



Original Article

Variation in *chick-a-dee* call sequences, not in the fine structure of *chick-a-dee* calls, influences mobbing behaviour in mixed-species flocks

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When animals vocalize under the threat of predation, variation in the structure of calls can play a vital role in survival. The *chick-a-dee* calls of chickadees and titmice provide a model system for studying communication in such contexts. In previous studies, birds' responses to *chick-a-dee* calls covaried with call structure, but also with unmeasured and correlated parameters of the calling sequence, including duty cycle (the proportion of the calling sequence when a signal was present). In this study, we exposed flocks of Black-capped Chickadees (*Poecile atricapillus*) and heterospecific birds to playback of *chick-a-dee* calls and taxidermic models of predators. We quantified birds' responses to variation in number of D notes and duty cycle of the signalling sequence. Chickadees and heterospecific birds responded more intensely to high-duty-cycle treatments, and equally to treatments where duty cycle was held constant and the number of D notes varied. Although our study does not disentangle the effects of call rate and duty cycle, it is the first to investigate independently the behavioural responses of birds to variation in structural and sequence-level parameters of the *chick-a-dee* call during a predator confrontation. Critically, our results confirm that the pattern previously observed in a feeding context holds true in a mobbing context: variation in calling sequences, not in call structure, is the salient acoustic feature of *chick-a-dee* calls. These results call into question the idea that *chick-a-dee* call structure carries allometric information about predator size, suggesting instead that sequence-level parameters play a central role in communication in a mobbing context.

Key words: alarm calls, chickadee, duty cycle, mobbing, *paridae*, *Parus*, *Poecile*, tit.

INTRODUCTION

Predation plays a powerful role in animal evolution, with a heavy influence on the behavioural decisions of animals (Chase et al. 2002). While animals have many different adaptations that minimize the risk of predation (e.g., cryptic coloration: Stevens and Merilaita 2009; alarm calling: Gill and Bierema 2013), many animals produce antipredator vocalizations that attract group members to the signaller's location where they harass the predator (e.g., vervet monkeys, *Chlorocebus pygerythrus*, Seyfarth et al. 1980; Formosan squirrels, *Callosciurus erythraeus*, Tamura 1989). In prey species, mobbing occurs when one or more animals congregate around and harass or even attack a predator (Curio 1978; Sordahl 1990). Mobbing behaviours usually include close approaches to

predators, frequent postural changes, stereotypical physical movements, and the production of loud and conspicuous vocalizations (Curio 1978).

Mobbing behaviour has been described in diverse groups of animals, including insects (Seeley et al. 1982), fish (Dominey 1983; Ishihara 1987), mammals (Owings and Coss 1977; Gursky 2005; Graw and Manser 2007), and, especially, birds (Curio 1978; Cunha et al. 2017; Pawlak et al. 2019). Mobbing behaviour entails both costs and benefits to fitness (Curio et al. 1978; Sordahl 1990), but, for mobbing behaviour to be maintained, the fitness benefits gained must exceed fitness costs incurred (Dugatkin and Godin 1992). Mobbing is time-consuming, energetically costly, and makes an individual conspicuous to predators (Collias NE and Collias EC 1978; Sordahl 1990). Further, predators may kill prey during these confrontations (Dugatkin and Godin 1992), and mobbing behaviour may attract additional predators to the vicinity (Sordahl 1990). Harassing predators may be beneficial, however, because predators are sensitive to disturbance, becoming injured or killed,

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leaving an area more quickly, or taking longer to return to an area as a result of being mobbed (Owings and Coss 1977; Seeley et al. 1982; Dominey 1983; Ishihara 1987; Pavey and Smyth 1998; Gursky 2005). Field studies make it clear that exposure to mobbing vocalizations can have similarly distressing effects on predators (Flasskamp 1994). Animals may also use mobbing vocalizations to inform group members of a threat or to convey information regarding a specific type of threat (Manser 2001; Templeton et al. 2005; Graw and Manser 2007).

The ability to communicate information concerning the perceived type of predator, or the urgency of a predation threat, may be beneficial if receivers can glean information about a predator (e.g., predator size, type, or proximity) and adapt their behaviour in response (Hauser 1996). Animals are capable of conveying information through acoustic signals via two mechanisms. First, they can produce a signal multiple times and encode information into sequence-level parameters, for example through changing signalling rate, consistency in timing of signal production, syntax, or duty cycle (i.e., the proportion of the calling sequence when the signal is present; Marler et al. 1986). Richardson's ground squirrels (*Urocitellus richardsonii*), for example, produce alarm calls repeatedly at a high rate when predators are close, but repeatedly at a low rate when predators are distant (Warkentin et al. 2001). Second, animals can encode information about predators by varying the fine structure of individual signals, including the signal's duration, amplitude, or frequency characteristics. Vervet monkeys, for example, produce acoustically distinct alarm calls in response to three different types of predators (snakes, eagles, and leopards) that correspond with three different types of response (Seyfarth et al. 1980). Similarly, male fowl (*Gallus gallus*) produce louder, shorter, more tonal alarm calls in response to larger, faster, and closer threats (Wilson and Evans 2012). The two encoding mechanisms are not mutually exclusive. For example, Richardson's ground squirrels, in addition to encoding predator proximity through variation in the rate of alarm call production, simultaneously encode predator proximity by adjusting the fine structure of individual calls (Sloan et al. 2005; see also Suzuki 2014).

The *chick-a-dee* call of the *paridae* family (chickadees and titmice) is one of the best-studied mobbing vocalizations produced by songbirds (Krams et al. 2012). Produced by both sexes, it is a structurally complex signal comprising four basic note types (A, B, C, and D) produced in a fixed order (Hailman et al. 1985; Hailman 1989; Charrier et al. 2004; Krams et al. 2012). The *chick-a-dee* call is usually produced repeatedly and considerable variation exists in the rate of signalling, the number of notes per call, and the ratio of note types (Hailman 1989; Baker and Becker 2002). In addition to being produced in a predator context, *chick-a-dee* calls are produced when a new food source is discovered, during territorial conflicts, and when individuals become separated from a mate or flock (Smith 1997; Lucas and Freeberg 2007). The calls communicate information about the caller's species, sex, group affiliation, and individual identity (Mammen and Nowicki 1981; Freeberg et al. 2003; Charrier et al. 2004; Charrier and Sturdy 2005; Lucas and Freeberg 2007), as well as information about extrinsic factors such as predators (Baker and Becker 2002; Templeton et al. 2005; Soard and Ritchison 2009; Bartmess-LeVasseur et al. 2010; Courter and Ritchison 2010; Avey et al. 2011; Hetrick and Sieving 2012; Freeberg et al. 2014; Congdon et al. 2016) and food (Freeberg and Lucas 2002; Mahurin and Freeberg 2008).

Previous research has revealed that the *chick-a-dee* call contains a high level of sophistication in a predator context (e.g., Baker

and Becker 2002), including an inverse relationship between the number of D notes in the *chick-a-dee* call and a predator's wingspan (Templeton et al. 2005; Templeton and Greene 2007; Soard and Ritchison 2009; Courter and Ritchison 2010; Avey et al. 2011; Hetrick and Sieving 2012). Predators with a shorter wingspan are thought to be more dangerous to small songbirds because of increased maneuverability (Ficken and Witkin 1977; Templeton et al. 2005). Furthermore, previous research involving the playback of *chick-a-dee* calls has shown that conspecific and heterospecific birds that often participate in winter flocks with chickadees (e.g., *Sitta* and *Picoides* species) exhibit high levels of discrimination in response to the number of D notes in the *chick-a-dee* call, where more individuals engage in mobbing (or respond more intensely) in response to calls with more D notes, suggesting sophisticated discrimination of these signals within and across species (Templeton et al. 2005; Templeton and Greene 2007; Soard and Ritchison 2009; Courter and Ritchison 2010; Hetrick and Sieving 2012; Congdon et al. 2016). These playback experiments, however, used un-manipulated recordings, which did not control for variation in the overall calling sequence (e.g., call rate or duty cycle) while testing behavioural response to variation in structural properties. Calls with more D notes are longer and, when played repetitively at a constant rate, yield sequences with a higher duty cycle than calls with fewer D notes. As a result, it is difficult to discern whether a sequence-level encoding mechanism or a structural encoding mechanism is used to encode information within these signals (Wilson and Mennill 2011).

Recognizing the uncertainty regarding which encoding mechanism chickadees use to convey information, a recent playback study used *chick-a-dee* call stimuli that varied independently in terms of call structure and the duty cycle of the playback stimuli; this allowed the investigators to differentiate between the two information-encoding mechanisms (Wilson and Mennill 2011). During this experiment, chickadees and other species responded more intensely to playback treatments with a high duty cycle, and they ignored variation in the signal structure of individual calls (Wilson and Mennill 2011). This study, however, was conducted in a food-provisioning context, not the mobbing context of previous investigations of *chick-a-dee* call function (Templeton et al. 2005; Templeton and Greene 2007; Soard and Ritchison 2009; Courter and Ritchison 2010; Avey et al. 2011; Hetrick and Sieving 2012; Congdon et al. 2016). As a result, it remains unknown whether birds respond to variation in the note composition or duty cycle of this call during a predator-mobbing interaction.

In this study, we used playback of mobbing calls accompanied with presentation of a predator model to determine whether Black-capped Chickadees and other species respond to variation in the call structure of *chick-a-dee* calls, to variation in the duty cycle of *chick-a-dee* call sequences, or to both. To test these alternative hypotheses, we independently manipulated the fine signal structure and duty cycle of *chick-a-dee* call recordings, and then paired their playback with the presentation of a taxidermic model of a Sharp-shinned Hawk (*Accipiter striatus*). Calls used in our playback treatments (i.e., 2 introductory notes proceeded by either 2 or 10 D notes) were consistent with the natural range of variation observed in *chick-a-dee* calls (Hailman et al. 1985). We observed and quantified the mobbing responses of both conspecific and heterospecific birds. If chickadees respond to variation in duty cycle, we predicted subjects would exhibit more intense behavioural responses when exposed to playback treatments containing call sequences with high duty cycles, regardless of variation in the note composition of individual calls. Alternatively, if chickadees respond to variation in

the note composition of individual calls, then we predicted that subjects would respond more strongly to treatments containing more D notes and ignore variation in duty cycle. To our knowledge, this is the first study to independently investigate the behavioural responses to variation in note composition of the *chick-a-dee* call and the duty cycle of the associated calling sequence in a simulated predator confrontation. Our intention was to elucidate the mechanism chickadees use to encode predator-related information in their *chick-a-dee* call and to provide insight into the fundamental nature of communication in this species.

METHODS

General field methods

We conducted this research at the Queens University Biological Station (44°34'N, 76°19.5'W) north of Kingston, Ontario, Canada. The study site includes several small lakes and diverse habitats ranging from old agricultural lands to mature mixed-woods forests. We conducted playback experiments in February 2017, when chickadees were still in their winter flocks. Sites for all playback locations were snow-covered and comprised mixed secondary forest habitats.

Black-capped Chickadees frequently participate in predator mobbing throughout the year, including during winter months (Shedd 1983). Chickadees will join winter foraging flocks of six to eight conspecific members (Ficken et al. 1990). Similar to many *paridae* species, Black-capped Chickadees maintain a complex social hierarchy with flock members while participating in these social groups (Ratcliffe et al. 2007), which is facilitated, in part, by their complex communication system (Charrier et al. 2004; Mennill and Otter 2007). Many other species regularly join these winter foraging flocks with chickadees (Dolby and Grubb 1998), and over 20 species have been shown to respond to the mobbing vocalizations of chickadees (Hurd 1996).

Black-capped Chickadees were the focal species of this experiment, yet numerous species are known to participate in mixed-species winter foraging flocks with chickadees (Krebs 1973), and we measured the behavioural responses of heterospecific species known to respond to *chick-a-dee* calls (Hurd 1996; Dolby and Grubb 1998). We did not colour-band individual birds in this study, and instead ensured that different flocks were sampled in each trial by moving >500 m between playback locations. This minimum separation distance is larger than the diameters of the estimated mean home ranges of most of the species that responded to our playbacks: Black-capped Chickadee (home-range diameter: 431 m; Odum 1942; minimum intertrial distance in this study: 519 m); Downy Woodpecker (*Picoides pubescens*: 462 m; Kellam et al. 2006; minimum intertrial distance in this study: 1164 m); Hairy Woodpecker (*Picoides villosus*: 1365 m; Covert-Bratland et al. 2006; minimum intertrial distance in this study: 3172 m); White-breasted Nuthatch (*Sitta carolinensis*: 359 m; Butts 1931; minimum intertrial distance in this study: 596 m); and Red-breasted Nuthatch (*Sitta canadensis*: 252 m; Matthysen et al. 1992; minimum intertrial distance in this study: 529 m).

Playback experiment

The experimental design for this study was adapted from a similar playback study on Black-capped Chickadees (Wilson and Mennill 2011), but, whereas that study was conducted in a food-provisioning context, this paper investigates how chickadees respond to variation in call structure and duty cycle during a simulated predator

confrontation. We conducted 40 trials of four experimental treatments, with 10 trials per experimental treatment. The order of treatments followed a randomized block design. We conducted trials between 08:00 and 15:45 based on the design of previous playback studies (Mahurin and Freeberg 2008; Wilson and Mennill 2011). Each trial was 20 min in duration.

Each trial involved broadcasting acoustic stimuli along with the simultaneous presentation of a taxidermic model of a Sharp-shinned Hawk. Due to their small size, maneuverability, and diet primarily of songbirds, chickadees and other small songbirds should perceive Sharp-shinned Hawks as a high threat (Apel 1985; Soard and Ritchison 2009). We used three different taxidermic specimens and randomly assigned one of three models to each trial. All predator models were adults, of the same sex (male), and were mounted in similar perched positions. Taxidermic specimens were provided by Holiday Beach Migration Observatory and the Queen's University Biological Station.

Playback stimuli

We used four experimental treatments: 1) 2-D *chick-a-dee* calls broadcast at a low signal rate ("2-D low duty cycle"; Figure 1a); 2) 2-D *chick-a-dee* calls broadcast at a high signal rate ("2-D high duty cycle"; Figure 1b); 3) 10-D *chick-a-dee* calls broadcast at a low signal rate ("10-D high duty cycle"; Figure 1c); and 4) a silent control treatment. We broadcast each treatment during the simultaneous presentation of the predator model. Treatments consisted of 1 min of playback followed by 4 min of silence, repeated for a total of 20 min. All calls were broadcast at rates consistent with the natural range of repetition observed in wild chickadees (Wilson and Mennill 2011). We included the silent treatment to assess the natural response of animals to the presence of an observer and the playback apparatus, including a taxidermic model of a Sharp-shinned Hawk. The "2-D high duty cycle" and "10-D high duty cycle" treatments have identical duty cycle, but differ in their fine signal structure, which allowed us to test for differences in receiver response to variation in signal structure. The "2-D low duty cycle" and "2-D high duty cycle" treatments have identical call structure, but differ in their duty cycle, which allowed us to test for differences in receiver response to variation in duty cycle. We used this design instead of a full-factorial design (which would have also included a "10-D low duty cycle" treatment) because the three selected experimental treatments still provided a direct test of each hypothesis while minimizing the number of predator simulations required.

Our experiment was designed to disentangle the effects of variation in the structure of individual calls from variation in the duty cycle of calling sequences on the mobbing responses of birds. It is possible that variation in calling rate (another sequence-level parameter) could influence mobbing responses. In this case, we would predict that responses to the "2-D low duty cycle" and "10-D high duty cycle" treatments would be indistinguishable, since they have identical calling rates, and that responses to the "2-D high duty cycle" treatment would be stronger than responses to the "2-D low duty cycle" or "10-D high duty cycle" treatments, since the calling rate of the "2-D high duty cycle" treatment is nearly four times higher (Figure 1).

We used the same acoustic stimuli as in Wilson and Mennill (2011). Acoustic stimuli were recordings of *chick-a-dee* calls produced by Black-capped Chickadees in Ontario, Canada in 2009 (settings: 44.1 kHz sampling rate, 16-bit accuracy, and WAVE format; Wilson and Mennill 2010) or taken from the Macaulay Library at Cornell

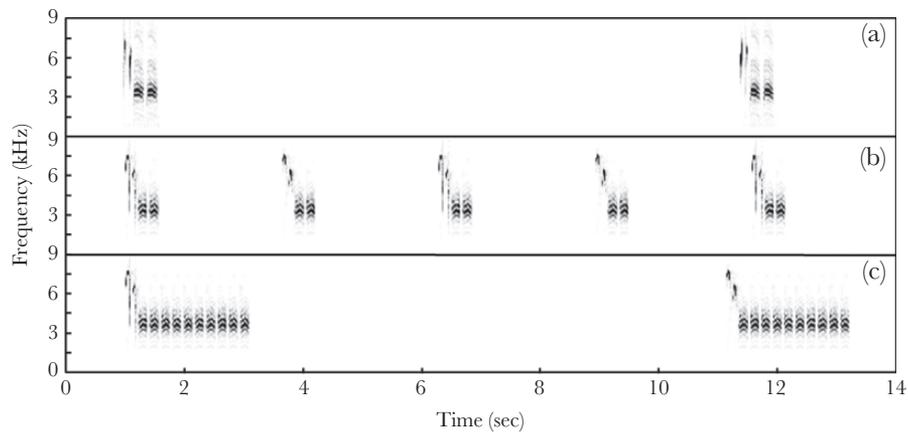


Figure 1

Sound spectrograms for three experimental treatments used to investigate the mechanism used to convey information in *chick-a-dee* calls: (a) 2-D low duty cycle; (b) 2-D high duty cycle; (c) 10-D high duty cycle. The 2-D low duty cycle and 2-D high duty cycle treatments contained calls with identical call structure, but differed in duty cycle (the proportion of the calling sequence when the signal is present, calculated by dividing the duration of one call by the time elapsed between the start of one call and the start of the next). The 2-D low duty cycle treatment (0.06) was approximately one quarter the duty cycle of the 2-D high duty cycle treatment (0.23). The 2-D high duty cycle and 10-D high duty cycle treatments had the same duty cycle (0.23), but contained differences in call structure (i.e., number of D notes per call). The 2-D low duty cycle and 10-D high duty cycle treatments had identical call rates (1 call every 10 s), whereas the calling rate of 2-D high duty cycle treatment was approximately four times higher. Playback treatments were broadcast together with the simultaneous presentation of a predator model to simulate a mobbing context. The stimuli shown here depict only 14 seconds of the one-minute stimuli.

Lab of Ornithology. We used 10 calls representing different individuals to create 10 blocks of playback stimuli. For each block, a single vocalization was used to construct a 2-D low duty cycle, a 2-D high duty cycle, and a 10-D high duty cycle stimulus (i.e., a single call was used to create three different treatments), which were then used, together with a silent control, to complete the block. We created the 2-D and 10-D versions of each call by removing all but the final two introductory notes and all but the first D note, and then repeating the remaining D note at a natural rate: we repeated the D note a single time to create a 2-D call; we repeated the D note nine times to create a 10-D call. Both call structures (i.e., 2 introductory notes followed by either 2 or 10 D notes) are within the natural range of structural variation observed in *chick-a-dee* calls (Hailman et al. 1985). Full details of stimulus creation are presented in Wilson and Mennill (2011).

Playback technique

We conducted 40 trials over the course of 5 days with similar weather conditions (clear, cold winter days). We selected playback locations based on the density of vegetation to provide potential perches for birds at a variety of distances from the taxidermic model and clear visibility for the observer. The same observer (B.L.) conducted all trials to avoid interobserver variation.

At each location, we hung a wireless loudspeaker (model: Foxpro Scorpion X1-B) in vegetation 1 m above the ground. We affixed a taxidermic Sharp-shinned Hawk model, perched on a 30-cm-long branch, to the top of a 1.5 m metal pole. The metal pole was driven into the snow or ground with the model positioned within 0.5 m above the speaker. We kept the predator concealed under a camouflage sheet until the initiation of playback, when we removed the sheet with an attached fishing line. We used flagging tape to mark 1, 5, and 10 m distances from the predator model in four cardinal directions to aid in estimating distances of subjects from the predator model. We started playback trials when chickadees were not in the immediate area, so that we could measure latency of response. Throughout each trial, the observer was located 15 m away

from the predator model and dictated vocal and physical behaviours of birds into a microphone (Audio-Technica AT8015 microphone and Marantz PMD660 digital recorder; settings: 44.1 kHz sampling rate, 16-bit accuracy).

We began each trial by broadcasting the predetermined playback treatment for 1 min at 80 dB sound pressure level (RadioShack 33–4050 sound level meter placed 1 m from the speaker; “C” weighting; fast response), followed by 4 min of silence. This sequence of 1-min playback followed by 4-min silence was repeated for the duration of the 20-min trial. We noted the time, species, and distance from model (estimated resolution: 0.5 m) each time a bird entered or changed locations within the playback area, which we defined as the area within 10 m of the predator model.

Analysis

We measured the behavioural responses of 96 Black-capped Chickadees and 39 heterospecific individuals across 40 trials. We quantified the behavioural response of chickadees and heterospecific birds using two response variables: 1) maximum number of individuals within the playback area at one time, which is considered a reliable estimate of the total number of respondents (Bartmess-LeVassuer et al. 2010) and is not influenced by birds making multiple trips into the playback area (Wilson and Mennill 2011); and 2) a variable representing mobbing intensity, calculated using a principal components analysis of three intercorrelated measures of response (see below). Focusing on these two response variables allowed us to draw direct comparisons to a previous investigation using the same technique but in a foraging context, rather than a mobbing context (Wilson and Mennill 2011). Behavioural responses were calculated separately for chickadees and heterospecific individuals, which provided four response variables.

Our principal component variable summarizing mobbing intensity was based on three measurements: latency of the first individual to enter the playback area, total time that at least one individual was present in the playback area, and distance of closest approach to the predator model. If no animals responded to the

playback treatment, we assigned values of 20 min for latency of individual to enter the playback area, 0 min for total time spent in the playback area, and 10 m for minimum distance of the closest animal. Of the 40 trials conducted, 6 trials did not elicit a response from conspecific individuals and 14 trials did not elicit a response from heterospecific individuals. The three measurements were highly intercorrelated for both Black-capped chickadees and heterospecific birds (Table 1), and Bartlett's test for sphericity indicated that a principal components analysis was appropriate ($P \leq 0.01$). For Black-capped Chickadees, principal components analysis yielded one principal component with an eigenvalue above 1 (eigenvalue: 2.64) which explained 87.8% of the variation in the three measurements; this principal component score, which we call "conspecific response intensity," had loadings of -0.96 from latency of approach, 0.94 from total time in the area, and -0.91 from distance of closest approach. For heterospecific birds, principal components analysis yielded one principal component with an eigenvalue above 1 (eigenvalue: 2.61) which explained 87.1% of the variation in the three measurements; this principal component score, which we call "heterospecific response intensity," had loadings of -0.96 from latency of approach, 0.92 from total time in the area, and -0.93 from distance of closest approach.

Due to the nonnormal distribution of the data, we used non-parametric Kruskal–Wallis tests to investigate the effects of the experimental treatments on our response variables. Using the non-parametric procedure for multiple comparisons (Dunn 1964), we conducted post hoc pairwise comparisons on all significant overall models ($\alpha = 0.05$), using the sequential Bonferroni method to maintain overall type I error rate (Rice 1989). All statistical analyses were conducted in JMP version 13 (SAS Institute Inc., Cary, NC).

RESULTS

Black-capped Chickadee playback responses

Both Black-capped Chickadees and heterospecific birds responded to playback of *chick-a-dee* calls accompanied by presentation of a predator model. During five trials, subjects flew directly at the predator model, attacking the hawk (three Black-capped Chickadees; two White-breasted Nuthatches), demonstrating that

Table 1

Correlation matrices for three measurements of birds' responses to playback of *chick-a-dee* calls and presentation of a predator model, as well as a principal component score summarizing these three measurements, for both Black-capped Chickadee responses and heterospecific bird responses

	Latency of approach (sec)	Total time in area (s)	Closest approach (m)
Black-capped Chickadee responses			
Latency of approach (s)		-0.89	0.81
Total time in area (s)	-0.89		-0.76
Closest approach (m)	0.81	-0.76	
Conspecific response intensity (PC1)	-0.95	0.92	-0.91
Heterospecific responses			
Latency of approach (s)		-0.84	0.84
Total time in area (s)	-0.84		-0.76
Closest approach (m)	0.84	-0.76	
Conspecific response intensity (PC1)	-0.96	0.92	-0.93

birds responded strongly to treatments and perceived the model as a predator.

Black-capped Chickadees responded differently to the four experimental treatments (Figure 2), showing differences for both the maximum number of individuals detected (Kruskal–Wallis test: $H = 28.2$, $P_{\text{adj}} < 0.01$, $n = 40$; Figure 2a) and in their conspecific response intensity scores ($H = 31.1$, $P_{\text{adj}} < 0.01$, $n = 40$; Figure 2b). When call structure was held constant, chickadees responded more strongly to the high duty cycle treatment than to the low duty cycle treatment (maximum number of individuals: 2-D low duty cycle vs. 2-D high duty cycle, $\zeta = 3.8$, $P_{\text{adj}} < 0.001$, $n = 20$; mobbing intensity: 2-D low duty cycle vs. 2-D high duty cycle, $\zeta = 4.2$, $P_{\text{adj}} < 0.0001$, $n = 20$). In contrast, when duty cycle was held constant, chickadees showed similar responses (maximum number of individuals: 2-D high duty cycle vs. 10-D high duty cycle, $\zeta = 1.3$, $P_{\text{adj}} = 1.0$, $n = 20$; mobbing intensity: 2-D high duty cycle vs. 10-D

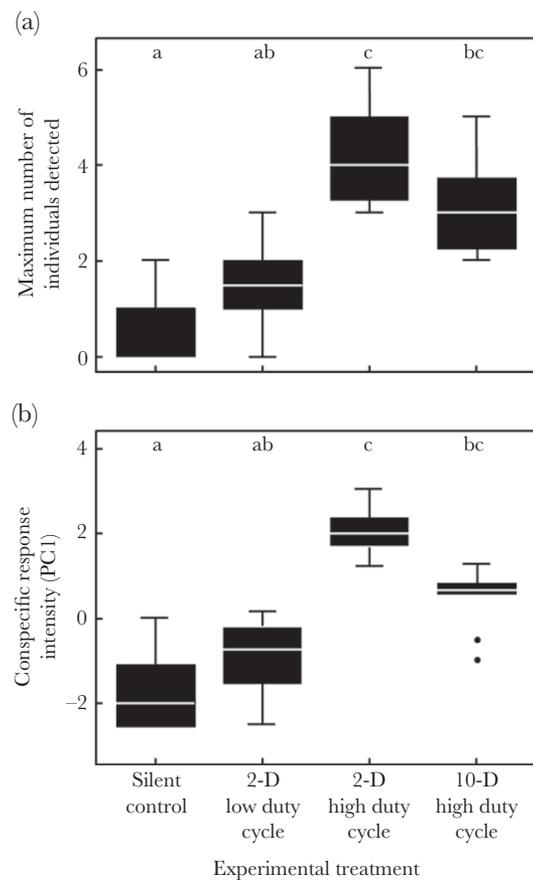


Figure 2

Black-capped Chickadee responses to playback treatments of *chick-a-dee* calls during 40 trials, quantified using two response variables: (a) maximum number of individuals detected within 10 m of the predator model, (b) intensity of mobbing behaviour represented as a principal component calculated with three measures of chickadee behaviour, including latency to respond, minimum distance from model, and total duration of response. Treatments consisted of three playback treatments ($N = 10$ trials per treatment) varying in structural and sequence-level parameters and a silent control ($N = 10$ trials). Corresponding treatments that are significantly different from each other are represented by different letters (post hoc test: $P_{\text{adj}} \leq 0.05$). Boxplots display median value (horizontal white line), upper and lower quartile (top and bottom of the box), 95% confidence intervals (whiskers), and outliers (dots).

high duty cycle, $\zeta = 2.2$, $P_{\text{adj}} = 0.19$, $n = 20$). When call rate was held constant, chickadees also showed similar responses (maximum number of individuals: 2-D low duty cycle vs. 10-D high duty cycle, $\zeta = 2.5$, $P_{\text{adj}} = 0.08$, $n = 20$; mobbing intensity: 2-D low duty cycle vs. 10-D high duty cycle, $\zeta = 2.0$, $P_{\text{adj}} = 0.24$, $n = 20$). Chickadees showed stronger responses to the high duty cycle treatments versus the silent control treatment (maximum number of individuals: 2-D high duty cycle vs. silent control, $\zeta = 4.7$, $P_{\text{adj}} < 0.0001$, $n = 20$; 10-D high duty cycle vs. silent control, $\zeta = 3.3$, $P_{\text{adj}} = 0.005$, $n = 20$; mobbing intensity: 2-D high duty cycle vs. silent control, $\zeta = 5.1$, $P_{\text{adj}} < 0.0001$, $n = 20$; 10-D high duty cycle vs. silent control, $\zeta = 2.9$, $P_{\text{adj}} = 0.02$, $n = 20$), but no significant differences in response to the low duty cycle treatment versus silent control treatment (maximum number of individuals: 2-D low duty cycle vs. silent control, $\zeta = 0.9$, $P_{\text{adj}} = 1.0$, $n = 20$; mobbing intensity: 2-D low duty cycle vs. silent control, $\zeta = 0.8$, $P_{\text{adj}} = 1.0$, $n = 20$).

Heterospecific birds' playback responses

Four other species of birds responded to playback: Downy Woodpecker ($n = 11$ trials; three 10-D high duty cycle; seven 2-D high duty cycle; one 2-D low duty cycle), Hairy Woodpecker ($n = 5$ trials; two 10-D high duty cycle; three 2-D high duty cycle), White-breasted Nuthatch ($n = 13$ trials; three 10-D high duty cycle; five 2-D high duty cycle; four 2-D low duty cycle; one silent control), and Red-breasted Nuthatch ($n = 6$ trials; one 10-D high duty cycle; three 2-D high duty cycle; one 2-D low duty cycle; one silent control).

Heterospecific birds showed a similar pattern of responses to playback treatments, as compared to Black-capped Chickadees; they responded differently to the treatments (Figure 3) both in terms of the maximum number of individuals detected (Kruskal–Wallis test: $H = 23.5$, $P_{\text{adj}} < 0.01$, $n = 40$; Figure 3a) and heterospecific response intensity scores (Kruskal–Wallis test: $H = 24.5$, $P_{\text{adj}} < 0.01$, $n = 40$; Figure 3b). When call structure was held constant, heterospecific birds responded more strongly to the high duty cycle treatment than to the low duty cycle treatment (maximum number of individuals: 2-D low duty cycle vs. 2-D high duty cycle, $\zeta = 3.4$, $P_{\text{adj}} < 0.004$, $n = 20$; mobbing intensity: 2-D low duty cycle vs. 2-D high duty cycle, $\zeta = 3.6$, $P_{\text{adj}} = 0.002$, $n = 20$). In contrast, when duty cycle was held constant, heterospecific birds exhibited similar responses to the 2-D and 10-D high duty cycle treatment in terms of maximum number of individuals (2-D high duty cycle vs. 10-D high duty cycle, $\zeta = 2.5$, $P_{\text{adj}} = 0.07$, $n = 20$) and showed a significantly higher response to 2-D high duty cycle treatments in terms of mobbing intensity (2-D high duty cycle vs. 10-D high duty cycle, $\zeta = 2.7$, $P_{\text{adj}} < 0.05$, $n = 20$). When call rate was held constant, chickadees showed similar responses (maximum number of individuals: 2-D low duty cycle vs. 10-D high duty cycle, $\zeta = 0.8$, $P_{\text{adj}} = 1.0$, $n = 20$; mobbing intensity: 2-D low duty cycle vs. 10-D high duty cycle, $\zeta = 0.9$, $P_{\text{adj}} = 1.0$, $n = 20$). Heterospecific birds showed a stronger response to the high duty cycle versus silent control treatments in terms of the maximum number of individuals that responded (2-D high duty cycle vs. silent control, $\zeta = 4.7$, $P_{\text{adj}} < 0.0001$, $n = 20$) and mobbing intensity (2-D high duty cycle vs. silent control, $\zeta = 4.7$, $P_{\text{adj}} < 0.0001$, $n = 20$). Heterospecific species did not show a difference in response to 10-D high duty cycle and silent control treatments (maximum number of individuals: 10-D high duty cycle vs. silent control, $\zeta = 2.1$, $P_{\text{adj}} = 0.21$, $n = 20$; mobbing intensity: 10-D high duty cycle vs. silent control, $\zeta = 2.0$, $P_{\text{adj}} = 0.25$, $n = 20$). There was no significant difference in the response of heterospecific birds to the 2-D low duty cycle and silent control treatments (maximum number of individuals: 2-D low duty cycle vs. silent control, $\zeta = 1.2$, $P_{\text{adj}} = 1.0$, $n = 20$; mobbing intensity: 2-D low duty cycle vs. silent control, $\zeta = 1.1$, $P_{\text{adj}} = 1.0$, $n = 20$).

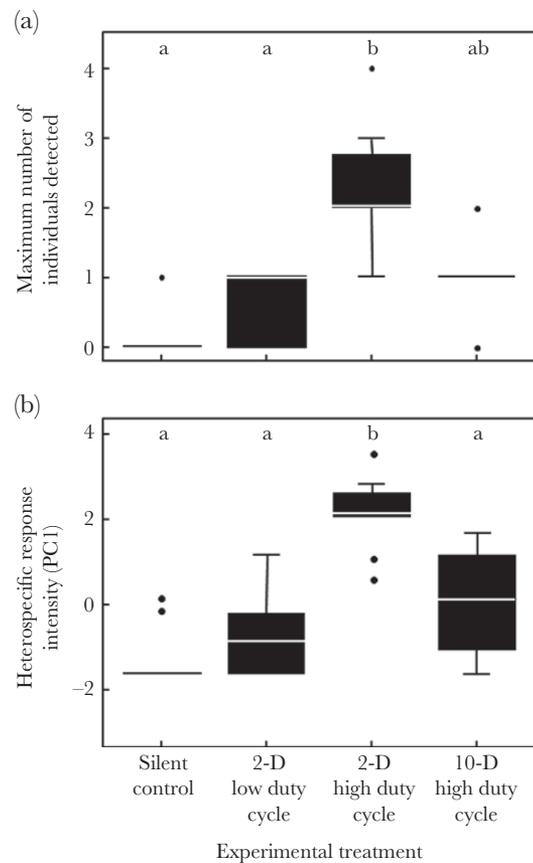


Figure 3

Heterospecific species' responses to experimental treatments of *chick-a-dee* calls during 40 trials, measured using two response variables: (a) Maximum number of individuals within 10 m of the predator model, (b) intensity of mobbing behaviour represented as a principal component of three measures of behaviour, including latency to respond, minimum distance from model, and total duration of response. Treatments consisted of three playback treatments ($N = 10$ trials per treatment) varying in structural and sequence-level parameters and a silent control ($N = 10$ trials). Corresponding treatments that are significantly different from each other are represented by different letters (post hoc test: $p_{\text{adj}} \leq 0.05$). Boxplots display median value (horizontal white line), upper and lower quartile (top and bottom of the box), 95% confidence intervals (whiskers), and outliers (dots).

The responses of Black-capped Chickadees and the responses of heterospecific birds were correlated, both for the maximum number of individuals responding ($r = 0.75$, $P < 0.0001$, $n = 40$) and for the intensity of response principal component scores ($r = 0.81$, $P < 0.0001$, $n = 40$). Across the 40 trials, Black-capped Chickadees arrived first in 25 cases (mean delay in arrival from first conspecific to first heterospecific: 223 s), heterospecific birds arrived first in 9 cases (mean delay in arrival from first heterospecific to first conspecific: 99 s), and Black-capped Chickadees and heterospecific birds arrived simultaneously in 6 cases.

DISCUSSION

Variation in *chick-a-dee* call sequences affected the intensity of mobbing responses by Black-capped Chickadees and the heterospecific birds that share their winter flocks. When call structure was held constant, chickadees and heterospecific birds showed stronger responses to experimental treatments containing high duty cycles compared to low duty cycles for both maximum numbers of

individuals to respond and the intensity of mobbing behaviour. When duty cycle remained uniform, chickadees exhibited no difference in response to variation in call structure (i.e., number of D notes per call), although, contrary to our prediction, heterospecific birds showed stronger responses to 2-D calls versus 10-D calls for the intensity of mobbing behaviour. When calling rate remained uniform, chickadees and heterospecific birds showed similar responses between treatments, despite differences in the note composition and duty cycle of the treatment stimuli. These results reveal that previously reported correlations between variation in mobbing responses and variation in call structure are better explained by correlated variation in sequence-level parameters, including duty cycle, calling rate, or both. Our results and experimental design do not allow us to disentangle the effects of duty cycle and calling rate, but show that some combination of these two factors affect receiver responses.

The question of how chickadees encode information in their *chick-a-dee* call is controversial (Templeton et al. 2005; Wilson and Mennill 2011). Templeton et al. (2005) showed, through presentation of live predators, that chickadees produced more D notes in their calls when exposed to predators with shorter wingspans (i.e., predators with increased maneuverability, and therefore of greater threat). However, Wilson and Mennill (2011) independently manipulated the call structure and duty cycle of *chick-a-dee* calls played back in a foraging context and showed no differences in conspecific or heterospecific responses to variation in note composition when duty cycle was uniform. The current study provides further support for the idea that variation in conspecific and heterospecific responses to *chick-a-dee* calls reflects variation in the overall signalling bout, rather than correlated variation in the structure of individual calls. However, unlike in Wilson and Mennill (2011), where behavioural responses could be explained by variation in duty cycle but not call rate, behavioural responses in the current study can be explained by variation in either duty cycle or call rate. Importantly, our study brings a new social context to the experimental design of Wilson and Mennill (2011)—that of a predator-mobbing context rather than a foraging context—allowing direct comparisons to Templeton et al. (2005) and Templeton and Greene (2007).

Heterospecific birds that form mixed foraging flocks with chickadees showed stronger mobbing intensity responses to the 2-D high duty cycle treatment than 10-D high duty cycle. However, our sample size for this analysis is quite limited; it is half the sample size of Wilson and Mennill's (2011) original food-provisioning study. A larger sample size may have reduced differences in heterospecific response between the two high duty cycle treatments and is a worthwhile avenue for future research to explore the interspecific communication system of chickadees and heterospecific flock-mates. An alternative explanation is that the intensity of heterospecific mobbing responses is influenced by variation in call rate rather than duty cycle, since the calling rate of the 2-D high duty cycle treatment was nearly four times higher than in the other calling treatments. We did not control for call rate in this study and this may be useful to investigate in future studies.

Like chickadees, heterospecific birds exhibited a stronger response to high duty cycle treatments than to low duty cycle or silent control treatments. Previous studies have indicated that several species are able to discern information from variation in the *chick-a-dee* call of *Poecile* species (e.g., White-breasted Nuthatch; Templeton and Greene 2007), showing substantial discrimination between these acoustic signals (Templeton and Greene 2007; Wilson and Mennill 2011). Templeton and Greene (2007) proposed

that White-breasted Nuthatches were responding to variation in note composition (i.e., number of D notes); however, as noted above, Wilson and Mennill (2011) points to variation in sequence-level parameters as the mechanism of information transfer between chickadees and heterospecific birds. Since heterospecific birds responded significantly more to 2-D high duty cycle treatments than 2-D low duty cycle treatments in this study, we suggest that heterospecific birds are gaining information through sequence-level parameters of the *chick-a-dee* call, such as duty cycle or call rate, suggesting a more parsimonious explanation than previously suggested for the discrimination between interspecific signals by these species (Templeton and Greene 2007). We found a correlation between the responses of conspecific and heterospecific birds to the playback, with conspecific animals leading the responses in the majority of cases. This correlation suggests an alternative explanation for the responses of heterospecific birds in our study: the heterospecific birds may have been following the responses of Black-capped Chickadees to the simulated predators, rather than responding to variation in sequence-level parameters of *chick-a-dee* call bouts. Our experimental design does not allow us to disentangle these two interpretations.

In this study, we independently manipulated the structure of *chick-a-dee* calls and the duty cycle of *chick-a-dee* call sequences to better understand how this long-studied vocalization conveys predator information. We show that chickadees and heterospecific birds respond to sequence-level variation in signals and do not respond to variation in the number of D notes in individual *chick-a-dee* calls. To our knowledge, this is the first study to test independently which mechanism, variation in call structure, or variation in calling sequences, Black-capped Chickadees and their heterospecific flock-mates use to convey information during a confrontation with a predator.

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