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Songbird mates change their call structure and intra-pair communication at the nest in response to environmental noise

Avelyne S. Villain * a, Marie S.A. Fernandez a,b, Colette Bouchut a, Hédi A. Soula b,c, Clémentine Vignal a

a Université de Lyon/Saint-Etienne, Neuro-PSI/ENES CNRS UMR 9197, France
b EPI BEAGLE INRIA F69621 Villeurbanne
c INSERM U1060 INSA LYON F69621 Villeurbanne

- Corresponding author: avelyne.villain@univ-st-etienne.fr
Highlights:

- Both zebra finch parents incubate and they use coordinated call duets when they meet at the nest.
- In their natural habitat, birds face variable wind noise that may constrain their communication at the nest.
- In response to an experimental noise, pairs adjusted their duets and increased their effort to communicate.
- Duets’ flexibility in response to noise depended on the context of meeting at the nest.
- Males and females produced louder calls with an up-shifted frequency spectrum in response to experimental noise.
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**Summary**

The coordination of behaviours between mates is a central aspect of the biology of the monogamous pair bonding in birds. This coordination may rely on intra-pair acoustic communication, which is surprisingly poorly understood. Here we examined the impact of an increased level of background noise on intra-pair acoustic communication at the nest in the zebra finch. We monitored how partners adapted their acoustic interactions in response to a playback of wind noise inside the nestbox during incubation. Both zebra finch parents incubate and use coordinated call duets when they meet at the nest. The incubating parent can vocalize to its partner either outside the nestbox (sentinel duets) or inside the nestbox (relief and visit duets),
depending on the context of the meeting. Pairs use these duets to communicate on predation threats (sentinel duets), incubation duties (relief), and other nesting activities (visit duets). Each of these duets likely represents a critical component of pair coordination. In response to the noise playback, partners called less and more rapidly during visit and relief duets. Male and female calls were more regularly and precisely alternated during relief duets. Mates increased the number of visit duets and their spatial proximity during sentinel duets. Furthermore, both males and females produced louder, higher-frequency, and less broadband calls. Taken together our results show that birds use several strategies to adjust to noise during incubation, underlining the importance of effective intra-pair communication for breeding pairs.

Keywords

Monogamy,

Vocal duet,

Breeding,

Acoustic flexibility,

Zebra finch,

Introduction

Monogamy in birds represents a partnership where the male and female adjust their behaviour to each other and synchronize many of their activities (Black, 1996). Many long-term monogamous species show an increase in reproductive success with pair bond duration, which may be due to the improvement in partners’ coordination over time (mate familiarity effect, (Black, 2001; Coulson, 1966; Forslund & Pärt, 1995)). In some species, partners synchronize their foraging trips or their nest visits to feed
the chicks (Lee, Kim, & Hatchwell, 2010; van Rooij & Griffith, 2013), and their
degree of synchrony can correlate with their reproductive success (Mariette &
Griffith, 2012, 2015). In species in which both partners incubate, hatching success
may be increased when parents better coordinate incubation bouts (Spoon, Millam, &
Owings, 2006). Partners’ coordination during parental care may reflect their
coordination in other situations: mates may defend their resources as a team by
alarming for danger (Krams, Krama, & Igaune, 2006), repelling predators or intruders
on their territory (Black, 2001; Regelmann & Curio, 1986), or alternating vigilance
periods (McGowan & Woolfenden, 1989).
Communication and especially acoustic communication may play a key role in
mate coordination. Whereas birdsong has been studied in the context of mate choice
extensively in males (Catchpole & Slater, 2008) and more rarely in females (Cooney
& Cockburn, 1995; Langmore, 1998; Odom, Hall, Riebel, Omland, & Langmore,
2014; Riebel, 2003; Riebel, Hall, & Langmore, 2005), much less is known about
vocal interactions after pair formation between the male and female of a breeding pair
(Gorissen, Eens, & Nelson, 2004) with the exception of acoustic duets. Duets are joint
acoustic displays of partners that alternate or partly overlap vocal or non-vocal sounds
(Dahlin & Wright, 2009; Farabaugh, 1982; Hall, 2004, 2009). Although rare (ca. 4% of bird species), they have attracted much interest, and the highly coordinated and
conspicuous song duets of tropical bird species have been particularly well studied
(Hall, 2004, 2009). But intra-pair communication may be more widespread and
involve simpler or low-amplitude vocalizations such as calls (Lamprecht, Kaiser,
Peters, & Kirchgessner, 1985; Morton & Derrickson, 1996; Todt, Hultch, & Duvall,
1981; Wright & Dahlin, 2007). Females can produce sounds at the nest (Beletsky &
Orians, 1985; McDonald & Greenberg, 1991; Yasukawa, 1989) that may be used in
interactive communication with their mate (Gorissen et al., 2004). Such vocal
interactions can facilitate a pair’s coordination during breeding (Halkin, 1997;
Ritchison, 1983). For instance, nest relief and greeting ceremonies have been
described in several bird species but their functions remain unclear (Wachtmeister,
2001).

If vocal interactions around the nest allow coordination of behaviours between
mates, they must remain efficient despite environmental constraints on acoustic
communication. Noise is a common constraint on acoustic communication. By
decreasing signal to noise ratio, background noise makes the signal harder to extract
for the receiver (Brenowitz, 1982; Wiley & Richards, 1982). Noise particularly
constrains acoustic communication if spectral components of the noise and the signal
partly overlap (Barber, Crooks, & Fristrup, 2010; Francis & Barber, 2013; Halfwerk
& Slabbekoorn, 2015; Slabbekoorn, 2004). Birds have evolved many adaptive
strategies to cope with background noise and to increase signal reception efficacy
(Blumenrath, Dabelsteen, & Pederson, 2004). The effects of background noise have
been primarily studied on songs and other long-range vocalisations, and thus little is
understood about the effects of background noise on private, short range vocalisations (Leonard & Horn, 2005, 2008). Importantly, short range vocalisations are less affected by degradation during sound propagation and thus likely require very different adjustment strategies than long-range vocalisations. Noise likely hinders intra-pair communication around the nest during breeding, which could explain the observed impairment of reproductive success by noise (Barber et al., 2010; Slabbekoorn & Ripmeester, 2008).

The zebra finch (*Taeniopygia guttata*) provides an excellent study system to test whether partners adapt short-range intra-pair communication to noise constraints. Zebra finches form life-long pair bonds and are highly coordinated partners, starting incubation on the same day (Gilby, Mainwaring, & Griffith, 2013), sharing incubation time equally (Delesalle, 1986; Gilby et al., 2013; Gorman, Arnold, & Nager, 2005; Zann & Rossetto, 1991), and synchronizing visits to the nest and to foraging patches during the nestling period (Mariette & Griffith, 2012, 2015). Each time they meet around the nest during incubation or the nestling period, mates perform a call duet that likely participate in coordination (Boucaud, Mariette, Villain, & Vignal, 2015; Elie et al., 2010) Zebra finches live in semi-arid zones of Australia, an unpredictable environment where windy conditions are highly variable on an hourly basis. Because zebra finch calls, and particularly nest calls, have a spectrum in the low range (Elie et al., 2010; Elie & Theunissen, 2015; Zann, 1996), they are very likely to overlap in frequency with wind noise. To our knowledge no experiment testing this effect has been conducted in zebra finches.

In the present study, we exposed incubating zebra finch pairs to a natural wind noise playback inside their nestbox. Because the male and female take turns incubating, both partners were exposed to the noise. After 15 hours of noise, intra-pair
communication and partners’ behaviour were monitored and compared to the control condition. Because call duets are supposed to participate in partners’ coordination during incubation, we expect birds to show strategies to maintain signal efficacy in response to the noise playback. We monitored three duets types – incubation relief/nest visit/sentinel – and we studied four aspects of this intra-pair communication
1) the temporal structure of duets 2) the male-female dynamic during the duet, 3) the number of vocal interactions between partners and the spatial proximity of partners during interactions, and finally 4) the acoustic structure of the calls used during interactions. In response to this experimental increase of noise, we expected the partners to either avoid communicating or to display strategies to cope with it e.g. via increasing signal redundancy (longer duets and/or duets composed of longer calls), increasing partners’ proximity during vocal interactions, and/or changing signal structure (frequency range and/or amplitude).

Materials and methods

Subjects and housing conditions

Eighteen male-female pairs of zebra finches (Taeniopygia guttata) were used in this study, from October 2013 to December 2013. All birds came from our breeding colony (ENES laboratory, University of Saint-Etienne). They were all the same age (between 24 and 28 months at the start of the experiment) and the experiment was conducted on the third reproductive event of their lifetime for every pair. Before the experiment, pairs were housed separately in cages (40 x 40 x 40 cm) equipped with perches and a pool for environmental enrichment. All birds were kept under the same environmental conditions (temperature between 24 and 26 °C, light conditions 14:10 h light-dark). Birds were fed with finch seed cocktail, egg paste, water and cuttlefish
bones *ad libitum* and supplemented with salad once a week. For the experiment, pairs were transferred to an indoor breeding aviary (6.5 x 5.5 x 3.5 m, temperature between 19 and 24 °C, light conditions 14:10 h light-dark). Twenty-seven nestboxes were installed (dimensions 13 x 12 x 17 cm).

During the experiment, all 18 pairs were allowed to breed freely in the aviary. Pairs were provided with dry grass and cotton *ad libitum*. Birds were identified with two plastic colour bands.

As the experiment was performed during incubation, pairs were captured a few days after hatching (from day 1 to 5 post hatching) and put back in their initial home cage with their nestbox containing the chicks. Other pairs were released in the aviary to replace the outgoing ones, so that the aviary always contained 12 breeding pairs, keeping the conspecific background noise at a stable level.

**Ethical Note**

Experiments were performed under the authorization no. 42-218-0901-38 SV 09 (ENES Lab, Direction Départementale des Services Vétérinaires de la Loire) and were in agreement with the French and European legislation regarding experiments on animals.

**Experimental manipulation of noise**

The experiment was performed during incubation. Each nest was recorded on two consecutive morning sessions, so the design was within-pair and all analyses were thus done using within-subject statistics. On one morning, the noise inside the nest had previously been artificially increased for 15 hours and the other morning was used as control treatment. The order of treatment days was defined at random. Because of the proximity between nestboxes in the aviary, we chose to experimentally modify the noise inside the nestbox, so that only pairs recorded during the session were subjected...
to the noise treatment, without disturbing other pairs around. This treatment mimicked
the noise underwent by incubating birds in artificial nestboxes (that are readily used
by wild zebra finches (Simon C. Griffith, 2008)), inside which wind noise is
reverberated, but also in natural nests which are woven bottle-shaped nests (Zann,
1996) that very weakly attenuate wind noise.
Noise was played back using a modified headphone (Sennheiser, HD 25-1) serving as
a speaker and specifically designed for short-range diffusion of sound. This speaker
was installed the day before the first day of recording (control or noise).
To mimic noise naturally encountered by the species, we used wind noise recorded in
the field from a breeding area of wild zebra finches (Arid Zone Research Station,
Fowlers Gap, New South Wales, Australia) with an ultra-directional microphone
(Sennheiser, MKH 70). We used a sequence of 15 seconds of wind noise (sound 0)
repeated for a total duration of 15 hours of playback before the recording session
(from 17:30 the day before to 10:00-12:00 the day of recording). The recordings took
place within the last 20% of the total playback time (from 8:30 to 12:00). So if birds
habituated to the particular noise snippet repeated during the playback, we assumed
habitation was largely completed after 15 hours, and all the behaviours and
vocalizations analysed in our results were equally affected.
The sound pressure level inside the next box was measured in the two treatments
using a sound level meter (Rion NL-42, with additional NX-42WR package,
frequency weighting ‘Z’, temporal weighting ‘Fast’). Wind noise playback increased
sound pressure level from 58.9 dB SPL to 63.7 dB SPL (~5 dB increase) compared to
control, which is a relevant increase in sound pressure level for zebra finches in the
wild (Fig. S2). This treatment represented an almost doubled acoustic pressure, which
is a strong change in background noise conditions (see spectral comparison of
background noise during treatments, Fig. S1). Because the noise was played inside the nestbox, the background noise level outside remained the same in both treatments i.e 58.1dB SPL in control and 58.7dB in noise (measured at 20cm from the box, with basal bird activity in the aviary). Note that the basal background noise in control treatment is created by the activity and vocalizations of the 12 pairs of birds breeding at the same time in the aviary.

**Recording of mates’ acoustic communication at the nest**

Mates’ communication at the nest was recorded with a tie-microphone (Audio Technica, AT 803) placed in the top of the nestbox, connected to a digital audio recorder (Zoom H4N, 44.1 kHz, 16 bit). An additional tie microphone was placed outside of the nestbox at 20 cm of the entrance to record vocal activity in the vicinity of the nest. Microphone calibrations were previously performed with a 10-seconds white noise and a sound level meter (Rion NL-42, with additional NX-42WR package). Duets between partners were assessed both around and inside the nest.

**Behavioural monitoring**

During all recording sessions, an observer sat in a hide inside the aviary and recorded partners’ behaviour. The location of both partners relative to the nest was monitored during vocal interactions. One recording session consisted of two consecutive incubation reliefs so that the two categories of reliefs were monitored (male returns vs. female returns to the nest). As a consequence, the duration of one session depended on the observed pair and could last from one to three and a half hours. For each session, duets were counted, classified and extracted.

**Definition of duets between mates**

Two types of duets were analysed: ‘meeting duets’ and ‘sentinel duets’. ‘Meeting duets’ are vocal greetings performed by the pair when one mate returns to the nest and
meets its partner, as described by (Elie et al., 2010). At the end of a meeting, the returning mate can relieve its partner in the nest or not, leading to two subtypes of ‘meeting duets’ defined by their outcome: the ‘relief duets’ (R) when the returning mate stays in the nest and takes its turn incubating the eggs, or the ‘visit duets’ (V) when the returning mate just visits its mate at the nest, for instance bringing nest material, but do not take its turn incubating and leaves the nest at the end of the interaction. During a ‘visit duet’ (V), the returning mate can either enter the nestbox or stay at the entrance but eventually departs.

A meeting duet was defined as a sequence of at least two calls, produced by both sexes and separated by less than 10 seconds (Elie et al., 2010). When the returning mate was far from the nestbox, its calls were not accurately detected among vocalizations of other birds of the aviary. As a consequence, we considered that a meeting duet started after the returning mate perched within 20 cm of the nestbox and when the partner inside the nest uttered a call less than 5 s before or after a call of its mate or if the returning mate entered the box. A meeting duet stopped either when at least one mate left the nest area, or when both birds stopped calling for a period of 10 s. During a meeting duet, the calls of the partners could either perfectly alternate or partly overlap.

‘Sentinel duets’ (S) are vocal interactions performed with one mate inside the nest and the other staying outside, located between 20 cm and 5 m from the nest (Elie et al., 2010). Again, calls from the outside mate could not be accurately detected among vocalizations of other birds, so we used the sequence of calls of the incubating partner as a proxy of the sentinel duet. The same 10 seconds rule as above was applied to decide the end of a sentinel duet. The incubating partner rarely produced isolated calls, i.e. calls not included in a sentinel duet.
Analysis of duet structure

Parameters

All duets (N= 323, from N= 18 pairs, table S1) were extracted and analysed using Praat software (www.praat.org). The location of the birds in or outside the nestbox during the duet was scored and all calls were manually labelled using Praat ‘annotate’ function as time intervals. Each call was labelled as male or female and assigned to one of the three following call types (see Fig. S3 for spectrograms of duets and call types and sounds 1 to 5):

- Short calls are primarily tet calls, i.e. soft and short harmonic stacks (57.7±19.2 ms) with almost no frequency modulations (Elie et al., 2010; Zann, 1996). Cackle and thuck calls were rarely produced and thus are pooled in this category (Zann, 1996).

- Whines are soft and high-pitched moans, with variable but usually long duration (182.3±109.3 ms). This ‘pleading’ sound is a vocalization specifically uttered at the nest site (Elie et al., 2010; Zann, 1996). It can be flanked with beak-nibbling sounds.

- Arks are intermediate calls (89.4±23.0 ms) with a downsweep component (Zann, 1996).

When duets were performed with both birds inside the nestbox, we reported the time partners spent together in the nest. The latency of the incubating mate to answer the calls of the returning partner was calculated as the delay between the start of the first call uttered by the returning partner and the start of the first call uttered in response by the incubating partner. Using annotations on duets, the following characteristics of the duets were automatically calculated: number of calls, call rate, number and proportion of each call category, duet duration (time from the start of the first call to the end of
Inter-Call Intervals (ICI) were calculated as the time between two call
starts, and the overall ICI, calculated over the whole duet, described the tempo of the
duet.

Statistics.

Distribution of parameters were checked and only parameters showing a symmetrical
distribution (after transformation if necessary) were kept to build composite scores of
the structure of each duet using Principal Components analyses (PCA) (McGregor,
1992). PCs with eigenvalue above 1 were kept for the analysis.

Since R and V duets are defined by their outcome, no clear acoustic basis was found
to analyse them separately, so they were pooled before running PCA. The PCA
described global structure of the duets: numbers and proportions of the different call
types, total number of calls, tempo (call rate and overall ICI) and duration (table 1).

Linear models (‘lmer’ function of ‘lme4’ R package) were then performed on PC
values to assess the effect of the treatment. The following model was applied: model 1
<- lmer (PC values ~ Treatment + Returning partner + Duet type + Noise treatment:
Returning partner + Noise Treatment: Duet type, random=~1|pair identity), where
Treatment had two levels (Noise vs. Control), Returning partner had two levels (Male
vs. Female) and Duet type had two levels (R vs. V). This model was built to test for
specific fixed factors and their interaction with the treatment, although not all the
interactions between the factors were tested they were either considered irrelevant or
biologically difficult to interpret (Forstmeier & Schielzeth, 2011). P-values were
assessed using the ‘Anova’ function (‘car’ R package). S duets were analysed
separately because parameters were measured on vocalizations of the incubating bird
only (see above, definition of duets and variable loadings, table 2). The following
model was used to assess the effect of the treatment on PC values: model 2 <- lmer
(PC values ~ Treatment * Returning partner, random=~1|pair identity), where Treatment had two levels (Noise vs. Control) and Returning partner had two levels (Male vs. Female). P-values were assessed using the ‘Anova’ function (‘car’ R package).

**Analysis of male-female dynamic during relief and visit duets**

Male-female dynamic during duets was assessed using two complementary methods: first using delays of response of each bird to the calls of its partner (ICI analysis), second using cross-correlation of male and female signals (Cross-correlation analysis).

**ICI analysis**

For this analysis, we assessed male and female tempos using means and standard deviations of intra-sex ICI s (time between two call starts of the same individual) and mean inter-sex ICI s, i.e. transitions between sexes (M-F and F-M delays) representing the reaction time of one bird to the calls of its partner. A PCA was run to build composite scores of male-female dynamic during the duet (table 3). The effect of the treatment was assessed with a linear model run on PC values (see model 1).

**Cross-correlation analysis**

In this analysis we focused on the temporal synchrony (or lack of it) in calling activity between mates by computing the cross-correlation between male and female calling signals. A calling signal is a temporal description of the calling emission and is defined as a function of time $t$ that is 1 if the bird was producing a sound at $t$ and 0 otherwise. The sampling frequency was set to 200Hz (5 ms bins). For example if, for one part of a calling signal of 75 ms, we obtained 0 0 0 1 1 1 1 1 1 1 1 0 0, it means that during the first 15 ms (3*5 ms) the bird was silent, then this bird produced
a call of 50 ms (10*5 ms) length, before it went back to silence for 10 ms. \( S_{\text{male}} \) stands for the male signal and \( S_{\text{female}} \) for the female signal. We computed the cross-correlation (cc) of these two signals \( (S_{\text{male}} \text{ and } S_{\text{female}}) \) with the following formula:

\[
cc(T) = \text{mean} \left( (S_{\text{male}}(t) - \text{mean}(S_{\text{male}}(t))) \times (S_{\text{female}}(t+T) - \text{mean}(S_{\text{female}}(t+T))) \right).
\]

With the normalization step, we have: \( CC(T) = \frac{cc(T)}{sd(S_{\text{female}}) \times sd(S_{\text{male}})} \), where CC is the normalized cross-correlation, T the time delay, and \( S_{\text{male}} \) and \( S_{\text{female}} \) the male and female signals as functions of t (time). On a cross-correlation curve, a peak on the right of the x-axis (positive time values) gives information about the time between a male call and the previous female call (F->M), and a peak on the left of the x-axis (negative time values) gives information about the time between a female call and the previous male call (M->F) (Perez, Fernandez, Griffith, Vignal, & Soula, 2015).

To compare cross-correlation between treatments, we measured the curve’s maximum peak height, which signs the strength of the cross-correlation, as well as the height of each peak (positive peak: F->M, negative peak: M->F). Because cross-correlation used the calculation of two means, we used only duets having more than 8 calls per individual. We first tested duets with the best correlation scores, \textit{i.e} greater than 0.1, according to Perez et al. (2015). In this case, cross-correlation was thus compared between 33 relief duets (16 in control and 17 in noise) from 14 pairs, and for 18 visit duets (12 in control and 6 in noise) from 10 pairs. We then used all the duets to confirm the results; cross-correlation was thus computed for 43 relief duets (24 in control and 19 in noise) from 16 pairs, and for 24 visit duets (18 in control and 6 in noise) from 10 pairs. The same model as above was used to assess the effect of the treatment (see model 1).

\textbf{Analysis of the numbers of visit duets and sentinel duets, and the spatial proximity between partners during sentinel duets}
Parameters

Occurrences of V duets and S duets were counted during a reference period (see below). In addition, the distance between partners during sentinel duets was analysed as a proxy of the spatial proximity between partners. Three distance categories were considered (from 0 to 1 meter, from 1 to 2 meters and more than 2 meters) and the number of sentinel duets in each distance category was counted.

One recording session consisted of two consecutive incubation reliefs (male return vs. female return to the nest). The duration of a recording session thus depended on pair identity, as some birds have shorter incubation shifts than others. As the observer started the recording session in the middle of an incubation shift, the first incubation shift could be more or less completed after the session’s start. To obtain comparable data for all pairs and for both sexes (as male return or female return could happen first depending on recording session), we quantified behaviours during a defined reference period. The duration of this reference period was defined as the last 40% of the time between two reliefs, an interval concentrating most of the birds’ vocal and behavioural activity. For one given pair recorded in one given treatment, two reference periods were defined (the first one being just before the first relief, the second being just before the second relief). In total, 72 reference periods were defined (four reference periods from 18 pairs). But for 15 recordings out of the total data set, the time between the start of the recording session and the first relief was shorter than this reference period (seven in noise treatment, eight in control treatment). Thus, all 18 pairs remained in the final dataset but for some of them, the first reference period was missing. The data set was then composed of 28 reference periods in control (12 with the female incubating and 16 with the male incubating) and 29 reference periods in noise (14 with the female incubating, 15 with the male incubating).
The effect of the treatment on total counts was tested separately on the number of V duets, the number of S duets and the number of S duets performed in each distance category, using generalized linear models for Poisson distribution (‘glmer’ function of ‘lme4’ R package). The following model was applied: model 3 <- glmer (total behavioural count ~ Treatment * Returning partner + (1| Pair), family=’poisson’).

Analysis of the acoustic features of the calls used during duets

Parameters

Calls uttered by the incubating partner with no additional noise overlapping the calls (from the partner, from birds movements inside the nest or other birds in the aviary) were manually selected using the ‘annotate’ function of Praat software. A spectral analysis was performed using custom-written codes using the Seewave R package (Sueur, Aubin, & Simonis, 2008) implemented in R (R Core Team, 2014). After bandpass filtering (0.5kHz-20kHz corresponding to the zebra finch vocalizations spectrum, Seewave ‘fir’ function), the following parameters of the call frequency spectrum were calculated (Seewave ‘specprop’ function, FFT using a Hamming window, window length=512, overlap=50%): mean, median, first (Q25) and third (Q75) quartiles, inter-quartile range (IQR), standard deviation (Sd) and mode (all in Hertz). One additional frequency parameter was calculated from 50% overlapping FFTs (window length=512): the call dominant frequency (in kHz), which is the mean over the call duration of the frequencies of highest level of energy (Seewave ‘dfreq’ function). Last, the call amplitude was measured using the root-mean-square (RMS) of the call envelope.

To compare the structure of calls used by birds in control and noise treatments, we mixed recorded calls in control with exemplars of noise. All measures were averaged
with 10 mixes obtained using 10 different exemplars of noise. A detailed explanation of the procedure is available as supplementary material (see Fig.S4).

Statistics.

Calls from all duet types (relief duets, visit duets or sentinel duet) were analysed together (1320 calls from 36 individuals). Acoustic parameters were log-transformed to fit a Gaussian distribution and used in a PCA. Principal components (PCs) with eigenvalue above 1 were selected (table 4).

To assess the effect of the treatment on calls’ structure, a linear model (‘lmer’ function of ‘lme4’ R package) was then performed on PC values. The following model was applied: model 4 <- lmer (PC values~ Treatment + Sex of the caller + Call type + Duet type + Treatment: Sex of the caller + Treatment: Duet type + Treatment: Call type +(1|subject identity)), where Treatment had two levels (Noise vs. Control), Sex of the caller had two levels (Male vs. Female), Call type had three levels (Ark, Whine and Short calls) and Duet type had two levels (‘V or R’ vs. ‘S’). Again, only relevant and interpretable interactions were kept in the full model. P-values were assessed using the ‘Anova’ function (‘car’ R package). When interaction between factors were significant, post hoc test were performed using ‘lsmeans’ function (‘lsmeans’ R package).

Statistical validation

To reduce the incidence of multiple testing on type I error, we computed PC scores using PCA on raw parameters as much as possible. We did not use the Bonferroni correction because its assumption of a universal null hypothesis (all null hypothesis being true simultaneously) was not verified in our case (Perneger, 1998). But Tukey correction was used in posthoc tests. For all linear models, residuals equi-variance and distribution were checked using ‘plotresid’ function (‘RVAideMemoire’ R package).
The influential data points were tested using ‘influence’ function of ‘Influence.ME’ R package (Nieuwenhuis, Grotenhuis, & Pelzer, 2012). Validity of binomial models was checked using custom-written codes based on Atkinson (1981) and Collett (2002) (see Fig. S5 for detailed description). For linear models using Poisson distribution, residuals over-dispersion was tested using ‘overdisp.glmer’ function (‘RVAideMemoire’ R package). All models were validated and presented after removing influential random factors that changed the results. To quantify the variance of the data explained by the models, a conditional coefficient of determination of each model was calculated with ‘r.squaredGLMM’ function (‘MuMIn’ R package).

RESULTS

Structure of relief (R) and visit (V) duets

During noise, partners meeting inside the nest (54% of the meetings) tended to spend less time together in the nest ($X^2=3.5$, $p=0.06$, Fig. 1a), but the number of meetings performed inside or outside the nest did not differ between treatments (binomial model, $X^2=0.64$, $p=0.42$). The latency of response of the incubating partner to the calls of its outside mate increased in relief duets (post hoc test, $X^2=4.1$, $p=0.04$, Fig. 1b).

During noise, both R and V duets were shorter and composed of fewer calls, since the first composite score PC1 of the PCA was significantly lower in noise than in control ($X^2=10.1$, $p=0.001$, Fig. 1c). Both R and V duets were also performed at a higher tempo and composed of a higher proportion of short calls, since PC2 was also significantly lower in noise than in control ($X^2=11.2$, $p=0.001$, Fig. 1d).
Table 1: Principal component analysis of the global structure of R and V duets.

Percentage of each parameter composing the PC, a percentage of explained variance and eigenvalues of each PC are indicated. Transformations are indicated in parentheses.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
<th>PC4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variance (% cumulative)</td>
<td>39%</td>
<td>59%</td>
<td>74%</td>
<td>87%</td>
</tr>
<tr>
<td>Eigenvalue</td>
<td>4.7</td>
<td>2.3</td>
<td>1.7</td>
<td>1.6</td>
</tr>
<tr>
<td>Duet duration (ln)</td>
<td>13.6</td>
<td>4.4</td>
<td>-8.28</td>
<td>2.41</td>
</tr>
<tr>
<td>Total number of calls (ln)</td>
<td>20.04</td>
<td>-0.95</td>
<td>-0.08</td>
<td>-0.08</td>
</tr>
<tr>
<td>Call rate (box-cox)</td>
<td>0.18</td>
<td>-7.63</td>
<td>25.73</td>
<td>-12.29</td>
</tr>
<tr>
<td>Overall ICI (box-cox)</td>
<td>-0.69</td>
<td>13.67</td>
<td>-28.17</td>
<td>0.43</td>
</tr>
<tr>
<td>Number of short calls (ln)</td>
<td>11.02</td>
<td>-12.83</td>
<td>-3.48</td>
<td>-0.25</td>
</tr>
<tr>
<td>Number of whine calls (ln)</td>
<td>12.73</td>
<td>1.09</td>
<td>2.31</td>
<td>12.57</td>
</tr>
<tr>
<td>Number of ark calls (ln)</td>
<td>11.42</td>
<td>6.83</td>
<td>1.3</td>
<td>-8.44</td>
</tr>
<tr>
<td>Number of female calls (ln)</td>
<td>14.56</td>
<td>-1.82</td>
<td>0.05</td>
<td>0.34</td>
</tr>
<tr>
<td>Number of male calls (ln)</td>
<td>14.98</td>
<td>-0.11</td>
<td>-0.49</td>
<td>-1.15</td>
</tr>
<tr>
<td>Proportion of short calls</td>
<td>-0.37</td>
<td>-27.82</td>
<td>-13.13</td>
<td>-0.06</td>
</tr>
<tr>
<td>Proportion of whine calls (square root)</td>
<td>0.08</td>
<td>1.8</td>
<td>16.33</td>
<td>37.76</td>
</tr>
<tr>
<td>Proportion of ark calls (square root)</td>
<td>0.49</td>
<td>21.05</td>
<td>0.64</td>
<td>-24.22</td>
</tr>
</tbody>
</table>

1: ICI= Inter-Call Interval

Table 2: Principal component analysis of R and V duets – male-female dynamic.

Percentage of each parameter composing the PC, percentage of explained variance

Male-female dynamic during relief (R) and visit (V) duets

During noise, R duets were performed with shorter intra-sex ICIs and inter-sex transitions, since PC1 was significantly higher, whereas intervals did not change in V duets (interaction treatment:duet type: $X^2=4.1$, $p=0.04$; relief duets: $X^2=8.5$, $p=0.003$; visit duets: $X^2<0.001$, $p=0.99$, Fig. 2a). As a consequence, partners changed their calling dynamic in noise only during relief duets.
and eigenvalues of each PC are indicated. Transformations are indicated in parentheses.

<table>
<thead>
<tr>
<th>PC1</th>
<th>Variance (% cumulative)</th>
<th>65%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Eigenvalue</strong></td>
<td></td>
<td>3.9</td>
</tr>
<tr>
<td>M to F transition (box-cox¹)</td>
<td></td>
<td>-14.02</td>
</tr>
<tr>
<td>F to M transition (box-cox¹)</td>
<td></td>
<td>-16.08</td>
</tr>
<tr>
<td>F to F ICI (box-cox¹)</td>
<td></td>
<td>-17.47</td>
</tr>
<tr>
<td>M to M ICI (box-cox¹)</td>
<td></td>
<td>-17.38</td>
</tr>
<tr>
<td>M to M ICI standard deviation (box-cox¹)</td>
<td></td>
<td>-18.42</td>
</tr>
<tr>
<td>F to F ICI standard deviation (box-cox¹)</td>
<td></td>
<td>-16.63</td>
</tr>
</tbody>
</table>

¹: The box cox transformation computes one parameter transformation using the following formula:

\[ \text{parameter}^{(\lambda)} = \text{parameter}^{(0)} - 1 / \lambda, \text{if} \lambda \neq 0 \text{and } \ln(\text{parameter}^{(\lambda)}) \text{if } \lambda = 0. \]

The ‘boxcox’ function of the ‘Mass’ R package automatically finds the appropriate \( \lambda \) value to reach a distribution as close as possible to the Gaussian distribution.

Furthermore, the cross-correlation between male and female calling signals showed that the regularity of the duet increased in response to the treatment. Noise affected differently the most regular R and V duets (i.e. duets with cross-correlation curves that showed peaks above 0.1), with significant interactions between treatment and duet types (Fig. 2b and 2c). The strength of the cross correlation (maximum peak height) and the precision of male delays to answer female calls (positive peak height) both increased in R duets (Fig. 2b and 2c, \( X_1=2.7, \ p=0.09 \) and \( X_1=5.9, \ p=0.02 \) respectively) whereas they decreased in V duets (\( X_1=4.8, \ p=0.03 \) and \( X_1=2.9, \ p=0.09 \) respectively). When considering all the duets, the strength of the cross-correlation and the precision of male delays were still higher in noise than in control (\( X_1=5.8, \ p=0.02 \) and \( X_1=4.9, \ p=0.03 \) respectively), but did not differentiate duet types anymore (no significant interaction treatment:duet type; \( X_1=1.8, \ p=0.17 \) and \( X_1=0.03, \ p=0.85, \) respectively). Overall, this analysis revealed higher regularity in male-female calling in noise compared to control (Fig. 2d), especially during R duets. In particular, male delays to answer female calls were more precise during noise.
S duets showed fewer calls and lower tempo (higher overall ICI) in noise than control, since PC2 was significantly lower in noise than control ($X_1=7.6$, $p=0.007$, Fig. 3b). The total duration of the duet was not affected (PC1, $X_1=0.94$, $p=0.33$, Fig. 3a).

**Table 1: Principal component analysis of the global structure of S duets.**

Percentage of each parameter composing the PC, percentage of explained variance and eigenvalues of each PC are indicated. Transformations are indicated in parentheses.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PC1</th>
<th>PC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variance (% cumulative)</td>
<td>64%</td>
<td>95%</td>
</tr>
<tr>
<td><strong>Eigenvalue</strong></td>
<td>2.6</td>
<td>1.2</td>
</tr>
<tr>
<td><strong>Total number of calls (box-cox)</strong></td>
<td>-10.08</td>
<td>58.99</td>
</tr>
<tr>
<td><strong>Sequence duration (ln)</strong></td>
<td>-34.83</td>
<td>6.52</td>
</tr>
<tr>
<td><strong>Call rate (ln)</strong></td>
<td>32.17</td>
<td>6.03</td>
</tr>
<tr>
<td><strong>Overall ICI (square root)</strong></td>
<td>-22.91</td>
<td>-28.46</td>
</tr>
</tbody>
</table>

**Occurrence of visit (V) and sentinel (S) duets and spatial proximity between partners.**

In noise, the total number of V duets increased by 2.6 ± 1.9 ($X_1=6.9$, $p=0.008$, Fig. 4a). The number of S duets did not differ between treatments ($X_1=0.8$, $p=0.34$, Fig. 4a).

During S duets, the returning partner perched significantly closer to the nest in noise than in control, increasing the spatial proximity between partners (Fig. 4b). In noise, S duets took place slightly more often with the outside mate at 0 to 1 meter from the nest (Fig. 4b, $X_1=2.4$, $p=0.12$) and significantly less at 1 to 2 meters ($X_1=9.0$, $p=0.003$).

**Acoustic features of calls produced inside the nest**
During noise, calls produced inside the nest were louder with an up-shifted and less broadband frequency spectrum (lower PC1, table 4), and this was true for both sexes (Fig. 5a) and all call types (Fig. 5b). Furthermore, female calls tended to be more affected by noise than male calls (interaction treatment: sex: $X_1 = 28.2$, $p < 0.001$; posthoc female vs male calls in noise: $T_{27.6} = -2.6$, $p = 0.06$, Fig. 5a) and short calls tended to be more affected by noise than whine calls (interaction treatment: call type: $X_1 = 18.4$, $p < 0.001$; post hoc short vs whine calls in noise: $T_{105.8} = 2.8$, $p = 0.06$, Fig. 5b), see examples of call spectra Fig. 5c.

**Table 4: Principal component analysis of call structure.** Percentage of each parameter composing the PC, percentage of explained variance and eigenvalues of each PC are indicated. Transformations are indicated in parentheses.

<table>
<thead>
<tr>
<th>Explained variance (%)cumulative</th>
<th>PC1</th>
<th>PC2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Eigenvalue</strong></td>
<td>43%</td>
<td>77%</td>
</tr>
<tr>
<td><em>Call duration</em></td>
<td>3.9</td>
<td>3.1</td>
</tr>
<tr>
<td><em>Mean frequency</em></td>
<td>0</td>
<td>-1.08</td>
</tr>
<tr>
<td><em>Sd</em></td>
<td>3.23</td>
<td>-26.78</td>
</tr>
<tr>
<td><em>Median frequency</em></td>
<td>20.29</td>
<td>-1.62</td>
</tr>
<tr>
<td><em>Q25</em></td>
<td>-9.18</td>
<td>-16.05</td>
</tr>
<tr>
<td><em>Q75</em></td>
<td>-15.34</td>
<td>-10.74</td>
</tr>
<tr>
<td><em>IQR</em></td>
<td>4.66</td>
<td>-24.42</td>
</tr>
<tr>
<td><em>Dominant frequency</em></td>
<td>13.37</td>
<td>-12.67</td>
</tr>
<tr>
<td><em>Amplitude</em></td>
<td>-16.31</td>
<td>-5.65</td>
</tr>
<tr>
<td></td>
<td>-17.62</td>
<td>-0.99</td>
</tr>
</tbody>
</table>

**Discussion**

We examined how zebra finch partners cope with a strong acoustic constraint on their intra-pair communication using a playback of wind noise inside the nestbox. All duets recorded in noise were shorter and quicker, and relief duets showed changes in male-female dynamic of calling (inter-call intervals and increased precision in response
timing to each other). Partners increased their effort in vocal interactions in noise (more visit duets and increased proximity during sentinel duets). Last, calls produced in the nest in noise were louder, with an up-shifted and less broadband frequency spectrum.

**Response to noise reveals potential functions of call duets**

Recent reviews on avian duetting underlined the lack of experimental evidence testing the relationship between duet structure and function (Dahlin & Benedict, 2013; Hall, 2009). Our results bring new insights on this perspective.

An increase in background noise partly impaired mate communication at the nest. First, the latency to initiate the duet tended to increase and duets were shorter (in time and in number of calls) and quicker in noise than in control. These results show that the noise treatment significantly constrained intra-pair communication. But even under this strong acoustic constraint, zebra finch mates continued to perform vocal duets each time they met at the nest, either during visit or during incubation relief. Although altered, continued duets under difficult acoustic conditions may confirm their biological significance.

The treatment did not affect visit duets and relief duets the same way. Specifically male-female dynamic was more significantly affected during relief duets, as duets performed in noise were more regular and precise, particularly when the male answered the female. This may reflect the different roles of visit and relief duets in the zebra finch intra-pair communication system. Nest relief in species with bi-parental incubation is a crucial step. A failure of relief would leave the nest unattended and could have irremediable impact on the clutch. Coordination between mates is thus essential, and could rely at least partly on call duets associated with nest relief, as
suggested by Boucaud et al. (2015). Therefore, it may be important to maintain sufficient information in relief duets.

The number of visit duets increased in noise. This could represent a strategy of signal redundancy, as many species dealing with masking background noise use redundancy to maintain signal efficacy (Brumm, Schmidt, & Schrader, 2009; Brumm & Slater, 2006). Short duets in response to noise might be compensated by increased redundancy. Visit duets might be involved either in contact maintenance and/or pair bond maintenance (Malacarne, Cucco, & Camanni, 1991; Wickler, 1980). Because incubation implies long periods of separation between mates, it may be important to keep contact. In particular, sentinel duets are hypothesized to be reassuring vocal interactions between the incubating bird, unable to detect the approach of a potential threat, and its partner showing anti-predator vigilance outside the nest, as suggested by Elie et al. (2010) and Mainwaring & Griffith (2013). Under this hypothesis, even with an acoustic constraint on their duetting activity, partners would keep duetting and may change their behaviour to facilitate vocal exchanges. Birds did not significantly modify the number of sentinel duets in response to the noise treatment, but the returning bird perched closer to the nest during these sequences. This strategy was previously observed during intra-pair communication in response to noise in the great tit (Halfwerk et al., 2012): when an increased level of background noise was broadcast inside the nest to the incubating female, the male perched closer to the nest to sing, showing that the male can use the feedback from the female to adjust his behaviour and maintain signal efficacy. We found similar results here but because both partners were subjected to the noise alternatively, we cannot conclude about the mechanism: either feedback from the partner inside the nest or previous experience with the noise
could explain the behavioural changes of the partner outside the nestbox (male or female).

Last, the fact that partners increased the number of visit duets may be a sign of an extreme increase of proximity between partners during vocal interactions. In this case we would expect a switch in duet interaction type in noise: an increase in the number of visit duets would be associated with a decrease in the number of sentinel duets. We did not find such a switch in our data; the number of visit duets increased but the occurrence of sentinel duets was not changed in noise, which emphasizes the fact that partners may reinforce vocal interactions in noise.

Noise impacts quiet vocalizations

Many studies have already demonstrated that birds modify the pitch and the amplitude of their vocalizations in response to noise, but they largely focused on loud and/or long-range vocalizations - display calls in king penguins, *Aptenodytes patagonicus* (Aubin & Jouventin, 2002), separation calls in fowls, *Gallus gallus domesticus* (Brumm et al. 2009), distance calls in common marmosets *Callithrix jacchus* (Brumm, Voss, Köllmer, & Todt, 2004), or territorial songs in several bird species (e.g. blackbirds, *Turdus merula* (Nemeth & Brumm, 2009), great tits (*Parus major*) (Brumm, 2006a; Slabbekoorn & den Boer-Visser, 2006). Studies focusing on the impact of noise on vocalizations used in more private contexts are rare and concern the quite conspicuous begging calls used in parent-offspring communication (Leonard & Horn, 2001, 2005, 2008). Our results confirm that birds modify the structure of their acoustic signals in response to background noise even if the signal is not aimed at a transmission over a long distance. The changes in acoustic features (frequency range, amplitude, or call type) that may facilitate reception efficacy under noisy
conditions seem to be the same for quiet signals used at short-range as for long-range communication signals.

Maintaining duet efficacy in response to noise may have predation costs

During an experimental increase of background noise, zebra finch pairs adjusted their behaviour and some of these adjustments (louder call duets, increased number of visit duets or change of posts of the returning partner) may have costs for their reproductive success. Increasing the number of visit duets may increase predation risk by facilitating nest site localisation. Furthermore, the adjustment made to calls (increasing amplitude and frequency), may also make the nest more vulnerable to predators, as low amplitude vocalizations are considered to represent an anti-predator strategy (Dalbelsteen, McGregor, Lampe, Langmore, & Holland, 1998). This could explain why duets recorded in noise were shorter and why partners spent less time together inside the nestbox: shorter duets and rapid relief might represent a trade-off with louder vocalizations to maintain low vulnerability to predation.

Noise impacts unlearned vocalizations in both females and males.

Male zebra finches learn their song and one of their calls (the distance call) during a juvenile phase, whereas females do not (Simpson & Vicario, 1990; Zann, 1996). This dimorphism in vocal learning has been linked to a dimorphism in brain song nuclei (the so-called song system), which atrophy in females and increase in males during development under steroid control (Bottjer, Glaessner, & Arnold, 1985). For this reason, vocal flexibility has been thought to be limited in females, and this could lead to the prediction of greater changes in males than in females during our experiment. We actually observed greater changes in females’ call structure than in males’. Moreover, most of the call types used during the vocal interactions monitored in the present study and whose structure changed in response to noise are unlearned
vocalizations in both sexes. Taken together, these results suggest that vocal flexibility
does not depend on the capacity of vocal learning and do not need a developed song
system. Indeed, vocal flexibility in response to noise has already been described in
vocal non-learners (Aubin & Jouventin, 2002; Brumm et al., 2009).

**Female and male response to noise differ**

Whereas major changes in duet structure, interaction dynamic and call structure in
response to noise were observed for both sexes, some changes were sex specific,
especially changes in male-female dynamic during the duet and in call structure.
These sex-specific changes may result from our protocol because both sexes were not
exposed to the noise playback for the same duration. The noise playback started the
day before the recording session. Because partners share incubation equally and take
turn on average once per hour, both partners experienced the noise playback and had
the time to habituate. However, zebra finch females generally spend the night in the
nest and thus incubate the eggs overnight (Zann & Rossetto, 1991). In our population,
females incubated alone in 89.5 % (±0.2) of the nights (15 pairs monitored for 136
nights, unpublished data). As a consequence, females were more subjected to the
playback than males and had perhaps more time to habituate. This may explain why
female call structure was more changed in noise than male call structure. Last, during
duets, male responses to female calls showed less variable delays in noise than in
control. Because duets are joint vocal interactions, it seems difficult to explain
separately female and male responses during the duet. The effects observed on male
or female responses to mate calls during the duet may not be sex-specific but a result
of a complex interactive communication.

To conclude, we experimentally tested the impact of elevated background
noise on intra-pair call duets at the nest in zebra finches. Even under difficult acoustic
conditions, partners maintained the three types of call duets (visit, relief and sentinel duets). This was achieved through several changes in partners’ behaviour: changes in acoustic features of the calls, in the structure of the duets, in the number of duets and in the spatial proximity between partners. Regularity and precision of partners’ interaction were enhanced only during relief duets, which may sign the importance of these duets in coordinating partners during the crucial moments of incubation shifts.

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http://doi.org/10.7717/peerj.232


Figure legends

Figure 1: Effect of treatment on the time together in nest (a), the latency to answer and the global structure of duets during relief (blue) and visit (grey).

Figure 2: Effect of treatment on the male-female temporal dynamic in duets. (a) Median, Inter-Quartile-Range and extreme values of PC1 are displayed in visit (grey) and relief (blue) duets. (b) Maximum cross-correlation peak height (mean ±se) in relief (blue circles) and visit (grey triangle) duets (significant treatment: duet type interaction $X^2$=5.5, p=0.02). (c) Cross-correlation curves between male and female signals in relief (blue circles) and visit (grey triangles) duets, showing the height of the negative and positive peaks in control (dashed lines) and noise (solid lines). Curves correspond to the mean (±se) of each peak over all duets. (d) Mean (±se)
cross-correlation over all duets in control (dashed line) and noise (solid line) for visit (grey) and relief (blue) duets. Since all data are averaged, no clear right and left peaks can be observed, because different peaks can represent different pairs. Model estimates are available in table S4. *** : p<0.001, ** : p<0.01, * : p<0.05, ´ : p<0.1.

**Figure 3: Effect of treatment on sentinel duets structure.** Median, Inter-Quartile-Range and extreme values of PCs are displayed in noise and control for: (a) PC1 and (b) PC2 of the PCA on sentinel duets. Model estimates are available in table S5. *** : p<0.001, ** : p<0.01, * : p<0.05, ´ : p<0.1.

**Figure 4: Effect of treatment on the total number of visit duets and sentinel duets (a) and location of the returning partner during sentinel duets (b) in control and noise.** Numbers above each bar indicate the number of pairs involved in the total count. Model estimates are available in table S6 and S7. *** : p<0.001, ** : p<0.01, * : p<0.05, ´ : p<0.1.

**Figure 5: Effect of treatment on calls’ structure.** (a,b) Results are medians, Inter-Quartile-Range and extreme values of PC1, presented for males and females (a) and in each call type (b) separately following significant interactions. Post hoc multiple comparisons (with Tukey correction) showed that both sexes were affected by noise (control vs noise: in females, T\textsubscript{704.0}= 11.1, p<0.001 and in males T\textsubscript{701.9}= 4.9, p<0.001) and all call types were affected by noise (control vs noise: in short calls, T\textsubscript{700.7}= 4.7, p<0.001, in ark calls T\textsubscript{694.3}= 6.9, p<0.001, in whine calls T\textsubscript{700.9}= 9.6, p<0.001). (c) Example of changes that can occur on a call spectrum (example with a short call from the same individual recorded in control and noise). Call spectrum of the control call has been corrected (see detailed procedure Fig. S4). Model estimates are available in table S8. *** : p<0.001, ** : p<0.01, * : p<0.05, ´ : p<0.1.
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Figure 1

(a) Time together in nest  (b) Latency to answer  (c) Duet global structure - PC1  (d) Duet global structure - PC2

- **Control**  - **Noise**

**PC1** - Longer duets with more calls

**PC2** - Slower duets with less short calls
Figure 2

(a) ICI Analysis

(b) Maximum peak height (scores > 0.1)

(c) Positive and negative peak heights (scores > 0.1)

(d) Male-female mean cross-correlation curve (all scores)
Figure 3
Sentinel duet global structure

(a) PC1 - faster and longer duet

(b) PC2 - faster duet with more calls

Control  Noise  Control  Noise

**
Figure 4

(a) Occurrence of duets

(b) Returning partner location

- **Sentinel Visit**
- **Sentinel duets**
- **Total number of events**
- **(a) Occurrence of duets**
- **Total number of events**
- **(b) Returning partner location**

- **Control**
- **Noise**

![Graphs showing occurrence of duets and returning partner location](#)
(a) Call structure in males and females

PC1 - Softer calls and lower frequency spectrum

(b) Call structure of each call type

PC1 - Softer calls and lower frequency spectrum

Ark calls
Short calls
Whine calls

(c) Call spectrum - one example

Corrected control call
Noise call

Figure 5
Click here to download Related material: 0.wind_snippet.wav
sound1
Click here to download Related material: 1.duet_extract_control.wav
Click here to download Related material: 3.whine_call_example.wav
Click here to download Related material: 4.short_control.wav
Click here to download Related material: 5.ark_call_example.wav