Chickadee behavioural response to varying threat levels of predator and conspecific calls

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Abstract

Chickadees produce many vocalizations, including *chick-a-dee* calls which they use as a mobbing call in the presence of predators. Previous research has shown that chickadees produce more D notes in their mobbing calls in response to high-threat predators compared to low-threat predators, and may perceive predator and corresponding mobbing vocalizations as similar. We presented black-capped chickadees with playbacks of high- and low-threat predator calls, high- and low-threat conspecific mobbing calls, non-threatening heterospecific calls, and reversed conspecific mobbing calls to examine vocal and movement behavioural responses. Chickadees produced more *chick-a-dee* calls in response to playback of calls produced by a high-threat predator compared to calls produced by a low-threat predator, and to reversed high-threat mobbing calls compared to normal (i.e., non-reversed) high-threat mobbing calls. Chickadees also vocalized more in response to all playback conditions consisting of conspecific mobbing calls compared to a silent baseline period. The number of D notes produced was similar to previous findings; chickadees produced approximately one to three D notes per call in response to low-threat mobbing calls, and produced more calls containing four to five D notes in response to high-threat mobbing calls, although this difference in the number of D notes per call was not significant. The difference in chickadees' production of *tseet* calls across playback conditions approached significance as chickadees called more in response to conspecific mobbing calls, but not in response to heterospecific calls. General movement activity decreased in response to playback of conspecific-produced vocalizations, but increased in response to heterospecific-produced vocalizations, suggesting that chickadees may mobilize more in response to predator playback in preparation for a “fight or flight” situation. These results also suggest that chickadees may produce more mobbing calls in response to high-threat predator vocalizations as an attempt to initiate mobbing with conspecifics, while they produce fewer mobbing calls in response to a low-threat predator that a chickadee could outmaneuver.

Keywords: animal behaviour; black-capped chickadee; predator alarm; mobbing call; communication; playback; songbird

Alarm and mobbing calls allow social animals to inform conspecifics, and reciprocal heterospecifics about the presence of predators (Sherman, 1977). For example, vervet monkeys (*Cercopithecus aethiops*) live in troops which produce unique alarm calls to three different types of predators. Each alarm call results in a different behavioural reaction by troop members (i.e., diving into a bush, climbing a tree, or searching the ground to initiate mobbing) (Struhsaker, 1967). In the Paridae family, great tits (*Parus major minor*) have been known to produce two discrete alarm calls to different predators; ‘jar’ calls are produced in response to snakes, while ‘chicka’ calls are produced to crows and martens (Suzuki, 2015). When a ‘jar’ call is made, nestlings jump out of the nest to escape from a snake, but when a ‘chicka’ call is made it is more appropriate to hide in the nest cavities since crows and martens attack nestlings from outside (Suzuki, 2011). Some avian species such as domestic chickens (*Gallus gallus domesticus*) produce acoustically different alarm calls after seeing an aerial versus terrestrial predator (Gyger, Marler, & Pickert, 1986), and chickens respond differentially to hearing these two types of alarm calls (i.e., crouching vs. erect posture; Evans, Evans, & Marler, 1993). All predators are not an equal threat, and these previous studies suggest that the perception of risk varies which directly influences anti-predator responses. Birds will attend to heterospecific vocalizations, not simply due to sounding similar to their own alarm calls (Fallow, Pitcher, & Magrath, 2013), but instead because they learn fear (see Sturdy & Proppe, 2015). For example, Magrath, Haff, McLachlan, and Igic (2015) demonstrated that superb fairy-wrens (*Malurus cyaneus*) originally ignored unfamiliar sounds, but would flee following only two days of training that paired the unfamiliar sounds with predator models. While alarm calls are produced in response to a predator, mobbing calls are used to coordinate nearby species to attack the predator to drive it away from the area (Pettifor, 1990). The survival of the receiver is based on their successful response to heterospecific and conspecific vocalizations (Magrath et al., 2015); and how birds respond to both predator and mobbing calls is the question we attempted to address with this study.

Black-capped chickadees (*Poecile atricapillus*), part of the Paridae family, are non-migratory North American songbirds (Smith, 1991). Chickadees are social animals that produce numerous vocalizations, including one of the most simple, but frequently used calls - the *tseet* call. This is a one-note call that is used as a contact call to other conspecifics within the flock or mated pairs (e.g., Odum, 1942). Chickadees of both sexes also produce *chick-a-dee* calls year-round (e.g., Odum, 1942). The *chick-a-dee* call is comprised of four note types: A, B, C, and D, which can be separated into a '*chick-a*' portion (composed of A, B, and/or C notes) and a '*dee*' portion (composed of D notes). The *chick-a-dee* call is a signal used to coordinate flock movements and chickadees use D notes to recognize flock-mates (Mammen & Nowicki, 1981). In addition, *chick-a-dee* calls, and specifically D notes, are used to recruit and mobilize chickadees and other avian species to attack and harass a nearby predator (Hailman, Ficken, & Ficken, 1987) and in these instances, *chick-a-dee* calls are referred to as 'mobbing calls'.

Chickadees are prey to many avian (e.g., owls, hawks) and mammalian (e.g., cats, weasels) predators. Small owls, which can easily maneuver through dense trees, are a higher threat to a chickadee's survival compared to larger owls (Howland, 1974). The number of D notes produced in black-capped chickadees' *chick-a-dee* mobbing calls are positively correlated with the degree of size, and thus threat level (Templeton et al., 2005). Specifically, more D notes are produced in response to smaller, higher-threat predators, creating a negative correlation between predator body length and D note production. Carolina chickadees (*P. carolinensis*), a close relative to black-capped chickadees, produced more '*chick-a*' notes and fewer D notes to larger, lower-threat predators, and few or no '*chick-a*' notes and significantly more D notes in response to smaller, higher-threat predators (Soard & Ritchison, 2009). Another parid, tufted titmice (*Baeolophus bicolor*) produced longer mobbing bouts with more D notes per call to mounts of smaller, higher-threat predators, and took longer to return to feeding after playback of these mobbing vocalizations in comparison to control calls (Courter & Ritchison, 2010). Billings, Greene, and Jensen (2015) found that black-capped and mountain (*P. gambeli*) chickadees produced more *chick-a-dee* calls to playback of small, high-threat predators (northern pygmy-owl, *Glaucidium gnoma*, and sharp-shinned hawk, *Accipiter striatus*) than a large, low-threat predator (northern goshawk, *Accipiter gentilis*), indicating that chickadees discriminate and respond differentially to predator calls based on threat level. Overall, many chickadee species alter vocal responses based on perceived threat, including producing more mobbing calls, typically containing a higher number of D notes, to more dangerous predators.

Now that we understand how chickadees alter their vocal behaviour in the presence of a predator and in response to predator calls, how do chickadees perceive acoustically distinct predator calls and chickadee mobbing calls? Avey, Hoeschele, Moscicki, Bloomfield, and Sturdy (2011) measured the amount of immediate early gene (IEG) expression in chickadee auditory forebrain areas following playback of various vocalizations in order to investigate whether neural responses varied with the threat level conveyed by black-capped chickadee mobbing calls, and whether neural response to mobbing calls was the same as the neural response evoked by the actual predators' calls. Avey et al. presented subjects with low- and high-threat auditory stimuli, including predator-elicited mobbing calls and the corresponding predator calls, and then compared levels of IEG expression among the playback groups. Higher levels of IEG were observed in the high-threat condition and, within the same threat level, there was no significant difference between the amount of IEG expression in response to predator-elicited mobbing calls compared to the original predator calls. This suggests that wild-caught chickadees perceived owl calls and mobbing calls that indicated the presence of that species of owl similarly, despite acoustic differences between the vocalizations.

Black-capped and Carolina chickadees mob longer and more intensely, and more individuals approach a hidden speaker during playback of small predator alarm mobbing calls (Templeton, Greene, & Davis, 2005; Soard & Ritchison, 2009), suggesting that chickadees mob when they hear high-threat mobbing calls. Templeton and Greene (2007) found that red-breasted nuthatches (*Sitta canadensis*) also approached more closely during heterospecific chickadees' mobbing calls indicating a high-threat predator. Taken together, these studies suggest that hearing mobbing calls influence songbirds' movement behaviour.

Previous experiments examined vocal production in the presence of a live or taxidermy mounted predator, but no studies to our knowledge have investigated vocal production in response to audio recordings of both predator calls and predator-elicited mobbing calls in the same study. Further, no previous research has examined how chickadees respond behaviourally (i.e., movement, such as perch hops, feeding, approaching the predator, etc.) to predator calls versus mobbing calls. The current study examined how chickadees respond to information regarding predator threat: specifically, we investigated chickadees' vocal and movement behavioural responses to predator calls and conspecific mobbing calls that vary based on threat level. Our playback experiment included six conditions: 1) low-threat predator calls (i.e., great horned owl calls, *Bubo virginianus*, GHOW), 2) low-threat predator-elicited conspecific mobbing calls (i.e., black-capped chickadee mobbing calls made in response to the presentation of a great horned owl mount, MOB GHOW), 3) high-threat predator calls (i.e., northern saw whet owl calls, *Aegolius acadicus*, NSWO), 4) high-threat predator-elicited conspecific mobbing calls (i.e., black-capped chickadee mobbing calls made in response to a northern saw-whet owl mount, MOB NSWO), 5) control non-chickadee vocalizations (i.e., red-breasted nuthatch vocalizations, RBNU), and 6) control reversed conspecific mobbing calls (i.e., reversed black-capped chickadee mobbing calls made to a northern saw-whet owl mount REV MOB NSWO).

Based on previous research (e.g.,Courter & Ritchison, 2010; Templeton et al., 2005) we predicted that chickadees would: 1) show a greater increase (compared to baseline) of *chick-a-dee* call production following playback of *chick-a-dee* mobbing calls compared to predator vocalizations; our first prediction was based on the notion that chickadees will produce more *chick-a-dee* calls in response to conspecific calls than predator calls as an attempt to join in on mobbing; 2) produce more *chick-a-dee* calls compared to other vocalizations in high-threat conditions (i.e., following playback of a high-threat predator or high-threat mobbing calls); our second prediction is based on the notion that since *chick-a-dee* calls are associated with mobbing behaviour, these calls would be the main vocalization produced in the context of high threat; 3) emit fewer non-mobbing call vocalizations (e.g., *tseet* calls) during any experimental playback; we predicted that chickadees will not produce non-mobbing call vocalizations during playback as other vocalizations (e.g., *fee-bee* songs, *tseet* calls) are not used for mobbing; 4) produce more D notes in response to high-threat vocalizations compared to low-threat vocalizations, for both predator calls and the corresponding mobbing calls (i.e., stimuli of the same threat); our fourth prediction was driven by Templeton et al.'s (2005) findings that chickadees produce more D notes to smaller, high-threat predators in comparison to large, low-threat ones; since the visual predator resulted in this acoustic response, it seems logical that predator calls, and the mobbing calls of the same threat level, would result in similar vocalizations; 5) suppress movement more in the presence of high-threat predator calls compared to low-threat predator calls; our fifth prediction was based on the notion that movement (e.g., flying, eating, pecking, etc.) could make chickadees more visible or audible to potential predators; therefore, we predicted that after hearing calls of a high-threat predator, chickadees should decrease all movement behaviour to stay inconspicuous, compared to calls of a low-threat predator, as a chickadee could more easily outmaneuver a larger, low-threat predator; this is in line with the results of Courter and Ritchison (2010), which found that tufted titmice took longer to return to feeding after playback of high-threat mobbing vocalizations in comparison to control calls; and 6) suppress movement more in response to predator calls than to mobbing calls; our last (sixth) prediction was based on the notion that birds would suppress movement in the presence of a predator (i.e., hiding) in comparison to conspecific mobbing calls, as mobbing calls should elicit mobbing behaviour.

Materials and Methods

Subjects

We used six adult black-capped chickadees (three males, three females). Subjects were captured from two regions in Edmonton, Alberta, Canada (North Saskatchewan River Valley, 53.53N, 113.53W; Mill Creek Ravine, 53.52N, 113.47W) between January 2010 and February 2012. At time of capture, chickadees were identified as adults by examining the colour and shape of the rectrices (Meigs, Smith, & Van Buskirk, 1983; Pyle, 1997). Sex was determined by DNA analysis (Griffiths, Double, Orr, & Dawson, 1998). Before the experiment, chickadees were housed in individual cages (30 × 40 × 40 cm, Rolf C. Hagen, Inc., Montreal, Quebec, Canada) allowing both visual and auditory contact with conspecifics. Home cages either had nesting boxes or barriers that birds could seek cover inside or behind. Birds were held under the natural light cycle for Edmonton, Alberta. Birds had *ad libitum* access to food (Mazuri Small Bird Maintenance Diet; Mazuri, St. Louis, MO, USA), water (vitamin supplemented three times a week; Prime vitamin supplement; Hagen, Inc.), grit (Rolf C. Hagen Inc., Montreal, Quebec, Canada), and cuttlebone. Birds were also provided three to five sunflower seeds daily, one superworm (*Zophobas morio*) three times a week, and a mixture of eggs and greens (spinach or parsley) twice a week. During the experiment, birds were monitored daily, provided *ad libitum* access to food (i.e., Mazuri), water (vitamin supplemented three times a week), grit, and cuttlebone, and given two superworms per day.

Apparatus

During the experiment, subjects were individually housed in a cage in a sound-attenuating chamber (inner dimensions 58 × 168 × 83 cm; Industrial Acoustics Corporation, Bronx, New York, USA). The cage contained two water bottles, two food cups, three equally-spaced plastic perches, and a small cardboard rodent house. The sound-attenuating chamber door was opened once daily to top up food and water and provide a supplemental worm to each bird. To prevent excessive noise disturbances, all birds (including those not being recorded) had food and water topped up following the entirety of the playback trials. All subjects were monitored twice daily (1000 and 1700) via video camera accessed externally.

Playback Stimuli

Avey et al. (2011) obtained mobbing calls by presenting black-capped chickadees with mounts of a northern saw-whet owl (high-threat predator) and a great horned owl (low-threat predator). These mobbing calls, along with northern saw-whet, great-horned owl, and red-breasted nuthatch calls, and computer-manipulated reversed northern saw-whet induced mobbing calls, used by Avey et al. (2011), were used in the current study (see Avey et al., 2011 for full details on obtaining the playback stimuli). Two different sets were generated for each stimulus category (e.g., two sets of northern saw-whet owl calls) to ensure that any differences in responding across conditions was due to the threat level of the stimulus, and not the length of the stimulus or individuals' vocalizations used to generate the stimulus. Playback stimuli contained vocalizations played for 15 s followed by 45 s of silence, repeated 15 times, for a total of 15 minutes. The number of calls presented within each 15-s window varied across conditions, but were as natural as possible for the species selected (see Table 1; Figure 1).

Playback Procedure

Prior to and during playback, each subject was housed in their home cage located within one of six randomly-assigned sound-attenuating chambers. Each bird was given 24 hr to acclimatize to the chamber before hearing one of the playback conditions. Subjects were exposed to a randomly-assigned playback condition every other day (i.e., three subjects per day, alternating days), with approximately 48 hours between each bird's playback sessions. Start times were constant for each bird (i.e., 12:45, 13:15, or 13:45). The order that the subjects were run was randomly assigned on day one of playback and remained the same throughout the experiment. We randomly assigned the order that each subject would hear playback stimuli using a 6×6 Latin square; all six subjects heard all six playback conditions. Each subject was recorded for a total of 30 minutes a day (15 minutes of silence, 15 of playback). Playback sessions were carried out sequentially, to one individual at a time.

The experiment was conducted August 15-21, 2014, before the fall equinox in mid-September, when both *chick-a-dee* calling and *fee-bee* song production are low (Avey et al., 2008). In each chamber, stimuli were played through an amplifier (Cambridge Audio, Azur 640A Integrated Amplifier; London, UK) to a speaker (Fostex

FE108 Σ or Fostex FE108E Σ full-range speaker; Fostex Corp., Japan; frequency response range 80-18,000 Hz) using an mp3 player (Creative ZEN; Singapore). Amplitude was measured at the level of the perches from the centre position of the cage and playback amplitude was set to approximately 75 db with a Brüel & Kjær Type 2239 sound level meter (Brüel & Kjær Sound & Vibration Measurement A/S, Nærum, Denmark; A weighting, slow response). Audio recordings of the subjects were obtained using six AKG C 1000S condenser microphones (frequency response: 50-20,000 Hz; AKG Acoustics, Vienna, Austria), and six solid-state recorders (Marantz PMD670, D&M Professional, Itasca, IL, USA). Video recordings of the playbacks were obtained using a video camera (Sony Handycam DCR-SX45, Sony Electronics Asia Pacific Pte Ltd., Tokyo, Japan, or Canon VIXIA HF R500, Canon Canada Inc., Mississauga, Ontario, Canada) and video capture software (EZ Grabber, Geniatech, Beijing, China) installed on a personal computer.

Re-recordings

During building renovations, background construction noise occurred when conducting the playback of one subject and the baseline period of another subject. Playback trials for these subjects were re-run 48 hours later to obtain the subjects' behavioural responses without interruption. For the subject whose playback condition (i.e., MOB GHOW) was re-run, there was no significant difference in vocal behaviour compared to the first session before interruption (t_{14} = .475, p = .642, d = .046). The other subject's baseline period was interrupted, so only heard the playback when the condition was re-run.

Response Measures

Audio and video files were scored separately using SIGNAL sound analysis software (Engineering Design, Version 5.10.24, RTS, Berkeley, California, USA) to identify chickadee vocalizations, and VLC Media Player (VideoLAN, 2.1.3 Rincewind, Paris, France) to quantify movement behaviour. The first author analyzed all audio files for vocal responses, while two undergraduate volunteers (blind to the playback conditions and predictions) examined the video files for movement responses. The first author then verified the response quantification conducted by the volunteers to ensure scoring was consistent and resolved any disparities; this response quantification was used for analysis. We quantified behaviours in the 15 minutes of baseline (prior to hearing the first playback stimulus) and in the 15 minutes of playback. We quantified five classes of vocal behaviours: *chick-a-dee* calls (categorized by the number of D notes; D note composition included *chicka*s and *chick-a-dee* calls with 1 D, 2 D, 3 D, 4 D, 5 D, 6 D notes), *gargle* calls, *fee-bee* songs (including *fee* only songs), and *tseet* calls. We quantified eight classes of movement behaviours: general activity (i.e., perch hops), food visits, water visits, ruffles, pecking bouts, beak wipes, approaches. See Table 2 for a description of the behaviours we quantified. Behavioural data from the six experimental conditions of each individual were separated into two phases: baseline and playback. For each individual, we subtracted baseline behaviours from the behaviours during playback to obtain a difference from baseline measure for each behaviour in every condition.

Statistical Analyses

We conducted repeated measures ANOVAs for each vocal and movement behaviour across the six playback conditions (n = 6 chickadees). Paired-samples t-tests were run to investigate significant differences in *chick-a-dee* call production across playback conditions. Huynh-Feldt correction was used on all repeated measures tests to correct for any possible violations in sphericity. Alpha levels were set at 0.05. We based our sample size on previous behavioural studies conducted in our lab (Hoeschele, Moscicki, Otter, van Oort, Fort, Farrell, Homan, Robson, & Sturdy, 2010). No animals were excluded from analyses. Recordings that were impacted by noise from building renovations were not included as described above.

Ethical Note

Birds remained in the sound chamber throughout testing, minimizing the transport and handling of each bird. Following the experiment, birds were returned to the colony room for use in future experiments. All procedures were conducted in accordance with the Canadian Council on Animal Care 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 (ABS) 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 a City of Edmonton Partners in Parks permit.

Results

Overall Vocal Output

Prediction 1. Figure 2 illustrates the difference from baseline in vocal responses of *chick-a-dee* calls (broken down by D note composition) made to each stimulus set. This graph shows that chickadees produced fewer *chick-a-dee* calls during playback of GHOW compared to baseline. Chickadees also decreased production of *chick-a* calls during playback of NSWO compared to baseline, but there was a slight increase in production of *chick-a-dee* calls containing one to six or more D notes. In addition, in comparison to heterospecific-produced playback conditions (i.e., owl and nuthatch calls), chickadees produced more *chick-a-dee* calls in response to all conspecific-produced playback conditions (Fig. 2). In general, chickadees vocalized more in response to conspecific stimuli. Chickadees produced fewer *chick-a-dee* calls, compared to baseline, containing four or more D notes in response to the MOB GHOW condition, but a one-way repeated measures ANOVA indicated that there were no significant differences in D note production between baseline and playback ($F_{2,9}$ = 1.99, p = .194, η_p^2 = 0.28). However, there was a significant difference in the *chick-a-dee* call production between NSWO (M = 9.50, SD = 11.20) and GHOW (M = -15.67, SD = 24.04) conditions, t_5 = -2.61, p = .048, d = 1.34, with chickadees producing more calls in response to the high-threat owl calls (NSWO) than the low-threat owl calls (GHOW). There was also a significant difference in the *chick-a-dee* call production between MOB NSWO (M = 23.00, SD = 50.93) and REV MOB NSWO (M = 55.83, SD = 52.044) conditions, t_5 = -3.51, p = .017, d = 6.38, with chickadees producing fewer calls in response to the high-threat mobbing calls (MOB NSWO) than the control condition (REV MOB NSWO). No other comparisons were significant (all ps $\geq$ .058).

Prediction 2. Chickadees produced slightly more *chick-a-dee* calls, over other vocalizations, in the NSWO condition in comparison to the GHOW playback condition. However, a 4 × 6 repeated measures ANOVA indicated no significant differences in the production of *chick-a-dee* calls in comparison to other vocalizations ($F_{1,5} = 3.53$, $p = .12$, $\eta_p^2 = 0.41$).

Prediction 3. Figure 3 shows that chickadees produced more *tseet* calls in response to chickadee-produced vocalizations, regardless of threat level. The difference in *tseet* production across playback conditions approached significance (one-way repeated measures ANOVA; $F_{2,11} = 3.46$, $p = .06$, $\eta_p^2 = 0.41$). *Gargles* (one-way repeated measures ANOVA; $F_{2,12} = 1.20$, $p = .34$, $\eta_p^2 = 0.19$) and *fee-bee* songs (one-way repeated measures ANOVA; $F_{5,25} = 1.45$, $p = .24$, $\eta_p^2 = 0.23$) did not differ across conditions.

Prediction 4. Last, the difference in D note composition across playback conditions (e.g., high-threat vs. low-threat) was not significant (7 × 6 repeated measures ANOVA; $F_{2,12} = 1.27$, $p = .32$, $\eta_p^2 = 0.20$). Despite this, there appear to be differences in the D note composition of *chick-a-dee* calls produced as chickadees produced more calls with four D notes per call to high-threat (i.e., NSWO and MOB NSWO) than to low-threat conditions (i.e., GHOW and MOB GHOW) (Fig. 2).

Overall Movement Behaviour

Predictions 5 & 6. General movement behaviour was significantly different across playback conditions (one-way repeated measures ANOVA; $F_{5,25} = 3.45$, $p = .02$, $\eta_p^2 = 0.41$). Chickadees exhibited less general activity relative to baseline in response to chickadee-produced calls (i.e., MOB GHOW, MOB NSWO, and REV MOB NSWO) regardless of threat level (low versus high). In contrast, chickadees exhibited more general activity relative to baseline in response to non-chickadee produced calls (i.e., GHOW, NSWO, and RBNU; see Figure 5). As a simple demonstration of this, we *post hoc* combined the averages of birds' movement difference scores for each conspecific playback($M = -195.39$, $SD = 86.22$); MOB GHOW, MOB NSWO, REV MOB NSWO) and heterospecific playback ($M = 85.06$, $SD = 21.92$; GHOW, NSWO, RBNU) and conducted a paired-samples t-test which showed a significant difference in the behaviour to these pooled classes of stimuli ($t_2 = 4.65$, $p = .043$, $d = 4.46$).

Figure 6 illustrates the difference from baseline of non-perch hop movement behaviour across the six playback conditions. Almost all non-perch hop movements decreased during playback across all six conditions, however these were not significantly different from baseline (one-way repeated measures ANOVAs; food visits: $F_{5,24} = 1.25$, $p = .32$, $\eta_p^2 = 0.20$; water visits: $F_{2,9} = 2.20$, $p = .17$, $\eta_p^2 = 0.31$; pecking bouts: $F_{2,11} = 0.80$, $p = .49$, $\eta_p^2 = 0.14$; and beak wipes: $F_{3,14} = 1.04$, $p = .40$, $\eta_p^2 = 0.17$.

Ruffles and approaches are plotted together in Figure 7. A one-way repeated measures ANOVA indicated no significant difference in the production of ruffles across playback conditions ($F_{3,13} = 1.79$, $p = .20$, $\eta_p^2 = 0.26$). A repeated measures ANOVA indicated that approaches did not differ significantly across playback ($F_{3,17} = 1.21$, $p = .34$, $\eta_p^2 = 0.20$).

Discussion

Black-capped chickadees were presented with playback of high- and low-threat predator calls and conspecific mobbing calls. By examining vocal and movement responses, the results here indicated that *chick-a-dee* mobbing call production and general movement activity (i.e., perch hops) varied depending on threat-level and producer (i.e., heterospecific vs. conspecific). Chickadees produced significantly more *chick-a-dee* calls in response to high-threat owl calls than low-threat owl calls. Chickadees also produced significantly more *chick-a-dee* calls to the control condition (i.e., REV MOB NSWO) than high-threat predator-elicited mobbing calls (i.e., NSWO). Chickadees exhibited more general activity to conspecific than heterospecific playbacks. Once a predator is detected, anti-predatory behaviours can assist birds in defending themselves; for example, *chick-a-dee* calling helps recruit conspecifics to mob the nearby predator, whereas moving from location to location, could prepare a bird to fight off the predator or fly away. These two behaviours (i.e., *chick-a-dee* calling and general activity) varied the most among playback conditions, suggesting that these behaviours are most related to anti-predatory responses.

Vocal Behaviour

The *chick-a-dee* call is an acoustically complex vocalization that can convey predator-related information to nearby conspecifics and heterospecifics (e.g., Templeton et al., 2005). Despite being a well-studied vocalization common among Parid species, some aspects of how the call communicates specific information (e.g., acoustic variation, including note composition and rate of calling; contextual aspects, such as the presence of a predator or a mate) are unclear (Wilson & Mennill, 2011). Wilson and Mennill (2011) manipulated the signaling rate (i.e., duty cycle) and structural variation of *chick-a-dee* calls and found that, regardless of acoustic structure, signaling sequences with a high duty cycle attracted more conspecific and heterospecific receivers that approached the speaker more quickly, closely, and remained near for longer. Here we found that the rate of *chick-a-dee* call production by our chickadees was higher to NSWO than to GHOW playback, which would likely result in attracting more receivers during contexts of high threat; this finding is supported by both Templeton et al. (2005), that found chickadees produced more mobbing calls to smaller, high-threat live predators than to larger predators or controls, and Billings et al. (2015), that found chickadees mobbed more during the playback of high-threat than low-threat raptors. We also found that the frequency of *chick-a-dee* calls was higher to REV MOB NSWO than to MOB NSWO playback; the reversed calls could be considered a type of foreign vocalization indicating unknown danger that chickadees should respond to with a high frequency of mobbing calls.

We predicted that chickadees would emit more *chick-a-dee* calls following playback of *chick-a-dee* mobbing calls compared to predator vocalizations. Although we did not find differences in vocal responses to conspecific- versus heterospecific-produced vocalizations within threat level (e.g., playbacks of high-threat), significant differences were found in the *chick-a-dee* call production between GHOW and NSWO conditions, with chickadees producing more calls to high-threat owl calls (NSWO) than low-threat ones (GHOW). The higher production of *chick-a-dee* calls in the NSWO condition in comparison to the GHOW condition may be a result of chickadees calling for ‘help’ in response to a quick, high-threat owl, whereas they opt not to recruit conspecifics when faced with a slower, low-threat owl that they can easily outmaneuver (Fig. 2). Chickadees also produced significantly more *chick-a-dee* calls in response to the chickadee-produced control condition (i.e., REV MOB NSWO) compared to the high-threat predator-elicited chickadee mobbing calls (i.e., MOB NSWO). It is unclear why chickadees called more to reversed chickadee calls than the identical ‘normal’ calls. Again, the reversed *chick-a-dee* call may be considered a foreign conspecific vocalization and threatening to a chickadee as if a conspecific is in some sort of unknown danger. No other playback conditions in our study were found to result in significantly different *chick-a-dee* call production. Our finding that within threat level (i.e., low-threat GHOW and MOB GHOW, high-threat NSWO and MOB NSWO) there were no significant differences in chickadees’ vocal responses is in line with Avey et al. (2011), which found that within threat level, there was similar neural expression regardless of whether the playback was chickadee- or predator-produced. Thus, IEG expression in caudomedial mesopallium (CMM) and caudomedial nidopallium (NCM), and vocal behaviour, both increase in response to both high-threat playback conditions. It seems that these results demonstrate a strong connection between auditory input, vocal output, and neural expression in auditory brain regions.

Second, we predicted that chickadees would produce more *chick-a-dee* calls compared to other vocalizations following high-threat playback (i.e., NSWO and MOB NSWO). This prediction was not supported as chickadees did not produce more *chick-*

a-dee calls compared to other vocalizations in high-threat conditions. Chickadees produced other vocalizations as often as they produced *chick-a-dee* calls during a high-threat context, including *tseet* calls that are typically used as contact calls.

Third, we predicted that during experimental playback chickadees would emit fewer non-mobbing call vocalizations (e.g., *tseet* calls). Chickadees actually produced more *tseet* calls in response to chickadee-produced vocalizations than predator vocalizations, regardless of threat. *Tseet* calls are a contact call for chickadees: chickadees produce this vocalization when they hear other chickadees (Odum, 1942). When investigating vocal differences across playback conditions, no significant results were found for *gargles* or songs. Juveniles typically produce *gargle* calls to establish themselves in the flock and gain access to food (Smith, 1991). It is unlikely that this vocalization would be useful in the presence of a predator. Chickadees use their *fee-bee* song to attract mates and maintain territory; Figure 4 indicates that song (both *fee* and *fee-bee* vocalizations) production decreased, relative to baseline, in response to high- and low-threat owl calls. Again, it would be appropriate to sing in the presence of a conspecific and abstain when a predator is nearby.

Fourth, we predicted that chickadees would produce calls with more D notes in response to high-threat compared to low-threat vocalizations, for both predator calls and the corresponding mobbing calls (i.e., stimuli of the same threat level). Templeton et al. (2005) found that chickadees produced more D notes when detecting a high-threat saw-whet owl (approximately four D notes per call) than to a low-threat great horned owl (approximately two to three D notes per call). Avey et al. (2011) found more IEG expression in auditory brain regions in response to high threat predator- and chickadee-produced calls than low threat predator- and chickadee-produced calls. Despite the acoustic differences of the stimuli, IEG levels were similar across stimuli of the same threat level, and we thus predicted that we would observe a similar pattern in a behavioural task. In the current study, *chick-a-dee* mobbing calls produced in response to MOB GHOW typically contained one to three D notes per call; chickadees also produced more calls in response to MOB NSWO that typically contained four to five D notes (Fig. 2). Again, within threat level (e.g., low-threat GHOW and MOB GHOW, and high-threat NSWO and MOB NSWO), vocal production did not differ significantly, in line with previous findings of inducing similar neural expression.

Movement Behaviour

We predicted that chickadees would suppress movement more in the presence of high-threat than low-threat stimuli, as chickadees could easily outmaneuver the large low-threat predator, and that movement would be suppressed more in response to predator calls (i.e., hiding) than to chickadee-produced mobbing calls, as mobbing calls should elicit mobbing behaviour (Predictions 5 & 6, respectively). We recorded perch hops as a general measure of movement response, similar to previous playback studies (e.g., Hoeschele et al., 2010). It is clear that chickadees exhibited less general activity relative to baseline in response to chickadee-produced calls (i.e., MOB GHOW, MOB NSWO, and REV MOB NSWO) regardless of threat. In contrast, chickadees exhibited more general activity relative to baseline in response to non-chickadee produced calls (i.e., GHOW, NSWO, and RBNU). These findings were in direct contrast to our prediction that chickadees would suppress movement more in response to predator calls than to mobbing calls (Fig. 6; Prediction 6). There was a trend toward low-threat playback resulting in larger deviations from baseline for general activity (i.e., increased perch hopping to GHOW and decreased to MOB GHOW) in comparison to high-threat playback, but this result was not significant (Fig. 6; Prediction 5). There was a negative relationship between *tseet* call production and general activity; this result may indicate that chickadees typically vocalize when stationary, and vocal production or movement frequency is affected by the context of their environment (i.e., who is producing vocalizations). It is possible that chickadees increase in general activity in response to predator playback is in preparation for a “fight or flight” situation. Increased general activity could be due to the initiation of mobbing behaviour, or alternatively results from birds changing positions in an effort to visually locate a potential predator or stay vigilant. Subsequent studies could equip cages with nest boxes to determine if the reduction of general activity is actually chickadees’ way of hiding when signaled about the presence of a predator by conspecifics.

Non-perch hop movements did not differ significantly across playback conditions. Despite this, food and water visits, and pecking bouts generally did decrease from baseline during most playback conditions (Fig. 7). Chickadees would decrease food and water visits in the presence of threat, regardless whether indicated by the predator or conspecifics. Previously, Nowicki (1983) found that chickadees foraged significantly less when they heard foreign flocks’ calls; a foreign flock would conceivably pose a threat to resources (e.g., territory or foraging) in the way that a predator would to survival, although not at the same level of consequence to individual fitness. Without proper syntax, the reversed mobbing call could be responded to as a "foreign" call or perhaps from a foreign flock. Even pecking bouts (conducted to break open seeds) could make birds vulnerable to predation. Chickadees may have moved less in the presence of a red-breasted nuthatch as they consume similar food to chickadees and could be perceived as competition.

Chickadees produce ruffles towards conspecifics as an aggressive behaviour and to establish dominance and gain access to food. However, chickadees did not appear to produce ruffles in response to high-threat predator- or chickadee mobbing calls. This could be a result of chickadees not ruffling in high-threat conditions to avoid being noticed by predators; ruffles and *gargles* are typically produced consecutively and could result in higher risk of being noticed by a predator (Smith, 1991).

Templeton and colleagues (2005) found that more chickadees approached a hidden speaker during the playback of high-threat mobbing calls than low-threat or control mobbing calls. In our experiment, approaches were defined as landing on the cage wall closest to the speaker; we predicted that chickadees would show similar approach behaviour by perching on the front wall more frequently in response to high-threat playback conditions. Although non-significant, approaches appear to have been produced more in response to the high-threat mobbing condition (i.e., MOB NSWO) in comparison to baseline. Therefore, approaches are most likely connected with mobbing behaviour, which is initiated by conspecific mobbing calls in the presence of high predator threat.

Conclusions

In an attempt to understand the behaviour, cognition, and communication of social animals, Stan Kuczaj recognized the value of studying animals both in the wild and captivity. One area of Stan's research focused on understanding the communication of highly social animal species, specifically the Atlantic bottlenose dolphins (*Tursiops truncates*). We found that chickadees, a highly social species, produced significantly more *chick-a-dee* mobbing calls in response to high-threat owl calls versus low-threat owl calls. Chickadees also produced significantly more *chick-a-dee* calls in response to reversed high-threat mobbing calls versus the original high-threat mobbing calls. *Tseet* production across playback conditions approached a significant difference between conspecific and heterospecific calls, with chickadees producing more contact calls in response to conspecific calls. Chickadees exhibited more general activity in response to heterospecific-produced calls than conspecific-produced calls. Overall, chickadees appeared to produce more *tseet* calls in response to the playback of conspecific calls but move less. However, no significant differences in *tseet* calling or general activity behaviour were found for high- versus low-threat conditions for either hetero- or conspecific playback. Stan and colleagues also found that dolphins' movement behaviour was altered in the presence of a high-speed personal watercraft - dolphins significantly reduced dive duration, the clustering of individuals, and breathing synchrony (Miller, Solangi, & Kuczaj, 2008). Although not predators, *per se*, boats pose a real danger to dolphins as interaction with them can cause serious injury or death. These results indicate that imminent danger can drastically affect animals' behaviour. Once a predator is detected, anti-predatory behaviours can assist birds in defending themselves; for example, *chick-a-dee* calling helps recruit conspecifics and heterospecifics (e.g., nuthatches) to mob the nearby predator, whereas increased mobility could prepare the bird for a “fight or flight” scenario. These results are noteworthy since vocal behaviour did not differ significantly within threat level, but movement behaviour did, contrary to previous findings of predator and corresponding mobbing playback inducing similar IEG expression (Avey et al., 2011); although auditory input, vocal output, and IEG expression in auditory areas appear to be connected, the movement behaviour of birds varies dependent on who is signaling the information.

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References

Avey, M. T., Hoeschele, M., Moscicki, M. K., Bloomfield, L. L., & Sturdy, C. B. (2011). Neural correlates of threat perception: Neural Equivalence of conspecific and heterospecific mobbing calls in learned. *PLoS ONE*, *6*, 1-7.

Billings, A. C., Greene, E., & Jensen, S. M. D. L. L. (2015). Are chickadees good listeners? Antipredator responses to raptor vocalizations. *Animal Behaviour*, *110*, 1-8.

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

Evans, C. S., Evans, L., & Marler, P. (1993). On the meaning of alarm calls: functional reference in an avian vocal system. *Animal Behaviour*, *46*, 23-38.

Fallow, P. M., Pitcher, B. J., & Magrath, R. D. (2013). Alarming features: Birds use specific acoustic properties to identify heterospecific alarm calls. *Proceedings of the Royal Society*, *28*, *1-9.*

Griffiths, R., Double, M. C., Orr, K., & Dawson, R. J. (1998). A DNA test to sex most birds. *Molecular ecology*, *7*, 1071-1075.

Gyger, M., Marler, P., & Pickert, R. (1987). Semantics of an avian alarm call system: The male domestic fowl, Gallus domesticus. *Behaviour*, *102*, 15-39.

Hailman, J. P., Ficken, M. S., & Ficken, R. W. (1987). Constraints on the structure and combinatorial “*Chick-a-dee*” calls. *Ethology*, *75*, 62-80.

Hoeschele, M., Moscicki, M. K., Otter, K. A., van Oort, H., Fort, K. T., Farrell, T. M., Homan, L., Robson, S. W.J., & Sturdy, C. B. (2010). Dominance signalled in an acoustic ornament. *Animal Behaviour*, *79*, 657-664.

Howland, H. C. (1974). Optimal strategies for predator avoidance: The relative importance of speed and manoeuvrability. *Journal of Theoretical Biology*, *47*, 333-350.

Mammen, D. L. & Nowicki, S. (1981). Individual differences and within-flock convergence in chickadee calls. *Behavioural Ecology and Sociobiology*, *9*, 179-186.

Magrath, R. D., Haff, T. M., McLachlan, J. R., & Igic, B. (2015). Wild birds learn to eavesdrop on heterospecific alarm calls. *Current Biology*, *25*, 2047-2050.

Meigs, J. B., Smith, D. C., & Van Buskirk, J. (1983). Age determination of Black-capped Chickadees. *Journal of Field Ornithology*, *54*, 283-286.

Miller, L. J., Solangi, M., & Kuczaj, S. A. (2008). Immediate response of Atlantic bottlenose dolphins to high-speed personal watercraft in the Mississippi Sound. *Journal of the Marine Biological Association of the UK*, *88*, 1139-1143.

Nowicki, S. (1983). Flock-specific recognition of chickadee calls. *Behavioural Ecology and Sociobiology*, *12*, 64-73.

Odum, E. P. (1942). Annual cycle of the black-capped chickadee. *Auk*, *59*, 499-531.

Pyle, P. (1997). Molt limits in North American passerines. *North American Bird Bander*, *22*, 49-89.

Sherman, P. W. (1977). Nepotism and the evolution of alarm calls. *Science*, *197*, 1246-1253.

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

Soard, C. M. & 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.

Struhsaker, T.T. (1967). Auditory communication among vervet monkeys (*Cercopithecus aethiops*). *Social communication among primates*, 281-324.

Sturdy, C. B. & Proppe, D. S. (2015). Hearing is believing: Birds learn fear. *Learning & Behavior*, *44*, *205-206*.

Suzuki, T. N. (2011). Parental alarm calls warn nestlings about different predatory threats. *Current Biology*, *21*, R15-R16.

Suzuki, T. N. (2014). Communication about predator type by a bird using discrete, graded and combinatorial variation in alarm calls. *Animal Behaviour*, *87*, 59-65.

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

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

Wilson, D. R. & Mennill, D. J. (2011). Duty cycle, not signal structure, explains conspecific and heterospecific responses to the calls of black-capped chickadees (*Poecile atricapillus*). *Behavioral Ecology*, *22*, 784-790.

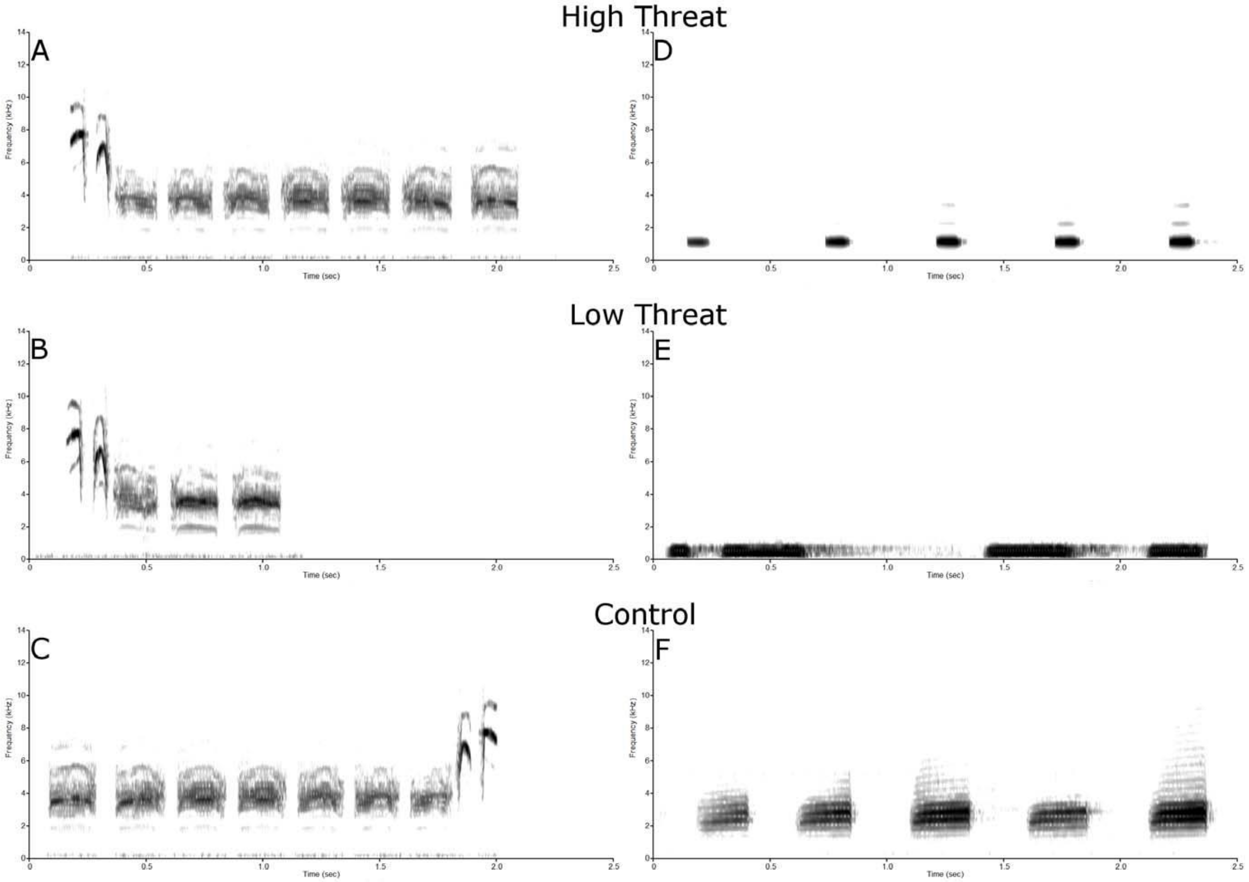

Figure 1. Figure from Avey et al. (2011) depicting sound spectograms (y-axis = frequency (0-14 kHz); x-axis = time (0-2.5 s) of examples of the six playback conditions: (A) black-capped chickadee mobbing calls made in response to a northern saw-whet owl mount; (B) black-capped chickadee mobbing calls made in response to the presentation of a great horned owl mount; (C) reversed black-capped chickadee mobbing calls made to a northern saw-whet owl mount; (D) northern saw-whet owl calls; (E) great horned owl calls; and (F) red-breasted nuthatch calls. doi:10.1371/journal.pone.0023844.g001

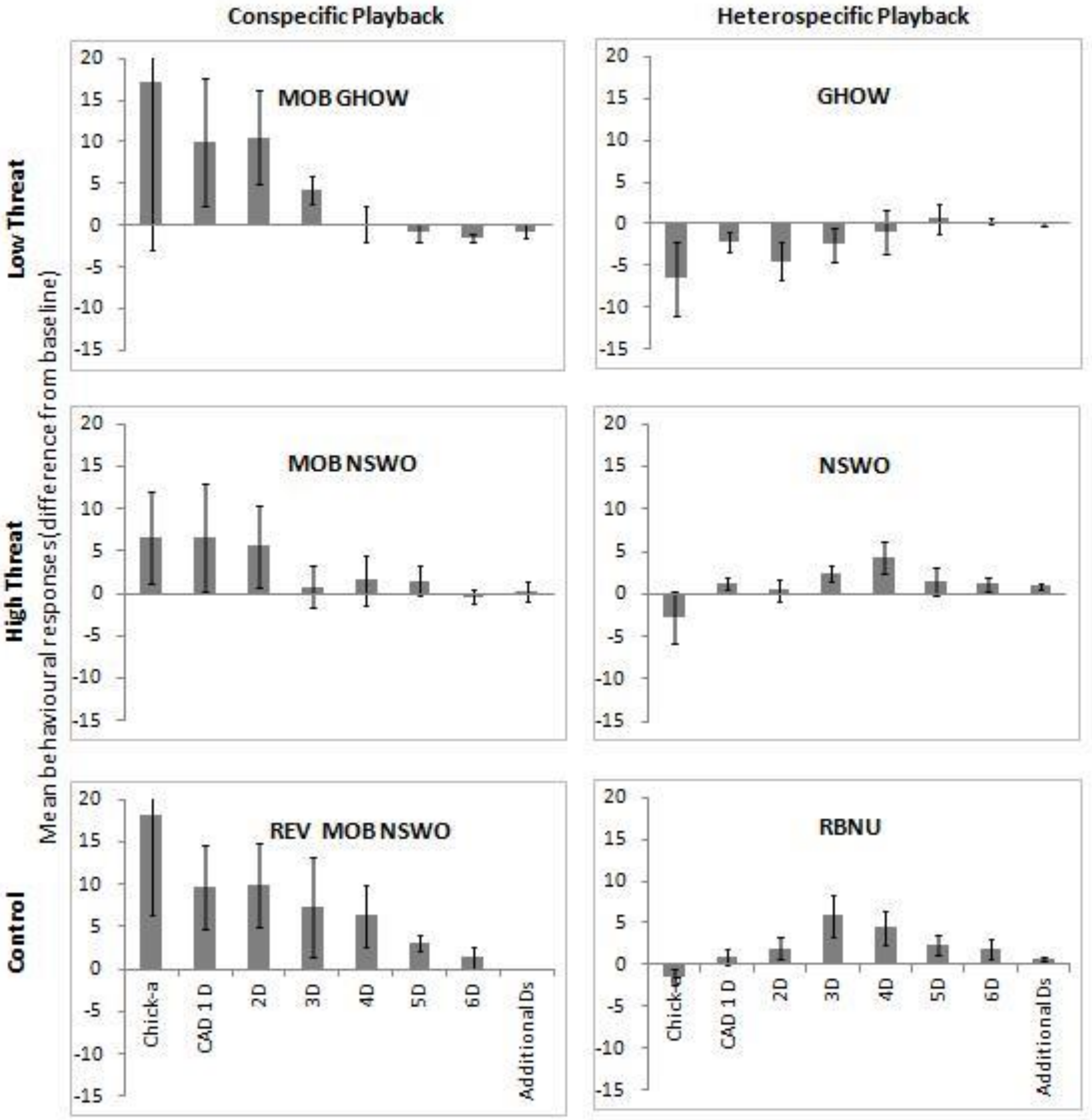

Figure 2. Mean ± SE difference from baseline in vocal responses (*chick-a*s, *chick-a-dee* (CAD) calls with 1 D, 2 D, 3 D, 4 D, 5 D, 6 D notes, and additional D notes (i.e., 7+ D notes) of black-capped chickadees (n = 6) after hearing six playback conditions. (GHOW = great horned owl calls; MOB GHOW = black-capped chickadee mobbing calls made in response to the presentation of a great horned owl mount; NSWO = northern saw-whet owl calls; MOB NSWO = black-capped chickadee mobbing calls made in response to a northern saw-whet owl mount; RBNU = red-breasted nuthatch calls; and REV MOB NSWO = reversed black-capped chickadee mobbing calls made to a northern saw-whet owl mount.)

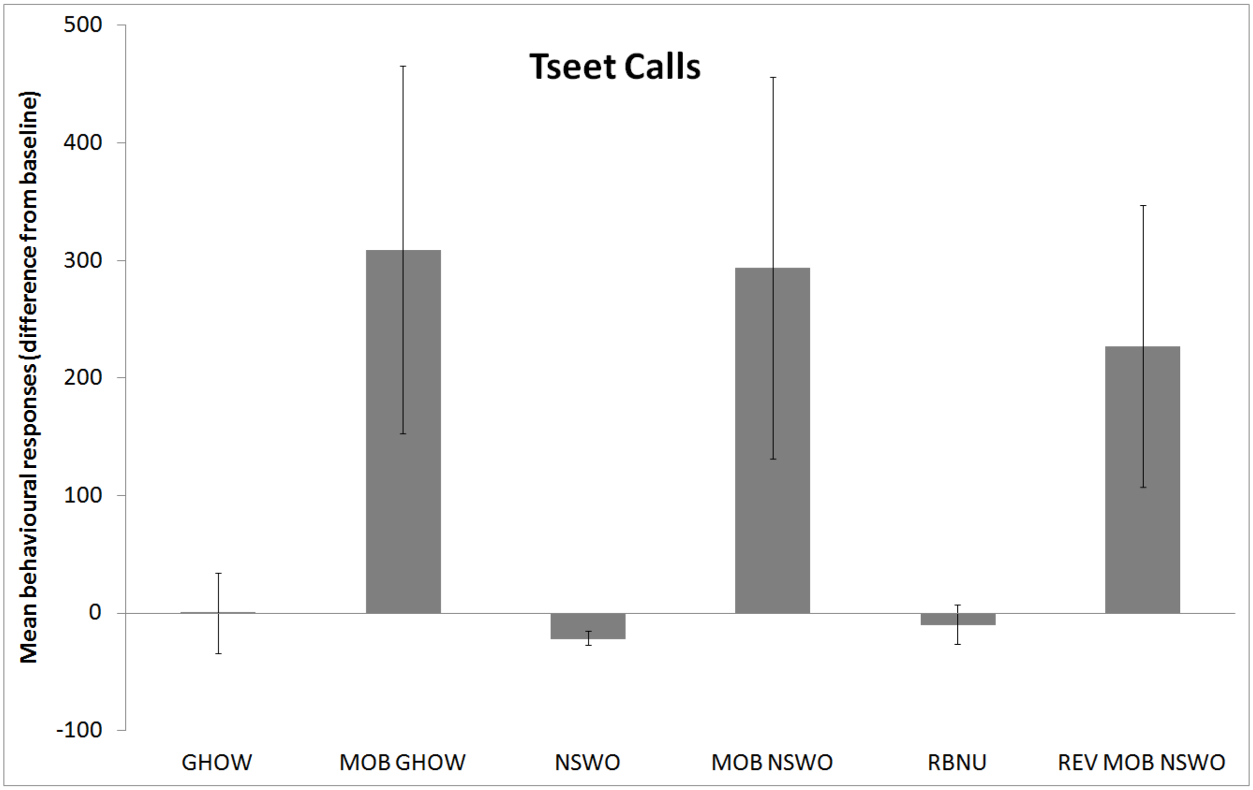

Figure 3. Mean ± SE difference from baseline in *tseet* calls produced by black-capped chickadees (n = 6) following playback of great horned owl calls (GHOW), black-capped chickadee mobbing calls made in response to a great horned owl mount (MOB GHOW), northern saw-whet owl calls (NSWO), black-capped chickadee mobbing calls made in response to a northern saw-whet owl mount (MOB NSWO), red-breasted nuthatch calls (RBNU), and reversed black-capped chickadee mobbing calls made to a northern saw-whet owl mount (REV MOB NSWO).

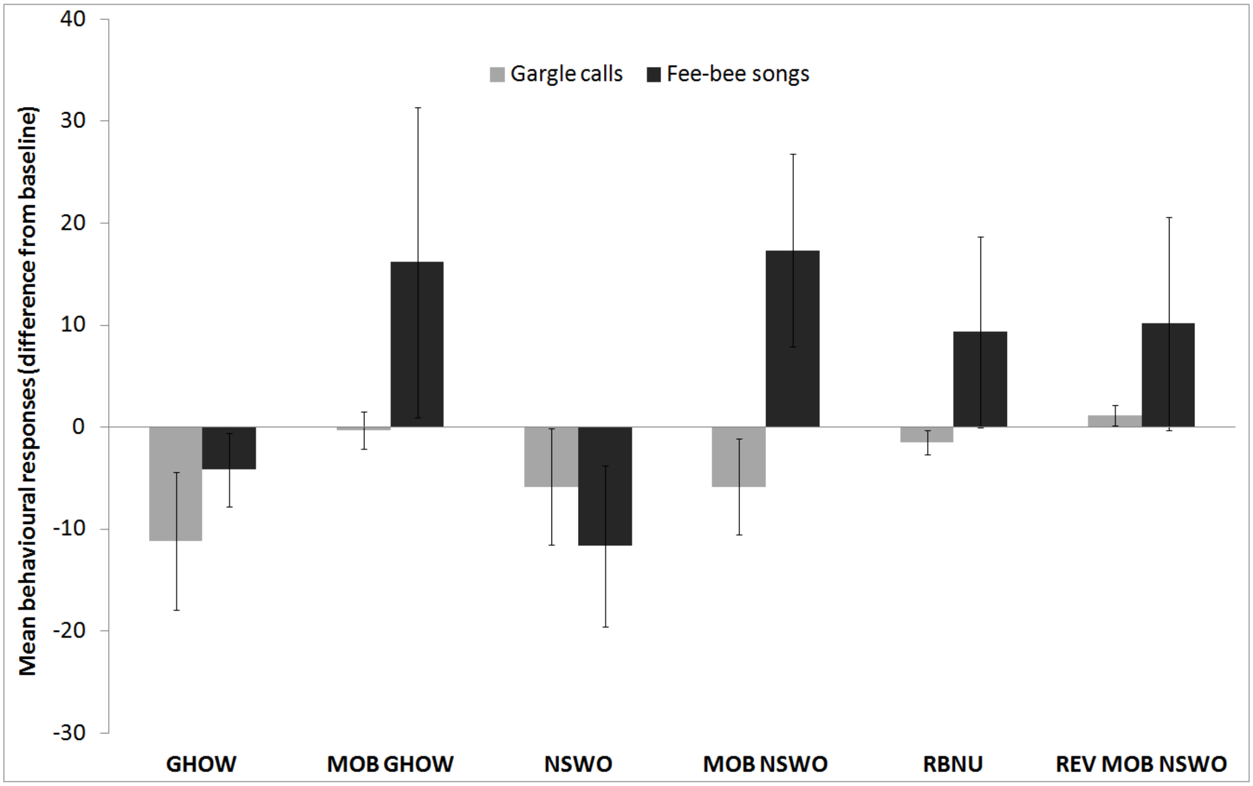

Figure 4. Mean ± SE difference from baseline in vocal responses (*gargle* calls and *fee-bee* songs) of black-capped chickadees (n = 6) after hearing six playback conditions. (GHOW = great horned owl calls; MOB GHOW = black-capped chickadee mobbing calls made in response to the presentation of a great horned owl mount; NSWO = northern saw-whet owl calls; MOB NSWO = black-capped chickadee mobbing calls made in response to a northern saw-whet owl mount; RBNU = red-breasted nuthatch calls; and REV MOB NSWO = reversed black-capped chickadee mobbing calls made to a northern saw-whet owl mount.)

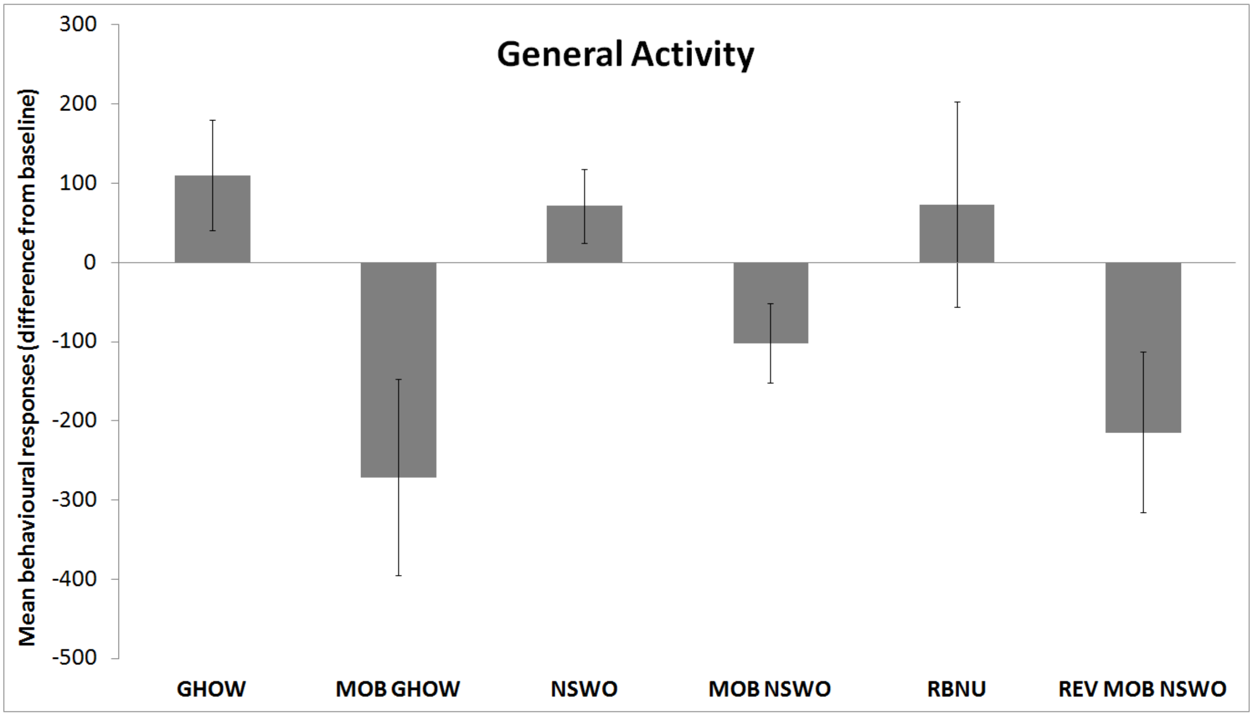

Figure 5. Mean ± SE difference from baseline in perch hops (a general measure of movement response) produced by black-capped chickadees (n = 6) following playback of great horned owl calls (GHOW), black-capped chickadee mobbing calls made in response to a great horned owl mount (MOB GHOW), northern saw-whet owl calls (NSWO), black-capped chickadee mobbing calls made in response to a northern saw-whet owl mount (MOB NSWO), red-breasted nuthatch calls (RBNU), and reversed black-capped chickadee mobbing calls made to a northern saw-whet owl mount (REV MOB NSWO).

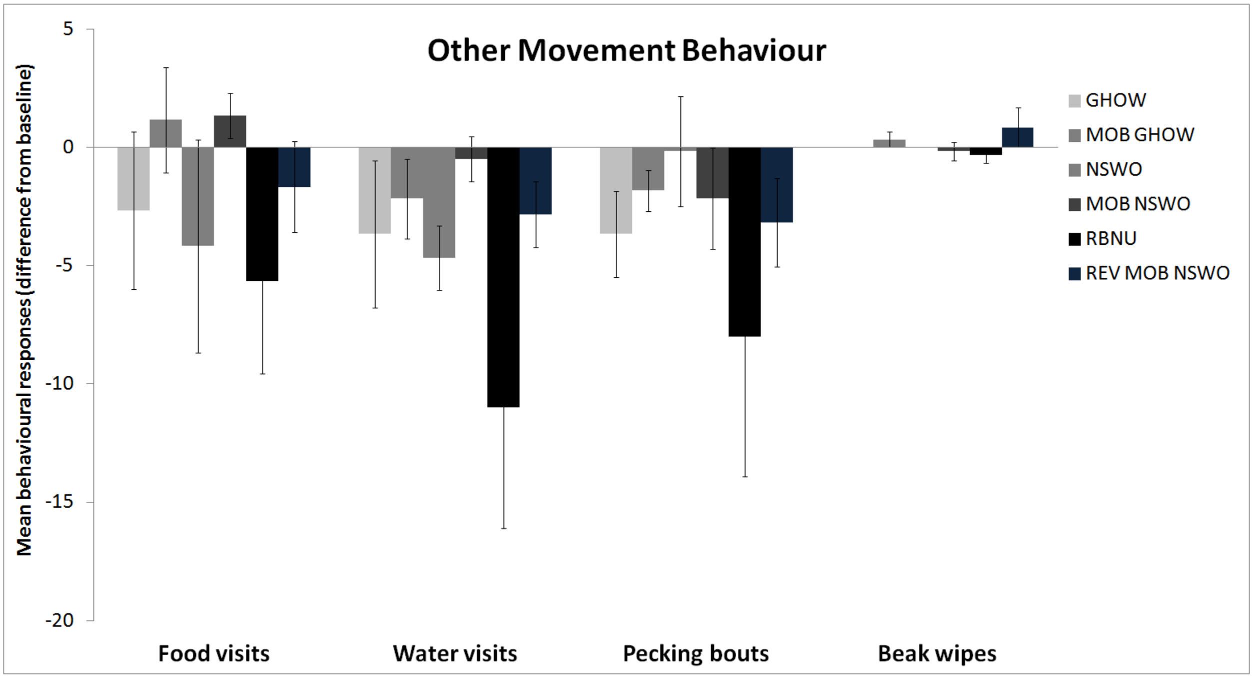

Figure 6. Mean ± SE difference from baseline in movement responses (food visits, water visits, pecking bouts, and beak wipes) produced by black-capped chickadees (n = 6) following playback of great horned owl calls (GHOW), black-capped chickadee mobbing calls made in response to the presentation of a great horned owl mount (MOB GHOW), northern saw-whet owl calls (NSWO), black-capped chickadee mobbing calls made in response to a northern saw-whet owl mount (MOB NSWO), red-breasted nuthatch calls (RBNU), and reversed black-capped chickadee mobbing calls made to a northern saw-whet owl mount (REV MOB NSWO).

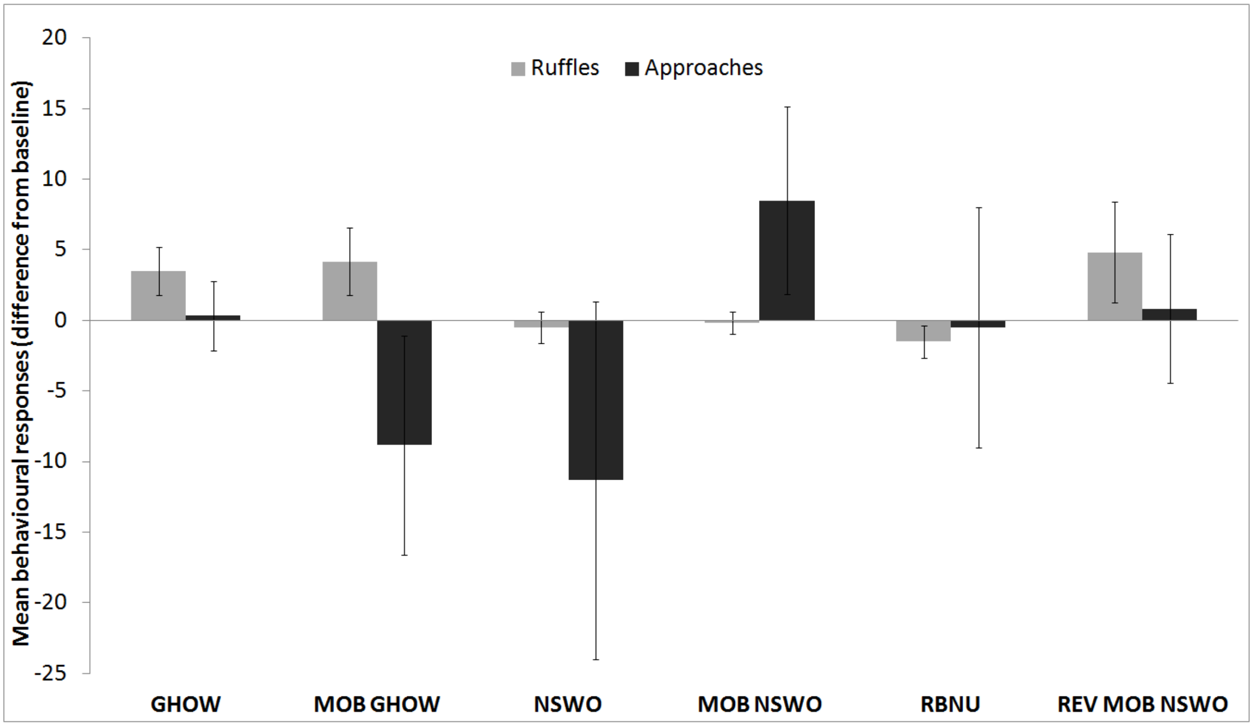

Figure 7. Mean ± SE difference from baseline in movement responses (ruffles and approaches) produced by black-capped chickadees (n = 6) following playback of great horned owl calls (GHOW), black-capped chickadee mobbing calls made in response to the presentation of a great horned owl mount (MOB GHOW), northern saw-whet owl calls (NSWO), black-capped chickadee mobbing calls made in response to a northern saw-whet owl mount (MOB NSWO), red-breasted nuthatch calls (RBNU), and reversed black-capped chickadee mobbing calls made to a northern saw-whet owl mount (REV MOB NSWO).

Table 1

Playback Stimuli

Stimulus set	Vocalization type (abbreviated)	Number of calls per 15s of playback
Set A	GHOW	3 hooting bouts
	MOB GHOW	2 *chick-a-dee* calls (2 D notes), 3 *chick-a* calls
	NSWO	31 whistled toots
	MOB NSWO	6 *chick-a-dee* calls (1-4 D notes), 2 *chick-a* calls
	RBNU	12 *yank* notes
	REV MOB NSWO	reversed MOB NSWO A
Set B	GHOW	3 hooting bouts
	MOB GHOW	4 *chick-a-dee* calls (3-4 D notes)
	NSWO	25 whistled toots
	MOB NSWO	5 *chick-a-dee* calls (3-7 D notes)
	RBNU	13 *yank* notes
	REV MOB NSWO	reversed MOB NSWO B

Note: Playback stimuli from Avey et al. (2011) were used. Vocalizations were recorded and collected to comprise two sets of stimuli. Each set contains three chickadee-produced stimuli and three heterospecific-produced stimuli. (GHOW = great horned owl calls; MOB GHOW = black-capped chickadee mobbing calls made in response to the presentation of a great horned owl mount; NSWO = northern saw-whet owl calls; MOB NSWO = black-capped chickadee mobbing calls made in response to a northern saw-whet owl mount; RBNU = red-breasted nuthatch calls; and REV MOB NSWO = reversed black-capped chickadee mobbing calls made to a northern saw-whet owl mount.)

Table 2

Recorded Behaviours

Behaviour Type	Behaviour	Behavioural Description
Vocal	*Chick-a-dee* call	Audible (nonstimulus) *chick-a-dee* call detected
	Gargle call	Audible *gargle* call detected
	Fee-bee song	Audible *fee* or *fee-bee* song detected
	Tseet call	Audible *tseet* call detected
Movement	General activity	Lands on new perch/moves to a new location
	Food visit	Pecks at food in cup
	Water visit	Pecks at water in bottle
	Ruffle	Shakes feathers
	Pecking bout	Performs four or more pecks in succession
	Beak wipe	Swipes wing across beak
	Approach	Lands on the wall closest to the speaker (Note: This movement is recorded twice as it is also defined as general activity.)

Note: Vocal and movement behaviours of male and female black-capped chickadees that were scored from audio and video files, respectively, and used in the analysis of chickadee behavioural responses to varying threat levels of predator threat. Adapted from Hoeschele et al. (2010).