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7 Fowl communicate the size, speed and proximity of avian stimuli through graded structure in referential alarm
8 calls

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27 Many animals produce alarm calls that warn conspecifics about predators. In some species, alarm calls
28 communicate continuous traits associated with a predator encounter, such as its level of threat. In other species,
29 alarm calls communicate categorical traits, such as predator class (e.g. avian versus terrestrial), and are
30 consequently considered functionally referential. In theory, functionally referential alarm calls can
31 simultaneously communicate continuously distributed traits, though examples of such calls are rare. Such dual-
32 function calls could be adaptive because they would enable receivers to tailor their responses to a specific
33 predator class, as well as to more subtle characteristics of individual attacks. Here, we tested whether male fowl
34 (*Gallus gallus*) communicate continuous variation in avian stimuli through graded structure in their functionally
35 referential aerial alarm calls. In the first experiment, we held male fowl in an indoor test cage and allowed them
36 to view wild birds flying past a window. We recorded their alarm calls and compared the structure to the size,
37 speed, and proximity of the eliciting stimuli. Stimuli that appeared closer, larger, and faster elicited alarm calls
38 that were shorter, louder, clearer, and lower in frequency. In the second experiment, we broadcast alarm calls to
39 foraging females and compared their responses to the graded structural changes documented earlier. Females
40 exhibited greater initial responses and finished feeding later in response to louder alarm calls. Together, these
41 results show that fowl communicate the size, speed and proximity of avian stimuli through graded variation in
42 their functionally referential aerial alarm calls.

43

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45 alarm

46 ground squirrel

47 information

48 meerkat

49 motivational signal

50 nonlinearity

51 referential signal

52 response urgency

53 suricate

54 vervet monkey

55

56 Many animals produce alarm calls that alert conspecifics to the presence of predators (reviewed in
57 Zuberbühler 2009). In some species, alarm calls also communicate continuous variation in some aspect of the
58 predator encounter, such as the caller's motivational state or the predator's proximity, size or speed of attack
59 (Darwin 1872; Morton 1977; Blumstein & Amitage 1997; Templeton et al. 2005). These traits can be important
60 correlates of a predator's hunting success, so they may be especially important for determining a receiver's
61 antipredator response (Howland 1974; FitzGibbon 1989). These traits can also be measured for all types of
62 predators and thus may be important to receivers in a wide variety of predator contexts.

63

64 In some communication systems, callers produce acoustically distinct alarm calls that correspond to specific
65 external referents (production specificity), such as different types of predators (e.g. Seyfarth et al. 1980) or
66 different types of predator behaviours (e.g. Griesser 2008). Receivers, upon hearing such calls, show
67 antipredator behaviours that are appropriate for the specific external referent that evoked the alarm (perception
68 specificity). Alarm calls that show both production and perception specificity are termed 'functionally
69 referential' (Macedonia & Evans 1993; Evans 1997), and they have been documented in primates, suricates
70 (*Suricata suricatta*), Gunnison's prairie dogs, *Cynomys gunnisoni*, and birds (Seyfarth et al. 1980; Evans et al.
71 1993a; Zuberbühler et al. 1999; Manser 2001; Manser et al. 2001; Fichtel & Kappeler 2002; Kiriazis &
72 Slobodchikoff 2006). In general, functionally referential alarm calls are associated with species that use different
73 escape strategies for different types of predators (Macedonia & Evans 1993). In some species, social factors,
74 such as the need to coordinate group movements during foraging, can also contribute to the evolution of
75 functionally referential alarm calls (Furrer & Manser 2009).

76

77 Theory predicts that functionally referential alarm calls can simultaneously communicate continuous
78 variation in some aspect of a predator encounter (Marler et al. 1992; Macedonia & Evans 1993). Furthermore,
79 there are no obvious mechanistic constraints. Predator class can be communicated by producing structurally
80 discrete types of alarm calls, whereas continuous traits associated with a predator attack can be communicated
81 through graded features of a particular call type (Evans 1997). In principle, there are several ways in which a
82 signal could be designed to simultaneously encode predator class and other information. For example, an animal

83 could produce a functionally referential alarm call repeatedly and encode a continuous trait, such as predator
84 distance, through a sequence-level parameter, such as calling rate. Alternatively, callers could encode a
85 continuous trait in the graded structure of a functionally referential call. For example, highly aroused individuals
86 generally produce harsh, low-frequency calls, whereas less aroused individuals generally produce calls that are
87 clearer and higher in frequency (Morton 1977). Regardless of the specific encoding mechanism, functionally
88 referential alarm calls that simultaneously communicate continuous traits could be highly adaptive because they
89 would allow receivers to tailor their antipredator responses to a specific predator class, as well as to more subtle
90 characteristics of individual predator attacks.

91
92 Functionally referential alarm calls that simultaneously communicate continuous traits have only been
93 documented definitively in suricates (Manser 2001; Manser et al. 2001). Callers in this species produce
94 acoustically distinct alarm calls in response to avian and terrestrial predators, and they simultaneously encode the
95 predator's distance into the graded structure of calls. When alarm calls are broadcast in the absence of predators,
96 receivers show antipredator behaviours that are appropriate for both the class and distance of predator that
97 evoked the call (Manser 2001; Manser et al. 2001). Other species, such as tufted capuchin monkeys, *Cebus*
98 *apella nigrinus*, produce functionally referential alarm calls in some contexts and separate alarm calls that
99 communicate continuous traits in others (Wheeler 2010). However, capuchins are not known to produce
100 functionally referential alarm calls that simultaneously communicate continuous traits (Wheeler 2010). Such
101 dual-function calls probably exist in other species, but the necessary experiments have yet to be conducted. For
102 example, white-browed scrubwrens, *Sericornis frontalis*, communicate the distance to aerial predators by
103 grading the number of elements in their trilled aerial alarm calls (Leavesley & Magrath 2005). Whether trilled
104 aerial alarm calls are functionally referential, however, remains unclear because formal tests of their production
105 specificity have yet to be conducted; calls may be elicited specifically by avian predators, or by all predators
106 more generally (Leavesley & Magrath 2005). Similarly, the mobbing calls of Siberian jays, *Perisoreus infaustus*,
107 encode the type of threat (hawk, owl) and the level of threat (low to high) that is associated with perched raptors
108 (Griesser 2009). Playback experiments necessary for assessing call perception have yet to be conducted,

109 however, so it remains unclear whether mobbing calls communicate predator type and predator threat to
110 receivers in jays (Griesser 2009).

111

112 Fowl (*Gallus gallus*) are ideal for studying alarm calling behaviour because they produce two functionally
113 referential alarm calls (Evans et al. 1993a). The ‘terrestrial alarm call’ is a loud series of broadband pulses that is
114 produced by both sexes specifically in response to predators approaching on the ground, such as foxes (*Vulpes*
115 *vulpes*) or racoons (*Procyon lotor*). In response to terrestrial alarm calls, fowl assume an erect posture and scan
116 the horizontal plane (Evans et al. 1993a). The ‘aerial alarm call’ is acoustically distinct from the terrestrial alarm
117 call and has a highly variable structure (Table 1, Fig. 1, Supplementary Material; see also Figure 4 in Evans et al.
118 1993a). It is produced only by males in response to a broad class of objects moving overhead, including insects,
119 airplanes and predatory and nonpredatory birds (Gyger et al. 1987; Evans & Marler 1995). Larger and faster
120 aerial stimuli that are shaped like raptors have the greatest probability of evoking these calls (Evans et al. 1993b;
121 Evans & Marler 1995). In response to aerial alarm calls, fowl crouch, scan the sky and seek shelter (Evans et al.
122 1993a).

123

124 The objective of the current study was to determine whether the functionally referential aerial alarm calls of
125 male fowl also communicate continuous traits associated with avian stimuli. In the first experiment (i.e. call
126 production), we held males in an indoor test cage and allowed them to view wild birds flying past a window. In
127 the past, we have noticed males housed indoors producing aerial alarm calls in response to wild birds flying past
128 outside. We therefore took advantage of this opportunity and compared the structure of males’ alarm calls to the
129 size, speed and proximity of the eliciting avian stimuli. In the second experiment (i.e. call perception), we played
130 back aerial alarm calls to females and compared variation in their antipredator responses to gradation in the
131 structure of the eliciting calls. We predicted that female responses would be explained best by the acoustic
132 parameters that were correlated with avian stimulus attributes in the call production experiment. By assessing
133 both call production and call perception, we were able to test whether fowl communicate continuous variation in
134 avian stimuli through gradation in their referential aerial alarm calls.

135

136

137 **METHODS**

138

139 *Experiment 1: Call Production*

140

141 In the first experiment, we placed male fowl into an indoor test cage and permitted them to view wild birds
142 and other objects flying past a window outside. To monitor and quantify the naturally occurring stimuli observed
143 by the subjects, we videorecorded the window from the male's perspective and quantified the apparent size,
144 speed and proximity of the stimuli. We also audiorecorded the subject's vocal response, so that we could test for
145 relationships between variation in avian stimulus attributes and gradation in alarm call structure.

146

147 *Subjects*

148 Subjects were 24 sexually mature male golden Sebright fowl that were between 1 and 3 years of age. When
149 not being tested, they were kept in a climate-controlled holding facility (temperature: 21 °C; light: overhead
150 incandescent lighting for 12 h, beginning at 0600 hours, as well as natural light from surrounding windows).
151 Each male was paired with one female in a separate wire cage (1 m long, 1 m wide, 0.8 m high) that contained
152 food, water, wooden perches and straw bedding.

153

154 *Apparatus*

155 During recording sessions, we held subjects in a wire test cage (1.12 m long, 0.45 m wide, 0.73 m high) that
156 had an artificial grass mat, wooden perches and a continuous supply of food and water. The test cage was housed
157 inside a climate-controlled test room (temperature: 21 °C; lighting: incandescent lights for 12 h, beginning at
158 0600 hours each day) that had a single window to the outside (1.11 m wide, 0.82 m high). The test cage was
159 positioned on a small table, such that its floor was level with the bottom of the window. The longitudinal axis of
160 the test cage was aligned with the centre of the window, and the narrow end of the cage was placed facing the
161 window at a distance of 1.7 m. To reduce acoustic interference from outside the test room, as well as
162 reverberation from within the test cage, we attached 10 cm thick 'Sonex' sound-attenuating foam baffles

163 (Illbruck Acoustic, Inc., Minneapolis, MN, U.S.A.) to the back and both sides of the cage, as well as to the wire
164 overhead.

165

166 The view from the test cage through the test room window was dominated by several large trees
167 (*Eucalyptus*) in the foreground (0–15 m from window), three small buildings in the mid-ground (15–30 m from
168 window) and several large trees in the background. Sky was visible between tree branches in the upper half of
169 the field of view, and green grass was visible between trees and buildings in the lower half of the field of view.
170 When a subject was in the end of the cage nearest the window (i.e. 1.7 m from window), its horizontal and
171 vertical fields of view through the window were approximately 36° and 27°, respectively. When a subject was in
172 the opposite end of the cage (i.e. 2.8 m from window), its horizontal and vertical fields of view through the
173 window were approximately 22° and 17°, respectively.

174

175 We videorecorded aerial stimuli from the subject's perspective using a Sony Handicam (model: HDR-HC7;
176 format: HDV 1080i50; shutter speed: 1/100 s). The camera was mounted on a tripod at approximately the
177 subject's eye-height (27 cm above the floor of the test cage) and was placed outside the subject's cage midway
178 along the side of the cage that faced the test room window. We adjusted the focal length so that the camera's
179 field of view included the entire window for the remainder of the experiment, so that the apparent size, speed
180 and proximity of stimuli could be compared across recording sessions. Because the camera was slightly closer to
181 the window, it captured the subject's complete field of view through the window, regardless of his position
182 within the cage. Video was recorded digitally (format: HDV 1080i50) to the hard drive of a Macintosh computer
183 using QuickTime Pro software (version 7; Apple, Inc., Cupertino, CA, U.S.A.).

184

185 We recorded the subject's vocalizations with a Sennheiser MHK 40-P48 microphone (cardioid pickup
186 pattern; 40–20 000 Hz frequency response, \pm 1 dB deviation) that was suspended from the centre of the subject's
187 cage. Calls were digitized using a MOTU UltraLite-mk3 digital interface (48 kHz sampling rate; 24-bit
188 amplitude encoding) and were recorded to the digital sound track (WAVE format, 48 kHz sampling rate, 16-bit
189 amplitude encoding) of the video file that was recording the aerial stimuli. By recording to the same digital file,

190 we ensured precise temporal synchronization of the audio and video tracks. We calibrated our system by
191 recording 30 s of continuous white noise, which we broadcast at 76 dB(C) SPL (measured at a distance of 1 m
192 with a RadioShack sound level meter, model 33-4050, slow response) through a Nagra Kudelski DSM monitor,
193 which was placed on the centre of the test cage floor. After this initial calibration procedure, we did not adjust
194 the gain on the audio-recording system for the remainder of the experiment, which ensured that all vocalizations
195 were recorded at the same level.

196

197 *Procedure*

198 We recorded subjects between 13 February and 7 May 2008. We began a recording session at 1200 hours
199 by transferring a subject and his female cagemate from the holding facility to the test cage inside the test room.
200 The female was included because males only produce alarm calls in the presence of a conspecific audience
201 (Karakashian et al. 1988; Evans & Marler 1992). The pair was given until 3 h before sunset to acclimate to the
202 test cage (time of sunset determined at $-33^{\circ}50'00''$ latitude; $151^{\circ}15'00''$ longitude). Audio and video recording
203 began at that time and continued until sunset, at which time the birds went up to roost and became silent. We
204 resumed recording the next morning at sunrise and continued for an additional 4 h, resulting in a total of 7 h of
205 audio and video recording per subject. We programmed all recordings to begin and end automatically, thus
206 eliminating the need for a human observer to enter the test room during a recording session. Following a
207 recording session, we returned the subject and his mate to the holding facility, replaced the food, water and
208 artificial grass mat in the test cage, and cleaned the test room window.

209

210 Our goal was to record at least 10 alarm calls per subject. To achieve this, we reviewed the audio recordings
211 following the first round of 24 recording sessions (see Sound Analysis details below). Males that had not
212 produced 10 alarm calls in the first round were recorded again in a second round. The recording procedure for
213 the second round was identical to that of the first, and subjects were recorded in the same order. Of the 24
214 subjects recorded in the first round, 16 were recorded in the second round, resulting in a total of 280 h of audio
215 and video recording.

216

217 *Quantifying stimulus attributes and alarm call structure*

218 We identified alarm calls on the original recordings using Soundtrack Pro software (version 2.0.2; Apple,
219 Inc., Cupertino, CA, U.S.A.). For each recording, we listened to the audio track at approximately natural
220 amplitude while simultaneously viewing the video track and a scrolling real-time spectrogram of the audio track
221 (512-point fast Fourier transform (FFT), 87.5% overlap, Hamming window). When we detected an alarm call
222 (see example spectrograms in Fig. 1; example audio files are provided in the Supplementary Material), we noted
223 the exact time (40 ms resolution) on a permanent time code that we had superimposed on the video track, and
224 then saved the alarm call into two separate digital files. The first file contained the video track only (format:
225 HDV 1080i50) and was used to characterize the aerial stimuli that evoked the alarm calls. The second file
226 contained the audio track only (WAVE format, 44.1 kHz sampling rate, 16-bit amplitude encoding) and was
227 used to quantify alarm call structure. Both files included 30 s of the original recording before and after the alarm
228 call. Females do not produce aerial alarm calls, so we were confident that the male subject produced all recorded
229 calls.

230

231 Extracted video clips were displayed on a high-resolution external video monitor (1920 x 1080 pixels
232 resolution) that we controlled with Final Cut Pro software (version 6.0.6; Apple, Inc.). For each of the 695 video
233 clips, we searched frame by frame for aerial stimuli during a 5 s measurement window that immediately
234 preceded the alarm call. The beginning and end of the measurement window were selected relative to the video's
235 permanent time code, thus keeping the observer blind to the structure and sound of the corresponding alarm call.
236 After detecting a stimulus, we categorized it as avian or nonavian and excluded all nonavian stimuli (e.g. flying
237 invertebrates, falling leaves) from subsequent analysis ($N = 217$ clips). We also excluded clips in which an avian
238 stimulus was airborne for less than two video frames (i.e. < 80 ms) during the measurement window ($N = 39$
239 clips) because it was not possible to measure the speed of such stimuli. If more than one avian stimulus was
240 present during the measurement window ($N = 52$ clips), we measured only the one closest in time to the alarm
241 call.

242

243 We measured the following three variables for every avian stimulus: (1) maximum diameter (a measure of
244 the size of the avian stimulus from the subject's perspective), (2) average speed and (3) proximity to subject. To
245 determine maximum diameter, we measured the largest diameter of the stimulus in every video frame in which it
246 was visible during the 5 s measurement window, and then noted the maximum value of these measures.
247 Measurements were made by placing a transparent ruler directly onto the video monitor. To determine average
248 speed, we divided the linear distance travelled by the stimulus during the 5 s measurement window by the period
249 of time for which it was visible. Linear distance was measured by placing a transparent ruler directly onto the
250 monitor and stepping frame by frame through the video; the measure did not incorporate deviations from a linear
251 flight path. To determine the proximity to the subject, we compared the position of the stimulus to landmarks in
252 the video (e.g. trees, buildings). Based on known distances between the landmarks and the video camera, we
253 could approximate the minimum distance between the stimulus and the camera. The resolution of this method
254 was limited by the spacing of landmarks and the difficulty of estimating depth from a two-dimensional video;
255 consequently, we quantified proximity using an ordinal scale that was based on landmark positions (1 = 0–15 m;
256 2 = 15–30 m; 3 = 30+ m).

257

258 For each avian stimulus that we measured, we also measured the fine structure of the corresponding alarm
259 call using SASLab Pro software (version 4.40; Avisoft Bioacoustics, Berlin, Germany). We filtered each
260 extracted audio file with a band-pass filter (200–12 000 Hz), which removed background noise without affecting
261 the structure of the alarm call (Wilson & Evans 2010). We then generated a spectrogram (1024-point FFT,
262 87.5% overlap, Hamming window, 2.9 ms temporal resolution, 43 Hz frequency resolution; Fig. 1) and used the
263 'automatic parameter measurements' feature (settings: holdtime, 100 ms; threshold, -30 dB relative to maximum
264 amplitude) to select the alarm call and measure its structure. If the subject produced more than one alarm call in
265 response to a particular avian stimulus, then we selected and measured only the first. We measured four
266 structural features, including (1) call length, (2) amplitude, (3) dominant frequency and (4) entropy. Amplitude is
267 the root-mean-square amplitude of the entire call, and dominant frequency is the frequency with the highest
268 amplitude. Entropy is a measure of sound purity that approaches 0 for pure tones and 100 for white noise; it is
269 the ratio of the geometric mean to the arithmetic mean of the spectrum, multiplied by 100. Dominant frequency

270 and entropy were measured from every FFT within the alarm call and were then averaged (separately for each
271 variable) for statistical analysis.

272

273 *Statistical analysis*

274 Preliminary analyses revealed that the three independent variables (i.e. maximum diameter, average speed,
275 proximity to subject) were highly intercorrelated. To avoid potential problems associated with multicollinearity,
276 we conducted a principal components analysis on the three independent variables (see Table 2) and used only the
277 derived factor scores in subsequent statistical analyses.

278

279 We used linear mixed model analysis to test for relationships between avian stimulus attributes and alarm
280 call structure. A single principal component derived from the three original avian stimulus variables was entered
281 as a covariate with fixed effects, and male identity was entered as a subject variable with random effects to
282 account for repeated measures of the same individuals. A separate model was constructed for each of the four
283 measures of alarm call structure. For each model, we estimated fixed effects using the restricted maximum
284 likelihood method and modelled the subject effect by assuming a variance components covariance structure.
285 Residuals were not normally distributed for three of the four models, but were corrected by applying a square-
286 root transformation to call length and a log transformation to dominant frequency and entropy. All other model
287 assumptions were satisfied. Tests were two tailed, and we considered results to be statistically significant when P
288 ≤ 0.05 . All analyses were conducted in PASW (version 18.0 for Macintosh; Chicago, IL, U.S.A.).

289

290 *Experiment 2: Call Perception*

291

292 In the second experiment, we tested whether female fowl respond appropriately to gradation in the structure
293 of aerial alarm calls. We played back alarm calls, and then compared female responses to variation in call
294 structure, which we quantified according to the structural measures described in experiment 1.

295

296 *Subjects*

297 Subjects were 32 sexually mature female golden Sebright fowl that were between 2 and 7 years of age. Of
298 the 32 females tested, 24 had served as a conspecific audience in the first experiment. When not being tested,
299 subjects were paired with males and kept in the same conditions and climate-controlled holding facility as in the
300 call production study.

301

302 *Apparatus*

303 During playback trials, we held subjects in a wire test cage (1.12 m long, 0.45 m wide, 0.73 m high) that
304 was placed on the floor in the centre of a sound-attenuating chamber (Ampliscience, model 10070;
305 Robassomero, Italy). The chamber measured 2.38 m wide x 2.38 m long x 2.15 m high and was lined with 10 cm
306 thick ‘Sonex’ foam baffles on the walls and 15 cm thick baffles on the ceiling to prevent reverberation. The cage
307 had an artificial grass mat, a continuous supply of water, and light provided by two incandescent lamps (60 W).
308 A remotely operated food dispenser was placed above the cage and was used to deliver fresh corn to the centre
309 of the cage floor during trials. To broadcast alarm call stimuli, we placed a Nagra Kudelski DSM monitor
310 midway along the length of the test cage, abutting the side. The monitor was connected through a conduit panel
311 in the chamber wall to a Behringer digital-to-analogue converter (model FCA202, 24 bits/96 kHz) and a
312 Macintosh computer that played stimuli using QuickTime Pro software (version 7; Apple, Inc.). Subjects were
313 monitored with a Panasonic video camera (model WV-CL320) and a Sennheiser microphone (model MHK 40-
314 P48) connected to a Canopus analogue-to-digital converter (model ADVC110) through the conduit panel. This
315 was connected to a second Macintosh computer, which recorded trials using QuickTime Pro software.

316

317 Prior to commencing playbacks, we calibrated the playback system by broadcasting the white noise that we
318 had recorded during the call production study. We adjusted the playback level so that the white noise measured
319 precisely 76 dB(C) SPL at a distance of 1 m (i.e. the same level used during recording). The playback level was
320 not adjusted for the remainder of the playback experiment, which ensured that each alarm call was broadcast at
321 the same amplitude at which it had been produced (mean \pm SD = 70 \pm 8 dB(C) SPL at a distance of 1 m).

322

323 *Stimuli*

324 Playback stimuli were 20 high-quality aerial alarm calls recorded during the call production study (Table 3,
325 Fig. 1, Supplementary Material). For each of four males, we selected five calls that were, as far as possible,
326 evenly distributed across the range of variation observed in the call production study (Table 1, 3, Fig. 1,
327 Supplementary Material). Using Raven Interactive Sound Analysis software (version 1.3 Pro, Cornell Lab of
328 Ornithology Bioacoustics Research Program, Ithaca, NY, U.S.A.), we extracted the calls from the original
329 recordings, plus 100 ms of silence before and after each call. We removed background noise with a band-pass
330 filter (200–12 000 Hz; Wilson & Evans 2010), and then saved the calls as separate digital files (WAVE format,
331 48 kHz sample rate, 16-bit amplitude encoding). We did not normalize the sound files because we wanted to
332 preserve natural amplitude gradation among calls.

333

334 *Procedure*

335 We tested subjects between 2 August and 9 September 2008 using a randomized complete block design. We
336 assigned the 32 hens at random to four equal-sized groups (i.e. 8 per group), and then tested each group daily
337 during a different 5-day period. Each group was tested with alarm calls derived from different stimulus males
338 (i.e. 4 stimulus males corresponding to 4 groups of subjects, with the constraint that subjects were not tested with
339 alarm call stimuli recorded from their cagemates), and each subject within the group was tested with the same set
340 of five alarm calls (i.e. one alarm call per day in a random order). Subjects were tested individually each day in
341 either the morning (0800–1100 hours) or the afternoon (1500–1800 hours) to correspond to peak foraging
342 periods. A given hen was always tested at the same time each day.

343

344 Prior to testing a group, we habituated each subject in the group to the test apparatus. We placed one of the
345 eight subjects into the test cage and allowed her to move freely around the cage for approximately 15 min.
346 During that time, we delivered five kernels of fresh corn to the centre of the test cage floor using the remotely
347 operated food dispenser. We repeated this habituation procedure each day until every subject in the group
348 walked readily around the cage, did not become startled by the food dispenser, and consumed all of the corn that
349 was delivered (range 3–11 habituation cycles per subject).

350

351 We began testing a group on the day after all birds had habituated to the test apparatus. A trial began by
352 placing one of the eight subjects into the test cage, closing the chamber door and initiating the recording
353 procedure. The observer controlled the experiment from outside the chamber, viewing the subject remotely on
354 the computer monitor. When the subject began moving around the cage, we delivered five kernels of fresh corn
355 to the centre of the test cage floor. As soon as the subject pecked at the corn, we broadcast an alarm call stimulus
356 and continued recording the subject until she consumed all of the remaining corn (4–59 s), or for 10 min if she
357 did not resume feeding. We then returned the subject to the holding facility, replaced the mat in the test cage and
358 reloaded the food dispenser with fresh corn.

359

360 *Quantifying female responses*

361 Prior to scoring female responses, we viewed the trial recordings using QuickTime Pro software. When the
362 corn was delivered, we stepped frame by frame through the video and noted the exact time at which the alarm
363 call stimulus was played, relative to the video's time code (40 ms resolution). We then scored female responses
364 to alarm call stimuli by viewing the recordings again with the audio track muted. This method allowed us to
365 score female responses relative to when the alarm call was played, but ensured that the observer was not
366 influenced by listening to the eliciting call.

367

368 We measured two dependent variables from every trial: (1) initial response and (2) time to finish feeding.
369 Initial response was scored as the immediate reaction to the onset of the alarm call and was quantified according
370 to the six-level ordinal scale defined by Evans et al. (1993b) as follows.

371

- 372 (1) No visible response.
- 373 (2) Looking upwards (typically by rolling the head to fixate with one eye).
- 374 (3) Looking upwards and flexing the neck, so as to draw the head towards the body.
- 375 (4) Responses 2 and 3, together with perceptible crouching.
- 376 (5) Responses 2 and 3, together with pronounced crouching, so that the body makes contact with the floor.
- 377 (6) Responses 2, 3 and 5, together with running in a crouched posture.

378

379 Time to finish feeding was designed to reflect the trade-off between foraging and vigilance. It was defined as the
380 time from the onset of the alarm call stimulus to when the subject consumed the last kernel of corn. If a subject
381 did not consume all of the corn after the alarm call was played, we set time to the maximum value observed
382 among the 32 subjects on that particular test day (i.e. test days 1–5). Subjects did not consume all of the corn in 7
383 of the 160 playback trials.

384

385 *Statistical analysis*

386 We used linear mixed model analysis to test for relationships between alarm call structure (independent
387 variables) and female response (dependent variables). Our measures of alarm call structure were derived directly
388 from the call production study (see above), and included call length, amplitude, dominant frequency and entropy.
389 These four measures, as well as test day (i.e. 1–5), were entered into the model as covariates with fixed effects.
390 Female identity was entered as a subject variable with random effects to account for repeated measures of the
391 same individuals. We constructed a separate model for each measure of female response. For each model, we
392 estimated fixed effects using the restricted maximum likelihood method and modelled the subject effect by
393 assuming a variance components covariance structure. Preliminary analyses revealed a two-way interaction
394 between test day and amplitude in the model explaining initial response. Therefore, in the final model explaining
395 initial response, we included as covariates with fixed effects all two-way interactions between test day and the
396 four measures of alarm call structure. No interaction effects were detected in the model explaining time to finish
397 feeding, so interactions were not included in this model. Residuals were not normally distributed in the model
398 describing time to finish feeding, but were corrected by applying a log transformation to the dependent variable.
399 All other model assumptions were satisfied. Tests were two tailed, and we considered results to be statistically
400 significant when $P \leq 0.05$.

401

402 **RESULTS**

403

404 *Experiment 1: Call Production*

405

406 Of 695 aerial alarm calls produced by 24 subjects during 280 h of recording, 373 (54%) were associated
407 with a clear avian stimulus, 217 (31%) with a nonavian stimulus (e.g. invertebrates, falling leaves) and 105
408 (15%) with no discernable aerial stimulus. Of the 373 calls that were associated with an avian stimulus, we
409 excluded 39 because the stimulus was not present on two or more video frames. Analyses were therefore based
410 on 334 aerial alarm calls that were associated with a clear avian stimulus during the 5 s immediately preceding
411 the call (mean \pm SD = 1.3 ± 0.9 calls/subject/h; range 0.1–4.0 calls per subject per h). In general, we could not
412 ascertain the species of avian stimuli because the lighting conditions (i.e. dim light inside the test room, bright
413 light outside the test room) caused the avian stimuli to appear very dark on the video recordings. The few stimuli
414 that we could identify included predatory birds, such as Australian magpies, *Cracticus tibicen*, kookaburras,
415 *Dacelo novaeguinae*, and unidentified raptors, as well as nonpredatory birds, such as parrots (Cacatuidae,
416 Psittacidae) and honey-eaters (Meliphagidae). The brown goshawk, *Accipiter fasciatus*, was the only raptor that
417 was observed regularly at our study site.

418

419 Avian stimuli were highly variable in terms of maximum diameter, average speed and proximity to subject,
420 as measured from the video on an external monitor (Table 1). In addition, the three variables were highly
421 intercorrelated and thus loaded heavily onto a single principal component that explained 82% of the variance in
422 the original 3 variables (Table 2). Maximum diameter and average speed loaded positively onto the principal
423 component, whereas proximity to subject loaded negatively onto the principal component. Consequently, a high
424 principal component score reflects a close, large and fast-moving avian stimulus.

425

426 The principal component describing the avian stimuli accounted for a significant amount of the gradation in
427 alarm call structure (Fig. 2). In response to stimuli that appeared larger, closer and faster moving, subjects
428 produced shorter alarm calls (linear mixed model analysis: $F_{1,327} = 40.8$, $P < 0.001$) with higher amplitude ($F_{1,323}$
429 = 38.5 , $P < 0.001$), lower dominant frequency ($F_{1,322} = 39.4$, $P < 0.001$) and less random energy distribution
430 ($F_{1,325} = 8.7$, $P = 0.003$). In contrast, stimuli that appeared smaller, more distant and slower moving elicited

431 longer alarm calls that had lower amplitude, higher dominant frequency and more randomly distributed energy
432 (Fig. 2).

433

434 *Experiment 2: Call Perception*

435

436 Females usually responded to the playback of male alarm calls by crouching, rolling their head to the side
437 and looking upwards with one eye. They also stopped consuming corn and remained still for up to 1 min. Alarm
438 call structure and test day both had a significant effect on female response (Fig. 3). The initial response was
439 stronger in response to louder alarm call stimuli, but declined significantly over the 5-day test period (Fig. 3a).
440 Furthermore, the effect of amplitude on initial response diminished over time, as reflected by a significant
441 interaction between test day and amplitude (linear mixed model analysis: test day: $F_{1,138} = 8.1$, $P = 0.005$;
442 amplitude: $F_{1,142} = 16.3$, $P < 0.001$; test day x amplitude interaction: $F_{1,139} = 14.5$, $P < 0.001$; Fig. 3a). Initial
443 response was not affected by the other measures of alarm call structure, or by their interactions with test day
444 (linear mixed model analysis: call length: $F_{1,145} = 0.0$, $P = 0.903$; dominant frequency: $F_{1,139} = 0.5$, $P = 0.494$;
445 entropy: $F_{1,140} = 0.4$, $P = 0.510$; test day x call length interaction: $F_{1,147} = 0.3$, $P = 0.571$; test day x dominant
446 frequency interaction: $F_{1,139} = 0.6$, $P = 0.439$; test day x entropy interaction: $F_{1,136} = 0.0$, $P = 0.900$). As with
447 initial response, the time to finish feeding was longer in response to louder alarm call stimuli, but declined
448 significantly over the 5-day test period (linear mixed model analysis: test day: $F_{1,124} = 33.8$, $P < 0.001$;
449 amplitude: $F_{1,150} = 8.0$, $P = 0.005$; Fig. 3b). Time to finish feeding was not affected by the other measures of
450 alarm call structure (linear mixed model analysis: call length: $F_{1,140} = 1.9$, $P = 0.166$; dominant frequency: $F_{1,154}$
451 $= 2.5$, $P = 0.114$; entropy: $F_{1,153} = 2.3$, $P = 0.128$).

452

453 **DISCUSSION**

454

455 Male fowl encoded continuous traits associated with avian stimuli into several graded parameters of their
456 functionally referential aerial alarm calls. Stimuli that appeared closer, larger and faster elicited alarm calls that
457 were shorter, louder, clearer and lower in frequency. Female receivers ignored gradation in the duration,

458 frequency and entropy of alarm calls, but responded appropriately to gradation in alarm call amplitude. In
459 response to louder alarm calls, females showed stronger initial responses and took longer to finish feeding.
460 Together, these results show that fowl communicate continuous variation in avian stimuli through graded
461 structure in their functionally referential aerial alarm calls.

462

463 Gradation in the structure of alarm calls correlated with continuous variation in both stimulus attributes and
464 receiver responses. This pattern is consistent with urgency-based calling, which has been described in ground
465 squirrels, birds, suricates and primates (Owings & Hennessy 1984; Blumstein & Armitage 1997; Manser 2001;
466 Manser et al. 2001; Warkentin et al. 2001; Fichtel & Hammerschmidt 2002; Leavesley & Magrath 2005;
467 Templeton et al. 2005; Fallow & Magrath 2010). As in these other systems, however, the precise cause of
468 gradation in alarm call structure is unclear (Evans 1997). It could reflect the size, speed or proximity of avian
469 stimuli, or the risk of predation associated with these physical properties. Another possibility is that gradation in
470 call structure instructs receivers about how to respond (i.e. imperative), rather than denoting stimulus
471 characteristics per se (i.e. denotative; Cheney & Seyfarth 1990; Marler et al. 1992). Finally, gradation could
472 reflect the caller's affective state, which logically correlates with those stimulus characteristics that predict attack
473 (Morton 1977; Evans 1997). Future studies could address this latter possibility by testing whether predator
474 attributes affect physiological measures that are associated with the caller's affective state (Cabanac & Aizawa
475 2000; Walker et al. 2006).

476

477 Gradation in the amplitude of alarm calls affected female responses and thus had communicative value. In
478 addition, receivers appeared to respond adaptively to this gradation, since they resumed feeding sooner in
479 response to quieter calls that were putatively associated with less dangerous predators (FitzGibbon 1989).
480 Female responses also diminished over time, which could reflect habituation to a novel environment. We have
481 observed similar effects in male fowl, whose alarm calling rates declined steadily for several weeks following
482 their introduction to a novel outdoor environment (Wilson & Evans 2008; Wilson et al. 2010). Alternatively,
483 reduced female responsiveness could reflect caller reliability. In our experimental design, we repeatedly
484 broadcast alarm calls from the same male in the absence predators, which made him progressively less reliable.

485 Since the alarm calls of fowl are individually distinctive (Bayly & Evans 2003), it is possible that females
486 became less responsive as the alarm calls of an individual male consistently failed to predict aerial predators.
487 Richardson's ground squirrels, *Spermophilus richardsonii*, yellow-bellied marmots, *Marmota flaviventris*, and
488 vervet monkeys, *Chlorocebus aethiops*, all discriminate between the alarm calls of reliable and unreliable
489 individuals (Cheney & Seyfarth 1988; Hare & Atkins 2001; Blumstein et al. 2004).

490

491 Amplitude was the only acoustic feature we measured that correlated with both stimulus attributes and
492 receiver responses. In general, animals produce louder calls when they are highly aroused (Darwin 1872; Driver
493 & Humphries 1969; Scherer 1985; Conover 1994; Jurisevic & Sanderson 1998; Rendall 2003; Soltis et al. 2009;
494 but see Searcy & Nowicki 2006). Furthermore, louder calls are generally more evocative than quiet calls
495 (Brenowitz 1989; Blumstein & Armitage 1997; Fichtel & Hammerschmidt 2002; Lampe et al. 2010; Brumm &
496 Ritschard 2011). Gradation in call amplitude thus provides a simple, noncognitive mechanism for adapting
497 receiver responses to those predator attributes that directly influence a caller's affective state. A potential
498 disadvantage of using amplitude gradation for communication is that amplitude can vary as a function of wind
499 gusts, topography, vegetation, movement of the caller's head, and other, presumably irrelevant, factors.
500 Furthermore, calls necessarily attenuate as they travel from caller to receiver (Bradbury & Vehrencamp 1998). In
501 fowl, however, the effects of attenuation and these other miscellaneous factors are probably minimal because
502 males only produce aerial alarm calls when accompanied closely by a conspecific audience (Karakashian et al.
503 1988; Evans & Marler 1992). In social groups, most aerial alarm calls are given by alpha males, which associate
504 closely with hens and keep other males at a distance (Wilson et al. 2008, 2009; Kokolakis et al. 2010).

505

506 Avian stimuli affected several acoustic parameters of alarm calls that failed to predict receiver responses.
507 These relationships potentially can be explained by mechanisms that also are unrelated to receiver responses. For
508 example, the apparent effect of avian stimulus attributes on call length could be an artefact of varying call
509 amplitude. In order to produce a loud, continuous call, the caller must expel the air in its air sacs at a high rate,
510 which rapidly depletes its air supply and results in a short call (Plummer & Goller 2008). Similarly, the effect of
511 stimulus attributes on dominant frequency can be explained by Morton's (1977) motivation-structural rules,

512 which predict that animals will produce lower-frequency sounds in highly arousing situations. In contrast to
513 dominant frequency, the observed effect of stimulus attributes on alarm call entropy contradicts Morton's (1977)
514 motivation-structural rules, which predict that animals will produce noisier calls (i.e. greater entropy) when they
515 are highly aroused. Surprisingly, fowl produced alarm calls with lower entropy in response to stimuli that we
516 assume were highly arousing. Similar results have also been found in other species, suggesting that highly
517 aroused individuals may produce clearer vocalizations more generally. For example, yellow-bellied marmots (D.
518 T. Blumstein & Y. Y. Chi, unpublished data), piglets, *Sus scrofa* (Puppe et al. 2005) and goats, *Capra hircus*
519 (Siebert et al. 2011) all produce clearer vocalizations when they are highly aroused.

520

521 By grading the structure of alarm calls in relation to predator distance, callers can potentially mitigate the
522 predation costs associated with calling. For example, Richardson's ground squirrels remain cryptic by producing
523 short-range ultrasonic alarm calls in lieu of long-range audible alarm calls when predators are distant and
524 unlikely to have detected them (Wilson & Hare 2006). Our results suggest that fowl use a similar strategy for
525 reducing predation risk. When predators were distant and unlikely to have noticed potential callers, males
526 produced low-amplitude, high-frequency alarm calls that are known to be cryptic (Marler 1955; Klump &
527 Shalter 1984; Wood et al. 2000). This finding is consistent with a recent study on risk management, which
528 showed that male fowl have a greater probability of producing alarm calls when concealed under cover
529 (Kokolakis et al. 2010). In contrast, when predators were close and likely to have already noticed the caller,
530 males produced loud, low-frequency alarm calls. These characteristics typically indicate a caller's willingness to
531 defend itself and may consequently function as threat signals (Morton 1977). Furthermore, these calls were
532 similar to the 'distress calls' produced by many birds. Distress calls are thought to startle predators during the
533 final stages of attack (Driver & Humphries 1969; Conover 1994), so this may be an additional function of the
534 loud, low-frequency alarm calls observed in our study.

535

536 In conclusion, fowl have a remarkably complex alarm communication system in which they produce
537 acoustically distinct alarm calls in response to aerial and terrestrial predators. Receivers, upon hearing alarm
538 calls, respond with categorically distinct antipredator behaviour that is specific to the type of predator that

539 evoked the call. By recording males' alarm-calling responses to naturally occurring avian stimuli, and by
540 comparing variation in females' responses to gradation in alarm call structure, the current study reveals
541 additional complexity in this system. Specifically, we show that male fowl encode continuous variation in avian
542 stimuli through gradation in the fine structure of their functionally referential aerial alarm calls. Stimuli that
543 appear closer, larger and faster elicit alarm calls that are shorter, louder, clearer and lower in frequency. We also
544 show that females ignore gradation in the duration, frequency and entropy of alarm calls, but respond
545 appropriately to gradation in call amplitude. In response to louder alarm calls, females show stronger initial
546 responses and take longer to finish feeding. Together, these results provide the first definitive evidence that a
547 bird can communicate continuous variation in avian stimuli through gradation in the fine structure of their
548 functionally referential alarm calls.

549

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551

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559

560 **Supplementary Material**

561

562 Supplementary material for this article is available, in the online version, at doi:

563

564 **References**

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695 **Table 1**

696 Description of 334 aerial alarm calls produced by male fowl and the avian stimuli that evoked them in the call
 697 production experiment

Variable	Minimum		Maximum		Average		Coefficient of variation (%)	
Alarm call structure								
Length (ms)	403	(349)	2154	(882)	1048	(308)	53	(19)
Amplitude (dB(C))	60	(5)	78	(7)	69	(5)	9	(3)
Dominant frequency (Hz)	673	(196)	1473	(370)	992	(218)	26	(10)
Entropy (%)	21	(3)	33	(4)	27	(3)	15	(3)
Avian stimuli								
Diameter (mm)	1.5	(1.3)	35.2	(15.2)	14.2	(5.8)	81	(14)
Speed (cm/s)	4.3	(1.8)	68.1	(34.6)	23.1	(10.2)	88	(21)
Proximity (index)	1.1	(0.4)	2.9	(0.3)	1.9	(0.4)	41	(11)
PC1	-1.1	(0.2)	1.6	(1.1)	0.0	(0.5)	—	

698 For each variable, we calculated four parameters for each male (minimum, maximum, average, coefficient of
 699 variation). Shown is the average (standard deviation) of each parameter from among the 24 males. ‘PC1’ is a
 700 principal component that incorporates the maximum size, average speed and proximity to subject. Coefficient of
 701 variation could not be calculated for PC1 because it involved division by zero. All values are shown prior to
 702 transformation.

703

704 **Table 2**

705 Details of the principal components analysis used to describe 334 avian stimuli recorded in the call production
706 experiment

Variable	PC1
Diameter (mm)	0.94
Speed (cm/s)	0.88
Proximity (index)	-0.89
Eigenvalue	2.45
Variance explained (%)	81.7

707

708 Component loadings are provided for the single extracted principal component (PC1). Analysis was based on the
709 correlation matrix and unrotated components were extracted when eigenvalues exceeded 1. Sampling adequacy
710 was assessed using Bartlett's test, and the hypothesis that the correlation matrix contained only zero correlations
711 was rejected ($\chi^2_3 = 595.8$, $P < 0.001$). Component scores were generated using the regression method.

712

713 **Table 3**

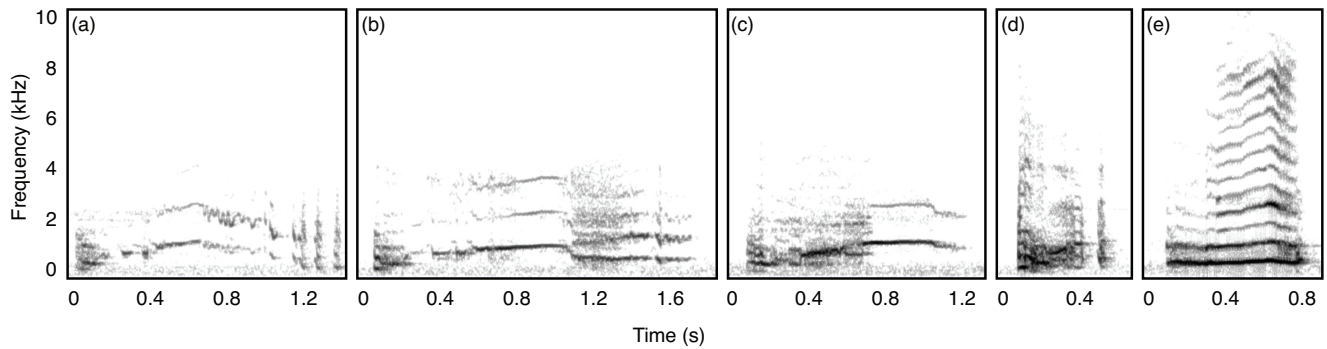
714 Description of the 20 aerial alarm calls that were used as stimuli in the call perception experiment

Variable	Minimum		Maximum		Average		Coefficient of variation (%)	
Length (ms)	473	(48)	1361	(180)	958	(75)	38	(7)
Amplitude (dB(C))	61	(4)	81	(4)	70	(1)	11	(2)
Dominant frequency (Hz)	655	(99)	1165	(214)	926	(30)	22	(1)
Entropy (%)	24	(3)	33	(3)	28	(1)	13	(4)

715 For each variable, we calculated four parameters for each male (minimum, maximum, average, coefficient of

716 variation). Shown is the average (standard deviation) of each parameter from among the four stimulus males.

Figure 1



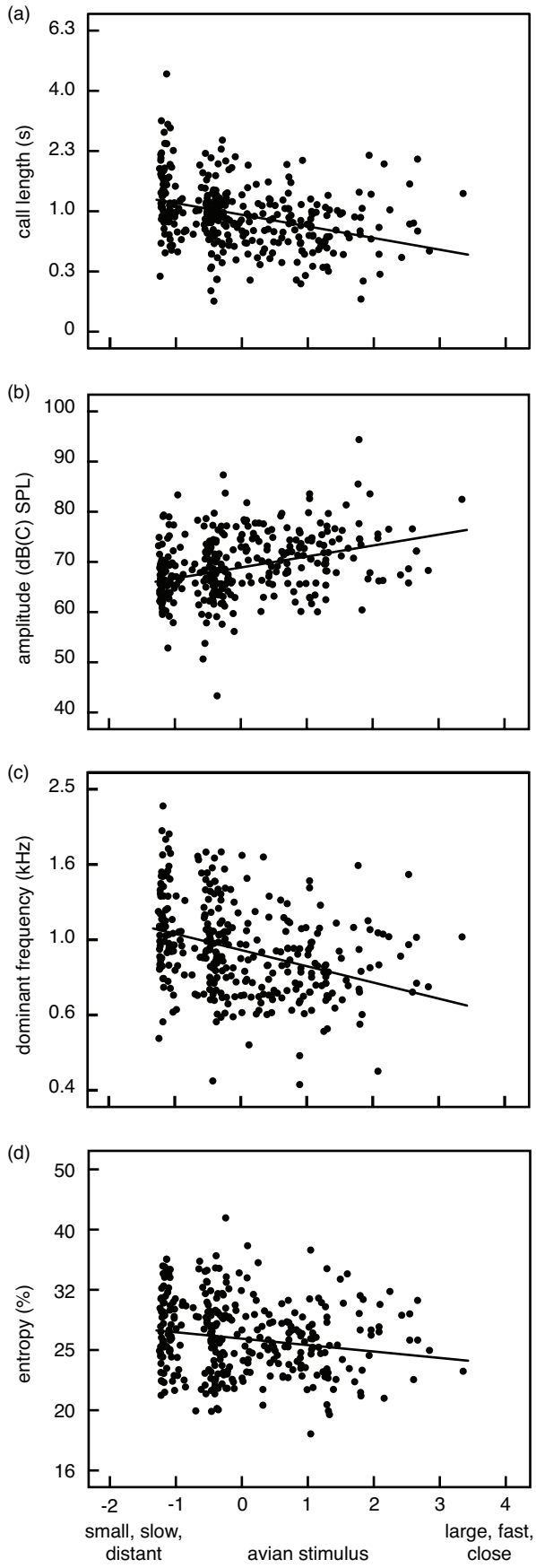
717

718

719 **Figure 1.** Graded structure of aerial alarm calls. The five calls shown here (a–e) were produced by a single male
720 in the call production experiment, and were used as one of the four sets of playback stimuli in the call perception
721 experiment. Calls are arranged in order of ascending amplitude because amplitude was the only acoustic feature
722 that correlated with both avian stimulus attributes and receiver responses. Spectrograms were generated using a
723 1024-point FFT, 87.5% overlap, and a Hamming window, which resulted in a frequency resolution of 43 Hz and
724 a temporal resolution of 2.9 ms. Calls were filtered with a bandpass filter (200–12 000 Hz) and are shown at
725 original amplitude. Greyscale represents an amplitude range of 50 dB.

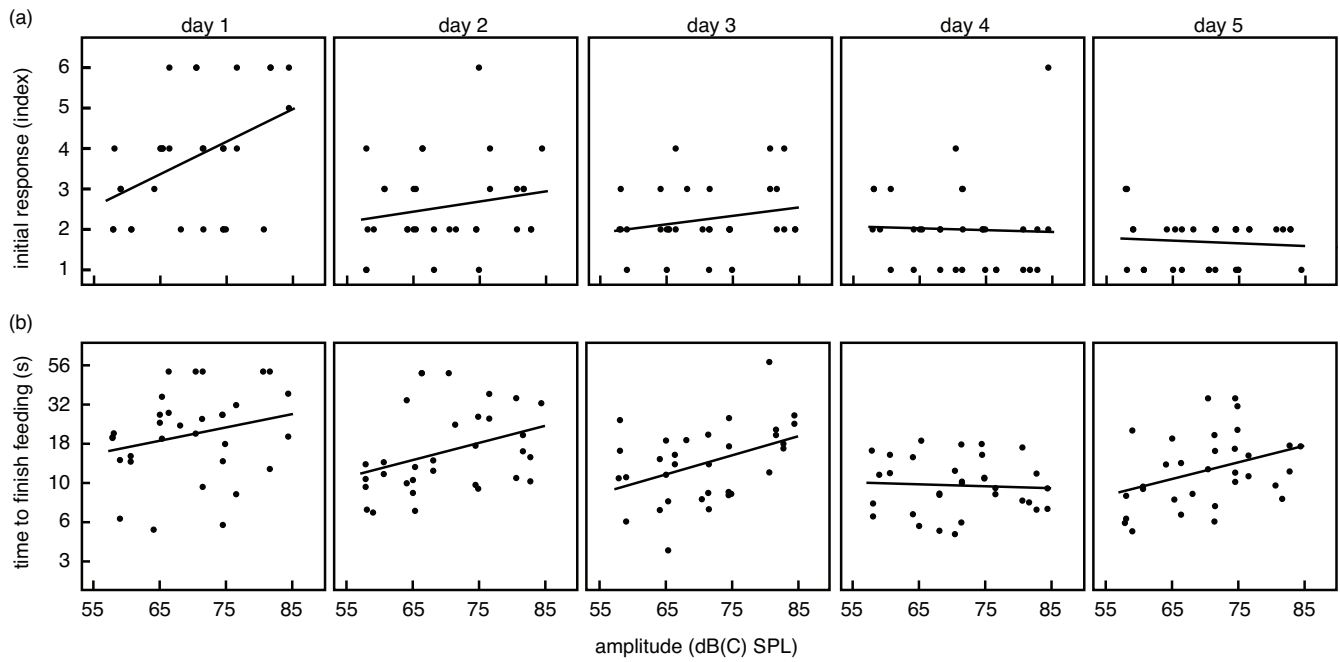
726

Figure 2



728 **Figure 2.** Relationship between avian stimulus attributes and alarm call structure. Shown on the abscissa is a
729 principal component that incorporates the size, speed and proximity of 334 avian stimuli. Shown on the ordinates
730 are the (a) call length, (b) amplitude, (c) dominant frequency and (d) entropy of the 334 corresponding alarm
731 calls ($N = 24$ males). Note the nonlinear ordinate scales for call length, dominant frequency and entropy (see text
732 for details of associated transformations). To elucidate the relationship between avian stimulus attributes and
733 within-male gradation in alarm call structure, we removed between-male differences in alarm call structure by
734 centering each male's measurements for a given variable on that variable's overall sample mean. Regression
735 lines were calculated from the slope and intercept coefficients generated by the linear mixed model analyses.
736

Figure 3



737

738

739 **Figure 3.** Relationship between alarm call structure and female response over a 5-day test period ($N = 32$).

740 Shown on the abscissa is the amplitude of the eliciting alarm call stimulus. Shown on the ordinates are (a) initial

741 response and (b) time to finish feeding. Note the nonlinear ordinate scale for time to finish feeding.