Young Eurasian Scops Owls produce individually distinctive vocalizations

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Abstract. Vocal distinctiveness is expected to occur more often in colonial-breeding species as the parents need to recognize their offspring in a large group of conspecifics. In this study, I examined the food-begging calls of the young of a territorial nocturnal raptor, the Scops Owl, shortly before and after fledging. The calls showed complex spectral structure not described in previous studies. I found a high degree of repeatability in many acoustic features, particularly call duration, spectral centre of gravity, and spectral skewness. Discriminant analysis assigned 52.7% of the calls to the correct individual, and 69.1% when restricting analysis within broods. The calculated information capacity H_S was 4.69 bits. Differences in acoustic features between siblings appeared before fledging and could be detected over up to ten days despite temporal variation. The results suggest that the vocalizations of young Scops Owls could potentially act as identity signals. However, the functional significance of acoustic signatures remains unclear. A few hypotheses are discussed. Call distinctiveness could simply reflect the development of vocal individuality typical of the adults; it could help parents recognize their offspring preventing misdirected parental care when breeding density is high; it could mediate preferential feeding; or it could be used by siblings communicating between each other. Further studies are needed to determine whether parents and offspring assess identity signals in vocalizations.

Keywords: Acoustic signature, food-begging, fledglings, individuality, information capacity, nestlings, Otus scops, repeatability.

Statements and Declarations

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Data availability. Data supporting the results are available on a repository [Link to figshare].

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INTRODUCTION

The ability to discriminate among conspecifics is an important characteristic in many animals and is often required for mate and kin recognition, and parent-offspring communication (Tibbetts & Dale, 2007). Studies have cumulated evidence that vocal distinctiveness is widespread across taxa including birds (Falls, 1982; Lambrechts & Dhondt, 1995; Carlson et al., 2020), and show the importance of distinctiveness in contexts such as mate and kin recognition (e.g. Nakagawa & Waas, 2004; Sharp et al., 2005; Vignal et al., 2008) neighbour-stranger discrimination (Stoddard, 1996), and parent-offspring communication (Beer, 1971; Tibbetts & Dale, 2007). Mechanism of recognition between parents and their offspring has often been found in bird species that breed in dense and noisy colonies, like penguins and seabirds (Buckley & Buckley, 1972; Lefevre et al., 1998; Jouventin & Aubin, 2002). In colonial swallow species, nestlings make individually distinctive calls that parents recognize (Beecher et al., 1981; Stoddard & Beecher, 1983; Loesche et al., 1991; Strickler, 2013). On the contrary, calls of nestlings of non-colonial species show weak individuality (Medvin & Beecher, 1986; Medvin et al., 1992, 1993; Leonard et al. 1997; but see Barg & Mumme, 1994). The observation that parent-offspring recognition is better developed in colonial than in noncolonial species led to the hypothesis that coloniality is a strong selection pressure for the evolution and maintenance of the distinctiveness of calls (Beecher, 1990). In colonially breeding species, the parents must identify their own young among many unrelated ones, particularly after their young leave the nest and become mobile, so that their location is an unreliable indicator of identity. In those cases, the risk of misdirected parental care is high.

Individual variation in vocalizations is a prerequisite for the ability to discriminate between conspecifics provided that visual or other types of cues are not available (Falls, 1982), and when the parents need to find their offspring in a crowded area far from the nest. Features of the signal that is sent out, like its dominant frequency, or duration, must show weak intra-individual variability combined with high inter-individual variability. However, vocal traits often vary along with physical or behavioural conditions of

the individual. To be a reliable indicator of identity, a signal must also be temporally stable, that is, it must remain unaltered along significant time scales (Gilbert & McGregor, 1994; Peake et al., 1998; Lengagne, 2001; Grava et al., 2008; Klenova et al., 2009, 2012). Among *Strigiformes*, vocal signatures have often been found in adult birds (e.g. Eagle owls: Lengagne, 2001; Great horned owls, Odom et al., 2013; Barred owls, Freeman, 2000; Tawny owls, Galeotti & Pavan, 1991; Pigmy owl: Galeotti et al., 1993; Western screech-owls, Tripp & Otter, 2006; Ural owl, Zhou et al., 2020; Northern spotted owl, Fitton, 1991; Eastern screech owl Cavanagh & Ritchison, 1987), suggesting that identity signals are favoured in this group of birds, particularly in the context of territorial interactions and communication with the breeding partner. In contrast with the ample evidence of individuality of adult vocalizations, little is known about individuality in earlier life stages, in owls and other groups in general, with some well-known exceptions, for example in penguins, seabirds, and swallows (Carlson et al., 2020). In owls, the only species where individual variation of juvenile calls has been established is, to date, the Barn Owl (Dreiss et al. 2014). The authors investigated interactions between siblings in the absence of the parents, and found that older Barn Owl nestlings produced less variable and lower-pitched calls. They found that nestlings 22 to 44-days old (that is, before fledging), produced calls with repeatable acoustic parameters like call duration and mean frequency. However, because each nestling was recorded in two successive nights, it remains unclear whether potential cues of individuality were stable for a longer time during development. To improve our understanding of individual variability in vocalizations, and its function, it is therefore crucial to know more about vocal ontogeny in species.

The Eurasian Scops Owl (*Otus scops*, hereafter ‘Scops Owl’) is a small (body length 19-20 cm), nocturnal, primarily insectivorous owl, that breeds in open habitats in the Mediterranean zone and Central-Eastern Europe (Mikkola, 1983; Cramp, 1985). The species is mainly territorial, although it sometimes forms “loose colonies” of breeding pairs with inter-nest distances as short as 15 m (Grieco 2018; for an overview see Cramp 1985). The advertising call of the adult is highly repeatable (Koenig, 1973; Dragonetti, 2007) and can be used to track individuals unobtrusively (Galeotti & Sacchi, 2001;

Denac & Trilar, 2006; Grieco, 2018). The young leave their nest about 21 days after hatching (Koenig, 1973), and on the following nights move further and further away from the nest. The parents keep feeding the young regularly up to 40 days after hatching, then increasingly less often. At 45 days the young are fully able to catch prey (Koenig 1973), and become gradually independent and disperse.

Since the first day of their life, young Scops owls produce short (0.15 s) “tschp” calls at intervals of 0.5-1 second, interpreted as food solicitation calls (Koenig 1973). Besides the spectrograms reported by Koenig (1973), there is no quantitative data available on the call structure, and very little is known about the development of the vocalizations, their inter-individual variation, and their stability over time.

When formulating hypotheses around the function of identity signals early in life, between-species variation in individuality is often linked to breeding biology. The Scops Owl is predominantly territorial, meaning that, after fledging, the young do not join large groups of conspecifics that pose the problem of parent-offspring recognition. In this densely nesting population, young owls have never been observed to mix with other, unrelated ones ([author], pers. obs.). This leads to the expectation that the parents do not need to recognize their offspring individually after they leave the nest. Therefore, the degree of individuality in the vocalizations of the young should be low, within the range found in other species that are non-colonial or breed in small groups (Medvin et al., 1993). Before the offspring leaves the nest, individual signatures would seem redundant, unless some form of recognition occurs, for example when the parents tend to provide relatively more resources to some nestlings (parental favouritism, e.g. Lessells 2002).

This observational study had two aims: (1) To provide the first detailed description of the food-begging calls of the young Scops Owls and their inter-individual variation based on spectrographic analysis; (2) To determine whether individuals produce calls that are consistent in their structure, and temporally stable. For this purpose, I recorded the vocalizations of young owlets both before and after nest departure. Especially information about post-fledging behaviour is particularly relevant since most of the studies of vocalizations and begging behaviour of young birds have focused on nestlings, while the post-fledging

phase is underrepresented (Draganoiu et al., 2006; Middleton et al., 2007), particularly for nocturnal bird species (Kouba et al., 2014).

METHODS

Study area

The study was carried out at the State Reserve Metaponto, Italy (40°22'24"N, 16°50'19"E), a reforested area dominated by Aleppo pine *Pinus halepensis* along the sea coast. Forest plots are mixed with human settlements, especially recreational resorts and camping parks. The Scops Owl is a primarily cavity-nesting species; however, tree cavities are virtually absent in this area, and breeding pairs occupy old twig nests, particularly those of Magpies (*Pica pica*).

Sound recording and call selection

I recorded vocalizations of 20 Scops Owl nestlings and fledged young from six broods between July and August 2021 and July 2022. Recording sessions were carried out between 19:30 and 21:30 and between 00:00 to 04:30 and were made opportunistically. The study area was actively searched for nests and fledged broods. To find active nests, Magpie twig nests were watched in the evening and in the morning for at least half an hour to detect adult owls bringing food (see also Grieco 2018). Fledged young were found by listening to their calls at a distance. To record sound, I used a Telinga Pro 8 MK2 stereo microphone mounted on a parabolic dish with a diameter of 52 cm, and a Marantz PMD 661 MK2 digital sound recorder. By pointing the microphone toward a vocalizing subject, it was possible to amplify its voice while keeping the voice of the neighbouring subjects in the background. This allowed to track the owls unobtrusively but at close range, provided that their voice somewhat differed. The beeline distance between the microphone and the owls was estimated to range between four and 15 meters. In addition,

siblings tended to stick together and this reduced the within-brood variation in distance to the microphone. As a result, any observed difference in the spectral structure between subjects was unlikely to be due to distance-related attenuation of higher frequencies (see Dragonetti 2007 for the effect of distance on the measurements of calls in adults). Furthermore, there was no evidence that the young and the adults altered their behaviour when I stood near them. Scops owls in the study area were used to the presence of humans – most of the nests were located within a camping area, usually at no more than 10 m from the ground. The adults gave alarm calls only when they detected cats or foxes around the nests. As soon as they did so, the young stopped calling for some time, depending on how long the alarm calls were given. The fact that I could record so many calls at short distances strongly indicates that the adults were not stressed. Also, the fledglings stayed on the spot for many minutes without moving away from me, and the adults kept bringing food to the nest or fed the young regularly during the recording sessions. All this suggests that the recording procedure did not affect the behaviour of the birds. Each recording lasted at least one minute and was stored in a WAV file at a sampling rate of 44.1 kHz, and with 16-bit depth. In most cases, recordings were made a few meters from the nest tree or the fledged young.

Among all the recordings available, I selected those that contained the calls of all the individuals of the brood that could be measured: brood 1-3 (3 individuals, 1 recording each); brood 4 (4 individuals, 2 recordings); brood 5 (4 individuals, 5 recordings), and brood 6 (3 individuals of which one disappeared; 13 recordings in total). An additional recording was selected for broods 5 and 6 to illustrate the changes in the calls' structure during the visits by the adults. The total length of the audio analysed was 30996 s (8 h 36 min 36 s). Recordings lasted on average 1291 ± 812 (SD) seconds (range 186-2653; n = 24).

Because hatching was not observed directly, the exact age of the nestlings was unknown. Following Koenig (1973) fledging was estimated to occur on average 24 days after hatching (range 21-30). For four of the six broods, the fledging date was known, and the first recording was made two days before fledging to 10 days after fledging (see Supplementary Information Table 1). Additional recordings were made earlier in the nestling stage but the quality of the sound was too poor to measure parameters properly. For

the other two broods with an unknown fledging date, their frequent movements observed between subsequent recordings suggest that they were older, and had left their nest at least one week before.

For each of the 20 owlets and each recording, I selected the 10 loudest calls that were free of background noise and did not overlap calls of their mates or adults. Calls were assumed to be of the type “food-begging *sensu* Koenig (1973). Other utterances like bill-snapping and trills that occur mostly during parental visits were ignored. Calls were selected that occurred at least 30 s before and 30 s after a parental visit. That criterion was necessary because previous observations suggested that young owls altered their calls’ structure and rhythm in the presence of the adults. Effort was made to pick calls that covered the recording length as much as possible. However, this was not always possible, for the following reasons: (1) the young calling at lower intensity - sometimes they could hardly be heard even when staying a few meters from them, (2) the microphone being pointed to a specific individual while others were far or in the opposite direction; (3) background noise (including cicada and cricket choruses). The third factor made entire sections of recordings unusable since insect sounds overlapped with calls across a wide range of frequencies and could not be filtered out with classic methods. In short, it was preferred to select louder calls rather than more equidistant, faint calls, since the latter would have heavily influenced the measurements of acoustic variables, for example, overestimating entropy and underestimating spectrum skewness. Overall, the median time between consecutive calls was 13.1 s (interquartile range 3.8-57.4 s).

The main data set consisted of 490 calls. Because the birds were not ringed, the calls were assigned to individuals after comparing them, both visually (i.e. using the spectrograms) and acoustically, i.e. by repeated listening to the calls. For this reason, one cannot be 100% sure that an individual labelled as “ID1” was the same labelled as “ID1” later in that recording or in a subsequent recording. Assigning labels because of similarity and later claiming that the calls were similar within individuals is open to circular reasoning. This issue needs to be tackled. In Online Resource S1 (Supplementary Information) I have described the method used to label the calls. I summarize the arguments here:

- In most cases, spectrogram shapes were “discrete”, with virtually no intermediate forms, at least within recordings. There were always as many shapes as the number of individuals observed.
- Within recordings, each shape recurred rather regularly with a rhythm independent of the rhythm of other shapes, indicating that a specific shape came from one single source. So, assigning labels within recordings is unlikely to have suffered from circularity.
- The time gap between one recording and the next was in most cases one day (range 0-3; Online Resource 1, Table 1). It is therefore unlikely that one shape changed completely to another one in such a short time.
- When an individual was followed separately, it always produced calls of one shape. Switches of call shapes between siblings were never observed. Separate individuals called with the same shape for at least 30 minutes, which should also exclude the chance that a particular shape was caused by transient arousal states.
- If individuals switched to another spectrogram shape, there should have been a fairly high chance to observe, at some point, two similar shapes next to each other, i.e. when two individuals casually called at the same time with the same shape. However, that was never observed.

The labelling method is obviously not error-free. Particularly in the nestling stage, when the young sat in the nest, their calls could not be separated visually and acoustically, since the sound sources overlapped. In particular, the very first recordings of brood 6 may have been problematic because two young called with very similar call structures (Figure 2). Therefore, spectrograms could have been accidentally swapped between the “true” individuals. However, most of the recordings were made in the post-fledging stage. Because the conclusions of the work apply almost entirely to the post-fledging stage, I am more confident that the chance of assigning the same ID to different individuals in different recordings was fairly low.

To analyse the effect of parental visits on the call’s acoustic properties, I compared 10 calls taken randomly at least 30 s before and 30 s after a visit (“Random calls”) with the calls that occurred in the

interval from 5 s before to 10 s after a visit ("Visit calls"). This was done for all the individuals and recordings where visit calls were available and not overlapping with those of brood mates (n = 9 young, all after fledging).

The temporal stability of the calls' acoustic features and between-sibling differences could only be analysed when a sufficient number of high-quality recordings were available. In this work, I analysed two broods, one of two young (brood 6, with 13 recordings over 10 days), and one of four (brood 5, with five recordings over five days). For brood 6, I could only analyse the calls of two young out of three, because one disappeared shortly after leaving the nest.

Acoustic analysis

Sound analysis was carried out in Audacity 2.1 (https://www.audacityteam.org), Praat 6.0 (https://www.fon.hum.uva.nl/praat/), and Sound Analysis Pro (http://soundanalysispro.com/) depending on the acoustic features examined (see below). Recordings were first filtered with a 1000-Hz high-pass filter in Praat 6.0 to remove low-frequency background noise. The following FFT settings were used: FFT window size 2048 samples, Hanning window, temporal resolution 46 ms, frequency resolution 21.5 Hz. Two temporal features and eight spectral features were measured:

1. Call duration (s). The time from the onset to the end of the call, measured from the waveform in Praat.
2. Time from the previous call (s). The interval between the onset time of the previous call and that of the current call, measured from the waveform in Praat.
3. Peak frequency (Hz). The frequency at maximum amplitude. To measure it, the entire call duration was selected in Audacity and the function Plot Spectrum was used with the following settings: algorithm Spectrum, function Hanning window, window size 2048.

4. Peak frequency 1/3 (Hz). The frequency at maximum amplitude within the first third of the call duration, measured the same way as Peak frequency.
5. Peak frequency 3/3 (Hz). The frequency at maximum amplitude within the last third of the call duration, measured the same way as Peak frequency. Peak frequency 1/3 and Peak Frequency 3/3 were chosen because the initial and the ending parts of the calls were often very different - whereas the initial part is generally noisy, the ending part is more structured.
6. Centre of gravity (Hz). The average frequency over the entire frequency domain, weighted by the sound amplitude. It was measured in Praat by selecting the call waveform and calling the function Spectrum > View spectral slice, then Query > Get centre of gravity, with power setting 2.
7. Pitch (Hz). The average pitch across the call duration. It was measured in Praat by selecting the waveform and calling the function Pitch > Get Pitch, with the following settings: frequency range 1000-10000, Analysis method Autocorrelation, Silence threshold 0.03, Voicing threshold 0.1. The intended use of Pitch was to describe the perceived tone of a sound.
8. Average entropy. The average Wiener entropy across the call duration, measured with Sound Analysis Pro. Entropy measures the extent to which the sound contains a mixture of frequencies, as opposed to a single frequency or tone. The noisier a sound is, the more energy is smeared within a wide range. Entropy is a pure number and varies between 0 to 1, but here it is reported on a logarithmic scale, ranging between minus infinite (pure tones, complete order) and zero (white noise, complete disorder).
9. Minimum entropy. The minimum Wiener entropy within the call duration (see Average Entropy above for details). Minimum Entropy aims at capturing the more structured sound, usually at the end of the call (see an example in Online Resource S2).
10. Spectrum skewness. A measure of the symmetry of the spectrum, that is. It was measured in Praat by selecting the waveform and calling the function Spectrum > View spectral slice, then Query > Get skewness, with power setting 2. Skewness is a pure number; it is negative when frequencies

are concentrated on the right of the spectrum, and positive when the frequencies are concentrated on the left of the spectrum. If skewness is zero, the spectrum is perfectly symmetrical.

Statistical analysis

For each of the acoustic features defined above, I tested whether the 10 measurements taken for each individual were more similar to each other than to those of the other brood mates. Data were first tested for normality. For each recording, I considered the residuals of the measurements after fitting the individual means (i.e., each measurement diminished by the mean within the recording). Kolmogorov-Smirnov tests were applied to the sets of measures in each recording (10 acoustic features and 23 recordings). After Bonferroni correction for multiple testing, where alpha was reduced by dividing 0.05 by the number of recordings, a few features showed signs of non-normality. The null hypothesis of normality was rejected in five out of 23 cases for Peak frequency and Peak Frequency 1/3 and in two out of 23 for Peak Frequency 3/3. Overall, the effects of non-normality should not be fatal for the ANOVA but the results of Peak Frequency and Peak Frequency 1/3 should be taken with caution.

To assess the repeatability of acoustic features, I performed a one-factor ANOVA for each recording and calculated the Repeatability (*R*) using the formula by Lessells & Boag (1987). In the main data set, each brood resulted in one to five values of R for each acoustic feature, depending on how many recordings were available for that brood. Furthermore, 95% Confidence intervals were calculated from the individual R values from the six broods using the method described by Nakagawa & Schielzeth (2010). Similarly, the second data set provided multiple values of R for each acoustic feature. For this data set, which should be considered illustrative as it was based on one brood (brood 6), the confidence intervals were not calculated.

Another way to look at individual differences in vocalizations is to use linear discriminant analysis (LDA) to test whether a specific call could be attributed to an individual based on its acoustic features. To keep a balanced design with an equal number of calls per individual, I selected 10 calls per recording, per individual. Of the 10 acoustic features, seven were entered as explanatory variables. The other three (Time from the previous call, Peak frequency, and Average entropy) were excluded because the former showed low repeatability (see Results) and the other two were highly correlated with other features of the same nature: Peak frequency was very similar to Peak frequency 1/3 as much of the amplitude was often concentrated in the first part of a call amplitude, and Average entropy correlated with Minimum entropy (see Table 2). Other variables were correlated (e.g., Centre of gravity correlated with Spectrum skewness and with Peak frequency 1/3; see Table 2), however they were kept in the model as the aim of the analysis was to discriminate between individuals based on most of the information available rather than detect independent sources of variation (for this, see PCA below). LDA was performed in XLSTAT for Excel (Lumivero, 2023). Three types of analysis were run. (1) Single recording, (2) Multiple recordings, and (3) First vs. Last recording. In (1) Single recording (20 individuals, 200 calls, leave-one-out cross-validation), each individual was represented by 10 calls all taken from one recording. This LDA has the least power because the signal in one recording could contain noise rather than true features of distinctiveness – for example when a specific individual is aroused and therefore shows some false distinctiveness. However, I ran this analysis since it was based on the maximal number of animals available (20). In addition, it has practical utility as it could help estimate the number of fledglings during surveys based on single recordings per brood found. In (2) Multiple recordings (11 individuals, 110 calls, leave-one-out cross-validation), three broods were included that had two or more recordings (see Supplementary Information). Ten calls were selected representing equally the recordings available. For example, with five recordings available, two calls per recording were selected. However, with four recordings available, 2, 3, 2 and 3 calls were selected, respectively. For both (1) and (2), classification accuracy was obtained with leave-one-out cross-validation, that is, each call was classified using a discriminant function computed from the remaining calls of the data set. In (3) First vs. Last recordings (11 individuals, 110 calls for testing, 110

calls for validation), the 10 calls per individual in the earliest recording available were used to compute the discriminant function, while the 10 calls in the last recording were used for validation. The time between the first and the last recording ranged from two to five days. This is the LDA that should provide more insights into the stability of the calls. A special case of (3) was run for brood 6 where two individuals were recorded over 10 days. The test set included 20 calls per individual from the first two recordings, while the validation set included 20 calls per individual from the last two recordings (see Supplementary Information, Table 2). In all cases, the Discrimination score (DS) was the percentage of calls classified correctly.

One important limitation of the DS is that it much depends on sampling; its meaning is limited to the specific number of individuals used. DS generally decreases with larger samples (Linhart et al., 2019) making comparisons between studies difficult. Beecher (1989) proposed the use of a statistic derived from the theory of information, H_S, the estimated number of bits that can describe an identity signal. Contrary to DS, this statistic is considered a population estimate of the extent to which a signature system is capable of correctly identifying individuals. From H_S one can directly derive the size 2^{H_S} of the group in which individuals can be identified with a certain degree of accuracy (Beecher, 1989). Although H_S is not completely independent of sampling (Linhart & Šálek, 2017), it enables meaningful comparisons between studies and across species (Linhart et al., 2019). H_S is obtained from the multivariate analysis of variance, provided that the acoustic variables considered are uncorrelated. To calculate H_S following Beecher (1989), I used the variables that exhibited significant inter-individual variation after standardization (i.e., each variable was divided by its standard deviation estimate σ_w) and Principal Component Analysis transformation (nine out of ten factors, all $P < 0.001$). After performing an ANOVA on each PCA factor, the individual values H_s obtained from the between and within mean squares were summed up to give the information statistic H_S.

To test the temporal stability of the calls' acoustic measures, and quantify the amount of variance accounted for by subjects and recordings, I analysed the two broods that were followed for the longest

time (brood 5 and brood 6, with five and 13 recordings available covering 5 and 10 days, respectively). I used linear mixed models in R (R Core Team, 2023) with the *lme4* package (Bates et al., 2015) where subject and recording were random factors. P-values were obtained by likelihood ratio tests of the model with the effect in question against the model without that effect. The proportion of variance explained by a factor was calculated from the figures given by the summary() function of the full model. Furthermore, Repeated Measures ANOVA was used to perform pairwise comparisons between subjects within broods. In this analysis, acoustic variables were averaged per recording and subject, reducing the sample size and therefore its power. Because multiple response variables were tested from the same sample, all results were corrected with the Benjamini-Hochberg procedure (FDR = 0.05).

RESULTS

The calls of young Scops Owls

Food calls of young Scops Owls showed marked variability in all the temporal and spectral features measured (Table 1). First, some call types exhibited a wide spectrum, with peak frequency around 4000-5000 Hz, while others showed a more asymmetric spectrum, with the loudest frequency components around 2000 Hz (Figure 1A). Second, calls of similar form were also consistent in their duration and occurred regularly with a certain rhythm (Figure 1A, B). Third, fine-scale spectral structure, in the form of spots or scribbles in the spectrograms, could be easily detected through visual inspection (Figure 1C). When looking at the call spectrogram superficially, differences may go unnoticed. However, when listening to them repeatedly, differences emerge. It is the case, for example, of two young of brood 6 which produced calls of very similar structure, including the “dot” patterns in the spectrogram, but sounded different to the human ear (Online Resource S3). A second video (Online Resource S4) illustrates the association between spectrogram shapes and the perceived sound in the calls of four siblings. Figure 2 shows a summary of the spectrogram types for each of the six broods examined.

Covariation between vocal features

Several correlations were found between the calls’ acoustic features (Table 2). Frequency measurements co-varied in general with each other, as expected. However, Peak frequency 3/3, which measured the dominant frequency at the end of the call, did not correlate significantly with any other measure. This may indicate that the signal at the end of the call was somewhat independent of the calls’ general

qualities. Young owls that gave higher-frequency calls also produced calls with a more symmetric spectrum, as indicated by the negative relationship between Peak frequency and Spectrum skewness (Table 2 and Online Resource S5). This relationship was also apparent when considering individuals within broods (Online Resource S6), suggesting that different broods shared the same acoustic space. Call duration and time from the previous call did not co-vary with spectral measures, although longer calls tended to be low-frequency and more skewed (Online Resource S5). Lastly, both Average entropy and Minimum entropy were not associated with any other measure.

Distinctiveness of calls

Repeatability estimates within broods were significantly greater than zero for all the measured features (Table 3). Call duration showed the highest repeatability with the lowest uncertainty of the estimate, suggesting that it was the best indicator of individual distinctiveness. Frequency features and spectral skewness showed moderate to high repeatability, while average and minimum entropy were somewhat less repeatable. On the contrary, the time from the previous call, a measure of the calling rhythm, showed the lowest repeatability.

Discriminant analysis confirmed a substantial level of individuality in the calls. When using just one recording per individual, 52.5% of the 200 calls were correctly assigned to an individual owl, more than expected by chance (Table 2 in Online Resource 1). Individual-based classification accuracy ranged from 30% to 90% (n = 20), suggesting that the calls of some individuals were more distinctive than those of others. When using multiple recordings per individual, classification accuracy did not change much (57.3%; range 40% to 80%). When classifying calls of the last recordings using the discrimination function calculated with the calls of the first recordings, the rate of correct classification was still relatively high (52.7%) and higher than chance-expected (9.1% with 11 individuals; Table 2 in Online

Resource 1). This suggests that some degree of distinctiveness could be traced for at least a few days. However, classification accuracy showed important variation between individuals (0% to 100%; Table 2 in Online Resource 1). When LDA was repeated within broods, classification accuracy increased as expected, since validation was based on fewer individuals per group (3 or 4, depending on the brood size, instead of 20 or 11). However, it was always significantly higher than chance-expected (Table 2 in Online Resource 1). A special case of within-brood LDA was run for brood 6 using recordings that covered the longest period (10 days). There, classification accuracy was just under 100%, suggesting that distinctiveness could last for several days (see also Figure 4).

Analysis of calls of the 20 individuals indicated an information capacity H_S of 4.69. The largest contribution to this value was by the first PCA factor (F1, H_s = 1.47), which expressed Centre of gravity (correlation between axis and variable: r = 0.96), Peak frequency (r = 0.90), PF 1/3 (r = 0.86) and Spectrum skewness (r = -0.82). This factor reflects the position of the dominant frequencies along the spectrum, especially at the onset of the call. The second largest contribution (F2, H_s = 0.99) expressed Minimum entropy (r = 0.83), PF 3/3 (r = -0.73) and Average entropy (r = 0.72). This factor reflects the amount of structure of the last part of the call.

Effect of parental visits on the call structure

Young owls in the post-fledging phase vocalized continually, also in the absence of the parents. It was therefore possible to compare the acoustic structure of the calls in the absence of the parents with that during parental visits. Figure 3 shows the changes in the calls' acoustic structure between random calls in the absence of the parents and those recorded around a visit. Compared with random calls, visit calls were (1) noisier, i.e., with higher average and minimum entropy, (2) more frequent, as the time from the previous call dropped significantly, (3) generally higher in frequency, as Centre of gravity increased and

Spectrum skewness decreased. Calls also tended to be shorter during visits by the parents, but the difference with the random calls was below significance (Figure 3). For an example of the temporal changes in the call structure for two brood mates before and around parental visits, see Online Resource S7, which also shows that some spectral features like Centre of gravity and Peak frequency 3/3 still differed between individuals during a visit. However, because of the small number of subjects and calls measured during parental visits, I could not assess whether vocal distinctiveness changed in those circumstances.

Stability of between-siblings differences

A separate analysis was carried out in two broods for which multiple recordings were available, covering periods of five and 10 days, respectively. Individual differences in spectrograms appeared seven days before fledging (Online Resource S8). However, at such early ages, the signal-to-noise ratio was too low to allow a proper quantitative comparison with later recordings. Linear mixed models revealed that all call features exhibited significant inter-individual differences (Table 4). In particular, subject identity explained more than 80% of the variation in call duration in both broods. Measures of frequency gave more ambiguous results, with the proportion of variance explained being between 2% and 70% depending on the measure considered and the brood.

Call features also showed significant variation between recordings (Table 4). The effect was different between features of frequency and those describing spectral shape. Features expressing frequency and pitch showed 2-13% of variation explained by recordings, confirming that such features may be important in maintaining the stability of the signal. Conversely, features like spectral entropy and skewness showed some more variability between recordings (11 to 19% of variance explained; Table 4). This is exemplified by the up-and-down pattern of entropy measures in both broods (Figure 4), suggesting that

the amount of noise and spectral shapes were less constant. This result confirms the finding that the repeatability of entropy was low in the data set with six broods (Table 3). Entropy could correlate with the ever-changing state of the subjects. In contrast, call duration and time from the previous call showed low variability between recordings. However, the former could almost perfectly separate the siblings (Figure 4), while the latter showed low to moderate between-individual variability (Table 4), confirming that calling rhythm was not a good indicator of identity.

Pairwise comparisons revealed some degree of unicity within broods. In the two-young brood, individual 2 gave longer calls and with higher frequency at the end (PF 3/3), lower Centre of gravity, lower Entropy, and higher Spectrum skewness. In the four-young brood, individual 3 produced the longest calls, while individual 4 had the highest Peak frequency and a higher Centre of gravity than individual 3. In contrast, individual 2 tended to have the lowest Pitch and the shorter Call duration among the four brood mates.

DISCUSSION

The results of this study, which should be considered preliminary because of the sample size and the observation period, can be summarized as follows: (1) The main call of young Scops Owls was found to be more complex than previously described. The only study of vocalizations of young Scops Owls is that by Koenig (Koenig, 1973) who reported a few spectrograms and a constant duration of the calls (i.e., about 0.15 s) and the interval between calls varying between 0.5 and 1 s. My study has not only revealed substantial quantitative variation in several acoustic and temporal parameters, but also details of the call spectrograms in the form of dots, S-shapes and other patterns consistent both within and between recordings, previously unknown in this species. (2) Inter-individual differences were found in all temporal and spectral features examined, although none could fully discriminate between siblings. Despite significant variation in some acoustic features between different recording sessions, individual differences persisted. Temporal changes in those features do not exclude distinctiveness *a priori*, if the parents can attend to those features day by day (e.g. with calls gradually becoming more tonal) and "track" the individual calls (McArthur, 1982). (3) Signs of distinctiveness could be detected a few days before fledging and traced up to a week after fledging. Because of the low number of recordings made in the nestling stage, it is not possible to ascertain when the distinctiveness of calls began to develop. (4) The information capacity statistic H_S based on variation in non-correlated variables was 4.69, which seems in line with what has been found in loosely-colonial species (see below). (5) Some call features changed markedly during parental visits, but whether that translates to lower distinctiveness remains an open question. Taken together, these findings suggest that vocalizations of young Scops Owls might act as signals of identity, and would potentially allow the parents to recognize their own offspring, or at least their brood as a unit. Mechanism of recognition would probably be important in the very first weeks after fledging, a very critical period for the survival of the young, and still poorly known (Cox et al., 2014; Naef-Daenzer & Grüebler, 2016; Perrig et al., 2017).

Acoustic parameters important in call distinctiveness

When looking at the variability in the call features between individuals across broods, at least two main factors contributed to call distinctiveness: (1) the position of high-amplitude frequencies along the spectrum, particularly at the onset of the calls where much of the energy is concentrated, and (2) the amount of structure at the end of the call. This latter contribution is particularly interesting because the ending part of the call, characterized by low peak frequency and low entropy, seemed to vary independently of other acoustic features.

The role of call duration is also complex. On the one hand, the length of calls exhibited the highest degree of within-individual repeatability, and a remarkable proportion of variance explained by subject identity, as revealed by mixed model analysis for the broods with most repeated measurements. Call duration alone could almost separate individuals within broods (Figure 4) but played a minor role when trying to use call duration to statistically separate individuals across broods. Analysis based on a larger sample is therefore needed to clarify whether call duration is potentially an identity cue within broods.

That several temporal and spectral parameters varied between individuals is not new. In nestlings of bird species, various acoustic parameters of food-begging vocalizations exhibit individual variation (Little Blue Penguin: pitch, Nakagawa et al. (2001); Zebra Finch: Call duration, fundamental frequency and frequency modulation among several others, Levréro et al. (2009); Black Redstart, various frequency measures of the low harmonic, Draganoiu et al. (2006); Jackson Golden-backed Weaver: call duration, frequency modulation, mean frequency and entropy, Reers and Jacot (2011); Spanish Sparrow: call duration, maximum frequency and proportion of time with harmonic structure, Marques et al (2011); Little Auk, Maximum frequency, call duration, entropy, and measures of various points of the spectrum, (Kidawa et al., 2023). While the frequency and its modulation are dominant in the literature as identity

cues, most authors suggest that individuality is not based on one single component, but on a wide range of components acting simultaneously or in sequence, which presumably reduces discrimination error and reaction time (Rowe, 1999).

The only study of individual signatures at early life stages in Strigiformes is that by Dreiss et al (2014) of nestling Barn Owls *Tyto alba*. Repeatability estimates of acoustic features found in Scops Owls were generally comparable with those found in Barn Owls, except that of Call duration which was higher in Scops Owls (average ± SE: 0.91 ± 0.01 vs. 0.71 ± 0.05 for Barn Owls). Like in Barn Owl nestlings, Call duration had the highest repeatability than any frequency measure. In contrast with my study, Dreiss et al. (2014) did experimental work where the orientation and distance of the microphone from the subjects were fixed, allowing to reliably record the loudness of the calls. They found that call loudness was also repeatable. An interesting analogy between the behaviour of nestling Scops Owls and Barn Owls can be found when looking at changes in structure. In Scops Owls, several acoustic features did contribute to individual variation, yet some of those features also changed significantly during parental visits (Figure 3). Changes in call structure could reflect the level of motivation to obtain food. Dreiss et al. (2014) found that Call duration and frequency measures in the calls of Barn Owl nestlings were also affected by food state (deprived or not). This seems in contrast with the expectation that signals of identity should be independent of condition (Dale et al., 2001; Reers & Jacot, 2011). My study shows some similarities with that in Barn Owls in that distinctiveness was achieved with multiple traits, which were nonetheless sensitive to the current state of the subject (presence vs. absence of provisioning parent).

Variation in discrimination success

In this study, the potential for statistical discrimination between calls of different individuals was rather uniform when the discriminant function was built upon single recordings or a mix of recordings per individual. LDA based on a single recording per brood says little about the stability of signals; it can,

however, provide useful information in monitoring studies, where one aims at estimating the number of fledglings produced in a local area with minimal effort. In contrast, multiple recordings should give information about the stability of identity signals. Analysis based on multiple recordings is not free of pseudoreplication because a second factor – in this case recording – is by definition not controlled for in LDA (Mundry & Sommer, 2007). Here, the discrimination scores were only slightly higher than those obtained with single recording analysis, so the effect of uncontrolled replicates might have been small. When classifying subjects from the last recording using the discrimination function of the first recording, which should provide the most meaningful results, the classification accuracy showed greater individual variability. Some individuals were identified correctly 10 times out of 10, while others were never identified correctly. A simple explanation for this result could be that in some individuals the signal changes with time more rapidly than in others. Individually-specific call features do change over short time scales (Ellis, 2008; Marques et al., 2011; Smeele et al., 2023). However, there may have been other reasons why statistical discrimination was low for some individuals. First, the discriminant function may have been biased towards features that occur in some “easy-to-find” spectrogram patterns, not others; for example, the frequency PF3/3 was chosen in the attempt to characterize spectrograms with “dots” at the end of the calls. Not all individuals exhibited such evident, tonal patterns. Second, the calls analyzed were not selected completely randomly (see Methods), therefore calls within a recording could have been more correlated to each other than those available if sampling had been truly random. This may have made individual recognition across recordings more difficult. Another reason could be that some siblings exhibited similar calls, like young 1 and 2 in brood 6 (Online Resource 3). Last, some calls may have been just too faint to be compared across recordings. Variability in discrimination success should therefore be considered with caution.

Acoustic distinctiveness in parent-offspring communication

The first question that arises is how the level of individuality in vocalizations of young Scops Owls compares with that of other bird species. The signature information capacity estimated in this study is comparable to that found in non-colonial and loosely colonial breeding species, such as the Barn Swallow (H_S = 4.57; Medvin et al. 1993)), the Tree swallow (H_S = 3.20; Leonard et al., 1997). In contrast, strictly colonial species exhibit, on average, higher H_S values; for example, the Bank Swallow (H_S = 10.2; Beecher, 1991) and the Cliff Swallow (H_S = 8.74; Medvin et al. 1993). An increase in H_S by 1 means that the number of individuals that can be potentially discriminated, keeping recognition accuracy constant, is doubled. Although H_S has been recognized as a valid method to compare identity signals across studies (Linhart et al., 2019), values of H_S are still rare in the literature, particularly for juvenile vocalizations. From the study of call signatures in Barn Owl nestlings (Dreiss et al. 2014) values of discrimination success of 62 % for six-chick broods can be converted to an H_S of 3.40 obtained with the R package IDmeasurer (Linhart et al., 2019). A low value is consistent with what is expected when looking at the position of the species along the territorial - colonial breeding gradient, since Barn Owls only rarely breed in colonies (Smith et al., 1974), and the chance that juveniles mix with unrelated conspecifics during the period of dependence on the parents is relatively low. In contrast, Scops Owls are known to form loose colonies with distances between nests as short as 12 m and partially overlapping territories of males (Cramp, 1985; Vrezec, 2001; Grieco, 2018, 2022). Although fledged broods tend not to mix with others, in this study area they have been observed entering other pairs' territories, and there is evidence that their parents forage in those territories as they follow the fledged brood (unpubl. data). This suggests that in this species the parents face a higher chance to come in contact with unrelated young, making recognition mechanisms more important for this species. The statistic H_S estimated in this study implies that a maximum of 26 individuals could be identified at the given degree of accuracy. This number corresponds to 6-8 broods of average size present in a certain area, which is not too far from reality in a semi-colonial context like that of this study (and presumably elsewhere in areas of high breeding density in Southern Europe). There, a wandering group of fledglings may enter several neighbouring territories in a period of a few weeks. Identifying offspring could therefore be critical for this species. Note that even in territorial

birds some degree of intermingling of young after fledging could explain the occurrence of parent-offspring recognition (Barg and Mumme, 1994).

An important difference between Barn Owls and Scops Owls is that in the former individuality seems to be limited to the nestling stage, as there is no evidence so far that Barn Owls after fledging, and in general as adults, make distinctive calls. Dreiss et al. (2014) proposed that the individuality of nestling calls plays a role in competition between siblings for food, rather than parent-offspring recognition.

The second question that arises is how individuality in early life stages is related to that in adults. The advertising call ("hoot") of adult Scops Owls shows remarkable individual variation and stability through time, such individuals can be recognized statistically using temporal and acoustic features of the calls (see the Introduction). Linhart et al. (2022) reported a H_S of 7.0 for adult Scops Owls obtained by converting the discrimination score in Galeotti & Sacchi (2001). We can therefore estimate that a maximum of 128 adult individuals can be unambiguously identified. This does not seem so unrealistic; in my study area, where the density of calling males is high, it is not uncommon to compile a list of 40-50 individuals in a radius of less than 1 km, by simply looking at key measures of dominant frequency, inter-note time intervals, and unique details of the spectrograms (unpubl. data; see Grieco 2018 for a sub-sample thereof). According to Koenig (1973), the hoot of adult Scops Owls derives from the begging calls of the young through a gradual transition, as it is also known in other species (Redondo & Exposito, 1990; Brittan-Powell et al., 1997; Marques et al., 2011). It is therefore tempting to speculate that the results of this study are just a snapshot of the maturation process in which the degree of distinctiveness increases. In that case, the young owls might not be “broadcasting” their individuality to be recognizable, rather their voice would merely reflect a maturation process, with no net advantage early in life.

There are other, not necessarily mutually exclusive, functional explanations for the occurrence of acoustic signatures in young Scops Owls, that go beyond the classic parent-offspring recognition problem in a crowded world. First, the parents could use acoustic signatures to discriminate between their own nestlings and alien (parasitic) nestlings, as found in other species (Colombelli-Négrel et al., 2012; Yang et

al., 2015). However, Scops Owls are not subject to any known brood parasites (Mikkola, 1983; Cramp, 1985), and laying eggs by multiple females in the same nest is a rare event (Bavoux et al., 1991). Therefore, the acoustic distinctiveness of nestlings is unlikely to be of adaptive value in the context of the rejection of unrelated nestlings.

In another, intriguing scenario, the parents could be using acoustic signatures of their offspring to feed preferentially some individuals over others, as suggested earlier for this species (Parejo et al., 2010). Theory (Lessells, 2002) and an increasing number of empirical studies suggest that in some circumstances the parents do not distribute food equally among nestlings, based on nestling size, body condition, sex, or age (Krebs, 2001; Smiseth et al., 2003; de Ayala et al., 2007; Dickens & Hartley, 2007; Dugas, 2009; Avilés et al., 2011; Ersoy et al., 2021). For example, female and male parents differ in the amount of care given to the smallest nestlings (Slagsvold, 1997), although it is not always clear whether nestling size is the primary cue used in this discrimination. Condition-dependent parental favouritism mediated by visual cues has been suggested to occur in several species (Jourdie et al., 2004; Bize et al., 2006; Galván et al., 2008; Avilés et al., 2011), including nocturnal raptors (Avilés & Parejo, 2013). In an experimental study on Scops Owls, Parejo et al. (2010) found that when the ultraviolet reflectance of the cere (i.e., the skin above the beak) of the nestlings was experimentally reduced, thereby simulating lower body condition, those nestlings gained more weight than their control siblings. The authors concluded that the parents somehow perceived the lower brightness of the cere and fed preferentially those nestlings over the untreated siblings. The study suggested that parent Scops Owls do attend to cues of quality of nestlings. Visual discrimination between offspring is efficient in the nestling-rearing phase, where both parent and offspring are confined in the small environment of the nest. However, when the young leave the nest and become mobile, a recognition system based on visual cues of limited size like the beak cere could be aided by acoustic distinctiveness (Draganoiu et al., 2006). The parents could learn to associate cues of quality in the offspring, like their body size, sex, or hatching order, with the vocal signatures learnt during the nestling-rearing phase so that they could adjust their food-provisioning decisions. With an important

limitation though – that the cues of quality and the vocal features of an individual remain stable in the short/medium term (i.e., over days to weeks) so that they can be associated with each other.

In a rather different, though plausible scenario, young Scops Owls could perceive the identity cues of their siblings, which enable them to communicate with each other. Support for this idea comes from the fact that the young spent significant time calling when the parents were absent (see for example Online Resource 7). Vocalizations in the absence of parents are known in other owl species (Roulin et al., 2000; Olsen et al., 2020). Identity cues may help the fledglings to keep contact with each other (Ligout et al., 2016). Alternatively, they could play a role in sibling competition over the next feed brought by the parents, as it occurs in Barn Owl nestlings (Roulin et al., 2000; Dreiss et al., 2014).

In conclusion, this work has revealed that vocalizations of juvenile Scops Owls potentially carry individual information, at least within broods. This should encourage researchers to address the many unanswered questions outlined above and gain insights into the relationships between nesting ecology, social/territorial organization, and communication in this still poorly known species. I also hope that the methods illustrated in this work can be useful in assessing and monitoring the productivity of Scops Owl populations.

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TABLES

Table 1

Descriptive statistics of ten acoustic variables of 670 vocalizations of 20 young Scops Owls after they left the nest. The standard deviation (SD) was calculated using the individual averages. (a) Minimum and maximum of individual averages. (b) Minimum and maximum of the single measurements (n=670).

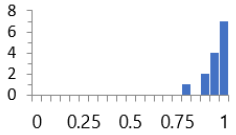
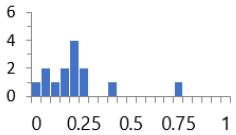
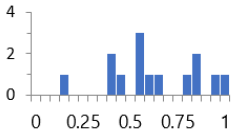
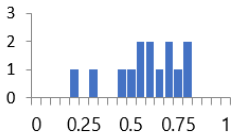
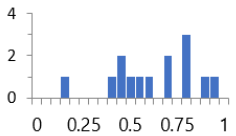
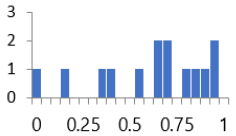
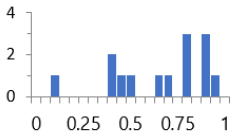
	Average	SD	Range (individuals) (a)	Range (calls) (b)
Call duration (s)	0.182	0.045	0.117 – 0.279	0.094 – 0.423
Time from previous call (s)	1.775	0.244	1.410 – 2.302	0.770 – 6.490
Peak frequency (Hz)	1924.9	701.7	1316.4 – 4082.1	1112 – 5963
Peak frequency 1/3 (Hz)	1984.6	690.1	1321.8 – 3937.6	1113 – 5393
Peak frequency 3/3 (Hz)	1577.6	235.0	1221.5 – 2093.6	1074 – 5459
Centre of gravity (Hz)	2296.6	560.2	1722.7 – 3717.9	1468 – 4924
Pitch (Hz)	1820.3	484.2	1334.8 – 3551.0	1202 – 5046
Average entropy	-2.73	0.50	-3.92 – -1.80	-4.80 – -1.18
Minimum entropy	-3.89	0.73	-5.12 – -2.39	-7.25 – -1.45
Spectrum skewness	2.24	1.13	-0.04 – 4.11	-1.04 – 9.36

Table 2

Pearson's correlation coefficients of temporal and acoustic variables in calls of 20 young Scops Owls, based on the per-individual averages. Values in bold indicate a significant correlation after Bonferroni correction (alpha = 0.001). * P < 0.05; ** P < 0.001. See also Online Resource 5.

	Time from previous call	Peak frequency	Peak frequency 1/3	Peak frequency 3/3	Centre of gravity	Pitch	Average entropy	Minimum entropy	Spectrum skewness
Call Duration	0.27 ^{ns}	-0.43 ^{ns}	-0.43 ^{ns}	0.06 ^{ns}	-0.52 *	-0.37 ^{ns}	-0.24 ^{ns}	-0.41 ^{ns}	0.47 *
Time from previous call		-0.002 ^{ns}	-0.08 ^{ns}	0.42 ^{ns}	-0.09 ^{ns}	-0.14 ^{ns}	-0.07 ^{ns}	-0.18 ^{ns}	0.34 ^{ns}
Peak frequency			**0.99** **	0.52 *	**0.93** **	**0.84** **	0.38 ^{ns}	0.43 ^{ns}	**-0.77** **
Peak frequency 1/3				0.50 *	**0.92** **	**0.85** **	0.38 ^{ns}	0.42 ^{ns}	**-0.77** **
Peak frequency 3/3					0.48 *	0.56 *	-0.05 ^{ns}	-0.17 ^{ns}	-0.22 ^{ns}
Centre of gravity						**0.84** **	0.49 *	0.55 *	**-0.90** **
Pitch							0.14 ^{ns}	0.17 ^{ns}	-0.66 *
Average entropy								**0.92** **	-0.50 *
Minimum entropy									-0.61 *

Table 3. Estimates of repeatability of acoustic features within six Scops Owl broods. Significance refers to the F tests for each of the 14 recordings. Some broods are represented more often than others (see Methods). Significance was established after Bonferroni correction (alpha = 0.004).

	Average	CI	Significant	
Call duration (s)	0.91	0.87 – 0.96	14 out of 14	
Time from previous call (s)	0.16	0.06 – 0.26	2 out of 14	
Peak frequency (Hz)	0.60	0.41 – 0.79	11 out of 14	
Peak frequency 1/3 (Hz)	0.54	0.48 – 0.61	12 out of 14	
Peak frequency 3/3 (Hz)	0.55	0.36-0.74	13 out of 14	
Centre of gravity (Hz)	0.56	0.22 – 0.91	11 out of 14	
Pitch (Hz)	0.59	0.37 – 0.81	12 out of 14	

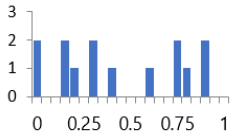
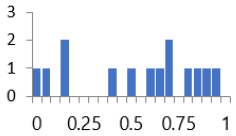
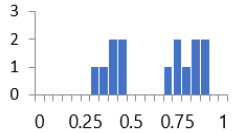
Average entropy	0.36	0.05 – 0.68	7 out of 14	
Minimum entropy	0.39	0.08 – 0.71	10 out of 14	
Spectrum skewness	0.62	0.41 – 0.83	11 out of 14	

Table 4. Results of Linear Mixed Models of call features in young Scops owls in brood 5 (four young, five recordings over five days, 200 calls) and brood 6 (two young, 13 recordings over 10 days, 260 calls). Values of acoustic features were averaged per recording, resulting in n = 5 for brood 5, and n = 13 for brood 6. Effects of individual (ID) and recording (Rec) are shown. χ^2_1 : a test of Likelihood Ratio after comparison between the model with both factors and the model with the other factor only. * Significant after Benjamini-Hochberg correction for multiple testing (FDR = 0.05, 10 tests per brood). Last column: Proportion of variance explained by the two random factors.

	Brood 5				Brood 6			
	Effect	χ^2_1	Sig.	Prop. of Var. (%)	Effect	χ^2_1	Sig.	Prop. of Var. (%)
Call duration	ID	337.9	*	83.1	ID	430.6	*	86.6
	Rec	44.0	*	4.8	Rec	65.4	*	4.3
Time from prev. call	ID	27.7	*	20.6	ID	6.8	*	8.0
	Rec	4.47	*	4.6	Rec	0.41	n. s.	1.4
Peak frequency	ID	70.1	*	37.5	ID	14.9	*	13.7
	Rec	13.7	*	7.8	Rec	1.5	n. s.	2.6
Peak frequency 1/3	ID	54.5	*	29.9	ID	9.4	*	9.9
	Rec	23.0	*	12.7	Rec	0.99	n. s.	2.2
Peak frequency 3/3	ID	195.8	*	69.4	ID	10.1	*	8.9
	Rec	13.3	*	3.7	Rec	16.5	*	11.8
Centre of gravity	ID	66.4	*	36.6	ID	118.1	*	48.4
	Rec	11.3	*	6.9	Rec	47.0	*	13.3
Pitch	ID	246.1	*	70.3	ID	7.2	*	7.2
	Rec	62.5	*	10.8	Rec	11.8	*	9.7

Average entropy	ID	28.3	*	19.1	ID	72.9	*	34.1
	Rec	16.0	*	11.3	Rec	55.1	*	18.9
Minimum entropy	ID	68.4	*	33.4	ID	149.2	*	51.0
	Rec	34.3	*	16.0	Rec	86.9	*	18.9
Spectrum skewness	ID	54.5	*	29.8	ID	150.1	*	54.0
	Rec	23.9	*	13.0	Rec	63.1	*	14.4

FIGURE LEGENDS

Fig. 1

Example of spectral and temporal variation in vocalizations of young Scops Owls. A. Spectrogram of the calls of two individuals, a and b, one week after leaving the nest. (a) A short call with a wide spectrum plotted on the left. (b) A longer call with a narrower bandwidth, and a main component around 2 kHz (see the spectrum plotted on the right). B. Spectrogram of a train of calls by the same two individuals as in A. The symbols below indicate the intervals between subsequent calls. See also Figure 4 for an overview of the stability of between-sibling differences over multiple days. C. Spectrogram of the calls of two individuals a and b in another brood. Although they look similar superficially, there are consistent differences in the location of frequency peaks at the beginning and the end of the calls (i.e., “dots” in the spectrograms indicated with the arrows; see also A). The time step is one second.

Fig. 2

Representative spectrograms of the calls of young Scops Owls of six broods (1-2 from 2021, 3-6 from 2022). The letters indicate features that help recognition of individuals. (a) Differences in call duration. (b) S-shape patterns, usually at the lower end of the frequency range. (c) Wider frequency band in the first part of the call, resulting in a higher Centre of gravity and lower Spectrum skewness. (d) Mass of frequency concentrated at specific points, especially at the end of the call (the “dots” in the spectrograms indicated with arrows). (e) The dominant frequency is generally lower than in calls of other brood mates. (f) More structured sound, with high spectral skewness

Fig. 3

Within-subject changes in acoustic variables of the calls of young Scops Owls (all after fledging) during a parental visit. Each pair of circles represents one subject. Random: The average value over ten random calls at least 30 s before the visit and 30 s after a previous visit. Visit: The average of calls between 5 s before and 10 s after the visit. Asterisks indicate statistical significance after Benjamini-Hochberg correction of multiple testing (False Discovery Rate set to 0.05, 10 paired t-tests, adjusted alpha = 0.025). Significant differences based on paired t-tests: Time from previous call, 1.89 ± 0.47 vs 1.03 ± 0.27 s, t_8 = 5.33, Cohen's d = 1.78; Centre of gravity, 2496.9 ± 498.3 vs 3301.1 ± 684.9 Hz, t_8 = -3.94, d = 1.31; Average entropy, -2.50 ± 0.38 vs -1.75 ± 0.37, t_8 = -11.75, d = 3.92; Minimum entropy, -3.80 ± 0.65 vs -2.65 ± 0.57, t_8 = -5.92, d = 1.97; Spectrum skewness, 2.30 ± 1.33 vs 0.99 ± 0.88, t_8 = 3.24, d = 1.08. Changes in Call duration approached significance (0.18 ± 0.05 vs 0.15 ± 0.02 s, t_8 = 2.52, P = 0.036).

Fig. 4

Temporal stability of the ten acoustic variables of the calls of young scops owls in two broods, one of two young (13 recordings over 10 nights; see the left in each half of the figure), and one of four young (five recordings over five nights; at the right). The horizontal bars in the top-left charts indicate the number of days covered by the measurements. Letters indicate the significance of between-individual comparisons. For the 2-young brood, see the ANOVA results in Table 4. For the four-young brood, the letters are based on a Tukey's HSD test after Benjamini-Hochberg correction with FDR = 0.05 (60 tests, adjusted alpha = 0.018). Values were averaged per recording, resulting in five data points for each individual. Individuals that do not share letters were significantly different; individuals sharing one letter were indistinguishable. The arrow indicates the fledging date of the first brood. The fledging date of the second brood was unknown.

Figure 1

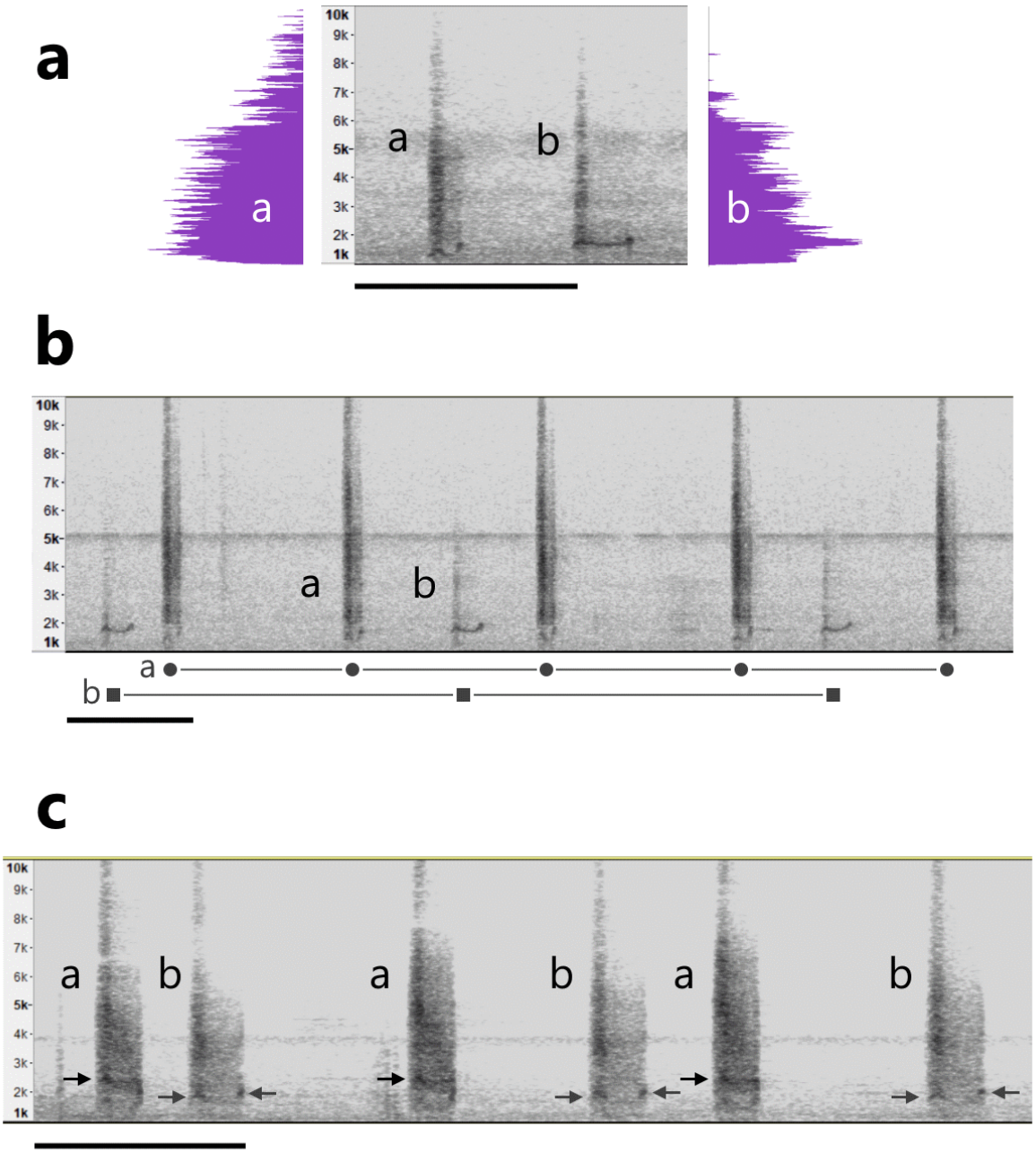


Figure 2

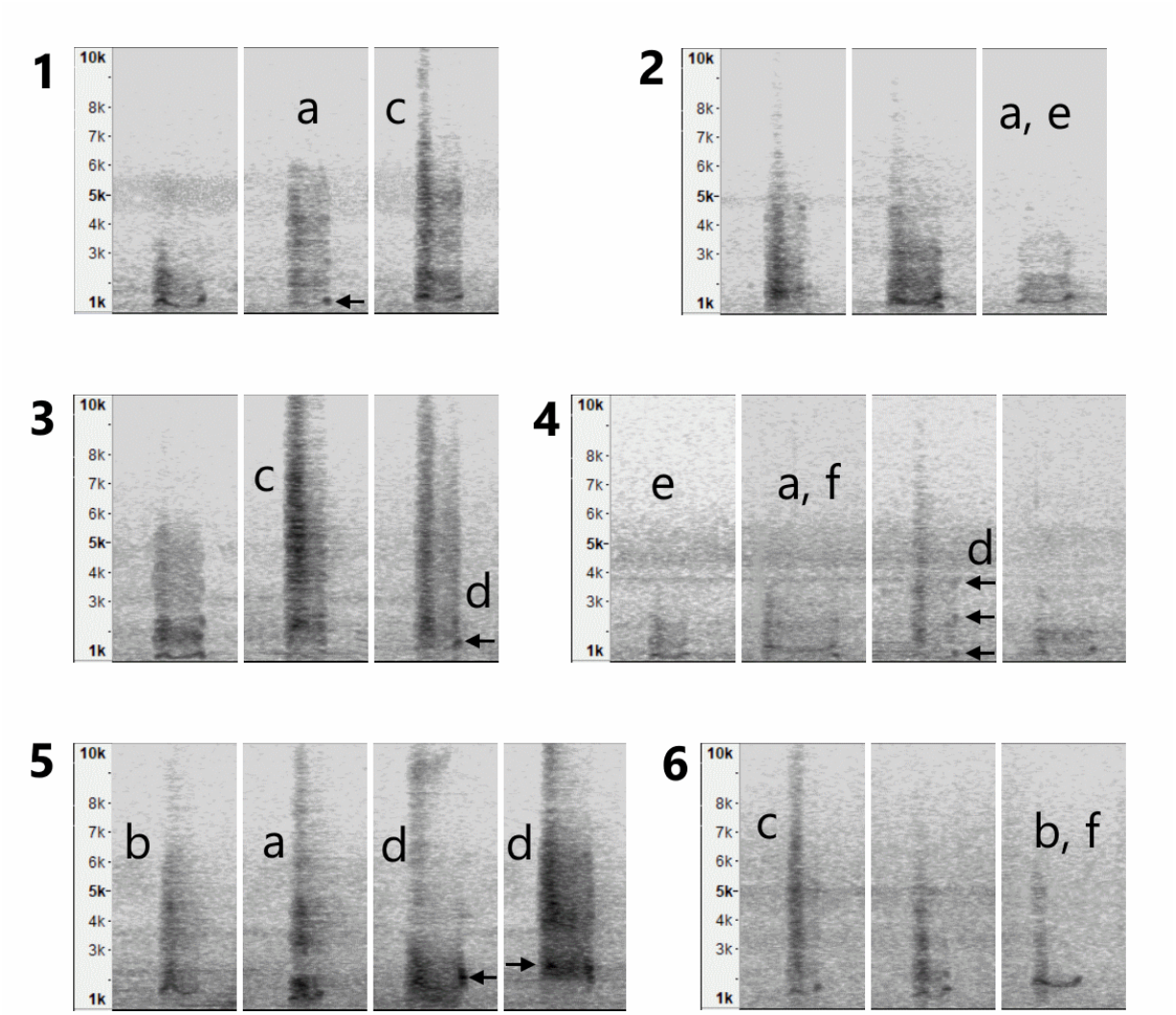


Figure 3

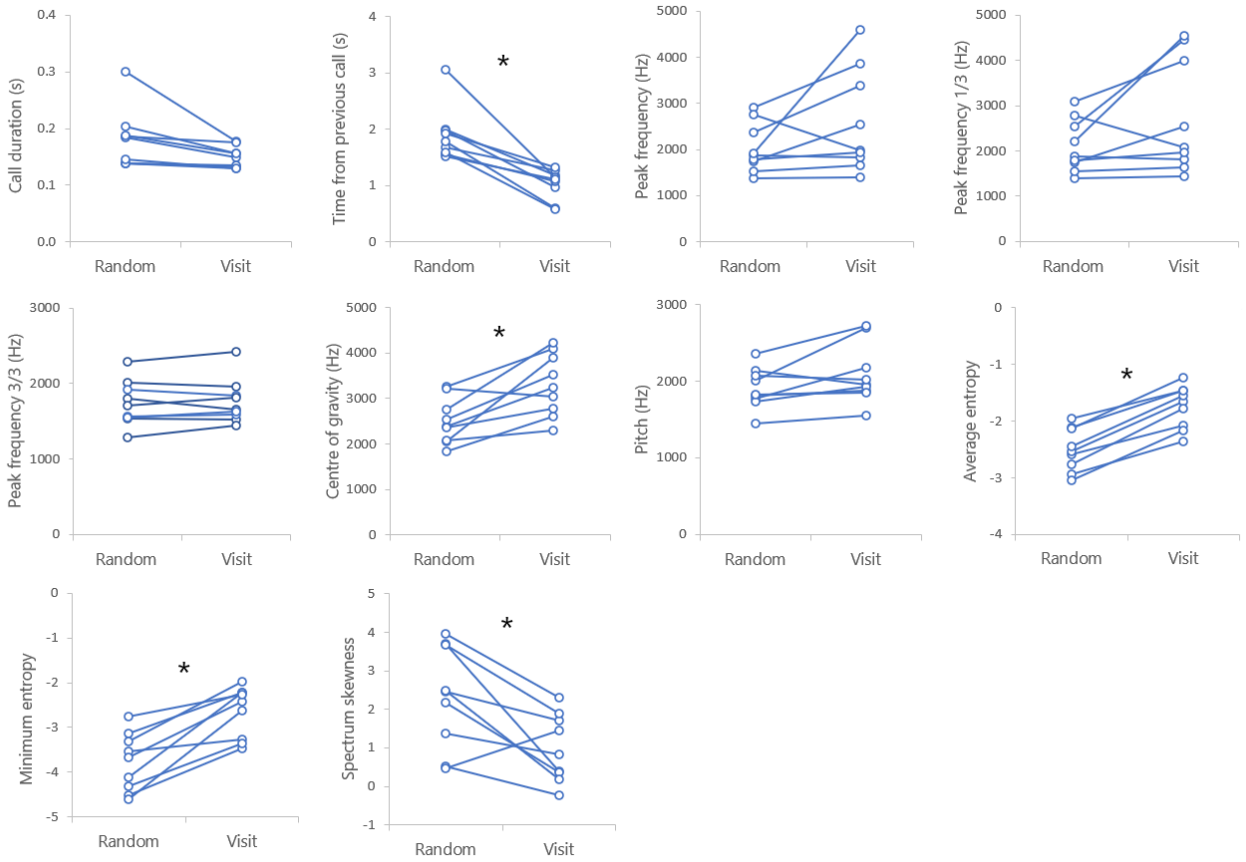
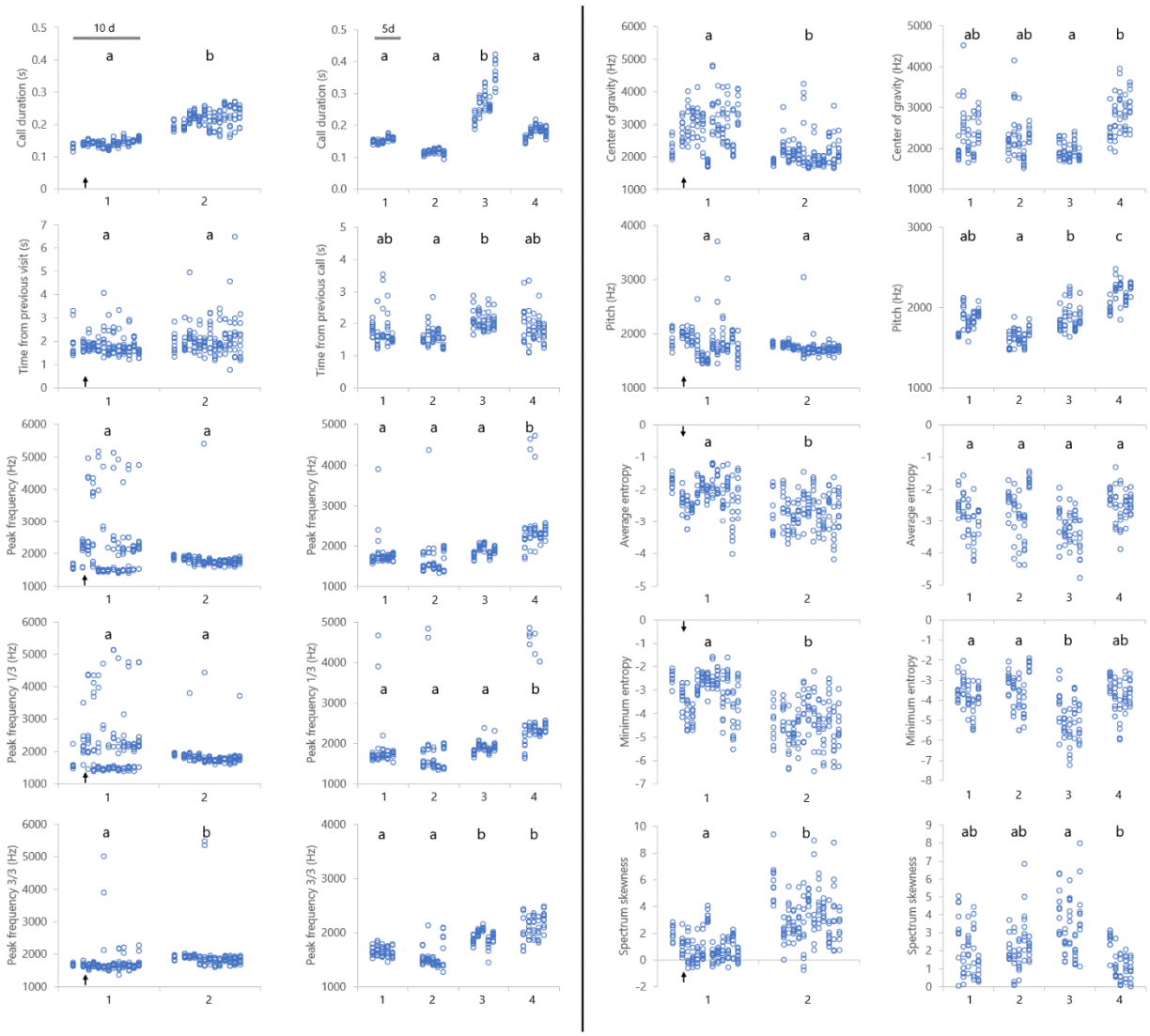

Call duration (s)
Time from previous call (s)
Peak frequency (Hz)
Peak frequency 1/3 (Hz)
Peak frequency 3/3 (Hz)
Centre of gravity (Hz)
Pitch (Hz)
Average entropy
Minimum entropy
Spectrum skewness
Random
Visit
*

Figure 4


10 d
5d
Call duration (s)
Time from previous visit (s)
Time from previous call (s)
Peak frequency (Hz)
Peak frequency 1/3 (Hz)
Peak frequency 3/3 (Hz)
Center of gravity (Hz)
Pitch (Hz)
Average entropy
Minimum entropy
Spectrum skewness

ELECTRONIC SUPPLEMENTARY MATERIAL

Online Resource 1 – ESM_1.pdf

This document describes the method used to label the calls of young Scops Owls by visual and acoustic inspection, within recordings and between recordings. It also contains Table 1 with a summary of the broods studied, and Table 2 with an overview of individual-based classification success of LDA performed with different datasets.

Online Resource 2 – ESM_2.tiff

Spectrogram of the calls of two young Scops Owls. (a) A low-frequency, long call. (b) A high-frequency, shorter call (b). The blue line represents the temporal changes of the Wiener entropy. The more a sound contains a mixture of frequencies, the higher the entropy. Entropy is at a minimum when sound contains a single frequency. Note the drop in entropy in the calls of individual a.

Online Resource 3 – ESM_3.m4v

Fragment of a recording of alternated vocalizations of two brood mates (brood 6 in Figure 2). Although the call spectrograms are similar, the two young make distinctive sounds. The sequence is as follows (a and b indicate the two individuals, b slightly lower-pitched than a): 15:07 a-b-a-b-a, 15:11 b-a-b-a-b-a, 15:16 b-a-b-a-b-a. Note the “dots” in the spectrograms at specific frequencies. In the background, calls of adult Scops Owls and crickets. This and similar fragments were not included in the analysis because of the pervasive background noise

Online Resource 4 – ESM_4.mp4

Fragment of a recording of vocalizations of four brood mates (brood 5). Calls are labelled with numbers based on the spectrograms' visual appearance and sound similarity. Note that each sound pattern recurs with a rather regular rhythm. For details, see ESM_1.pdf.

Online Resource 5 – ESM_5.tiff

Correlation matrix for the ten acoustic variables measured in 20 young Scops Owls. The straight line indicates a significant correlation after Bonferroni correction (n= 45 correlation tests; $P < 0.001$).

Online Resource 6 – ESM_6.tiff

Spectrum skewness plotted against the spectrum's Centre of gravity of calls of 20 young Scops Owls of six broods. Points represent individual averages; symbols represent different broods. The insets show examples of spectrograms of calls at the two extremes of the range. Top: low-frequency, highly skewed calls. Bottom: calls with a less skewed spectrum and more frequencies in the 4-6 kHz range. Pearson correlation, r = -0.90, n = 20, $P < 0.001$.

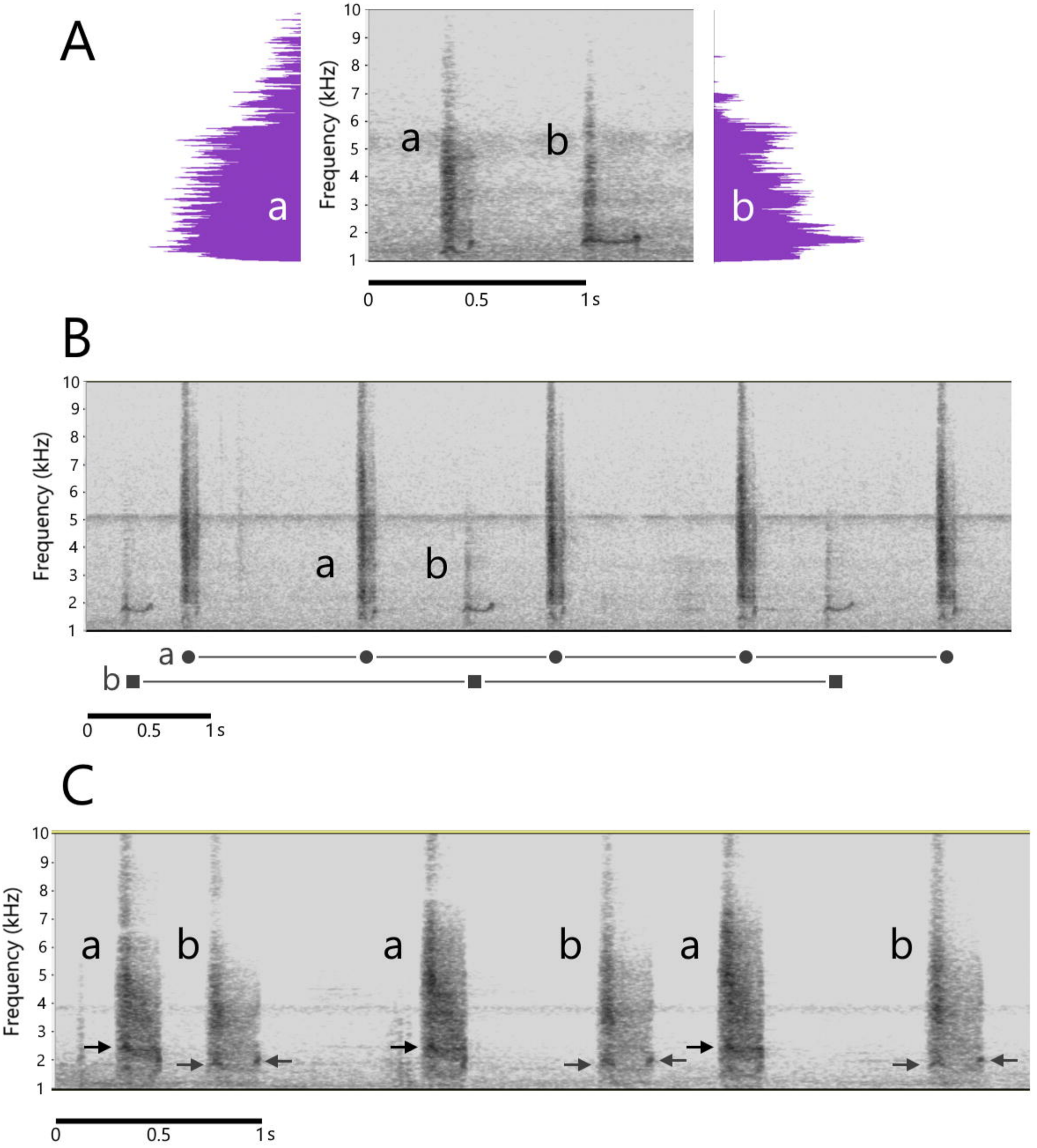
Online Resource 7 – ESM_7.tiff

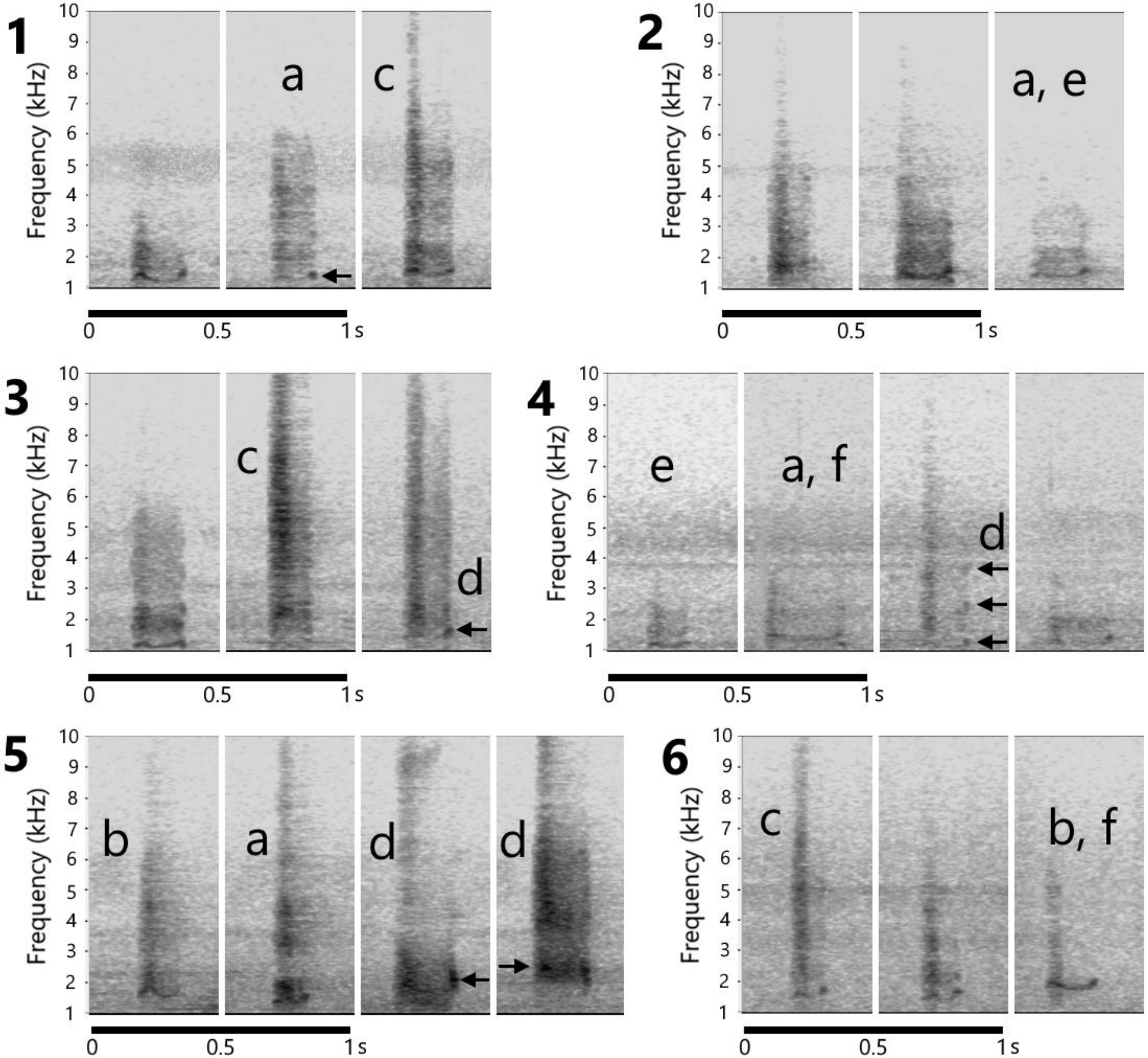
Temporal variation of the calls' acoustic features in two fledglings (see also the last two spectrograms in Figure 2, brood 5) during a 31-minute recording, which included two visits of one of their parents (indicated by the owl symbols). During this period the owlets were always separated and therefore could be identified. Time is expressed as the time remaining to the first visit (0 s). Each point represents the loudest call selected in a 30-second interval. The thin lines represent the running average calculated with three values per data point. On the right: representative spectrograms of the calls at five time points within the recording time (numbers are indicated in the top-left chart). The first spectrogram refers to the

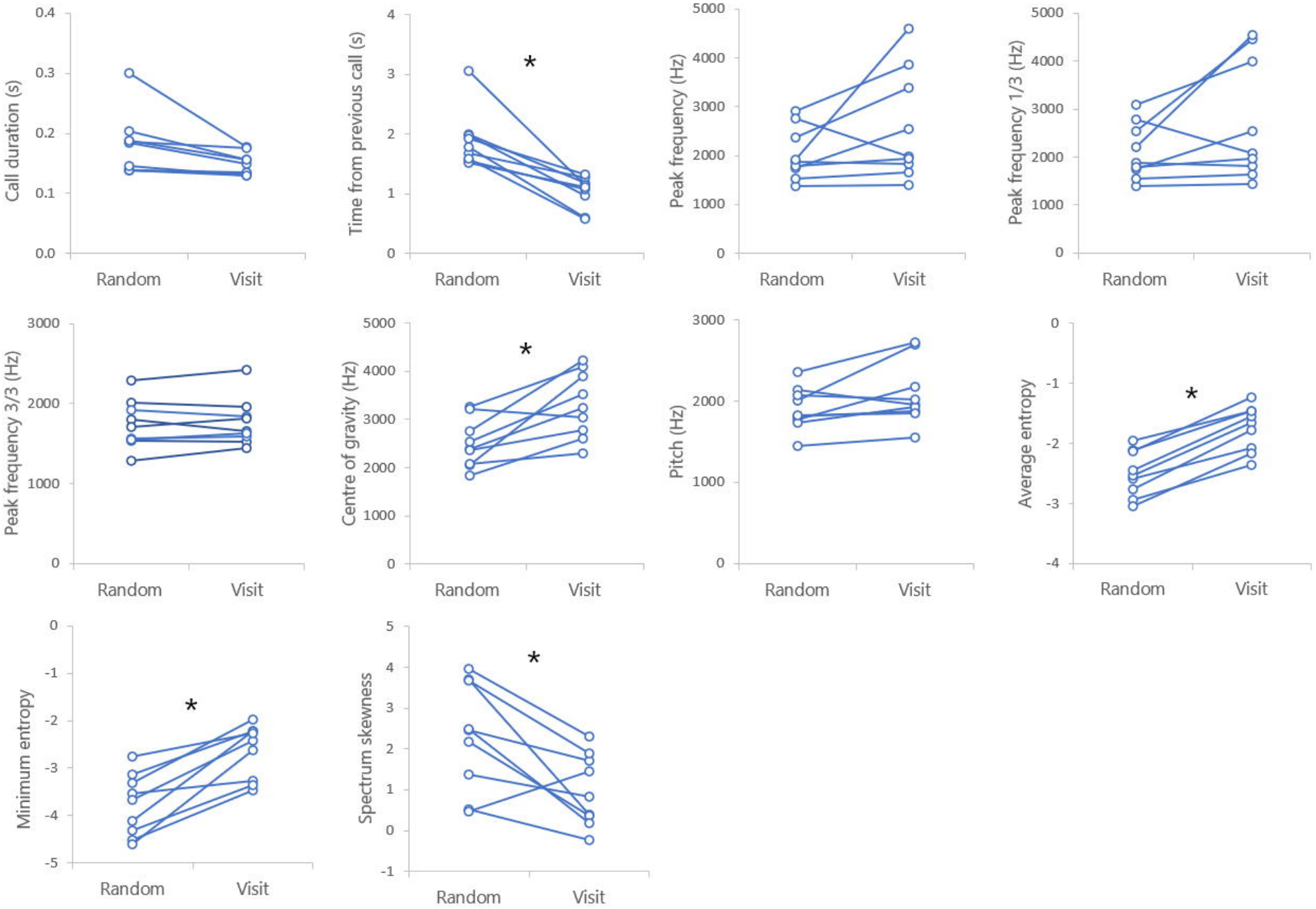
individual represented with open circles in the charts.

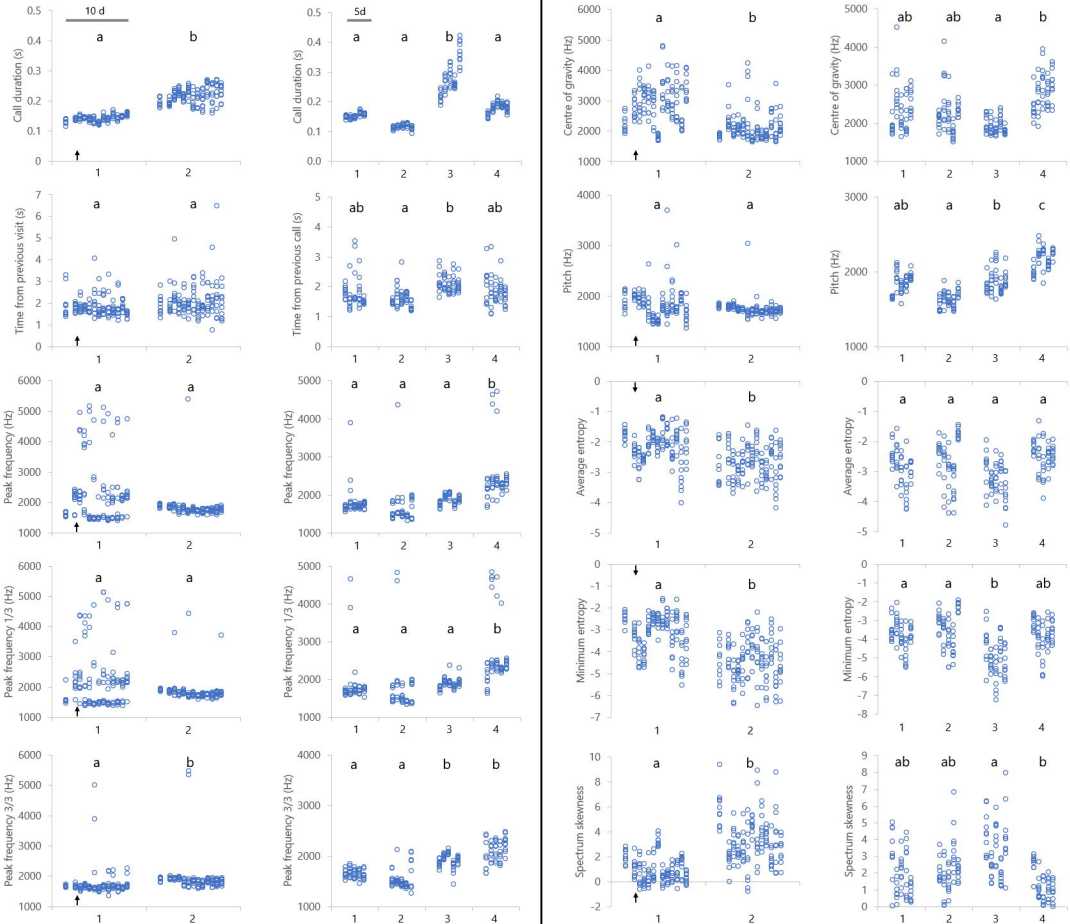
Online Resource 8 – ESM_8.tiff

Call spectrograms of young Scops Owls in brood 6, seven days before fledging. The first fragment includes three calls, the others two calls each. The arrows indicate the call with a more structured sound, similar to that detected later in the same brood (compare Figure 2, brood 6) which remained distinct from the other call types in the following recordings, for 10 days, suggesting that some call structures originate before fledging.


A
Frequency (kHz)
10
9
8
7
6
5
4
3
2
1
a
b
0
0.5
1 s
B
Frequency (kHz)
a
b
0
0.5
1 s
C
Frequency (kHz)
a
b
a
b
a
b
0
0.5
1 s


1
a
c
2
a, e
3
c
d
4
e
a, f
d
5
b
a
d
d
6
c
b, f
Frequency (kHz)
10
9
8
7
6
5
4
3
2
1
0
0.5
1 s


Call duration (s)
0.4
0.3
0.2
0.1
0.0
Random
Visit
Time from previous call (s)
4
3
2
1
0
*
Random
Visit
Peak frequency (Hz)
5000
4000
3000
2000
1000
0
Random
Visit
Peak frequency 1/3 (Hz)
5000
4000
3000
2000
1000
0
Random
Visit
Peak frequency 3/3 (Hz)
3000
2000
1000
0
Random
Visit
Centre of gravity (Hz)
5000
4000
3000
2000
1000
0
*
Random
Visit
Pitch (Hz)
3000
2000
1000
0
Random
Visit
Average entropy
0
-1
-2
-3
-4
*
Random
Visit
Minimum entropy
0
-1
-2
-3
-4
-5
*
Random
Visit
Spectrum skewness
5
4
3
2
1
0
-1
*
Random
Visit


10 d
5d
Call duration (s)
Time from previous visit (s)
Time from previous call (s)
Peak frequency (Hz)
Peak frequency 1/3 (Hz)
Peak frequency 3/3 (Hz)
Centre of gravity (Hz)
Pitch (Hz)
Average entropy
Minimum entropy
Spectrum skewness