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Acoustic communication in female songbirds: functions, flexibility and plasticity in calls

Avelyne Villain

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Communication Acoustique chez les Passereaux

Femelles:

Fonctions, Flexibilité et Plasticité des cris



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Acoustic Communication in Female

Songbirds:

Functions, flexibility and plasticity in calls

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General introduction

1. The design and modulation of acoustic signals

A. Animal communication

a. What is communication?

Communication is a process of information transfer involving four components: a signal, carrying information, transmitted through an environment, from a sender to a receiver. Defining concepts is a key aspect to study a biological phenomenon and the definition of communication has been debated. Since a communication implies a relationship between at least two individuals (sender and receiver), several definitions emerged depending on how beneficial the transmission of information must be for the sender and/or the receiver to define communication (Bradbury and Vehrencamp, 2011). According to Bradbury and Vehrencamp (2011), both senders (first criterion) and receivers (second criterion) have to benefit from a communication. The first criterion implies that a communication involves signals that are aimed to be sent: so an animal which leaves scents behind as it moves or makes noises that attract predators are not considered as communications. The first criterion also implies that senders can increase the efficacy of their signals and that evolution may refine the structure of communication signals. On the other side, receivers also have to benefit from a communication (second criterion) which is interesting because (1) it may decrease the probability of misinformation from the sender and tend to the production of honest signals and (2) diverging evolution paths may be shaped depending on interests of senders and receivers. Converging interests would lead to the magnification of a signal, whereas diverging interest would lead to a succession of deceits of both parties and lead to a communication arm race (Kilner et al., 1999). As examples of signal magnification, we can notice secondary sexual characters (peacock's tail, birdsong, Andersson, 1994). As an example of communication arm race, we can notice the existence of host-parasite systems, in which parasites tend to enhance their signals to profit from their host whereas hosts tend to increase their ability to discriminate between parasitic and non-parasitic signals (ex: cuckoos-hosts arm race, (Davies et al., 1998)). The definition of

communication involving these two criteria, called 'true communication' (Marler, 1977) (Bradbury and Vehrencamp, 2011) is well spread and is very useful to study functions, mechanisms and evolution of animal communication.

Animals use several communication channels (visual, tactile, chemical or acoustic) and depending on species, one may or may not be predominant. The predominant use of a given communication channel depends on (1) the capacity of the sender to code a signal (2) the capacity of the receiver to decode this signal and (3) the environment in which the signal is propagated. In particular, the environment constrains signal propagation: signals may not propagate as quickly or as far depending on the environment. On an evolutionary perspective, environmental constraints are likely to play a key role in shaping a communication signal.

b. Why is communication so important?

Signals allow the transmission of various pieces of information: identity, sex, signalling what is likely to happen, alerting about predator or food availability, signalling physiological state... All social interactions involve communication: mate attraction, breeding, parental care, group movements, establishment and maintenance of hierarchy... Communication is the glue that holds animal societies together.

c. Why acoustics?

Among all communication channels, acoustics is widespread in the animal kingdom: from insects to vertebrates. Acoustics has the particularity to spread rapidly in the environment which allows an effective communication at short distance [between parents and their young in the egg: in crocodiles (Vergne and Mathevon, 2008), or birds (Mariette and Buchanan, 2016)] and long distance [communication calls in whales (Madsen et al., 2002), in elephants (McComb et al., 2003)]. Acoustics is also transient and do not leave evidence, contrary to chemicals (Davies et al., 2012). But, as all communication channels, acoustics has disadvantages and selection pressures act on acoustic communication. Conspicuous acoustic signals can be easily located by predators and some components of an acoustic signal degrade rapidly in the environment (atmospheric attenuation, spreading loss) or are changed by the properties of the environment (reflection and refraction on obstacles) (Bradbury and Vehrencamp, 2011). The design of an acoustic signal is thus constrained by multiple aspects and is the result of a compromise to assure efficacy and benefits for both senders and receivers.

B. The variety of pieces of information carried by acoustic signals

An acoustic signal allows the coding of information in three domains: amplitude, frequency (high or low frequency sounds, complex or pure tones), and time (duration and rhythm of vocal sequences, amplitude or frequency modulations) (more information in Box2, at the end of the general introduction). Playing with these characteristics of the sound allows fine coding of information in vocalizations.

Species identity. Acoustics signals may carry a specific signature, which may help avoiding non-adaptive inter specific communications and/or maintain reproductive barriers between closely related species living in the same habitat. A variety of parameters may be used. For example, it is possible to distinguish between five species of dolphins using only the minimum and maximum frequencies from the beginning to the end of a whistle (Steiner, 1981). In songbirds, specific signature may be coded in the song [syllable duration, frequency modulation, rhythm (Charrier and Sturdy, 2005; Mathevon and Aubin, 2001; Payne, 1986)].

Sex difference. Acoustic signals may differ between females and males. For example, the calls of blue-footed boobies, *Sula nebouxii*, or zebra finches, *Taeniopygia guttata*, differ between females and males: males have frequency-modulated calls whereas females have flat and harmonic calls. In kittiwakes, *Rissa tridactyla*, or in baboons, *Papio spp.*, female and male calls have different frequencies (Aubin et al., 2007; Rendall et al., 2004). In the African clawed frog, *Xenopus laevis*, both temporal and frequency parameters allow the discrimination of females and males (Vignal and Kelley, 2007). In species with low visual dimorphism (kittiwakes, blue-footed boobies) or in which visual cues are not relevant (nocturnal frogs living in muddy waters) identifying the sex of a conspecific using acoustic cues may be crucial to assess a mate.

Individual identity. Vocalizations also carry information about individual identity ('individual signature') and allow recognition: between parents and offspring [marine mammals, (Charrier et al., 2001, 2009), birds (Beecher et al., 1981; Levréro et al., 2009; Mulard et al., 2010), pigs (Illmann et al., 2002), sheep (Sèbe et al., 2010)], between mates [in birds: zebra finches, (Hernandez et al., 2016; Miller, 1979; Vignal et al., 2004, 2008), blue-footed boobies, (Dentressangle et al., 2012), yelkouan shearwaters, *Puffinus yelkouan* (Curé et al., 2011)], between siblings (in zebra finches, (Ligout et al., 2016)]. Acoustic parameters involved in recognition vary between species, degrees of sociality or

habitat, and closely related species may not use the same code. Identity coding and recognition between mates or between parent and chick in penguins provide a remarkable example. Nesting penguins, which have a stable meeting point rely on few parameters in their calls to identify each other [like the pitch of the calls, in Adélie penguin, *Pygoscelis adeliae*, and the gentoo penguin, *Pygosceli papua* or the harmonic structure of the calls in macaroni penguin, *Eudyptes chrysolophus* (Jouventin and Aubin, 2002; Searby et al., 2004)] whereas non nesting penguins rely on a more sophisticated information coding [two voices systems, with frequency modulations, in king penguins, *Aptenodytes patagonicus*, or emperor penguins, *Aptenodytes forsteri* (Aubin and Jouventin, 2002; Aubin et al., 2000)]. Loss of territoriality may have shaped recognition systems in non-nesting penguins.

Transient information. Within a vocalization type, the structure of the signal may be refined to precisely code a specific situation. For example, in mammals [vervet monkeys, *Chlorocebus pygerythrus*, (Seyfarth, 1980), suricates, *Suricata suricatta*, (Manser et al., 2002)] or birds [domestic fowls, *Gallus gallus*, (Evans et al., 1993)] different types of alarm calls are used to signal different types of predators (=referential coding) (Townsend and Manser, 2013). In white-browed scrubwrens, *Sericornis frontalis*, the aerial trill alarm calls also varies according to the distance from danger (Leavesley and Magrath, 2005). Vocalization structure may code physiological state [reproductive state (Elliott and Kelley, 2007), hunger level or thermal state (Leonard and Horn, 2001; Reers and Jacot, 2011)] or emotions (Briefer, 2012). For example, in mammals, an increase in vocalization rate, fundamental and peak frequencies, or energy distribution are good indicators of the intensity of an emotion and similar results were shown in birds (Perez et al., 2012).

C. Environmental effects on vocal production – main mechanisms

One important problematic regarding the study of vocal signals concerns their degree of plasticity: is the acoustic structure of a signal innate or does life experience plays a role in the design of a signal? Vocal plasticity is generally seen as a long-term process and is a prerequisite to vocal production learning.

Another important problematic regarding the study of sound signals is their degree of flexibility. Vocal flexibility occurs at very short term in response to the environment (biotic or abiotic). To what extent external factors drive vocal behaviour and signal

structure on the short-term? Does context-dependent modulation of signal structure participate in information coding?

a. Plasticity and vocal production learning

In most animal taxa, vocalizations were thought to be genetically determined and to develop without any effect of the environment (Simmons et al., 2003) and two exceptions to this rule were well described: humans and birds, in which three orders (hummingbirds, parrots and songbirds) learn some of their vocalizations (Catchpole and Slater, 2008). There is now evidence that vocal production learning occurs in more taxa than thought, particularly in mammals [in pinnipeds (reviewed in Reichmuth and Casey, 2014), in cetaceans (Janik, 2000), in bats (Knörnschild et al., 2012), in goats, (Briefer and McElligott, 2012), or in elephants (Poole et al., 2005) (and reviewed in Janik and Slater, 1997)].

Experimental procedures such as isolation from conspecifics during the development, cross-fostering experiments, acoustic tutoring or operant conditioning allowed to show that two non exclusive mechanisms are involved in vocal plasticity and vocal production learning: imitation and social reinforcement (Janik and Slater, 2000). Social reinforcement involves changes in a vocalization through social interactions whereas imitation involves copying a sound that has been heard (produced by a conspecific or not). Both mechanisms allow shaping the structure of vocal signals and one major example is song learning in songbirds (Catchpole and Slater, 2008; Marler et al., 1972). Besides birdsong learning, plasticity and learning are involved in more contexts, for example, in convergence of vocal types between individuals. Territorial neighbours in birds may share song or syllable types which allows appeasing social interactions by the ‘dear enemy effect’ (Draganoiu et al., 2014). Within social groups, the establishment of a common vocal signature may allow recognition: in birds [group signature in begging calls in zebra finches or swallows (Ligout et al., 2016; Reers et al., 2014), call convergence in parrots (Balsby and Bradbury, 2009)] but also in mammals [whales (synthesis in Mercado et al., 2004), goats (Briefer and McElligott, 2012) or bats (Knörnschild et al., 2012)].

b. Vocal flexibility

Vocal flexibility allows short-term modifications of the structure of vocal signals in response to changes in the environment (biotic or abiotic). The expression of transient information in vocalizations relies on vocal flexibility. Different social contexts may

involve the use of different variants of the same vocalization. Changes in the audience may change the structure of a vocal signal: for example, in unmated male bengalese finches, *Lonchura striata domestica*, the structure of the song differs when addressed to different unmated females, which may signal mate choice (Heinig et al., 2014). In skylarks, *Alauda arvensis*, song structure differs between spontaneous and aggressive situations (Geberzahn and Aubin, 2013). The abiotic environment may also influence the structure of a vocal signal. For example, in response to low frequency background noise, birds use vocalizations with upshifted spectrum (Bermúdez-Cuamatzin et al., 2010), or switch syllable types (Halfwerk and Slabbekoorn, 2009).

Songbirds have been model of choice for the study of acoustic communication because (1) they highly rely on acoustic communication in many social contexts (Catchpole and Slater, 2008; Marler and Slabbekoorn, 2004), (2) they exhibit high degree of plasticity and flexibility in their vocal signals (Brenowitz and Beecher, 2005), (3) their vocal repertoire is composed of both learned (songs) and non-learned (calls) vocalizations. Although the conspicuous singing behaviour of male songbirds has been well investigated, female vocal production has been neglected. In the next section, I introduce a state of the art of female vocal production in songbirds and how we can study vocal flexibility/plasticity and functions of females' vocalizations.

2. Female vocal production in songbirds

A. Female vocal production has been poorly investigated

a. Historical biases led to the negligence of female vocal production

As a mate-attracting signal, male birdsong has received most of the interest in the study of avian communication: it allows honest signalling of quality (Gil and Gahr, 2002) and song characteristic has been linked to reproductive success (Botero et al., 2009; Hasselquist et al., 1996; Soma and Garamszegi, 2011). Male song is a typical example of sexually selected trait, which has major individual fitness costs in terms of survival but also fitness advantages through the access to mates (Andersson, 1994; Catchpole and Slater, 2008; Macdougall-Shackleton, 1997; Searcy and Yasukawa, 1996). In this paradigm, females have been considered as signal receivers (Karlsson Green and Madjidian, 2011) and their vocal production has been less studied (Riebel, 2003; Riebel et al., 2005). This lack of investigation may be due to a bias toward studies of temperate species –in which females have more rarely been reported to sing (Kroodsma et al., 1996). The few records of female song in temperate zone species were considered as physiological by-products (Byers and King, 2000) or ‘isolated cases [for which] there is no need to seek a general, functional explanation’ (p123, Catchpole and Slater, 2008).

However, the vast majority of songbird species are located in the tropics, where female song is common (Kroodsma et al., 1996). Odom et al (2014) found phylogenetic evidence to support the hypothesis that female song is an ancestral trait in songbirds (Garamszegi et al., 2007). Female song was lost in several bird species of temperate zones compared to tropical zones where it was maintained. Several correlates have been proposed to explain why female song has been maintained or lost. The presence of female song correlates with social patterns [year round territoriality (Robinson, 1948), convergence in sex roles (Slater and Mann, 2004), social monogamy (Price, 2009)]. The absence of female song correlates with particular life history traits [migration (Price, 2009)] or sexual selection [correlation with sexual dimorphism in carotenoid-based plumage colouration (Garamszegi et al., 2007)].

b. Some functions of female vocalizations

The few studies that focused on the functions of female song in both temperate and tropical species showed similar functions to male song (reviewed in Langmore et al 1998), with only recent experimental evidence. Female song plays a key role in territorial defence or female-female competition (Cooney and Cockburn, 1995; Krieg and Getty, 2016). In several species, female song occurs around nest building, when the competition for breeding territories is the highest (song sparrows, *Melospiza melodia*, (Arcese et al., 1988)) or between neighbouring females (yellow warblers, *Dendroica petechial* (Hobson and Sealy, 1990)). Experiments showed that female song occurred in response to a male and/or female song playback (in the white-crowned sparrow, *Zonotrichia leucophrys*, (Baptista et al., 1993), house wrens, *Troglodytes aedon* (Krieg and Getty, 2016), superb fairy-wrens, *Malurus cyaneus*, (Cain and Langmore, 2015), stripe-headed sparrows, *Aimophila ruficauda*, (Illes and Yunes-Jimenez, 2009)), or after intrusion or simulation of intrusion of unpaired females (great reed warblers, *Acrocephalus arundinaceus*, (Kluyver, 1955), starlings, *Sturnus vulgaris*, (Sandell and Smith, 1997)). Female song can also act as a mate-attracting signal. In dusky antbirds, *Cercomacra tyrannina*, ‘courtship song’ of female and male can both play a role in mate choice. In alpine accentor, *Prunella collaris*, females sing during their fertile period and playbacks of female song attracted males (Langmore et al., 1996). It was interpreted regarding the polygynandrous breeding system in which both females and males compete to assess mates. In the polygynous reg-winged blackbirds, *Agelaius phoeniceus*, females have two types of song that may be involved in two specific contexts (territory defence vs. mate communication) (synthesis from Beletski work in Catchpole and Slater, 2008). Female song may also play a role in the coordination of breeding activities, for example functioning as a group maintenance signal (Ritchison, 1983). In the New Zealand bellbird, *Anthornis melanura*, female song rate and structure were predictors of reproductive success (Brunton et al., 2016) but it remained to be tested whether it was linked to female quality, as it has been shown in male song. Beside songs, females produce calls that may play an important role in the breeding season: territory defence (Beletsky and Orians, 1985), organisation of breeding activities (Inman, 1986).

c. Limited vocal plasticity and flexibility in female songs?

Most of the experimental work on vocal plasticity and learning was carried out in species in which females do not sing. For example, the zebra finch has been intensively used in laboratories to study vocal learning and it is one of the species in which the vocal and brain dimorphism is the most obvious (MacDougall-Shackleton and Ball, 1999). This led to the dogmatic idea that females would not express vocal plasticity in their vocalizations. However, as males do, females may learn their song and exhibit high degrees of plasticity and flexibility however experimental evidence is scarce (Riebel, 2003; Riebel et al., 2005). Some direct evidence of plasticity and learning in female song is the incorporation of heterospecific sounds, as seen in hand raised starlings, *Sturnus vulgaris*, or magpies, *Gymnorhina tibicen*, (Brown et al., 1988; West et al., 1983) or natural mimicry in starlings (Hausberger and Black, 1991), or superb lyrebirds, *Menura novaehollandiae* (Dalziell and Welbergen, 2016). In many species the female and the male of a pair share some syllable types in their song, which provide indirect evidence of plasticity and learning (reviewed in Riebel, 2003). Isolation experiments showed that, as for males, isolated females developed abnormal songs (in cardinals, *Cardinalis cardinalis*, white-crowned sparrows, *Zonotrichia leucophrys*), however tape tutoring experiments showed that females were less likely to learn from tape-tutoring than males (Baptista and Petrinovich, 1986; Cunningham and Baker, 1982), meaning that in these species females might learn from other stimuli than auditory stimuli and may require social interactions. Not enough studies of female song learning and plasticity exist in the literature to draw general conclusions.

d. More systematic studies of vocal production in both sexes are needed

Although widespread and recently demonstrated as a multifunctional vocalization, female song remains to be fully investigated. In particular, possibilities of vocal plasticity and flexibility in female vocal production have been understudied. This may come from the fact that research in songbirds has been focused on very few contexts of vocal communication (territory defence and mate attraction), in which (1) sex roles were theoretically attributed and (2) vocalizations studied were very dimorphic, in many targeted species. Under this classic paradigm, no comparison of female and male vocal productions was carried out. Studying vocal communication using similar protocols in females and males may help us understand degrees of sex differences in vocal production, potential mechanisms driving these differences and consequences (on both functional and evolutionary perspectives) (Riebel, 2016). Since research on birdsong tended to focus on

obvious and/or extravagant vocal behaviour, we may have missed contexts in which female vocalisations are as common as males’.

In the two next sections, I present to what extent (B) birdcalls may allow us to address questions of vocal plasticity and flexibility in females and males and (C) vocal communication between mates during the breeding season may allow us to investigate other functions of females and males vocal signals.

B. Birdcalls: a possibility to study vocal plasticity and flexibility in females and males

Contrary to songs mainly used for mate choice and territory defence, birdcalls are used in a wider range of contexts: alerting about predator, mobbing, parent-offspring communication, contact maintenance (Marler, 2004). In most species of birds, females and males share call types, which allow the study of vocal production in both sexes. Contrary to song, isolated birds develop their call repertoire in many species, and they do not seem to be learned (Marler, 2004).

However, several studies showed evidence for call convergence: within pairs [crossbills, *Loxia curvirostra* (Groth, 1993), several species of goldfinches (Mundinger, 1970), twites, *Acanthus jlavirostris*, (Marler and Mundinger, 1975), budgerigars, *melopsittacus undulates*, (Hile and Striedter, 2000; Hile et al., 2000)] or within flocks (the ‘chick-a-dee’ call of black-capped chickadees, *Parus atricapillus*, (Nowicki, 1989). In black-capped chickadees the development of the ‘chick-a-dee’ call is influenced by both auditory feedbacks and social experience (Hughes et al., 1998). Cross-fostering experiments in crossbills revealed that chicks develop flight calls that resemble the ones of their foster parents (Sewall, 2011), which participate in species divergence. Juveniles of some species of cuckoos mimic the begging calls of their host and their calls are shaped by the behavioural response of host parents (Langmore et al., 2008). In host species, vocal learning in calls plays a key role in parasitism detection and avoidance. For example, in Superb Fairywrens, *Malurus cyaneus*, as well as in Red-backed fairywrens, *Malurus melanocephalus*, females call to their eggs during incubation and nestling calls resemble the one of their mother, functioning as a password and allowing females to discriminate hetero specific brood parasite from offspring. The more similar the calls

between mother and young, the more food provisioning from mother to young (Colombelli-Négrel et al., 2012, 2016).

Birdcalls may also show flexibility: signalling physiological stress (male distance calls, (Perez et al., 2012) or begging calls (Perez et al., 2016) in zebra finches), thermal state (begging calls (Leonard and Horn, 2001)) or hunger levels (begging calls, Leonard and Horn, 2001; Reers and Jacot, 2011).

C. Female-male vocal production in the monogamous pair bond during breeding

a. The pair bond as a social partnership

In birds 90% of species are socially monogamous (compared to 10% in mammals) (Black, 1996). Monogamy in birds is a good example of a partnership in which female and male synchronize their activities and participate in parental care as a team (Black, 1996; Helm et al., 2006). Many long-term monogamous species show an increase in reproductive success with pair bond duration, which may be achieved by the improvement in partners' coordination (mate familiarity effect, (Black, 2001; Coulson, 1966; Forslund and Pärt, 1995). In some species, partners synchronize their foraging activities or nest visits during parental care (Lee et al., 2010; van Rooij and Griffith, 2013), and these coordinated activities may correlate with higher reproductive success (Mariette and Griffith, 2012). In cases where both partners incubate, hatching success may increase with synchronization of incubation bouts (Spoon et al., 2006). Partners may defend their resources as a team as well: alarming for danger (Krams et al., 2006), repelling predators or intruders on their territory (Black, 2001; Regelmann and Curio, 1986), alternating vigilance (McGowan and Woolfenden, 1989).

The breeding success of a pair is likely to rely on the success of pair members as a team; also, it may be crucial for individuals to succeed in behavioural adjustments and reach coordination as a pair. Because acoustic communication plays an important social role in birds, intra-pair acoustic communication during breeding is a good candidate to reach coordination.

b. Intra-pair communication during breeding: forms and functions

Interactive female-male acoustic communication has been first investigated with the analysis of song duets. Duets, performed by members of a monogamous pair, are joint acoustic displays when partners alternate or partly overlap their vocal or non-vocal sounds (Dahlin and Wright, 2009; Farabaugh, 1982; Hall, 2004, 2009). Duetting has been described over 220 avian species from 44 different families, mostly in tropical songbirds (Dahlin and Wright, 2009; Farabaugh, 1982; Hall, 2009; Hall and Peters, 2008) but also in temperate zones passerines (Benedict, 2008). Song duets performed by tropical bird species have been particularly studied and are thought to fulfil many different functions like joint resource defence, contact and recognition between partners, pair bonding and maintenance, mate guarding or reproductive synchrony [reviewed in (Hall, 2004, 2009)]. Song duets are interesting displays but are only present in 4% of bird species, mainly tropical species. More generally, intra-pair communication may involve simpler and less conspicuous vocalizations such as calls (Boucaud et al., 2016a; Gorissen et al., 2004; Halfwerk et al., 2011a; Lamprecht et al., 1985; Wright and Dahlin, 2007) or low-amplitude vocalizations (Morton and Derrickson, 1996). Studying intra-pair communication in more private contexts (at the nest for example) would allow testing for other forms and functions.

Female-male communication at the nest may allow the organization of breeding tasks or adjustments of behaviours during breeding. In northern cardinals, *Cardinalis cardinalis*, the males were more likely to visit the nest when females produced songs from the nest (Halkin, 1997). In New Zealand bellbird, *Anthornis melanura*, female song rate was correlated with male feeding (to incubating females or chicks) at the nest (Brunton et al., 2016). In breeding zebra finches, partners perform soft call duets at the nest that participate in coordinating parental care activities (Boucaud et al., 2016b; Elie et al., 2010). In great tits, *Parus major*, partners engage in interactive communication at the nest at different stages of the breeding season (Boucaud et al., 2016a). Vocal interactions between mates may lead to female feeding by the male inside the nest (Boucaud et al., 2016a). In a recent study, Boucaud et al (in press) tested the hypothesis that females could encode their physiological needs in their calls. The authors experimentally supplemented incubating females with mealworms in the nest and observed that both the temporal organization of female-male vocal interactions and the acoustic structure of their calls differed between the control and food-supplemented treatments. Their results

bring interesting insights in potential functions of intra-pair communication during breeding in a temperate zone species in which females are rarely reported to sing.

Varying the properties of the environment and studying consequences on vocal production is a key tool to assess degrees of plasticity and flexibility in vocalizations. As environmental conditions also constrain acoustic communication, birds may rely on vocal flexibility to maintain the efficacy of information transfer.

3. Background noise: a major environmental constraint on vocal communication

A. Noise and masking effect

For a given communication channel, we can define as noise everything that is not the signal. The relevance of a signal transmitted through the environment is directly linked to the signal to noise ratio (SNR, Klump 1996; Warren et al. 2006). Acoustic background noise is permanently present in natural habitats and has (1) several sources: either abiotic (wind, rainfalls, water stream) or biotic (insect or bird choruses) and (2) several characteristics: either continuous or variable, more or less predictable (Slabbekoorn, 2004). Increasing background noise leads to a decrease of the signal to noise ratio, making a signal harder to detect and/or interpret for a receiver, a phenomenon called 'masking' (Wiley and Richards, 1982) (illustration of masking effect in Box 3: examples of non-masked and masked calls, p. 41). Background noise is particularly constraining when the frequency range of the noise partly or totally overlap the frequency range of the signal (Brumm and Slabbekoorn, 2005; Slabbekoorn, 2004).

B. Negative impacts of noise: the study case of urban noise

Urbanization occurred rapidly over the past 150 years with the accumulation of disturbances (light, chemical and noise pollution, human activities) and habitat fragmentation (Barber et al., 2010). Urbanization drastically changed many aspects of behaviour, life-history and populations dynamics at the scale of species and communities. It is a new and complex environment and animals have to deal with new constraints to settle and maintain themselves in this habitat. This is why some authors qualified it a 'natural experiment' (Patricelli and Blickley, 2006) and important research effort has been made to study the impact of urbanization on wildlife, both on evolutionary and conservation perspectives.

One of the major consequences of urbanization and human activities is an increasing background noise, with rare equivalent in natural habitat. It is now known as a major pollutant for both terrestrial and aquatic life (Barber et al., 2010; Popper and Hawkins, 2011; Wright et al., 2007). Urban noise may be responsible for a decrease in species richness and changes in avian communities (Francis et al., 2009; Stone, 2000), changes in prey-predator interactions (Francis et al., 2009) and may have fitness costs [lower mating success (Habib et al., 2007), reduced clutch size (Halfwerk et al., 2011b), fewer young with lower body condition (Schroeder et al., 2012)]. Several aspects of urban noise may explain its effect on animals. First, noise directly impacts the physiology (hearing, stress levels, DNA integrity for example, reviewed by Kight and Swaddle, 2011). Second, anthropogenic noise is a low frequency noise, with most of the energy from 0 to 2 kHz that partly overlaps with the frequency range of animal vocal signals.

Social acoustic communication is impaired by urban noise and may impact reproductive success. In birds, parent-offspring communication may be disrupted (Leonard and Horn, 2005; Leonard et al., 2015) and female-male communication may also be impaired by noise. Laboratory experiments showed noise impairs pair preferences in zebra finches, *Taeniopygia guttata*, (Swaddle and Page, 2007) and female preferences in canaries, *Serinus canaria* (Huet des Aunay et al., 2014). In great tits, males' low frequency songs, used before laying, lose their potency under noisy conditions (Halfwerk et al., 2011a). However, strong relationships between disruption of acoustic communication and reproductive success are difficult to establish (Leonard et al., 2015; Meillère et al., 2015). This may result from the variety of adjustments from both senders

and receivers of acoustic signals. In particular senders use several adjustment strategies in response to the noise constraint that counteract the masking effect of noise and maintain signal efficacy.

C. Vocal adjustments in response to noise

Animals have been reported to change their vocal behaviour in response to background noise. In this section, I list examples of noise-dependant changes in vocal production (see table 1). All of these vocal adjustments tend to avoid the masking effect of noise and increase signal efficacy under noisy conditions.

a. Vocalizing louder

In response to a high level of noise, animals may increase the amplitude of their vocalizations, an effect known as the Lombard effect (Lombard, 1911). Initially demonstrated in humans, the Lombard effect has been shown in many vertebrates (see table 1 for species, reviewed in Brumm and Zollinger, 2011). The Lombard effect occurs as an immediate response to high background noise levels. Many studies on the Lombard effect investigated the response to artificial white noise or urban noise, even when carried out in a natural setting (Brumm, 2004). In this study, nightingales, *Luscinia megarhynchos*, living in areas with higher levels of anthropogenic background noise produced louder territorial songs. Only one study to my knowledge investigated the Lombard effect in response to natural noise of the habitat, in hummingbirds, *Lampornis clemenciae* (Pytte et al., 2003).

Table 1: Synthesis of the four categories of effect of background noise on vocal production depending on the study site (Natural, Urban or Laboratory), the type of constraint studied (when studies separated effects from noise vs. urban habitats), the type of study (B = correlational between population, W = correlational within population, E = Experimental within population). ‘?’ indicated when information unsure. References at the end of the manuscript.

Type of vocalizations	Study site	Type of constraint studied	Type of study	Effect	Species
Modulation of amplitude (Lombard effect)					
Long-range (?)	Natural	Creek noise	W + E	Louder vocalizations	Blue-throated hummingbird, <i>Lampornis clemenciae</i> ¹ ; torrent frog, <i>Odorrana tormota</i> , ² ;
Long-range	Natural	Urban noise	W	Louder songs	Nightingales, <i>Luscinia megarhynchos</i> ³
Long-range	Laboratory	White noise	E	Louder songs and calls	Zebra finch, <i>Taeniopygia guttata</i> ⁴ , Cotton-top tamarins, <i>Saguinus oedipus</i> ⁵
Short-range	Laboratory	White noise	E	Louder calls	Japanese quail, <i>Coturnix coturnix japonica</i> ⁶ ; Domestic fowls, <i>Gallus gallus</i> ⁷ ; Marmoset, <i>Callithrix jacchus</i> ⁸
Speech	Laboratory	White noise	E	Louder speech	Humans, <i>homo sapiens</i> ^{4,9}
Variation in the frequency range					
Long-range	Urban	Anthropogenic noise	W	Higher minimum frequency	Great tit, <i>Parus major</i> ¹⁰ ; song sparrow, <i>Melospiza melodia</i> ¹¹
Long-range	Urban-Non urban	Urban habitats	B	Higher minimum frequency in songs and calls, in song: lower syllable rate, shorter notes, shorter song	Silvereyes, <i>Zosterops lateralis</i> ¹² , Great tits <i>Parus major</i> ¹³ ; several species of oscines and non oscine birds ¹⁴ , Black birds, <i>Turdus merula</i> ¹⁵ ; Chiffchaff, <i>Phylloscopus collybita</i> ¹⁶
Short-range	Urban-Non urban	Urban habitats	B	Higher (min) frequency	Silvereyes, <i>Zosterops lateralis</i> ¹² ; California ground squirrel, <i>Spermophilus beecheyi</i> ¹⁷
Long-range (?)	Natural	Natural forest or stream noise	W	Higher min frequency, pure tones, Ultrasonic vocalizations	Little greenbul, <i>Andropadus virens</i> ¹⁸ , birds genus, <i>Phylloscopus</i> , Frogs genus (<i>Rana</i>) ¹⁹ ; Torrent frog, <i>Amolops tormotus</i> ²⁰ ; Old World frog, <i>Amolops tormotus</i> , Rufous-faced warbler,

						<i>Abroscopus albogularis</i> ²¹
						Torrent frog, <i>Amolops tormotus</i> ²
Long-range (?)	Natural	Natural stream noise	E	Higher F0 in ultrasonic vocalizations		Great tits, <i>Parus major</i> ²² , Chiffchaff, <i>Phylloscopus collybita</i> ¹⁶
Long-range	Natural	Antropogenic noise	E	Higher minimum frequency		Cotton-top tamarins, <i>Saguinus oedipus</i> ^{5,23}
Long-range	Laboratory	White-noise	E	Higher minimum frequency		House finches; <i>Carpodacus mexicanus</i> ²⁴
Long-range	Laboratory	Antropogenic noise	E	Higher minimum frequency		Humans, homo sapiens ²⁵
Speech	Laboratory	?	?	Higher pitched speech		
Redundancy						
Long-range	Natural	Natural stream noise	W	Longer songs		Chaffinch, <i>Fringilla coelebs</i> ²⁶
Long-range	Natural	Wind noise	W	More calls, more syllables per calls		King penguin, <i>Aptenodytes patagonicus</i> ²⁷
Short-range	Laboratory	White-noise	E	Longer bout of calls and increased rate		Japanese quail, <i>Coturnix c. japonica</i> ⁶
Long-range	Natural	Antropogenic noise	W	Longer calls		Killer whale, <i>Orcinus orca</i> ²⁸
Long-range	Laboratory	Antropogenic noise	E	Longer songs, longer syllables		Zebra finch, <i>Taeniopygia guttata</i> ²⁹ House finche; <i>Carpodacus mexicanus</i> ²⁴
Long-range (?)	Laboratory	White noise	E	Longer calls		Marmoset, <i>Callithrix jacchus</i> ⁸ ; cotton-top tamarin, <i>Saguinus oedipus</i> ²³
Speech	?	?	E	Longer words		Human, <i>Homo sapiens</i> ²⁵
Timing changes						
Long-range	Laboratory	Heterospecific noise	E	Changes in timing of vocalizations		Nightingale, <i>Luscinia megarhynchos</i> ³⁰
Long-range	Urban-non urban	Urban habitats	W	Earlier down chorus, night singings		Several oscine birds species ^{31,33} ; European robin, <i>Erithacus rubecula</i> ³²

b. Changing the frequency range of vocalizations

Animals may also change the frequency range of their vocalizations to avoid the masking effect of background noise. Birdsongs produced in cities have an increased minimum frequency (see species table 1), making them higher pitched. This change has been described in many contexts: in urban vs. non-urban correlational studies (Potvin et al., 2011; Slabbekoorn and den Boer-Visser, 2006), within urban areas with different levels or background noise (Slabbekoorn and Peet, 2003; Wood et al., 2006) or in the laboratory (Hotchkin et al., 2015; Leonard and Horn, 2008). One study on the calls and songs of several species of oscine and non-oscine birds showed that some species were more likely to have upshifted minimum frequency in urban habitats than others. In particular, species with already high-pitched vocalizations were less likely to show a spectral shift in cities (Hu and Cardoso, 2010). Similar phenomena have been observed in natural habitats. For instance, in the particularly loud and broadband background noise of torrents and waterfalls, the torrent frog uses ultrasonic calls (Feng et al., 2006). An observational study hypothesized a convergence between birds and frogs living near torrents that both use high pitched and pure tone vocalizations (Dubois and Martens, 1984). Authors suggested that concentrating the energy in a very narrow frequency band could provide greater amplitude to the call and thus favour the propagation of pure notes over distances.

c. Making the signal redundant

In response to background noise, animals may also increase the probability for a receiver to get the message by making the signal longer or repeating it. Increased duration is common in mammals (Brumm et al., 2004; Foote et al., 2004; Hotchkin et al., 2015), but apparently not in avian species. In tree swallows, Leonard et al (2005) found that the duration of begging calls increased with the level of ambient noise in the field but this correlative result could not be reproduced experimentally in the laboratory (Leonard and Horn, 2008). In domestic fowls, *Gallus gallus*, Brumm et al found no evidence of an increase in signal duration (2009). However, birds do increase signal redundancy by increasing the number of vocalization units per bout in response to noise (king penguins' calls, zebra finches' songs, Japanese quails' calls, chaffinches' song, table 1).

d. Changing timing of vocalization

Instead of modifying vocal signals, another way to increase the probability for a signal to be detected by a receiver is to avoid vocalizing when or where the constraint is too strong. For examples, songbirds living near airports advance their dawn chorus and avoid masking noise from aircrafts (Gil et al., 2014), nightingales avoid heterospecific noise from other bird species by singing between noisy time intervals (Brumm, 2006) (table 1).

D. Studying vocal signals in response to variations of natural background noise

As we saw with the short review of the noise-dependent vocal adjustments reported in the literature (table 1), many of them were described in response to urban noise. According to some authors, urban habitats may be the setting of microevolution processes in bird species (reviewed in Slabbekoorn and Ripmeester, 2008). One hypothesis is that urban noise may accelerate reproductive divergence between urban and non-urban populations (through adaptive vocal adjustments and social feedbacks).

However, species exposed to urban noise also deal with a new habitat diverse constraints (light and chemical pollution, food provisioning, human activities) and it is still difficult to disentangle the effect of noise from other confounding factors. To study the impact of elevated noise on vocal production we may profit from studying species that either evolved under constantly noisy conditions or experience unpredictable noisy conditions in their natural habitat.

E. Studying acoustic communication in noise on short-range vocal signals

Because the vast majority of social interactions rely on short-range signals (Marler, 2004), noise-dependent disruption of this communication may be particularly costly. What would be the adjustments of these signals in response to noise?

Various studies interpreted vocal adjustments in response to noise using propagation constraints. Louder and higher pitched vocalizations are less degraded in noisy

environments and increase the probability of detection (Nemeth and Brumm, 2015; Patricelli and Blickley, 2006; Warren et al., 2006). Short-range signals are less subjected to degradation. If vocal adjustments in response to noise are adaptations to maximise signal transmission, we expect either no signal modification or different modifications in short-range vocal signals. However, if vocal adjustments are driven by other factors (physiological, hearing feedbacks), we may see similar changes in short range signals as in long-range signals. In addition, since senders may adapt their vocal amplitude to the distance from the receiver (Brumm and Slater, 2006), short-range signals are expected to be softer than long-range signals. In that case, short-range signals may be particularly affected by increasing background noise level. Only few studies focused on these short-range signals and described noise dependent variations of their structure [contact calls within social groups (Potvin et al., 2011), begging calls during parent-offspring communication (Leonard and Horn, 2008) or calls in non-oscine birds (Hu and Cardoso, 2010)]. More studies are needed on short-range vocal signals (Wong and Lowry, 2016), to (1) describe noise-dependent vocal adjustments in more social contexts and (2) to highlight the possible mechanisms driving these adjustments.

The possibility of vocal adjustments may depend on the capacity of the sender of vocal flexibility and plasticity. Learn and non-learned vocalizations may show different vocal adjustments. For example, non-human primates or non-oscine birds are thought to have limited vocal plasticity in signal spectral structure and rely more on temporal redundancy and Lombard effect than spectral shifts in response to increased noise level. The social context of production may also play a role in the use of a particular strategy in noise-dependent vocal adjustments.

4. Questions, hypotheses, scientific approach

As seen in previous sections, female vocal production in songbirds has been neglected and this thesis aims at bringing more knowledge about the functions and the degree of plasticity and flexibility of female vocalizations. I explored female vocal production in two contexts: female-male vocal communication at the nest during breeding and parent offspring communication during early life. I asked three different questions:

- Does female and female-male vocal communication at the nest reflect parental care activities during the breeding season in a socially monogamous songbird?
- How do females and males of a monogamous pair adapt their vocal signals at the nest in response to natural abiotic constraints?
- Is call development of female and male nestlings influenced by early social environment?

Q1: Does female and female-male vocal communication at the nest reflect parental care activities during the breeding season in a socially monogamous songbird?

Female vocalizations could play a role in the organization of breeding activities (either using solo songs, calls or during interactive communication with their mate). So far, intrapair communication was extensively studied in duetting species and not particularly during breeding. Investigating female-male communication during breeding offers the possibility to test this hypothesis. Intrapair communication at the nest during breeding has been explored in only few species (zebra finches, tits, northern cardinals). In particular, intrapair communication at the nest in a species in which females have been reported to sing would be interesting to disentangle the functions of female solo vocalizations and female vocalizations produced in interaction with their mate.

Our working hypothesis is that female-male communication at the nest allows the organization of breeding activities and we have two predictions. If female-male communication participates in organizing breeding tasks, vocal communication should

(1) depend on breeding stages (incubation, chicks rearing) and change as the breeding season progresses and (2) correlate with partners' behaviour during breeding stages. I tested these predictions in a seasonal monogamous species of the temperate zone, in which both females and males sing: the white-throated dipper, *Cinclus cinclus*.

Q2: How do females and males of a monogamous pair adapt their vocal signals at the nest in response to natural abiotic constraints?

Background noise is a major constraint on vocal communication in birds and a good tool to investigate expression of vocal flexibility. Several strategies of vocal adjustments in response to elevated noise have been shown in the literature and provide a strong theoretical framework. Unfortunately the vast majority of studies address the question on male birdsong and in response to anthropogenic noise. There is thus a lack of investigation of (1) the effect of noise on less conspicuous signals, such as short-range vocal signals (2) the effect of noise on female vocal signals (3) the effects of natural background noise. In particular, to study long term and short-term effects of elevated noise, we need to study species that evolved under constraining environmental noise in their natural habitat.

The context of intrapair communication at the nest offers the possibility to investigate the effects of elevated background noise on short-range vocal signals in females and in males. Because intrapair communication is likely to be a key factor of the pair's success, vocal signals used during vocal interactions at the nest are expected to resist to environmental constraints and show noise-dependent adjustments. Regarding pair responses to noise, we had several predictions.

First, in a species that evolved under continuously high background noise, we had two predictions: either birds evolved vocalizations that avoid the acoustic constraints of the background noise (particularly loud vocalizations with frequency range outside the frequency range of the background), or they adjust their vocal signals to the local characteristics of the noise (selection of vocalizations, vocal adjustments). I tested these predictions in the white-throated dipper, a species dependent on fast running rivers.

Secondly, in a species that evolved under discontinuous elevation of background noise, pairs would either adjust their vocalizations on the short-term to continue their vocal interactions even under difficult conditions, or they would wait for the conditions to go

back to normal and vocalize after. I tested these predictions in the zebra finch, a species experiencing highly variable wind conditions in its natural habitat.

Q3: Is call development of female and male nestlings influenced by early social environment?

In species in which females do not sing, sex differences in vocal plasticity are clear. Two questions arise: what about plasticity in other vocal signals than songs? How early sex specific plasticity is expressed? Although birdcalls were supposed to be non-learned, they nevertheless show potential for plasticity. In most species, both females and males share the same call types. Birdcalls are thus excellent models to investigate sex differences in vocal production and development. To investigate sex differences in vocal development, one approach is (1) to study vocalizations that are produced before the period of song learning in females and males and (2) to artificially modify the social environment to elicit vocal plasticity. Cross fostering is a good tool to test vocal plasticity, if foster parents have different social behaviour or different vocalizations. If vocal plasticity abilities are expressed during early life, call development should differ in response to a cross fostering experiment. Using the zebra finch as a study species, I investigated vocal plasticity in begging call development in response to variations in social environment.

I used both correlative and experimental approaches on two target species: the zebra finch, *Taeniopygia guttata*, in the lab and the white-throated dipper, *Cinclus cinclus*, in the wild. In the next sections, I present these two species regarding their interest to address my questions.

5. The white-throated dipper



Figure 1: A pair of white-throated dippers, *Cinclus cinclus*, sexually monophormic and territorial species of temperate zones. Both females and males sing. [Photograph taken in Chartreuse mountains, France, credits: Alain Blanc].

A. A naturally noisy habitat

a. Dependent on noisy running waters

The white-throated dipper, *Cinclus cinclus*, is a medium-sized passerine (fig. 1). Dippers depend on fast-flowing rivers for foraging in which they mainly feed on aquatic invertebrates (Tyler and Ormerod, 1994). Dippers are territorial, with breeding territories ranging from 300-400 m up to 2500m along a river. More than altitude, the gradient is one of the main factors explaining density with gradients from 2.5 to 20m/km (Tyler and Ormerod, 1994). The abundance and presence of breeding pairs partly depend on the presence of rocks, shallow water and pools (Tyler and Ormerod, 1994). River noise has a typical pink noise (Marler and Slabbekoorn, 2004) with a peak at low frequencies and attenuated higher frequencies, which overlap with the frequency range of vocalizations of the vast majority of songbirds. Dippers live under constant and constraining environmental noise. Noise characteristics (gradient, presence of rocks) may vary

between rivers and various types of territories may exist (various noise level and frequency range of noise).

b. Nest sites are also expected to be noisy

Dippers unvariably nest over the water (Shaw, 1978; Smiddy et al., 1995). In mountainous streams they use natural cavities on rocks, tree roots and even behind natural waterfalls. In more man-modified rivers they use mainly human structures such as bridges, dams and walls (Shaw, 1978; Tyler and Ormerod, 1994). They also readily accept nest boxes placed under bridges (Smith L. and Cross T., 2012) (Box 1 for information on our study site). Because of the variety of nest sites, noise characteristics at the nest may be more diverse than pink noise. For example, waterfall noise is known to be closer to a white noise (with energy in all frequencies) than a pink noise; river noise may be reverberated under bridges. Nest sites are also likely to be at least as noisy as other parts of their territory and dippers breed in constant noise.

B. A monogamous species with asymmetric sex roles

Dippers are socially monogamous with rare cases of polygyny (when several females build a nest within a single male territory) (Marzolin, 1988). Pairs form from January to March. After pair formation, females choose a nest site and males defend the territory (Tyler and Ormerod, 1994). Only females incubate eggs and brood hatchlings but both partners participate in feeding the offspring.

Most surviving birds stay on the same territory from one year to the next (i.e. breeding dispersal is rare, less than 5%, personal comm. B. Doligez) (Tyler et al., 1990), and pairs may stay together in winter. Pairs breeding in altitude may migrate to winter territories (Tyler and Ormerod, 1994). Considering survival rates in adults, which is about 0.52 in both sexes (Loison et al., 2002), pairs may stay together for several breeding seasons.

Most of pair bonding behaviours occur at pair formation on winter territories and are inconspicuous when the pair is already established (Tyler and Ormerod, 1994).

Box 1: Field study on Dippers. A long-term study of a wild population of white-throated dippers started in 2014, in and around the Parc Naturel Régional de Chartreuse, FRANCE (46.20N, 5.40E, fig. 2) (Blandine Doligez’s CRPBO Personal Program 655). The site comprises relatively mountainous habitat and contains a series of watersheds suitable for dippers’ breeding ranging from 200m to 1100m. Nest boxes, made of PVC tubes (20cm diameter, 30cm long) were installed under bridges (by B. Doligez, colleagues and students) (fig. 2) their occupation started quickly and the number of monitored breeding dippers increased with the years, with an occupation rate of nest boxes from 1 to 25% over the three years (table 2).

Figure 2: Dippers field site (summer 2016). Location in France (a) (Grenoble is pointed) and geographic maps with nest boxes (squares) and natural cavities (circles and stars depending on their occupation) (a to c, source: geoportail), water network has been highlighted in blue. Two zones were zoomed to illustrate low altitude (east) and mountainous habitats (west) (respectively b and c). Photos of different nest box sites, with various types of rivers and natural environmental noise (d to f).

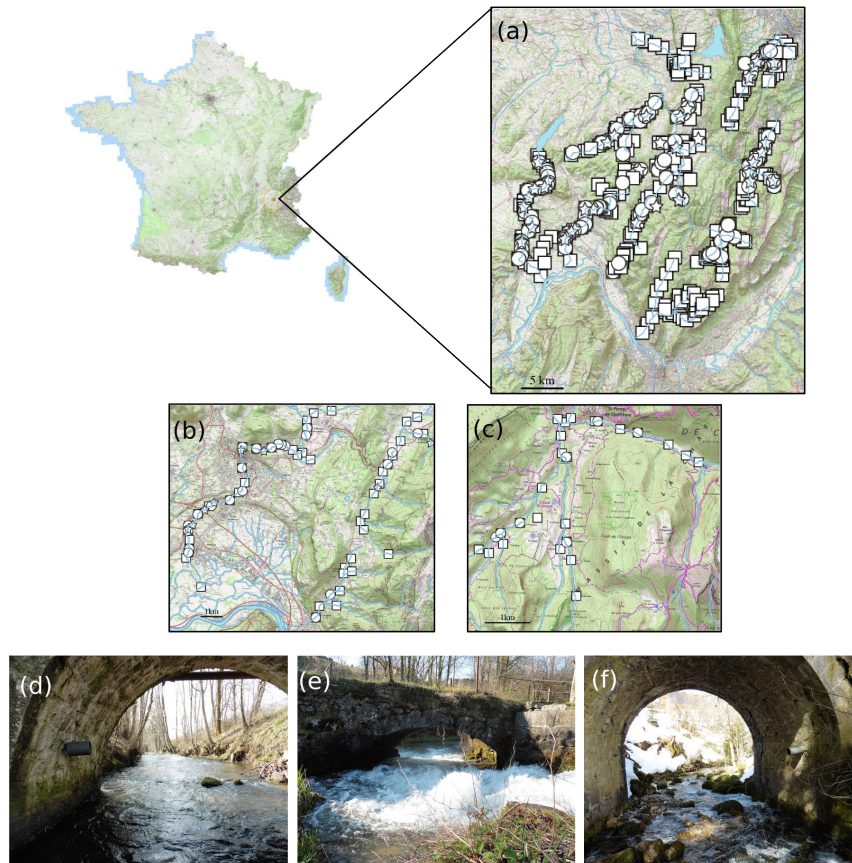


Table 2: Nest boxes installation and breeding pairs monitoring. Counts of only active nest sites (i.e with at least one egg laid), one site (natural or nest box) could host several breeding events during a given season.

Nest boxes installation periods	Cumulative number of nest boxes in study site	Breeding season	Active nests sites	Active nest sites in boxes
February to March 2014	58	2014	46	1
September to November 2014	208	2015	86	42
September to November 2015	267	2016	117	69
July to October 2016	340	2017	?	?

C. Vocal communication remains to be investigated

Dippers are one of the rare temperate zone songbirds in which both females and males have been reported to sing during courtship and pair formation. Some studies mention that both sexes sing equally, others mention that the male is more vocal [synthesized in (Tyler and Ormerod, 1994)]. Descriptions of the vocal repertoire in both sexes are however rare and reduced to literal description of the courtship song that could be sung alone or as part of a display (p 91, Tyler and Ormerod, 1994):

‘The song of the male includes a variety of notes, in any order with short phrases repeated in single units. By contrast, the female’s song is less sweet, being a series of whistles and disconnected units [...] Female’s song is less melodious and more scratchy than that of the male.’

Dippers sing all year long except during moult in summer and very cold winter episodes, and song rates are particularly strong during settlement and territory defence. Males may sing more than females, during nest building and incubation. Females may sing off the nest when their mate is approaching (Tyler and Ormerod, 1994). Pair formation and pair bonding behaviour was described as associated with “rattling call”, ‘err’ or ‘zuurrr’” (Tyler and Ormerod, 1994) but no detailed acoustic characteristics and repertoire structure of these calls are available to my knowledge. Part of this description is the aim of chapter 1. In figure 3 three vocalization types are illustrated: a trill-like call (which may or may not be the “rattling call”), a flight call and some song syllables. Overall, vocal communication in dippers is poorly studied even though this species represents a good model to study vocal production in both females and males in temperate zones. Dippers are also a good model to study how natural environmental background noise may constrain vocalization structure. Nevertheless, to my knowledge only one report compared the frequency spectrum of dipper flight calls to river noise (fig. 4). Authors illustrated the use of a ‘silent window’: ‘Birds evade acoustical masking of their vocalizations by calling at frequencies higher than the typical background noise of their habitat’ (Brumm and Slabbekoorn, 2005).

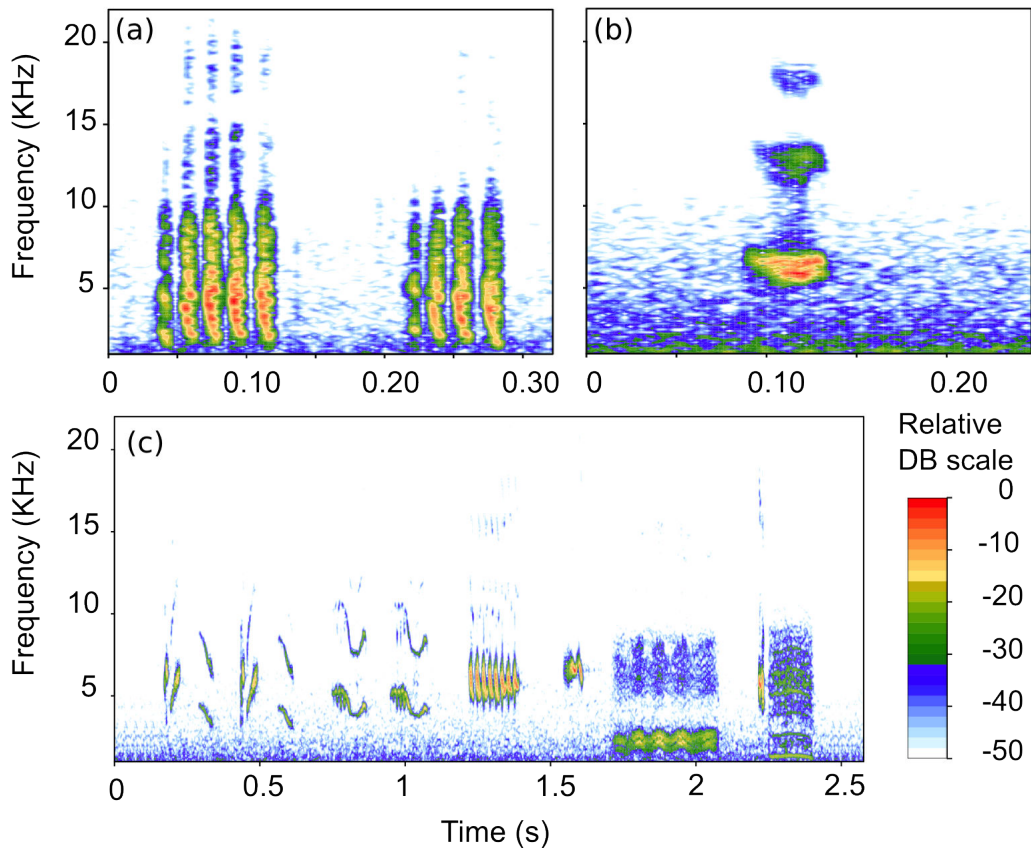


Figure 3: Examples of dippers' vocalizations illustrating the common vocal repertoire of females and males, spectrogram of a Trill call (a) (which may or may not be the 'rattling' calls described in Tyler, 2002.), a Flight call (b) and an extract of a song illustrating different types of Notes (c). All vocalizations illustrated here are females' and produced from the nest where a microphone was installed (see chapter 1,2,3) (sound files: chapter1-3, from #1 to #3).

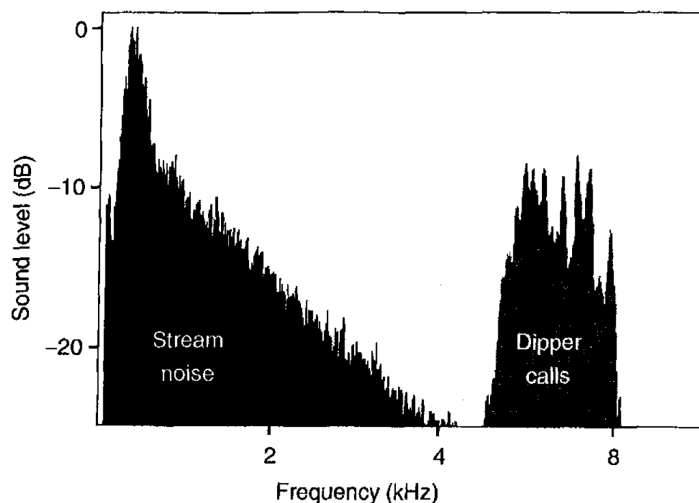


Figure 4: Spectrum of Dipper calls (flight calls probably) and water stream noise, from Brumm and Slabbekoorn (2005). This figure is part of a more general review of acoustic communication in noise.

6. The zebra finch



Figure 5: A pair of zebra finches, *Taeniopygia guttata*, sexually dimorphic and gregarious songbirds. Female above the nest box, male in the hole. Only males sing, both sexes produce calls. [Photo taken in the wild, Fowlers Gap Arid Zone, Australia. Credits: Marie Fernandez]

A. A laboratory model from behaviour to genetics and neuro-endocrinology.

The domesticated zebra finch (fig. 5), a gregarious songbird, has become a worldwide study system to investigate vocal behaviour in the laboratory. It is an opportunistic breeder and sexual maturity is acquired rapidly, which allows rapid establishment of laboratory colonies. Vocal learning occurs in a limited period of time, from day 10 to day 90 post hatching (Zann, 1996). Male song received great interest to understand the processes of vocal learning in songbirds (Slater et al., 1988): from the imitation of an adult tutor (Zann, 1990) to the social influence of the group on song structuration (Derégnaucourt and Gahr, 2013). The neuronal (Scharff and Nottebohm, 1991; Sohrabji

et al., 1990; Theunissen et al., 2004a) and genetic basis (Forstmeier et al., 2009) of song learning have also been investigated and the zebra finch was selected as the first songbird genome to be sequenced (<http://www.songbirdgenome.org>). It allows now the integration of behaviour, genetics and brain study to understand vocal plasticity and learning in songbirds (Clayton et al., 2009).

B. A common call repertoire in both sexes

Although only zebra finch males sing, both sexes use the same categories of calls in diverse behavioural contexts (fig 6 and Zann, 1996): contact maintenance within the group (Tet, Distance calls), social bonds (Cackles, Ark, Whine) or aggressive behaviour (Wsst). In a recent paper revisiting the zebra finch's vocal repertoire, Elie and Theunissen (2015) were able to clearly distinguish each of the 11 call types from one another by both trained ears and quantitative analyses. Some zebra finch calls already received interest in terms of vocal plasticity, flexibility and function (The Distance and the Begging call). In other calls, only the context of occurrence has been described but the function, the potential vocal flexibility and plasticity have not yet been investigated (Distress, aggressive, Nest and Tet calls).

Distance calls. They allow members of a group or a pair to keep contact over distances. They are sexually dimorphic (see fig. 6) and the vocal development differs between sexes. Males learn their Distance calls concomitantly with the song by imitation of an adult tutor (Zann, 1990). Using nerve sections, Simpson and Vicario (1990) showed the development of the Distance call involves the activation of song specific brain pathways in males and not in females. The female Distance call, on the contrary, is supposed to be stable along the vocal development. This study was performed on only seven birds (one out of four males did not vocally respond and one out of three females showed a difference in call duration after the nerve section) and no further experiments were carried out in females. Distance calls carry an individual signature stronger in males than in females (Forstmeier et al., 2009), but stable after propagation over long distances in both sexes (Mouterde et al., 2014a). This specific vocal signature allows conspecific recognition in both sexes, especially between mates. Laboratory experiments showed that mates recognized each other through Distance calls (Hernandez et al., 2016; Vignal et al., 2004, 2008) and that females learned to recognize individual Distance calls even

degraded (Mouterde et al., 2014b). The equivalent experiment with males has not been carried out. Beyond the intrinsic individual features of Distance calls, the acoustic structure is flexible and carries transient information. Stress level in males is expressed in the acoustic structure of their Distance calls –increased fundamental frequency, duration and spectral noisiness (Perez et al., 2012), and females can perceive the stress level of their mate through his Distance call (Perez et al., 2015a). So far, a similar experience on females has not been published.

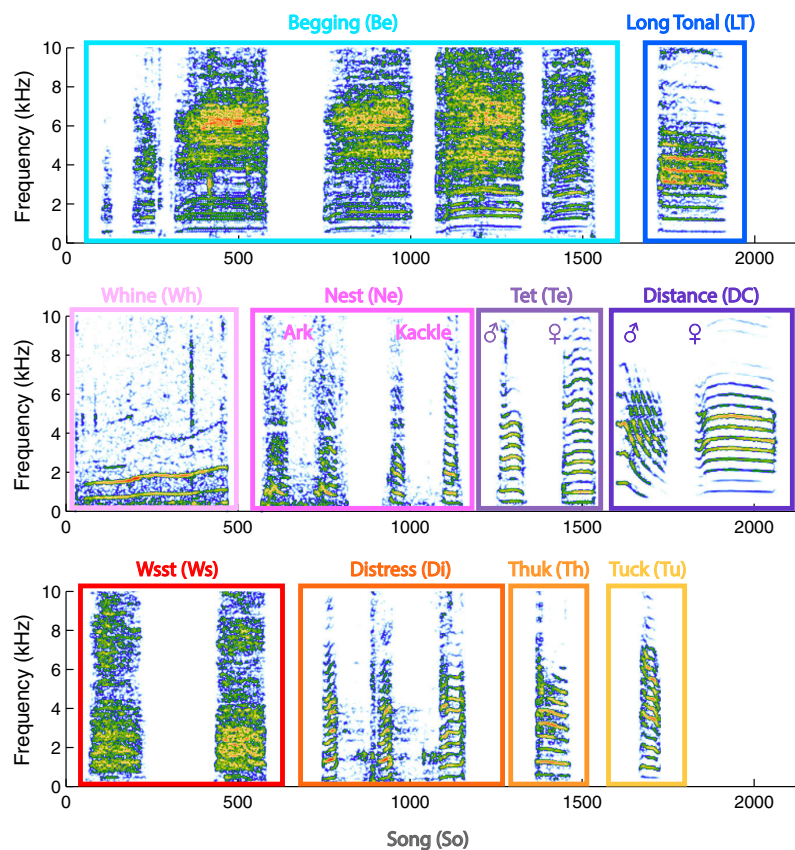


Figure 6: Call repertoire common to female and male zebra finches, adapted from (Elie and Theunissen, 2015). Spectrogram of each call category of the zebra finch repertoire, with juvenile specific calls (first row), adult affiliative calls (second row) and non-affiliative calls (third row). Distance and Tet calls show a sexual dimorphism [sound files for whine, ark and tet: chapter4 from #3 to #5].

Begging calls. They are produced by juveniles to get fed by their parents, from about day three to day 40 after hatching. Begging calls carry an individual signature that allows parent-offspring recognition (Levréro et al., 2009) but also a nest-mate recognition after fledging (Ligout et al., 2016). In fledglings, begging calls also carry a brood signature that may or may not be acquired through social shaping. Begging calls also carry labile

information –such as stress levels (Perez et al., 2016). Potential plasticity has not been tested yet.

Other adult calls. Although the characteristics, development and functions of Distance calls seem clear, the other adult calls (Tet, Whines, Ark, Cackles...) have been poorly studied so far. They may reflect activities of the group: tets are supposed to be the most generic calls, functioning as contact calls and a playback experiment showed they allow mate recognition (Elie et al., 2010) whereas Whine, Ark and Cackles are related to breeding. Recently, Gill et al. (2015) showed that switching from neutral social to breeding contexts was associated with a decrease in the number of Distance calls and an increase in the number of Cackles and Whines. Tet production remained unchanged. The calling dynamic changed in the group and call types used in response to one another showed specific combinations: Cackles were used in response to Cackles for example. Studies have started to focus more on these calls only recently. Functions, potential plasticity and flexibility remain to be investigated.

C. A strong and symmetrical monogamous social bond

Zebra finches are social birds, living in flocks, and the primary social unit is the life-long monogamous pair bond. When paired, partners exhibit specific pro-social behaviours (allopreening, clumping) with a lack of agonistic behaviours towards each other (Elie et al., 2011). They spend the majority of their time together year-round (McCowan et al., 2015; Zann, 1996). Pair disruption mainly happens when one of the partners disappears from the colony, probably after dying (Immehmann, 1966; Zann, 1996). Experimental separation of partners leads to an increase in stress hormone level that goes back to baseline when pair members are reunited (Ramage-Healey et al., 2003). Extra-pair copulation is rare in the wild compared to other songbird species: less than 2%, (Griffith et al., 2010), compared to 11% on average in other socially monogamous passerine species (Griffith et al., 2008).

During the breeding season, partners have reciprocal roles: both partners chose the site together, build the nest, incubate eggs and provide food to the young. Partners are also highly coordinated: they start incubation on the same day (Gilby et al., 2013) and share incubation time equally (Delesalle, 1986; Gilby et al., 2013; Gorman et al., 2005; Zann and Rossetto, 1991). Incubation is the only breeding stage when partners are separated

from each other (when one partner incubates the eggs, the other partner forages). After hatching, they synchronize visits to the nest and to foraging patches (Mariette and Griffith, 2012). Brood size manipulation experiments showed foraging and nest synchrony varied with brood size and were predictors of nestlings mass (Mariette et al., 2015). Behavioural coordination in this species seems to be a strong determinant of breeding success. However, the behavioural mechanisms involved in the setting and the maintenance of this coordination is not known. As acoustic communication is central in social interactions in this species, and particularly in the pair bond: intra pair acoustic communication is a good candidate to reach such coordination.

D. Constant vocal communication may support the pair bond

Zebra finch mates keep constant visual and/or acoustic contact, using Tets for short-range communication, or Distance calls when visually separated (Zann, 1996). Separation-reunion experiments showed that partners adapt their calling activity and increase precision in their vocal interactions when visually isolated (Perez, Fernandez et al., 2015a). Socially isolated females may also use the Distance call of their mate as acoustic reward (Hernandez et al., 2016). Females are also able to perceive acute stress in their mate's Distance call, which will provoke an increase in their stress hormone levels (emotional contagion, (Perez et al., 2015a)). Therefore, vocal interaction between mates may allow the transfer of precise information. In 2010, Elie et al., first described private vocal displays between mates at the nest during the breeding season. On free-living zebra finches, during incubation and chicks rearing, they described call exchanges occurring either when partners met at the nest or when one partner was a few meters away from the nest and the other at the nest. Results showed that partners can both initiate the call exchange and participate equally to the exchange and that call alternation was not random (fig. 7). These vocal exchanges could thus be seen as call duets. Two behavioural contexts were identified: 'meeting' duets when partners were both at the nest and 'sentinel' duets when one partner was a few meters apart from the nest. Among meeting duets performed during incubation, some may end in one partner relieving the other and taking its turn incubating (relief duets) while others end up without relief (visit duets). Because these call duets occur when partners take turn incubating or brooding, they can function as greeting ceremonies, or allow contact maintenance between partners. But the

main hypothesis the authors proposed is that duets may participate in the coordination of breeding activities during incubation / brooding. Recently, artificially modifying partners' incubation coordination, we found that calling rates during relief duets were good predictors of incubation share between partners (Boucaud et al., 2016b).

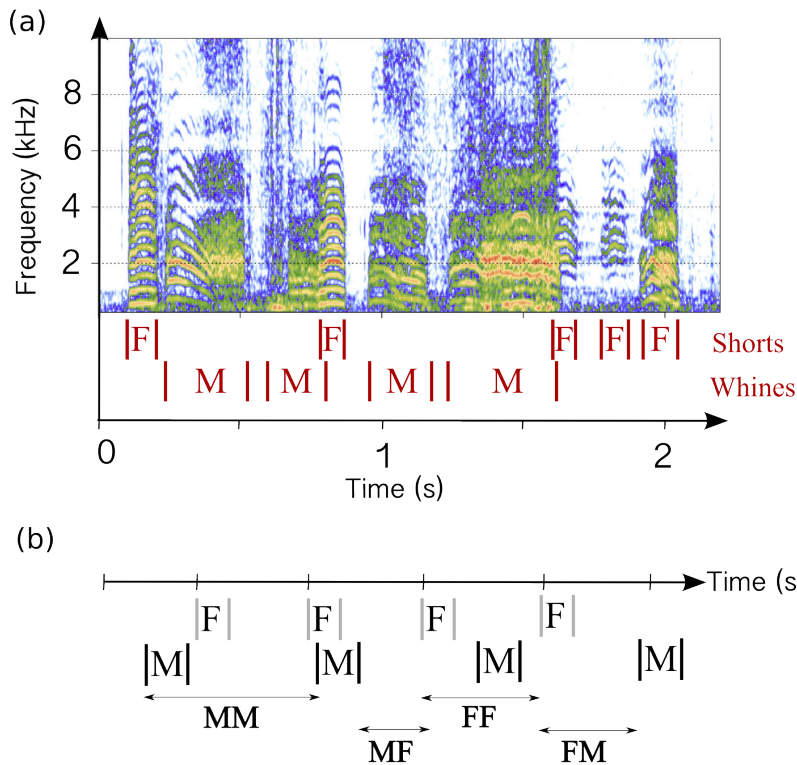


Figure 7: Extract of a zebra finch call duet inside the nest. (a) Spectrogram and call manual annotation. ‘Shorts’ refer to ‘Nest calls’ and ‘Tet calls’ pooled together in the same call category for duet analyses. Sex of the caller (F=Female, M=Male) is attributed according to the position of the birds (returning and leaving partner) rather than individual or sex specific signature. (b) Average dynamic of a duet –rebuilt from personal raw data in which intra-sex inter-call intervals (FF=Female to Female and MM= Male to Male) and between-sex inter-call intervals (FM= Female to Male, MF= Male to Female) were calculated (18 pairs, 36 duets) –showing a global alternation of calls between mates and a slight asymmetry between female and male answer delays, leading to some overlapping calls. [sound file: chapter4_1].

E. An unpredictable natural environment

Zebra finches live in arid to semi arid zones of Australia. This habitat is characterised by high variability (high maximum and low minimum temperatures) and unpredictability with respect to several main environmental factors: rainfalls and, more importantly with regards to this study, alternating calm and windy days. Wind conditions can also change on a hourly basis, and maximum wind speed can reach high values (20m/s), which correlate with high noise levels [typically 65 DB SPL vs. 35-40 DB SPL with no wind (Mouterde et al., 2014a)] (fig. 8). Natural wind noise frequency, ranging from 0 to 5 kHz

is likely to mask vocal signals and alter soft communication between partners either outside or inside the nest. Indeed, nests are often woven and bottle shaped (Zann, 1996) and very weakly attenuate wind noise. This unpredictable constraint on nest duetting is useful to (1) address the question of vocal flexibility in response to noise on soft short-range and non-learned vocalizations in both sexes and (2) test the significance of call duets for the breeding pair. If call duets are important for transmission of information during the breeding season, partners should adjust their behaviour to maintain duet efficacy under noisy conditions.

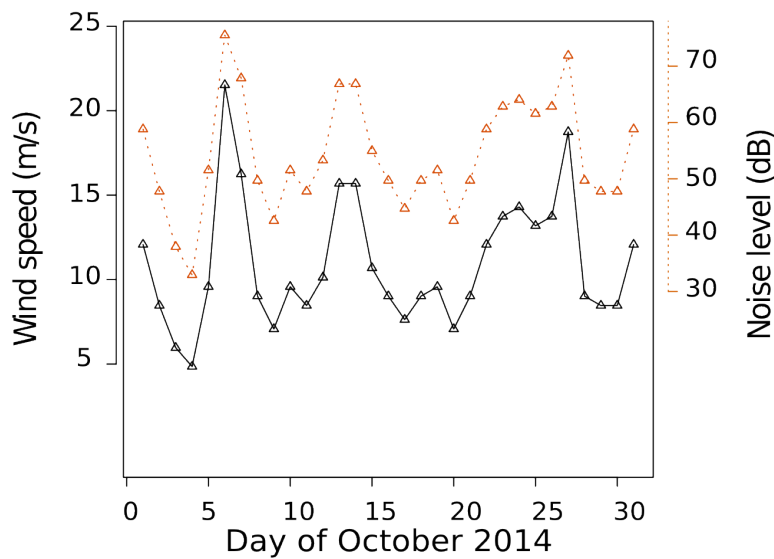


Figure 8: Wind noise in natural habitat of zebra finches. Maximum wind gust per day during a month of the breeding season at Fowlers Gap scientific field station (GPS), Australia, solid black lines (wind data available:

www.bom.gov.au/climate/dwo/201410/html/IDCJDW2155.201410.shtml). Correlational noise level calculated from a model reporting the correspondence between wind speed and noise levels in an open field (Lightstone et al., 2010), using the following model the authors fitted: $y=0.0028x^3 - 0.2225x^2 - 6.9199x + 0.875$, $R^2=0.99$, dashed orange lines.

7. Content of the thesis

This thesis is composed of five chapters. I detail below the questions I addressed in each of them.

Chapter 1: Vocal behaviour of mates at the nest in the White-Throated Dipper, *Cinclus cinclus*: context and structure of vocal interactions, pair-specific acoustic signature

Rare studies investigated forms and potential functions of vocal communication at the nest in birds and these questions have never been asked on dippers. This chapter presents the basis for future studies on vocal communication between dippers' mates at the nest during breeding. Do mates vocalize at the nest during breeding? What are the contexts in which they vocally interact? What is the acoustic structure of the vocalizations used and how are they organized in vocal interactions? In addition, if female-male vocal interactions are important and participate in the organization of breeding, their structure should reflect changes in parental care activities. Do vocal interactions (occurrence, structure) vary depending on the breeding stage?

This chapter is currently under review in Journal of Ornithology.

Chapter 2: Linking female incubation behaviour and vocal activity at the nest in Dippers: female vocalizations are predictors of female behaviour.

If communication between mates participates in the organization of breeding in dippers, behavioural activity should be linked to mates' vocal activity. In this chapter, we used passive daylong monitoring of both female incubation behaviour (using nest thermometers) and vocal behaviour (in the vicinity of the nest). Is female incubation behaviour linked to female-male or female vocal activity at the nest? Does vocal behaviour predict behavioural events occurring during incubation?

Chapter 3: Impact of natural and experimentally elevated noise on vocal communication between mates in dippers.

As Dippers live and breed in a particularly noisy environment, female and female-male vocalizations are likely to be constrained. Two non-exclusive hypotheses arise to explain vocalization structure. Either vocalizations structure avoids the acoustic constraints of the background noise. In that case, vocalizations structure will not vary between pairs or in response to experimentally increased noise level. Or dippers rely on vocal flexibility and adjust their vocalizations to variations of the background noise. Using both between pairs comparison (between different nest sites) and within pair experimental manipulation of background noise, we tested this hypothesis on pair vocal production at the nest.

Chapter 4: Songbird mates change their call structure and intrapair communication at the nest in response to environmental noise.

In zebra finches, vocal communication between mates at the nest has already been described. During incubation, mates meet and duet at the nest in two different contexts: during a relief or during simple visits. One hypothesis is that the relief duets may allow coordination of breeding tasks. Do visit and relief duets have different functions? If relief duets are particularly important for breeding we expect that under a constraining acoustic condition pairs adjust their vocal behaviour to maintain an effective information transmission. In this chapter, we asked how zebra finch pairs deal with acoustic constraints on their vocal communication at the nest. Do females and males respond the same way? What do responses to noise tell us about the potential functions of duetting at the nest (during relief or visit duets)?

This chapter was published in *Animal behaviour* and is presented in the journal format.

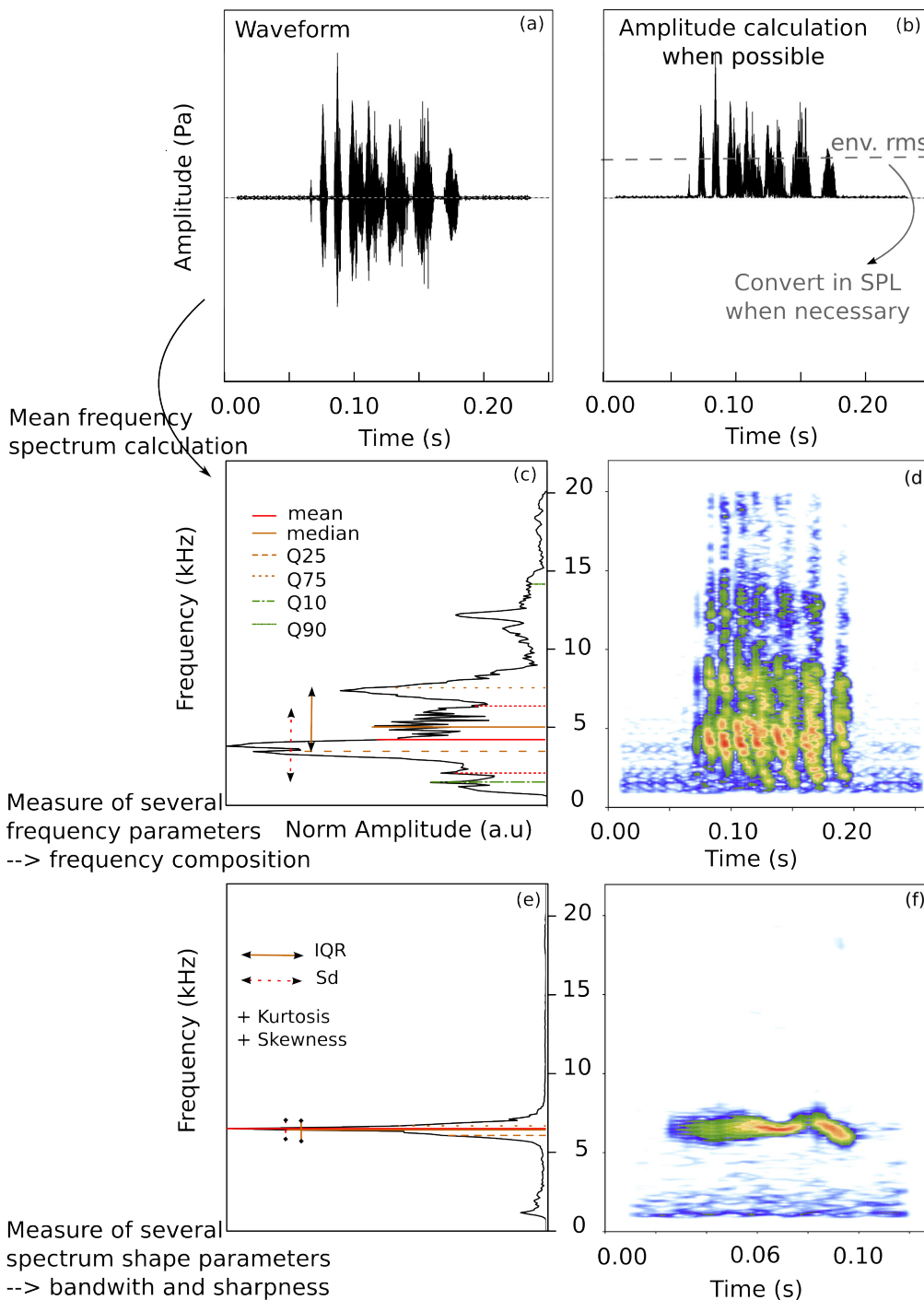
Chapter 5: Parental influence on begging call structure in zebra finches, *Taeniopygia guttata*: evidence of early vocal plasticity

Although vocalizations of many vertebrate species are individualized and allow fine coding of information, how vocalizations develop is a major question: does social experience play a role in vocal development? This question of developmental vocal

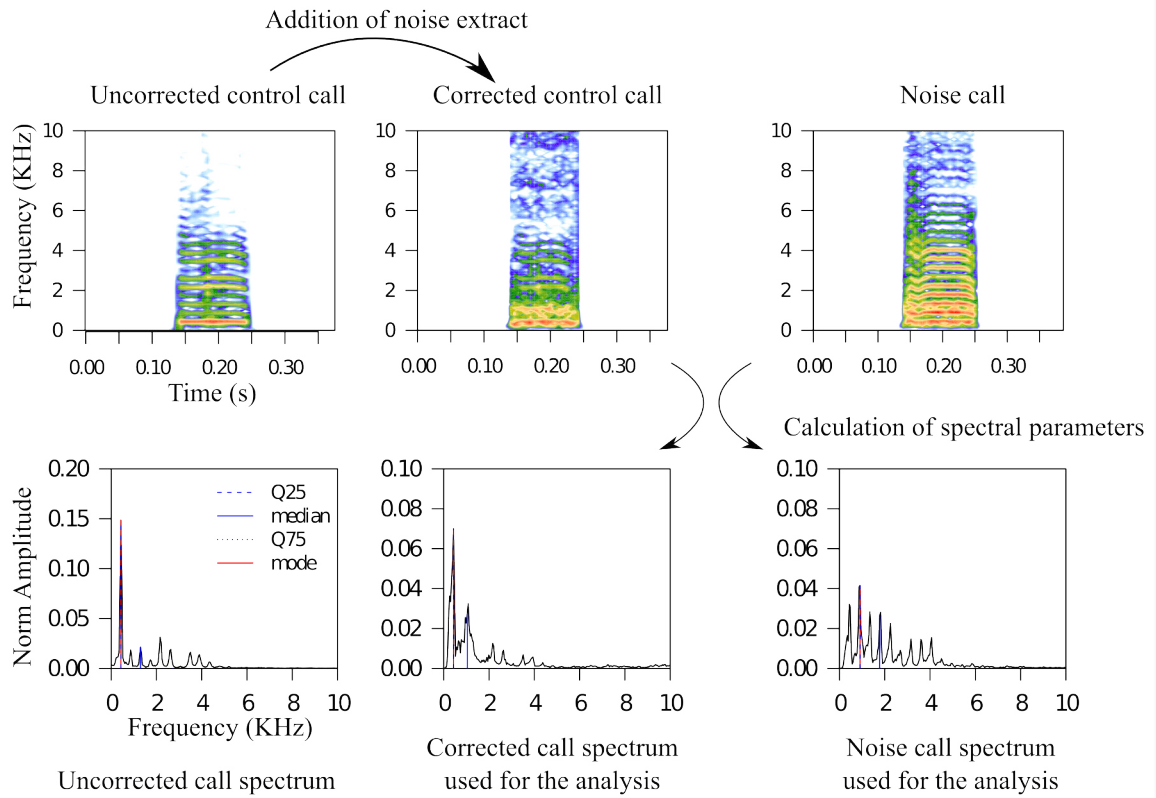
plasticity and vocal learning has been deeply investigated in birdsong. Studies on model species like the zebra finch showed that females and males have different developmental trajectories which explain that males sing and females do not. Birdcalls used by both females and males received less interest. To what extent early social experience may or may not drive the vocal development of birdcalls? Does call development differ between females and males and how early does it occur? Both females and males use begging calls in early life, before the period of expression of plasticity in males. Using a hetero specific cross-fostering experiment, in this chapter, we studied the impact of early life experience on the development of the begging calls of female and male zebra finches.

This chapter was published in Royal Society Open Science and is presented in the journal format.

Box 2: Acoustic analyses. From the waveform (a) several parameters were measured. First, when the distance from the bird to the microphone was standardized (ex: recording in the nest) the amplitude of the signal was measured calculating the root-mean-square of the signal envelope [env. rms, (b)]; it was converted in sound pressure level (DB scale) when possible. Second, the mean spectrum was calculated from several computations of spectra along the signal using time windows (Fast Fourier Transform windows). Several spectral measurements were measured on the mean spectrum: the frequency composition [Q10 and Q90 = 10 and 90% deciles, Q25 and Q75 = first and third quartiles, median and mean of the mean spectrum, (c)] and the shape of the spectrum [IQR= Inter-Quartile-Range, Sd= standard deviation of the mean, shewness and kurtosis of the frequency spectrum, (e)]. No measures were carried out on the spectrogram (d,f), except for temporal analysis of vocal sequences.



Box 3: Acoustic analyses in control VS noise playback treatments. Playback treatments artificially added noise of a given amplitude and with a particular frequency spectrum (wind noise and water stream noise frequency spectrum). To control acoustic measures and allow comparison of the acoustic structure of vocalizations between treatments, all control vocalizations were corrected adding noise extract of the same duration as the vocalization. For each control calls, ten different noise extracts were added and spectral measurements were performed on the average of the ten mixes.



-Chapter 1-

Vocal Behaviour of Mates at the Nest in the White-Throated Dipper *Cinclus cinclus*: context and structure of vocal interactions, pair-specific acoustic signature

Avelyne S. Villain, Mahamoud-Issa M., Doligez B., Vignal C.

Key words: Acoustic communication, Birdcalls, Breeding, Female song, Monogamy.

Submitted to Journal Of Ornithology.

Overview

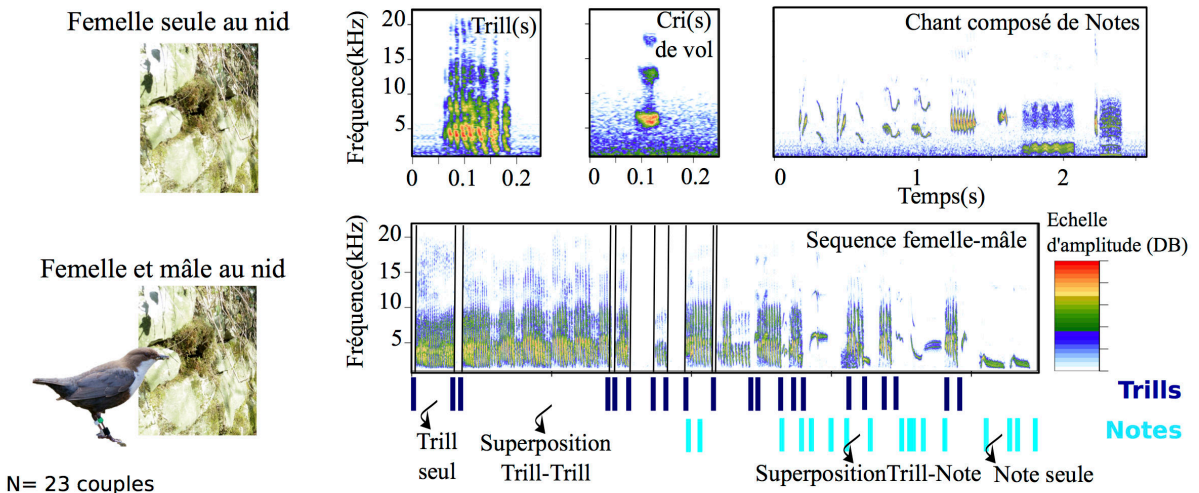
Dans quels contextes les couples de cincles vocalisent-ils autour du nid ?
 Quelle est la structure des vocalisations et des séquences vocales ?
 Comment les séquences vocales varient-elles selon les stades de reproduction ?

Méthodes

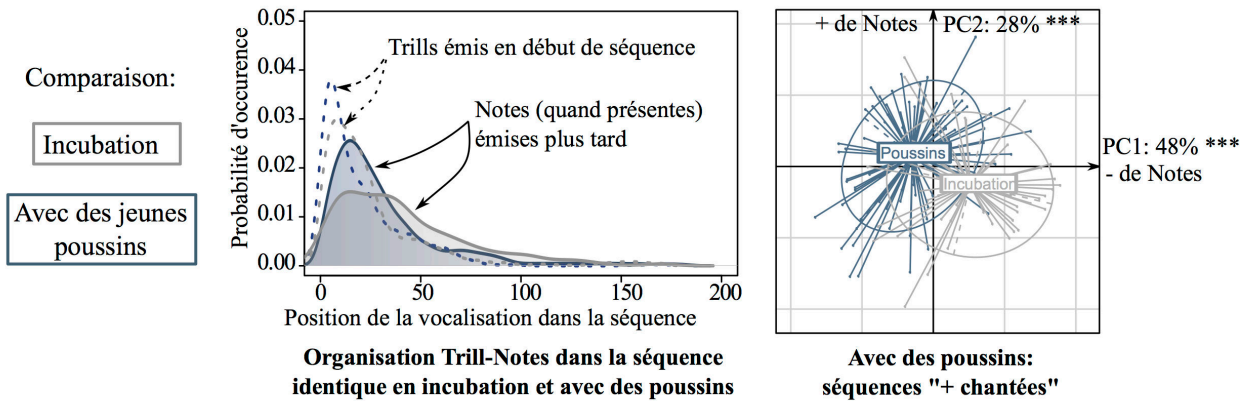
Enregistrements de couples au nid pendant (1) l'incubation (2) avec jeunes poussins
 Observations comportementales pendant 3 à 4 h
 Analyses semi-manuelles des séquences vocales femelle-mâle (150 séquences)



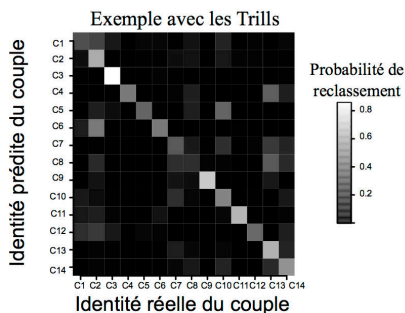
Contextes et types de séquences vocales



Structure des séquences femelle-mâle / variation selon le stade de reproduction



Signature acoustique du couple dans les vocalisations



Cris bien reclassés sur la diagonale

Conclusions

- Au nid:
- Les couples utilisent trois types de vocalisation:
 - * Les cris de vol
 - * Les Trills typiques du nid (séries de pulses rapides et très larges en bandes de fréquence)
 - * Les Notes de chant (très diversifiées!)
 - Les couples sont identifiables juste avec la structure des vocalisations
 - La structure des séquences reflète les stades de reproduction: rôle dans l'organisation des tâches ?

Abstract

Contrary to male song, female song and more generally female vocalizations have been neglected, and their biological functions and evolutionary history have just recently gained interest. Despite analyses of conspicuous vocal duets of tropical species, we still lack descriptions of intrapair vocal communication in most species, which can be of different forms, and more widespread than thought. In this paper, we describe the vocal behaviour of mates at the nest in the White-throated dipper *Cinclus cinclus*, a European species in which females have been reported to sing during pair formation but intrapair communication has never been investigated. We describe contexts of vocalizations during incubation and while brooding hatchlings (N=23 pairs). Vocal interactions were mainly composed of two vocalization types: 'Trills' and 'Notes', Trills being more common at the beginning of the vocal sequence. Both the acoustic features of vocalizations and the temporal organization of sequences changed between breeding stages. In particular, Trills and Notes produced while brooding were lower pitched and female-male vocal sequences were composed of more Notes and songs, with a lower overlap rate. This may reflect changes in parental activities. Trills and Notes carried a pair-specific acoustic signature. This first description of intrapair acoustic communication during breeding in Dippers lays the essential basis for the investigation of their functions.

Introduction

As a mate-attracting signal, birdsong is under sexual selection, through intra-sexual competition and/or inter-sexual choice (Andersson 1994). So far, male song has received most interest, maybe because most studies were performed in temperate zones, on species where females have rarely been reported to sing (Kroodsma et al. 1996; Garamszegi et al. 2007). Consequently, female vocalizations have largely been neglected, even though female song is common in the tropics – where the vast majority of songbird species is located – and may have similar functions as male song (Cooney and Cockburn 1995). Since the recent demonstration that the presence of song in both sexes is likely to be the ancestral trait in songbirds (Odom et al. 2014), we may benefit from studying vocal signals used by both sexes both during and after pair formation to better understand the functions and the evolution of vocal communication in avian species.

Reciprocal female-male vocal interactions have rarely been investigated with the exception of vocal duets. Duets are joint acoustic displays of partners who alternate or partly overlap vocal or non-vocal sounds (Farabaugh 1982; Hall 2004; Hall 2009; Dahlin and Wright 2009). Although rare (ca. 4% of bird species, Farabaugh 1982), the highly coordinated and conspicuous song duets of tropical bird species have attracted much interest. Functions of song duets are numerous, including joint territorial defence, mate guarding or pair bonding (Hall and Peters 2008; Benedict 2010; Hall 2000). But intrapair acoustic communication may be more widespread and involve simpler or lower-amplitude vocalizations such as calls (Todt et al. 1981; Lamprecht et al. 1985; Morton and Derrickson 1996; Wright and Dahlin 2007). In zebra finches (*Taeniopygia guttata*), female and male take turns incubating the eggs and perform soft call-duets during nest reliefs (Elie et al. 2010). These duets adapt to environmental noise (Villain et al. 2016) and participate in coordinating incubation bouts between partners (Boucaud et al. 2016a). In species in which only the female incubates the eggs, females have been reported to sometimes emit sounds at the nest (Beletsky and Orians 1985; Yasukawa 1989; McDonald and Greenberg 1991) that may be used in interactive communication with their mate (Gorissen et al. 2004; Boucaud et al. 2016b). However little is known about the occurrence and potential functions of these female-male vocal communications at the nest in songbirds.

In the present paper we describe female-male communication at the nest in a wild population of White-throated dippers (*Cinclus cinclus*). Dippers are medium-sized passerine birds that live along streams and riverine habitats of temperate zones. They form monogamous pairs and parents have different roles, with the female incubating and brooding hatchlings alone. Despite both sexes have been reported to sing during courtship and pair formation, the rare studies on acoustic communication in dippers mainly described courtship songs (Tyler and Ormerod 1994) or adaptation of vocal signals to noisy habitats (Brumm and Slabbekoorn 2005). Particularly, female-male acoustic communication after pair formation and its potential importance in breeding has never been described.

Using microphones placed inside the nest, we recorded acoustic communication between mates at and around the nest at two different key stages of reproduction (i.e. incubation and brooding), when the female spends most of her time in the nest. We visually monitored the location of both partners relatively to the nest while vocalizing to describe spatial contexts of occurrence. We quantified occurrences of female-male vocal sequences along the day during incubation and described diel variations. We compared (1) the organization of female-male vocal sequences and (2) the acoustic structure of vocalizations used in the sequences between the two breeding stages. Last, we tested whether vocalizations used in female-male vocal sequences could bear a pair-specific acoustic signature.

Methods

Study site and subjects

This study was conducted on a wild population of White-throated Dippers (*Cinclus cinclus*), in Parc Naturel Régional de Chartreuse, FRANCE (46.20N, 5.40E) (CRBPO Personal program 655, 2014). The site is made of relatively mountainous habitat (from 200m to 1100m) and contains several watersheds suitable for dippers' breeding.

The study was conducted from February to May 2014 on 23 pairs breeding in natural nests. Pairs were monitored from nest building to fledging.

Recording of vocal sequences at the nest.

Vocal sequences were recorded using a digital recorder (Zoom H4N, 44.1 kHz, 16 bit) and a tie microphone (Audio Technica, AT 803) hidden in the moss of the nest. The

recording equipment was installed in the morning, 15 min before the beginning of the recording session. One recording session consisted in 03:30 ($\pm 00:30$) hours of recording and started at 09:00 ($\pm 00:30$) in the morning. A hidden observer was placed 15 to 20 m away from the nest to monitor birds' behaviour and location.

Two breeding stages were studied:

- 'incubation': the recording day during this breeding stage varied between pairs from day 3 to day 14 of incubation.
- 'brooding': the recording day during this breeding stage varied between pairs from day 2 to day 5 after hatching.

Over the 23 pairs recorded, 14 were recorded during both breeding stages.

Vocalization types used in vocal sequences

We defined three call types based on their spectrograms (see Fig.1):

- The 'Trill' is a rapid series of broadband pulses. Trills could be produced either by one bird or the two partners at the same time (Fig. 1a).
- The 'Flight call' is a short and high-pitched harmonic stack used during/prior to/after a flight (Fig. 1b).
- 'Notes' are diverse vocalizations used in songs (song syllables) (Fig. 1d).

Spatial contexts of vocal interactions near and at the nest

Using all data (from both incubation and brooding stages), three categories of vocal sequences were defined: 'TwoBirds', 'Call series' and 'Songs'. 'Call series' and 'Songs' refer to sequences produced by one bird with no answer from its partner. 'Call series' are composed of series of Trills and/or Flight calls, and 'Songs' are series of Notes. 'TwoBirds' refers to vocal interactions between mates. Several spatial contexts of production of vocal sequences were observed. Contexts were defined using female and male locations relatively to the nest: 'Nest' = the bird was inside the nest, 'Around' = the bird was visually observable by the experimenter around the nest, 'x' = the bird was absent from the nest area. Eight spatial locations of partners were defined to describe spatial contexts of vocal interactions at the nest: 'FAround-MAround', 'FNest-MNest', 'FNest-MAround', 'FNest', 'FAround', 'MAround', 'MNest', 'FAround-MNest'. Because the dataset was particularly unbalanced (a high number of contexts with few observations in each of them), the effects of the vocal sequence category (Three levels), the context (Eight levels) and the breeding stage (Two levels) on the number of vocal

sequences could not all be tested at the same time. Only the number ‘TwoBirds sequences’ was analysed using a generalized mixed effect model on sequence counts (see detailed procedure Appendix Text 1a).

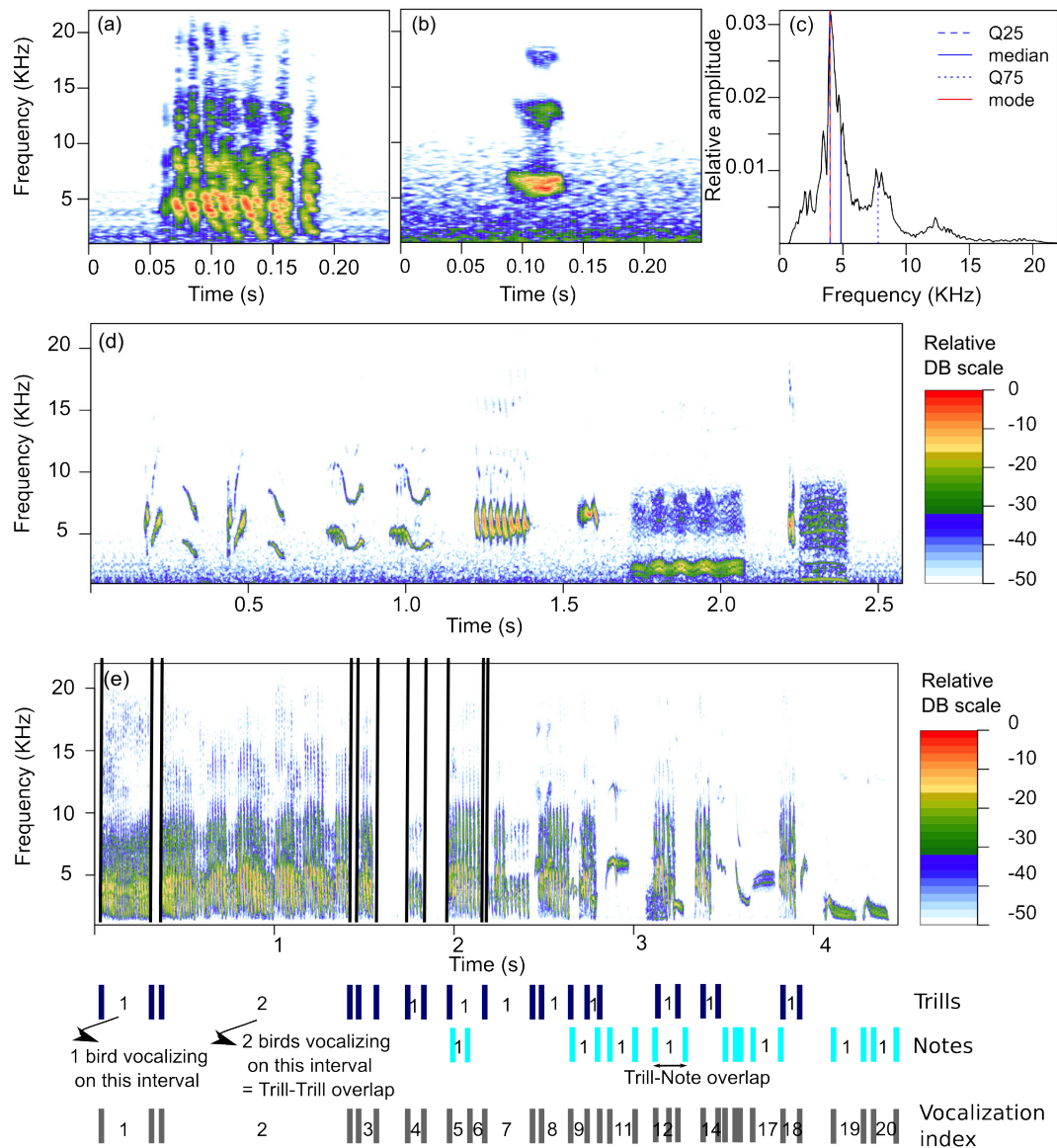


Figure 1: Vocalization types and annotation method. (a,b,d,e) Spectrograms of Trill call (a) flight call (b), extract of song and extract of female-male vocal sequence [wl=512, overlap=75%]) (b) Mean spectrum and acoustic parameters—median, first (Q25) and third (Q75) quartiles, mode – (e) Manual annotation of female-male vocal sequences. Each vocal interval was classified either as ‘Trills’ or as ‘Note’. Since birds could overlap each other’s vocalizations, the number of birds vocalizing on each vocal interval was also labelled, as either one – the vocal interval was then an individual call- or two –the vocal interval was then a block of overlapping vocalizations produced by both birds. Two kinds of overlap could then happen, either ‘Trill-Note’ overlaps –when one bird produced a ‘Trill’ and the other one a ‘Note’ as the same time – or ‘Trill-Trill’ overlaps – when the two birds produced ‘Trills’ at the same time. The figure also shows the definition of the vocalization index, which is the chronological rank of each vocalization in the sequence.

Organization of female-male vocal sequences

Definitions of temporal parameters

Vocal sequences were manually extracted from the recordings. To maximise the quality of the recordings, only sequences produced inside the nest were kept ('FNest-MNest' context, 87% of female-male vocal sequences).

In each vocal sequence, the three vocalization types ('Trills' or 'Notes' or 'Flight calls') were manually labelled as time intervals using the 'Annotate function' of Praat software (www.praat.org). Songs were secondly defined as a suite of at least three Notes, with no more than one second of silence in between. Since they were rare, 'Flight calls' were pooled with 'Notes'. Because birds were close to one another and the observer could not visually identify callers when at the nest, the identification of the caller/singer was not possible but the overlaps between two birds were easily distinguished on recordings from vocalizations by a single bird, either when the two birds produced Trills simultaneously (Trill-Trill overlaps) or when one bird produced a Trill and the other an overlapping Note (Trill-Note overlaps) (Fig. 1e). Using the labels, the following parameters were automatically calculated on each vocal sequence:

- Duration of the sequence (in s) defined from the start of the first vocalization of the sequence to the end of the last one.
- The number Trills (produced by one or two birds)
- The number of Notes
- The duty cycle (s), describing the proportion of time spent vocalizing, defined as the ratio between the total duration of vocalizations (sum of 'Trills' and 'Notes') and the duration of the sequence.
- The proportion of time spent producing Trills over the duration of the sequence
- The proportion of time spent producing Notes over the duration of the sequence
- The number of overlapping vocalizations.
- The vocalization index (either 'Trill' or 'Note'), as the chronological rank of each vocalization along the sequence (Fig. 1e).

Among the initial 23 pairs, three never vocalized at the nest during the recording sessions and two did not produce female-male vocal sequences in the 'FNest-MNest' context. This analysis was therefore performed on 18 pairs.

Analyses.

We successively described (i) the acoustic composition and activity, (ii) the occurrence of overlaps and songs and (iii) the temporal organization of female-male sequences, and we also compared these aspects between the two breeding stages.

(i) Acoustic composition and activity of the sequence

Using a set of six parameters (duty cycle, sequence duration, number of Notes and Trills, proportion of time spent using Trills or Notes), a Principal Component Analysis (PCA) was computed to reduce the number of variables and build scores describing the acoustic composition and activity of vocal sequences (McGregor 2005). Only PCs having an Eigen value above one were kept in the analysis ('ade4' library from R software). The effect of the breeding stage on the acoustic composition and activity of female-male vocal sequences at the nest was tested using a within-pair linear mixed effect model on PCs (see detailed procedure Appendix Text 1b).

(ii) Occurrence of overlaps and songs

The number of overlapping intervals (either Trill-Trill or Trill-Note) was counted in each sequence and divided by the sequence duration to measure the overlap rate in the sequence. The overlap rate was compared between breeding stages using a within pair linear mixed effect model on 'Overlap rate'. The probability of having one song in the sequence ('Song occurrence': '1'=at least one song in sequence and '0'= no song) was compared between breeding stages using a within-pair generalized mixed effect model for binomial distribution on 'Song occurrence' (see detailed procedure Appendix Text 1b).

(iii) Temporal organization

The temporal distribution of Trills and Notes in the sequence was analysed using the index of each vocalization. Vocalization indexes were then analysed and compared between breeding stages a within-pair linear mixed effect model (see detailed procedure Appendix Text 1b)

Variations in vocalizations' acoustic structure across breeding stages

Acoustic parameters calculations

Vocalizations produced at the nest with no additional noise (from the partner, from birds' movements or chicks begging calls) were manually selected using the 'annotate' function of Praat software (Appendix table A1 for data composition). A spectral analysis was

performed with custom-written codes using the Seewave R package (Sueur et al. 2008). After bandpass filtering (1-20 kHz, 'fir' function), the following parameters of the vocalization's 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 (all in Hz, see example Fig. 1c) and the spectral flatness (Sfm). Sfm is a measure of the signal's noisiness. Sfm of a noisy signal tends towards 1, whereas Sfm of a pure tone tends towards 0. Last, the Acoustic Complexity Index (ACI), a measure of the spectrogram complexity (Pieretti et al. 2011), was also calculated.

Analyses

The two vocalization types were defined according to the global shape of their spectrogram, thus a common analysis pooling these two vocalization types was not relevant and would have led to obvious differences. Instead, each type was analysed separately using a Principal Component Analysis (PCA) on all the acoustic parameters defined above to build acoustic scores of each vocalization type. Only PCs having an Eigen value above 1 were kept for the analyses. Variations in acoustic structure of Notes and Trills between breeding stages was tested on PCs using a within-pair linear mixed effect model (see detailed procedure Appendix Text 1c).

Analysis of pair-specific acoustic signature in vocalizations

Potential for identity coding and repeatability

The variability in acoustic structure of the vocalizations produced by a pair was analyzed using the coefficient of variation (CV) of each acoustic parameter. For each parameter, the within-pair CV (CV_i) and between-pair CV (CV_b) were quantified and used to calculate the ratio CV_b/meanCV_i (over all vocalizations from the pair). This ratio may be used as a proxy of the potential for identity coding (PIC) of the parameter (Robisson et al. 1993). Repeatability of acoustic parameters within pairs was also calculated from variance components (between-pair vs. within-pair variation) using the mean squares of a one-way ANOVA (Lessells and Boag 1987).

Pair-specific acoustic signature in vocalizations

Raw values of acoustic parameters were centered and scaled (i.e., transformed into z-scores) to ensure correct weighting because acoustic parameters had different units. We analyzed the potential pair acoustic signature in vocalizations used in female-male vocal

sequences using a Discriminant Function Analysis (DFA) ('lda' function, 'MASS' R library). The DFA was composed of two steps: in the first step, a set of discriminant functions was obtained from a subset of the data (the training data set); in the second step, these functions were used to test the classification on a validation set. This cross-validation step gives a measure of the percent correct and of the statistical significance by comparing to chance the percent correct assignment of 100 random selections of the original data set divided into a training and a testing set. The training set consisted of 2/3 of the total data of all pairs in each of the 100 runs. To validate the analyses, as addressed by Mundry and Sommer (Mundry and Sommer 2007) and Mathevon et al. (Mathevon et al. 2010), it is possible to compare the percent correct obtained in the DFA to the distribution of percent correct values obtained by randomly assigning pair identity (Mundry and Sommer 2007; Mathevon et al. 2010). We did so by randomly creating 100 data sets where the pair identity was permuted in each set (permuted DFA).

Results

Spatial contexts of production of vocal sequences near and at the nest.

The three sequence categories ('OneBird Call series', 'OneBird Songs' and 'TwoBirds' sequences) occurred in various spatial contexts (Fig. 2a) but some context-category association never occurred. For example, the 'FNest-MNest' context always led to a 'TwoBirds' vocal sequence and never to solo vocalizations; females around the nest ('FAround') or males at the nest ('MNest' or 'FAround-MNest') never produced songs. We can notice that these last two contexts were specific of the brooding stage, because males were never seen at the nest without the female during incubation. As explained in methods (Appendix Text 1b), due to a very unbalanced dataset, no statistics could be performed on these observations.

Variation of female-male vocal sequences across breeding stages.

Female-male vocal sequences occurred more often during brooding than during incubation ($X^2_1=8.60$, $P=0.003$, Fig. 2b), with a significant interaction between the Breeding stage and the Context ($X^2_2=10.50$, $P=0.005$, Fig. 2b). This increase was explained by an increase in vocal sequences in the 'FNest-MNest' context (during a visit

of the male to the female at the nest), whereas the number of ‘FNest-MAround’ and ‘FAround-MAround’ sequences did not change (post hoc, Tukey contrast, ‘Incubation’ vs ‘Brooding’ for ‘Nest’: $Z=3.50$, $P=0.006$; ‘Nest-out’: $Z=0.82$, $P=0.96$; ‘Out’: $Z=1.91$, $P=0.39$). Within breeding stages, the occurrence of ‘FNest-MNest’ sequences was predominant (‘FNest-MNest’ vs ‘FNest-MAround’ in ‘Incubation’: $Z=3.98$, $P=0.001$ and in ‘Brooding’: $Z=7.45$, $P<0.001$; ‘FNest-MNest’ vs ‘FNest-MAround’ in ‘Incubation’: $Z=4.87$, $P<0.001$ and in ‘Brooding’ $Z=7.18$, $P<0.001$), explaining the significant effect of the ‘Context’ ($X^2_1=114.1$, $P<0.001$). The occurrence of ‘FNest-MAround’ and ‘FAround-MAround’ vocal sequences did not differ within breeding stages (‘FNest-MAround’ vs ‘FAround-MAround’ in ‘Incubation’: $Z=1.01$, $P=0.91$ and in ‘Brooding’: $Z=-0.79$, $P=0.97$). This analysis also showed that the number of ‘FNest-MNest’ sequences was a good proxy of the number of female-male vocal sequences (87% of the total).

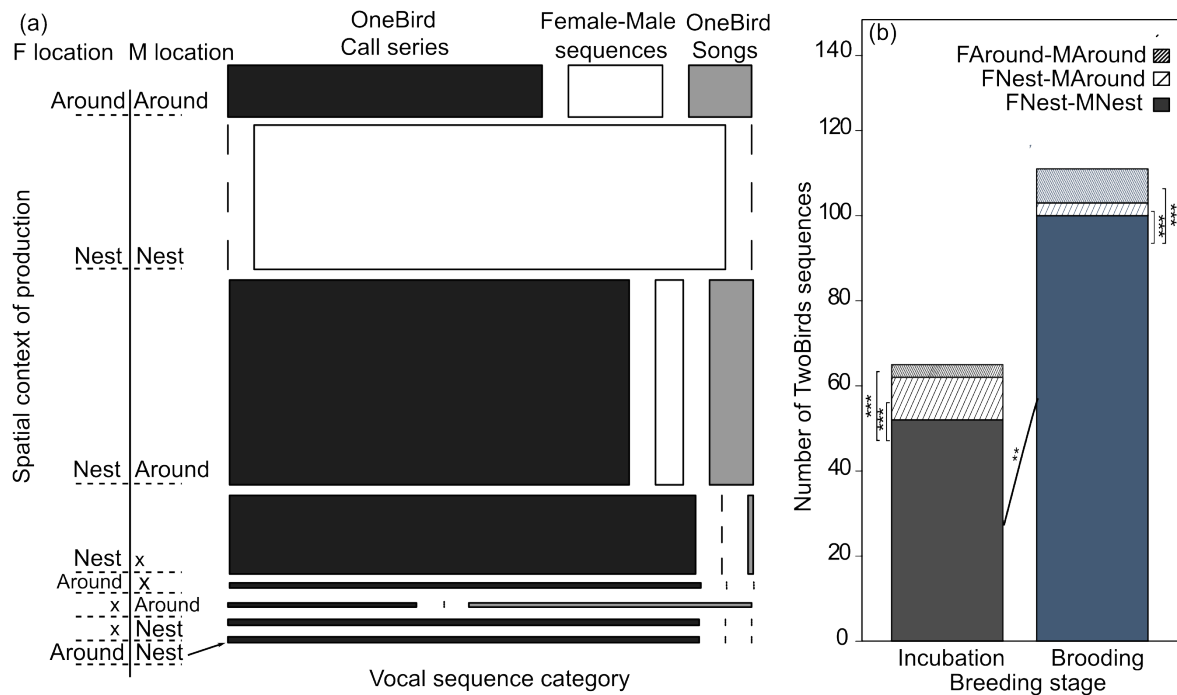


Figure 2: Spatial contexts of female and/or male vocalizations near and at the nest (a) and variation in occurrence of female-male vocal sequences around the nest across breeding stages (b). (a) Mosaic plot of the relative numbers of vocal sequences depending on the category of the sequence (horizontal axis) and the spatial context (vertical axis). Spatial contexts were defined according to the spatial location of the female and the male relatively to the nest (‘Nest’, ‘Around’, ‘x=Away’). (b) Effect of the spatial context (‘FNest-MNest’: male visiting the female, ‘FNest-MAround’: female at the nest and the male around or ‘FAround-MAround’: both partners around the nest) and the breeding stage (‘Incubation’ and ‘Brooding’) on the number of ‘TwoBirds’ sequences (produced by the female and the male). Results are on 23 pairs (13 recorded during both breeding periods). See Appendix table A2 for detailed statistics. ***: $P<0.001$, **: $P<0.01$, *: $P<0.05$.

Organization of female-male vocal sequences across breeding stages

The organization of female-male vocal sequences at the nest differed between breeding stages. Songs were more frequent in vocal sequences during brooding than incubation ($X^2_1=13.9$, $P<0.001$, Fig. 3a). During both breeding stages, vocal sequences were more likely to start by Trill calls and end by Notes (significant effect of the vocalization type on the call index, $X^2_1=69.5$, $P<0.001$, Fig. 3b). This was more pronounced during the incubation stage (significant interaction $X^2_1=15.3$, $P<0.001$, post hoc, Tukey contrast, ‘Notes’ vs ‘Trills’ for ‘Incubation’: $Z=8.03$, $P<0.001$; and for ‘Brooding’: $Z=4.5$, $P<0.001$). Overlap rate was higher in vocal sequences produced during incubation than brooding ($X^2_1=48.0$, $P<0.001$, Fig. 3c). The PCA on the acoustic composition and activity revealed that vocal sequences produced during incubation were richer in vocalizations (higher duty cycle) and composed of more Trills than Notes (table 1), since PC1 was significantly higher (PC1: 48% the variance, $X^2_1=56.0$, $P<0.001$, Fig. 3d and 3e) and PC2 was significantly lower (PC2: 28% the variance, $X^2_1=6.7$, $P=0.01$, Fig. 3d and 3f).

Table 1: Variable loadings of the PCA on the acoustic composition and activity of the sequences. Contribution of each parameter, percentage of variance explained and Eigen value for the PCs. The transformation used for each parameter to reach symmetrical distribution is given in parentheses.

	PC1	PC2	PC3
Variance explained (% cumulative)	48	75	95
Eigen value	2.6	2.1	1.03
Duty cycle	0.68	0.48	-0.52
Number of Trills (ln)	0.01	-0.82	-0.53
Number of Notes (ln)	-0.81	0.30	-0.45
Sequence duration (ln)	-0.69	-0.62	-0.25
Proportion of Trills (Box-Cox ¹)	0.91	-0.18	-0.34
Proportion of Notes (Box-Cox)	-0.42	0.84	-0.31

¹: The Box-Cox transformation computes one parameter transformation using the following formula: $\text{parameter}^{(\lambda)} = \text{parameter}^{(\lambda)} - 1 / \lambda$, if $\lambda \neq 0$ and $\ln(\text{parameter}^{(\lambda)})$ if $\lambda=0$. The ‘boxcox’ function (‘Mass’ R package) automatically finds the appropriate λ value to reach a distribution as close as possible to the Gaussian distribution.

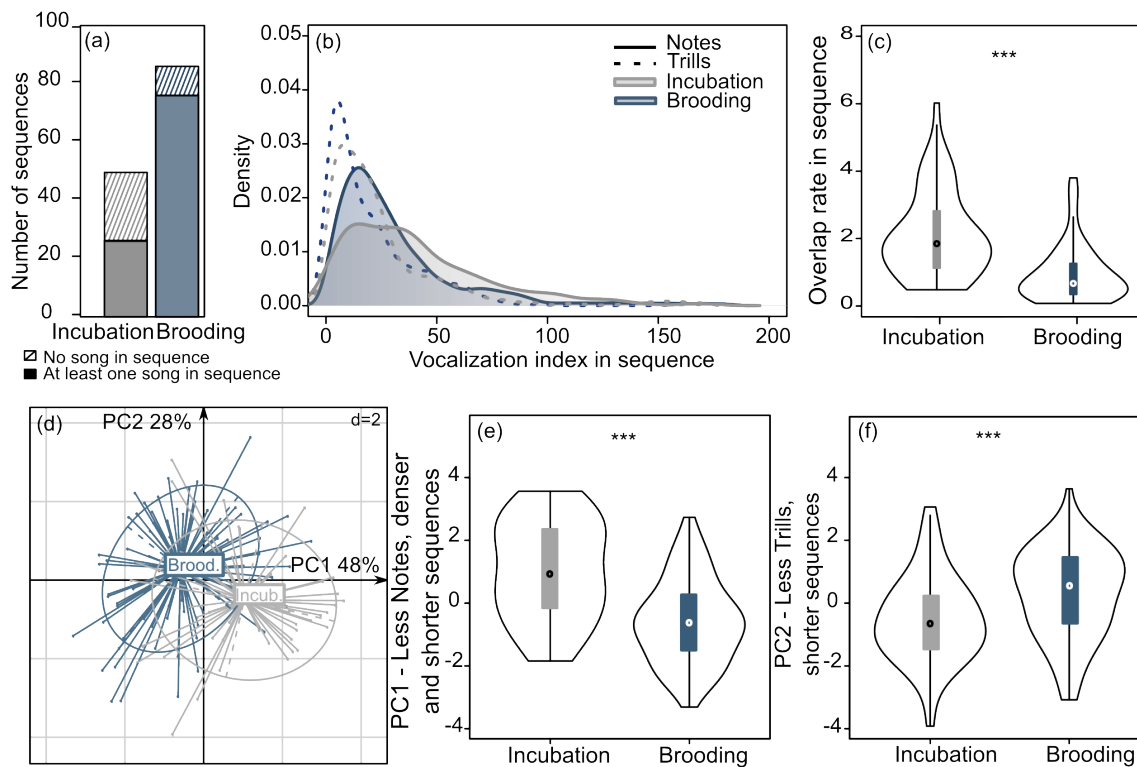


Figure 3: Effect of the breeding stage on the organization of female-male vocal sequences ('Incubation' in grey or 'Brooding' in blue). (a): Number of vocal sequences having 'at least one song' or 'no song'. (b): Density distributions of vocalization indexes for Trills (dotted lines) or Notes (bold lines). (c): Violin plots of the overlap rate in sequences. (d) Scatter plot the acoustic composition and activity of the sequence, PC1 and PC2 of each sequence (points) for the two breeding stages. Ellipses represent 1.5 of the inertia and centre are centroids of each breeding stage. The percentage of explained variance is given for each PC. (e,f) Violin plots of PC1 (e) and PC2 (f) of the acoustic composition and activity of the sequence for both breeding stages. Results are on 18 pairs (8 with sequences in both breeding stages). See Appendix tables A3 and A4 for detailed statistics ***: $P < 0.001$, **: $P < 0.01$.

Acoustic structure of vocalizations across breeding stages

The PCA analyses on Notes and Trills (table 2) revealed changes in acoustic structure depending on both the breeding stage and the vocalization index in the sequence. Notes produced during brooding were in lower frequencies than during incubation –since PC1 was higher (PC1: 43% of variance, $X^2_1=153.1$, $P < 0.001$, Fig. 4a). Notes produced later in the sequence had lower frequencies since PC1 increased with increasing vocalization index ($X^2_1=10.4$, $P=0.001$, Appendix Fig. A1a). The PCA analysis on Trills revealed similar results: Trills had lower frequencies during brooding –since PC1 was higher (PC1: 52% of variance, $X^2_1=25.1$, $P < 0.001$, Fig. 4b). Trills produced later in the sequence also had lower frequencies ($X^2_1=17.3$, $P < 0.001$, Appendix Fig. A1b). Mean values of the

eight acoustic parameters used in the PCA analyses are presented within vocalization types and breeding stage in Appendix, table A6.

Table 2: Variable loadings of the PCA analyses on the acoustic structure of Notes and Trills. Contribution of each parameter, percentage of variance explained and Eigen value for the PCs. The transformation used for each parameter to reach symmetrical distribution is given in parentheses.

	PC1	PC2
PCA on Notes		
Eigen Value	3.44	2.39
Variance explained (% cumulative)	43	73
Mean (ln)	-0.93	0.34
Median (ln)	-0.89	0.32
Q25 (ln)	-0.91	-0.10
Q75 (ln)	-0.67	0.62
Skewness (ln)	-0.49	-0.81
Kurtosis (ln)	-0.48	-0.79
Sfm	0.18	0.70
ACI	-0.15	-0.11
PCA on Trills		
Eigen Value	4.16	1.83
Variance explained (% cumulative)	52	75
Mean (ln)	-0.97	0.21
Median (ln)	-0.92	0.16
Q25 (ln)	-0.82	0.37
Q75 (ln)	-0.91	0.04
Skewness (ln)	0.48	0.87
Kurtosis (ln)	0.40	0.90
Sfm	-0.57	-0.03
ACI	-0.39	0.25

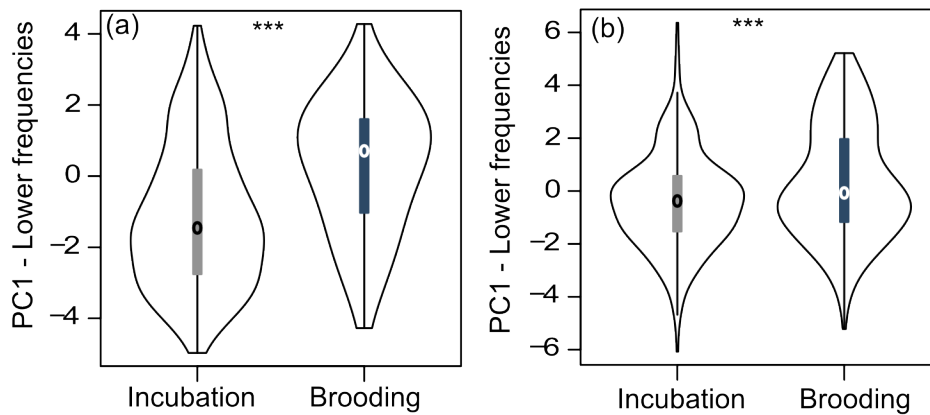


Figure 4: Variation of vocalizations' acoustic structure between breeding stages. Results are expressed after computation of a PCA on acoustic parameters within each vocalization type - Notes and Trills. Violin plots of PC1 of the analysis of Notes (a) and Trills (b) in Incubation (in grey) and Brooding (in blue). See table A5 for detailed statistics ***: $P < 0.001$, **: $P < 0.01$.

Pair acoustic signature in vocalizations

When the Potential for Individuality Coding (PIC) index is higher than one, then the interindividual variation is higher than the intraindividual variation and this suggests that the acoustic parameters are individualized. All measured acoustic parameters of Trills and Notes were individualized at the level of the pair (Table 3), but PIC values were variable and moderate. This was confirmed by the values of intra-pair repeatability (mean \pm SD = 0.32 ± 0.20 , min = 0.02, max = 0.63) (Table 3).

DFAs on Trills and Notes identified significant acoustic differences between pairs (Fig. 5a and b respectively), with a cross-validation success rate on 100 iterations significantly above chance (53.8% correct classifications vs. 31.8% for Trills and 58.0% vs 26.4% for Notes). Classification success increased when analysing only Notes produced during brooding, for which sample sizes were the highest (Appendix table A1), (67.7% correct classification vs 29.0%).

Table 3: Analysis of the variability in acoustic structure of Trills and Notes produced in female-male vocal sequence during incubation and brooding stages.

Breeding stage	Vocalization type	Mean	Median	Q25	Q75	Skewness	Kurtosis	Sfm	ACI	Mean for all	
										acoustic	parameters
Incubation	Notes	0.14	0.02	0.08	0.07	0.07	0.06	0.53	0.05	0.13	
	Trills	0.56	0.43	0.63	0.33	0.24	0.21	0.63	0.40	0.43	
Brooding	Notes	0.47	0.33	0.52	0.18	0.20	0.15	0.63	0.17	0.33	
	Trills	0.61	0.52	0.57	0.51	0.14	0.09	0.38	0.35	0.39	
PIC=CVb/mean(CVi)											
Incubation	Notes	1.90	1.66	1.85	1.72	1.28	1.40	1.38	1.22	1.55	
	Trills	1.51	1.40	1.59	1.28	1.31	1.40	1.47	1.32	1.41	
Brooding	Notes	1.30	1.13	1.34	1.19	1.07	1.11	1.30	1.04	1.18	
	Trills	1.26	1.20	1.17	1.23	1.11	1.15	1.82	1.21	1.27	

For each parameter, the coefficients of variation CVi (within individual) and CVb (between individuals) were assessed and used to calculate the ratio CVb/meanCVi (potential of individuality coding (PIC)) (Robisson et al. 1993). Repeatability of acoustic parameters within individuals was calculated from variance components (between-individual variation vs. within-individual variation) using the mean squares of a one-way ANOVA (Lessells and Boag 1987).

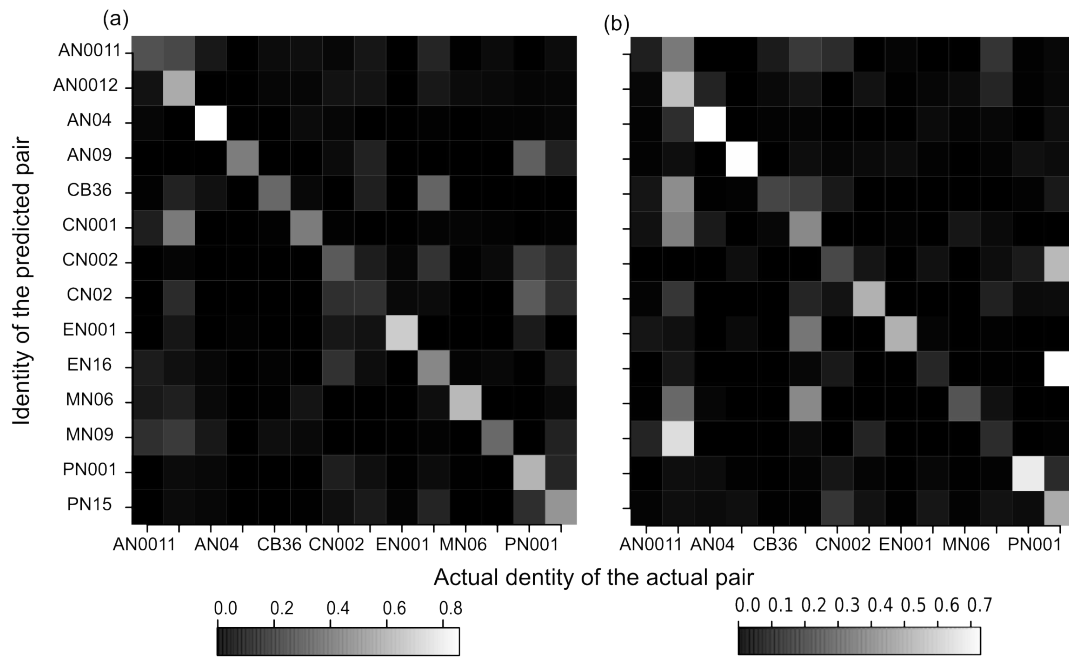


Figure 5: Pair acoustic signatures in Trills (a) and Notes (b) used in female-male vocal sequences at the nest. Confusion matrices obtained from the two DFAs on the cross-validation data set. Using a gray scale, cell $[i,j]$ shows the conditional probability of guessing that the test call came from pair j when in fact it was produced by i . Note that these predictions are made using all discriminant functions obtained from the DFA. Row labels indicate pair identity.

Discussion

White-throated dippers produced female-male vocal sequences around the nest in three contexts: (1) the male visiting the female at the nest –which was predominant, (2) the male staying relatively close to the nest and the female inside or (3) both partners being outside and relatively close to the nest.

Potential functions of female-male vocal sequences

Functions of female-male vocal sequences have been mainly investigated in duetting species, using structural analyses and contexts of production. The main hypotheses for duet functions are: mate guarding, mutual recognition, pair bond maintenance and territory defence (Hall 2004; Hall 2009). Female-male vocal sequences recorded in dippers cannot be interpreted as duets, since the coordination between the vocalizations of the female and the male could not be investigated in our data set. Nevertheless, we described a vocal interaction between mates and we can raise hypotheses about its functions in this theoretical framework. Since we could not distinguish the vocalizations

produced by the female or the male, we cannot quantify sex specific vocal behaviour or individual vocal signature. Consequently, neither the mate-guarding hypothesis nor the mutual recognition hypothesis (Hall 2000; Hall 2004) can be discussed here. Duetting has been related to territorial defence (Hall and Peters 2008), but in our case, the vocal interactions occurred during incubation or brooding, i.e. when territories have already been established. Nevertheless, dippers have been reported to steal conspecific nest during the breeding season (Tyler and Ormerod 1994) so vocal sequences produced inside the nest (either during male visit or female song) may signal to conspecifics that the nest and the territory are already occupied. Female-male vocal sequences were produced in a private context and might thus be involved in pair bond maintenance (Wickler 1980; Malacarne et al. 1991). These signals could also be involved in coordinating parental care or mate feeding behaviour (Halkin 1997; Ritchison 1983). For example, in zebra finches, the duet structure during nest reliefs (when partners take turns incubating) predicts partners' incubation share (Boucaud et al. 2016a). In great tits (*Parus major*), the structure of vocal interactions between mates during incubation differs depending on whether the vocal exchange occurs prior to a mate feeding. Mate feeding (during courtship or incubation) is common in songbirds (Donazar et al. 1992; Helfenstein et al. 2003) and several hypotheses have been proposed to explain it, in particular by considering it as a honest signal of parental abilities (Nisbet 1973; Korpimäki 1989), a way to strengthen pair bond (Lack 1940) or a compensatory energetic strategy, particularly in species in which the female builds the nest and incubates alone (Galván and Sanz 2011). To the best of our knowledge, mate feeding and associated vocal exchanges have not been reported in dippers. This hypothesis remains to be investigated; video recordings in the vicinity of the nest would allow monitoring males' activity during nest visits during eggs incubation.

Variations of vocal interactions across breeding stages

More vocal sequences were recorded during brooding than incubation, especially when males visited females at the nest. Since males' nest visits to the female always led to vocal sequences, this increase in the number of nest vocal sequences may reflect an increase of visits during this stage, in particular to feed the nestlings.

Repertoire and temporal analysis of female-male vocal sequences produced at the nest showed that some parameters were stable between breeding stages: in both incubation

and brooding, Trills were more likely to occur at the beginning of the sequence and notes after. Other parameters varied across breeding stages: vocal sequences produced during brooding were more likely to contain a song and were composed of more notes than Trills, with a lower overlap rate, compared to incubation. This underlines a change in temporal vocal dynamic and repertoire from incubation to brooding. Female-male vocal sequences were also produced more often during brooding. A change in the effort of communication and the structure of vocal sequences may reflect a change in the information exchanged as the breeding season progresses. A similar pattern has been showed in great tits, in which the structure of vocal exchanges between mates around the nest differ between breeding stages (laying vs. incubation) (Boucaud et al. 2016b). The acoustic structure of vocalization types used during vocal sequences also changed across breeding stages: notes and Trills were lower pitched during brooding than incubation. Several hypotheses can be proposed to explain these acoustic changes in vocalizations' structure. First, the identity of the caller/singer may change between breeding stages: vocalizations would be mainly produced by one bird during incubation and by the other one during brooding (for example male singing during incubation and female singing during brooding or vice versa). Second, changes in acoustic structure could be due to an increase in diversity within each vocalization type. This is particularly relevant for notes, since they (1) had lower repeatability than Trills and (2) were produced more often during brooding than incubation. Third, each individual could change the acoustic structure of each of its vocalization types. In this case, vocal flexibility could be due to changes in physiological or motivational state along the breeding season. Previous studies have shown that the structure of vocalizations can reflect and/or signal thermal state (Leonard and Horn 2001), hunger level (Kacelnik et al. 1995), motivation (Morton 1977) or emotions (Perez et al. 2012; Perez et al. 2015). This hypothesis could explain that some acoustic parameters changed in the same direction in both call types (e.g. lower frequency calls during brooding). All these hypotheses can partly explain the results obtained here, are non-exclusive and remain to be tested.

Vocalization types and pair signature

Two vocalization types were distinguished: Trills and Notes. Trills are rapid series of broadband pulses characterised by relatively higher within-pair repeatability than notes. Notes are diverse song syllables. Both vocalization types showed potential for pair-

individuality coding (PIC higher than 1) for most acoustic parameters and carried an acoustic pair signature. Nevertheless, this signature was moderate, as showed by percentage of correct classification of the DFA on Trills and notes. Because we do not know which bird produced each particular vocalization, we cannot conclude if this acoustic pair signature results from an actual pair signature (the sum of the individual vocal signatures of both partners) or the individual signature of only one partner in the sequence. This information could be important to formulate hypotheses on the functions of this signature. Some species show between-pair differences in vocal types used in duets, mates using preferred syllables among the species repertoire for example in the red-crowned crane (*Grus japonensis*) (Klenova et al. 2008), or in Australian magpie-lark (*Grallina cyanoleuca*) (Hall 2006). In the latter, pairs respond differently to the duets of neighbor and unfamiliar conspecific pairs (Hall 2000), so pair specificity in joint vocal signals may play a role in territoriality.

In summary, our study provides the first detailed description of the vocal behaviour of dippers' mates around the nest during breeding. We showed that pairs produced vocal interactions in different contexts and at two breeding stages. During incubation, vocal interactions occurred all day long and more than half of the females produced songs at the nest. Vocal sequences produced by mates when the male visited the female at the nest were composed of Trills and notes and their structure varied with the breeding stage. They also carried a moderate pair acoustic signature. These findings are valuable additions to the description of the variety of vocal behaviour of females, males and mated pairs, but also provide a basis for further functional analyses.

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Appendix

Appendix text 1: Detailed statistical procedures

1.a. Spatial contexts of vocal interactions near and at the nest across breeding stages.

Because the dataset was particularly unbalanced (a high number of contexts with few observations in each of them), the effects of the vocal sequence category (three levels), the context (eight levels) and the breeding stage (two levels) on the number of vocal sequences could not all be tested at the same time. Only the number of ‘TwoBirds sequences’ was analysed using the following model (‘lme4’ library of R software (R Core Team 2014)): `model1 = glmer (Number of vocal sequences ~ offset (Zduration of the recording session) + Context*Breeding stage+(1|WithinPairContexts) +(1|WithinPairBreedingStage), family='poisson')` using two interacting fixed factors ‘Breeding stage’ (2 levels: ‘Incubation’ and ‘Brooding’) and ‘Contexts’ (3 levels: ‘FNest-MNest’, ‘FNest-MAround’ and ‘FAround-MAround’) and two random factors to deal with pseudo replication since the three contexts were repeated on 23 pairs (WithinPairContexts) and two breeding stages were studied per pair (WithinPairBreedingStage). A random factor ‘Pair identity’ would not take all repetitions into account. An ‘offset’ controlled for the potential effect of the duration of the recording session on counts. The duration of the recording was centered and scaled as recommended for continuous covariates (Schielzeth 2010).

1.b Organization of female-male vocal sequences

The effect of the breeding stage on (1) the acoustic composition and activity of female-male vocal sequences at the nest, (2) the overlap rate and (3) the occurrence of songs in sequences was tested using the following model: `model4 = lmer/glmer(PC~Breeding stage +(1|Pair identity))`, using the ‘Breeding stage’ as fixed factor (2 levels: ‘Incubation’ and ‘Brooding’) and controlling for pseudo replication (‘Pair identity’ random factor, 18 levels). Since the dataset was partly paired (eight pairs with sequences in both breeding stages), the same model was applied on the whole dataset as well as on a reduced dataset containing only the eight pairs having produced vocal sequences in both breeding stages.

Because results were statistically similar in both cases, we present here only results for the whole dataset. Models applied on PC3 were not stable (marginal effects of random factors, see methods section 1.d ‘statistical validation and quantification’) we then present only results obtained on the two first PCs.

The temporal distribution of Trills and Notes in the sequence was analysed using the index of each vocalization. Vocalization indexes were then analysed using the following linear mixed effect model: $\text{model5} = \text{lmer}(\text{ZCall index} \sim \text{Vocalization type} * \text{Breeding stage} + (1|\text{Sequence Identity}) + (1|\text{Pair identity}))$, using the vocalization type (2 levels: Note and Trill) and the ‘Breeding stage’ (2 levels: ‘Incubation’ and ‘Brooding’) as fixed factors and ‘Sequence Identity’ (138 levels) and ‘Pair’ (18 levels) as random factors (controlling respectively for the facts that several vocalizations belonged to the same vocal sequence and pairs were recorded twice). The vocalization index was centered and scaled.

1.c Variation of vocalizations’ acoustic structure across breeding stages

Variations in acoustic structure of Notes and Trills between breeding stages was tested on PCs. Since the rank order of a given vocalization in the temporal sequence could impact its acoustic structure (Villain et al. 2015), the vocalization index was also tested as an explanatory variable in this analysis. The effect of the breeding stage on the PCs was then tested using the following model: $\text{model6} = \text{lmer}(\text{PC} \sim \text{Breeding stage} + \text{ZVocalization index} + (0 + \text{ZVocalization index} | \text{Sequence Identity}) + (0 + \text{ZVocalization index} | \text{Pair identity}))$, using the ‘Breeding stage’ (2 levels: ‘Incubation’ and ‘Brooding’) as a fixed factor and the vocalization index as a continuous covariate and two random factors to control for pseudo replication: ‘Sequence Identity’ (109 levels, since several vocalizations per sequence were analysed) and ‘Pair identity’ (16 levels, since each pair was recorded twice). The Vocalization index was used as a random slope (centered and scaled) with both random factors as recommended for within subjects designs with a covariate (Forstmeier and Schielzeth 2011). Problems in model validation and convergence led us to remove correlations between random factors (since this does not increase type I error (Barr et al. 2013)) as well as the interaction between the two fixed factors. Because the dataset was partly paired (799 Notes from six pairs, 540 Trills on 8 pairs), the same model was applied on the whole dataset and on a reduced dataset containing only pairs having vocal sequences in both breeding stages. Only validated models with consistent results on both datasets were kept (analysis on PC1 only).

1.d Statistical validation and quantification

Linear and generalized mixed effect model were computed using ‘lmer’ and ‘glmer’ functions respectively (‘lme4’ R package ,Bates et al. 2014)). Equivariance and normality of residuals and absence of over dispersion were checked respectively using ‘plotresid’ and ‘overdisp.glmer’ functions (‘RVAideMemoire’ R package). Conditional and marginal coefficients of determination of the linear or generalized models were computed using ‘r.squaredGLMM’ function (‘MuMIn’ R package). The influential random factors were tested using the ‘influence’ function (‘Influence.ME’ R package, Nieuwenhuis et al. 2012). P-values were computed using the ‘Anova’ function run on models (Type II Wald Chisquare tests on which it is possible to interpret single effects despite significant interactions between explanatory variables, ‘car’ R library). Model estimates were computed using the ‘summary’ function (R ‘base’ package).

Appendix results part

Table A1: Data composition in Trills and Notes vocalizations.

Breeding stage	Call type	Total N calls	N pairs	Mean	SD	SE	Min	Max
Incubation	Notes	311	10	31.10	34.07	10.77	7	116
Incubation	Trills	339	14	24.21	27.88	7.45	1	93
Brooding	Notes	1329	11	120.82	133.05	40.12	3	404
Brooding	Trills	391	11	35.55	33.71	10.16	1	105

Mean number per pair, Standard deviation (Sd), Standard error (Se), minimum and maximum are indicated.

Table A2: Occurrence of female-male vocal interactions depending on the ‘Context’ (‘FNest-MNest’= ‘Nest’, ‘FNest-MAround’ = ‘Nest-Around’ or ‘FAround-MAround’= ‘Around’) and the ‘Breeding stage’ (‘Incubation’ or ‘Brooding’).

Contrast	Estimate	SE	z.ratio	P.value
Brooding,Nest - Incubation,Nest	1.402	0.401	3.498	0.006
Brooding,Nest-out - Incubation,Nest-out	0.390	0.474	0.823	0.963
Brooding,out - Incubation,out	0.905	0.470	1.928	0.385
Brooding,Nest - Brooding,Nest-out	1.855	0.249	7.455	<0.001
Brooding,Nest - Brooding,out	1.609	0.224	7.180	<0.001
Brooding,Nest-out - Brooding,out	-0.245	0.309	-0.794	0.969
Incubation,Nest - Incubation,out	1.112	0.228	4.870	<0.001
Incubation,Nest - Incubation,Nest-out	0.842	0.212	3.982	0.001
Incubation,Nest-out - Incubation,out	0.270	0.267	1.013	0.914
Brooding,Nest - Incubation,Nest-out	2.245	0.424	5.295	<0.001
Brooding,Nest - Incubation,out	2.515	0.432	5.814	<0.001
Incubation,Nest - Brooding,Nest-out	0.452	0.454	0.997	0.919

Incubation,Nest - Brooding,out	0.207	0.441	0.470	0.997
Brooding,Nest-out - Incubation,out	0.660	0.482	1.370	0.745
Incubation,Nest-out - Brooding,out	-0.635	0.462	-1.376	0.742

Table of estimates of the model computed with ‘lmeans’ function (‘lmeans’ R package) and multiple comparisons with ‘Tukey’ adjustments for multiple testing.

Table A3: Analysis of the organization of female-male vocal sequences at the nest.

	Estimate	SE	Chisq	Df	P-value
Occurrence of songs (binomial) [R2c=0.40]					
(Intercept)	2.31	0.53	-	-	-
Breeding stage (Incubation)	-2.10	0.56	13.87	1	<0.001
Zcall index (linear model) [R2c=0.36]					
(Intercept)	-0.26	0.11	-	-	-
Vocalization type (Trills)	-0.14	0.03	69.52	1	<0.001
Breeding stage (Incubation)	0.14	0.09	0.01	1	0.91
Vocalization type (Trills): Breeding stage (incubation)	-0.20	0.05	15.30	1	<0.001
Overlap rate in sequence (linear model) [R2c=0.41]					
(Intercept)	-0.53	0.14	-	-	-
Breeding stage (Incubation)	1.09	0.16	48.03	1	<0.001
Acoustic composition and activity - PC1 (linear model) [R2c=0.58]					
(Intercept)	-0.85	0.30	-	-	-
Breeding stage (Incubation)	1.87	0.25	56.00	1	<0.001
Acoustic composition and activity - PC2 (linear model) [R2c=0.20]					
(Intercept)	0.33	0.23	-	-	-
Breeding stage (Incubation)	-0.68	0.26	6.72	1	0.01

Within pair linear mixed-effect models testing the effect of ‘Breeding stage’ (2 levels: ‘Incubation’ vs ‘Brooding’) on parameters describing the structure of nest vocal sequences. For vocalization indexes, the vocalization type was also tested as an interactive explanatory variable (2 levels: ‘Notes’ vs ‘Trills’). Models’ estimates and standard errors (first two columns) and results from the Anova (three last columns). R2c values, represent conditional coefficients of determination of models.

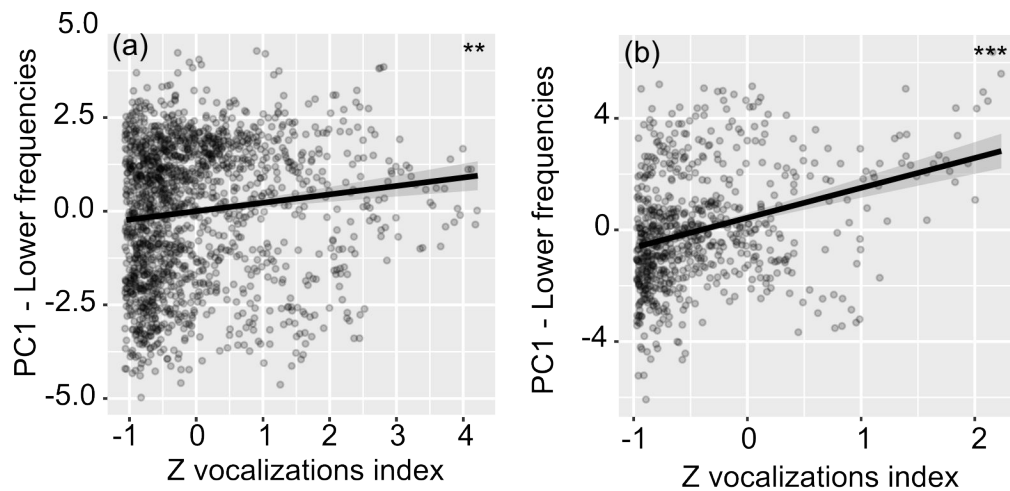


Figure A1: Variation of vocalizations’ acoustic structure along a sequence. Effect of the vocalization index in the sequence was accessed on PCs on Notes (a) and Trills (b). Regression slopes of model estimates with confidence intervals for each fixed factor. Here the vocalization index (centered and scaled) was plotted against the response variable (linear relationship between fixed term and response). See ESM table A5 for detailed statistics ***: $P < 0.001$, **: $P < 0.01$.

Table A4: Analysis of vocalization index in sequence following significant interaction between ‘Vocalization type’ (2 levels: ‘Notes’ vs ‘Trills’) and ‘Breeding stage’ (2 levels: ‘Incubation’ vs ‘Brooding’).

Contrast	Estimate	Standard Error	DF	t-ratio	p.value
Notes, Brooding - Trills, Brooding	0.136	0.030	5057.8	4.493	<0.001
Notes, Brooding - Notes, incubation	-0.138	0.090	181.1	-1.537	0.418
Notes, Brooding - Trills, incubation	0.200	0.085	146.0	2.367	0.088
Trills, Brooding - Notes, incubation	-0.273	0.090	187.6	-3.026	0.015
Trills, Brooding - Trills, incubation	0.065	0.085	151.9	0.759	0.873
Notes, incubation - Trills, incubation	0.338	0.042	5010.0	8.029	<0.001

Table of estimates of the model computed with ‘lmeans’ function (‘lmeans’ R package) and multiple comparisons with ‘Tukey’ adjustments for multiple testing.

Table A5: Analysis of vocalization acoustic structure across breeding stages.

	Estimate	Std. Error	Chisq	DF	P-value
Notes - PC1 [R2c=0.51]					
(Intercept)	0.532	0.058	-	-	-
Breeding stage (Incubation)	-1.559	0.126	153.080	1	<0.001
Zvocalization index	0.848	0.263	10.403	1	0.001
Trills - PC1 [R2c=0.53]					
(Intercept)	0.775	0.113	-	-	-
Breeding stage (Incubation)	-0.841	0.168	25.083	1	<0.001
Zvocalization index	1.401	0.337	17.264	1	<0.001

Within pair linear mixed-effect models testing the effect of ‘Breeding stage’ (2 levels: ‘Incub’ vs ‘Brooding’) and ‘Vocalization index’ centered and reduced continuous covariate) on PCs describing vocalization structures, controlling for repeated measures. Estimates and standard error

of models (first two columns) and results from the Anova (last three columns). R2c values represent conditional coefficients of determination of models.

Table A6: Values of the eight acoustic parameters used in the PCAs (Trills and Notes).

Acoustic parameter	Call type	Breeding stage	Mean	Sd	CV
Mean	Notes	Brooding	4634.45	826.94	0.18
		Incubation	5233.45	1077.69	0.21
	Trills	Brooding	4435.99	675.87	0.15
		Incubation	4673	609.5	0.13
		Brooding	4197.21	1147.24	0.27
		Incubation	4640.63	1350.24	0.29
Median	Notes	Brooding	3746.41	763.4	0.2
		Incubation	4052.3	634.43	0.16
	Trills	Brooding	2872.19	1217.35	0.42
		Incubation	3831.53	1359.56	0.35
		Brooding	2667.53	688.47	0.26
		Incubation	2900	658.69	0.23
Q25	Notes	Brooding	5495.66	991.87	0.18
		Incubation	5903.69	1412.63	0.24
	Trills	Brooding	5343.69	936.25	0.18
		Incubation	5686.67	865.06	0.15
		Brooding	2.94	1.16	0.39
		Incubation	3.95	1.39	0.35
Q75	Notes	Brooding	1.96	0.51	0.26
		Incubation	1.95	0.49	0.25
	Trills	Brooding	13.4	10.17	0.76
		Incubation	21.86	13.41	0.61
		Brooding	6.39	2.98	0.47
		Incubation	6.42	2.78	0.43
Skewness	Notes	Brooding	0.26	0.09	0.36
		Incub	0.21	0.1	0.48
	Trills	Brooding	0.27	0.08	0.29
		Incub	0.26	0.07	0.28
		Brooding	176.74	23.97	0.14
		Incub	184.47	29.72	0.16
Kurtosis	Notes	Brooding	199.36	24.47	0.12
		Incub	206.89	26.24	0.13
	Trills	Brooding	0.26	0.09	0.36
		Incub	0.21	0.1	0.48
		Brooding	0.27	0.08	0.29
		Incub	0.26	0.07	0.28
Sfm	Notes	Brooding	176.74	23.97	0.14
		Incub	184.47	29.72	0.16
	Trills	Brooding	199.36	24.47	0.12
		Incub	206.89	26.24	0.13
		Brooding	0.26	0.09	0.36
		Incub	0.21	0.1	0.48
ACI	Notes	Brooding	0.27	0.08	0.29
		Incub	0.26	0.07	0.28
	Trills	Brooding	176.74	23.97	0.14
		Incub	184.47	29.72	0.16
		Brooding	199.36	24.47	0.12
		Incub	206.89	26.24	0.13

Mean, standard deviation (Sd) and coefficient of variation (CV) are given for each parameter in Trills and Notes, from incubation and brooding. Frequency spectrum description: mean, median, first (Q25) and third (Q75) quartile are all in Hz, skewness, kurtosis and spectral flatness (Sfm) are in arbitrary units. The acoustic Complexity Index (ACI) is in arbitrary unit.

Appendix references

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-Chapter 2-

Linking vocal behaviour at the nest and parental care: female vocal activity predicts behaviour during incubation in dippers.

Villain A.S., Fernandez M.S.A, Doligez B., Soula H.A, Vignal C.

Key words: Acoustic communication, breeding, *Cinclus cinclus*, female song, Intra-pair communication, Monogamy

In prep.

Overview

Peut-on relier le comportement d'incubation des femelles à l'activité vocale au nid chez le cincle plongeur ?



Méthodes

Enregistrements de l'activité vocale au nid pendant l'incubation :

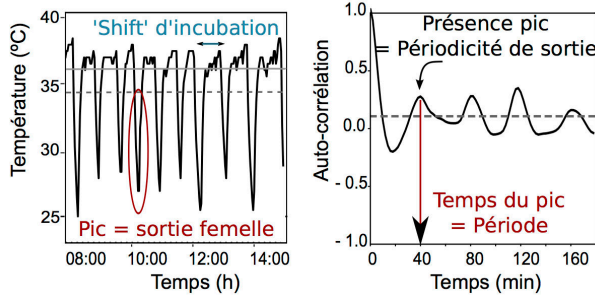
Séquences de cris des femelles, femelle seule ou avec le mâle

Chants femelles, femelle seule ou avec le mâle

Séquences vocales femelle-mâle au nid

Suivi du comportement au nid avec des thermomètres miniatures au milieu des oeufs

Pendant l'incubation, les femelles sortent du nid de manière périodique



Suivi de température sous les oeufs

Sortie (assez longue) --> Pic négatif de température

Auto-corrélation = mesure de périodicité

'Shift d'incubation' = temps entre deux sorties

Incubation

77% des sessions d'enregistrement --> périodicité

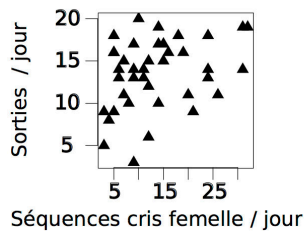
Périodes : moyenne 56 min

minimum 20 min

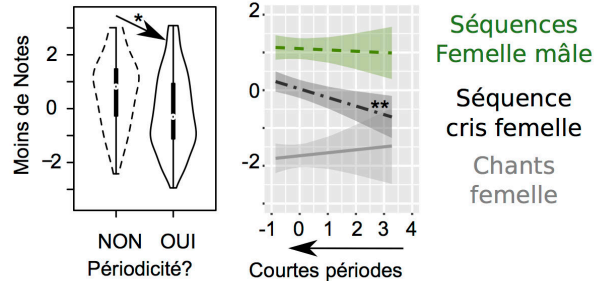
maximum 150 min

L'activité vocale des femelles au nid prédit des paramètres de l'incubation

Le nombre de séquences de cris femelle est corrélée au nombre de sorties (tendance)

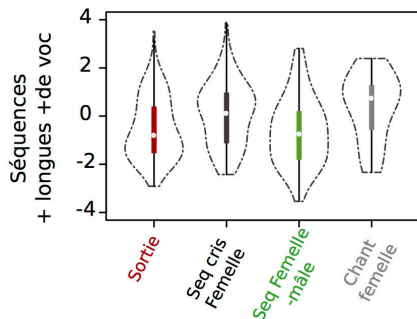


La composition des séquences de cris femelle reflète la périodicité des sorties

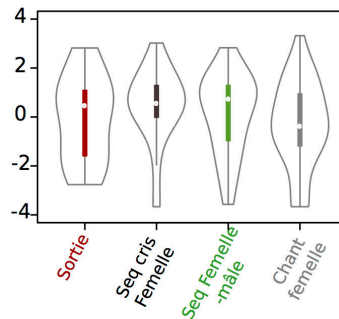


Les séquences vocales femelles reflètent les événements au cours des 'shift d'incubation'

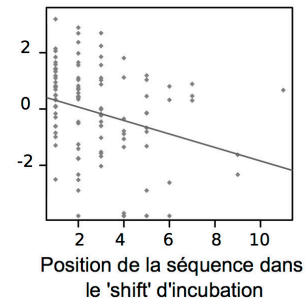
Structure des séquences de cris femelle, dépend de l'évènement suivant



Structure des chants femelle, dépend de l'évènement suivant



Structure des chants femelle, dépend de l'activité vocale



Conclusion

Différentes séquences vocales ne sont pas liées aux mêmes paramètres de comportement --> ≠ fonctions ?

- Séquences de cris femelle sont reliées à des paramètres de l'incubation --> signaux des allers-retours au nid ?

- La structure des chants femelle change avec l'activité vocale au cours du 'shift d'incubation' --> motivation ?

- Les séquences femelle-mâle ne prédisent aucun paramètre de l'incubation --> autre fonction ?

Abstract

In birds, incubation is both crucial and costly: nest predation and metabolic costs have major impacts on the success of incubation. In species with single-sex incubation, incubating parents must balance the thermal needs of the eggs and protection against predators with their own energetic needs. Non-incubating partners may participate to the incubation effort during nest defence or mate feeding. With regards to the intense vocal communication in birds, we may expect vocal communication to play an important role in the organization of breeding activities, among which incubation (coordination of nest trips and defence, mate feeding). We monitored incubation behaviour and vocal activity at the nest of white-throated dippers, *Cinclus cinclus*, in which only females incubate. During incubation, females produced call series and songs, and both partners engage in a joint vocal display when meeting at the nest. But the function of these vocalizations is poorly understood. If vocal sequences produced at the nest participate in coordinating female nest trips and periods of nest attentiveness, we can predict that their occurrence and structure should reflect female incubation behaviour. Using nest thermometers we were able to show that during incubation, females regularly and generally periodically leave the nest to forage. We showed that contrary to female songs or female-male sequences at the nest, female calls series (occurrence and composition) reflected female incubation behaviour and may signal female nest trips. Female song structure was mainly linked to the female vocal activity and female song may thus function as a territorial signal or during interactions with their mate. The structure of female-male sequences could not be predicted by any incubation behavioural pattern or parameter and thus seemed more likely to play a role in mate feeding and/or pair bond maintenance. Our results highlight potentially different functions of vocal sequences produced at the nest in dippers.

Introduction

In birds, incubation is a crucial and constraining stage that strongly influences reproductive success (Tinbergen & Williams, 2002; Williams, 1996). On the one hand, increasing nest attentiveness may shorten the incubation period and ensure high hatching success (via reduced duration of development) and high offspring body condition (Lyon & Montgomerie, 1985; Reid, Monaghan, & Ruxton, 2002). On the other hand, incubating parents are vulnerable to predators (Conway & Martin, 2000; Ghalambor & Martin, 2002; Magrath, 1988; Martin, Scott, & Menge, 2000) and incubation is energetically costly (Thomson, Monaghan, & Furness, 1998). Incubating parents must thus balance the thermal needs of the eggs with their own energetic needs (Reneerkens, Grond, Schekkerman, Tulp, & Piersma, 2011; Tinbergen, Drent, & Biebach, 1984). This energetic trade-off might be especially challenging in species with single-sex incubation, since the eggs are left unattended when the incubating parent leaves for foraging (Williams, 1996).

In species with single-sex incubation (generally female), the incubating parent may show different incubation strategies depending on the environmental constraints: under high predation risk, females may limit trips outside the nest using longer incubation shifts (longer time spent incubating between two foraging trips) and longer foraging time (Conway & Martin, 2000). In colder environments, females may adopt shorter incubation shifts and foraging time to avoid eggs cooling down (Conway & Martin, 2000). Because monogamy is the general reproductive strategy in birds (Cockburn, 2006), both parents benefit from a successful incubation. While females incubate, males may participate to the reproductive effort in other ways. Male may defend the nest / territory or feed their mates at the nest during incubation, which may relieve the physiological cost of

incubation and has been shown to increase breeding success (Galván & Sanz, 2011). Males also increased mate feeding during incubation when predation risk was experimentally manipulated (Fontaine & Martin, 2006; Ghalambor & Martin, 2002) or when their mate's ability to forage had been artificially decreased (Cantarero, López-Arrabé, Palma, Redondo, & Moreno, 2014). Communication either between mates or towards conspecifics may be crucial for a successful incubation. Incubating birds, in particular birds nesting in cavities, may not be able to receive visual information from outside the nest and they may therefore rely on acoustic information instead.

Vocal signals at the nest may be used by the non-incubating partner to alert about the presence of predators (Elie et al., 2010; L. S. Johnson & Kermott, 1991; Mainwaring & Griffith, 2013; Ziolkowski, Johnson, Hannam, & Searcy, 1997). They may also function as territorial signals (Small & Boersma, 1990, Inman, 1986; Ritchison, 1983). Another possibility is that incubating birds may use calls when leaving the nest to signal their absence (Beletsky & Orians, 1985) and increase nest attendance by their non-incubating partner in return (Yasukawa, 1989). Interactive vocal communication between mates may therefore allow coordination of incubation activities by both partners. In northern cardinals, *Cardinalis cardinalis*, (Halkin, 1997) and in the New Zealand bellbird, *Anthornis melanura*, (Brunton, Roper, & Harmer, 2016), female song rate at the nest was correlated with the visiting rate of the male at the nest for mate feeding. In great tits, *Parus major*, the structure of female-male vocal exchanges and female calls structure differ depending on the outcome of the interaction (female remaining alone in the nest, female flying off the nest, or mate feeding the female in the nest, Boucaud et al., 2016). This may be because female great tits may signal their needs to their mate in their vocalizations (I.C.A Boucaud, Aguirre Smith, Valère, & Vignal, In press). Links between incubation behaviour and vocal activity at the nest is unexplored in many species.

In the present study, we monitored both female incubation behaviour (using nest thermometers inside the nest cup) and vocal activity inside the nest on a population of white-throated dippers, *Cinclus cinclus*. Dippers are monogamous songbirds and parents have asymmetric roles, with the female incubating and brooding hatchlings alone. They are early breeders (Shaw, 1978), which may start incubation when air temperatures are low and should constrain thermal costs. During incubation and hatchling brooding periods, females spend most of their time alone in the nest, but they may (1) leave the nest for foraging, and (2) be visited by their mate at or around the nest. Females regularly call (female call sequences) and sing (female song) from and around the nest and both partners engaged in joint acoustic displays every time males visit their mate at the nest (female-male sequences). A previous study showed that, from incubation to brooding, the organization of female-male vocal sequences reflected changes in parental activities (Villain, Mahamoud-Issa, Doligez, & Vignal, Chapter 1). However, the function of vocal activity at the nest in dippers and potential links with females' incubation behaviour remains unexplored. If female vocal activity or female-male vocal interactions at the nest participate in coordinating female incubation shifts or male attendance during trips away from the nest, we expect these vocal signals to change according to female incubation behaviour (i.e. whether the female leaves the nest). In addition, if several types of vocal sequences have different functions, we predict they relate differently to incubation activities.

Material and methods

Study site

This study was conducted on a wild population of White-throated dippers, in and around the Parc Naturel Régional de Chartreuse, France (46.20N, 5.40E) (CRBPO Personal program 655, 2014). The study site (approx. 40 x 30 km) comprises relatively mountainous and hilly habitat and valleys (from 200m to 1100m a.s.l.). About 250 nest boxes –consisting in 30cm long and 20cm diameter PVC tubes- were installed from February 2014 to January 2015.

The study was conducted during the 2015 breeding season (from February to May). Breeding pairs were monitored from nest building to fledging. 38 pairs were monitored, and among them, 33 occupied nest boxes that were installed under bridges between spring and autumn 2014 (210 nest boxes, PVC tubes, 20 cm diameter and 30 cm long).

Monitoring of vocal sequences at the nest.

Equipment and procedure

Vocal sequences were recorded using a digital recorder (Zoom H4N, 44.1 kHz, 16 bit) and a tie microphone (Audio Technica, AT 803) hidden in the moss of the nest. The recording equipment was placed the day before the recording session and did not disturb birds' behaviour. One recording session consisted in a whole day of passive monitoring – a 12-hour recording that started at 06:10 ($\pm 00:34$). To maximize the number of recorded vocal sequences and deal with intra-pair variability, each pair was recorded on two consecutive days.

Automatic extraction of vocal sequences

Sequences of vocalizations were extracted from recordings using in-house softwares. These programs were written in python (www.python.org) by authors H.A.S. and M.S.A.F using open-source libraries. The accuracy of these softwares was tested, confirmed and used in previous studies (J. E. Elie, Soula, Mathevon, & Vignal, 2011; Perez, Fernandez, Griffith, Vignal, & Soula, 2015). Vocalization detection consisted of two stages. First, sounds were detected based on a simple threshold high-pass filtered energy envelope (1024 samples FFT; 441 Hz sampling; cut-off frequency: 500Hz). Each detected event was reconstructed in order to maintain an amplitude range of 90% compared to the maximum amplitude, and overlapping events were merged. Second, noises that were not dipper vocalizations (water, wing noises or other bird species vocalizations) were automatically removed using a machine learning process. We trained a supervised classifier using a data set composed of 750 random extracted sounds from all of our data. Each sound was classified by one expert (MSAF) as “vocalization” or “non-vocalization”. We trained a Random Forest classifier (Breiman, 2001) using 500 sounds as a training set. The validation set was composed of the remaining 250 sounds. This classifier had an overall rate of error below 3% (error on single vocalizations). Because one vocal sequence contains several vocalizations, all sequences from dippers were detected, with no false negative. Some false positives were manually removed. This program largely facilitated the detection of vocal sequences over hours of recordings on several days (around 820 hours of recording in total for 2015), without missing information.

Manual classification of vocal sequence categories

Nests were exposed to different levels of background noise depending on the local characteristics of the water stream. The vocalizations produced outside the nest were easier to detect at quieter nest sites than noisier ones. On the contrary, noise did not affect

the detection of the vocalizations produced inside the nest since birds were very close to the microphone. Thus, only vocalizations produced inside the nest were kept and three categories of vocal sequences were distinguished based on the acoustic activity of the female and the male at the nest.

- When two voices were heard in a vocal sequence, a ‘TwoBirds’ sequence was defined, which defines the context of a male visiting an incubating female at the nest.

- When only one voice was heard, it could be a series of Trills and flight calls (Calls series), or a series of Notes (Songs). These sequences were assumed to be female sequences, since only females incubate (Tyler & Ormerod, 1994), and when males visited females at the nest during incubation, it always led to a ‘TwoBirds’ vocal sequence. No male vocalized at the nest alone during incubation. ‘FemaleCalls’ and ‘FemaleSong’ were then respectively defined to describe these two categories of one-bird vocal sequences.

During ‘FemaleCalls’ and ‘FemaleSong’ sequences, the male could be around the nest and produce vocalizations in interaction with the female but because the detection depended on the noise level at each site, these vocalizations could not be taken into account. ‘FemaleCalls’ and ‘FemaleSong’ could thus be produced by the female alone or during interactions with the male located few meters away. The number of ‘TwoBirds’ sequences is therefore underestimated in this analysis. For each extracted sequence, the sequence type and the time of the day were recorded. Out of 38 pairs recorded, 33 produced ‘TwoBirds’ vocal sequences, 37 produced ‘FemaleCalls’ vocal sequences and 21 produced at least one ‘FemaleSong’.

Vocal sequences analysis

Automatic detection of vocalization types in vocal sequences

The vocal repertoire was simply divided into two vocalization types: Trills and Notes (fig. 1). Trills are broadband signals, composed of a series of pulses (fig. 1a). Notes are diverse and are composed both of flight calls (fig. 1b) and song syllable (fig. 1c).

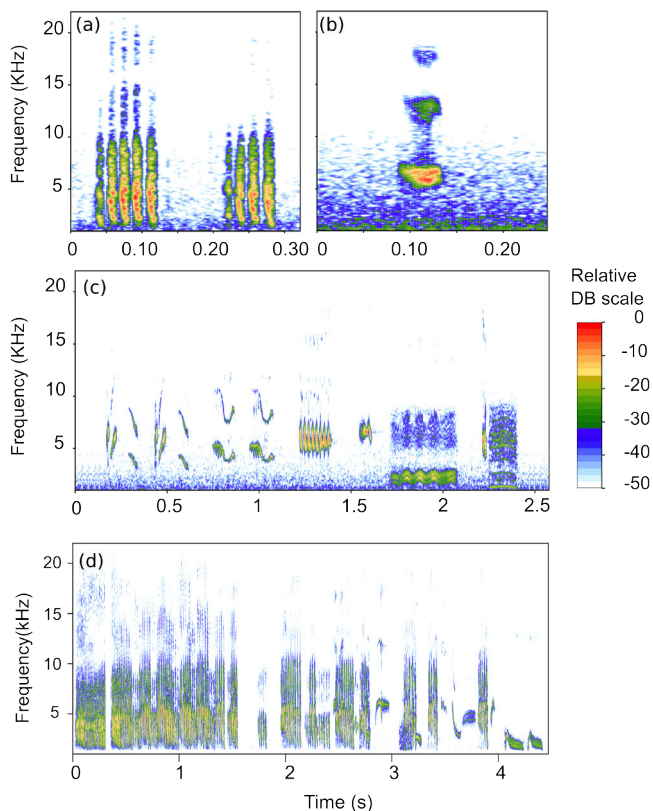


Figure 1: Vocal repertoire at the nest. Spectrograms of extracts of a ‘FemaleCalls’ sequence with Trill calls (a) and Flight call (classified as Notes) (b), a FemaleSong with Notes mainly (c), a ‘TwoBirds’ sequence with overlapping Trills and Notes produced by both the female and the male. Spectrograms produced from normalized wave sounds, using the same DB scale, window length= 512, overlap=50%, ‘spectro’ function, ‘seewave’ R package (Sueur, Aubin, & Simonis, 2008).

Trills and Notes have very different acoustic structures that allowed automatic detections and classifications of vocalization types along vocal sequences. Using a set of manually

annotated sequences, a machine-learning program classified each time interval as Trill, Note or noise using an analysis of images on spectrograms. After checking the accuracy of the program (about 10% of misclassified vocalizations), it was generalized to all vocal sequences. Flight calls resembled more to Notes than Trills and were classified as Notes by the program. It should be noted that Notes detected in FemaleCalls sequence are more likely to be Flight calls than song syllables (programmed developed by author HAS).

Proxies of temporal organization and composition of vocal sequences

The following parameters describing the sequence were measured: the number of vocalizations, the number of Trills and Notes, the proportion of Notes, the proportion of time spent using Trills over the duration of the sequence, the duration of the sequence and the duty cycle (the proportion of time spent vocalizing over the duration of the sequence). A PCA was computed to build scores of the temporal organization and composition of vocal sequences. The two first PCs were kept (SeqPC1 and SeqPC2, 46% and 35% of explained variance respectively) and were used in further statistical analyses (see variable loading table 1).

Table 1: Variable loading of the PCA on temporal organization and composition of vocal sequences (variable transformation in parentheses).

	SeqPC1	SeqPC2
Explained variance (%cumulative)	46.35	81.31
Eigen Value	3.24	2.45
Number of vocalizations (ln)	-0.98	0.07
Number of Notes (ln)	-0.81	-0.39
Sequence Duration (ln)	-0.95	-0.12
Duty cycle	0.32	0.47
Proportion of Notes	0.04	-0.91
Number of Trills (Boxcox)	-0.79	0.56
Proportion of time spent using Trills (boxcox)	-0.04	0.96

Diel variations of female incubation behaviour

Data collection

Female incubation behaviour was monitored using ibutton® thermometers (1cm diameter, 3mm height) placed inside the nest (below the eggs), which recorded the nest temperature every two minutes with a precision of 0.5°C. Since incubation temperature and outside temperature largely differed (difference from 15 to 30 °C), every time the female left the nest during a significant time interval, a rapid decrease of the nest temperature was recorded (fig. 1a). The nest temperature was monitored on 71 daily sessions (33 nests), from the start of the acoustic recording and for 15 hours per session (corresponding to the 12 hours of acoustic recording plus the last three hours before midnight used as a standard of low outside temperature and high nest temperature (with no exit of the female). On 9 sessions (three nests) the thermometer was removed from the nest by the female, the analyses were then carried out on 65 sessions (32 nests). The audio recording was available for 39 sessions (25 nests) which could thus be used to study the relationships between vocal activity at the nest and female incubation behaviour.

Analyses

Two analyses were performed: (1) a peak detection and (2) an auto-correlation computation over the time series of temperatures. In this section, we also explain the validity and the limits of the parameters describing the incubation behaviour obtained with these two analyses (3).

(1) Peak detection analysis

The mean and standard deviation of the incubation temperature were calculated as the mean and sd of the temperature over 15 hours of the recording session (12 hours during

the recording et three hours at night, after sunset). A peak was detected if the temperature fell below a threshold defined as ‘mean incubation temperature– sd(incubation temperature)’ (fig .1a). Each peak was registered as an event of female exit and the time of occurrence of a peak was recorded as the estimated time of exit (point 1 fig. 1a). The time when the peak reached its minimum temperature registered the estimated time of female entrance (point 2, fig. 1a). Between those two points we can define the peak amplitude (as the temperature difference between point 1 and 2) and the duration of the peak (as the time difference between point 1 and 2). These parameters are features of female exits. The daily pattern of female exits was estimated using the mean inter-peak interval (Inter-Exit interval) over the session and its standard deviation (point 3, fig. 1a), and the number of peaks per day (Number of exits).

An incubation shift was defined as the time between a female entrance and the next female exit.

(2) Auto-correlation computation

To quantify the regularity of female exits, the autocorrelation of the temperature signal was computed and three parameters were extracted: the occurrence of at least one auto-correlation peak (an auto-correlation peak is defined as an auto-correlation above 0.1, describing a significant periodicity of female exits, fig. 1b), the amplitude of the first auto-correlation peak (=Female periodicity strength, point 4 fig. 1b) and the time of the first auto-correlation peak (=Female period of exit, point 5 fig. 1b).

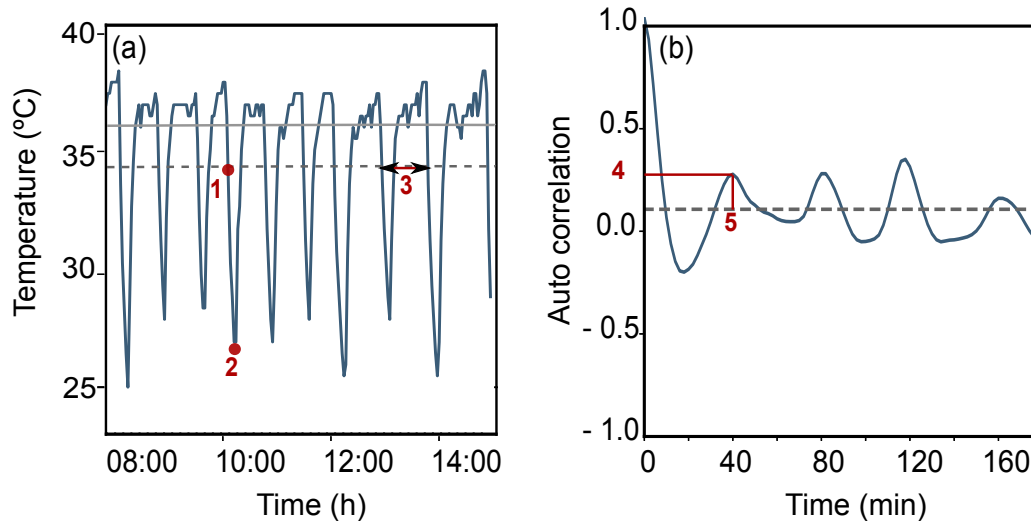


Figure 2: Monitoring of female incubation behaviour and parameters calculation. (a) Extract of temperature monitoring in one nest with indication of the ‘incubation temperature’ (grey solid line), peak detection threshold (grey dashed line). Examples of estimated time of exit (1), minimum peak temperature/estimated time of entrance (2) and inter-exit interval (3). (b) Autocorrelation curve of one female, threshold for presence of autocorrelation peak (0.1, grey dashed line), period (4) and strength of the periodicity (5).

(3) Method validation and limits

The time of the first auto-correlation peak (so the period of exit of females) was correlated positively with the mean Inter-Exit Interval (logarithm transformations for both parameters, $X_T=16.4$, $P<0.001$, slope [95% confidence interval]: 0.30 [0.15: 0.45], $R^2c=0.45$), and negatively with the number of exits (logarithm transformations for both parameters, $X_T= 21.9$, $P<0.001$, slope [95% confidence interval]: -0.33 [-0.46: -0.19], $R^2c=0.46$). So the time of the first auto-correlation peak is a good proxy of female exit behaviour and validates the use of the autocorrelation analysis.

It was not possible to use the temperature data to measure the exact time spent incubating, or the proportion of the day spent incubating by the female. To do so, we would expect that the longer the female stayed outside (time difference between point 2 and 1 on fig. 1a), the lower the peak temperature (point 2, fig. 1a). We would then expect a correlation between the estimated time spent outside and the amplitude of the peak,

which was not verified by the data ($X_j=1.6$, $P=0.20$, slope [95%confidence interval]: $0.30[-0.27: 0.06]$, $R^2=0.18$). In addition, the mean (\pm SE) estimated time spent outside recorded in our data was 3.4 min (± 0.3), considering the temperature sample rate at 0.01Hz, it is unlikely that this estimated time spent outside represents the actual time spent by the female outside the nest. Possible explanations to these discrepancies are: (1) the temperature inside the nest may not decrease immediately after the female left the nest (the nest and eggs might have some temperature inertia), (2) our system detected only exits from the nest that were long enough for the temperature to decrease below the chosen threshold. For these reasons, we will only consider female exit as punctual events without taking into account the estimated time spent outside the nest.

Relationships between vocal activity at the nest and female incubation behaviour: approach

Relationships between vocal activity at the nest and female incubation behaviour were studied using temperature recordings and acoustic recordings.

(1) We tested if female incubation behaviour could be linked to the daily vocal activity (daily number of each category of vocal sequence). For this analysis we used the daily number of exits as a proxy of female incubation behaviour, so that periodic and non-periodic females could be included. Linear mixed effect models and type II Wald X^2 tests were computed (Bates, Mächler, Bolker, & Walker, 2014).

(2) We secondly tested links between the structure of vocal sequences at the nest and parameters of female incubation periodicity (occurrence of an autocorrelation peak, period and strength of the periodicity). Linear mixed effect models and type II Wald X^2 tests were computed (Bates et al., 2014).

(3) Last, we looked at the scale of one vocal sequence produced during an incubation shift and tested if female incubation behaviour (production of vocal sequences and female exits) could predict the temporal organization of the sequence. A model selection procedure was used to assess the best predictors of the temporal organization and composition of the sequence (Burnham & Anderson, 2002; J. B. Johnson & Omland, 2004).

Statistical analyses

All statistics were performed using R (R Core Team, 2015). All continuous covariates were scaled (z-scored) in models to increase interpretability (Schielzeth, 2010), scaled covariates appear with a 'Z' before their name.

(1) Daily vocal activity and female incubation behaviour

From the four daily measures of behaviour (number of female exits, number of 'FemaleCalls', 'FemaleSong' and 'TwoBirds' sequences), pairwise correlations were calculated using spearman correlation (figure 3). The number of female exits and the number of 'TwoBirds' sequences corresponded to two unambiguous behaviours (the female leaving for foraging and the male visiting the female at the nest), contrary to 'FemaleCalls' and 'FemalesSong', that could be produced either alone or in interaction with their mate few meters away. The daily number of female exits and 'TwoBirds' sequences were thus used as response variables into two generalized mixed effect models for Poisson distribution testing for relationship between female behaviour and nest vocal activity. The following models were computed: `model1 <- glmer(Number of female exits~Znumber of TwoBirds + Znumber of FemaleCalls + Znumber of FemaleSong +(1|PairID))`, and `model2<-lmer(Number of TwoBirds~ Znumber of female`

exits+Znumber of FemaleCalls + Znumber of FemaleSong +(1|PairID)), the random factor 'PairID' dealt with several measures per session and per pair.

(2) Periodicity and temporal organization and composition of vocal sequences.

We tested whether the two proxies of temporal organization and composition of vocal sequences (SeqPC1 and SeqPC2) could depend on the three parameters of female incubation periodicity cited above and the type of sequence ('FemaleCalls', 'FemaleSong' and 'TwoBirds'). The following models were computed: model3<- lmer (SeqPC~ Sequence type * occurrence of an autocorrelation peak+(1|PairID/SessionID), on all data) and model4<-lmer (SeqPC~ Sequence type*ZFemale period of exit + Sequence type*ZFemale periodicity strength + (1|PairID/SessionID), on the subset of data of females showing an autocorrelation peak), the random factor 'PairID/SessionID' dealt with several measures per session and per pair.

(3) Sequence organization and composition during an incubation shift

Each sequence produced inside the nest was included in an incubation shift of the female (time between an entrance and the next exit) and several vocal sequences could occur during a given incubation shift. To test whether the temporal organization and composition of a given vocal sequence could reflect the organization of incubation (succession of vocal sequences and female exits), we studied the three types of vocal sequence ('FemaleCalls', 'FemaleSong', 'TwoBirds') and the female's exits as a time series of events. The following parameters were registered for each sequence: its type, the delay after the previous sequence, the upcoming event (one of the sequence types of a female exit), the delay to the next sequence, the timing of the vocal sequence in the incubation shift (as a percentage of the incubation shift (= 'PercentInShift')) and the index of the sequence among the series of events during the incubation shift (= 'IndexInshift').

The duration of the incubation shift was also noticed for each sequence (=‘ZShiftDuration’). A full model was built with either SeqPC1 or SeqPC2:

```
fullmodel<-lmer(-SeqPC1orSeqPC2~UpcomingEvent+ZPercentInIncubShift+
ZshiftDuration+ZTimeFromPreviousSeq+ZTimeToNextSeq+ZIndexInShift+(1|PairID/S
ession)), the ‘PairID/Session’ random factor controlled for repeated measures in each
recording session of each pair. A model selection was performed (‘dredge’ function
‘MuMIn’ R package (Bartoń, 2016)), which compares all possible models built using
subsets of the initial explanatory variables of the full model, including null model.
Models were compared using Akaike Information Criteria corrected for small sample size
(AICc). When more than one model had some support (i.e. Delta AICc < 2, (Burnham &
Anderson, 2002), the importance of explanatory terms was evaluated by calculating the
predictor weight for each term (i.e. the sum of the Akaike weights for each model that
contained that variable). Because the three types of vocal sequences had very different
organization (SeqPC1 and SeqPC2) this procedure was performed in each vocal sequence
type separately.
```

Statistical validation and quantification

The validity of all models was checked for residual equivariance and symmetrical distribution (‘plotresid’ function, ‘RVAideMemoire’ R package (Hervé, 2016)). When models fitted Poisson distribution, residual over dispersion was tested (‘overdisp.glm’ function, ‘RVAideMemoire’ R package). When interactions between fixed factors were significant, post-hoc tests between interacting factors were computed with Tukey correction for comparisons (‘lsmeans’ function, ‘lsmeans’ R package (Lenth, 2016)) and post-hoc tests between a factor and a continuous variable were computed with Hommel correction for multiple comparisons (‘testInteraction’ function, ‘phia’ package (De

Rosario-Martinez, 2015)). All model estimates were computed using 'lsmeans'. To quantify the variance of the data explained by the models, a conditional coefficient of determination of each model was calculated with the 'r.squaredGLMM' function ('MuMIn' R package).

Results

Daily occurrence of vocal sequences is not strongly related to incubation behaviour

The number of female exits per day tended to vary with the daily number of FemaleCalls sequences ($X_f=3.4$, $P=0.07$, effect size [95%CI], increase of 1.04[0.94,1.15] per unit, $R^2c=0.35$, fig. 3), but not with the daily number of FemaleSong or TwoBirds sequences ($X_f=0.45$, $P=0.50$, $X_f<0.01$, $P>0.99$ respectively, $R^2c=0.35$). The daily number of TwoBirds was correlated to the daily number of FemaleCalls ($X_f=4.3$, $P=0.04$, effect size [95%CI], increase of 1.3 [0.99,1.63] per unit, $R^2c=0.79$, fig. 3) and tended to be correlated with the daily number of FemaleSong ($X_f=3.6$, $P=0.06$, effect size [95%CI], increase of 0.97[0.74,1.27] per unit, $R^2c=0.79$, see fig. 3) but no effect of the daily number of female exits was found ($X_f=0.06$, $P=0.81$, $R^2c=0.79$).

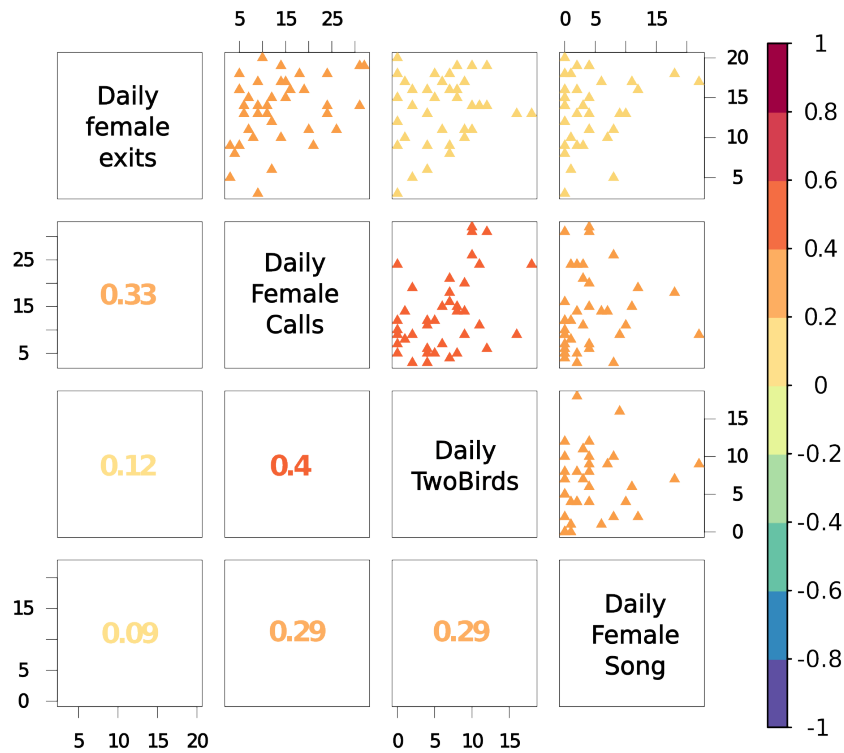


Figure 3: Relationships between daily numbers of each vocal sequence and daily numbers of female exits of the nest. The four behavioural measures are on the diagonal, the upper triangle shows scatter plots, and the lower triangle shows the respective spearman's values, for information, since statistics were performed (1) to take into account repeated measures per nest (2) using Poisson distributions. All values are total number per day (39 daily sessions of recording from 25 pairs). Colour scale of spearman's values is indicated.

The structure of female calls sequences depends on female incubation periodicity

We found that female incubation behaviour was most of the time periodic: 77% of sessions presented an autocorrelation peak (65 sessions, from 32 pairs). Periods ranged from 28 to 178 min (mean \pm SD: 56 \pm 28 min).

We found significant links between incubation periodicity and proxies describing the temporal organization and composition of vocal sequences (SeqPC2 –describing the ratio between Notes and Trills). The occurrence of female periodicity had a significant effect on SeqPC2 ($X_j=4.3$, $P=0.04$, table A2) and this effect was mainly explained by 'FemaleCalls' sequences (post hoc test following significant interaction: Absence vs

Presence of Periodicity in ‘FemaleCalls’, $ratio = 3.0$, $P=0.05$, fig. 4a). When females were periodic, ‘FemaleCalls’ sequences had a significantly higher proportion of Notes. No effect was found in ‘FemaleSong’ and ‘TwoBirds’ sequences (post hoc tests, Absence vs Presence of Periodicity: $ratio=0.016$, $P=1.0$ and $ratio = 0.44$, $P=1.0$ respectively, fig. 4a). When considering only sessions during which females were periodic, the period of exit had a significant effect on SeqPC2 and again ‘FemaleCalls’ mainly explained this effect: the longer the period, the higher the proportion of Notes in sequences (post hoc tests on slope: $X_7=8.7$, $P=0.003$, fig. 4b). No effect was found in ‘FemaleSong’ and ‘TwoBirds’ sequences (post hoc tests: $X_7=0.29$, $P=0.59$ and $X_7=0.13$, $P=0.72$ respectively, fig. 4b).

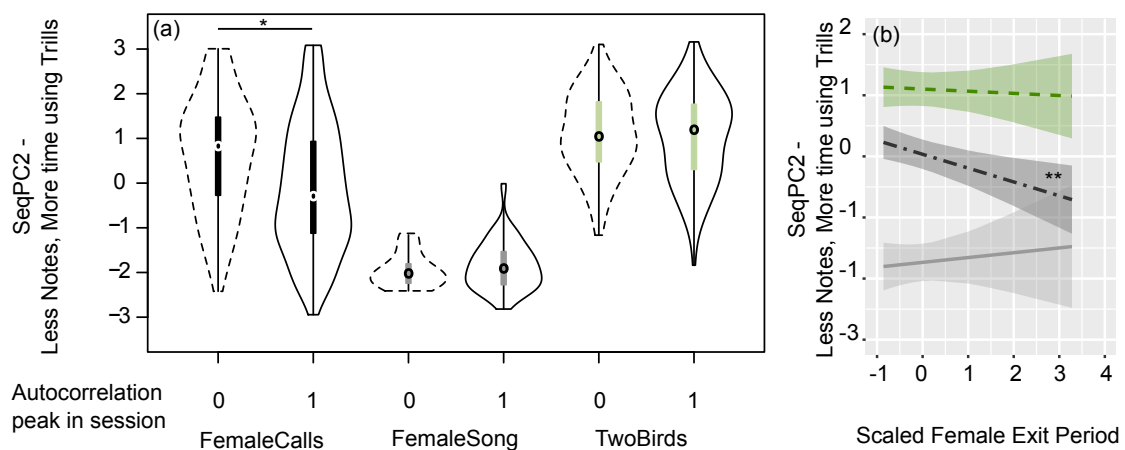


Figure 4: Vocal activity and female incubation periodicity. (a) Violin plot (median, min, max, first and third quartiles and contour of data distribution) of SeqPC2 according to sequence type (‘FemaleCalls’ in black, ‘FemaleSong’ in light grey and ‘TwoBirds’ in green) and occurrence of an autocorrelation peak during the day (0: no periodicity, dashed lines, 1: periodicity, solid lines). (b) Model estimates and confidence interval: ‘FemaleCalls’ (irregularly dashed black lines), ‘FemaleSong’ (solid grey lines) and ‘TwoBirds’ (regularly dashed green lines). All model estimates are available in Appendix tables A2-A6, * $P<0.05$.

No effect of the female periodicity strength was found on SeqPC2 ($X_1=0.98$, $P=0.32$, see table A5). SeqPC1 –describing the duration of the sequence and the number of vocalizations of each types, did not depend neither on the presence of female periodicity nor on parameters describing the periodicity (period and strength) (see table A5 and A6 for statistics). So female incubation periodicity was reflected in the composition of FemaleCalls sequences, in particular, females with longer periods of exit produced ‘FemaleCalls’ with higher proportion of Notes.

Characteristics of the incubation shift are good predictors of the structure of female vocal sequences

A model selection approach was used to identify the best predictors of the structure of female vocal sequences among a set of potential predictors (table 3). Model selections were performed in each sequence type separately on proxies of the structure of the sequence (–SeqPC1 and SeqPC2). We identified predictors of the structure of ‘FemaleCalls’ and ‘FemaleSong’ using –SeqPC1 (models with $\Delta AIC_c < 2$), but all other analyses found no predictor better than the null model (‘TwoBirds’ sequence using –SeqPC1 and all sequences using SeqPC2, table 3). The best predictors (highest Akaike weights) of the temporal organization and composition (–SeqPC1) of ‘FemaleCalls’ sequences were the upcoming event and the duration of the incubation shift (table 2). Namely, FemaleCalls sequences seemed shorter when followed by a female exit or a ‘TwoBirds’ sequence and when the incubation shift was longer (fig. 5a and 5b). The best predictors of the temporal organization and composition of ‘FemaleSong’ were: the upcoming event and the index of the sequence during the incubation shift (table 2, fig 5c and 5d). FemaleSong were shorter when followed by another FemaleSong and when produced after several other vocal sequences during the incubation shift.

Table 2: Akaike weights of predictors of temporal organization and composition of vocal sequences. Only models weighting more than null model were considered. Best predictors in bold.

	FemaleCalls	FemaleSong
Upcoming Event	0.626	0.449
Zincubation Shift Duration	0.626	0
Zpercent In Shift	0	0.094
Zsequence Index In Shift	0.337	0.449
Ztime From Previous Sequence	0.231	0.084
Ztime To Next Sequence	0.296	0.085

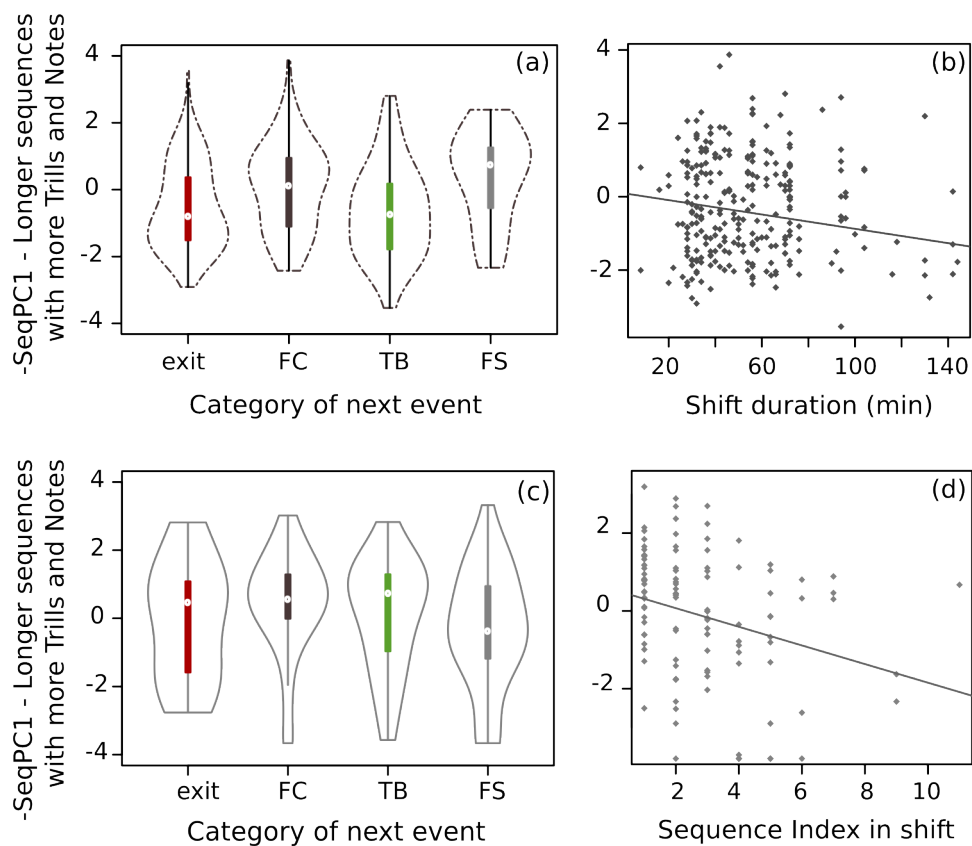


Figure 5: Relationship between temporal organization and composition of vocal sequences (-SeqPC1 and SeqPC2) and their best predictors after model selection procedure (exit, FC= FemaleCalls, FS= FemaleSong, TB= TwoBirds, duration of the incubation shift and sequence index in shift). Predictors of the structure of ‘FemaleCalls’ (a,b) and ‘FemaleSong’ (c,d). (a,c) Violin plot (median, min, max, first and third quartiles and contour of data distribution) for each category of event considered. (b,d) Scatterplot of raw data, model estimates of the simple model between x and y variable with the PairID/Session random factor (solid line)

Table 3: Model selection table. Only the equivalent best models after selections are shown (i.e Delta AICc<2) as well as the null model. All models are ranked. Estimates of each covariate when present in a given model, DF, LogLik, AICc, Delta AICc and weight of models. Model selection were computed in each sequence type separately (FemaleCalls: 273 sequences from N=21 pairs, FemaleSong: 111 sequences from N=14 pairs, TwoBirds sequences: 152 sequences from N=18 pairs)

	Intercept	Upcoming Event	Zincubation Shift	Zincubation Duration	Zpercent In Shift	Zsequence Index In Shift	Ztime From Previous Sequence	Ztime To Next Sequence	df	logLik	AICc	delta	weight
Response: -SeqPC1													
FemaleCalls													
1	-0.541	+	-0.284			0.130			9	-444.613	907.910	0.000	0.121
2	-0.609	+	-0.304			0.139		-0.110	10	-443.599	908.038	0.128	0.113
3	-0.562	+	-0.273						8	-445.968	908.482	0.573	0.091
4	-0.623	+	-0.290					-0.098	9	-445.164	909.013	1.103	0.070
5	-0.526	+	-0.254				-0.109		9	-445.200	909.084	1.175	0.067
6	-0.591	+	-0.273				-0.121		10	-444.213	909.266	1.356	0.061
7	-0.592	+	-0.292				-0.072		11	-443.307	909.626	1.716	0.051
8	-0.524	+	-0.271				-0.062		10	-444.399	909.637	1.727	0.051
Null	-0.393								4	-454.905	917.960	10.050	0.001
FemaleSong													
1	-0.010	+				-0.632			8	-204.929	427.270	0.000	0.187
2	-0.085	+			0.169	-0.650			9	-204.434	428.651	1.381	0.094
3	-0.044	+				-0.622		-0.574	9	-204.537	428.856	1.586	0.085
4	0.039	+				-0.591	0.129		9	-204.546	428.875	1.605	0.084
Null	-0.068								4	-213.386	435.149	7.879	0.004
TwoBirds													
Null	1.479								4	-199.388	407.049	0.000	0.100
2	1.463		0.108						5	-198.546	407.504	0.455	0.080
3	1.508							0.084	5	-198.699	407.808	0.759	0.068
4	1.493								5	-199.042	408.495	1.446	0.049
5	1.490		0.097					0.074	6	-198.014	408.608	1.559	0.046
6	1.472						0.035		5	-199.254	408.920	1.871	0.039

Response: SeqPC2											
FemaleCalls											
Null	0.122								941.829	0.000	0.154
2	0.104	0.086						942.696	0.868	0.100	0.100
3	0.113		-0.045					943.616	1.787	0.063	0.063
4	0.113			0.046				943.658	1.830	0.062	0.062
5	0.119				-0.031			943.737	1.908	0.059	0.059
FemaleSong											
1	-1.878	-0.086						183.247	0.000	0.126	0.126
Null	-1.857							183.537	0.290	0.109	0.109
3	-1.891	-0.087		-0.060				183.889	0.643	0.091	0.091
4	-1.870			-0.059				184.228	0.981	0.077	0.077
TwoBirds											
Null	1.133							397.515	0.000	0.085	0.085
2	1.091				-0.100			397.529	0.013	0.084	0.084
3	1.109		-0.099					398.016	0.501	0.066	0.066
4	1.069		-0.097					398.104	0.589	0.063	0.063
5	1.091	-0.063			-0.102			398.965	1.450	0.041	0.041
6	1.133	-0.060						398.994	1.478	0.040	0.040
7	1.137			-0.058				399.094	1.579	0.038	0.038
8	1.096			-0.047				399.331	1.816	0.034	0.034

Discussion

In the present paper we showed that, in dippers, females' nest trips occurred generally periodically. Interestingly the three types of vocal sequence recorded at the nest were not related to the same behavioural parameters during incubation. The daily number of female exits was positively correlated to the daily number of female call sequences and the temporal organization and composition of female call sequences depended on the periodicity of incubation patterns. On the contrary, female songs were not related to parameters of female incubation periodicity or shifts. However, the structure of female song was linked to parameters of the vocal activity during incubation shifts: female song seemed shorter when produced after several vocal sequences during a shift or followed by another female song. The daily number of female-male vocal sequences was correlated to the daily number of female songs and calls, but their structure did not relate to any of the parameters describing incubation behaviour. Taken together, these results suggest several hypotheses that can be raised regarding the potential functions of each vocal sequence produced at the nest during incubation.

Female call sequences may participate in signalling incubation behaviours

The temporal organization and composition of female call sequences was related to several aspects of female incubation behaviour. Our measures of female incubation behaviour were general (daily number of exits, periodicity patterns) and may have blurred fine variations in incubation behaviour. Nevertheless, female calls composition was quite strongly related to these general proxies of incubation behaviour. Female call sequences were composed of a higher proportion of Notes than Trills when incubation bouts were periodic and the longer the period, the more Notes in the sequence. Similarly to other songbird species, female call

sequences may signal when females leave and come to back to the nest (McDonald & Greenberg, 1991). Because their composition varied with the periodicity (long vs short incubation shifts), they could function as timing signals by the female, allowing their mate to defend the territory whenever needed (Yasukawa, 1989). If female call sequences signal trips away from the nest, we would expect a correlation between the daily number of female call sequences and the daily number of female exits. We found a trend consistent with this prediction. We still do not know whether vocal activity is intrinsically linked to characteristics of females (for example, body condition) or whether it can be adjusted in response to changes in incubation behaviour (linked to territory quality or outside temperature). In other bird species, it is possible to artificially increase the quality of a territory using feeders. Although dippers dive in rivers to forage, it may be possible to add underwater feeders with invertebrates close to the nest. In addition, several studies successfully managed to experimentally modify incubation parameters by manipulating outside temperatures. For example, in pectoral sandpiper, *Calidris melanotos*, heating the nest led to an increase in the total proportion of time spent attending the eggs (Cresswell et al., 2004). An explanation for this result is that artificially heating nests reduced the metabolic costs of incubation and thus allowed the birds to stay longer in the nest without foraging. Similar results were obtained in great tits, *Parus major* (Bryan & Bryant, 1999). Because dippers start breeding early, when outside temperatures are low, artificially modifying nest temperature during incubation would be a useful way to test whether females signal the time spent on the nest using calls.

During an incubation shift, the score for the duration and number of vocalizations of the female call sequence (SeqPC1) was predicted both by the duration of incubation shift (the longer the shift, the shorter the sequence) and by the category of the upcoming event (but not its Notes-Trills composition – SeqPC2). In particular, female call sequences seemed

shorter when followed either by a female exit or a female-male sequence (TwoBirds), two events that may be associated to either foraging behaviour or mate feeding during visits, (Engstrand, Ward, & Bryant, 2002). Spending more time in the nest without foraging may be associated with a change in physiological state and two hypotheses arise to explain shorter female calls sequence: either the duration of the sequence is a by-product of physiological state or it is a signal of need addressed to the mate. In the first case, the signal would not be addressed to the mate and females would suffer from a lack of food, resulting in shorter sequences. In the second case, females may signal their physiological need to their mate. In other contexts in which physiological needs are coded by signals, vocalizations or vocal sequences were longer, for example in nestling begging calls (Leonard & Horn, 2001; Sacchi, Saino, & Galeotti, 2002) or in incubating great tits. In great tits, *Parus major*, female-male vocal exchanges at the nest during incubation differed in their acoustic structure depending on the outcome (Boucaud et al., 2016): when the outcome was a female exit or a mate feeding after the male entered the nest, the vocal exchange was longer than when the exchange ended by a female exit or a male visit. Females also changed their vocal production during exchanges with their mate when being experimentally fed leading to shorter female-male vocal sequences (Boucaud et al, in press). Thus, female great tits signal their needs to their mate during vocal exchanges at the nest using longer sequences. We found here the opposite pattern. Therefore, female call sequence duration may not be a signal of need from females to their mate but rather a mere product of their physiological state. However we did find a weak but significant positive correlation between the number of female calls sequence and the number of female-male sequences (potentially associated to mate feeding). In our case, female calls sequences may be produced either by females alone, or in interaction with their mate located few meters away (Villain et al., Chapter 1). Our passive acoustic monitoring did not allow us to distinguish these two contexts of vocal production. This may explain why the occurrence of female call

sequences was not strongly correlated with the number of female exits whereas their composition was. Pooling recordings in which (i) female vocalize alone and (ii) female vocalize with her mate may have hidden some patterns. We still need to explore the occurrence of the two contexts of production of female call sequences and whether we could acoustically distinguish between them.

Female songs at the nest are related to vocal activity

Contrary to female call sequences, the structure of female song was linked neither to the periodicity of incubation nor to the duration of an incubation shift. However, the vocal activity during the incubation shift was a good predictor of the organization of female song since songs were shorter when produced after several vocal sequences of an incubation shift or when produced before another female song. In addition, the number of female songs tended to correlate with the number of female-male sequences (TwoBirds).

Female songs inside the nest during incubation may be related to female-male behavioural interactions. Such female songs have been described in several species of birds (Brunton et al., 2016; Halkin, 1997; Inman, 1986; Ivor, 1944) and one hypothesis is that they may be related to parental investment. In northern cardinal and in New Zealand bellbirds, female song rate was correlated to mate feeding rate during incubation (Halkin, 1997; Brunton et al., 2016). Here, we found a correlative trend between the daily numbers of female-male sequences at the nest and female songs: female song may stimulate male visits in dippers, during which 23% lead to a clear mate feeding (Engstrand et al., 2002). Nevertheless, the correlation was weak: as for female calls, female songs may be produced by the female alone in the nest or in interaction with her mate being few meters away, and pooling vocal signals produced in these two contexts may have blurred correlations.

Several studies showed that female songs may function as territorial signals (Cooney & Cockburn, 1995; Krieg & Getty, 2016; Langmore, 1998) and singing conspicuously may not be necessary to carry an aggressive message. Soft songs and calls have indeed also been demonstrated to represent aggressive signals in several species of birds (Akçay, Anderson, Nowicki, Beecher, & Searcy, 2015). Singing soft songs from the nest may function as an aggressive signal in dippers. Indeed, dippers are territorial and intra-specific competition for nests sites and mates between males is high, with cases of usurpation involving infanticide (B. Doligez, pers. comm.; see also (Wilson, Nrgo, & Hiraldo, 1992). Because females typically remain alone in the nest for most of the incubation period, it may be advantageous to signal territory occupancy from the nest. This has been shown in other species calling from the nest (e.g. Small & Boersma, 1990). However, because singing from the nest may increase predation risk (Kleindorfer, Evans, & Mahr, 2016; Yasukawa, 1989), shortening female song after the production of several vocal sequences or before another female song may limit the cost of singing in terms of predation. Female may trade singing against remaining inconspicuous inside the nest. Alternatively, shortened female song after serial repetition may result from motor fatigue or increased physiological needs.

Female song may also function as self-stimulation during incubation. The repeated performance of a vocalization could alter motivational state by modulating neuroendocrine functions (Cheng & Durand, 2004). This hypothesis has been tested in ringdoves, *Streptopelia risoria*, during courtship prior to egg laying. Experimentally preventing females to vocalize inhibited follicular growth despite active courtship by the male (Cheng, 1992). Since incubation is metabolically costly, females may stimulate themselves by singing in the nest. In that case, female song rate should increase with incubation effort, for instance when outside temperature is low or for larger clutches.

Female-male vocal sequences at the nest may be associated to mate feeding

None of the parameters of female-male sequences (TwoBirds), which took place during male visits to the incubating female, were correlated to incubation parameters (either the daily number of female exits or incubation periodicity). In several bird species, males feed the incubating female during their visits (Donázar, Negro, & Hiraldo, 1992; Helfenstein, Wagner, Danchin, & Rossi, 2003). Therefore, female-male sequences may here be associated to mate feeding. In dippers, mate feeding has been reported, although feedings were not systematic at each visit (Engstrand et al., 2002) and females do not rely solely on mate feeding and forage by themselves. Our observations seem consistent with this idea because the number of female-male sequences was not negatively correlated with the number of female exits. Female-male sequences thus do not replace female foraging trips away from the nest. The daily number of female-male sequences was positively correlated to the number of female calls and marginally correlated to the number of female songs. If female calls and female songs are produced during interactions with their mates, females may stimulate male visit to the nest by singing (see Brunton et al., 2016; Halkin, 1997). Mate feeding may also be a honest signal of paternal investment (Korpimäki, 1989; Lyon & Montgomerie, 1985) or a way to strengthen the pair bond (Lack, 1940). In many species, mate feeding rate was associated to higher reproductive success (Pearse, Cavitt, & Cully, 2004; Klatt, Stutchbury, & Evans, 2008; Matysioková & Remeš, 2010) and it would be interesting to test whether the number of female-male sequences at the nest is linked to the breeding success in our study species.

Beside mate feeding, female-male sequences may also function in pair bond maintenance. Greeting ceremonies when meeting at the nest have been described in several bird species and they sometimes involve vocal signals (Wachtmeister, 2001). This hypothesis could be

tested by investigating mate fidelity in relation to the number and characteristics of female-male sequences.

To conclude, we showed that only female call sequences were linked to general parameters describing female incubation patterns and may thus signal trips away from the nest during incubation. Female songs were only linked to overall female vocal activity and may function as territorial or self-stimulation signals. Female-male sequences at the nest were not related to parameters of incubation behaviour either, but may be associated to mate feeding and participate in pair bond maintenance. The relationships reported here between incubation behaviour and vocal activity at the nest are nevertheless correlative. Experimental modifications of clutch size or nest temperature could be useful to manipulate incubation behaviour and test whether vocal behaviour is impacted in response to this change. In addition, how other phenotypic traits (morphometric traits and body condition) or birds' age may affect behaviour at the nest needs to be explored to better understand what parameters drive the organization of the crucial of incubation in birds.

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Appendix

Table A1: Daily vocal activity and female incubation periodicity. Estimates (first three columns) and Anova table (last three columns) of models explaining the daily vocal activity score by the presence of female periodicity (Presence or not of an autocorrelation peak). R2c refers to conditional coefficient of determination of each model.

	Estimate	Std. Error	t value	Chisq	Df	Pr(>Chisq)
Response: -DailyVocActivity [r2c=0.68] (all dataset)						
(Intercept)	-0.606	0.578	-1.048	-	-	-
Presence of an autocorrelation peak	0.910	0.649	1.403	1.969	1	0.161
Response: -DailyVocActivity [r2c=0.70] (dataset with autocorrelation peak)						
(Intercept)	0.151	0.408	0.369	-	-	-
Scaled Female Period of exit	0.238	0.275	0.863	0.745	1	0.388
Scaled Female Periodicity strength	0.139	0.368	0.378	0.143	1	0.705

Table A2: Vocal sequence temporal organization and composition and occurrence of female incubation periodicity. Anova table.

	Chisq	Df	Pr(>Chisq)
Response: -SeqPC1			
Sequence type	331.080	2	<0.001
Presence of an autocorrelation peak	0.185	1	0.667
Sequence type: Presence of an autocorrelation peak	4.686	2	0.096
Response: SeqPC2			
Sequence type	511.861	2	<0.001
Presence of an autocorrelation peak	4.343	1	0.037
Sequence type: Presence of an autocorrelation peak	7.660	2	0.022

Table A3: Vocal sequence temporal organization and composition and occurrence of female incubation periodicity (0: no periodicity, 1: periodicity) depending on sequence type - Post hoc test table with Tukey correction for multiple comparisons.

contrast	estimate	SE	df	t.ratio	p.value
FemaleCalls,0 - FemaleSong,0	2.334	0.247	865.102	9.431	<0.001
FemaleCalls,0 - TwoBirds,0	-0.596	0.167	910.408	-3.580	0.005
FemaleCalls,0 - FemaleCalls,1	0.566	0.192	59.278	2.951	0.049
FemaleCalls,0 - FemaleSong,1	2.338	0.215	88.083	10.862	<0.001
FemaleCalls,0 - TwoBirds,1	-0.497	0.204	76.065	-2.440	0.156
FemaleSong,0 - TwoBirds,0	-2.930	0.258	907.675	-11.372	<0.001
FemaleSong,0 - FemaleCalls,1	-1.767	0.271	181.185	-6.530	<0.001
FemaleSong,0 - FemaleSong,1	0.005	0.288	214.670	0.016	1.000
FemaleSong,0 - TwoBirds,1	-2.831	0.279	199.402	-10.160	<0.001
TwoBirds,0 - FemaleCalls,1	1.163	0.214	83.919	5.426	<0.001
TwoBirds,0 - FemaleSong,1	2.935	0.235	115.115	12.473	<0.001
TwoBirds,0 - TwoBirds,1	0.099	0.224	100.537	0.443	0.998
FemaleCalls,1 - FemaleSong,1	1.772	0.127	912.913	13.996	<0.001
FemaleCalls,1 - TwoBirds,1	-1.063	0.111	910.667	-9.558	<0.001
FemaleSong,1 - TwoBirds,1	-2.835	0.145	910.871	-19.586	<0.001

Table A4: Vocal sequence temporal organization and composition and occurrence of female incubation periodicity (0: no periodicity, 1: periodicity) depending on sequence type – Model estimates tables. R2c refers to conditional coefficient of determination of each model.

Sequence type	Presence of an autocorrelation peak=Periodicity	lsmean	SE	df	lower.CL	upper.CL
Response: -SeqPC1 [r2c=0.36]						
FemaleCalls	0	-0.398	0.220	46.449	-0.840	0.044
FemaleSong	0	-0.288	0.325	189.531	-0.928	0.353
TwoBirds	0	1.418	0.252	78.484	0.916	1.919
FemaleCalls	1	-0.692	0.152	31.971	-1.000	-0.383
FemaleSong	1	0.083	0.192	73.838	-0.299	0.465
TwoBirds	1	1.474	0.173	54.202	1.127	1.822
Response: =SeqPC2 [r2c=0.45]						
FemaleCalls	0	0.615	0.165	49.932	0.284	0.945
FemaleSong	0	-1.719	0.253	219.783	-2.218	-1.220
TwoBirds	0	1.211	0.192	87.532	0.830	1.592
FemaleCalls	1	0.048	0.114	33.483	-0.183	0.279
FemaleSong	1	-1.724	0.148	84.177	-2.019	-1.429
TwoBirds	1	1.111	0.133	61.552	0.846	1.377

Table A5: Vocal sequence temporal organization and composition and occurrence of female incubation periodicity parameters (Period of Exit and Periodicity strength) depending on sequence type –Anova table and post hoc test following significant interaction (‘Hommel’ correction for multiple comparison on covariates). R2c refers to conditional coefficient of determination of each model.

	Chisq	Df	Pr(>Chisq)
Response: -SeqPC1 [R2c=0.37]			
Sequence type	241.431	2	<0.001
Scaled Female Periodicity strength	0.362	1	0.548
Scaled Female Period of exit	0.873	1	0.350
Sequence type:Scaled Female Periodicity strength	8.597	2	0.014
Sequence type:Scaled Female Period of exit	1.708	2	0.426
Response: SeqPC2 [R2c=0.46]			
Sequence type	383.759	2	<0.001
Scaled Female Periodicity strength	0.983	1	0.321
Scaled Female Period of exit	4.333	1	0.037
Sequence type:Scaled Female Periodicity strength	1.737	2	0.420
Sequence type:Scaled Female Period of exit	6.075	2	0.048
-SeqPC1 - post hoc test slopes comparisons			
FemaleCalls-FemaleSong	4.750	1	0.059
FemaleCalls-TwoBirds	1.839	1	0.175
FemaleSong-TwoBirds	8.576	1	0.010
-SeqPC1 - post hoc tests, slopes significance			
FemaleCalls	0.264	1	0.608
FemaleSong	2.473	1	0.116
TwoBirds	2.588	1	0.108
SeqPC2 - post hoc test slopes comparisons			

FemaleCalls-FemaleSong	4.150	1	0.125
FemaleCalls-TwoBirds	3.276	1	0.141
FemaleSong-TwoBirds	0.491	1	0.483
SeqPC2 - post hoc tests, slopes significance			
FemaleCalls	8.682	1	0.003
FemaleSong	0.285	1	0.593
TwoBirds	0.127	1	0.722

Table A6: Vocal sequence temporal organization and composition and occurrence of female incubation periodicity parameters (Period of Exit and Periodicity strength) depending on sequence type –Model estimates table. R2c refers to conditional coefficient of determination of each model.

Sequence type	lsmean	SE	df	lower.CL	upper.CL
Response: -SeqPC1 [R2c=0.37]					
Factor estimates at mean value for covariates					
FemaleCalls	-0.675	0.154	18.671	-0.998	-0.351
FemaleSong	0.089	0.196	43.531	-0.306	0.483
TwoBirds	1.495	0.176	31.500	1.135	1.854
Slopes estimates within factors					
Scaled Female Periodicity strength					
FemaleCalls	0.068	0.149	22.649	-0.240	0.376
FemaleSong	-0.301	0.203	91.440	-0.705	0.103
TwoBirds	0.256	0.172	44.123	-0.091	0.603
Scaled Female Period of exit					
FemaleCalls	0.135	0.135	17.284	-0.151	0.420
FemaleSong	0.249	0.208	104.759	-0.164	0.661
TwoBirds	0.004	0.155	34.645	-0.311	0.319
Response: SeqPC2 [R2c=0.46]					
Factor estimates at mean value for covariates					
FemaleCalls	0.034	0.124	19.627	-0.224	0.293
FemaleSong	-1.735	0.157	45.704	-2.052	-1.418
TwoBirds	1.102	0.142	33.223	0.814	1.390
Slopes estimates within factors					
Scaled Female Periodicity strength					
FemaleCalls	-0.046	0.096	10.686	-0.258	0.166
FemaleSong	-0.002	0.148	77.074	-0.297	0.293
TwoBirds	-0.174	0.118	30.267	-0.414	0.066
Scaled Female Period of exit					
FemaleCalls	-0.226	0.083	11.322	-0.408	-0.045
FemaleSong	0.079	0.152	126.651	-0.222	0.379
TwoBirds	-0.035	0.104	33.184	-0.247	0.176

- Chapter 3 -

Pair communication at the nest in Dippers: effects of natural and experimentally elevated environmental noise.

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Key words: Acoustic flexibility, Intra-pair communication, Lombard effect, Acoustic Adaptation Hypothesis, Female song, Incubation

In prep.

Overview

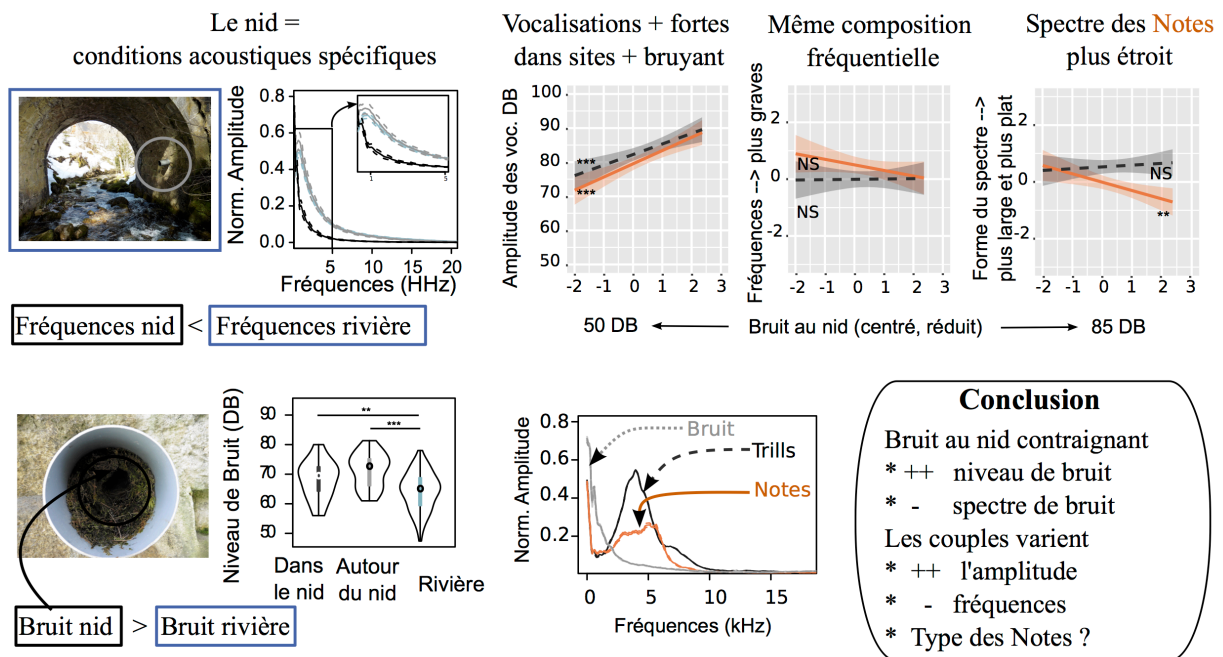
Comment les couples de cincles adaptent-ils leurs vocalisations au nid selon le bruit de fond de l'environnement ?

Méthodes et hypothèses

Etape 1: Enregistrement de couples au nid pendant l'incubation selon les niveaux de bruit naturels du site
Vocalisations toujours au-dessus des caractéristiques du bruit ? Ajustement au bruit du site ?

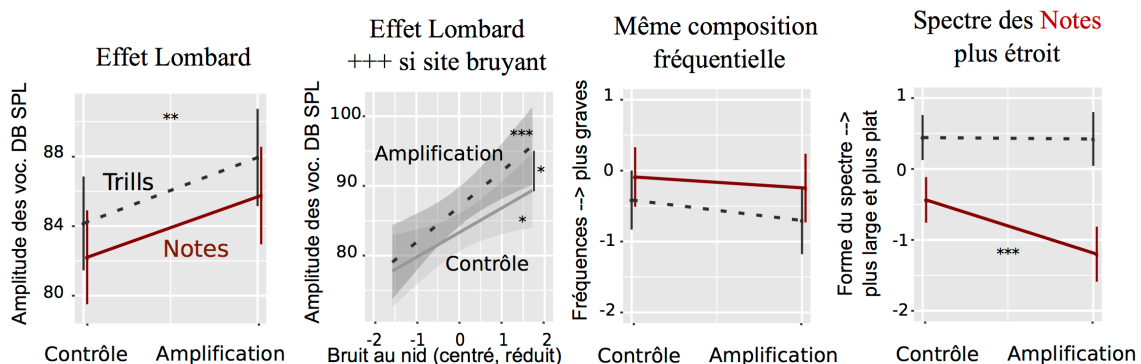
Etape 2: Augmenter artificiellement le bruit autour du nid.
Les couples ajustent-ils leurs vocalisations au bruit additionnel ?

Etape 1: Bruit naturel au nid et communication des couples



Etape 2: Augmentation expérimentale du bruit autour du nid

Playback de bruit de rivière +8 DB à chaque nid, pendant 6h, analyses des vocalisations les plus fortes



Conclusion

Effet Lombard à court terme, sur tous les types de vocalisation
Composition fréquentielle générale non flexible --> Fréquences déjà optimisées à l'environnement ?
Flexibilité des Notes mais pas des Trills

Abstract

Background noise is a major constraint on vocal communication and is likely to shape the acoustic structure of signals. Although urban noise has received a great interest, studying noise-dependent vocal adjustments in species that evolved under continuously constraining natural environmental noise may be useful to understand the evolution of communication and signal structure. White-throated dippers, *Cinclus cinclus*, depend on, and invariably nest over fast flowing water. They nest under continuous water stream noise that can reach high sound pressure levels (from 55 to 85 DB SPL). Female and male vocally interact during incubation in this constraining environment using nest typical Trill calls and song Notes. We explored the influence of natural background noise at the nest on the pair's vocal activity and signal structure using both a correlative approach (between-pair design) and an experimental approach by amplifying water stream noise around the nest (within-pair design). We showed that dippers vocalized at frequencies that do not overlap with the frequency range of the background noise and that frequency composition of vocalizations did not change in response to the noise amplification. This suggests that water stream noise has favoured the evolution of high frequency short-range signals. Pairs breeding in louder sites vocalized at higher amplitude. In response to noise amplification, birds increased the amplitude of their vocalizations, providing strong evidence for a Lombard effect in this species. Pairs also showed vocal flexibility in response to the background noise level at their nest site and in response to noise amplification, using Notes with a sharper and narrowed frequency spectrum in response to high noise level. Dippers' specifically noisy breeding habitat offers new possibilities to investigate the effect of noise on breeding success in naturally noisy environments.

Introduction

The efficacy of an acoustic signal directly depends on its probability to be detected by a receiver. Therefore, background noise is a major constraint on acoustic communication. Elevated background noise decreases the signal-to-noise ratio (Klump, 1996) and is particularly constraining when its frequency range overlaps the frequency range of vocalizations (Slabbekoorn, 2004). Because urban noise is a chronic constraint imposed by human activities, its impact on acoustic communication in animals has attracted strong interest. Birds particularly rely on acoustic communication for social interactions and reproduction (Catchpole & Slater, 2008; Marler & Slabbekoorn, 2004). An impairment of their acoustic communication may therefore partly explain bird species decline in cities (reviewed in Barber, Crooks, & Fristrup, 2010), which generated a major research effort on the impact of anthropogenic noise on vocal production in birds. In response to elevated noise, birds exhibit multiple strategies and all of them tend to either maintain the signal-to-noise ratio or avoid masking effects from noise (Brumm & Slabbekoorn, 2005). Birds can increase the amplitude of their vocalizations, a strategy called the Lombard effect (Lombard, 1911), already described in many vertebrate species (Brumm & Todt, 2002; Brumm, 2004; Cynx, Lewis, Tavel, & Tse, 1998; reviewed in Brumm & Zollinger, 2011). Birds may also use frequencies outside the noise range to avoid spectral masking (Dubois & Martens, 1984; Halfwerk & Slabbekoorn, 2009; Nemeth & Brumm, 2009; Potvin, Parris, & Mulder, 2011; Slabbekoorn & den Boer-Visser, 2006; Slabbekoorn & Peet, 2003; Verzijden, Ripmeester, Ohms, Snelderwaard, & Slabbekoorn, 2010). They may also increase the duration of their vocalization bouts or songs (Brumm & Slater, 2006a; Potvin & MacDougall-Shackleton, 2015). Finally, birds may also avoid signal masking by changing the timing of their vocalizations to more quiet time periods (Gil, Honarmand, Pascual, Pérez-Mena, & Garcia, 2014).

Overall, these results clearly show that urban noise drives major changes in vocal production in birds. Nevertheless, changes in noise levels are only one of the many constraints imposed by urban habitats, which are also usually characterized by increased levels of chemical and light pollution, anthropogenic perturbations, habitat fragmentation, etc. (Barber et al., 2010; Gil & Brumm, 2013). To avoid confounding factors, we need to

study species that have evolved under continuously high noisy conditions in their natural habitat.

In addition, most studies so far have described noise-dependent vocal adjustments on long-range vocal signals, in particular bird songs (Mockford & Marshall, 2009; Nemeth & Brumm, 2009; Robisson, Aubin, & Bremond, 1993; Wood, Yezerinac, & Dufty, 2006) but also localisation calls (Lengagne, Aubin, Lauga, & Jouventin, 1999). However, social acoustic communication also relies on short-range vocal signals, such as those used in parent-offspring communication [(e.g. begging calls (M. Leonard & Horn, 1996)), communication between monogamous partners [e.g. soft duets; (Elie et al., 2010; Halkin, 1997)] and group interactions [e.g. distress and alarm calls; (Marler, 2004)]. If vocal adjustments are driven by adaptations to propagation through the environment, short-range signals that are not aimed at being propagated may show different changes in response to noise compared to long-range vocal signals. Only few studies investigated the effect of background noise on short-range vocalizations and overall, they found noise-dependent vocal adjustments comparable to those described in long-range vocalizations [juvenile begging calls: Leonard & Horn, 2005; Leonard, Horn, Oswald, & McIntyre, 2015), contact calls (Hu & Cardoso, 2010; Potvin et al., 2011), nest adult calls (Villain, Fernandez, Bouchut, Soula, & Vignal, 2016)]. The context of intra-pair communication during breeding has been overlooked. In species with biparental care, which represent the vast majority of bird species (Black, 1996, 2001), pair members adjust their parental behaviour to each other (Coulson, 1966; Lee, Kim, & Hatchwell, 2010; Mariette & Griffith, 2012; Mariette, Griffith, Adkins-Regan, & Kalisz, 2015; Morris, 1987; Spoon, Millam, & Owings, 2006). This adjustment may rely on intra-pair communication at the nest (Boucaud, Mariette, Villain, & Vignal, 2016; Halkin, 1997), but such intra-pair communication has only been described in few species of songbirds (Boucaud, Valère, et al., 2016; Elie et al., 2010; Gorissen, Eens, & Nelson, 2004). If intra-pair vocal communication allows pair members to coordinate and adjust their parental behaviour during breeding, noise may negatively impact reproductive success. Therefore, noise may be a major environmental factor shaping individual fitness in noisy environments.

In the present study, we monitored the vocal activity of pairs at the nest in a wild population of White-throated dippers *Cinclus cinclus* living under naturally varying environmental noise level. Dippers are an excellent study system to describe how environmental noise may shape short-range vocal signals, because they nest by or over

running water. Dippers thus live in a continuously noisy environment. Water stream noise has a typical pink frequency spectrum (Marler & Slabbekoorn, 2004) and noise at dippers' nest sites often reaches high sound pressure levels. Additionally, snow melting and unpredictable rainfalls can raise water level and increase background noise for short periods of time. Whether dippers' adapt their intra-pair communication to the constraint of a continuous background noise is unknown. We studied characteristics of the vocal activity of pair members (occurrence and temporal organization of vocal sequences, acoustic structure of vocalizations) according to (1) the natural background noise at the nest site (between-pair design) and (2) during and after an experimental increase of background noise level around the nest (within-pair design). If dippers adapt their within-pair communication to the local acoustic conditions of their nest site, we predict that pairs' vocal activity will differ between sites with different background noise and that pairs will adjust their activity to the experimental short-term noise increase. If on the contrary, the dippers' particular environment has selected for vocalisations that emerge from all possible water stream noise conditions, we predict that the vocal behaviour of pairs will not differ between nest sites of different noise levels and that pairs will not adjust their vocal activity to the experimental increase in noise level.

Methods

Study species and site

Dippers are monogamous songbirds breeding on rock ledges by the river, sometimes close to or behind waterfalls, in crevices or in roots by the riverbank (Shaw, 1978). They also readily use cavities under bridges or walls in human-modified areas and accept nest boxes when provided under bridges (Tyler & Ormerod, 1994). In dippers, both sexes have been reported to sing during pair formation (Tyler & Ormerod, 1994). During incubation and the young nestling phase, females spend most of their time alone in the nest, but they may be visited by males at/around the nest and during these visits, mates perform joint acoustic displays. Females also produce calls and songs when alone in the nest (Villain et al., Chapter 1).

This study was conducted on a wild population of White-throated dippers, in and around the Parc Naturel Régional de Chartreuse, France (46.20N, 5.40E) (CRBPO Personal

program 655, 2014). The study site (approx. 40 x 30 km) comprises relatively mountainous and hilly habitat and valleys (from 200m to 1100m a.s.l.). About 250 nest boxes –consisting in 30cm long and 20cm diameter PVC tubes- were installed from February 2014 to January 2015. This study was conducted from March to May in 2015 and in 2016.

Environmental noise recordings

The environmental noise was recorded both at the nest and at different distances from the nest along the river (sound-level meter Rion NL-42, with additional NX-42WR package, frequency weighting 'Z', temporal weighting 'Fast'). At the nest, recordings were performed inside the nest ('InNest') and at 50 cm from the nest ('AroundNest'). Six recordings were done along the river ('River') at one meter high above the water and at different distances from the nest: 40, 100 and 200 meters from the nest, both upstream and downstream. Repeated measures were kept in the analysis.

All environmental noise recordings were conducted during incubation. Fifty-three sites were recorded (not all used for the recording of pairs' vocal activity: 31 pairs in nest boxes, 14 natural nests under bridges and eight natural nests in open areas), among them, 27 were recorded at the three locations ('InNest', 'AroundNest' and 'River').

Pair recordings

The vocal activity at the nest was recorded using a digital recorder (Zoom H4N, 44.1 kHz, 16 bit in 2015 and Wildlife Song meter SM2+, 96 kHz, 16 bit in 2016) and a microphone (Audio Technica, AT 803 in 2015 and Wildlife SMX-US in 2016) either hidden in the moss of the nest or hidden inside the nest box. The recording equipment was placed the day before the recording session and did not disturb the birds' behaviour. One recording session consisted in a whole day of passive monitoring – a 12-hour recording that started at 06:10 ($\pm 00:34$). All recordings took place during incubation.

Dataset #1: Natural environmental noise at nest sites and pair communication

In our population, nesting dippers chose freely their nest site (either a natural cavity or a nest box). Nest sites may differ in environmental noise characteristics. To assess the effects of environmental noise at the nest site on pair communication, 36 pairs were recorded (sound pressure level at the nest from 45 to 85 DB). All recordings took place in

2015 and a between pair analysis was conducted. Pairs were recorded during two recording sessions.

Dataset #2: Experimental increase of the local background noise and pair communication

Nesting dippers can experience short-term variations in background noise at their nest site, mainly after spring rainy days or snow melting. An increase in water stream levels implies an increase in background noise level that can last one or two days. To assess the effects of a short-term increase of background noise on pair communication, we built a within pair playback experiment. The local background noise at the nest was previously recorded (Rion NL-42, with additional NX-42WR package, frequency weighting 'Z', temporal weighting 'Fast') and used to build a one-minute playback track, so that the experimental increase of background noise levels used the actual water stream noise of each nest site. The playback (Tag, Premio amplifier) amplified the background noise (8 DB SPL) for six hours (\pm 30min). Different nest sites had different initial levels of background noise but all nest sites were subjected to the same relative increase of background noise (six-fold increase in sound pressure).

Each pair was recorded two days during the control treatment (visual artefacts of amplifiers were used during the control treatment) and two days during the noise amplification treatment. Days order was randomized. On each day, two periods were defined:

- 'PB' corresponds to the 6 hours of playback and the equivalent period during the control treatment (six hours in the morning)
- 'PostPB' corresponds to the post playback period and the equivalent period during the control treatment (six hours in the afternoon).

Using these two periods we assessed the responses during and after the noise amplification treatment.

Twenty-one pairs composed this dataset, all nesting under bridges and mainly in nest boxes (only 2 natural nests).

Extraction and classification of vocal sequences recorded at the nest

Automatic extraction of vocal sequences on long sound files

Sequences of vocalizations were extracted from recordings using an in-house software. This program was written in python (www.python.org) by authors H.A.S. and M.S.A.F using open-source libraries. This software accuracy was tested, confirmed and used in previous studies. Briefly, vocalization detection consisted of two stages. The first process was a simple threshold-based sound detection based on a high-pass filtered energy envelope (1024 samples FFT; 441 Hz sampling; cut-off frequency: 500Hz). Each detected event was reconstructed in order to maintain an amplitude range of 95% compared to the maximum amplitude, and overlapping events were merged. The second stage automatically removed noises that were not dippers' vocalizations (water, wings, other bird species) using a machine learning process. We trained a supervised classifier using a data set composed of 750 and 2500 (for 2015 and 2016 respectively) random extracted sounds from all of our data. Each sound was classified by one expert (MSAF) as "vocalization" or "non-vocalization". We trained a Random Forest classifier (Breiman, 2001) using 650 and 2000 (for 2015 and 2016 respectively) sounds as a training set. The validation set was composed of the remaining 75 and 500 sounds (for 2015 and 2016 respectively). This classifier had an overall rate of error below 5%, on each single vocalization. Considering that vocal sequences were mainly composed of several vocalizations, the program generated no false negative, but false positive could be extracted. This program largely facilitated and accelerated the detection of vocal sequences over hours of recordings on several days (around 820 hours of recording for 2015 and 570 hours of recording for 2016).

Manual classification of vocal sequences

Nests were exposed to different levels of background noise depending on the local characteristics of the water stream. The vocalizations produced outside the nest were easier to detect at quieter nest sites than noisier ones. On the contrary, noise did not affect the detection of the vocalizations produced inside the nest since birds were very close to the microphone. Thus, only vocalizations produced inside the nest were kept and three categories of vocal sequences were distinguished based on the acoustic activity of the female and the male at the nest.

- When two voices were heard in a vocal sequence, a 'TwoBirds' sequence was defined, which corresponds to the context of a male visiting an incubating female at the nest.

- When only one voice was heard, it was either a series of Trills and flight calls (Calls series), or a series of Notes (Songs) (Villain et al., Chapter 1). These sequences were assumed to be female sequences, since only females incubate (Tyler & Ormerod, 1994), and in our population, when males visited females at the nest during incubation, it always led to a 'TwoBirds' vocal sequence. In our population, no male came to vocalize alone at the nest during incubation (Villain et al., Chapter 1). 'FemaleCalls' and 'FemaleSong' were then respectively defined to describe these two categories of one-bird vocal sequence.

During 'FemaleCalls' and 'FemaleSong' sequences, the male could be around the nest and produce vocalizations in interaction with the female but because the detection depended on the noise level at each site, these vocalizations could not be taken into account. 'FemaleCalls' and 'FemaleSong' could thus be produced by the female alone or during interactions with the male located few meters away. The number of 'TwoBirds' sequences is therefore underestimated in this analysis. For each automatically extracted sequence, the sequence type and the time of the day were recorded.

Analysis of vocal sequences

Automatic classification of vocalization types in sequences

The vocal repertoire was simply divided into two vocalization types: Trills and Notes (fig. 1). Trills are broadband signals, composed of a series of pulse (fig. 1a), Notes are diverse and are composed both of flight calls (fig. 1b) and song syllable (fig. 1c).

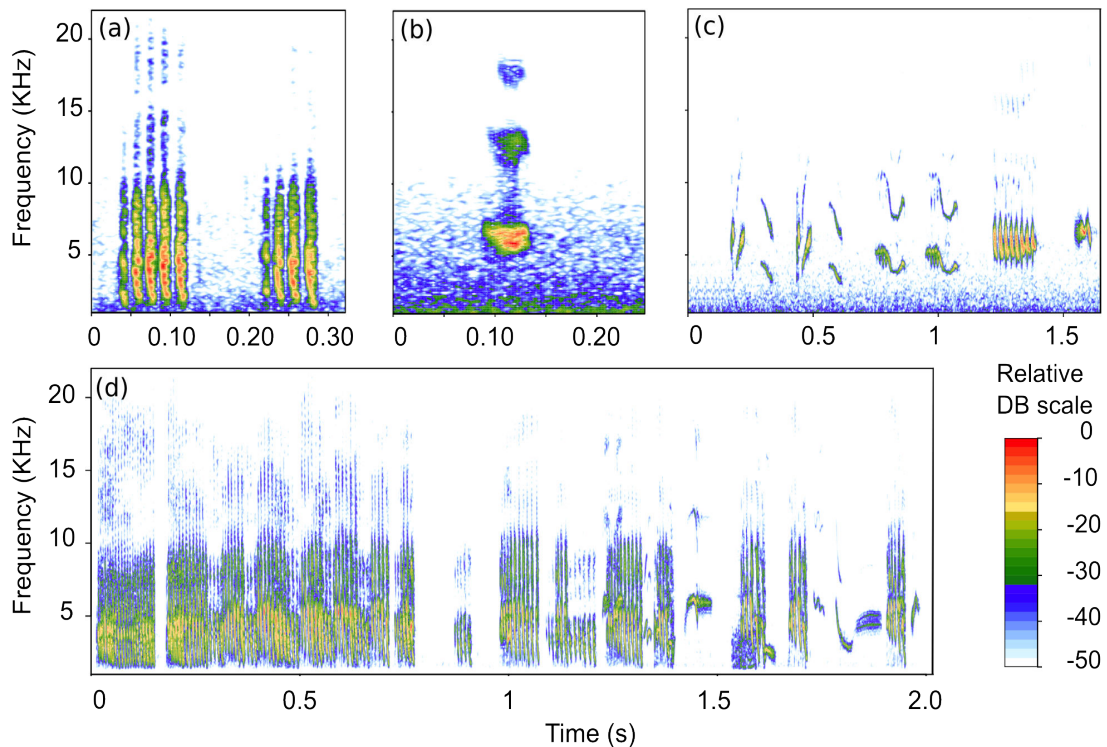


Figure 1: Vocal repertoire at the nest. Spectrograms of extracts of: a ‘FemaleCalls’ sequence: with two Trill calls (a) and Flight call (classified as Notes) (b), a FemaleSong: with Notes mainly (c), a ‘TwoBirds’ sequence: with overlapping Trills and Notes produced by both the female and the male. Spectrograms produced from normalized wave sounds, using the same DB scale, window length= 512, overlap=50%, ‘seewave’ R package (Sueur, Aubin, & Simonis, 2008).

Trills and Notes have very different acoustic structures that allowed automatic detections and classifications of vocalization types along vocal sequences. Using a set of manually annotated sequences, a machine-learning program classified each time interval as Trill, Note or noise using an analysis of images on spectrograms. After checking the accuracy of the program (about 10% of misclassified vocalizations), it was generalized to all vocal sequences. Flight calls resembled more to Notes than Trills and were classified as Notes by the program. It should be noted that Notes detected in FemaleCalls sequence are more likely to be Flight calls than song syllables (programmed developed by author HAS).

Scores of temporal organization and composition of vocal sequences

The following parameters describing the sequence were calculated: the number of vocalizations, the number of Trills and Notes, the proportion of Notes, the proportion of time spent using Trills over the duration of the sequence, the duration of the sequence and the duty cycle (the proportion of time spent vocalizing over the duration of the sequence). A PCA was computed to build scores of the temporal organization and composition of

vocal sequences. The two first PCs were kept (SeqPC1 and SeqPC2, 46% and 35% of explained variance respectively) were used in further statistical analyses (see variable loading table 1).

Table 1: Variable loadings of the PCA on temporal organization and composition of vocal sequences. Transformations used to reach symmetrical distributions of variables are indicated.

	SeqPC1	SeqPC2
Explained variance (%cumulative)	46.35	81.31
Eigen Value	3.24	2.45
Number of vocalizations ¹	-0.98	0.07
Number of Notes ¹	-0.81	-0.39
Sequence Duration ¹	-0.95	-0.12
Dutycycle	0.32	0.47
Proportion of Notes	0.04	-0.91
Number of Trills ²	-0.79	0.56
Proportion of time spent using Trills ²	-0.04	0.96

1: ln, 2: Box-cox. The Box-Cox transformation computes one parameter transformation using the following formula: parameter $(\lambda) 1/4$ (parameter $\lambda e 1)/\lambda$, if $\lambda s 0$ and parameter $(\lambda) 1/4$ $\ln(\text{parameter})$ if $\lambda 1/4 0$. The 'boxcox' function of the 'Mass' R package automatically finds the appropriate λ value to reach a distribution as close as possible to the Gaussian distribution.

Acoustic analyses

All acoustic analyses were performed with custom-written codes using the Seewave R package (Sueur et al., 2008).

Acoustic analysis of environmental noise

From each recording of environmental noise, five intervals of five seconds were manually labelled as intervals containing only the water stream noise, with no anthropogenic noise and no biotic noise. An acoustic analysis was performed on each five-second interval, and the five measures were then averaged to have one measure per recording. After low pass filtering (0-20 kHz, 'fir' function), five spectral parameters were computed ('specprop' function, FFT using a Hamming window, window length 1024, overlap 50%): mean, median, Q25, Q75, Q90 and used in a PCA. Only the first PC was kept to build a frequency composition score of the noise (=NoisePC1, 92% of the explained variance, see variable loading table 2). The amplitude of the background noise was directly averaged from the sound level meter data (100 measures of sound pressure level per second, in DB SPL).

Table 2: Variable loadings of the PCA on frequency composition of the noise. Transformations used to reach symmetrical distributions of variables are indicated

	NoisePC1
Explained variance (%cumulative)	92.60
Eigen Value	4.63
Mean ¹	-0.99
Median ¹	-0.98
Q25 ²	-0.90
Q75 ¹	-0.98
Q90 ¹	-0.95

1: ln, 2: box-cox

Acoustic analysis of vocalizations and acoustic scores

All vocalizations automatically detected were extracted for further acoustic analysis. The amplitude was assessed after microphone calibration (Rion NL-42) using the root mean square of vocalizations. Since birds were at around ten centimetres from the microphone and this distance did not vary (in-nest vocalizations), the absolute amplitude calculated is then the source level (DB SPL). Before spectral measurements, vocalizations were bandpass filtered to avoid calculation in the frequency composition of the background noise (3-20 kHz, 'fir' function). Then the following parameters of the frequency spectrum of the vocalizations were calculated ('specprop' function, FFT using a Hamming window, window length 512, overlap 50%): mean, median, 25% (Q25) and 75% (Q75) quartiles, standard deviation (Sd), skewness, Kurtosis, Inter-quartile-Range (IQR= Q75-Q25). Additionally, the 10% and 90% quantiles (respectively Q10 and Q90) as well as the Inter-Decile-Range (IDR= Q90-Q10) were also calculated.

Two acoustic scores were built to describe the frequency spectrum of the vocalizations: the frequency composition score and the spectral shape score, using two Principal Component Analyses (PCA). The frequency composition PCA used: mean, median, Q25, Q75 and Q90 and the spectral shape PCA used: Sd, IQR, IDR, skewness, and kurtosis. Only the first PC was kept for each PCA: PC1 of the frequency composition PCA (=VocFreqPC, 80% of explained variance) and PC1 of the spectral shape PCA (=VocShapePC, 56% of explained variance) (see variable loading table 3).

The natural background noise at nest sites can reach high sound pressure levels and its frequency range can partly overlap the spectrum of the vocalizations. In order to measure

as precisely as possible the acoustic structure of vocalizations, only vocalizations with a signal to noise ratio above 6 DB SPL were kept, corresponding to vocalizations at least four times louder than the background noise. This selection concerned all the vocalizations from the control recordings (dataset #1) and the vocalizations recorded after the noise amplification playback (post playback dataset#2). We statistically controlled that this sampling was not biased towards one vocalization type or specific bird pairs (Appendix fig. A1). To analyse recordings with experimental manipulations of background noise level and to be able compare the structure of vocalizations produced with and without noise addition we used a procedure of noise addition on all control vocalizations. We mixed recorded vocalizations in the control with exemplars of noise from the same nest, recorded during the playback. All measures were averaged with 10 mixes obtained using 10 different exemplars of noise (method validated in Villain et al., 2016). This protocol concerned vocalizations recorded during the playback period (playback dataset #2).

Table 3: Variable loading of the PCAs on the acoustic structure of vocalizations. Transformations used to reach symmetrical distributions of variables are indicated.

	Control Recordings	Playback	Post-playback
VocFreqPC – Frequency composition			
Explained variance			
(%cumulative)	80.21	75.63	78.52
Eigen Value	3.21	3.03	3.14
Mean ¹	-0.98	-0.98	-0.98
Median ¹	-0.83	-0.79	-0.82
Q25 ²	0.00	0.00	0.00
Q75 ¹	-0.93	-0.92	-0.93
Q90 ¹	-0.83	-0.77	-0.80
VocShapePC – Spectral shape			
Explained variance			
(%cumulative)	59.00	60.79	58.30
Eigen Value	2.95	3.04	2.91
IQR (Q75-Q25) ³	-0.88	-0.87	-0.87
Sd ^{3,1,1}	-0.77	-0.74	-0.74
Kurtosis ^{3,1,1}	0.62	0.70	0.65
Skewness ^{3,1,1}	0.68	0.74	0.69
IQR1 (Q90-Q10) ^{3,1,1}	-0.85	-0.83	-0.84

¹:ln ²: Boxcox ³:square root transformations

Statistical analyses

Three different analyses were conducted. First, we described the properties (spectral composition and amplitude) of the environmental noise at the nest, compared to the rest of the territory and extracted the right proxy to describe the environmental noise at the nest (1). Then, we studied whether vocal activity (occurrence and temporal organisation of vocal sequences, structure of vocalizations) varied with the natural environmental noise at the nest (2) and in response to the playback (3). All statistics were performed on R (R Core Team, 2015), using linear mixed effect models (Bates, Mächler, Bolker, & Walker, 2014). Continuous covariates used as explanatory variables were scaled to increase model interpretability (Schielezeth, 2010), a 'Z' indicated this transformation.

(1) Properties of environmental noise at nest sites.

Firstly, we compared the noise levels (Sound Pressure levels) at three different 'Location': along the river ('River'), outside the nest ('AroundNest') and inside the nest ('InNest') in three different 'Nest types': nest boxes under bridges, natural nests under bridges, natural nests in open areas. The following linear mixed effect model was computed: $\text{lmer}(\text{mean DB} \sim \text{NestType} * \text{Location} + (1|\text{nestID}))$. Secondly, the effect of the same two factors and of the mean noise level (ZmeanDB) on the frequency composition of the environmental noise (NoisePC1) was tested with the following model: $\text{lmer}(\text{NoisePC1} \sim \text{NestType} + \text{Location} + \text{ZmeanDB} + \text{NestType} : \text{ZmeanDB} + \text{Location} : \text{ZmeanDB} + \text{NestType} : \text{Location} + (1|\text{nestID}))$. Both models used the identity of the nest site as random factor to deal with pseudo replication.

(2) Impact of natural environmental noise on pair vocal activity (dataset #1)

The effect of the noise level on the vocal activity of the pair (occurrence and structure of vocal sequences) was tested using the following model: $\text{lmer}(\text{Param} \sim \text{Sequence type} * \text{ZmeanDB} + (1|\text{nestID}/\text{session}))$, where 'Param' was either the number of vocal sequences or scores of their structure (SeqC1, SeqPC2), 'Sequence type' had three levels ('FemaleCalls', 'FemaleSong' or 'TwoBirds'). The 'nestID/session' random factor dealt with repetitions within pairs (two recording sessions per pair) and repetitions of sequences within recording sessions.

The effect of the noise level at the nest on the acoustic structure of the vocalizations was tested using three proxies: the vocalization amplitude, the vocalization frequency composition (VocFreqPC) and the vocalization frequency spectral shape (VocShapePC),

depending on the vocalization type ('Notes' vs 'Trills'). The following linear model was used: $\text{lmer}(\text{Acoustic proxy} \sim \text{vocalization type} * \text{ZmeanDB} + (1 | \text{sequenceID}) + (1 | \text{nestID}))$, using two random factors to deal with the fact that several vocalizations came from the same vocal sequence and/or from the same pair. 'ZmeanDB' was the scaled noise level (DB SPL).

To assess the variability of vocal production relatively to the environmental noise, the effect of noise level on vocalizations amplitude was tested on all vocalizations, on sample of the 25% loudest vocalizations and on sample of the 25% quietest vocalizations per pair.

(3) Impact of noise amplification on pair vocal activity (dataset #2)

The magnitude of the noise amplification was equivalent in all nests, but the initial noise level at the nest in control could differ. To test whether the response to the amplification treatment could depend on the noise level in control, this variable was used as a continuous covariate in all analyses and was scaled (ZMeanDB).

First, we tested the effect of the noise amplification on the vocal activity at the nest (i.e. number of vocal sequences per hour). Because the number of each vocal sequence type could change depending on the treatment ('Control' VS 'Amplification') and the Period (during the playback 'PB' or after 'PostPB') these factors were studied as interacting explanatory variables. The following model was computed, with only interpretable interactions: $\text{lmer}(\text{sqrt}(\text{Number of vocal sequences per hour}) \sim \text{Sequence type} + \text{Treatment} + \text{Period} + \text{ZMeanDB} + \text{Treatment} : \text{ZMeanDB} + \text{Sequence type} : \text{Treatment} + \text{Sequence type} : \text{Period} + \text{Treatment} : \text{Period} + \text{Treatment} : \text{Period} : \text{ZMeanDB} + (1 | \text{nestID}/\text{session}))$. The random factor dealt with repeated measures per recording session and different sessions per pair.

Then, we tested the effect of the experimental noise amplification on the structure of the vocalizations. The automatic detection of vocalizations may increase the number of false negatives in vocal sequences recorded during the noise amplification (due to a decrease in signal-to-noise ratio) and may detect only the loudest vocalizations. For this reason: (1) the impact of noise amplification on the temporal organization of vocal sequences was not studied, (2) we chose to study only the 25% loudest vocalizations per treatment, per pair and per vocalization type. To compare the acoustic structure of vocalizations

produced in control and during the playback in noise amplification, we added noise extracts on control vocalizations (see method paragraph ‘*Acoustic analysis of vocalizations and acoustic scores*’), thus the playback (‘PB’) and post-playback (‘PostPB’) periods were analysed separately. The following model was built for both periods: $\text{lmer}(\text{Acoustic parameter} \sim \text{Vocalization type} * (\text{Treatment} + \text{ZMeanDB} + \text{ZTime In Treatment}) + \text{Treatment} : \text{ZTime In Treatment} + \text{Treatment} : \text{ZMeanDB} + (1|\text{SequenceID}) + (1|\text{nestID}/\text{session}))$, where vocalization types had two levels (‘Notes’ and ‘Trills’). Three response variables were analysed (‘Vocalization amplitude’, ‘VocFreqPC’ and ‘VocShapePC’), Treatment had two levels (‘Amplification’ and ‘Control’). The covariate ‘ZTime In Treatment’ tested whether time during the noise amplification treatment affected the structure of the vocalizations. Two random factors controlled for the fact that several vocalizations were sampled in the same vocal sequences and that several recording sessions per pair were conducted.

Statistical quantification and validation

The validity of all models was checked for residual equivariance and symmetrical distribution (‘plotresid’ function, ‘RVAideMemoire’ R package (Hervé, 2016)). All P values were computed using the ‘Anova’ function (‘car’ R package). When interactions between fixed factors were significant, post-hoc tests between interacting factors were computed with Tukey correction for multiple testing (‘lsmeans’ function, ‘lsmeans’ R package (Lenth, 2016)) and post-hoc tests between a factor and a continuous variable were computed with Hommel correction for multiple testing (‘testInteraction’ function, ‘phia’ package (De Rosario-Martinez, 2015)). All model estimates were computed using ‘lsmeans’. To quantify the variance of the data explained by the models, a conditional coefficient of determination of each model was calculated with the ‘r.squaredGLMM’ function (‘MuMIn’ R package (Bartoń, 2016)).

Results

Acoustic environment of nesting dippers

The analysis of the environmental noise of nesting dippers’ territories revealed that the acoustic environment of the nest differed from the background noise of the river. First, in

all nest types tested, the noise level was higher at the nest than along the river (fig 2a, post hoc tests AroundNest-River and InNest-River, nest boxes under bridges: $t_{ratio}=8.7$, $P<0.001$; $t_{ratio} = 4.4$, $P=0.001$; natural nests under bridges: $t_{ratio} =3.5$, $P=0.02$, $t_{ratio} =2.3$, $P=0.32$; natural nests in open areas: $t_{ratio} =5.9$, $P<0.001$, $t_{ratio} =6.6$, $P<0.001$). In all nest types, no difference in noise level was found between recordings inside and outside the nest (fig. 2a, post hoc tests InNest-AroundNest, $t_{ratio} = -2.5$, $P=0.26$; $t_{ratio} =-0.80$, $P=0.99$; $t_{ratio} =0.4$, $P=1.0$ for nest boxes under bridges, natural nests under bridges and natural nests in open areas respectively). The noise level inside the nest was positively correlated to the noise level along the river (slope estimate [95% CI]: 3.3 [1.1:5.6], $X_1= 8.2$, $P=0.004$). So dippers' nest sites were louder than the rest of the territory and the noise level was not attenuated inside the nest. Secondly, in all nest types, the noise had significantly more energy in lower frequencies inside than outside the nest or along the river (fig. 2b, post hoc InNest-River comparison: $t_{ratio} =-16.6$, $P<0.001$; $t_{ratio} =-6.0$, $P<0.001$; $t_{ratio} =4.7$, $P<0.001$ for nest boxes under bridges, natural nests under bridges and natural nests in open areas respectively). So the nests changed the spectrum of the noise towards lower frequencies (fig. 2b). The interaction between the location of the recording and the noise level was significant ($X_7=14.6$, $P=0.006$, fig 2c). The score of frequency composition of the noise increased with the noise level only in river recordings (post hoc test on river slope, $X_7=15.3$, $P<0.001$) and the noise level did not affect the frequency composition outside and inside the nest (fig. 2c post hoc test, $X_7=0.6$, $P=0.45$ and $X_7=0.2$, $P=0.63$ for AroundOut and InNest location respectively).

Thus, the noise level at dippers' nest sites is higher than along the river, and is not attenuated inside the nest cavity. The frequency spectrum of the noise is downshifted inside the nest regardless the noise level, as illustrated in figure 2.d. Consequently, all subsequent analyses will take into account the noise level inside the nest but not the noise spectrum.

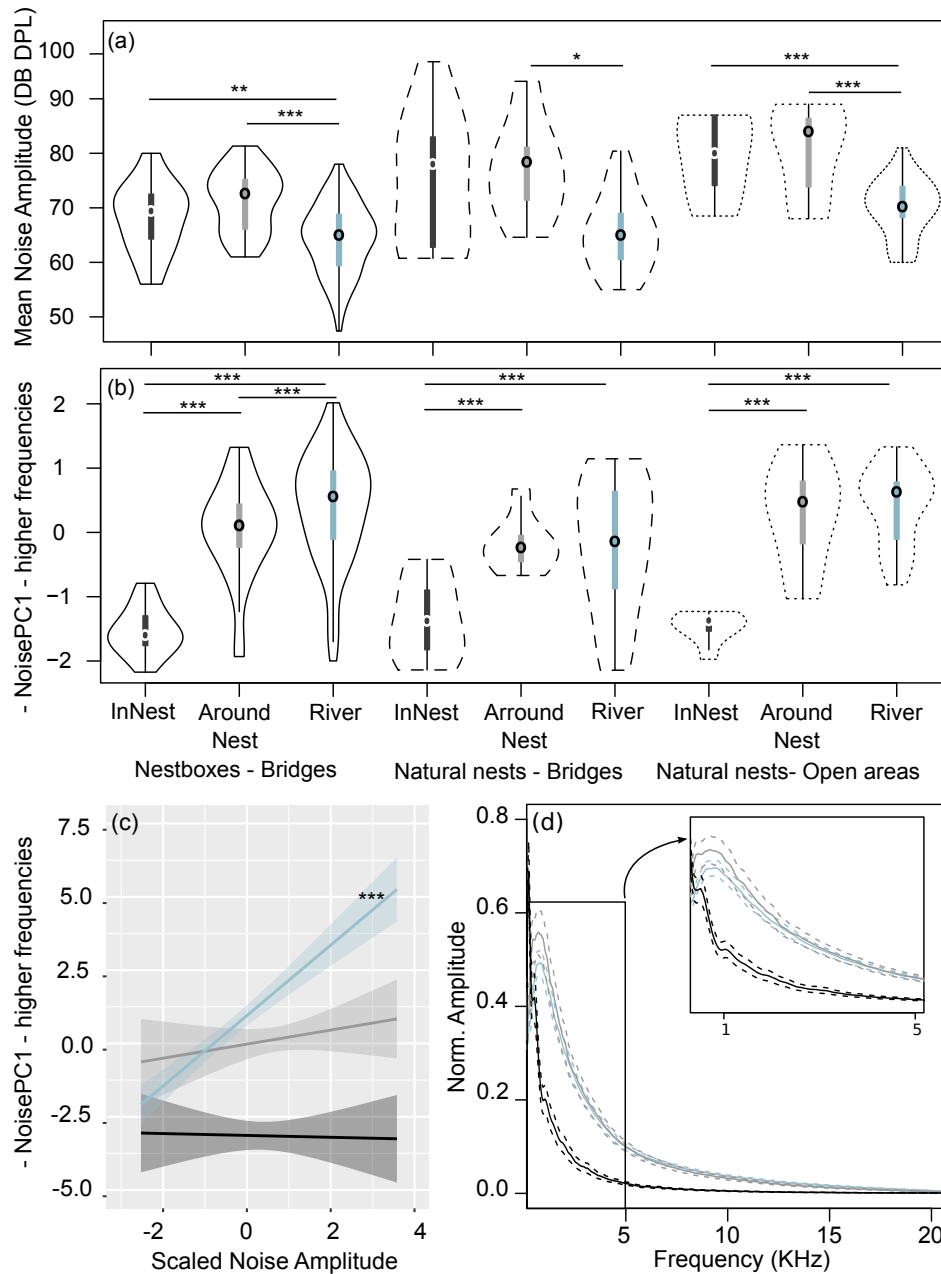


Figure 2: Environmental noise at the nest and along the river of dippers' territory, violin plots (a,b) (median, first and third quartiles, minimum, maximum and shape of data distribution) (c) and model estimates with 95% confidence interval plot. Effect of the nest type (nest-boxes under bridges (solid lines), natural nest under bridges (dashed lines) and natural nest in open areas (pointed dashed line)) and the location of the recording ('River' in light blue, 'Around the nest' in light grey and 'Inside the nest' in black) on (a) the noise level (sound pressure levels, DB SPL) and (b) the noise frequency composition (-NoisePC1). (c) Effect of noise level on the frequency composition of the noise. (d) Mean frequency spectrum (solid lines) and standard deviations (dashed lines) of each of the tested locations ('River' in light blue, 'AroundNest' in grey and 'InNest' in black), with a zoom in on low frequencies (up to 5 KHz) (mean spectra over five second signals, band pass filter: 0.1-20 KHz, FFT: 1024, overlap: 50%). *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, all model estimates are available Appendix tables A2, A3, and A4.

Impact of natural environmental noise on pair vocal activity

We found no evidence of an effect of the noise level at the nest on the daily number of vocal sequences in any of the sequence type (see Appendix table A5 and A6). The effect of the noise level on the temporal organization and composition of the sequence depended on the sequence type (noise level*sequence type interaction on SeqPC1: $X_2=27.0$, $P<0.001$). SeqPC1 (46% of variance), which was correlated with less vocalizations per sequence and shorter sequences, showed a significant increase in ‘FemaleCalls’ and ‘FemaleSong’ sequences (Post hoc tests on slopes: $X_7=13.2$, $P<0.001$ and $X_7=26.0$, $P<0.001$ respectively) but not in ‘TwoBirds’ ($X_7=0.4$, $P=0.54$, fig. 2). SeqPC2 (35% of variance), which correlated with more time spent using Trills and sequences with less Notes, was not affected by the noise level (see Appendix table A7 and A8 for statistics). So ‘FemaleCalls’ and ‘FemaleSong’ sequences were shortened in loud environments but had the same global composition.

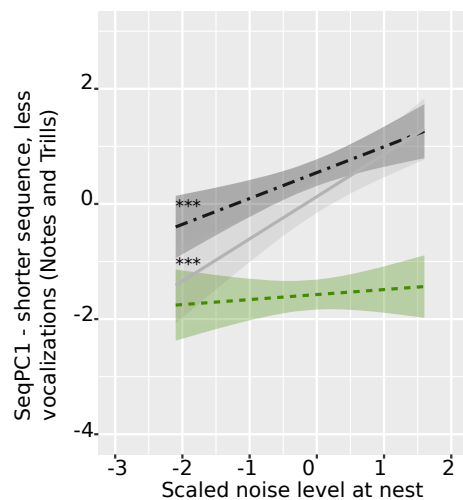


Figure 3: Impact of natural environmental noise on the temporal structure and composition of vocal sequences. Effect of the noise level at the nest (scaled) on SeqPC1 (model estimates with 95% confidence interval) of each sequence type (‘FemaleCalls’= non regularly dashed black line, ‘Female Song’= solid light grey line and ‘TwoBirds’= regularly dashed green line). Significance of slopes after post hoc tests, ***: $p<0.001$. All model estimates are available in Appendix tables A7 and A8.

The acoustic structure of vocalizations used in vocal sequences depended on the noise level at the nest. The amplitude of both Trills and Notes was higher in louder environments and the changes were stronger in Notes than in Trills (post hoc tests on slopes following significant interaction: $X_7=22.4$, $P<0.001$ and $X_7=14.3$, $P<0.001$, fig. 4a).

Similar results were found when subsetting the dataset and taking only the 25% quietest vocalizations per pair (see Appendix tables A9 and A10) but the effect was not significant when taking the 25% loudest vocalizations per pair (post hoc tests on slopes following significant interaction: $X_j=3.1$, $P=0.08$ and $X_j=1.9$, $P=0.16$, fig. 4b). These results emphasize that dippers nesting in louder environments use louder vocalizations by selecting vocalizations of higher amplitude and discarding quieter vocalizations. We found no evidence of an effect of the noise level at the nest on the proxy of the frequency composition of the vocalizations (PC score VocFreqPC, post hoc tests on slopes following significant interaction: $X_j=2.3$, $P=0.13$ and $X_j=0.009$, $P=0.92$ respectively for Notes and Trills, fig. 4c). But a significant effect was found on the proxy of the spectral shape (PC score VocShapePC): Notes, but not Trills, produced in louder environments had a narrower and sharper frequency spectrum (post hoc test on slopes following significant interaction: $X_j=7.6$, $P=0.006$ and $X_j=0.3$, $P=0.56$ respectively fig. 4d). The average frequency spectrum of Notes and Trills in our population (fig. 5) show that dippers use a ‘silent window’: regardless of the natural noise level at the nest site, the vocalizations have most of their energy in frequencies with no overlap with the spectrum of the background noise (fig. 5).

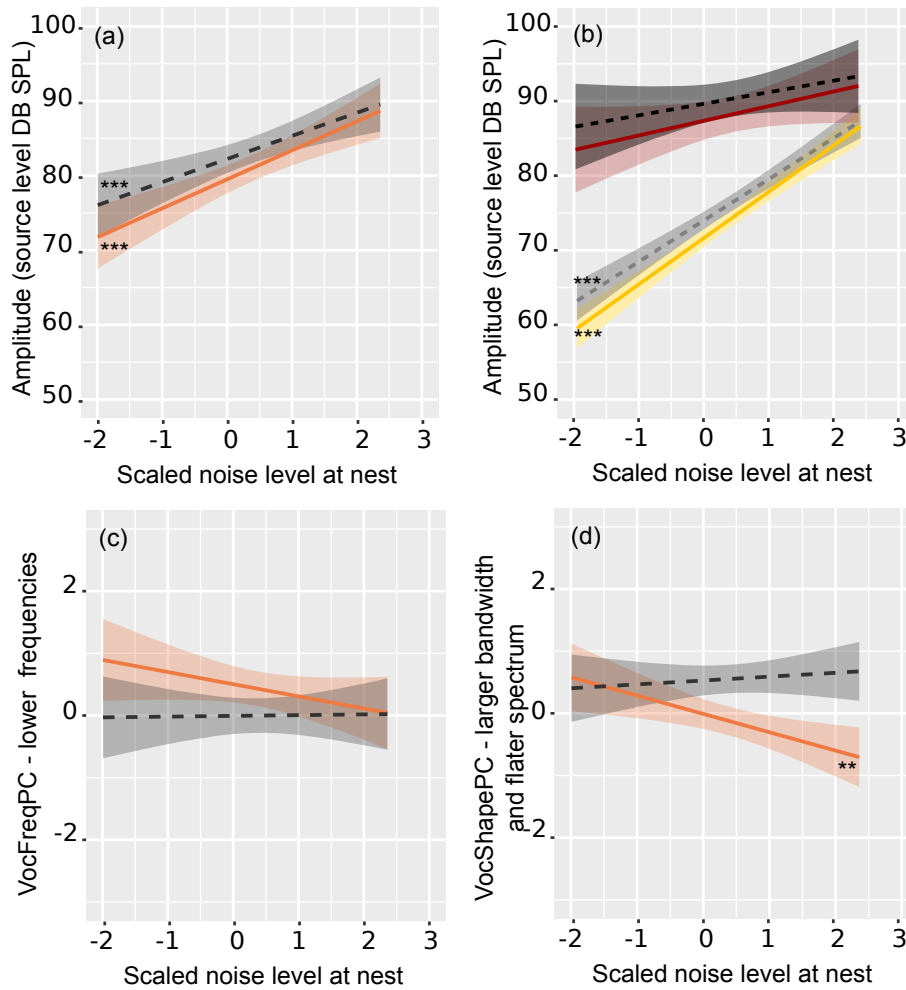


Figure 4: Impact of the level of natural environmental noise (scaled) on the acoustic structure of the vocalizations produced at the nest. Model estimates and 95% confidence interval for each call type (Notes: orange, yellow or red solid lines and Trills: grey or black dashed lines) of vocalization amplitude (source level, DB SPL) (a and b), frequency composition PC score (c) and the spectral shape PC score (d). In b, model estimates of vocalization amplitude for the 25% loudest vocalizations per nest (red and black lines) and for the 25% quietest vocalizations per nest (light grey and yellow lines). Significance of slopes after post hoc tests, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. All model estimates are available in tables Appendix A9 and A10.

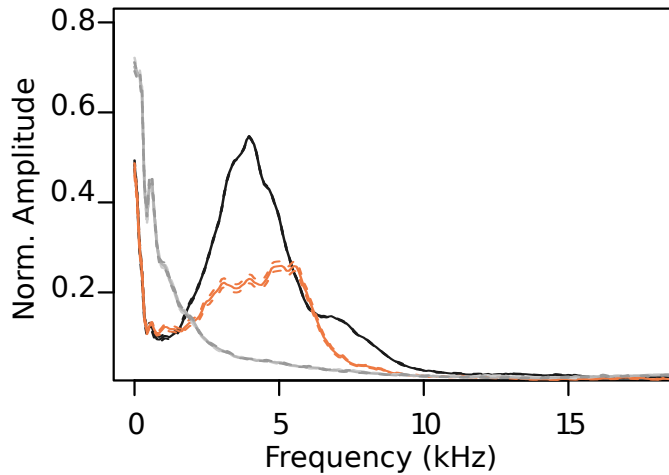


Figure 5: Use of a ‘silent window’ (as proposed in Brumm & Slabbekoorn, 2005) in dippers’ nest vocalizations. Mean normalized frequency spectrum (solid lines) and standard errors (dashed lines) of the two vocalization types (Notes in orange and Trills in black) and the noise inside the nest (in grey). To compute the spectrum of the noise, we used ten random samples of background noise of the same duration as each one of the vocalizations used to compute the spectra of the vocalization types (band pass filter: 0.1-20 KHz, FFT: 512, overlap: 50%). About 4000 vocalizations of each vocalization type were used from a subset of 10 pairs nesting in different noise levels (from 55 to 75 DB SPL).

Impact of experimental noise amplification on pair vocal activity

Independently of the treatment, the vocal activity was higher earlier in the day, so it was higher during the playback period (first six hours) than after (main effect of Period: $X_7=98.7$, $P<0.001$, fig. 6). During the playback only, the vocal activity at the nest decreased compared to control (significant interaction Treatment: Period $X_7=4.1$, $P=0.04$, comparison Control vs Amplification during and after the playback: $t=-3.0$, $P=0.02$, $t=-0.228$, $P=0.9$ respectively, fig. 6). This response did not depend on the initial noise level at the nest site (no interaction Treatment: ZMeanDB $X_7=0.115$, $P=0.7$, Appendix table A11). All sequence types were affected, since no interaction between Treatment and Sequence type was found ($X_2=0.07$, $P=0.9$). So, during the noise amplification, both pair and female vocal activities decreased in the nest, and this was not compensated after the playback.

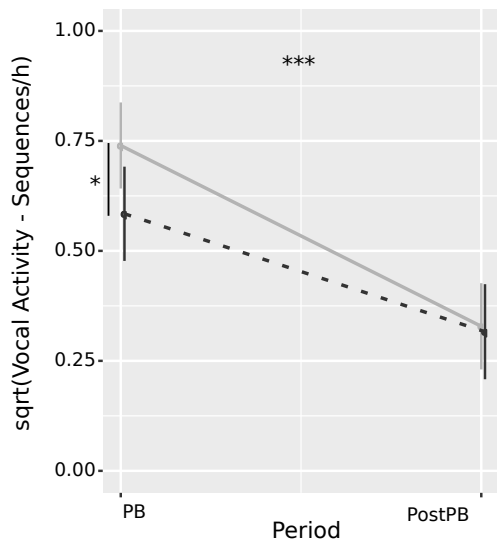


Figure 6: Effect of the noise amplification on vocal activity at the nest. Model estimates and 95% confidence interval following significant interaction. Dashed black line: noise amplification, solid grey line: control. ‘PB’: during the playback, ‘PostPB’: after the playback. N= 27 pairs. *: $P<0.05$, ***: $P<0.001$. Detailed statistical tables and model estimates are available in appendix (Appendix table A11 to A13).

During the noise amplification, the amplitude of the 25% loudest vocalizations (per pair and vocalization type) increased compared to control, in Trills and in Notes (main effect of Treatment, $X_1=29.3$, $P<0.001$, fig. 7a) and this effect depended on the initial noise level at the nest site: pairs living in louder environments increased more the amplitude of their loudest vocalizations during the noise amplification (Treatment: ZMeanDB interaction, $X_1=4.677$, $P=0.03$, post hoc tests on slopes, Control: $X_1=6.4$, $P=0.01$, Amplification: $X_1=12.2$, $P<0.001$, fig. 7b). The frequency composition of vocalizations did not change ($X_1=1.7$, $P=0.2$, fig 7c) but the bandwidth was smaller and the spectrum sharper in Notes produced in response to the noise amplification (Vocalization type: Treatment interaction, $X_1=52.5$, $P<0.001$ post hoc test Control vs. Amplification: $t=4.1$, $P=0.001$ fig. 7d). All effects were independent of the time at which vocalizations were produced during the treatment since no significant interaction between Treatment and ZTime in treatment was found (see Appendix table A14).

The effect of the noise amplification on vocalization amplitude was not persistent after the playback (Vocalization type: Treatment $X_1=7.3$, $P=0.007$, post hoc tests Control vs. Amplification: $ratio=0.6$, $P=0.9$ for Notes and $ratio=-0.9$, $P=0.8$ for Trills, Appendix fig. A2a). During the post-playback period, no change in the frequency composition of vocalizations was detected either ($X_1=0.721$, $P=0.4$, Appendix fig. A2b) but the frequency

spectrum of Notes remained sharper and less broadband after the noise amplification (Vocalization type: Treatment, $X_1=16.138$, $P<0.001$, post hoc tests Control vs. Amplification days: $ratio = 3.3$, $P=0.008$ for Notes and $ratio = 0.4$, $P=0.9$ for Trills, Appendix fig. A2c).

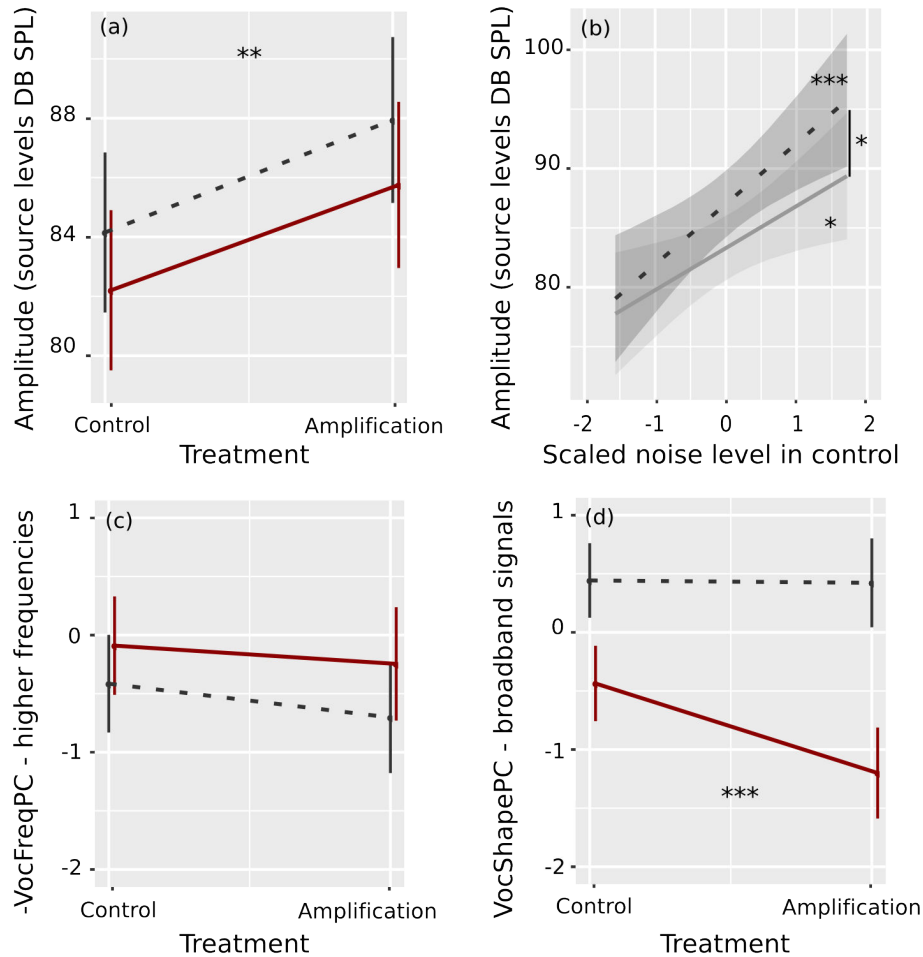


Figure 7: Effect of noise amplification on the acoustic structure of vocalizations. Interaction plot of the Vocalization amplitude (a), frequency composition score (c) and spectral shape score (d) depending on the Treatment, the vocalization type (Notes: solid red lines and Trills: dashed black lines). (b) Differential effects of treatment depending the initial noise level at nest site on vocalization amplitude (Amplification: dashed black line and Control: solid grey line). Model estimates and 95% confidence intervals are represented. N=19 pairs. *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$. Detailed statistical tables and model estimates are available in appendix (Appendix table A14 to A16).

Discussion

In the present paper, we showed that the natural environmental noise at dippers' nest sites reached high sound pressure levels (mean [min: max]= 72 [55: 85] DB SPL). The noise level at dippers' nest sites was higher than along the rivers, and is not attenuated inside the nest. Independently from the noise level, the frequency spectrum of the noise was downshifted inside the nest. We showed that dippers vocalized at frequencies that do not overlap with the frequency range of the background noise and that frequencies did not change in response to the noise amplification. However pairs breeding in louder nest sites vocalized at higher amplitude and increased the amplitude of their loudest vocalizations in response to noise amplification. The shape of the frequency spectrum also changed, but only in Notes and not Trills. Pairs breeding in louder nest sites produced Notes with a sharper and less broadband frequency spectrum. In response to noise amplification, pairs also produced Notes with a sharper and less broadband frequency spectrum and the effect lasted after the playback. The noise level impaired vocal activity at the nest: females breeding in louder nest sites produced shortened call sequences and songs, but female-male sequences were not affected. In response to the noise amplification, pairs produced less vocal sequences per hour, and did not compensate after the perturbation.

Response to noise reveals Lombard effect in short range vocalizations

Dippers nesting in louder environments use louder vocalizations by selecting vocalizations of higher amplitude. Following this result we can raise two hypotheses: either each individual produces vocalizations at a given amplitude and chooses a habitat that matches its vocal abilities, or individuals adjust their vocalization amplitude to the noise level of their habitat. Our results are in favour of the second hypothesis: in response to a short-term noise amplification, pairs increased the amplitude of their loudest vocalizations and this response was (1) more pronounced in pairs nesting in already loud sites, (2) immediate (since no effect of the timing of production during the treatment was found) and (3) exclusive to the playback period. These results show that, despite the loud background noise of some nest sites, dippers did not vocalize at their full performance level and still express flexibility. This brings another evidence of the Lombard effect in natural habitats (Pytte, Rusch, & Ficken, 2003; Brumm, 2004). However, in the present study we focused on short-range signals. Short-range signals are less subjected to

degradation. If vocal adjustments in response to noise are adaptations to maximise signal transmission, we expect no increase of the amplitude of short-range signals. However, if vocal adjustments are driven by other factors (physiological, hearing feedbacks), we expect an increased amplitude in noise (H. Brumm & Todt, 2002; Cynx et al., 1998; Manabe, Sadr, & Dooling, 1998). Our results are consistent with the second explanation because of the proximity between the caller and the receiver in our case and because signals studied here were not aimed at being propagated (especially Trills, recorded only in short-range communication contexts).

The frequency spectrum of vocalizations is above noise characteristics at the nest

We found that the frequency composition of vocalizations did not depend on the noise level at the nest, either in response to the natural background noise level at the nest or in response to noise amplification. The frequency composition of dippers' vocalizations at the nest was already outside the frequency spectrum of the noise inside the nest, and no significant spectral masking occurred in this context. Evolution may have shaped the frequency spectrum of dipper's vocalizations towards higher frequencies, allowing them to avoid masking from noise. The 'Acoustic Adaptation Hypothesis' predicts that the evolution of the structure of vocalizations is driven by properties of the acoustic habitat and the structure of vocalizations is optimized for propagation (Morton, 1975). Several studies and observations support this hypothesis in birds and anurans (Boncoraglio & Saino, 2007; Brenowitz, 1982; Dubois & Martens, 1984; meta-analysis in Ryan & Brenowitz, 1985; Slabbekoorn & Smith, 2002). The relevance and possible generalizations of this hypothesis to all acoustic habitats has received great interest but led to mixed results (Boncoraglio & Saino, 2007; Ey & Fischer, 2009). In our case, it is likely that the natural pink noise of water stream may have constrained the evolution of higher frequency signals in dippers, leading to minimal masking effects. We studied short-range vocal signals: although Notes may be used in long-range contexts of communication (in territorial or courtship songs), Trills were not described outside short-range social context. We conclude that the adaptation of the frequency spectrum of vocal signals to the frequency spectrum of the background may be generalizable to short-range signals and not restricted to signals propagated through distances (Morton, 1975). The characteristics of the short-range signals might also be a physiological by-product of the selected characteristics of the long-range signals.

Dippers rely on vocal flexibility in Notes but not in Trills

We showed different degrees of vocal adjustments in Notes and Trills. Notes produced by pairs breeding in nest sites where noise level is high had a sharper and less broadband frequency spectrum. Pure tones and narrow vocal signals are expected to be used in loud environments (Dubois & Martens, 1984; reviewed in Warren et al., 2006). Our results on Notes are thus in line with this expectation. Two hypotheses can be raised: either birds living in loud nest sites already produce optimized vocalizations regarding the environmental noise they nest in, or they rely on vocal flexibility and adjust their signals to the noise level. Here, in response to the noise amplification playback, the loudest Notes also shifted to sharper and less broadband signals. Therefore, we can conclude that birds rely on vocal flexibility in Notes. These changes were maintained after the playback, which suggests that vocal flexibility can perpetuate on longer periods of time. Two behavioural mechanisms may be at scene to explain these changes: either the same Note types are used in quiet and loud habitats but their spectral structure is changed in response to the noise level or birds switch Note types. In a previous study we showed that pairs exhibited an acoustic signature in Notes (Villain et al., Chapter 1), so different pairs may use different Note types. However since they also shifted their Note structure we cannot conclude about the mechanism in response to the playback. A detailed analysis of the Notes repertoire may be useful to clearly test these two hypotheses.

Trills showed no change depending on the noise level, either between nest sites or during and after the noise amplification playback. Notes and Trills may differ in their expression of vocal flexibility: either no flexibility is possible in Trills (due to anatomical or physiological constraints) or vocal flexibility is not necessary in the context they are produced. First, Notes may be learned together with the song, whereas Trills may be non-learned, as most birdcalls (Marler, 2004). Different neuronal pathways may be involved during the production of learned and non-learned vocalizations (Simpson & Vicario, 1990) and may explain differential flexibility. Second, different contexts of vocal interactions may drive different noise-dependent vocal flexibility through selection. Trills were recorded only during short-range communication ('rattling calls' in Tyler & Ormerod, 1994; Villain et al., Chapter 1). Trills are broadband signals, which are usually less easily detected than pure tones under noisy conditions, which may not matter, since the receiver is located close to the caller.

Frequency shifts: side effects of vocalizing louder?

In many species, changes in the distribution of spectral energy of the signal are likely to be biomechanically linked to vocal amplitude, and are, thus, driven by the Lombard effect (Osmanski & Dooling, 2009; Suthers & Zollinger, 2004). Some authors raised the idea that noise-dependent frequency shifts showed in many bird species in cities may be side effects of vocalizing louder (Brumm & Bee, 2016; Nemeth et al., 2013). We showed that dippers responded to noise amplification with louder vocalizations but the frequency composition was not changed and the shape of the frequency spectrum was changed only in Notes and not in Trills. Our results showed that amplitude and spectral components are at least partly uncorrelated in dippers' vocalizations.

Intrapair communication in dippers: adaptation to background noise and significance

Pair vocal activity depends on background noise level at nest site

We showed that despite high levels of environmental noise, all pairs produced the same number of vocal sequences per day in all nest sites (between pair design). However, the temporal organization and composition of female calls and female song sequences were shortened in louder sites. Female sequences can be produced either during vocal interactions with the male located few meters away or by the female alone. If produced during vocal interactions, changes in the sequence structure in response to the noise level may reveal impairments of vocal communication between mates. Vocal sequences were shorter in noisy sites perhaps because mates failed to detect each other's vocalizations, as it was showed on duets around the nest under noisy conditions in zebra finches, *Taeniopygia guttata* (Villain et al., 2016).

We showed that a higher noise level did not affect all types of vocal sequence the same way, in particular, the temporal organization and composition of female-male vocal sequences (TwoBirds) changed depending on environmental noise level. As all sequence types have the same general acoustic characteristics and are thus under the same acoustic constraints, different responses may reveal different functions. Maintaining the organization of female-male vocal sequences may underline either stereotypic behaviour in all pairs or that information is contained in their temporal organization and composition and pairs may benefit from keeping it unchanged.

Experimental increase of background noise changed the vocal activity at the nest

In response to noise amplification, pairs decreased the number of vocal sequences of all types (females calls and song or female-male sequences) (within pair design). A similar experiment in zebra finches showed that pairs increased their effort of vocal communication at the nest in response to experimentally elevated noise level (Villain et al., 2016). Dippers avoided vocal activity at the nest during the playback. They perhaps changed their meeting points somewhere else on their territory but our passive acoustic monitoring did not allow us to monitor vocal activity away from the nest. An additional monitoring of female incubation might help testing this hypothesis.

Intrapair communication in noise and cost of vocal adjustments

Pairs living in loud nest sites vocalized louder but females shortened their sequences. In response to the noise amplification playback, they avoided vocal activity at the nest. Several studies demonstrated the metabolic cost of louder vocalizations (Oberweger & Goller, 2001; Zollinger, Goller, & Brumm, 2011). Motor fatigue may be responsible for shortened females' vocal sequences in dippers exposed to high levels of noise, as already shown in chaffinches, *Fringilla coelebs*, (Brumm & Slater, 2006). Vocalizing louder may also have predation costs. Increased predation was shown as a result of singing from the nest in superb fairy-wren, *Malurus cyaneus* (Kleindorfer, Evans, & Mahr, 2016). We can hypothesize that females produced shortened vocal sequences in loud nest sites as a trade-off of increased amplitude that may counteract the potential predation cost.

Nesting in loud places: choice or side effect?

Our analyses showed that the environmental noise at the nest of breeding dippers was correlated to but also louder than the river noise. In most nest sites, the background noise may be amplified by sound reflection (by the bridge or the rock cavity) or by the proximity of rushing water (rapids and waterfalls). The nest itself changed the spectral properties of the background noise. The frequency spectrum was downshifted, perhaps by the nest materials used (mostly moss). We showed that dippers nested in louder sites than other parts of their territory. Birds might select their nest site on their acoustic characteristics, or the acoustic characteristics might be by-products of other advantages provided by the site (food quantity and quality (Engstrand, Ward, & Bryant, 2002),

protection from predators or disturbances). The impact of noise on nesting birds has been mainly investigated on urban noise and results lead to diverging conclusions. First, authors have suggested that elevated anthropogenic noise is similar to a predation threat (Frid & Dill, 2002; Gill, Sutherland, & Watkinson, 1996) and noisy places may be avoided by birds with previous experience of breeding (Habib, Bayne, & Boutin, 2007). Studies also showed that nesting in louder places had a strong impact on reproductive success: in great tits, *Parus major*, smaller clutches and less fledglings (independently from clutch size) were produced at louder nest sites (Halfwerk, Holleman, Lessells, & Slabbekoorn, 2011). In house sparrows, *Passer domesticus*, nesting in louder places impaired chicks development: parents had fewer young, with lower body mass, explained by less food provisioning by parents (Schroeder, Nakagawa, Cleasby, & Burke, 2012). On the contrary, other studies showed that birds breeding in louder areas had a higher reproductive success that may be linked to changes in prey-predator dynamics (Francis, Ortega, & Cruz, 2009). No study investigated the consequences of breeding in loud places in species naturally experiencing high noise levels.

To conclude, our study is the first to investigate noise-dependent vocalization structure in a naturally noisy environment measuring both amplitude and spectral properties of vocalization in birds. We found strong evidence that dippers (1) adjust their vocalization amplitude (Lombard effect) on the short-term and (2) rely on vocal flexibility in their Notes, producing Notes with a sharper and less broadband frequency spectrum in response to the high noise level of their habitat. However they vocalize at frequencies already above the frequency range of the background noise. Marginal masking occurs and no changes in the frequency composition were shown in response to elevated noise level. Intrapair communication at the nest was impaired under high noise levels, which may impact breeding success. Dippers' particularly noisy breeding habitat offers new possibilities to investigate noise-dependent breeding success in naturally noisy environments.

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Appendix

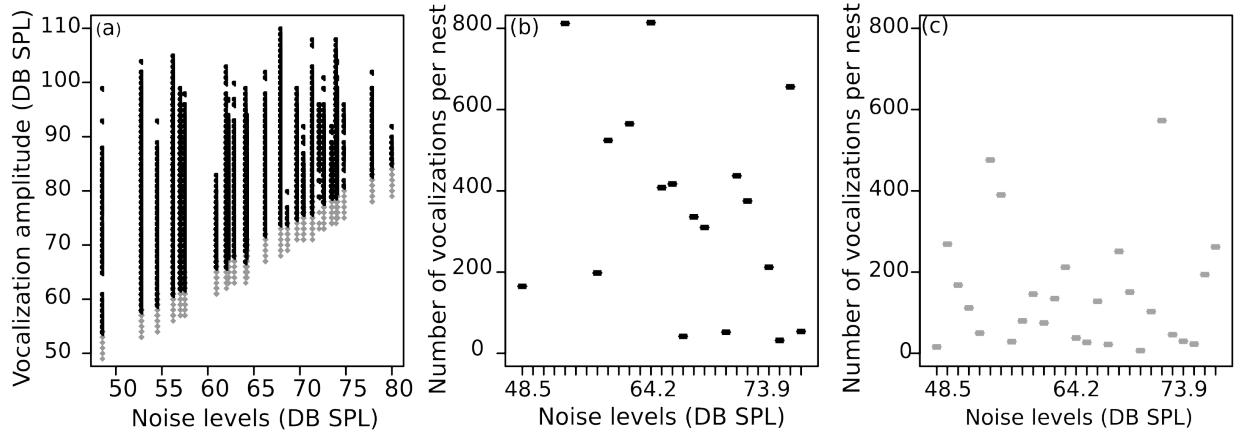


Figure A1: Scatter plot of vocalization amplitude depending on noise level at the nest (a), with vocalization having a signal to noise ratio above 6 DB SPL (black), kept for the analyses and vocalizations removed (grey) (b,c) number of vocalizations kept (b) or removed (c) per nest depending noise level. Analyses of control recording were performed only on vocalizations having a signal to noise ratio above 6 DB SPL. In order to control for possible bias towards the removal of more vocalization in louder nest sites, the effect of the noise level at the nest on the number of vocalization removed was tested in a linear model using the call type as interacting explanatory variable and the pair identity as random factor. No effect of noise was found on the number of vocalization removed (post hoc test following significant interactions: Notes: slope estimates [95%CI] = -0.21 [-0.70:0.26], $X_1=0.81$, $P=0.36$; Trills: slope estimates [95%CI] = 0.31 [-0.17:0.80], $X_1=1.74$, $P=0.18$).

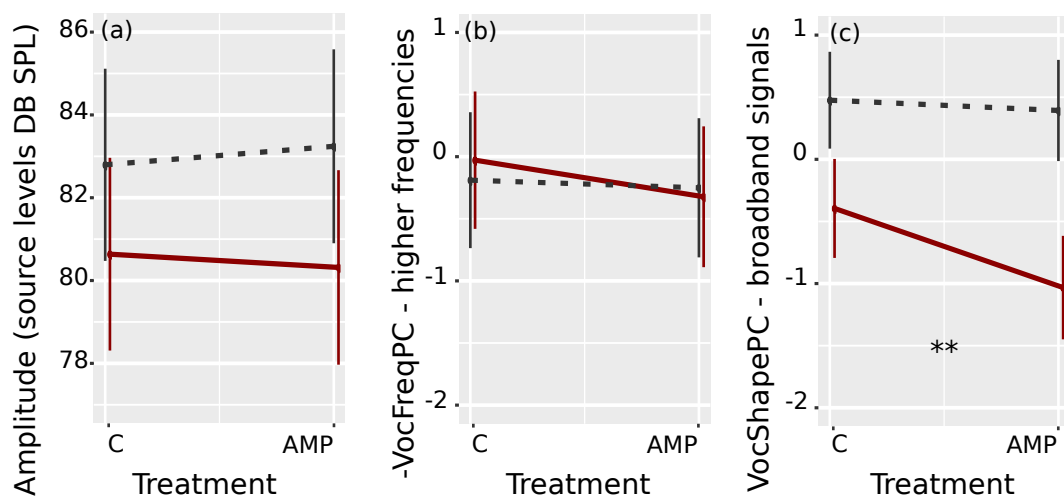


Figure A2: Effect of noise amplification on vocalizations structure – post perturbation effects. Interaction plot of the Vocalization amplitude (a), frequency composition score (b) and spectral shape score (c) depending on the Treatment, the vocalization type (Notes: solid red lines and Trills: dashed black lines). Model estimates and 95% confidence intervals are

represented. N=20 pairs. **: P<0.01. Detailed statistical tables and model estimates are available in appendix (Appendix table A14 to A16).

Table A2: Analysis of environmental noise according to Nest type ('BoxB' = nestboxes, 'NatB'=natural nests under bridges, 'NatO'=natural nests in open areas) and Location of the recording ('NestIn' = inside the nest, 'NestOut'=at 50cm from the nest, 'River'=along the river), impact of noise level on frequency composition of the noise - Anova Table –

	Chisq	Df	Pr(>Chisq)
On mean noise level			
Location	137.912	2	<0.001
Nest Type	11.237	2	0.004
NestType:Location	20.308	4	<0.001
On -NoisePC1			
Nest Type	7.450	2	0.024
Location	360.619	2	<0.001
ZmeanDB	45.287	1	<0.001
NestType:ZmeanDB	1.313	2	0.519
Location:ZmeanDB	29.118	2	<0.001
NestType:Location	14.585	4	0.006
Post hoc tests on slope following significant interaction between Nest type and ZmeanDB			
Comparisons between slopes			
NestIn-NestOut	1.052	1	0.305
NestIn-River	25.144	1	<0.001
NestOutRriver	15.302	1	<0.001
Significance of slopes			
NestIn	0.222	1	0.637
NestOut	0.571	1	0.450
River	53.342	1	<0.001

Table A3: Analysis of environmental noise according to Nest type ('BoxB' = nestboxes, 'NatB' = natural nests under bridges, 'NatO' = natural nests in open areas) and Location of the recording ('NestIn' = inside the nest, 'NestOut' = at 50cm from the nest, 'River' = along the river), multiple comparison table following significant NestType: Location interaction.

contrast	Response= meanDB level			Response= -NoisePC1		
	estimate	SE	df	estimate	SE	df
Comparisons among nestboxes						
NestIn,BoxB - NestOut,BoxB	-2.783	1.135	261.441	-3.216	0.322	265.752
NestIn,BoxB - river,BoxB	4.332	0.983	265.679	-4.601	0.276	277.517
NestOut,BoxB - river,BoxB	7.114	0.814	265.673	-1.385	0.265	292.296
Comparisons among natural nest underbriges						
NestIn,NatB - NestOut,NatB	-1.426	1.783	265.762	-2.362	0.550	267.719
NestIn,NatB - river,NatB	4.063	1.762	288.408	-2.744	0.491	287.300
NestOut,NatB - river,NatB	5.489	1.570	288.613	-0.382	0.480	278.227
Comparisons among natural nest in open area						
NestIn,NatO - NestOut,NatO	1.022	2.503	293.115	-3.688	0.768	293.131
NestIn,NatO - river,NatO	13.499	2.050	283.920	-3.568	0.756	289.919
NestOut,NatO - river,NatO	12.477	2.100	302.166	0.120	0.756	280.163
Comparisons among recording inside the nest						
NestIn,BoxB - NestIn,NatB	-7.429	2.587	113.371	-0.318	0.588	181.918
NestIn,BoxB - NestIn,NatO	-10.972	3.078	126.662	0.048	0.827	220.348
NestIn,NatB - NestIn,NatO	-3.543	3.341	167.148	0.366	0.891	206.944
Comparisons among recording outside the nest						
NestOut,BoxB - NestOut,NatB	-6.073	2.426	90.138	0.535	0.561	148.652
NestOut,BoxB - NestOut,NatO	-7.167	2.809	117.331	-0.424	0.769	216.561

NestOut,NatB - NestOut,NatO -1.094 2.984 165.734 -0.367 1.000 -0.960 0.843 209.557 -1.138 0.968

Table A5 continues

Comparisons among recording of rivers										
river,BoxB - river,NatB	-7.698	2.338	70.563	-3.292	0.039	1.539	0.448	67.663	3.431	0.027
river,BoxB - river,NatO	-1.804	2.679	74.987	-0.673	0.999	1.081	0.512	69.560	2.111	0.475
river,NatB - river,NatO	5.894	3.014	104.584	1.956	0.577	-0.458	0.604	74.998	-0.758	0.998

Table A4: Analysis of environmental noise according to Nest type ('BoxB' = nestboxes, 'NatB'=natural nests under bridges, 'NatO'=natural nests in open areas) and Location of the recording ('NestIn' = inside the nest, 'NestOut'=at 50cm from the nest, 'River'=along the river), model estimates in all groups and slopes estimates of the effect of noise level.

Location	Nest Type	Ismean	SE	df	lower.CL	upper.CL
Model on noise level (DB SPL) according to NestType and Location of the recording [R2c=0.76]						
NestIn	BoxB	68.222	1.468	97.551	65.309	71.135
NestOut	BoxB	71.004	1.367	75.038	68.282	73.727
River	BoxB	63.890	1.233	49.991	61.414	66.366
NestIn	NatB	75.651	2.130	122.015	71.435	79.867
NestOut	NatB	77.077	2.004	98.551	73.100	81.054
River	NatB	71.588	1.987	81.676	67.635	75.541
NestIn	NatO	79.194	2.706	137.194	73.843	84.544
NestOut	NatO	78.171	2.454	136.253	73.318	83.025
River	NatO	65.694	2.378	84.641	60.966	70.423
Model on NoisePC1 according to NestType, Location of the recording and DB levels [r2c=0.71]						
Groups estimates						
NestIn	BoxB	-3.193	0.304	168.865	-3.794	-2.593
NestOut	BoxB	0.022	0.287	136.111	-0.545	0.590
River	BoxB	1.408	0.227	58.932	0.952	1.863
NestIn	NatB	-2.875	0.506	187.884	-3.873	-1.878
NestOut	NatB	-0.513	0.516	170.016	-1.532	0.506
River	NatB	-0.131	0.391	72.533	-0.910	0.647
NestIn	NatO	-3.241	0.772	229.644	-4.763	-1.719
NestOut	NatO	0.447	0.743	238.443	-1.018	1.911
River	NatO	0.327	0.456	71.872	-0.583	1.237
Slopes estimates of ZmeanDB in groups						
NestIn	-	-0.103	0.219	302.658	-0.534	0.328
NestOut	-	0.169	0.225	282.477	-0.273	0.611
river	-	1.130	0.156	304.634	0.824	1.437

Table A5: Effect of natural noise level on occurrence of vocal sequences, response variable: ‘sqrt(Number of sequences)’ – Anova

	Chisq	Df	Pr(>Chisq)
Sequence type	73.275	2	<0.001
ZmeanDB	0.950	1	0.330
Sequence type:ZmeanDB	0.318	2	0.853

Table A6: Effect of natural noise level on occurrence of vocal sequence, response variable: ‘sqrt(Number of sequences)’ – Model estimates

Sequence type	lsmean	SE	df	lower.CL	upper.C L
Factors Estimates at mean(meanDB) in dataset					
FemaleCalls	2.574	0.152	53.284	2.270	2.879
FemaleSong	1.262	0.152	53.284	0.958	1.566
TwoBirds	1.778	0.152	53.284	1.474	2.083

Table A7: Effect of natural noise level on the temporal organization and composition of vocal sequences –Anova table

	Chisq	Df	Pr(>Chisq)
Anova(model) SeqPC1			
Sequence type	456.824	2	<0.001
ZmeanDB	12.568	1	<0.001
Sequence type:ZmeanDB	27.012	2	<0.001
Anova(model) SeqPC2			
Sequence type	677.001	2	<0.001
ZmeanDB	1.066	1	0.302
Sequence type:ZmeanDB	0.180	2	0.914
Posthoc test on SeqPC1 following significant interaction between Sequence type and ZmeanDB			
Slopes comparisons			
FemaleCalls-FemaleSong	7.641	1	0.006
FemaleCalls-TwoBirds	11.754	1	0.001
FemaleSong-TwoBirds	26.906	1	<0.001

Table A8: Effect of natural noise level on the temporal organization and composition of vocal sequences – model estimates

	lsmeans estimates	SE	df	lower.CL	upper.CL	Chisq	Df	Pr(>Chisq)
Model estimates: SeqPC1 explained by Noise level inside the nest and sequence type [r2c=0.41]								
Slope Estimates within factors (following significant interaction between ZmeanDB and Sequence type)								
FemaleCalls	0.449	0.124	27.912	0.196	0.703	13.201	1	<0.001
FemaleSong	0.734	0.144	49.728	0.444	1.024	26.029	1	<0.001
TwoBirds	0.087	0.143	48.899	-0.201	0.375	0.370	1	0.543
Model estimates: SeqPC2 explained by Noise level inside the nest and sequence type [r2c=0.47]								
Factors Estimates (following no interaction between ZmeanDB and sequence type and no effect of ZmeanDB)								
FemaleCalls	0.318	0.112	28.469	0.089	0.547	-	-	-
FemaleSong	-1.596	0.131	51.347	-1.858	-1.334	-	-	-
TwoBirds	0.973	0.122	40.090	0.726	1.219	-	-	-

Table A9: Effect of natural environmental noise level on the acoustic structure of vocalizations – anova table

	Chisq	Df	Pr(>Chisq)
Response : Vocalization amplitude all dataset			
Vocalization type	669.076	1	<0.001
ZmeanDB	17.096	1	<0.001
Vocalization type:ZmeanDB	54.805	1	<0.001
Response : Vocalization amplitude 25% quietest vocalizations/pair			
Vocalization type	376.391	1	<0.001
ZmeanDB	127.070	1	<0.001
Vocalization type:ZmeanDB	32.268	1	<0.001
Response : Vocalization amplitude 25% loudest vocalizations/pair			
Vocalization type	532.726	1	<0.001
ZmeanDB	2.354	1	0.125
Vocalization type:ZmeanDB	17.090	1	<0.001
Response : VocFreqPC all dataset			
Vocalization type	379.764	1	<0.001
ZmeanDB	0.246	1	0.620
Vocalization type:ZmeanDB	61.134	1	<0.001
Response : VocShapePC all dataset			
Vocalization type	414.954	1	<0.001
ZmeanDB	0.429	1	0.513
Vocalization type:ZmeanDB	203.509	1	<0.001

Table A10: Effect of natural environmental noise level (ZmeanDB variable) on the acoustic structure of vocalization types (Notes and Trills) – slope estimates and confidence within vocalization types ('lsmeans' function, first four columns), post hoc tests on slopes ('testInteraction' function, 'phia' package, last three columns).

Slope estimates		SE	df	lower.CL	upper.CL	Df	Chisq	Pr(>Chisq)
Response : Vocalization amplitude all dataset [r2c=0.57]								
Notes	3.887	0.821	40.029	2.226	5.547	1	22.389	<0.001
Trills	3.101	0.820	39.664	1.444	4.758	1	14.317	<0.001
Response : Vocalization amplitude 25% quietest vocalizations/pair [r2c=0.82]								
Notes	6.202	0.518	31.739	5.147	7.258	1	143.403	<0.001
Trills	5.520	0.515	30.960	4.470	6.570	1	115.104	<0.001
Response : Vocalization amplitude 25% loudest vocalizations/pair [r2c=0.89]								
Notes	1.960	1.113	35.787	-0.298	4.218	1	3.102	0.078
Trills	1.547	1.112	35.653	-0.709	3.803	1	1.935	0.164
Response : VocFreqPC all dataset [r2c=0.36]								
Notes	-0.196	0.130	40.040	-0.458	0.066	1	2.276	0.131
Trills	0.012	0.129	39.164	-0.249	0.273	1	0.009	0.927
Response : VocShapePC all dataset [r2c=0.31]								
Notes	-0.295	0.107	38.478	-0.512	-0.079	1	7.610	0.006
Trills	0.062	0.106	37.415	-0.153	0.277	1	0.344	0.558

Table A11: Effect of experimental noise amplification on vocal activity at the nest – Anova table.

	Chisq	Df	Pr(>Chisq)
Response: sqrt(number of vocal sequences/h) [R2c=0.51]			
Sequence type	178.696	2	<0.001
Treatment	5.048	1	0.025
Period	98.679	1	<0.001
ZMeanDB In Control	0.018	1	0.894
Treatment:ZmeanDB	0.115	1	0.735
Sequence type: Treatment	0.073	2	0.964
Sequence type: Period	30.177	2	<0.001
Treatment: Period	4.120	1	0.042
Treatment: Period: ZMeanDB	5.098	2	0.078

Table A12: Effect of experimental noise amplification on vocal activity at the nest – post hoc multiple comparisons (‘Tukey’ contrasts) following significant interaction.

contrast	estimate	SE	df	t.ratio	p.value
Multiple comparison following Sequence Period significant interaction					
FemaleCalls,pb - FemaleSong,pb	0.549	0.061	324.122	8.981	<0.001
FemaleCalls,pb - TwoBirds,pb	-0.249	0.062	326.157	-4.029	0.001
FemaleCalls,pb - FemaleCalls,postpb	0.345	0.060	320.491	5.738	<0.001
FemaleCalls,pb - FemaleSong,postpb	0.650	0.062	325.166	10.526	<0.001
FemaleCalls,pb - TwoBirds,postpb	0.336	0.063	327.209	5.354	<0.001
FemaleSong,pb - TwoBirds,pb	-0.798	0.063	322.474	-12.752	<0.001
FemaleSong,pb - FemaleCalls,postpb	-0.204	0.061	324.077	-3.319	0.013
FemaleSong,pb - FemaleSong,postpb	0.101	0.062	321.421	1.634	0.577
FemaleSong,pb - TwoBirds,postpb	-0.212	0.064	323.665	-3.344	0.012
TwoBirds,pb - FemaleCalls,postpb	0.594	0.062	325.978	9.562	<0.001
TwoBirds,pb - FemaleSong,postpb	0.899	0.063	321.453	14.215	<0.001
TwoBirds,pb - TwoBirds,postpb	0.585	0.064	321.614	9.205	<0.001
FemaleCalls,postpb - FemaleSong,postpb	0.305	0.061	325.090	4.965	<0.001
FemaleCalls,postpb - TwoBirds,postpb	-0.009	0.062	327.127	-0.141	1.000
FemaleSong,postpb - TwoBirds,postpb	-0.314	0.063	322.553	-4.946	<0.001
Multiple comparison following Treatment: Period significant interaction					
Amp,pb - Control,pb	-0.155	0.052	156.982	-2.991	0.017
Amp,pb - Amp,postpb	0.272	0.055	321.522	4.911	<0.001
Amp,pb - Control,postpb	0.260	0.052	157.739	5.016	<0.001
Control,pb - Amp,postpb	0.427	0.052	157.507	8.185	<0.001
Control,pb - Control,postpb	0.415	0.046	320.894	8.978	<0.001
Amp,postpb - Control,postpb	-0.012	0.052	157.930	-0.228	0.996

Table A13: Effect of experimental noise amplification on vocal activity at the nest – model estimates in groups.

Factor 1	Factor2	lsmean	SE	df	lower.CL	upper.CL	real Mean	real SE.inf	real SE.sup
sqrt									
Estimates of Sequence types within Periods									
FemaleCalls	pb	0.766	0.057	65.852	0.653	0.879	0.587	0.503	0.677
FemaleSong	pb	0.218	0.058	69.976	0.102	0.333	0.047	0.026	0.076
TwoBirds	pb	1.015	0.059	73.503	0.898	1.132	1.031	0.915	1.153
FemaleCalls	postpb	0.421	0.057	65.856	0.308	0.535	0.177	0.133	0.228
FemaleSong	postpb	0.116	0.058	71.546	0.000	0.232	0.014	0.003	0.030
TwoBirds	postpb	0.430	0.059	76.221	0.312	0.548	0.185	0.137	0.240
Estimates of Sequence types within Treatments									
FemaleCalls	Amplification	0.545	0.059	76.761	0.427	0.663	0.297	0.236	0.365
FemaleSong	Amplification	0.129	0.062	88.110	0.005	0.252	0.017	0.004	0.036
TwoBirds	Amplification	0.684	0.063	92.139	0.559	0.809	0.468	0.386	0.558
FemaleCalls	Control	0.642	0.055	56.894	0.533	0.752	0.412	0.345	0.486
FemaleSong	Control	0.205	0.055	56.894	0.096	0.314	0.042	0.023	0.067
TwoBirds	Control	0.761	0.056	60.866	0.650	0.873	0.579	0.498	0.667
Estimates of Treatments within Periods									
Amp	pb	0.589	0.055	55.303	0.479	0.698	0.347	0.285	0.414
Control	pb	0.744	0.050	39.885	0.643	0.844	0.553	0.482	0.630
Amp	postpb	0.317	0.055	56.967	0.206	0.427	0.100	0.068	0.138
Control	postpb	0.329	0.050	40.211	0.228	0.429	0.108	0.078	0.143
Slope estimates of ZmeanDB in control within Treatments									
	Amplification	-0.013	0.047	31.901	-0.109	0.082	-	-	-
	Control	-0.001	0.044	24.728	-0.091	0.089	-	-	-

Table A14: Effect of experimental noise amplification on acoustic structure of vocalizations (Vocalization amplitude, Frequency composition and spectrum bandwidth and shape) during and after the playback. – Anova table.

	DURING PLAYBACK			POST PLAYBACK		
	Chisq	Df	Pr(>Chisq)	Chisq	Df	Pr(>Chisq)
Response: Vocalization Amplitude (DB SPL) [R2c=0.90]				[R2c=0.89]		
Vocalization type	507.785	1	<0.001	301.674	1	<0.001
Treatment	29.326	1	<0.001	0.085	1	0.770
ZMeanDB in control	8.439	1	0.004	17.404	1	<0.001
Ztime in Treatment	2.158	1	0.142	0.166	1	0.683
Vocalization type: Treatment	1.270	1	0.260	7.286	1	0.007
Vocalization type: ZMeanDB in control	15.609	1	<0.001	80.536	1	<0.001
Vocalization type: Ztime in Treatment	2.918	1	0.088	17.660	1	<0.001
Treatment day: Ztime in Treatment	0.163	1	0.686	0.049	1	0.825
Treatment day: ZMeanDB in control	4.677	1	0.031	0.029	1	0.865
Slopes comparison following significant interaction 'Treatment day: ZMeanDB in control'						
Control-Amplification	4.677	1	0.031	-	-	-
Control	6.388	1	0.011	-	-	-
Amplification	12.150	1	<0.001	-	-	-
Response: -VocFreqPC [r2c=0.42]				[R2c=0.53]		
Vocalization type	53.968	1	<0.001	0.712	1	0.399
Treatment day	1.692	1	0.193	0.721	1	0.396
ZMeanDB in control	1.835	1	0.176	0.606	1	0.436
Ztime in Treatment	2.956	1	0.086	0.113	1	0.737
Vocalization type: Treatment day	1.340	1	0.247	3.028	1	0.082
Vocalization type: ZMeanDB in control	28.096	1	<0.001	8.508	1	0.004
Vocalization type: Ztime in Treatment	1.551	1	0.213	0.240	1	0.624
Treatment day: Ztime in Treatment	0.317	1	0.573	0.365	1	0.546
Treatment day: ZMeanDB in control	0.047	1	0.828	0.006	1	0.940
Response: VocShapePC [r2c=0.43]				[R2c=0.43]		
Vocalization type	601.814	1	<0.001	263.744	1	<0.001
Treatment day	2.787	1	0.095	2.823	1	0.093
ZMeanDB in control	1.873	1	0.171	0.147	1	0.701
Ztime in Treatment	6.833	1	0.009	0.646	1	0.422
Vocalization type: Treatment day	52.538	1	<0.001	16.138	1	<0.001
Vocalization type: ZMeanDB in control	0.228	1	0.633	0.740	1	0.390
Vocalization type: Ztime in Treatment	0.101	1	0.751	4.486	1	0.034
Treatment day: Ztime in Treatment	0.139	1	0.709	0.736	1	0.391
Treatment day: ZMeanDB in control	0.517	1	0.472	0.303	1	0.582

Table A15: Effect of experimental noise amplification on acoustic structure of vocalizations (Vocalization amplitude, Frequency composition and spectrum bandwidth and shape) during and after the playback – post hoc multiple comparisons (“Tukey” contrasts) following significant interaction.

contrast	estimate	SE	df	t.ratio	p.value
Response: Vocalization Amplitude, post playback dataset					
Notes,Control - Trills,Control	-2.159	0.191	2406.905	-11.272	<0.001
Notes,Control - Notes,Amp	0.320	0.542	65.755	0.591	0.935
Notes,Control - Trills,Notes	-2.606	0.533	61.572	-4.890	<0.001
Trills,Control - Notes,Amp	2.479	0.535	62.468	4.636	<0.001
Trills,Control - Trills,Amp	-0.447	0.525	58.003	-0.851	0.830
Notes,Amp - Trills,Amp	-2.926	0.212	2405.135	-13.825	<0.001
Response: VocShapePC, playback dataset					
Notes,Control - Trills,Control	-0.879	0.051	5340.087	-17.140	<0.001
Notes,Control - Notes,Amp	0.764	0.185	68.004	4.141	0.001
Notes,Control - Trills,Notes	-0.859	0.179	59.910	-4.806	<0.001
Trills,Control - Notes,Amp	1.644	0.183	65.671	8.993	<0.001
Trills,Control - Trills,Amp	0.020	0.177	58.196	0.114	0.999
Notes,Amp - Trills,Amp	-1.623	0.088	4884.929	-18.463	<0.001
Response: VocShapePC, post playback dataset					
Notes,Control - Trills,Control	-0.871	0.093	2230.444	-9.376	<0.001
Notes,Control - Notes,Amp	0.637	0.193	65.478	3.300	0.008
Notes,Control - Trills,Notes	-0.788	0.188	58.604	-4.203	0.001
Trills,Control - Notes,Amp	1.508	0.188	60.056	8.008	<0.001
Trills,Control - Trills,Amp	0.083	0.182	52.147	0.454	0.969

Table A16: Effect of experimental noise amplification on acoustic structure of vocalizations (Vocalization amplitude, Frequency composition and spectrum bandwidth and shape) during and after the playback – Model estimates in groups.

Type	Treatment	DURING PLAYBACK				POST PLAYBACK					
		Ismean	SE	df	lower.CL upper.CL	Ismean	SE	df	lower.CL upper.CL		
Response: Vocalization Amplitude (DB SPL) [R2c=0.90]											
Notes	Control	82.204	1.376	32.454	79.402	85.006	80.636	1.187	31.337	78.217	83.056
Trills	Control	84.150	1.375	32.371	81.350	86.951	82.795	1.184	31.002	80.381	85.209
Notes	Amplification	85.752	1.428	36.517	82.857	88.648	80.316	1.198	32.384	77.877	82.756
Trills	Amplification	87.939	1.425	36.224	85.049	90.829	83.242	1.194	31.986	80.809	85.675
Response: -VocFreqPC [r2c=0.42]											
Notes	Control	-0.090	0.214	28.472	-0.529	0.349	-0.028	0.282	29.899	-0.604	0.548
Trills	Control	-0.415	0.213	27.575	-0.851	0.021	-0.189	0.279	28.663	-0.761	0.382
Notes	Amplification	-0.246	0.247	44.499	-0.744	0.253	-0.323	0.289	32.539	-0.912	0.266
Trills	Amplification	-0.703	0.243	41.348	-1.193	-0.213	-0.250	0.286	31.065	-0.834	0.333
Response: VocShapePC [r2c=0.43]											
Notes	Control	-0.436	0.164	30.731	-0.771	-0.101	-0.395	0.204	33.997	-0.809	0.019
Trills	Control	0.443	0.163	29.400	0.111	0.775	0.476	0.199	31.175	0.070	0.882
Notes	Amplification	-1.200	0.199	52.142	-1.599	-0.801	-1.033	0.213	38.790	-1.464	-0.601
Trills	Amplification	0.423	0.194	47.649	0.032	0.813	0.393	0.208	35.505	-0.030	0.816

Table A17: Effect of experimental noise amplification on acoustic structure of vocalizations (Vocalization amplitude, Frequency composition and spectrum bandwidth and shape) during and after the playback – Slope estimates in treatments and vocalization types.

factor	DURING PLAYBACK				POST PLAYBACK					
	Ismeans trend	SE	df	lower.CL	upper.CL	Ismeans trend	SE	df	lower.CL	upper.CL
Response: Vocalization Amplificationlitude (DB SPL) [R2c=0.90]										
Slopes estimates of ZMeanDB in Control within vocalization types										
Notes	4.508	1.388	31.637	1.679	7.337	5.557	1.166	29.650	3.174	7.939
Trills	4.139	1.388	31.564	1.311	6.967	4.317	1.165	29.543	1.937	6.698
Slopes estimates of Ztime in Treatment day within vocalization types										
Notes	0.040	0.105	1000.971	-0.166	0.247	0.468	0.169	554.030	0.137	0.800
Trills	0.190	0.094	782.679	0.005	0.376	-0.161	0.147	345.105	-0.449	0.127
Slopes estimates of Ztime in Treatment day within Treatment days										
Control	0.079	0.097	591.197	-0.111	0.270	0.123	0.186	278.109	-0.242	0.489
Amplification	0.151	0.150	647.584	-0.144	0.446	0.184	0.205	305.491	-0.219	0.587
Slopes estimates of ZmeanDB in Control within Treatment days										
Control	3.542	1.402	32.475	0.689	6.396	4.891	1.184	30.984	2.475	7.306
Amplification	5.105	1.465	37.124	2.137	8.073	4.983	1.205	33.003	2.531	7.436
Response: -VocFreqPC [r2c=0.42]										
Slopes estimates of ZMeanDB in Control within vocalization types										
Notes	0.433	0.214	26.865	-0.006	0.872	-0.104	0.271	26.212	-0.661	0.454
Trills	0.166	0.212	26.180	-0.270	0.602	-0.295	0.270	25.889	-0.851	0.261
Slopes estimates of Ztime in Treatment day within vocalization types										
Notes	0.014	0.048	944.884	-0.079	0.108	-0.043	0.070	598.057	-0.180	0.094
Trills	0.073	0.042	860.163	-0.009	0.155	-0.009	0.058	354.309	-0.122	0.105
Slopes estimates of Ztime in Treatment day within Treatment days										
Control	0.065	0.041	533.837	-0.015	0.145	0.006	0.071	264.136	-0.134	0.146
Amplification	0.023	0.064	605.501	-0.103	0.148	-0.057	0.079	302.744	-0.213	0.098
Slopes estimates of ZmeanDB in Control within Treatment days										
Control	0.321	0.217	27.594	-0.124	0.766	-0.192	0.279	28.019	-0.763	0.379
Amplification	0.278	0.250	41.900	-0.228	0.783	-0.207	0.292	33.151	-0.801	0.388

Response: VocShapePC [r2c=0.43]		[R2c=0.43]	
Slopes estimates of ZMeanDB in Control within vocalization types			
Notes	0.201	0.162 28.125	-0.131
Trills	0.179	0.161 27.133	-0.151
Slopes estimates of Ztime in Treatment day within vocalization types			
Notes	0.075	0.042 915.587	-0.007
Trills	0.088	0.036 873.717	0.016
Slopes estimates of Ztime in Treatment day within Treatment days			
Control	0.069	0.035 522.353	0.000
Amplification	0.093	0.055 594.575	-0.015
Slopes estimates of ZmeanDB in Control within Treatment days			
Control	0.256	0.166 29.300	-0.083
Amplification	0.124	0.202 47.945	-0.282
		0.189 26.293	-0.415
		0.188 25.665	-0.471
		0.068 592.226	-0.274
		0.054 355.892	-0.098
		0.066 251.829	-0.154
		0.074 298.182	-0.253
		0.198 29.143	-0.512
		0.216 39.054	-0.443
			0.360
			0.300
			-0.008
			0.116
			0.105
			0.038
			0.297
			0.432

- Chapter 4 -

Songbird Mates Change their Call Structure
and Intrapair Communication at the Nest in
Response to Environmental Noise

Villain A.S, Fernandez M.S.A, Bouchut C., Soula H.A, Vignal C.

Key words: acoustic flexibility, breeding, monogamy, vocal duet, zebra finch

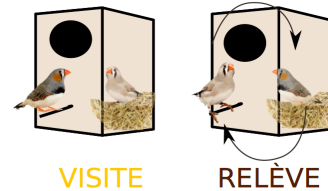
Overview

Quels sont les effets du bruit sur la communication acoustique à courte distance ?
Quelles informations les réponses au bruit nous apportent-elles sur les fonctions de cette communication privée ?

Diamant mandarin, *Taeniopygia guttata*



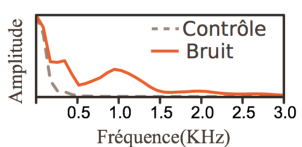

- * Partage de l'incubation entre femelle et mâle
- * Duos vocaux à chaque rencontre au nid
- * Deux contextes de rencontre:
 - des visites ou
 - des relèves

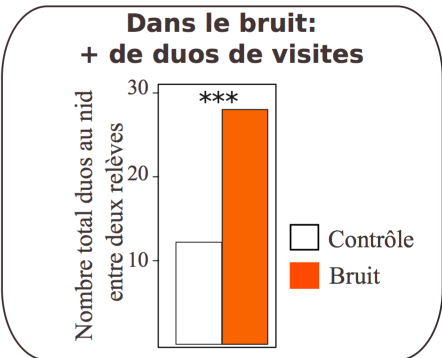


Si les duos ont des fonctions importantes pour le partage de l'incubation alors les partenaires devraient les ajuster en réponse au bruit

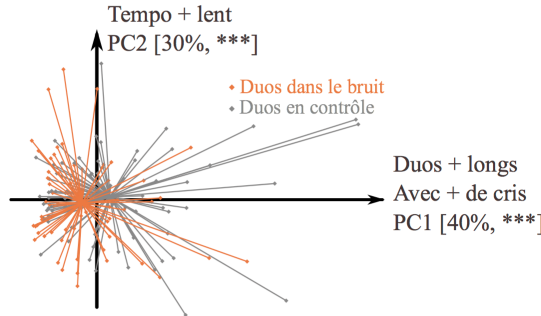
Expérience : ajout expérimental de bruit au nid

- * 2 jours d'enregistrement par couple
- * Repasse de bruit dans le nid
- * Enregistrements des duos

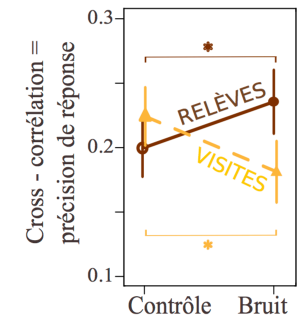



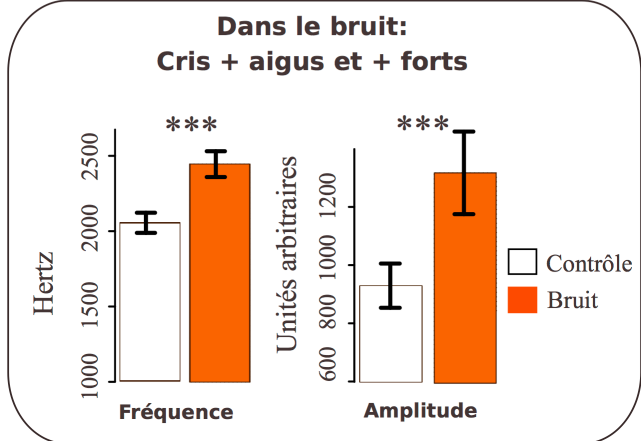


Dans le bruit: les duos sont + courts et + rapides en visite et en relèvements



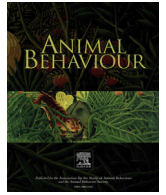
Seuls les duos de relèvements sont produits avec + de précision dans le bruit





Conclusions

- * Même à très courtes distances, le bruit a des effets majeurs sur le comportement vocal:
 - duos raccourcis
 - variation de la structure des cris chez les femelles et chez les mâles
- * Visites vs. Relèvements: réponses ≠
 - > visites: stratégie de redondance (augmentation du nombre)
 - > relèvements: duos plus précis
- * Confirmation de l'hypothèse du rôle central du duo pendant la relève.



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



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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 intrapair acoustic communication, which is surprisingly poorly understood. Here we examined the impact of an increased level of background noise on intrapair acoustic communication at the nest in the zebra finch, *Taeniopygia guttata*. 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 probably represents a critical component of pair coordination. In response to the noise playback, partners called less and more rapidly during visit and relief duets. Female and male 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 females and males 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 intrapair communication for breeding pairs.

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Monogamy in birds represents a partnership in which the female and male 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 alarm calling for danger (Krams, Krama, & Igaune, 2006), repelling predators or intruders on their territory (Black, 2001; Regelman & 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 female and male 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 nonvocal 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

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and conspicuous song duets of tropical bird species have been particularly well studied (Hall, 2004, 2009). But intrapair communication may be more widespread and involve simpler or low-amplitude vocalizations such as calls (Lamprecht, Kaiser, Peters, & Kirchgessner, 1985; Morton & Derrickson, 1996; Todt, Hultsch, & 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 (Brumm & Slabbekoorn, 2005). Senders can use different frequencies to avoid spectral overlap between signal and noise (Slabbekoorn & Peet, 2003). They can increase signal amplitude (the 'Lombard effect'; Brumm & Todt, 2002; Cynx, Lewis, Tavel, & Tse, 1998; Potash, 1972) or signal redundancy (Brumm & Slater, 2006; Lengagne, Aubin, Lauga, & Jouventin, 1999), as well as change the timing of their vocalizations to avoid noise (Brumm, 2006b; Dreiss, Ruppli, Faller, & Roulin, 2015; Gil, Honarmand, Pascual, Pérez-Mena, & Garcia, 2014). Senders and receivers can also adjust their location during communication, such as moving closer to each other (Halfwerk, Bot, & Slabbekoorn, 2012) or stay outside/inside the nest cavity (Blumenrath, Dabelsteen, & Pederson, 2004). The effects of background noise have been primarily studied on songs and other long-range vocalizations, and thus little is understood about the effects of background noise on private, short-range vocalizations (Leonard & Horn, 2005, 2008). Importantly, short-range vocalizations are less affected by degradation during sound propagation and thus probably require very different adjustment strategies from long-range vocalizations. Noise probably hinders intrapair 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 intrapair 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 probably aids coordination (Boucaud, Mariette, Villain, & Vignal, 2016; Elie et al., 2010). Zebra finches live in semiarid zones of Australia, an unpredictable environment in which 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 female and male take turns incubating, both partners were exposed to the noise. After 15 h of noise, intrapair communication and partners' behaviour were monitored and compared to the control condition. Because call duets are thought to aid partners' coordination during incubation, we expected birds to show strategies to maintain signal efficacy in response to the noise playback. We monitored three duet types, incubation relief/nest visit/sentinel, and we studied four aspects of this intrapair communication: (1) the temporal structure of duets; (2) the female–male 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 in noise, we expected the partners either to avoid communicating or to display strategies to cope with it, for example by 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).

METHODS

Subjects and Housing Conditions

Eighteen pairs of zebra finches 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 × 40 × 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 × 5.5 m and 3.5 m high, temperature between 19 and 24 °C, light conditions 14:10 h light:dark). Twenty-seven nestboxes were installed (13 × 12 cm and 17 cm high).

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 h and the other morning was used as a 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 them. This treatment mimicked the noise heard by incubating birds in artificial nestboxes (which are readily used by wild zebra finches, Griffith, Pryke, & Mariette, 2008), inside which wind noise reverberates, but also in natural nests, which are woven and bottle shaped (Zann, 1996), and 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 ultradirectional microphone (Sennheiser, MKH 70). We used a sequence of 15 s of wind noise (Supplementary Material sound 0) repeated for a total duration of 15 h of playback before the recording session (from 1730 hours the day before to 1000–1200 hours the day of recording). The recordings took place within the last 20% of the total playback time (from 0830 to 1200 hours). So if birds habituated to the particular noise snippet repeated during the playback, we assumed habituation was largely completed after 15 h, and all the behaviours and vocalizations analysed in our results were equally affected.

The sound pressure level inside the nestbox 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 (ca. 5 dB increase) compared to the control, which is a relevant increase in sound pressure level for zebra finches in the wild (Fig. A1). 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. A2). Because the noise was played inside the nestbox, the background noise level outside remained the same in both treatments, i.e. 58.1 dB SPL in the control and 58.7 dB SPL in noise (measured at 20 cm 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 the nestbox 20 cm from the entrance to record vocal activity in the vicinity of the nest. Microphone calibrations were previously performed with 10 s of white noise and a sound level meter (Rion NL-42, with additional NX-42WR package to have the audio file and the corresponding dB file). 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 (female returns versus male returns to the nest). As a consequence, the duration of one session depended on the observed pair and could last from 1 to 3.5 h. 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 does 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 s (Elie et al., 2010). When the returning mate was far from the nestbox, its calls were not accurately detected among vocalizations of other birds in 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 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 s 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 A1) 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 the Praat 'annotate' function as time intervals. Each call was labelled as female or male and assigned to one of the three following call types (see Fig. A3 for spectrograms of duets and call types and Supplementary Material sounds 1–5).

(1) 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).

(2) 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.

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

When duets were performed with both birds inside the nest-box, we measured 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 the last one). Intercall 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 the PCA. The PCA described the 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 \leftarrow lmer (PC values ~ Treatment + Returning partner + Duet type + Noise treatment: Returning partner + Noise Treatment: Duet type, random = ~1|pair identity). This model was built to test for three

Table 1
Principal component analysis of the global structure of relief and visit duets

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

^a Absolute contributions of the decomposition of inertia for each PC ('inertia.dudi' function from 'ade4' R package), divided by 100 to get the percentage. Signs are the signs of the coordinate.

^b Transformations used before the PCA.

^c The Box-Cox transformation computes one parameter transformation using the following formula: parameter $(\lambda) = (\text{parameter}^{\lambda} - 1)/\lambda$, if $\lambda \neq 0$ and parameter $(\lambda) = \ln(\text{parameter})$ if $\lambda = 0$. The 'boxcox' function of the 'Mass' R package automatically finds the appropriate λ value to reach a distribution as close as possible to the Gaussian distribution.

^d ICI = intercall interval.

fixed factors (Treatment (two levels: Noise versus Control), Returning partner (two levels: Female versus Male) and Duet type (two levels: R versus V)) and two interactions (Noise treatment: Returning partner, Noise Treatment: Duet type), taking into account the within-subject design using a random factor (pair identity). Not all the interactions between the fixed factors were tested, as some 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 \leftarrow lmer (PC values ~ Treatment * Returning partner, random = ~1|pair identity), to test two interacting fixed factors: Noise treatment (Noise versus Control) and Returning partner (Female versus Male). *P* values were assessed using the 'Anova' function ('car' R package).

Analysis of Duets' Dynamic During Reliefs and Visits

The female–male 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) and second using cross-correlation of female and male signals (cross-correlation analysis).

ICI analysis

For this analysis, we assessed female and male tempos using means and standard deviations of intrasex ICIs (time between two call starts of the same individual) and mean intersex ICIs, 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 female–male 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 female and male calling signals. A calling signal is a temporal description of the calling output 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 200 Hz (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_{female} stands for the female signal and S_{male} for the male signal. We computed the cross-correlation (cc) of these two signals (S_{female} and S_{male}) with the following formula:

Table 2
Principal component analysis of the global structure of sentinel duets

	PC1	PC2
Variance explained (% cumulative)	64	95
Eigenvalue	2.6	1.2
Total number of calls (Box-Cox)	-10.08	58.99
Sequence duration (ln)	-34.83	6.52
Call rate (ln)	32.17	6.03
Overall ICI (square root)	-22.91	-28.46

Table 3
Principal component analysis of relief and visit duets: female–male dynamic

	PC1
Variance explained (% cumulative)	65
Eigenvalue	3.9
M to F transition (Box-Cox)	–14.02
F to M transition (Box-Cox)	–16.08
F to F ICI (Box-Cox)	–17.47
M to M ICI (Box-Cox)	–17.38
M to M ICI standard deviation (Box-Cox)	–18.42
F to F ICI standard deviation (Box-Cox)	–16.63

$$cc(T) = \frac{\text{mean}((S_{\text{male}}(t) - \text{mean}(S_{\text{male}})) \times (S_{\text{female}}(t + T) - \text{mean}(S_{\text{female}})))}{\text{SD}(S_{\text{male}}) \times \text{SD}(S_{\text{female}})}$$

With the normalization step, we have: $CC(T) = cc(T) / (\text{SD}(S_{\text{female}}) \times \text{SD}(S_{\text{male}}))$, where CC is the normalized cross-correlation, T the time delay, and S_{female} and S_{male} the female and male 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 ($F \rightarrow M$), 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 ($M \rightarrow F$) (Perez, Fernandez, Griffith, Vignal, & Soula, 2015).

To compare cross-correlation between treatments, we measured the curve's maximum peak height, which indicates the strength of the cross-correlation, as well as the height of each peak (positive peak: $F \rightarrow M$, negative peak: $M \rightarrow F$). Because cross-correlation used the calculation of two means, we used only duets having more than eight calls per individual. We first tested duets with the best correlation scores, 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 six 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 six in noise) from 10 pairs. The same model as above was used to assess the effect of the treatment (see model 1).

Occurrence of Duets and Spatial Proximity Between Partners

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 m, from 1 to 2 m and more than 2 m) and the number of sentinel duets in each distance category was counted.

One recording session consisted of two consecutive incubation reliefs (female return versus male return to the nest). The duration of a recording session thus depended on pair identity, as some birds had 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 female return or male 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 being just before the first relief, the second 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 data set but, for some of them, the first reference period was missing. The data set was then composed of 28 reference periods in the 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).

Statistics

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 \leftarrow glmer (total behavioural count ~ Treatment * Returning partner + (1 | Pair), family = 'poisson'), to test two interacting fixed factors: Noise treatment (Noise versus Control) and Returning partner (Female versus Male).

Acoustic Analysis of Calls used in 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 (0.5 kHz–20 kHz 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, interquartile range (IQR), standard deviation (SD) and mode (all in Hz). 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 the 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 the control with exemplars of noise. All measures were averaged with 10 mixes obtained using 10 different exemplars of noise (For a detailed explanation of the procedure see Fig. A4).

Table 4
Principal component analysis of call structure

	PC1	PC2
Variance explained (%cumulative)	43	77
Eigenvalue	3.9	3.1
Call duration	0	–1.08
Mean frequency	3.23	–26.78
SD	20.29	–1.62
Median frequency	–9.18	–16.05
Q25	–15.34	–10.74
Q75	4.66	–24.42
IQR	13.37	–12.67
Dominant frequency	–16.31	–5.65
Amplitude	–17.62	–0.99

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 \leftarrow 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)), to test four fixed factors: Noise treatment (Noise versus Control), Sex of the caller (Female versus Male), Call type (Ark, Whine and Short calls) and Duet type ('V or R' versus 'S') and three interactions (Noise treatment: Sex of the caller, Noise treatment: Call type and Noise treatment: Duet type). 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 tests 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 post hoc tests. For all linear models, residuals equivariance and distribution were checked using the 'plotresid' function ('RVAideMemoire' R package). The influential data points were tested using the '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); for a detailed description see Fig. A5. For linear models using Poisson distribution, residuals overdispersion was tested using the 'overdisp.glm' 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 the 'rsquaredGLMM' 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 ($\chi^2_1 = 3.5$, $P = 0.06$; Fig. 1a), but the number of meetings inside or outside the nest did not differ between treatments (binomial model: $\chi^2_1 = 0.64$, $P = 0.42$). The latency of response of the incubating partner to the calls of its outside mate increased in relief duets (post hoc test: $\chi^2_1 = 4.1$, $P = 0.04$; 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 the control ($\chi^2_1 = 10.1$, $P = 0.001$; 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 the control ($\chi^2_1 = 11.2$, $P = 0.001$; Fig. 1d).

Female–Male Dynamic during Relief (R) and Visit (V) Duets

During noise, R duets were performed with shorter intrasex ICIs and intersex transitions, since PC1 was significantly higher, whereas intervals did not change in V duets (interaction treatment*duet type: $\chi^2_1 = 4.1$, $P = 0.04$; relief duets: $\chi^2_1 = 8.5$, $P = 0.003$; visit duets: $\chi^2_1 < 0.001$, $P = 0.99$; Fig. 2a). As a consequence, partners changed their calling dynamic in noise only during relief duets.

Furthermore, the cross-correlation between female and male 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, c). 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, c; $\chi^2_1 = 2.7$, $P = 0.09$ and $\chi^2_1 = 5.9$, $P = 0.02$, respectively) whereas they decreased in V duets (Fig. 2b, c, $\chi^2_1 = 4.8$, $P = 0.03$ and $\chi^2_1 = 2.9$, $P = 0.09$, 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 the control ($\chi^2_1 = 5.8$, $P = 0.02$ and $\chi^2_1 = 4.9$, $P = 0.03$, respectively), but no longer differentiated duet types (no significant interaction treatment*duet type; $\chi^2_1 = 1.8$, $P = 0.17$ and $\chi^2_1 = 0.03$, $P = 0.85$, respectively). Overall, this analysis revealed higher regularity in female–male calling in noise than in the control (Fig. 2d, e), especially during R duets. In particular, male delays in answering female calls were more precise during noise.

Structure of Sentinel Duets (S)

The total duration of S duets was not affected (PC1: $\chi^2_1 = 0.94$, $P = 0.33$; Fig. 3a). Duets showed fewer calls and lower tempo (higher overall ICI) in noise than in the control, since PC2 was significantly lower in noise ($\chi^2_1 = 7.6$, $P = 0.007$; Fig. 3b).

Occurrence of Duets/Spatial Proximity Between Partners

In noise, the total number of V duets increased by 2.6 ± 1.9 ($\chi^2_1 = 6.9$, $P = 0.008$; Fig. 4a). The number of S duets did not differ between treatments ($\chi^2_1 = 0.8$, $P = 0.34$; Fig. 4a).

During S duets, the returning partner perched significantly closer to the nest in noise than in the control, increasing the spatial proximity between partners (Fig. 4b). In noise, S duets took place slightly more often with the outside mate at 0–1 m from the nest (Fig. 4b; $\chi^2_1 = 2.4$, $P = 0.12$) and significantly less at 1–2 m ($\chi^2_1 = 9.0$, $P = 0.003$).

Acoustic Features of Calls Produced Inside the Nest

During noise, calls produced inside the nest were louder with an upshifted 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: $\chi^2_1 = 28.2$, $P < 0.001$; post hoc female versus 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: $\chi^2_1 = 18.4$, $P < 0.001$; post hoc short versus whine calls in noise: $t_{105,8} = 2.8$, $P = 0.06$; Fig. 5b, see examples of call spectra in Fig. 5c).

DISCUSSION

We examined how zebra finch partners cope with a strong acoustic constraint on their intrapair communication using a

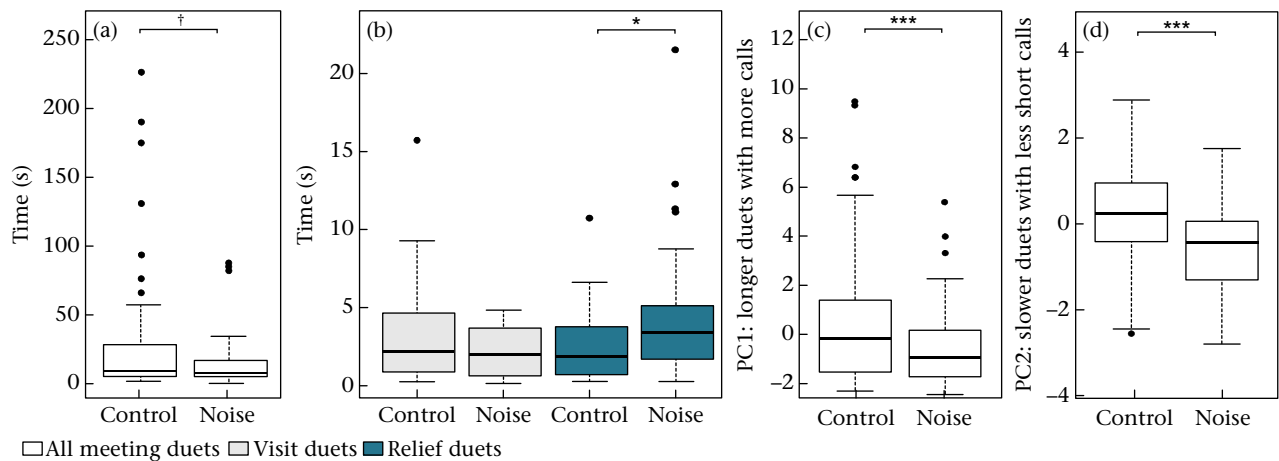


Figure 1. Effect of treatment on (a) the time together in the nest, (b) the latency of the incubating partner to answer to its partner, (c) PC1 and (d) PC2 of the global structure of relief and visit duets in noise and the control. Boxes are median, first and third quartiles (Q1 and Q3, respectively). The upper whisker is located at the *smaller* of the maximum \times value and $Q3 + 1.5$ interquartile range (IQR); the lower whisker is located at the *larger* of the smallest \times value and $Q1 - 1.5$ IQR. Individual points more extreme in value than $Q3 + 1.5$ IQR are plotted separately at the high end, and those below $Q1 - 1.5$ IQR are plotted separately at the low end (circles). Model estimates are available in [Tables A2 and A3](#). † $P < 0.1$; * $P < 0.05$; *** $P < 0.001$.

playback of wind noise inside the nestbox. All duets recorded in noise were shorter and quicker, and relief duets showed changes in the female–male dynamic of calling (intercall 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 upshifted and less broadband frequency spectrum.

Response to Noise Reveals Potential Call Duets' Functions

Recent reviews on avian duetting underlined the lack of experimental evidence testing the relationship between duet structure and function ([Dahlin & Benedict, 2014](#); [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 the control. These results show that the noise treatment significantly constrained intrapair communication. But even under this strong acoustic constraint, zebra finch mates continued to perform vocal duets each time they met at the nest, during either visits or incubation reliefs. 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, the female–male 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 intrapair communication system. Nest relief in species with biparental 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. \(2016\)](#). 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 for by increased redundancy. Visit duets might be involved 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 maintain 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 antipredator vigilance outside the nest, as suggested by [Elie et al. \(2010\)](#) and [Mainwaring and 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 intrapair communication in response to noise in the great tit, *Parus major* ([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 the partners were subjected to the noise alternately, we cannot conclude anything 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 (female or male).

Last, the fact that partners increased the number of visit duets may be a sign of an extreme increase in 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 that partners may reinforce vocal interactions in noise.

Noise Impacts Quiet Vocalizations

Many studies have already demonstrated that birds and mammals modify the pitch and the amplitude of their vocalizations in response to noise, but they have largely focused on loud and/or

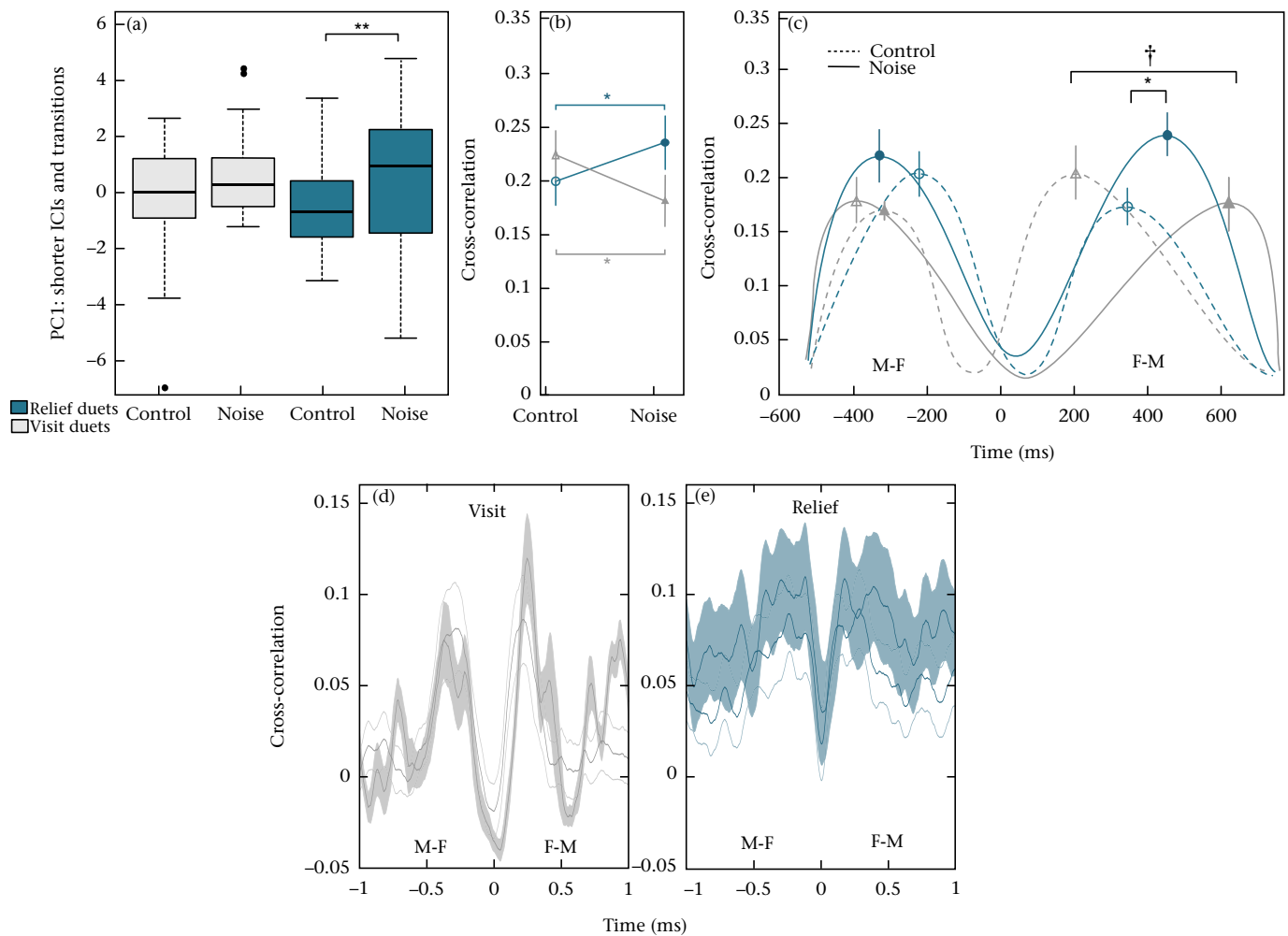


Figure 2. Effect of treatment on the female–male temporal dynamic in duets. (a) Boxes of PC1 are displayed in visit and relief duets. Boxes are median, first and third quartiles (Q1 and Q3, respectively). The upper whisker is located at the “smaller” of the maximum \times value and $Q3 + 1.5$ interquartile range (IQR); the lower whisker is located at the “larger” of the smallest \times value and $Q1 - 1.5$ IQR. Individual points more extreme in value than $Q3 + 1.5$ IQR are plotted separately at the high end, and those below $Q1 - 1.5$ IQR are plotted separately on the low end (circles). (b) Maximum cross-correlation peak height (mean \pm SE) in relief (circles) and visit (triangles) duets (significant treatment \times duet type interaction: $\chi^2_1 = 5.5$, $P = 0.02$). (c) Cross-correlation curves between female (F) and male (M) signals in relief (circles) and visit (triangles) duets, showing the height of the negative and positive peaks in the control and noise. Curves correspond to the mean (\pm SE) of each peak over all duets. (d, e) Mean (\pm SE) cross-correlation over all duets in the control and noise for (d) visit and (e) relief 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 A4. $^{\dagger}P < 0.1$; $^{*}P < 0.01$; $^{***}P < 0.001$.

long-range vocalizations: display calls in king penguins, *Aptenodytes patagonicus* (Aubin & Jouventin, 2002), separation calls in fowl, *Gallus gallus domesticus* (Brumm et al. 2009), distance calls in common marmosets, *Callithrix jacchus* (Brumm, Voss, Köllmer, & Todt, 2004) and territorial songs in several bird species (e.g. blackbirds, *Turdus merula*: Nemeth & Brumm, 2009; great tits: 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 very 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 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 Noise may be Costly

During an experimental increase in 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 localization. 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 antipredator strategy (Dalbelsteen, McGregor, Lampe,

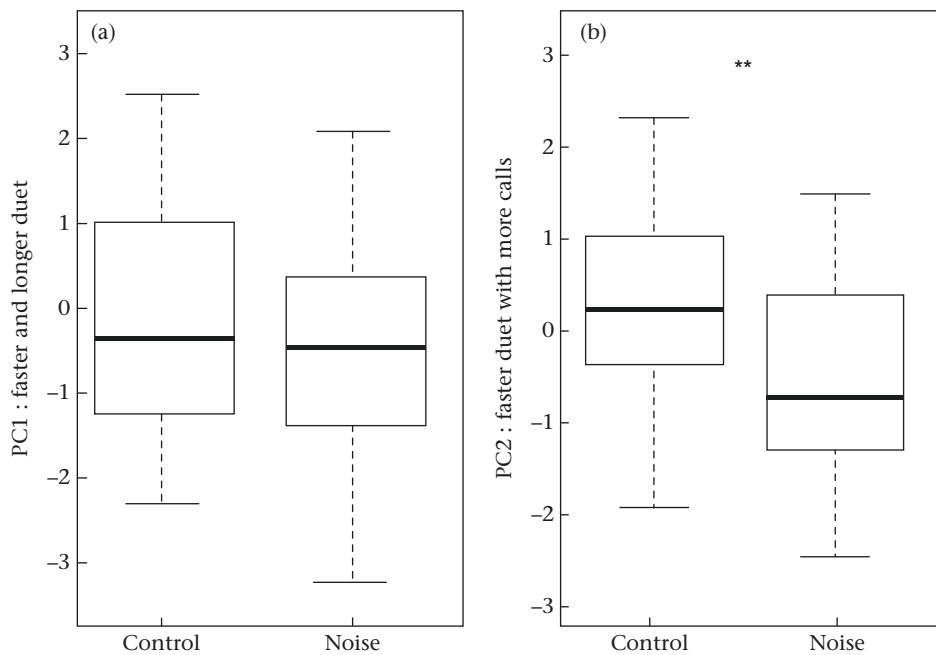


Figure 3. Effect of treatment on sentinel duet structure. Boxes of PCs are displayed in noise and the control for (a) PC1 and (b) PC2 of the PCA on sentinel duets. Boxes are median, first and third quartiles (Q1 and Q3, respectively). The upper whisker is located at the *smaller* of the maximum \times value and $Q3 + 1.5$ interquartile range (IQR); the lower whisker is located at the *larger* of the smallest \times value and $Q1 - 1.5$ IQR. Model estimates are available in Table A5. ** $P < 0.01$.

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 Sexes

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

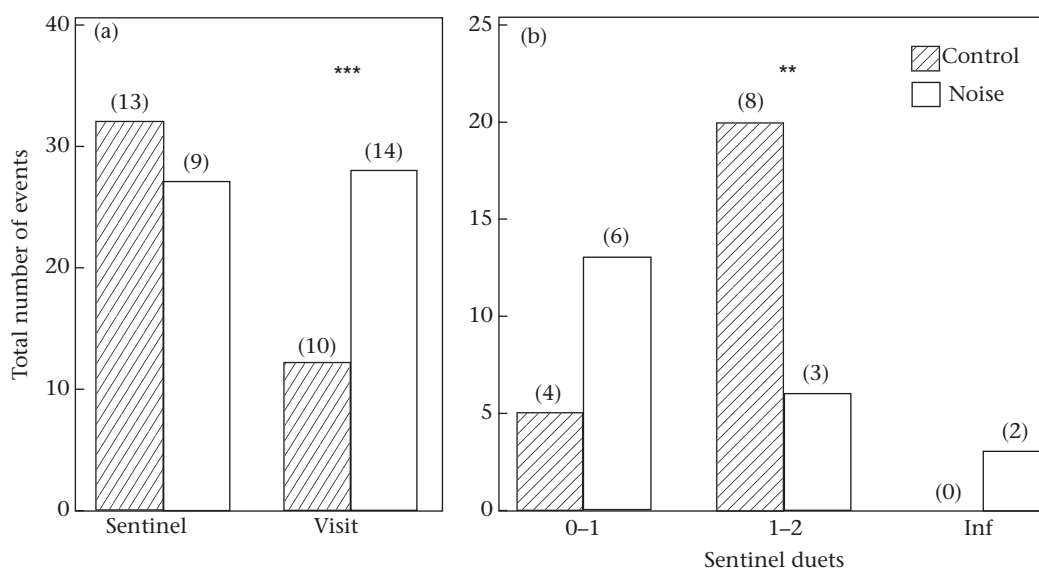


Figure 4. Effect of treatment on (a) the total number of visit and sentinel duets and (b) the location of the returning partner during sentinel duets in the control and noise (location is in metres from the nest; 'Inf' refers to calls produced while the partner was not around). Numbers above each bar indicate the number of pairs involved in the total count. Model estimates are available in Tables A6 and A7. ** $P < 0.01$; *** $P < 0.001$.

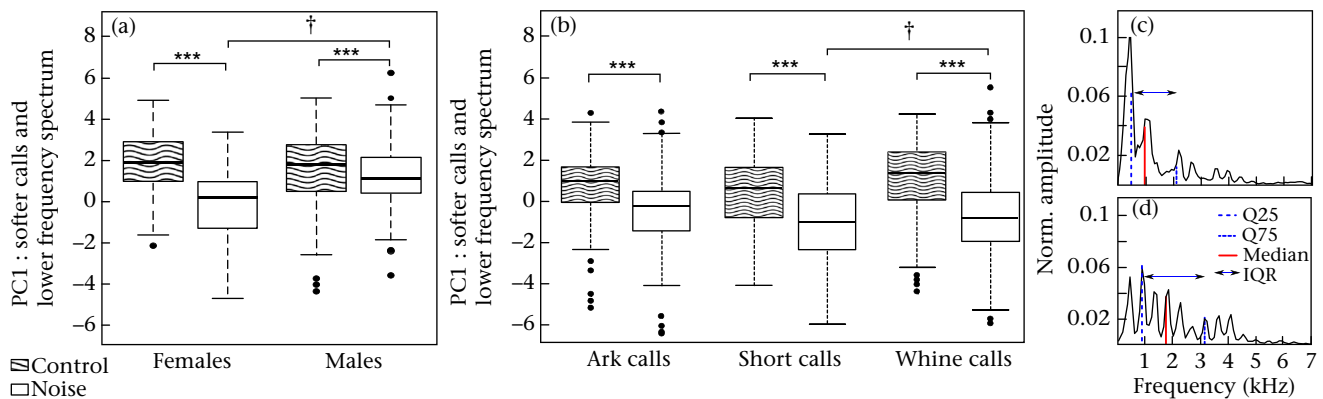


Figure 5. Effect of treatment on calls' structure. (a, b) Boxes of PC1 are displayed in noise and the control for (a) females and males and (b) in each call type separately following significant interactions. The upper whisker is located at the *smaller* of the maximum \times value and $Q3 + 1.5$ interquartile range (IQR); the lower whisker is located at the *larger* of the smallest \times value and $Q1 - 1.5$ IQR. Individual points more extreme in value than $Q3 + 1.5$ IQR are plotted separately at the high end, and those below $Q1 - 1.5$ IQR are plotted separately on the low end (circles). Post hoc multiple comparisons (with Tukey correction) showed that both sexes were affected by noise (control versus noise: in females: $t_{704.0} = 11.1$, $P < 0.001$ and in males $t_{701.9} = 4.9$, $P < 0.001$) and all call types were affected by noise (control versus noise: in short calls: $t_{700.7} = 4.7$, $P < 0.001$; in ark calls: $t_{694.3} = 6.9$, $P < 0.001$; in whine calls: $t_{700.9} = 9.6$, $P < 0.001$). (c, d) Example of changes that can occur on a call spectrum (example with a short call from the same individual recorded in (c) the control and (d) noise). Call spectrum of the control call in (c) has been corrected (detailed procedure is given in Fig. A4). Model estimates are available in Tables A8–A14. † $P < 0.1$; *** $P < 0.001$.

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 does not need a developed song system. Indeed, vocal flexibility in response to noise has already been described in vocal nonlearners (Aubin & Jouventin, 2002; Brumm et al., 2009).

Female and Male Response to Noise Differs

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 the female–male 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 h, 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, Boucaud, Fernandez, Villain, & Vignal, n.d.). As a consequence, females were subjected to the playback more 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 the control. Because duets are joint vocal interactions, it seems difficult to explain separately female and male responses during the duet. The effects observed on female or male 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 intrapair 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 indicate the importance of these duets in coordinating partners during the crucial moments of incubation shifts.

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Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.anbehav.2016.03.009>.

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APPENDIX

Table A1

Data composition

	Relief duets		Visit duets		Sentinel duets	
	M returning	F returning	M returning	F returning	M returning	F returning
Control	18	16	36	9	66	31
Noise	19	16	23	11	47	19

Total number of duets in each treatment (Control versus Noise) and each duet type (Reliefs, Visits or Sentinel duets). At the beginning of a duet, one mate is inside the nestbox, incubating the eggs, and the other is outside, returning near the nest (F = female and M = male).

Table A2

Effect of treatment on latency to answer, number of meeting duets (relief and visit) performed inside the nest and the time spent inside the nest

	Estimate	SE	χ^2	df	P
Latency to answer (log) (N=16 pairs) [$R^2c=0.05$]					
(Intercept)	0.614	0.284	–	–	–
Noise treatment (Noise)	-0.037	0.385	0.610	1	0.435
Returning partner (M)	0.111	0.285	0.026	1	0.873
Duet type (Relief)	-0.159	0.269	0.899	1	0.343
Noise treatment*Returning partner	-0.280	0.399	0.493	1	0.483
Noise treatment*Duet type	0.714	0.388	3.377	1	0.066
Latency to answer (log) post hoc test (relief duets) (16 pairs) [$R^2c=0.09$]					
(Intercept)	0.664	0.276	–	–	–
Noise treatment (Noise)	0.605	0.390	4.076	1	0.043
Returning partner (M)	-0.295	0.384	1.801	1	0.180
Noise treatment*Returning partner	-0.124	0.535	0.053	1	0.817
Latency to answer (log) post hoc test (visit duets) (16 pairs) [$R^2c=0.04$]					
(Intercept)	0.062	0.396	–	–	–
Noise treatment (Noise)	0.335	0.532	0.006	1	0.940
Returning partner (M)	0.656	0.444	1.981	1	0.159
Noise treatment*Returning partner	-0.428	0.620	0.476	1	0.490
Meeting inside the nest (binomial model) (18 pairs) [$R^2c=0.74$]					
(Intercept)	2.06	0.825	–	–	–
Noise treatment (Noise)	-0.973	0.994	0.640	1	0.424
Returning partner (M)	-1.022	0.702	1.460	1	0.227
Duet type (Relief)	-2.732	0.774	23.006	1	<0.001
Noise treatment*Returning partner	0.823	0.962	0.731	1	0.393
Noise treatment*Duet type	0.166	0.943	0.031	1	0.860
Time together in nest (log) (N=18 pairs) [$R^2c=0.24$]					
(Intercept)	2.956	0.497	–	–	–
Noise treatment (Noise)	-0.112	0.702	3.570	1	0.059
Returning partner (M)	-0.411	0.362	1.242	1	0.265
Duet type (Relief)	-0.233	0.423	2.890	1	0.089
Noise treatment*Returning partner	0.249	0.506	0.243	1	0.622
Noise treatment*Duet type	-0.607	0.629	0.930	1	0.335

Linear mixed-effect models ('lmer' function from 'lme4' R package) tested the effect of the treatment (Control versus Noise), the sex of the partner returning to the nest (Female versus Male) and the duet type (Relief versus Visit), with pair identity as random factor. P values were assessed using the Anova function ('car' R package). The number of pairs included in the analysis after removal of overly influential levels of the random factor is indicated ('influence' function 'influence.ME' R package). R^2c value, which represents the conditional coefficient of determination of the model, is indicated for each model ('rsquaredGLMM' function of 'MuMIn' R package). Estimates were extracted from the summary of the model.

Table A3

Statistical results on PCs from PCAs performed separately on global features of the duet and female–male dynamic during the duet

	Estimate	SE	χ^2	df	P
Global duet structure: statistics on PCs					
PC1(log(+3))=duet duration and call quantity [$R^2c=0.45$]					
(Intercept)	0.704	0.147	–	–	–
Noise treatment (Noise)	-0.030	0.173	10.893	1	0.001
Returning partner (M)	0.034	0.127	0.831	1	0.362
Duet type (Relief)	0.651	0.122	39.092	1	<0.001
Noise treatment*Returning partner	-0.220	0.175	1.587	1	0.208
Noise treatment*Duet type	-0.209	0.171	1.491	1	0.222
PC2=tempo and call repertoire [$R^2c=0.29$]					
(Intercept)	0.228	0.352	–	–	–
Noise treatment (Noise)	-0.604	0.429	11.235	1	0.001
Returning partner (M)	0.186	0.318	0.124	1	0.725
Duet type (Relief)	-0.261	0.307	1.243	1	0.265
Noise treatment*Returning partner	-0.208	0.440	0.223	1	0.637
Noise treatment*Duet type	0.027	0.437	0.004	1	0.950

Table A3 (continued)

	Estimate	SE	χ^2	df	P
PC3 [R²c=0.14]					
(Intercept)	0.151	0.330	—	—	—
Noise treatment (Noise)	-0.251	0.441	0.242	1	0.623
Returning partner (M)	-0.126	0.326	0.269	1	0.604
Duet type (Relief)	0.031	0.307	0.188	1	0.664
Noise treatment*Returning partner	0.467	0.451	1.076	1	0.300
Noise treatment*Duet type	0.129	0.437	0.087	1	0.768
PC4 [R²c=0.18]					
(Intercept)	0.159	0.314	—	—	—
Noise treatment (Noise)	0.343	0.417	1.123	1	0.289
Returning partner (M)	0.386	0.306	0.100	1	0.751
Duet type (Relief)	-0.652	0.291	15.674	1	<0.001
Noise treatment*Returning partner	-0.617	0.424	2.119	1	0.145
Noise treatment*Duet type	-0.336	0.411	0.669	1	0.413
Female—male dynamic during the duet: statistics on PCs					
PC1=intercall intervals and transitions [R²c=0.21]					
(Intercept)	-0.387	0.499	—	—	—
Noise treatment (Noise)	0.072	0.695	5.499	1	0.019
Returning partner (M)	-0.177	0.483	0.270	1	0.603
Duet type (Relief)	0.328	0.465	7.505	1	0.006
Noise treatment*Returning partner	-0.022	0.714	0.001	1	0.975
Noise treatment*Duet type	1.422	0.695	4.182	1	0.041
PC1=intercall intervals and transitions post hoc tests (relief duets) [R²c=0.18]					
(Intercept)	-0.727	0.514	—	—	—
Noise treatment (Noise)	1.630	0.775	8.534	1	0.003
Returning partner (M)	1.021	0.704	3.119	1	0.077
Noise treatment*Returning partner	-0.242	1.031	0.055	1	0.815
PC1=intercall intervals and transitions post hoc tests (visit duets) [R²c=0.33]					
(Intercept)	0.459	0.574	—	—	—
Noise treatment (Noise)	0.028	0.751	0.000	1	0.989
Returning partner (M)	-1.544	0.645	9.984	1	0.002
Noise treatment*Returning partner	-0.033	0.945	0.001	1	0.972

Linear mixed-effect models ('lmer' function from 'lme4' R package) tested the effect of the noise treatment (Control versus Noise), the sex of the partner returning to the nest (Female versus Male) and the duet type (Relief versus Visit), with pair identity as random factor. *P* values were assessed using the Anova function ('car' R package). For PC2 from the global features PCA, three pairs were found as overly influential ('influence' function, 'influence.ME' R package), the results are thus on $N = 15$ pairs. $N = 18$ pairs for all other results. R^2c value, which represents the conditional coefficient of determination of the model, is indicated for each model ('r.squaredGLMM' function of 'MuMIn' R package). Estimates were extracted from the summary of the model.

Table A4

Statistical results on cross-correlation between female and male signals during duets

	Estimates	SE	χ^2	df	P
Data set with cross-correlation > 0.1 only					
Maximum peak height * N=16 pairs [R²c=0.43]					
(Intercept)	0.202	0.024	—	—	—
Noise treatment (Noise)	0.031	0.031	0.321	1	0.570
Returning partner (M)	-0.008	0.024	0.030	1	0.861
Duet type (Relief)	-0.031	0.018	0.200	1	0.654
Noise treatment*Returning partner	0.011	0.037	0.086	1	0.768
Noise treatment*Duet type	0.063	0.026	5.497	1	0.019
Maximum peak height: post hoc (Relief duets): 14 pairs [R²c=0.47]					
(Intercept)	0.176	0.026	—	—	—
Noise treatment (Noise)	0.032	0.033	2.741	1	0.097
Returning partner (M)	0.036	0.030	2.920	1	0.087
Noise treatment*Returning partner	0.004	0.043	0.010	1	0.918

Table A4 (continued)

	Estimates	SE	χ^2	df	P
Maximum peak height: post hoc (Visit duets): 10 pairs [R²c=0.55]					
(Intercept)	0.289	0.034	—	—	—
Noise treatment (Noise)	-0.047	0.046	4.836	1	0.027
Returning partner (M)	-0.087	0.039	10.262	1	0.001
Noise treatment*Returning partner	-0.024	0.061	0.154	1	0.694
F → M peak height (log): N=16 pairs [R²c=0.16]					
(Intercept)	-0.774	0.053	—	—	—
Noise treatment (Noise)	0.192	0.093	2.510	1	0.113
Returning partner (M)	-0.022	0.064	0.710	1	0.399
Duet type (Relief)	-0.050	0.046	0.244	1	0.621
Noise treatment*Returning partner	-0.052	0.103	0.258	1	0.611
Noise treatment*Duet type	0.169	0.073	5.291	1	0.021
F → M peak height: post hoc (Relief duets): 14 pairs [R²c=0.30]					
(Intercept)	-0.846	0.054	—	—	—
Noise treatment (Noise)	0.176	0.098	5.856	1	0.015
Returning partner (M)	0.102	0.070	1.942	1	0.163
Noise treatment*Returning partner	-0.067	0.117	0.323	1	0.569
F → M peak height: post hoc (Visit duets): 9 pairs [R²c=0.58]					
(Intercept)	0.287	0.030	—	—	—
Noise treatment (Noise)	0.060	0.042	2.919	1	0.087
Returning partner (M)	0.125	0.038	16.121	1	<0.0001
Noise treatment*Returning partner	0.022	0.057	0.160	1	0.688
M → F peak height: N=14 pairs [R²c=0.41]					
(Intercept)	0.183	0.024	—	—	—
Noise treatment (Noise)	0.023	0.030	0.160	1	0.688
Returning partner (M)	0.035	0.026	2.038	1	0.153
Duet type (Relief)	0.009	0.018	0.941	1	0.331
Noise treatment*Returning partner	-0.017	0.038	0.209	1	0.647
Noise treatment*Duet type	0.015	0.030	0.265	1	0.606
Complete data set					
Maximum peak height: N=18 pairs [R²c=0.33]					
(Intercept)	0.159	0.025	—	—	—
Noise treatment (Noise)	0.059	0.035	5.754	1	0.016
Returning partner (M)	-0.004	0.026	0.000	1	0.997
Duet type (Relief)	-0.018	0.019	0.076	1	0.782
Noise treatment*Returning partner	0.011	0.042	0.071	1	0.789
Noise treatment*Duet type	0.043	0.032	1.814	1	0.177
F → M peak height (sqrt): N=18 pairs [R²c=0.24]					
(Intercept)	0.146	0.026	—	—	—
Noise treatment (Noise)	0.018	0.039	4.862	1	0.027
Returning partner (M)	-0.018	0.029	0.016	1	0.897
Duet type (Relief)	0.011	0.028	0.000	1	0.988
Noise treatment*Returning partner	0.086	0.064	1.351	1	0.244
Noise treatment*Duet type	-0.014	0.048	0.033	1	0.853
M → F peak height: N=18 pairs [R²c=0.30]					
(Intercept)	0.115	0.026	—	—	—
Noise treatment (Noise)	0.031	0.036	1.748	1	0.186
Returning partner (M)	0.024	0.028	0.293	1	0.588
Duet type (Relief)	0.007	0.020	0.307	1	0.579
Noise treatment*Returning partner	0.031	0.045	0.481	1	0.488
Noise treatment*Duet type	0.053	0.034	2.42	1	0.119

Three parameters describing the cross-correlation between female and male signals were tested: the maximum peak height, the female to male peak height (F → M) and the male to female peak height (M → F). Linear mixed-effect models ('lmer' function from 'lme4' R package) tested the effect of the noise treatment (Control versus Noise), the sex of the partner returning to the nest (Female versus Male) and the Duet type (Relief versus Visit), with pair identity as random factor. *P* values were assessed using the Anova function 'car' R package. When the interaction between the duet type and the noise treatment was significant, post hoc tests were performed within duet type categories. The number of pairs used in the analysis is specified for each model (see Cross-correlation section in Methods), as well as the R^2c value, which represents the conditional coefficient of determination of the model ('r.squaredGLMM' function of 'MuMIn' R package). Estimates were extracted from the summary of the model.

Table A5

Statistical results on PCs from a PCA describing the temporal structure of sentinel duets

	Estimate	SE	χ^2	df	P
PC1 ($R^2c=0.19$)					
(Intercept)	-0.509	0.509	—	—	—
Noise treatment (Noise)	1.247	0.716	0.936	1	0.333
Returning partner (M)	0.602	0.592	0.000	1	0.997
Noise treatment*Returning partner	-1.309	0.903	2.100	1	0.147
PC2 ($R^2c=0.17$)					
(Intercept)	0.120	0.303	—	—	—
Noise treatment (Noise)	-1.012	0.456	7.259	1	0.007
Returning partner (M)	0.247	0.365	2.406	1	0.121
Noise treatment*Returning partner	0.447	0.575	0.605	1	0.437

Linear mixed-effect models ('lmer' function from 'lme4' R package) tested the effect of the noise treatment (Control versus Noise) and the sex of the returning partner (Female versus Male), with pair identity as random factor. *P* values were assessed using the Anova function ('car' R package). For each model, some pairs were found to be overly influential and changed the results ('influence' function of 'Influence.ME' R package), so results are presented after removal of these pairs. For PC1, the results are on 52 sequences (31 in control and 21 in noise) from 13 pairs; for PC2, the results are on 67 sequences (42 in control and 25 in noise) from 15 pairs. For each model, the R^2c value is the conditional coefficient of determination of the model (calculated with 'r.squaredGLMM' function of 'MuMIn' R package).

Table A6

Statistical results on the number of visit and sentinel duets

	Estimate	SE	χ^2	df	P
Number of visit duets					
(Intercept)	-1.388	0.579	—	—	—
Noise treatment (Noise)	0.821	0.679	6.944	1.000	0.008
Returning partner (M)	0.682	0.684	4.987	1.000	0.026
Noise treatment*Returning partner	0.163	0.800	0.041	1.000	0.839
Number of sentinel duets					
(Intercept)	-0.716	0.441	—	—	—
Noise treatment (Noise)	0.445	0.472	0.845	1.000	0.358
Returning partner (M)	0.788	0.449	0.269	1.000	0.604
Noise treatment*Returning partner	-1.121	0.591	3.594	1.000	0.058
Number of sentinel duets post hoc test (M returning)					
(Intercept)	-0.051	0.383	—	—	—
Noise treatment (Noise)	-0.658	0.364	3.267	1.000	0.071
Number of sentinel duets post hoc test (F returning)					
(Intercept)	-0.805	0.500	—	—	—
Noise treatment (Noise)	0.380	0.500	0.573	1.000	0.500

Linear mixed-effect models for Poisson distribution tested the effect of the noise treatment (Control versus Noise) and the sex of the returning partner (Female, F, or Male, M) (glmer function, 'lme4' R package). When fixed factors presented a significant interaction, post hoc tests were run on a subset of data for each sex (Female or Male returning). *P* values were assessed using the Anova function ('car' R package). Estimates were extracted from the summary of the model.

Table A7

Statistical results on the location of the returning partner during sentinel duets

	Estimate	SE	χ^2	df	P
Number of sentinel duets performed while the returning partner was 0–1 m from the nest					
(Intercept)	-2.632	0.935	—	—	—
Noise treatment (Noise)	1.841	0.844	2.364	1.000	0.124
Returning partner (Male)	0.215	0.924	2.606	1.000	0.106
Noise treatment*Returning partner	-1.821	1.177	2.392	1.000	0.122
Number of sentinel duets performed while the returning partner was 1–2 m from the nest					
(Intercept)	-1.270	0.643	—	—	—
Noise treatment (Noise)	-1.551	0.869	9.023	1.000	0.003
Returning partner (Male)	0.355	0.594	0.675	1.000	0.411
Noise treatment*Returning partner	0.177	1.041	0.029	1.000	0.865

Linear mixed-effect models for Poisson distribution tested the effect of the treatment (Control versus Noise) and the sex of the returning partner (Female or Male) (glmer function, 'lme4' R package). When fixed factors presented a significant interaction, post hoc tests were run on a subset of data for each sex (Female or Male incubating). *P* values were assessed using the Anova function ('car' R package). Estimates were extracted from the summary of the model.

Table A8Statistical results on calls' acoustic features: ANOVA of PC1 [$R^2c = 0.41$]

	χ^2	df	Pr(> χ^2)
Noise treatment	144.045	1	<0.001
Sex of the caller	1.037	1	0.309
Call type	1.411	2	0.494
Duet type	0.293	1	0.588
Noise treatment*Sex of the caller	28.205	1	<0.001
Noise treatment*Context of call production	5.339	1	0.021
Noise treatment*Call type	18.470	2	<0.001

For details see Table A14.

Table A9

Mean PC1 value per category from post hoc test: pairwise noise treatment: call type)

Call type	Noise treatment	lsmean	SE	df
Ark	Control	1.318	0.227	53.969
Short calls	Control	1.152	0.230	56.195
Whine	Control	1.745	0.230	57.838
Ark	Noise	-0.186	0.236	63.607
Short calls	Noise	0.156	0.232	58.617
Whine	Noise	-0.418	0.240	66.689

For details see Table A14.

Table A10

Contrasts on PC1 from post hoc test: pairwise noise treatment: call type)

Contrast	Estimate	SE	df	<i>t</i> ratio	P
Ark,C–Short calls,C	0.166	0.200	697.9	0.830	0.962
Ark,C–Whine,C	-0.427	0.182	703.9	-2.347	0.177
Short calls,C–Whine,C	-0.593	0.197	703.0	-3.017	0.032
Ark,C–Ark,N	1.504	0.217	694.3	6.941	<0.001
Ark,C–Short calls,N	1.162	0.216	703.9	5.392	<0.001
Ark,C–Whine,N	1.736	0.220	698.8	7.886	<0.001
Short calls,C–Ark,N	1.338	0.221	696.6	6.065	<0.001
Short calls,C–Short calls,N	0.997	0.212	700.7	4.711	<0.001
Short calls,C–Whine,N	1.570	0.223	700.8	7.050	<0.001
Whine,C–Ark,N	1.931	0.223	700.3	8.653	<0.001
Whine,C–Short calls,N	1.590	0.227	707.0	7.009	<0.001
Whine,C–Whine,N	2.163	0.224	700.9	9.638	<0.001
Ark,N–Short calls,N	-0.341	0.188	702.6	-1.817	0.455
Ark,N–Whine,N	0.232	0.189	696.9	1.226	0.824
Short calls,N–Whine,N	0.573	0.205	705.8	2.799	0.059

For details see Table A14.

Table A11

Mean PC1 value per category from post hoc test: pairwise noise treatment: sex of the caller)

Sex of the caller	Noise treatment	lsmean	SE	df
Female	Control	1.524	0.291	29.284
Male	Control	1.286	0.260	31.502
Female	Noise	-0.648	0.297	31.866
Male	Noise	0.349	0.266	34.343

For details see Table A14.

Table A12

Contrasts on PC1 from post hoc test: pairwise noise treatment: sex of the caller)

Contrast	Estimate	SE	df	<i>t</i>	P
Female,C–Male,C	0.238	0.380	27.3	0.627	0.922
Female,C–Female,N	2.172	0.195	704.0	11.149	<0.001
Female,C–Male,N	1.175	0.394	31.3	2.983	0.027
Male,C–Female,N	1.934	0.393	31.4	4.924	<0.001
Male,C–Male,N	0.937	0.190	701.9	4.920	<0.001
Female,N–Male,N	-0.997	0.381	27.6	-2.616	0.064

For details see Table A14.

Table A13

Mean PC1 value per category from post hoc test: pairwise noise treatment: duet type)

Context of call production	Noise treatment	lsmean	SE	df
Meeting duet	Control	1.203	0.191	27.701
Sentinel duet	Control	1.607	0.257	86.227
Meeting duet	Noise	0.026	0.193	28.576
Sentinel duet	Noise	-0.325	0.283	120.308

For details see Table A14.

Table A14

Contrasts on PC1 from post hoc test: pairwise noise treatment: duet type)

Contrast	Estimate	SE	df	t	P
M,C–S,C	-0.404	0.211	697.7	-1.912	0.224
M,C–M,N	1.177	0.123	706.9	9.579	<0.001
M,C–S,N	1.528	0.240	705.6	6.362	<0.001
S,C–M,N	1.581	0.206	696.5	7.691	<0.001
S,C–S,N	1.932	0.293	701.4	6.604	<0.001
M,N–S,N	0.351	0.249	706.9	1.412	0.492

Linear mixed-effect models ('lmer' function from 'lme4' R package) were performed on PC1 to test the effect of the treatment (Control, C, versus Noise, N), the sex of the caller (Female versus Male), the call type (Arks, Short calls or Whines) and the duet type ('meeting duets', M, versus 'sentinel duets', S) with subject identity as random factor. *P* values were computed using the 'Anova' function of 'car' R package. Interactions between call type, sex of the caller or duet type with the noise treatment were also tested in the model. Table depicts the results of the Anova. When interactions were significant, post hoc tests were performed using 'lsmeans' function of the 'lsmeans' R package, with Tukey adjustment for multiple comparisons. Table depicts estimates (table of mean PC1 value) of each factor and results from the comparison of interacting factors (table of contrasts). Results are presented after removal of influential levels of the random factor ('influence' function, 'Influence.ME' R package). Final data set composition: 25 individuals (11 females and 14 males with on average 15 (±3) calls per individual per treatment). The *R*² value represents the conditional coefficient of determination of the model (calculated with 'r.squaredGLMM' function of 'MuMIn' R package).

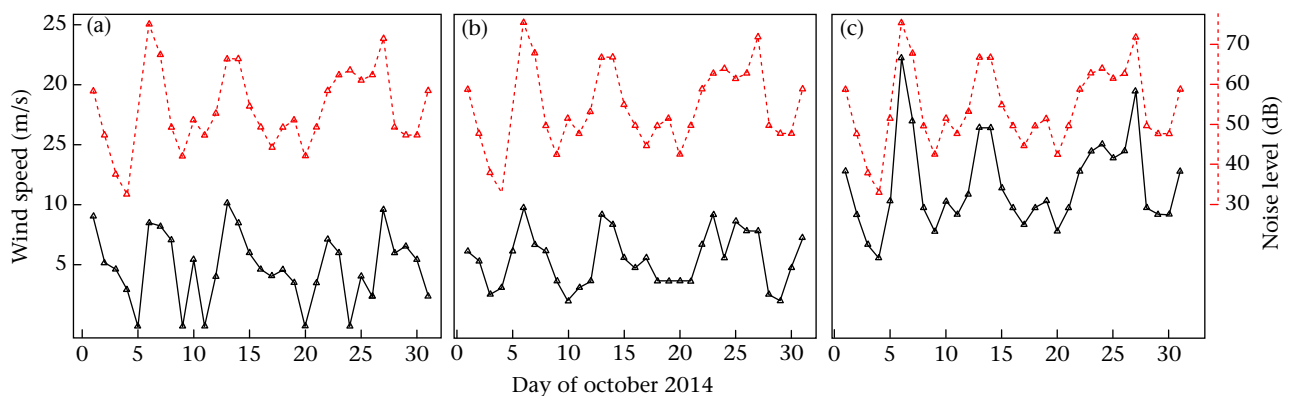


Figure A1. Additional information on natural wind noise in the zebra finch's habitat. To check the relevance of our experimental background noise level we used records of wind speed over a representative month of a breeding season at the field station of Fowlers Gap (www.bom.gov.au/climate/dwo/201410/html/IDCJDW2155.201410.shtml), at (a) 0900 and (b) 1500 hours and for the (c) maximum wind gust. We then referred to a paper reporting the correspondence between wind speed and noise levels in an open field (Lightstone, Du, & Matthew, 2010), which is comparable to the zebra finch's breeding environment with very scarce vegetation. We used the following model the authors fitted: $y = 0.0028x^3 - 0.2225x^2 + 6.9199x + 0.875$, $R^2 = 0.99$. The results show a high variability in wind speed between days (bold black line, when the term 'Calm' was used to describe the wind speed, the value was set at zero) and inferred noise levels ranging from 35 to 75 dB (dotted red line). Our treatment, 63 dB SPL, is thus representative of noise levels frequently happening during the breeding season.

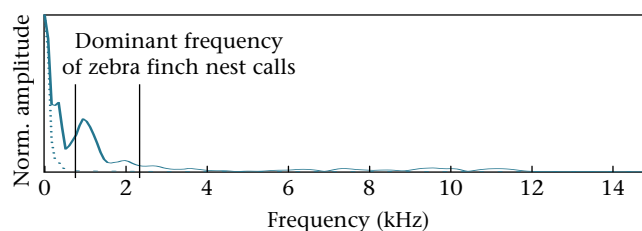


Figure A2. Spectral comparison of background noise between the control (dashed line) and noise (solid line) treatments. Spectra were calculated from a sequence of 15 s of background noise (Supplementary Material sound 0) recorded with the nest microphone using 'specprop' function of 'Seewave' R package (FFT using a Hamming window, window length = 512, overlap = 50%). The range of frequencies of zebra finch calls used in call duets is also shown. The difference in sound pressure level between control and noise treatments was about 5 dB. As a reference, an increase of 6 dB refers to doubling the acoustic pressure level or decreasing by half the distance from the source.

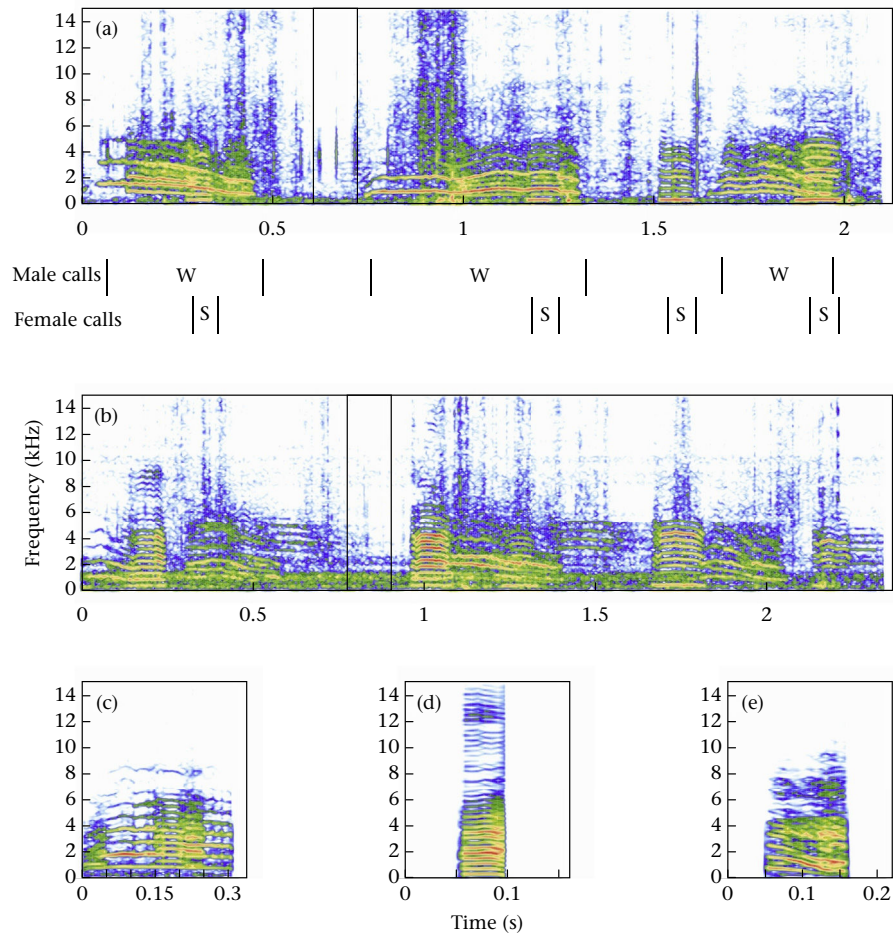


Figure A3. (a, b) Spectrograms of duets recorded in (a) the control (Supplementary Material sound 1) and (b) noise treatments (Supplementary Material sound 2), using the same graphic parameters. The call annotation method is shown below the spectrogram in (a). Calls produced by the female and the male are annotated separately, as well as two of the three call types (W: whine calls; S: short calls; only W and S calls were produced during the chosen extract). (c, d, e) Spectrograms of the three call types considered in the study: (c) whine call (Supplementary Material sound 3), (d) short call (Supplementary Material sound 2) and (e) ark call (Supplementary Material sound 5). The two black rectangles show the relative background noise in the control and noise treatments when no calls are produced.

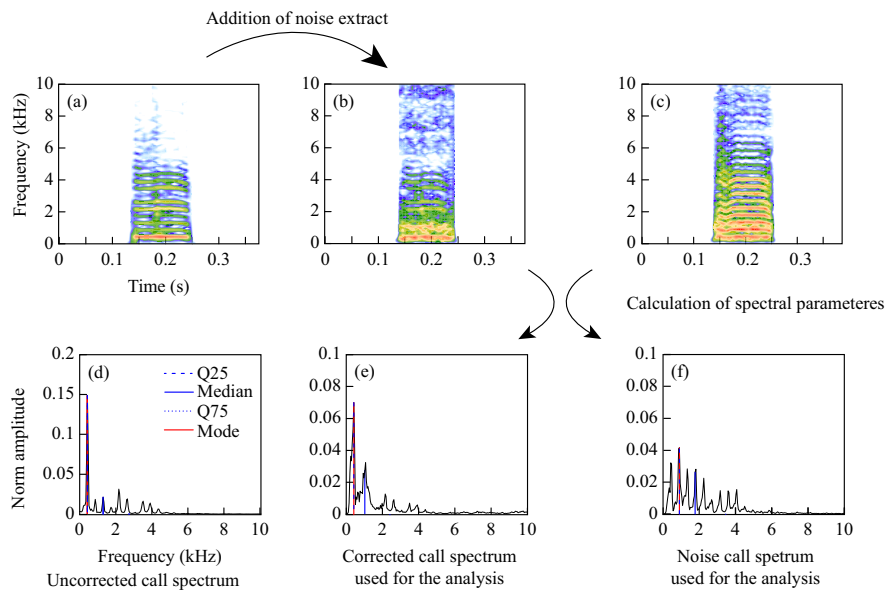


Figure A4. Method of comparison of call structure between calls recorded in noise and control treatments. To compare the structure of calls used by birds in these treatments, we needed to control for the increase in amplitude and the additional spectral components added by the experimental playback of wind noise on recordings. The wave files of calls recorded during the control treatment were corrected by adding a sample of identical duration extracted from experimental recordings of the noise playback sequence. Because the playback of noise was built from natural wind noise recordings, it was temporally variable. To deal with this variability, the correction of each call recorded in the control treatment was done 10 times, with 10 different randomly chosen samples of noise. The final measurements are means of these 10 repetitions. Calls recorded during the noise treatment were analysed without any modification. The figure illustrates this correction. Spectrograms show (a) an uncorrected control call, (b) a corrected control call and (c) a noise call and (d, e, f) their respective spectra.

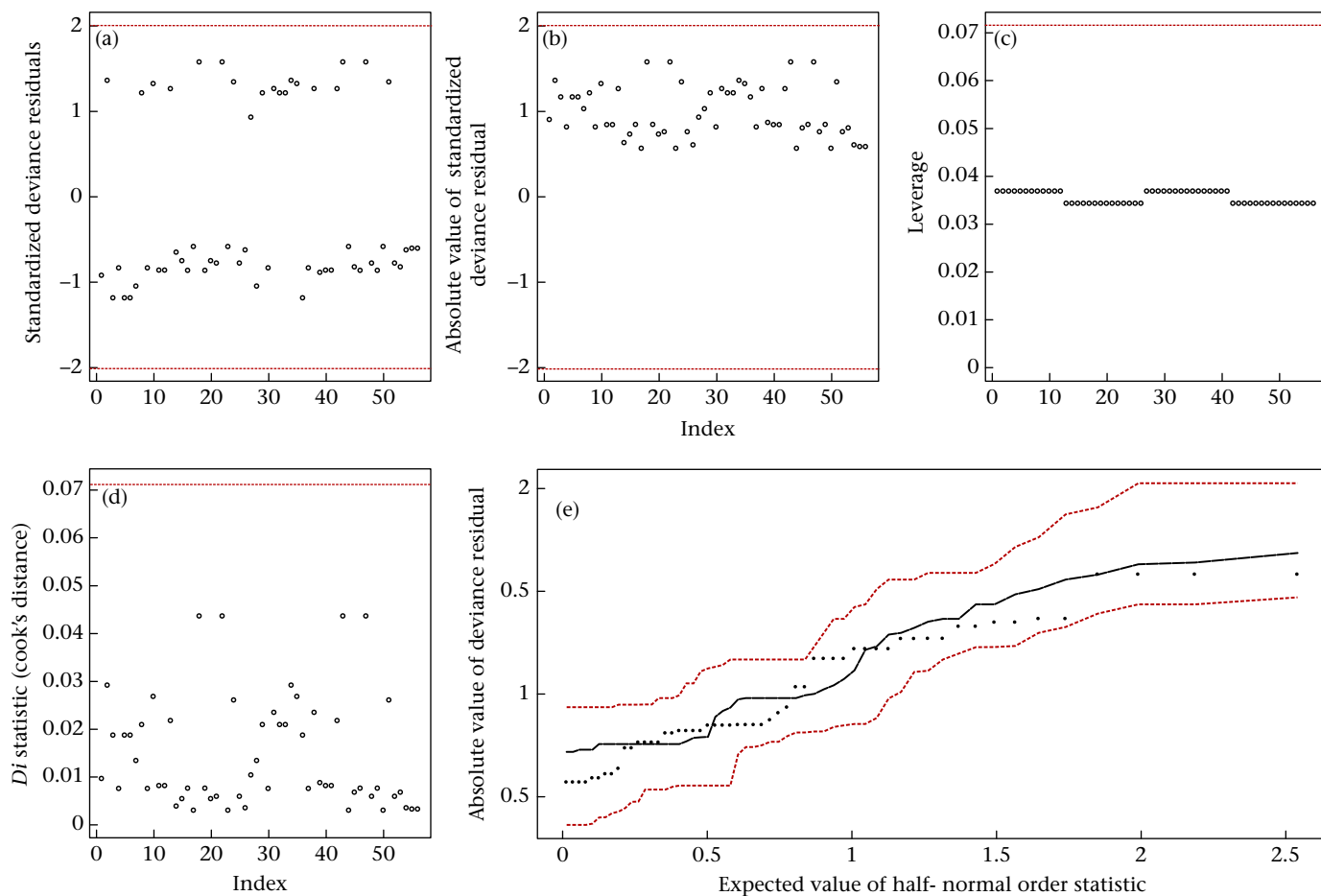


Figure A5. Validation of binomial generalized linear model. Before being interpreted each model was checked, paying particular attention to its residuals. For binomial models, we used five relevant plots from custom-written codes (Atkinson, 1981; Collett, 1991) to test the validity. First, with the graph of standardized deviance residuals (a) we checked the residuals mean homogeneity, and with the graph of absolute value of standardized deviance residuals (b) we checked the residuals variance homogeneity. For both plots we only checked if the residuals were between -2 and 2 : because of the binary nature of the data (and contrary to classical linear models), nonhomogeneously distributed residuals do not necessarily reflect an inappropriate model. The model hat matrix was then extracted and its diagonal coefficients (h_i) enabled us to check the general influence of observations on the model fit to data (c). The threshold for h_i values is $2 \times \text{mean}(h_i)$. The Cook's distance gave (d) us information about the influence of each observation on the parameter estimation, and had to be lower than $4/n$ with n the number of observations. Finally, we build the half-normal plot (e) (Atkinson, 1981), i.e. standardized deviance residuals as a function of the half-normal distribution quantiles with simulated envelope (red dotted line). If data points were included in the envelope, the linear predictor was correct.

- Chapter 5 -

Parental influence on Begging Call
Structure in Zebra Finches (*Taeniopygia
guttata*): Evidence of Early Vocal Plasticity.

Villain A.S, Boucaud I.C.A, Bouchut C., Vignal C.

Key words: Songbird, Nestling, Parent–offspring communication, Vocal production learning, Cross-fostering, Sex differences

Overview

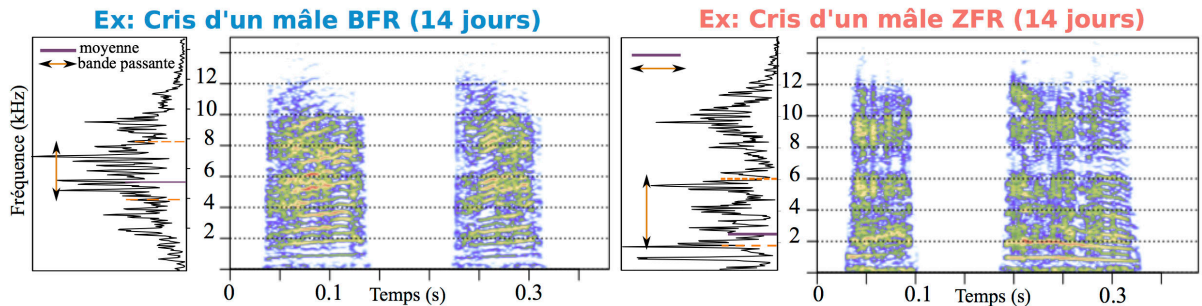
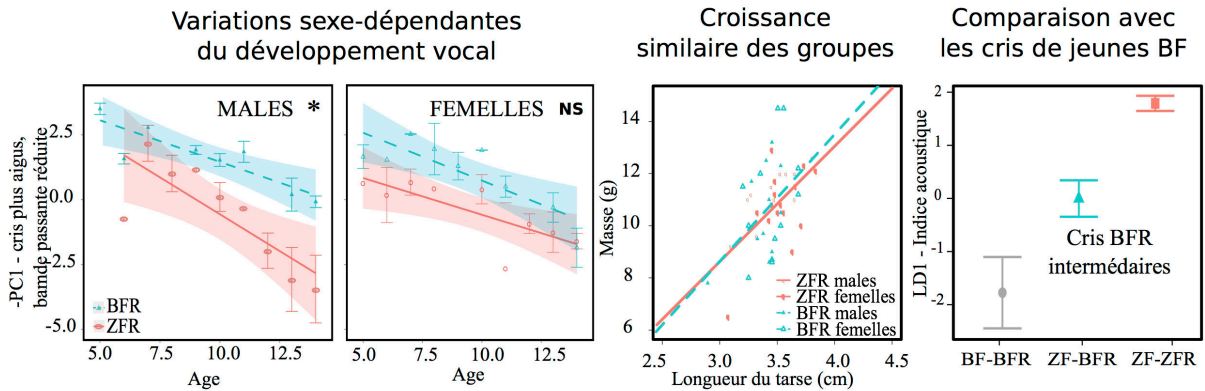
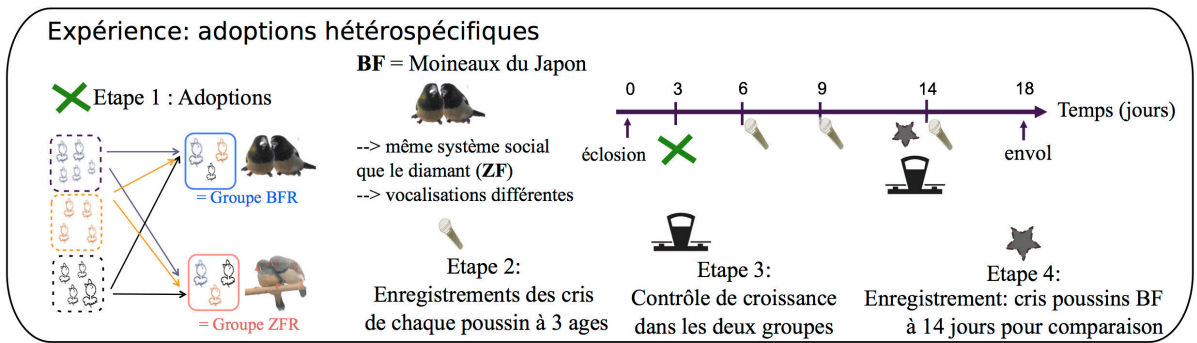
Influence parentale sur le développement des cris de quémante alimentaire:
Plasticité vocale précoce chez le diamant mandarin.



Diamant mandarin, *Taeniopygia guttata*

- * Mâles apprennent leur **chant** --> plasticité et apprentissage
- * Femelles ne chantent pas --> plasticité ?
- * Femelles et mâles émettent des **cris**
- * Les cris --> plasticité ?
- * Les cris de quémante: produits **avant** apprentissage par femelles et mâles

Le développement des cris de quémante alimentaire est-il influencé par l'environnement parental ?
Existe-t-il des degrés de plasticité différents chez les jeunes femelles et mâles ?



Conclusion:
Cris plus aigus et bande de fréquence moins large à la fin du développement au nid chez les **mâles BFR**
Cris intermédiaires avec ceux des jeunes BF, non expliqué pas des variations de croissance
Pas d'effet sur les cris femelles



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Parental influence on begging call structure in zebra finches (*Taeniopygia guttata*): evidence of early vocal plasticity

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Begging calls are signals of need used by young birds to elicit care from adults. Different theoretical frameworks have been proposed to understand this parent–offspring communication. But relationships between parental response and begging intensity, or between begging characteristics and proxies of a young’s need remain puzzling. Few studies have considered the adjustment of nestling begging features to previous experience as a possible explanation of these discrepancies. In this study, we tested the effect of a heterospecific rearing environment on individual developmental trajectories of the acoustic structure of nestling begging calls. Fifty-two zebra finch chicks were fostered either to Bengalese finch or to zebra finch parents, and begging calls were recorded at several stages of nestling development. Acoustic analyses revealed that the development of the spectral features of the begging calls differed between experimental conditions: chicks reared by Bengalese finches produced higher pitched and less broadband begging calls than chicks reared by conspecific parents. Differences were stronger in males than females and were not explained by differences in growth rate. We conclude that nestling begging calls can be plastic in response to social interactions with parents.

1. Introduction

Young mammals and birds solicit care from parents using complex begging behaviours [1]. Begging signals are conspicuous and intense performances that involve visual and acoustic cues [2]. Several theoretical frameworks have been used to understand the design of begging signals. Parent–offspring conflict theory suggests that begging behaviour is the result of an evolutionary conflict of interests over resource allocation [3,4]. From this

perspective, begging displays are signals by which offspring manipulate parents to provide more care than would be optimal [1] or through which siblings compete for a larger share of parental care [5]. Alternatively, begging behaviours are considered by honest signalling models to be costly [6] and as such, to be reliable signals of needs that allow parents to make decisions on the appropriate amount of care needed by their offspring [7,8]. Accordingly, the intensity of begging by bird nestlings increases with their level of hunger and can stimulate parental feeding [9–13]. However, inconsistencies in the impact of begging intensity on parental response have been puzzling [14,15], and relationships between begging characteristics and several proxies of nestlings' needs seem complex [12,16–18].

Some of the discrepancies between models and measurements of begging's correlates might be because of the dynamic of parent–offspring communication. Environmental conditions and learning processes might change the value of a signal on the receiver side and might shape the characteristics of a signal on the emitter side. Parents, on the one hand, may learn to ignore or pay particular attention to some aspects of their offspring's begging. Parents show varied levels of sensitivity to offsprings' signals, both between and within species [7,19]. Nestlings, on the other hand, may learn from their previous experience which aspects of their begging display were more rewarding and change their begging behaviour accordingly [16,18,20,21]. Nestlings show a preference for particular positions in the nest previously associated with food provisioning [22,23] and are able to learn to beg at the most rewarding begging intensity [16,24]. This behavioural adjustment in response to experience could allow nestlings to cope with changing conditions, such as variations in parental provisioning or sibling competition and would modify offspring signalling of needs to the parents. Getting a better estimation of the extent of learning in begging is thus important to further our understanding of the evolution of parent–offspring communication.

Surprisingly, learning in nestling begging calls has rarely been investigated. Begging calls are known to carry multiple pieces of information such as individual signature [25] or information about sex [26], hunger level [20,21] or thermal state of the individual [13]. A change in the acoustic structure of begging calls in response to experience has been examined in the particular context of host–parasite interactions. In brood parasitic bird species like the Horsfield's bronze-cuckoo (*Chalcites basalis*) [27], the common cuckoo (*Cuculus canorus*) [28] or the African indigobirds (*Vidua* species) [29], chicks mimic the begging call structure of their host, and this structural change seems to be shaped by the behavioural response of host parents. In this recognition arms race, one host species (the superb fairy wren, *Malurus cyaneus*) has been shown to use a parent-specific password: females call to their eggs at the end of incubation, and upon hatching nestlings produced begging calls with high similarity to their mother's call [30]. Parasitic nestlings of the brown-headed cowbird (*Molothrus ater*) exaggerate the host's display and thus increase food provisioning by host parents. In response to nest parasites, the host nestlings of song sparrow (*Melospiza melodia*) change their begging call to match the parasite [31]. Apart from these examples in host–parasite systems, one study in tree swallow nestlings (*Tachycineta bicolor*) suggested that brood signature in begging calls just before fledging results from interactions between environment and genetic/maternal effects [32]. However, to our knowledge, no study has tracked individual ontogeny of begging call structure to demonstrate vocal adjustment in response to social experience.

In this study, we tested the possibility of learning of the begging calls of male and female nestling zebra finches (*Taeniopygia guttata*). To do so, nestlings were all cross-fostered to control for genetic effects, and raised either by zebra finch parents or heterospecific parents, Bengalese finches (*Lonchura striata* var. *domestica*), which are classically used as social parents in cross-fostering experiments [33]. We recorded begging calls at several stages of nestling development. As chicks get older and heavier, begging calls are expected to shift to lower values of the frequency spectrum [34,35] as well as to increase their spectral bandwidth and duration [35]. If chicks adjust their begging call structure in response to the cross-fostering condition, we expect deviations from this normal developmental trend and differences between cross-fostering groups at a given age. On the one hand, parental feeding could act as a conditioning process [24,36] and chicks could hone in on the acoustic cues that get the greatest response from the parents [28,37]. In this case, we expect zebra finch chicks reared by Bengalese finch parents to call at higher frequencies and with reduced spectral bandwidth, which are acoustic cues of Bengalese finch begging calls and vocal repertoire [38]. On the other hand, the cross-fostering treatment could represent a developmental stress. If chicks reared by Bengalese finch parents are less fed and thus hungrier, begging calls are expected to be longer, produced at a higher call rate [13,34,39] and with more spectral noise [40]. Hungrier chicks may also be more stressed, and from motivation-structural rules [41] and work on adult zebra finches [42], their calls are predicted to be at higher frequencies. If begging calls differ between cross-fostering conditions because of differences in levels of parental care, this should result in growth differences between experimental groups that can be quantified by measuring weight and tarsus length

of the young at the end of the nestling period. As several studies suggested that female zebra finches show higher vulnerability to conditions of restricted food [43–45] this hypothesis suggests that females should be more affected than males by the cross-fostering treatment.

2. Material and methods

2.1. Subjects and housing conditions

Fifty-two chicks of zebra finches (*Taeniopygia guttata*) were used in this study. Zebra finch chicks were produced by 20 parental pairs and were all fostered to male–female pairs of either zebra finches ($N = 12$ pairs) or Bengalese finches (*Lonchura striata* var. *domestica*; $N = 8$ pairs).

All adult zebra finches came from our breeding colony (ENES Laboratory, University of Saint-Etienne), whereas adult Bengalese finches were purchased from commercial providers, seven weeks before the beginning of the experiments. All pairs were freely formed in aviaries, were between 1 and 2 years old and had previous breeding experience. Pairs were housed separately in breeding cages ($80 \times 40 \times 40$ cm) equipped with perches, a nest-box and a pool for environmental enrichment. All the birds were kept under the same environmental conditions (temperature between 24°C and 26°C , light conditions 14L:10Dh). Birds were fed finch seed cocktails, egg paste, water and cuttlefish bones *ad libitum* and supplemented with salad once a week.

2.2. Cross-fostering procedure and experimental groups

Chicks were cross-fostered at 2 or 3 days old by transferring them from their parental nest to the nest of their foster parents. Foster parents were either zebra finches (chicks reared by ZF = ZFR) or Bengalese finches (chicks reared by BF = BFR). No chick was reared by its genetic parents and genetic siblings were split between different foster families: cross-fostered social broods thus included chicks from different genetic parents. Ages of the chicks in an experimental brood differed from 0 to 4 days, as can be observed in the wild [46]. To avoid an effect of brood size on development [47], experimental brood size was fixed at three chicks per nest, but depending on chicks hatching date and synchrony between nests of origin, it was sometimes not possible to cross-foster three chicks in all foster nests, so some pairs reared only two chicks (see brood composition, electronic supplementary material, table S2). Experimental groups were similar in brood size (mean \pm s.d.: 2.82 ± 0.6 and 3.13 ± 0.35 for ZFR (16 broods) and BFR (eight broods) groups, respectively, Wilcoxon rank sum test, $W = 32$, $p = 0.2189$) and in chicks' mortality (21.8% in BFR, 20.33% in ZFR, $\chi^2_1 < 0.001$, $p = 0.92$).

The two experimental groups (zebra finch parents or Bengalese finch parents) were housed in separate and acoustically isolated rooms. So the chicks' auditory experience was restricted to their experimental condition.

Chicks' sex was determined by molecular sexing (Genindexe, <http://www.genindexe.com/>) using feather samples. The intra-brood sex-ratio was calculated as relative proportion of females and males in the brood (electronic supplementary material, table S2). Sex-ratio did not differ between the two groups (mean \pm s.d.: 0.59 ± 0.19 and 0.51 ± 0.19 for ZFR (16 broods) and BFR (eight broods) groups, respectively, Wilcoxon rank sum test, $W = 51.5$, $p = 0.5406$).

The overall sex composition of the groups was as follows:

- (1) BFR: 12 females and 10 males.
- (2) ZFR: 15 females and 13 males.

2.3. Analysis of begging call development

2.3.1. Data collection

All chicks were recorded at three different developmental stages (6 ± 1 , 9 ± 1 , 14 ± 1 days post-hatching (DPH)) after removing the chick from the nest temporarily.

Because begging call structure strongly depends on the level of hunger of the chick [40], we controlled for chicks' hunger level at the time of recording by recording them at their maximum level of motivation. To do so, we submitted the chicks to a short food deprivation during which they remained in the nest in the breeding cage, but the access to the nest was blocked for the parents. We increased the time

of deprivation (60 min at 6 DPH, 90 min at 9 DPH, 120 min at 14 DPH), mimicking the natural inter-visit interval of the parents [46]. We also checked that only a few seeds remained in the chick's crop (visible through the skin) before the recording. To induce begging calls from the nestlings, we used beak stimulations with a small red stick that mimicked an adult beak. Until 9 DPH, the chick's eyes are closed, so begging calls were predominantly triggered by the tactile stimulation. After this age, stick stimulation was still equally efficient in both experimental groups. After 9 DPH, 4297 calls were recorded in BFR and 4352 in ZFR.

Each chick was placed in an experimental nest furnished with cotton located in a room adjacent to the breeding colony and recorded using an Audio Technica 803 tie-microphone placed at 10 cm from the chick and connected to a Marantz PMD 671 recorder.

2.3.2. Acoustic analysis

Depending on individuals, 10–50 begging bouts (each bout being a chain of several repeated calls, on average 4.43 ± 4.24 per individual) were extracted at 6, 9 and 14 DPH. A total of 10 621 calls (5364 in BFR, 5653 in ZFR) were extracted and analysed.

Acoustic analysis was performed using custom-written codes using the Seewave package [48] implemented in R [49]. After bandpass filtering (250–15 000 Hz, Seewave 'fir' function) and intensity normalization, the call duration was measured via the Seewave 'timer' function and the following spectral parameters were computed using the Seewave 'specprop' function (FFT using a Hamming window and a window length of 512):

- The mean, the median, the first (=Q25) and third (=Q75) quartiles, the inter-quartile range (=IQR), the standard deviation (=s.d.) and the mode of the call frequency spectrum (all in Hertz).
- The spectral flatness (=Sfm) of the frequency spectrum—a measure of the signal's noisiness. Sfm of a noisy signal tends towards 1, whereas Sfm of a pure tone tends towards 0.
- The skewness—a measure of the distribution symmetry of the frequency spectrum of the call. Skewness tends to 0 when the spectrum is symmetric, is positive when the spectrum is skewed to right and negative when the spectrum is skewed to left.

2.3.3. Statistical analysis

All statistical analyses were performed using R software [50]. Because the dataset contained missing values, statistics were computed on 9423 calls (4273 in ZFR and 5150 in BFR), from 51 chicks. Electronic supplementary material, table S3 gives the number of calls per session for each subject of each group.

We first performed a principal component analysis (PCA) on acoustic parameters of the calls (1), and then built a linear mixed effects model to test the effect of the experimental treatment on the principal components (PCs) resulting from the PCA (2). The effect of the experimental treatment on each parameter separately is presented in the electronic supplementary material. Finally, we tested the effect of the experimental treatment on chicks' body condition using a linear mixed model (3). All models were validated by checking residuals' equivariance and symmetrical distribution. Model stability was checked using a custom-written function, written by Roger Mundry.

PCA on acoustic parameters. PCA is commonly used in behavioural analysis to reduce the number of variables by eliminating redundancy caused by intercorrelation and emphasizes mutual dependencies between them. It is particularly useful because subjects can display different combinations of behaviours to express the same functional response [51]. Before the PCA, parameters with non-symmetrical distributions were transformed. The PCA was performed on 10 acoustic parameters ('dudi.pca' function of 'ade4' R package): mean, s.d., median, mode, Q25, Q75, skewness, Sfm, call duration (for variable composition, see table 1). The two first PCs of the PCA, which had eigenvalues above one, were kept.

Linear mixed effect models on principal components. Each PC was analysed using a linear mixed effects model ('lmer' function from 'lme4' R package [52]). We built a model including fixed and random factors having a potential effect on call structure considering the design of the experiment. To increase the interpretability of the results, all continuous variables included in the model were z-transformed [53] (indicated with a 'z' before the factor's name in the model formula).

Table 1. Variable compositions of the PCA on acoustic parameters. Transformations are indicated in parentheses. Percentage of each parameter composing the PC,^a percentage of explained variance and eigenvalues of each PC are indicated.

	PC1	PC2
explained variance (% cumulative)	50.7	79.6
eigenvalue	5.1	2.9
mean	-18.65	-0.03
s.d.	-0.08	31.37
median	-17.9	-1.01
mode	-9.48	-8.29
Q25	-14.1	-7.05
Q75	-16.41	3.83
IQR	-1.88	28.79
Sfm	-6.89	16.66
skewness (square root)	10.43	0
call duration (log)	-4.17	-2.97

^a Absolute contributions of the decomposition of inertia for each PC ('inertia.dudi' function from 'ade4' R package), divided by 100 to get the percentage. Signs are the signs of the coordinate.

The following model was computed:

```
model <- lmer(PC ~ Group *zAge* Sex + zcall index + zsex-ratio + zBCI + (1|Subject) + (0 + zAge|Subject) + (1|Nest of Origin) + (0 + SexF|Nest of Origin) + (0 + GroupFR|Nest of Origin) + (0 + zAge|Nest of Origin) + (0 + SexF: zAge|Nest of Origin) + (1|Foster Nest) + (0 + sexF|Foster Nest) + (0 + zAge|Foster Nest) + (0 + SexF: GroupFR|Foster Nest) + (0 + SexF: zAge|Foster Nest) + (1|sessionID).
```

'Group', 'Sex' and 'Age' and their interactions were used as fixed factors to test (i) the effect of the treatment throughout chicks' development and (ii) potential differential effects in male and female chicks. Because 'Age' was analysed as a continuous covariate, the effect of the cross-fostering treatment was not analysed for each age point separately but on changes of call acoustic structure over the development. During the recording session, the motivation to beg of the chicks could change due to the absence of food. We thus included the call index in the sequence ('call index') as an additional fixed factor. Intra-brood sex-ratio ('sex-ratio') or chicks' body condition index ('BCI', see §2.4) could also have had an effect on call acoustic structure and were added as fixed factors as well. Because our experimental design used repeated recordings of calls per subject and per age, two random factors were added. First, we used a 'sessionID' random factor, which is a unique term of the session of recording for a given chick at a given age. Second, a 'subject' random factor was used to deal with repeated measures on each chick. As recommended for within-subjects design with a covariate [51], 'Age' was also included as a random slope within the subject. To control for genetic and social effects on call structure, the 'Nest of Origin' and the 'Foster Nest' were added as random factors. Again, as recommended for within-subjects design with a covariate [51], experimental group ('GroupFR') and sex ('SexF') were included as random slopes after being manually dummy coded (binary code: 1 if true, 0 if false). Interactions between random slopes were considered only when the sample size in each corresponding subset of data was sufficient.

P-values were assessed using the 'drop1' function ('stats' R package). 'Drop1' computes likelihood ratio test statistics and *P*-values for all single terms, fits those models and computes a table of the changes in fit. Only relevant interactions then compose the statistical table. When the three-way interaction was significant (Group: Sex: zAge), post hoc models were run either in each sex separately or in two categories of age (old nestlings: Age ≥ 12 or young nestlings: Age ≤ 7). Estimates of fixed factors for each model were computed with the 'lsmeans' function ('lmerTest' R package) and are available in the electronic supplementary material.

2.4. Analysis of chicks' body condition

2.4.1. Data collection

To control potential effects of cross-fostering treatment on chicks' body condition, two morphological features of the chicks were measured once at 14 (±1) DPH. Tarsus and weight were measured using a

calliper (± 0.1 mm) and a Pesola scale (± 0.1 g), respectively. All measures were done at the same time of the day (noon), after acoustic recordings.

We calculated the BCI as residuals of the linear regression model between tarsus length and weight at day 14 (± 1).

All of the 52 nestlings were measured, but only 23 of them were recorded at 14 (± 1) DPH. Results of the BCI analyses on the complete dataset (52 nestlings) or on the subset of data restricted to the nestlings recorded at 14 (± 1) DPH (23 nestlings) did not differ, so only the first is presented.

2.4.2. Statistical analysis

To test the effect of the treatment and take into account the fact that male and female zebra finches can have initial differences in growth rate [43], we used the following linear model:

```
modelBCI <- lmer(BCI values ~ Group * Sex + Age at measurement + zsex-ratio + (0 + groupFR|Foster Nest) + (0 + GroupFR|Nest of Origin) + (0 + sexF|Foster Nest) + (0 + sexF|Nest of Origin).
```

'Group', 'Sex' and their interaction were included as fixed factors. The 'Age at measurement' (unique per chick) was added to control the effect of between-individuals variation in age at measurement (14 ± 1). The intra-brood sex-ratio ('sex-ratio') was added to control the effect of brood composition on chick body condition and this continuous parameter was z-transformed. The Nest of Origin and the Foster Nest were used as random factors and dummy coded Sex and Group were used as random slopes.

P-values were assessed using the 'drop1' function. Estimates of fixed factors for each model were computed with the 'lsmeans' function and are available in the electronic supplementary material.

The model was validated by checking residuals' equivariance and symmetrical distribution ('plotresid' function from 'RVAideMemoir' R package). Model stability was checked using a custom-written function, written by Roger Mundry.

3. Results

3.1. Cross-fostering to heterospecific parents differentially affected the development of male and female begging calls

The cross-fostering treatment affected the structure of begging calls produced by nestlings. PC1 (explaining 50.7% of the total variance; table 1) was significantly affected by the triple interaction between group, age and sex of the chicks but not PC2 (explaining 28.9% of the total variance; table 2; electronic supplementary material, table S4a). Post hoc tests were run on PC1: (i) in males and females separately and (ii) in young and old nestlings separately.

The interaction between group and age was significant in males but not in females (figure 1 and table 2; electronic supplementary material, table S4b). The call spectrum shifted to lower frequencies during development (increasing PC1 values). But this developmental shift was influenced by the cross-fostering group in males only: it was slower in BFR chicks than in ZFR chicks. Thus, male chicks fostered to Bengalese finch parents produced calls with more energy in higher frequency bands later in development than control chicks (figure 3; and electronic supplementary material, figure S3).

In young nestlings ($5 \leq \text{Age} \leq 8$ DPH), the cross-fostering treatment had no effect on PC1 whereas in old nestlings ($12 \leq \text{Age} \leq 15$ DPH), we found a significant interaction between cross-fostering group and sex (figure 2 and table 2; and electronic supplementary material, table S4b). So the effect of the cross-fostering treatment observed in males appeared during development and was more pronounced at the end of the nestling period, whereas female calls were not affected by the treatment.

The call index affected PC2 in both sexes and PC1 in males only (table 2). Calls produced later during the recording session had an up-shifted and less broadband frequency spectrum but this result did not seem to interact with the cross-fostering treatment (see the electronic supplementary material, additional figure S2).

3.2. Divergent growth trajectories do not explain begging call structure differences

To determine the effect of the cross-fostering group on begging call structure development, the potential side effects on growth of the cross-fostering to heterospecific parents needed to be controlled for. Bengalese finches could feed their chicks more or less leading to differences in chicks' physiology and condition that could be responsible for the observed changes in acoustic structure of begging calls. There

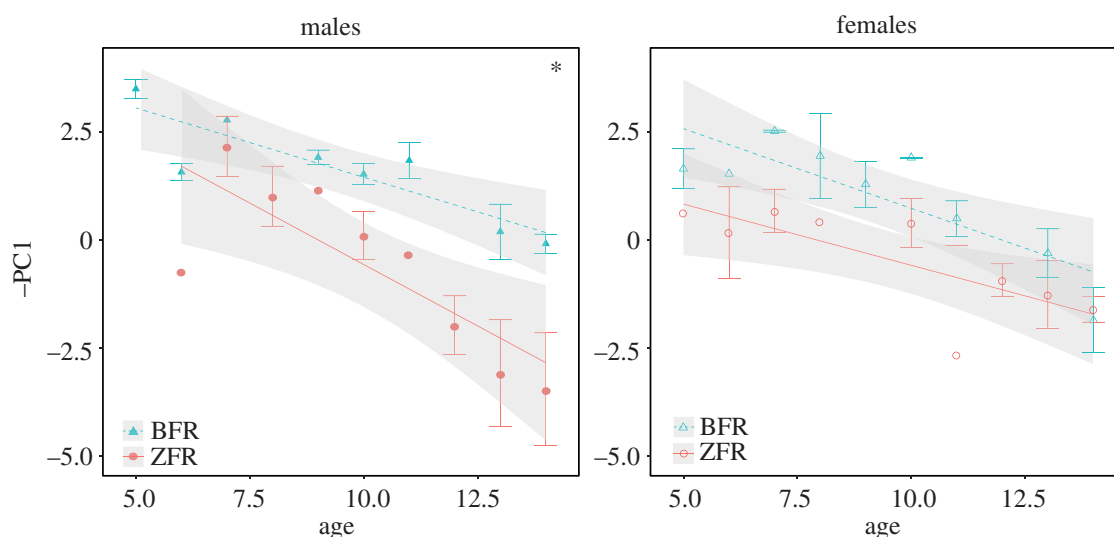


Figure 1. Modifications of begging call acoustic structure over the development of zebra finches reared either by zebra finches (ZFR, $N = 24$, 13 females and 11 males) or by Bengalese finches (BFR, $N = 21$, 12 females and nine males). PCI is the first principal component from a PCA on acoustic parameters. Points are mean (\pm s.e.) when the corresponding subset of data contains several chicks, and mean only (without error bar) when the data point represents only one chick. The dataset for one chick at a given age still represents multiple calls, so the corresponding data point is the mean of all these calls. Grey shades are 95% CIs of the linear regression. Because the three-way interaction was significant (cross-fostering group: sex: age; table 1), post hoc tests were performed for each sex. Significance of the interaction between cross-fostering group and age as a covariate is indicated in insert: $*p \leq 0.05$.

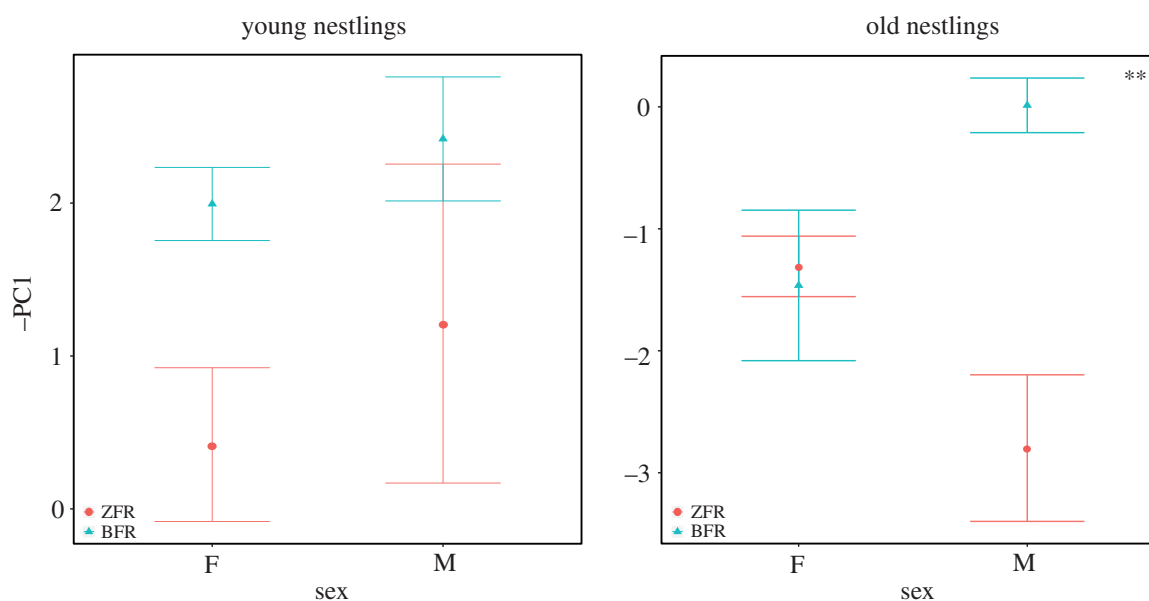


Figure 2. Modifications of begging call acoustic structure of young nestlings ($5 \leq \text{Age} \leq 7$ DPH) and old nestlings ($12 \leq \text{Age} \leq 14$ DPH) fostered either to zebra finch parents ($N = 14$, seven males and seven females) or Bengalese finch parents ($N = 15$, seven males and eight females). Points are means (\pm s.e.) of all chicks. When the cross-fostering group: sex interaction was significant, post hoc tests were performed in each sex separately (table 2). In females, post hoc tests were all non-significant; in males, significance is indicated in insert: $**p \leq 0.01$.

was no significant difference in BCI at 14 DPH between BFR chicks and ZFR chicks (figure 4 and table 3; electronic supplementary material, table S9).

4. Discussion

In this study, we found that an early cross-fostering event to Bengalese finch parents affected the development of acoustic features of begging calls of young zebra finches. As chicks get older and heavier, begging calls are expected to shift to lower values of the frequency spectrum [34,35], as well

Table 2. Statistical results from the 'drop1' function ('lmerTest' R package) computed on the full model (a) and post hoc models following significant interactions (b).

	d.f.	LRT	Pr(Chi)
<i>(a) results from 'drop1' function on all data</i>			
<i>PC1—all data</i>			
call number	1	0.356	0.551
intra-brood sex-ratio	1	0.393	0.531
BCI	1	0.000	0.993
Social Group : Sex : Age	1	10.529	0.001
<i>PC2—all data</i>			
call number	1	65.212	0.000
intra-brood sex-ratio	1	2.810	0.094
BCI	1	0.001	0.972
Social Group : Sex : Age	1	2.866	0.090
<i>(b) results from 'drop1' function on post hoc models on PC1</i>			
<i>(i) analysis of the Social Group: Age interaction</i>			
<i>PC1—post hoc test on males</i>			
call number	1	4.432	0.035
intra-brood sex-ratio	1	0.019	0.889
BCI	1	0.034	0.853
Social Group: Age	1	4.399	0.036
<i>PC1—post hoc test on females</i>			
call number	1	3.383	0.066
intra-brood sex-ratio	1	0.939	0.332
BCI	1	0.096	0.757
Social Group: Age	1	1.609	0.205
<i>(ii) analysis of the Social Group: Sex interaction</i>			
<i>PC1—post hoc test on young nestlings</i>			
call number	1	0.000	0.989
intra-brood sex-ratio	1	0.286	0.593
BCI	1	0.126	0.723
Social Group: Sex	1	0.253	0.615
<i>PC1—post hoc test on old nestlings</i>			
call number	1	0.000	0.995
intra-brood sex-ratio	1	0.139	0.709
BCI	1	0.062	0.804
Social Group: Sex	1	7.056	0.008
<i>PC1—post hoc test on old male nestlings</i>			
Social Group	1	10.545	0.001
call number	1	2.333	0.127
intra-brood sex-ratio	1	0.741	0.389
BCI	1	0.009	0.926

(Continued.)

Table 2. (Continued.)

	d.f.	LRT	Pr(Chi)
<i>PC1—post hoc test on old female nestlings</i>			
Social Group	1	0.028	0.868
call number	1	4.473	0.034
intra-brood sex-ratio	1	0.082	0.775
BCI	1	0.015	0.903

as to increase their spectral bandwidth and duration [35]. If chicks adjust their begging call structure to the cross-fostering condition, we expected deviations from this normal developmental trend. Several features of the frequency spectrum differed between BFR chicks and control ZFR chicks. In males only, the normal developmental shift of the frequency spectrum to lower frequencies happened more slowly in BFR chicks. Changes observed in the begging call acoustic features were greater in old nestlings than in young nestlings, confirming an effect of the cross-fostering treatment on the ontogeny of begging calls.

Several hypotheses can be raised to interpret the effect of the heterospecific cross-fostering on begging call ontogeny. A first explanation could be physiological: changes in begging call characteristics could be a side effect of the cross-fostering event on the physiology and the growth rate of the chicks. For example, differences in stress and satiety levels, due to low levels of food provisioning by the parents, could have influenced begging call structure. BFR chicks could have had calls with more energy in high-frequency bands, because they were smaller or hungrier as demonstrated in nestling tree swallows (*Tachycineta bicolor*) or in nestling Jackson's golden-backed weaver (*Ploceus jacksoni*) [27–29]. But if they were hungrier, their begging calls would have been longer, produced at a higher call rate [13,34,39] and with more spectral noise [40]. We found that call duration and spectral noise (spectral flatness) loaded weakly on PCs, and separate analysis of each parameter (electronic supplementary material, tables S6–S8) showed no effect of the cross-fostering group. Moreover, the two experimental groups did not differ in body condition at 14 days of age, when the acoustic differences between groups were the most pronounced. Finally, several studies have suggested that female zebra finches show higher vulnerability to developmental stress, growth rate and body mass being lower in females than males after food restriction [43–45]. If the cross-fostering to Bengalese finch parents represented a developmental stress, we would expect females to be more affected than males. Females did not differ from males in body condition at 14 DPH, and female begging calls were less affected by the cross-fostering treatment. So differences in acoustic structure of begging calls do not seem to be explained by differences between experimental groups in body condition or in motivation to beg linked to different levels of parental care. Nevertheless, it remains to be investigated whether different patterns of care in the two species could lead to differences in physiology that are not manifested neither in standard measures of body condition nor in our measures of begging acoustic features.

Alternatively, the changes in call structure could be shaped by feeding and brooding responses of the parents: chicks would adjust their begging call structure as a result of the reinforcement they get from food or care delivered by the parents. Bengalese finch parents could be more sensitive to specific cues in the begging call (natural begging call cues of Bengalese finch chicks) and would positively reinforce begging calls that fit these cues. As a consequence, chicks would modify their begging calls by reinforcement learning to resemble Bengalese finch begging calls. Unfortunately, no description of Bengalese finch begging calls is available in the literature. Compared to zebra finch calls, adult Bengalese finch calls show a reduced spectral bandwidth and a higher pitch [38]. A preliminary comparison of Bengalese finch and zebra finch begging calls at 14 DPH (electronic supplementary material, figure S5) shows that the former have a much smaller spectral bandwidth centred around the mean frequency. The spectral noisiness of the signal was also lower. Our results show that BFR nestlings (particularly males) decreased the spectral bandwidth of their calls (lower s.d.) and increased their frequency (higher mean, median, Q25 and Q75). A preliminary comparison of composite scores of the acoustic structure of begging calls from control Bengalese finches, control ZFR zebra finches and BFR zebra finches shows that the latter present intermediate scores between the two control groups (electronic supplementary material, table S10 and figure S6). Taken together, all these results are in favour of the hypothesis that chicks converged with Bengalese finch begging calls. This would be consistent with the reinforcement learning hypothesis, chicks homing in on what stimulates parents best. This mechanism has been described in some cuckoo species [27–29]. Following this hypothesis of begging call structure being

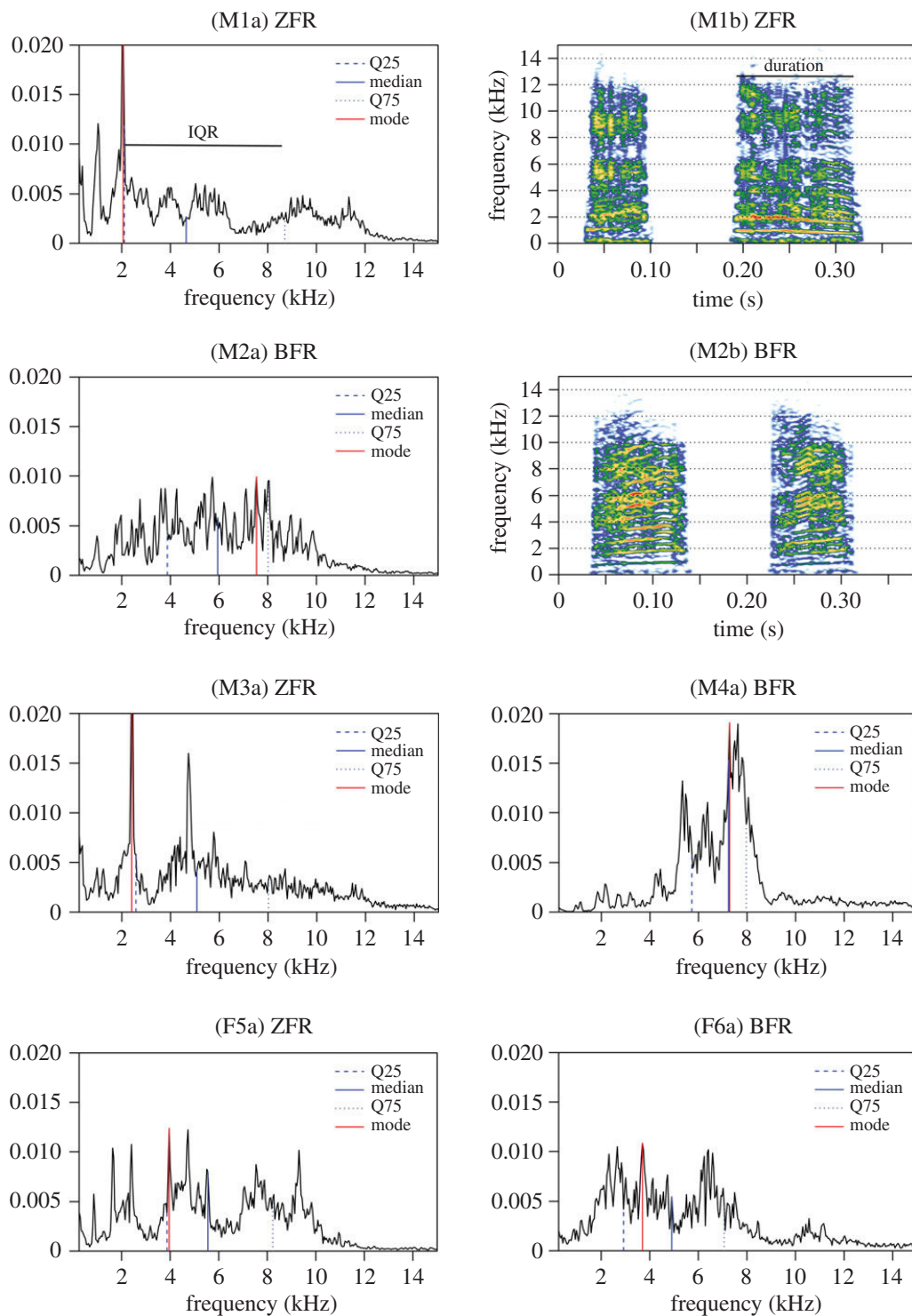


Figure 3. Effect of the cross-fostering treatment on spectro-temporal features of begging calls. Examples of begging calls recorded at 14 DPH on six different subjects (M = males, F = females), either fostered to zebra finch parents (ZFR) or to Bengalese finch parents (BFR). Two representations of the calls are used: ‘a’ labels refer to call spectra with annotations of several spectral parameters (Q25, Q75, median and mode) and ‘b’ labels to spectrograms of begging bouts of two calls with annotation of call duration.

shaped by parental response, it is possible that male and female chicks take a different developmental trajectory if they get different food reinforcement. Previous studies demonstrated parental favouritism in zebra finches, male nestlings receiving more food than females [54] especially under poor environmental conditions [55]. Other studies also show that male nestlings beg more strongly during the first days after hatching [56]. If this is the case during cross-fostering to Bengalese finch parents, it means that young males get more occasions to test the efficiency of their begging call structure by trial and error, and thus more occasions to adjust. However, this should translate into sex differences in growth, which we did not observe at the end of the nestling period.

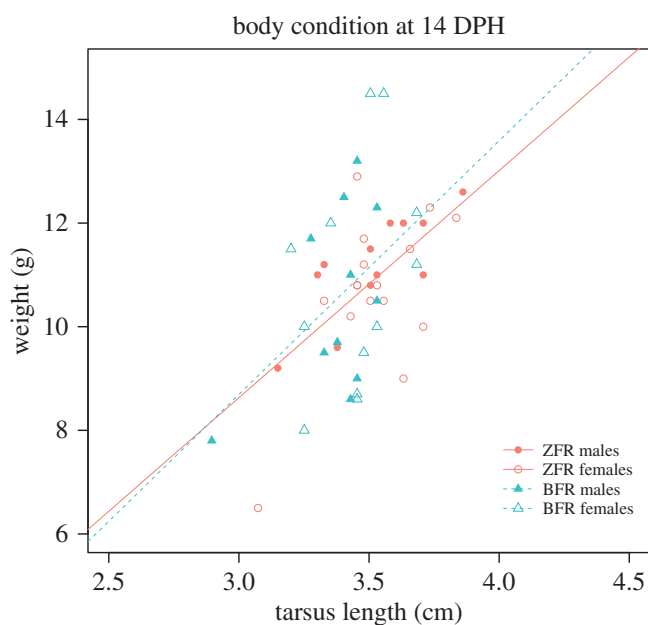


Figure 4. Effect of the cross-fostering group and sex on the BCI of zebra finch chicks fostered either to zebra finch parents (ZFR) or to Bengalese finch parents (BFR).

Table 3. Results from the 'drop' function ('lmerTest' R package) computed on the full model.

	d.f.	LRT	Pr(Chi)
<i>BCI—all data (51 subjects)</i>			
intra-brood sex-ratio	1	0.317	0.573
day of measurement (day 14 ± 1)	1	0.070	0.792
Group: Sex	1	0.404	0.525

A third possibility is that vocal plasticity could be the result of either social acoustical stimulation or vocal imitation. In the former hypothesis, the global acoustic context could be responsible for the changes in begging call structure by vocal improvisation. When hand-reared in groups, Oregon juncos *Junco oreganus* develop larger song repertoire than when reared singly as a result of vocal improvisation without imitation [57,58]. So the simple fact of being acoustically stimulated by social sounds can influence vocal development. As Bengalese finches have higher pitched vocalizations than zebra finches [38], chicks would produce higher pitched calls in this higher pitched context. Following this latter hypothesis, chicks would modify their call structure by imitation of the external model represented by the vocalizations of the adults. This is the mechanism known for song learning, which involves two phases: the memorization of an adult tutor song during the sensory phase followed by the production of initially immature vocalizations that gradually become similar to the tutor song during the sensorimotor phase [59]. In the zebra finch, these two phases happen in succession from day 15 to day 90, with some overlap between day 25 and 60. Our recordings took place from day 5 to day 14, before the onset of the sensorimotor period of song learning. It is thus unlikely that young zebra finches changed their begging call structure by imitation in our experiment, but it remains to be tested.

In all these hypotheses (vocal improvisation, vocal imitation or vocal adjustment by social shaping), hearing and auditory feedback are paramount for the structuration of begging calls. Previous studies bolster this idea. Liu *et al.* [60] used a deafening method in chipping sparrow (*Spizella passerina*) chicks to investigate the importance of the auditory feedback in begging call structure. Deafened male chicks lost begging call structuration, emphasizing the importance of auditory feedback. The authors concluded that different pathways for begging call production could exist: one hearing dependent, one not hearing dependent. They also proposed that auditory-sensitive vocal variability during food begging calls could be the first step leading to vocal imitation, as deafened females did not lose begging call structuration and male begging calls were more variable, showing more vocal exploration. Our results confirm that

begging calls could participate in early sensorimotor exploration, and it is interesting to note that we found a significantly stronger effect of the cross-fostering on male begging calls than on female begging calls.

Vocal plasticity at such an early age can be surprising. In the zebra finch, song control brain nuclei develop from day 9 in both sexes and start to become sexually dimorphic from day 12 [61]. So, the differential changes of male and female begging calls in our experiment, which happened between day 5 and day 14, are unlikely to be related to the nervous control of song control nuclei. But it could be an early dimorphism in the vocal organ. Adult zebra finch males have modified sound generating structures of the syrinx, which are associated with larger muscles contracting at higher rates [12,13,62]. Together with dimorphism in neural control, these are thought to allow the male to generate vocalizations in a larger frequency range. As dimorphism of the vocal organ starts to develop in the first weeks after hatching [63], begging calls could represent a form of vocal experimentation associated to syrinx maturation.

Furthermore, a fully matured vocal control system may not be necessary to express degrees of vocal plasticity and vocal imitation. Indeed, it has been recently described that social influences on vocal production can act during embryonic stages: in a host species of some cuckoos (the superb fairy wren, *Malurus cyaneus*), females call to their eggs at the end of incubation, and upon hatching nestlings produced begging calls with high similarity to their mother's call that are used as a password in female-offspring recognition [30].

Our results give new insights into early vocal plasticity in begging calls, and in male–female differences in degrees of plasticity. We show evidences of an adjustment of nestling begging call acoustic characteristics in response to experience during parental care in a non-parasitic passerine species. This adds to the complexity of offspring signalling of needs to the parents. It may help in understanding some of the discrepancies in the relationships between begging intensity and parental response [14,15] as well as between begging characteristics and several proxies of nestlings' needs [12,16–18]. To evaluate the impact of reinforcement learning on parents–offspring conflict, future studies might benefit from analysing parents–offspring interactions as time series or using dynamical systems theory.

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

Data accessibility. Data available from Dryad at <http://dx.doi.org/10.5061/dryad.9pn24>.

Authors' contributions. A.V. participated in the design of the study, carried out the experiments and the data analysis, and drafted the manuscript; C.B. and I.B. carried out the experiments and drafted the manuscript; C.V. designed the study, participated in the data analysis, and drafted the manuscript. All authors gave final approval for publication.

Competing interests. We declare we have no competing interests.

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Chapter 5: Appendix

Methods

Table ESM1: Composition of experimental groups, ZFR = chicks reared by zebra finch parents and BFR = chicks reared by Bengalese finch parents. Number of subjects of each sex recorded at each age (F= Females, M= Males).

Age	5	6	7	8	9
ZFR	1 F - 0 M	3 F - 1 M	2 F - 2 M	1 F - 2 M	1 F - 1 M
BFR	4 F - 2 M	1 F - 3 M	2 F - 1 M	3 F - 0 M	4 F - 4 M

Age	10	11	12	13	14
ZFR	5 F - 3 M	2 F - 1 M	2 F - 3 M	2 F - 3 M	3 F - 2 M
BFR	2 F - 2 M	6 F - 3 M	--	2 F - 2 M	6 F - 4 M

Table ESM2: Sex composition of each nest. ZFR = chicks reared by zebra finch parents and BFR = chicks reared by Bengalese finch parents. The sex ratio was calculated for each brood as the ratio of the number of females over the total number of chicks.

Group	Foster Nest	nF	nM	Brood Size	Intra-Brood Sex-ratio
ZFR	1	2	2	4	0.5
	3	2	1	3	0.67
	4	2	1	3	0.67
	6	2	1	3	0.67
	7	1	2	3	0.33
	8	1	2	3	0.33
	9	2	1	3	0.67
	10	1	1	2	0.5
	11	2	1	3	0.67
	15	1	1	2	0.5
	16	2	0	2	1
BFR	1	1	2	3	0.33
	2	1	2	3	0.33
	3	2	1	3	0.67
	4	1	2	3	0.33
	5	2	1	3	0.67
	6	3	1	4	0.75
	7	1	2	3	0.33
	8	2	1	3	0.67

Table ESM3: Composition of the begging call dataset. a-ZFR = chicks reared by zebra finch parents and b-BFR = chicks reared by Bengalese finch parents. NCalls = number of calls per session for each chick. Age in days post-hatching.

a- ZFR Group				b- BFR Group			
Subject	Sex	Age	Ncalls	Subject	Sex	Age	Ncalls
318	M	12	38			10	14
319	F	11	72	427	M	13	111
321	F	12	10	429	F	8	30
323	M	12	159			10	74
	M	13	137	430	M	10	70
339	F	6	87			14	147
		10	88			7	62
340	F	5	46	432	F	9	46
341	M	8	56			13	82
342	M	9	147			9	18
		13	105	433	F	10	81
343	F	6	62			7	29
345	M	8	108	434	M	9	74
347	F	10	113			13	96
348	F	7	95			5	59
		13	170	435	F	9	54
349	M	7	64			14	194
		13	52	437	M	5	44
350	M	10	79			9	116
352	M	7	88	438	F	9	41
		14	99			8	152
354	F	8	68	439	F	11	158
		10	143			6	204
355	M	12	157	440	M	9	83
		8	57			8	180
356	M	10	84	441	F	13	50
		12	217			5	53
357	F	11	126			7	111
		14	61	442	F	11	113
361	F	11	113			14	25
		14	188	443	F	11	59
363	F	6	98			14	82
		10	109	444	M	9	93
364	F	10	166			5	38
		14	186	445	F	11	336
365	M	9	26			14	264
		13	166			5	148
365	M	6	93	446	M	11	178
		10	173			14	296

366	F	7	70			5	77
		14	97	447	F	11	146
						14	42
						6	29
				448	M	11	54
						14	127
						6	49
				450	M	11	102
						14	148
						6	30
				451	F	11	105
						14	176

Results

Table ESM4: Statistical table of models on principal components of the PCA on acoustic parameters. Estimates, standard errors and confidence intervals, generated with ‘lsmeans’ function (‘lmerTest’ R package) for each model. a-Values resulting from the initial full model, composed of three fixed factors (Group, Age and Sex). b- When the triple interaction was significant, post hoc models were run in subsets of the data with two fixed factors (in males and females separately to test for the Group: Age interaction, and in young and old nestlings separately to test for the Group: Sex interaction).

a- INITIAL FULL MODEL : Group*Age*Sex

	Estimate	Standard Error	DF	Lower CI	Upper CI
<i>PC1- all data</i>					
ZFR group	0.925	0.260	16.400	0.376	1.474
BFR group	-0.862	0.277	9.800	-1.480	-0.244
Females	0.304	0.289	14.100	-0.315	0.923
Males	-0.241	0.211	11.700	-0.701	0.220
ZFR group: Females	1.074	0.396	17.800	0.241	1.906
BFR group: Females	-0.466	0.422	11.800	-1.387	0.456
ZFR group: Males	0.777	0.286	13.700	0.162	1.391
BFR group: Males	-1.258	0.296	8.000	-1.942	-0.574
<i>PC2- all data</i>					
ZFR Group	0.5601	0.2498	12.1	0.0163	1.1039
BFR Group	-0.2665	0.2903	8.6	-0.9274	0.3944
Females	0.4696	0.2209	22	0.0114	0.9277
Males	-0.176	0.2408	27.9	-0.6693	0.3173
ZFR Group: Females	0.7618	0.3032	20	0.1292	1.3944
BFR Group: Males	0.1773	0.3201	11.9	-0.5204	0.8751
ZFR Group: Males	0.3584	0.3155	21.6	-0.2966	1.0134
BFR Group: Males	-0.7103	0.3528	17.5	-1.453	0.0324

b- POST HOC MODELS FOLLOWING SIGNIFICANT GROUP*AGE*SEX INTERACTION

	Estimate	Standard Error	DF	Lower CI	Upper CI
<i>PC1- post hoc test on males</i>					
ZFR group	0.846	0.258	16.900	0.301	1.390
BFR group	-1.249	0.265	12.900	-1.822	-0.676

PCI- post hoc test on females

ZFR group	1.010	0.397	17.300	0.174	1.845
BFR group	-0.476	0.410	8.500	-1.412	0.460

PCI- post hoc test on young nestlings

ZFR group	-0.768	0.519	8.100	-1.963	0.428
BFR group	-2.182	0.497	6.200	-3.391	-0.973
Females	-1.007	0.397	9.100	-1.903	-0.111
Males	-1.942	0.444	13.900	-2.896	-0.989
ZFR group: Females	-0.184	0.560	9.900	-1.433	1.066
BFR group: Females	-1.831	0.558	8.700	-3.100	-0.563
ZFR group: Males	-1.352	0.688	15.100	-2.816	0.113
BFR group: Males	-2.533	0.555	10.400	-3.763	-1.303

PCI- post hoc test on old nestlings

ZFR group	2.042	0.350	12.200	1.280	2.805
BFR group	0.667	0.399	5.200	-0.345	1.680
Females	1.372	0.397	4.100	0.282	2.461
Males	1.338	0.360	11.200	0.548	2.128
ZFR group: Females	1.340	0.515	14.800	0.241	2.439
BFR group: Females	1.404	0.596	1.600	-1.789	4.597
ZFR group: Males	2.745	0.470	11.200	1.714	3.776
BFR group: Males	-0.069	0.548	11.400	-1.271	1.133

Figure ESM1: Individual developmental trajectories of begging call acoustic features in males and females. Each point represents the individual mean of the first principal component (PC1) calculated from all the calls recorded for this individual at this age. The error bars are standard error of the mean (related to the number of calls analysed). Each point has error bars, usually very small illustrating the stability of begging call structure for an individual at a given age stage. Lines connect points at different ages of the same individual. Linear mixed effect models were performed on PC1. Because triple interaction of sex, group and age was significant, post hoc models were then performed separating males and females (see table ESM4). The models showed differential developmental trajectories (Age: Group interaction) between cross-fostering groups in males but not in females. The significance of Age:Group interaction is indicated in insert. *: $P \leq 0.05$.

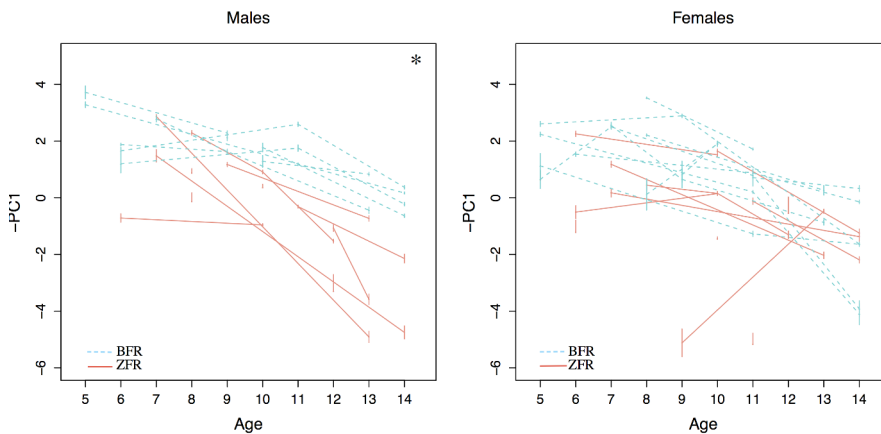


Figure ESM 2: Effect of call index during recording session on principal components of the PCA on acoustic parameters. Statistics showed an effect of the call index on PC2 (table ESM4) but also on PC1 in males only. Call index was centered and reduced. Since no effect of the cross-fostering group was found on PC2, all data are pooled together. On the contrary, because PC1 was significantly affected by the cross-fostering group in males, groups are plotted separately to check possible interactions. The significance of the effect of the call index on PC values is indicated in insert. ***: $P \leq 0.001$, *: $P \leq 0.05$, . : $P \leq 0.1$.

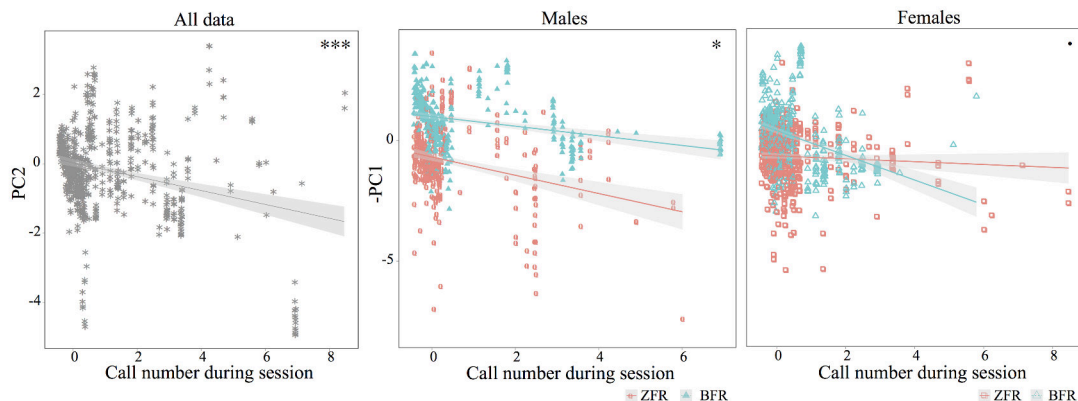


Figure ESM3: Examples of begging call spectro-temporal features of zebra finch chicks either fostered to zebra finch parents (ZFR) or Bengalese finch parents (BFR). Spectra (left column) and spectrograms (right column) of two females at day 5 (F1 and F2) and two males at day 8/9 (M1 and M1). Note the decrease in call frequency bandwidth between 5 and 8/9 DPH.

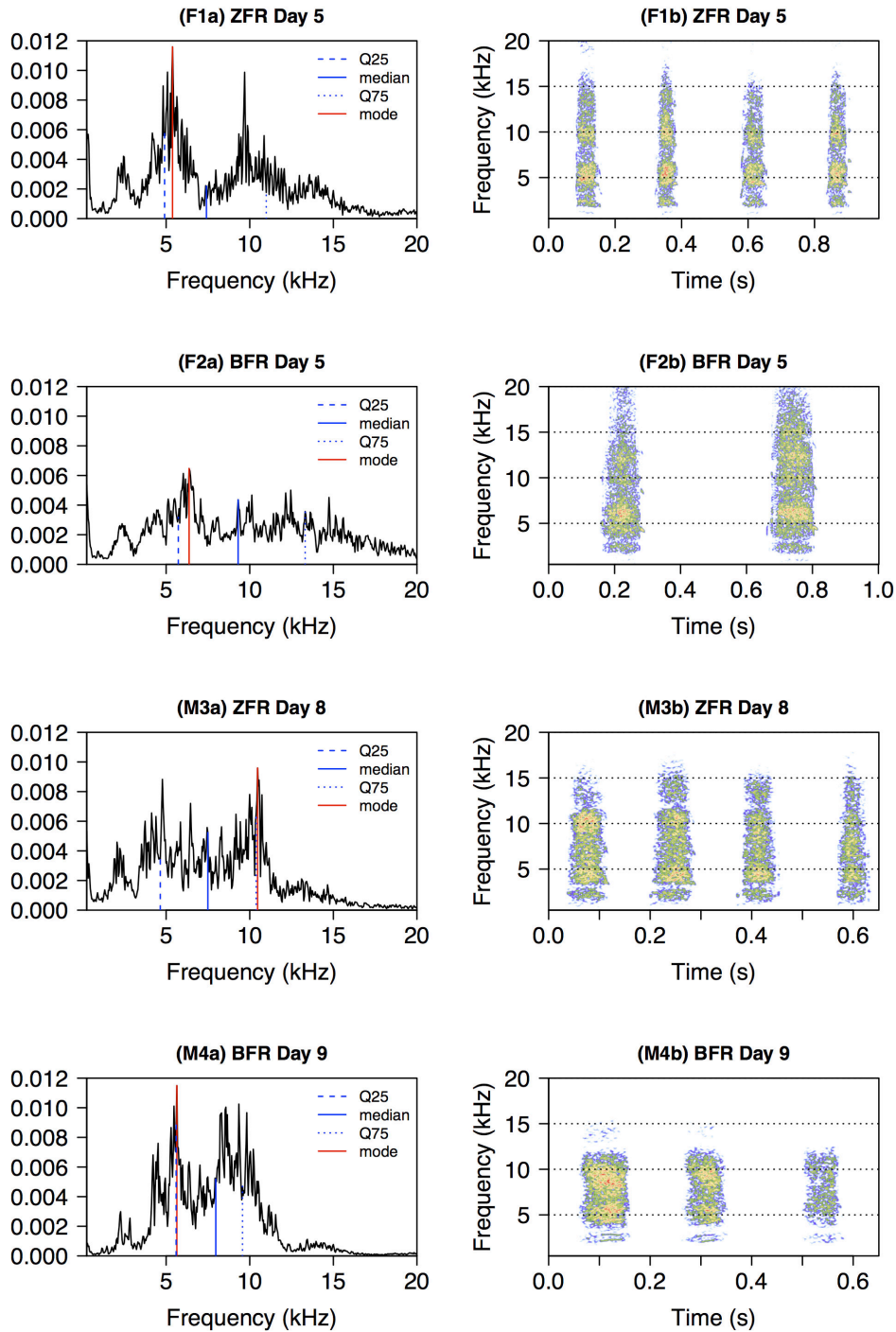


Table ESMS5: Acoustic features of begging calls of chicks reared by zebra finch parents (=ZFR) or chicks reared by Bengalese finch parents (=BFR), recorded from 6 to 14 DPH. Values are means of the mean values obtained per individual \pm Se, standard error calculated using the number of subjects in the subset of data, a NA as standard error indicates that only one individual composed the subset. The number of individuals included per age, sex and group is specified in table 1. Only age classes presenting a complete comparison (both sexes and both cross-fostering groups) are presented. ¹=in kHz, ² = in ms

Age	Group	Sex	Mean ¹	Se	Sd ¹	Se	Median ¹	Se	Mode ¹	Se	Q25 ¹	Se	Q75 ¹	Se	IQR ¹	Se	Skewness	Se	Sfm	Se	Duration ²	Se		
6	ZFR	F	6.83	0.58	3.50	0.01	6.28	0.61	4.70	0.59	4.19	0.58	9.46	0.80	5.27	0.23	1.79	0.25	0.72	0.02	58.41	11.9		
		M	6.51	NA	3.24	NA	5.94	NA	4.08	NA	4.07	NA	8.57	NA	4.50	NA	4.50	NA	2.18	NA	0.72	NA	44.07	NA
	BFR	F	7.41	NA	3.52	NA	6.58	NA	5.32	NA	4.72	NA	10.17	NA	5.45	NA	5.45	NA	1.05	NA	0.80	NA	96.24	NA
		M	7.50	0.12	3.62	0.02	7.04	0.18	4.97	0.31	4.59	0.08	10.50	0.15	5.91	0.17	5.91	0.17	1.47	0.20	0.80	0.02	83.38	6.41
7	ZFR	F	6.98	0.23	3.37	0.08	6.57	0.37	5.46	0.88	4.42	0.26	9.37	0.24	4.96	0.03	4.96	0.03	1.10	0.25	0.77	0.01	52.38	1.08
		M	7.98	0.48	3.44	0.08	7.67	0.54	5.85	0.00	5.33	0.44	10.73	0.63	5.40	0.19	5.40	0.19	1.17	0.07	0.75	0.03	74.01	8
	BFR	F	7.98	0.01	3.37	0.11	7.97	0.02	7.55	0.72	5.26	0.10	10.68	0.01	5.41	0.10	5.41	0.10	0.86	0.06	0.75	0.04	69.64	0.53
		M	8.35	NA	3.43	NA	8.54	NA	6.56	NA	5.49	NA	11.18	NA	5.69	NA	5.69	NA	1.04	NA	0.74	NA	58.05	NA
10	ZFR	F	6.85	0.31	3.12	0.12	6.67	0.41	5.86	0.78	4.44	0.35	8.98	0.36	4.53	0.26	4.53	0.26	1.08	0.10	0.70	0.03	52.96	5.80
		M	6.71	0.31	2.91	0.18	6.53	0.42	6.27	0.73	4.62	0.26	8.60	0.45	3.98	0.31	3.98	0.31	1.34	0.21	0.63	0.05	65.05	1
	BFR	F	7.62	0.07	3.33	0.17	7.62	0.21	7.13	0.17	5.05	0.41	10.20	0.06	5.15	0.47	5.15	0.47	0.91	0.02	0.75	0.05	56.64	1.64
		M	7.52	0.18	3.28	0.31	7.62	0.22	6.51	0.16	5.00	0.22	9.88	0.60	4.88	0.82	4.88	0.82	1.14	0.02	0.73	0.03	57.86	19.2
11	ZFR	F	5.27	1.16	3.25	0.15	4.65	1.55	3.90	2.24	2.88	1.19	7.24	1.45	4.36	0.26	4.36	0.26	2.62	1.58	0.58	0.11	42.27	0.30
		M	6.44	NA	3.10	NA	6.17	NA	4.58	NA	4.04	NA	8.71	NA	4.67	NA	4.67	NA	1.15	NA	0.67	NA	52.28	NA
	BFR	F	6.97	0.27	3.10	0.16	6.75	0.37	5.99	0.59	4.62	0.35	9.05	0.31	4.44	0.36	4.44	0.36	1.28	0.06	0.68	0.04	62.27	7.88
		M	7.72	0.17	3.04	0.35	7.75	0.13	7.11	0.51	5.49	0.37	9.92	0.63	4.43	0.92	4.43	0.92	1.02	0.21	0.66	0.09	77.37	8

13	ZFR	F	5.92	0.51	3.12	0.46	5.52	0.90	3.94	1.75	3.48	1.14	8.27	0.05	4.80	1.19	1.71	0.25	0.59	0.08	70.05	8.44
		M	5.20	0.63	3.15	0.26	4.62	0.99	3.43	1.53	2.85	0.90	7.16	0.56	4.32	0.64	3.24	0.86	0.52	0.03	45.96	4.08
		F	6.34	0.14	2.98	0.31	6.19	0.03	5.71	0.28	4.23	0.19	8.19	0.70	3.95	0.89	1.33	0.38	0.66	0.08	70.85	0.79
		M	6.68	0.51	2.89	0.11	6.57	0.50	5.91	0.73	4.69	0.90	8.57	0.17	3.88	0.73	1.03	0.17	0.63	0.01	72.32	10.99
14	ZFR	F	5.68	0.15	3.06	0.26	5.38	0.17	4.08	0.83	3.33	0.24	7.93	0.51	4.61	0.72	1.50	0.21	0.59	0.05	49.53	2.73
		M	4.91	0.65	3.41	0.44	3.98	1.16	2.54	1.27	2.36	1.08	6.89	0.55	4.53	0.53	3.16	0.74	0.58	0.02	55.52	7.34
		F	5.71	0.35	3.18	0.08	5.16	0.47	3.81	0.57	3.32	0.40	7.83	0.47	4.52	0.20	2.16	0.56	0.61	0.04	50.29	7.29
		M	6.57	0.20	2.81	0.11	6.35	0.20	5.93	0.52	4.53	0.36	8.44	0.18	3.92	0.41	1.23	0.31	0.59	0.02	79.77	14.41

Table ESM6: Statistical results from models on each acoustic parameter separately. The table depicts values resulting from the ‘drop1’ function (‘lmerTest’ R package) computed on the full model testing the following interaction explanatory variables: Group*Age*Sex. ‘Drop1’ computes all the single terms that can be added to or dropped from the model, fits those models and computes a table of the changes in fit. Only relevant interactions then compose the table. When a significant triple interaction was found, post hoc tests were run first in the two sexes (Test of the group : Age interaction, table ...) and on two age classes (test of the group : Sex interaction, table...).

	DF	LRT	Pr(Chi)		DF	LRT	Pr(Chi)
<u>Mean frequency</u>				<u>Third Quartile frequency (Q75)</u>			
Call number	1	1.193	0.275	Call number	1	3.622	<u>0.057</u>
Intra-brood sex-ratio	1	0.290	0.591	Intra-brood sex-ratio	1	0.048	<u>0.826</u>
BCI	1	0.047	0.828	BCI	1	0.894	0.344
Social Group: Age: Sex	1	9.707	0.002	Social Group: Age: Sex	1	8.151	0.004
<u>Standard deviation (Sd)</u>				<u>Inter-quartile-Range (IQR)</u>			
Call number	1	66.385	0.000	Call number	1	50.015	0.000
Intra-brood sex-ratio	1	3.035	<u>0.081</u>	Intra-brood sex-ratio	1	0.888	0.346
BCI	1	0.095	0.758	BCI	1	0.001	0.975
Social Group: Age: Sex	1	4.086	0.043	Social Group: Age: Sex	1	0.056	0.812
<u>Median frequency</u>				<u>Spectral Skewness</u>			
Call number	1	1.853	0.173	Call number	1	1.928	0.165
Intra-brood sex-ratio	1	1.529	0.216	Intra-brood sex-ratio	1	1.909	0.167
BCI	1	0.047	0.828	BCI	1	1.748	0.186
Social Group: Age: Sex	1	8.646	0.003	Social Group: Age: Sex	1	8.019	0.005
<u>Mode frequency</u>				<u>Spectral flatness</u>			
Call number	1	8.053	0.005	Call number	1	23.084	0.000
Intra-brood sex-ratio	1	3.468	<u>0.063</u>	Intra-brood sex-ratio	1	0.663	0.416
BCI	1	0.255	0.613	BCI	1	0.014	0.904
Social Group: Age: Sex	1	6.665	0.010	Social Group: Age: Sex	1	0.063	0.801
<u>First Quartile frequency (Q25)</u>				<u>Call duration</u>			
Call number	1	33.912	0.000	Call number	1	2.098	0.147
Intra-brood sex-ratio	1	1.678	0.195	Intra-brood sex-ratio	1	1.008	0.315
BCI	1	0.320	0.571	BCI	1	1.205	0.272
Social Group: Age: Sex	1	8.606	0.003	Social Group: Age: Sex	1	5.145	0.023

Table ESM7: Statistical results of models on each acoustic parameter, within sexes post hoc models.

	MALES			FEMALES		
	DF	LRT	Pr(Chi)	DF	LRT	Pr(Chi)
<i>Mean frequency</i>						
Call number	1	1.030	0.310	1	3.072	<u>0.080</u>
Intra-brood sex-ratio	1	-0.048	1.000	1	0.704	<u>0.402</u>
BCI	1	0.004	0.950	1	0.011	0.915
Social Group: Age	1	3.165	<u>0.075</u>	1	1.072	0.301
<i>Standard deviation (Sd)</i>						
Call number	1	130.367	0.000	1	6.063	0.014
Intra-brood sex-ratio	1	0.826	0.364	1	3.816	<u>0.051</u>
BCI	1	0.047	0.828	1	0.410	0.522
Social Group: Age	1	6.304	0.012	1	0.027	0.870
<i>Median frequency</i>						
Call number	1	0.274	0.600	1	3.181	<u>0.074</u>
Intra-brood sex-ratio	1	0.225	0.636	1	1.261	0.261
BCI	1	0.392	0.531	1	0.458	0.498
Social Group: Age	1	4.512	0.034	1	0.885	0.347
<i>Mode frequency</i>						
Call number	1	2.135	0.144	1	5.861	0.015
Intra-brood sex-ratio	1	0.095	0.758	1	4.149	0.042
BCI	1	0.338	0.561	1	0.770	0.380
Social Group: Age	1	6.577	0.010	1	0.564	0.453
<i>First Quartile frequency (Q25)</i>						
Call number	1	32.334	0.000	1	11.609	0.001
Intra-brood sex-ratio	1	0.219	0.640	1	2.237	0.135
BCI	1	0.222	0.637	1	0.078	0.779
Social Group: Age	1	6.199	0.013	1	0.334	0.563
<i>Third Quartile frequency (Q75)</i>						
Call number	1	16.581	0.000	1	0.028	0.868
Intra-brood sex-ratio	1	0.946	0.331	1	0.127	0.722
BCI	1	0.171	0.679	1	0.031	0.861
Social Group: Age	1	0.759	0.384	1	3.754	<u>0.053</u>
<i>Spectral Skewness</i>						
Call number	1	2.439	0.118	1	6.411	0.011
Intra-brood sex-ratio	1	0.608	0.435	1	1.545	0.214
BCI	1	0.426	0.514	1	2.013	0.156
Social Group: Age	1	6.217	0.013	1	1.183	0.277
<i>Call duration</i>						
Call number	1	3.540	<u>0.060</u>	1	0.249	0.618
Intra-brood sex-ratio	1	0.157	0.692	1	5.731	0.017
BCI	1	5.171	0.023	1	0.931	0.335
Social Group: Age	1	0.269	0.604	1	4.280	0.039

Table ESM8: Statistical results from models on each acoustic parameter, post hoc models separating age classes (young nestlings : $5 \leq \text{Age} \leq 7$, old nestlings : $12 \leq \text{Age} \leq 15$). When the two way interaction between Group and Sex was significant, which was the case in old nestling for seven of the eight parameters (a) post hoc test were run separating sexes (b).

a- POST HOC MODELS SEPARATING YOUNG AND OLD NESTLINGS				b- POST HOC MODELS IN OLD NESTLING			
YOUNG				MALE OLD NESTLING			
DF	LRT	Pr(Chi)	DF	LRT	Pr(Chi)	DF	LRT
OLD				FEMALE OLD NESTLINGS			
DF	LRT	Pr(Chi)	DF	LRT	Pr(Chi)	DF	LRT
<i>Mean frequency</i>							
1	0.016	0.898	1	0.443	0.506	1	8.286
1	0.431	0.512	1	0.014	0.907	1	0.587
1	0.406	0.524	1	0.054	0.817	1	0.651
1	0.728	0.393	1	6.966	0.008	1	0.409
<i>Social Group: Sex</i>							
<i>Standard deviation (Sd)</i>							
1	0.292	0.589	1	86.962	0.000	1	8.163
1	1.023	0.312	1	1.048	0.306	1	0.009
1	2.128	0.145	1	0.020	0.888	1	0.804
1	1.949	0.163	1	1.757	0.185	1	0.151
<i>Median frequency</i>							
1	0.303	0.582	1	0.833	0.361	1	8.163
1	0.405	0.524	1	0.201	0.654	1	0.009
1	2.239	0.135	1	0.054	0.816	1	0.804
1	0.117	0.732	1	6.831	0.009	1	0.151
<i>Mode frequency</i>							
1	0.002	0.968	1	13.361	0.000	1	11.396
1	0.053	0.818	1	3.251	<u>0.071</u>	1	6.019
1	0.862	0.353	1	1.860	0.173	1	0.082
1	0.094	0.759	1	4.843	0.028	1	0.407
<i>Social Group: Sex</i>							

<u>First Quartile frequency (Q25)</u>				<u>First Quartile frequency (Q25)</u>									
Call number	1	0.004	0.952	1	42.136	0.000	Social Group	1	8.174	0.004	1	0.494	0.482
Intra-brood sex-ratio	1	0.129	0.719	1	0.242	0.623	Call number	1	31.137	0.000	1	17.017	0.000
BCI	1	0.296	0.587	1	0.149	0.699	Intra-brood sex-ratio	1	0.177	0.674	1	0.000	0.993
Social Group: Sex	1	1.013	0.314	1	4.551	0.033	BCI	1	1.242	0.265	1	0.096	0.757
<u>Third Quartile frequency (Q75)</u>				<u>Third Quartile frequency (Q75)</u>									
Call number	1	0.000	0.988	1	5.626	0.018	Social Group	1	6.400	0.011	1	0.870	0.351
Intra-brood sex-ratio	1	0.822	0.365	1	0.060	0.807	Call number	1	10.041	0.002	1	0.234	0.629
BCI	1	0.010	0.920	1	0.057	0.811	Intra-brood sex-ratio	1	0.737	0.391	1	0.802	0.370
Social Group: Sex	1	2.785	0.095	1	5.958	0.015	BCI	1	0.131	0.717	1	0.578	0.447
<u>Spectral Skewness</u>				<u>Spectral Skewness</u>									
Call number	1	0.072	0.788	1	0.019	0.890	Social Group	1	12.093	0.001	1	0.275	0.600
Intra-brood sex-ratio	1	0.068	0.794	1	0.183	0.669	Call number	1	1.962	0.161	1	5.016	0.025
BCI	1	0.142	0.706	1	2.338	0.126	Intra-brood sex-ratio	1	0.093	0.760	1	0.013	0.910
Social Group: Sex	1	0.113	0.737	1	8.705	0.003	BCI	1	2.620	0.106	1	0.657	0.418
<u>Call duration</u>				<u>Duration</u>									
Call number	1	0.313	0.576	1	6.631	0.010	Social Group	1	13.330	0.000	1	0.040	0.842
Intra-brood sex-ratio	1	0.076	0.783	1	2.190	0.139	Call number	1	7.974	0.005	1	0.199	0.656
BCI	1	1.629	0.202	1	0.462	0.497	Intra-brood sex-ratio	1	4.337	0.037	1	0.675	0.411
Social Group: Sex	1	1.460	0.227	1	7.640	0.006	BCI	1	3.701	<u>0.054</u>	1	0.151	0.698

Figure EMS4: Modifications of begging calls' median frequency, first quartile (Q25), standard deviation (Sd), and skewness of the frequency spectrum over the development of zebra finches reared either by zebra finches (ZFR) or by Bengalese finches (BFR). Data points are mean (\pm se) when the corresponding subset of data contains several chicks, and mean only (without error bar) when the data point represents only one chick. The data set for one chick at a given age still represents multiple calls, so the corresponding data point is the mean on all these calls of the considered parameter. Grey shades are 95% Confidence Interval of the linear regression. Figures from results on post hoc models testing the group: Age interaction in males and females, the significance of the interaction between group and Age as a covariate is indicated in insert. * $P \leq 0.05$.

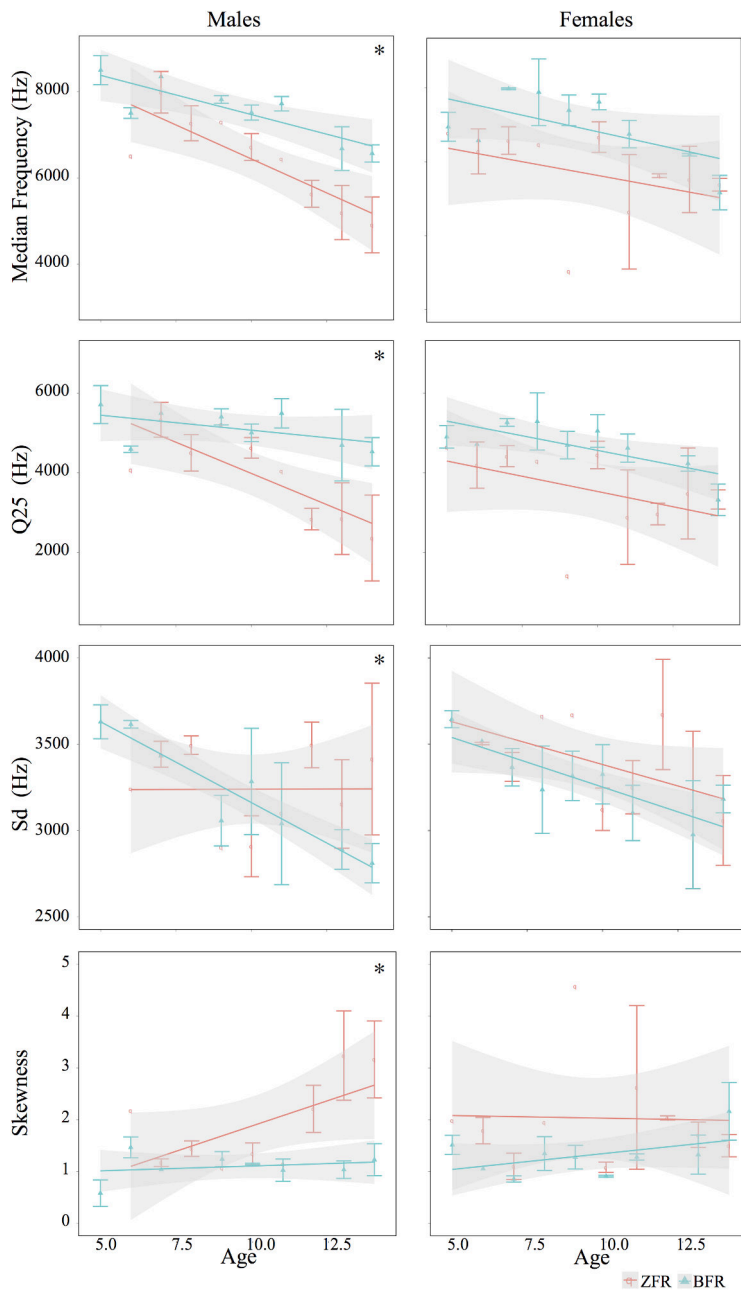


Table ESM9: Statistical table for the analysis of chicks' body condition at 14 DPH. a- Results from the 'drop1' function of the model testing the main effects of the treatment without the non-significant interaction between sex and group. b- Results from the 'drop1' function ('lmerTest' R package) computed on the reduced data set (composed only of chicks for which we had recordings at 14 DPH exactly). 'Drop1' computes all the single terms that can be added to or dropped from the model, fits those models and computes a table of the changes in fit. Only relevant interactions then compose the table. c-Estimates, standard errors and confidence intervals, generated with 'lsmeans' function ('lmerTest' R package) of models on the complete and reduced dataset

	Df	LRT	Pr(Chi)			
a-						
<i>BCI - additional model without the non-significant interaction between sex and group (51 subjects)</i>						
group	1	0.017	0.896			
sexe	1	1.159	0.282			
Zday_14	1	0.353	0.552			
Intra-Brood sex-ratio	1	0.089	0.766			
b-						
<i>BCI - reduced dataset (23 subjects)</i>						
Intra-Brood sex-ratio	1	2.234	0.135			
Day of measurement (Day 14 ±1)	1	1.210	0.271			
Group: Sex	1	1.047	0.306			
c-						
	Estimate	Standard Error	DF	t-value	Lower CI	Upper CI
<i>BCI - all data (51 subjects)</i>						
ZFR Group	-0.078	0.195	26.400	-0.400	-0.478	0.323
BFR Group	-0.044	0.472	13.400	-0.090	-1.061	0.972
Females	-0.200	0.335	23.600	-0.600	-0.891	0.491
Males	0.078	0.260	17.800	0.300	-0.469	0.626
ZFR Group : Females	-0.310	0.335	15.300	-0.930	-1.022	0.402
BFR Group: Females	-0.090	0.583	21.300	-0.150	-1.300	1.121
ZFR Group: Males	0.155	0.206	19.000	0.750	-0.276	0.585
BFR Group: Males	0.002	0.474	13.300	0.000	-1.020	1.023
<i>BCI - reduced dataset (23 subjects)</i>						
ZFR Group	-0.456	0.313	5.200	-1.460	-1.251	0.339
BFR Group	-0.099	0.675	6.200	-0.150	-1.738	1.540
Females	-0.227	0.485	11.100	-0.470	-1.292	0.839
Males	-0.329	0.393	7.400	-0.840	-1.248	0.591
ZFR Group : Females	-0.562	0.553	4.600	-1.020	-2.018	0.894
BFR Group: Females	0.109	0.802	9.900	0.140	-1.680	1.897
ZFR Group: Males	-0.351	0.304	1.600	-1.150	-1.996	1.294
BFR Group: Males	-0.307	0.724	6.400	-0.420	-2.049	1.436

Figure ESM5: Spectro-temporal features (spectra on the left column and spectrograms on the right column) of Bengalese finch begging calls at 14 (± 1) DPH. Six Bengalese finch nestlings (three males and three females) reared by three Bengalese finch pairs were recorded at several stages of their development using the same protocol as in the heterospecific cross fostering experiment (see methods), except that chicks were reared by their genetic parents. Here are presented three examples of calls from three different individuals. Note almost the same median frequency as in zebra finch begging calls of the same age but a much smaller spectral bandwidth. Bengalese finch calls are also more tonal than zebra finches' ones (see spectrograms).

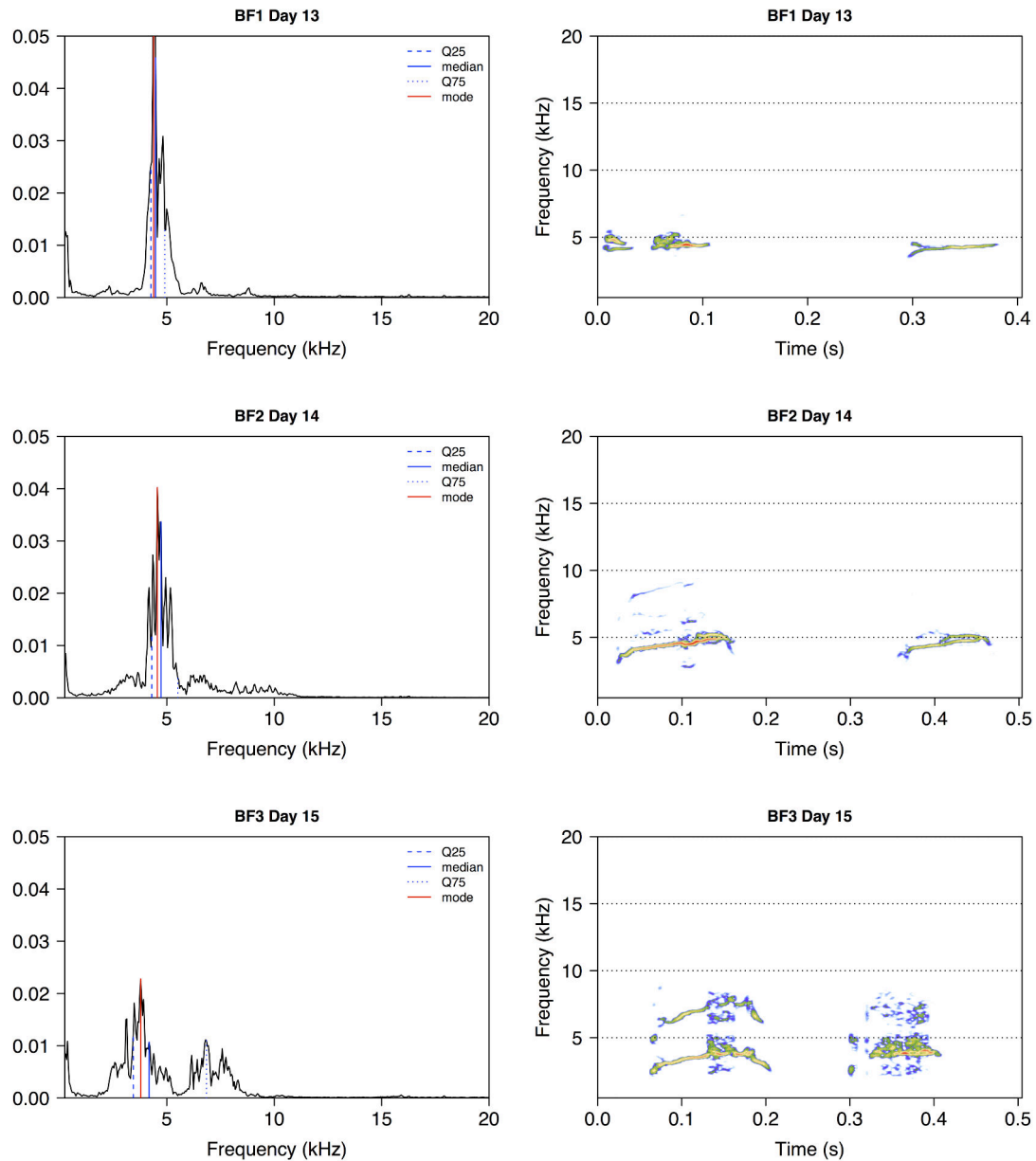
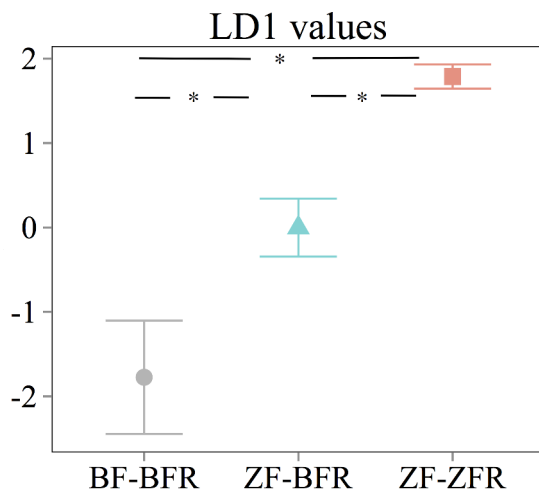


Table ESM10: Comparison of begging call features of Bengalese finch chicks reared by Bengalese finch parents (BF-BFR), zebra finch chicks reared by Zebra finch parents (ZF-ZFR), and zebra finch chicks reared by Bengalese finch parents (ZF-BFR): step 1. A Linear Discrimination Analysis (LDA) was computed on the two control groups (BF-BFR and ZF-ZFR)

using 11 acoustic parameters. All nestlings were from 12 to 15 DPH. The first linear discriminant function (LD1) was then used to compare the acoustic structure of BF-BFR, ZF-ZFR and ZF-BFR (see Figure 5). The table gives the coefficients of LD1 and mean LD1 values of each parameter in each group ('LDA.format' function of 'RVAideMemoire' R package).

	LD1 coefficients	Mean LD1 values	
		BF-BFR	ZF-ZFR
Mean	-0.099	-0.622	0.180
Sd	0.765	-1.150	0.332
Median	0.499	-0.467	0.332
Mode	0.018	0.214	-0.062
Q25	0.116	0.486	-0.140
Q75	-0.766	-1.070	0.309
Skewness	-0.667	1.147	-0.331
Kurtosis	0.616	1.030	-0.298
Sh	1.542	-1.449	0.419
Duration	-0.419	0.668	-0.193
Ici	-0.023	0.404	-0.117

Figure ESM6: Comparison of begging call features of Bengalese finch chicks reared by Bengalese finch parents (BF-BFR), zebra finch chicks reared by Zebra finch parents (ZF-ZFR), and zebra finch chicks reared by Bengalese finch parents (ZF-BFR): step 2. BF-BFR: N=6 (three females, three males), ZF-ZFR: N=14 (seven females, seven males), ZF-BFR: N=15 (seven males and eight females). All nestlings were from 12 to 15 PDH. LD1 values for BF-BFR and ZF-ZFR calls resulted from the LDA in Table 13, LD1 values for ZF-BFR calls were calculated using the 'predict' function of 'MASS' R package. Each point represents the mean (\pm SE) of the LD1 value of all individuals of a group. Normality and variance homogeneity of the data were not respected. Results of non parametric multiple comparisons following a significant Kruskal and Wallis test ('kruskalmc' function of 'pgirmess' R package): BF-BFR vs. ZF-ZFR: Observed $\text{diff}_{\text{critical diff.}} = 2308.7_{144.8}$, BF-BFR vs. ZF-BFR: Observed $\text{diff}_{\text{critical diff.}} = 927.4_{144.01}$, ZF-ZFR vs. ZF-BFR Observed $\text{diff}_{\text{critical diff.}} = 1381.3_{96.1}$. * = True difference found by the post hoc test.



General discussion

1. Vocal flexibility and plasticity in female songbirds

A. Female vocal flexibility in response to noise during intra-pair communication at the nest

In chapter 3 (dippers) and chapter 4 (zebra finches) we studied the effect of elevated noise on vocal production at the nest: vocalization amplitude, frequency composition and shape of the frequency spectrum. In both studies, birds responded to noise by louder vocalizations. Narrower frequency spectra were found in zebra finches as well as in Notes in dippers (but not in Trills). Up-shifts in frequency composition were found in zebra finches but not in dippers.

In dippers, females sing as well as males and abilities in vocal flexibility may not be different between sexes in this species. And indeed, when comparing vocalizations between nest sites, with varying noise levels, no difference was found in amplitude, frequency composition or shape of the frequency spectrum between ‘female’ and ‘female-male’ vocalizations.

In zebra finches, we investigated sex differences in call vocal flexibility produced by both sexes (chapter 4). Contrary to songs, birdcalls are supposed to be non-learned vocalizations. In this theoretical framework, we would expect limited spectral changes in response to noise. In both sexes and in all call types; calls had an upshifted and narrower frequency spectrum in response to noise. Since birds also vocalized louder, the spectral shift in this case may be a side effect of louder vocalizations, as demonstrated in (Nemeth et al., 2013). Nevertheless, females showed greater changes in call structure than males, supposedly because they were subjected to the noise playback for a longer period of time. Despite obvious differences in vocal production, females are capable of as much flexibility in their calls as males in response to noise. These results lead to two non exclusive hypotheses: either vocal flexibility do not rely on abilities of vocal plasticity and learning, as proposed in (Patricelli and Blickley, 2006) or female abilities of vocal plasticity and learning in calls have been underestimated. In any case, it might be helpful to test for female vocal flexibility and plasticity in other types of calls and/or in different contexts.

B. Preliminary results: female call flexibility in response to social isolation

Perez et al. (2012) investigated the possibility that male zebra finches express their stress in their calls. They showed that males in social isolation produced distance calls that were higher pitched (higher fundamental frequency, higher dominant frequency), less broadband and longer than calls produced with an audience. In this social bird, isolation is a powerful stressor. Using oral administration of corticosterone (the main stress hormone in birds), the authors showed that changes were partly driven by corticosterone. These results were consistent with results obtained in mammals (Briefer, 2012). However, Perez et al. did not perform the experiment in females. Since male zebra finches learn their distance call conjointly with their song (Zann, 1990), vocal flexibility was expected. Contrary to males, females do not seem to learn their distance calls. I conducted a similar experiment to Perez et al. using females. I showed that female distance calls produced during social isolation were longer, higher pitched, more broadband and had a less harmonic structure (Box 4) than calls produced with an audience. So, in response to a similar protocol, females showed similar changes in their call structure to males, emphasizing their capacity of flexibility. However differences between female and male responses have to be noticed. The range of variation of spectral components in female calls was smaller than in male calls: contrary to males, the fundamental frequency of female calls was not affected. In addition, females showed wider energy distribution whereas it was narrowed in males (Box4). However, the range of variation in call duration was equivalent, which may mean that call duration and spectral components have different physiological correlates of production (Box4). Two non exclusive mechanisms may explain these sex differences: either females were less sensitive to social stress and expressed a lower response than males (no measure of corticosterone was taken) or the results reflect sex differences in spectral flexibility abilities.

Box 4: Social isolation protocol in mated female zebra finches. Playbacks of mate distance calls to evoke female calling. Acoustic analysis of female calls recorded in isolation (stressful) and audience (two females). Parameters presented are standard deviation of mean spectrum (SD), call duration, spectral flatness (harmonic calls have a spectral flatness close to 0), dominant and fundamental frequencies measured on each FFT window. Figures depict mean (\pm se) per females (black or grey) in the two social conditions and average over all females (red). Examples of two spectra and spectrograms.

Expression of social isolation stress in female distance calls in zebra finches,
Evidence for female vocal flexibility in calls

Background

Birdcalls are supposed to be unlearned vocalizations.

Evidence of call flexibility/plasticity and learning has been shown in a few species

Female zebra finches do not sing and flexibility in calls is not expected

Social isolation is a powerful stressor: in males, stress is expressed in distance calls and recognized by mates

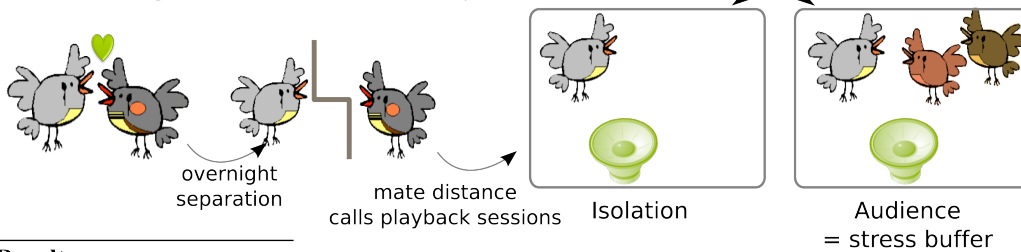
Perez et al 2012, 2015

Experimental procedure

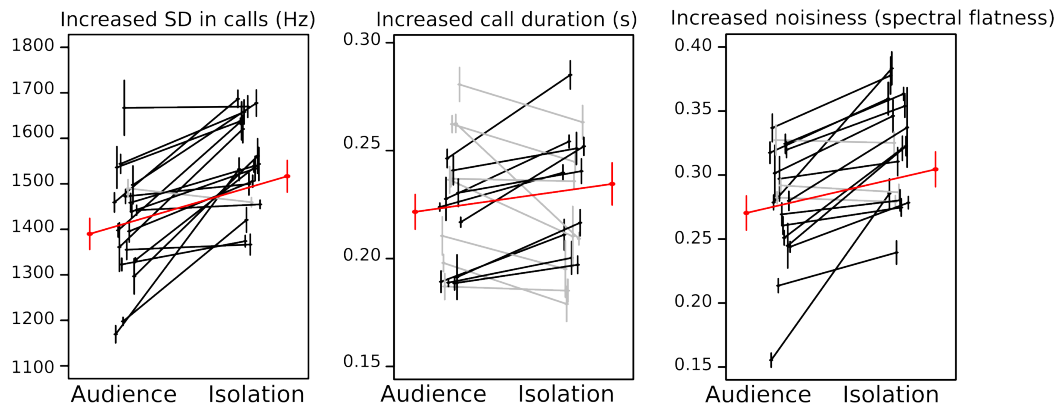
30 mated pairs

Previous recording of male distance calls, evoked by unfamiliar female calls

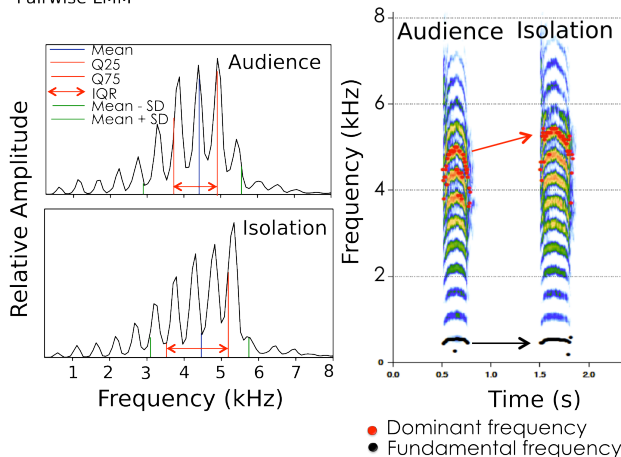
7 days
random order



Results



Pairwise LMM



Conclusion

Isolation vs Audience

Females vs males (Perez et al, 2012)

% variation of parameters

	Females	Males
Dominant frequency	+ 2.6	+ 8.2
Duration	+ 5.8	+ 5.7
Fundamental frequency	0	+10.2
Energy distribution	+ 8.5	- 6.9
Noisiness (SFM)	+ 12.6	?

C. Physiological correlates of sex difference in adult vocal flexibility

The sexual dimorphism related to vocal production in zebra finches is among the strongest in songbirds: females do not sing at all. This major difference in vocal behaviour may be explained by several differences in physiology and anatomy involved in vocal production: brain structures that control vocal production [song control nuclei atrophy during development and are smaller in females (Bottjer et al., 1985; Gahr, 2007; MacDougall-Shackleton & Ball, 1999; Ball & Macdougall-Shackleton, 2001)], syrinx anatomy [with fewer muscle fibers in females (Riede et al., 2010)] and steroid hormone system [fewer receptors (Veney and Wade, 2004)].

These differences may result in a lower ability of spectral modulation of vocalizations in females. However, recent studies have shown that having smaller song control nuclei may not be a limit for female vocal plasticity. In Forest weavers, *Ploceus bicolor*, both members of a sexual pair learn the same unison vocalization, so both the male and the female sing together. In this songbird, the male has larger song control brain nuclei, but the female produces higher levels of mRNA of synapse-related proteins in the song control nuclei HVC and RA. So smaller song control nuclei might be compensated by more synapse-associated proteins in females, allowing them to learn the same song as their mate (Gahr et al., 2008). In addition, contrary to other songbird species in which females show no song nuclei (Bengalese finches for example (Ball and Macdougall-Shackleton, 2001; MacDougall-Shackleton and Ball, 1999)), song nuclei remain in adult female zebra finches and the song control system may play a role in the regulation of vocal production of adult females. A recent study is in favour of this hypothesis. Mated zebra finches have coordinated call exchanges in several social contexts (Elie et al., 2010; Perez, Fernandez, Griffith, Vignal, & Soula, 2015, chapter 4). In a recent study, Benichov et al. (2016), studied vocal timing in call responses between a partner bird and a bird robot and showed similar stereotyped responses. The robot was programmed to generate calls disrupting usual latencies and create jamming calls. Both females and males learned to avoid jamming with robot calls and adjusted their responses to the one of the robot. Females showed more precise adjustments than males. Blocking the song system cortical output dramatically reduced the precision of birds' response timing and abolished their ability to avoid jamming. The authors concluded that the regulation of the timing of calls involved descending forebrain pathways that include the song system, in both females

and males. This result shows that female song control is used during vocal production and allows vocal flexibility in timing. This emphasizes the need to go further in the analysis of vocal production in female and male calls. The song control system may also be involved in call spectral flexibility. The study from Simpson & Vicario, (1990) which analysed brain pathways of learned and non-learned vocalizations in males and females were never reproduced. Among the three females tested, only two responded to their prediction, which may stress the need to revisit call vocal development and potential plasticity.

D. Female vocal plasticity in response to early social environment during parent-offspring communication

We showed that in response to a cross fostering treatment, the development of begging calls was changed, bringing evidence for early vocal plasticity in a non-learned vocalization. We also showed sex differences since only male begging calls showed significant deviations to the normal development. Thus, females are either not able to modify their calls or they did not express vocal plasticity. Previous studies demonstrated parental favouritism in zebra finches, male nestlings receiving more food than females (Mainwaring et al., 2011) especially under poor environmental conditions (Foster and Burley, 2007). These mechanisms may explain male-biased sex ratio at fledging when food is scarce (Martins, 2004). We hypothesized that sex difference in vocal plasticity may be the result of differential social reinforcement by parents. However this hypothesis remains to be tested. As song learning is shaped by social interactions, call vocal development may also be driven by social interactions (with sibling, parents or juveniles). Following the study on nestling begging calls, the cross fostering experiment was continued during the post fledgling period to study the call vocal development in females and males. Six recordings were carried out between 20 and 100 days post hatching to revisit social influences on distance call development in both sexes. Analyses of this experiment would allow us to have a more complete view of female and male call vocal plasticity.

2. Vocal communication at the nest during breeding: communicative value

A. Responses to background noise perturbations: a test to assess the communicative value of vocal behaviour at the nest?

During my PhD project, I had one prediction regarding pair vocal behaviour at the nest during breeding: if it allows a transfer of information and the organization of breeding tasks, it should adjust in response to a constraint on vocal communication. I tested this prediction on my two study species, using a similar protocol: a short-term increase of the natural background noise at the nest during incubation. In dippers, I used a playback of the water stream noise each pair experienced at their nest sites and conducted an amplification of the background noise (+8 DB SPL on average) around the nest. This treatment mimicked a raise in water level and noise following rainy days. It started in the morning and lasted 6 hours (chapter 3). In domesticated zebra finches, I used a playback of wind noise in the vicinity of the nest that mimicked a windy day (+5 DB SPL increase). The treatment started before night and continued during the recording session (chapter 4). A similar protocol did not result in the same behavioural responses in both species.

In zebra finches, although duets between mates were shortened, pairs increased their effort of communication at the nest by increasing the number of meeting duets, which can be interpreted as a redundancy strategy. In addition, they continued duetting during incubation reliefs. In dippers, pairs decreased their vocal activity (fewer female-male vocal sequences at the nest, fewer female call sequences and songs).

Diverging responses to noise: diverging breeding strategies between species?

We can interpret diverging responses to the playback regarding the social systems of the two study species: two-sex vs. single-sex incubation. In zebra finches, both sexes incubate the eggs. Failing a relief and leaving the nest unattended may have serious impacts on the hatching success. Effective information transfer may be required to successfully achieve incubation share. Recently, relief duets have been shown to carry

important information about incubation sharing between partners (Boucaud et al., 2016b). As a consequence, in response to the noise playback, partners benefited from continuing duetting. By adjusting their duets to the noise constraint, they maintain the information transfer necessary to incubation sharing. In dippers, only females incubate the eggs (see section below). In chapter 2, we showed female incubation behaviour relates only to the structure of female vocal sequences and not female-male vocal sequences at the nest. Females also forage by themselves. A decrease in vocal activity during incubation, as observed during the playback, may not significantly impact incubation efficiency in a single sex incubation species.

In the sections below, I discuss what we recently learnt about the functions and communicative value of vocal behaviour at the nest in zebra finches and in dippers.

B. In zebra finches, duetting at the nest allows coordination of incubation shifts

In chapter 4, we showed that visit and relief duets were not changed the same way by the noise playback and it may stress that these duets have different functions during the breeding season. Indeed, contrary to visit duets, relief duets were performed with more precision in noise than in control. Increasing precision in vocal interactions is a strategy used by zebra finches in response to other constraints on their social communication. For example, a separation-reunion protocol showed that in absence of visual contact, non-breeding partners also increased the precision of their call exchanges (Perez et al., 2015). Recently, we published an experimental study testing the hypothesis that relief duets allow the sharing of incubation duties between partners. In this experiment, Boucaud et al. (2016) delayed the return of the male to his nest to take his turn on the eggs. The treatment thus artificially increased the duration of the female's incubation shift. This delay impacted the next duet: when the male was late, the subsequent duet was shortened. This in turn impacted the duration of the next incubation shift: the shorter the duet, the longer the next male incubation shift. Interestingly, the best predictor of this behaviour was the male calling rate during the duet: the more the male called, the shorter his following incubation bout. This study showed the first experimental evidence of a 'vocal negotiation' over parental care. This function could explain why, during our experiment, partners showed an increased care in the production of relief duet in noise ('precision

strategy’). We also showed that in noise, partners produced more visit duets (‘redundancy strategy’) and the outside partner came closer to the nest during sentinel duets (‘proximity strategy’). Interestingly, the three categories of duets showed different strategies in response to noise and may reflect different functions: coordination of incubation (relief duets), contact and pair bond maintenance (visit duets), contact and nest defence (sentinel duets (Mainwaring and Griffith, 2013)).

We also found a high asymmetry in the number of visit duets initiated by the female or the male: males initiated visit duets more often and this result was also found in a recent study in the wild (Boucaud, Perez, Griffith, & Vignal, under review). One hypothesis not discussed in chapter 4 is that visit duets may be failed relief duets. This phenomenon can be compared to what was observed in ring doves, *Streptopelia risoria* (Ball and Silver, 1983). In this species both partners also incubate the eggs and incubation shifts may last several days before they exchange. It was observed that while females were incubating, males usually visit several times before the visit ended by a relief. The opposite was not observed. Additionally, preventing males from visiting between the usual times of nest reliefs did not alter the timing of the next incubation shift. Meaning that in this species, visits from the male may be additional behaviour not related to the coordination of incubation shifts. During our experiment, all our birds were equipped with RFID pit-tags. On a subset of pairs, we followed nests/feeders trips of partners (from the beginning of the treatment the day before, to the end of the recording session the next morning).

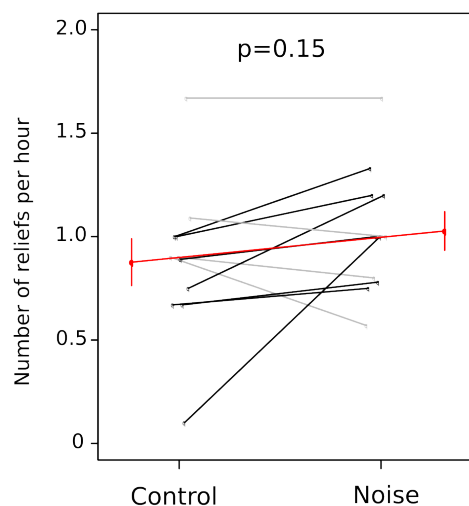


Figure 9: Effect of noise playback on the number of reliefs per hour (data from RFID pit-tags, counts of detection at the entrance of nest-boxes and at feeders). Paired t-test.

We found no effect of the treatment on the number of reliefs per hour (fig. 9) meaning that the organization of incubation shifts is unlikely to have changed in response to the noise playback. We can conclude that, visits were not failed attempts of nest relief. Similarly to male nest visits in ringdoves, male visits (and so visit duets) in zebra finches are independent from reliefs (and so relief duets). This confirms that visit duets may function in contact or pair bond maintenance during periods in which mates are separated.

In zebra finches, we accumulated correlational and experimental evidence and we start to understand (1) the functions of the different vocal interactions between mates during breeding and (2) how these interactions allow the coordination of breeding tasks. The zebra finch is a fantastic study species on which we can easily experiment, however, partners establish a strong pair bond (Zann, 1996), compare to other species of songbirds and the year-round and long-life social system is particular. Studying more species, exhibiting different social systems would allow us to draw more general conclusions on how vocal communication participates in partners' coordination during the breeding season.

C. In dippers, functions of female and female-male vocal sequences at the nest remain to be tested

a. Hypotheses on the functions of female-male vocal sequences at the nest in dippers

In dippers, the vocalization types, their structure and use were not described before this project. My work on acoustic communication between mates in this species brings correlative evidence of their functions. In chapter 2 and 3, we saw that different types of vocal sequences produced at the nest (1) were linked to different behavioural events; in particular their structure was not linked to the same behavioural parameters. I discuss below to what extent vocal sequences may be used in interactive communication between mates or considered as signals produced for other purposes.

To what extent mates communicate at the nest in dippers

In chapter 1, we visually monitored partners' behaviour around the nest during incubation and brooding, two stages in which females stay in the nest most of the time but males sometimes visit them. These visits always led to a female-male vocal sequence. During my field observations, males sometimes fed their mate or chicks during visits

(although the conditions were not optimized for detailed observations of behaviours inside the nest). Engstrand, Ward, & Bryant, (2002) also reported mate provisioning during male visits (23% of 81 observed visits, although less obvious feedings may have occurred). So female-male sequences are likely to be either simple visits or mate feeding events. Mate feeding or nest reunions have been interpreted as honest signals of parental investment (Korpimäki, 1989; Lyon and Montgomerie, 1985). The temporal organization and composition of female-male sequences also changed with the breeding stages (chapter 1), which may reflect that they play a role during parental care activities.

During my field observations, incubating or brooding females were rarely seen interacting with their mate located few meters away from the nest (chapter 1). However we found positive correlation between the daily number of female-male vocal sequences and the daily numbers of female calls sequences or female song (chapter 2). Females may stimulate male visits using calls or songs, as seen in northern cardinal (Halkin, 1997) or New-Zealand bellbirds (Brunton et al., 2016). In great tits, showing sex roles of partners similar to dippers, the structure of vocal exchanges between mates depends on the context of interaction. Exchanges were longer when followed by a mate feeding in the nest or a female exit, and these longer exchanges signal the needs of the female (Boucaud et al., In press, 2016a). In dippers on the contrary, female call sequences were shorter when followed by a male visit or a female exit. Two hypotheses can be raised: either female call sequences have similar functions in dippers and in great tits (coding physiological needs) but the encoding is different between the two species (needs are coded in different acoustic parameters), or females calls in dippers have another function. Because physiological needs are often coded with longer calls and or longer bout of vocalizations (Leonard and Horn, 2001), the second hypothesis seems probable.

Great tits and dippers show similarities in the acoustic structure of the calls most commonly used by females in the nest: dippers' Trill calls and great tits' chattering calls (fig. 10). Both were recorded only inside the nest. Both are broadband compared to syllables used in songs, and are produced in bouts, i.e. series with short inter-call interval. Similar acoustic structure may reveal similar functions. For example, distress calls of several species of birds share similar acoustic features and playback experiments also showed similar decoding processes (Aubin, 1991). Here, we can hypothesize that Trills and bout of chattering calls are involved in similar functions associated to nest activities, which explains a convergence in their structure. As pair communication at the nest in

birds may be more common than already described, it would be interesting to record more bird species showing similar sex roles during incubation to compare the structure of nest calls.

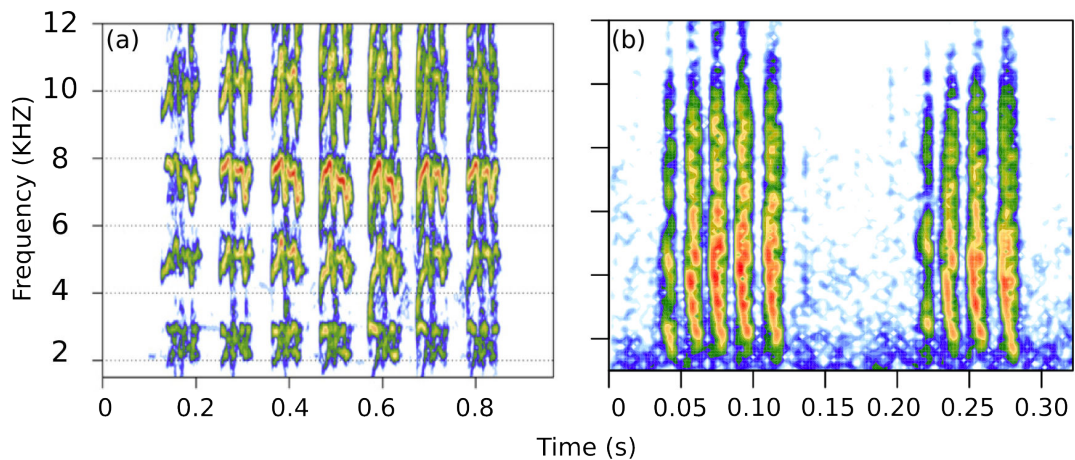


Figure 10: Spectrograms of female calls at the nest in (a) great tits : bout of chattering calls (adapted from Boucaud et al, 2016) [one call is one pulse] and in (b) dippers: two Trill calls [one call is a serie of pulse]. Color scales are different.

Female calls may also be linked to characteristics of female incubation behaviour. Indeed, the number of female exits tended to be correlated to the number of female calls sequences per day, and the Note/Trill composition of female calls sequences was linked to incubation periodicity patterns (chapter 2). Taking all these results together, we hypothesized that females may signal their trips in/out of the nest to their mate, perhaps to stimulate his nest-defence behaviours during her absence (Yasukawa, 1989). The use of playback experiments may allow us to test this hypothesis: female calls sequences should attract the mate around the nest and/or increase his nest-defence behaviours.

Female sequences: territorial or motivational sequences?

If not directed to their mate, female calls and songs may also be territorial signals. In red-winged blackbirds, two types of female songs were identified and associated to different contexts: one type addressed to the male and another to rivals (Beletsky and Orians, 1985; Catchpole and Slater, 2008). In dippers, close to the nest, we could use playbacks of mate vs. non-mate songs or female intruder vs. male intruder songs and study female responses. Female sequences (calls and songs) may also function as self-motivational sequences (chapter 2). In ringdoves, it has been shown that preventing females to vocalize decrease their sensitivity to male courtship (Cheng, 1992), showing that vocalizations may be important not only for communication but also for motivation.

Incubating female dippers may use vocalizations as self-reward during this energetically costly stage.

With female calls sequences, we face the problem of having one category of vocal sequence that could reflect several behavioural contexts (calling alone *vs* calling with the mate) and we may need to find other acoustic proxies to disentangle different contexts. Because of the river noise, we cannot count on detecting male vocalizations produced outside the nest. Female calls sequences are composed both of flight calls and Trill calls. If flight calls are produced around the females' nest trips (exit or entrance), we could subset female call sequences into two subcategories: call sequences with at least one flight calls *vs* call sequences without any flight call. Since sequences with more Notes (flight calls in that case) were linked to periodicity patterns, we may strengthen some relationships we found.

d. Measuring parental activities at other stages of the breeding season in dippers

Chicks feeding

In this thesis, we focused on incubation to study vocal production at the nest and link vocal and incubation behaviours. However, in dippers, incubation is a particular stage during which sex roles are very different. In order to have better proxies of parental activities we could use other stages in which parents have supposedly symmetrical roles: the post hatching period. Several measures of parental activities have been taken in our dippers population during the 2014, 2015 and 2016 breeding seasons that are not included in this thesis. In particular, chicks feeding rates by parents were monitored using video cameras. Post hatching parental care in dippers has not been described yet. Measuring parental care through chicks feeding may be of importance to describe both parental investment and coordination between partners. Do females and males participate equally in feeding the chicks? Do partners synchronize their feeding visits?

Preliminary results show that partners have positively correlated feeding rates (fig. 11). Further analyses (2015 and 2016 breeding seasons) should help better describe the temporal occurrence of female and male feeding events and the coordination between them. In addition, dipper parents clean faeces from the nest and it might be an interesting measure of parental care.

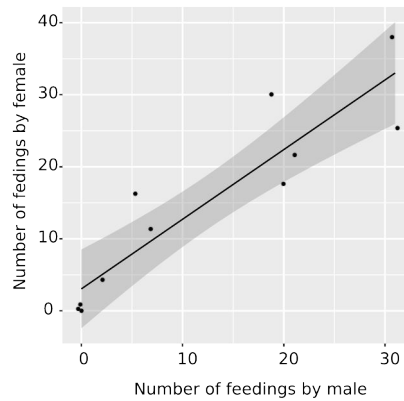


Figure 11: Preliminary results on the relationship between female and male number of chicks feeding visits. Points are numbers of events over two hours of video recording, in the morning (start between 9:00 to 12:00), at 12 ± 1 days post hatching. Only 11 pairs composed this data set from 2014. Three pairs did not come to the nest during the video recording, this might be because recordings were done late in the morning, when less visits occur. Model regression line and 95%CI, $R^2=0.87$, $\rho_{\text{spearman}}=0.92$.

Pair's response to predation threat

During the 2015 and 2016 breeding seasons, experiments were also carried out to test parental response to predators. Dummy blue jays were used to mimic the presence of a nest predator close to the nest. Do both partners participate to nest defence? Do pairs differ in their response to a predation threat?

Adding several measures of parental behaviour would allow us to build scores of pair behaviour during breeding. To go further we would test for relationships between these scores and pair vocal behaviour during incubation. Ultimately, we would answer the question: Does vocal behaviour during incubation reflect parental investment in future stages of breeding?

To have a better idea of the functions of vocal signals at the nest in dippers, future studies should experimentally manipulate parental investment using for example manipulations of clutch size or female energetic expenditure during incubation (by heating the nest to modify nest attentiveness).

D. Linking vocal behaviour at the nest and breeding success

a. Communication, coordination and breeding success

In species providing bi parental care to their offspring, coordination between partners may be an important factor explaining the breeding success of a pair. An efficient

incubation (high nest attentiveness) may shorten the period of incubation and increase the body condition of the chicks (Lyon & Montgomerie, 1985). In species in which both partners incubate the eggs, failing an incubation relief, leaving the eggs unattended may have serious impacts on the hatching success. During the post hatching period, coordination of parental care may also be crucial: for example, Shen et al (2010), showed that in joint-nesting Taiwan yuhinas, *Yuhina brunneiceps*, adults visit the nest to feed nestlings mainly in groups and rarely in solo. When large groups of adults came to feed, fewer chicks begged and food was more evenly distributed among nestlings that when smaller groups of adults came to feed. Synchronization of visits may thus reduce competition between siblings and decrease the occurrence of conspicuous begging calls (Shen et al., 2010). Synchronizing nest trips has been shown to avoid being detected by a potential predator (Martin et al., 2000; Raihani et al., 2010). In several species, the degree of coordination between parents correlated with the breeding success of the pair (Mariette and Griffith, 2012; Raihani et al., 2010; Shen et al., 2010). Mariette, et al., (2015) demonstrated experimentally in zebra finches the adaptive significance of synchronizing nest visits and foraging trips. Using clutch size manipulations, authors artificially manipulated parental investment. They showed both partners increased their workload in response to the treatment, synchrony at the nest was best explained by the number of nestlings in the nest and nestling mass was best explained by foraging synchrony. Communication between mates is a good candidate to achieve coordination (and this is our working hypothesis) but so far, no published data have linked acoustic communication between mates and reproductive success.

In zebra finches, we have strong evidence that vocal communication between mates at the nest during incubation plays a key role in coordinating incubation bouts, the next step would be to test whether parameters of vocal communication are predictors of the breeding success of a pair. In chapter 4, we used a within pair design to quantify changes in vocal communication at the nest in response to a noise constraint. Intensity of changes in duet features may reflect partners' abilities to vocally negotiate and influence the breeding success of pairs. As a preliminary test (18 pairs), I statistically tested, on relief duets, whether degrees of responses to the treatment are linked to the hatching success of pairs, but I found no relationship between playback response intensity and hatching success. My sample size was low to test this hypothesis and a study of breeding success may be more relevant in natural settings. Using a similar experimental design as in

Mariette et al. (2015), it would be interesting to link reproductive effort, communication and breeding success.

During October and November 2015, I carried out an experiment on wild zebra finches at Fowlers Gap field station (Australia) (see further sections below). I recorded pairs during incubation on two days after a clutch size manipulation. Half of the nests had three eggs, and the other half had seven eggs. If communication at the nest allows coordination of incubation, we predict that partners should adjust their vocal communication in response to clutch size manipulation. The analysis of these duets is still in progress.

b. Preliminary results: vocal production at the nest and breeding success in dippers.

During my PhD project, in 2015, the vocal behaviour of dippers at the nest was monitored on 36 pairs, with a total of more than 1400 vocal sequences (chapter 2). In parallel, breeding data were collected. As a preliminary analysis, I calculated simple proxies of the breeding success: success vs. failure (success = rearing at least one fledgling) and the number of fledglings (normalized by clutch size, normalized number of fledglings = number of fledglings / clutch size). I correlated these proxies with proxies of vocal production at the nest during incubation (identical to those used in chapter 2 and 3): daily number of each type of vocal sequence (female calls sequence, female song and female-male vocal sequences) and proxies of their structure (SeqPC1, SeqPC2).

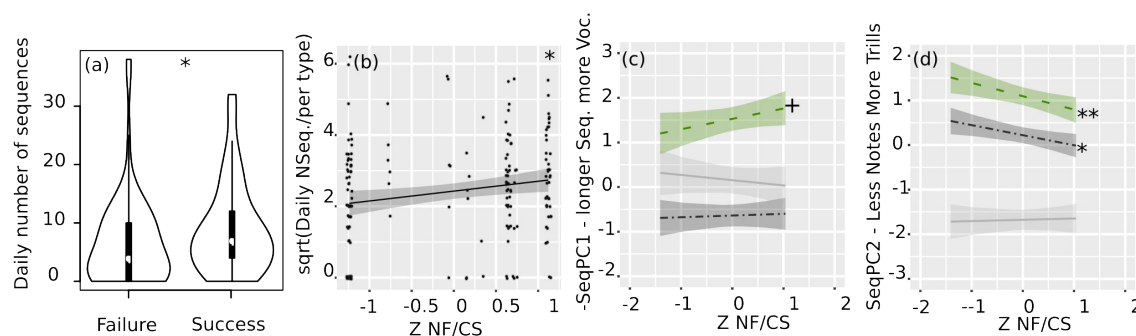


Figure 12: Preliminary analysis of the links between vocal behaviour at the nest and reproductive success in dippers. Relationships between the daily number of vocal sequences and (a) success vs failure of rearing at least one fledgling or (b,c,d) the normalized number of fledglings (scaled 'Z' NF/CS = number of fledglings / clutch size). (a) violin plot (median, first and third quartile, min, max and distribution contours), (b) raw data and linear estimates [95%CI] (c,d) linear estimates [95%CI] of proxies of vocal sequence structure in each sequence type (female calls: unregularly dashed black lines, female song: solid light grey lines and female-male vocal sequences: regularly dashed green lines). Post hoc tests on slopes and factors were performed with 'Hommel' and 'Tukey' corrections respectively. +: $P < 0.10$, *: $P < 0.05$, **: $P < 0.01$.

Successful nests had significantly more vocal sequences of all types (LMM, success vs. failure: $X_1=5.5$, $P=0.02$, fig. 12a, no interaction with sequence type: $X_1=2.9$, $P=0.23$). A significant positive correlation was found between the normalized number of fledglings and the daily number of vocal sequences (LMM, ZNF/CS: $X_1=4.2$, $P=0.04$, fig 12b, no interaction with sequence type: $X_1=2.3$, $P=0.31$). Proxies of the temporal organization and composition of sequences (SeqPC1 and SeqPC2) were not linked to the success vs. failure of breeding. However, in nest with higher number of fledglings, female-male vocal sequences tended to be longer (LMM, post hoc test on slope following significant interaction with sequence type, $X_1=2.9$, $p=0.08$, fig.12c) and were showed higher Notes/Trills ratio (post hoc test on slopes following significant interaction with sequence type: $X_1= 8.8$, $P=0.003$, fig.12d). Contrary to female-male sequences, female calls sequences did not change in duration with the number of fledglings (LMM, post hoc test on slope: $X_1=0.09$, $P=0.75$, fig. 12c) but showed higher Notes/Trills ratio (LMM, post hoc test on slope: $X_1=5.2$, $P=0.03$, fig 12c). These preliminary results show that the more the pair vocalizes at the nest, the higher the breeding success. Furthermore, longer sequences with higher Notes/Trills ratio may be linked to higher reproductive success. Contrary to Brunton et al., (2016), we found no relationship between the structure of female song from the nest and the number of fledglings (fig 12c and d). However, we did not measure the same parameters on songs: syllable diversity and number of transitions score significantly explained the number of fledglings (Brunton et al 2016), parameter we did not measure yet.

This emphasizes that different vocal sequences produced at the nest during incubation in dippers may fulfil different functions. In particular, female-male sequences, sometimes associated to mate feeding during visits (Engstrand et al., 2002) had a stable structure in chapter 2 and 3 and showed here significant changes. These preliminary results are in line with the hypothesis that mate feeding and vocal communication associated with male visits may play a role in the breeding success of a pair (Klatt et al., 2008; Lyon and Montgomerie, 1985; Matysioková and Remeš, 2010).

These preliminary results need to be confirmed with more controls (hatching date for example) and we need to disentangle effects of vocal behaviour from intrinsic characteristics of the parents (biometry, body condition) or variation in their social characteristics as a pair (occurrence of extra-pair paternity for example). They are nonetheless promising for further analyses.

3. Consequences of high noise levels on breeding birds: the dipper case

A. Advantages of studying noise impacts on breeding birds in dippers

In chapter 3, we studied the properties of the environmental noise (both amplitude level and frequency range) at nest sites in breeding dippers. We found that natural nest sites in open areas (close to waterfalls, or under vegetation roots close to the water), natural nest sites under bridges and nest boxes under bridges showed similar properties of environmental noise: the noise level at the nest reached high sound pressure levels (measures from 53 nest sites: 55 DB SPL to 90 DB SPL), the noise level was higher at the nest than in other part of the territory (on average +8, +6, +13 DB SPL when comparing noise level along the river and noise level around the nest respectively for nest boxes, for natural nest sites under bridges and natural nest sites in open areas). This allows us to draw two conclusions.

First, nest boxes do not significantly modify the acoustic environment of natural nest sites. This was important for our study, so that our conclusions can be generalized to the population.

Second, dippers have particularly loud nest sites compared to other species. Urban noise typically ranges from 55 to 65 DB SPL from 30 to 0 km from city centres [example according to measurements at Phoenix Arizona (Warren et al., 2006)], although specific industrial areas can reach higher sound pressure levels. Chronic noise exposure protocols tended to mimic similar sound pressure level and treatments generally range from 60 to 65 DB SPL, for example in (Leonard et al., 2015): experimental nests were at about 65 DB SPL (for nests initially ranging from 41 to 67 DB SPL in the field), in (Meillère et al., 2015), experimental nests were at about 63 DB SPL (compared to 43 DB SPL in controls). Natural environmental noise levels at dipper nest sites offer higher ranges of variations and higher absolute sound pressure levels between nest sites than the ones measures in cities or mimicked during published experimental treatments. Dippers are thus interesting model systems to address questions on the impacts of elevated noise on breeding birds in natural habitat. In addition, river noise is typically a pink noise, with higher energy in low frequencies, and it is downshifted inside the nest. The natural properties of the background

noise at the nest thus tend to mimic low frequency noise of urban habitats. This might offer an opportunity to test hypotheses generally tested in urban areas in a more natural setting.

B. Consequences on female and female-male vocal production at the nest

a. Adaptations and adjustments of vocal signals to noise characteristics

As dippers vocalize in a naturally noisy habitat, we had two predictions: either vocal signals are constantly above the constraining aspects of the noise (louder, with a frequency range outside noise spectral properties) or birds adjust when necessary their vocalizations to the changing properties of the background noise. We showed that birds responded to noise amplification by increasing the amplitude of their vocalizations (Lombard effect) and changed the spectral shape of their Notes by producing Notes closer to pure tones. Birds could use the same categories of Notes that change in structure, or birds could switch to different note types in response to noise, as it has been shown in great tits (Halfwerk and Slabbekoorn, 2009). Because the response lasted after the end of the playback perturbation, vocal flexibility may play a key role in shaping the structure of vocal signals in dippers. However, we found no evidence of change in the frequency composition of Notes and Trills, perhaps because the frequency range of these calls is already optimized to the acoustic characteristics of the habitat. All vocalizations in dippers have a frequency range above the frequency range of the low frequency environmental noise, which is in favour of the 'Acoustic Adaptation Hypothesis' (Morton, 1975). We found that this spectral composition do not show any flexibility in response to noise modifications. This might reveal physiological constraints on vocal production in the species.

During my thesis, I mainly studied responses to noise on the sender side. On the receiver side, selective pressures may have shaped the auditory system in dippers. Although the signal-to-noise ratio is the limiting criterion for communication, birds' auditory system may adapt to background noise. For example, urban and non-urban populations of birds have different auditory thresholds (Patricelli and Blickley, 2006; Pohl et al., 2012). Neuronal filters in the auditory system could be sharpened to adapt to the spectrum of the local background noise (Nieder and Klump, 1999). Several adjustments of the auditory system may lead to precise detections in noisy environments. For example, in king penguin, chicks are able to recognize their parents' calls despite the noisy condition of a breeding colony and

detect calls with negative signal to noise ratio [‘cocktail party effect’, (Aubin and Jouventin, 1998)]. It is possible that similar mechanisms occur in dippers.

b. High noise levels may constrain vocal communication at the nest

Interactive communication at the nest between mates has been studied in only few species [zebra finches (Boucaud et al., 2016b; Elie et al., 2010; Gill et al., 2015), great tits (Boucaud et al., 2016a; Gorissen and Eens, 2005; Gorissen et al., 2004; Halfwerk et al., 2011a), blue tits (Gorissen and Eens, 2005)]. Contrary to zebra finches, tits and dippers present a similar social system (asymmetrical roles during incubation) and we can compare forms of vocal communication between mates in these two species. In great and blue tits, vocal exchanges between mates start with exchanges of female calls (from the nest) and song or call from the male (outside) and this sometimes leads to a male visit (Gorissen & Eens, 2005; Boucaud et al., 2016). However, in dippers, the vast majority of female-male vocal interactions occurred when the male visited the female at the nest without vocal exchanges before the male’s entrance (87% of interactions, chapter 1). In addition, in great tits, vocal exchanges often involved several bouts of vocalizations produced by both partners (Boucaud et al., 2016). In dippers, the rare vocal exchanges that occurred with the female inside the nest and the male outside involved one bout of vocalizations per partner, either a song or calls from both partners (pers. observations): one partner starting and the other answering once. Although more data are needed, one hypothesis we can raise is that the number of vocal interactions at the nest in dippers is limited by the extremely constraining environmental background noise.

Within our dipper population, we showed that although the same daily numbers of vocal sequences were produced in quiet and loud nest sites, female sequences (calls and songs) were shortened when produced at louder nest sites (chapter 3). If produced in interaction with their mate, shortened sequences may stress an impairment of communication between mates, similarly to what we showed on sentinel duets in zebra finches (sentinel sequences produced in noise were shortened, chapter 4). In addition, short-term experimental noise amplification had a major impact on the vocal activity at the nest (chapter 3): fewer vocal sequences of all types per hour, so pairs avoided vocalizing during the perturbation. Pairs may suffer from the metabolic cost of singing/calling louder (Brumm and Zollinger, 2011; Oberweger and Goller, 2001) or may counteract potential predation costs of conspicuousness (Kleindorfer et al., 2016) by shortening their sequences or avoid vocalizing when the noise level is too high.

C. Preliminary results: noise levels and breeding success in dippers

a. Noise level at the nest impairs reproductive success

In chapter 3, we showed that dippers chose noisy nest sites. Birds might select their nest site on their acoustic characteristics, or the acoustic characteristics might be by-products of other advantages provided by the site (protection from disturbances, food supply close to the nest). An analysis of the reproductive success might be useful to answer this question. Using data from the monitoring of the 2015 and 2016 breeding seasons and noise recordings at the nest, I tested whether the breeding success of dippers' pairs depended on the noise level at their nest site. Breeding success was measured using 3 proxies: the success in rearing at least one fledgling (success *vs.* failure), the number of fledglings (normalized by the initial clutch size) and the body condition of the chicks at 12 days post hatching.

First, results from generalized mixed effect models showed that dippers nesting at loud nest sites were less likely to succeed in rearing at least one fledgling (effect of noise levels on factor success *vs.* fail: $X_7=8.7$, $P=0.003$, fig. 13a), but no effect was found neither of the clutch size, the breeding attempt (first or second brood in the season) or the hatching date, all included as controls in the model ($X_7=0.27$, $P=0.60$, $XI=0.34$, $P=0.55$, $X_7=0.54$, $P=0.46$ respectively).

Second, model selections were computed to test whether the noise level was a good predictor of the normalized number of fledglings. I used the following variables in the model: clutch size, number of hatchlings, hatching success, breeding attempt, noise level. Hatching success and number of hatchlings were highly correlated ($\rho=0.82$), so only the hatching success was kept in the final model. Three predictors of the normalized number of fledglings were found: hatching success (the higher hatching success, the more fledglings, Akaike weight= 0.51), noise level (the higher the less fledglings Akaike weight= 0.51) and the interaction between hatching success and noise level (the higher the less fledglings, Akaike weight= 0.28) (see estimates of selected models table 3 and fig. 13b) So the noise level has a negative impact on the number of fledglings reared by a pair. Since the interaction between hatching success and noise level was also a predictor, the noise level may impact the number of fledglings through a decrease in hatching success. In that case, incubation may be a crucial stage and acoustic communication between mates a potential mechanism impacted by noise.

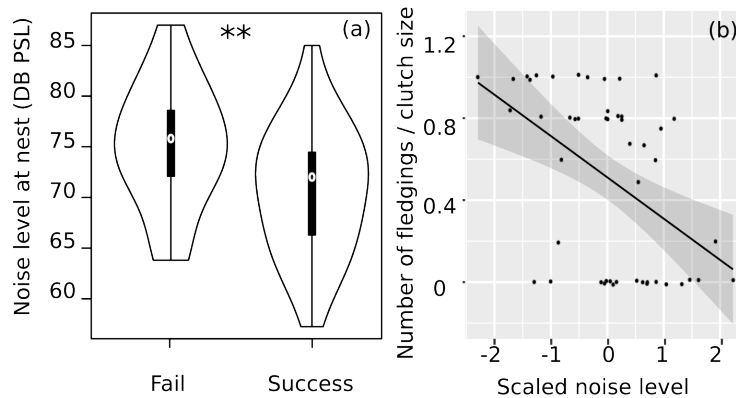


Figure 13: Noise level at the nest and breeding success in dippers. (a) Violin plot (median, first and third quartile, min and max) of the noise level depending on the success of a breeding attempt (success= at least one fledgling reared) [49 nests in total]. (b) Data points and model estimates with 95%CI of the effect of noise level on the number of fledglings per nest normalized by the clutch size (NF/CS ratio), estimates of the full model before model selection [177 chicks over 46 nests in total].

Table 3: Model selections. Only best models are presented (i.e delta AIC<2) and null models. For each model selection, the coefficient of determination of the full model is provided (R2c). Estimates of predictors in models are indicated in parentheses.

	Intercept	Model formula	DF	LogLit	AICc	Delta	weight
Response: NF/CS ratio [R2c=0.51]							
1	2.53	Zhatching success (1.15) + ZmeanDB (-0.89)+ Zhatching success: ZmeanDB (-0.41)	5.00	-87.34	186.07	0.00	0.28
2	2.53	Zhatching success (1.14) + ZmeanDB (-0.95)	4.00	-88.81	186.53	0.46	0.22
Null	2.53		2.00	-106.12	216.50	30.44	0.00
Response: chicks BCI [r2c=0.54]							
1	-0.17	Zhatching success (0.18)	4.00	-136.40	281.11	0.00	0.27
Null	-0.18		3.00	-137.92	282.02	0.91	0.17
3	-0.16	Zclutch size (0.01) + Zhatching success (0.21)	5.00	-136.21	282.91	1.80	0.11
4	-0.16	Zhatching success (0.17) + ZmeanDB (0.06)	5.00	-136.24	282.96	1.85	0.11

Finally, the body condition index of the chicks (BCI) was measured using the residuals of the linear regression between mass and tarsus length (method used in chapter 5). Model selections were computed to test which of the following variables were predictors of the BCI: clutch size, number of fledglings, number of hatchlings, hatching success and noise level. The hatching date could not be added due to missing values that would reduce the dataset. The number of hatchlings was highly correlated to hatching success ($\rho=0.72$) and was removed from the analysis. The number of fledglings and the noise level were also highly correlated ($\rho=-0.59$), so only the noise level was kept. The final model contained the noise level, the clutch size, the hatching success and interactions between the noise level and the two latter variables. No good predictor of the BCI was found using this set of parameters, since the null model was selected among the best models (table 3). Nesting in loud places in dippers may not impact the body condition of the nestlings at 12 days post-hatching. Other parameters may explain the BCI of the chicks (parental care, like feeding rate for example)

b. Several factors may explain lower breeding success in noisy nest sites

Several studies investigated impacts of noise on breeding birds, most of the time in response to anthropogenic noise. Urban noise has fitness costs: lower mating success (Habib et al., 2007), reduced clutch size (Halfwerk et al., 2011b), fewer young with lower body condition (Schroeder, Nakagawa, Cleasby, & Burke, 2012; Kight, Saha, & Swaddle, 2012). Our results are in line with some of these results: pairs living in louder nest sites reared fewer young. Both behavioural and intrinsic explanations may explain this result.

Changes in female incubation behaviour in noise

First, as shown by several authors, noise (anthropogenic noise in particular) may be similar to a predation threat, which may explain several behavioural changes in response to noise. Experimental studies showed higher vigilance in response to noise: great tits (Klett-Mingo et al., 2016), or chaffinches (L. Quinn et al., 2006) spent less time foraging in noise. Changes in time allocated to vigilance or foraging may have fitness costs: animals increasing vigilance may end up with lower body condition. At the nest, chronic noise exposure in house sparrow, showed that during breeding, parents also increased their vigilance: they flushed from the nest at further distances when disturbed (Meillère et al., 2015). However authors did not find any effect of their treatment on breeding success. In our case, we saw that hatching success explained the number of fledglings. Hatching

success may be linked to female incubation behaviour. For example, an increased vigilance may lead to lower nest attentiveness. I tested whether female incubation behaviour was different at quiet and loud places.

No relationship was found between noise level at the nest and the daily number of female exits ($XI=0.28$, $P=0.59$, fig.14) or parameters of incubation periodicity [ln(period): LMM estimates [95%CI] = -0.01 [$-0.04:0.01$], $XI=0.77$, $P=0.38$; ln(periodicity strength): 0.02 [$-0.16:0.19$], $XI=0.03$, $P=0.85$]. So either there is no effect of noise level on incubation behaviour or our behavioural measures do not reveal the effect. Developing another way to monitor female incubation behaviour more precisely would be helpful. At the end of the season 2015 and in 2016, adults were caught before they start breeding and were equipped with RFID pit-tag rings. Coupling nest temperature recording with RFID detections at the entrance of the nest will allow us to have a precise measure of female nest trips and nest attentiveness.

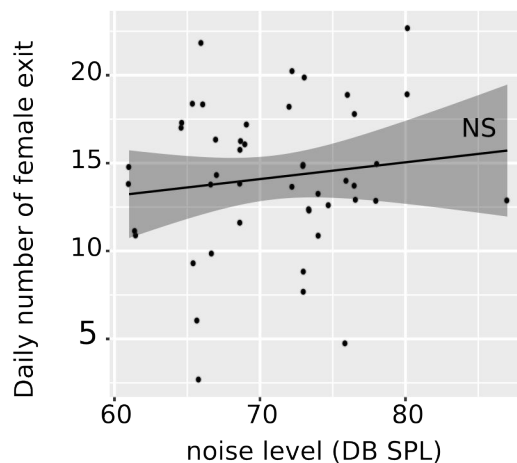


Figure 14: Relationship between noise level at the nest and the daily number of female exits from the nest. Data collected from 22 nests on a total of 46 recording sessions (LMM: estimates [95%CI] = 0.07 [$-0.19:0.33$]).

Disruption of pair communication at the nest

Disruption of pair vocal communication in noise may also lead to a decrease in breeding success. In great tits, an experimental noise exposure decreased female sensitivity to her mate's low frequency songs and females exited the nest less often before laying, which may impact breeding success (Halfwerk et al., 2011). Dippers pairs nesting in loud habitats produced louder vocalizations (chapter 3). Since vocalizing from the nest may have predation costs (Kleindorfer et al., 2016), it is possible that noise dependent

vocal adjustments increase predation rate. However, these pairs also changed their vocal activity: females produced shortened sequences and this may counteract potential predation costs of vocalizing louder. In addition, in section 2Db of this general discussion we saw that some parameters of vocal activity were also linked to higher breeding success (more vocal sequences, longer female-male sequences with more Notes, female calls with more Notes as well). It remains unclear to what extent vocal activity at the nest may drive lower breeding success in louder habitats and more analyses are needed to conclude.

Birds of different 'quality' live in quiet and loud nest sites

Another hypothesis formulated by authors on the effect of noise (again, anthropogenic noise) on breeding birds is that noisier sites may be interpreted as lower quality areas. This hypothesis received some support by a study from (Habib et al., 2007) which showed that birds nesting in louder areas experienced a lower pairing success (77% vs 92%) and were also younger than the ones nesting in noiseless areas. So, experienced individuals avoided noisy nest sites, leaving younger and less reproductive ones in noisier areas (Habib et al., 2007). Because the individuals who occupy quiet and loud sites are different, this represents a confounding factor, which may explain the observed fitness cost of breeding in noisy areas (body condition, for example). A similar mechanism may explain low reproductive success in louder nest sites. To disentangle these effects, it would be useful to assess the body conditions and age of adults. All adults were caught and several biometrical and physiological measurements were performed, as well as an estimation of the age of the individuals. These data were not included in this thesis but the next step is to link body condition of parents and noise data with reproductive success to disentangle the effects of intrinsic characteristics of parents from noise effects to explain reproductive success.

4. Going further in the study of the effects of natural background noise on short range communication

Wind is an abiotic constraint on communication in the zebra finch habitat. Zebra finches breed in colony and calls from conspecific may also constrain the private vocal communication between mates at the nest. Vocal activity from conspecifics may be constraining on two aspects: this background noise with similar frequency range may decrease the signal to noise ratio of mates' calls, but it may also represent a distraction because of the social information it carries. During the 2015 field season, in Fowlers Gap, Australia, we built a two-step playback experiment to disentangle these aspects. We compared acoustic communication between mates at the nest in a control condition (no manipulation of the background noise) and during two different playbacks. One playback consisted of a colony noise. We previously recorded vocalizations of zebra finches in social trees (Zann, 1996). Birds were counted every minute and only recordings with on average 9 birds in the three over 30 min of recording were kept. Songs were removed to build a 10 min playback track (fig 15a,c,d). The second playback consisted in a control noise. The playback track of the colony noise was transformed to keep only the frequency range, the temporal and amplitude variations of the playback but without any social information (a ripple playback (Theunissen et al., 2004b) fig. 15 b,d). The two noise playbacks (colony and ripple) were broadcasted at the same amplitude (58 DB SPL on average vs 35-40 DB in control without wind), 33 pairs were tested for five hours per treatment. On a subset of nests, we monitored partners' behaviour inside the nest box using video cameras to assess changes in vigilance behaviour, nest attentiveness, or incubation sharing between partners (10 pairs).

If the colony noise is simply a constraint on the signal to noise ratio of pair vocalizations at the nest, we expect equivalent responses to both playback conditions. Different responses to the two playback treatments would show that partners are sensitive to some information in the colony noise. Pre-processing and processing of audio and videos recordings are still in progress.

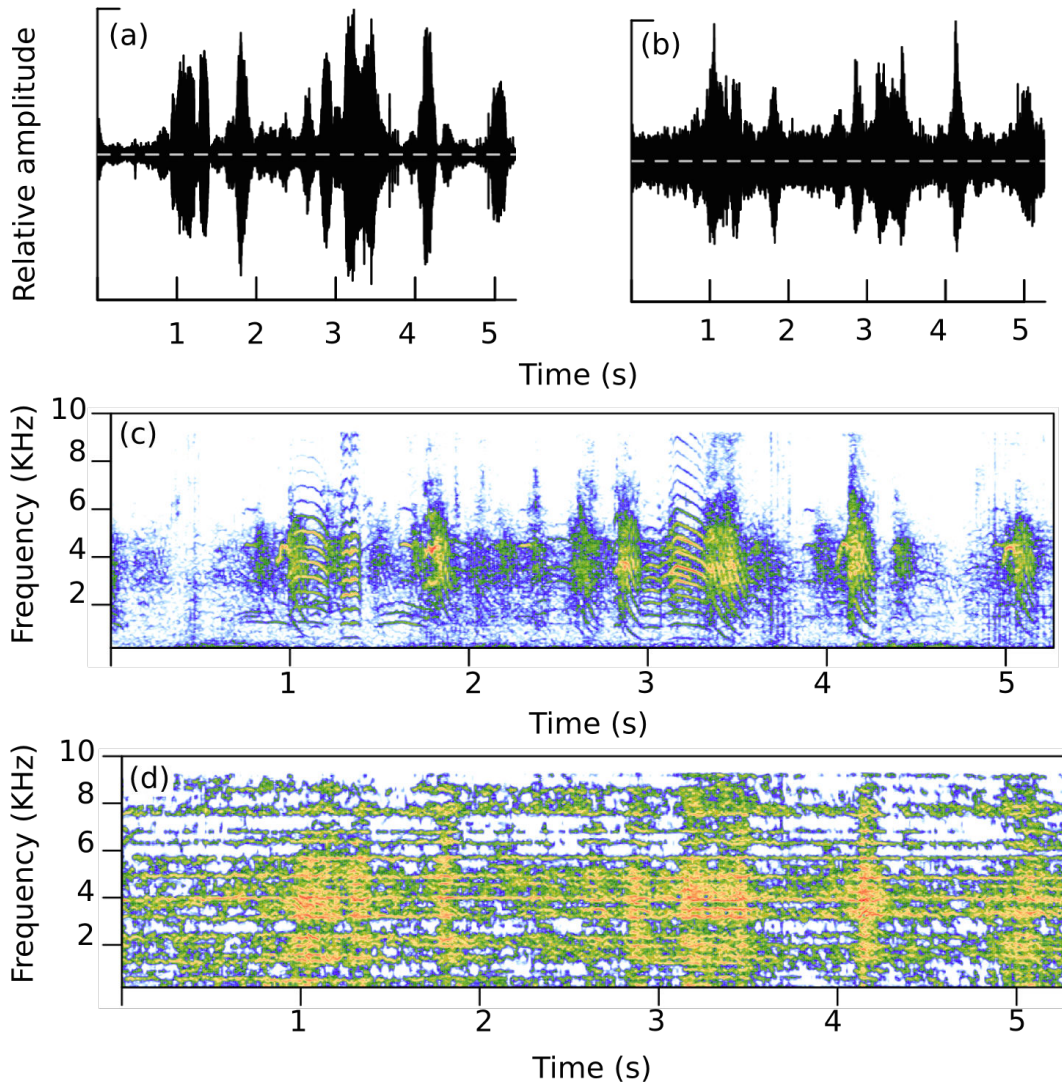


Figure 15: Playback design of the experiment testing the effect of colony noise on pair vocal communication at the nest in zebra finches. (a,b) oscillograms, (c,d) Spectrograms of an extract of colony noise playback (a,c) and ripple noise playback designed from the colony noise extract (b,d). In the ripple noise, the temporal variations and the frequency range of the colony noise are preserved. (spectrograms: $F_s=44100$, $wl=1024$, $overlap=50\%$).

General conclusion

During my PhD, I participated in filling the gap of knowledge on vocal production in female songbirds, studying vocalization types produced by both sexes (calls in zebra finches, calls and songs in dippers) and choosing contexts in which both sexes vocalize (intra pair communication and parent offspring communication). I studied vocal flexibility and vocal plasticity in response to the environment (biotic and abiotic). In the context of intrapair communication at the nest, I showed that both adult females and males displayed flexibility in their vocalizations in response to elevated background noise (even when produced at very short distances, even when the vocalizations are non-learned) or in response to the social context (expression of stress in female distance calls). In the context of parent-offspring communication, I showed that only males expressed developmental vocal plasticity in response to a change in social environment. This may reveal that females either have differential abilities in vocal plasticity or experience different social reinforcement explaining diverging developmental trajectories. I would like to look further into this hypothesis in future work.

Because I studied a species of temperate zones rarely investigated and in which both sexes sing, the white-throated dipper, I had the opportunity to describe for the first time the structure and possible functions of female and female-male vocal signals at the nest. In the framework of cooperation between partners of a monogamous pair, the results of this work bring another evidence that intra pair communication may be of importance during breeding and may drive pair reproductive success. Future work should describe in more details parental care in this species and experimentally test hypotheses on functions of vocal signals during breeding.

The particularly noisy habitat of dippers has rare equivalents in other vertebrate species. This allowed me to address the question of adaptations and adjustments of vocal signals in response to continuously noisy natural habitats. The dipper is a perfect example of species in which highly constraining background noise has shaped the frequency range of vocal signals. Future work on dippers should address the question of the impact of elevated noise on the breeding success, in particular, what are the factors explaining the negative impact I described in this thesis: cost of vocal adjustments in noise? Disruption of intra pair communication? Body condition of the birds? Predation pressure? A better understanding of the effects of naturally noisy habitats on breeding birds will allow a better understanding of the consequences of anthropogenic noise on avian species, and play a key role in the application of future conservation policies.

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Title: Acoustic communication in female songbirds: functions, flexibility and plasticity in calls

The theory of sexual selection has drastically oriented research on acoustic communication in birds: males learn and sing conspicuous songs and females choose. Consequently, (1) female vocal production has been neglected, (2) birdcalls (most bird social communication) have been understudied. Birdcalls were supposed to be non-learned and no effect of the environment was expected on their structure (no flexibility, no learning). I thus focused my thesis on vocal flexibility (short-term) and vocal plasticity (developmental) of female vocalizations (mainly calls). I studied two contexts in which both sexes produce vocalizations: intrapair communication at the nest and parent-offspring communication. Do pairs express vocal flexibility in their calls in response to environmental noise? Is call development influenced by social environment? I studied two species: the white-throated dippers, *Cinclus cinclus*, (in which both sexes produce calls and songs) and the zebra finch, *Taeniopygia guttata*, (in which only males sing but both sexes use the same calls). I showed in both species, that in response to environmental noise, pairs increased the amplitude of their calls or song notes. In dippers, spectral flexibility was observed in song notes but not in calls. However, zebra finch calls showed spectral flexibility in response to noise. Both sexes showed similar changes in their calls: call spectral flexibility is not sex specific. Last, I showed that the structure of male begging calls changed in response to the early social environment, bringing evidence of early vocal plasticity in males. No change was found in females, showing that they either differ in their plasticity abilities or do not express plasticity because they receive different social feedbacks. My work showed that females and males show vocal flexibility but their vocal developmental trajectories may differ. Calls are thus good study objects to investigate sexual dimorphism in vocal behaviour.

Titre: Communication acoustique chez les passereaux femelles: fonctions, flexibilité et plasticité des cris.

La théorie de la sélection sexuelle a drastiquement orienté l'effort de recherche sur la communication acoustique chez les oiseaux: les mâles apprennent et produisent des chants élaborés et les femelles choisissent. Par conséquent (1) la production vocale chez les femelles a été négligée, (2) les cris (la majorité de la communication sociale) ont été peu étudiés. Contrairement aux chants, les cris ont été considérés comme innés et aucun effet de l'environnement sur leur structure n'était attendu. J'ai donc posé la question de la flexibilité vocale (court-terme) et de la plasticité vocale (au cours du développement) chez les femelles, en étudiant les cris majoritairement. J'ai étudié deux contextes où les deux sexes vocalisent: la communication dans le couple au nid et la communication parent-jeunes. Les vocalisations produites au nid par les couples montrent-elles de la flexibilité en réponse au bruit? Le développement des cris est-il influencé par l'environnement social? J'ai travaillé sur deux espèces: le cincle plongeur, *Cinclus cinclus* et le diamant mandarin, *Taeniopygia guttata*. Chez les deux espèces, en réponse au bruit, les couples augmentent l'amplitude de leurs vocalisations. Chez le cincle une variation de la structure spectrale est observée dans les notes de chant mais pas dans les cris. Chez le diamant mandarin, les cris montrent des changements de leur structure spectrale: ils peuvent donc être flexibles en réponse au bruit. Les changements sont similaires chez les femelles et les mâles : la flexibilité n'est pas spécifique du sexe. Enfin, j'ai montré que l'environnement social précoce influence le développement des cris de quémante alimentaire chez le diamant mandarin : il existe une plasticité précoce des cris chez les mâles. J'ai montré que les femelles expriment des degrés de flexibilité similaires aux mâles mais que leur développement vocal peut prendre des trajectoires différentes. Les cris sont de bons objets de recherche pour étudier des variations de comportement vocal liées au sexe.

Discipline : éthologie, écologie comportementale, bio-acoustique

Mots clés : Bruit, Cri, Chant, Monogamie, Cincle, *Cinclus cinclus*, Diamant mandarin, *Taeniopygia guttata*

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