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Unusual social calls of Nathusius’ pipistrelle (Vespertilionidae, Chiroptera) recorded outside the mating season

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Abstract. The advertisement calls of Nathusius’ pipistrelle (Pipistrellus nathusii) show an unified structure: three main motifs which can be supplemented with additional two motifs especially during mating season. In May and June, a continuous social vocalization of quite unusual structure and composition was recorded apart from these standard calls. Most of these calls were designed in a long “wavy line” pattern with the peak frequency of 14–36 kHz, and were accompanied by standard or modified echolocation calls. In one case we succeeded to supplement the acoustic records with a complete record of behavioural details: two bats slowly flew and followed one another in a circular path in front of the roost, where they repeatedly performed false landings, and during approaching one of them emitted the unusual social calls. This behaviour lasted for about 40% of the whole-night activity. The possible meaning is discussed.

Key words: Pipistrellus nathusii, vocalization, modified calls

Introduction
Nathusius’ pipistrelle (Pipistrellus nathusii, Keyserling & Blasius, 1839) is a small insectivorous bat distributed throughout Europe. In contrast to sibling pipistrelles, in which the seasonal dispersal is limited mostly to the local scale (Gaisler et al. 2003, Taake & Vierhaus 2004), Nathusius’ pipistrelle is a migratory species well known for its long-distance seasonal migrations for over 1900 km (Masing 1988, Petersons 1990, Ahlén 1997, Hutterer et al. 2005). In Western and Central Europe, the mass appearance of advertising males in late summer is conspicuous and the peak of mating season falls just into the period between August and September (Arnold et al. 1996, Vierhaus 2004). Nathusius’ pipistrelle is a polygynous bat species with a resource defence polygyny mating system (Gerell-Lundberg & Gerell 1994). The advertising males actively defend their roosts and adjacent territories, exhibit high roost fidelity and return to the same area each year (Rachwald 1992). In contrast, females return to their nursery areas (Schmidt 1984).

Males of Nathusius’ pipistrelles are well known for their complex acoustic advertisement behaviour performed as a songflight or sedentary display. The advertisement calls are usually composed of three motifs (Lundberg 1989). The fourth and the fifth motifs can follow after the standard three motifs, and the complexity of advertising calls increases in the peak of the mating season (Jahelková et al. 2008). The occurrence of several stable parameters in advertising calls enables individual recognition (Jahelková 2003, Russ & Racey 2007, Jahelková et al. 2008). These calls can be recorded throughout the whole season (Barlow & Jones 1996, Russ et al. 1998), but vocalization activity is much lower in spring than in late summer to autumn (Furmankiewicz 2003).

During a long-term study of advertising behaviour of Nathusius’ pipistrelle in Southern Bohemia, social calls of a quite different structure and modified echolocation calls among typical social calls of this species were recorded during May and June 2006. The aim of this report is to describe this type of unusual social calls.
Material and Methods

The study area covers 5 km² and is situated in the Třeboňsko reserve, Southern Bohemia, in the vicinity of the town Veselí nad Lužnicí (49°03' N, 14°50' E). The acoustic survey in this study area was undertaken from 1999 to 2007; the unusual social calls were recorded in May and June 2006 at three sites (1, 6, 17, cf. Jahelková et al. 2008). During whole night observation at site 17 on May 13, the respective behaviour was observed in bright moonlight and red light illumination. To assess the amount of vocalization activity during this observation, “a positive 15 seconds” unit was used (instead of the routinely used “a positive minute” unit) for better resolution. The acoustic analysis was performed with outputs of the bat detector D240x (10x time expansion), recorded by the digital recorder Sony MZ-RH10, transferred to the computer and examined using the Bat Sound 1.2 and Avisoft-SasLabPro 4.23 software (sampling frequency 22050 Hz, Hamming window, FFT size 256 and FFT overlap 87.5%; frequency resolution 1120 Hz, time resolution 0.1451 ms). Mann-Whitney U statistics was used to compare the W- and I-type of unusual calls.

Results

During observations of the activity of Nathusius’ pipistrelle (Pipistrellus nathusii) in May and June 2006, standard echolocation calls and advertising calls of standard structure ABC (Fig. 1) were recorded together with modified echolocation calls and unusual social calls with a wavy-line character (Fig. 2). Short observations and recordings were made during regular transect at site 1 on May 4 (2:15–2:45) and during an occasional observation at site 6 on June 6 (22:00–23:45). Sites 1, 6 and 17 are known to be traditional advertising sites of males (Jahelková et al. 2008). Site 1 (roost in a roof crevice) is quite far from both maternity colonies in the area (1250 m, 2180 m), whereas sites 6 (roost in tree hole) and 17 (roost in roof crevice) are in moderate distance from one of them (250 m, 2520 m and 2040 m, 520 m).

In all situations, the calls were emitted in the presence of more individuals of Nathusius’ pipistrelle. A maximum of four individuals were observed during the collective flight. Usually, only two individuals were observed. The circular flight path was about 20 m in diameter and a false landing or approaching to short distance (0.5 m) towards the roosts was always noticed. On May 13, 2006, a whole-night observation revealed the night activity pattern of this unusual vocalization behaviour. A resident male (male 17, cf. Jahelková et al. 2008) produced typical advertising calls with ABC motifs in total for 57.5 minutes. Between 1:00 and 2:05 was recorded also very short songflight of an alien male which lasted in total 1.75 minutes (Fig. 3A). The total amount of activity corresponding with the emission of unusual social calls was 173.5 minutes and the increased flight activity of two and more individuals without vocalization lasted for 67.7 minutes (Fig. 3B).

The observed behaviour was as follows: Two bats (resident male and unknown individual, more likely female) slowly flew in a circular path in front of the roost and followed one another closely (0.5–1 m). They did not necessarily produce echolocation calls during circling, except for the approaching phase of false landings, during which their echolocation calls were always recorded. The unusual vocalization was produced only when one of the circling individuals approached the roost, while advertising calls
were emitted both during approaching and circling (songflight behaviour). At 1:32, this behaviour was interrupted by sudden arrival of a third individual. Consequently, advertising calls of the resident male and the alien male were recorded, two bats separated and one bat chased the other (most likely resident male pursued the alien male) till 1:37. After two minutes the resident male returned, joined its partner and they both continued in a circular flight. In a period between 2:05 and 4:40, one or two new bats, supposedly females, randomly arrived and circled together with the couple, and no aggressive behaviour was recorded.

Unfortunately, no continual sequence of unusual social calls developing from the advertising calls was recorded; so the sex and identity of the individual that emitted these unusual calls was unknown. The unusual calls could be thus produced by resident male, second individual (presumably female) or by both of them.

During approaching, some echolocation calls were also modified and formed an increasing steep frequency-modulated part at the start of the call. The bandwidth of the increasing steep frequency-modulated part ranged from 4.3 to 15.8 kHz. These modified echolocation calls could lose their quasi-constant part at the end and be incorporated into the unusual calls (I-type calls).

In the unusual social vocalization calls with a “wavy line” pattern (W-type calls) prevailed; the others found more often were: steep frequency-modulated calls and steep frequency-modulated calls with an increasing steep frequency-modulated part (I-type calls); quasi-constant calls with slight undulation; inverted “V-syllable”; and syllables known from both male and female – juvenile vocalization (Fig. 4). Typical syntax of these unusual calls was one or two (maximum five) W-type calls supplemented by shorter types (mainly I-type calls).

Because the structure was complicated and variable, only basic parameters of W- and I-type calls were measured (Table 1). Individual differences in all surveyed frequency characteristics of W-type calls were statistically significant, although the minimal and maximal values overlap. The best illustration is given by the bandwidth of the calls, where calls obtained at vocalization site 17 had much lower bandwidth than those of site 6 (Mann-Whitney U test, N₁₇ = 47, N₆ = 16; Z = -4.36, P < 0.001). Differences in the duration were not significant (Mann-Whitney U test, N₁₇ = 47, N₆ = 16; z = -0.17, P = 0.86). In contrast, in steep frequency-modulated type, only slight differences in duration were found (Mann-Whitney U test, N₁₇ = 36, N₆ = 16; z = 2.44, P < 0.05).

**Discussion**

Social calls can be classified into four major categories: (a) agonistic or aggressive calls (noisy broad-band frequency calls), (b) distress calls (trill-like calls), (c) isolation and directive calls (tonal calls) and (d) advertisement or mating calls (song-like calls) (Pfalzer & Kusch 2003).

The unusual social calls were formed by combination of (c) and (d) calls. Category (c) includes the tonal calls which produce males during advertising especially in the peak of mating period, and which are individually specific (motif D). Similar calls produce also mothers and juveniles in maternity colonies (Jahelková et al. 2008). Pattern of modified echolocation calls which start with frequency modulated “hook” resembles to social/echolocation calls of *Myotis daubentonii* with the same modification (Pfalzer & Kusch 2003).
Table 1. Measured parameters of two most common components recorded in unusual social calls: n, number of analyzed calls; stf, start frequency in kHz; lof, lowest frequency of waves in kHz; upf, highest frequency of waves in kHz; fmaxe, frequency of maximal energy in kHz; enf, end frequency in kHz; bw, bandwidth of the call in kHz; dur, duration in ms.

<table>
<thead>
<tr>
<th>Site</th>
<th>Date, n</th>
<th>stf</th>
<th>lof</th>
<th>upf</th>
<th>fmaxe</th>
<th>enf</th>
<th>bw</th>
<th>dur</th>
</tr>
</thead>
<tbody>
<tr>
<td>W-type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>13.5.2006, n = 47</td>
<td>12.9–78.4</td>
<td>15.6–32.7</td>
<td>18.9–68</td>
<td>14.3–36.1</td>
<td>12.9–60.3</td>
<td>4.28–50.8</td>
<td>19.6–87.5</td>
</tr>
<tr>
<td></td>
<td>mean ± SD</td>
<td>32.5 ± 14.5</td>
<td>20.8 ± 3.8</td>
<td>30.6 ± 10.5</td>
<td>22.7 ± 4.9</td>
<td>21.9 ± 10.7</td>
<td>18.4 ± 10.7</td>
<td>44.6 ± 15.4</td>
</tr>
<tr>
<td></td>
<td>median</td>
<td>31.0</td>
<td>20.0</td>
<td>27.7</td>
<td>21.9</td>
<td>16.4</td>
<td>17.2</td>
<td>42.6</td>
</tr>
<tr>
<td>6</td>
<td>6.6.2006, n = 16</td>
<td>18.1–68</td>
<td>13.8–59.6</td>
<td>24.1–70.6</td>
<td>18.1–35</td>
<td>18.1–38.8</td>
<td>16.3–50.0</td>
<td>11.9–95.4</td>
</tr>
<tr>
<td></td>
<td>mean ± SD</td>
<td>43.8 ± 13.4</td>
<td>23.5 ± 10.1</td>
<td>50.5 ± 12.2</td>
<td>18.3 ± 6.0</td>
<td>28.4 ± 6.6</td>
<td>33.5 ± 10.1</td>
<td>45.4 ± 20.4</td>
</tr>
<tr>
<td></td>
<td>median</td>
<td>42.6</td>
<td>22.1</td>
<td>50.4</td>
<td>30.1</td>
<td>27.6</td>
<td>33.4</td>
<td>47.2</td>
</tr>
<tr>
<td>I-type</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>17</td>
<td>13.5.2006, n = 36</td>
<td>41.3–94.7</td>
<td></td>
<td>25.6–50.8</td>
<td>13.8–37.9</td>
<td>20.7–65.5</td>
<td>1.9–11.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mean ± SD</td>
<td>66.4 ± 13.4</td>
<td></td>
<td>35.9 ± 6.3</td>
<td>26.4 ± 5.8</td>
<td>40.0 ± 11.8</td>
<td>4.0 ± 2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>median</td>
<td>68.9</td>
<td></td>
<td>35.3</td>
<td>26.7</td>
<td>42.0</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6.6.2006, n = 16</td>
<td>55.1–77.5</td>
<td></td>
<td>29.6–43</td>
<td>21.5–37.9</td>
<td>33.4–47.4</td>
<td>1.9–4.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mean ± SD</td>
<td>65 ± 6.9</td>
<td></td>
<td>36.7 ± 5</td>
<td>29.3 ± 3.8</td>
<td>35.6 ± 8.4</td>
<td>2.8 ± 0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>median</td>
<td>64.2</td>
<td></td>
<td>37.4</td>
<td>29.3</td>
<td>34.9</td>
<td>2.5</td>
<td></td>
</tr>
</tbody>
</table>
The category (d) contains the most complex species-specific calls. Trills as a sequence of “V-shaped signals” or tied trills of “wavy lines” can be also found in other species as a part of calls emitted during “excitement”: e.g., the territorial and courtship song of *Saccopteryx bilineata* (Behr & von Helversen 2004), harem-associated calls “whines, warblers and trills” of *Carrollia perspicillata* (Porter 1979), calls of *Nyctalus noctula* (Weid 1994) and *Myotis myotis* (Zahn & Dippel 1997). 

Unfortunately in the described observation of Nathusius’ pipistrelle (*Pipistrellus nathusii*), neither a sex of vocalizing individual nor purpose of production of these unusual calls is known. During slow flight around his roost, both the resident male and the unknown individual (presumably female) could emit unusual calls. It seems that the production of calls is limited to short period and specific conditions. Since 2006, the calls have not been recorded in 2007 (23.4., 10.5., 11.5., 8.6., 9.6., 10.6.), 2009 (12.6.) and 2010 (11.6.). Moreover, the unusual calls were neither recorded in both maternity colonies (70 hours of observation, 1231 tonal calls analyzed). All unusual calls were always recorded at vocalization sites that are known to be very busy advertising sites during mating season (unpublished data). If a female is the sex which produce these unusual calls (or at least major part of them), one of speculative explanation is that the respective behaviour and calls are directed to a resident male, and could refer to unmated status of the vocalizing female. In the long-distance migratory species Nathusius’ pipistrelle it is unknown whether a part of the females that mate unsuccessfully in the autumn, may take part in additional mating during the spring period. In this species it is also remarkable that parturitions are not very synchronous and both weaned and newborn pups may occur together in one roost (Heise 1984, Schmidt 1985). This fact could be explained both by the effect of migration (combination of temperature and humidity in the fetus developement) and additional spring mating. Although harem formation was found during spring periods, there is a lack of direct evidence for mating (Heise 1982, Schmidt 1994 a, b). To uncover the real purpose of this unusual vocalization and respective behaviour, playback experiments are needed. The above described observations suggest convincingly that our information on the actual acoustic repertoire and its behavioural background is rather incomplete, even in a species investigated in this respect in great details.

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