Effectiveness of mist-netting of bats (Chiroptera, Mammalia) during the non-hibernation period in oak forests of Eastern Ukraine

Alona GUKASOVA and Anton VLASCHENKO

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Abstract. We tested how the effectiveness of capturing bats with mist nets varied by month (April-September), period in the bat life cycle, habitat and capture effort. A total of 898 bats of 10 species were captured in the National Nature Park "Gomolshansky lessy" from 2006 to 2009. The most successful month of mist-netting was July with a median value of 4.0 (range 0-16.7) b/h (bats per hour) index and bats caught on 94% of nights. The ratio of different bat species changed in different periods of the bat life cycle. The highest number of bat species was caught in July (n=9). Mist nets placed along an ecotone – the border between forest and river – were the most efficient. The period when young-of-the-year bats become volant is concluded to be the most effective period for mist-netting.

Key words: Vespertilionidae, mist-netting, survey, methodology, habitat, phenology, protected areas.

Alona GUKASOVA, Department of Vertebrate Zoology, Saint-Petersburg State University, Universitetskaya emb. 7/9, 199034, Saint-Petersburg, Russia.

E-mail: alenagukasova@gukasov.net

Anton VLASCHENKO, Interdepartmental Research Laboratory "Study of biodiversity and development of nature reserve management", Svobody sq. 4, 61077, Kharkov, Ukraine. E-mail: vlaschenko@yandex.ru

I. INTRODUCTION

Bats are recognized as important indicators of biodiversity (JONES et al. 2009). These animals have high conservation status and many species are threatened (MICKLEBURGH et al. 2002). As with all groups of threatened species, bats need a clear assessment of changes taking place in their populations, which can be done via monitoring. In spite of a great number of guides for field bat researchers (KUNZ 1990; ZAGORODNIUK et al. 2002; PARSONS et al. 2007) a unitized concept of bat population monitoring is undeveloped. As far as we know, two monitoring approaches are applied in Europe: acoustic monitoring, using bat detectors (including point counts, car- or bike-based transect counts and waterway sur-

veys), and visual monitoring with regular survey of potential summer and winter roosts (WALSH et al. 2001). However, these methods are remote techniques and provide only fragmentary data about species richness and relative abundance, without data on the population structure. Bat monitoring in hibernacula is more standardized and similar standards are often used in monitoring of summer bat residences (LESIŃSKI et al. 2005). Winter surveys involve greater risk of bat mortality and do not give reliable data about population structure or condition of populations in summer.

There are no large caves on the plains of Ukraine and Russia. Moreover, only a small proportion of forest bat species hibernate in caves, while several of them (*Nyctalus, Pipistrellus, Vespertilio*) migrate from Ukraine to the south and west. Therefore summer mist net surveys are considered the best method for studying bats in the woodlands of Eastern European countries (Ukraine, Russia and Belarus) (VEHNIK & SACHKOV 2005; VLASCHENKO & GUKASOVA 2009).

Due to these factors, mist netting has recently become the method of choice in Ukraine and has proved to be highly effective in bat fauna research (PETRUSHENKO et al. 2001; TYSHCHENKO et al. 2005) relative to other countries where mist netting has been applied for decades (KUNZ & BROCK 1975). Currently, however, there is no standardized methodology for mist net surveys. Previously (VLASCHENKO & GUKASOVA 2009; GUKASOVA & VLASCHENKO 2010) we developed a method for bat fauna inventory using mist nets in woodlands. Despite acoustic techniques being the most generally employed method for monitoring of bats in many countries (AHLEN & BAAGOE 1999; O'FARRELL & GANNON 1999; WALSH et al. 2001; RUSSO & JONES 2002), our findings indicate that mist netting is also effective for this purpose (GUKASOVA & VLASCHENKO 2010). Additionally, mist netting is a relatively inexpensive and accessible technique for the majority of bat-researchers.

Little is known about bats from most of the territories of Ukraine, Russia and Belarus. No primary information on bat species composition (KURSKOV 1981; ILYIN et al. 2002; ILYIN 2003; GODLEVSKA 2007) or more fundamental data on distribution and population sizes are available. Lack of data is a barrier to the implementation of suitable bat conservation methods and the development of management action plans in these territories. Hence, it is important to identify the periods of the year when bat capture will be the most effective for species inventories.

It remains unclear whether it is necessary to distribute capture points over the whole study area or if it suffices to catch bats in one standard point during the whole non-hibernation period (or few periods) to capture all species present.

The objectives of this paper are 1) to evaluate mist net capture success in the same points during the non-hibernation period of the year; 2) to assess but species composition changes over different periods of the but life cycle by mist-netting.

II. STUDY AREA

Investigations were conducted in the National Nature Park "Gomolshansky lessy", Zmeev district, Kharkov region, Ukraine (49°35′ N, 36°15′ E). The National Nature Park (14 000 ha) was established in 2004. It includes pine forest, the floodplain of the Seversky Donetz River and oak forest. The altitude varies between 80 and 215 m a. s. l. Climate is

temperate with the mean temperature in January -7.3°C (minimum - 35°C) and \pm 20.8 °C in July (with maximum reaching between \pm 37 and 40°C). The mean annual rainfall is approximately 495 mm.

The forest is represented by 80-120 year old oak forest (*Quercus robur*) with ash-trees (*Fraxinus excelsior*) and lime (*Tilia cordata*). Also, maples (*Acer platanoides* and *A. campestre*), aspens (*Populus tremula*), pears (*Pyrus communis*), apple-trees (*Malus sylvestris*) and elms (*Ulmus laevis*) could be found.

Ten species of bats have been recorded in the territory of the National Nature Park (VLASCHENKO 2005; VLASCHENKO & GUKASOVA 2009).

Phenology of the bat life cycle during the non-hibernation period in the study area

We determined stages of bat life cycle according to phenological seasons worked out for the Kharkov region by BOOT (1971). Spring (the period from the change of average daily temperature over 0° C until its change over +15°C) begins in the region on the 20th of March. Already at the end of March, using ultrasound detectors (Pettersson D200), we heard the first foraging bats: Eptesicus serotinus SCHREBER, 1774, and Myotis daubentonii KUHL, 1817 (VLASCHENKO 2006). Due to cold spells, bat activity is often unstable until the middle of April. By the middle of April sedentary bat species and facultative migrants (Myotis brandtii EVERSMANN, 1845, M. daubentonii, Myotis dasycneme BOIE, 1825, E. serotinus, and Plecotus auritus LINNAEUS, 1758) move to summer roosts. In last third (ten-day period) of April the first migratory species arrive: Nyctalus noctula SCHRE-BER, 1774, and Pipistrellus nathusii KEYSERLING & BLASIUS, 1839. The complete structure of the summer bat assemblage is formed by the middle of May, when Pipistrellus pygmaeus LEACH, 1825, and Nyctalus leisleri KUHL, 1817, arrive (VLASCHENKO 2006). Phenological summer begins on the 19th of May in the region (the period with stable daily temperature over +15° C) (BOOT 1971). Bat births begin in the first ten days of June in the study territory and continue till the beginning of the first ten days of July in some species. From the beginning of July the first young-of-the-year bats start to fly (VLASCHENKO 2006, 2009). Mass flights of young-of-the-year are recorded from the beginning of the second third of July (GUKASOVA et al. 2008). Summer assemblages remain stable to the end of the first third of August, when autumn swarming and migration begins. At the end of August P. pygmaeus migrate to the south. N. leisleri and Vespertilio murinus LINNAEUS, 1758, postpone their migration to the end of the first third of September. Mating colonies of N. noctula are fixed on the study territory until mid October. Autumn begins on 11th September and lasts until 14th November (the period between the dates of stable fall of daily average temperature below +15° C and its further stable fall to nearly 0° C and lower) (BOOT 1971).

III. METHODS

Captures were carried out during 2006-2009 from April to September. Bats were caught with nylon mist nets (7 and 12 m in length and 3 m high; mesh size-15 mm) at exactly the same locations each time. Mist netting was conducted mainly on the west river

bank with oak forest, with one capture site in the centre of the forest near the Gomolsha River. We selected 6 sites for mist nettings – 4 main and 2 additional sites (Table I).

Usually, netting at the same site night after night leads to a decrease in the number of bats captured (KUNZ & BROCK 1975). To avoid this we netted at 3-5 day intervals. Mist nets were open from sunset until sunrise, weather permitting. In spring mist nets were open for several hours, and in other seasons we tried to prolong the exposure period until sunrise. Duration of mist-netting (h) was recorded from the moment when the first bats (usually $N.\ noctula$) appeared in the air (defined using an ultrasound bat-detector Pettersson D200 set at 25 kHz) until the time at which the nets were taken down (accurate to 0.25 h = 15 min).

Captured bats were put into textile bags and kept near the net during the night. In May and June pregnant and lactating females were released a few hours before sunrise. In other months bats were released the next evening after capture. All methods were ethical and respectful for animal welfare and conservation of protected species, according to GANNON et al. 2007.

Species, sex, age, weight and forearm measurement of each bat were recorded. All captured bats were also banded (using "Kiev, Ukraine" rings) and then released.

P. pygmaeus was identified on the base of general appearance, fur and membrane coloration, and also by the pattern of the venation in the wing membranes (SCHOFIELD 2002, HELVERSEN & HOLDERIED 2003). Penis shape and coloration were not taken into account, because there were no adult males in our study region.

Table I

Characteristics of mist-netting sites in National Nature Park "Gomolshansky lessy"

Site No.	Name	Coordinates	Characteristics					
1	Beach near stone stairs	49°37′21′′ N, 36°19′40 E	S.Donetz river bank, bend near edge of oak forest, net at a distance of 1.5 m from water and 20 m from forest					
2	Alanskiy beach	49°36′56′′ N, 36°19′38′′ E	S.Donetz river bank, small clearing surrounded with forest, net at open bank, at a distance of 1.5 m from the water					
3	Tortoise lake	49°36′17′′ N, 36°20′05′′ E	Overflow wooden footbridge over S.Donetz river bay, net over water directly near forest clearing					
4	Bridge over the Gomolsha River	49°33′25′′ N, 36°20′35′′ E	Wooden footbridge over the Gomolsha River, 3 km to the West from place of falling into the S.Donetz River. Marshland, reed bush, the only opened space in a radius of 3 km is near bridge. Oak forest on both banks situated at a distance of 300-400 m from the bridge. Net placed on the bridge perpendicularly to the river flow					
5	Road to the Turbenka beach	49°37′02′′ N, 36°19'27 E	Old road under the oak forest canopy, at a distance of 100 m to the river bank					
6	Teacher's beach	49°37′30′′ N, 36°20′00′′ E	A plot of opened floodplain of the S.Donetz River. Net on sandy beach near water edge, 700 m from the forest edge					

As was noted above (in phenological characterization of the non-hibernation period) the important period of bat life cycles is the middle of May when summer assemblages emerge. Therefore for statistical comparisons the non-hibernation period was divided according to calendar months (used for testing of capture effectiveness and activity of bats) and phenological periods of bat life cycle (used for comparison of species composition). Percentage of positive nights (when at least one bat was caught) was used as a measure of capture efficiency.

The b/h (bats per hour) index was used to assess bat activity and capture success. The index was calculated independently of mist net length. The difference in net length (12 and 7 m) was hypothesized to be unappreciable and verified statistically. To check the correlation between the duration of mist netting and number of captured individuals, we applied the Spearman rank R test (nonparametric). We used a nonparametric Kruskal-Wallis H test to reveal differences in b/h index between months and between mist-net points. To test for differences between particular months (pairwise comparison) we applied non-parametric Mann-Witney U tests with Bonferroni correction.

We analyzed differences in species composition between phenological periods with a χ^2 test (pairwise comparison). Rare species were combined into one group for χ^2 tests. Statistical analyses were conducted with Statistica 7.0 and Excel 2003.

IV. RESULTS

Number of individuals and activity index

In total 898 bats of 10 species (*M. brandtii*, *M. dasycneme*, *M. daubentonii*, *N. noctula*, *N. leisleri*, *E. serotinus*, *P. nathusii*, *P. pygmaeus*, *V. murinus* and *Pl. auritus*) were captured during 49 nights. Total time of mist net exposure was 283.75 h (Table II).

In April 55% of nights (n=9) were unsuccessful (no bats were caught). In May (n=5), and September (n=5) capture efficiency was similar (60% positive nights). The most successful months (more than 80% positive nights) were June (n=6), July (n=18) and August (n=6) with the highest number of positive nights in June – 100%.

The median value of the b/h index increased from April to July and decreased in August and September (Fig. 1). Kruskal-Wallis H test revealed significant differences between months (df=5, n=49; H=16.10, p=0.007). A pairwise comparison (Mann-Whitney U test) showed significant differences between: June - April: U=7, p=0.028; June - September: U=4, p=0.044; July - April: U=20, p=0.009; July - May: U=10, p=0.016; July - September: U=20, p=0.007). However, according to Bonferroni correction only the differences between July-April and July-September are valid (p=0.01)

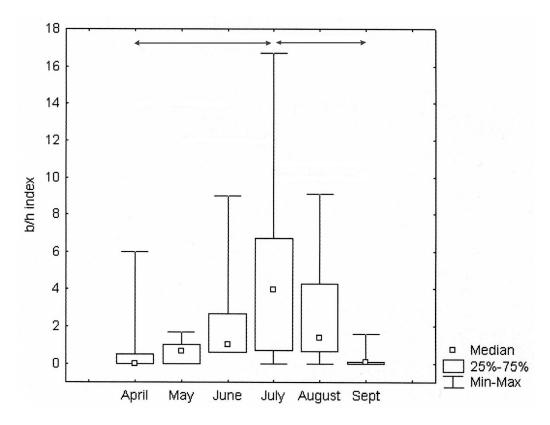
The high b/h index value in July seems to be linked with the increased duration of mist netting (Table II). The positive correlation between the duration of mist netting and number of captured bats was statistically significant for all months combined (49 nights; R=0.40, p=0.004). But for particular months there was no statistically significant correlation (April R =0.305, p=0.33; June R=0.032, p=0.95). In July and August the correlation was negative (R=-0.384, p=0.11; R=-0.057, p=0.91).

Table II

Mist-netting effort during April-September 2006-2009 at six sites in National Nature Park "Gomolshansky lessy"

Mist-netting site	April	May	June	July	August	September	Total		
1 Beach near stone stairs	4*/0**	6/7	14/8	36/112	8/73	10.5/1	78.5/201		
2 Alanskiy beach	7.5/22	_	7/63	20.25/234	25.5/47	8.5/1	68.75/367		
3 Tortoise lake	5.25/4	3.5/0	5.5/6	14.25/120	7/0	9/0	44.5/130		
4 Bridge over Gomolsha	8.5/1	2.75/3	8.25/16	3.25/41	8.5/12	8/13	39.75/86		
5 Road to the Turbenka beach	_	1.75/0	_	29.25/47	_	_	31/47		
6 Teacher's beach	_	_	_	21.25/67	_	_	21.25/67		
Total	25.25/27	14/10	35.25/93	124.25/621	49/132	36/15	283.75/898		

^{*} duration of mist-netting (hours); ** number of captured individuals.



 $Fig.\ 1.\ Seasonal\ dynamics\ of\ b/h\ index\ in\ the\ National\ Nature\ Park\ "Gomolshansky\ lessy"\ (horizontal\ arrow:\ statistically\ significant\ differences\ (p\ values\ in\ text).$

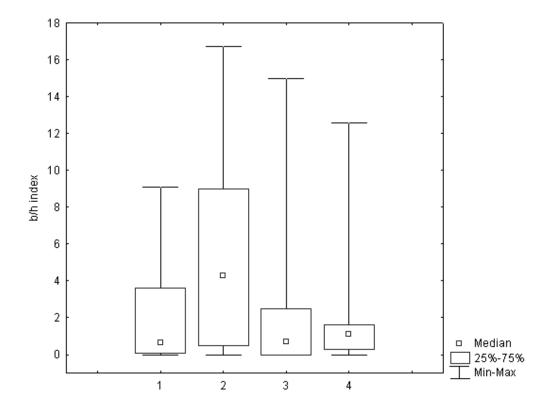
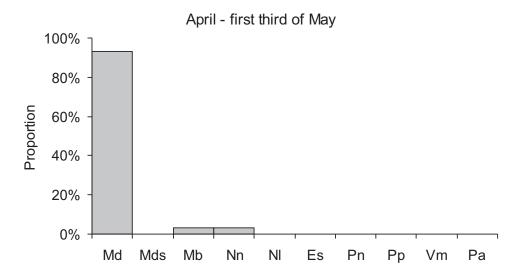


Fig. 2. B/h index value at four main mist-netting sites during the non-hibernation period in the National Nature Park "Gomolshansky lessy".

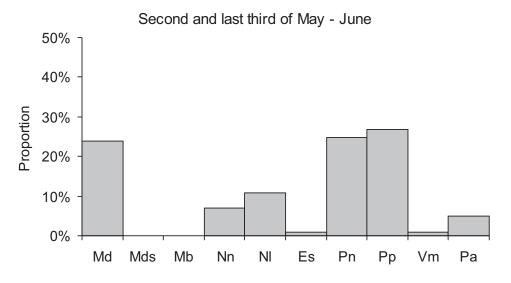
Seasonal changes in species composition and species richness

The combined data on species composition for all sites together are presented in Fig. 3a-e. Only three bat species (*M. daubentonii*, *M. brandtii*, *N. noctula*) were captured during the end of the spring migration (April-first third of May). *M. daubentonii* was the most abundant species during this period (93.1%). In the first part of the summer – the period of pregnancy and lactation, *P. pygmaeus* (26.7%), *P. nathusii* (24.7%) and *M. daubentonii* (23.8%) dominated. Most bat species (n= 9) were captured in the mist nets in July – the period when young-of-the-year bats begin to fly. *N. noctula* (45%), *M. daubentonii* (19.8%) were the most abundant species and two species of the genus *Pipistrellus* (12% and 17%) were also common. In August the number of species decreased to eight, with *N. noctula* (49.3%) and *P. nathusii* (23.6%) dominating. In September (Fig. 3e) five species of bats were mist-netted with the highest proportion of *P. nathusii* (66.7%) and *V. murinus* (13.2%).

Statistical analysis revealed significant variation of species composition between five phenological periods. April-first third of May and second-last third of May-June (χ^2 =105.0, p<0.001), April-first third of May and July (χ^2 =113.9, p<0.001); species com-

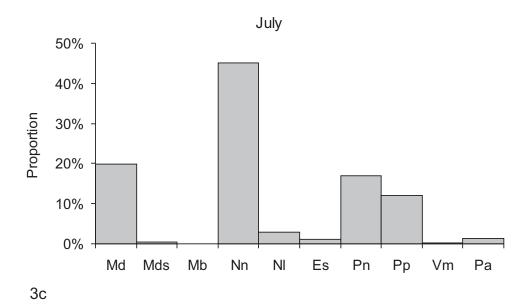


3a



3b

Fig. 3 a-b. Percentage composition of species of bats captured in different phenological periods; a – Final days of spring migration (n=29); b – Period of pregnancy and lactation (n=101). Abbreviated names: Md - Myotis daubentonii, Mds - Myotis dasycneme, Mb - Myotis brandtii, Nn - Nyctalus noctula, Nl - Nyctalus leisleri, Es - Eptesicus serotinus, Pn - Pipistrellus nathusii, Pp - Pipistrellus pygmaeus, Vm - Vespertilio murinus, Pa - Plecotus auritus.



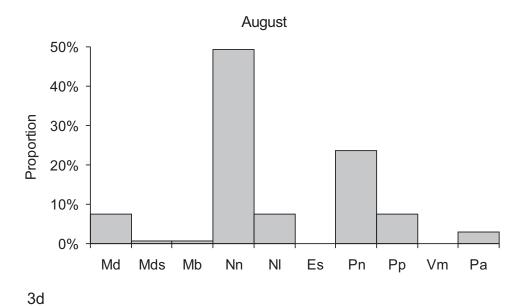


Fig. 3 c-d. Percentage composition of species of bats captured in different phenological periods; c – Period of the beginning of independent flight of bats born during the year of capture (n=621); d – Period of the beginning of autumn migration (n=132). Abbreviated names: Md – Myotis daubentonii, Mds – Myotis dasycneme, Mb – Myotis brandtii, Nn – Nyctalus noctula, Nl – Nyctalus leisleri, Es – Eptesicus serotinus, Pn – Pipistrellus nathusii, Pp – Pipistrellus pygmaeus, Vm – Vespertilio murinus, Pa – Plecotus auritus.

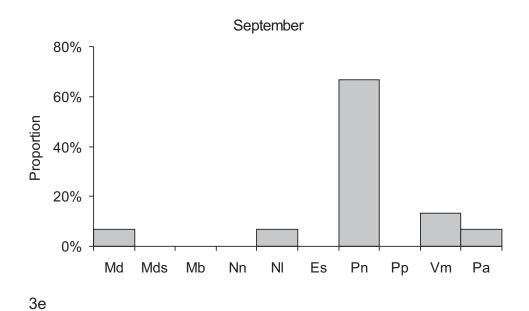


Fig. 3 e. Percentage composition of species of bats captured in different phenological periods; e – Period of peak of autumn migration (n=15). Abbreviated names: Md – Myotis daubentonii, Mds – Myotis dasycneme, Mb – Myotis brandtii, Nn – Nyctalus noctula, Nl – Nyctalus leisleri, Es – Eptesicus serotinus, Pn – Pipistrellus nathusii, Pp – Pipistrellus pygmaeus, Vm – Vespertilio murinus, Pa – Plecotus auritus.

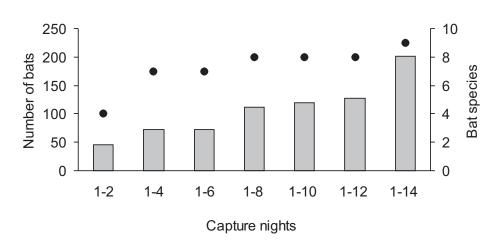
position in August differed from all other periods (p<0.000) except for July (χ^2 =9,303, p=0.054); species composition in September differed from all other periods (p<0.000).

Figure 4a-d shows the accumulation of bat species in relation to the number of captured individuals at four main mist-netting sites. We did not detect all ten species known for this region at any of the sites. The first rapid increase in number of individuals was from 40-100 bats; 6-8 bat species were already caught at the first three mist-netting sites (Fig. 4 a-c). The next increase was to 200 bats and the number of species had risen to nine at the first and second sites (Fig. 4a-b). At the first mist-netting site the ninth species was captured. However, at the remaining sampling sites a subsequent increase of capture effort (number of capture nights) and number of captured individuals respectively, did not result in new bat species.

Species composition and effectiveness of bat capture at different mist-netting sites

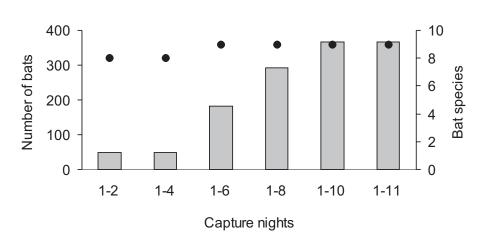
The four main mist netting sites were situated in different types of habitats (Table I). The first and second sites were the most similar (both ecotones, border between the forest and river – an important foraging area for the majority of bat species). At the first site V. murinus was not caught, and at the second one – M. brandtii was not detected (Table III).





4a

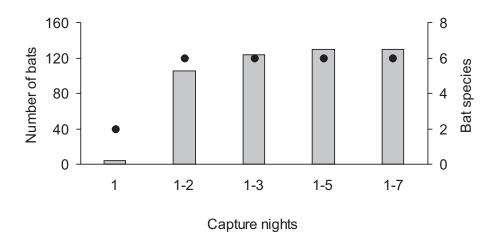
Mist-netting site 2



4b

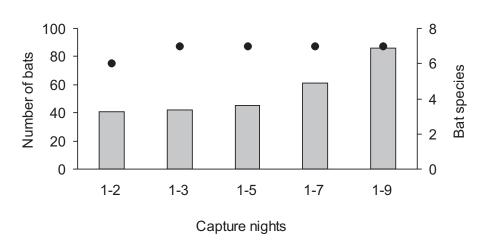
Figure. 4a-b. Dynamics of accumulation of number of bat species (circles) in relation to number of captured bats (columns) in four main mist-netting sites; $a-site\ 1$; $b-site\ 2$.

Mist-netting site 3



4c

Mist-netting site 4



4d

Figure. 4c-d. Dynamics of accumulation of number of bat species (circles) in relation to number of captured bats (columns) in four main mist-netting sites; $c-site\ 3$; $d-site\ 4$.

The third mist netting site was located on the open flood plain of the S. Donetz River and four species were not found there (*E. serotinus*, *N. leisleri*, *V. murinus* and *Pl. auritus*).

The fourth site was the most remote and distinct, *E. serotinus*, *M. brandtii* and *M. dasycneme* were not detected (Table III). *M. dasycneme* was not captured in the centre of the forest (in relation to three other sites near the river). At the same time the absence of such species as *E. serotinus* and *V. murinus* at some sites could be considered a chance result and in further study they might be captured. The second site was most effective for mistnetting. During the second capture night 48 bats of 8 species were caught there (Table III, Fig. 4b).

Table III
Species composition and number of individuals in particular mist-netting sites

Bat species	Mist-netting site							
Dat species	1	2	3	4	5	6		
M. brandtii	1	0	1	0	0	0		
M. dasycneme	2	1	1	0	0	0		
M. daubentonii	70	82	9	1	4	17		
N. noctula	54	183	3	29	35	49		
N. leisleri	7	21	0	11	3	0		
E. serotinus	2	3	0	0	2	1		
P. nathusii	42	27	77	25	1	0		
P. pygmaeus	18	46	39	7	1	0		
V. murinus	0	1	0	4	0	0		
Pl. auritus	5	3	0	9	1	0		

At the other additional two sites bats were only caught in July 2006-2008 and there was large variation in number of captured bats from night to night. Seven and three species were caught at each of these sites, respectively (Table III).

B/h index value did not differ between the four main mist-netting sites (Kruskal-Wallis H test: df=3, n= 41; H=2.67, p=0.44; Fig 2).

V. DISCUSSION

The presented results show that the effectiveness of capturing bats using mist nets correlated with the period of the summer. There is no correlation with the duration of mistnetting for each month. For example, in August the hours of netting increased and at the same time the number of captured bats decreased (Fig. 1).

July is the best calendar month for bat mist netting (for conditions in Eastern Ukraine). In this period of the bat life cycle young-of-the-year bats become volant. July is also the period of maximum bat activity (RUSS et al. 2003). The b/h index reflects the activity of bats, but we hypothesize that an important factor is the attraction of free flying bats to captured ones. Bats are known to be attracted to nets with captive bats (BORISSENKO 1999). One of the recent methods of attracting other foraging bats to mist nets is the use of an acoustic lure (e.g. Autobat). Playing back echolocation calls near mist nets has been demonstrated to be an effective capture method (HIILL & GREENAWAY 2005). We assume that the living bats in our study provided more attractive sounds than an electronic playback. Furthermore, in July relationships between mothers and pups still exist (PANYUTIN 1970), and they can attract one another to mist nets.

There was no statistical difference between b/h index for mist netting sites with 7 and 12 m mist nets (Fig. 2). Although the b/h index was higher at the site with a 12 m net (at three points 7 m nets were installed and at one point a 12 m net) than points with 7 m nets, the effectiveness of capture is determined by the attraction of captured bats. For this reason we counted the b/h index per one net not per length of the net (as shown in DIETZ & PIR 2009).

Considering that the timing of bat birth in the temperate zone has a clear correlation with the weather in spring and in the beginning of summer (PANYUTIN 1970, STRELKOV & ILYIN 1990, LUČAN & HANÁK 2008) we recommend not to follow July as calendar month as such, but rather the period when young-of-the-year bats begin to fly. This period could differ within one week from the south to the north due to variation in climate in Eastern Europe (VLASCHENKO 2006).

In none of study months were all ten species known for this territory captured. But if we increase the number of capture sites and types of habitat we would likely capture all species in July (VLASCHENKO & GUKASOVA 2009). The more important argument for July as an optimal month for the inventory of bat species is that during this period the resident bat fauna remains stable. We detected resident breeding bats, but not migrants that were captured in the end of August and September. In June females give birth and tend to remain in roosts for a few days which may also impact the capture results.

Our results are comparable with temporal bat activity patterns based on ultrasound detection obtained in N Poland (CIECHANOWSKI et al. 2010). The proportion of *Nyctalus* and *Pipistrellus* was higher during the summer phenological period and similar to the acoustic data in CIECHANOWSKI et al. (2010). *M. daubentonii* gradually decreased in number during the phenological period, however in Poland its activity had two peaks (in April and August-September). The proportion of captured *N. leisleri* was high in two periods (during pregnancy and lactation and at the beginning of the autumn migration) in contrast to echolocation calls which were recorded almost exclusively during spring and summer (CIECHANOWSKI et al. 2010).

Our results are also interesting when compared with mist-net data obtained in Białowieża Primeval Forest by RACHWALD et al. (2001). They also had maximum species diversity in July, compared to June and August. But our data differs in the change of species composition in August. There was a marked change in the relative frequencies of sedentary species in August, when *M. daubentonii* was the most numerous, followed by *Pl. auritus* (RACHWALD et al. 2001). This seems to reflect the timing of autumn migration of bats in different parts of Europe.

A decrease in the proportion of *M. daubentonii* in our captures in August and September give evidence of earlier migration of *M. daubentonii* in Eastern Europe than Western Europe. This comparison demonstrates how we can use data obtained by mist-netting for the study of bat phenology. Earlier, VEHNIK and SACHKOV (2005) proposed to use mist nets for the study of bat phenology. But we consider this capture technique not to be appropriate for recording the time of arrival and departure of bats from the summer part of their range (VLASCHENKO & GUKASOVA 2009) because effectiveness of mist-netting in spring and autumn is low (Fig.1). For this purpose bat detectors are more useful. Mist nets could be used for revealing the changes in structure of a local bat assemblage, especially in protected nature areas.

We assume, relying on our previous investigations (VLASCHENKO & GUKASOVA 2009), that to detect all ten species present in this study territory it is required to capture no less than 430 bats at a single site. We did not capture this many in our study. The tenth species might be captured at some of these sites in the future, if the number of bats increased to 400 specimens.

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