

# Ontogeny of pup isolation-induced ultrasonic calls in a highly social rodent, the Harting's vole (*Microtus hartingi*)

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## ABSTRACT

We examined the ontogeny of pup ultrasonic isolation-induced calls (USVs) in a highly social Arvicolinae rodent species, the Harting's vole (*Microtus hartingi*). We recorded, with two-days intervals, the USVs of 55 individual pups from 11 litters from 1 to 12 days of age and measured body mass and the linear body size variables of each subject. The number of pups producing USVs decreased from 100 % at 1–2 days of age to 11.5 % at 11–12 days of age. Call rate changed with age, with maximum at 3–4 d of age. It ranged from 121.8 to 94.2 USVs/min between 1–2 and 7–8 days of age, decreased to 36.4 USVs/min at 9–10 days of age and to 1.5 USVs/min at 11–12 days of age. Overall, pup USVs were becoming simpler in their contour shapes with increase of age. Pup age class significantly affected all acoustic variables for the exclusion of the start and maximum fundamental frequencies, which values did not display significant changes with age. Body mass negatively correlated with USV acoustic variables, for the exclusion of call rate. A high percent of USVs contained nonlinear phenomena. We discuss the differences and similarities of pup Harting's vole USV ontogeny with other vole species and summarize the traits which make pup USVs of the Harting' vole a convenient model for biomedical research.

## 1. Introduction

In most mammalian species, infants emit vocalizations to promote parental care (Blumberg and Sokoloff, 2001; Lingle et al., 2012). Pups of rodents are commonly highly vocal when outside the nest and produce ultrasonic isolation-induced calls (USVs, above 20 kHz), stressed by being alone and by cooling temperatures (Okon, 1972; Ehret, 2005). Pup USVs evoke in parents the behavior of searching the pup and its retrieval to the nest (Brunelli et al., 1994; Rabon et al., 2001; Boulanger-Bertolus et al., 2017). For example, mouse and rat pups that vocalize at high rates are retrieved more rapidly than less vocal pups (Bowers et al., 2013). Thus, USVs provide important communicative feedback between pups and conspecific caregivers in rodents (Branchi et al., 2001; Caruso et al., 2018).

Call rate of USVs depends on a few factors: most important of them are pup body temperature, possibility of contact with caregivers/littermates, handling, and smell (Okon, 1972; Ehret, 2005). The low temperatures during isolation-induced tests evoke the higher calls rates of USVs in comparison with the higher temperatures (Okon, 1970, 1971; Szentgyörgyi et al., 2008). Contact with conspecific caregivers (Yu et al., 2011; Boulanger-Bertolus et al., 2017) and handling (Sales and Smith, 1978) changes the production of USVs. The odor from the home nest bedding may increase (Geyer, 1979) or decrease call rates of USVs (Kapusta et al., 1995; Szentgyörgyi et al., 2008).

Call rate of isolation-induced USVs in rodent pups is age-dependent. As a rule, rodent pups produce no or little USVs at birth, and then increase the numbers of USVs in the following days up to the peak value at 6–10 days (d) of age. At later ages, call rate of USVs decreases and finally

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calling ceases at about 20 d of age (Brooks and Banks, 1973; Blake, 1992; Motomura et al., 2002; Yu et al., 2011). In many species of rodents, pups are born without fur and sustainable thermoregulation, and with closed ears and eyes (Okon, 1972; Yoshinaga et al., 1997; Blake, 2002, 2012). Thus, changes of USV call rate at pup early ontogeny are predetermined by their physical development and milestones of development of sensory systems: establishing own thermoregulation, ear and eye opening, progress of locomotory activity (Blake, 1992, 2002, 2012; Vieira and Brown, 2002; Mandelli and Sales, 2004). In most rodents, after establishing these traits, call-eliciting procedure including isolation and pup cooling from being outside of the nest, does not further evoke emission of USVs. Exclusions from this common rule are rare, e.g. the yellow steppe lemming *Eolagurus luteus*, which continue producing isolation-induced USVs up to maturation (Yurlova et al., 2020).

Acoustic variables of rodent pup USVs may change along ontogeny with body growth, but trends of their changes may differ from those of the sonic (below 20 kHz) calls. While the sonic calls, produced by rodent larynx, show a decrease of call pitch with age (Fischer et al., 2004; Volodin et al., 2017, 2018), for rodent USVs, the ontogenetic pathways of acoustic parameters may be inconsistent among species and still poorly studied. For example, in singing mice of the genus *Scotinomys* and in the yellow steppe lemming, pup USVs are higher-frequency than in adults (Campbell et al., 2014; Yurlova et al., 2020). In the Brandt's and mandarin voles (*Lasiopodomys brandtii* and *L. mandarinus*), pup and adult USVs are equally high-frequency (Dymskaya et al., 2022), but in the domestic mouse *Mus musculus* and fat-tailed gerbil *Pachyuromys duprasi*, pup USVs are lower-frequency than in adults (Liu et al., 2003; Zaytseva et al., 2019). Studying vocal ontogeny of other rodent species will help to find the common rules for the ontogenetic pathways of acoustic parameters of USVs.

The voles of Arvicolinae family are usual for studying pup USVs (e.g. Colvin, 1973; Mandelli and Sales, 2004; Warren et al., 2022). The subfamily Arvicolinae includes many species, which are different by body size, lifestyles (above-ground or subterranean, solitary or social), systems of breeding (promiscuous or monogamous) and systems of caregiving (by mother only or collective) (Kryštufek and Shenbrot, 2022). There is a relationship between the degree of parental care and amounts of pup USVs in some vole species (Shapiro and Insel, 1990; Rabon et al., 2001; Blake, 2002, 2012).

The Harting's vole *Microtus hartingi* is a highly social vole species within the "guentheri" group of the social voles of the Arvicolinae subgenus *Sumeriomys* (Golenishchev et al., 2002, 2003; Kryštufek et al., 2012; Abramson et al., 2021; Kryštufek and Shenbrot, 2022; Selçuk et al., 2024). The Harting's vole was previously considered within the Guenter's vole *Microtus guentheri*, but now the Harting's vole is recognized as a taxonomically separate species, inhabiting the Balkans and the Anatolia peninsula, while the Guenther's vole is found in Turkey east of the Anatolian Diagonal, as well as in Lebanon, western Syria, Jordan and Israel (Thanou et al., 2020; Kryštufek and Shenbrot, 2022; Selçuk et al., 2024). In captivity, the Harting's voles live in family groups consisting of a parental pair and a few of their successive litters (Volodin et al., 2024). For natural populations, information about behavioral biology of this species is unavailable. Body mass of adult Harting's voles is up to 50 g in either sex (Çolak et al., 1998; Kryštufek and Shenbrot, 2022).

Adult Harting's voles of both sexes produce squeaks during dyad interactions on a neutral territory (Rutovskaya, 2019) and alarm calls in response to threat, with maximum pitch of about 17 kHz on average (Pandourski, 2011; Volodin et al., 2024). The USVs have yet to be investigated for pup and adult Harting's voles. The aim of this study was to describe the acoustic variation of the Harting's vole pup ultrasonic isolation-induced USVs along pup early ontogeny up to 10 d of age.

## 2. Material and methods

### 2.1. Study site, subjects and dates

Ultrasonic isolation-induced calls (USVs) of 55 individual pups from 11 litters of the Harting's vole *Microtus hartingi ankaraensis* (Golenishchev et al., 2022) were recorded at the Joint Usage Center "Live collection of wild species of mammals" at A.N. Severtsov Institute of Ecology and Evolution (the biological station "Tchernogolovka"), Moscow Region, Russia, located 50 km NE from Moscow city. At birth, the litters contained 3–6 pups, Mean  $\pm$  SD  $5.0 \pm 1.1$  pups per litter. Of the 55 study pups, 52 survived to 12 d of age; two pups died at 2 d of age and one pup died at 4 d of age by reasons not related to experiments. Pups were recorded for USVs 5–6 times per pup, at 1–2, 3–4, 5–6, 7–8, 9–10 and 11–12 d of age, from 27 March to 26 May 2023.

Nine litters were first litters of a parental pair, two litters were second in order and were kept with pups of a preceding litter. The litters were delivered between 27 March to 14 May 2023 by 10 different parental pairs, one pair delivered 2 litters. All subject pups were descendants of 25 (15 female, 10 male) Harting's voles from a captive population of Zoological Institute of Russian Academy of Sciences (Saint Petersburg, Russia), transported to "Tchernogolovka" biological station on 5 March 2023. This laboratory population of Harting's voles originated from 7 (3 female and 4 male) adults obtained in 2003 from surroundings of Ankara city: Kirsehir, Turkey (39°9'56.38"N, 34°6'6.99"E). This population was bred in captivity for generations without signs of inbred depression or incest-tabu (Zorenko et al., 2016; Golenishchev et al., 2022; Zorenko, 2023; Zorenko and Kaija, 2024).

At the "Tchernogolovka" station, the vole families were kept under a natural light regime in a room of 20 m<sup>2</sup> at 22–24°C. The plastic home cages of 55 x 35 x 20 cm, one per family, contained sawdust, shelters and hay as bedding and enrichment and were checked every day for new litters. The voles were fed with custom-made small rodent chow, mineral supplements, fruits, grass and vegetables and water ad libitum. During this study, the composition of the pairs was unchanged, each male was constantly sitting with its female and pups.

### 2.2. USV eliciting and recording

Pup USVs were recorded in a room neighboring with the room where the animals were kept, without other animals present. This allowed recording USVs reliably assigned to the focal pup. Callers vocalized at 13–14 °C and electrical equipment turned off to avoid the presence of background ultrasonic noise. The 2-min test trials (following Zaytseva et al., 2019; Klenova et al., 2021; Dymskaya et al., 2022), started when a focal pup was placed in the experimental plastic cylinder (diameter 170 mm, high 120 mm). The focal pup produced USVs in response to cooling due to the imperfect thermoregulation and isolation from the nest; after recording the USVs for about 2 min, the caller was weighed accurate to 0.01 g and measured for body length, head length, foot length, the forepaw length and tail length, accurate to 0.1 mm. We measured body length (from the tip of the muzzle to anus), head length (from tip of muzzle to occiput), foot length (from the heel to the tip of the middle toe), forepaw length (from the elbow to the tip of fingers) and tail length from the anus to the tip of the tail. The body variables and body mass were used as proxies of body size for comparisons with the USV acoustic variables.

After than the focal pup was tested, it was temporally placed outside of the experimental room in a small plastic box with sawdust standing on a chair near the heater, providing the temperature in the box area of 30–33 °C. Experimental trials with all other littermates were done consequently in the same manner and then all of them were simultaneously returned to their home cage to their parents; the time of pup stay out of the nest was within 30 min. Before each test trial, the experimental setup was cleaned with water, dried with rubbing by clean cotton napkin and then rubbed with cotton washed with 40 % ethanol, because

a higher concentration of ethanol may affect rodent behavior (Lopez-Salesansky et al., 2021).

During each test trial, lasting 2 min, we continuously recorded the focal pup USVs (256 kHz sampling frequency, 16-bit resolution) with Echo Meter Touch 2 PRO (Wildlife Acoustics, Inc., Maynard, MA USA) attached to a smartphone. The frequency response range of the microphone was 20–30 kHz ± 15 dB and 30–60 kHz ± 5 dB. The microphone was mounted at 10–15 cm over the table in the focal pup area, providing a high signal-to-noise ratio during recording. Pup recordings were made only up to 12 d of age, because 13–14-day old pups were silent during the call-eliciting procedure, probably because they already had own thermoregulation and did not need in the heating from the family members. We did not identify the pups individually within litters, but USVs of each pup in each test were stored as a separate wav-file. In total, we conducted 316 call-eliciting test trials.

### 2.3. USV samples

Using visual inspection of spectrograms of wav-files of the audio recordings, created with Avisoft SASLab Pro software (Avisoft Bioacoustics, Berlin, Germany), we selected for acoustic analyses up to 10 USVs per test trial. If the recording (stored as one wav file per individual) contained more than 10 USVs, we took calls randomly among those considered eligible, of high sound-to-noise ratio and without superimposed noise from different parts of each 2-min recording, avoiding taking calls following each other. If the recording contained 10 or less USVs, we included in analysis all available USVs. Call frequency contour and presence of nonlinear phenomena were not considered as selection criteria. We defined an ultrasonic call as frequency contour either continuous without breaks or with breaks shorter than 10 ms (Dou et al., 2018; Zaytseva et al., 2019; Kozhevnikova et al., 2021). If the separation break exceeded 10 ms, we considered that the contours belonged to two different calls.

To avoid the bias in selection of calls for measurements and to measure the calls evenly throughout the test trial, we visually estimated in each trial the area of the test "covered" with USV calls and separated this area into 10 sections. For instance, if all the 120 s of the test contained USVs, we separated the test to 10 sections of 11–12 s long. Then, we took for measurements the USVs which were most close to the places of sectioning. If the call of the place of selection was of bad quality, we took for the measurements a neighboring call.

From 264 test trials conducted with pups at ages of 1–2, 3–4, 5–6, 7–8, 9–10 d, 232 audio files (one file per trial) contained USVs (Table 1). These audio recordings made at the first five age classes, contained USVs from 55 to 26 pups of all 11 litters. From 52 test trials conducted with 11–12-d pups, only 6 audio files contained USVs. These 6 trials were

**Table 1**

Distribution of subjects (test trials), litters and ultrasonic calls of the Harting's vole pups across age classes examined with 2-d intervals between 1–12 d of age. Designations: *n* pups (test trials) – number of tested individuals; *n* callers – number of individuals which provided ultrasonic calls; *n* litters – number of tested litters; *n* vocal litters – number of litters which provided ultrasonic calls; *n* USVs – number of analysed ultrasonic calls.

Variable	Age class (days)						Total
	Age class	Age class	Age class	Age class	Age class	Age class	
	1–2	3–4	5–6	7–8	9–10	11–12	
<i>n</i> pups (test trials)	55	53	52	52	52	52	316
<i>n</i> callers	55	53	50	48	26	6	238
<i>n</i> litters	11	11	11	11	11	11	11
<i>n</i> vocal litters	11	11	11	11	11	3	11
<i>n</i> USVs	500	530	470	477	229	28	2234

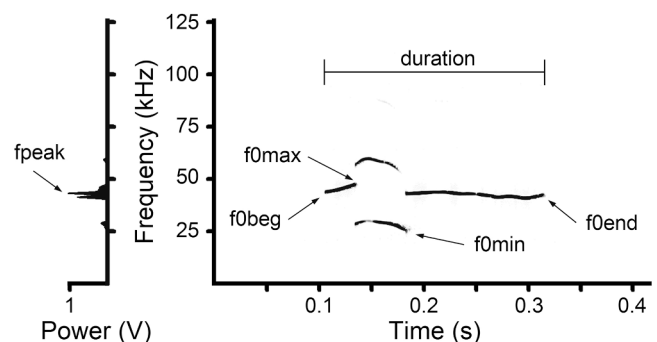
recorded from pups of only 3 litters (Table 1). To the 13th d of life, the pups completely stopped producing USVs in the context of isolation. For analysis of call rate and parameters of body size, we used all 316 trials from all the six age classes. For acoustic analyses, we selected 2206 USVs from 232 recordings obtained from pups of the first five age classes (1–2, 3–4, 5–6, 7–8, 9–10 d). We selected 28 USVs from 6 recordings from pups at 11–12 d of age, but did not include them in the analysis of age-related changes, because the sample was insufficient for conducting the valid comparison.

### 2.4. USV analysis

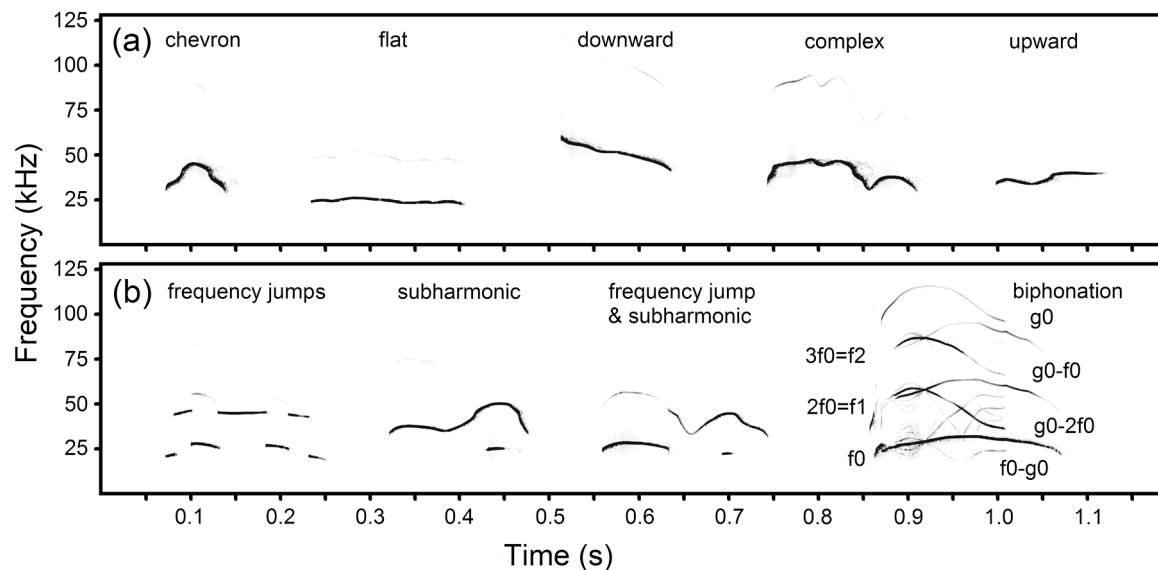
Acoustic variables of pup USVs have been conducted with Avisoft and exported to Microsoft Excel (Microsoft Corp., Redmond, WA, USA). The measurements were made with the following settings: sampling frequency 256 kHz, Hamming window, FFT 1024 points, frame 100 %, overlap 87.5 %, providing frequency resolution 250 Hz and time resolution 0.5 ms. For each of 316 recordings from pups of all age classes, we calculated the number of USVs per 2 min and calculated call rate as the number of USVs per 1 min. For recordings without USVs call rate was zero. For 2206 USVs from pups of five age classes of 1–2, 3–4, 5–6, 7–8, 9–10 d (Table 1), we measured in the spectrogram window of Avisoft the duration with the standard marker cursor, and the maximum fundamental frequency (f0max), the minimum fundamental frequency (f0min), the fundamental frequency at the onset of a call (f0beg), the fundamental frequency at the end of a call (f0end) with the reticule cursor (Fig. 1). For each USV, we measured, in the power spectrum window of Avisoft, the frequency of maximum amplitude (fpeak) from the call's mean power spectrum (Fig. 1). As f0min of USVs always exceeded 10 kHz, before measurements all wav-files were subjected to 10 kHz high-pass filtering, to remove low-frequency noise.

### 2.5. USV contours and nonlinear phenomena

By visual inspection in Avisoft, we classified 2206 USVs from pups of five age classes of 1–2, 3–4, 5–6, 7–8, 9–10 d to one of five contours (Fig. 2): flat, chevron, upward, downward and complex (following Yurlova et al., 2020; Kozhevnikova et al., 2021; Dymaskaya et al., 2022). Flat contour was determined if the difference between f0min and f0max was less than 6 kHz. When the difference between f0min and f0max was equal or larger than 6 kHz, a call contour was classified as chevron (up-and down), upward (ascending from start to end), downward (descending from start to end) or complex (up-and-down a few times or U-shaped) (Fig. 2).



**Fig. 1.** Measured variables for pup Harting's vole ultrasonic calls (USVs), spectrogram (right) and mean power spectrum of a call (left). Designations: duration – call duration; f0beg – the fundamental frequency at the onset of a call; f0end – the fundamental frequency at the end of a call; f0max – the maximum fundamental frequency; f0min – the minimum fundamental frequency; fpeak – the frequency of maximum amplitude. The spectrogram was created at 256 kHz sampling frequency, FFT length 1024, Hamming window, frame 50 %, overlap 87.5 %.



**Fig. 2.** Spectrogram illustrating (a) five contour shapes and (b) nonlinear phenomena of the Harting's vole pup ultrasonic calls (USVs). Designations:  $f_0$  – the low fundamental frequency band (about 30 kHz);  $2f_0$ ,  $3f_0$  – harmonic of  $f_0$ ;  $g_0$  – the high fundamental frequency band (95–115 kHz);  $g_0-f_0$ ,  $g_0-2f_0$ ,  $f_0-g_0$  – combinatorial frequency bands. The spectrogram was created at 256 kHz sampling frequency, FFT length 1024, Hamming window, frame 50 %, overlap 87.5 %. The audio file of these calls is available as Supplementary Audio A1.

For each USV, we noted the presence of nonlinear phenomena (Fig. 2): frequency jumps, biphonations and subharmonics (Wilden et al., 1998; Yurlova et al., 2020; Kozhevnikova et al., 2021; Dymyskaya et al., 2022; Rutovskaya et al., 2024). Frequency jump was noted when  $f_0$  suddenly changed for  $\geq 10$  kHz up or down. Biphonation was noted when two independent fundamental frequencies, the low ( $f_0$ ) and the high ( $g_0$ ) and their combinatorial frequency bands ( $g_0-f_0$ ;  $g_0-2f_0$ ; etc.) were present. Subharmonics were noted when the intermediate frequency bands of  $1/2$  or  $1/3$  of  $f_0$  were present between harmonics (Fig. 2). For calls with frequency jumps, we identified the contour shape by virtual smoothing the contour as if frequency jump was lacking and the fundamental frequency contour was continuous (Yurlova et al., 2020). The biphonic calls with two different fundamental frequency contours were classified based on the lowest frequency contour. In the biphonic calls in which the high fundamental frequency ( $g_0$ ) contour was well visible, we additionally measured the maximum high fundamental frequency ( $g_0\text{max}$ ).

## 2.6. Statistical analyses

Statistical analyses were made with STATISTICA, v. 8.0 (StatSoft, Tulsa, OK, USA) and R 4.1.0 (R Development Core Team, 2023). Means were given as mean  $\pm$  SD, all tests were two-tailed and differences were considered significant whenever  $p < 0.05$ .

For each subject pup in each of the 232 test trials (Table 1), the averaged values of each acoustic variable of USV calls were used for the statistic comparisons, to take into account the effect of multiple measurements of parameters from the same pup. Only 1 of 72 distributions of body size and acoustical parameters differed significantly from the normal (Kolmogorov-Smirnov test,  $p > 0.05$ ), so we could apply the parametrical tests. We used GLM with Tukey HSD (Honestly Significant Difference) post-hoc test to estimate the effects of age, introduced as fixed factor, and litter identity (litter ID) introduced as random factor, on the parameters of body size and the acoustics of the USVs. We used Principal Component Analysis (PCA) to estimate the degrees of correlation between the body size variables and for calculating the body size index on the basis of these variables.

Due large number of zero values in call rate and the skewed shape of the distributions we used permutation ANOVA test in the R library *lmPerm* with 5000 permutations (Wheeler and Torchiano, 2010) with

pairwise permutation tests as post-hoc in the package *rcompanion* (Mangiafico, 2024) to estimate the effect of age on call rate. For comparison of occurrence of different USV contours and nonlinear phenomena in USVs, we presented each contour and kind of nonlinear phenomenon as 0 or 1 value, where 0 (no) means absence and 1 (yes) presence in the call. Then we used Generalized Linear Models (GLM) with binomial distribution and logit link function with post-hoc test to estimate the effects of age and ID litter on the occurrence of different types of contours and different kinds of nonlinear phenomena in USVs. We used Pearson correlation with Bonferroni correction to estimate potential correlation between age, body size index and the acoustic variables of the USV calls.

## 3. Results

### 3.1. Body variables

We found a significant effect of age class on pup body mass and body size variables (Table 2). From 1–12 d of age, body mass gain was 0.5 g per day on average. For each age class, significantly higher values than that of the younger age classes were observed for body mass (Table 2,  $p < 0.001$ , Tukey *post hoc*) and for all measured body variables (Table 2,  $p < 0.01$ , Tukey *post hoc*), for the exclusion of tail length between 5 and 6 age classes (Table 2,  $p = 0.26$ , Tukey *post hoc*).

We found a positive correlation between age class and body mass ( $r = 0.854$ ,  $p < 0.001$ ), body length ( $r = 0.843$ ,  $p < 0.001$ ), head length ( $r = 0.899$ ,  $p < 0.001$ ), foot length ( $r = 0.898$ ,  $p < 0.001$ ), forepaw length ( $r = 0.896$ ,  $p < 0.001$ ) and tail length ( $r = 0.828$ ,  $p < 0.001$ ). Thus, body mass and all body variables provided clear correlates of pup age.

Body mass and all other body variables were correlated with the first PCA axis very highly, with correlation coefficients from 0.91 to 0.97. The first PCA factor accounted for 89.8 % of the variation. As soon as the first PCA axis responded for 89.8 % of variation, then, we used the values of the first PCA factor for each pup as a generalizing body size index.

### 3.2. Call rate and USV categories

The number of pups producing USVs during test trials exceeded 92 %

**Table 2**

Values (Mean  $\pm$  SD) of body mass and size variables of the Harting's vole pups across age classes examined with 2-d intervals between 1–12 d of age and GLM results for the effect of age class on their values. Litter ID was introduced in analysis as random factor. Designations: *n* individuals – number of pups weighted and measured in each age class.

Body size variable	Age class (days)						GLM
	Age class 1–2	Age class 3–4	Age class 5–6	Age class 7–8	Age class 9–10	Age class 11–12	
<i>n</i> individuals	55	53	52	52	52	52	316
Body mass (g)	3.26 $\pm$ 0.65	4.49 $\pm$ 0.92	5.67 $\pm$ 1.10	6.84 $\pm$ 1.31	8.10 $\pm$ 1.41	8.85 $\pm$ 1.49	$F_{5300}= 508.4, p < 0.001$
Body length (mm)	37.6 $\pm$ 2.8	41.8 $\pm$ 3.4	44.7 $\pm$ 3.1	46.3 $\pm$ 3.4	50.8 $\pm$ 3.5	53.9 $\pm$ 4.1	$F_{5300}= 226.5, p < 0.001$
Head length (mm)	15.3 $\pm$ 1.1	17.1 $\pm$ 1.3	18.8 $\pm$ 1.5	20.6 $\pm$ 1.2	22.0 $\pm$ 1.6	23.5 $\pm$ 1.4	$F_{5300}= 433.6, p < 0.001$
Foot length (mm)	7.4 $\pm$ 0.8	8.6 $\pm$ 0.9	10.4 $\pm$ 1.1	11.9 $\pm$ 1.5	13.6 $\pm$ 1.3	14.6 $\pm$ 1.6	$F_{5300}= 415.3, p < 0.001$
Forepaw length (mm)	11.4 $\pm$ 1.6	13.3 $\pm$ 1.1	15.3 $\pm$ 1.2	17.1 $\pm$ 1.4	18.4 $\pm$ 1.2	19.3 $\pm$ 1.4	$F_{5300}= 383.3, p < 0.001$
Tail length (mm)	7.5 $\pm$ 0.9	8.7 $\pm$ 1.0	10.1 $\pm$ 1.3	11.4 $\pm$ 1.4	13.0 $\pm$ 1.7	13.5 $\pm$ 2.0	$F_{5300}= 176.0, p < 0.001$

between 1 and 8 d of age, decreased to 50 % at 9–10 d of age and then abruptly fell to 11.5 % at 11–12 d of age (Fig. 3). Age class significantly affected call rate (permutation ANOVA test,  $F_{5305} = 40.15, p < 0.001$ ). From 1–2–7–8 d of age, call rate ranged between 121.8 and 94.2 USVs/min, with maximum of call rate at 3–4 d of age (Fig. 3). Then call rate significantly decreased to 36.4 USVs/min at 9–10 d of age and then significantly decreased to 1.5 USVs/min at 11–12 d of age (Fig. 3). The average call rate from 1 to 12 d of age was (mean and min-max values)  $75.4 \pm 67.1$  (0–233) USVs/min.

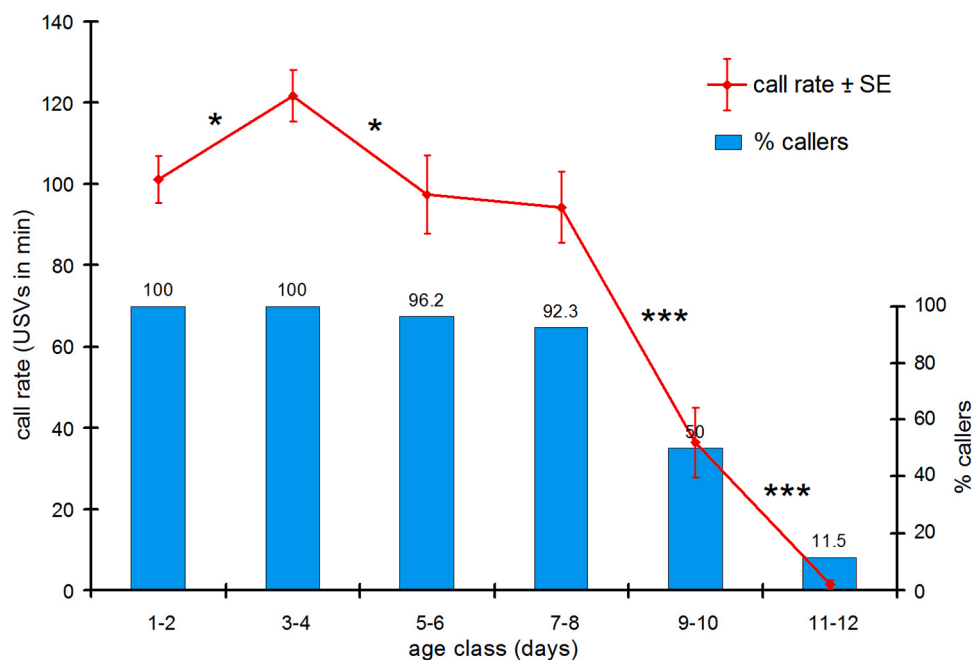
Percentages of USVs with different contours only at small extent changed along ontogeny (Fig. 4). In the total sample of 2206 USVs of all subjects at the 5 age classes, the most widespread was the chevron contour: 1097 USVs (49.7 %), then in order flat: 448 USVs (20.3 %), downward: 401 USVs (18.2 %), complex: 232 USVs (10.5 %) and upward contour: 28 USVs (1.3 %).

GLZ revealed significant effects on percentage of chevron contour (Wald stat = 43.6,  $p < 0.001$ ), flat contour (Wald stat = 53.5,  $p < 0.001$ ), downward contour (Wald stat = 64.1,  $p < 0.001$ ), and complex contour (Wald stat = 34.4,  $p < 0.001$ ), but not of upward contour (Wald stat = 9.3,  $p = 0.055$ ). The chevron contour comprised from 42 % to 61 % of USVs and became more often to 3–4 d of age (Fig. 4). The downward contour decreased its percentage from 1–2 d to 3–6 d of age, whereas the occurrence of the flat contour increased from

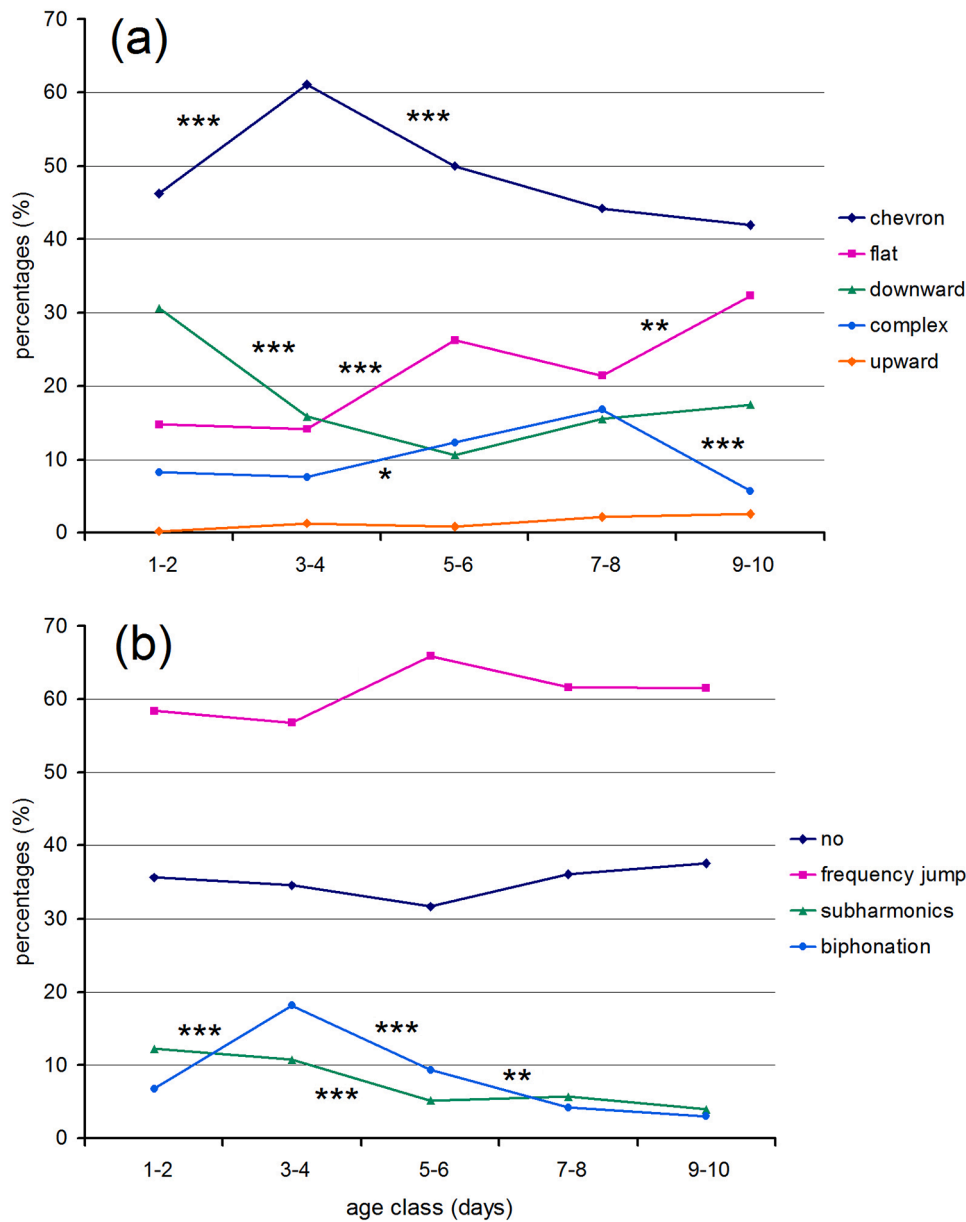
1–4 d to 9–10 d of age (Fig. 4). The complex contour became more often to 7–8 d of age and then became rarer to 9–10 d of age. The upward contour was least frequent at any age (Fig. 4). We can conclude that USVs contour shapes are becoming more simplified with pup age, as to 9–10 d of age, the percentage of USVs with flat contours increases and percent of USVs with complex contours decreases.

Nonlinear phenomena occurred at all the 5 age classes, in 1438 (65.2 %) USVs from the total of 2206 USVs. Most frequent were frequency jumps, occurring in 1338 (60.7 %) of USVs. Biphonation was noted in 201 (9.1 %) USVs and subharmonics in 178 (8.1 %) USVs. Many USVs (266 USVs, 12.1 %) contained two nonlinear phenomena, and 13 (0.6 %) USVs contained all the three nonlinear phenomena: frequency jump, biphonation and subharmonics.

GLZ revealed significant effects on percentage of frequency jumps (Wald stat = 10.1,  $p = 0.039$ ), biphonations (Wald stat = 70.9,  $p < 0.001$ ), and subharmonics (Wald stat = 31.0,  $p < 0.001$ ). Percent of USVs without nonlinear phenomena did not depend on age (Wald stat = 1.3,  $p = 0.85$ ) and varied from 32 % to 37 % between age classes (Fig. 4). At all age classes, more than the half of USVs (from 57 % to 66 %) contained frequency jumps, which steadily increased to 5–6 d of age (Fig. 4). A remarkable rise of USVs with biphonations up to 18 % at age class of 3–4 d was observed, with the subsequent fall to a previous level to 7–8 d of age. The number of USVs with subharmonics was the



**Fig. 3.** Call rate (Mean  $\pm$  SE) and percentages of pups (% callers) producing USVs during call-eliciting test trials at the 6 age classes (of 1–2, 3–4, 5–6, 7–8, 9–10 and 11–12 days). Above the pillars, percentages of pup callers are given. Only the results of comparisons of neighboring age classes (1 and 3, 3 and 5, 5 and 7, 7 and 9) with permutation ANOVA test are shown. Stars (\*\*\*) –  $p < 0.001$ , \* –  $p < 0.05$  indicate statistically different values (pairwise permutation post-hoc test).



**Fig. 4.** Percentages of (a) five different USV contours and (b) nonlinear phenomena in the total sample of 2206 USVs of the Harting's vole pups at the 5 age classes (of 1–2, 3–4, 5–6, 7–8 and 9–10 days). Only the results of comparisons of neighboring age classes (1 and 3, 3 and 5, 5 and 7, 7 and 9) with GLZ are shown. Stars (\*\*\*) –  $p < 0.001$ , \*\* –  $p < 0.01$ , \* –  $p < 0.05$ ) indicate statistically different values (post-hoc test).

**Table 3**

Values (Mean  $\pm$  SD) of USV acoustic variables of the Harting's vole pups at five examined age classes (of 1–2, 3–4, 5–6, 7–8 and 9–10 days of age) and GLM results for the effect of age class on their values. Litter identity (ID) was introduced in the analysis as random factor. Designations:  $n$  – number of individuals; duration – call duration;  $f_{0\text{beg}}$  – the fundamental frequency at the onset of a call;  $f_{0\text{end}}$  – the fundamental frequency at the end of a call;  $f_{0\text{max}}$  – the maximum fundamental frequency;  $f_{0\text{min}}$  – the minimum fundamental frequency;  $f_{\text{peak}}$  – the frequency of maximum amplitude. Different superscripts (<sup>a</sup>, <sup>b</sup>, <sup>c</sup>) indicate statistically different values (Tukey HSD test,  $p < 0.05$ ).

Acoustic variable	Age class (days)					GLM
	Age class 1–2	Age class 3–4	Age class 5–6	Age class 7–8	Age class 9–10	
$n$ individuals	55	53	50	48	26	232
duration (ms)	16.7 $\pm$ 5.9 <sup>a</sup>	16.5 $\pm$ 5.2 <sup>a</sup>	13.2 $\pm$ 5.1 <sup>b</sup>	14.0 $\pm$ 4.0 <sup>a,b</sup>	12.8 $\pm$ 6.0 <sup>b</sup>	$F_{4217} = 6.56, p < 0.001$
$f_{0\text{beg}}$ (kHz)	34.8 $\pm$ 5.3	33.1 $\pm$ 6.1	32.7 $\pm$ 5.4	32.4 $\pm$ 4.7	33.3 $\pm$ 5.2	$F_{4217} = 1.37, p = 0.24$
$f_{0\text{max}}$ (kHz)	43.7 $\pm$ 5.5	42.2 $\pm$ 6.6	42.5 $\pm$ 7.0	41.0 $\pm$ 5.5	40.7 $\pm$ 6.5	$F_{4217} = 1.70, p = 0.15$
$f_{0\text{end}}$ (kHz)	35.0 $\pm$ 5.0 <sup>a</sup>	31.3 $\pm$ 4.2 <sup>b</sup>	29.4 $\pm$ 4.6 <sup>b,c</sup>	28.3 $\pm$ 4.5 <sup>c</sup>	29.0 $\pm$ 4.3 <sup>b,c</sup>	$F_{4217} = 22.50, p < 0.001$
$f_{0\text{min}}$ (kHz)	26.6 $\pm$ 5.2 <sup>a</sup>	23.7 $\pm$ 3.0 <sup>b</sup>	22.9 $\pm$ 4.9 <sup>b</sup>	22.3 $\pm$ 3.8 <sup>b</sup>	23.7 $\pm$ 3.4 <sup>b</sup>	$F_{4217} = 8.24, p < 0.001$
$f_{\text{peak}}$ (kHz)	35.3 $\pm$ 4.1 <sup>a</sup>	33.3 $\pm$ 4.9 <sup>b</sup>	31.3 $\pm$ 4.0 <sup>b,c</sup>	30.9 $\pm$ 4.2 <sup>c</sup>	32.4 $\pm$ 4.5 <sup>b,c</sup>	$F_{4217} = 8.77, p < 0.001$

highest at 1–2 d of age and steadily decreased to 5–6 d of age (Fig. 4).

### 3.3. Acoustic variables

The USVs of all 232 pups calculated over the five age classes (1–2, 3–4, 5–6, 7–8, 9–10 d) had the following mean (min–max) values of acoustic variables: duration of  $149 \pm 54$  (7–333) ms,  $f_{0\max}$  of  $42.2 \pm 6.3$  (20.7–62.2) kHz,  $f_{0\min}$  of  $23.9 \pm 4.6$  (16.8–47.7) kHz,  $f_{0\text{beg}}$   $33.3 \pm 4.6$  (19.7–61.3) kHz,  $f_{0\text{end}}$   $30.8 \pm 5.1$  (16.8–47.5) kHz and  $f_{\text{peak}}$   $32.7 \pm 4.6$  (20.5–58.7) kHz. Age class significantly affected all acoustic variables for the exclusion of  $f_{0\text{beg}}$  and  $f_{0\max}$ , which values did not display significant changes with age (Table 3). The duration of USVs significantly decreased from 1–4 d to 5–6 d of age. Since the age 5–6 d onwards, pup USV duration was becoming undistinguishable between age classes (Table 3). The  $f_{0\text{end}}$ ,  $f_{0\min}$  and  $f_{\text{peak}}$  of USVs also decreased with age, displaying significantly highest values at 1–2 d of age and showing undistinguishable values since 3–4 d of age onwards (Table 3).

The USVs of 6 pup callers at age of 11–12 d had the duration of  $66 \pm 31$  ms,  $f_{0\max}$  of  $41.5 \pm 8.5$  kHz,  $f_{0\min}$  of  $32.0 \pm 10.9$  kHz,  $f_{0\text{beg}}$   $38.4 \pm 9.3$  kHz,  $f_{0\text{end}}$   $36.5 \pm 9.9$  kHz and  $f_{\text{peak}}$   $38.1 \pm 10.7$  kHz. However, these values should be interpreted cautiously, because the number of litter ( $n = 3$ ), individuals ( $n = 6$ ) and USVs ( $n = 28$ ) at 11–12 d of age was disproportional small compared to other age classes (Table 1). We did not include these USVs in the analysis of age-related changes (Table 3), because this sample was insufficient for conducting valid comparison.

We measured the maximum values of the high fundamental frequency ( $g_{0\max}$ ) for 51 biphonic USVs, which were present at all age classes. Calculated all over the age classes, the mean  $g_{0\max}$  of the biphonic USVs was  $104.8 \pm 8.8$  kHz and varied (min–max) from 84.7 to 122.0 kHz. The value of  $g_{0\max}$  did not depend on age class ( $F_{4,38} = 0.93$ ,  $p = 0.46$ ). The minimum value of  $g_{0\max}$  (84.7 kHz) was higher than the maximum value of  $f_{0\max}$  (62.2 kHz) calculated for the total sample of USVs (2206 USVs).

Body size index significantly negatively correlated with all USV acoustic variables (Table 4). However, age class was not significantly negatively correlated with  $f_{0\text{beg}}$  and  $f_{0\max}$  after Bonferroni correction (Table 4). Thus, only duration,  $f_{\text{peak}}$ ,  $f_{0\min}$  and  $f_{0\text{end}}$  decreased with increasing age and body growth.

## 4. Discussion

This study showed that pup Harting's voles start calling USVs in the isolation test trials from their first day of life and most pups stop producing USVs to 11–12 d of life. Pup Harting's voles produce at isolation a lot of USVs (over 100 calls/min), with a peak on 3–4 d of age and a significant decrease of call rate from 11–12 d of life and completely stop to spontaneously produce USVs at isolation on the 13th d of life. Pups of

**Table 4**

Pearson's correlation coefficients between age class, body size index and acoustic variables of ultrasonic calls (USVs). Threshold for significant values after Bonferroni correction comprises  $p < 0.008$ . Designations:  $n$  – number of individuals; duration – call duration;  $f_{0\text{beg}}$  – the fundamental frequency at the onset of a call;  $f_{0\text{end}}$  – the fundamental frequency at the end of a call;  $f_{0\max}$  – the maximum fundamental frequency;  $f_{0\min}$  – the minimum fundamental frequency;  $f_{\text{peak}}$  – the frequency of maximum amplitude.

Acoustic variable	Age class	Body size index
$n$ individuals	232	232
duration (ms)	$r = -0.26$ , $p < 0.001$	$r = -0.21$ , $p = 0.001$
$f_{0\text{beg}}$ (kHz)	$r = -0.12$ , $p = 0.08$	$r = -0.20$ , $p = 0.002$
$f_{0\max}$ (kHz)	$r = -0.16$ , $p = 0.02$	$r = -0.21$ , $p = 0.001$
$f_{0\text{end}}$ (kHz)	$r = -0.43$ , $p < 0.001$	$r = -0.53$ , $p < 0.001$
$f_{0\min}$ (kHz)	$r = -0.25$ , $p < 0.001$	$r = -0.36$ , $p < 0.001$
$f_{\text{peak}}$ (kHz)	$r = -0.29$ , $p < 0.001$	$r = -0.38$ , $p < 0.001$

other vole species also stop producing USVs at isolation to the age of 15–20 d of life (Brooks and Banks, 1973; Blake, 1992; Szentgyörgyi et al., 2008; Yu et al., 2011), what is related to body growth and development of thermoregulation (Okon, 1972; Blake, 2002, 2012). In pup Harting's voles, the start of a strong decrease of the percentage of callers and call rate (Fig. 3) coincided with the age of eyes opening (10.8 d of age, Zorenko et al., 2016). Similar relationships were also reported for other vole species (De Ghatt, 1977; Shapiro and Insel, 1990; Yu et al., 2011).

Calling of numerous USVs from the first day of life in the test for isolation the pup from the nest was described, aside from the Harting's vole, for the prairie vole *Mynomes ochrogaster* (Shapiro and Insel, 1990; Rabon et al., 2001; Blake, 2002; Warren et al., 2022) and the pine vole *Mynomes pinetorum* (Geyer, 1979; Blake, 2012). Pups of these vole species also display a very high call rate of USVs (over 100 calls/min) and a very high call rate of isolation-induced calls just after birth, in the first days of life. A similar pattern for producing USVs was also found in pups of the mandarin vole, displaying a very high call rate from the first day of life (160 calls/min) with a small increase to the 11th d of age (240 calls/min) and subsequent rapid decrease of call rate (Yu et al., 2011). For pups of the Japanese grass vole *Alexandromys montebelli*, an intense producing USVs from the first day of life with a small peak on the third day followed by a rapid fall after it was noted, however, the current rate was not reported (Yoshinaga et al., 1997).

As the Harting's vole, the prairie voles, pine voles and mandarin voles are all social species with monogamous system of breeding, biparental care about offspring, living in extended family groups (McGuire and Novak, 1984; Powell and Fried, 1992; Getz et al., 1993; Smorkatcheva, 1999). Producing the large amounts of USVs by pups is adaptive for these social species, because the parents or other group members are often nearby and thus can rapidly help to a pup fallen out of the nest before predator can hear it (Blake, 2002, 2012).

In contrast to social species, in the solitary species of voles (in which pups are raised by a mother only), call rate is substantially lower (on average, not higher than 10–20 calls/min), amounts of USVs produced soon after birth are relatively very small, and the peak of call rate is at the age of 8–10 d of life, which coincides with development of sensory and locomotory systems of pups (Blake, 2002, 2012). Such pattern of calling was shown for bank voles *Myodes glareolus* (Blake, 1992), water voles *Arvicola amphibius* (Blake, 1992), field voles *Agricola agrestis* (Blake, 1992), meadow voles *Mynomes pennsylvanicus* (Blake, 2012) and montane voles *Mynomes montanus* (Shapiro and Insel, 1990; Rabon et al., 2001; Blake, 2002, but see De Ghatt, 1977). In the collared lemming *Dicrostonyx groenlandicus*, pups produce little USVs at the first day of life; the peak for producing USVs is on the 5th d of life, however, at this peak, the pups produce over 100 calls/min (Brooks and Banks, 1973). Producing the small amounts of USVs by pups is adaptive for these solitary species, because it promotes less attention to the pups from the predators (Blake, 2002, 2012), e.g., sorcids of the genus *Blarina* (Getz et al., 1992).

Pups of the Harting's vole at the age of 1–10 d produced USVs of duration 128–167 ms, with  $f_{0\max}$  of 41–44 kHz,  $f_{0\min}$  22–27 kHz and  $f_{\text{peak}}$  31–35 kHz (Table 3). The values of acoustic variables of USVs in pup Harting's voles at the test of isolation were very similar with those of other vole species (Table 5). For all studied vole species, the duration of pup USVs varied from 25 to 156 ms,  $f_{0\max}$  varied from 25 to 56 kHz,  $f_{0\min}$  varied from 20 to 42 kHz, and  $f_{\text{peak}}$  varied from 25 to 41 kHz (Table 5). The USVs of pup Harting's vole had the values between these margins. However, the duration of USVs of pup Harting's vole was one of the longest among the studied vole species (Table 5) in spite of that duration of the shortest USV included in analyses in this study, was 7 ms.

Acoustic variables of Harting's vole pup USVs changed only slightly during the first 10 days of pup lives (Table 3). Duration decreased from 1–4–5–10 d of age, the  $f_{\text{peak}}$ ,  $f_{0\min}$ , and  $f_{0\text{end}}$  also decreased from 1–2–3–4 d of age and onwards; the  $f_{0\max}$  and  $f_{0\text{beg}}$  did not show the age-related changes (Table 3). This pathway of a slight decrease of

**Table 5**

Summary metadata table for values of acoustic variables for isolation-induced pup vole USVs across Arvicolinae species and references to the original studies. Average values are provided where possible. Designations: duration – call duration; f0max – the maximum fundamental frequency; f0min – the minimum fundamental frequency; fpeak – the frequency of maximum amplitude.

Species	Latin name	Age (days)	Duration (ms)	f0max (kHz)	f0min (kHz)	fpeak	Reference
Harting's vole	<i>Microtus hartingi</i>	1–10	149	42	24	33	This study
eastern meadow vole	<i>Mynomes pennsylvanicus</i>	1–10	79	39			Colvin (1973)
California vole	<i>Mynomes californicus</i>	1–10	71	40			Colvin (1973)
long-tailed vole	<i>Mynomes longicaudus</i>	1–10	77	41			Colvin (1973)
montane vole	<i>Mynomes montanus</i>	1–10	74	39			Colvin (1973)
montane vole	<i>Mynomes montanus</i>	1–14	23				De Ghett (1977)
montane vole	<i>Mynomes montanus</i>	6–8	125	36	26	35	Shapiro and Insel (1990)
prairie voles	<i>Mynomes ochrogaster</i>	1–10	121	32			Colvin (1973)
prairie voles	<i>Mynomes ochrogaster</i>	6–8	156	33	26	34	Shapiro and Insel (1990)
prairie voles	<i>Mynomes ochrogaster</i>	6–16	25–100	42–45	27–28	33–34	Warren et al. (2022)*
pine vole	<i>Mynomes pinetorum</i>	1	63	56	40		Geyer (1979)**
common field vole	<i>Agricola agrestis</i>	1–14	31–50	43–52	36–42		Mandelli and Sales (2004)***
common vole	<i>Microtus arvalis</i>	3				38	Motomura et al. (2002)
Japanese grass vole	<i>Alexandromys montebelli</i>	1–8				25	Yoshinaga et al. (1997)
Brandt's vole	<i>Lasiopodomys brandtii</i>	2–5	82	40	25	30	Dymkaya et al. (2022)
mandarin vole	<i>Lasiopodomys mandarinus</i>	2–5	55	45	29	34	Dymkaya et al. (2022)
mandarin vole	<i>Lasiopodomys mandarinus</i>	2–14	100–130			25–30	Yu et al. (2011)
bank vole	<i>Myodes glareolus</i>	6–7	54	29	26		Kapusta et al. (1995)
bank vole	<i>Myodes glareolus</i>	5–6	55	40			Szentgyörgyi et al. (2008)
collared lemming	<i>Dicrostonyx groenlandicus</i>	3–8	61–88	25–29	20–24		Brooks and Banks (1973)
yellow steppe lemming	<i>Eolagus luteus</i>	1–12	37–70	49–53	29–31	36–41	Yurlova et al. (2020)

\*Instead of fpeak, the median fundamental frequency is provided.

\*\* Data are provided for a single individual pup

\*\*\* Median values are provided

duration and fundamental frequency of USVs was strongly reminiscent of those during 1–10 d of age in five species of the genus *Mynomes* (Colvin, 1973). Shortening the duration, decrease of f0max, lack of changes in the median fundamental frequency, but increase of f0min from the age of 6–16 d of life was found in the prairie vole (Warren et al., 2022). However, in pups of common field voles, despite some apparent changes with age, no significant changes with age were found for any call characteristics from the age of 1–14 d of life (Mandelli and Sales, 2004).

A small shortening of duration of USVs from the age of 2–11 d of life was shown for pups of the mandarin vole, but the range of fpeak remained unchanged (Yu et al., 2011). The duration and fundamental frequency of USVs also decreases in the first 9 d of life in the yellow steppe lemming and then remains unchanged up to the adulthood (Yurlova et al., 2020). The duration and fundamental frequency of USVs slightly but significantly decreased between the age of 3–8 d of life in pups of the collared lemming (Brooks and Banks, 1973). Thus, we can conclude that there is a common pathway for age-related changes of USV acoustic structure in vole species in the first 10–14 d of life, appearing in the shortening of call duration and small decrease of call fundamental frequency.

While the most frequent contour shape in pup Harting's vole was chevron (Fig. 4), in pups of other vole species, the most widespread contours were flat, as in the common field vole (simple pulse, Mandelli and Sales, 2004) and in the five vole species of the genus *Mynomes* (plain whistle, Colvin, 1973), chevron and upward in the yellow steppe lemming (Yurlova et al., 2020), chevron and flat in the Brandt's vole and upward and flat in the mandarin vole (Dymkaya et al., 2022). As in pup Harting's voles, frequency jump was the most frequent nonlinear phenomenon in pup yellow steppe lemming, Brandt's vole and mandarin vole (Yurlova et al., 2020; Dymkaya et al., 2022). Second by the occurrence in all the four species were biphonations and then subharmonics (Yurlova et al., 2020; Dymkaya et al., 2022). Another nonlinear phenomenon, the deterministic chaos was never present in vole USVs, although it could be usual for pup USVs of other rodent species (Klenova et al., 2021; Riede et al., 2022; Rutovskaya et al., 2024).

In general, this study revealed the traits which make the Harting's vole pup USVs a perspective wild-type model for biomedical research

and testing drugs, as prairie vole (Warren et al., 2022). Among these traits are the high pup vocal activity in the ultrasonic range of frequencies; sustainability of captive populations of the Harting's vole for many generations over 20 years without any signs of inbred depression or incest-tabu, and the high intensity of breeding without strict seasonality and low aggressiveness to conspecifics (Zorenko et al., 2016; Golenishchev et al., 2022; Zorenko, 2023; Zorenko and Kaija, 2024; Volodin et al., 2024).

#### CRedit authorship contribution statement

**Rutovskaya Marina V.:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Volodin Ilya A.:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Golenishchev Fedor N.:** Writing – review & editing, Writing – original draft, Resources, Conceptualization. **Volodina Elena V.:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Investigation, Formal analysis, Conceptualization.

#### Declaration of Competing Interest

The authors declare no competing or financial interests.

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#### Appendix A. Supporting information

Supplementary data associated with this article can be found in the



online version at [doi:10.1016/j.beproc.2025.105161](https://doi.org/10.1016/j.beproc.2025.105161).

## Data Availability

Data will be made available on request.

## References

- Abramson, N.I., Bodrov, S.Y., Bondareva, O.V., Genelt-Yanovskiy, E.A., Petrova, T.V., 2021. A mitochondrial genome phylogeny of voles and lemmings (Rodentia: Arvicolinae): evolutionary and taxonomic implications. *PLoS ONE* 16, e0248198. <https://doi.org/10.1371/journal.pone.0248198>.
- Blake, B.H., 1992. Ultrasonic vocalization and body temperature maintenance in infant voles of three species (Rodentia: Arvicolidae). *Dev. Psychobiol.* 25, 581–596. <https://doi.org/10.1002/dev.420250805>.
- Blake, B.H., 2002. Ultrasonic calling in isolated infant prairie voles (*Microtus ochrogaster*) and montane voles. *M. Mont.* J. Mammal. 83, 536–545. [https://doi.org/10.1644/1545-1542\(2002\)083<0536:UCIIP>2.0.CO;2](https://doi.org/10.1644/1545-1542(2002)083<0536:UCIIP>2.0.CO;2).
- Blake, B.H., 2012. Ultrasonic calling in 2 species of voles, *Microtus pinetorum* and *M. pennsylvanicus*, with different social systems. *J. Mammal.* 93, 1051–1060. <https://doi.org/10.1644/11-MAMM-A-356.2>.
- Blumberg, M.S., Sokoloff, G., 2001. Do infant rats cry? *Psychol. Rev.* 108, 83–95. <https://doi.org/10.1037/0033-295x.108.1.83>.
- Boulanger-Bertolus, J., Rincón-Cortés, M., Sullivan, R.M., Mouly, A.M., 2017. Understanding pup affective state through ethologically significant ultrasonic vocalization frequency. *Sci. Rep.* 7, 13483. <https://doi.org/10.1038/s41598-017-13518-6>.
- Bowers, J.M., Perez-Pouchoulen, M., Edwards, N.S., McCarthy, M.M., 2013. Foxp2 mediates sex differences in ultrasonic vocalization by rat pups and directs order of maternal retrieval. *J. Neurosci.* 33, 3276–3283. <https://doi.org/10.1523/JNEUROSCI.0425-12.2013>.
- Branchi, I., Santucci, D., Alleva, E., 2001. Ultrasonic vocalisation emitted by infant rodents: a tool for assessment of neurobehavioural development. *Behav. Brain Res.* 125, 49–56. [https://doi.org/10.1016/S0166-4328\(01\)00277-7](https://doi.org/10.1016/S0166-4328(01)00277-7).
- Brooks, R.J., Banks, E.M., 1973. Behavioural biology of the collared lemming [*Dicrostonyx groenlandicus* (Trail)]: An analysis of acoustic communication. *Anim. Behav. Monogr.* 6 (1), 1–83. [https://doi.org/10.1016/0003-3472\(73\)90003-1](https://doi.org/10.1016/0003-3472(73)90003-1).
- Brunelli, S.A., Shair, H.N., Hofer, M.A., 1994. Hypothermic vocalizations of rat pups (*Rattus norvegicus*) and direct maternal search behavior. *J. Comp. Psychol.* 108, 298–303. <https://doi.org/10.1037/0735-7036.108.3.298>.
- Campbell, P., Pasch, B., Warren, A.L., Phelps, S.M., 2014. Vocal ontogeny in Neotropical singing mice (*Scotinomys*). *PLoS One* 9, e113628. <https://doi.org/10.1371/journal.pone.0113628>.
- Caruso, A., Sabbioni, M., Scattoni, M.L., Branchi, I., 2018. Quantitative and qualitative features of neonatal vocalizations in mice. In: Brudzynski, S.M. (Ed.), *Handbook of Ultrasonic Vocalization: A Window into the Emotional Brain*. Elsevier Academic Press, pp. 139–147. <https://doi.org/10.1016/B978-0-12-809600-0.00013-5>.
- Çolak, E., Sözen, M., Yiğit, N., Özkurt, S., 1998. A study on ecology and biology of *Microtus guentheri* Danford and Alston. 1880 (Mamm.: Rodentia) *Turk. Turk. J. Zool.* 22, 289–295.
- Colvin, M.A., 1973. Analysis of acoustic structure and function in ultrasounds of neonatal *Microtus*. *Behaviour* 44, 234–262. <https://doi.org/10.1163/156853973x000418>.
- De Ghett, V.J., 1977. The ontogeny of ultrasonic vocalization in *Microtus montanus*. *Behaviour* 60, 115–121. <https://doi.org/10.1163/156853977x00306>.
- Dou, X., Shirahata, S., Sugimoto, H., 2018. Functional clustering of mouse ultrasonic vocalization data. *PLoS ONE* 13, e0196834. <https://doi.org/10.1371/journal.pone.0196834>.
- Dymyskaya, M.M., Volodin, I.A., Smorkatcheva, A.V., Vasilieva, N.A., Volodina, E.V., 2022. Audible, but not ultrasonic, calls reflect surface-dwelling or subterranean specialization in pup and adult Brandt's and mandarin voles. *Behav. Ecol. Sociobiol.* 76, 106. <https://doi.org/10.1007/s00265-022-03213-6>.
- Ehret, G., 2005. Infant rodent ultrasounds — a gate to the understanding of sound communication. *Behav. Genet.* 35, 19–29. <https://doi.org/10.1007/s10519-004-0853-8>.
- Fischer, J., Kitchen, D., Seyfarth, R., Cheney, D., 2004. Baboon loud calls advertise male quality: acoustic features and relation to rank, age, and exhaustion. *Behav. Ecol. Sociobiol.* 56, 140–148. <https://doi.org/10.1007/s00265-003-0739-4>.
- Getz, L.L., Larson, C.M., Lindstrom, K.A., 1992. *Blarina brevicauda* as a predator on nestling voles. *J. Mammal.* 73, 591–596. <https://doi.org/10.2307/1382030>.
- Getz, L.L., McGuire, B., Pizzuto, T., Hofmann, J.E., Frase, B., 1993. Social organization of the prairie vole (*Microtus ochrogaster*). *J. Mammal.* 74, 44–58. <https://doi.org/10.2307/1381904>.
- Geyer, L.A., 1979. Olfactory and thermal influences on ultrasonic vocalization during development in rodents. *Am. Zool.* 19, 420–431. <https://doi.org/10.1093/icb/19.2.419>.
- Golenishchev, F.N., Sablina, O.V., Borodin, P.M., Gerasimov, S., 2002. Taxonomy of voles of the subgenus *Sumeriomys* Argyropoulou, 1933 (Rodentia, Arvicolinae, *Microtus*). *Russ. J. Theriol.* 1, 43–55. <https://doi.org/10.15298/rusjtheriol.1.1.03>.
- Golenishchev, F.N., Malikov, V.G., Nazari, F., Vaziri, A.S., Sablina, O.V., Polyakov, A.V., 2003. New species of vole of “*guentheri*” group (Rodentia, Arvicolinae, *Microtus*) from Iran. *Russ. J. Theriol.* 1, 117–123. <https://doi.org/10.15298/rusjtheriol.1.2.05>.
- Golenishchev, F.N., Zorenko, T.A., Petrova, T.V., Voyta, L.L., Kryuchkova, L.Y., Atanasov, N., 2022. Evaluation of the “bottleneck” effect in an isolated population of *Microtus hartingi* (Rodentia, Arvicolinae) from the Eastern Rhodopes (Bulgaria) by methods of integrative analysis. *Diversity* 14, 709. <https://doi.org/10.3390/d14090709>.
- Kapusta, J., Marchlewska-Koj, A., Sales, G.D., 1995. Home bedding modifies ultrasonic vocalization of infant bank voles. *J. Chem. Ecol.* 21, 577–582. <https://doi.org/10.1007/BF02033702>.
- Klenova, A.V., Volodin, I.A., Volodina, E.V., Ranneva, S.V., Amstislavskaya, T.G., Lipina, T.V., 2021. Vocal and physical phenotypes of calyntenin2 knockout mouse pups model early-life symptoms of the autism spectrum disorder. *Behav. Brain Res.* 412, 113430. <https://doi.org/10.1016/j.bbr.2021.113430>.
- Kozhevnikova, J.D., Volodin, I.A., Zaytseva, A.S., Ilchenko, O.G., Volodina, E.V., 2021. Pup ultrasonic isolation calls of six gerbil species and the relationship between acoustic traits and body size. *R. Soc. Open Sci.* 8, 201558. <https://doi.org/10.1098/rsos.201558>.
- Kryštufek, B., Shenbrot, G.I., 2022. *Voles and lemmings (Arvicolinae) of the Palaearctic region*. University of Maribor Press, Maribor, Slovenia. <https://doi.org/10.18690/um.fnm.2.2022>.
- Kryštufek, B., Zorenko, T., Buzan, E., 2012. New insights into taxonomy and phylogeny of social voles inferred from mitochondrial cytochrome *b* sequences. *Mamm. Biol.* 77, 178–182. <https://doi.org/10.1016/j.mambio.2011.11.007>.
- Lingle, S., Wyman, M.T., Kotrba, R., Teichroeb, L.J., Romanow, C.A., 2012. What makes a cry a cry? A review of infant distress vocalizations. *Curr. Zool.* 58, 698–726. <https://doi.org/10.1093/czoolo/58.5.698>.
- Liu, R.C., Miller, K.D., Merzenich, M.M., Schreiner, C.E., 2003. Acoustic variability and distinguishability among mouse ultrasound vocalizations. *J. Acoust. Soc. Am.* 114, 3412–3422. <https://doi.org/10.1121/1.1623787>.
- Lopez-Salesansky, N., Wells, D.J., Chancellor, N., Whitfield, L., Burn, C.C., 2021. Handling mice using gloves sprayed with alcohol-based hand sanitiser: acute effects on mouse behaviour. *Anim. Technol. Welf.* 20, 11–20.
- Mandelli, M.-J., Sales, G., 2004. Ultrasonic vocalizations of infant short-tailed field voles, *Microtus agrestis*. *J. Mammal.* 85, 282–289. [https://doi.org/10.1644/1545-1542\(2004\)085<0282:UVOISF>2.0.CO;2](https://doi.org/10.1644/1545-1542(2004)085<0282:UVOISF>2.0.CO;2).
- Mangiafico, S.S., 2024. rcompanion: Functions to support extension education program evaluation. R package version 2.4.36. (<https://cran.r-project.org/package=rcompanion/>).
- McGuire, B., Novak, M., 1984. A comparison of maternal behavior in the meadow vole (*Microtus pennsylvanicus*), prairie vole (*M. ochrogaster*) and pine vole (*M. pinetorum*). *Anim. Behav.* 32, 1132–1141. [https://doi.org/10.1016/S0003-3472\(84\)80229-8](https://doi.org/10.1016/S0003-3472(84)80229-8).
- Motomura, N., Shimizu, K., Shimizu, M., Aoki-Komori, S., Taniguchi, K., Serizawa, I., Saito, T.R., 2002. A comparative study of isolation-induced ultrasonic vocalization in rodent pups. *Exp. Anim.* 51, 187–190. <https://doi.org/10.1538/expanim.51.187>.
- Okon, E.E., 1970. The effect of environmental temperature on the production of ultrasounds in non-handled albino mouse pups. *J. Zool.* 162, 71–83. <https://doi.org/10.1111/j.1469-7998.1970.tb01258.x>.
- Okon, E.E., 1971. The temperature relations of vocalization in infant Golden hamsters and Wistar rats. *J. Zool.* 164, 227–237. <https://doi.org/10.1111/j.1469-7998.1971.tb01308.x>.
- Okon, E.E., 1972. Factors affecting ultrasound production in infant rodents. *J. Zool.* 168, 139–148. <https://doi.org/10.1111/j.1469-7998.1972.tb01344.x>.
- Pandouraki, I., 2011. Case of alarm vocalization in a colony of *Microtus guentheri* (Danford & Alston, 1880) (Mammalia, Rodentia, Arvicolidae) from Southern Bulgaria. *Z. oonotes* 20, 1–3.
- Powell, R.A., Fried, J.J., 1992. Helping by juvenile pine voles (*Microtus pinetorum*), growth and survival of younger siblings, and the evolution of pine vole sociality. *Behav. Ecol.* 3, 325–333. <https://doi.org/10.1093/beheco/3.4.325>.
- R Development Core Team, 2023. R: A Language and Environment for Statistical Computing. Vienna, R Foundation for Statistical Computing. (<https://www.R-project.org/>).
- Rabon, D.R., Sawrey, D.K., Webster, Wm.D., 2001. Infant ultrasonic vocalizations and parental responses in two species of voles (*Microtus*). *Can. J. Zool.* 79, 830–837. <https://doi.org/10.1139/cjz-79-5-830>.
- Riede, T., Kobrina, A., Bone, L., Darwaiz, T., Pasch, B., 2022. Mechanisms of sound production in deer mice (*Peromyscus* spp.). *J. Exp. Biol.* 225, jeb243695. <https://doi.org/10.1242/jeb.243695>.
- Rutovskaya, M.V., 2019. Sound communication in social voles (subgenus *Sumeriomys*). *Bioacoustics* 28, 503–521. <https://doi.org/10.1080/09524622.2018.1482787>.
- Rutovskaya, M.V., Volodin, I.A., Feoktistova, N.Y., Surov, A.V., Gureeva, A.V., Volodina, E.V., 2024. Acoustic complexity of pup isolation calls in Mongolian hamsters: 3-frequency phenomena and chaos. *zoad36 Curr. Zool.* 70. <https://doi.org/10.1093/cz/zoad036>.
- Sales, G.D., Smith, J.C., 1978. Comparative studies of the ultrasonic calls of infant murid rodents. *Devel. Psychobiol.* 11, 595–619. <https://doi.org/10.1002/dev.420110609>.
- Selçuk, A.Y., Kaya, A., Kefeliöglu, H., 2024. Integrative studies of *Microtus hartingi* and *Microtus guentheri* (Cricetidae, Arvicolinae) and taxonomic problem of *Microtus ebeylei*. *J. Comp. Zool.* 311, 131–144. <https://doi.org/10.1016/j.jcz.2024.07.002>.
- Shapiro, L.E., Insel, T.R., 1990. Infant's response to social separation reflects adult differences in affiliative behavior: a comparative developmental study in prairie and montane voles. *Devel. Psychobiol.* 23, 375–393. <https://doi.org/10.1002/dev.420230502>.
- Smorkatcheva, A.V., 1999. The social organization of the Mandarin vole, *Lasiopodomys mandarinus*, during the reproductive period. *Z. Saugtierf.* 64, 344–355.
- Szentgyörgyi, H., Kapusta, J., Marchlewska-Koj, A., 2008. Ultrasonic calls of bank vole pups isolated and exposed to cold or to nest odor. *Physiol. Behav.* 93, 296–303. <https://doi.org/10.1016/j.physbeh.2007.09.015>.
- Thanou, E., Paragamian, K., Lymberakis, P., 2020. Social but lonely: species delimitation of social voles and the evolutionary history of the only *Microtus* species living in Africa. *J. Zool. Syst. Evol. Res.* 58, 475–498. <https://doi.org/10.1111/jzs.12325>.

- Vieira, M.L., Brown, R.E., 2002. Ultrasonic vocalizations and ontogenetic development in California mice (*Peromyscus californicus*). *Behav. Process.* 59, 147–156. [https://doi.org/10.1016/S0376-6357\(02\)00089-X](https://doi.org/10.1016/S0376-6357(02)00089-X).
- Volodin, I.A., Efremova, K.O., Frey, R., Soldatova, N.V., Volodina, E.V., 2017. Vocal changes accompanying the descent of the larynx during ontogeny from neonates to adults in male and female goitred gazelles (*Gazella subgutturosa*). *Zoology* 120, 31–41. <https://doi.org/10.1016/j.zool.2016.09.001>.
- Volodin, I.A., Sibiryakova, O.V., Vasilieva, N.A., Volodina, E.V., Matrosova, V.A., García, A.J., Pérez-Barbería, F.J., Gallego, L., Landete-Castillejos, T., 2018. Old and young female voices: effects of body weight, condition and social discomfort on the vocal aging in red deer hinds (*Cervus elaphus*). *Behaviour* 155, 915–939. <https://doi.org/10.1163/1568539X-00003513>.
- Volodin, I.A., Rutovskaya, M.V., Golenishchev, F.N., Volodina, E.V., 2024. Startle together, shout in chorus: collective bursts of alarm calls in a social rodent, the Harting's vole (*Microtus hartingi*). *Behaviour* 161, 587–611. <https://doi.org/10.1163/1568539X-bja10274>.
- Warren, M.R., Campbell, D., Borie, A.M., Ford, C.L., Dharani, A.M., Young, L.J., Liu, R.C., 2022. Maturation of social-vocal communication in prairie vole (*Microtus ochrogaster*) pups. *Front. Behav. Neurosci.* 15, 814200. <https://doi.org/10.3389/fnbeh.2021.814200>.
- Wheeler, R.E., Torchiano, M., 2010. lmp: Permutation tests for linear models in R. R package version 1.1-2. (<https://cran.r-project.org/web/packages/lmp/>).
- Wilden, I., Herzel, H., Peters, G., Tembrock, G., 1998. Subharmonics, biphonation, and deterministic chaos in mammal vocalization. *Bioacoustics* 9, 171–196. <https://doi.org/10.1080/09524622.1998.9753394>.
- Yoshinaga, Y., Ohno, W., Shiraishi, S., 1997. Postnatal growth, development and ultrasonic vocalization, of young Japanese field voles, *Microtus montebelli*. *Mamm. Study* 22, 53–70. <https://doi.org/10.3106/mammalstudy.22.53>.
- Yu, P., Wang, J., Tai, F., Broders, H., An, S., Zhang, X., He, F., An, X., Wu, R., 2011. The effects of repeated early deprivation on ultrasonic vocalizations and ontogenetic development in mandarin vole pups. *Behav. Process.* 88, 162–167. <https://doi.org/10.1016/j.beproc.2011.09.001>.
- Yurlova, D.D., Volodin, I.A., Ilchenko, O.G., Volodina, E.V., 2020. Rapid development of mature vocal patterns of ultrasonic calls in a fast-growing rodent, the yellow steppe lemming (*Eolagurus luteus*). *PLoS One* 15, e0228892. <https://doi.org/10.1371/journal.pone.0228892>.
- Zaytseva, A.S., Volodin, I.A., Ilchenko, O.G., Volodina, E.V., 2019. Ultrasonic vocalization of pup and adult fat-tailed gerbils (*Pachyuromys duprasi*). *PLoS One* 14, e0219749. <https://doi.org/10.1371/journal.pone.0219749>.
- Zorenko, T., Kaija, L.P., 2024. Inbreeding tolerance in two isolated populations of Harting's vole *Microtus hartingi* (Rodentia, Arvicolinae). *Turk. J. Zool.* 48, 140–153. <https://doi.org/10.55730/1300-0179.3168>.
- Zorenko, T.A., 2023. Communal reproduction of females of two subspecies of Harting's vole *Microtus (Sumeriomys) hartingi* (Rodentia, Arvicolinae) under experimental conditions. *Biol. Bull. Russ. Acad. Sci.* 50, 1603–1614. <https://doi.org/10.1134/S1062359023070336>.
- Zorenko, T.A., Atanasov, N., Golenishchev, F.N., 2016. Behavioral differentiation and hybridization of the European and Asian forms of Harting's vole *Microtus hartingi* (Rodentia, Arvicolinae). *Russ. J. Theriol.* 15, 133–150. <https://doi.org/10.15298/rusjtheriol.15.2.06>.