

Nestling use of alternative acoustic antipredator responses is related to immune condition and social context



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Birds present a variety of antipredator responses, including the use of acoustic signals that may be directed to other individuals or to the predator. We tested competing hypotheses regarding antipredator responses by analysing patterns of variation in the use of distress calls in campo flicker, *Colaptes campestris campestris*, nestlings. More specifically, we tested whether immune response and social context (presence or absence of other individuals of the social group) were associated with variation in use of acoustic signals in response to human handling. Individuals with higher immunocompetence used proportionally more harsh, low-pitched calls ('scream' notes) than individuals with lower immunocompetence, while the latter used mostly tonal, high-pitched calls ('week' notes); in both cases there was no influence of the social context on call type. Individuals responded to the social context by giving fewer scream notes and week notes in the presence of adults of the social group than when the adults were absent. Although playbacks are necessary to determine the function of the calls, our results indicate that campo flicker nestling antipredator calls could be used to attract other individuals of the group to help defend the nestling. Also, the use of proportionally more scream notes by healthier individuals suggests that these notes could act as an honest signal of the nestlings' physiological condition.

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The use of acoustic signals is a versatile form of communication, with some species of birds having a diverse range of calls in their repertoire (reviewed in Marler, 2004). This diversity in call types is thought to be shaped by selective pressures acting upon both signallers and receivers, as both parties may benefit from the information exchange when there is consistency between the signal and the context in which it is presented (Bradbury & Vehrencamp, 2011; Font & Carazo, 2010; Maynard Smith & Harper, 1995, 2003). Information encoded in signals may be related to the environmental or social context perceived by an individual, its physiological condition or motivation, or a combination of these (Bradbury & Vehrencamp, 2011; Carazo & Font, 2010; Font & Carazo, 2010; Marler, 2004).

One of the contexts wherein signal emission is commonly observed is in the presence of predators. In birds, acoustic antipredator responses are mainly characterized by alarm and distress calls (or fear screams). Alarm calls are vocalizations given in a

context of danger, such as in the presence of a predator (Brémond & Aubin, 1990; Marler, 2004). Although distress calls can be considered a type of alarm call, they present certain distinctions such as being given when the signaller and the predator are in close proximity, including when the signaller has been captured (Hogstedt, 1983; Inglis, Fletcher, Feare, Greig-Smith, & Land, 1982). Distress calls are also extremely loud and harsh, usually with long and broadband notes, while other alarm calls usually have narrower frequency bands and a higher pitch (Hogstedt, 1983; Inglis et al., 1982; reviewed in Marler, 2004). These characteristics provide obvious means to identify these calls for most species. The information contained in these signals and the selective pressures involved in their evolution, however, are unclear.

One of the many possible functions of antipredator calls is to warn the predator that it has been detected, discouraging it from pursuing the prey (Bergstrom & Lachmann, 2001). Another suggested role is to warn kin of the presence of a predator, so they can seek cover (Sherman, 1977). These calls may function to attract other individuals to the site, including the parents of juveniles (Perrone, 1980), predators other than the one causing distress (Hogstedt, 1983; Koenig, Stanback, Hooge, & Mumme, 1991; Perrone, 1980), and heterospecifics (Aubin, 1991; Chu, 2001; Greig-Smith, 1984), thus generating confusion or distraction and

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allowing the prey to escape its predator. Furthermore, distress calls could function as an honest signal of quality of the potential prey, indicating to the predator its condition and ability to flee (Laiolo, Tella, Carrete, Serrano, & Lopez, 2004; Laiolo et al., 2007). Finally, distress calls could startle the predator, causing it to release the prey (Conover, 1994).

The use of alarm and distress calls is widespread among birds (Hogstedt, 1983; Marler, 2004), although there is considerable variation in the use and acoustic structure of the calls. In some species, alarm and distress calls vary in note composition, pitch or rate of production according to the type or distance of a predator (e.g. Branch & Freeberg, 2012; Ficken, 1990; Leavesley & Magrath, 2005; Stone & Trost, 1991; Suzuki, 2012; Zachau & Freeberg, 2012), consequently carrying additional information to conspecifics about the degree of risk (Ficken, 1990). This flexibility in fine-tuning signals has great potential for adaptive evolution, since inappropriate signalling in this context can be extremely costly for an individual's fitness (Caro, 1995).

Nestlings of many bird species utter distress calls when handled by humans (e.g. Perrone, 1980). In our study species, the campo flicker, *Colaptes campestris campestris*, nestlings being handled by humans use antipredator calls that present notes that typically characterize distress calls (loud and harsh 'screams') and notes that sound very similar to a 'week' call (tonal, harmonically structured, and with slightly modulated fundamental frequency) presented by adults of the species (Goedert 2010) and by other congeneric species (Short, 1972). Such flexibility provides an ideal opportunity to test the adaptive significance of these calls, as the study of plasticity in behaviours is important to understand and predict their evolution (Mazer & Damuth, 2001). Although most of the studies on antipredator strategies are focused on adults, nestlings are particularly vulnerable to predators. In tropical areas, nest predation is the overall main cause of nest failure, whereas adults have high survival rates (reviewed in Stutchbury & Morton, 2001).

In this study, we investigated how acoustic signals are used as antipredator responses by campo flicker nestlings. We focused on two questions. First, is the use of differently structured calls a condition-dependent response? Previous studies have suggested that the harshness of distress calls can be an honest signal of the signaller's condition (e.g. Laiolo et al., 2004; Laiolo et al., 2007). If this is the case for campo flicker nestlings, we expected individuals in better condition to use more scream notes (harsh, broadband notes) relative to week notes (tonal notes) in their calls.

Second, is calling rate dependent on the social context? If calls are directed to conspecifics, we would expect nestlings to adjust the rate of calling in relation to the presence of adults from their social group. If calls are directed to other receivers, possibly the predator or other predators in the vicinity, then we would not expect a difference in calling rates when adults from the social group were nearby and when they were not.

Considering the high degree of sociality of campo flickers (Dias, Webster, Goedert, & Macedo, 2013) and the high degree of relatedness among individuals of a social group (Dias, Macedo, Goedert, & Webster, 2013), there are at least two possible interpretations for the function of the calls if these are directed to other individuals of the social group: (1) to signal the presence of predators so that the genetically related individuals can seek cover (warn kin hypothesis; Inglis et al., 1982; Sherman, 1977); and (2) to attract other individuals that could mob the predator (cry for help hypothesis; Hogstedt, 1983; Perrone, 1980; Rohwer, Fretwell, & Tuckfield, 1976). As these two possible interpretations generate competing predictions for calling rates depending on the presence of conspecifics (as proposed by Branch & Freeberg, 2012), the suggested functions can be tested: if calls function to warn kin of the presence of predators, nestlings should show higher calling rates when adults

Table 1

Characterization of campo flickers in this study, indicating number and sex of nestlings per social group, number of adults in the social groups and the age at which nestlings were recorded

Social group	Number of adults	Number of offspring			Age (days) of nestlings when recorded
		Males	Females	Total	
1	3	2	3	5*,†	23
2	2	1	1	2*	23
3	3	1	1	2	24
4	2	1	2	3‡	24
5	2	1	0	1	24
6	2	3	1	4‡	25
7	2–3	1	3	4	24
8	2	1	2	3	24
9	3	0	1	1	24
10	4	1	1	2	24
11	2	1	2	3	24
	Total	13	17	30	

* Audio recording was missing for one offspring in the group and the individual was excluded from the analyses.

† Phytohaemagglutinin (PHA) data were missing for two offspring and both individuals were excluded from the first analysis.

‡ PHA data were missing for one offspring and the individual was excluded from the first analysis.

of the social group are nearby; however, if calls function to attract other individuals to the nest, we would expect nestlings to present lower calling rates when adults of the social group are nearby.

METHODS

Study Species and Field Site

The campo flicker is a terrestrial Neotropical woodpecker endemic to South America (Short, 1975, 1982; Sick, 1997), recognized as the subspecies *campestris* at the northeastern portion of its distribution (northeast Brazil to central Paraguay; Short, 1972). The campo flicker is a facultative cooperative breeder, with a highly complex social structure (Dias, Macedo, et al., 2013). Social groups range from two to five individuals during the breeding season, with all individuals engaging in reproductive activities and defence of group territories, which range in size from approximately 20 to 80 ha (Dias, Webster, et al., 2013).

We conducted this study during the breeding season (June to December) of 2009 at Fazenda Água Limpa (15°57'S, 47°56'W) in central Brazil, a site composed of a variety of typical cerrado (Brazilian savannah) vegetation types. The study population had been monitored for 2 years prior to this study, during which time we mist-netted and marked individuals with unique combinations of colour bands and identified social groups and territories (see Dias, Webster, et al., 2013 for details). A social group was considered as two or more individuals inhabiting a territory and showing territorial defence behaviours such as group displays.

Fieldwork

During the breeding season, we searched territories for active nests. Campo flickers are cavity nesters, nesting preferentially in termite mounds, but using tree cavities in territories lacking these structures (Dias, Webster, et al., 2013). We monitored nests every 2–3 days to check for the presence of eggs, and daily when eggs were close to hatching. Nestlings were captured and handled 23–25 days after the first chick hatched (nestling period lasts 29 days on average; Dias, Webster, et al., 2013). We measured the cell-mediated immunocompetence response of each nestling as an estimate of individual condition, evaluated based on skin swelling in

response to an injection of phytohaemagglutinin (PHA) solution (Smits, Bortolotti, & Tella, 1999). The PHA-test has been shown to correlate not only with individual health and condition (e.g. Alonso-Alvarez & Tella, 2001; Gleeson, Blows, & Owens, 2005; Tella, Bortolotti, Forero, & Dawson, 2000), but also with survival (Tella, Bortolotti, Dawson, & Forero, 2000) and life-history traits (Tella, Scheuerlein, & Ricklefs, 2002). Birds were injected in the patagium of the left wing with a solution of 0.2 mg of PHA (Sigma-Aldrich) dissolved in 0.04 ml of sterile phosphate-buffered saline and marked with one coloured band for identification. We measured patagium thickness to the nearest 0.01 mm with a pressure-sensitive micrometre (Vonder[®]) at the point of injection immediately before and 24 h after application, following Smits et al. (1999). On both the day of PHA application and the day after, we took four consecutive measures of patagium thickness to calculate the repeatability of these measurements.

Acoustic recording of nestlings took place on the second day of manipulation (24 h after PHA injection) and before processing, with the exception of nestlings of group 1 (Table 1), which were recorded during banding on the first day. We removed all nestlings from the nest at the same time and kept them in cloth bags, because they usually attempted to flee when their nestmates were taken from the cavity. One person (R.I.D.) then removed a single nestling from the bag and held it with a 'bander's grip' (The North American Banding Council, 2001, p. 9), while a second person (D.G. or a field assistant) recorded calls uttered by the nestlings for a total of 70 s (10 s for adjustments of the microphone and 60 s for data collection), at a distance of approximately 1 m. We recorded the calls using a digital recorder (Marantz PMD660) equipped with a unidirectional microphone (Sennheiser ME66/K6), at 16 bits and 44.1 kHz, and saved them in wav format. Immediately after the recording, we marked nestlings with two additional colour bands and one metallic band, providing a unique colour combination, and returned them to the nest.

We recorded presence or absence of members of the social group during the recording of vocal calls of each nestling. We considered adults to be present if there were visual and/or auditory cues, including mobbing behaviour, alarm and mobbing calls, or territorial calls within 20 m of the nest during the recording. Nestlings had no obvious cue relative to the presence of members of the social group before being removed from the nest, since adults were not calling or mobbing upon our arrival at the nest site, and only older nestlings (28 days) were ever observed looking out of the cavity (D. Goedert, personal observation). We did not quantify variation in behaviour of adults of the social group, but they generally mobbed and called intensively almost immediately upon the start of our recordings (whether they were present before or flew in as a response to the nestlings' calls). On only one occasion did adults leave the vicinity of the nest while we were still handling the nestlings.

Thirty nestlings from 11 different groups were included in the analyses (Table 1). We repeated the analyses excluding group 1, since these nestlings were recorded differently, but there were no changes in results (results not shown).

Sound Analyses

We measured acoustic properties of calls using Raven Pro 1.4 (Cornell Lab of Ornithology, Ithaca, NY, U.S.A.). Spectrograms were visualized in Hann window (DFT: 1024 Hz, 512 window size, with 80.1% overlap for the time axis of the matrix and a hop size of 102). We eliminated the first 10 s of the recording to account for possible variation in calling rate and call type while nestlings were being released from the bag and while they were being held by the researcher, and also allowing for adjustments in the gain of the

microphone for the recordings. We considered notes as individual elements isolated by at least 60 ms (Brown, Farabaugh, & Veltman, 1988). In a preliminary inspection, we selected every visual element in the spectrogram using the 'selection' tool in Raven Pro as precisely as possible. We calculated the time between notes as the difference between the end time of one element and the beginning time of the next element. For any two elements separated by less than 60 ms, we adjusted the selection boundaries in a waveform. We then recalculated the time between elements and considered as single notes any apparent individual elements that did not extrapolate the 60 ms threshold. Notes that started during the first 10 s of the recording were not measured, even when the note exceeded this time limit. Notes that started during the 70 s of recording were counted even if they exceeded the 70 s limit.

All recordings were visually and acoustically inspected and each note was classified as a 'scream' or 'week' note based on its acoustic structure. We considered a note to be a week if it was narrow-banded and harmonically structured, with a slightly modulated fundamental frequency, and had a central frequency that ranged from approximately 1.5 to 3 kHz, as described for week calls of adults (Goedert, 2010; Supplementary Audio S1). Adults have been observed to utter week calls when they are approached by an observer or a potential predator, as well as when they are caught in a mist net (Goedert, 2010). Alternatively, we considered a note to be a scream if it had characteristics commonly described for distress calls of several species of birds, such as those having a broad frequency band and a harsh component (frequency modulation; e.g. Marler, 2004; Mathevon, Aubin, & Brémond, 1997; Supplementary Audio S2). In contrast, adults rarely produce scream notes, and on the few occasions in which it has been observed, they produced scream notes only when captured in a mist nest (D. Goedert, personal observation). Only notes that presented acoustic structures that could be assigned to one of these two descriptions were counted.

Finally, we inspected calls for distortions and clipping, selecting the ones without these issues for structural characterization of the notes ($N = 512$ notes, from 15 individuals). For each note, we measured the peak (or dominant) frequency, the average entropy (distribution of energy between the upper and lower frequency bounds, averaged over time), the total energy and the duration of the note considering boundaries containing 90% of the total energy of the call (duration 90%). The latter was chosen because the usual method of measuring duration relies on visual determination of the boundaries and is therefore bound to reflect subjective variation among researchers. Duration 90% is a more robust measure, as small alterations in the delimitations of a selection will not affect its value (Charif, Waack, & Strickman, 2010). Average entropy indicates the pattern of distribution of energy along the frequency domain within a note, with high values indicating a greater distribution of energy along the frequency domain, while a value of zero indicates a sound where all energy is concentrated in a single frequency bin (Charif et al., 2010). For statistical comparison of acoustic characteristics of the notes, we included in analyses only calls of individuals that contained both alarm and distress notes ($N = 199$ notes from 6 individuals).

Statistical Analyses

We used the statistical software R (R Development Core Team, 2013) for all statistical analyses. We calculated the mean and standard deviation for each acoustic variable of the notes, and used mixed effects models with each of the acoustic variables as the response variable and the type of note as the fixed effects, including individual identity as a random effect. Duration 90%, peak frequency and total energy were log transformed to meet assumptions

of the models. We used a Bonferroni correction to account for multiple comparisons between the two types of call, considering $P = 0.0125$ as the significance level (Cabin & Mitchell, 2000). In addition, we calculated effect sizes (d) for each of the variables, following Nakagawa and Cuthill (2007). We obtained confidence intervals (CI) for each of the effect sizes through a bootstrapping procedure modified from R codes provided at www.bio.bris.ac.uk/research/behavior/efficientsize.htm (see Nakagawa & Cuthill, 2007). In summary, for each acoustic variable, we added the fitted values to resampled residuals of the mixed model, obtaining new response variables. We then used each new response variable in a model with the same structure as the original models. We repeated this process 10 000 times for each acoustic variable, obtaining a distribution of 10 000 values of effect size, from which the region within the 2.5% quantile to the 97.5% quantile represented the 95% CI.

In addition, we performed a discriminant function analysis (DFA) to assess whether scream and week call notes could be discriminated based on their acoustic structure. Since DFA requires data to be balanced, we removed one individual that had a single scream note in its call, and used three randomly selected notes of each type per individual. Because the number of parameters in the analysis should not exceed one less than the sample size in the smallest unit, we only included two of the acoustic variables measured in the analyses. We chose to include peak frequency and average entropy as our variables of interest, as pitch and harshness of calls are known to affect propagation and ease of source location (reviewed in Marler, 2004). Moreover, peak frequency signals predation risk in Mexican chickadees, *Parus sclateri* (Ficken, 1990) and white-browed scrubwrens, *Sericornis frontalis* (Leavesley & Magrath, 2005), for example, as well as influences the identification of heterospecific alarm calls in the superb fairy-wren, *Malurus cyaneus* (Fallow, Pitcher, & Magrath, 2013). Therefore, peak frequency seems to be an important acoustic property in the discrimination of calls. Because the selected notes could have a significant impact on the results, we repeated the random selection of notes and the DFA 1000 times (Mundry & Sommer, 2007). Peak frequency was log transformed to meet the assumptions of normality, and prior probabilities were set as equal for each of the note types. For each of the DFA, then, we used the leftover notes ($N = 150$) to cross-validate our discriminant functions (hold-out-sample cross-validation), obtaining a distribution of proportion of correctly classified notes from the original data.

Finally, because of the nonindependence of multiple notes belonging to the same individual, we also performed a permuted DFA (pDFA), following Mundry and Sommer (2007). The pDFA differs from the regular DFA because notes of each individual are randomized between the two types of notes before the DFA is run. We also repeated this 1000 times, and used the left-over notes for cross-validation, obtaining a distribution of the proportion of correctly classified notes from the permuted data. We then used this distribution of permuted values to calculate the 95% upper quantile, which corresponds to the proportion of correctly classified notes expected with a probability of 5% or lower.

We estimated the repeatability of the patagium thickness measurements from the intraclass correlation coefficient (ICC) of a one-way ANOVA, using the package *irr* (Gamer, Lemon, & Singh, 2012). Because of the high repeatability of the measures (pre-exposure: $ICC = 0.807$, $F_{28,87} = 17.7$, $P < 0.001$; postexposure: $ICC = 0.747$, $F_{28,87} = 12.8$, $P < 0.001$), we considered the difference between pre- and postexposure mean thickness as a measure of the cell-mediated immunocompetence response of each individual (Smits et al., 1999).

The use of a week note or a scream note are mutually exclusive for any given note, resulting in a negative correlation between the relative use of different note types since the recording time was

kept constant. Thus, we performed a principal component analysis on the correlation matrix for the number of notes given for each of the calls, after log transforming the number of week call notes. This resulted in a first principal component (PC1) containing 77.86% of the variance in the data that was positively correlated with the number of week call notes (eigenvalue = 0.71). Therefore, we considered this PC1 to be a measure of the vocal call used, with positive loadings denoting individuals that presented relatively more week call notes and negative loadings denoting individuals that used relatively more scream call notes. We avoided using the proportion to represent call type use because it generated a truncated distribution of residuals.

To investigate whether the behavioural response of campo flicker nestlings was condition dependent, we conducted a mixed effects model analysis with call type use (PC1) as the response variable. As fixed predictive effects, we included response to PHA (log transformed to meet model assumptions of normality) and social context (presence/absence of group members, as a binary variable). Social context was included in this model since, if the use of scream call notes over week call notes is a condition-dependent strategy, we expected call type use to be independent of the social context. As siblings could hear each other calling, and the presence of parents during the handling of each sibling was nonindependent, we included social group and the order in which nestlings were recorded as random effects. We then constructed a second model to investigate whether individuals' call rates differed based on social context. Since total duration of the recording was the same for all nestlings (i.e. 60 s), the total number of notes produced represents the calling rate of notes per minute. Social context and PC1, as well as their interaction, were included as fixed effects.

For both models, sex was initially included as a covariate, but was not significant. Therefore, it was removed from subsequent analyses to avoid overparameterization of analyses. Similarly, we compared the complete models with those that did not include the order of recording as a random factor, all fitted with restricted maximum likelihood (REML), using likelihood ratio tests (LRTs), to select the best structure of random factors for the data (Bates, 2010; Pinheiro & Bates, 2000; Zuur, Ieno, Walker, Saveliev, & Smith, 2009). Significance level when testing for random effects was considered at 0.10, as this comparison using LRT is known to be conservative (Bates, 2010; Pinheiro & Bates, 2000). We removed the effect due to order in which individuals were recorded if it did not significantly improve model fit. Social group was always maintained in the model to account for nonindependence.

For each model, we visually inspected the normal probability plots of the residuals, the plots of the standardized within-group residuals by the within-group fitted values, and the normal probability plots for each of the levels of the random effects to verify whether the assumptions of normality of the within-group errors, homogeneity of variance and normality of random effects, respectively, were met, and for the presence of outliers (Pinheiro & Bates, 2000). We also checked for patterns of nonlinearity by plotting residuals against the explanatory variables (Pinheiro & Bates, 2000). Because of the unbalanced design, we obtained confidence intervals and P values for the estimates of fixed effects with Markov-chain Monte Carlo (MCMC) simulations ($N = 1000\ 000$) using the package *languageR* (Baayen, 2011). Finally, we calculated Cook's distance to verify whether extreme observations represented influential points using the package *influence.ME* (Nieuwenhuis, te Grotenhuis, & Pelzer, 2012).

Ethical Note

This study was conducted in accordance with the current laws in Brazil (permits 14368 and 2056, from Instituto Brasileiro de

Recursos Renováveis, IBAMA). We attempted to minimize disturbance by keeping handling of the nestlings to a minimum, and leaving the nearby area immediately once procedures were finished. Nestlings were removed from and returned to the nest all together, and kept in cloth bags until they were processed. Keeping nestlings in bags during their siblings' handling seemed to result in less adverse impacts than removing and returning nestlings one at a time, as previous experience with returning one nestling while still handling its siblings resulted in one case of premature fledging (D. Goedert, personal observation). At age 28 days, we checked nests for the presence of nestlings and conducted observations on parental activity as part of a different study (Dias, Webster, et al., 2013). All nestlings used in this study survived until fledging, and none of the nests were abandoned by the adults.

RESULTS

Differences Between Call Types

Campo flicker nestlings gave week notes that were narrow-banded compared to scream notes and that showed frequency modulation, with the frequency decreasing from the beginning to the end of the note, forming a ladder shape (Fig. 1a). The notes ($N = 202$ notes, 11 individuals) were harmonically structured with a mean \pm SD peak frequency of 3.48 ± 0.43 kHz (range 2.41–5.86 kHz) and an average entropy of 4.89 ± 0.33 . Scream notes

($N = 309$ notes, 10 individuals), in turn, were broadband, with peak frequency ranging from 1.16 to 6.76 kHz (mean \pm SD: 3.01 ± 0.69 kHz; Fig. 1b) and an average entropy of 5.67 ± 0.49 . Week notes had a higher peak frequency ($P_{\text{MCMC}} = 0.001$; $d = 0.477$; 95% CI: 0.234, 0.894; $N = 198$ notes, 6 individuals) and a lower average entropy ($P_{\text{MCMC}} < 0.001$; $d = -1.562$; 95% CI: -1.759, -1.379; $N = 199$ notes, 6 individuals) than scream notes.

Moreover, week notes ranged in duration 90% from 0.01 to 0.10 ms (mean \pm SD: 0.07 ± 0.01 ms) and were shorter than scream notes ($P_{\text{MCMC}} < 0.001$; $d = -3.387$; 95% CI: -3.968, -3.020; $N = 198$ notes, 6 individuals), which ranged from 0.05 to 1.28 ms (0.33 ± 0.15 ms). Total energy of week and scream notes were 126.40 ± 3.34 and 126.79 ± 5.13 , respectively, and did not differ ($P_{\text{MCMC}} = 0.34$; $d = 0.142$; 95% CI: -0.122, 0.344; $N = 198$ notes, 6 individuals).

DFA indicated that scream and week notes could be distinguished based on their peak frequency and average entropy, with a cross-classification indicating an average of 88.96% calls correctly classified (range 84.11–93.38%; Fig. 1c). This classification average was significantly greater than that expected by chance (pDFA, 95% quantile: 69.53%, range 23.18–75.50%; Fig. 1c).

Variation in Use of Call Types

Vocal call type used by nestlings was significantly associated with cell-mediated immunocompetence response (estimate \pm SE:

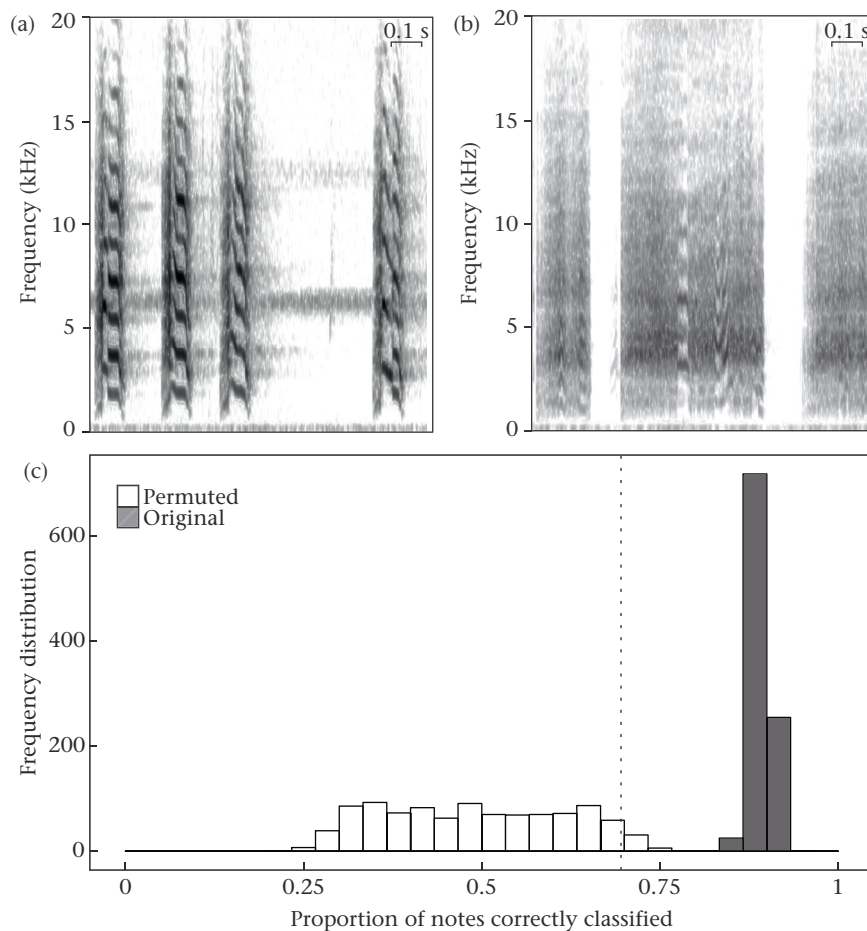


Figure 1. Examples of (a) a week note and (b) a scream note of a campo flicker nestling (also see [Supplementary Audio S1 and S2](#), respectively). (c) Notes can be distinguished based on structural characteristics, as shown by the distributions of the proportion of correctly classified notes for the original and permuted data obtained from hold-out-sample cross-classification from a DFA and a pDFA, respectively. The dashed line in (c) indicates the limit of the 95% quantile for the distribution of the permuted data.

-1.2908 ± 0.3158 ; 95% CI: $-1.992, -0.3231$; $P_{\text{MCMC}} = 0.0084$) and unrelated to the social context (estimate \pm SE: -1.2334 ± 0.6290 ; 95% CI: $-1.145, 1.0647$; $P_{\text{MCMC}} = 0.9764$). Individuals in better condition used relatively more scream notes (Fig. 2). Removing social context from the model did not affect the relationship between call usage and cell-mediated immunocompetence response (estimate \pm SE: -1.1745 ± 0.3521 ; 95% CI: $-1.978, -0.3504$; $P_{\text{MCMC}} = 0.0072$).

We found significant effects of call type used (estimate \pm SE: -11.264 ± 3.339 ; 95% CI: $-18.04, -4.111$; $P_{\text{MCMC}} = 0.0031$) and social context (estimate \pm SE: -24.628 ± 7.790 ; 95% CI: $-41.47, -7.302$; $P_{\text{MCMC}} = 0.0084$) on total number of calls uttered per minute. Individuals that predominantly used scream call notes had higher calling rates than individuals that predominantly used week call notes, and all individuals called at a higher rate when adults were not present (Fig. 3). Total number of calls uttered by nestlings was not affected by the interaction between call type used and social context ($P_{\text{MCMC}} > 0.05$), so the interaction was removed from the final model.

One observation presented a distinctly low value for the immunocompetence response variable (0.04 mm), and this same observation had a large Cook's distance (1.16), indicating it had a large effect on the slope of the regression. Also, including this observation resulted in residuals that suggested a pattern of nonlinearity when plotted against the fitted values, a pattern not seen when the observation was removed. Therefore, we considered it an outlier and removed it from the first analysis (Fig. 2). The results were qualitatively identical for the models with and without this observation. In the second analysis, one observation presented a large Cook's distance value (0.94, more than twice that of the next most influential observation), and was removed from the analysis (Fig. 3). Including this influential point in the analysis did not qualitatively change the effect of social context, but the effect of PC1 became nonsignificant ($P_{\text{MCMC}} = 0.10$). Recording order was removed from both models, as it did not significantly improve the fit (LRT, $P > 0.1$). Variance for social group identity was estimated as 1.496 in the first model, which represented 50.7% of the total variance. The final model for calling rates, on the other hand, accounted for less than 1% of the total variance associated with social group identity.

DISCUSSION

Our findings support the hypothesis that the use of scream notes over week notes is a condition-dependent response. Individuals

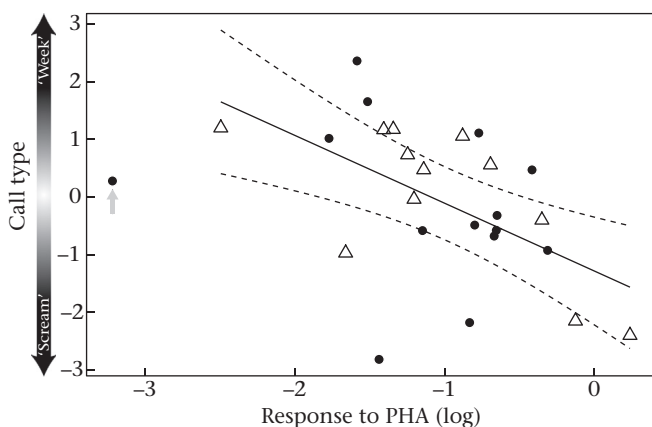


Figure 2. Type of call produced by nestlings in relation to cell-mediated immunocompetence response (response to phytohaemagglutinin, PHA, injection) in the presence (triangles) or absence (circles) of adults, with the estimate represented by a solid line and the 95% confidence interval indicated by the dotted lines. The arrow indicates an influential outlier that was removed from the analyses.

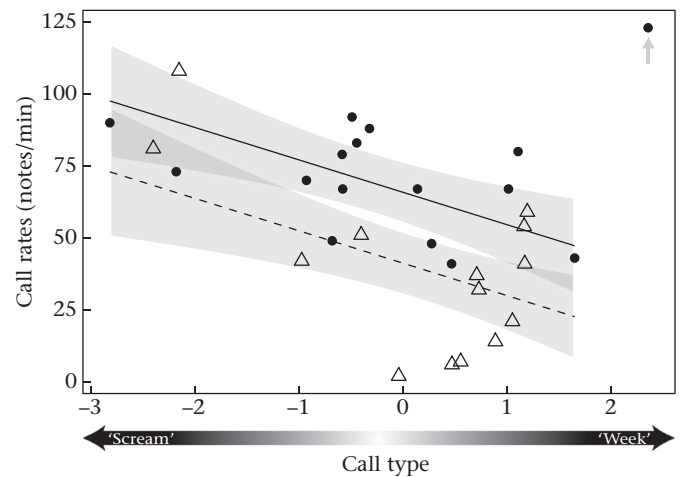


Figure 3. Call rates (notes per min) by type of call produced under two social contexts: when adults of the social group were present (triangle; dotted line) or absent (circle; solid line); with 95% confidence intervals indicated by the shaded areas. The arrow indicates an influential outlier that was removed from the analyses.

with stronger cell-mediated immune responses gave more scream notes than individuals with lower immune responses, and the latter were more likely to use week notes. Individuals that used more scream notes, in addition, had higher calling rates than individuals that gave more week notes, with calling rates of both scream and week calls being dependent upon the external context faced by the caller: individuals had lower calling rates when adults were present. Both calls, therefore, seem to be directed to the adults of the social group, possibly acting as a 'call-for-help' signal. Additionally, the use of more scream notes than week notes possibly acts as an honest signal of the nestlings' health status to the social group and/or to the predator.

Honesty of distress calls could be evolutionarily maintained through its association with the individual's quality, as a 'performance' signal (Bradbury & Vehrencamp, 2011; Maynard Smith & Harper, 1995, 2003) if predators respond to distress calls by moving on to more vulnerable prey that may yield a higher capture success (Bradbury & Vehrencamp, 2011; Cresswell & Quinn, 2004; Pole, Gordon, & Gorman, 2003). Although this hypothesis may explain the use of distress calls in other species of birds or in situations where the predator has not yet captured the bird, we argue that it is unlikely that campo flicker nestlings, once captured, could elicit a release response from the predator. Snakes, which are a common nest predator, have sound vibration sensitivity at very low frequencies (e.g. 80–160 Hz; Christensen, Christensen-Dalsgaard, Brandt, & Madsen, 2012). Based on reports of Mustelidae (e.g. Fargallo, Blanco, Potti, & Viñuela, 2001) and Felidae (e.g. Altamirano, Hernández, de la Maza, & Bonacic, 2013) preying on cavity nests, other possible predators of campo flicker nestlings found in the Cerrado are medium-sized mammals, such as the tayra, *Eira barbara*, and the jaguarundi, *Puma yagouaroundi* (Marinho-Filho, Rodrigues, & Juarez, 2002). Therefore, captured nestlings are most likely always vulnerable to such predators, which should not be selected to choose among the nestlings on the basis of performance signals.

Honesty could also be maintained by a high cost in the production of this signal, such that individuals in poorer condition are unable to meet this cost (handicap signals; Bradbury & Vehrencamp, 2011; Maynard Smith & Harper, 1995, 2003; Zahavi, 1975). One possibility is that vocal activities, including singing, are activities with high energetic demands. Evidence of this, however, is still equivocal (Chappell, Zuk, Kwan, & Johnsen, 1995; Eberhardt, 1994;

Thomas, 2002; Ward, 2004; Ward, Speakman, & Slater, 2003; see also Gaunt, Bucher, Gaunt, & Baptista, 1996). In starlings (*Sturnus vulgaris*), an increase in 16 dB in song intensity results in a 16% increase in oxygen consumption (Oberweger & Goller, 2001), indicating that sounds containing more energy could be more energetically costly. In campo flickers, we found no difference in total energy between scream notes and week notes. Although total energy may not be the only important variable representing costs (e.g. see Gil & Gahr, 2002), we have no evidence that scream notes are more energetically costly to produce than week notes.

Alternatively, physiological constraints could be acting to impair an individual's ability to produce scream calls. In mammals, individuals with higher glucocorticoid levels have been shown to produce more tonal (i.e. less harsh) alarm calls in the context of fear reactions (Blumstein & Chi, 2012). In addition to tonality, the pitch of the calls seems to be under physiological constraint. Higher-pitched begging calls have been associated with body condition in nestlings of Wilson's storm-petrels, *Oceanites oceanicus* (Gladbach, Buesser, Mundry, & Quillfeldt, 2009) and barn swallows, *Hirundo rustica* (Sacchi, Saino, & Galeotti, 2002), for example. Also, yellow-legged gull, *Larus michahellis*, embryos developing in eggs that had been injected with a glucocorticoid produced higher-pitched embryonic vocalizations compared with those in the control treatment (Rubolini et al., 2005). Glucocorticoid levels have been found to increase in birds subjected to poor environmental conditions such as food deprivation (Breuner, Patterson, & Hahn, 2008; Kitaysky, Kitaiskaia, Wingfield, & Piatt, 2001; Saino, Suffritti, Martinelli, Rubolini, & Møller, 2003; Wingfield & Kitaysky, 2002), and also to suppress immune function (e.g. reviewed in Apanius, 1998; Martin, Gilliam, Han, Lee, & Wikelski, 2005; Rubolini et al., 2005; Saino et al., 2003). Therefore, stress levels may mediate the relationship between individual condition and the use of distress calls, such that individuals in worse immune condition cannot produce harsh, low-pitched calls. Our results are consistent with this proximate mechanism, and further studies could test the hypothesis that stress constrains production of harsh vocal calls by experimentally manipulating the physiological condition of nestlings.

The association between calling rate and social context indicates that these calls are motivational signals directed to other individuals of the social group. More specifically, the decrease in signalling rates in the presence of adults supports the hypothesis that both call types function to attract the group to the nest and, consequently, could stimulate adults to mob the predator. In this species, we observed both parents as well as other individuals from the social group actively mobbing opportunistic predators (e.g. toco toucan, *Ramphastos toco*, southern crested caracara, *Caracara plancus*) and other cavity-nesting birds near the nest. Considering the high degree of relatedness among group individuals (Dias, Macedo, et al., 2013), the benefit to receivers of responding to these signals and rescuing nestlings would include gains through indirect fitness (Hamilton, 1964a, 1964b). Moreover, evolution should favour parents that adjust investment according to nestling quality (reviewed in Montgomerie & Weatherhead, 1988), and future studies should focus on the response of adults to variation in nestlings' use of calls to test this hypothesis.

Finally, we cannot reject the secondary predator attraction hypothesis (Hogstedt, 1983; Koenig et al., 1991; Perrone, 1980). Distress calls of other woodpeckers have been shown to attract predators (Koenig et al., 1991), and we observed birds of prey flying over the area when nestlings gave scream calls on two occasions, providing anecdotal evidence that predator attraction is one possible function of campo flickers' distress calls. Playback experiments are necessary to test whether secondary predation attraction is a function of these calls.

We speculate, however, that a predation attraction function would most likely be associated with scream calls and not week calls, based on differences in the acoustic structural properties of these two call types. Structural characteristics of the campo flicker scream notes include a broad frequency spectrum, sudden breaks and repetitiveness, which possibly result in long-distance signal propagation, and a relatively easy identification of the source location (Hogstedt, 1983; reviewed in Marler, 2004). In contrast, week notes have a narrower frequency band and a higher peak frequency, which possibly results in decreased propagation distance and increased difficulty in source location (reviewed in Marler, 2004). Considering the stable social structure presented by the campo flicker, it is reasonable to expect that parents and other individuals of the social group will be closer to the nest more often than potential predators. Given that group members know the nest location, they can rely on cues derived from the acoustic properties of calls when deciding whether to attend the nest, even when the acoustic structure does not allow the source to be easily located. For example, a call structure that results in fast attenuation may provide individuals with a cue that the source is nearby, indicating that the signal is coming from the group's territory. Therefore, both scream and week calls could function to attract the social group, while only scream calls would possibly attract secondary predators, which do not have previous knowledge of the nest location. The use of scream calls, then, could be advantageous over the use of week calls by increasing the number of receivers that can respond. This suggests that week calls may represent 'the best-of-a-bad-job' response, and that the flexibility in antipredator responses may be adaptive (see Conover, 1994; Pigliucci, 2005) if individuals that use week calls have higher survival rates than individuals that show no antipredator response.

In conclusion, our results demonstrate that the use of different acoustic antipredator responses in campo flicker nestlings is related to both the internal and external condition of the signaller. Therefore, although the receiver's behaviour in response to these calls still needs to be evaluated, our results shed light on possible functions of the calls from the sender's perspective (see Vehrencamp, Hall, Bohman, Depeine, & Dalziell, 2007). Moreover, we show that communication between individuals of a social group in an antipredatory context can be complex, in agreement with the idea that sociality and communication complexity coevolve (reviewed in Freeberg, Dunbar, & Ord, 2012). Finally, our results suggest that the flexibility in use of calls could be adaptive, providing a starting point for future studies on the function of these calls and the evolutionary causes of variation in antipredator behaviour in campo flickers and other species with alternative antipredator strategies.

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Supplementary Material

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References

- Aapanius, V. (1998). Stress and immune defense. *Advances in the Study of Behavior*, 27, 133–153.
- Alonso-Alvarez, C., & Tella, J. L. (2001). Effects of experimental food restriction and body-mass changes on the avian T-cell-mediated immune response. *Canadian Journal of Zoology*, 79, 101–105.
- Altamirano, T. A., Hernández, F., de la Maza, M., & Bonacic, C. (2013). Güiña (*Leopardus guigna*) preys on cavity-nesting nestlings. *Revista Chilena de Historia Natural*, 86, 501–504.
- Aubin, T. (1991). Why do distress calls evoke interspecific responses? An experimental study applied to some species of birds. *Behavioural Processes*, 23, 103–111.
- Baayen, R. H. (2011). *languageR: Data sets and functions with analyzing linguistic data: A practical introduction to statistics*. Retrieved from <http://CRAN.R-project.org/package=languageR>.
- Bates, D. (2010). *lme4: Mixed-effects modelling with R*. Retrieved from <http://lme4.r-forge.r-project.org/book/>.
- Bergstrom, C. T., & Lachmann, M. (2001). Alarm calls as costly signals of antipredator vigilance: the watchful babbler game. *Animal Behaviour*, 61, 535–543.
- Blumstein, D. T., & Chi, Y. Y. (2012). Scared and less noisy: glucocorticoids are associated with alarm call entropy. *Biology Letters*, 8, 189–192.
- Bradbury, J. W., & Vehrencamp, S. L. (2011). *Principles of animal communication*. Sunderland, MA: Sinauer.
- Branch, C. L., & Freeberg, T. M. (2012). Distress calls in tufted titmice (*Baeolophus bicolor*): are conspecifics or predators the target? *Behavioral Ecology*, 23, 854–862.
- Brémond, J. C., & Aubin, T. (1990). Responses to distress calls by black-headed gulls, *Larus ridibundus*: the role of non-degraded features. *Animal Behaviour*, 39, 503–511.
- Breuner, C. W., Patterson, S. H., & Hahn, T. P. (2008). In search of relationships between the acute adrenocortical response and fitness. *General and Comparative Endocrinology*, 157, 288–295.
- Brown, E., Farabaugh, S., & Veltman, C. (1988). Song sharing in a group-living songbird, the Australian magpie, *Gymnorhina tibicen*. Part I. Vocal sharing within and among social groups. *Behaviour*, 104, 1–28.
- Cabin, R. J., & Mitchell, R. J. (2000). To Bonferroni or not to Bonferroni: when and how are the questions. *ESA Bulletin*, 81, 246–248.
- Carazo, P., & Font, E. (2010). Putting information back into biological communication. *Journal of Evolutionary Biology*, 23, 661–669.
- Caro, T. M. (1995). Pursuit-deterrence revisited. *Trends in Ecology & Evolution*, 10, 500–503.
- Chappell, M. A., Zuk, M., Kwan, T. H., & Johnsen, T. S. (1995). Energy cost of an avian vocal display: crowing in red junglefowl. *Animal Behaviour*, 49, 255–257.
- Charif, R. A., Waack, A. M., & Strickman, L. M. (2010). *Raven Pro 1.4 user's manual*. Ithaca, NY: Cornell Lab of Ornithology. Retrieved from <http://www.birds.cornell.edu/brp/raven/Raven14UsersManual.pdf>.
- Christensen, C. B., Christensen-Dalsgaard, J., Brandt, C., & Madsen, P. T. (2012). Hearing with an atympanic ear: good vibration and poor sound-pressure detection in the royal python, *Python regius*. *Journal of Experimental Biology*, 215, 331–342.
- Chu, M. (2001). Heterospecific responses to scream calls and vocal mimicry by phainopepla (*Phainopepla nitens*) in distress. *Behaviour*, 138, 775–787.
- Conover, M. R. (1994). Stimuli eliciting distress calls in adult passerines and response of predators and birds to their broadcast. *Behaviour*, 131, 19–37.
- Cresswell, W., & Quinn, J. L. (2004). Faced with a choice, sparrowhawks more often attack the more vulnerable prey group. *Oikos*, 104, 71–76.
- Dias, R. L., Macedo, R. H., Goedert, D., & Webster, M. S. (2013). Cooperative breeding in campo flickers. II: Patterns of reproduction and kinship. *Condor*, 115, 855–862.
- Dias, R. L., Webster, M. S., Goedert, D., & Macedo, R. H. (2013). Cooperative breeding in campo flickers. I: Breeding ecology and social behavior. *Condor*, 115, 847–854.
- Eberhardt, L. S. (1994). Oxygen consumption during singing by male Carolina wrens (*Thryothorus ludovicianus*). *Auk*, 111, 124–130.
- Fallow, P. M., Pitcher, B. J., & Magrath, R. D. (2013). Alarming features: birds use specific acoustic properties to identify heterospecific alarm calls. *Proceedings of the Royal Society B: Biological Sciences*, 280, 20122539.
- Fargallo, J. A., Blanco, G., Potti, J., & Viñuela, J. (2001). Nestbox provisioning in a rural population of Eurasian kestrels: breeding performance, nest predation and parasitism. *Bird Study*, 48, 236–244.
- Ficken, M. S. (1990). Acoustic characteristics of alarm calls associated with predation risk in chickadees. *Animal Behaviour*, 39, 400–401.
- Font, E., & Carazo, P. (2010). Animals in translation: why there is meaning (but probably no message) in animal communication. *Animal Behaviour*, 80(4), e1–e6. <http://dx.doi.org/10.1016/j.anbehav.2010.05.015>.
- Freeberg, T. M., Dunbar, R. I. M., & Ord, T. J. (2012). Social complexity as a proximate and ultimate factor in communicative complexity. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 367, 1785–1801.
- Gamer, M., Lemon, J., & Singh, I. F. P. (2012). irr: Various coefficients of interrater reliability and agreement. Retrieved from <http://CRAN.R-project.org/package=irr>.
- Gaunt, A. S., Bucher, T. L., Gaunt, S. L. L., & Baptista, L. F. (1996). Is singing costly? *Auk*, 113, 718–721.
- Gil, D., & Gahr, M. (2002). The honesty of bird song: multiple constraints for multiple traits. *Trends in Ecology & Evolution*, 17, 133–141.
- Glabach, A., Buesser, C., Mundry, R., & Quillfeldt, P. (2009). Acoustic parameters of begging calls indicate chick body condition in Wilson's storm-petrels *Oceanites oceanicus*. *Journal of Ethology*, 27, 267–274.
- Gleeson, D. J., Blows, M. W., & Owens, I. P. F. (2005). Genetic covariance between indices of body condition and immunocompetence in a passerine bird. *BMC Evolutionary Biology*, 5, 61.
- Goedert, D. (2010). *Comunicação acústica em pica-pau-do-campo (Colaptes campestris): caracterização estrutural e contextos sociais e ecológicos de sinais sonoros*. Master's thesis. Brasília, Brazil: Universidade de Brasília.
- Greig-Smith, P. W. (1984). Distress calling by woodland birds: seasonal patterns, individual consistency and the presence of conspecifics. *Zeitschrift für Tierpsychologie*, 66, 1–10.
- Hamilton, W. D. (1964a). The genetical evolution of social behaviour. I. *Journal of Theoretical Biology*, 7, 1–16.
- Hamilton, W. D. (1964b). The genetical evolution of social behaviour. II. *Journal of Theoretical Biology*, 7, 17–52.
- Hogstedt, G. (1983). Adaptation unto death: function of fear screams. *American Naturalist*, 121, 562–570.
- Inglis, I. R., Fletcher, M. R., Feare, C. J., Greig Smith, P. W., & Land, S. (1982). The incidence of distress calling among British birds. *Ibis*, 124, 351–355.
- Kitaysky, A., Kitaiskaia, E., Wingfield, J., & Piatt, J. (2001). Dietary restriction causes chronic elevation of corticosterone and enhances stress response in red-legged kittiwake chicks. *Journal of Comparative Physiology B*, 171, 701–709.
- Koenig, W. D., Stanback, M. T., Hooge, P. N., & Mumme, R. L. (1991). Distress calls in the acorn woodpecker. *Condor*, 93, 637–643.
- Laiolo, P., Serrano, D., Tella, J. L., Carrete, M., Lopez, G., & Navarro, C. (2007). Distress calls reflect poxvirus infection in lesser short-toed lark *Calandrella rufescens*. *Behavioral Ecology*, 18, 507–512.
- Laiolo, P., Tella, J. L., Carrete, M., Serrano, D., & Lopez, G. (2004). Distress calls may honestly signal bird quality to predators. *Proceedings of the Royal Society B: Biological Sciences*, 271(Suppl.), S513–S515.
- Leavesley, A. J., & Magrath, R. D. (2005). Communicating about danger: urgency alarm calling in a bird. *Animal Behaviour*, 70, 365–373.
- Marinho-Filho, J., Rodrigues, F. H. G., & Juarez, K. M. (2002). The cerrado mammals: diversity, ecology, and natural history. In P. S. Oliveira, & R. J. Marquis (Eds.), *The cerrados of Brazil: Ecology and natural history of a Neotropical savanna* (pp. 266–284). New York, NY: Columbia University Press.
- Marler, P. (2004). Bird calls: a cornucopia for communication. In P. Marler, & H. Slabbekoorn (Eds.), *Nature's music: The science of birdsong* (pp. 132–177). San Diego, CA: Elsevier.
- Martin, L. B., II, Gilliam, J., Han, P., Lee, K., & Wikelski, M. (2005). Corticosterone suppresses cutaneous immune function in temperate but not tropical house sparrows, *Passer domesticus*. *General and Comparative Endocrinology*, 140, 126–135.
- Mathewon, N., Aubin, T., & Brémond, J. C. (1997). Propagation of bird acoustic signals: comparative study of starling and blackbird distress calls. *Comptes rendus de l'Académie des sciences, Série III, Sciences de la vie*, 320, 869–876.
- Maynard Smith, J., & Harper, D. (1995). Animal signals: models and terminology. *Journal of Theoretical Biology*, 177, 305–311.
- Maynard Smith, J., & Harper, D. (2003). *Animal signals*. New York, NY: Oxford University Press.
- Mazer, S. J., & Damuth, J. (2001). Nature and cause of variation. In C. W. Fox, D. A. Roff, & D. J. Fairbairn (Eds.), *Evolutionary ecology: Concepts and case studies* (pp. 3–15). New York, NY: Oxford University Press.
- Montgomerie, R. D., & Weatherhead, P. J. (1988). Risks and rewards of nest defence by parent birds. *Quarterly Review of Biology*, 63, 167–187.
- Mundry, R., & Sommer, C. (2007). Discriminant function analysis with nonindependent data: consequences and an alternative. *Animal Behaviour*, 74, 965–976.
- Nakagawa, S., & Cuthill, I. C. (2007). Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biological Reviews*, 82, 591–605.
- Nieuwenhuis, R., te Grotenhuis, M., & Pelzer, B. (2012). Influence.ME: tools for detecting influential data in mixed effects models. *R Journal*, 4, 38–47.
- Oberweger, K., & Goller, F. (2001). The metabolic cost of birdsong production. *Journal of Experimental Biology*, 204, 3379–3388.
- Perrone, M. (1980). Factors affecting the incidence of distress calls in passerines. *Wilson Bulletin*, 92, 404–408.
- Pigliucci, M. (2005). Evolution of phenotypic plasticity: where are we going now? *Trends in Ecology & Evolution*, 20, 481–486.
- Pinheiro, J. C., & Bates, D. (2000). *Mixed effects models in S and S-Plus*. New York, NY: Springer.
- Pole, A., Gordon, I. J., & Gorman, M. L. (2003). African wild dogs test the 'survival of the fittest' paradigm. *Proceedings of the Royal Society B: Biological Sciences*, 270(Suppl.), S57.
- R Development Core Team. (2013). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from <http://www.R-project.org/>.
- Rohwer, S., Fretwell, S. D., & Tuckfield, R. C. (1976). Distress screams as a measure of kinship in birds. *American Midland Naturalist*, 96, 418–430.
- Rubolini, D., Romano, M., Boncoraglio, C., Ferrari, R. P., Martinelli, R., Galeotti, P., et al. (2005). Effects of elevated egg corticosterone levels on behavior, growth, and immunity of yellow-legged gull (*Larus michahellis*) chicks. *Hormones and Behavior*, 47, 592–605.
- Sacchi, R., Saino, N., & Galeotti, P. (2002). Features of begging calls reveal general condition and need of food of barn swallow, *Hirundo rustica*, nestlings. *Behavioral Ecology*, 13, 268–273.

- Saino, N., Suffritti, C., Martinelli, R., Rubolini, D., & Møller, A. P. (2003). Immune response covaries with corticosterone plasma levels under experimentally stressful conditions in nestling barn swallows (*Hirundo rustica*). *Behavioral Ecology*, *14*, 318–325.
- Sherman, P. W. (1977). Nepotism and the evolution of alarm calls. *Science*, *197*, 1246–1253.
- Short, L. L. (1972). Systematics and behavior of South American flickers (Aves, *Colaptes*). *American Museum of Natural History*, *149*, 1. Retrieved from <http://hdl.handle.net/2246/1198>.
- Short, L. L. (1975). A zoogeographic analysis of the South American Chaco avifauna. *American Museum of Natural History*, *153*, 163–352.
- Short, L. L. (1982). *Woodpeckers of the world*. Greenville, DE: Delaware Museum of Natural History.
- Sick, H. (1997). *Ornitologia Brasileira*. Rio de Janeiro, Brasil: Editora Nova Fronteira.
- Smits, J., Bortolotti, G., & Tella, J. (1999). Simplifying the phytohaemagglutinin skin-testing technique in studies of avian immunocompetence. *Functional Ecology*, *13*, 567–572.
- Stone, E., & Trost, C. H. (1991). Predators, risks and context for mobbing and alarm calls in black-billed magpies. *Animal Behaviour*, *41*, 633–638.
- Stutchbury, B. J. M., & Morton, E. S. (2001). *Behavioral ecology of tropical birds*. San Diego, CA: Academic Press.
- Suzuki, T. N. (2012). Referential mobbing calls elicit different predator-searching behaviours in Japanese great tits. *Animal Behaviour*, *84*, 53–57.
- Tella, J. L., Bortolotti, G. R., Dawson, R. D., & Forero, M. G. (2000). The T-cell-mediated immune response and return rate of fledgling American kestrels are positively correlated with parental clutch size. *Proceedings of the Royal Society B: Biological Sciences*, *267*, 891–895.
- Tella, J., Bortolotti, G. R., Forero, M., & Dawson, R. D. (2000). Environmental and genetic variation in T-cell-mediated immune response of fledgling American kestrels. *Oecologia*, *123*, 453–459.
- Tella, J. L., Scheuerlein, A., & Ricklefs, R. E. (2002). Is cell-mediated immunity related to the evolution of life-history strategies in birds? *Proceedings of the Royal Society B: Biological Sciences*, *269*, 1059–1066.
- The North American Banding Council. (2001). *The North American bander's study guide*. Retrieved from <http://www.nabanding.net/other-publications/>.
- Thomas, R. J. (2002). The costs of singing in nightingales. *Animal Behaviour*, *63*, 959–966.
- Vehrencamp, S. L., Hall, M. L., Bohman, E. R., Depeine, C. D., & Dalziell, A. H. (2007). Song matching, overlapping, and switching in the banded wren: the sender's perspective. *Behavioral Ecology*, *18*, 849–859.
- Ward, S. (2004). Singing is not energetically demanding for pied flycatchers, *Ficedula hypoleuca*. *Behavioral Ecology*, *15*, 477–484.
- Ward, S., Speakman, J. R., & Slater, P. J. B. (2003). The energy cost of song in the canary, *Serinus canaria*. *Animal Behaviour*, *66*, 893–902.
- Wingfield, J. C., & Kitaysky, A. S. (2002). Endocrine responses to unpredictable environmental events: stress or anti-stress hormones? *Integrative and Comparative Biology*, *42*, 600–609.
- Zachau, C. E., & Freeberg, T. M. (2012). Chick-a-dee call variation in the context of 'flying' avian predator stimuli: a field study of Carolina chickadees (*Poecile carolinensis*). *Behavioral Ecology and Sociobiology*, *66*, 683–690.
- Zahavi, A. (1975). Mate selection: a selection for a handicap. *Journal of Theoretical Biology*, *53*, 205–214.
- Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R*. New York, NY: Springer.