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TESTING THE EFFECTIVENESS OF TWO METHODS OF SUMMER BAT FAUNA INVENTORY

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Testing the Effectiveness of Two Methods of Summer Bat Fauna Inventory. Prylutska, A., Kravchenko, K., Vlaschenko, A. — Effective techniques of fauna inventory and biodiversity monitoring are essential for obtaining reliable data on species composition, population structure, and habitat use. Bats remain one of the least studied groups of mammals, yet they are important indicators of natural habitat condition. We present a comparison of the results of two survey methods, mist-netting and acoustic recordings, conducted at the same locations to assess summer bat assemblages in the National Nature Park "Homilsha Forest" (Kharkiv Region, Ukraine). Mist-netting yielded 440 individuals of 10 bat species, while acoustic recordings allowed the detection of 8 species, with a total of 3,468 echolocation sequences treated as individual records. Two species (*Myotis brandtii* and *Pipistrellus kuhlii*) were detected exclusively through mist-netting, whereas all other species were detected by both survey methods. The Jaccard index ranged from 0.70 to 0.80, indicating partial overlap of the two survey methods in terms of species detection. Species relative abundance differed significantly between the two methods (Chi-square test, $p < 0.001$). Mist-netting and acoustic recordings yielded different estimates of relative abundance for several species, suggesting method-specific biases in a bat species abundance assessment. The survey method also had a significant effect on species richness and relative abundance (two-way ANOVA, $p < 0.05$). In summary, we recommend a mist-netting survey as the best method for species identification (and population structure) and acoustic recording as a monitoring tool for summer bat assemblages.

Key words: biodiversity monitoring, Eastern Europe, species richness, bats, protected area, conservation.

Introduction

Accurately identifying species composition in natural and human-modified landscapes is the first and most essential step in biodiversity monitoring (Yoccoz et al., 2001; Schmeller et al., 2017). A comprehensive species inventory is the foundation for long-term ecological studies, conservation planning, and environmental management. Without precise data on species occurrence, assessing population trends, evaluating ecosystem health, or developing effective conservation strategies is impossible.

Ukraine ranks among the leading European countries in terms of habitat preservation and species diversity, owing to its extensive network of natural landscapes, including forests, wetlands, steppe ecosystems, and mountain regions. For the preservation of these habitats and species diversity, natural reserves, national nature parks and regional landscape parks are continuously being established. Currently, Ukraine has more than 100 natural protected areas on national (National Nature Parks, Natural and Biosphere Reserves) and regional levels (Regional Landscape Parks) (e. g., Petrovych et al., 2024). However, despite this rich biodiversity, species inventories and systematic biodiversity monitoring have not yet reached sufficient levels, particularly in comparison to European Union countries, in both unprotected and protected areas.

Bats are widely recognized as a bioindicator group among vertebrates (Jones et al., 2009; Russo & Jones, 2015). In Ukraine, a minimum of 28 bat species are recorded (Prylutska et al., 2020; Annex 1: List of animal species to be included in the Red Data Book of Ukraine (fauna), 2021), all of which are protected under national legislation (Akimov, 2009; Annex 1: List of animal species to be included in the Red Data Book of Ukraine (fauna), 2021) and international agreements (Agreement on the Conservation of Populations of European Bats (EUROBATS), 1991). These legal frameworks require continuous and specific monitoring to assess population trends and species status. Some European bat species (e. g. *Nyctalus lasiopterus*, *Barbastella barbastellus*, *Myotis bechsteinii*) are strongly associated with well-preserved natural woodlands mixed with wetlands and exhibit high sensitivity to habitat degradation (Russo et al., 2016; Vlaschenko et al., 2022). Such species can serve as indicators of ecosystem integrity, making their presence or absence a key factor in assessing the conservation status of natural areas. Understanding bat species composition and distribution provides a scientific basis for conservation planning, habitat protection, and the development of long-term biodiversity monitoring strategies in Ukraine (Vlaschenko, 2010).

In this study, we tested and compared the results of bat mist-netting and acoustic recording within the National Nature Park "Homilsha Forest". Our goal was to assess the efficiency of each method in detecting different bat species and their relative abundance to provide insights into their application for future bat monitoring programs in Ukraine.

Material and Methods

Study site

National Nature Park "Homilsha Forest" (NPHF) (49.58, 36.25; Chuhuiv District (Zmyiv District till 2020), Kharkiv Region, Ukraine) is one of the largest broadleaf forested areas in Kharkiv Region, covering 14,314.8 ha, with approximately 10,000 ha of forest. Established in 2004, it lies within the forest-steppe natural zone. It features a diverse landscape, including upland mature oak forests (*Quercus robur* with *Fraxinus excelsior*, *Acer platanoides*, *Populus tremula*, and *Tilia cordata* in the canopy) on the right bank of the Severskyi Donets River and pine forests (*Pinus sylvestris*) on the left bank. The temperate continental climate has a mean annual temperature of +6.9 °C and precipitation of 540 mm/year (Gukasova & Vlaschenko, 2011).

The survey was conducted on a 400-ha area in the NPHF's northeastern section (Fig. 1), adjacent to the Severskyi Donets River. Forest habitats were primarily mid-aged maple-linden upland oak forests, with dominant stands aged 80–120 years.

Extensive surveys over the last 25 years (in the NPHF) have revealed 11 bat species (e. g., Vlaschenko, 2010; Vlaschenko et al., 2022): *Myotis brandtii* Eversmann, 1845; *Myotis dasycneme* Boie, 1825; *Myotis daubentonii* Kuhl, 1817; *Nyctalus noctula* Schreber, 1774; *Nyctalus leisleri* Kuhl, 1817; *Eptesicus serotinus* Schreber, 1774; *Pipistrellus nathusii* Keyserling and Blasius, 1839; *Pipistrellus kuhlii* Kuhl, 1817; *Pipistrellus pygmaeus* Leach, 1825; *Vespertilio murinus* Linnaeus, 1758; and *Plecotus auritus* Linnaeus, 1758. It provides a reference framework of bat species composition for this territory.

Mist-netting and recording

The field survey was conducted in July 2011. Seven sampling sites were selected for double mist-netting and six for acoustic recording (Fig. 1); one acoustic recording sampling site was located along a forest road between two mist-netting sites that were combined for further analysis. The first mist-netting session took place from 2 to 11 July, and the second from 12 to 20 July, while acoustic recordings were conducted from 1 to 12 July (Table 1).

The mist-netting protocol, as well as the determination of sex, age, and reproductive status of captured bats, followed methods previously described in detail by Vlaschenko et al. (2016 a, b, 2022). Bat capture and handling adhered to ethical standards and ensured animal welfare following international guidelines (Sikes, 2016) and Ukrainian national legislation (Vlaschenko et al., 2021). This fieldwork was part of a

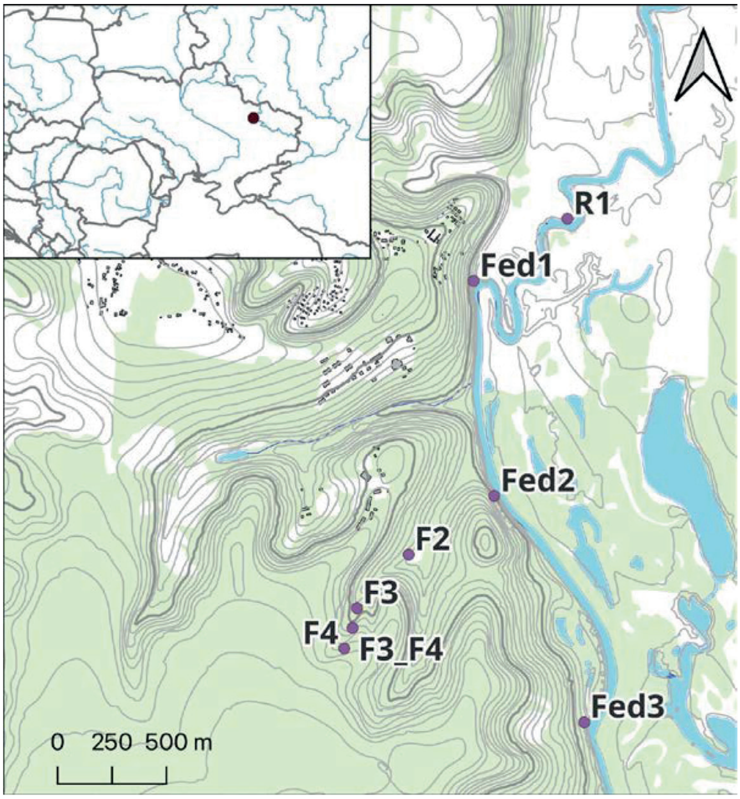

Fig. 1. Sampling sites in the territory of the National Nature Park "Homilsha Forest" (the North-Eastern Ukraine); R1, F2, 3, 4 and Fed1, 2, 3 — mist-netting sites, R1, F2, 3–4 and Fed1, 2, 3 — acoustic recording sites

multi-year biodiversity monitoring program in the NPHF, known as "Litopys Pryrody". In total, 14 mist-netting sessions were conducted for the current study, with a combined netting effort of 101.5 hours. To avoid interference, mist-netting and acoustic monitoring at each sampling site were carried out on separate nights.

Echolocation signals of bats were recorded using a Tranquillity Transect ultrasonic detector (recording mode — 320 ms, time expansion factor — 10) in conjunction with a ZOOM H2 recording device, which recorded soundtracks in WAV format. Recordings were carried out in three separate sessions: 30 minutes after sunset, at midnight (01:00–01:30), and 30 minutes before sunrise. Each recording session lasted 25 minutes, divided into five-minute segments to facilitate analysis. In total, 450 minutes of bat echolocation signals were recorded and analyzed.

Processing of Bat Acoustic Signals

The acoustic analysis was conducted using BatSound 4.4 software (Pettersson Elektronik AB) (Appendix, Fig. Ap1). Species identification was performed manually by measuring the following acoustic parameters: pulse duration, peak frequency (Fpeak), inter-pulse interval (time interval between two consecutive pulses), frequency range (minimum and maximum frequencies: Fmin and Fmax), signal structure (FM, QCF, FM-QCF, QCF-FM), and pulse repetition rate (Appendix, Fig. Ap2).

This methodology allowed for the identification and characterization of bat species based on their echolocation call parameters. For bat species identification based on acoustic signals, we used the identification keys the “Limits of Echolocation Calls of European Bats” table (Russ, 1999, 2012), the iBats program identification key, and a reference guide with sound examples (Barataud, 1996). Each sequence of echolocation pulses recorded at a consistent frequency within a single 320-ms interval was treated as an individual record, under the assumption that it originated from a single bat, and was therefore counted as one individual in further analyses.

Limitations in species identification based on acoustic signals

Bat echolocation calls are highly variable, and within species, are influenced by factors such as individual age and habitat type (Kalko & Schnitzler, 1993; Russ, 1999; Obrist et al., 2004). In particular, *P. nathusii* and *P. kuhlii* cannot be reliably distinguished in recordings made with ultrasonic detectors, as they share overlapping pulse parameters (Fpeak = 37–42 kHz) (Zsebők et al., 2012). Therefore, all sequences with such characteristics were attributed to *P. nathusii*, which has been consistently the most abundant species according to mist-netting data from previous years (Vlaschenko & Gukasova, 2009; Vlaschenko et al., 2022).

In 3.6% of acoustic sequences, species-level identification was not possible due to low signal quality or overlapping noise; these were assigned to Vespertilionidae spp. Additionally, nine sequences with 44–48 kHz peak frequencies, characteristic of *Pipistrellus pipistrellus* (Schreber, 1774), were recorded (sites Fed1 and Fed2, see Fig. 1). However, all mist-netted individuals of the *P. pipistrellus/pygmaeus* complex from 2006–2018 in the NPHF were morphologically identified as *P. pygmaeus* (Schofield, 2002; von Helversen & Holderied, 2003), consistent with findings across whole Kharkiv Region (e. g., Gukasova & Vlaschenko, 2011; Vlaschenko et al., 2021). Moreover, the recently published Europe-wide review (Godlevska & Gazaryan, 2024) further confirms the absence of *P. pipistrellus* s. s. in central and eastern Ukraine. Therefore, these sequences were conservatively attributed to Vespertilionidae spp.

Data Analysis

Data were compiled and organized in Microsoft Excel, analyses were initially performed in Statistica 6.0 (StatSoft, Inc.). The Jaccard similarity index was used to quantify the overlap in species composition between two mist-netting sessions and between each session and the acoustic dataset (unidentified species were counted as absent). The chi-square test was applied to identify the differences in bat species abundance in different sessions of mist-netting and acoustic recordings. To assess differences in relative species abundances between survey methods, we performed Z-tests for each species, comparing their proportional representation in the combined mist-netting data (two sessions) and acoustic recordings (except for *P. kuhii*).

To assess the influence of survey method and site on bat diversity and activity, we applied two-way ANOVA focused on two response variables: Species richness (number of species), and Total abundance (sum of individuals/sequences across all species). The explanatory variables were Method (mist-netting vs acoustic) and Sampling site. Prior to analysis, richness and abundance values were aggregated across all detected species for each combination of method and site.

Results

A total of 440 individuals representing 10 bat species were captured using mist-netting during two survey sessions. In parallel, acoustic monitoring yielded 3468 echolocation sequences, from which 8 bat species were identified.

Two species, *Myotis brandtii* and *P. kuhlii*, were detected exclusively through mist-netting (Table 2) and were not recorded acoustically. *Myotis brandtii*, despite having distinct acoustic characteristics compared to other species of the genus *Myotis* (Russ, 1999), was not identified in our acoustic recordings. Conversely, all species identified acoustically were also represented among the mist-net captures. The number of individuals of bat species based on mist-net captures and sequences from acoustic recordings detection is presented in Table 2.

The Jaccard index between the two mist-netting sessions was 0.70. The index between the first mist-netting session and the acoustic recordings was 0.78, while the index between the second mist-netting session and the acoustic recordings was 0.70.

Table 1. **Results of bat mist-netting and recording (only identified by species) on different sampling sites (Fig. 1) in National Nature Park "Homilsha Forest" in July 2011**

Point ID	Date	Mist-netting		Acoustic	
		Number of species	Number of individuals	Number of species	Number of sequences
R1	02–03.07.2011	–	–	7	489
	07–08.07.2011	4	19	–	–
	14–15.07.2011	3	3	–	–
F2	05–06.07.2011	–	–	6	586
	06–07.07.2011	1	1	–	–
	12–13.07.2011	3	2	–	–
F3	10–11.07.2011	2	2	–	–
	19–20.07.2011	3	34	–	–
F3_F4	07–08.07.2011	–	–	7	57
F4	10–11.07.2011	3	19	–	–
	19–20.07.2011	2	4	–	–
Fed1	01–02.07.2011	–	–	7	552
	02–03.07.2011	5	39	–	–
	17–18.07.2011	6	96	–	–
Fed2	03–04.07.2011	–	–	7	1165
	05–06.07.2011	4	37	–	–
	13–14.07.2011	8	163	–	–
Fed3	11–12.07.2011	–	–	7	619
	09–10.07.2011	0	0	–	–
	15–16.07.2011	4	20	–	–

Finally, the Jaccard index between the mist-netting data and the acoustic recordings was 0.80.

Chi-square tests revealed significant differences in species composition between all pairs of survey approaches: MN I vs MN II (χ^2 = 69.32), MN I vs Acoustic (χ^2 = 210.06), MN II vs Acoustic (χ^2 = 74.81), and MN_total vs Acoustic (χ^2 = 119.24) all $p < 0.001$. The strongest divergence was observed between mist-netting sessions and acoustic data compared to divergence between the two mist-netting sessions (Table 2).

Based on the Z-tests results, species with significantly higher relative abundance in mist-netting samples compared to acoustics were *M. brandtii*, *M. daubentonii*, *P. pygmaeus* and *Pl. auritus* (Z = 4.86, 5.16, 3.18, 3.00; $p < 0.01$). Species with significantly higher relative abundance in acoustic recordings were *E. serotinus* and *N. leisleri* (Z = –5.68 and –3.45, respectively, $p < 0.01$). The remaining species (*M. dasycneme* Z = –0.7, p = 0.482; *N. noctula* Z = –0.87, p = 0.386; *P. nathusii* Z = 1.61, p = 0.107) showed no statistically significant differences in relative abundance between methods, suggesting both mist-netting and acoustic monitoring provided comparable estimates for these taxa.

Table 2. **Number of mist-netted (MN) individuals and recorded sequences of different bat species in National Nature Park "Homilsha Forest" in July 2011**

Bat species	MN I session	MN II session	Acoustic Sequences	MN Total
M. brandtii	1	2	0	3
M. dasycneme	0	1	16	1
M. daubentonii	36	15	186	51
N. noctula	58	239	2411	297
N. leisleri	1	8	210	9
E. serotinus	1	1	269	2
P. nathusii	5	16	114	21
P. kuhlii	0	1	0	1
P. pygmaeus	12	40	259	52
Pl. auritus	3	0	3	3
Vespertilionidae spp.	–	–	137	–
Total: species/ind.	8/117	9/323	8/3605	10/440

Table 3. **Results of two-way ANOVA testing the effects of survey method (mist-netting vs. acoustic recording) and sampling point on species richness and bat abundance (total number of individuals in mist-netting and sequences in acoustics)**

Response	Factor	F-value	p-value *
Species richness	Method	9.7493	**0.0354**
Species richness	Point	1.7007	0.3098
Abundance	Method	7.8470	**0.049**
Abundance	Point	1.6090	0.3270

* Significant effects ($p < 0.05$) are shown in bold.

Two-way ANOVA revealed that the survey method had a statistically significant effect on both species richness ($p = 0.035$) and bat abundance ($p = 0.049$), whereas the sampling point did not significantly influence either variable ($p > 0.3$) (Table 3).

Discussion

The study evaluates the effectiveness of two bat survey methods (mist-netting and acoustic recordings) made for the first time in Ukraine. Both methods were applied simultaneously at the same sampling sites within a single summer season, allowing for a robust, paired assessment of their performance. In total, 10 bat species were recorded by mist-netting and 8 species through acoustic recording, with two species (*M. brandtii* and *P. kuhlii*) detected exclusively by mist-nets. Species composition between methods showed substantial overlap (Jaccard index: 0.70–0.80), yet statistical tests revealed significant differences in relative abundance estimates. These differences reflect species-specific biases related to call intensity, detectability, and flight behavior. To our knowledge, this is the first comprehensive effort in Ukraine to evaluate survey methodologies for bats using a combined, site-specific approach. Our case study represents an attempt to propose a more effective methodological way of assessing bat diversity and further monitoring in protected areas (not only) of Ukraine.

Despite comparable species lists, the two methods showed divergence in their estimates of relative abundance. While *N. noctula,* the most common and abundant bat species in summer bat assemblages of Eastern European deciduous forests (Vlaschenko et al., 2016a, 2022) consistently dominated both mist-net captures and acoustic detections. *Myotis dasycneme* and *P. nathusii* are two species that exhibited similar abundance patterns under both methodologies. Other species exhibited greater variability between methods, for example *E. serotinus* and *N. leisleri* were recorded in higher proportions via acoustic monitoring, presumably due to their louder echolocation calls and greater detection range (Russ, 1999), as well as potential behavioural traits that make them less likely to be captured in mist-nets. Likely, the relative abundance of these species is somewhat underestimated by mist-netting. Especially in the case of *E. serotinus*, which is known to be widespread and abundant in the central and northern regions of Ukraine (Buzunko et al., 2014). Species that were more effectively captured using mist-netting (*M. brandtii*, *M. daubentonii*, *P. pygmaeus* and *Pl. auritus*) emitted short-range, high-frequency calls (Russ, 1999). These echolocation signals, while suitable for precise navigation in cluttered environments (mostly *M. brandtii* and *Pl. auritus*, less *M. daubentonii* and *P. pygmaeus*), tend to be less detectable by passive acoustic monitoring systems (Russ, 1999). *Vespertilio murinus* was not detected by either mist-netting or acoustic recordings in 2011. However, the species has been occasionally recorded in both earlier and subsequent years, typically represented by single individuals. Its consistent absence in both capture and acoustic data in 2011 further supports the species' rarity in the study area and more broadly within the Kharkiv Region (Vlaschenko et al., 2022).

Our comparative study underscores the strengths and limitations of both mist-netting and acoustic monitoring as survey methods for bat assemblages. Mist-netting remains the most reliable approach for species-level identification, as all captured individuals were handled and identified based on morphological characteristics. In cases of cryptic species when morphological traits are insufficient, wing biopsy samples obtained from mist-netted individuals can be subjected to genetic analysis for definitive identification (e. g., Bashta et al., 2018; Vlaschenko et al., 2023). Importantly, mist-netting allows researchers to obtain data on sex, age, reproductive status (Vlaschenko et al., 2022), and morphometric traits — information that acoustic methods cannot provide. Additionally, approximately 4% of echolocation sequences in our study could not be assigned to species with confidence, and some species could not be reliably identified acoustically due to overlapping call structures or low-intensity signals. Although general patterns of species dominance were consistent between the two methods, differences in detection efficiency suggest that relative abundance should be compared within, rather than between, survey methods. These method-specific biases highlight the importance of using a single approach when assessing relative abundance to avoid misinterpretation.

It is well established that bats are distributed unevenly across landscapes, with many species favoring ecotones and riparian zones (e. g., Salvarina, 2016; Salvarina et al., 2018). Based on this, we hypothesized that sampling sites located along the river corridor (Fed1–3) would yield higher species richness and abundance. However, analysis showed that the survey method alone had a stronger influence on both species richness and abundance than the location (sampling site) itself (Table 3). This underscores the importance of method choice when assessing bat assemblages.

There is an ongoing debate on the advantages and limitations of acoustic surveys versus mist-netting. Advocates of acoustic methods emphasize their non-invasive nature but acknowledge that population parameters cannot be reliably inferred without physical captures (O'Farrell & Gannon, 1999). For example, Hayes et al. (2009) argue that high acoustic activity may not accurately reflect population size. Moreover, acoustic activity does not always correspond with actual population density (Milchram et al., 2020). Furthermore, presence of bat echolocation calls in the soundscape does not necessarily indicate the habitat value; distinguishing feeding buzzes and other behavioral indicators is essential (Jones et al., 2009). Acoustic activity alone also does not provide insight into population structure, such as the sex, reproductive status, or age of individuals, which are essential for understanding habitat use. Mist-netting allows for determining these factors, which are crucial for assessing habitat quality. Females, especially during the breeding season, require higher habitat quality than males, making habitats that support reproduction more important for conservation prioritization than those used by males alone.

Historically, bat research in Ukraine before 2000 primarily relied on bat roost searches (e. g., (Kondratenko, 1998; Ruzhilenko et al., 1998). Since the early 2000s, mist-netting has become increasingly widespread, alongside the introduction of ultrasonic detectors — initially, simple heterodyne devices (Zagorodniuk & Godlevska, 2000), followed by more advanced models capable of recording and analyzing echolocation calls (e. g., Bashta et al., 2010; Godlevska & Rebrov, 2018). In recent years, Ukrainian bat researchers have increasingly employed a wide range of ultrasonic detector models (e. g., Koval & Bashta, 2018; Volokh et al., 2021; Godlevska et al., 2022) that in combination with classical methods of roosting sites search and mist-netting allowing for a more comprehensive assessment of summer bat fauna (including in protected areas). However, mist-netting remains the only effective method for the detection and reliable identification (or future genetic identification) of rare or cryptic species (e. g., Bashta et al., 2011; Gashchak et al., 2013; Vlaschenko et al., 2025).

In conclusion, based on the outcomes of this study, we recommend that baseline bat surveys in protected areas of Ukraine begin with a combination of intensive mist-netting and simultaneous acoustic monitoring. Mist-netting, following the methodological guidelines previously outlined (Vlaschenko & Gukasova, 2009; 2010), ensures accurate species identification and allows for the detection of a maximum number of species, including those less likely to be detected acoustically. At the same time, early acoustic recordings provide the first reference data that can be used to calibrate further acoustic monitoring. Once a comprehensive species list is obtained, acoustic methods can then be relied upon for long-term monitoring of bat activity and, in combination with other approaches, may support inferences on population trends.

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Appendix

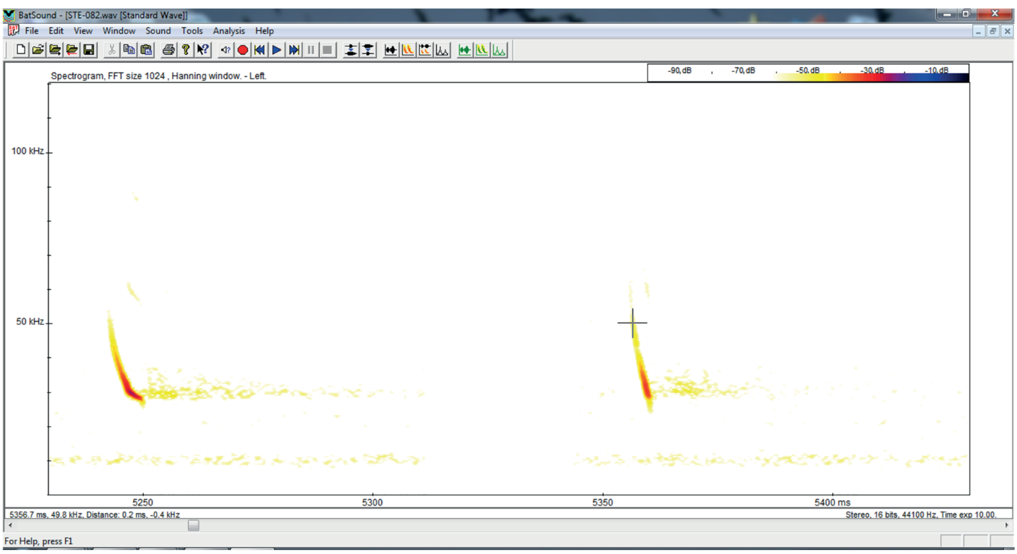

Fig. Ap1. An example of two echolocation pulses from a single individual was analyzed using BatSound 4.4 software. The x-axis represents time (ms), the y-axis represents frequency (kHz), and colour indicates sound intensity (dB)

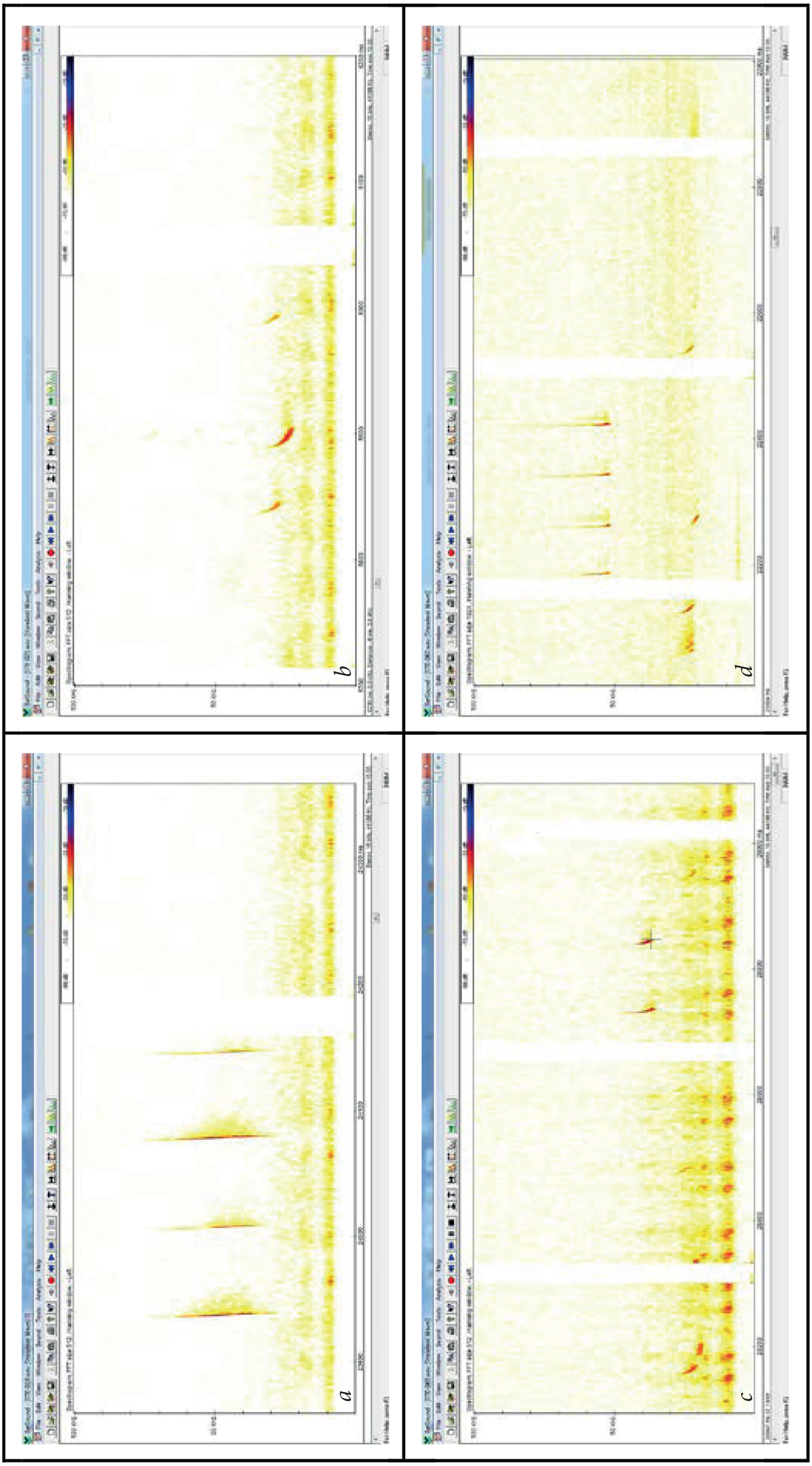

Fig. Ap2. Examples of echolocation pulses from different bat species: *a* — *M. daubentonii*, *b* —*N. noctula* and *N. leisleri*, *c* — *P. nathusii* and *N. noctula*, *d* — *P. pygmaeus* and *N. noctula*