Title: **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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Lay Abstract: Many animals use vocalizations when mobbing predators. Vocal behaviour may communicate information about the threat posed by the predator. Using playback of *chick-a-dee* calls during a simulated predator confrontation, we show that birds respond more intensely to treatments with greater vocal output, but do not

respond differently to calls that differ in the number of *dee* notes. Our results

reveal that variation in calling sequences plays a central role in communication in

a mobbing context.

Abstract

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.

Keywords: Alarm calls; chickadee; duty cycle; mobbing; *paridae*; *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 colouration: Stevens & Merilaita 2009; alarm calling: Gill & 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 & Coss 1977; Gursky 2005; Graw & 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 & Collias 1978; Sordahl 1990). Further, predators may kill prey during these confrontations (Dugatkin & 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, leaving an area more quickly, or taking longer to return to an area as a result of being mobbed (Owings & 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 & 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 & 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 &

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 & Freeberg 2007). The calls communicate

information about the caller's species, sex, group affiliation, and individual identity (Mammen &

Nowicki 1981; Freeberg et al. 2003; Charrier et al. 2004; Charrier & Sturdy 2005; Lucas & Freeberg

2007), as well as information about extrinsic factors such as predators (Baker & Becker 2002;

Templeton et al. 2005; Soard & Ritchison 2009; Bartmess-LeVasseur et al. 2010; Courter &

Ritchison 2010; Avey et al. 2011; Hetrick & Sieving 2012; Freeberg et al. 2014; Congdon et al.

2016) and food (Freeberg & Lucas 2002; Mahurin & Freeberg 2009).

Previous research has revealed that the *chick-a-dee* call contains a high level of

sophistication in a predator context (e.g. Baker & 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 & Ritchison 2009; Courter & Ritchison 2010; Avey et

al. 2011; Hetrick & Sieving 2012). Predators with a shorter wingspan are thought to be more

dangerous to small songbirds because of increased maneuverability (Ficken & Witkin 1977;

99   Templeton et al. 2005). Furthermore, previous research involving the playback of *chick-a-dee* calls

100   has shown that conspecific and heterospecific birds that often participate in winter flocks with

101   chickadees (e.g. *Sitta* and *Picoides* species) exhibit high levels of discrimination in response to the

102   number of D notes in the *chick-a-dee* call where more individuals engage in mobbing (or respond

103   more intensely) in response to calls with more D notes, suggesting sophisticated discrimination of

104   these signals within and across species (Templeton et al. 2005; Templeton & Greene 2007; Soard

105   & Ritchison 2009; Courter & Ritchison 2010; Hetrick & Sieving 2012; Congdon et al. 2016). These

106   playback experiments, however, used un-manipulated recordings, which did not control for

107   variation in the overall calling sequence (e.g. call rate or duty cycle) while testing behavioural

108   response to variation in structural properties. Calls with more D notes are longer and, when

109   played repetitively at a constant rate, yield sequences with a higher duty cycle than calls with

110   fewer D notes. As a result, it is difficult to discern whether a sequence-level encoding mechanism

111   or a structural encoding mechanism is used to encode information within these signals (Wilson

112   and Mennill 2011).

113   Recognizing the uncertainty regarding which encoding mechanism chickadees use to

114   convey information, a recent playback study used *chick-a-dee* call stimuli that varied

115   independently in terms of call structure and the duty cycle of the playback stimuli; this allowed

116   the investigators to differentiate between the two information-encoding mechanisms (Wilson and

117   Mennill 2011). During this experiment, chickadees and other species responded more intensely to

118   playback treatments with a high duty cycle, and they ignored variation in the signal structure of

119   individual calls (Wilson and Mennill 2011). This study, however, was conducted in a food-

120   provisioning context, not the mobbing context of previous investigations of *chick-a-dee* function

121   (Templeton et al. 2005; Templeton and Greene 2007; Soard & Ritchison 2009; Courter & Ritchison

122   2010; Avey et al. 2011; Hetrick & Sieving 2012; Congdon et al. 2016). As a result, it remains

123   unknown whether birds respond to variation in the note composition or duty cycle of this call

124   during a predator-mobbing interaction.

125   In this study, we used playback of mobbing calls accompanied with presentation of a

126   predator model to determine whether Black-capped Chickadees and other species respond to

127   variation in the call structure of *chick-a-dee* calls, to variation in the duty cycle of *chick-a-dee* call

128   sequences, or to both. To test these alternative hypotheses, we independently manipulated the

129   fine signal structure and duty cycle of *chick-a-dee* call recordings, and then paired their playback

130   with the presentation of a taxidermic model of a Sharp-shinned Hawk (*Accipiter striatus*). Calls

131   used in our playback treatments (i.e. 2 introductory notes proceeded by either 2 or 10 D notes)

132   were consistent with the natural range of variation observed in *chick-a-dee* calls (Hailman et al.

133   1985). We observed and quantified the mobbing responses of both conspecific and heterospecific

134   birds. If chickadees respond to variation in duty cycle, we predicted subjects would exhibit more

135   intense behavioural responses when exposed to playback treatments containing call sequences

136   with high duty cycles, regardless of variation in the note composition of individual calls.

137   Alternatively, if chickadees respond to variation in the note composition of individual calls, then

138   we predicted that subjects would respond more strongly to treatments containing more D notes

139   and ignore variation in duty cycle. To our knowledge, this is the first study to independently

140   investigate the behavioural responses to variation in note composition of the *chick-a-dee* call and

141   the duty cycle of the associated calling sequence in a simulated predator confrontation. Our

142   intention was to elucidate the mechanism chickadees use to encode predator-related information

143   in their *chick-a-dee* call and to provide insight into the fundamental nature of communication in

144   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 6-8 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 & Otter 2007). Many other species regularly join these winter foraging flocks with chickadees (Dolby & 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 & 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 >500m

166   between playback locations. This minimum separation distance is larger than the diameters of the

167   estimated mean home ranges of most of the species that responded to our playbacks: Black-

168   capped Chickadee (home-range diameter: 431m; Odum 1942; minimum inter-trial distance in this

169   study: 519m); Downy Woodpecker (*Picoides pubescens*: 462m; Kellam et al. 2006; minimum inter-

170   trial distance in this study: 1164m); Hairy Woodpecker (*Picoides villosus*: 1365m; Covert-Bratland

171   et al. 2006; minimum inter-trial distance in this study: 3172m); White-breasted Nuthatch (*Sitta*

172   *carolinensis*: 359m; Butts 1931; minimum inter-trial distance in this study: 596m); Red-breasted

173   Nuthatch (*Sitta canadensis*: 252m; Matthysen et al. 1992; minimum inter-trial distance in this

174   study: 529m).

175   *Playback experiment*

176   The experimental design for this study was adapted from a similar playback study on

177   Black-capped Chickadees (Wilson and Mennill 2011), but, whereas that study was conducted in a

178   food-provisioning context, this paper investigates how chickadees respond to variation in call

179   structure and duty cycle during a simulated predator-confrontation. We conducted 40 trials of

180   four experimental treatments, with 10 trials per experimental treatment. The order of treatments

181   followed a randomized block design. We conducted trials between 08:00-15:45 based on the

182   design of previous playback studies (Mahurin & Freeberg 2008; Wilson & Mennill 2011). Each trial

183   was 20 minutes in duration.

184   Each trial involved broadcasting acoustic stimuli along with the simultaneous presentation

185   of a taxidermic model of a Sharp-shinned Hawk. Due to their small size, maneuverability, and diet

186   primarily of songbirds, chickadees and other small songbirds should perceive Sharp-shinned

187   Hawks as a high threat (Apel 1985; Soard & 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 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 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 & 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 inter-observer variation.

At each location, we hung a wireless loudspeaker (model: Foxpro Scorpion X1-B) in vegetation 1m above the ground. We affixed a taxidermic Sharp-shinned hawk model, perched on a 30cm-long branch, to the top of a 1.5m metal pole. The metal pole was driven into the snow or ground with the model positioned within 0.5m 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 1m, 5m, and 10m 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 15m 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 one minute at 80 dB sound pressure level (RadioShack 33-4050 sound level meter placed 1 m from the speaker; 'C' weighting; fast response), followed by four minutes 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: 0.5m) each time a bird entered

254 or changed locations within the playback area, which we defined as the area within 10m of the

255 predator model.

256 *Analysis*

257 We measured the behavioural responses of 96 Black-capped Chickadees and 39

258 heterospecific individuals across 40 trials. We quantified the behavioural response of chickadees

259 and heterospecific birds using two response variables: (1) maximum number of individuals within

260 the playback area at one time, which is considered a reliable estimate of the total number of

261 respondents (Bartmess-LeVassuer et al. 2010) and is not influenced by birds making multiple trips

262 into the playback area (Wilson and Mennill 2011), (2) a variable representing mobbing intensity,

263 calculated using a principal components analysis of three intercorrelated measures of response

264 (see below). Focusing on these two response variables allowed us to draw direct comparisons to a

265 previous investigation using the same technique but in a foraging context, rather than a mobbing

266 context (Wilson and Mennill, 2011). Behavioural responses were calculated separately for

267 chickadees and heterospecific individuals, which provided four response variables.

268 Our principal component variable summarizing mobbing intensity was based on three

269 measurements: latency of the first individual to enter the playback area, total time that at least

270 one individual was present in the playback area, and distance of closest approach to the predator

271 model. If no animals responded to the playback treatment, we assigned values of 20 minutes for

272 latency of individual to enter the playback area, 0 minutes for total time spent in the playback

273 area, and 10m for minimum distance of the closest animal. Of the 40 trials conducted, six trials

274 did not elicit a response from conspecific individuals and 14 trials did not elicit a response from

275 heterospecific individuals. The three measurements were highly inter-correlated for both Black-

276   capped chickadees and heterospecific birds (Table 1), and Bartlett's test for sphericity indicated

277   that a principal components analysis was appropriate ($p \leq 0.01$). For Black-capped Chickadees,

278   principal components analysis yielded one principal component with an eigenvalue above 1

279   (eigenvalue: 2.64) which explained 87.8% of the variation in the three measurements; this

280   principal component score, which we call "conspecific response intensity," had loadings of -0.96

281   from latency of approach, 0.94 from total time in the area, and -0.91 from distance of closest

282   approach. For heterospecific birds, principal components analysis yielded one principal

283   component with an eigenvalue above 1 (eigenvalue: 2.61) which explained 87.1% of the variation

284   in the three measurements; this principal component score, which we call "heterospecific

285   response intensity," had loadings of -0.96 from latency of approach, 0.92 from total time in the

286   area, and -0.93 from distance of closest approach.

287   Due to the non-normal distribution of the data, we used non-parametric Kruskal-Wallis

288   tests to investigate the effects of the experimental treatments on our response variables. Using

289   the nonparametric procedure for multiple comparisons (Dunn 1964), we conducted post hoc

290   pairwise comparisons on all significant overall models ($\propto$ = 0.05), using the sequential Bonferroni

291   method to maintain overall type I error rate (Rice 1989). All statistical analyses were conducted in

292   JMP version 13 (SAS Institute Inc., Cary, North Carolina).

293   **Results**

294   *Black-capped Chickadee playback responses*

295   Both Black-capped Chickadees and heterospecific birds responded to playback of *chick-a-*

296   *dee* calls accompanied by presentation of a predator model. During five trials, subjects flew

297   directly at the predator model, attacking the hawk (three Black-capped Chickadees; two White-

298   breasted Nuthatches), demonstrating that birds responded strongly to treatments and perceived

299   the model as a predator.

300   Black-capped Chickadees responded differently to the four experimental treatments

301   (Figure 2), showing differences for both the maximum number of individuals detected (Kruskal-

302   Wallis test: adjusted H = 28.2, p_{adj} < 0.01, n = 40; Figure 2.a) and in their conspecific response

303   intensity scores (adjusted H = 31.1, p_{adj} < 0.01, n = 40; Figure 2.b). When call structure was held

304   constant, chickadees responded more strongly to the high duty cycle treatment than to the low

305   duty cycle treatment (maximum number of individuals: 2-D low duty cycle vs. 2-D high duty cycle,

306   Z = 3.8, p_{adj} < 0.001, n = 20; mobbing intensity: 2-D low duty cycle vs. 2-D high duty cycle, Z = 4.2,

307   p_{adj} < 0.0001, n = 20). In contrast, when duty cycle was held constant, chickadees showed similar

308   responses (maximum number of individuals: 2-D high duty cycle vs. 10-D high duty cycle, Z = 1.3,

309   p_{adj} = 1.0, n = 20; mobbing intensity: 2-D high duty cycle vs. 10-D high duty cycle, Z = 2.2, p_{adj} =

310   0.19, n = 20). When call rate was held constant, chickadees also showed similar responses

311   (maximum number of individuals: 2-D low duty cycle vs. 10-D high duty cycle, Z = 2.5, p_{adj} = 0.08, n

312   = 20; mobbing intensity: 2-D low duty cycle vs. 10-D high duty cycle, Z = 2.0, p_{adj} = 0.24, n = 20).

313   Chickadees showed stronger responses to the high duty cycle treatments versus the silent control

314   treatment (maximum number of individuals: 2-D high duty cycle vs. silent control, Z = 4.7, p_{adj} <

315   0.0001, n = 20; 10-D high duty cycle vs. silent control, Z = 3.3, p_{adj} = 0.005, n = 20; mobbing

316   intensity: 2-D high duty cycle vs. silent control, Z = 5.1, p_{adj} < 0.0001, n = 20; 10-D high duty cycle

317   vs. silent control, Z = 2.9, p_{adj} = 0.02, n = 20), but no significant differences in response to the low

318   duty cycle treatment versus silent control treatment (maximum number of individuals: 2-D low

319   duty cycle vs. silent control, $Z = 0.9$, $p_{adj} = 1.0$, $n = 20$; mobbing intensity: 2-D low duty cycle vs.

320   silent control, $Z = 0.8$, $p_{adj} = 1.0$, $n = 20$).

Heterospecific birds' playback responses

322   Four other species of birds responded to playback: Downy Woodpecker (n = 11 trials;

323   three 10-D high duty cycle; seven 2-D high duty cycle; one 2-D low duty cycle), Hairy Woodpecker

324   (n = 5 trials; two 10-D high duty cycle; three 2-D high duty cycle), White-breasted Nuthatch (n = 13

325   trials; three 10-D high duty cycle; five 2-D high duty cycle; four 2-D low duty cycle; one silent

326   control), and Red-breasted Nuthatch (n = 6 trials; one 10-D high duty cycle; three 2-D high duty

327   cycle; one 2-D low duty cycle; one silent control).

328   Heterospecific birds showed a similar pattern of responses to playback treatments, as

329   compared to Black-capped Chickadees; they responded differently to the treatments (Figure 3)

330   both in terms of the maximum number of individuals detected (Kruskal-Wallis test: adjusted $H =$

331   23.5, $p_{adj} < 0.01$, $n = 40$; Figure 3a) and heterospecific response intensity scores (Kruskal-Wallis

332   test: adjusted $H = 24.5$, $p_{adj} < 0.01$, $n = 40$; Figure 3b). When call structure was held constant,

333   heterospecific birds responded more strongly to the high duty cycle treatment than to the low

334   duty cycle treatment (maximum number of individuals: 2-D low duty cycle vs. 2-D high duty cycle,

335    $Z = 3.4$, $p_{adj} < 0.004$, $n = 20$; mobbing intensity: 2-D low duty cycle vs. 2-D high duty cycle, $Z = 3.6$,

336    $p_{adj} = 0.002$, $n = 20$). In contrast, when duty cycle was held constant, heterospecific birds exhibited

337   similar responses to the 2-D and 10-D high duty cycle treatment in terms of maximum number of

338   individuals (2-D high duty cycle vs. 10-D high duty cycle, $Z = 2.5$, $p_{adj} = 0.07$, $n = 20$) and showed a

339   significantly higher response to 2-D high duty cycle treatments in terms of mobbing intensity (2-D

340   high duty cycle vs. 10-D high duty cycle, $Z = 2.7$, $p_{adj} < 0.05$, $n = 20$). When call rate was held

341 constant, chickadees showed similar responses (maximum number of individuals: 2-D low duty

342 cycle vs. 10-D high duty cycle, Z = 0.8, p_{adj} = 1.0, n = 20; mobbing intensity: 2-D low duty cycle vs.

343 10-D high duty cycle, Z = 0.9, p_{adj} = 1.0, n = 20). Heterospecific birds showed a stronger response

344 to the high duty cycle versus silent control treatments in terms of the maximum number of

345 individuals that responded (2-D high duty cycle vs. silent control, Z = 4.7, p_{adj} < 0.0001, n = 20) and

346 mobbing intensity (2-D high duty cycle vs. silent control, Z = 4.7, p_{adj} < 0.0001, n = 20).

347 Heterospecific species did not show a difference in response to 10-D high duty cycle and silent

348 control treatments (maximum number of individuals: 10-D high duty cycle vs. silent control, Z =

349 2.1, p_{adj} = 0.21, n = 20; mobbing intensity: 10-D high duty cycle vs. silent control, Z = 2.0, p_{adj} =

350 0.25, n = 20). There was no significant difference in the response of heterospecific birds to the 2-D

351 low duty cycle and silent control treatments (maximum number of individuals: 2-D low duty cycle

352 vs. silent control, Z = 1.2, p_{adj} = 1.0, n = 20; mobbing intensity: 2-D low duty cycle vs. silent control,

353 Z = 1.1, p_{adj} = 1.0, n = 20).

354   The responses of Black-capped Chickadees and the responses of heterospecific birds were

355 correlated, both for the maximum number of individuals responding (r = 0.75, p < 0.0001, n = 40)

356 and for the intensity of response principal component scores (r = 0.81, p < 0.0001, n = 40). Across

357 the 40 trials, Black-capped Chickadees arrived first in 25 cases (mean delay in arrival from first

358 conspecific to first heterospecific: 223 seconds), heterospecific birds arrived first in 9 cases (mean

359 delay in arrival from first heterospecific to first conspecific: 99 seconds), and Black-capped

360 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 & 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 & 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

384   cycle was uniform. The current study provides further support for the idea that variation in

385   conspecific and heterospecific responses to *chick-a-dee* calls reflects variation in the overall

386   signalling bout, rather than correlated variation in the structure of individual calls. However,

387   unlike in Wilson and Mennill (2011), where behavioural responses could be explained by variation

388   in duty cycle but not call rate, behavioural responses in the current study can be explained by

389   variation in either duty cycle or call rate. Importantly, our study brings a new social context to the

390   experimental design of Wilson and Mennill (2011) – that of a predator mobbing context rather

391   than a foraging context – allowing direct comparisons to Templeton et al. (2005) and Templeton

392   and Greene (2007).

393   Heterospecific birds that form mixed foraging flocks with chickadees showed stronger

394   mobbing intensity responses to the 2-D high duty cycle treatment than 10-D high duty cycle.

395   However, our sample size for this analysis is quite limited; it is half the sample size of Wilson and

396   Mennill's (2011) original food-provisioning study. A larger sample size may have reduced

397   differences in heterospecific response between the two high duty cycle treatments and is a

398   worthwhile avenue for future research to explore the inter-specific communication system of

399   chickadees and heterospecific flock-mates. An alternative explanation is that the intensity of

400   heterospecific mobbing responses is influenced by variation in call rate rather than duty cycle,

401   since the calling rate of the 2-D high duty cycle treatment was nearly four times higher than in the

402   other calling treatments. We did not control for call rate in this study and this may be useful to

403   investigate in future studies.

404   Like chickadees, heterospecific birds exhibited a stronger response to high duty cycle

405   treatments than to low duty cycle or silent control treatments. Previous studies have indicated

406   that several species are able to discern information from variation in the *chick-a-dee* call of

407   *Poecile* species (e.g. White-breasted Nuthatch; Templeton & Greene 2007), showing substantial

408   discrimination between these acoustic signals (Templeton & Greene 2007; Wilson & Mennill

409   2011). Templeton and Greene (2007) proposed that White-breasted Nuthatches were responding

410   to variation in note composition (i.e. number of D notes); however, as noted above, Wilson &

411   Mennill (2011) points to variation in sequence-level parameters as the mechanism of information

412   transfer between chickadees and heterospecific birds. Since heterospecific birds responded

413   significantly more to 2-D high duty cycle treatments than 2-D low duty cycle treatments in this

414   study, we suggest that heterospecific birds are gaining information through sequence-level

415   parameters of the *chick-a-dee* call, such as duty cycle or call rate, suggesting a more parsimonious

416   explanation than previously suggested for the discrimination between interspecific signals by

417   these species (Templeton & Greene 2007). We found a correlation between the responses of

418   conspecific and heterospecific birds to the playback, with conspecific animals leading the

419   responses in the majority of cases. This correlation suggests an alternative explanation for the

420   responses of heterospecific birds in our study: the heterospecific birds may have been following

421   the responses of Black-capped Chickadees to the simulated predators, rather than responding to

422   variation in sequence-level parameters of *chick-a-dee* call bouts. Our experimental design does

423   not allow us to disentangle these two interpretations.

424   In this study, we independently manipulated the structure of *chick-a-dee* calls and the

425   duty cycle of *chick-a-dee* call sequences to better understand how this long-studied vocalization

426   conveys predator information. We show that chickadees and heterospecific birds respond to

427   sequence-level variation in signals and do not respond to variation in the number of D notes in

428   individual *chick-a-dee* calls. To our knowledge, this is the first study to test independently which

429   mechanism, variation in call structure or variation in calling sequences, Black-capped Chickadees

430   and their heterospecific flock-mates use to convey information during a confrontation with a

431   predator.

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References

Apel KM. 1985. Antipredator behavior in the black-capped chickadee (*Parus atricapillus*). Doctoral dissertation, University of Wisconsin-Milwaukee.

Avey MT, Hoeschele M, Moscicki MK, Bloomfield LL, Sturdy CB. 2011. Neural correlates of threat perception: neural equivalence of conspecific and heterospecific mobbing calls is learned. Public Library of Science ONE, 6:e23844.

Baker MC, Becker AM. 2002. Mobbing calls of black-capped chickadees: effects of urgency on call production. The Wilson Bulletin, 114:510-516.

Bartmess-LeVasseur J, Branch CL, Browning SA, Owens JL, Freeberg TM. 2010. Predator stimuli and calling behavior of Carolina chickadees (*Poecile carolinensis*), tufted titmice (*Baeolophus bicolor*), and white-breasted nuthatches (*Sitta carolinensis*). Behavioral Ecology and Sociobiology, 64:1187-1198.

Butts WK. 1931. A study of the chickadee and white-breasted nuthatch by means marked individuals. part III: the white-breasted nuthatch (*Sitta carolinensis cookei*). Bird-Banding, 2:59-76.

Charrier I, Sturdy CB. 2005. Call-based species recognition in black-capped chickadees. Behavioural Processes, 70:271-281.

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

Chase JM, Abrams PA, Grover JP, Diehl S, Chesson P, Holt RD, Richards S, Nisbet R, Case TJ. 2002. The interaction between predation and competition: a review and synthesis. Ecology letters, 5:302-315.

Collias NE, Collias EC. 1978. Cooperative breeding behavior in the white-browed sparrow weaver. The Auk, 95:472-484.

Congdon JV, Hahn AH, McMillan N, Avey MT, Sturdy CB. 2016. Chickadee behavioural response to varying threat levels of predator and conspecific calls. International Journal of Comparative Psychology, 29.

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

Covert-Bratland KA, Block WM, Theimer TC. 2006. Hairy woodpecker winter ecology in ponderosa pine forests representing different ages since wildfire. Journal of Wildlife Management, 70:1379-1392.

Cunha FCR, Fontenelle JCR, Griesser M. 2017. Predation risk drives the expression of mobbing across bird species. Behavioral Ecology, 28:1517-1523.

Curio E. 1978. The adaptive significance of avian mobbing: I. Teleonomic hypotheses and predictions. Zeitschrift für Tierpsychologie, 48:175-183.

Curio E, Ernst U, Vieth W. 1978. The adaptive significance of avian mobbing. Ethology, 48:184-202.

Dolby AS, Grubb Jr, TC. 1998. Benefits to satellite members in mixed-species foraging groups: an experimental analysis. Animal Behaviour, 56:501-509.

Dominey WJ. 1983. Mobbing in colonially nesting fishes, especially the bluegill, *Lepomis macrochirus*. Copeia, 1983:1086-1088.

Dugatkin LA, Godin JGJ. 1992. Prey approaching predators: a cost-benefit perspective. In Annales Zoologici Fennici (pp. 233-252). Finnish Zoological Publishing Board (pp. 233-252). Finnish Zoological Publishing Board.

Dunn OJ. 1964. Multiple comparisons using rank sums. Technometrics, 6:241-252.

Ficken MS, Witkin SR. 1977. Responses of black-capped chickadee flocks to predators. The Auk, 94:156-157.

Ficken MS, Weise CM*, p*opp JW. 1990. Dominance rank and resource access in winter flocks of black-capped chickadees. The Wilson Bulletin,102:623-633.

Flasskamp A. 1994. The adaptive significance of avian mobbing V. An experimental test of the 'move on' hypothesis. Ethology, 96:322-333.

Freeberg TM, Lucas JR. 2002. Receivers resond differently to chick-a-dee calls varying in note composition in Carolina chickadees*, poecile carolinensis*. Animal Behaviour, 63:837-845.

Freeberg TM, Lucas JR, Clucas B. 2003. Variation in chick-a-dee calls of a Carolina chickadee population*, poecile carolinensis*: Identity and redundancy within note types. Journal of the Acoustical Society of America, 113:2127-2136.

Freeberg TM, Krama T, Vrublevska J, Krams I, Kullberg C. 2014. Tufted titmouse (*Baeolophus bicolor*) calling and risk-sensitive foraging in the face of threat. Animal Cognition, 17:1341-1352.

Gill SA, Bierema AMK. 2013. On the meaning of alarm calls: a review of functional reference in avian alarm calling. Ethology, 119:449-461.

Graw B, Manser MB. 2007. The function of mobbing in cooperative meerkats. Animal Behaviour, 74:507-517.

Gursky S. 2005. Predator mobbing in *Tarsius spectrum*. International Journal of Primatology, 26:207-221.

Hailman JP, Ficken MS, Ficken RW. 1985. The chick-a-dee calls of *Parus atricapillus*: a recombinant system of animal communication compared with written English. Semiotica, 56:191-224.

Hailman JP. 1989. The organization of major vocalizations in the *paridae*. The Wilson Bulletin, 101:305-343.

Hauser M. 1996. The evolution of animal communication. MIT Press, Cambridge, Massachusetts.

Hetrick SA, Sieving KE. 2012. Antipredator calls of tufted titmice and interspecific transfer of encoded threat information. Behavioral Ecology, 23:83-92.

Hurd CR. 1996. Interspecific attraction to the mobbing calls of black-capped chickadees (*Parus atricapillus*). Behavioral Ecology and Sociobiology, 38:287-292.

Ishihara M. 1987. Effect of mobbing toward predators by the damselfish *Pomacentrus coelestis* (Pisces: Pomacentridae). Journal of Ethology, 5:43-52.

Kellam JS, Lucas JR, Wingfield JC. 2006. The role of testosterone in male downy woodpeckers in winter home range use, mate interactions and female foraging behaviour. Animal behaviour, 71:695-707.

Krams I, Krama T, Freeberg TM, Kullberg C, Lucas JR. 2012. Linking social complexity and vocal complexity: a parid perspective. Philosophical Transactions of the Royal Society B: Biological Sciences, 367:1879-1891.

Krebs JR. 1973. Social learning and the significance of mixed-species flocks of chickadees (*Parus* spp.). Canadian Journal of Zoology, 51:1275-1288.

Lucas JR, Freeberg TM. 2007. "Information" and the *chick-a-dee* call: Communicating with a complex vocal system. In: Otter KA, editor. Ecology and behavior of chickadees and titmice: an integrated approach. Oxford: Oxford University Press. p. 199-213.

Mahurin EJ, Freeberg TM. 2008. Chick-a-dee call variation in Carolina chickadees and recruiting flockmates to food. Behavioral Ecology, 20:111-116.

Mammen DL, Nowicki S. 1981. Individual differences and within-flock convergence in chickadee calls. Behavioral Ecology and Sociobiology, 9:179-186.

Manser MB. 2001. The acoustic structure of suricates' alarm calls varies with predator type and the level of response urgency. Proceedings of the Royal Society of London B: Biological Sciences, 268:2315-2324.

Marler P, Dufty A, *p*ickert R. 1986. Vocal communication in the domestic chicken: II. Is a sender sensitive to the presence and nature of a receiver? Animal Behaviour, 34:194-198.

Matthysen E, Cimprich D, Grubb Jr, TC. 1992. Is social organization in winter determined by short- or long-term benefits? A case study on migrant red-breasted nuthatches *Sitta canadensis.* Ornis Scandinavica, 23:43-48.

Mennill DJ, Otter KA. 2007. Status signaling and communication networks in chickadees: complex communication with a simple song. Ecology and behavior of chickadees and titmice (Otter, KA, ed.). Oxford University Press, Oxford, 215-233.

Odum EP. 1942. Annual cycle of the black-capped chickadee: 3. The Auk, 59:499-531.

Owings DH, Coss RG. 1977. Snake mobbing by California ground squirrels: adaptive variation and ontogeny. Behaviour, 62:50-69.

Pavey CR, Smyth AK. 1998. Effects of avian mobbing on roost use and diet of powerful owls, *Ninox strenua*. Animal Behaviour, 55:313-318.

Pawlak P, Kwieciński Z, Hušek J. 2019. Mobbing of the top predator: a correlation between avian community richness and the number of mobbing species. Journal of Ornithology, 160:665-672.

Ratcliffe L, Mennill DJ, Schubert KA. 2007. Social dominance and fitness in black-capped chickadees. Ecology and behavior of chickadees and titmice: an integrated approach. Oxford University Press, Oxford, 131-147.

Rice WR. 1989. Analyzing tables of statistical tests. Evolution, 43:223-225.

Seeley TD, Seeley RH, Akratanakul P. 1982. Defense strategies of the honeybees in Thailand. Ecological Monographs, 52:43-63.

Seyfarth RM, Cheney DL, Marler P. 1980. Vervet monkey alarm calls: semantic communication in a free-ranging primate. Animal Behaviour, 28:1070-1094.

Shedd DH. 1983. Seasonal variation in mobbing intensity in the black-capped Chickadee. The Wilson Bulletin, 95:343-348.

Sloan JL, Wilson DR, Hare JF. 2005. Functional morphology of Richardson's ground squirrel, *Spermophilus richardsonii,* alarm calls: the meaning of chirps, whistles and chucks. Animal Behaviour, 70:937-944.

Smith SM. 1997. Black-capped chickadee: behavioural ecology and natural history. Cornell Univ. Press, Ithaca, NY.

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

Sordahl TA. 1990. The risks of avian mobbing and distraction behavior: an anecdotal review. The Wilson Bulletin, 102:349-352.

Stevens M, Merilaita S. 2009. Animal camouflage: current issues and new perspectives. Philosophical Transactions of the Royal Society of London B: Biological Sciences, 364:423-427.

Suzuki TN. 2014. Communication about predator type by a bird using discrete, graded and combinatorial variation in alarm calls. Animal Behaviour, 87:59-65.

571   Tamura N. 1989. Snake-directed mobbing by the Formosan squirrel *Callosciurus erythraeus*
572   *thaiwanensis*. Behavioral Ecology and Sociobiology, 24:175-180.

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

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

577   Warkentin KJ, Keeley ATH, Hare JF. 2001. Repetitive calls of juvenile Richardson's ground squirrels
578   (*Spermophilus richardsonii*) communicate response urgency. Canadian Journal of Zoology, 79:569-
579   573.

580   Wilson DR, Evans CS. 2012. Fowl communicate the size, speed and proximity of avian stimuli
581   through graded structure in referential alarm calls. Animal Behaviour, 83:535-544.

582   Wilson DR, Mennill DJ. 2010. Black-capped chickadees*, poecile atricapillus*, can use individually
583   distinctive songs to discriminate among conspecifics. Animal Behaviour, 79:1267-1275.

584   Wilson DR, Mennill DJ. 2011. Duty cycle, not signal structure, explains conspecific and
585   heterospecific responses to the calls of black-capped chickadees (*Poecile atricapillus*). Behavioral
586   Ecology, 22:784-790.

587

Table 1: Correlations 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 (sec)	Closest approach (m)
Black-capped Chickadee responses			
Latency of approach (sec)	-	-0.89	0.81
Total time in area (sec)	-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 (sec)	-	-0.84	-0.76
Total time in area (sec)	-0.84	-	0.84
Closest approach (m)	-0.76	0.84	-
Conspecific response intensity (PC1)	0.9	-0.93	-0.93

593
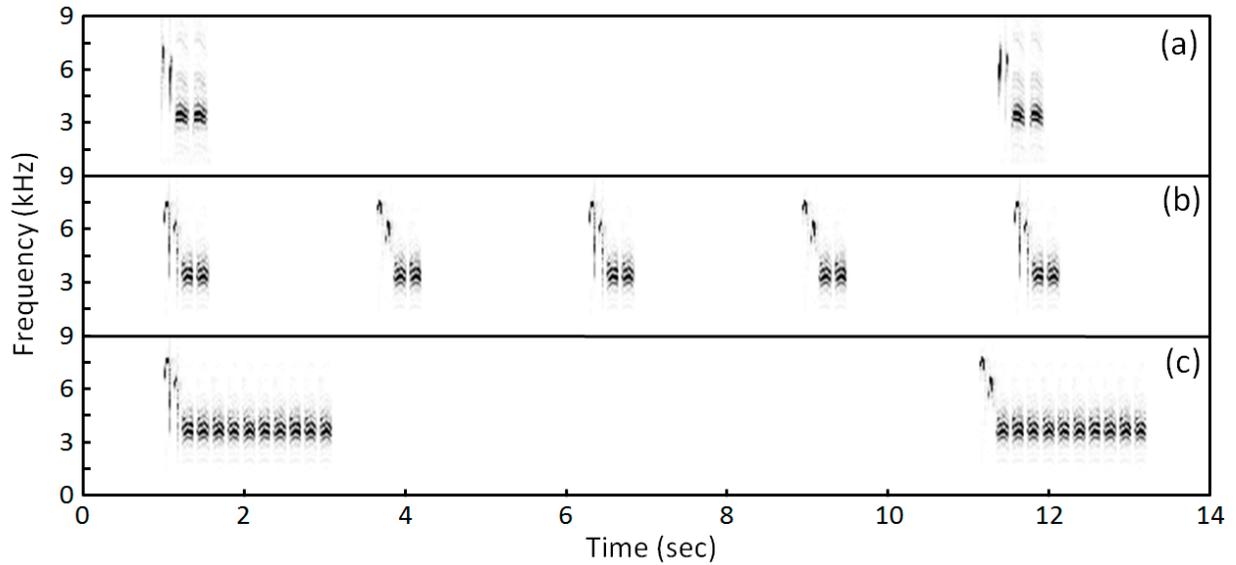
Figure 1. Sound spectrograms for three experimental treatments used to investigate the mechanism used to convey information in *chick-a-dee* calls: (a) 2-D low duty cycle; (b) 2-D high duty cycle; (c) 10-D high duty cycle. The 2-D low duty cycle and 2-D high duty cycle treatments contained calls with 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.

(a)

(b)
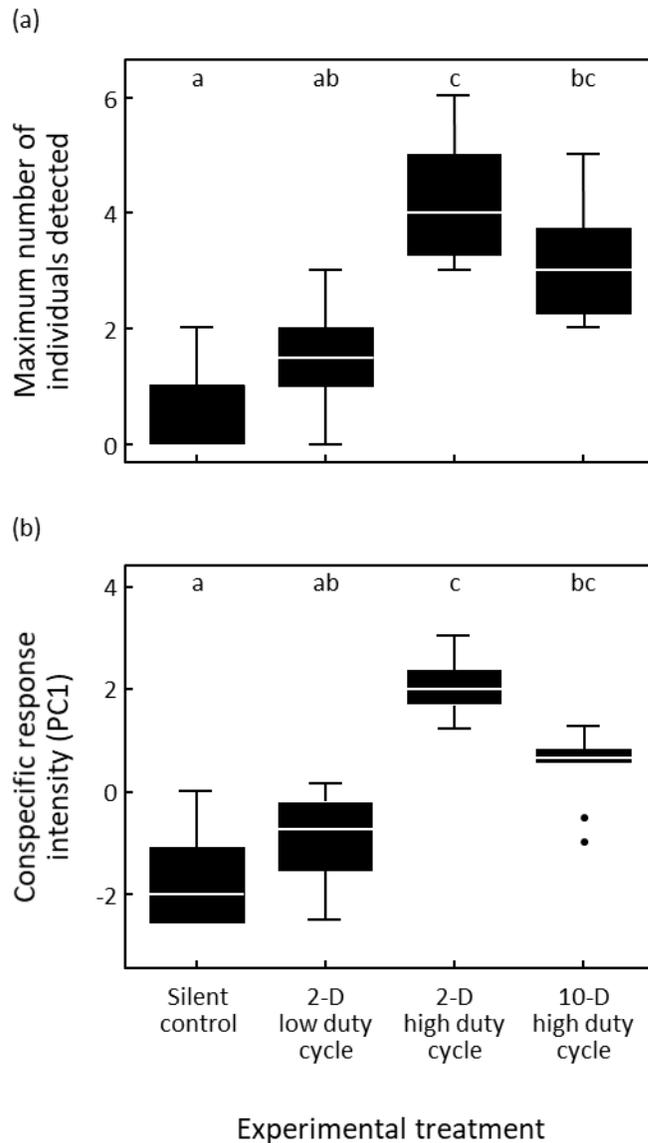
Figure 2. Black-capped Chickadee responses to playback treatments of *chick-a-dee* calls during 40 trials, quantified using 2 response variables: (a) maximum number of individuals detected within 10 m of the predator model, (b) intensity of mobbing behaviour represented as a principal component calculated with 3 measures of chickadee behaviour, including latency to respond, minimum distance from model, and total duration of response. Treatments consisted of 3 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: $p_{adj}= \leq 0.05$). Boxplots display median value (horizontal white line), upper and lower quartile (top and bottom of the box), 95% confidence intervals (whiskers), and outliers (dots).

(a)

(b)
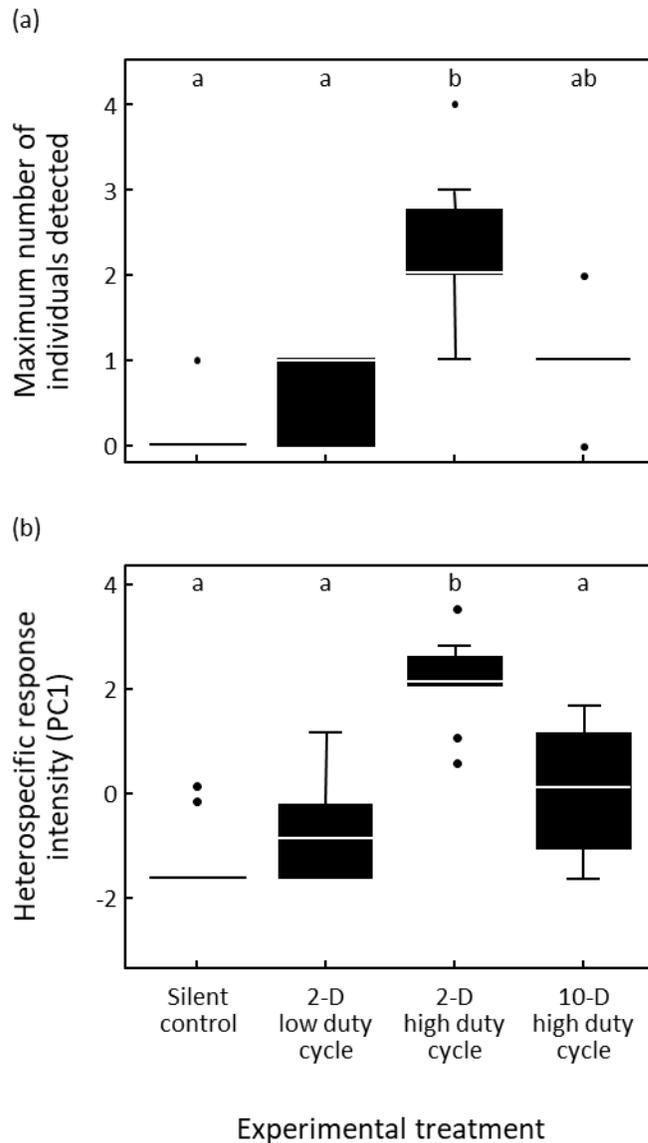
Experimental treatment

Figure 3. Heterospecific species' responses to experimental treatments of *chick-a-dee* calls during 40 trials, measured using 2 response variables: (a) Maximum number of individuals within 10 m of the predator model, (b) intensity of mobbing behaviour represented as principal component of 3 measures of behaviour, including latency to respond, minimum distance from model, and total duration of response. Treatments consisted of 3 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_{adj}= ≤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).