

## Clustering behaviour and acoustic activity of *Myotis myotis* (Mammalia: Chiroptera) in an underground hibernaculum

Markéta STAŇKOVÁ, Radek LUČAN & Ivan HORÁČEK

Department of Zoology, Faculty of Science, Charles University, Viničná 7, CZ–128 00 Praha 2, Czech Republic;  
e-mails: marketa.stankova@natur.cuni.cz, rlucan@centrum.cz, horacek@natur.cuni.cz

Received 15 April 2023; accepted 16 June 2023  
Published 27 August 2023

**Abstract.** The present paper provides a descriptive survey of the clustering behaviour of *Myotis myotis* (Borkhausen, 1797) in underground galleries of Alkazar quarry in Central Bohemia consisting of two parts distinctly differing in the pattern of bat clustering. The long-term record (56 years of standardized monitoring) is confronted with a detailed study from two recent seasons, which besides sequences of weekly visual controls provided a large number of acoustic recordings associated with seasonal dynamics of clustering behaviour. The course of hibernation reveals five successive stages which differ in the distribution of bats within hibernaculum and degree of clustering (number and sizes of clusters, fraction of clustered bats etc.) as well as in the composition of social vocalization and diversity of acoustic spectrum recorded in the site of cluster formation.

**Key words.** clustering behaviour, social calls, hibernation, bats, *Myotis myotis*.

### INTRODUCTION

Hibernation, surviving the winter period in deep lethargy, is an essential component of the life cycle of the temperate bats. No wonder that a considerable part of studies on the biology of temperate bats has been devoted just to the natural history of hibernation in particular species (Krzanowski 1959, Bezem et al. 1964, Daan & Wichers 1968, Harmata 1969, Davis 1970, Gaisler 1970, 1979, Bogdanowicz & Urbanczyk 1983, Řehák et al. 1994, Webb et al. 1996, Kokurewicz 2004, Boyles et al. 2007, 2017, 2020, Boratynsky et al. 2012) and/or related physiological adaptations (Davis 1970, Thomas et al. 1990, Speakman & Thomas 2003, Wojciechowski et al. 2007, Czenze et al. 2017). In terms of ecology, hibernation used to be traditionally looked upon as a simple interface between microclimate selection and energy expenditure (Thomas et al. 1990).

The recent survey of theoretical assumptions on optimal hibernation (Boydes et al. 2020) stressed a complexity of the factors setting hibernation under natural conditions and turned attention to non-energetic costs of hibernation (such as evaporative loss, arousal dynamics with costs of euthermia and/or cold arousals and arousal cascades demonstrated by Bartonička et al. 2017 or Blažek et al. 2019, etc.). Regarding these conceptual shifts, a detailed information is urgently needed on behavioural drivers by which optimal hibernation is achieved and the hibernation costs reduced. Despite little attention paid to that topic until now (Boratynski et al. 2012), two of them are explicit: (i) selection of roosting site and site replacements during the arousal bouts (driven by social tradition, social learning, and previous individual experiences; Horáček 1985), and (ii) aggregating into hibernating clusters.

Hibernation in clusters is expected to promote the maintenance of optimal body temperature for hibernation torpor and buffering individuals against fluctuations in ambient temperature. Boyles et al. (2008) demonstrated the essential effect of clustering for decreases of heat loss during brief

euthermic periods and argued that clustering may lessen body mass loss during hibernation in stable environments, may be minimized during hibernation when there are large fluctuations in the thermal environment, and that the tendency to cluster may be negatively related to body condition. A significant decrease in energetic expenditure and evaporative loss was convincingly demonstrated even for rather small clusters of five bat species (Brown 1999, Boyles & Brack 2009, Thomas & Geiser 1997, Boratyński et al. 2012, 2015).

Nevertheless, hibernation in mass clusters fixed by social tradition represents at the same time quite a risky hibernation strategy. As exemplified by mass extinctions in the bat hibernacula of NE North America caused by the White Nose Syndrome (*Geomyces (Pseudogymnoascus) destructans*), it can play a role of a detrimental factor both for rapid spillover of the pathogen and for repeated cascade awaking of all cluster members in response to arousals of few infected bats (Reeder et al. 2012). Preference for solitary hibernation and dispersal among a wide range of smaller hibernacula characterizing the hibernation strategy in most of the European bats sensitive to geomycosis infection (*Myotis* spp. in particular) was thus interpreted as an adaptive response to the repeated past influence of the respective agent which has been of the European origin (Martínková et al. 2010, Horáček et al. 2014, Harazim et al. 2018).

In short, for many European bats, including *Myotis myotis* (Borkhausen, 1797) – a model species of many studies including this one, solitary roosting has been a default way of hibernation while clustering appeared rather as an accessory strategy formerly met in few hibernacula only (Gauckler & Kraus 1963). Yet, with extensive increase of bat populations during recent decades the situation changed. A detailed analysis based on data from standard annual monitoring (a complete visual census of hibernating bats at a January/February term) in a large hibernaculum of the Býčí skála cave (Moravian karst) was undertaken by Martínková et al. (2020). They demonstrated a highly significant progressive enlarging of cluster size with abundance increase while the mean fraction of solitary roosting bats varied around 14% of the total population notwithstanding the exponential abundance increase during 41 years of covered period (1977–2018).

Here we present results of similar analyses in a traditional hibernaculum in Central Bohemia and confront them with results of continuous observations on the course of clustering behaviour during two winter seasons (2021/2022, 2022/2023) supplemented with preliminary analyses of instrumental recording of associated acoustic behaviour.

Besides variation in amount of acoustic activity during hibernation period we focused particularly to appearance of social vocalisation which represents an essential source of information on behavioral context of particular events of social biology (Zahn & Dippel 1997, Pfalzer & Kusch 2003, Knörnschild 2007, Jahelková et al. 2008, Furmankiewicz et al. 2013, Wright et al. 2014, Walter & Schnitzler 2019, Russ 2021, Middleton et al. 2022).

## MATERIAL AND METHODS

The investigations were undertaken in a system of galleries in the abandoned Alkazar quarry (Kozel, Hostim II) near Srbsko in Bohemian Karst (49°57'01.5"N, 14°07'28.7"E). Besides a small gallery aside (part A) with no or few bats only as a rule, the hibernaculum is composed of two parts: B (with diverse temperature and humidity conditions) and C (with continuously higher humidity; Fig. 1).

Standard visual controls (at January/February term) have been performed here from the beginning of the monitoring project in 1969 (Horáček 2010) supplemented by corresponding data from controls in other terms, representing in total of 45 checks in 1967–1998 and 69 checks in 1999–2020. At each control all bats were visually identified, and their position was indicated on the map of the site with particular attention to clustered individuals and sizes of clusters.

The same procedure was applied also in the winter seasons 2021/2022 and 2022/2023 when controls were performed once a week. Then the study was supplemented with a continuous automated recording of bat calls by SM4BAT device (Wildlife Acoustic) and video recording by photo trap UOVision UV 535 Panda, installed in part C nearby the site of frequent cluster formation (Fig. 2).

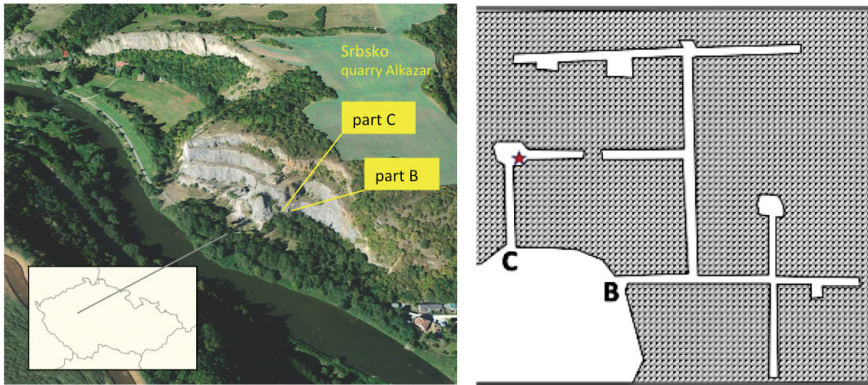


Fig. 1. Position of the studied hibernaculum and a sketch map of its corridors in the parts B and C.

Results of visual controls were stored in an Excel database and supplemented with computed values of a number of solitary individuals and clusters of particular sizes, fractions of the total abundance corresponding to solitary roosting bats and those contributing clusters of particular sizes, and numbers of between control changes in these characteristics. The identical arrangement was separately applied also for inhabitants of the parts B and C of the hibernaculum. Basic statistics for particular subsets were computed in Excel, statistical testing, correlations and multivariate comparisons (PCA, UPGMA clustering etc.) were undertaken with aid of Past 4 software.

The acoustic recordings and video sequences were stored in SD cards replaced weekly. Screening of acoustic records and detailed sonographic analyses were undertaken in Kaleidoscope Pro (Wildlife Acoustics). From each month, 200 recor-



Fig. 2. Setting of the instrumental device in the main clusters chamber of the part C. Photo by I. Horáček.

dings were randomly chosen and analyzed. The selected recordings were automatically identified by software and then manually controlled. Voices were classified into echolocation and social calls. Social calls were further manually divided according to structural characteristics and similarity into individual classes and their subclasses. Previously published literature (Zahn & Dippel 1997, Gillam & Fenton 2016, Walter & Schnitzler 2019, Russ 2021, Middleton et al. 2022) was used as a classification model, yet we modified it to cover the voice types recorded during the study. For the purpose of a rough comparisons undertaken in frame of the present paper we subdivided the recorded social calls into six voice classes with further subclasses: A (A1, A2), B (B1–B8), C (C1–C4), E (E1–E3), F, G (G1–G8). The detailed account of the particular categories and the sonographic characteristics distinguishing them are available in Appendix.

Data on the ambient temperature in part C were obtained from an internal thermometer of the SM4BAT instrumental equipment, external climatic currents were taken from public records of the meteorological station in Dobřichovice situated 11 km from the site available at [www.chmi.cz](http://www.chmi.cz).

## RESULTS

### Clustering in a long-term perspective

The data referring to the situation recorded by regular monitoring controls (January/February term, 1983–2022) are summarized in Figs. 3 and 4. The population history in the covered period include three distinct steps of abundance increase in the years 1995–1998, 2011–2015, and 2019–2023 (Fig. 3a). Bats were almost equally distributed in parts B and C of the hibernaculum (Fig. 3a, f) notwithstanding small temporal variation (without significant time trend: Mann-Kendal Trend test:  $S=-29$ ,  $Z=-0.397$ ,  $p>0.5$ ), and weak bimodal tendencies (Fig. 3f). A highly significant decrease in percentage of solitary roosting bats (Mann-Kendal Trend test:  $S=448$ ,  $Z=6.627$ ,  $p<0.001$ ) corresponds well to a greatly pronounced linear relation between total abundance and number of bats roosting in clusters (Fig. 3e). Overall fraction, however, of solitary roosting individuals and those in clusters are significantly correlated with total abundance ( $r=0.566$ ,  $p=0.0005$ ) while only weak and insignificant correlations were found between total abundance and distribution of bats among clusters of diverse sizes. Fraction of bats hibernating in small clusters (2–5 inds.) was negatively correlated with that in very large clusters ( $>10$  inds.):  $r=-0.698$ ,  $p<0.0001$ .

Comparing three stages of abundance development (1983–1998 vs. 1999–2013 vs. 2014–2023) we found no significant differences in the fraction of bats hibernating in small clusters of 2–5 individuals (34.4% vs. 36.3% vs. 36.2% of total population) but in the last period both fraction of solitary roosting bats (34.5% vs. 35.0% vs. 20.4%) and those assembling in larger clusters ( $>5$  individuals) increased (31.7% vs. 28.8% vs. 43.4%).

Distribution of hibernating bats sorted by particular cluster sizes (Fig. 3d) shows a highly significant bimodal pattern with a random assembling into smaller clusters conforming to a power distribution function scaled by total abundance and a well-pronounced tendency to form large clusters. The former pattern characterizes the hibernation strategy prevailing in part B of the hibernaculum (Fig. 4a, b) while the latter one clearly predominated in the part C (Fig. 4c, d) independently upon variation in the total abundance of hibernating population. Worth mentioning is that these differences in hibernation strategy observed between parts B and C, respectively, have been well pronounced both in the recent stage of high abundances (1999–2020:  $n=141.6$  on average) and in the preceding period of rather low total abundances (1967–1998:  $n=35.1$  on average; Fig. 5). Compared to 1983–2020 January/February record with indistinct correlation between parts B and C in a fraction of clustered bats ( $r=0.327$ ,  $p=0.05$ ), the complete dataset 1967–2020 (113 checks) shows a robust correlation ( $r=0.625$ ,  $p<0.0001$ ) similarly as with overall number of clusters ( $r=0.755$ ,  $p<0.0001$ ), which varied from 0 to 28 (average 8.47) in part B, and from 0 to 17 (average 4.90) in part C. At the same time, the fraction of clustered bats varied from 0% to 85.7% (average 43.37%, with mean cluster size 3.03 inds.) in parts B and from 0% to 100% (average 63.96%, mean cluster size 6.03 inds.) in part C.

### Clustering in course of a winter season

Primary data obtained by visual controls of the hibernaculum during the winter seasons 2021/2022 and 2022/2023 are summarized in Figs. 6 and 7. The major pattern of seasonal dynamics of all main characteristics (total abundance, fraction of solitary roosting and clustered individuals and number of clusters) was nearly identical in both seasons. It included a gradual abundance increase

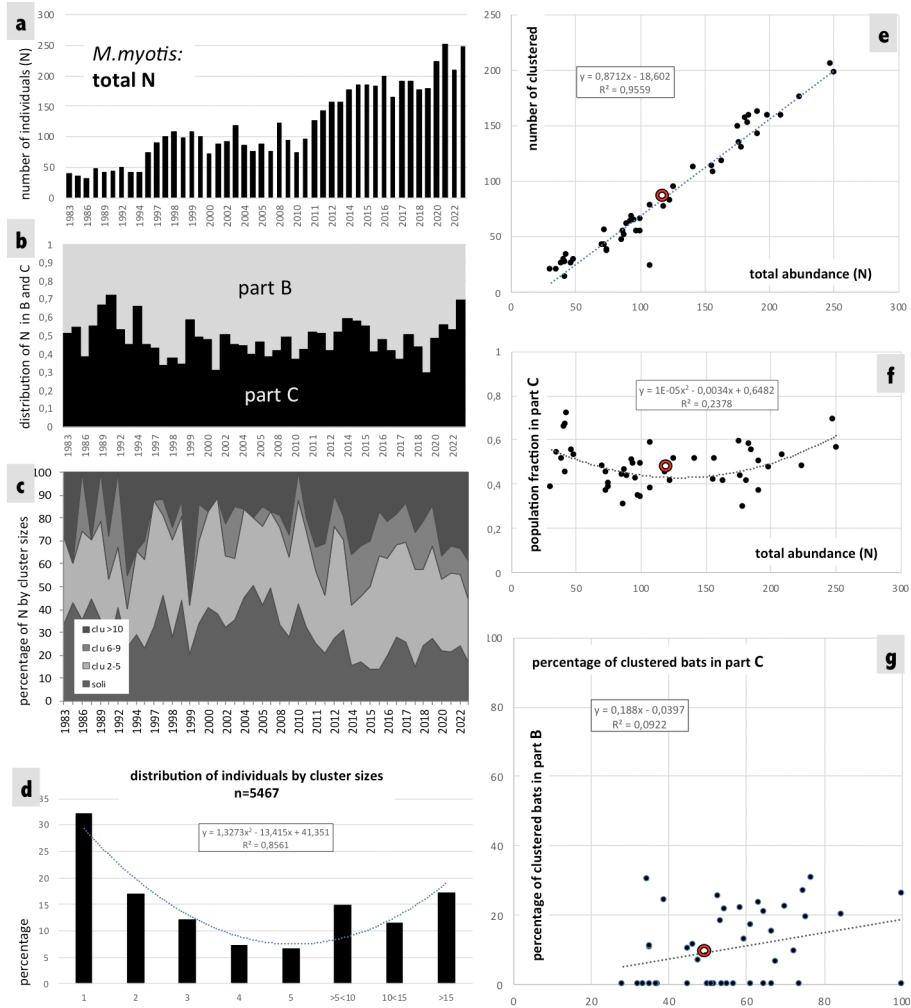


Fig. 3. Survey of bat population of the hibernaculum in a 40 years sequence of monitoring records (a single visual control in a season in the January/February term) 1983–2023; a – total abundances, b – distribution of bats between the parts B and C, c – fractions of solitary bats, and those hibernating in small, medium-sized and large clusters, d – mean fractions of solitary bats and those attributing clusters of indicated sizes, e – overall relations between the number of clustered bats and total abundance, f – relations between the fraction of population roosting in part C and total abundance, g – relations between parts B and C in fractions of clustered bats. Red circles in e–g – centroid values.

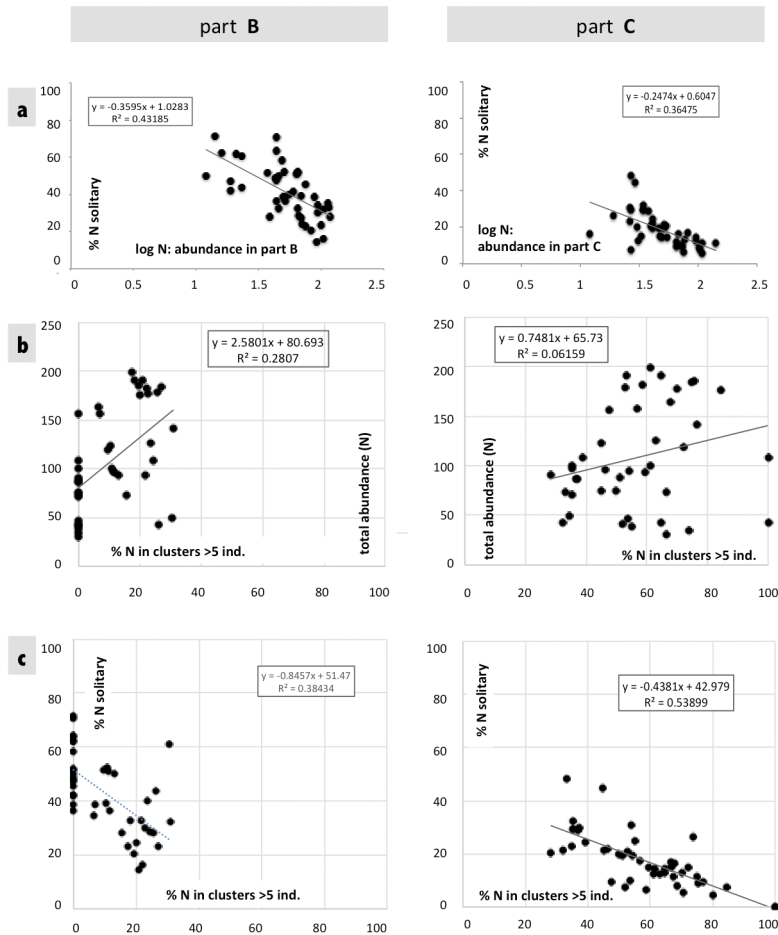


Fig. 4. Comparison of situations in parts B and C of the hibernaculum based on data from monitoring (January/February) controls 1983–2020; a – fraction of solitary roosting bats compared to log N (abundances in parts B and C, respectively), b – fraction of bats roosting in larger clusters (>5 inds.) compared to total abundances in whole hibernaculum, c – fraction of bats roosting in larger clusters (>5 inds.) compared to the fraction of solitary bats.

in September and October, a stage of a minute abundance increases in November and in December associated with an increase in the number of clusters and the onset of decreasing trend in the amount of solitary roosting bats which further continued until the end of the season. The A fraction of clustered bats exhibited a pronounced increase from the second half of December reaching the maximum values in February and the first half of March. Particularly in 2022/2023 season a well pronounced additional increase in total abundance and in the fraction of bats composing large clusters (>10 ind.) was apparent roughly from the beginning of February. In both seasons, there were distinct differences between parts B and C (Figs. 6, 7) in course of abundance development, the number of solitary roosting individuals and particularly in the fraction of clustered bats. While

during the second half of the hibernation period the abundance in part B showed a gradual decrease, abundance in part C rapidly increases particularly by enlarging sizes of clusters. In contrast to the part B, the fraction of clustered bats in part C amounted to more than 90% of the population hibernating in that part with about half of it being assembled in large clusters (>10 inds.) which did not appear at all in the part B. At the same time, the between-control differences in dislocation of hibernating bats during that period did not exceed there 10% suggesting no essential variation

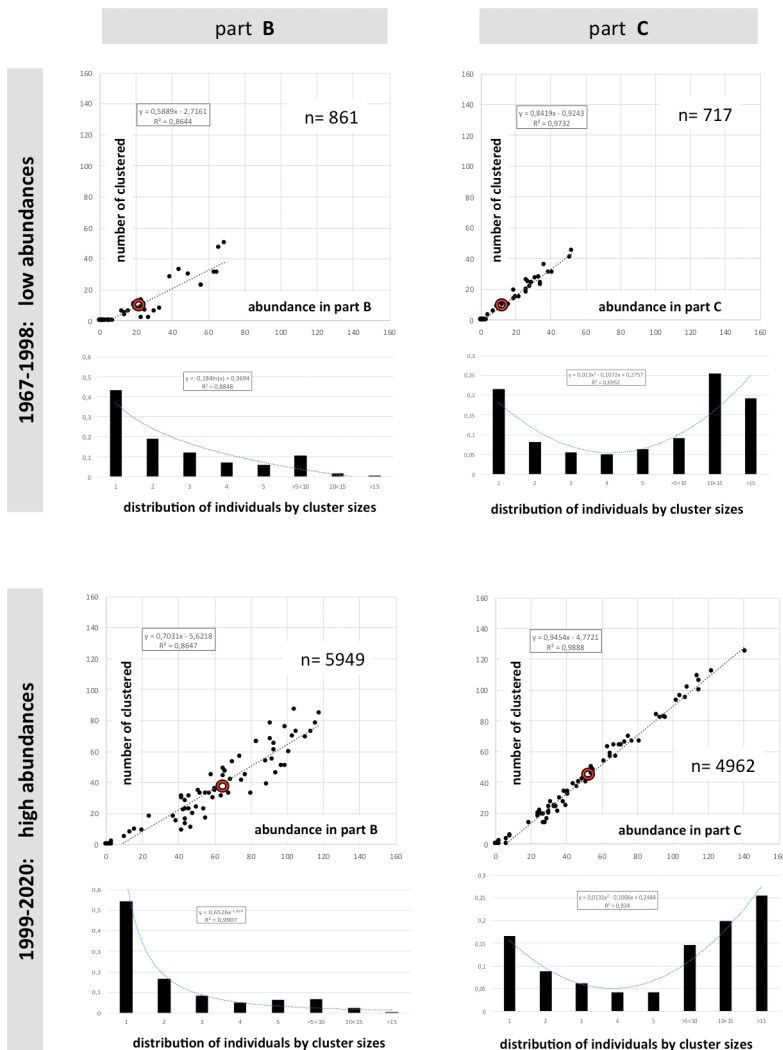


Fig. 5. Comparison of situations in parts B and C of the hibernaculum based on all records from a period of low abundances (45 checks 1967–1998) and that of high abundances (67 checks 1999–2020): relation between the number of clustered bats and abundances in the parts B and C, respectively, and distribution of abundances by particular cluster sizes.

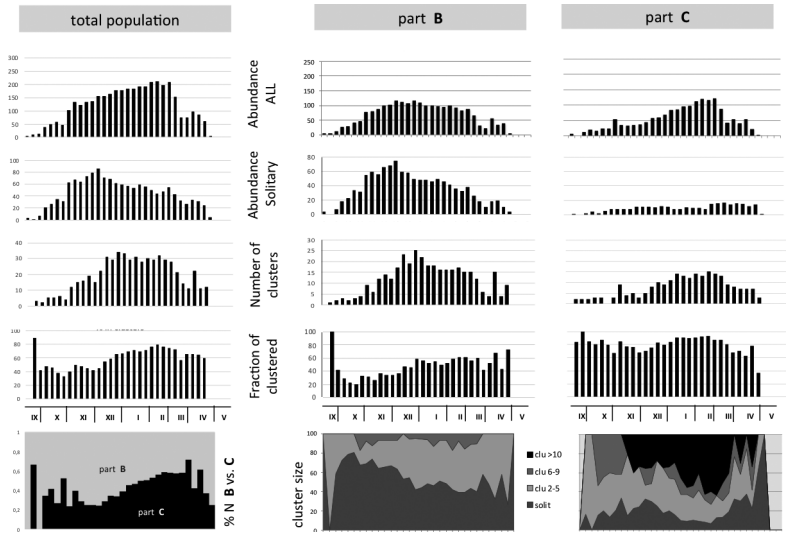


Fig. 6. Results of particular controls in hibernaculum during the season 2021/2022 reporting for the whole hibernaculum (total population) and in its parts B and C actual abundances, abundances of solitary roosting individuals, number of clusters, fraction of clustered bats, distribution of abundances between parts B and C (%N B vs. C), and percentages of solitary individuals and those assembled in the cluster of particular sizes respectively (cluster sizes).

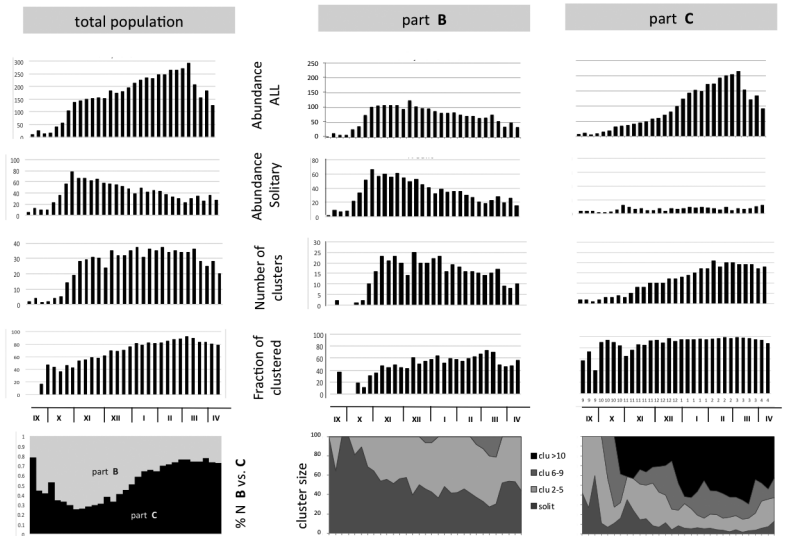


Fig. 7. Results of particular controls in hibernaculum during the season 2022/2023 reporting for the whole hibernaculum (total population) and in its parts B and C actual abundances, abundances of solitary roosting individuals, number of clusters, fraction of clustered bats, distribution of abundances between parts B and C (%N B vs. C), and percentages of solitary individuals and those assembled in the cluster of particular sizes respectively (cluster sizes).



in the clustering pattern. Even more distinctly, this pattern was recorded in the season 2022/2023 when a significant abundance increase in the part C was obviously contributed by arrival of individuals not resident in the studied hibernaculum during the former stage of the season.

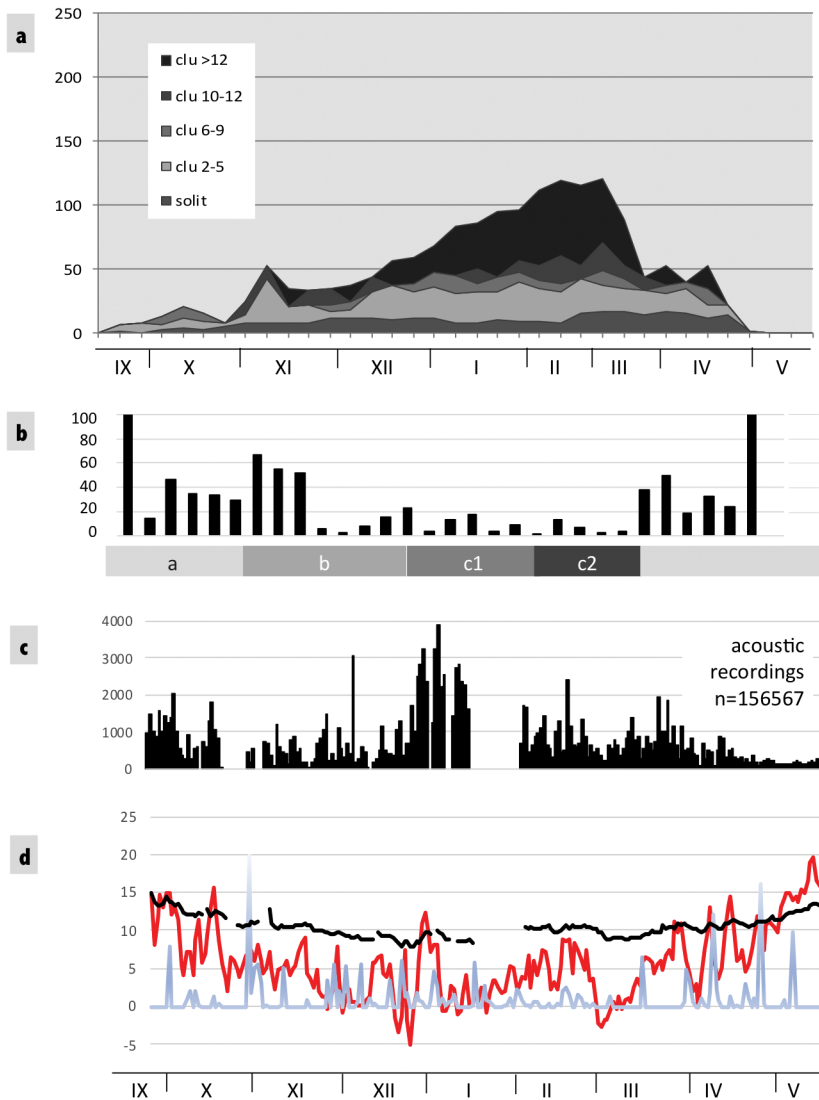


Fig. 8. Detailed data on the course of hibernation in the part C of the hibernaculum during the season 2021/2022: a – abundance and distribution of bats in clusters of indicated sizes, b – relative amount of between-control changes in dislocation of hibernating bats, stages a–d of cluster development, c – amount of daily acoustic recordings, and d – daily course of ambient temperature (°C, black line), external temperature (°C, red line) and precipitation (mm, blue line).

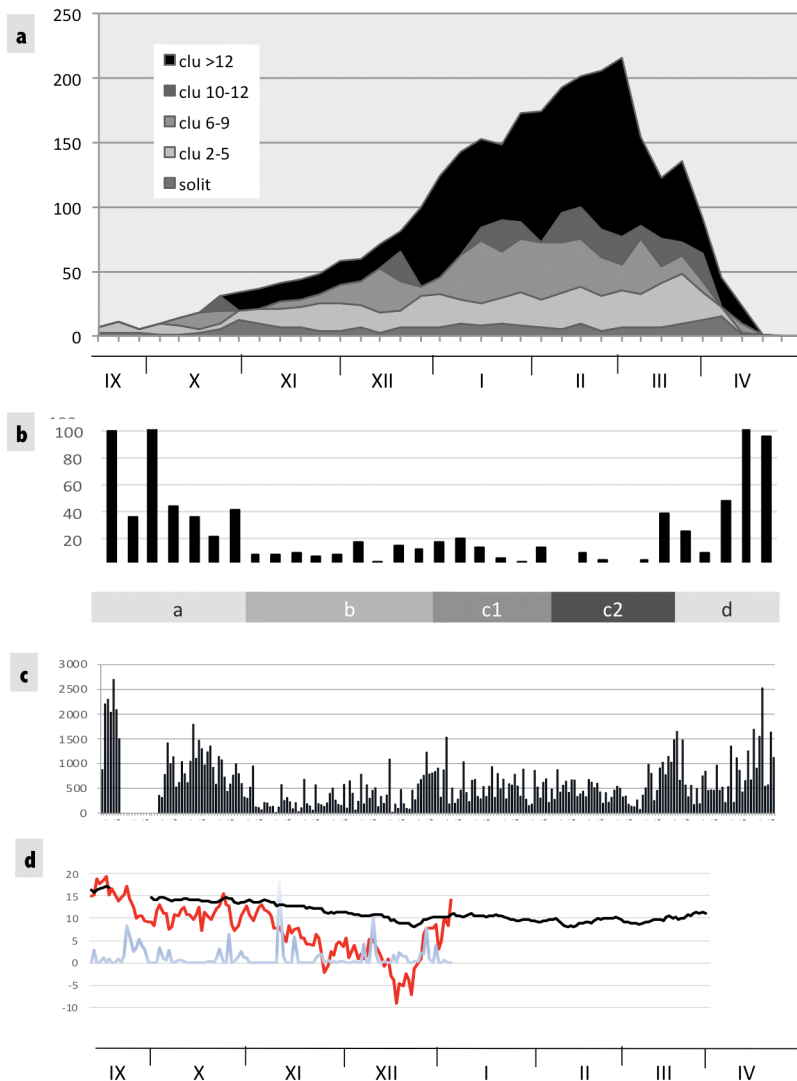


Fig. 9. Detailed data on the course of hibernation in the part C of the hibernaculum during the season 2022/2023: a – abundance and distribution of bats in clusters of indicated sizes, b – relative amount of between-control changes in dislocation of hibernating bats, stages a-d of cluster development, c – amount of daily acoustic recordings, and d – daily course of ambient temperature (°C, black line), external temperature (°C, red line) and precipitation (mm, blue line).

In regard to the clustering dynamics, the course of the winter season can be subdivided into several distinct stages: **(a)** slow abundance increase with intensive rearrangements of spatial dislocation, about 40% of individuals in small clusters (mostly pairs or triples), **(b)** an abrupt abundance increase in the part B which hosted nearly 70% of the total population, subsequent

Table 1. Survey of mean values of particular characteristics of clustering behaviour, mean ambient temperatures and amounts of acoustic recordings during particular stages of hibernation period (a, b, c1, c2, c) in the winter seasons 2021/2022 and 2022/2023.

season	2021 / 2022				2022 / 2023			
	a	b	c1	c2	a	b	c1	c2
stage of dispersal dynamics	total population				total population			
N	30.29	133.29	178.83	203.40	37.71	180.00	219.60	264.33
solit	17.57	71.29	59.33	50.00	21.43	55.57	44.00	32.33
% clustered	41.98	46.15	66.65	75.35	38.13	67.07	79.85	87.63
no clu	3.57	18.57	30.67	29.60	4.57	31.00	34.80	35.00
avg clu	3.40	3.45	3.92	5.19	3.32	3.93	5.06	6.64
avg diff%	36.16	15.96	3.39	4.19	61.91	6.53	5.51	3.92
	part B				part B			
N	22.57	96.14	104.17	90.80	24.14	98.43	86.00	71.00
solit	16.29	62.00	49.50	38.60	18.86	48.57	36.20	25.67
% clustered	34.75	34.91	52.34	57.58	13.73	50.93	57.83	64.07
no clu	2.14	13.29	19.67	15.80	2.14	20.00	19.60	15.50
avg clu	2.88	2.69	2.79	3.30	2.95	2.50	2.55	2.93
avg diff%	30.05	12.11	5.47	5.72	63.66	8.45	4.45	7.24
	part C				part C			
N	11.14	37.14	74.67	112.60	13.57	81.57	133.60	193.33
solit	2.25	9.29	9.83	11.40	2.57	7.00	7.80	6.67
% clustered	73.40	73.91	86.08	89.93	74.91	88.63	94.15	96.51
no clu	1.71	5.29	11.50	13.80	2.43	11.00	15.20	19.50
avg clu	4.23	5.58	5.59	7.35	4.02	6.43	8.24	7.17
avg diff%	42.99	29.40	11.74	5.60	57.24	10.79	12.17	5.82
acoust	959.08	566.46	1692.00	755.07	961.02	287.63	522.33	445.36
sd acoust	509.75	509.42	978.47	450.61	610.89	200.87	314.19	212.75
t	12.72	9.92	9.09	9.84	14.38	11.74	9.81	9.40
sd t	0.83	0.84	0.87	0.69	0.89	0.97	0.80	0.54
mean acoustic records / mean abundance	86.07	15.25	22.66	6.71	70.81	3.53	3.91	2.30
								7.64

Table 2. A number of individual acoustic sequences (classes and subclasses of social vocalisation and/or echolocation sequences) in samples of 200 recordings per each moth of the season 2022/2023

month	ix	x	xi	xii	i	ii	iii
A1	16	42	92	94	103	84	82
A2	4	12	16	22	28	15	19
B1	8	17	34	28	21	20	32
B2	0	3	12	4	5	9	2
B3	4	7	12	5	7	1	4
B4	1	12	37	63	43	43	42
B5	2	6	11	16	8	1	6
B6	1	0	0	2	1	0	0
B7	0	1	1	2	3	2	3
B8	0	1	0	3	11	8	16
B9	6	2	2	0	6	1	9
C1	6	4	0	0	0	0	2
C2	27	11	5	3	5	0	7
C3	1	0	1	0	0	1	4
C4	0	0	2	0	0	0	2
E1	3	8	17	5	12	6	2
E2	0	1	4	5	1	4	3
E3	1	3	6	1	0	0	1
F1	11	4	11	2	3	0	1
G1	130	53	2	3	1	0	1
G2	53	18	3	2	1	0	2
G3	24	19	0	1	0	0	0
G4	28	11	0	0	0	0	2
G5	12	9	1	0	0	0	0
G6	65	49	1	1	0	0	0
G7	19	17	0	0	0	0	0
G8	1	5	0	0	0	0	0
H1	0	0	0	0	1	0	0
echolocation total	74	87	91	90	118	138	115
	497	402	363	352	379	333	373

gradual abundance increase in the part C with establishing core aggregations of larger hibernation clusters, **(c1)** increase in the number of clusters and the fraction of clustered bats in both parts of the hibernaculum, well-pronounced abundance increase in the part C with the gradual enlargement of the fraction of clustered bats, **(c2)** decrease in abundance and the number of clusters in the part B, the major part of total population concentrated in the part C composing mostly the largest clusters, attributed also by the arrival of alien individuals not previously resident in the hibernaculum, **(d)** spring abundance decrease with a pronounced decrease in the number of clusters and initial resistance and later decomposition of the largest clusters.

The timing of particular stages and the corresponding development of abundance and clustering pattern observed in the part C are summarized in Figs. 8 and 9. Mean values of particular characteristics in the total population and in both parts of the hibernaculum are surveyed in Table 1.

Multivariate comparisons of similarity among individual controls in the distribution of populations in clusters of a particular size (Fig. 10) revealed their grouping perfectly corresponding to particular stages (a–d) of clustering dynamics.

### Acoustic behaviour recorded in part C

The set of 156,567 and 123,161 acoustic recordings obtained during the seasons 2021/2022, and 2022/2023 respectively in the part C shows in both the season a considerably high amount of vocalization (about 1000 records per day) during the stages of (a) and (d), a clear drop during the stage (b) and comparatively smaller amounts during stage (c) with pronounced inflation at the beginning of the stage (c1); comp. Figs. 8, 9. No day was without any record, the number of records per day varied from 44 to 3915 with 670 on average.

Preliminary analyses of the acoustic recordings (covering 1400 randomly selected records from all particular stages of the season 2022/2023) confirmed that vast majority of voices (97.6%)

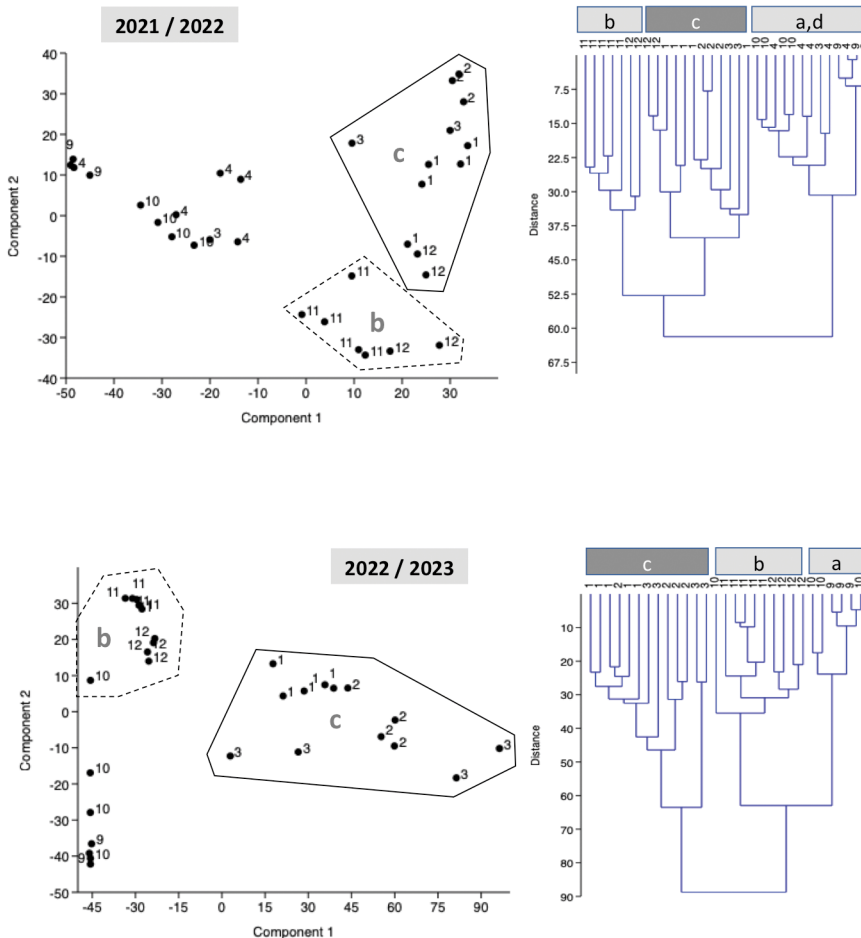


Fig. 10. Multivariate comparisons of similarity among particular controls (numbers refer to particular months) in the distribution of total hibernating population in clusters of different sizes in the season 2021/2022, and 2022/2023, respectively: plot of PCA I vs. II scores (with hulls indicating the controls representing b and c stages), and UPGMA clustering based on Euclidean distances.

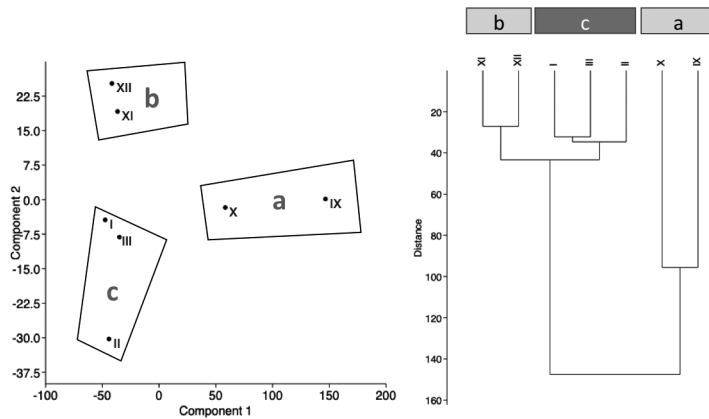


Fig. 11. Multivariate comparisons of similarity among particular months in the appearance of particular voice classes and subclasses in part C of the hibernaculum in the season 2022/2023: plot of PCA I vs. II scores (with hulls indicating the controls representing the b and c stages), and UPGMA clustering based on Euclidean distances.

belong to *M. myotis*, and that most of the records represent diverse social calls. Echolocation calls represented only 29.4% on average, except for stage (c2) when they amounted to 41.4% on average, supposedly due to alien bats arriving during that stage. As concerns the social calls (separated by sonographic characteristics into six classes and further subclasses – see Appendix for details), the preliminary analyses suggest that: (i) the autumn stage (a) distinctly differs from the rest of the season by the dominant appearance of class G social calls representing on average 57.2% of the acoustic spectrum (69.6% of social calls). G-calls are obviously males’ display songs attracting females (“chirping” and “W-note” calls by Zahn & Dippel 1997). This type of calls is almost

Table 3. Percentage of particular voice classes in the acoustic spectrum of particular stages of hibernation in the season 2022/2023

	a	b	c1	c2	d
<b>% of all calls</b>					
echol	17.91	25.45	31.13	41.44	30.83
A	8.23	31.61	34.56	29.73	27.08
B	7.90	32.29	27.44	25.53	27.08
C	5.34	1.82	1.58	0.30	8.85
E+F	3.45	5.87	4.49	3.00	4.02
G	57.17	1.96	0.79	0	2.14
<b>% of social calls only</b>					
A	10.03	42.40	50.19	50.77	39.15
B	5.62	44.65	39.85	43.59	39.15
C	6.50	2.44	2.30	0.51	12.79
E+F	4.20	7.88	6.51	5.13	5.81
G	69.65	2.63	1.15	0	3.10

absent in the next stages of the hibernation period, (c) in particular, while in a small percentage (>2%) they were recorded in the stage (d). **(ii)** During the following stages of hibernation (b–c), the acoustic spectrum was contributed particularly by the calls of A and B classes. The A-calls, representing supposedly an aversive response of resident bats to alien intruders, composed about one third of the acoustic spectrum (about 50% of social calls), the B-calls supposedly related to semantic context of A-calls, composed about 40% of social calls on average, but prevailed over A-calls (44.7% to 42.4%) during the stage of the initial formation of clusters (b) (Zahn & Dippel 1997, Walter & Schnitzler 2019). **(iii)** Overall amount of social calls (including A and B) was somewhat reduced during stage (c2) compared to stages (b) and (c1). **(iv)** The acoustic spectrum recorded in the terminal stage of hibernation (d) was characterized by an increased diversity of social calls covering almost all call subclasses, some in the highest representation compared to other stages (B1, B8, C3). For further details see Tables 2, 3 and Figs. 11, 12.

The multivariate comparisons of the vocalization spectrum revealed in particular month samples aggregated the month records into groups perfectly corresponding to the above declared stages of hibernation dynamics, viz. (a)–(d), see Fig. 11.

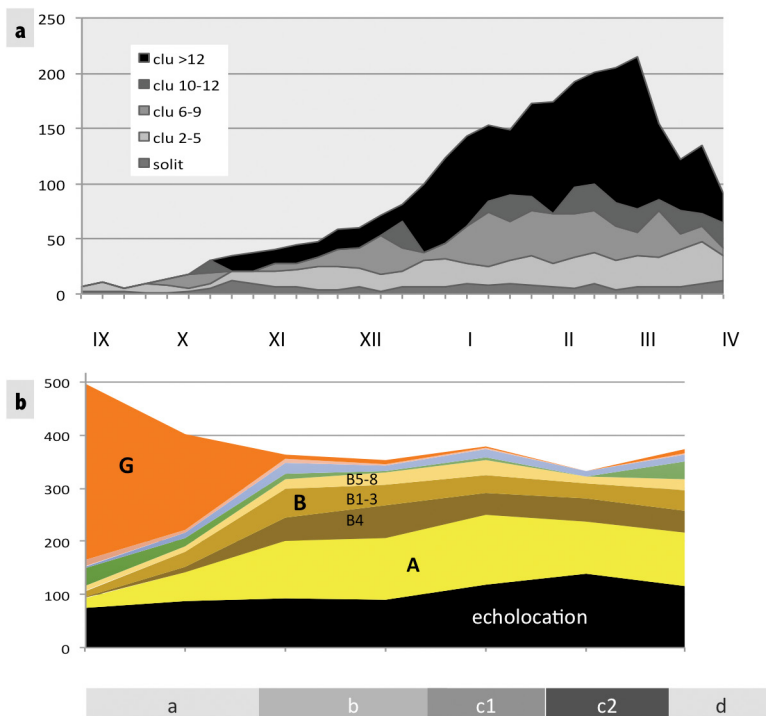


Fig. 12. Comparison of clustering (a – number of individuals) with patterns of vocalization spectrum (b – number of acoustic records of particular call classes) in part C of the hibernaculum during the season 2022/2023.

## DISCUSSION

The above-surveyed results demonstrated that the bats colonizing the studied hibernaculum perform a clear discrimination between parts B and C as concerns the mode of the hibernation strategy. Forming larger clusters has been traditionally restricted to part C. This discrimination has been retained throughout whole the 56 years of the covered period (1967–2023), including the stage of quite low abundances (1967–1998). The long-term record further indicated that the percentage of solitary roosting bats was negatively correlated with the total abundance of the hibernating population (contrary to observations in a mass hibernaculum in the Moravian Karst – Martínková et al. 2020) while the fraction of clustered bats was significantly correlated with total abundances only in the case of the largest clusters (>10 inds.). This particularly concerned part C of the hibernaculum where the distribution of bats by cluster size exhibited instantly a greatly pronounced bimodal pattern while in part B it approached a random distribution scaled by a negative exponential function.

The detailed data obtained from weekly controls throughout the course of two winter seasons revealed unexpectedly pronounced stability in the performance of clustering behaviour within the hibernating population. During the early autumn period, a considerable between-control variation in abundance and spatial dislocations of bats was observed. In that time, i.e. in stage (a) of the hibernation period, but also in the following stage (b – November – beginning of December), the distribution of bats between parts B and C was roughly proportional to the square of B and C corridors, yet during December the abundance in both parts grew almost equal, and since the beginning of February (stage c2) the fraction of bats hibernating in the part C, aggregated mostly in larger clusters, clearly outnumbered that of the part B.

The position of the largest clusters (obviously fixed by social tradition) was identical in both seasons and was preformed by small clusters already during the stage (a). The preforming groups represented mostly the harem aggregations as suggested by associated vocalization with considerable predominance of G-calls, not appearing in the latter stages. Slow enlargement of these core aggregations characterizing the stage (b) was accompanied by an antiphonal exchange of the A- and B-calls with dominance of B-calls probably emitted by individuals entering the clusters (Walter & Schnitzler 2019). The multi-harmonic long FM E-calls obviously emitted by roosting bats were also relatively common at that stage as well as during stages (c) of rapid increase of cluster sizes. During late November and December (late (b) and (c1) stages) the large clusters formed in the part C of the hibernaculum increased their sizes most probably by the arrival of bats roosting until then solitary in the part B (where the abundance began to decrease at that time) – similar trend was also described by Boratyński et al. (2012). Yet, the rapid increase of clusters during the peak stage of hibernation (c1: from middle January to middle March), associated with a reduced amount of social vocalization and increased amount of echolocation calls, was for obvious reasons attributed mostly by the arrival of alien individuals from less suitable hibernacula in the vicinity, as observed e.g. by Boyles et al. (2008). Judging from the acoustic record, their incorporation into existing clusters was not accompanied by more pronounced social interactions. At that stage, the mean number of daily acoustic records relative to the number of individuals was even less than half of that in the previous stage (c1) and reached its minimum within the whole hibernation period. Decomposition of hibernating aggregations during stage (d) from mid-March was associated with significant increase of social vocalization exhibiting a peak diversity as concern representation of particular acoustic subclasses, including considerably increased appearance of the multi-harmonic contact B1-, B8- and C3-calls, and even a re-appearance of the G-calls suggesting mating efforts of some males (Zahn & Dippel 1997). The smaller clusters exhibited at that stage an enlarged turnover while core assemblages of the largest clusters remained intact until early to middle April



despite continuous disappearances of the individuals from cluster margins. The trends summarized above exhibited certain between-season differences both in the timing of particular stages and in the extent of associated rearrangements. Such kind of differences could possibly explain also the well-marked fluctuations in clustering characteristics pertinent for the long-term record of standard monitoring terms (January/February) – comp. e.g., Fig. 3c.

In this study, we did not test the effects of external climatic variation upon abundance and clustering dynamics. Anyhow, our data suggest that fluctuations in microclimatic variables were only little expressed in the part C; comp. Figs. 8, 9 and Table 1. Yet, the seasonal course of external conditions might supposedly influence the timing of particular stages of hibernation dynamics and the performance of clustering behaviour in a considerable way. Martínková et al. (2020) tried to express such an effect by introducing a winter season severity index, yet they found its effect negligible compared to the general trend of exponential increase in January/February abundance during the study period.

Correspondingly, the obvious differences between the two seasons of our study, 2021/2022 and 2022/2023, expressed in an increase in abundance and the fraction of clustered bats in part C (comp. Figs. 6–9, Table 1) cannot be easily explained by slight differences in the course of the season climatic currents. More likely it seems that successful hibernation under the clustering strategy in the part C strengthened the social signature of this site and in the season 2022/2023 attracted more individuals hibernating under the suboptimal conditions to join the assemblage of clustered bats in that site.

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## APPENDIX

### Sonographic classification of the social vocalization recorded in the part C

#### A-Calls

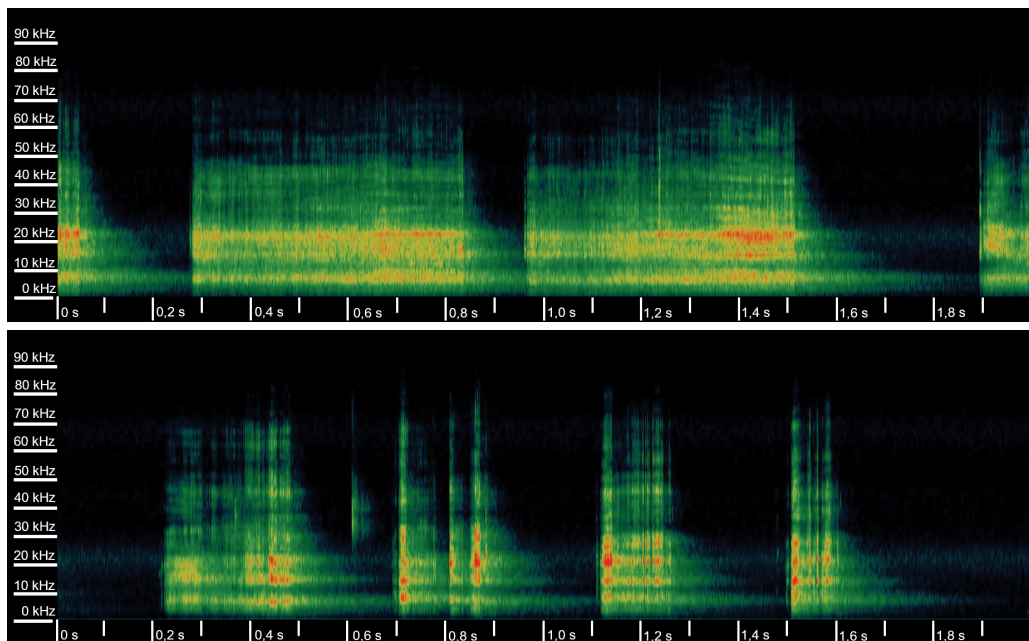
Prolonged loud, broadband (2–75 kHz) social calls with fundamental frequency <10 kHz and pronounced low audible components (maximum energy around 20 kHz).

##### Subclass A1

A common non-melodic social call, also described in Walter & Schnitzler (2019) (screams) and Zahn & Dippel (1997) as squawk or scolding (maximum frequency is around 20 kHz. Long voices with duration from 100 to 700 ms (it can also have 2000 ms). The broadband frequency is 75 kHz (Fig. 1).

##### Subclass A2

Short non-melodic social calls with duration from 15 to 100 ms. Maximum frequency is around 20 kHz. The broadband frequency is 75 kHz (Fig. 2).



Figs. 1, 2. Recording of the A-calls of social call of *Myotis myotis*. 1 (top) – A1 subclass; 2 (below) – A2 subclass.

### B-calls

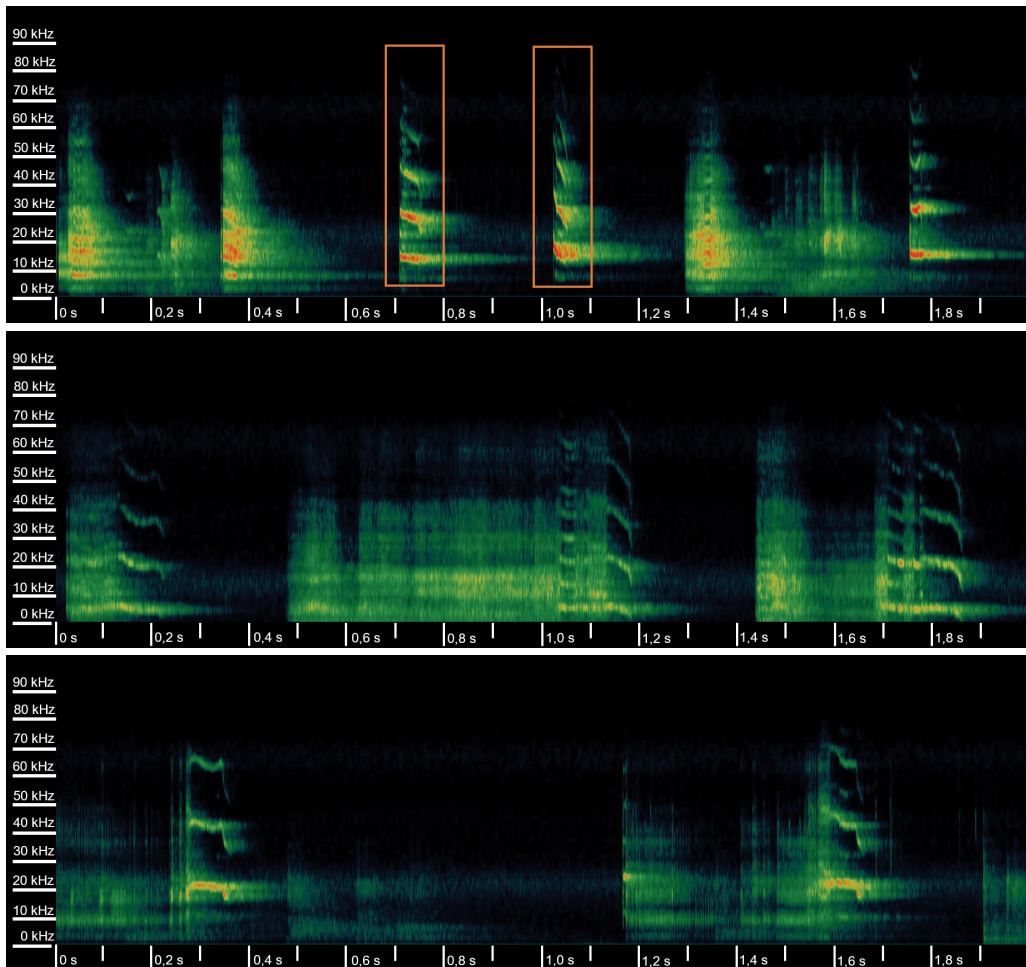
A class of multi-harmonic trill social calls usually with distinctly separated aCF or qFM pure harmonics.

#### Subclass B1

FM type of social call with 3 to 6 harmonic frequencies from which the 2nd is the strongest harmonic frequency with a maximum of around 20 kHz. The third and fourth harmonic frequencies are also strong (30 kHz and 45 kHz). Duration of the call is usually around 22 ms, but it can vary from 10 to 40 ms. The broadband frequency is 60 kHz (Fig. 3).

#### Subclass B2

Multi-harmonic type (3–5 frequencies) of social call consisting of a longer wave component and terminating with an FM sweep. The B2 type can be preceded by the A1 call type. The strongest is the 1st (16–20 kHz) or 2nd (30 or 40 kHz) harmonic frequency. The duration of the signal is usually around 100 ms (range 80–200 ms). The broadband frequency is 65 kHz (Figs. 4, 5).



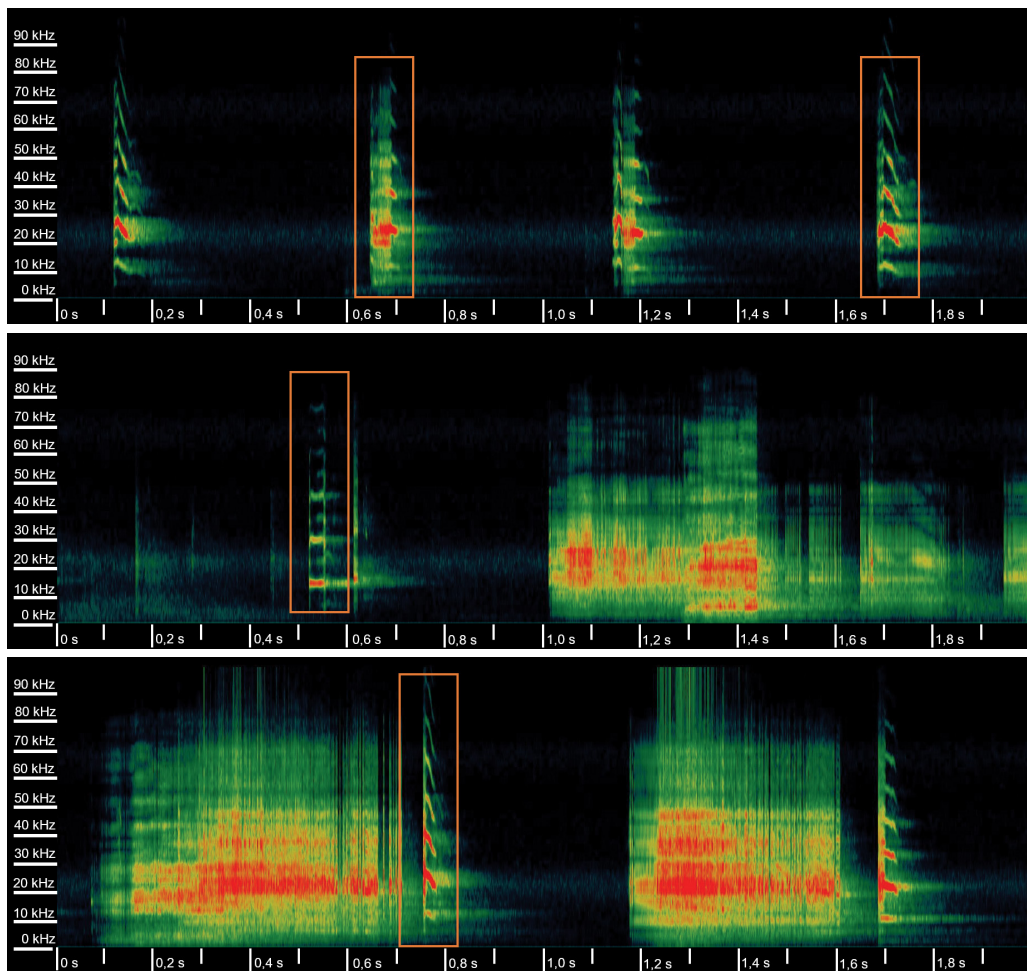
Figs. 3–5. Recording of the B-calls of social call of *Myotis myotis*. 3 (top) – B1 subclass; 4 (middle) – B2 subclass; 5 (below) – B2 subclass.

### Subclass B3

Multi-harmonic social call containing five or more frequencies - the maximum is usually on the second harmonic frequency (20–25 kHz), sometimes on the first one (15 kHz). The duration of the signal is most often around 30 ms (maximum 50 ms). The broadband frequency is 70 kHz (Figs. 6, 7).

### Subclass B4

Multi-harmonic signal which is almost identical in structure to B1. It always occurs in context of A1. FM character of social call with less fragmentation than B2 with five or more harmonic frequencies. The maximum frequency is at the 2nd or 3rd (approximately 20 kHz and 30 kHz) harmonic frequency. The duration of the signal is usually 22 ms (20–40 ms). The broadband frequency is 70 kHz (Fig. 8).



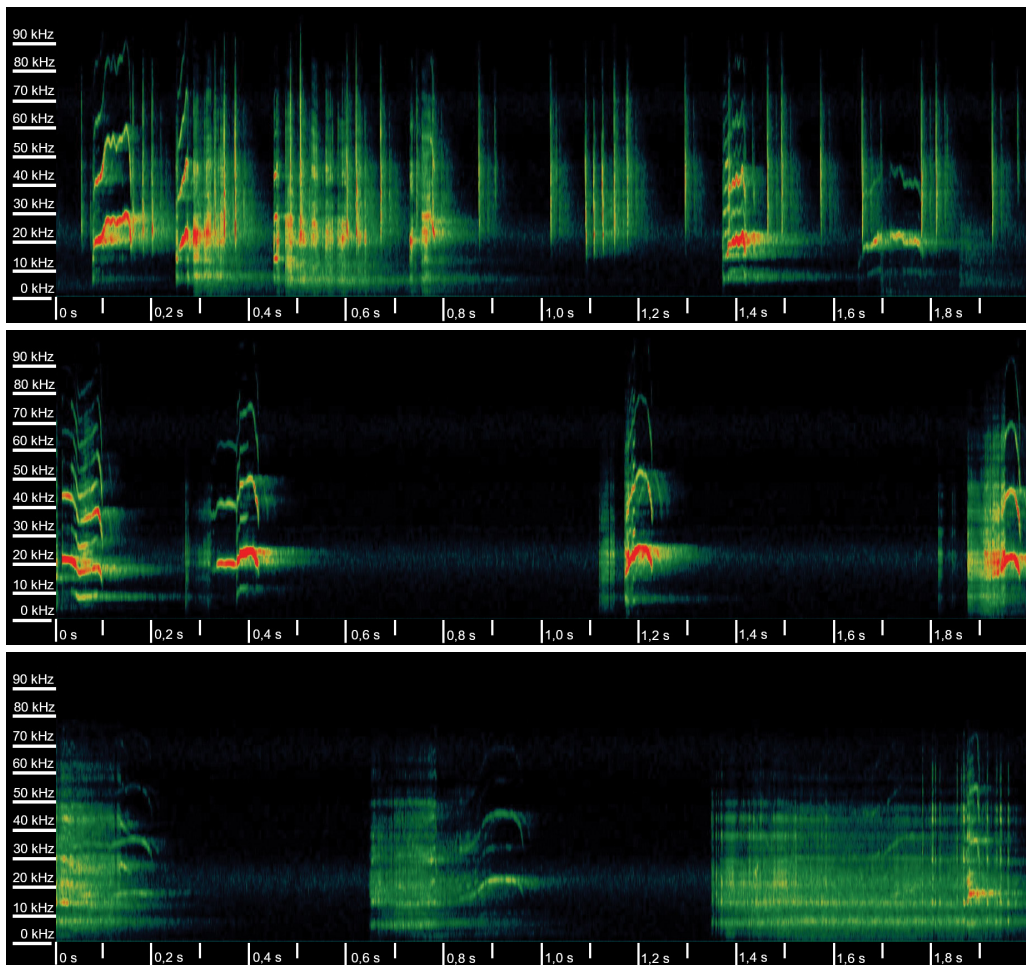
Figs. 6–8. Recording of the B-calls of social call of *Myotis myotis*. 6 (top) – B3 subclass; 7 (middle) – B3 subclass; 8 (below) – B4 subclass.

### Subclass B5

It is an arched type of social call usually with at least 3 harmonic frequencies. It consists of two subtypes – smooth and trillloid. The maximum frequency is around 25 kHz – the 1st or 2nd harmonic frequency. The duration of the signal is 40–70 ms. The broadband frequency is 70 kHz (Figs. 9, 10).

### Subclass B6

The arched social call with a melodic sweep from lower to higher frequency continuing in a steep frequency drop within a broadband of ca. 10 kHz. It contains 3 to 5 harmonic frequencies. The maximum frequency is at the first (at the highest point of the arc it is 14 kHz or 25 kHz) or at the second harmonic frequency (at the highest point of the arc it is 25 kHz). The duration of the signal is around 200 ms. The total broadband frequency is 65 kHz (Figs. 11, 12). It can attract landing of flying bat.



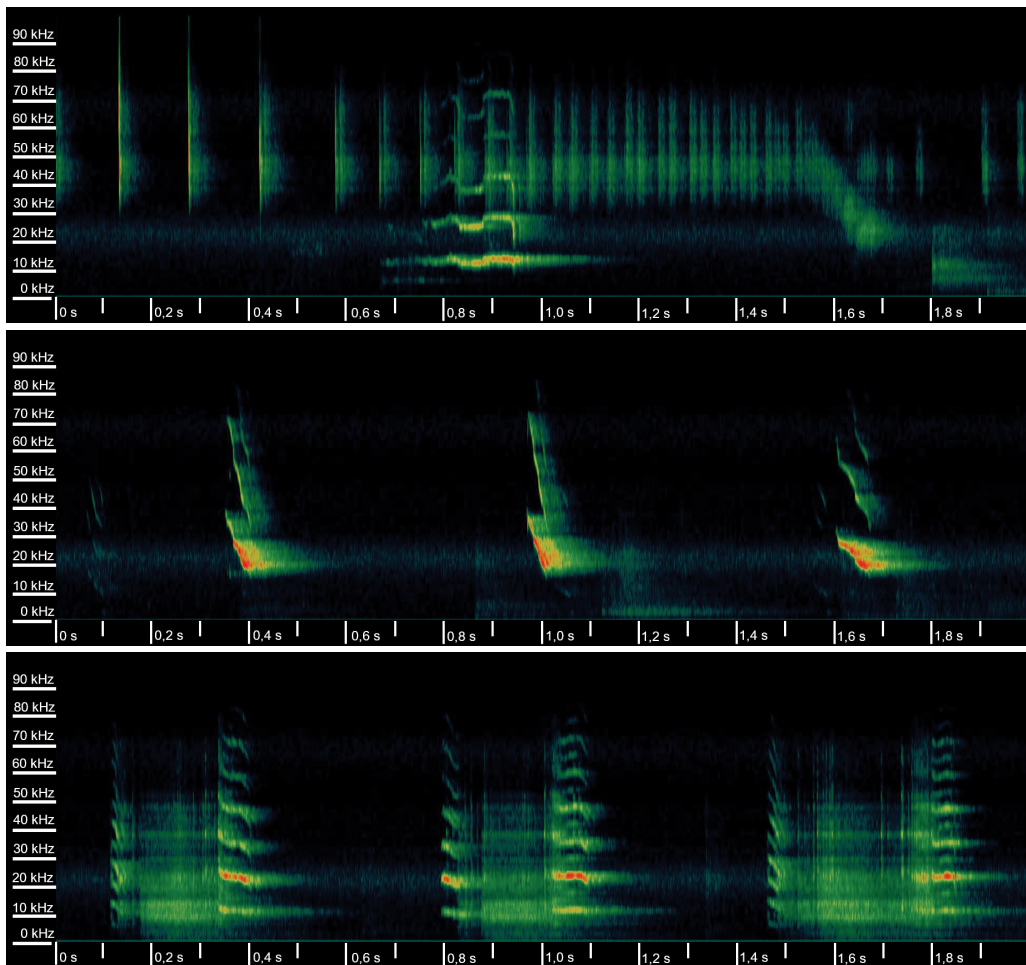
Figs. 9–11. Recording of the B-calls of social call of *Myotis myotis*. 9 (top) – B5 subclass; 10 (middle) – B5 subclass; 11 (below) – B6 subclass.

*Subclass B7*

It is an FM-type of social call with three harmonic frequencies. The first harmonic frequency is the strongest, with a maximum of around 25 kHz and a broadband frequency of 25 kHz. The duration of the call is between 45–70 ms. The total broadband frequency is around 60 kHz (Fig. 13).

*Subclass B8*

It is a multi-harmonic call with 6 frequencies – the strongest is usually the second harmonic frequency which has 25 kHz. Simple structure similar to the B4 type. However, the signal is longer (50–70 ms) and is connected to the A1 type at the end, but also at the beginning, or occurs without it. The total broadband frequency is 70 kHz, the broadband frequency of one harmonic is 6 kHz (Fig. 14).



Figs. 12–14. Recording of the B-calls of social call of *Myotis myotis*. 12 (top) – B6 subclass; 13 (middle) – B7 subclass; 14 (below) – B8 subclass.

## C-calls

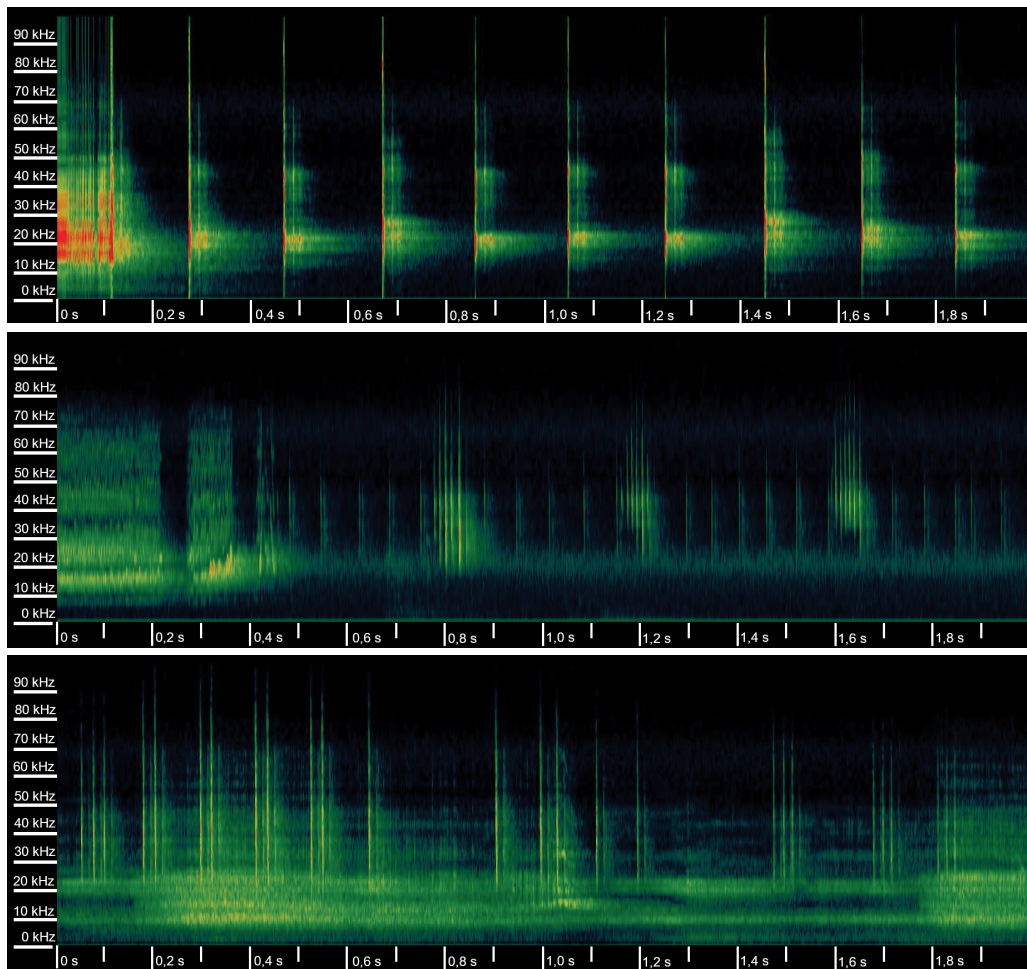
Short FM social calls associated with echolocation pulses.

### Subclass C1

Steep FM signals with a maximum frequency of around 25 kHz and a duration of 4 ms. The broadband frequency of the signal is 40 kHz. The distance between individual calls is 200 ms (Fig. 15).

### Subclass C2

Steep FM signals in tight irregular bursts of two or more calls (mostly up to ten calls). Frequency maximum is 22–25 kHz. Signal duration is 5 ms. The broadband frequency is 60 to 70 kHz (Figs. 16, 17).



Figs. 15–17. Recording of the C-calls of social call of *Myotis myotis*. 15 (top) – C1 subclass; 16 (middle) – C2 subclass; 17 (below) – C2 subclass.



### *Subclass C3*

A narrow variable FM call type derived from echolocation occurs in the presence of several individuals. The maximum frequency is around 37 kHz. The duration of the call is 3–4 ms. The broadband frequency is 60–70 kHz (Fig. 18).

### *Subclass C4*

It is an A1 type fully or partially phased into individual calls. The range between calls is 4 to 20 ms. The maximum frequency is around 22 kHz. The length of the scream is approximately 2 ms. The frequency range is around 65 kHz (Fig. 19).

## **E-calls**

Multi-harmonic FM-qCF social calls terminating with a prolonged phase of a constant frequency.

### *Subclass E1*

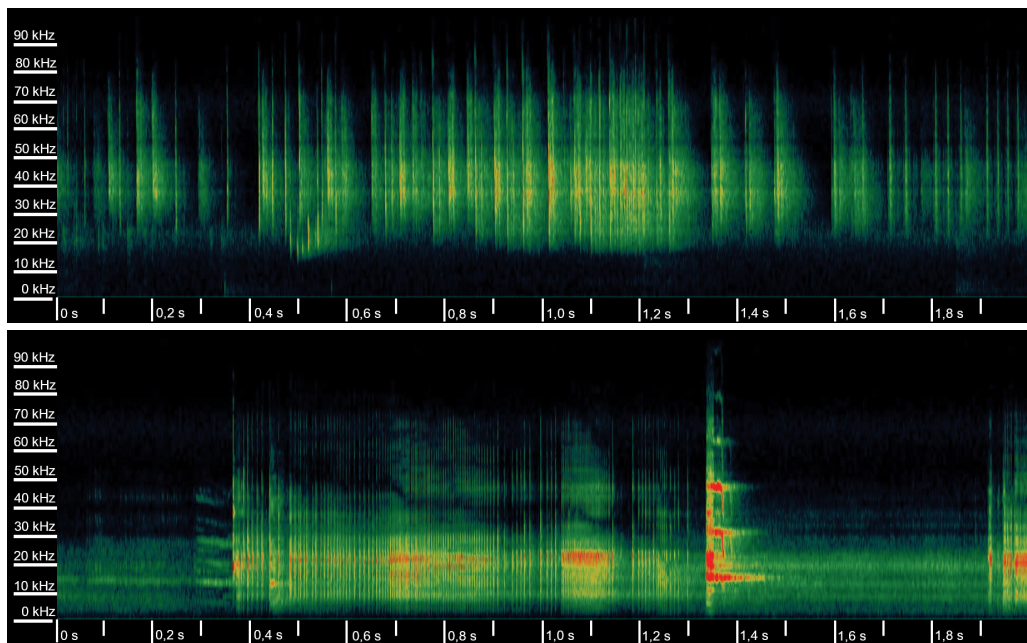
A prolonged multi-harmonic social call almost entirely composed of the qCF phase with maximum energy around 10 kHz. The length of the signal is 75–30 ms. The total broadband frequency is 30–45 kHz. The broadband frequency of one harmonic is 5–10 kHz (Fig. 20).

### *Subclass E2*

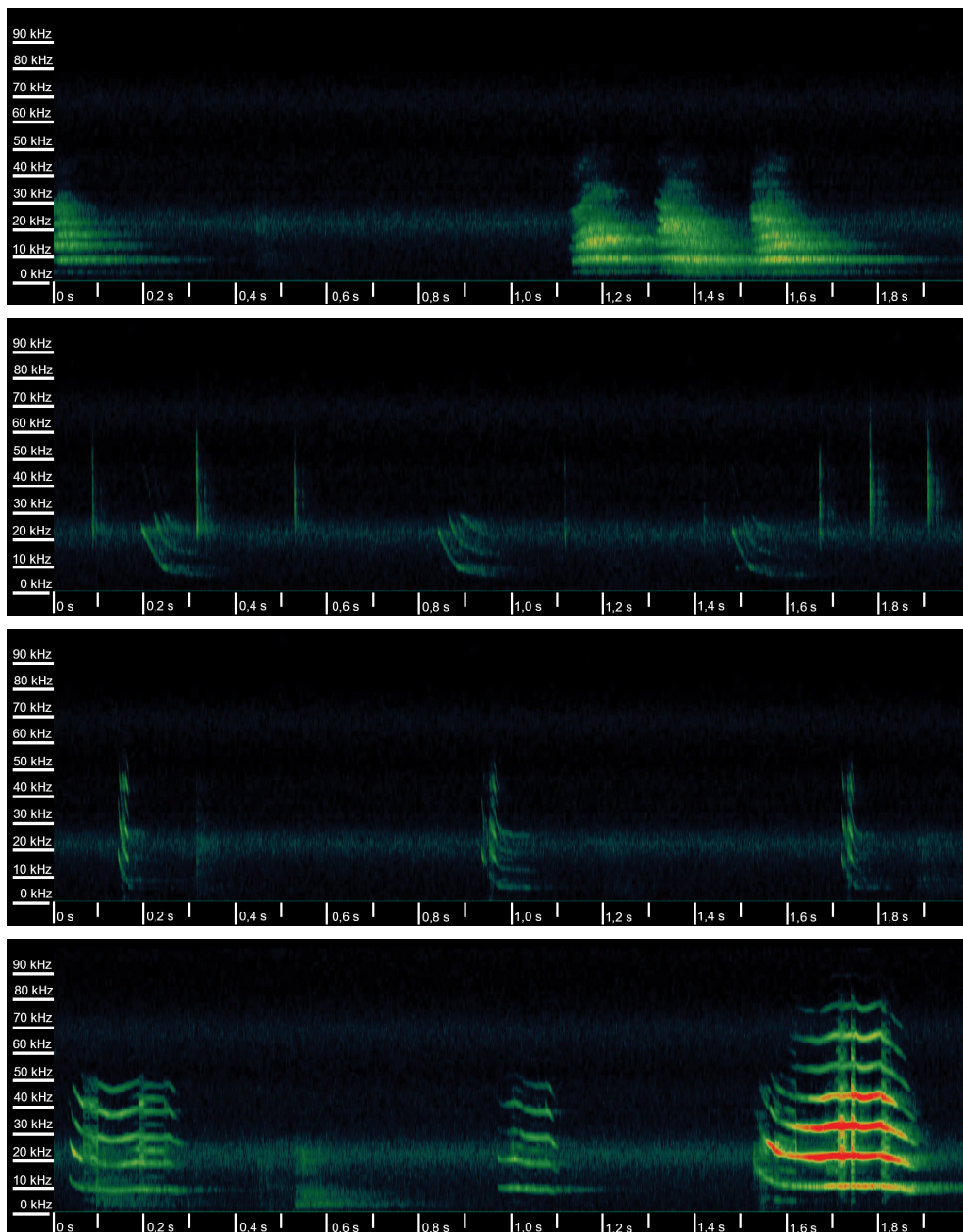
A multi-harmonic FM social call with a steep frequency drop at the beginning that transitions to a constant frequency at the end. The call has three to five harmonic frequencies – the strongest being the second harmonic at around 22 kHz. The duration of the call is 70–200 ms. The total broadband frequency is 20–40 kHz. The broadband frequency of one harmonic is 12–20 kHz (Figs. 21, 22).

### *Subclass E3*

A prolonged social call with five harmonic frequencies and maximum energy in the 2nd and 3rd harmonics (20–25 kHz and 30–40 kHz). The duration of the call is 100–350 ms. The total broadband frequency is around 40 kHz (up to 70 kHz). The broadband frequency of individual harmonic frequencies is 10–15 kHz (Fig. 23).



Figs. 18, 19. Recording of the C-calls of social call of *Myotis myotis*. 18 (top) – C3 subclass; 19 (below) – C4 subclass.



Figs. 20–23. Recording of the E-calls of social call of *Myotis myotis*. 20 (top) – E1 subclass; 21 (middle top) – E2 subclass; 22 (middle below) – E2 subclass; 23 (below) – E3 subclass.

### F-call

A social call with a steep FM character of the signal. It usually occurs in the form of a series of 2–8 calls in 100–200 ms. It most often follows types A and G. The maximum frequency is in the region of 30–40 kHz and 15–25 kHz. The broadband frequency is 60–70 kHz. The social call is similar to the C2 type. Related to echolocation calls, yet obviously produced by a non-flying individual (Fig. 24).

### G-calls

The social calls of a more complex trill-shaped structure showing a great variation in their sonographic characteristics. Diverse performance of G-calls distinguished below as subclasses G1–G8 often appear in various mutual combinations. Vast majority of G-calls were recorded during the swarming period.

#### Subclass G1

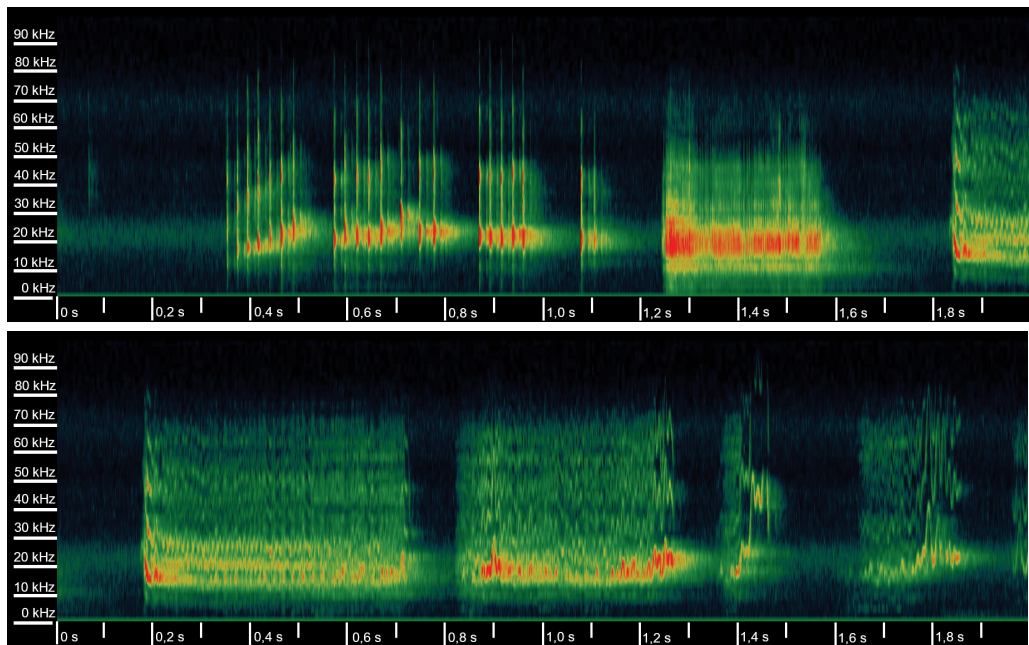
A trill-shape social call has up to six harmonic frequencies. The maximum frequency is around 20 kHz. The duration of the signal is 200–700 ms. The frequency range is 60 kHz (Fig. 25).

#### Subclass G2

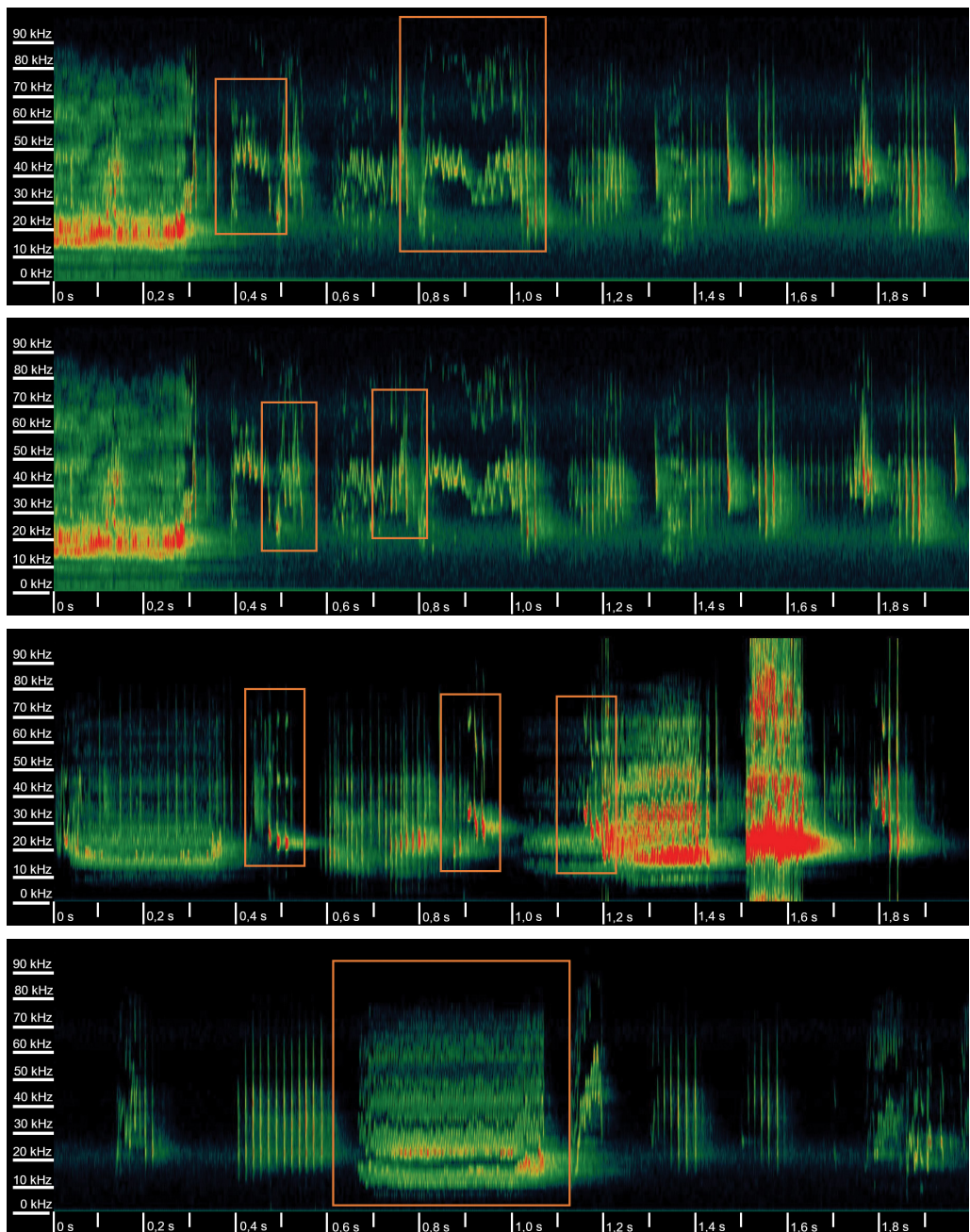
A trill-shape social call usually with two harmonic frequencies. Arched character of the call with different number of protrusions. The upper part of the arc and at the same time the longest phase is around 35–45 kHz, it can reach up to 60 kHz. The duration is usually 70–200 ms. The total broadband frequency is around 70 kHz. The broadband frequency of the first harmonic is 35 kHz (Fig. 26).

#### Subclass G3

The sharp arc-like character of the social call in the form of an inverted V. The voice is not connected but divided into simple calls followed by an inverted V. The first harmonic frequency is prominent, the second one is faintly visible. Peak V is in the range of 60–70 kHz and the call duration around 50–90 ms. The broadband frequency of the first harmonic is around 50 kHz (Fig. 27).



Figs. 24, 25. Recording of the F- and G-calls of social call of *Myotis myotis*. 24 (top) – F class; 25 (below) – G1 subclass.



Figs. 26–29. Recording of the G-calls of social call of *Myotis myotis*. 26 (top) – G2 subclass; 27 (middle top) – G3 subclass; 28 (middle below) – G4 subclass; 29 (below) – G5 subclass.

#### Subclass G4

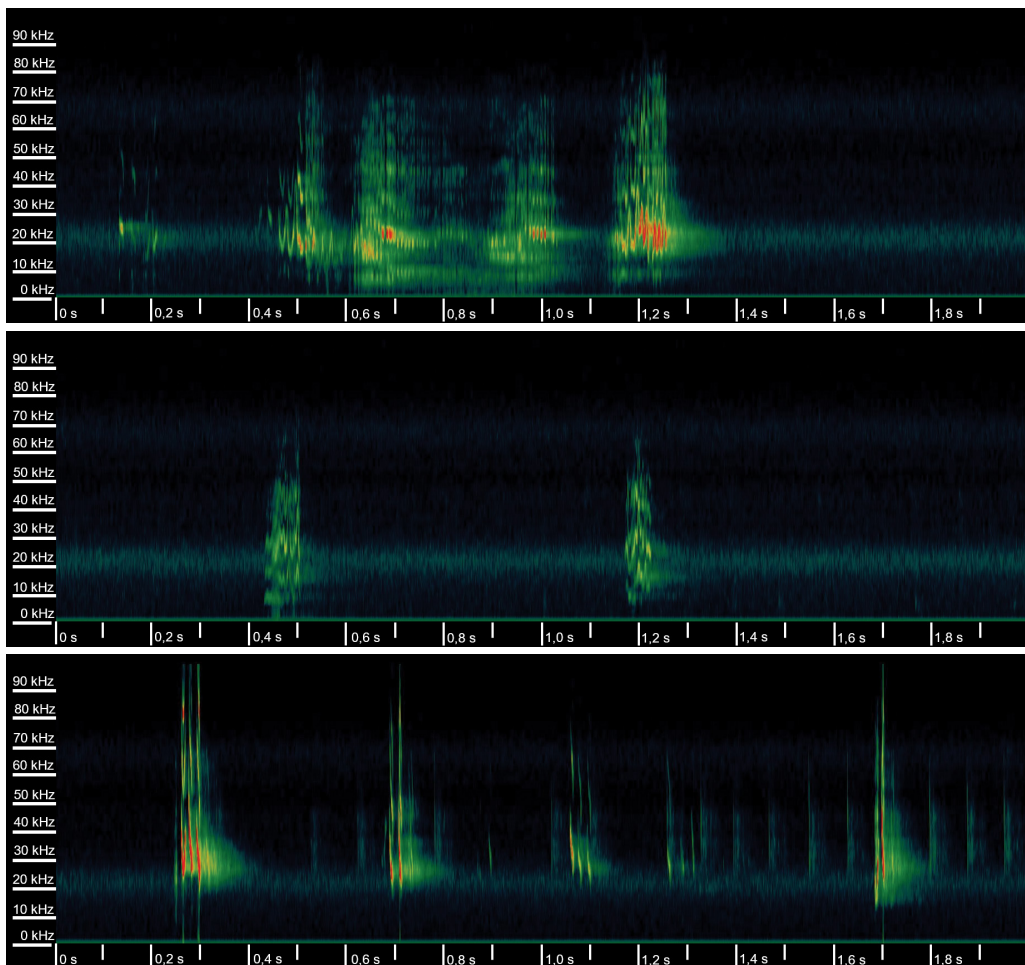
The mallet-shaped FM character of the social call with three harmonic frequencies. The first harmonic frequency is the strongest with a maximum around 25 kHz. Usually, the call is made up of a series of three voices. The duration of one voice is around 8 ms. The broadband frequency of the first harmonic frequency is 10–15 kHz (Fig. 28).

#### Subclass G5

A multi-harmonic trill-like social call similar to the G1 type, but with a larger frequency range of individual harmonics. The voice contains 3–4 harmonic frequencies. The maximum frequency of the first harmonic is around 23 kHz. The duration of the call is 200–700 ms. The broadband frequency is 70 kHz (Fig. 29).

#### Subclass G6

The trill-like character of the social call, most often with three harmonic frequencies. The strongest is the first or second harmonic frequency with a maximum of 20–25 kHz. The duration of the call is 70–150 ms. The broadband frequency of the main harmonic frequency is 25 kHz (Figs. 30, 31).



Figs. 30–32. Recording of the G-calls of social call of *Myotis myotis*. 30 (top) – G6 subclass; 31 (middle) – G6 subclass; 32 (below) – G7 subclass.

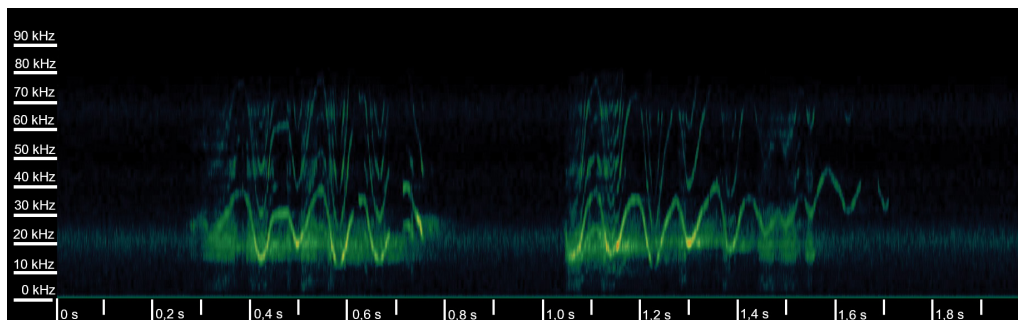


Fig. 33. Recording of the G8 subclass of social call of *Myotis myotis*.

#### *Subclass G7*

It is an FM social call with two harmonic frequencies. The first harmonic frequency is the most prominent with a maximum of around 25 kHz. It is often based on the G7 type. The duration is around 7 ms. The broadband frequency of the first harmonic is 10–15 kHz (Fig. 32).

#### *Subclass G8*

A trill-like social call with two or three harmonic frequencies. The most prominent is the first harmonic frequency with a maximum of around 25 kHz. The length is most often between 100 and 400 ms – variable, depending on the number of arcs. The frequency dispersion of one harmonic is 20–30 kHz (Fig. 33).