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Exploring the acoustic behaviour of the Bavarian Pine Vole (Microtus bavaricus)

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Elizabeth Rosalind Wilkinson

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Zusammenfassung

Die Bayerische Kurzohrmaus (*Microtus bavaricus)* zählt sowohl zu den am stärksten bedrohten sowie den am wenigsten beforschten Arten Europas. Die Spezies verbringt den überwiegenden Teil ihres Lebens unter der Erde und ist in freier Wildbahn nur selten anzutreffen. Die Untersuchung ihrer vokalen Kommunikation bietet insofern die Möglichkeiten eines besseren Verständnisses ihres schwer zu beobachtenden Verhaltens sowie der Ableitung von Implikationen für künftige Schutzmaßnahmen. Die vorliegende Studie wurde im Alpenzoo Innsbruck durchgeführt, wo 2021 ein entsprechendes Zuchtprogramm implementiert wurde. Zunächst wurden dort Aufnahmen von einzelnen Zuchtpaaren sowie von Paaren, die unbekannten Artgenossen ausgesetzt waren, angefertigt, um Vokalisationen in verschiedenen sozialen Kontexten zu erfassen. Die Spektrogramme der Aufnahmen wurden anschließend mit einem Klassifizierungssystem annotiert, welches auf dem vokalen Repertoire anderer Nager basiert. Abschließend wurde eine unüberwachte Cluster-K-Mittelwertanalyse durchgeführt. Die Ergebnisse zeigten, dass *M. bavaricus* hauptsächlich Vokalisationen im Ultraschallbereich mit mehreren Silben und Obertönen erzeugte. Beim Hinzutreten eines unbekannten Männchen oder Weibchens zu einem Brutpaar waren deutliche Veränderungen des akustischen Verhalten zu beobachten. Dies legt nahe, dass *M. bavaricus* seine Lautäußerungen an den jeweiligen sozialen Kontext anpassen könnte. Durch einen Vergleich der manuellen Klassifizierung mit der unüberwachten Analyse konnten zudem konkrete Ansatzpunkte für Verbesserungen bei der weiteren Beschreibung des vokalen Repertoires identifiziert werden. Zusammenfassend bietet unsere Studie eine richtungsweisende Grundlage für eine weitergehende Erforschung des akustischen Verhaltens von *M. bavaricus*.

Abstract

The Bavarian pine vole (*Microtus bavaricus*) is one of Europe's most endangered species whose behaviour has never before been studied. The species lives much of its life underground and has rarely been found in the wild. Therefore, studying its vocal communication provides an opportunity to understand behaviour that is hard to observe and could have useful implications for future conservation efforts. This study took place at Alpenzoo Innsbruck, where a breeding programme was established in 2021. We took recordings of breeding pairs and also exposed them to unfamiliar conspecifics to record vocalisations in different social contexts. We annotated spectrograms of these recordings with a classification system based on other rodent species vocal repertoires and ran an unsupervised cluster K-means analysis. The results revealed that *M. bavaricus* emits mainly ultrasonic vocalisations with multiple syllables and harmonics. Comparing our manual classification and unsupervised analysis highlighted where improvements could be made in further descriptions of the vocal repertoire. There was a significant change in acoustic behaviour when an unfamiliar male or female was introduced to a breeding pair, suggesting that *M. bavaricus* adapts its vocalisation according to context. To further the understanding of the acoustic behaviour of M. bavaricus, future research can use our study as a basis to inform directions.

Acknowledgements

I would like to express my deepest gratitude to everyone who contributed to this project.

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Contents

Introduction and Background

The loss of species as a consequence of human impact in recent centuries indicates a sixth mass extinction event (Dirzo et al., 2014). Whilst extensive efforts have already been taken to implement research and conservation initiatives for many well-known endangered species, less recognisable endangered species have often been overlooked (Colléony et al., 2017; Kennerley et al., 2021). For example, the order Rodentia makes up the largest taxon of mammals (Sales, 2010), yet is underrepresented in conservation research (Verde Arregoitia, 2016). Small mammals, such as rodents, provide important ecosystem services (Singleton et al., 2015) and serve as bioindicators for the health of their habitats (Bertolino et al., 2015). Increasing research focus on overlooked taxons is important for tackling the loss of biodiversity.

Studying the behaviour of a species has an integral part in its conservation. Behaviour is the direct interaction of an animal with its environment. As a central mediator of individual fitness, it affects population dynamics (Goymann & Küblbeck, 2020). Therefore, understanding how individuals respond to their environment and surrounding animals can be beneficial in protecting populations and species.

Bioacoustics provides insights into the behaviour of species that are difficult to observe (Teixeira et al., 2019), such as rodents, who are mostly small and live in inaccessible habitats to human researchers. Knowledge of acoustic behaviour can be used to inform captive breeding programs, for species counts and to discover how individuals communicate. Understanding how individuals communicate and what they are communicating can be used in a range of contexts from measuring habitat use to genetic fitness (Terry et al., 2005).

Discovering the vocal repertoire of a species is an essential precursor to studying its social interactions. Sound propagation depends on the characteristics of the habitat so

vocalisations will likely be shaped to reduce distortion by the local environment. This and the quality and type of interactions among group members may affect the diversity of signals in the repertoire (Range & Fischer, 2004). Describing a vocal repertoire, therefore, gives insights into the life-history of a species and allows us to distinguish one species from another for research in the field.

In sum, a greater focus on conservation is required on previously understudied species. Research into acoustic behaviour provides methods for undertaking this in rodent species where visual observation is limited.

Acoustic communication

Communication is the process through which signals are transmitted from a signaller to a receiver, resulting in the receiver acquiring information that influences their behaviour (Seyfarth et al., 2010). A signal can occur in several modalities: acoustic, visual, tactile, olfactory, electric or seismic. Acoustic communication thus refers to the process by which acoustic information, which in many cases is produced through vocal production mechanisms, is perceived through the auditory system.

An Overview

Acoustic communication is both long range and information dense which gives it a distinct advantage over other modalities (Podraza et al., 2024). Sound can propagate rapidly through various environments, whereas visual signals for example cannot travel through opaque mediums and olfactory signals may take much more time to cover the same distance. These advantages are especially useful for subterranean and fossorial animals who need to communicate in habitats where visibility is limited (Stein & Rachlow, 2023).

Functions of Acoustic Communication. Acoustic communication forms an important part of many social interactions including mate selection and attraction, social organisation and territory defense (Beery et al., 2021). It forms the foundation of all social relationships between animals (Brumm & Slabbekoorn, 2005). In the study of behaviour, understanding communication is therefore essential for uncovering the complexities of animal interactions (Bradbury & Vehrencamp, 1998).

Acoustic signals can carry more complex messages than other modalities of communication, facilitated by adjustments to the spectral, temporal, and spectrotemporal properties of vocalisations. For example, alarm calls from yellow-bellied marmots can contain information about the individual, including its age and sex through variations in the waveform, call duration and frequency and amplitude of the partials (Blumstein & Munos, 2005).

Sound production Mechanisms. In mammals, vocalisations are produced by the combination of a source and a filter (Fant, 1960). The source includes the larynx and all sub-laryngeal and laryngeal structures, and the filter refers to the vocal tract, the passage from the larynx to the mouth and nose (Taylor & Reby, 2010). The larynx consists of the thyroid, cricoid and arytenoid cartilages. The cricoid cartilage connects the larynx to the trachea and lungs which provide an air flow for sound production. The thyroid and arytenoid cartilages are connected by the vocal folds, three layer structures of muscle, vocal ligament and the epithelium (Taylor & Reby, 2010). The glottis refers to the vocal folds and space between them and is where sound is produced.

The mechanism of sound production at the source is explained by the myoelastic-aerodynamic theory (MEAD) of phonation (van der Berg, 1958). The theory describes the self-sustained ‘flow-induced oscillation’ of the vocal folds (Chan & Titze, 2006). Initially the vocal folds are brought together by muscular tension, this leads to a build

up of subglottal pressure. This compresses the folds into a convergent shape, they continue to be compressed until they open fully. This wave of compression is called a traveling or mucosal wave (Berke & Long, 2010). The air flows through the glottis and releases pressure across the folds, in accordance with the Bernoulli principle (as air increases in speed, the subglottal pressure is reduced; Titze, 1980). The elastic recoil of the muscles closes the folds in a divergent position, creating a jet in the flow of air and the process begins once again. The rate at which this oscillation occurs determines the fundamental frequency of the sound wave.

The vocal tract then filters this wave by damping or enhancing certain frequencies through resonances within the vocal tract (Taylor & Reby, 2010). These are termed formants, the dispersion of the formants relates to the length of the vocal tract (Fitch, 1997). Formants allow for more information to be encoded in the resulting vocalisation.

Ultrasonic Vocalizations

In addition to the mechanism described above, rodents produce high-frequency whistles, widely known as ultrasonic vocalisations (USVs) (Pasch et al., 2017; Riede, 2011; Riede & Pasch, 2020). A driver of the large diversity of rodent species may be the origin of this new vocal production mechanism for USVs, similar to the role of a new vocal organ (the syrinx) in promoting bird diversification (Fernández-Vargas et al., 2022).

Functions of Ultrasonic Vocalizations. USVs make up a major part of acoustic communication for many rodents. They have been reported from at least 50 species in 30 genera of the suborders Sciuromorpha and Myomorpha. They are mostly between 25 and 200 ms in duration and between 20 and 80 kHz in frequency (Sales, 2010). The behavioural significance of USVs has been studied most extensively in rats and mice (Simola & Granon, 2019).

In Rats. Evidence suggests that rats emit ultrasonic calls as an expression of emotional arousal and motivation to influence the situation causing this arousal (Brudzynski, 2021). Rats emit 50 kHz calls in situations of positive emotional valence, such as affiliative interactions and for social coordination (appetitive 50 kHz USV (Wöhr & Schwarting, 2013)). Short, flat, 50 kHz contact calls have been observed in interactions with familiar conspecifics and scents of other rats (Brudzynski & Pniak, 2002; Wöhr et al., 2008). 50 kHz vocalisations are also produced when rats are tickled by humans, which has a buffering effect on anxiety when being handled. The vocalisations themselves may also play a role in buffering anxiety, as has been seen in other mammals (Brudzynski, 2021).

In situations causing negative emotional valence, rats emit vocalisations at around 22 kHz (aversive 22 kHz USV (Wöhr & Schwarting, 2013)). These calls have been seen in intraspecies agonistic interactions (Luciano & Lore, 1975) and long 22 kHz calls are given as appeasement after defeat (Takahashi et al., 1983). They also serve as alarm calls to predators (Blanchard, 1991) or perceived danger (Kaltwasser, 1991). Saito et al. (2016) found that rats responded positively to novel ambiguous cues when presented alongside playbacks of 50 kHz vocalisations and responded negatively to the same cues with 22 kHz vocalisations. Infant rats also emit isolation calls when separated from their mother, this is common in many rodents (Hofer, 2010; Figure 1).

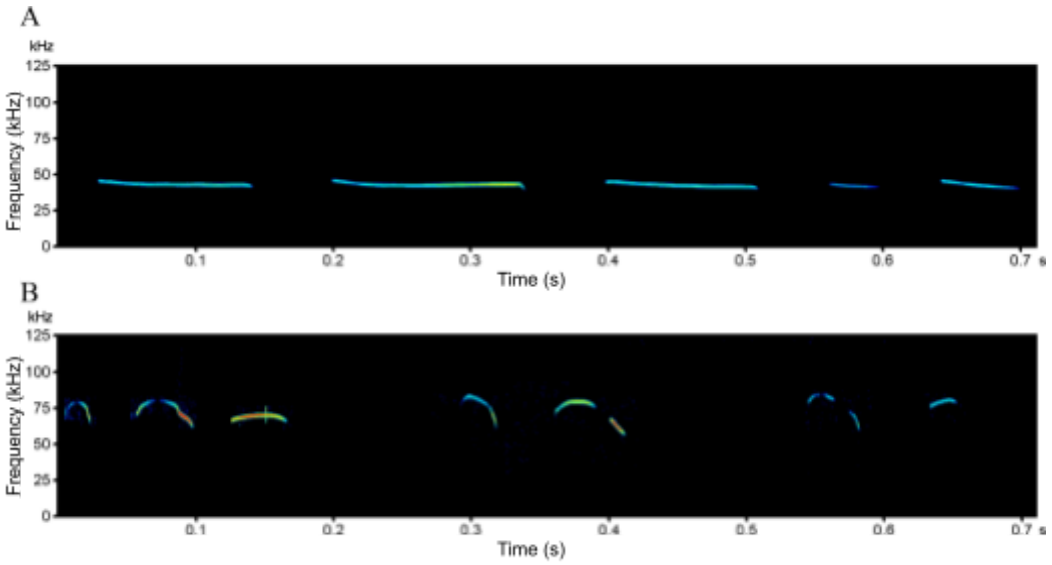


Figure 1. *Ultrasonic vocalisations of rat and mice pups.* A) Vocalisations of male rat pups. B) Vocalisations of male mice pups. Figure reproduced from Simola and Granon, 2019.

In Mice. Mice also emit USVs to communicate emotional states (Simola & Granon, 2019). However, the behavioural significance may not overlap with that of rats and some key acoustic parameters appear different (Portfors, 2007; Figure 1). Like rats, infant mice emit isolation calls (Figure 1), but, instead of appetitive and aversive, adult and juvenile vocalisations occur when adult males encounter females or female scents (female-induced) or during social interaction between conspecifics (interaction-induced; Wöhr & Schwarting, 2013).

Males emit USVs more abundantly when they sense a female than a male (Portfors and Perkel, 2014). They initially produce short syllables that become more frequent and complex during courtship and mounting (Zala et al., 2017). Females are attracted to vocalising males and prefer more complex USVs (Zala et al., 2020). Therefore, male USVs emitted by males are ascribed a role in courtship.

Territoriality also plays a significant role in mouse vocalisation. This has been investigated using the resident-intruder paradigm, in which individuals are housed in a cage

for a week to establish it as their territory, then an unfamiliar conspecific is introduced (Koolhaas et al., 2013). In these experiments the structure and volume of vocalisations of males and females were not found to be significantly different (Hammerschmidt et al., 2012), leading researchers to suggest that USVs emitted by males are used for more than just courtship and may have different meanings in different social contexts (Portfors & Perkel, 2014). These experiments also show different responses of residents to intruders of different sex, suggesting USVs have a role in establishing social hierarchies (Moles et al., 2007; Yao et al., 2023). This demonstrates how USVs in mice may have a variety of partly overlapping functions (Hammerschmidt et al., 2012).

Sound Production Mechanisms of Ultrasonic Vocalizations. The mechanism for the production of USVs was first investigated by Roberts (1975), who showed that the frequency of ultrasonic vocalisations produced by rats was greatly altered when breathing light gases but the audible vocalisations were not, suggesting different mechanisms of production.

The exact method of these whistles is still an active area of research (Boulanger-Bertolus & Mouly, 2021; Håkansson et al., 2022; Reide et al., 2022). Many observations have shown that sound production begins with airstream passing through a 1 to 2 mm gap at the back of the adducted vocal folds (Sanders et al., 2001; Reide et al., 2017). Measurements of the laryngeal muscle activity in rats suggests that the features of the ultrasonic sounds are controlled by the glottal shape (Riede, 2011; Riede 2013). Based on these initial observations researchers suggested a hole-tone whistle mechanism similar to that of a teakettle (Boulanger-Bertolus & Mouly, 2021). This would require two holes for sound production. The initial glottal opening disturbs the airflow then this disturbed flow passes

through a second hole between the epiglottis and base of the tongue, creating vortices responsible for the sound wave.

However, this was disproved by Mahrt et al. (2016) who showed that mice could still produce ultrasonic sounds when their epiglottis was removed. Instead they proposed the impinging jet mechanisms in which USVs could be produced by airflow from the glottal opening impinging on structures of the planar laryngeal wall (Figure 2, right). Riede et al. (2017) proposed instead that sounds were generated by an edge-tone whistle mechanism (Figure 2, left) when the airflow from the glottis hits the alar cartilage, a rodent-specific cartilage inside the larynx (Fernández-Vargas et al., 2022), and resonates around the ventral pouch, an air-sac-like cavity between the vocal folds and alar cartilage (Abhirami et al., 2023).

More recent research into USV production still fails to agree on the exact mechanism. Håkansson et al. (2022) created 3D models of the mouse and rat larynx without a ventral pouch and found that they could still produce USVs. However, Abhirami et al. (2023) found inconsistencies in the spectral properties of fictive and real USVs in models without the ventral pouch. Although the exact mechanism remains unclear, each of these studies has provided important steps towards a better understanding.

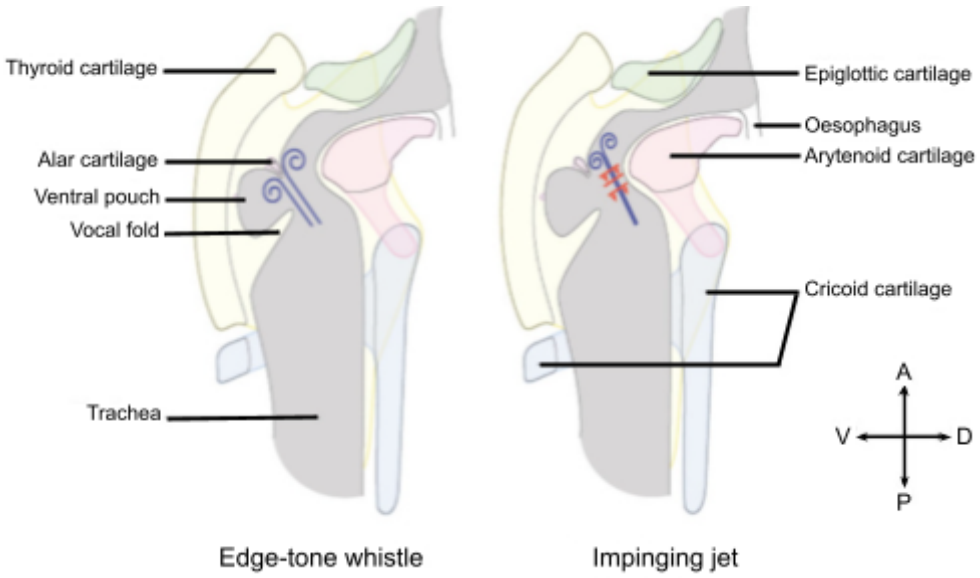


Figure 2. *Two potential mechanisms of high-frequency whistle production in the rodents.* The figure displays the proposed mechanisms of USV production, in which air flowing through a small hole in the dorsal side of the adducted vocal folds meets either the alar cartilage (Riede, 2017; edge-tone whistle) or the laryngeal wall (Mahrt et al., 2016; impinging jet). Figure adapted from Boulanger-Bertolus & Mouly (2021).

Acoustic communication in voles

The subfamily Arvicolinae (voles, lemmings and muskrats) shows a variety of degrees of kinship and acoustic system development. Therefore, it is well suited for studying communicative system evolution (Rutovskaya, 2020). Vocalisations in this subfamily have several functions. These include distress calls in agonistic interactions (genera: *Microtus, Lasiopodomys, Lagurus*, and *Clethrionomys*), male courtship of females (*Microtus, Alexandromys* and *Lasiopodomys*) and alarm calls (*Lasiopodomys brandti, Microtus gregalis* and *Microtus miurus*) (Rutovskaya, 2019). Although, the function of the alarm call of the singing vole (*Microtus miurus*) may instead serve as a territorial signal (Cole & Wilson, 2010).

Vole vocalisations range between 1 and 60 kHz (Table 1) and are less than 1 s long

(Mandelli & Sales, 2004; Blake, 2012; Rutovskaya, 2020; Warren et al., 2022; Table 1). USVs make up a large part of the calls used in the social behaviours of voles (Kapusta & Sales, 2009), with especially the 20 to 40 kHz range being used for social communication (Stewart et al., 2015).

Table 1. *Characteristics of the vocalisations of eight vole species.* The mean frequency (kHz) and duration (ms) of audible and ultrasonic calls of eight vole species. The studies of ultrasonic calls did not separate their subjects by sex, so do not provide separate male and female values.

Species	**Frequency (kHz)**	**Duration (ms)**	**Reference**
	Ultrasonic Range (>20 kHz)		
Common vole (*Microtus arvalis*)	29.2	67.1	Kapusta and Sales (2009)
Field vole (*Microtus agrestis*)	42.0	67.5	Kapusta and Sales (2009)
Bank vole (*Myodes glareolus*)	25.8	63.7	Kapusta and Sales (2009)
Prairie voles (*Microtus ochrogaster*)	34.067	1.20	Stewart et al. (2015)
	Audible Range (<20 kHz)		
Root vole (*Microtus oeconomus*)	female: 1.19 male: 1.46	female: 66 male: 64	Rutovskaya (2020)
Lacustrine vole (*Microtus limnophilus*)	female: 3.16 male: 3.49	female: 34 male: 39	Rutovskaya (2020)
Middendorf's vole (*Microtus middendorffii*)	female: 3.46 male: 3.57	female: 56 male: 41	Rutovskaya (2020)
Maximowicz's vole (*Microtus maximowiczii*)	female: 2.73 male: 3.03	female: 23 male: 23	Rutovskaya (2020)

Ultrasonic isolation calls have been observed in the pups of many vole species (Blake,

1992; Rabon et al., 2001; Blake, 2012). These calls have been linked to the inability to thermoregulate adequately when young (Blake, 2002). However, in North American *Microtus* species there appears to be a stronger relationship with the degree of parental care and the mating system (Mandelli & Sales, 2004).

Cherry and Lepri (1986) investigated ultrasonic vocalisation behaviour of pine voles (*Microtus pinetorum*) and found that males are the predominant vocalisers and emission of their USVs is regulated by hormones (androgens) produced in the gonads. They observed vocalisations in the audible range by both sexes in response to unfamiliar conspecifics, leading them to suggest that USVs are a component of sexual behaviour whilst audible vocalisations are a component of aggressive behaviour. This finding was supported by Kapusta et al. (2007) who concluded that USVs were more often produced by species with a gregarious and tolerant social organisation and did not appear in heterospecific, aggressive interactions. They suggested that USVs may decrease aggression and help maintain social structure. Males have also been found to emit the majority of USVs in prairie voles (*Microtus ochrogaster*; Lepri et al., 1988) and bank voles (*Myodes glareolus*; Marchlewska-Koj et al., 1994). A recent paper by Warren et al. (2024) showed that female prairie voles (*Microtus ochrogaster*) were able to identify their mate by USVs.

The Bavarian Pine Vole (*Microtus bavaricus)*

One of the rarest and least studied members of the subfamily Arvicolinae is the Bavarian Pine Vole (*Microtus bavaricus*). It was first discovered near Garmisch-Partenkirchen in Bavaria in 1962 (König, 1962). König described the voles having whitish whiskers, fur on top of their paws and as larger than the sympatric *Microtus subterraneus* but with slightly smaller ears (Figure 3). So far *M. bavaricus* has been found in moderately wet meadows on northern slopes (König, 1962) and in open woodland of pine

(Stille et al, 2024) and spruce (Martínková et al., 2007) forests. After the initial description *M. bavaricus* was not recorded in Germany for over 60 years. Man made changes to its terra typica, such as the construction of a hospital on the site, mean that this original population is most likely extinct (Pucek, 1985; Stille et al., 2024).

After the initial description of the species there have been very few reported sightings. In the year 2000, some individuals found in Northern Tyrol, initially assigned as *M. liechtensteini,* were identified as *M. bavaricus* by molecular genetic analysis of the mitochondrial control region (Haring et al., 2000). Further genetic analysis of an individual captured in the Rofan Mountains, Northern Tyrol in 2004 revealed that *M. bavaricus* and *M. liechtensteini* have the lowest observed divergence of any vole species, suggesting a very recent origin of both species (Martínková et al., 2007). In 2008, one individual was discovered in Croatia, in the area of Papuk Mountain. This led researchers to question the hypothesis that the Northern Alps were a glacial refuge for the species, as proposed by Spitzenberger et al. (2000). Instead they suggest a much larger Pleistocene distribution that was fragmented later on by competition with *M. subterraneus* (Tvrtković, 2010). Most recently, one individual was identified near Mittenwald, Bavaria, making this the first sighting in Germany since the original species description (Stille et al, 2024). Stille (2024) proposed that undiscovered populations in Bavaria are likely as their habitats are difficult to access and their lifestyle is secretive.

Today, the populations of *M. bavaricus* in Austria are closely monitored and land use has been adapted to their needs, however fewer individuals are observed each year (Stille et al., 2024). The species is considered to be one of the most endangered rodent species in Europe (Temple & Terry, 2007) and is classified as Critically Endangered on the IUCN Redlist (Meinig, 2019). Therefore, in September of 2021 André Stadler established a

breeding programme at Alpenzoo Innsbruck with some individuals captured in the Rofan Mountains (Stadler, 2023).

The first two offspring of the Alpenzoo inhabitants were born in 2022 and the programme has since successfully bred a captive population. The programme is thought to make up around 10% of the world's population, since the number of individuals in the Rofan Mountains is estimated to be less than 100 (Stadler, 2023). The programme provides opportunities to study the behaviour and communication of *M. bavaricus.* This could allow for the development of improved monitoring techniques and conservation measures specifically tailored to the species.

Figure 3. *A Bavarian Pine Vole (Microtus bavaricus) at Alpenzoo Innsbruck*. *Microtus bavaricus* of the suborder *Myomorpha*, family *Cricetidae*, subfamily *Arvicolinae*, subgenus *Terricola*. This individual is part of the captive breeding programme at Alpenzoo Innsbruck. Picture provided by André Stadler.

Research objectives

This is the first time the acoustic behaviour of *M. bavaricus* has been studied. Therefore, we aim to provide an initial description of the vocal repertoire and investigate the frequency range in which most of the vocalisations appear. We will also conduct an investigation into the social contexts that shape the vocal behaviour of *M. bavaricus* by monitoring the vocalisations produced in response to male and female conspecifics.

Based on other rodent vocal repertoires we hypothesis that:

(i) the majority of vocalisations will be situated in the ultrasonic range (>20 kHz),

(ii) the types of calls emitted will vary in different social contexts.

Our results will provide the essential basis for further directed investigations into the acoustic behaviour of *M bavaricus*.

Materials and Methods

Subjects and Housing

Our subjects were part of the *Microtus bavaricus* ex-situ breeding programme at Alpenzoo Innsbruck, established by zoo director André Stadler in September 2021 (Stadler, 2022). Most of the voles are kept behind the scenes of the zoo where they can be closely monitored as this was the first zoo in the world to keep the species. Therefore, captive individuals have been under ethological observation since they arrived to obtain basic data on their life history parameters. At the time of recording, the breeding programme consisted of 10 individuals (4 male, 4 female, 2 undetermined; Table 2).

Table 2. *Individuals housed at Alpenzoo Innsbruck in March 2024, their sex, place of birth, date of birth, sire and container number.* An overview of our study subjects sex, origin and current housing. The sire and exact birth date of the wild born individual is unknown. Individuals in container 5 were moved to Salzburg before official recordings started.

ID	Sex	Place of birth	Date of birth	Sire	Container
M1429	Female	Alpenzoo	15.03.2023	M1405	1
M1416	Male	Alpenzoo	07.09.2022	M1373	1
M1374	Female	Tyrol: Wild born	Estimated 15.06.2020	N/A	2
M1405	Male	Alpenzoo	13.06.2022	M1373	2
M1428	Undetermined	Alpenzoo	04.12.2022	M1405	3
M1457	Male	Alpenzoo	06.06.2023	M1405	3
M1458	Female	Alpenzoo	06.06.2023	M1405	4
M1459	Male	Alpenzoo	19.07.23	M1405	4
M1463	Female	Alpenzoo	16.09.2023	M1405	6

M1434	Undetermined	Alpenzoo	17.05.2023	M1406	6

They were housed in 5 plastic containers each housing a male-female breeding pair. In each container was a 10 to 15 cm layer of natural soil with bark, roots, stones and sticks both in and on it and some dry grasses planted in it. The voles use these materials to build subterranean tunnel systems as they would in the wild. They also had a small wooden house in the container to knaw and wear their teeth down on. These houses could not be too high as the voles can jump and climb very well. The containers were covered with a wire mesh. They had access to food (i.e., fruit, vegetables, leaves, berries and mealworms) and water ad libitum. Both were refreshed and their toilet corner was cleaned once a day between 12 pm and 1 pm. The room in which the containers lay was kept between 14 and 16.5°C with a humidity of 63 to 75 % and a light source simulating a constant day-night rhythm.

Data collection

Data was collected between the 8th and 23rd of March 2024 by Alice Mengarelli and me. We first arrived at the zoo on the 4th and spent 4 days determining our protocol. We positioned an ultrasonic condenser microphone CM16/CMPA (frequency range: 2 kHz – 200 kHz) at the top of a container through a hole in the metal mesh. The microphone was connected to an ultraSoundGate 116 (Avisoft Bioacoustics). We took recordings with the Avisoft RECORDER USGH (v. 4.4.1-30, Avisoft Bioacoustics; 300 kHz sampling rate, 16 bit format, 256 Hz FFT Size, permanent continuous recording, 5 minute files). To minimise disturbances that might discourage vocalisation, we used a 10 m XLR cable between the microphone and the SoundGate, connected to a laptop, so that we could initiate and monitor recordings from outside the vole's room. We used a soundproofing setup made from styrofoam and acoustic foam around the container being recorded to avoid picking up vocalisations from other containers.

Between the 8th and 18th of March 2024, we took recordings between 2 pm and 9 am. We used a random number generator to determine the container to be recorded each day. Each container was recorded on two non-consecutive days.

Manipulations

We introduced experimental manipulations between the 18th and 23rd of March 2024, following the resident-intruder paradigm. We implemented two conditions: introducing an unfamiliar male (condition 1) or an unfamiliar female (condition 2) to the breeding pair whose container we were recording. We then recorded between 9 am and 11 am. The unfamiliar conspecifics were placed into the main container in a smaller box for their protection. To minimise stress, they were removed after an hour. We also tested a manipulation (condition 3) in which we distributed faeces of unfamiliar voles around the container to be recorded. However, we decided to disregard this condition from further analysis. We implemented each of the three conditions on two non-consecutive days. Again the container to be recorded was determined by a random number generator. Baseline recordings (i.e. without the manipulation) resumed at 2 pm, after the daily cleaning and feeding.

Ethical approval

This study complies with all applicable Austrian laws and was conducted in accordance with the Guidelines for the Treatment of Animals in Behavioral Research and Teaching (ASAB/ABS, 2015). The research was approved by the Animal Ethics and Experimentation Board of the Faculty of Life Sciences, University of Vienna No./Nr. 2024-006.

Data preparation

The continuous recordings were sliced into 5 minute segments. These segments were saved as audio files in the WAV format.

First, to identify high-quality files with the richest call content, we screened all files in Praat (v. 6.4.18; view range 0-150 kHz, window length = 0.005 s, dynamic range 60 dB; Boersma and Weenink, 2024), leading to the selection of 50 potentially suitable files.

Next, based on a literature review of the number of calls used in studies of mammalian vocal repertoires (Mean = 3702; Table 3), we selected 9 files to analyse, 3 from the baseline recordings, 3 from condition 1 and 3 from condition 2. From our initial screening we estimated that these 9 files would contain a sufficient number of calls to give us a representative view of the vocal repertoire of *M. bavaricus*.

Table 3. *The number of calls used in studies of the vocal repertoire of mammals.* This table shows the study species and number of calls used in papers that aimed to investigate the vocal repertoire of these species. These papers were compiled from Google Scholar searches in August 2024 using the search term "vocal repertoire" and combinations of the additional search terms "size", "mammal" and "rodent".

Study animal	**Number of calls**	**Reference**
Degu (*Octodon degus*)	3535	Long (2007)
Female chacma baboons (*Papio cynocephalus ursinus*)	513	Fischer et al. (2008)
Common marmosets (*Callithrix jacchus*) (free living)	754	Bezzera & Souto (2008)
Capybara (*Hydrochoerus*	2069	Barros et al. (2010)

hydrochaeris)		
Barbary macaques (*Macaca sylvanus*)	8479	Hammerschmidt & Fischer (2010)
Giant mole-rats (*Fukomys mechowii*)	1175	Bednářová et al. (2012)
Western gorillas (*Gorilla gorilla*)	2163	Salmi et al. (2013)
Chacma baboons (*Papio ursinus*)	912	Wadewitz et al. (2015)
Common marmoset (*Callithrix jacchus*) (captive)	34629	Agamaite et al. (2015)
Mashona mole-rat (*Fukomys darlingi*)	932	Dvořáková et al. (2016)
Ring-tailed coati (*Nasua nasua*)	1457	Gasco et al. (2017)
North American flying squirrel (*Glaucomys sabrinus*)	815	Gilley et al. (2019; Species 1)
North American flying squirrel (*Glaucomys volans*)	486	Gilley et al. (2019; Species 2)
Female northern grey gibbon (*Hylobates funereus*)	933	Clink & Klinck (2020)
Northern Giant Mouse Lemur (*Mirza zaza*)	1493	Hending et al. (2020)
Grey mouse lemur (*Microcebus murinus*)	2123	Romero-Mujalli et al. (2021)
Diademed Sifaka (*Propithecus diadema*)	3814	Valente et al. (2022; Species 1)
Indri (*Indri indri*)	3360	Valente et al. (2022; Species 2)
Black-and-white ruffed lemur (*Varecia variegata*)	688	Batist et al. (2023)
Mean number of calls	3702	

Call detection and feature extraction

Spectrograms were created in STx, S_TOOLS-STx (v. 5.0.6, Acoustics Research Institute, Austria; range 50 dB, floor -80 dB, frame 4 ms, overlap 75%, window = hanning, frequency range 0-150 kHz). We used an automatic mouse ultrasound detector (A-MUD; v.

3.2; resolution 300 Hz, hopsize 0.5 ms; Zala et al., 2017), developed for wild-derived house mice (*Mus musculus musculus*) and adjusted for voles by Anton Noll, to detect ultrasonic vocalisations and extract acoustic parameters (Table 4). We manually reviewed all the calls, adding false negatives and removing false positives, and annotated each one with a specific call type using a custom built template.

Table 4. *The acoustic features obtained by the automatic mouse ultrasound detector (A-MUD) used in our analysis.* Descriptions of the acoustic features extracted for each ultrasonic call that were used in our analysis, based on Noll (2017; Description of the Algorithm) and Zala et al. (2017; 2020).

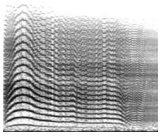
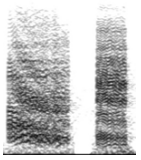
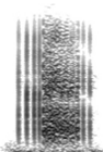
Parameter	Description	Unit
Length	Duration of the call	ms
fSlope	Frequency slope of all detected points in the call obtained by a linear regression	kHz/ms
fMean	Mean frequency of all detected points in the call	kHz
aMean	Mean amplitude of all detected points in the call	dB
fTmin	Frequency of the first time point	kHz
aTmin	Amplitude of the first time point	dB
fTmax	Frequency of the last time point	kHz
aTmax	Amplitude of the last time point	dB
fFmin	Minimum frequency of the call	kHz
aFmin	Amplitude at the point of the minimum frequency	dB
fFmax	Maximum frequency of the call	kHz
aFmax	Amplitude at the point of maximum frequency	dB
fAmax	Frequency at the point of maximum amplitude	kHz
aAmax	Maximum amplitude of the call	dB

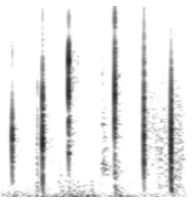
Call Classification

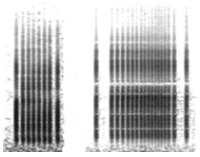
To annotate our spectrograms, we created a classification system based on the vocal repertoires of mice (Zala et al., 2017; Marconi et al., 2020; Zala et al., 2020) and other rodents (Wilden et al., 1998; Ma et al., 2014; Zaytseva et al., 2019, 2020; Yurlova et al., 2020; Table 5). We defined the inter-call distance as 10 ms, so elements separated by less than 10 ms were considered as part of one call. Firstly, calls were classified according to their frequency range: audible (0 - 20 kHz; AUD), mid-frequency vocalisation (appearing in both AUD and above, with > 50 % of the call below 20 kHz; MFV), ultrasonic vocalisation (> 20 kHz; USV) or clicks (appearing as a vertical line on the spectrogram that can cover both the AUD and USV range; CLICK). They were then assigned a call type within these categories. The USVs had multiple steps of classification so we established a hierarchy. We noted whether they had harmonic elements, then the number of syllables, and then the shape of the main syllable (the syllable lowest frequency or highest energy if this was not clear) or by time if the syllable was under 10 ms.

Rodent vocalisations also include nonlinear phenomena (NLP; Volodin et al., 2021; Reide et al., 2022). These are irregularities in the acoustic features of a call resulting from irregular vibrations of the vocal fold vibration (Fitch et al., 2002). In our classification we originally included two NLP: biphonation, the simultaneous production of two independent fundamental frequencies, and subharmonics, occurance of frequency bands at 1/n of the fundamental frequency (Yurlova et al., 2020; see Table 1 in the Supplementary Material). However, we found these phenomena hard to identify in practice so excluded them from our data analysis.

Table 5. *Our classification system, devised based on literature exploring the vocalisation types of other rodents.* We compiled a classification system based on other vole and closely related rodent species in order to annotate our recordings of *Microtus bavaricus* vocalisations.

Call type	Abbreviation	Definition	Spectrogram	Reference
Audible	AUD	Entirely < 20 kHz		
Normal harmonic		A fundamental frequency band with harmonics		Zaytseva et al., 2020 (Figure 2e)
Separated squeaks		Short calls with harmonics well visible		Zaytseva et al., 2020 (Figure 2c)
Mid-frequency squeaks		Pulses of the fundamental frequency band and harmonics, general appearance resembling noise		Zaytseva et al., 2020 (Figure 2b)
Mixed		Several AUD types merged into one call, still distinguishable from each other		Zaytseva et al., 2020 (Figure 2e)
Unclassified		Calls that could not be assigned to any AUD category		Zaytseva et al., 2020
Mid-frequency vocalisations	MFV	Calls in both the audible and ultrasonic range where > 50 % of the call is below 20 kHz and ≤ 1 harmonic		Grimsley et al., 2016
Ultrasonic vocalisations	USV	At least 50 % ≥ 20 kHz		Images from Zala et al., 2020 (Figure 1)
Ultrashort	us	< 5 ms		Hanson & Hurley, 2012
Short	s	< 10 ms		Hanson & Hurley, 2012

Flat	f	< 5 kHz frequency modulation		Hanson & Hurley, 2012
Down	d	Decrease in frequency > 5 kHz		Hanson & Hurley, 2012
Up	up	Increase in frequency > 5 kHz		Hanson & Hurley, 2012
U-shaped	u	First decrease then increase in frequency > 5 kHz each		Hanson & Hurley, 2012
Chevron	ch	First increase then decrease in frequency > 5 kHz each		Hanson & Hurley, 2012
Complex	c	Two or more directional changes in frequency > 5 kHz each		Hanson & Hurley, 2012
Complex variations	c2, c3, c4…	Two/more syllables separated by frequency jumps but without time separation	 	Hanson & Hurley, 2012
Harmonic	h	Calls with harmonic components		Hanson & Hurley, 2012
Unclassified		Calls that could not be assigned to any USV category		Scattoni et al., 2011; Grimsley et al., 2016
Click	CLICK	Clearly separated vertical lines that may be entirely in the audible, ultrasound, or both: fairly separate lines not attached as in “Click-like"	 Shown here in the audible range	Zaytseva et al., 2020 (Figure 2d)

Click-like	Series of irregularly spaced vertical lines grouped closely together in the audible range		Zaytseva et al., 2020 (Figure 2a)

Data analysis

All statistical analyses were performed in R (v. 4.2.2; R Core Team, 2022) using RStudio (v. 2024.12.0.467; RStudio Team, 2020). A significance level of $\alpha = 0.05$ was used for all statistical tests. For multiple comparisons, a Benjamini-Hochberg correction was applied.

We explored the distribution of call types and sub-call types across all annotated files. We used the acoustic parameters extracted by A-MUD to explore the mean acoustic features of the USVs. The distribution of mean call frequencies (fMean) was visualised in a combined Kernel Density Estimate (KDE) and histogram plot. For this analysis and visualisation of the results we used the packages: *dplyr, tidyr, knitr, ggplot2, RColorBrewer* and *gridExtra.*

Checking acoustic data obtained by A-MUD

To validate the acoustic features extracted by the A-MUD algorithm, we conducted a manual check of key frequency variables in STx. We selected a random sample of 10% of the USVs from the male and female datasets for this analysis. For each call, we collected the following parameters: the starting frequency, the ending frequency, the minimum frequency and the maximum frequency. Additionally, the mean frequency was calculated as the mean of the minimum and maximum frequencies.

The resulting data was explored using the following packages: *dplyr, tidyr, ggplot2, ggpubr* and *gridExtra*. We calculated Pearson's correlation coefficients (*R*) and the significance level (*p*) to assess the relationship between the frequency parameters extracted

by A-MUD and the corresponding manually determined values. These coefficients were used to evaluate the accuracy and reliability of the algorithm.

K-means cluster analysis

We used an unsupervised cluster model (K-means) to define call types based on the acoustic parameters extracted by A-MUD. Unsupervised cluster analysis allows us to explore categories within our data from an objective view and possibly highlight pitfalls in our classification system. We adapted the methods from Langehennig-Peristenidou et al. (2023), who provided R scripts used for their analysis. The analysis was run using 2963 USVs. Before running the model, Pearson's correlations were calculated between the acoustic parameters. For correlations higher than 0.7 only one parameter was used. The remaining data was standardised (z-score) to prevent the different range of units of measurement falsifying the model. We ran the model iteratively (500 repetitions, 100 maximum clusters, 100 random starts) using a function in the "tihoCluster" package from Romero-Mujalli et al. (2021; github R package: https://github.com/danielrm84/tihoCluster). The number of clusters was calculated using an elbow figure considering the ratio between total within-cluster Sum of Squared Errors (SSE) and the total SSE. The optimal number was determined by the model as the intersection of the two lines that best fit the elbow data; this process is known as the knee method. Each call was assigned a cluster number and clusters were visualised two dimensionally via a t-SNE (t-distributed stochastic neighbor embedding) analysis using the package *Rtsne*. We used the cluster numbers assigned to each call to explore how calls in each cluster looked. We compared our manual annotation to the clustering by the K-means analysis by calculating an adjusted Rand index using the *mclust* package.

Differences between conditions

We also explored the differences in calls between conditions. We compared the distribution of call types with a Pearson's chi-squared test. We compared the number of USVs per condition with a one-way ANOVA, followed by Tukey post-hoc tests between conditions. To ensure our data met the assumptions for an ANOVA a Shapiro-Wilk test for normality and Levene's test for homogeneity of variances, using the package *car,* were used.

We also explored the differences in acoustic parameters with Kruskal-Wallis tests between all conditions. For significant results we performed further pairwise Wilcoxon signed rank tests between each condition and the others. Both the Kruskal-Wallis and Wilcoxon tests were corrected for multiple comparisons with the Benjamini-Hochberg correction. To visualise these results we used the packages: *ggplot2, RColorBrewer, gridExtra* and *ggsignif*.

Further Kruskal-Wallis and Wilcoxon tests were carried out between the three annotated files within each condition to check whether differences between conditions could in fact be ascribed to differences in files.

Results

Call types

We identified 5605 calls in total (AUD = 106; CLICK= 2500; MFV = 25; USV = 2963; other = 11; Figure 4A). USVs constituted the majority of the acoustic repertoire (53% of identified calls), so they were the primary focus of our analysis. They consisted mainly of harmonic calls (1718; Figure 4D), complex variations (386; c2 = 306, c3 = 71, c4 = 8, c5 = 1; Figure 4D) and flat calls (366; Figure 4D). As shown in Table 6, USVs had a mean length of 96.31 ± 1.07 ms, a mean frequency of 30.66 ± 0.15 kHz (Figure 5) and a mean amplitude of 15.56 ± 0.05 dB.

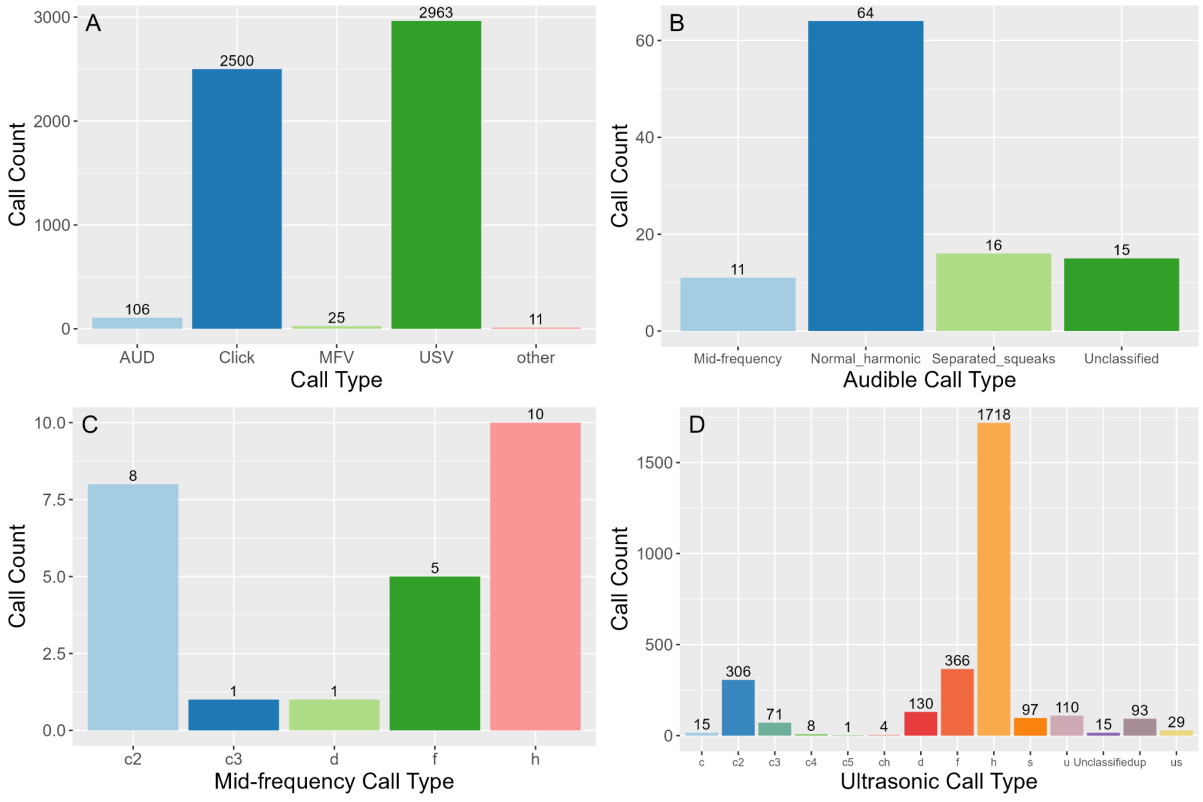


Figure 4. *Call counts of each call type and sub-call type.* A) The number of calls in the main call types within our data (AUD, CLICK, MFV and USV) and that could not be classified (other). B) The number of calls in each AUD call type. C) The number of calls in each MFV

call type. D) The number of calls in each USV call type. See Table 5 for classification of call types.

Table 6. *Acoustic parameters of USVs.* The mean and standard error of duration, frequency and amplitude parameters extracted by the A-MUD algorithm (ncall = 2963).

Parameter	**Mean**	**Standard Error**
Length (ms)	96.31	1.07
fSlope (kHz/ms)	0.00	0.02
fMean (kHz)	30.66	0.15
aMean (dB)	15.56	0.05
fTmin (kHz)	32.91	0.22
aTmin (dB)	11.53	0.02
fTmax (kHz)	32.16	0.21
aTmax (dB)	11.42	0.02
fFmin (kHz)	25.33	0.16
aFmin (dB)	12.65	0.05
fFmax (kHz)	40.31	0.31
aFmax (dB)	12.46	0.06
fAmax (kHz)	30.66	0.16
aAmax (dB)	22.11	0.11

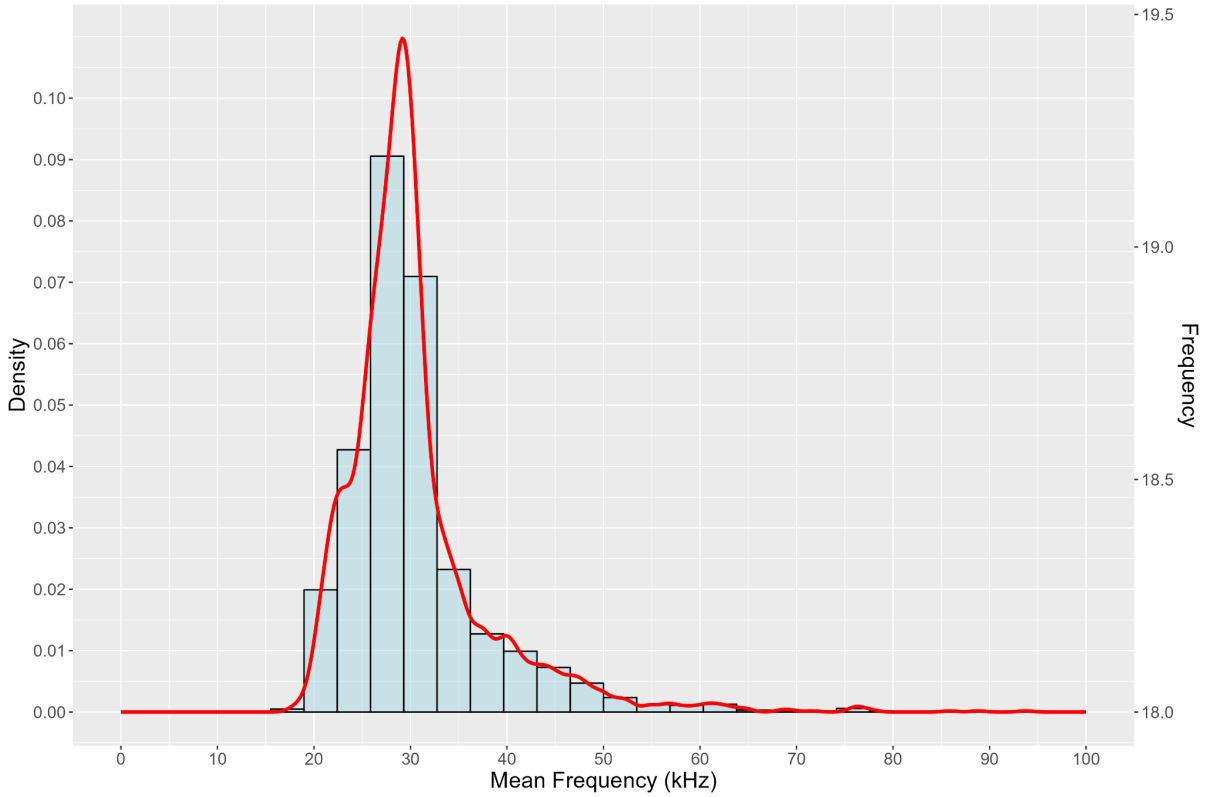


Figure 5. *Distribution of USV frequencies.* Combined Kernel Density Estimate (KDE) and histogram of the mean call frequencies (fMean) of the USVs identified.

We identified calls from all categories of our classification system (Figure 6). Audible calls had many harmonics, whilst USVs had distinct fundamental frequencies and few harmonics.

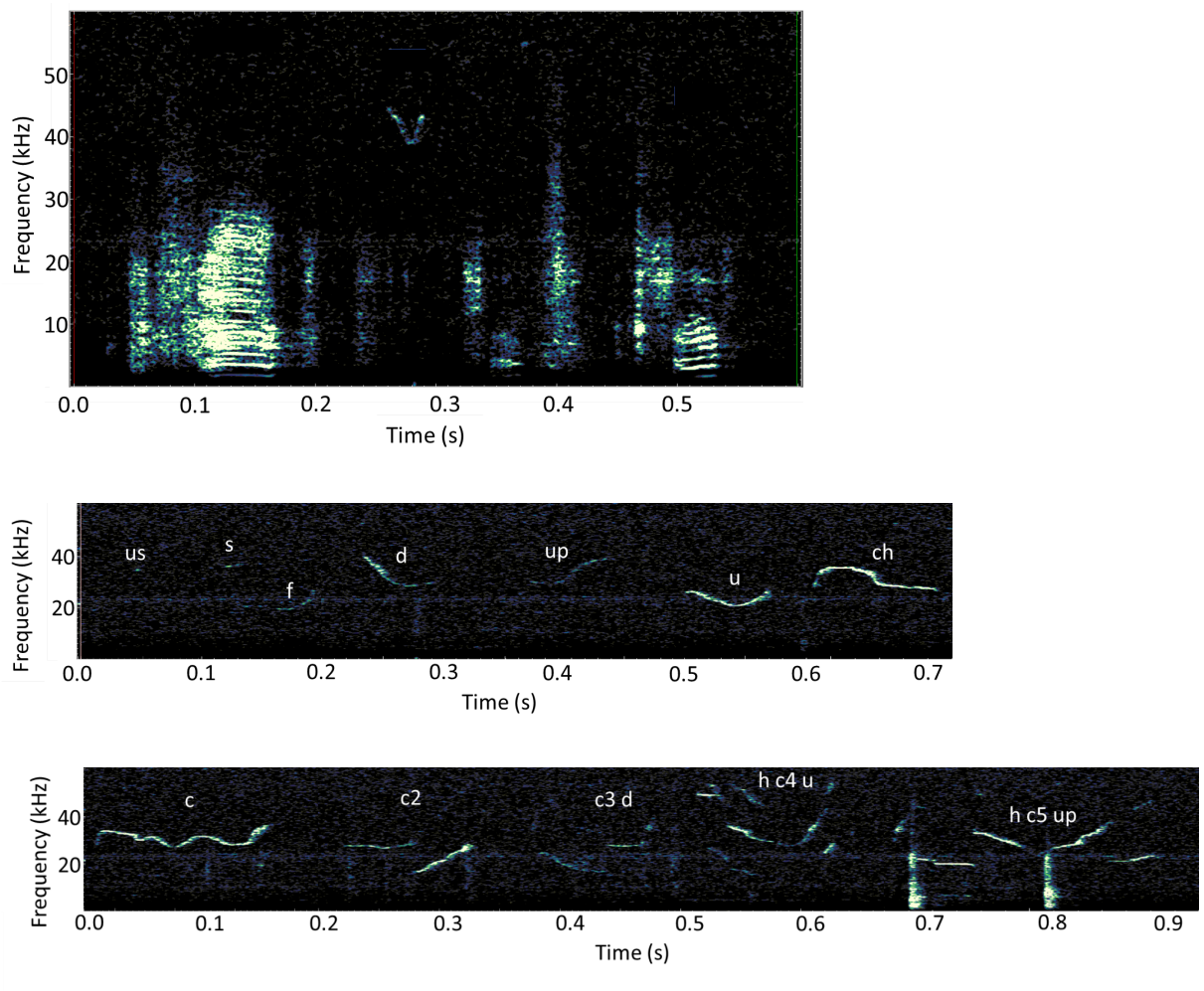


Figure 6. *Spectrograms of our identified call types.* Spectrograms extracted from STx show the calls we categorised according to our classification system (Table 5). The first spectrogram shows an audible 'normal harmonic' call, the next two show USV call types, all shown in windows between 0 and 60 kHz. Calls h c4 u and h c5 up demonstrate our hierarchy of classification as these calls contain harmonics (h) and complex variations (c4/c5).

Quality of acoustic data obtained by A-MUD

Pearson's correlations between manually checked parameters and those obtained by A-MUD were all significant ($p<0.001$; Figure 7).

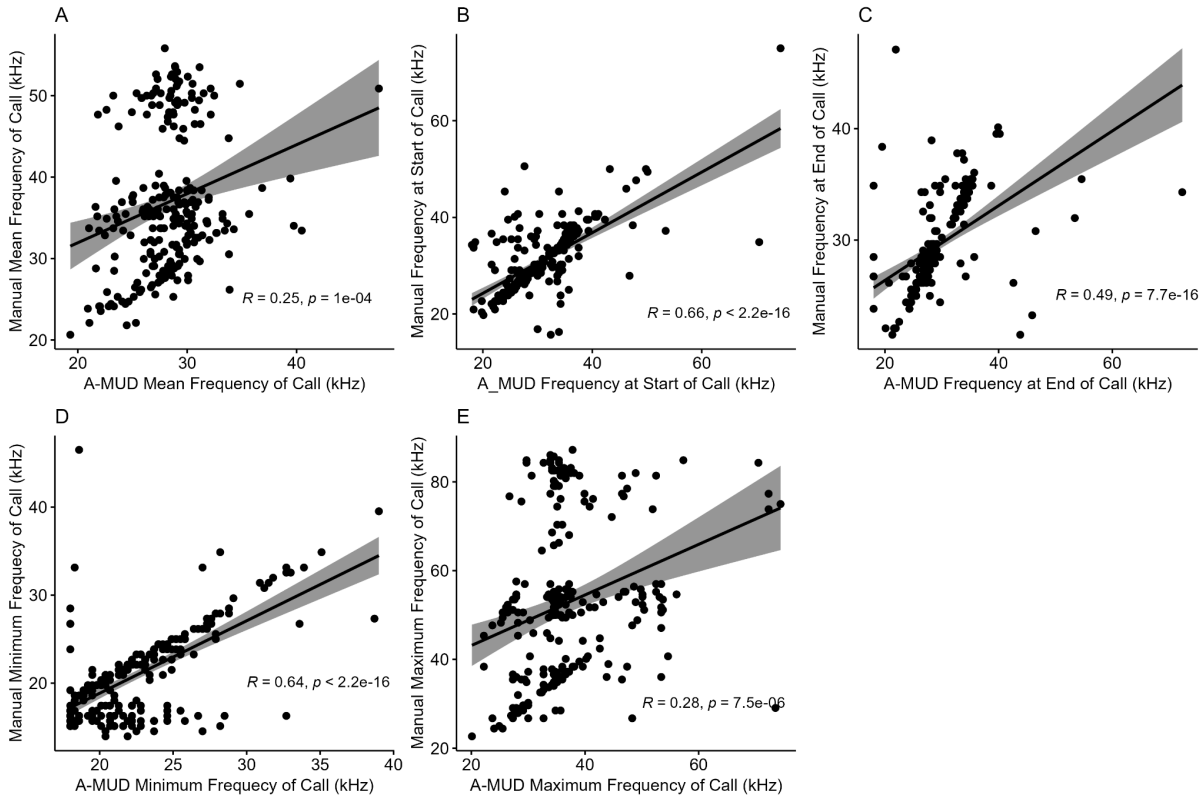


Figure 7. *Correlations between manually checked parameters and those detected by A-MUD.* Manually checked frequency parameters plotted against the corresponding A-MUD determined frequency parameter with Pearson's correlation coefficient (R) and significance (p) values.

Cluster analysis

We used 10 acoustic parameters for this analysis. We found four Pearson's correlation coefficients between parameters of over 0.7, and thus excluded one of the parameters in each correlation to avoid distorting cluster shapes (fTmax, fFmin, fAmax, aAmax; see Figure 1 in Supplementary Material). The K-means clustering algorithm and knee method determined 13 clusters (see Figure 2 in Supplementary Material), representing 13 different call types, that were visualised with the t-SNE analysis (Figure 8). Some call types of the clusters include: complex harmonic (cluster 1), u-modulated (cluster 2), complex u harmonic (cluster 6), simple harmonic (cluster 7) and flat (cluster 9; for mean acoustic features of each cluster see

Table 2 in Supplementary Material). The adjusted Rand index determined a low level of similarity between the manual classification and K-means clustering (ARI = 0.1).

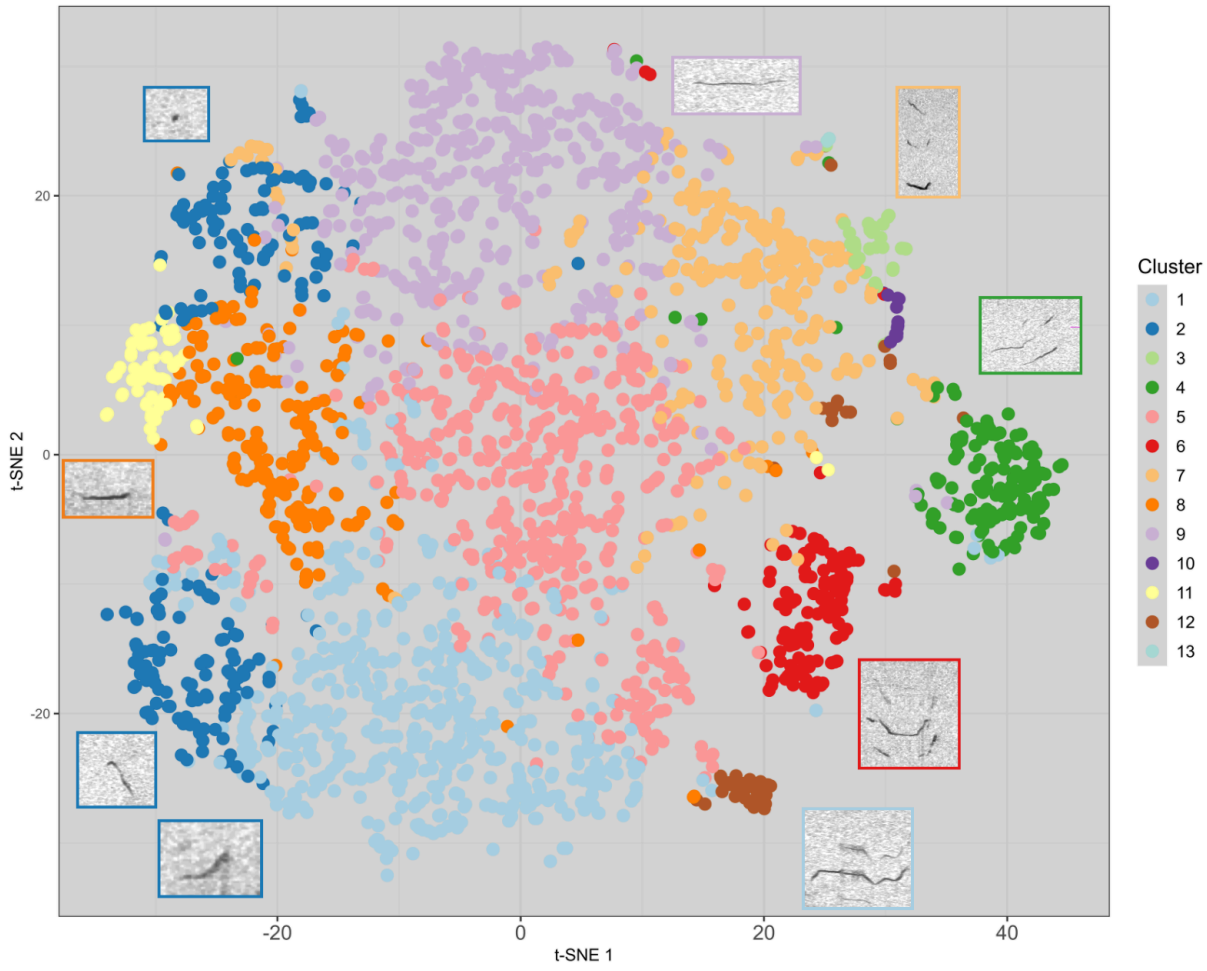


Figure 8. *Call types obtained from the unsupervised K-means cluster analysis.* Each colour represents a distinct cluster of calls determined by our unsupervised K-means cluster analysis. The results of the K-means analysis were visualised by t-SNE (t-distributed stochastic neighbor embedding) analysis, a technique for visualising highly dimensional data in two dimensions. Call types have been matched to clusters where possible.

Differences between conditions

The total number of calls and call types varied across conditions (Figure 9A). In the baseline recordings (Basic), 1182 calls were identified (AUD = 49; CLICK = 495; MFV= 15;

USV= 612; Other = 11; Figure 9B). For manipulation 1 (Male), in which an unfamiliar male was introduced to a breeding pair, 3479 calls were identified (AUD = 29; CLICK = 1152; MFV= 3; USV= 2295; Figure 9C). In manipulation 2 (Female), in which an unfamiliar female was introduced to a breeding pair, 944 calls were identified (AUD = 28; CLICK = 853; MFV= 7; USV= 56; Figure 9D). A Pearson's chi-squared test was conducted to determine whether the distribution of call types differed significantly across the three conditions. The results showed a significant association between call types and condition, χ^2 (df = 8, ncall = 5605) = 1183.8, $p < 0.001$.

Concerning USVs, a one-way ANOVA comparing the total number of USVs across the conditions found a significant difference between conditions (ncall = 2963, p = 0.016). Tukey post-hoc tests revealed that there was no significant difference between the Basic and the Female conditions (ncall = 668, M diff = -185.33, p = 0.598) or the Basic and the Male condition (ncall = 2907, M diff = 561.00, p = 0.051). The Male condition had significantly more calls than the Female condition (ncall = 2351, M diff = 746.33, p = 0.016).

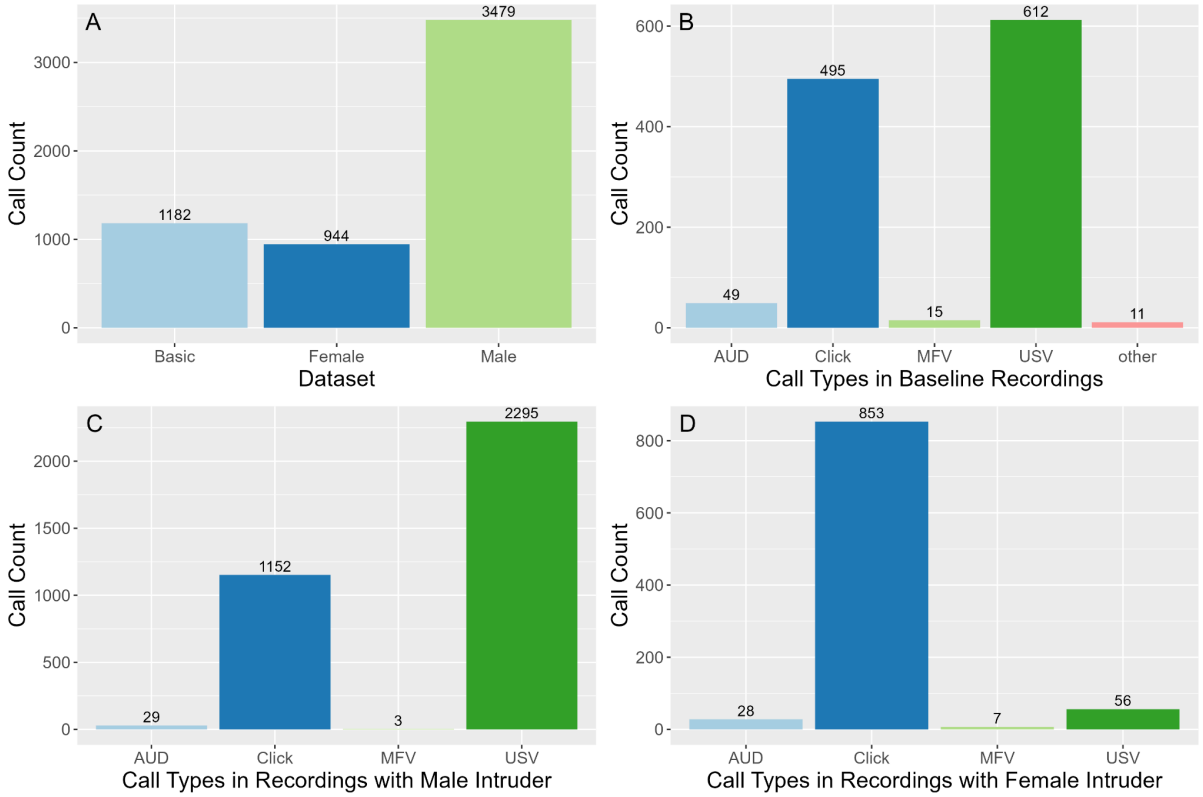


Figure 9. *Call counts and call types of each condition.* A) The number of calls identified in 3 files from the baseline recordings (Basic), in the first manipulation (Male) and in the second manipulation (Female). B) The number of calls in the main call types within our data (AUD, CLICK, MFV, USV and other) in the 'Basic' files. C) The number of calls in the main call types in the 'Male' files. D) The number of calls in the main call types in the 'Female' files.

As shown in Figure 10, the types of USVs also varied between conditions, with Basic and Male conditions containing mostly harmonic calls (Basic = 185; Male = 1527) and Female containing mostly flat calls (26; Figure 10C).

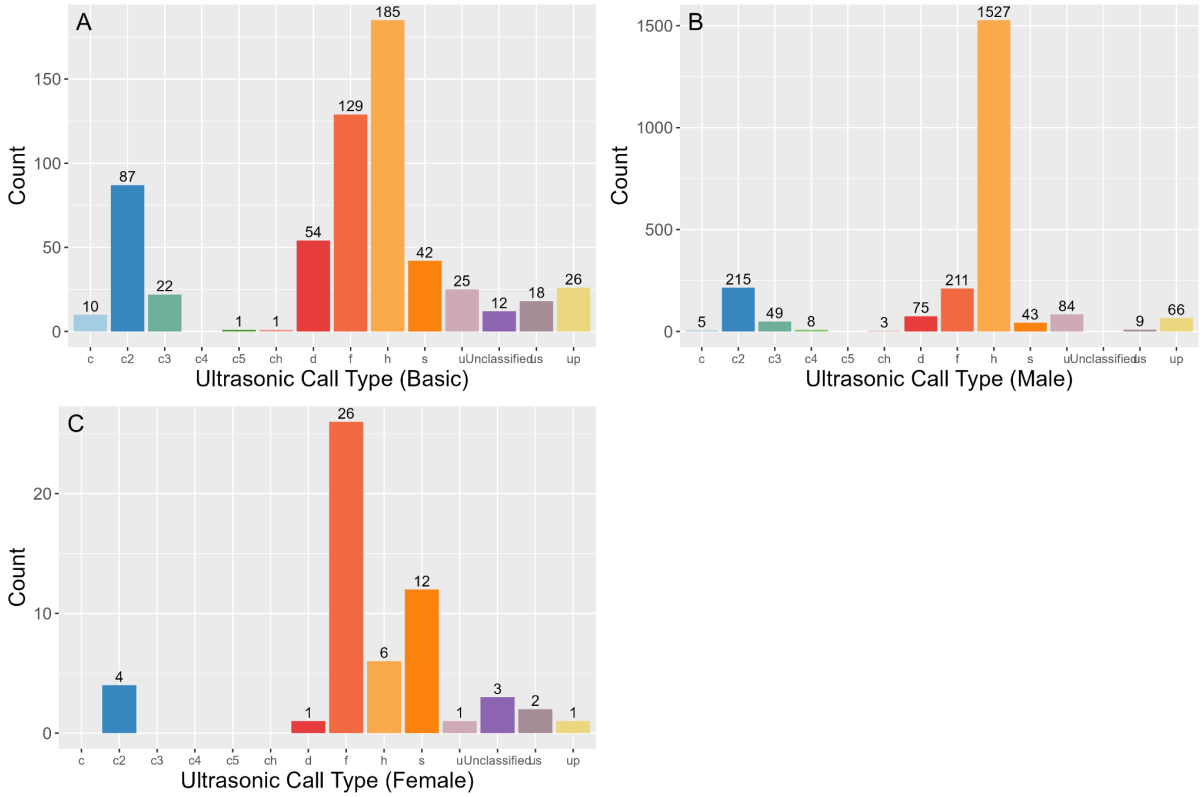


Figure 10. *USV call types across each condition.* A) The number of calls in each USV call type for calls in the Basic recordings. B) The number of calls in each USV call type for calls in the Male recordings. C) The number of calls in each USV call type for calls in the Female recordings.

Pairwise Wilcoxon tests between key acoustic parameters were conducted between conditions (Figure 11). Calls in the Basic and Male conditions differed significantly in all parameters. Calls in the Basic and Female conditions differed significantly in length, mean frequency and maximum frequency. Calls in the Male and Female conditions differed significantly in length, mean amplitude, maximum frequency and maximum amplitude.

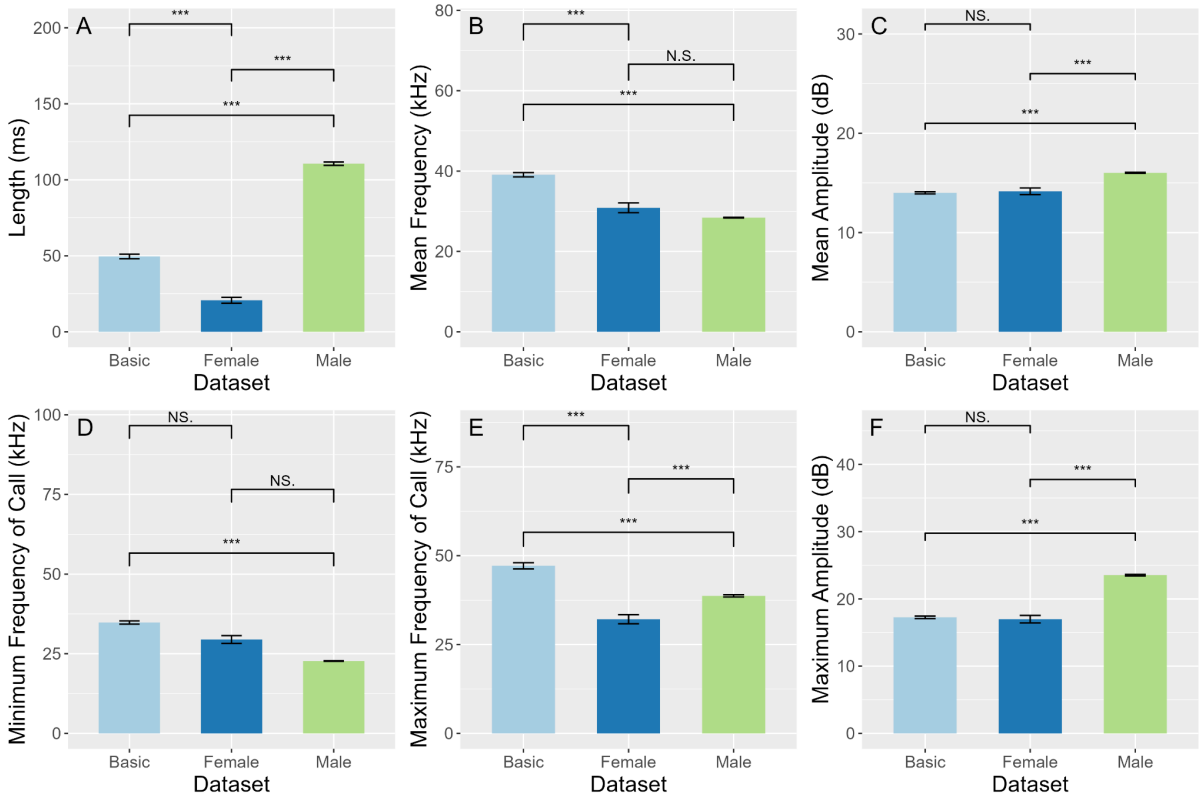


Figure 11. *Comparison of key acoustic parameters between conditions.* The mean values with standard error bars of A) call duration, B) mean frequency of a call, C) mean amplitude of a call, D) minimum frequency of a call, E) maximum frequency of a call, F) maximum amplitude of a call. Pairwise Wilcoxon-signed rank tests (with Benjamini-Hochberg corrections) display significant differences between datasets (*** = $p<0.001$, ** = $p<0.01$, * = $p<0.05$, NS. = $p>0.05$)

Further Kruskal-Wallis tests between files within each condition (Table 7) showed no significant difference across Basic or Female files. However, in Male files each parameter varied significantly. These differences were confirmed by pairwise Wilcoxon tests between each of the three Male files (supplementary material Table 3).

Table 7. *Kruskal-Wallis tests between files within the same dataset.* Kruskal-Wallis tests between the three files of each condition, with adjusted p-values according to the Benjamini-Hochberg correction ($p<0.05$ in bold type).

Parameter	**Condition**		
	Basic	**Male**	**Female**
Length (ms)	0.010	**<0.001**	0.719
fMean (kHz)	0.022	**<0.001**	0.794
aMean (dB)	0.128	**<0.001**	0.918
fFmin (kHz)	0.081	**<0.001**	0.794
fFmax (kHz)	0.252	**<0.001**	0.794
aAmax (dB)	0.081	**<0.001**	0.929

Discussion

This study is the first to provide an initial description of the vocal repertoire of *M. bavaricus.* Based on research on other rodent vocalisations, we expected to find the majority of vocalisations in the ultrasonic range. We investigated the repertoire with a manual classification system used to annotate spectrograms and an unsupervised K-means cluster analysis based on numerical acoustic data. To assess whether the vocalisations of *M. bavaricus* are influenced by the social context, we also investigated whether introducing a female or male to a breeding pair led to changes in acoustic behavior.

Our first key finding is that *M. bavaricus* mainly produces vocalisations between 20 and 60 kHz. These calls often contain multiple syllables and at least one harmonic element.

Our second key finding is that vocal behaviour changes in different social contexts and is dependent on the sex of the intruder. This suggests that social context significantly influences the vocal behaviour of *M. bavaricus*.

General repertoire

In the previously unknown vocal behaviour of M. bavaricus, the present study has revealed a broad vocal repertoire ranging from the audible to ultrasonic range, as seen in other closely related vole species (*Myodes glareolus*, Kapusta & Pochroń, 2011; *Microtus ochrogaster*, Warren et al., 2022; *Microtus pennsylvanicus* and *Microtus pinetorum*, Blake, 2012).

Exploring the frequency range of vocalisations

Identifying the frequency range in which the majority of vocalisations are produced can give an insight into where the frequency range of perceptual advantage lies in M. bavaricus. Mammals have evolved a complex middle ear structure which allows increased sensitivity to certain frequencies. The polyvagal hypothesis suggests that neural regulation of the autonomic nervous system, which regulates involuntary bodily functions, is tied to the production and perception of vocalisations in mammals (Porges & Lewis, 2009). A mammal's internal state is reflected in its vocalisations and signals to receivers what type of interaction to expect. Vocalisations in the frequency range of perceptual advantage serve to increase social engagement by signalling safety to down regulate the physiological state and promote positive interactions, such as grooming and play, that maintain social bonds (Stewart et al., 2015). The average frequency of USVs (30.66 ± 0.15 kHz) was found in this expected frequency range for voles (20 - 40 kHz; Stewart et al., 2015).

Identifying shifts in the frequency range of vocalisations in different contexts could provide insight into the behavioural motivations behind them. There was a significant difference in the mean frequency in Basic and in Male recordings. This could reflect a differing internal state between these conditions, reflected in the vocalisation frequency.

Exploring USVs

The spectrographic shapes of the calls we identified were similar to those seen in other rodent species. In rats, audible vocalisations consist of a fundamental frequency and many harmonics, whereas the USVs consist of a single frequency with little to no harmonics (Boulanger-Bertolus & Mouly, 2021). Our findings follow this general pattern, although *M.*

bavaricus' USVs are slightly more complex in number of syllables, frequency modulation and often have at least one harmonic.

The USV shapes of *M. bavaricus* are very similar to those of the prairie vole (*M. ochrogaster*). Ma et al. (2014) found that *M. ochrogaster* most commonly produces flat, harmonic and complex calls. We found similar results with different distributions. *M. bavaricus* had a higher proportion of harmonic and complex calls, whereas *M. ochrogaster* emitted mostly flat calls.

We may be able to draw some comparisons to the social structure of *M. ochrogaster* and *M. bavaricus*. *M. ochrogaster* is a monogamous and territorial species. They establish long-term pair bonds, engage in biparental care and maintain minimal overlap with neighbouring territories, with males showing aggression to intruding males (Madrid et al., 2020). From behaviour observed at Alpenzoo Innsbruck whilst establishing the breeding programme, *M. bavaricus* appears to prefer being housed in monogamous pairs and males show aggression towards other males but only females raise the offspring (per comms. André Stadler). The similarities in acoustic repertoire may mirror the similarities in social organisation, namely formation of monogamous pairs and male territoriality.

The unclear function of clicks

Clicks made up a large part of the vocal repertoire of *M. bavaricus*. Clicks are also found in the vocal repertoires of a range of rodent species from capybaras (*Hydrochoerus hydrochaeris*), during group movement or the return of an individual to the group, to domestic mice (*Mus musculus*), when pups are isolated or during handling (Zaytseva et al., 2020). However, clicks have not been well studied in terms of acoustic structure or context. Clicks were also highly prevalent across conditions, which suggests they are an important

part of the repertoire in different contexts but further investigation is required into their actual function.

Differences between conditions

One of our principal findings is that the vocalisations of *M. bavaricus* vary with social context. We observed a significant difference in number and distribution of calls between our conditions: baseline recordings, introducing an unfamiliar male to a breeding pair (manipulation 1) or introducing an unfamiliar female to a breeding pair (manipulation 2). This suggests that social context significantly influences the vocal behaviour of *M. bavaricus*. The highly significant chi-squared test ($p < 0.001$) confirms a strong association between call type and condition. This supports the idea that the presence of an unfamiliar conspecific alteres the vocal behaviour and that this is influenced by the sex of the conspecific.

The difference in the number of calls produced when an unfamiliar male was introduced and when an unfamiliar female was introduced indicates a much higher level of arousal in the presence of the male. Differing reactions to male and female intruders suggest that *M. bavaricus* can discriminate between sexes based on acoustic and/or olfactory cues, since the breeding pair and intruder individual were not in visual or physical contact.

Difference in USV production

Consistent with previous research on other *Microtus* species, we found a significant increase in USVs in the Male condition compared to the Female condition. Ma et al. (2014) found that *M. ochrogaster* males show a more robust increase in USV production in response to novel social cues, whereas females produced very few USVs in response to novel social stimulation. A number of studies of other *Microtus* species found that males are the dominant producers of USVs (*Microtus pinetorum,* Cherry & Lepri, 1986; *Microtus ochrogaster*; Lepri

et al., 1988; *Myodes glareolus*; Marchlewska-Koj et al., 1994). This could also be the case in *M. bavaricus,* however, due to our experimental setup, we were not able to determine whether these calls came from the male intruder, the male or female of the breeding pair or a combination of all.

The increased number of USVs in the Male condition could indicate that introduction of a male stimulates USV production. In mice, studies using a resident-intruder paradigm have found that USVs are predominantly produced by the resident (Hammerschmidt et al., 2012). If this was the case in our study then USV production could indicate territorial or competitive motivation from the resident male. Kapusta et al., (2007) found that bank voles (*Clethrionomys glareolus*) emitted USVs during aggressive encounters. However, they found that bank voles emitted significantly less USVs than more tolerant species, field voles (*Microtus agrestis*) and common voles (*Microtus arvalis*), in which USVs are used to reduce aggression and facilitate social interaction. Therefore, the increase in USVs may in fact be a sign of affiliative interactions. USVs may instead be produced by the intruder in an attempt to reduce aggression of the residents or to facilitate a courtship interaction with the resident female. Alternatively, USVs could indicate affiliative interactions between the resident pair. In California mice (*Peromyscus californicus*) pairs produce more USVs than individuals when confronted with intruders. They may use USVs to coordinate during territorial defense (Rieger et al., 2021). Identifying individual callers in further research could build on our findings and assign behavioural context to the vocal responses to intruders of different sexes.

Predominant call types. In the Basic and Male conditions harmonic calls were most frequent but in the Female condition flat calls were most frequent. This suggests a fundamental change in the information content of the vocalisations. Regardless of who is producing the calls, the change in USV type in the Female condition indicates a shift in the

vocal communication strategy. This could indicate that harmonic and flat calls are representative of different communication strategies in *M. bavaricus*.

Acoustic parameters

The Basic condition is likely to represent the baseline level of vocal activity. This condition showed the highest mean and maximum frequencies which could indicate a broader range of vocalisation used for various purposes in communication between a breeding pair. Calls in this condition had a duration higher than the Female condition and lower than the Male condition, which could suggest that *M. bavaricus* uses changes in duration to communicate different information.

The Male condition had longer calls and a higher mean and maximum amplitude. This could indicate a shift towards more intense vocal communication. The longer duration could indicate extended interactions. The increased amplitude could signal greater arousal in this condition.

Calls in the female condition had a shorter duration, lower mean frequency than the Basic condition and the lowest maximum frequency. The shorter calls and a lower amplitude than in the Male condition could indicate more localised communication. The lower frequency could indicate a decrease in the level of arousal.

The differences in acoustic parameters across conditions suggest *M. bavaricus* adjusts its vocal behavior depending on the social context. The introduction of a male seems to elicit longer, louder calls while the introduction of a female seems to be associated with shorter, quieter calls, potentially related to different social interactions. Since we cannot assign calls to individuals, the observed changes in acoustic parameters reflect the overall vocal output in each social context, not the vocalizations of specific individuals. Therefore, we cannot yet assign these changes to specific behavioural contexts.

Within-condition variation. To check the validity of the comparison of acoustic features we compared variation between the files within each condition. If all conditions showed no within-condition variation the observed differences would likely be due to the social context itself. If all conditions showed significant within-condition variation the differences could instead be due to other factors, such as individual differences or external environmental influences. However, only the Male condition showed within-condition variation. This suggests that the Male condition is somehow more susceptible to these other factors than the other conditions. This could reflect increased sensitivity to subtle changes in a situation that appears to increase levels of arousal. Alternatively, individual differences may be more pronounced, especially due to the higher number of calls produced. Replication of our experiments should consider using a larger sample size to explore these differences further.

Cluster analysis

We performed an unsupervised analysis to compare how calls were classified with our manual classification system. The K-means cluster analysis identified a similar number of USV call types to our manual system. However, there was very little agreement in the content of these call types between the unsupervised and manual methods. This could indicate limitations in the K-means analysis or a degree of subjectivity in our manual classification system.

The low level of agreement between the manual and unsupervised methods could indicate limitations in the acoustic features we used for our K-means analysis. Manual inspection of calls may allow more nuanced features to be considered in the classification. For example, frequency modulations in the fundamental frequency can be picked up by visual inspection but are represented by a single value (fSlope) in the unsupervised analysis. The

fSlope uses a linear regression to give the frequency slope of all detected points in the call. This parameter may oversimplify modulation patterns and does not consider temporal detail (ie. when the modulations occur). It could also be sensitive to distortions by the harmonics of a call. In future efforts to improve on our original description of the vocal repertoire of *M. bavaricus* we could increase detail in our acoustic parameters, for example by using wavelet analysis to explore frequency modulations.

A further limitation of the unsupervised K-means analysis is the assumption of spherical clusters. K-means analysis is useful for large datasets as it has a fast clustering speed, but it assumes that the clusters will be spherical and of roughly equal size (He et al., 2022). Therefore, complex call shapes or varying densities of call types may be slightly underrepresented by this analysis. Call types with lower densities are at risk of being merged into larger categories (Langehennig-Peristenidou et al., 2023). Our K-means analysis has provided a valuable overview of *M. bavaricus's* repertoire but further research could look at the suitability of other clustering algorithms to our data.

Disagreement between manual and unsupervised classification could also be caused by inherent subjectivity in our manual classification. We focused our classification on the shape of a call's main syllable. Despite assigning numerical values to call definitions (eg. down = decrease in frequency of > 5 kHz) manual classification introduces a degree of human error. Therefore, also running an unsupervised analysis highlights potential areas where subjectivity has skewed our judgement.

We can take findings from both manual and unsupervised methods to improve call classification in future research. Based on the results of the K-means analysis, we could consider some acoustic parameters. For example, rat calls generally fall into two categories based on their frequency (aversive, 22 kHz, or appetitive, 50 kHz; Wöhr and Schwarting,

2013). Grouping calls in similar frequency bands may identify patterns in the vocal behaviour of *M. bavaricus* that were overlooked in this study.

We could consider combining some call types in our manual classification. The K-means clusters appear to create clusters based on how complex and harmonic calls are; cluster 2 combines simple call types with no harmonics, including up, down and u-shaped; cluster 7 contains these simple call types and harmonics; cluster 1 contains complex and harmonic calls. We could use this perspective to improve our manual classification system.

Further research could also combine acoustic data with other information, such as behavioural context. In many rodent species, the context in which vocalisations are produced plays a significant role. For example, *Microtus agrestis* emits u-shaped calls with a harmonic component during sexual behaviour (Sales, 2010). Similarities and differences between vocalisations emitted in different contexts can highlight core elements of the vocal repertoire and how *M. bavaricus* makes use of these.

Future directions

From the results of our study, we can suggest potential avenues for future research. In the following paragraphs we highlight some promising directions for this research.

In the process of annotating our recordings we were able to identify some areas that require improvement with our classification system. We originally planned to identify nonlinear phenomena, which proved complicated in practice. For example, the presence of echos in parts of the recordings could be mistaken for biphonation, the simultaneous production of two independent fundamental frequencies. In further descriptions of the vocal repertoire of M. bavaricus, nonlinear phenomena could be an area of focus.

Our results could also inform adjustments to the A-MUD algorithm for future use with vole vocalisations. The original algorithm had to be adjusted to the frequency range of

vole USVs as mice produce USVs in a higher range. We investigated the quality of the acoustic data obtained by A-MUD prior to our statistical analysis and determined that it was sufficiently representative. The starting frequency and minimum frequency values obtained by A-MUD and manually showed high levels of correlation. However, for the maximum frequency the correlation was not as strong. The presence of more harmonics in the vocalisations of *M. bavaricus* than in mice may make these vocalisations harder for the algorithm to track. Now we have more information on the repertoire of *M. bavaricus,* further adjustments to the A-MUD algorithm would allow more reliable statistical analysis of large numbers of calls.

Our experimental setup meant that we could not assign calls to individuals so we cannot draw concrete conclusions into the motivation of the changes in vocalisation behaviour we observed. To improve on our methods future studies should focus on identifying individual callers. There are limitations when studying *M. bavaricus* to be navigated in order to do so. Firstly, *M. bavaricus* spends much of its time underground so behaviour is hard to observe. Secondly, our subjects for this study were part of a breeding programme designed to conserve the species. Any disturbances to individuals or between pairs must be minimal and not impact their breeding success. Using an array of microphones would allow the source of vocalisations to be identified without having to remove individuals from their housing. To separate the vocalisations of a breeding pair some visual observation may be required. Video recordings would not be suitable for a species often found underground but an infrared camera could overcome this. Infrared cameras provide lower resolution recordings but would only be necessary to match the path of movement of the vocalisations and individuals.

Additionally, if calls can be assigned to individuals, future research could investigate sex differences in acoustic behaviour. Exploring whether males and females have different

rates of vocalisation could provide a basis to explore intra- and intersex dynamics. For example, the discovery that *M. ochrogaster* males produce more USVs led to the discovery of individual recognition of mates by females (Warren et al., 2024). Further research into sex differences may also explore the difference in USV call types (flat or harmonic) that we observed between conditions. Our results could be used to suggest hypotheses for a more in depth description of how *M. bavaricus* makes use of its vocal repertoire.

Conclusion

This study provides the first detailed description of the vocal repertoire of the Bavarian pine vole, revealing a rich array of call types in both the audible and ultrasonic frequency ranges. Our findings demonstrate that *M. bavaricus* possesses a complex vocal system, comparable with closely related species, with USVs resembling those of *M. ochrogaster*. The observed differences in call number, type and acoustic parameters across different conditions (baseline, introduction of an unfamiliar male, introduction of an unfamiliar female) strongly suggest that the social environment significantly influences acoustic behaviour in this species. The shift in USV type from predominantly harmonic in Basic and Male conditions to primarily flat in the Female condition highlights a fundamental change in vocal communication strategy that warrants further investigation. While our experimental design, with its single microphone setup, limited our ability to assign calls to specific individuals, our results lay the groundwork for future research. Future work using techniques for identifying individual callers will be crucial for unraveling the specific roles of each individual in the observed vocal changes. Combining technical advancements with detailed observations of acoustic behaviour will allow for a more comprehensive understanding of the function and context-dependent usage of the diverse vocal repertoire of

M. bavaricus, ultimately shedding light on the complex social dynamics of this endangered species.

References

Abhirami, S., Agarwalla, S., Bhattacharya, A., & Bandyopadhyay, S. (2023). Contribution of the ventral pouch in the production of mouse ultrasonic vocalizations. *Physical Review E*, *107*(2), 024412. https://doi.org/10.1103/PhysRevE.107.024412

Agamaite, J. A., Chang, C.-J., Osmanski, M. S., & Wang, X. (2015). A quantitative acoustic analysis of the vocal repertoire of the common marmoset (*Callithrix jacchus*). *The Journal of the Acoustical Society of America*, *138*(5), 2906–2928. https://doi.org/10.1121/1.4934268

ASAB/ABS, Buchanan, K., Burt de Perera, T., Carere, C., Carter, T., Hailey, A., Hubrecht, R., Jennings, D., Metcalfe, N., Pitcher, T., Peron, F., Sneddon, L., Sherwin, C., Talling, J., Thomas, R., & Thompson, M. (2012). Guidelines for the treatment of animals in behavioural research and teaching. *Animal Behaviour*, *83*(1), 301–309. https://doi.org/10.1016/j.anbehav.2011.10.031']

Barros, K. S., Tokumaru, R. S., Pedroza, J. P., & Nogueira, S. S. C. (2011). Vocal Repertoire of Captive Capybara (*Hydrochoerus hydrochaeris*): Structure, Context and Function. *Ethology*, *117*(1), 83–94. https://doi.org/10.1111/j.1439-0310.2010.01853.x

Batist, C. H., Razafindraibe, M. N., Randriamanantena, F., & Baden, A. L. (2023). Bioacoustic characterization of the black-and-white ruffed lemur (Varecia variegata) vocal repertoire. *Primates*, *64*(6), 621–635. https://doi.org/10.1007/s10329-023-01083-8

Bednářová, R., Hrouzková-Knotková, E., Burda, H., Sedláček, F., & Šumbera, R. (2013). Vocalizations of the giant mole-rat (*Fukomys mechowii*), a subterranean rodent with the richest vocal repertoire. *Bioacoustics*, *22*(2), 87–107. https://doi.org/10.1080/09524622.2012.712749

Beery, A. K., Lopez, S. A., Blandino, K. L., Lee, N. S., & Bourdon, N. S. (2021). Social selectivity and social motivation in voles. *eLife*, *10*, e72684. https://doi.org/10.7554/eLife.72684

Berke, G. S., & Long, J. L. (2010). Chapter 10.1—Functions of the larynx and production of sounds. In S. M. Brudzynski (Ed.), *Handbook of Behavioral Neuroscience* (Vol. 19, pp. 419–426). Elsevier. https://doi.org/10.1016/B978-0-12-374593-4.00038-3

Bertolino, S., Colangelo, P., Mori, E., & Capizzi, D. (2015). Good for management, not for conservation: An overview of research, conservation and management of Italian small mammals. *Hystrix, the Italian Journal of Mammalogy*, *26*(1). https://doi.org/10.4404/hystrix-26.1-10263

Bezerra, B. M., & Souto, A. (2008). Structure and Usage of the Vocal Repertoire of Callithrix jacchus. *International Journal of Primatology*, *29*(3), 671–701. https://doi.org/10.1007/s10764-008-9250-0

Blake, B. H. (1992). Ultrasonic vocalization and body temperature maintenance in infant voles of three species (Rodentia: Arvicolidae). *Developmental Psychobiology*, *25*(8), 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. MONTANUS). *Journal of Mammalogy*, *83*(2), 536–545. https://doi.org/10.1644/1545-1542(2002)083<0536:UCIIIP>2.0.CO;2

Blake, B. H. (2012). Ultrasonic calling in 2 species of voles, *Microtus pinetorum* and *M. pennsylvanicus* , with different social systems. *Journal of Mammalogy*, *93*(4), 1051–1060. https://doi.org/10.1644/11-MAMM-A-356.2

Blanchard, R. J., Blanchard, D. C., Agullana, R., & Weiss, S. M. (1991). Twenty-two kHz alarm cries to presentation of a predator, by laboratory rats living in visible burrow systems. *Physiology & Behavior*, *50*(5), 967–972. https://doi.org/10.1016/0031-9384(91)90423-l

Blumstein, D. T., & Munos, O. (2005). Individual, age and sex-specific information is contained in yellow-bellied marmot alarm calls. *Animal Behaviour*, *69*(2), 353–361. https://doi.org/10.1016/j.anbehav.2004.10.001

Boersma, P., & Weenink, D. (2025). *Praat: Doing phonetics by computer* (Version 6.4.26) [Computer software]. http://www.praat.org/

Boulanger-Bertolus, J., & Mouly, A.-M. (2021). Ultrasonic Vocalizations Emission across Development in Rats: Coordination with Respiration and Impact on Brain Neural Dynamics. *Brain Sciences*, *11*(5), 616. https://doi.org/10.3390/brainsci11050616

Bradbury, J. W. (with Internet Archive). (2011). *Principles of animal communication*. Sunderland, Mass. : Sinauer Associates. http://archive.org/details/principlesofanim0000brad

Brudzynski, S. M. (2021). Biological Functions of Rat Ultrasonic Vocalizations, Arousal Mechanisms, and Call Initiation. *Brain Sciences*, *11*(5), 605. https://doi.org/10.3390/brainsci11050605

Brudzynski, S. M., & Pniak, A. (2002). Social contacts and production of 50-kHz short ultrasonic calls in adult rats. *Journal of Comparative Psychology*, *116*(1), 73–82. https://doi.org/10.1037/0735-7036.116.1.73

Brumm, H., & Slabbekoorn, H. (2005). Acoustic Communication in Noise. In *Advances in the Study of Behavior* (Vol. 35, pp. 151–209). Elsevier. https://doi.org/10.1016/S0065-3454(05)35004-2

Chan, R. W., & Titze, I. R. (2006). Dependence of phonation threshold pressure on vocal tract acoustics and vocal fold tissue mechanics. *The Journal of the Acoustical Society of America*, *119*(4), 2351–2362. https://doi.org/10.1121/1.2173516

Chen, Z., & Wiens, J. J. (2020). The origins of acoustic communication in vertebrates. *Nature Communications*, *11*(1), 369. https://doi.org/10.1038/s41467-020-14356-3

Cherry, J. (1986). Sexual dimorphism and gonadal control of ultrasonic vocalizations in adult pine voles, Microtus pinetorum. *Hormones and Behavior*, *20*(1), 34–48. https://doi.org/10.1016/0018-506X(86)90027-9

Clink, D. J., & Klinck, H. (2021). Unsupervised acoustic classification of individual gibbon females and the implications for passive acoustic monitoring. *Methods in Ecology and Evolution*, *12*(2), 328–341. https://doi.org/10.1111/2041-210X.13520

Cole, F. R., & Wilson, D. E. (2010). Microtus miurus (Rodentia: Cricetidae). *Mammalian Species*, *42*, 75–89. https://doi.org/10.1644/855.1

Colléony, A., Clayton, S., Couvet, D., Saint Jalme, M., & Prévot, A.-C. (2017). Human preferences for species conservation: Animal charisma trumps endangered status. *Biological Conservation*, *206*, 263–269. https://doi.org/10.1016/j.biocon.2016.11.035

Dawkins, R., & Krebs, J. R. (1978). Animal Signals: Information or Manipulation? In J. R. Krebs & N. B. Davies (Eds.), *Behavioural Ecology: An Evolutionary Approach* (pp. 282–309). Blackwell Scientific.

Dent, M. L., Fay, R. R., & Popper, A. N. (Eds.). (2018). *Rodent bioacoustics*. ASA Press, Springer.

Dirzo, R., Young, H. S., Galetti, M., Ceballos, G., Isaac, N. J. B., & Collen, B. (2014). Defaunation in the Anthropocene. *Science*, *345*(6195), 401–406. https://doi.org/10.1126/science.1251817

Dvořáková, V., Hrouzková, E., & Šumbera, R. (2016). Vocal repertoire of the social Mashona mole-rat (*Fukomys darlingi*) and how it compares with other mole-rats. *Bioacoustics*, *25*(3), 253–266. https://doi.org/10.1080/09524622.2016.1141117

Fant, G. (1960). *Acoustic Theory Of Speech Production*. https://www.semanticscholar.org/paper/Acoustic-Theory-Of-Speech-Production-Fant/5d79d86d4501b4a5381a7a8cc3562bf66cf975e6

Fernández-Vargas, M., Riede, T., & Pasch, B. (2022). Mechanisms and constraints underlying acoustic variation in rodents. *Animal Behaviour*, *184*, 135–147. https://doi.org/10.1016/j.anbehav.2021.07.011

Fischer, J., Hammerschmidt, K., Cheney, D. L., & Seyfarth, R. M. (2001). Acoustic Features of Female Chacma Baboon Barks. *Ethology*, *107*(1), 33–54. https://doi.org/10.1111/j.1439-0310.2001.00630.x

Fitch, W. T. (1997). Vocal tract length and formant frequency dispersion correlate with body size in rhesus macaques. *The Journal of the Acoustical Society of America*, *102*(2), 1213–1222. https://doi.org/10.1121/1.421048

Gasco, A., Ferro, H. F., & Monticelli, P. F. (2019). The communicative life of a social carnivore: Acoustic repertoire of the ring-tailed coati (*Nasua nasua*). *Bioacoustics*, *28*(5), 459–487. https://doi.org/10.1080/09524622.2018.1477618

Gilley, L. M., Diggins, C. A., Pearson, S. M., & Best, T. L. (2019). Vocal repertoire of captive northern and southern flying squirrels (Glaucomys sabrinus and G. volans). *Journal of Mammalogy*, *100*(2), 518–530. https://doi.org/10.1093/jmammal/gyz064

Goymann, W., & Küblbeck, M. (2020). The second warning to humanity—Why ethology matters? *Ethology*, *126*(1), 1–9. https://doi.org/10.1111/eth.12965

Grimsley, J. M. S., Sheth, S., Vallabh, N., Grimsley, C. A., Bhattal, J., Latsko, M., Jasnow, A., & Wenstrup, J. J. (2016). Contextual Modulation of Vocal Behavior in Mouse: Newly Identified 12 kHz "Mid-Frequency" Vocalization Emitted during Restraint. *Frontiers in Behavioral Neuroscience*, *10*. https://doi.org/10.3389/fnbeh.2016.00038

Håkansson, J., Jiang, W., Xue, Q., Zheng, X., Ding, M., Agarwal, A. A., & Elemans, C. P. H. (2022). Aerodynamics and motor control of ultrasonic vocalizations for social communication in mice and rats. *BMC Biology*, *20*(1), 3. https://doi.org/10.1186/s12915-021-01185-z

Hammerschmidt, K., & Fischer, J. (1998). The Vocal Repertoire of Barbary Macaques: A Quantitative Analysis of a Graded Signal System. *Ethology*, *104*(3), 203–216. https://doi.org/10.1111/j.1439-0310.1998.tb00063.x

Hammerschmidt, K., Radyushkin, K., Ehrenreich, H., & Fischer, J. (2012a). The structure and usage of female and male mouse ultrasonic vocalizations reveal only minor differences. *PloS One*, *7*(7), e41133. https://doi.org/10.1371/journal.pone.0041133

Hammerschmidt, K., Radyushkin, K., Ehrenreich, H., & Fischer, J. (2012b). The Structure and Usage of Female and Male Mouse Ultrasonic Vocalizations Reveal only Minor Differences. *PLoS ONE*, *7*(7), e41133. https://doi.org/10.1371/journal.pone.0041133

Hanson, J. L., & Hurley, L. M. (2012). Female Presence and Estrous State Influence Mouse Ultrasonic Courtship Vocalizations. *PLoS ONE*, *7*(7), e40782. https://doi.org/10.1371/journal.pone.0040782

Haring, E., Herzig-Straschil, B., & Spitzenberger, F. (2000). Phylogenetic analysis of Alpine voles of the Microtus multiplex complex using the mitochondrial control region. *Journal of Zoological Systematics and Evolutionary Research*, *38*(4), 231–238. https://doi.org/10.1046/j.1439-0469.2000.384139.x

He, H., He, Y., Wang, F., & Zhu, W. (2022). Improved K‐means algorithm for clustering non‐spherical data. *Expert Systems*, *39*(9), e13062. https://doi.org/10.1111/exsy.13062

Heckman, J., McGuinness, B., Celikel, T., & Englitz, B. (2016). Determinants of the mouse ultrasonic vocal structure and repertoire. *Neuroscience and Biobehavioral Reviews*, *65*, 313–325. https://doi.org/10.1016/j.neubiorev.2016.03.029

Hending, D., Seiler, M., & Stanger-Hall, K. F. (2020). The Vocal Repertoire of the Northern Giant Mouse Lemur (Mirza zaza) in Captivity. *International Journal of Primatology*, *41*(5), 732–763. https://doi.org/10.1007/s10764-020-00181-4

Hofer, M. A. (2010). Evolution of the infant separation call: Rodent ultrasonic vocalization. In *Handbook of Behavioral Neuroscience* (Vol. 19, pp. 29–35). Elsevier. https://doi.org/10.1016/B978-0-12-374593-4.00004-8

Kaltwasser, M. T. (1991). Acoustic startle induced ultrasonic vocalization in the rat: A novel animal model of anxiety? *Behavioural Brain Research*, *43*(2), 133–137. https://doi.org/10.1016/s0166-4328(05)80063-4

Kapusta, J., & Pochroń, E. (2011). Effect of gonadal hormones and sexual experience on vocalizations and behavior of male bank voles (*Myodes glareolus*). *Canadian Journal of Zoology*, *89*(11), 1117–1127. https://doi.org/10.1139/z11-087

Kapusta, J., Sales, G., & Czuchnowski, R. (2007). Aggression and Vocalization Behaviour of Three Sympatric Vole Species during Conspecific and Heterospecific Same-Sex Encounters on JSTOR. *Behaviour*, *144*(3), 283–305.

Kapusta, J., & Sales, G. D. (2009). Male-Female Interactions and Ultrasonic Vocalization in Three Sympatric Species of Voles during Conspecific and Heterospecific Encounters. *Behaviour*, *146*(7), 939–962.

Kenkel, W. M., Gustison, M. L., & Beery, A. K. (2021). A Neuroscientist's Guide to the Vole. *Current Protocols*, *1*(6), e175. https://doi.org/10.1002/cpz1.175

Kennerley, R. J., Lacher, T. E., Hudson, M. A., Long, B., McCay, S. D., Roach, N. S., Turvey, S. T., & Young, R. P. (2021). Global patterns of extinction risk and conservation needs for Rodentia and Eulipotyphla. *Diversity and Distributions*, *27*(9), 1792–1806. https://doi.org/10.1111/ddi.13368

König, C. (1962). Über eine neue Wühlmaus aus der Umgebung von Garmisch-Partenkirchen (Oberbayern): Pitymys bavaricus (Mammalia: Rodentia). *Senckenbergiana biologica*, *43*, 1–10.

Koolhaas, J. M., Coppens, C. M., de Boer, S. F., Buwalda, B., Meerlo, P., & Timmermans, P. J. A. (2013). The Resident-intruder Paradigm: A Standardized Test for Aggression, Violence and Social Stress. *Journal of Visualized Experiments : JoVE*, *77*, 4367. https://doi.org/10.3791/4367

Langehennig-Peristenidou, A., Romero-Mujalli, D., Bergmann, T., & Scheumann, M. (2023). Features of animal babbling in the vocal ontogeny of the gray mouse lemur (Microcebus murinus). *Scientific Reports*, *13*(1), 21384. https://doi.org/10.1038/s41598-023-47919-7

Lepri, J. J., Theodorides, M., & Wysocki, Ch. J. (1988). Ultrasonic vocalizations by adult prairie voles,Microtus ochrogaster. *Experientia*, *44*(3), 271–273. https://doi.org/10.1007/BF01941736

Long, C. V. (2007). VOCALISATIONS OF THE DEGU OCTODON DEGUS, A SOCIAL CAVIOMORPH RODENT. *Bioacoustics*, *16*(3), 223–244. https://doi.org/10.1080/09524622.2007.9753579

Luciano, D., & Lore, R. (1975). Aggression and social experience in domesticated rats. *Journal of Comparative and Physiological Psychology*, *88*(2), 917–923. https://doi.org/10.1037/h0076439

Ma, S. T., Resendez, S. L., & Aragona, B. J. (2014). Sex differences in the influence of social context, salient social stimulation and amphetamine on ultrasonic vocalizations in prairie voles. *Integrative Zoology*, *9*(3), 280–293. https://doi.org/10.1111/1749-4877.12071

Madrid, J. E., Parker, K. J., & Ophir, A. G. (2020). Variation, plasticity, and alternative mating tactics: Revisiting what we know about the socially monogamous prairie vole. In *Advances in the Study of Behavior* (Vol. 52, pp. 203–242). Elsevier. https://doi.org/10.1016/bs.asb.2020.02.001

Mahrt, E., Agarwal, A., Perkel, D., Portfors, C., & Elemans, C. P. H. (2016). Mice produce ultrasonic vocalizations by intra-laryngeal planar impinging jets. *Current Biology*, *26*(19), R880–R881. https://doi.org/10.1016/j.cub.2016.08.032

Mandelli, M.-J., & Sales, G. (2004). ULTRASONIC VOCALIZATIONS OF INFANT SHORT-TAILED FIELD VOLES, MICROTUS AGRESTIS. *Journal of Mammalogy*, *85*(2), 282–289. https://doi.org/10.1644/1545-1542(2004)085<0282:UVOISF>2.0.CO;2

Marchlewska-Koj, A., Kruczek, M., Kapusta, J., & Sales, G. D. (1994). Ultrasonic calls of bank voles in response to conspecific chemosignals. *Advances in Biosciences*, *93*.

Martínková, N., Zima, J., Jaarola, M., Macholán, M., & Spitzenberger, F. (2007). The origin and phylogenetic relationships of Microtus bavaricus based on karyotype and mitochondrial DNA sequences. *Folia Zoologica Praha*, *56*(1), 39–49.

Moles, A., Costantini, F., Garbugino, L., Zanettini, C., & D'Amato, F. R. (2007). Ultrasonic vocalizations emitted during dyadic interactions in female mice: A possible index of sociability? *Behavioural Brain Research*, *182*(2), 223–230. https://doi.org/10.1016/j.bbr.2007.01.020

Noll, A., & White, J. S. (n.d.). *S. Tools-STx* (Version 5) [Computer software]. OEAW. Retrieved 7 February 2025, from https://www.oeaw.ac.at/isf/das-institut/software/stx

Podraza, M. E., Moss, J. B., & Fischer, E. K. (2024). Evidence for individual vocal recognition in a pair-bonding poison frog, *Ranitomeya imitator*. *Journal of Experimental Biology*, *227*(3), jeb246753. https://doi.org/10.1242/jeb.246753

Porges, S. W., & Lewis, G. F. (2010). The polyvagal hypothesis: Common mechanisms mediating autonomic regulation, vocalizations and listening. In *Handbook of Behavioral Neuroscience* (Vol. 19, pp. 255–264). Elsevier. https://doi.org/10.1016/B978-0-12-374593-4.00025-5

Portfors, C. V. (2007). Types and functions of ultrasonic vocalizations in laboratory rats and mice. *Journal of the American Association for Laboratory Animal Science: JAALAS*, *46*(1), 28–34.

Portfors, C. V., & Perkel, D. J. (2014). The role of ultrasonic vocalizations in mouse communication. *Current Opinion in Neurobiology*, *28*, 115–120. https://doi.org/10.1016/j.conb.2014.07.002

Pucek, Z. (1989). A preliminary report on threatened rodents in Europe. In *Rodents: A world survey of species of conservation concern.* (Vol. 4, pp. 26–32).

R Core Team. (2022). *R: A language and environment for statistical computing.* [Computer software]. R Foundation for Statistical Computing. https://www.r-project.org/

Rabon Jr., D. R., Sawrey, D. K., & Webster, Wm. D. (2001). Infant ultrasonic vocalizations and parental responses in two species of voles (*Microtus*). *Canadian Journal of Zoology*, *79*(5), 830–837. https://doi.org/10.1139/z01-043

Range, F., & Fischer, J. (2004). Vocal Repertoire of Sooty Mangabeys (*Cercocebus torquatus atys*) in the Taï National Park. *Ethology*, *110*(4), 301–321. https://doi.org/10.1111/j.1439-0310.2004.00973.x

Riede, T. (2011). Subglottal pressure, tracheal airflow, and intrinsic laryngeal muscle activity during rat ultrasound vocalization. *Journal of Neurophysiology*, *106*(5), 2580–2592. https://doi.org/10.1152/jn.00478.2011

Riede, T. (2013). Stereotypic Laryngeal and Respiratory Motor Patterns Generate Different Call Types in Rat Ultrasound Vocalization. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *319*(4), 213–224. https://doi.org/10.1002/jez.1785

Riede, T., Kobrina, A., Bone, L., Darwaiz, T., & Pasch, B. (2022). Mechanisms of sound production in deer mice (*Peromyscus* spp.). *Journal of Experimental Biology*, *225*(9), jeb243695. https://doi.org/10.1242/jeb.243695

Rieger, N. S., Monari, P. K., Hartfield, K., Schefelker, J., & Marler, C. A. (2021). Pair-bonding leads to convergence in approach behavior to conspecific vocalizations in California mice (Peromyscus californicus). *PLOS ONE*, *16*(8), e0255295. https://doi.org/10.1371/journal.pone.0255295

Roberts, L. H. (1975). The rodent ultrasound production mechanism. *Ultrasonics*, *13*(2), 83–88. https://doi.org/10.1016/0041-624X(75)90052-9

Romero-Mujalli, D., Bergmann, T., Zimmermann, A., & Scheumann, M. (2021). Utilizing DeepSqueak for automatic detection and classification of mammalian vocalizations: A case

study on primate vocalizations. *Scientific Reports*, *11*(1), 24463. https://doi.org/10.1038/s41598-021-03941-1

RStudio Team. (2020). *RStudio: Integrated Development for R* [Computer software]. PBC. https://www.posit.co/

Rutovskaya, M. (2019). Sound communication in social voles (subgenus *Sumeriomys*). *Bioacoustics*, *28*(6), 503–521. https://doi.org/10.1080/09524622.2018.1482787

Rutovskaya, M. V. (2019). Vocal communication in Microtus (Terricola) schelkovnikovi and M. (T.) daghestanicus in the audible range of frequencies. *Journal of Ethology*, *37*(2), 187–195. https://doi.org/10.1007/s10164-019-00587-5

Rutovskaya, M. V. (2020). Acoustic communication in four species of subgenus Alexandromys (Rodentia, Cricetidae). *Russian Journal of Theriology*, *19*(1), 21–36. https://doi.org/10.15298/rusjtheriol.19.1.02

Sales, G. D. (2010). Ultrasonic calls of wild and wild-type rodents. In *Handbook of Behavioral Neuroscience* (Vol. 19, pp. 77–88). Elsevier. https://doi.org/10.1016/B978-0-12-374593-4.00009-7

Salmi, R., Hammerschmidt, K., & Doran‐Sheehy, D. M. (2013). Western Gorilla Vocal Repertoire and Contextual Use of Vocalizations. *Ethology*, *119*(10), 831–847. https://doi.org/10.1111/eth.12122

Scattoni, M. L., Ricceri, L., & Crawley, J. N. (2011). Unusual repertoire of vocalizations in adult BTBR T+tf/J mice during three types of social encounters. *Genes, Brain and Behavior*, *10*(1), 44–56. https://doi.org/10.1111/j.1601-183X.2010.00623.x

Seyfarth, R. M., Cheney, D. L., Bergman, T., Fischer, J., Zuberbühler, K., & Hammerschmidt, K. (2010). The central importance of information in studies of animal communication. *Animal Behaviour*, *80*(1), 3–8. https://doi.org/10.1016/j.anbehav.2010.04.012

Simola, N., & Granon, S. (2019). Ultrasonic vocalizations as a tool in studying emotional states in rodent models of social behavior and brain disease. *Neuropharmacology*, *159*, 107420. https://doi.org/10.1016/j.neuropharm.2018.11.008

Singleton, G. R., Jacob, J., Krebs, C. J., & Monadjem, A. (2015). A meeting of mice and men: Rodent impacts on food security, human diseases and wildlife conservation; ecosystem benefits; fascinating biological models. *Wildlife Research*, *42*(2), 83. https://doi.org/10.1071/WR15094

Spitzenberger, F., Brunet-Lecomte, P., Nadachowski, A., & Bauer, K. (2000). Comparative morphometrics of the first lower molar in Microtus (Terricola) cf. Liechtensteini of the Eastern Alps. *Acta Theriologica*, *45*, 471–483. https://doi.org/10.4098/AT.arch.00-47

Stadler, A. (2022). (PDF) Rarest mammal (Microtus bavaricus) arrives at Alpenzoo Innsbruck. *ResearchGate*. https://www.researchgate.net/publication/368667170_Rarest_mammal_Microtus_bavaricus_arrives_at_Alpenzoo_Innsbruck

Stadler, A. (2023). Pine Vole in WAZA News. *ResearchGate*. https://www.researchgate.net/publication/379956332_Pine_Vole_in_WAZA_News

Stein, R. M., & Rachlow, J. L. (2023). Acoustic ecology of terrestrial mammals: A new Signaller–Receiver Conceptual Framework. *Mammal Review*, *53*(3), 143–157. https://doi.org/10.1111/mam.12314

Stewart, A. M., Lewis, G. F., Yee, J. R., Kenkel, W. M., Davila, M. I., Sue Carter, C., & Porges, S. W. (2015a). Acoustic features of prairie vole (Microtus ochrogaster) ultrasonic vocalizations covary with heart rate. *Physiology & Behavior*, *138*, 94–100. https://doi.org/10.1016/j.physbeh.2014.10.011

Stewart, A. M., Lewis, G. F., Yee, J. R., Kenkel, W. M., Davila, M. I., Sue Carter, C., & Porges, S. W. (2015b). Acoustic features of prairie vole (Microtus ochrogaster) ultrasonic vocalizations covary with heart rate. *Physiology & Behavior*, *138*, 94–100. https://doi.org/10.1016/j.physbeh.2014.10.011

Stille, D., Rudolph, B.-U., & Ripperger, S. P. (2024). Rediscovery of the Bavarian pine vole (*Microtus bavaricus*) in Germany. *Mammalia*, *88*(6), 570–574. https://doi.org/10.1515/mammalia-2024-0075

Takahashi, L. K., Thomas, D. A., & Barfield, R. J. (1983). Analysis of ultrasonic vocalizations emitted by residents during aggressive encounters among rats (Rattus norvegicus). *Journal of Comparative Psychology (Washington, D.C.: 1983)*, *97*(3), 207–212.

Taylor, A. M., & Reby, D. (2010). The contribution of source–filter theory to mammal vocal communication research. *Journal of Zoology*, *280*(3), 221–236. https://doi.org/10.1111/j.1469-7998.2009.00661.x

Teixeira, D., Maron, M., & Van Rensburg, B. J. (2019). Bioacoustic monitoring of animal vocal behavior for conservation. *Conservation Science and Practice*, *1*(8), e72. https://doi.org/10.1111/csp2.72

Temple, H. J., & Terry, A. (2007). *The status and distribution of European mammals.* IUCN - The World Conservation Union.

Terry, A. M., Peake, T. M., & McGregor, P. K. (2005). The role of vocal individuality in conservation. *Frontiers in Zoology*, *2*(1), 10. https://doi.org/10.1186/1742-9994-2-10

Titze, I. R. (1980). Comments on the Myoelastic—Aerodynamic Theory of Phonation. *Journal of Speech, Language, and Hearing Research*, *23*(3), 495–510. https://doi.org/10.1044/jshr.2303.495

Tvrtković, N., Pavlinić, I., & Podnar, M. (2010). Microtus bavaricus discovered in Croatia: Southern refugium or geographical variation? *Mammalian Biology*, *75*(6), 561–566. https://doi.org/10.1016/j.mambio.2009.07.001

Valente, D., Miaretsoa, L., Anania, A., Costa, F., Mascaro, A., Raimondi, T., De Gregorio, C., Torti, V., Friard, O., Ratsimbazafy, J., Giacoma, C., & Gamba, M. (2022). Comparative Analysis of the Vocal Repertoires of the Indri (Indri indri) and the Diademed Sifaka (Propithecus diadema). *International Journal of Primatology*, *43*(4), 733–751. https://doi.org/10.1007/s10764-022-00287-x

van den Berg, J. (1958). Myoelastic-Aerodynamic Theory of Voice Production. *Journal of Speech and Hearing Research*, *1*(3), 227–244. https://doi.org/10.1044/jshr.0103.227

Verde Arregoitia, L. D. (2016). Biases, gaps, and opportunities in mammalian extinction risk research. *Mammal Review*, *46*(1), 17–29. https://doi.org/10.1111/mam.12049

Volodin, I. A., Yurlova, D. D., Ilchenko, O. G., & Volodina, E. V. (2021). Ontogeny of audible squeaks in yellow steppe lemming Eolagurus luteus: Trend towards shorter and low-frequency calls is reminiscent of those in ultrasonic vocalization. *BMC Zoology*, *6*(1), 27. https://doi.org/10.1186/s40850-021-00092-8

Wadewitz, P., Hammerschmidt, K., Battaglia, D., Witt, A., Wolf, F., & Fischer, J. (2015). Characterizing Vocal Repertoires—Hard vs. Soft Classification Approaches. *PLOS ONE*, *10*(4), e0125785. https://doi.org/10.1371/journal.pone.0125785

Wang, Y., Hu, Y., Meng, J., & Li, C. (2001). An Ossified Meckel's Cartilage in Two Cretaceous Mammals and Origin of the Mammalian Middle Ear. *Science*, *294*(5541), 357–361. https://doi.org/10.1126/science.1063830

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. *Frontiers in Behavioral Neuroscience*, *15*. https://www.frontiersin.org/journals/behavioral-neuroscience/articles/10.3389/fnbeh.2021.814200

Warren, M. R., Zha, J., Young, L. J., & Liu, R. C. (2025). Vocal recognition of partners by female prairie voles. *iScience*, *28*(2), 111796. https://doi.org/10.1016/j.isci.2025.111796

Wilden, I., Herzel, H., Peters, G., & Tembrock, G. (1998). SUBHARMONICS, BIPHONATION, AND DETERMINISTIC CHAOS IN MAMMAL VOCALIZATION. *Bioacoustics*, *9*(3), 171–196. https://doi.org/10.1080/09524622.1998.9753394

Wöhr, M., Houx, B., Schwarting, R. K. W., & Spruijt, B. (2008). Effects of experience and context on 50-kHz vocalizations in rats. *Physiology & Behavior*, *93*(4–5), 766–776. https://doi.org/10.1016/j.physbeh.2007.11.031

Wöhr, M., & Schwarting, R. K. W. (2013). Affective communication in rodents: Ultrasonic vocalizations as a tool for research on emotion and motivation. *Cell and Tissue Research*, *354*(1), 81–97. https://doi.org/10.1007/s00441-013-1607-9

Yao, K., Bergamasco, M., Scattoni, M. L., & Vogel, A. P. (2023). A review of ultrasonic vocalizations in mice and how they relate to human speech. *The Journal of the Acoustical Society of America*, *154*(2), 650–660. https://doi.org/10.1121/10.0020544

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*(2), e0228892. https://doi.org/10.1371/journal.pone.0228892

Zala, S. M., Nicolakis, D., Marconi, M. A., Noll, A., Ruf, T., Balazs, P., & Penn, D. J. (2020). Primed to vocalize: Wild-derived male house mice increase vocalization rate and diversity after a previous encounter with a female. *PLOS ONE*, *15*(12), e0242959. https://doi.org/10.1371/journal.pone.0242959

Zala, S. M., Reitschmidt, D., Noll, A., Balazs, P., & Penn, D. J. (2017). Automatic mouse ultrasound detector (A-MUD): A new tool for processing rodent vocalizations. *PLOS ONE*, *12*(7), e0181200. https://doi.org/10.1371/journal.pone.0181200

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*(7), e0219749. https://doi.org/10.1371/journal.pone.0219749

Zaytseva, A. S., Volodin, I. A., Ilchenko, O. G., & Volodina, E. V. (2020). Audible calls and their ontogenetic relationship with ultrasonic vocalization in a rodent with a wide vocal range, the fat-tailed gerbil (Pachyuromys duprasi). *Behavioural Processes*, *180*, 104241. https://doi.org/10.1016/j.beproc.2020.104241

Supplementary Material

Table 1. *An extract from our classification system defining nonlinear phenomena.* We compiled a classification system based on other vole and closely related rodent species in order to annotate our recordings of *Microtus bavaricus* vocalisations. This section was part of our original classification system, however, nonlinear phenomena were not considered in our results.

Nonlinear phenomena	NLP	Irregularities in the acoustic features of a call resulting from irregularities in the vocal fold vibration		Wilden et al., 1998; Fitch et al., 2002; Tokuda, 2018; Yurlova et al., 2020
Biphonation		Simultaneous production of two independent fundamental frequencies		Yurlova et al., 2020 (Figure 3)
Subharmonics		Frequency bands at 1/n of the fundamental frequency		Yurlova et al., 2020 (Figure 3)

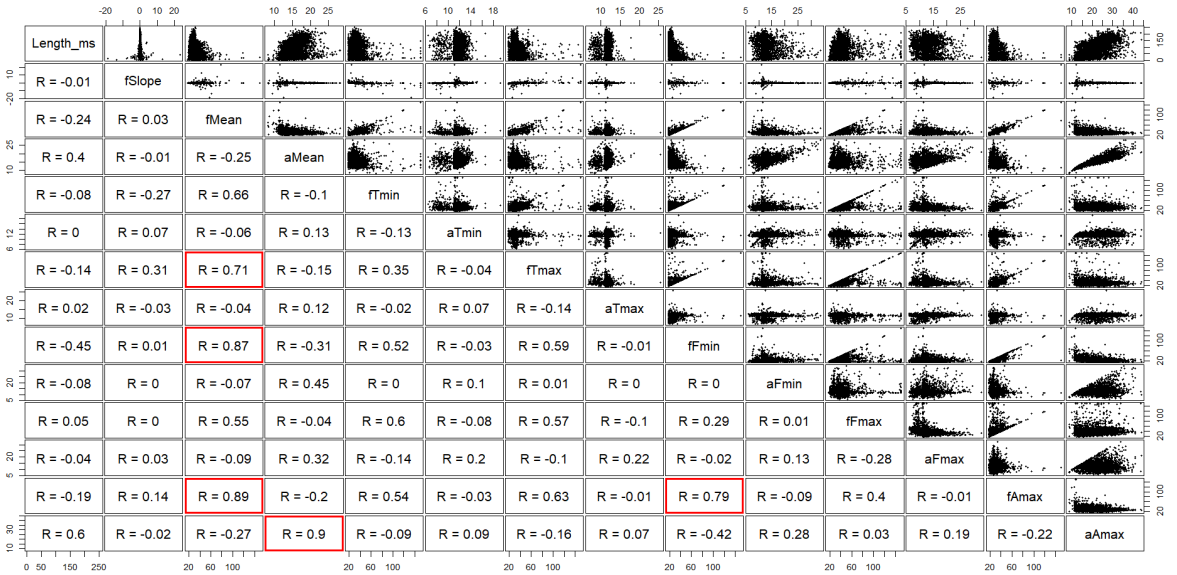


Figure 1. *Pearson's correlations of acoustic parameters considered for the unsupervised K-means cluster analysis.* Pearson's correlation coefficients were calculated prior to the unsupervised K-means cluster analysis to identify highly correlated parameters (R > 0.7; highlighted in red). For highly correlated parameters, one was excluded from the analysis (fTmax, fFmin, fAmax, aAmax).

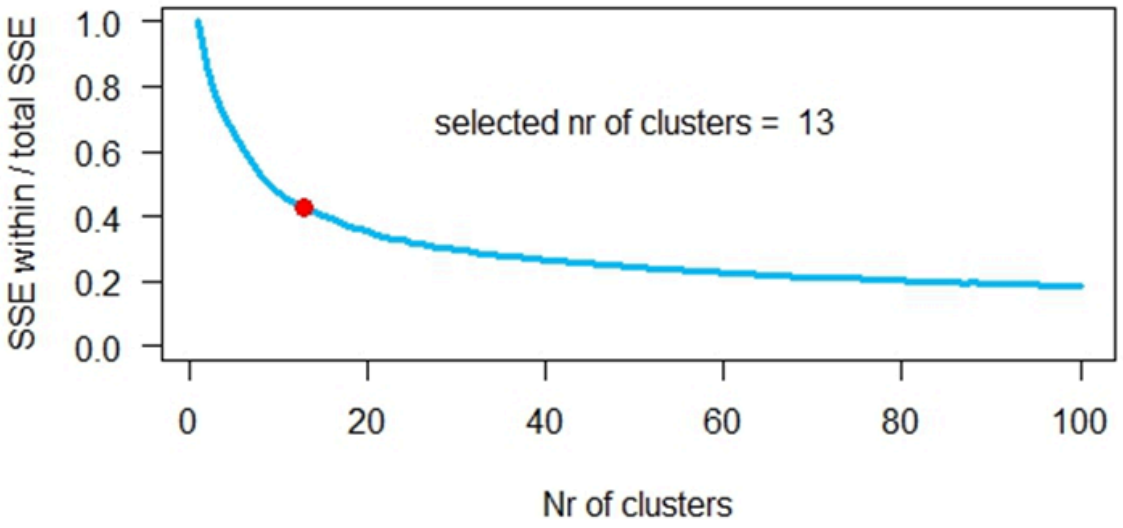


Figure 2. *The elbow figure generated from the K-means cluster analysis to determine the optimal number of clusters.* The number of clusters found in our USVs was calculated using

this elbow figure. It considered the ratio between total within-cluster Sum of Squared Errors (SSE) and the total SSE. The optimal number was determined by the model as the intersection of the two lines that best fit the elbow data; this process is known as the knee method.

Table 2. *Means and standard deviations of the acoustic features of each cluster.* The K-means analysis determined 13 distinct clusters in the USVs. The number of calls per cluster is noted in brackets below the cluster number. For each acoustic feature the mean and standard deviation was calculated.

Acoustic feature	1 (620)	2 (233)	3 (40)	4 (147)	5 (574)	6 (135)	7 (314)	8 (212)	9 (539)	10 (14)	11 (68)	12 (52)	13 (2)
Duration	162.49 ± 28.23	93.43 ± 57.78	32.11 ± 25.38	100.42 ± 48.1	114.57 ± 25	116.51 ± 50.55	49.64 ± 36.07	90.51 ± 52.63	34.02 ± 22.57	47.69 ± 43.04	75.83 ± 52.67	106.88 ± 48.45	26.25 ± 23.97
fSlope	-0.02 ± 0.05	0.03 ± 0.22	-0.13 ± 1.8	0.06 ± 0.36	0 ± 0.15	-0.09 ± 0.45	0.05 ± 0.68	0 ± 0.15	0.05 ± 0.4	-4.91 ± 5.59	-0.03 ± 0.18	0.22 ± 1.19	18.13 ± 7.16
fMean	28.83 ± 2.38	28.97 ± 4.68	71.69 ± 21.7	29.43 ± 5.51	28.35 ± 3.62	30.71 ± 6.07	41.97 ± 5.94	27.87 ± 4.9	26.94 ± 4.11	40.97 ± 10.73	29.61 ± 7.26	34.91 ± 9.09	60.27 ± 24.83
aMean	17.37 ± 1.31	17.55 ± 2.06	11.64 ± 1.92	15.08 ± 2.14	14.45 ± 1.32	15.97 ± 2.58	13.71 ± 1.76	17.64 ± 1.74	13.58 ± 1.5	13.88 ± 2.99	22.31 ± 2.35	15.96 ± 2.91	11.21 ± 0.44
fTmin	32.04 ± 5.6	29.22 ± 5.86	76.21 ± 22.78	30.75 ± 7.3	29.82 ± 5.61	37.84 ± 12.01	43.11 ± 7.83	30.94 ± 7.67	26.99 ± 4.63	125.23 ± 32.64	33.97 ± 12.78	34.85 ± 17.32	23.4 ± 0.42
aTmin	11.84 ± 0.69	11.88 ± 0.97	10.91 ± 1.41	11.67 ± 0.88	11.58 ± 0.49	8.49 ± 0.82	11.72 ± 0.7	11.75 ± 0.77	11.54 ± 0.54	10.46 ± 1.47	11.74 ± 1.25	11.44 ± 0.92	10.75 ± 0.55
aTmax	11.66 ± 0.6	11.88 ± 1.26	10.89 ± 1.35	8.54 ± 0.81	11.57 ± 0.43	11.37 ± 0.66	11.61 ± 0.83	11.63 ± 0.64	11.53 ± 0.52	11.29 ± 0.87	11.29 ± 0.93	11 ± 1.04	10.12 ± 1.79
aFmin	11.57 ± 1.48	12.68 ± 2.1	11.24 ± 1.81	12.38 ± 2.04	11.79 ± 1.22	12.06 ± 2.34	12.09 ± 1.53	17.58 ± 2.35	11.91 ± 1.11	11.68 ± 1.15	25.33 ± 3.52	12.88 ± 2.62	10.75 ± 0.55
fFmax	38.95 ± 6.86	33.28 ± 5.23	81.45 ± 22.31	40.47 ± 11.55	37.84 ± 8.5	43.67 ± 10.76	49.91 ± 10.11	36.87 ± 10.2	29.48 ± 5.53	126.19 ± 32.6	40.44 ± 17.05	114.71 ± 23.13	118.65 ± 44.34
aFmax	12.38 ± 2.11	19.32 ± 3.1	10.73 ± 1.61	10.64 ± 2.28	11.3 ± 1.72	10.49 ± 2.45	12.15 ± 1.81	12.37 ± 2.1	12.18 ± 1.38	10.44 ± 1.51	14.79 ± 4.44	9.66 ± 1.18	9.91 ± 2.59

Table 3. *Wilcoxon tests between Male files.* Wilcoxon tests between each of the three files (002, 007, 008) of the Male condition, with adjusted p-values according to the Benjamini-Hochberg correction ($p<0.05$ in bold type).

Parameter	**002 - 007**	**002 - 008**	**007 - 008**
Length (ms)	0.17	**<0.001**	**<0.001**
fMean (kHz)	**0.016**	**<0.001**	**<0.001**
aMean (dB)	**<0.001**	**<0.001**	**<0.001**
fFmin (kHz)	**<0.001**	**<0.001**	**<0.001**
fFmax (kHz)	**<0.001**	1	**<0.001**
aAmax (dB)	**<0.001**	0.59	**<0.001**