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Resting Frequency of Echolocation Calls within a Lesser Horseshoe Bat Population (Southern Poland) and its Relation to Body Size, Condition and Mass

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The echolocation calls emitted by stationary bats are characterised by their resting frequency (RF). The ecological role of RF has been widely discussed across the literature concerning the Rhinolophidae family, where it has been found that the RF may vary depending on many factors, although its role in shaping the variability of different populations remains unclear, and the data for many species – including *Rhinolophus hipposideros* – is scarce. In this study, we aimed to determine how sex, age and biometric parameters (body mass, forearm length and the body condition index) affected the RF in a *R. hipposideros* population and to investigate the individual variability in this parameter. Bats were captured in front of two Carpathian caves in Southern Poland during the mating season. The echolocation calls of the hand-held bats were recorded, and later their peak frequency was measured with computer software. The analyses showed higher RF values for females than for males, but (in contrast to previous reports) no differences between the age classes were identified. RF did not correlate with any biometric parameters in the studied population, which rules out the possibility of quality characteristic signalling through this parameter. However, we observed significant individual differences in RF within the sex-age groups, which might reflect some communication potential. The discrepancies among the research results available for this topic indicate the need for further studies aimed at investigating the variability of RF and its role across species distribution ranges and their phenology.

Key words: RF, *Rhinolophus hipposideros*, Chiroptera, intrapopulation variability, individual differences, BCI, mating season.

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Rhinolophidae is a monogeneric family of bats (Chiroptera) that possess a unique echolocation mechanism. Like Hipposideridae and Rhinonycteridae, they belong to the group of high duty cycle (HDC) echolocators, i.e. bats spending a high percentage of their time on producing sound (FAWCETT *et al.* 2015; FENTON *et al.* 1995). Hence, they emit echolocation calls of a long duration relative to the intervals between subsequent calls.

The rhinolophids' echolocation calls follow the FM-CF-FM structure (BARATAUD 2015): there is a prominent, pure-tone constant frequency (CF) part that concentrates the peak energy of the call and is flanked with brief frequency modulated (FM) sweeps. The CF component is particularly important in relation to foraging, as the hearing of horseshoe bats is sharply tuned to a narrow band of frequencies. This results from the acoustic fovea: the specific inner ear structure and neurons within the auditory pathway responsible for determining a response only to frequencies around the CF component of the emitted call. The area with the highest hearing sensitivity covers a frequency band width of around 1.5 kHz (SCHULLER & POLLAK 1979; SCHULLER 1980). During flight, bats emit echolocation of slightly lower frequencies than the frequency to which the acoustic fovea is tuned, in order to compensate for the Doppler shift (see review: FENTON *et al.* 2012). The flight speed also influences the peak frequency of the emitted echolocation calls: at a velocity of 5m/s, the difference between the frequency of stationary and flying bat calls is equal to around 3% (DIETZ *et al.* 2009). While the bat is resting, it does not need to compensate for the Doppler shift and it emits echolocation calls of a stable frequency. Therefore, to study the variability of the echolocation peak frequency and its possible role in the characteristics of Rhinolophidae, only the echolocation frequencies of stationary (usually hand-held) bats are considered and they are defined as the resting frequency (RF).

In rhinolophids, the RF may be characteristic for a species, particular populations or even individuals. On the species level, RF has been found to correlate negatively with the bat's body size (HELLER & VON HELVERSEN 1989; FRANCIS & HABERSETZER 1998; STOFFBERG *et al.* 2011) and the size of their prey (ANDREAS *et al.* 2013). Considering the properties of sound waves (frequency is inversely proportional to wavelength), small species preying on small insects should use high-pitched echolocation calls to forage effectively (KINGSTON *et al.* 2000). However, researchers have observed differences in RF between sympatric species that do not differ in terms of their prey repertoire. Under a high acoustic complexity resulting from multiple co-occurring species, the need for intraspecific communication could also shape the RF of each species. This phenomenon relies on sonar partitioning – shifting echolocation frequency bands apart to allow for the mutual recognition of individuals (JACOBS *et al.* 2007). Nonetheless, in various studies conducted on sympatric species populations, both disjointed and overlapping RF ranges were observed (SIEMERS *et al.* 2005; RUSSO *et al.* 2007).

The RF ranges for rhinolophid species are determined based on data collected from different populations across the distribution ranges. Each population may exhibit characteristic patterns in the RF values that are driven by various factors including: geographical isolation (e.g. YOSHINO *et al.* 2006; CHEN *et al.* 2009), genetic isolation (e.g. CHEN *et al.* 2009; STOFFBERG *et al.* 2012), climatic conditions (e.g. JIANG *et al.* 2010; JACOBS *et al.* 2017), sympatric species, and cultural drift emerging from call disparities accumulated during vocal learning (e.g. SUN *et al.* 2013; XIE *et al.* 2017). The contribution levels of the abovementioned factors depend on both the studied species and the size of the studied area.

Within a population, studies have demonstrated that in some rhinolophid species the RF might characterise – to some extent – the sex, age or a particular individual (JONES & RANSOME 1993; SIEMERS *et al.* 2005; FINGER *et al.* 2017). Occurring differences have been mainly linked to potential niche partitioning (diminishing competition) between the sexes or sex-age groups, as well as offspring development or the communicative function of echolocation in the colonies (JONES *et al.* 1992; RUSSO *et al.* 2001; FINGER *et al.* 2017). In some species, RF has also been found to correlate with biometric parameters (SIEMERS *et al.* 2005), and it might serve as an honest signal of the quality characteristic for individuals during the mating season (*R. mehelyi,* PUECHMAILLE *et al.* 2014).

Rhinolophus hipposideros (Bechstein, 1800) is a sedentary species, which is widely distributed in all European countries and the islands of the Mediterranean region. In the north, the species reaches Poland and Germany, and it also occurs in Great Britain and Ireland. This bat is present in North Africa and the Middle East, with some populations also found in Afghanistan, Tajikistan and Northern India. It forages within shrublands, broadleaf woodlands and riparian forests. It frequently roosts in underground sites; however, its summer nursery roosts are usually found in the attics of houses in the northern parts of the range. According to the International Union for Conservation of Nature, the population trend is decreasing (TAYLOR 2016), although recent studies have indicated increasing trends and the recovery of populations in Poland and other European countries (MARTÍNKOVÁ *et al*. 2020; TOFFOLI & CALVINI 2021; WĘGIEL *et al.* 2021). *R. hipposideros* is included in Annex II of the Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora. Therefore, broadening the knowledge about the ecology of this species is essential for its effective protection.

To date, data on the intra-population variability of RF in *R. hipposideros* has come from the United Kingdom, Hungary and Italy (the peninsula and Sardinia). These studies have focused on different aspects of the population's acoustic ecology (JONES *et al.* 1992; RUSSO *et al.* 2007; GYÖRÖSSY *et al.* 2020). However, the methodology and the results were not fully consistent between the works, which reveals the need for further studies. Our study is an attempt to complement the state of knowledge within this topic. We conducted analyses on individuals captured only within the mating period in a small area, to avoid the effects of environmental factors, phenology and the offspring development process. We investigated four sex-age groups and examined the variability within these groups, which constitutes a novel insight. Our aim was to identify which factors (sex, age and biometric parameters) influenced the RF variability in the studied *R. hipposideros* population and to what extent, as well as to verify the evidence for individual signalling through RF variability.

We hypothesised that:

– RF would differentiate the sex-age groups;

– RF would differentiate individuals within the sex-age groups;

– RF is correlated with the biometric parameters of specimens within the groups, potentially providing information about an individual's quality characteristics.

Material and Methods

All the activities were carried out under a permit from the General Directorate for Environmental Protection (DZP-WG.6401.86.2020.EB)

Study area

The surveys took place near the entrances of two caves: Zbójecka Cave in Łopień and Borsucza Dziura Cave in Mogielica, within the Beskid Wyspowy mountain range (Island Beskids). The area is a part of the Carpathian Mountains.

Zbójecka Cave in Łopień (with two openings at ca. 880 m a.s.l.: 49°42′N, 20°17′E; 49°42′N 20°17′E) is a non-karstic cave situated on the northern slope of Mt. Łopień. The cave passage is 433 m long and has a denivelation of 20.3 m. The cave itself serves as a crucial hibernaculum and swarming site for bats (PIKSA *et al.* 2011). The habitat around the cave consists mainly of a beech (*Fagus sylvatica*) forest and a mixed forest of beech, fir (*Abies alba*) and spruce (*Picea abies*), and it is situated within Natura 2000 Special Area of Conservation PLH120078 'Uroczysko Łopień'.

Borsucza Dziura Cave (with one opening at ca. 700 m a.s.l.; 49°39′N; 20°18′E), is a non-karstic cave situated on the eastern slope of Mt. Mogielica. Bats use this cave for both hibernation and as a swarming site. The cave passage is ca. 300 m long and has a denivelation of 10 m. The habitat around the cave consists mainly of a beech forest and it is not covered by any form of nature protection measures.

The caves are situated ca. 7 km from each other in the submontane belt.

Surveys

We limited our study to the mating period in order to maintain the consistency of the results, as some researchers have reported possible differences in the resting frequency (RF) resulting from the season (or temperature) in *Rhinolophus ferrumequinum* (JONES & RANSOME 1993). Others have reported a relationship between the RF and body mass in *R. mehelyi* males during the mating season (PUECHMAILLE *et al.* 2014). Based on that data, we anticipated the largest variability in RF, and hence the highest probability of demonstrating the existing relationships, during this season.

Lesser horseshoe bats were captured between 07.09.2021 and 28.10.2021. Our visits were limited to one every three weeks, to minimise the disturbance to the bats. Three mist-nets (one 3 m and two 6 m long, 3 m high, Ecotone, Poland, Denier netting: 0.08 mm) were placed in front of the cave openings. The captures generally started shortly before dusk and continued until dawn; however, in Borsucza Dziura the surveys finished at around midnight. The nets were supervised constantly. Specimens were marked with a non-toxic, durable colour marker to avoid retrapping and were released immediately after undergoing the procedures described below, normally within five to ten minutes.

The captured bats were sexed by inspecting the genitalia. Two categories of males were recognised: adults with a thicker penis and a fully developed scrotal rudiment; and juveniles (born that summer) with a thinner penis and the lack of a scrotal rudiment. Similarly to the males, two categories of females were recognised: adults with well-developed pectoral and pubic (anchoring) nipples- and juveniles with feebly--developed pectoral nipples and a lack of pubic nipples.

In determining the age category of these two sexes, additional features were taken into account: ossification of the long bones of the wings (with incomplete fusion of the phalange epiphysis in juveniles), pelage colour (greyish fur and skin of a similar colour on the ventral and dorsal areas in juveniles vs brown fur of the dorsal and a lighter fur colour of the ventral part of the body in adults) and the body structure (softer in juveniles vs robust in adults) (DIETZ & VON HELVERSEN 2004; GAISLER 1966).

The bats were weighed (PESOLA®: precision 0.1 g) and the right forearm length (FL) was measured (Limit precision calliper: precision 0.1 mm). The body condition index (BCI) was defined as the body mass to FL ratio (SPEAKMAN & RACEY 1986).

The echolocation calls of each captured, hand-held individual were recorded with an Dodotronic Ultramic 384K BLE ultrasonic microphone connected to an Android smartphone running the Bat Recorder application (ver. 1.0R172). The recording mode was triggered manually. The microphone was protected with a dedicated membrane, with two amplification switches that were turned on and held around 30 cm from a bat. For each specimen, around a one minute recording was obtained.

The recordings were analysed with Kaleidoscope Lite software (ver. 5.4.2) under the default settings (FFT size 256, window size 128). For each bat, the peak frequency of the recorded calls, i.e. the resting frequency (RF), was noted from the power spectrum plot for 15 representative, excellent-quality calls. Whole signals were taken into account during the measurements (SIEMERS *et al.* 2005). Only the specimens with a certain affiliation to the age groups qualified for the further analyses. These were comprised of 14 adult females (Fa), 15 juvenile females (Fj), 19 adult males (Ma) and 19 juvenile males (Mj).

Statistical analysis

A two-way ANOVA was applied for testing the differences in RF, FL, body mass and BCI, with the sex and age as fixed factors. The RF for this analysis was calculated as a mean of 15 measurements taken from each individual. The requirements concerning the normality of the data distribution (Kolmogorov-Smirnov test for normality with the Liliefors adaptation) and the equality of variances (Levene's test) were met. The analysis was performed in Statistica (ver. 13.3, TIBCO Software Inc.).

Kruskal-Wallis non-parametric test was conducted to test the differences in the RF between individuals (15 calls per individual) within the sex-age groups, due to the lack of normality in the data distribution of some individuals. The Mann-Whitney pairwise test with Bonferroni corrected p values was applied as a post hoc measure. The analyses were performed in PAST software (ver. 4.07b, HAMMER *et al.* 2001). Graphs presenting the differences between individuals were generated in Statistica.

A Pearson correlation was performed to test correlations between the RF (mean) and the biometric parameters for *R. hipposideros* on the population level (i.e. without dividing for the sex and age category) and for each sex-age group separately. The analyses were conducted in Statistica software and the graphic representation was generated in Microsoft Excel 2019.

A principal component analysis (PCA) was employed to explain the variability observed in the dataset, i.e. to reveal which of the analysed variables (RF, FL, BCI or body mass) might determine the differences between the sex and age groups and to what extent. Both the analysis and the diagram were created with PAST software.

The graphic processing of all the figures was performed in Inkscape (ver. 1.0.1) and Corel Draw 2021.

Results

Resting frequency and biometric parameter values in relation to the sex-age group affiliation

In the examined population of *Rhinolophus hipposideros,* we observed statistically significant differences between the sex-age groups in relation to the resting frequency (RF), forearm length (FL), body mass and body condition index (BCI) (Table 1). The females emitted echolocation calls of a higher RF than the males; while there were no differences in the RF between juveniles and adults. The females were also characterised by higher FL values than the males, but there was no difference between the age classes regarding this parameter. The body mass and BCI values were higher in the adults than in the juveniles; however, there were no differences between males and females. We did not observe any interaction between the sex and age factors (Table 1).

Table 1

Descriptive statistics and the results of the two-way ANOVA for the resting frequency (RF) and biometric parameters depending on sex and age in the studied *Rhinolophus hipposideros* population

Sex-age group	n	RF [kHz]		FL [mm]		Body mass [g]		BCI [g/mm]	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Fa	14	108.73	0.62	39.01	0.67	6.57	0.73	0.17	0.02
Fj	15	108.65	0.81	39.18	0.60	5.75	0.27	0.15	0.01
Ma	19	105.43	0.74	38.03	0.54	6.55	0.48	0.17	0.01
Mj	19	105.33	0.87	37.99	0.58	5.65	0.43	0.15	0.01
Two-way ANOVA for factors: sex, age and interaction									
Factor		F	p	F	p	F	p	F	p
sex		267.58	<0.001	49.1	<0.001	0.21	0.651	0.83	0.366
age		0.2	0.659	0.18	0.673	44.43	<0.001	48.94	<0.001
sex*age		0.003	0.957	0.44	0.511	0.1	0.757	0.1	0.753

Sex-age groups: Fa – adult females; Fj – juvenile females; Ma – adult males; Mj – juvenile males
Biometric parameters: FL – forearm length; BCI – body condition index.

RF differences between individuals within the sex-age groups

We observed statistically significant differences in the RF between individuals within all the sex-age groups. The post hoc analysis result divided the specimens into a few subgroups, in which the RF values did not differ, as well as several individuals with mean RF values that were distinct from the others (Fig. 1).

Correlation between the RF and biometric parameters

We found no significant relationship between the RF and any of the biometric factors within the sex-age groups (Table 2). The analyses performed for individuals on the population level (with no division into sex-age groups) indicated no correlation between the RF and the body mass or BCI index, but a positive correlation was found between the RF and FL (Fig. 2). The latter resulted from differences between the sexes within the population – the females, on average, had longer forearms and echolocated at higher frequencies (Fig. 2a). However, there was no significant correlation between the RF and the body mass or RF and BCI within the population (Fig. 2b, c).

Factors determining differences within the population

In the principal component analysis (PCA) (Fig. 3), the two first axes explain 91% of the variability presented in the ordination diagram. The first axis presents gradients of the RF and FL values and explains almost 50% of the variability in the analysed population. The positive values on this axis are associated with females, while the negative values are associated with males. The second axis presents gradients of the body mass and BCI values and explains 41% of the observed variability. Here, the positive values are asso-

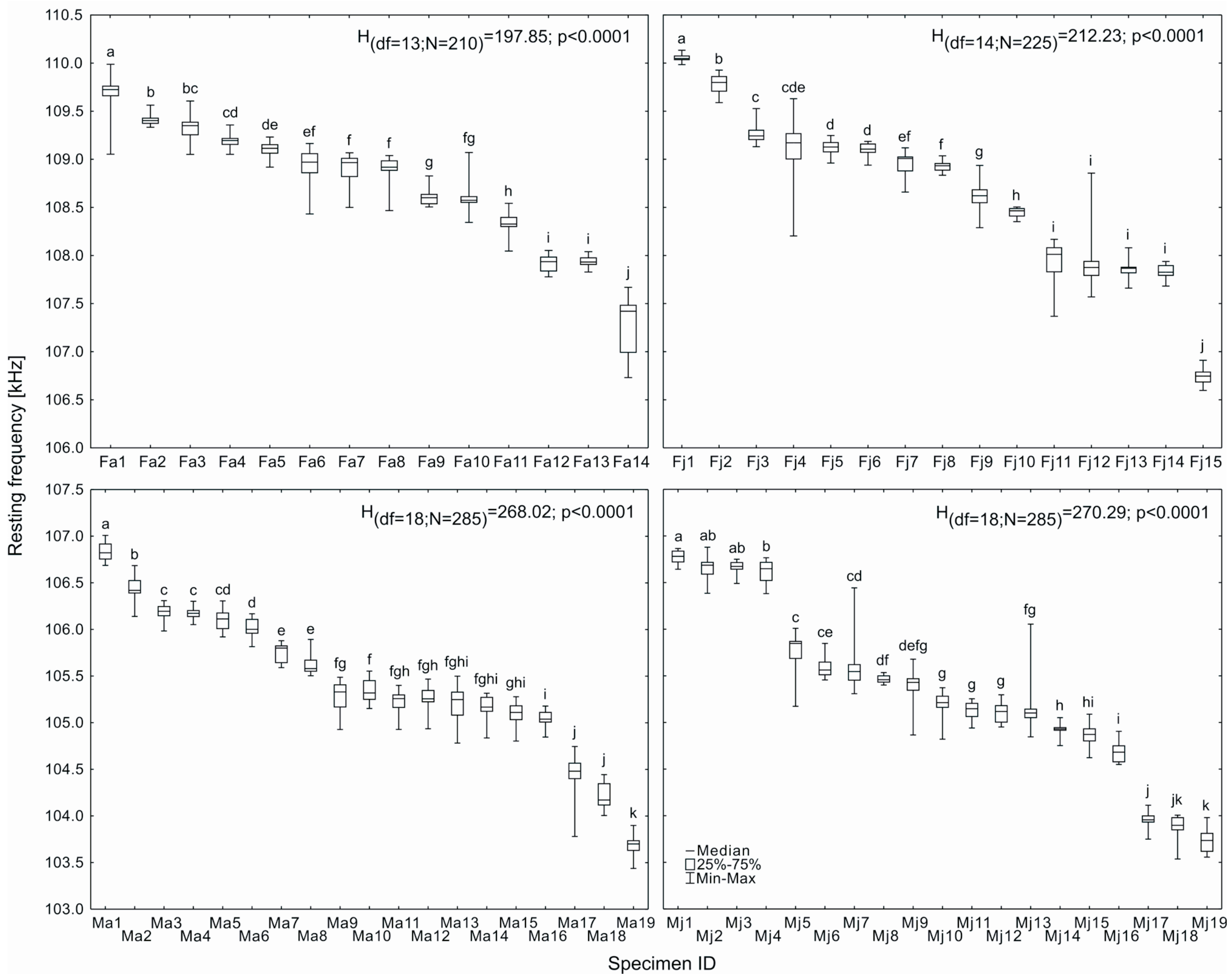

Fig. 1. Differences in the resting frequency (RF) between individuals within sex-age groups within the *Rhinolophus hipposideros* population (Kruskal-Wallis non-parametric ANOVA; post hoc Mann-Whitney test with a Bonferroni correction for p values). Diagrams shown clockwise: adult females (Fa); juvenile females (Fj); juvenile males (Mj); adult males (Ma). Bar charts marked with identical letters show no significant differences.

Table 2

Pearson correlation coefficients between the resting frequency and biometric parameters within sex-age groups of the studied *Rhinolophus hipposideros* population

Sex-age group	n	FL		Body mass		BCI	
		r	p	r	p	r	p
Fa	14	0.268	0.355	-0,188	0.520	-0.244	0.402
Fj	15	0.148	0.599	0.236	0.398	0.156	0.579
Ma	19	-0.007	0.978	0.090	0.715	0.100	0.695
Mj	19	0.142	0.561	-0.145	0.554	-0.176	0.471

Sex-age groups: Fa – adult females; Fj – juvenile females; Ma – adult males; Mj – juvenile males
Biometric parameters: FL – forearm length; BCI – body condition index

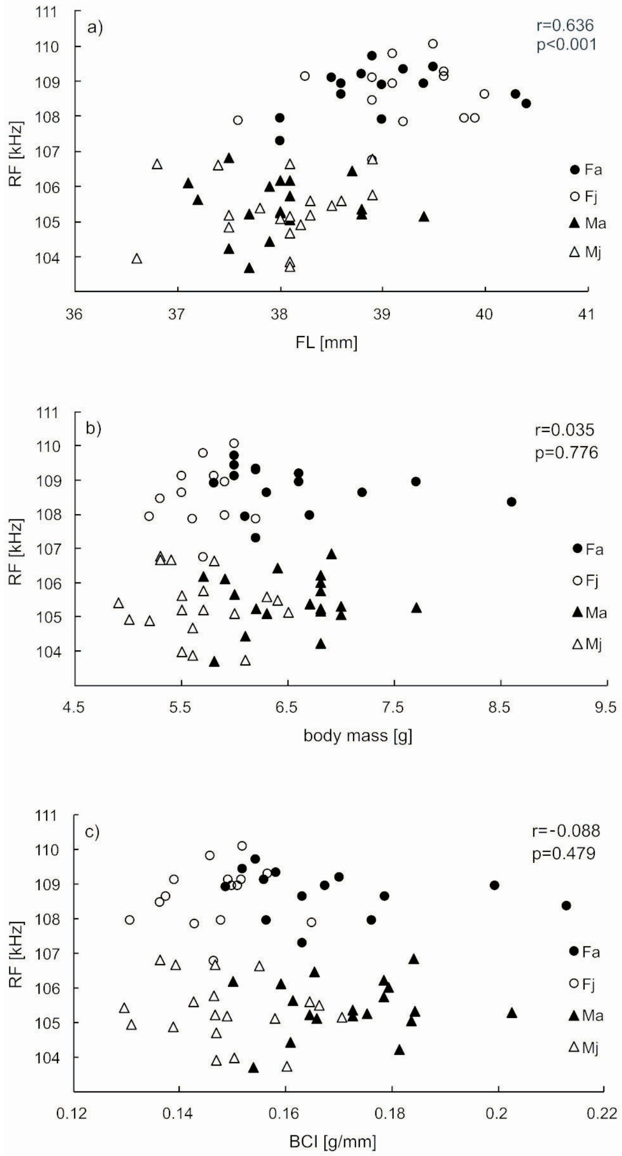

Fig. 2. Pearson correlations between the resting frequency (RF) and biometric parameters: a) forearm length (FL), b) body mass, and c) body condition index (BCI) – conducted for *Rhinolophus hipposideros* individuals without a division into sex-age groups (population-level analysis). Fa – adult females; Fj – juvenile females; Ma – adult males; Mj – juvenile males.

ciated with adults, and the negative values are associated with juveniles. Four pre-established sex-age groups are well separated on the ordination diagram: higher values of the RF and FL, and higher values of the body mass and BCI were characteristic of the adult females; higher RF and FL, and lower body mass and BCI were characteristic of the juvenile females; lower RF and FL, and higher body mass and BCI were characteristic of the adult males; and lower RF and FL, and lower mass and BCI were characteristic of the juvenile males.

Discussion

Resting frequency in sex-age groups within the population

Our results indicated significantly higher resting frequency (RF) values for females than for males in the *Rhinolophus hipposideros* population. Numerous patterns for the RF's variability between the sexes have been observed in rhinolophid populations: some papers have reported higher RF values in females (Yoshino *et al.* 2006; Chen *et al.* 2009; SUN *et al.* 2013; Xie *et al.* 2017), while others indicated no differences (Russo *et al.* 2001; Soisook *et al.* 2008; Liu *et al.* 2009; Finger *et al.* 2017) or reported higher RF values in males (statistically insignificant tendencies) (Mutumi *et al.* 2016). Within the distribution ranges of some species, researchers have observed populations presenting contrasting patterns – in some populations, differences in RF occurred between the sexes, while in others, they were absent or had opposite characteristics (Siemers *et al.* 2005; Jiang *et al.* 2010).

All of the studies concerning *R. hipposideros* populations available to date have reported a significantly higher RF among females. The means for the sexes differed by ≥ 2.65 kHz (Jones *et al.* 1992; Russo *et al.* 2007; Györössy *et al.* 2020). In our study, the ranges of RF utilised by each sex overlapped only

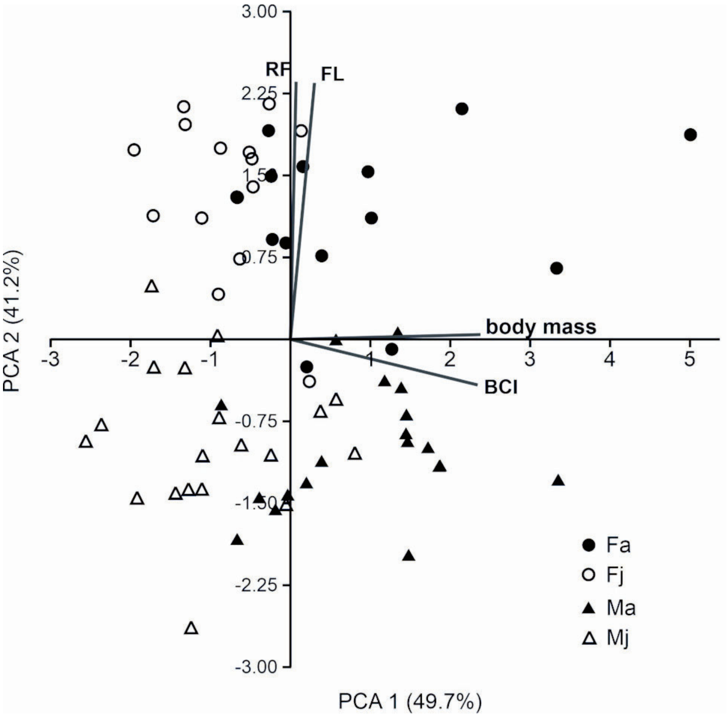

Fig. 3. Principal component analysis (PCA) diagram depicting the distribution of *Rhinolophus hipposideros* individuals belonging to the sex-age groups in relation to the RF and biometric parameters: forearm length (FL), body mass and body condition index (BCI). Fa – adult females; Fj – juvenile females; Ma – adult males; Mj – juvenile males.

within the 0.1 kHz frequency band, but studies conducted in Hungary and the United Kingdom reported broader overlaps (ca. 1.5 kHz) (JONES *et al.* 1992; GYÖRÖSSY *et al.* 2020). As we collected our data only during the autumn, and the cited studies took place during more than one season, those discrepancies may result from differences in the body temperature of individuals in different seasons and RF changes within the first year of life in juveniles (JONES & RANSOME 1993).

In the literature, three hypotheses are proposed to explain the sex-related differences in *R. hipposideros* RF: communication and mutual recognition, niche partitioning (JONES *et al.* 1992) and broadening the spectrum of potential prey in females (GYÖRÖSSY *et al.* 2020). In Poland and Great Britain, there are no rhinolophid species that use the same RF range as *R. hipposideros*. However, the occurrence of sympatric rhinolophid species with overlapping RF bands can shape the RF variability in local populations, and could potentially affect the ecological utility of this parameter. From the few studies that are available, it seems that differences in the RF between sexes may persist in populations regardless of whether there is an overlap with sympatric species or not (JONES *et al.* 1992; GYÖRÖSSY *et al.* 2020). To understand the ecological role of RF with regard to sex, more studies must be carried out throughout the species distribution range and they should include experiments to verify the hypotheses mentioned above.

In our study, adult and juvenile specimens of *R. hipposideros* utilised the same ranges in their RF. Contrary to our findings, most studies comparing RF values between sex-age groups in the Rhinolophidae family showed a lower RF for juveniles. This outcome has been noted for *R. monoceros* (CHEN *et al.* 2009), *R. ferrumequinum* (JONES & RANSOME 1993), *R. euryale* and *R. mehelyi* (RUSSO *et al*. 2001). However, another study did not reveal this pattern for the latter two species (SIEMERS *et al.* 2005). Authors have indicated differences in the times of the surveys (July/August vs September) and different stages of development in the juvenile bats as reasons for the contrasting results. In *R. hipposideros,* lower mean RF values for juveniles were also demonstrated (JONES *et al.* 1992). We collected our data from a larger sample and with younger individuals than in the cited work (respectively: 34 juveniles, 67 in total vs 17 juveniles, 41 in total; and September/October vs October/November, December/February and March/April). Differences in the phenology and sample sizes might explain the discrepancy in the obtained results; however, more studies with larger samples, repeated within and between seasons, are needed to fully understand the age-related RF variability in this species.

Differences in the resting frequency between individuals in the population

Our study showed differences in the RF of calls emitted by particular specimens in the studied population of *R. hipposideros.* To our knowledge, no studies have inspected the RF variability specifically within sex-age groups in Rhinolophidae. Some authors observed a lower individual variability of RF compared to the population variability, although no statistical analysis was provided (SIEMERS *et al.* 2005; CHEN *et al.* 2009). Other studies investigated the role of RF in the intraspecific recognition of individuals. Research conducted on *R. clivosus* showed that bats could discriminate between individuals based on echolocation characteristics, where the RF parameter apparently explained the variability to the largest extent. Since the RF ranges of individuals inevitably overlap together with an increasing population size, other echolocation call parameters are thought to play a role in individual recognition; however, this potential is limited by the primary function of echolocation, i.e. orientation and foraging (FINGER *et al.* 2017). We observed both single individuals differing from others in their RF range and groups of similar specimens up to six individuals. It is possible that this variability might reflect some communication effort within the population, where the RF serves as a component of an individual signature. Certainly, thorough behavioural observations are needed in order to verify this supposition.

Biometric parameters (forearm length, body mass and body condition index) and their relationship with resting frequency

In regard to the differences between sexes, our work demonstrated a higher forearm length (FL) and higher RF values in females than in males, with no differences in the body mass and body condition index (BCI). Analyses conducted for Italian populations only concerned the FL and RF parameter and showed higher values for females (RUSSO *et al.* 2007); while in British and Hungarian populations, larger mean values for females were detected in all the discussed biometric parameters (JONES *et al.* 1992; RUSSO *et al.* 2007; GYÖRÖSSY *et al.* 2020). The cited analyses were conducted for adults and juveniles pooled together, captured within at least two seasons. Since we collected our data only during the mating season and divided the individuals into age groups, it is perhaps for this reason that our findings are not fully congruent with the literature. On the intraspecific level, a sex-related positive correlation between FL and RF has been observed in all *R. hipposideros* populations studied to date. Opposite trends have been discovered on the interspecific level, where larger species usually utilise lower RF values (HELLER & VON HELVERSEN 1989). Many species have also presented deviations from this pattern for various reasons, such as disambiguation in social interactions (*R. capensis* and *R. clivosus,* JACOBS *et al.* 2007) and trophic niche differentiation or expansion (*R. macrotus* and *R. lepidus,* SHI *et al.* 2009). Presumably, similar explanations may apply for the variability observed within species, which could be another direction for future studies.

Regarding the age classes, we reported different patterns: the adults were characterised by higher body mass and BCI values than the juveniles, with no differences in the FL values. Only a few studies have mentioned such differences between the age groups. As in our research, analyses for *R. mehelyi* and *R. euryale* showed a lower BCI among juveniles compared to adults. We concur with the suggestion of those authors that this finding may be linked with lower foraging success and incomplete body growth (RUSSO *et al.* 2001).

We observed a statistically significant correlation between RF and FL for the analysed population, but it did not occur after dividing the sample into sex-age groups as both the FL and RF were higher in females. RF was not dependent on any of the other examined biometric parameters within the groups. Most studies investigating the relationship between RF and biometric parameters in Rhinolophidae have found either no correlation between RF and FL or a positive correlation, either in the population as a whole or within the sex groups (NEUWEILER *et al.* 1987; JIANG *et al.* 2010; MUTUMI *et al.* 2016; XIE *et al.* 2017). In a few studies providing analyses for juveniles, the observed correlations depended on the stage of development (RUSSO *et al.* 2001; SIEMERS *et al.* 2005). To our knowledge, four studies have considered the relationship between RF and mass or BCI in the context of quality characteristic signalling (JONES *et al.* 1992; RUSSO *et al.* 2001; SIEMERS *et al.* 2005; PUECHMAILLE *et al.* 2014); however, only one study reported an evident positive correlation between the RF and body mass, linking this observation with the females' choice of mate in *R. mehelyi* (PUECHMAILLE *et al.* 2014). Our findings did not provide evidence for the existence of such mechanisms in *R. hipposideros.*

Summary

In the *Rhinolophus hipposideros* population investigated in this study, the chosen parameter values strongly supported the division of the sex-age groups. The resting frequency (RF) variability seems to be shaped by sex, but also individual factors, indicating that it might serve as one of the components of an acoustic signature. Regarding the disparities between our findings and those of other works, it is likely that RF and its role is not constant throughout different seasons and populations; therefore, populations should be studied independently, considering the phenology. Concerning the array of factors influencing RF described in the literature, more research is needed to investigate its ecological functions under various sets of conditions.

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Author Contributions

Research concept and design: J.K., K.P.; Collection and/or assembly of data: A.K., K.P.; Data analysis and interpretation: J.K., E.P.; Writing the article: J.K., E.P; Critical revision of the article: J.K., E.P, A.K., K.P.; Final approval of article: J.K., E.P, A.K., K.P.

Conflict of Interest

The authors declare no conflict of interest.

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