Ultrasound social calls of greater horseshoe bats 
(Rhinolophus ferrumequinum) in a hibernaculum

MARGARET M. ANDREWS1, PETER T. ANDREWS2, DAVID F. WILLS3, and SYLVIA M. BEVIS3

1Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, United Kingdom 
E-mail: m.m.andrews@livjm.ac.uk
2Department of Physics, Liverpool University, Liverpool L69 5BX, United Kingdom
3Devon Bat Group, Exeter, Devon, United Kingdom

Ultrasound calls made by adult Rhinolophus ferrumequinum in a hibernaculum in early October were recorded using a time expansion detector during two 24 hrs periods. In addition to echolocation calls (83–84 kHz), ultrasound social calls were recorded, similar to those recorded previously in a nursery roost. The recordings also included social calls not found in the repertoire of the nursery roost colony from late April until early October. These were multiple component ultrasound social calls with six or seven components and prolonged trill calls. The trill call frequency varied periodically over a range of 2.9 ± 1.7 kHz about the mean with a period of 8.0 ± 3.1 ms. The trill calls and very long ultrasound social calls have not been reported previously. The possibility that the trill calls may be used by male bats as advertisement calls in the cave is discussed.

Key words: Rhinolophus ferrumequinum male bats, trill ultrasound social calls, hibernaculum

INTRODUCTION

Rhinolophid ultrasound social calls were first described by Möhres (1953, 1966), Long and Schnitzler (1975) and Matsumura (1979). Ultrasound social calls made by greater horseshoe bats R. ferrumequinum, in a nursery roost in Wales, provided evidence of extensive social communication in the colony at frequencies below those used for echolocation (Andrews and Andrews, 2003). The diversity of calls indicated extensive social activity within a colony of R. ferrumequinum bats similar to that studied by Rossiter et al. (2000, 2002) who suggested that social interaction was based on fidelity to mating sites and kin-biased associations. It was proposed by Andrews and Andrews (2003) that one or more of the frequency modulated (FM) ultrasound social calls identified in the nursery roost might represent male advertisement since mature male bats occupied the nursery roost (Andrews, 2000) but further investigations were needed to test this hypothesis. There have been many reports of social calls made by bats during the mating season (Sluiter and van Heerdt, 1966; Barclay and Thomas, 1979; Barclay et al., 1979; Thomas et al., 1979; Lundberg and Gerell, 1986; Racey et al., 1987; Gerell-Lundberg and Lundberg, 1994; Barlow and Jones, 1997a, 1997b; Russo and Jones, 1999; Locke, 2004). Mature male R. ferrumequinum bats habitually occupy their own mating territories with mature females from midsummer to
November (Ransome, 1991; Stebbings, 1991). The Chudleigh caves in Devon have been used over a long period as a *R. ferrum-equinum* hibernaculum (Hooper and Hooper, 1956; Hooper, 1962). An ultrasound call below the frequency range of echolocation of this species was observed during monitoring of *R. ferrumequinum* bats in the Chudleigh caves in July (Wills, 2003).

The aims of this study were: (1) to record any ultrasound calls made by bats during 24 hours periods in the hibernaculum during early October and July; (2) to identify species and to classify and compare ultrasound social calls made by *R. ferrumequinum* with calls identified previously in a nursery roost in South West Wales. Low frequency calls, < 10 kHz, were not included in the study since they were made at frequencies at which *R. ferrumequinum* hearing is not acute (Neuweiler, 1970, 2000; Long and Schnitzler, 1975; Vater, 1987). The number of low frequency *R. ferrumequinum* social calls was proportional to the number of echolocation calls (Andrews and Andrews, 2002). In this study the level of activity was monitored by counting the echolocation calls.

**MATERIALS AND METHODS**

**Sound Recording and Method of Analysis**

At the end of the second week in October 2004 an ultrasound bat detector was used to record bat cries. The detector was placed in a waterproof box in the cave. The Tranquility III time expansion bat detector (Bale, Courtpan Design Ltd., U.K.) was triggered when a call was received and a recording was stored in the bat detector in an 8 bit memory at a sampling rate of 409.6 kHz. After 320 ms it was replayed 32 times slower. The maximum frequency range of the detector was 10 kHz to 200 kHz. The detector output was stored in a Sony ICD-MS1 voice recorder (Sony Corporation, Japan) in a compressed format. The recorder was operated in SP (short play) mode with a bandwidth of 240 Hz to 4.8 kHz. With 32 times expansion this allowed bat cries in the range 10 kHz to 153 kHz to be recorded. The data on the Sony memory stick was later transferred to a computer and converted to 8-bit WAV files for storage and analysis. The output from a speaking clock announcing the hours was combined with the bat detector output at the input to the recorder. The ultrasound social calls occurred at times when *R. ferrumequinum* echolocation calls were also recorded. At the end of July 2005 a Tranquility III ultrasound bat detector (as above) was placed in the cave 1 hour before sunset and the output was stored on a Maxell, XL-II 74 mini disc (Maxell Europe, Telford, U.K.).

**Identification of Ultrasound Social Calls**

All the calls were analysed using BatSound with a Hanning window and a FFT size of 512. Frequency and duration measurements were made according to a method described by Pettersson (1991), Russ (1999) and Altringham (2003). Ultrasound social calls were identified according to the categories described by Andrews and Andrews (2003).

**Bat Activity**

In October bat activity was monitored by continuous recording during two consecutive 24 hour periods. The first recording was made near the main cave entrance and the second was made further inside the cave near the *R. ferrumequinum* colony. In July 2005 bat activity in the cave was observed for an hour before emergence and during emergence. Echolocation was monitored so that ultrasound social calls could be related to general activity. The rate at which echolocation calls were made in October and July was used as a measure of bat activity. In July *R. ferrumequinum* bats were observed during the emergence with a binocular image intensifier (Cobra Meteor, Alana Ecology, U.K.).

**Bat Occupancy and Cave Conditions**

The number of *R. ferrumequinum* roosting in the cave in October were counted at midday when the recording equipment was left and collected at the beginning and end of the recording session. The ambient temperature inside the cave at the beginning and end of recording was measured with a C.H.Y. 110 infrared non-contact thermometer (Maplin, U.K.). The internal cave conditions and weather in the vicinity were noted.

**Statistical Analysis**

Minitab version 14 (Minitab Ltd., Coventry, U.K.) was used for analysis of clusters of trill
ultrasound social calls. Combinations of linkage and distance options were tested for identification of the best separation of the agglomerative clusters (Everitt, 1977; Kaufman and Rousseeuw, 1990). The main criterion for initial identification of clusters was a clear separation of agglomerate clusters from the major dendrons. The best number of clusters was then judged according to the demonstration of statistically significant values for the parameters tested with each cluster set of calls. One-way analysis of variance (ANOVA) with the Tukey test was used to identify statistically significant parameters. Each parameter was tested with the factor, ‘cluster sets’. The Bonferroni correction was applied to minimize the probability of type 1 error (Rice, 1995). Where the $P$ value was the same for several parameters, e.g., $P \leq 0.001$, it was desirable to find a method of determining the relative importance of each parameter that characterised differences between sets of calls. A score was devised to place each parameter in a rank order of significance. The score was derived from pairwise comparisons among the means of cluster sets. The lower, centre and upper level values that ranged, for example, from $1.678$ to $9.007$ or $-6.082$ to $-1.025$ were allocated a significance score of 1. If the lower, centre and upper level values ranged from $-1.962$ to $+5.54$ then a score of 0 was given. A zero score denoted that the values for the parameter in cluster x were not significantly different from the values in cluster y. The values for a cluster designated set 1 were compared with values for cluster sets designated 2–10 then cluster set 2 was compared with 3–10 etc. until all cluster sets were compared with each other. A score sheet was devised in which, for example, clusters 1–10 were entered in the horizontal axis and vertical axis to form a grid. The maximum score would be 45 (100%) and a score of 23 would be rated at 51.1%. The highest score was rated as 1 and lower scores were placed in rank order 2 to 10.

RESULTS

Bat Occupancy and Cave Conditions

In the passage at the cave entrance during the first recording session in October there were no bats visible. At the beginning and end of the second session there were 80 torpid $R. ferrumequinum$ bats hanging in a loose cluster in a cavern at the junction between a chimney and a large internal passage. The ambient temperature in the cave was $10.2 \pm 0.6^\circ$C ($\bar{x} \pm$ SE) and the cave was generally damp but not wet. There was no rain or wind during the 24 hrs recording period at the cave entrance and some intermittent light rain during the consecutive 24 hrs period when recordings were made near the bat colony. In July there were no bats clustered in the cavern near the detector. $Rhinolophus ferrumequinum$ bats visited the cavern on their way towards the mouth of the cave. 79 bats emerged between 19:28 and 20:43 hrs and 15 bats remained in the entrance passage.

Greater Horseshoe Bat Activity Inside The Cave and at The Entrance

In October activity was higher inside the cave near the colony of 80 greater horseshoe bats than at the cave entrance (Fig. 1F and 1C). 1,017 echolocation calls were recorded at the main entrance compared with 3,812 echolocation calls inside the cave. Peak activity at the cave entrance occurred between 17:00–18:00 h GMT, which coincided with sunset at 17:15 h GMT. There was some activity during the night between 21:00–23:00 h but very little at dawn (06:15 h GMT). Inside the cave peak activity was just after sunset between 18:00–19:00 h. Activity continued during the night between 20:00–23:00 h and around dawn at 05:00–07:00 h GMT. A sample of 500 echolocation calls was analyzed in detail. The calls were typical FM-CF-FM calls of $R. ferrumequinum$ made at an average frequency of $83.8 \pm 0.4$ kHz and of $21.0 \pm 6.4$ ms duration. In July there were 1,104 echolocation calls made in the cave from 19:28–20:43 h GMT. Peak activity occurred at 21:25 h, just after sunset. The average frequency of a sample of 500 echolocation calls was $84.0 \pm 0.5$ kHz and they were $31.9 \pm 9.5$ ms long.
FIG. 1. Echolocation and ultrasound social calls made by *R. ferrumequinum* bats in a cave in early October. Recordings made during two 24 h periods timed at intervals of one hour. A–C — recordings made at the cave entrance. D–F — recordings made inside the cave in the vicinity of 80 roosting *R. ferrumequinum* bats. Echolocation calls (†). Ultrasound social calls; single component calls (■) multiple component calls (◇), modified echolocation calls (○), prolonged multiple component calls and trill calls (●). Details of ultrasound social calls in the text
Ultrasound Social Calls Recorded in October

In October ultrasound social calls were detected with fundamental frequencies in the range of 11–39 kHz, however, the majority of frequencies were between 15–29 kHz (92.6%). The length of the ultrasound social calls covered a wide range (4–237 ms) but the majority (70.3%) were 4–49 ms long.

Detailed Identification of Ultrasound Social Calls

Analysis of ultrasound social calls (n = 1,219) made in October showed that there were single component calls, multiple component calls, and modified echolocation calls but also prolonged oscillatory trill calls (Fig. 2, Tables 1 and 3). The parameters used to classify each category of calls were the frequency, duration and number of components measured. Roman numerals I–XIV were allocated to identify categories, which were also designated as CF (constant frequency) or FM (frequency modulated) calls. The names given to the ultrasound social calls facilitated sorting into categories and did not imply any interpretation of the meaning of the calls.

There was a marked difference between the type, number and proportion of ultrasound social calls recorded at the cave entrance and inside the cave (Fig. 1A–B, 1D–E). There were only nine ultrasound social calls recorded at the cave entrance compared with 1,210 ultrasound social calls inside the cave. The ratio of echolocation calls to ultrasound social calls made by greater horseshoe bats at the cave entrance was 113:1 compared with 3:1 inside the cave.

Single Component Ultrasound Social Calls

There were three categories of ultrasound social calls designated single component calls. The frequency was either constant (CF) or it rose (FM r), or it fell (FM f) (Table 1). Categories of calls were allocated Roman numerals I–II for classification. The occurrence of categories of calls designated CF I, FM II r and FM II f during the 24 hrs periods of recording at the cave entrance and inside the cave is shown in Fig. 1.

CF I, Constant Frequency Single Component Call (Monotone Whistle)

Inside the cave 255 CF I ultrasound social calls were the largest group recorded (Table 1) which occurred mainly just after sunset and at dawn. There were no CF I calls at the entrance to the cave.

FM II R, Single Component Calls, Rising Frequency Whistles

The single component rising frequency calls were observed at the cave entrance and inside the cave. There were 4 FM II r calls recorded at the entrance around sunset compared with 92 FM II r calls inside the cave, mainly just after sunset and at dawn (Fig. 1B and 1E).

FM II F, Single Component Calls, Falling Frequency Whistles

226 FM II f calls formed the second largest group of single component calls inside the cave. They occurred not only before and after sunset but also during the day (Fig. 1E). No FM II f calls were recorded at the cave entrance.

Multiple Component Calls

Multiple component calls were categorised according to Roman numerals (III–VII, XIII–XIV) and the number of components in which the frequency rose
Fig. 2. Sonograms of multiple component, trill and modified echolocation ultrasound social calls made by *R. ferrumequinum* in a hibernaculum in early October. CF — constant frequency, FM — frequency modulated, VII, XI–XIV — categories of calls (see the text and Tables 1–3 for details), EL — echolocation, t — trill calls, r — rising frequency, f — falling frequency, C1–C7 — components 1–7.
or fell. The components were referred to as the first component to the seventh component (Table 1; see also Fig. 1A and 1D).

**Double Component Calls**

The group of ultrasound social calls with two components were characterised by a rise in the frequency of the first component then the frequency of the second component either fell, FM III r–f, or was sustained, FM IV r–s (Table 1).

**FM II R–F, Double Component Rising and Falling Frequency Call (Two Tone Whistle)**

There was only one FM III call recorded at the cave entrance, at sunset, compared with 137 FM III calls inside the cave near the bat colony around sunset and at dawn (Fig. 1).

**FM IV R–S, Double Component Rising And Sustained Frequency Call (Screech)**

There were no FM IV calls recorded at the cave entrance and relatively few inside the cave ($n = 19$). The calls occurred at a minimal level after sunset and the largest proportion was recorded at dawn (Fig. 1).

**FM V, Triple Component Call (Three Tone Whistle)**

Variations in frequency in components 1–3 characterised the FM V call (Table 1). The occurrence was similar to the FM IV calls above since there were no FM V calls recorded at the cave entrance and relatively few inside the cave ($n = 17$). Between 17:00–21:00 h calls occurred at a minimal level then there was a small peak ($n = 5$) between 20:00–21:00 h and a few calls ($n = 4$) were recorded around dawn (Fig. 1).

**FM VI, Quadruple Component Call (Shriek)**

The rise and fall of frequency in components 1–4 characterised this call (Table 1). There was 1 call recorded at the cave entrance at sunset compared with 24 calls recorded inside the cave mainly after sunset ($n = 12$) then sporadically through the night with a small peak ($n = 4$) at dawn (Fig. 1).

**FM VII, Quintuple Component Call (Squeal)**

The call was characterised by rising or falling frequencies in components 1–5 (Table 1). There were only five calls recorded inside the cave, which were spread out between 18:00–20:00 h and there was one call at dawn. There were no FM VII calls recorded at the cave entrance (Fig. 1).

**FM XIII, Sextuple Component Call (Howl)**

The alternate rise and fall in frequency in components 1–6 identified the call (Table 1, Fig. 2). There were seven calls made inside the cave between 18:00–08:00 h and there were none at the cave entrance.

**FM XIV, Septuple Component Call (Bellow)**

Variations in frequency in each of the seven components, C1–C7, characterised the call (Table 1). There were no FM XIV calls recorded at the cave entrance and inside near the colony three calls occurred after sunset, one at dawn and two in the early morning (Fig. 1).

**Multiple Component Trill Calls**

Analysis of multiple component calls with 5–7 components showed that within the overall frequency variations in each component the frequency of some calls also varied to produce trills (Fig. 2). The
frequency varied by an average of 2.9 ± 1.7 kHz about the mean with a period of 8.0 ± 3.1 ms. These calls, classified as trill calls, were made exclusively inside the cave (Fig. 1D).

**FM VII T, Quintuple Component Trill Call**

There were 20 FM VII trill calls of which 11 were most frequent just after sunset (Fig. 1). These calls were very long (130.0 ± 48.2 ms), the average frequency of the fundamental was relatively high (23.5 ± 3.9 kHz), and there were mainly 2–3 harmonics.

**FM XIII T, Sextuple Component Trill Call**

There were 26 FM XIII trill calls of which 10 calls were most frequent just after sunset but there were also 10 calls between 19:00–20:00 h and a further six calls around dawn (Fig. 1, D). This category of calls was shorter than other trill calls (102.6 ± 42.2 ms), the average frequency of the fundamental was 20.4 ± 2.9 kHz, and there were mainly five harmonics.

**FM XIV T, Septuple Component Trill Call**

There were 16 FM XIV trill calls of which nine were recorded between 18:00–24:00 h and seven between 05:00–10:00 h (Fig. 1). The duration was 120.3 ± 56.3 ms and the average frequency of the fundamental was relatively low (19.0 ± 2.4). The majority of calls had 5–6 harmonics.

### TABLE 1. Analysis of the fundamental of single and multiple component ultrasound social calls made by *R. ferrumequinum* in a cave. Records made in early October during a 24 h period. Mean, SD, and minimum–maximum range of frequency (in kHz) or duration (in ms, in parentheses) are shown. I–XIV, r–f, r–s — categories of call (see text for details); CF – constant frequency; FM – frequency modulated. All frequencies given in kHz, duration in ms. a — frequency at the start of the call; b — frequency of the first component; r — rising frequency; f — falling frequency

<table>
<thead>
<tr>
<th>No.</th>
<th>Call category</th>
<th>n</th>
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<th>2nd component</th>
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<td></td>
<td>Frequency a</td>
<td>Frequency b</td>
<td>Duration</td>
</tr>
<tr>
<td>1</td>
<td>CF I</td>
<td>255</td>
<td>18.1, 3.6</td>
<td>18.1, 3.6</td>
<td>43.5, 27.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(12–21)</td>
<td>(4–26)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>FM II r</td>
<td>96</td>
<td>19.4, 3.9</td>
<td>25.8, 6.2</td>
<td>45.3, 24.1</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>(17–42)</td>
<td>(4–160)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>FM II f</td>
<td>226</td>
<td>19.9, 3.1</td>
<td>16.9, 3.0</td>
<td>49.1, 22.5</td>
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<td></td>
<td></td>
<td></td>
<td>(13–32)</td>
<td>(6–126)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>FM III double r–f</td>
<td>138</td>
<td>17.4, 3.1</td>
<td>22.2, 5.1</td>
<td>18.9, 10.5</td>
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<td></td>
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<td>(11–28)</td>
<td>(13–50)</td>
<td>(5–52)</td>
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<td>5</td>
<td>FM IV double r–s</td>
<td>19</td>
<td>17.6, 2.3</td>
<td>28.7, 6.3</td>
<td>30.2, 13.6</td>
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<td></td>
<td></td>
<td></td>
<td>(14–21)</td>
<td>(20–34)</td>
<td>(7–52)</td>
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<tr>
<td>6</td>
<td>FM V triple</td>
<td>17</td>
<td>17.6, 3.9</td>
<td>25.0, 8.3</td>
<td>28.7, 14.1</td>
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<td>7</td>
<td>FM VI quadruple</td>
<td>24</td>
<td>18.1, 3.9</td>
<td>23.6, 5.6</td>
<td>17.3, 11.8</td>
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<td>8</td>
<td>FM VII quintuple</td>
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<td>19.3, 4.4</td>
<td>24.5, 5.4</td>
<td>10.2, 7.8</td>
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<tr>
<td>9</td>
<td>FM XIII sextuple</td>
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<td>16.6, 2.6</td>
<td>23.0, 3.8</td>
<td>16.3, 13.2</td>
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<td>10</td>
<td>FM XIV septuple</td>
<td>6</td>
<td>16.7, 2.4</td>
<td>21.7, 4.2</td>
<td>19.8, 14.3</td>
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</table>
Statistical Analysis of Trill Calls

Since the oscillations of the trill calls were distinctive (Fig. 2) an analysis was made of the whole group \((n = 62)\). Multivariate cluster analysis, using a combination of Ward linkage and Euclidean distance, gave clear separation into nine clusters (Table 3) at the 5.53 distance level (maximum 43.71) and 38.99 similarity level. ANOVA was performed sequentially for each cluster set with each parameter using the Tukey test, at 95% confidence intervals (CI). The Bonferroni correction was applied and Tukey test was repeated with the family error rate reduced from the 5% level to 0.1%. The Tukey test performed with 99.9% CI confirmed that the characteristic frequencies of each of the nine clusters or sets of trill calls (Table 3) showed significant differences, \(P \leq 0.001\), (Table 2) and did not occur at random. The parameters in which the most significant differences occurred between the sets of calls were the number of components, average frequency, peak frequency and frequency at the start of the call. The frequency at the end of the fundamental, the variation in frequency of the oscillations and the number of harmonics showed smaller differences (Table 2). The total duration, number of oscillations per call and the period of the oscillations did not characterise differences between the sets of trill calls made in the cave (Table 2). Multivariate cluster analysis repeated, with Ward linkage and Euclidean distance, using the significant parameters gave clear separation into nine clusters (Table 3) at the at the same distance level and similarity level as the initial analysis.

Modified Echolocation CF and FM Calls

Modified echolocation calls were allocated to categories according to the central component of the call (CF or FM), and
whether the calls were separate or occurred in a cascade of calls (Fig. 2). The FM sweep at the start and end of the central part was present in all the CF modified echolocation calls but the FM sweep at the end of the call was more evident. The modification of the CF calls was the progressive reduction of frequencies in a stepwise manner. The conversion of the central constant frequency (CF) pulse to a frequency modulated (FM) pulse was the alternative modification (Fig. 2). The calls were allocated to sets according to the frequency range and, where appropriate, the sequence in which they occurred in a cascade of calls. Modified echolocation calls in category FM XI were classified according to the prolongation of the FM sweep at the start and end of the call and the removal of the CF component. The modified echolocation call frequencies were produced in the second harmonic to the sixth harmonic (Fig. 2).

FM XI F, Single Frequency Modulated Falling Frequency Modified EL Call

There was one FM XI f call recorded at the cave entrance compared with 243 FM XI f calls recorded inside near the bat colony (Fig. 1B and 1E). The majority of calls occurred after sunset and just before dawn. Most of 154 FM XI f calls were made between 18:00–21:00 h and the second smaller group of 24 calls around dawn coincided with the largest number of echolocation calls, indicating that the number of FM XI f calls correlated with general flight activity in the cave.

FM XI F and FM XI R Cascade, Frequency Modulated Cascade Calls

The falling frequency FM XI f cascade calls commonly followed the FM XI r rising frequency cascade calls when the sequence of cascade calls occurred in reverse order at progressively higher frequencies (Fig. 2). Calls were numbered [1]–[10] according to the sequence of each call in the cascade. The frequencies of all the FM XI cascade calls were below the echolocation frequency.

FM XI R, Frequency Modulated Rising Frequency Modified EL Call

The distribution of FM XI r calls was similar to the FM XI f calls above (Fig. 1B and 1E). There were 2 FM XI r calls made at the cave entrance compared with

<table>
<thead>
<tr>
<th>Parameter</th>
<th>x</th>
<th>SD</th>
<th>P</th>
<th>Rank</th>
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<tbody>
<tr>
<td>Total duration (ms)</td>
<td>116.0</td>
<td>48.8</td>
<td>0.304</td>
<td>*</td>
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<tr>
<td>Number of oscillations per call</td>
<td>15.0</td>
<td>5.5</td>
<td>0.002</td>
<td>*</td>
</tr>
<tr>
<td>Period of oscillations (ms)</td>
<td>8.0</td>
<td>3.1</td>
<td>0.436</td>
<td>*</td>
</tr>
<tr>
<td>Average frequency of the fundamental (kHz)a</td>
<td>21.0</td>
<td>3.6</td>
<td>&lt; 0.0001</td>
<td>2</td>
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<td>Start frequency of the fundamental (kHz)a</td>
<td>16.9</td>
<td>3.3</td>
<td>&lt; 0.0001</td>
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<tr>
<td>End frequency of the fundamental (kHz)a</td>
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<td>5.6</td>
<td>&lt; 0.0001</td>
<td>5</td>
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<td>Peak frequency of the fundamental (kHz)a</td>
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<td>5.3</td>
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<tr>
<td>Total excursion of frequency variation in oscillations (kHz)a, b</td>
<td>5.7</td>
<td>2.4</td>
<td>&lt; 0.0001</td>
<td>6</td>
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<tr>
<td>Number of components in each category of calla</td>
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<td>0.8</td>
<td>&lt; 0.0001</td>
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</tr>
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<td>Number of harmonics a</td>
<td>4.5</td>
<td>1.3</td>
<td>&lt; 0.0001</td>
<td>7</td>
</tr>
</tbody>
</table>

TABLE 2. Parameters of the trill ultrasound social calls made by R. ferrumequinum in a cave during a 24 h period in early October (n = 62). Parameters were used in multivariate cluster analysis, which identified nine clusters or sets of calls (Table 3). a — parameters used in multivariate analysis, b — change in frequency per oscillation. One-way analysis of variance (ANOVA) used to determine which parameters were significantly different in each cluster set (factor). P = P-value for the Tukey test, Bonferroni corrected. Rank = rank order of significance, * — not statistically significant (please see details of the tests in the text).
<table>
<thead>
<tr>
<th>Category and number of components</th>
<th>Set No.</th>
<th>Number of calls</th>
<th>Fundamental frequency (kHz)</th>
<th>Number of harmonics</th>
<th>Oscillations variation</th>
<th>Number of trill calls during 24 h</th>
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<tr>
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<td>Average</td>
<td>Start</td>
<td>End</td>
<td>Peak</td>
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<td>FM VII t</td>
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63 inside the cave. There was a peak of 12 calls made during the night (20:00–21:00 h) and a second larger peak of 40 calls made at dawn. The peaks of FM XI r calls coincided with the minor peaks in echolocation calls which indicated that the number of FM XI r calls also correlated with general flight activity of the colony in the cave.

**CF XII Cascade, Constant Frequency Modified EL Calls**

CF XII cascade was the category of ultrasound calls in which the frequency of the central component was constant but below the echolocation frequency (Fig. 2). The central CF pulse of each successive call, [1]–[4], showed a reduction in frequency (Fig. 2).

**Harmonics of Ultrasound Social Calls**

Analysis of the harmonics of single component and multiple component ultrasound social calls in the cave \((n = 857)\) showed that 6.4% had no harmonics. Although 93.6% of calls had harmonic 2 there was a progressive reduction in the number of calls with harmonics 3–7. The relative intensity of high frequency harmonics probably depended on whether the bat was facing the detector microphone or not. Harmonics provided variety to each category of calls in addition to variations in the frequency and duration of the fundamental. Harmonics 4 and 5 produced frequencies in range 83–84 kHz and harmonics 6 and 7 in the range 110–140 kHz (Fig. 2).

**Ultrasound Social Calls Recorded in July**

There were 68 ultrasound social calls made by *R. ferrumequinum* in the cave during emergence in July. The majority were single component calls \((n = 64, 94.1\%)\). The CF I category calls were predominant \((n = 62)\) and there were only 2 FM II f calls. The remaining multiple component calls were FM III \((n = 1)\) and FM XIV \((n = 1)\) category calls. The single component calls were concurrent with the general activity but the multiple component calls occurred only at 21:03 h and 21:18 h. The average frequencies of the CF I and FM II f calls were 20.9 ± 2.8 kHz and 23.6 ± 2.0 kHz, respectively. The average length of those calls was 76.2 ± 35 ms and 2.0 ± 0.0 ms, respectively. The frequency of the FM III call was 21.1 ± 0.0 kHz it was 138 ms long and the frequency of the remaining FM XIV call was 27.9 kHz and it was 105 ms long.

**DISCUSSION**

**Bat Activity and Hibernaculum Conditions**

The cave conditions and *R. ferrumequinum* bat colony size were similar to those found previously in winter in the Chudleigh Caves (Hooper and Hooper, 1956; Wills, 2004). A rapid reduction in the size of a nursery roost colony in West Wales on October 9th 2004 confirmed the onset of *R. ferrumequinum* hibernation (Andrews, 2004). The short period of flight activity at the cave entrance around dusk in early October was also similar to the short evening excursions from the *R. ferrumequinum* nursery roost in West Wales (monitored by PA). The pattern of emergence of *R. ferrumequinum* bats from the cave in July (Wills, 2005) was similar to the exit from the nursery roost in Wales at that time of year (monitored by PA). The larger number of echolocation calls and the relatively high ratio of ultrasound social calls to echolocation calls inside the cave compared with the cave entrance showed that *R. ferrumequinum* winter roost social
activity in this study was centered inside the cave.

**Ultrasound Social Calls, Cave Repertoire in July**

The single component ultrasound social calls, categories CF I and FM II f, were typical of the calls made during the exit of *R. ferrumequinum* bats from a nursery roost (Andrews and Andrews, 2002). However, the FM III multiple component call was longer in the cave repertoire and the FM XIV call was an addition to the repertoire which was not observed in a nursery roost (Andrews and Andrews, 2002). Direct observation of *R. ferrumequinum* bats in the cave in July 2005 confirmed that they used ultrasound calls at lower frequencies than those used for echolocation and that these were associated with behaviour in a similar way to the colony observed in a nursery roost.

**Ultrasound Social Calls, Hibernaculum Repertoire in October**

Although the majority of ultrasound social calls made by *R. ferrumequinum* in the cave were similar to calls made by adult *R. ferrumequinum* bats in a nursery roost (Andrews and Andrews, 2003) there was an additional repertoire of prolonged multiple component calls (FM XIII, FM XIV) and trill ultrasound social calls in the cave (FM VII t, FM XIII t, FM XIV t). The trill calls made inside the cave near the roosting *R. ferrumequinum* bats were distinct from other calls in the greater horseshoe bat repertoire. All the categories of ultrasound social calls identified merit further investigation with simultaneous observation of *R. ferrumequinum* bat behaviour since Rossiter et al. (2000, 2002, 2005) have shown that a similar colony of this species were closely related yet avoided incestuous mating.

**Harmonics of Single and Multiple Component Ultrasound Social Calls**

The frequencies of harmonics 4 and 5 in the cave calls were in the most sensitive range of *R. ferrumequinum* bat hearing (Bruns, 1976a, 1976b; Suga et al., 1976; Schuller and Pollack 1979; Vater, 1987). This would enable directional location of the source by other *R. ferrumequinum* visiting the cave. (Andrews, 1995). Harmonics 5–7 would also be heard by other bat species since they were in the sensitive range of hearing of either *R. hipposideros* or *Myotis* species such as *M. nattereri* (Vaughan et al., 1997). Both *R. hipposideros* and *M. nattereri* have long-established winter use of the Chudleigh Caves (Hooper and Hooper, 1956; Wills, 2004).

**Modified Echolocation Calls**

There were minor differences between modified echolocation calls made by *R. ferrumequinum* in the Welsh nursery roost (Andrews and Andrews, 2003) and in the cave. All modified echolocation calls in the nursery roost were produced as harmonic 2 of the fundamental in the range 15–40 kHz but in the cave similar calls were produced as harmonics 2–6 of the fundamental in the range 10–39 kHz. This may have been due to a higher probability of bats facing or flying towards the microphone in the cave. There was a greater proportion of single FM XI and CF XII modified echolocation calls in the cave and fewer cascades than in the nursery roost.

**Rhinolophid Breeding Sites and Male Advertisement Calls**

*Rhinolophus ferrumequinum* bats mate in caves from the autumn onwards and fertilization is delayed until spring. Mature males occupy territorial sites in hibernacula
during the winter and females consistently return to these sites to mate (Hooper and Hooper, 1956, 1967; Stebbings, 1964; Ransome, 1968, 1971, 1991). Ransome (1991) described a single mature male R. ferrumequinum with up to six breeding females occupying part of the same cave as a cluster of mainly immature bats of both sexes. In our study bats were not captured in order to sex them but previous work at the Chudleigh confirmed that predominantly male R. ferrumequinum occupied the caves in the summer (Billington, 2002). In other studies Chudleigh Caves have been found to be predominantly male hibernacula containing unsegregated clusters of from six to over 100 bats (Hooper and Hooper, 1956; Wills, 2004).

Male bats of many species are known to attract females by using ultrasonic or lower frequency trill advertisement calls characteristic of their species either while stationary or when flying (Bradbury, 1977; Porter, 1979; Ahlén, 1990; Barataud, 1996; Behr and Helversen, 2004). This seems to be a common way in which such vocal animals attract a mate. R. ferrumequinum trill calls have not been described previously. Cluster analysis resolved the 62 calls recorded in the cave into nine sets.

The most significant differences identified between the R. ferrumequinum trill calls were the average frequency, peak frequency and initial frequency of the fundamental. The reason for believing that the trill calls were probably advertisement calls is that they were associated with the echolocation calls of R. ferrumequinum in the cave at a time and place when mating was likely. Further investigation of the trill calls would be useful because R. ferrumequinum is listed in Annex II of the EU Habitats and Species Directive (Ransome, 1999; Appleton et al., 2002) and breeding sites need to be conserved. If the trill calls were shown to be male advertisement calls in established male territorial sites, such as the cave in Devon, then small or inaccessible sites could be initially located by sound.

This study has shown that R. ferrumequinum bats produce ultrasound calls within a hibernaculum, presumably social, at frequencies lower than those used for echolocation calls. Differences between the calls were sufficient for identification of groups of similar calls within a colony of the species. Harmonics of the calls were distributed in the frequency range in which the R. ferrumequinum bats normally echolocate and this would have allowed this species to locate each other accurately. This study has identified the season and the time of night when R. ferrumequinum trill calls were observed and the characteristics of these calls, analysed into nine distinguishable sets. The R. ferrumequinum trill calls, used in the breeding season in mainly male territory, merit further work to establish whether they are or are not male advertisement calls.

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