302 species; 10 stimuli total); NSW0 calls and GH0W calls recorded at six distances (i.e., 25m,  
303 50m, 75m, 100m, 150m, 200m; four stimuli per type and distance; 72 stimuli total); and  
304 synthetic high- and low-threat stimuli replicating all six distances (four stimuli per type and  
305 distance; 48 stimuli total).

306       Due to the number of testing stimuli, we created four rounds of Transfer Testing. First,  
307 birds completed at least three 322-trial blocks that included 10 additional NSW0 and GH0W  
308 recorded at short distances testing stimuli (i.e., 5 additional stimuli recorded at short distances  
309 per species). Next, birds completed at least three 342-blocks of Transfer testing rounds 2, 3, 4,  
310 and 5, in a random order; these testing rounds included 30 additional testing stimuli comprised of  
311 a random assortment of NSW0 and GH0W recorded at 25m, 50m, 75m, 100m, 150m, 200m  
312 (four stimuli per type and distance), and high-and low-threat synthetic stimuli replicating all six  
313 distances (four stimuli per type and distance). During Transfer Testing, the stimuli from  
314 Discrimination 85 training were presented thirteen times each, randomly-selected without  
315 replacement and the testing stimuli were each presented once during the 322- or 342-trial block  
316 (round 1 and 2-5, respectively).

317       Birds completed a minimum of three blocks for each round of Transfer Testing and these  
318 were included in the analysis. Between each round of Transfer, birds completed two 312-trial

319 blocks of Discrimination 85 with a  $DR \geq 0.80$ . Following the final round of Transfer Testing,  
320 birds were returned to the colony room.

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

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

337 **Statistical Analyses.** We conducted independent-samples  $t$ -tests on the number of trials  
338 to criterion for the True and Pseudo category groups during Discrimination training. To  
339 investigate responding to stimuli during Transfer Testing, we split stimuli into: natural stimuli  
340 and synthetic stimuli. First, we conducted a three-way repeated measures ANOVA for the True  
341 group with Condition (High Threat S+, Low Threat S+)  $\times$  Stimulus Species (NSWO, GHOW)  $\times$

342 Stimulus Distance (short distance, 25m, 50m, 75m, 100m, 150m, 200m) as fixed factors and the  
343 scaled proportion of responding to natural stimuli during Transfer Testing as the dependent  
344 variable; additional stimuli recorded at short distances were included with the distant stimuli to  
345 directly compare all natural stimuli. Second, we conducted a three-way repeated measures  
346 ANOVA for the True group with Condition (High Threat S+, Low Threat S+)  $\times$  Stimulus  
347 Species (NSWO, GHOW)  $\times$  Stimulus Distance (25m, 50m, 75m, 100m, 150m, 200m) as fixed  
348 factors and the scaled proportion of responding to synthetic stimuli during Transfer Testing as  
349 the dependent variable. Where applicable, significant analyses were followed by independent  
350 samples *t*-tests on responding to stimulus type across distance to determine which stimuli birds  
351 demonstrated transfer of training (i.e., to which stimuli birds responded).

352 **Ethical Note.** Throughout the experiment, birds remained in the testing apparatus to  
353 minimize the transport and handling of each bird and reduce stress. Following the experiment,  
354 birds were returned to the colony room for use in future experiments. With the exception of five  
355 birds that died of natural causes, birds remained healthy during the experiment. All procedures  
356 were conducted in accordance with the Canadian Council on Animal Care (CCAC) Guidelines  
357 and Policies with approval from the Animal Care and Use Committee for Biosciences for the  
358 University of Alberta (AUP 108), which is consistent with the Animal Care Committee  
359 Guidelines for the Use of Animals in Research. Birds were captured and research was conducted  
360 under an Environment Canada Canadian Wildlife Service Scientific permit (#13-AB-SC004),  
361 Alberta Fish and Wildlife Capture and Research permits (#56076 and #56077), and City of  
362 Edmonton Parks Permit.

## 363 **Results**

### 364 **Trials to Criterion**

365 To determine whether birds in the two True category groups differed in their speed of  
366 acquisition, we conducted an independent-samples  $t$ -test on the number of 312-trial blocks to  
367 reach criterion for the two True category conditions (High Threat S+:  $X+SEM = 143.000+8.881$ ,  
368  $N = 6$ ; Low Threat S+:  $X+SEM = 132.167+9.141$ ,  $N = 6$ ). There was no significant difference,  $t_{10}$   
369  $= 0.850$ ,  $p = 0.415$ ,  $d = .0538$ , 95% Confidence Interval [CIs] =  $-17.564, 39.230$ .

370 To determine whether birds in the two Pseudo category groups differed in their speed of  
371 acquisition, we conducted an independent-samples  $t$ -test on the number of 312-trial blocks to  
372 reach criterion for the two Pseudo category conditions (Pseudo 1 Group:  $X+SEM =$   
373  $502.667+167.185$ ,  $N = 3$ ; Pseudo 2 Group:  $X+SEM = 513.500+133.878$ ,  $N = 4$ ). There was no  
374 significant difference,  $t_5 = -0.039$ ,  $p = 0.970$ ,  $d = .025$ , 95% CIs =  $-723.075, 701.409$ .

375 To compare the acquisition performance of the True and Pseudo category groups and to  
376 determine if the True group learned to categorize in fewer trials than the Pseudo group, we  
377 conducted an independent-samples  $t$ -test on the number of 312-trial blocks to reach criterion for  
378 the True category and Pseudo category groups. Due to a violation of Levene's test, we used the  
379  $p$ -value that did not assume homogeneity of variance; there was a significant difference between  
380 the groups ( $t_{6.030} = -2.962$ ,  $p = 0.025$ ,  $d = -2.412$ , 95% CIs =  $-677.612, -64.935$ ) in that True birds  
381 learned to discriminate significantly faster than Pseudo birds. Thus, this suggests that NSW0 vs.  
382 GHOW calls are both perceptual and biologically-relevant categories according to our subjects.

### 383 **Analysis of Transfer Stimuli**

384 **Natural stimuli.** To determine if the pattern of learning was the same across calls from  
385 testing species in Transfer Testing, we conducted a three-way repeated measures ANOVA for  
386 the True group with Condition (High Threat S+, Low Threat S+)  $\times$  Stimulus Species (NSWO,  
387 GHOW)  $\times$  Stimulus Distance (short distance, 25m, 50m, 75m, 100m, 150m, 200m) as fixed

388 factors and the proportion of responding during Transfer Testing as the dependent variable.  
389 Using a Huynh-Feldt correction, there was a significant three-way interaction of Condition  $\times$   
390 Stimulus Species  $\times$  Stimulus Distance ( $F_{1, 1, 6} = 9.293, p < 0.001, \eta^2 = .650$ ), indicating that there  
391 was differential responding to stimulus species according to condition across all seven stimulus  
392 distances. The interaction of Condition  $\times$  Stimulus Species ( $F_{1, 6} = 36.109, p = 0.002, \eta^2 = .878$ ),  
393 and the two-way interaction of Stimulus Species  $\times$  Stimulus Distance were also significant ( $F_{1, 6}$   
394  $= 4.779, p = 0.002, \eta^2 = .489$ ), indicating that there was a significant difference in responding to  
395 Stimulus Species based on the Condition, and Stimulus Distance based on Stimulus Species. The  
396 Stimulus Species main effect was significant ( $F_1 = 62.038, p = 0.001, \eta^2 = .925$ ); however, the  
397 two-way interaction of Condition  $\times$  Species Distance and the main effects of Condition and  
398 Stimulus Distance were non-significant (all  $ps > 0.148$ ). To further investigate the three-way  
399 interaction, we conducted independent samples and paired-samples  $t$ -tests; see Table 3 and 4 for  
400 these statistical results, respectively. These results indicate that black-capped chickadees in both  
401 groups were able to transfer training to reward-contingency stimuli recorded at short distances,  
402 and that the Low Threat S+ subgroup responded significantly more to GHOW stimuli compared  
403 to the High Threat S+ subgroup across all seven distances. In contrast, the High Threat S+ group  
404 responded significantly more to stimuli recorded at short distances compared to stimuli recorded  
405 at 150m; see Figure 2. This suggests that there is a perceptual difference from the subjects'  
406 perspective, which is likely due to the physical difference between signals (i.e., a result of signal  
407 degradation).

408       **Synthetic stimuli.** To determine if the pattern of learning was the same across calls from  
409 testing species in Transfer Testing, we conducted a three-way repeated measures ANOVA for  
410 the True group with Condition (High Threat S+, Low Threat S+)  $\times$  Stimulus Type (synthetic

411 high-threat, synthetic low-threat)  $\times$  Stimulus Distance (short distance, 25m, 50m, 75m, 100m,  
412 150m, 200m) as fixed factors and the proportion of responding during Transfer Testing as the  
413 dependent variable. Using a Huynh-Feldt correction, there was a significant three-way  
414 interaction of Condition  $\times$  Stimulus Type  $\times$  Stimulus Distance ( $F_{1, 1, 5} = 4.420, p = 0.005, \eta^2 =$   
415  $.469$ ), indicating that there was differential responding to stimulus species according to condition  
416 across all seven stimulus distances. The two-way interaction of Condition  $\times$  Stimulus Type ( $F_{1, 5}$   
417  $= 37.465, p = 0.002, \eta^2 = .882$ ), and the two-way interaction of Condition  $\times$  Stimulus Distance  
418 were also significant ( $F_{1, 5} = 2.751, p = 0.044, \eta^2 = .355$ ), indicating that there was a significant  
419 difference in responding to Stimulus Type on the Condition and Stimulus Distance based on  
420 Condition. However, the two-way interaction of Stimulus Type  $\times$  Stimulus Distance and the  
421 main effects of Condition, Stimulus Type, and Stimulus Distance were non-significant (all  $ps >$   
422  $0.245$ ). To further investigate the three-way interaction, we conducted independent samples and  
423 paired-samples  $t$ -tests; see Table 5 and 6 for these statistical results. These results indicate that  
424 black-capped chickadees in the High Threat S+ group were able to transfer training to stimuli  
425 recorded at 25m and 50m, and responded significantly more to stimuli recorded at 25m  
426 compared to stimuli recorded at 100m. However, unlike responding to the natural stimuli, the  
427 Low Threat S+ subgroup did not respond significantly more to low-threat stimuli compared to  
428 the High Threat S+ subgroup across distances; see Figure 3. These results demonstrate that  
429 chickadees in the High Threat S+ group performed similarly to natural and synthetic stimuli,  
430 suggesting that chickadees trained to recognize and respond to highly threatening stimuli were  
431 capable due to the acoustic similarities.

432

## Discussion

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

#### 442 **Natural Stimuli: Short Distance**

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

#### 455 **Natural Stimuli: Distant**

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

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

471 A second hypothesis is that there may be an issue with transmission of high-threat  
472 NSWO signals over distance, as signal degradation may be a cause for the error in perception. It  
473 is likely that our higher-frequency, high-threat NSWO calls may be more affected by signal  
474 degradation than low-frequency, low-threat GHOW calls (see Figure 4). Upon transmission, the  
475 high-frequency notes contained in the black-capped chickadee *chick-a-dee* calls (i.e., A, B, C  
476 notes) attenuated most in dense coniferous forests compared to deciduous and mixed forests  
477 (Proppe et al. 2010). This is further supported by the analyses conducted on our stimuli set in  
478 which there were statistical differences between the original, short distance NSWO stimuli and

479 the stimuli at each distance; this was not true of GHOW stimuli (see Table 2). Yip et al. (2017)  
480 also demonstrated that sound attenuation appears to be frequency-dependent in that high-  
481 frequency songbird vocalizations had a lower effective detection radius compared to lower-  
482 frequency owl vocalizations, and that high-frequency vocalizations attenuated more when played  
483 back in the forest compared to roadside playback. Considering that our stimuli were re-recorded  
484 at multiple distances throughout the boreal forest, which is primarily coniferous, this could  
485 explain our results, suggesting a strong influence of signal degradation on high-frequency, high-  
486 threat NSWO calls compared to low-frequency, low-threat GHOW calls. In general, call  
487 propagation rates can vary depending on the species due to acoustic traits like call frequency and  
488 structure (i.e., NSWO could attenuate more quickly than GHOW) and signals will degrade at  
489 differing rates depending on those acoustic traits over distance (i.e., due to reverberation,  
490 refraction, and absorption; Yip et al., 2017). Thus, we propose that the degradation impacts  
491 signal perception by impairing category perception.

492         Nonetheless, we recommend further investigation to examine this lack of responding to  
493 originally high-threat NSWO calls at further distances, perhaps by including stimuli that have  
494 similar acoustic characteristics to high threat calls, yet are of low threat, we can tease apart if  
495 responding is based on the threat posed by the vocalizations or the effect of signal degradation  
496 across distance. However, large animals typically produce vocalizations with lower frequencies  
497 compared to small predators (e.g., Martin et al. 2011) so finding a stimulus that would satisfy  
498 both higher-frequency and low-threat characteristics might be difficult. Conversely, NSWOs and  
499 GHOWs pose varying threat levels to chickadees, but not to humans (*Homo sapiens*). Yip et al.  
500 (2017) had human observers determine at what distances high-frequency songbird and lower-  
501 frequency owl vocalizations were detectable and found that the results for NSWO and GHOW

502 calls to be comparable in that both stimuli were detectable or not detectable, dependent on the  
503 observer. We propose that an extension of the current experiment could be completed as a  
504 comparative go/no-go task with human participants to further investigate if lack of responding to  
505 NSW0 stimuli is based on threat perception or signal degradation.

### 506 **Synthetic Stimuli**

507         In the current study, synthetic stimuli were created to sound like high-threat NSW0 and  
508 low-threat GHOW calls. We predicted chickadees would respond to synthetic high-threat and  
509 low-threat tones similarly to the natural predator calls. Chickadees in the High Threat S+ and  
510 Low Threat S+ subgroups did in fact respond appropriately to high-threat and low-threat  
511 synthetic tones, respectively, but did so at lower and typically non-significant levels. The finding  
512 that our subjects responded to synthetic stimuli similarly, although at a lower level, to the  
513 original calls is important as it suggests that our synthetic signals contain some acoustic features  
514 of these owl calls that are related to the concept of high- and low-threat, but that the birds can  
515 still perceive them as different from owl calls. The most pivotal result is that the High Threat S+  
516 group outperformed the Low Threat S+ group at responding to appropriate stimuli across  
517 distance, which suggests a biologically-relevant finding, critical for survival: Chickadees  
518 recognized and responded similarly to stimuli that mimicked a natural, high-threat predator's  
519 call.

### 520 **Conclusions**

521         Overall, this experiment provides insights into songbird perception of predator threat, and  
522 how that perception is affected by distance and signal degradation. Our findings do not support  
523 our prediction that black-capped chickadees would continue to discriminate high-threat signals at  
524 further distances compared to low-threat signals. Again, we propose that chickadees in the High

525 Threat S+ subgroup responded in this way as they were discriminating threat not species as at  
526 further distances small predators may no longer pose a high threat. This would explain the  
527 difference between the High Threat S+ and Low Threat S+ results. Future studies are necessary  
528 to parse threat perception from the effects of signal degradation, including stimuli of high-threat  
529 and low-frequency and/or comparative trials with humans. Synthetic tones that were created to  
530 match frequency and duration of NSW0 and GHOW stimuli have demonstrated that chickadees  
531 will respond to tones similarly compared to natural predator calls. If chickadees had  
532 inappropriately responded to synthetic stimuli that were acoustically similar to high-threat  
533 predator calls, then the species may not survive. In general, an inability to discriminate between  
534 biologically-relevant and biologically-irrelevant acoustic stimuli may result in a loss of  
535 opportunities to feed or mate due to antipredator behaviours, or could instead result in  
536 habituation to the incorrect signals (i.e., not producing antipredator behaviours in the presence of  
537 a high-threat predator). Thus, this ability to perceive and respond to threat appropriately was  
538 likely selected for. In total, the natural and synthetic stimuli used in the current experiment  
539 provides many insights into the threat perception of songbirds, including the effects of distance  
540 and signal degradation, and the perceptual similarities between natural and synthetic stimuli

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### Acknowledgements

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

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571 **Table 1.**  
572 Average signal-to-noise ratio of all great horned owl (GHOW) and northern saw-whet owl  
573 (NSWO) calls at each distance of the original and rebroadcast stimuli, calculated using Audacity  
574 2.4.2. (The Audacity Team, Carnegie Mellon University, PA, USA).

<i>Distance</i>	<i>GHOW</i>	<i>NSWO</i>
<i>Short</i>	35.920	31.46
<i>25m</i>	26.075	13.835
<i>50m</i>	20.930	13.288
<i>75m</i>	19.943	13.135
<i>100m</i>	18.920	12.555
<i>150m</i>	15.988	12.030
<i>200m</i>	13.703	4.800

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599 **Table 2.**

600 Results of the paired samples *t*-tests comparing signal-to-noise ratios of the original, short  
 601 distance stimuli to the rebroadcast stimuli of the six additional distances. Bonferroni corrections  
 602 have been applied ( $p = .05/6$  tests = .0083).

<i>Paired Samples t-test Comparison</i>	<i>GHOW t-value (df=3) &amp; p-value</i>	<i>NSWO t-value (df=3) &amp; p-value</i>
<i>Short v. 25m</i>	2.183, .117	<b>8.063, .004</b>
<i>Short v. 50m</i>	2.408, .095	<b>8.314, .004</b>
<i>Short v. 75m</i>	2.636, .078	<b>7.104, .006</b>
<i>Short v. 100m</i>	2.775, .069	<b>8.224, .004</b>
<i>Short v. 150m</i>	2.854, .065	<b>6.432, .008</b>
<i>Short v. 200m</i>	2.885, .063	<b>10.452, .002</b>

603 **Bold** font indicates significance.

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631 **Table 3.**

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

635 Note: Negative *t*-values indicate that Low Threat S+ responded more than High Threat S+.

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

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641 **Table 4.**

642 Results of the paired-samples *t*-tests comparing subjects' responding in High Threat S+ and Low  
 643 Threat S+ groups to each natural stimulus between natural NSW0 and GHOW stimuli recorded  
 644 at short distances vs. distant stimuli during Transfer Testing, with Bonferroni corrections ( $p =$   
 645  $0.05/6 = 0.0083$ ).

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

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656 **Table 5.**

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

660 Note: Negative *t*-values indicate that Low Threat S+ responded more than High Threat S+.

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

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665 **Table 6.**

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

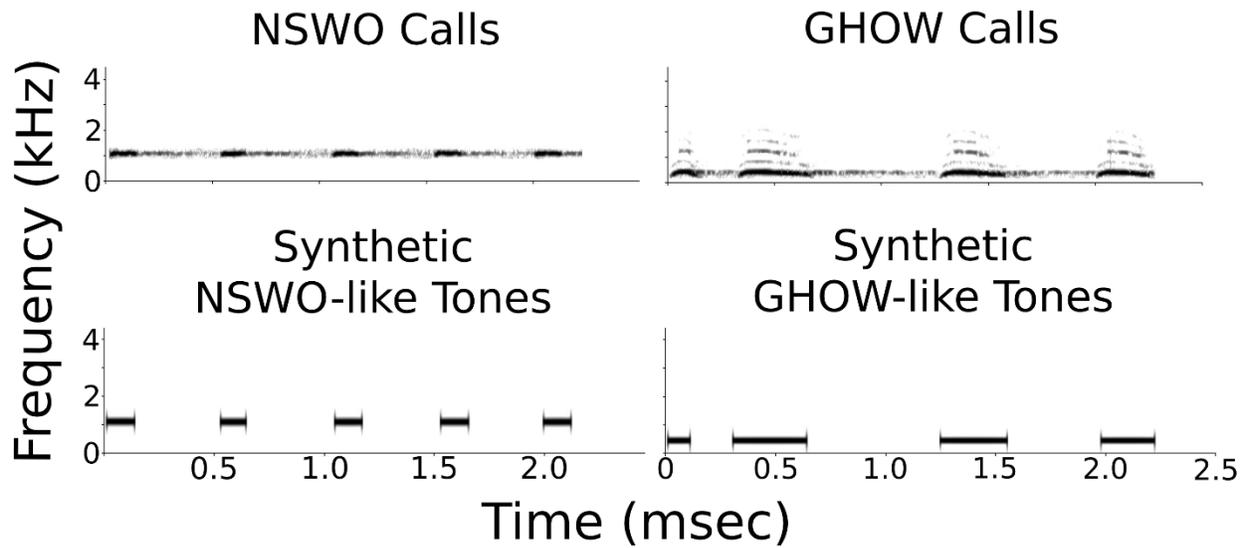
	<i>t</i> -test	<i>p</i> -value	<i>Cohen's d</i>	<i>Confidence Intervals</i>
<b>High Threat S+ (<i>df</i> = 5)</b>				
<i>high-threat 25 v. 50m</i>	3.038	0.029	1.073	0.026, 0.308
<i>high-threat 25 v. 75m</i>	2.236	0.076	0.879	-0.025, 0.358
<b><i>high-threat 25 v. 100m</i></b>	<b>9.400</b>	<b>&lt;0.001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

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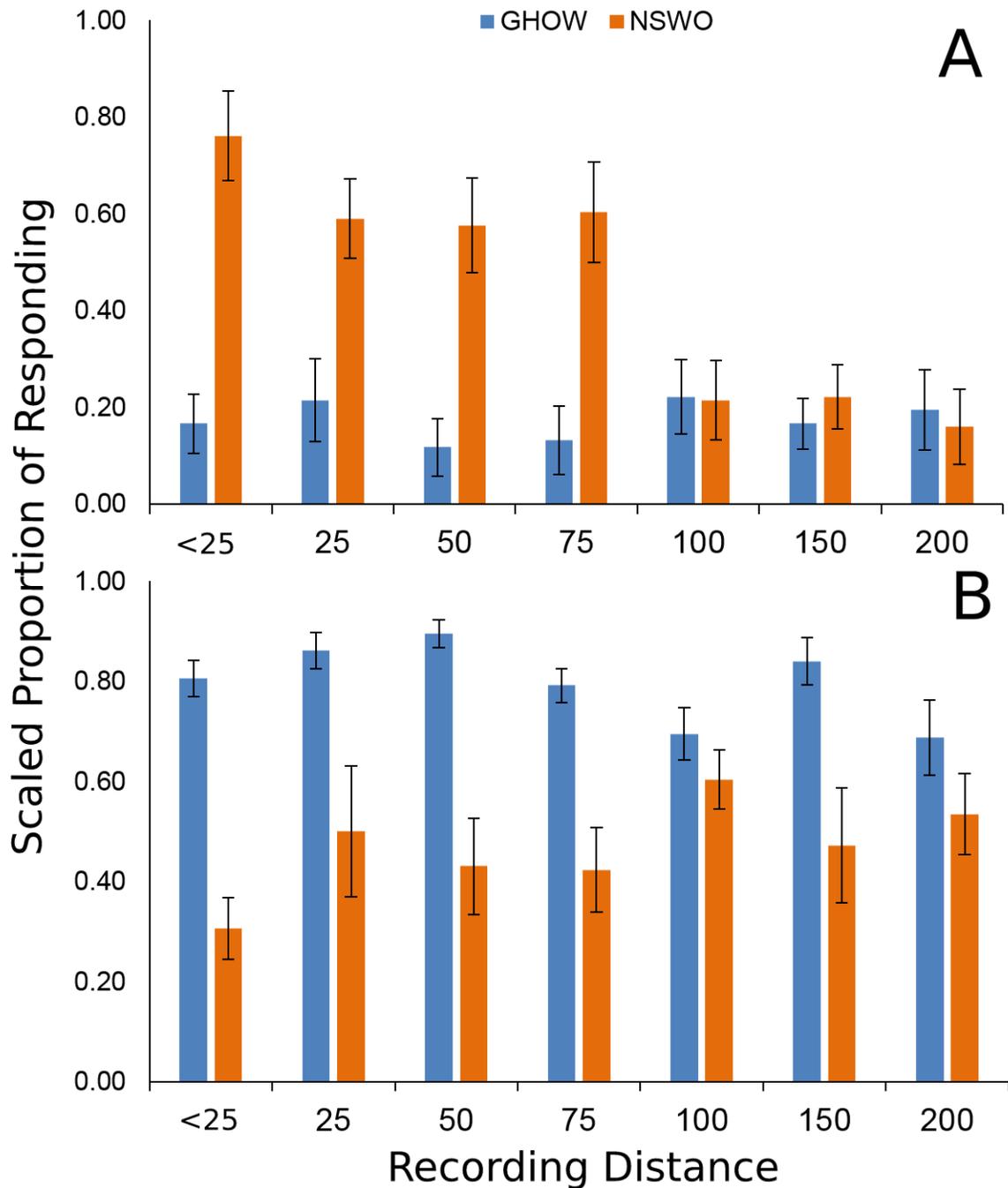
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*Figure 1.* Sample sound spectrograms of high-threat northern saw-whet owl (NSWO) and low-threat great horned owl (GHOW) calls, and synthetic high-threat and low-threat tones used as acoustic stimuli with time (msec) on the *x*-axis and frequency (kHz)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

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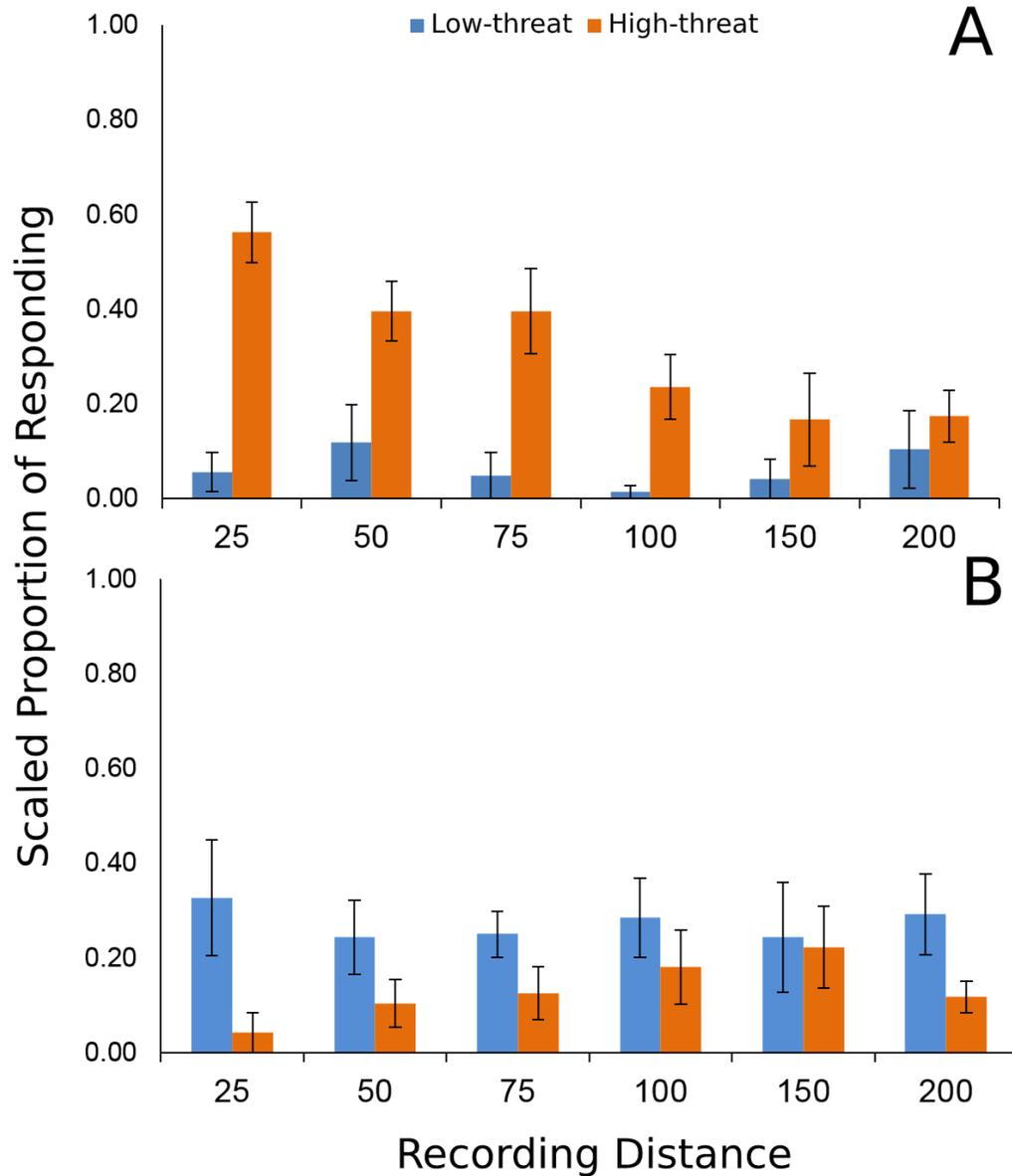


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681 *Figure 2.* Scaled proportion of responding to natural high-threat NSW and low-threat GHOW  
 682 calls by black-capped chickadees in the High Threat S+ subgroup (**A**;  $n = 6$ ) and Low Threat S+  
 683 subgroup (**B**;  $n = 6$ ) during the Transfer Testing phase  $\pm$  SEM across recording distances (short  
 684 distances/<25, 25, 50, 75, 100, 150, 200m).

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688 *Figure 3.* Scaled proportion of responding to synthetic high-threat and low-threat synthetic tones  
 689 by black-capped chickadees in the High Threat S+ subgroup (**A**;  $n = 6$ ) and Low Threat S+  
 690 subgroup (**B**;  $n = 6$ ) during the Transfer Testing phase  $\pm$  SEM across recording distances (25, 50,  
 691 75, 100, 150, 200m).

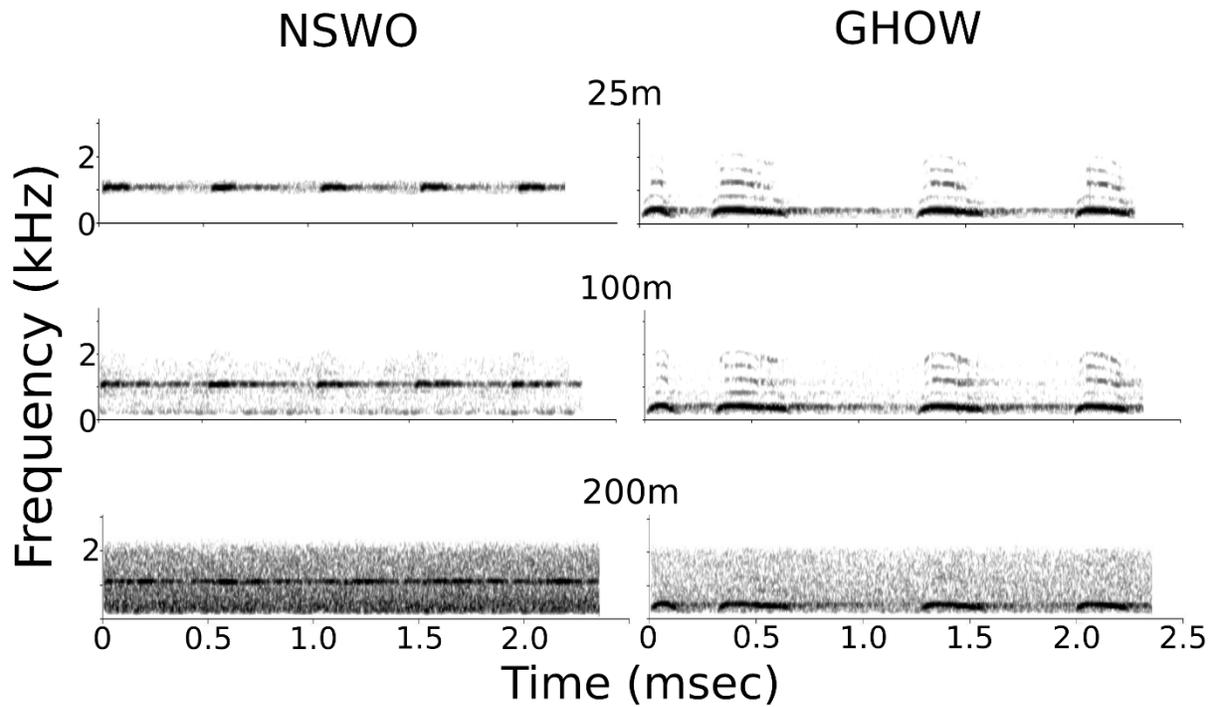
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698 *Figure 4.* Sample sound spectrograms of high-threat northern saw-whet owl (NSWO) and low-

699 threat great horned owl (GHOW) calls across distances (25m, 100m, and 200m), with time

700 (msec) on the *x*-axis and frequency (kHz) on the *y*-axis, to demonstrate the effect of signal

701 degradation on NSWO stimuli compared to GHOW stimuli.

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**References**

- 715 Avey MT, Hoeschele M, Moscicki MK, Bloomfield LL, & Sturdy CB (2011) Neural correlates  
716 of threat perception: Neural equivalence of conspecific and heterospecific mobbing calls is  
717 learned. *PLoS One* 6:e23844.  
718
- 719 Baker MC & Becker AM (2002) Mobbing calls of black-capped chickadees: Effects of urgency  
720 on call production. *The Wilson Journal of Ornithology* 114:510-517.  
721
- 722 Briggs DE (2015) The cambrian explosion. *Current Biology* 25:R864-R868.  
723
- 724 Chandler CR & Rose RK (1988) Comparative analysis of the effects of visual and auditory  
725 stimuli on avian mobbing behavior. *Journal of Field Ornithology* 59:269-277.  
726
- 727 Congdon JV, Hahn AH, Filippi P, Campbell KA, Hoang J, Scully EN, Bowling DL, Reber SA,  
728 & Sturdy CB (2019) Hear them roar: A comparison of black-capped chickadee (*Poecile*  
729 *atricapillus*) and human (*Homo sapiens*) perception of arousal in vocalizations across all  
730 classes of terrestrial vertebrates. *Journal of Comparative*  
731 *Psychology*:<http://dx.doi.org/10.1037/com0000187>.  
732
- 733 Dudley R (2002) Mechanisms and implications of animal flight maneuverability. *Integrative and*  
734 *Comparative Biology* 42:135-140.  
735
- 736 Griffiths R, Double MC, Orr K, & Dawson RJ (1998) A DNA test to sex most birds. *Molecular*  
737 *Ecology* 7:1071-1075.  
738
- 739 Hahn AH, Hoeschele M, Guillette LM, Hoang J, McMillan N, Congdon JV, ... & Ratcliffe LM.  
740 (2016) Black-capped chickadees categorize songs based on features that vary  
741 geographically. *Animal Behaviour* 112:93-104.  
742
- 743 Hahn AH, Campbell KA, Congdon JV, Hoang J, McMillan N, Scully EN, ... & Sturdy CB  
744 (2017) Discrimination of acoustically similar conspecific and heterospecific vocalizations  
745 by black-capped chickadees (*Poecile atricapillus*). *Animal Cognition* 20:639-654.  
746
- 747 Hoang J (2015). *Pitch perception is not unitary: Evidence for the perception of pitch chroma in*  
748 *black-capped chickadees* (Unpublished master's thesis). University of Alberta, Edmonton,  
749 AB, Canada.  
750
- 751 Kryštofková M, Haas M, & Exnerová A (2011) Nest defense in blackbirds *Turdus merula*: Effect  
752 of predator distance and parental sex. *Acta Ornithologica* 46:53-61  
753
- 754 Martin JP, Doucet SM, Knox RC, & Mennill DJ (2011) Body size correlates negatively with the  
755 frequency of distress calls and songs of Neotropical birds. *Journal of Field Ornithology*  
756 82:259-268.  
757

- 758 Njegovan M, Hilhorst B, Ferguson S, & Weisman R (1994) A motor-driven feeder for operant  
759 training in song birds. *Behavior Research Methods, Instruments, & Computers* 26:26-27.  
760
- 761 Palya WL & Walter DE (2001) *Document set for the high-performance experiment controller*.  
762 Retrieved 25 October 2014 from [http://www.jsu.edu/depart/psychology/sebac/Exp-](http://www.jsu.edu/depart/psychology/sebac/Exp-Ctl.html)  
763 [Ctl.html](http://www.jsu.edu/depart/psychology/sebac/Exp-Ctl.html).  
764
- 765 Proppe DS, Bloomfield LL, & Sturdy CB (2010) Acoustic transmission of the *chick-a-dee* call of  
766 the black-capped chickadee (*Poecile atricapillus*): Forest structure and note function.  
767 *Canadian Journal of Zoology* 88:788-794.  
768
- 769 Pyke GH, Pulliam HR, & Charnov EL (1977) Optimal foraging: a selective review of theory and  
770 tests. *The Quarterly Review of Biology* 52:137-154.  
771
- 772 Pyle (1997) Identification guide to North American birds. Slate Creek Press, Bolinas.  
773
- 774 Sturdy CB & Weisman RG (2006) Rationale and methodology for testing auditory cognition in  
775 songbirds. *Behavioural Processes* 72:265-272.  
776
- 777 Templeton CN & Greene E (2007) Nuthatches eavesdrop on variations in heterospecific  
778 chickadee mobbing alarm calls. *Proceedings of the National Academy of Sciences*  
779 104:5479-5482.  
780
- 781 Templeton CN, Greene E, & Davis K (2005) Allometry of alarm calls: Black-capped chickadees  
782 encode information about predator size. *Science* 308:1934-1937.  
783
- 784 Yip DA, Bayne EM, Sóllymos P, Campbell J, & Proppe D (2017) Sound attenuation in forest and  
785 roadside environments: Implications for avian point-count surveys. *The Condor:*  
786 *Ornithological Applications* 119:73-84.