

AUTUMN SWARMING OF BATS:

Phenology and vocal communication of *Myotis* bats during swarming at the Kalkberg Cave (Bad Segeberg, Germany) and implications for conservation



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Cover page: Two Natterer's bats (*Myotis nattereri*) in front of the speaker during playback experiment.

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DECLARATION OF AUTHORSHIP

I hereby declare that I alone am responsible for the content of my doctoral dissertation and that I have only used the sources or references cited in the dissertation.

TABLE OF CONTENTS

Acknowledgments	4
Declaration of Authorship	6
Table of Contents	7
Summary	9
Zusammenfassung	11
General Introduction.....	14
Non-invasive monitoring of animals.....	15
Bats and echolocation	16
Acoustic parameters for species identification from echolocation calls	17
Social calls	18
Autumn swarming.....	20
Study Site: Kalkberg Cave	20
Conservation of endangered bat species	21
Thesis outline	22
References	24
Chapter One.....	30
The soundscape of swarming: Proof of concept for a noninvasive acoustic species identification of swarming <i>Myotis</i> bats	
Chapter Two	47
Similarities in social calls during autumn swarming may facilitate interspecific communication between <i>Myotis</i> bat species	
Chapter Three.....	68
Prior familiarity plays a bigger role in detecting newly installed bat boxes than improved echo-reflective box properties	
General Discussion.....	83
The need for comprehensive monitoring of bats.....	84
Application of new methods for species identification	84
The variety of social calls during autumn swarming	86
Utilization of swarming sites for information transfer.....	88

How can bat identification be facilitated in the future? 89

Advantages and constraints of non-invasive monitoring 90

Conclusion..... 92

References 93

List of Publications..... 96

SUMMARY

Bats worldwide are experiencing severe declines, and a concerning percentage of bat species are considered endangered, with inadequate data for many others. This makes the application of meaningful conservation efforts challenging, as a comprehensive understanding of the behavior and habitat use of endangered species is crucial for successful conservation. The nocturnal and flying nature of bats additionally complicates observational studies. However, bats hold great potential for non-invasive acoustic monitoring as they are highly vocal and constantly emit high-frequency echolocation calls for orientation in their predominantly dark environment. While species discrimination via echolocation calls became more and more reliable over the years, similar calls evolved in some species based on habitat and prey preferences, complicating identification via echolocation calls. This is especially true within the genus *Myotis*. Besides calls for orientation, most species also employ a variety of social calls for communication. Social calls are far less studied than echolocation calls, especially when produced on the wing. As those calls are known to contain information about individual identity, sex, or age of the sender they should at the very least be species-specific, thus also holding great potential to be used for species identification.

I aimed to study bats' behavior and social calls during autumn swarming at a large hibernaculum in Northern Germany, the Kalkberg Cave in Bad Segeberg, whose main inhabitants are Daubenton's bats (*Myotis daubentonii*) and Natterer's bats (*Myotis nattereri*). Prior to hibernation, temperate zone bats gather in front of underground hibernacula (winter roosts) and participate in a behavior known as swarming. Swarming is characterized by intense flight activity and is accompanied by large amounts of both echolocation calls and social calls. Different functions of swarming are suggested, incorporating information transfer, mating, and the facilitation of gene flow between otherwise isolated colonies.

The small differences between the echolocation calls of the two predominant *Myotis* species become further attenuated in this swarming context. To tackle that problem, in **Chapter One**, I tested the possibility of species identification from overlapping echolocation call sequences recorded in a crowded swarming situation. With a combination of Linear Frequency Cepstral Coefficients (LFCCs) and classic acoustic parameters, I analyzed the "soundscape" rather than single echolocation calls. This combination enabled me to detect the predominant species at the time of recording. The method has great potential to facilitate species identification and long-term monitoring in situations with more than one individual calling, e.g. on so far understudied swarming sites. By gathering information on species assemblage during autumn swarming conclusions about winter populations in hibernacula can be drawn, reducing the necessity of disruptive winter controls.

Chapter Two was dedicated to further investigations of social call repertoire and function in a swarming context. I identified ten distinct call types based on differences in their spectro-temporal structure and confirmed the grouping via a discriminant function analysis. An additional analysis of the surrounding

echolocation calls (as developed in Chapter One) revealed that both species potentially present at the swarming site employed social calls with large structural similarities. However, a subsequent analysis of classic acoustic parameters revealed subtle but significant differences in the structure of such calls. For a better understanding of the call function, I conducted playback experiments in the vicinity of the swarming site. I broadcasted three different call types for each species and observed an increase in bat activity (approximated as echolocation call rates) during and after the playback in three out of six scenarios, indicating that bats inspected or approached the playback site. By simultaneously taking photographs with a camera trap, I sometimes managed to identify approaching bats to species level. The results suggest that one call type is used to maintain group cohesion within the same species, while another type is used for communication between different species. The results are in line with what we already knew about the autumn swarming phenomenon and further enhance our understanding of it.

The results from Chapter Two underlined the function of swarming sites for communication and in this situation, it could be possible to introduce a cue to the bats, which is subsequently connected to a suitable hibernaculum. Thus, for **Chapter Three**, I installed hollow hemispheres as strong echo-reflective cues at the swarming site, where bats first encountered them during swarming. Those cues are known to provide an equally strong echo over a broad range of angles which should be highly conspicuous to passing bats. To investigate whether bats subsequently prefer roosts with hollow hemispheres I installed 60 boxes (30 with and 30 without hollow hemispheres) across three study sites. To non-invasively measure bat activity I constructed a light beam system that recorded activity at boxes, and indeed, bat activity exhibited significant variation. However, I found that prior familiarity with bat boxes played a bigger role in roost occupation than the advanced echo-reflective properties did. Additionally, I developed an effective monitoring system for tracking activity rates at bat boxes. This system revealed activity at boxes weeks to months before bats or feces were detectable via traditional visual inspections.

To not interfere with the animals' natural behavior and not disturb them in their environment all investigations were made as non-invasive as possible. A reasonable combination of monitoring techniques has the potential to gain comprehensive overviews of species-specific behavior, thereby laying the basis for informed conservation decisions for endangered species. Consequently, this study enhances our understanding of bat behavior and establishes a foundation for comprehensive monitoring in the future: By integrating both soundscape analysis and social call monitoring we potentially will gain deeper insights into bat populations, including habitat usage, species assemblage, phenology, and seasonal variations.

ZUSAMMENFASSUNG

Weltweit erleben Fledermauspopulationen Rückgänge und viele Arten gelten als stark bedroht. Neben einem besorgniserregenden Prozentsatz an Arten, der als gefährdet eingestuft wird, reicht für viele weitere Arten die Datenlage nicht für eine zuverlässige Einschätzung des Gefährdungsstatus. Ein umfassendes Verständnis des Verhaltens und der Habitatnutzung gefährdeter Arten ist jedoch eine wichtige Grundlage für die Anwendung sinnvoller Schutzmaßnahmen. Typische Verhaltensweisen der Fledermäuse – Nachtaktivität und aktiver Flug – erschweren zusätzlich Beobachtungen im natürlichen Lebensraum. Da Fledermäuse sich in der Dunkelheit mittels hochfrequenter Echoortungsrufe orientieren und somit dauerhaft Rufe ausstoßen, bieten sie jedoch hohes Potenzial für die Anwendung nicht-invasiven akustischen Monitorings. Die Echoortungsrufe weisen artspezifische Unterschiede auf und die Artbestimmung mittels Echoortung ist im Laufe der Jahre immer zuverlässiger geworden. Basierend auf ähnlichen Habitat- und Beutepräferenzen haben sich jedoch innerhalb einiger Arten sehr ähnliche Rufe entwickelt, wodurch die Artbestimmung deutlich erschwert wird. Dies wird beispielsweise innerhalb der Gattung *Myotis* deutlich. Neben Rufen zur Orientierung verwenden die meisten Arten aber auch eine Vielzahl von Sozialrufen zur Kommunikation. Im Gegensatz zu Echoortungsrufen sind Sozialrufe deutlich weniger erforscht, insbesondere solche, die im Flug erzeugt werden. Es ist bekannt, dass Sozialrufe Informationen über Identität, Geschlecht oder Alter des Senders enthalten können, wodurch sie auch artspezifisch sein sollten und somit großes Potenzial für die Artbestimmung bieten.

An einem großen Winterquartier in Norddeutschland, der Kalkberghöhle in Bad Segeberg, habe ich schwärmende Fledermäuse beobachtet. Die Winterpopulation innerhalb der Höhle besteht zum überwiegenden Teil aus Wasserfledermäuse (*Myotis daubentonii*) und Fransenfledermäuse (*Myotis nattereri*). Fledermäuse, die in der gemäßigten Zone in Untergrundquartieren überwintern, sammeln sich während des Herbstes vor potenziellen Winterquartieren, wo sie über viele Nächte schwärmen. Dieses Schwärm-Verhalten ist durch intensive Flugaktivität gekennzeichnet und wird von einer Vielzahl von sowohl Echoortungs- als auch Sozialrufen begleitet. Dem Schwärmen werden dabei verschiedene Funktionen zugeschrieben, darunter Informationsübertragung, Paarung und Unterstützung des Genflusses zwischen Individuen verschiedener Kolonien.

In dieser Situation mit vielen zeitgleich rufenden Individuen werden die geringen Unterschiede zwischen den Echoortungsrufen von *Myotis*-Fledermäusen noch zusätzlich abgeschwächt. In **Kapitel Eins** habe ich eine Methode entwickelt, mit deren Hilfe mittels überlappender Echoortungssequenzen die dominante Art zur Zeit der Aufnahme bestimmt werden kann. Mit einer Kombination aus Linear Frequency Cepstral Coefficients (LFCCs) und klassischen akustischen Parametern wird dabei die „Geräuschkulisse“ anstelle von Einzelrufen analysiert. Diese Methode hat großes Potenzial die Artbestimmung in Situationen mit mehr als einem rufenden Individuum zu verbessern und so zum Beispiel das Monitoring an bisher wenig erforschten Schwärmquartieren zu erleichtern. Durch ein

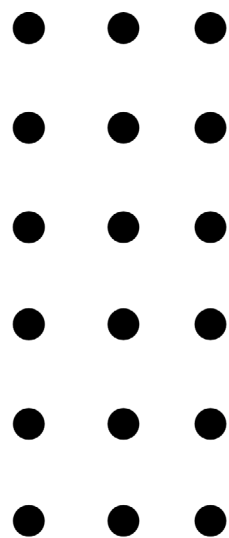
umfassendes Monitoring der Artzusammensetzung während des herbstlichen Schwärmens könnten zukünftig Rückschlüsse auf die Winterbewohner eines Quartiers gezogen werden, um so die Notwendigkeit störender Winterquartierkontrollen auf ein Minimum zu reduzieren.

In **Kapitel Zwei** habe ich das Repertoire von Sozialrufen und ihre Funktion während des Schwärmens untersucht. Basierend auf der spektro-temporalen Struktur der Rufe konnte ich zehn verschiedene Sozialruftypen spezifizieren. Diese Einordnung wurde mittels einer Diskriminanzanalyse bestätigt. Eine Analyse der umgebenden Echoortungssequenzen (wie in Kapitel Eins entwickelt) zeigte, dass die beiden dominierenden Arten strukturell ähnliche Ruftypen zur Kommunikation am Schwärmquartier nutzen. Durch eine nachfolgende Analyse der klassischen akustischen Parameter wurden jedoch kleine, aber signifikante Unterschiede in der Rufstruktur deutlich. Zum besseren Verständnis der Ruffunktion habe ich Playbackversuche nahe des Schwärmareals durchgeführt und jeweils drei Ruftypen beider Arten abgespielt. In drei von sechs Fällen kam es während und nach der Playback-Phase zu einer Zunahme der Fledermausaktivität (gemessen als Anzahl der Echoortungsrufe). Die gleichzeitige Dokumentation der anfliegenden Fledermäuse mittels einer Kamerafalle ließ in einigen Fällen eine artgenaue Identifikation der Individuen zu. Die Ergebnisse deuten darauf hin, dass ein Ruftyp der innerartlichen Koordination und Gruppenzusammenführung dient, was bisherige Kenntnisse über das Phänomen des Herbst-Schwärmens bestätigt. Ein weiterer Ruftyp scheint darüber hinaus auch die zwischenartliche Kommunikation am Schwärmquartier zu unterstützen.

Die Ergebnisse von Kapitel Zwei unterstreichen die Funktion des Schwärmquartiers als Platz des Informationsaustauschs. In dieser Situation sollte es auch möglich sein, den Fledermäusen einen Stimulus zu präsentieren, der mit der Funktionalität des Quartiers verknüpft wird. Für **Kapitel Drei** habe ich hohle Halbkugeln als „akustische Katzenaugen“ am Schwärmquartier angebracht, um die Fledermäuse in dieser Situation mit dem Stimulus vertraut zu machen. Die Kugeln haben ein hohes Echopotenzial und reflektieren über ein breites Spektrum an Einfallswinkeln ein gleichbleibend starkes Echo, wodurch sie für vorbeifliegende Fledermäuse sehr auffällig sind. Um herauszufinden, ob Fledermäuse Fledermauskästen mit einem solchen Echoreflektor in Folge der besseren Detektierbarkeit und Verknüpfung mit einem geeigneten Quartier bevorzugt beziehen, habe ich 60 Kästen (30 mit und 30 ohne Halbkugeln als Reflektor) in drei Studienwäldern ausgebracht. Mittels selbstgebauten Lichtschrankensystemen habe ich die Fledermausaktivität an den Kästen nichtinvasiv gemessen. Die Fledermausaktivität an den Kästen variierte stark – Ursache dafür war jedoch nicht die verbesserte Detektierbarkeit der Kästen, sondern vielmehr die vorherige Vertrautheit der Fledermäuse mit Kastenquartieren innerhalb eines Waldes. Die Lichtschrankensysteme haben darüber hinaus gezeigt, dass die Fledermäuse deutlich früher an den Kästen aktiv waren als durch visuelle Quartierkontrollen nachweisbar. Messungen der Fledermausaktivität mittels Lichtschranken stellen somit eine gute Alternative zu menschlichen Kontrollen dar.

Um gut angepasste Schutzmaßnahmen für bedrohte Arten ergreifen zu können, ist ein umfassendes Wissen über artspezifisches Verhalten notwendig. Nichtinvasive Beobachtungsformen unterstützen uns dabei, das nötige Wissen zu gewinnen, ohne Tiere in ihrem natürlichen Lebensraum zu stören. Alle vorliegenden Ergebnisse wurden so wenig invasiv wie möglich gewonnen. Diese Arbeit erweitert dabei unser Wissen über das Verhalten heimischer Fledermäuse und bildet ein Fundament für umfassendes Monitoring in der Zukunft. Wenn wir zukünftig sowohl die Analyse der „Geräuschkulisse“ als auch die Sozialrufe mit in Beobachtungen von Fledermäusen einbeziehen, können wir ein tieferes Verständnis von Populationsdynamiken, Artzusammensetzung und Habitatnutzung, sowie Phänologie und saisonalen Veränderungen gewinnen.

General Introduction



Non-invasive monitoring of animals

Extensive monitoring plays a crucial role in understanding the ecological dynamics or population trends of wild animals. By closely monitoring animal activities, valuable insights can be gained into key ecological processes, such as predator-prey relationships or reproductive patterns. Beyond its relevance in behavioral studies, extensive monitoring is also of utmost importance for conservation efforts, enabling informed conservation decisions and effective management strategies. Various monitoring techniques can be employed to gather information about animals, their behavior, and internal state. Thereby, capturing animals undoubtedly aids in gathering information about species identity, sex, age, or health status. Furthermore, once caught the utilization of bio-loggers or tracking devices offer insights into an animal's internal condition (reviewed by Cooke et al., 2004; Wilmers et al., 2015) or its external environment (Charrassin et al., 2002; Roquet et al., 2014). However, despite these benefits, capturing wild animals induces stress, which should be particularly avoided when studying the behavior of rare and endangered species (Cattet et al., 2008). To minimize direct interference with the animals, non-invasive monitoring serves as a valuable tool for acquiring unbiased information and understanding the natural behavior and population dynamics of wild animals.

Various non-invasive techniques can be employed depending on the study species, its habitat, and the research objectives. For instance, genetic sampling can be done without direct animal encounters by collecting feces (Kohn et al., 1999), hair (Ruibal et al., 2010), or saliva (Wheat et al., 2016). Likewise, urine samples are utilized to measure steroid hormones, providing insights into the behavioral ecology of animals, a commonly used method to understand the reproductive cycles of female primates (reviewed by Behringer & Deschner, 2017).

At times, visual observations become necessary. The use of cameras enables for more continuous monitoring of wildlife behavior. Especially automated camera traps have played a key role in observations since the development of the first systems in the 1980s (Savidge & Seibert, 1988). Camera traps can facilitate the estimation of population size (Griffiths, 1993; Gilbert et al., 2021) and home range span (Gil-Sánchez et al., 2011), reveal activity patterns of entire communities (C. P. van Schaik & Griffiths, 1996), and report specific behaviors (e.g., seed dispersal by rodents: Nyiramana et al., 2011, carcass scavenging by pumas: Bauer et al., 2005). This technology has been revolutionary for studying the behavior of species that are challenging to observe based on their habitat or lifestyle, rendering them almost invisible through direct observation.

The original camera systems have since evolved, now including the use of unmanned aerial vehicles (UAVs), e.g. for comprehensive population censuses (Guo et al., 2018) or behavioral studies (Koger et al., 2023). Additionally, satellite imagery is employed, aiding for instance in the study of marine mammals (Fretwell et al., 2014; Borowicz et al., 2019). Simultaneous acoustic monitoring further broadens the scope of observational possibilities (Frouin-Mouy et al., 2020), while the application of

acoustic monitoring alone has proven to provide comprehensive population information for more vocal animals (Oppel et al., 2014; Enari et al., 2017). Moreover, in addition to all these possibilities, which often result in large volumes of data, there is a rapidly growing field of automated, deep-learning-based techniques that enable us to handle and analyze such extensive datasets (Tabak et al., 2019; Carl et al., 2020; Krivek et al., 2023).

Bats and echolocation

Among the highly vocal animals are bats, which make up for 20 % of all mammals worldwide. With a combination of active flight and being nocturnal, they have conquered a broad variety of habitats across the globe. For information acquisition, orientation, and foraging in a predominantly dark environment bats evolved echolocation (Griffin et al., 1958), and by listening to the echoes of high-frequency calls bats gain detailed information about their environment and are capable of extraordinary spatial discrimination (e.g., Simmons et al., 1983). Differences in echolocation calls between species often reflect specific prey preferences or divergent foraging techniques. With various individual information being encoded in constantly emitted calls (e.g., colony membership: Jameson & Hare, 2009, sex: Knörnschild et al., 2012, individual identity: Yovel et al., 2009), there is great potential for acoustic monitoring, and species discrimination via echolocation calls is widely applied and has become more and more reliable over the years. Acoustic monitoring is especially helpful for information acquisition in challenging situations and for long-term monitoring. For instance, continuous ultrasonic acoustic monitoring across the North Sea revealed details about the autumnal offshore migration pattern of Nathusius' pipistrelle (*Pipistrellus nathusii*) in relation to weather conditions (Lagerveld et al., 2023). Additionally, migratory bat species at the Baltic Sea, mainly also Nathusius' pipistrelles, were found to aggregate at the coastline during migration season indicating migration across the sea (Ijäs et al., 2017). Ultimately, by combining such information from acoustic monitoring surveys a more detailed picture about migration patterns can be drawn, without the necessity to catch migrating bats or track individuals continuously. Besides, general population monitoring can lead to valuable insights, especially when applied over many consecutive years. Thus, acoustic data help to evaluate the impact of major outbreaks of diseases, such as the white-nose syndrome in North America (Ford et al., 2011; Nocera et al., 2019; Hicks et al., 2020). Another valuable application of acoustic monitoring is the surveillance of bat activity at wind turbines. While wind energy production is a growing sector, the impact on flying and especially migrating species bears some open questions, and collisions with the rotors almost always result in fatalities for bats and birds (Arnett et al., 2008). Acoustic monitoring allows for the reliable estimation of bat activity and thus potential mortality rates (Behr et al., 2023), forming the foundation for successful mitigation measures (Behr et al., 2017; Hayes et al., 2019).

Nevertheless, despite the essential need to extensively monitor the behavior and activity of endangered species, there is currently no spatially inclusive and comprehensive monitoring system in place.

Moreover, acoustic monitoring has not yet reached full automation, and for certain genera, the calls are challenging to distinguish for both human observers and existing algorithms.

Acoustic parameters for species identification from echolocation calls

Commonly used acoustic parameters for species identification via echolocation calls are frequencies, especially at the start and end of calls, call duration, and the specific patterns of frequency changes (Fenton & Bell, 1981). Over the years, advancements in digital signal processing have further facilitated the extraction and analysis of such parameters, enabling more efficient and accurate species identification (Jones et al., 2000). However, exclusively relying on classic acoustic parameters has limitations and may not always yield the desired results because calls can vary considerably between situations or even within sequences. Furthermore, similar calls evolved often with similar ecological niches (Schnitzler & Kalko, 2001). For instance, large similarities can be observed in the structure of European *Myotis* species' echolocation calls (Wimmer & Kugelschafter, 2015), which are mainly adapted to orientation close to background vegetation. However, despite these similarities, *Myotis* species possess the ability to discriminate between seemingly similar calls and even can recognize individual identities based on those (Kazial et al., 2008; Yovel et al., 2009). Nevertheless, species identification via echolocation calls in this genus can be challenging in the field, prompting the question, which additional parameters might contribute to reliable species recognition.

Indeed, not only does the spectro-temporal structure of single signals differ, but the general sound characteristics, such as timbre, also vary between species or even individuals. This fact is widely used in human voice recognition and speaker recognition algorithms of modern smartphones. The human voice has certain unique features, making it one of the most useful biometric signals, as individual as a fingerprint. For human speaker identification and recognition acoustic feature extraction techniques based on mel-frequency cepstral coefficients (MFCCs) are used (reviewed in Jain & Sharma, 2013). Mel scaling is linear below 1 kHz and logarithmic above, emphasizing the importance of low-frequency components in human speech. This feature extraction technique has found numerous applications besides speaker recognition; for instance, Cetin et al. (2004) developed an algorithm utilizing speech recognition technology to evaluate agricultural products by distinguishing pistachio nuts with closed shells from those with open shells. Moreover, MFCCs have been used for various other purposes including music modeling (Logan, 2000), instrument distinction (Loughran et al., 2008), classification of livestock vocalizations (Jahns, 2008), and classification of emotions from speech (Sato & Obuchi, 2007).

In contrast to humans, bats mostly communicate in higher frequencies, which renders a focus on lower frequencies less preferable. Consequently, a linear scale, as reached by the extraction of linear frequency cepstral coefficients (LFCCs) becomes a more suitable option (Zhou et al., 2011). LFCCs already have been shown to capture important acoustic characteristics of bat vocalizations. They carry information

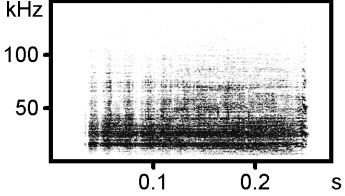
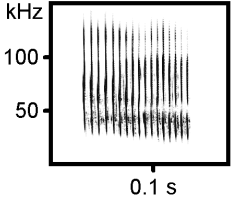
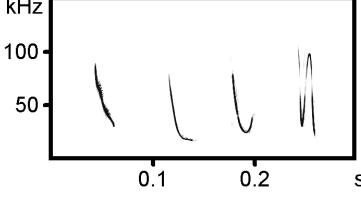
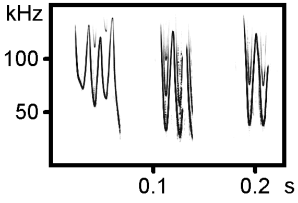
about group identity (Knörnschild et al., 2017) or can vary based on whether the intended receiver of a vocalization is an adult bat or a pup (Fernandez & Knörnschild, 2020). So taken together, both cepstral coefficients make the measurement of single call parameters expendable by representing entire signals in a compact form and the addition of cepstral coefficients can facilitate the analysis of acoustic signals by adding further information. A combination of LFCCs and classic spectral parameters has already been used for acoustic species classification in fish and insects (Noda et al., 2016, 2019). As it considers the “soundscape” of acoustic recordings, this combination should also hold the potential to facilitate non-invasive species discrimination when it comes to species whose echolocation calls are hardly distinguishable based on the exclusive utilization of classic acoustic parameters, such as *Myotis* bats.

Social calls

In addition to echolocation calls for orientation, bats also employ a broad variety of social calls. However, these calls have received significantly less attention in research compared to echolocation calls, especially when produced on the wing. Social calls are far more diverse and, unlike echolocation, social vocalizations are solely intended for communication, aiming to elicit specific behavioral responses from other individuals (Rendall et al., 2009; Bradbury & Vehrencamp, 2011). As an adaptation to long-distance transmission social calls of bats are usually of lower frequency and louder than echolocation calls (Pfalzer & Kusch, 2003). Considering the communicative purpose, social calls are expected to be highly species-specific. Indeed, bats show clear preferences for social calls of conspecifics when compared to calls of heterospecifics (Schöner et al., 2010). Social calls thus hold great potential to further facilitate species identification in situations where identification via echolocation calls may be complicated.

Furthermore, social vocalizations with known functions can offer valuable insights into a species’ biology (Bohn & Gillam, 2018; Chaverri et al., 2018), as their variety and characteristics vary depending on the social context and different situations elicit unique vocal responses. Overall, the investigation of social calls in bats can complement the research on echolocation and contribute to a more comprehensive understanding of bat communication and social dynamics. With a focus on temperate zone bats, previous studies have contributed to the description and classification of social calls (Pfalzer, 2002; Pfalzer & Kusch, 2003; Middleton et al., 2022). Most commonly, calls are divided into distinct groups based on their sonographic structure (see Table i). However, this classification approach overlooks the fact that call structure is not always directly linked to behavioral function, especially when observed interspecifically. While it provides a valuable overview of the diverse repertoire of vocalizations, understanding the full meaning and significance of these calls in social contexts requires additional observations and well-directed experiments.

Table i. Overview of recently defined groups of temperate zone bats' social calls based on their sonographic structure.

	Call Characterization	Pfalzer & Kusch (2003)	Middleton et al. (2022)
	Low frequency, noisy calls of long duration, broadband structure due to multiple harmonics	A – squawk	Type A – threatening/ aggressive
	High number of repeated FM-modulated pulses, forming long call sequences	B – repeated, trill	Type B – distress
	Frequency-modulated cheep-like, single pulses of short duration with wide bandwidth	C – curved cheep	Type C – isolation/ location connected to tandem flight or co-ordination/ cohesion/ chase flights
	More complex, song-like structures, often consisting of different component types	D – complex, song	Type D – advertisement (song-like) or agonistic

The annual cycle of temperate zone bats consists of three main phases: a winter hibernation phase, a summer maternity phase, and an autumn mating phase. For many species, these phases are coupled with the use of different habitats (e.g., forests in summer and underground sites in winter), and sometimes require local or long-distance migration between them (Segers & Broders, 2015; Lehnert et al., 2018). Despite the acknowledgment of these seasonal patterns, research in the fields of ecology and conservation biology often leans towards focusing primarily on the breeding season (Marra et al., 2015). Moreover, social calls are often studied in the context of roosting behavior, as bats are more accessible and easier to identify when roosting. However, it is worth noting that the diversity of social calls observed during roosting may be limited, and the composition of calls emitted in the vicinity of maternity roosts may differ from those in other situations (Schmidbauer & Denzinger, 2019), highlighting the need for more comprehensive observations.

Autumn swarming

One situation where a broad variety of social calls can be observed is autumn swarming. Prior to hibernation during autumn many temperate zone bat species aggregate at underground sites and engage in a behavior known as swarming (Davis, 1964; Roer & Egsbaek, 1966). Swarming is characterized by intense flight activity, chase or tandem flights, and circling in and around the entrances without entering (Fenton, 1969; Parsons et al., 2003). This behavior is accompanied by large amounts of not only echolocation but also social calls. Various observations have suggested different functions of autumn swarming so far. Behavioral studies showed that bats swarm where they hibernate (J. van Schaik et al., 2015) and thus swarming may serve to assess the suitability of hibernacula for both adult bats and their current offspring (Fenton, 1969; Stumpf et al., 2017). Furthermore, in both the Brown Long-eared bat (Furmankiewicz et al., 2013) and the Bechsteins' bat (Kerth & Reckardt, 2003) an enlargement of the epididymides at swarming sites indicates increased mating readiness. But while for the Greater Mouse-eared bat and the Whiskered bat, the main mating period coincides with the swarming period and ends when animals enter hibernation, for the Brown Long-eared bat, the epididymides remained enlarged while bats hibernated, suggesting that mating also occurs during hibernation and subsequent spring swarming (Pfeiffer & Mayer, 2013). Mating during swarming has also been observed in some individuals of other *Myotis* species. For most swarming species, males are solitary or live in small groups throughout the summer (Safi & Kerth, 2007), and swarming sites are one of a few locations where both sexes meet regularly.

Although swarming bats emit a broad variety of social calls besides echolocation calls, such vocalizations have only been described to a limited extent. Overall, knowledge of species-specific vocalizations during swarming is scarce. Social calls of a few species, including the Brown Long-eared bat (Furmankiewicz, 2005; Murphy, 2012), Natterer's bat (Schmidbauer & Denzinger, 2019), Whiskered bat, Bechstein's bat and Greater Barbastelle bat (Pfalzer, 2002) have been partially described. Also, for Natterer's bats differences between social calls of swarming bats in front of summer roosts and autumn swarming sites have been observed, indicating differences in call function (Schmidbauer & Denzinger, 2019). Further investigation of social calls during autumn swarming can lead to a better understanding of this behavior. Specifically, species-specific social calls can aid in species identification in crowded swarming situations, especially when individuals of different species converge. Additionally, studying the typical function of specific social calls allows for conclusions about the behavioral context, contributing to a more comprehensive understanding of the various functions of swarming.

Study Site: Kalkberg Cave

One of the largest natural hibernacula in Germany is the Kalkberg Cave in Bad Segeberg (Figure i). It is inhabited by a variety of *Myotis* species, with a predominance of Natterer's bats (*Myotis nattereri*)

and Daubenton's bats (*Myotis daubentonii*). Extensive monitoring of the cave has revealed that about 30,000 bats hibernate there each winter and before hibernation, the vicinity of the cave is extensively used for autumn swarming. The bats primarily, but not exclusively, swarm in front of the two cave entrances. In particular, the upper entrance resembles an arena-like setting, where bats circle for hours and over many nights.

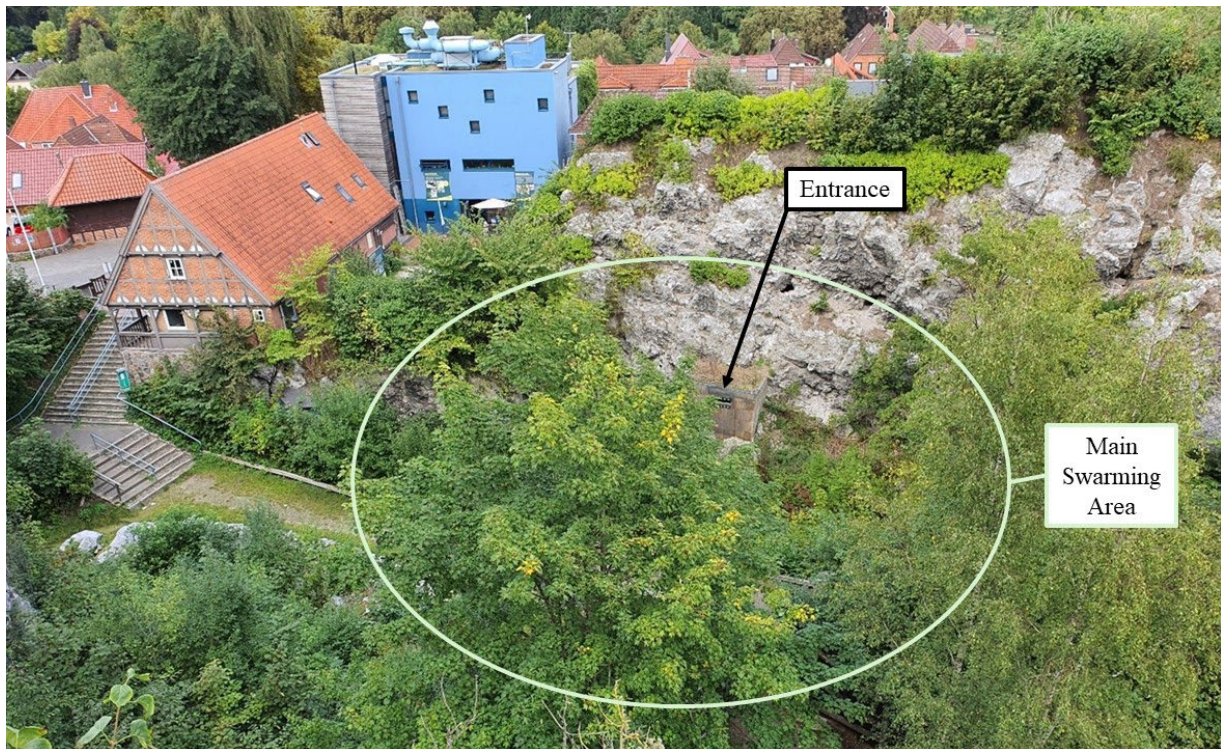


Figure i. One of two entrances and main swarming area of the Kalkberg Cave, Bad Segeberg, Germany.

Conservation of endangered bat species

While underground caves are relatively stable systems, other habitat structures in the annual life cycles of bats are more prone to changes, and especially during the 1960s and 70s, bat populations have significantly declined in Germany (e.g. Roer, 1977; Frank et al., 1980; Vierhaus, 1997). Despite protective measures, about half of the 25 species are still considered endangered (Meining et al., 2020) and all are protected by national law (BNatschG, 1992). One of the main threats is extensive habitat alteration, which impacts numerous aspects of bats' lives. For instance, newly constructed roads affect navigation behavior as key orientation points and guiding structures along traditional routes disappear (Fensome & Mathews, 2016). Additionally, low-flying bat species face the danger of colliding with road traffic (Lesiński et al., 2011). Another primary cause of population decline is the extensive loss of natural habitats, often accompanied by a decrease in roosting opportunities (Mickleburgh et al., 2002; Davidson-Watts et al., 2006). Factors contributing to habitat loss include deforestation and the early removal of dead and old trees, which reduces the availability of suitable tree cavities for bats. Unfortunately, the natural formation of tree cavities is a slow process (Vesk et al., 2008), and the availability of trees is

diminishing on a global scale (Lindenmayer & Laurance, 2017). For effective conservation measures, the protection of roosts thus is essential.

To mitigate the consequences of habitat loss, artificial roost boxes can expand suitable habitats for tree-dwelling bats. As different bat species have varying roosting preferences, there is a great variety of artificial roosts available. Even though such artificial roosts have been used since the 1950s (Issel & Issel, 1955), the details of how bats search for and find new artificial roost sites are not fully understood. For vespertilionid bats different sensory modalities may play a role in the detection of boxes, as the mostly small entrances are difficult to detect via echolocation in cluttered surroundings. At least for some species eavesdropping on echolocation calls of conspecifics within roosts leads to a decrease in search time for suitable roosts (Ruczyński et al., 2009) and also social calls emitted from the roost have the potential to lead to higher detection rates from conspecifics (Schöner et al., 2010; Furmankiewicz et al., 2011). Studies have shown that olfactory cues such as urine and guano typically do not play a significant role in roost location (Ruczyński et al., 2007, 2009; Brown & Carter, 2022). However, ultimately, the success of roost supplementation depends on the detection and occupancy of newly installed boxes. One approach to increase box detectability is to make them more conspicuous to passing bats, which might be achievable through the attachment of an echo-reflective cue to the box.

During autumn swarming bats intensively perceive their surroundings as the substantial amount of echolocation calls emitted during swarming provides a detailed picture of the swarming site. Stimuli which are presented to the swarming bats in this situation may enhance a positive association between the stimulus and the swarming site as a suitable roost. Hollow hemispheres work as acoustic reflectors by providing a strong echo over a broad range of angles and are thus highly conspicuous to passing bats (von Helversen & von Helversen, 1999; Simon et al., 2011). By equipping both the roost entrances at the swarming site and newly installed boxes as nearby summer roosts, it is possible to test whether a positive association was made. The hollow hemispheres as acoustic reflectors should lead to a decrease in search time and higher activity rates at the equipped boxes in comparison to conventional roost boxes.

Thesis outline

The better we understand the behavior of endangered species the better we can protect them. With this study, I aim to contribute to the understanding of the swarming behavior of temperate zone bat species, especially within the genus *Myotis*. My main study organisms were the predominant species at the Kalkberg Cave, Natterer's bats (*Myotis nattereri*) and Daubenton's bats (*Myotis daubentonii*). Echolocation calls of *Myotis* species exhibit large similarities in their classic acoustic parameters and the minor differences in these calls are additionally attenuated in a crowded swarming situation, where many individuals call simultaneously, making species identification considerably more challenging.

Chapter One deals with the problem of how to identify bat species during swarming acoustically, and thus non-invasively. I complemented the paradigm of species identification based on classic acoustic

parameters with the additional use of Linear Frequency Cepstral Coefficients (LFCCs). With this parameter combination, the “soundscape” of swarming bats is analyzed without the need to analyze single calls. Based on a set of unequivocally identified reference calls a classification of the predominant swarming species at the time of recording was possible based on sequences of overlapping echolocation calls.

In comparison to echolocation calls, considerably less research focused on social calls, particularly when emitted in-flight. However, a comprehensive description of social calls is necessary to enhance our understanding of bats’ behavior. In **Chapter Two**, I described the variety of social calls I observed during autumn swarming over two consecutive swarming seasons. To gain information about the calling species I applied the method developed in Chapter One to the echolocation calls that accompanied social calls. Seemingly similar social calls were assigned to both species respectively, showing that Natterer’s bats and Daubenton’s bats emit rather similar calls during autumn swarming. For a better understanding of the call function, I conducted playback experiments in the vicinity of the swarming site with three of the recorded call types of both species respectively. In three out of six experimental scenarios an increase in bat activity (approximated as echolocation call rates) during and after stimulus presentation indicated that bats inspected or approached the playback site. By simultaneously taking photographs with a camera trap, I sometimes managed to identify approaching bats to species level. Taken together, my results suggest that one call type facilitates interspecific communication while another one works for group cohesion intra-specifically. These findings align with and expand upon our understanding of autumn swarming.

With the swarming site being a place for information transfer between individuals or – as I showed in Chapter Two – even between species, I aimed to determine whether a cue presented to the bats in this context could establish a positive association between the cue and a suitable roost. Thus, for **Chapter Three** I installed hollow hemispheres as highly conspicuous echo-reflective cues at the swarming site. Bats first encountered these cues during swarming and encountered them again upon leaving the hibernaculum after hibernation. Afterwards, I equipped bat boxes with hollow hemispheres, to investigate whether bats would show a preference for such boxes over boxes without hemispheres. To non-invasively measure bat activity I constructed a light beam system that recorded activity at boxes, and indeed, bat activity (quantified as light beam interruptions) exhibited significant variation. However, the main driver was not the equipment of boxes with hollow hemispheres, but rather the bats’ familiarity with boxes as artificial roosting opportunities within the observed forest sites. Additionally, I developed a well-functioning monitoring system to measure activity rates at bat boxes, which showed that boxes often were inhabited many days before feces or even individuals could be observed during visual controls.

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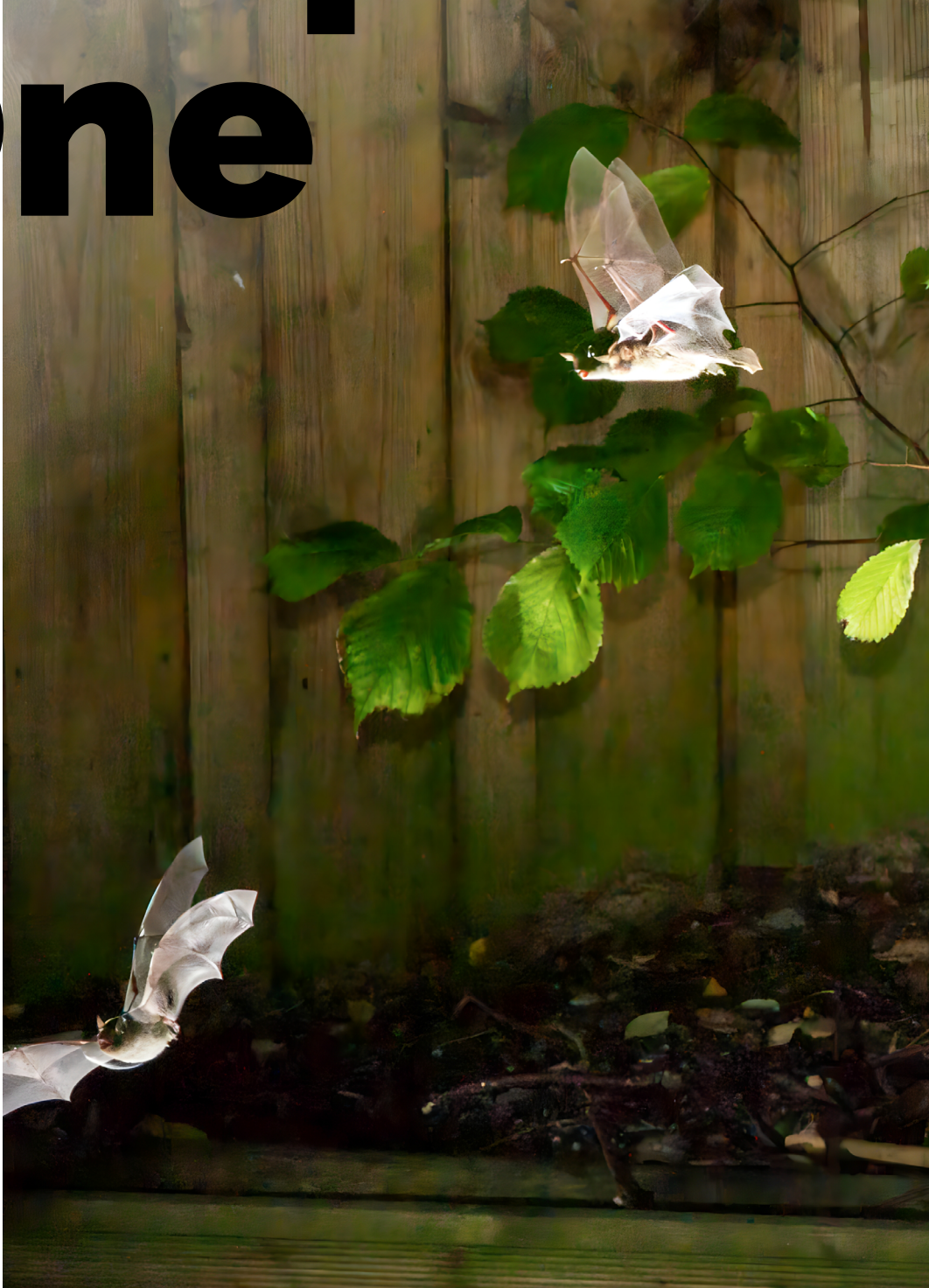
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Chapter One



The soundscape of swarming:
Proof of concept for a noninvasive acoustic
species identification of swarming *Myotis* bats

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Cover of Chapter One: Two Natterer's bats (*Myotis nattereri*) during autumn swarming at the Kalkberg Cave.

RESEARCH ARTICLE

The soundscape of swarming: Proof of concept for a noninvasive acoustic species identification of swarming *Myotis* bats

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Abstract

Bats emit echolocation calls to orientate in their predominantly dark environment. Recording of species-specific calls can facilitate species identification, especially when mist netting is not feasible. However, some taxa, such as *Myotis* bats can be hard to distinguish acoustically. In crowded situations where calls of many individuals overlap, the subtle differences between species are additionally attenuated. Here, we sought to noninvasively study the phenology of *Myotis* bats during autumn swarming at a prominent hibernaculum. To do so, we recorded sequences of overlapping echolocation calls ($N = 564$) during nights of high swarming activity and extracted spectral parameters (peak frequency, start frequency, spectral centroid) and linear frequency cepstral coefficients (LFCCs), which additionally encompass the timbre (vocal “color”) of calls. We used this parameter combination in a stepwise discriminant function analysis (DFA) to classify the call sequences to species level. A set of previously identified call sequences of single flying *Myotis daubentonii* and *Myotis nattereri*, the most common species at our study site, functioned as a training set for the DFA. 90.2% of the call sequences could be assigned to either *M. daubentonii* or *M. nattereri*, indicating the predominantly swarming species at the time of recording. We verified our results by correctly classifying the second set of previously identified call sequences with an accuracy of 100%. In addition, our acoustic species classification corresponds well to the existing knowledge on swarming phenology at the hibernaculum. Moreover, we successfully classified call sequences from a different hibernaculum to species level and verified our classification results by capturing swarming bats while we recorded them. Our findings provide a proof of concept for a new noninvasive acoustic monitoring technique that analyses “swarming soundscapes” by combining classical acoustic parameters and LFCCs, instead of analyzing single calls. Our approach for species identification is especially beneficial in situations with multiple calling individuals, such as autumn swarming.

Gloza-Rausch and Knörnschild joint senior authorship.

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KEYWORDS

bats, echolocation calls, linear frequency cepstral coefficients, *Myotis*, noninvasive acoustic monitoring, noninvasive species identification, swarming phenology

TAXONOMY CLASSIFICATION

Behavioural ecology, Biodiversity ecology, Conservation ecology

1 | INTRODUCTION

In our need to understand the behavior of animals, we often inadvertently affect it. Nevertheless, extensive monitoring is not only important for behavioral studies but also for conservation efforts. Without question, capturing animals facilitates information collection in terms of species identity, sex or age. In addition, bio-loggers or tracking devices can be applied and provide information about an animal's internal state (reviewed in Cooke et al., 2004; Wilmers et al., 2015) or its external environment (Charrassin et al., 2002; Roquet et al., 2014). However, despite these advantages, capturing wild animals causes stress, which is especially relevant in the context of behavioral studies and the observation of rare and endangered species (Cattet et al., 2008; Lane & McDonald, 2010). To avoid interfering with the animals directly, noninvasive monitoring is a powerful tool to gain information about the natural behavior of wild animals or population dynamics. When the focal species are nocturnal, fast-moving, or of small body size, visual observation becomes difficult and often is connected with high effort (Therriault et al., 2014). Therefore, depending on the focal species, its surroundings, and the goals of the observation, other techniques are applied, such as camera traps (Gilbert et al., 2021; Kalle et al., 2011), collection of feces (Kohn et al., 1999; Prugh et al., 2005) or acoustic monitoring (Enari et al., 2017; Oppel et al., 2014).

Acoustic monitoring is often used to detect bats, a crucial endeavor for conservation applications because more than half of all bat species occurring in Germany are endangered there and all are protected by national law (Meinig et al., 2020). In recent decades technical capabilities for detecting and analyzing bat sounds have developed rapidly (Grinnell et al., 2016), thus facilitating species identification via species-specific echolocation calls emitted in-flight. Those calls evolved for information acquisition, orientation and foraging in a predominantly dark environment (Griffin et al., 1958) and make bats capable of extraordinary spatial discrimination (e.g. Simmons et al., 1983). Besides encoding colony membership (Jameson & Hare, 2009; Masters et al., 1995), individual identity (Kazial et al., 2008; Voigt-Heucke et al., 2010; Yovel et al., 2009), sex (Jones et al., 1992; Knörnschild et al., 2012; Siemers et al., 2005) or age (Jones et al., 1992; Masters et al., 1995), echolocation calls facilitate species recognition, even interspecifically (Schuchmann & Siemers, 2010). Indeed, differences in echolocation calls may reflect specific prey preferences or divergent foraging techniques, and often ecologically similar bats employ similar echolocation calls (Neuweiler, 2003; Schnitzler & Kalko, 2001; Siemers

& Schnitzler, 2004). For instance, large similarities occur in the call structure of some European *Myotis* species, which are mainly adapted to orientation close to background vegetation. Such similarities complicate species identification through echolocation calls in this genus. Nevertheless, *Myotis* species are capable of discrimination between seemingly similar calls and even can recognize individual identity based on those (Kazial et al., 2008; Yovel et al., 2009), indicating the possibility of comprehensive species discrimination.

About 30,000 individuals of six different *Myotis* species hibernate at the Kalkberg cave in Northern Germany and among them are large numbers of Daubenton's bats, *Myotis daubentonii*, and Natterer's bats, *Myotis nattereri*, (estimations based on light barrier counts and camera traps; MELUND, 2019). Prior to hibernation *Myotis* bats and other temperate zone bats that hibernate in underground sites are often engaged in an activity known as "swarming." Following the first observation in North America (Davis, 1964) also European bats were found to swarm at underground roosts outside the period of hibernation (Roer & Egsbaek, 1966). Swarming is characterized by intense flight activity, chase flights and circling in and around the entrances of the hibernacula (winter roosts used for hibernation) without entering, accompanied by a large amount of both echolocation calls and social vocalizations (Fenton, 1969; Parsons, Jones, & Greenaway, 2003). Behavioral and genetic studies have revealed various functions of swarming so far. Swarming is important to assess hibernacula, both for experienced individuals and their current offspring (e.g. Fenton, 1969; Stumpf et al., 2017). In addition, gene flow between otherwise isolated colonies and promiscuous mating behavior is facilitated when bats of different colonies meet at the swarming sites (e.g. Burns & Broders, 2015; Kerth et al., 2003; Rivers et al., 2005). Overall, swarming at hibernacula facilitates various, not mutually exclusive social functions depending on the individual's species, sex or age.

Because swarming bats constantly emit echolocation calls, the calls strongly overlap, thus making small differences even more subtle and identification of some bat species very challenging (Rydell et al., 2017). While the acoustic species identification based on echolocation calls has made remarkable progress in recent years (Bas et al., 2017; Obrist & Boesch, 2018; Schwab et al., 2022), classifying the echolocation calls of many bats vocalizing at the same time (i.e. during swarming) still remains extremely difficult because it is often impossible to extract overlap-free single calls or call sequences for species identification. Here, we demonstrate a proof of concept how this problem could be solved by focusing on the swarming soundscape, i.e. the predominant acoustic impression at a

given time period during swarming instead of calls from single individuals (see [Figure A1](#)).

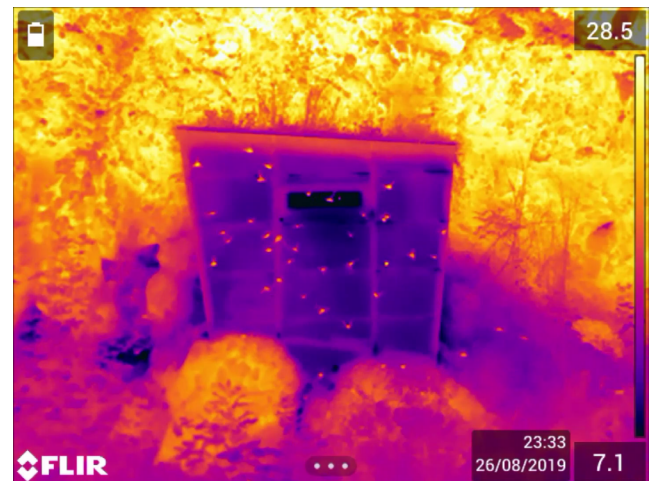
We discriminated two different *Myotis* species (*M. daubentonii* and *M. nattereri*) with the help of both classical acoustic parameters and derived parameters originally employed in human speaker recognition. Not only the spectro-temporal structure of single calls but also the general sound characteristics of calls (such as color of voice) differ between species or even individuals, a fact that is for instance exploited in the speaker recognition algorithms of modern smartphones. Often, acoustic feature extraction techniques based on mel frequency cepstral coefficients (MFCCs) are used (reviewed in Jain & Sharma, 2013). MFCCs use a mel scale, which is linear up to 1 kHz and logarithmic above to emphasize low frequencies, like human voice. For signals with a higher frequency, such as echolocation calls, this emphasis is not desirable and a linear scale can be applied yielding linear frequency cepstral coefficients (LFCCs) instead (Zhou et al., 2011). Both cepstral coefficients make the measurement of single call parameters expendable by representing entire signals in a compact form. During the process of feature extraction, the information of the whole signal is condensed in several steps of calculations (Cuong et al., 2012; Loughran et al., 2008). Cepstral coefficients in combination with classical acoustic parameters (e.g. peak frequency, duration, etc.) have been employed to facilitate species identification based on single calls for crickets and katydids (Noda et al., 2019) or fish (Noda et al., 2016). Furthermore, cepstral coefficients have been used to categorize call types of giant otters groups (Mumm & Knörnschild, 2017) or to discriminate between colony-specific signatures in territorial songs of male bats (Knörnschild et al., 2017).

The goal of our study was to test whether our approach would allow us to identify two swarming *Myotis* species based on the soundscape their echolocation calls created. We presumed that we could identify the predominantly swarming species during a given time period by comparing our recordings of overlapping echolocation call sequences (swarming soundscapes) to a set of reference data, i.e., previously identified echolocation call sequences, thus making the analysis of single call sequences obsolete. We used the second set of previously identified call sequences to validate our classification results. Moreover, we compared our acoustic species identification of swarming bats to the known swarming phenology of both species at our study site.

2 | METHODS

2.1 | Study site and bat activity

Recordings of echolocation calls were conducted at the Kalkberg cave (10°18'57"E; 53°56'09"N), one of the most important hibernacula of bats in central Europe. The natural cave is located in Bad Segeberg, Northern Germany, and shelters more than 30,000 bats per winter (MELUND, 2019). Both entrances of the cave have been monitored



VIDEO 1 Autumn swarming in front of one of the two entrances of the Kalkberg cave at the 26th August 2019, 23:32. The video was recorded using a thermal camera (FLIR E95, Teledyne FLIR LLC, Wilsonville, USA).

with light barriers (ChiroTEC, Lohra, Germany) since 1991, counting incoming and departing individuals. Among the hibernating bats are six *Myotis* species, with *M. nattereri* and *M. daubentonii* making up for about 90% of the winter population (estimations based on light barrier counts and camera traps; MELUND, 2019). In total, at least seven bat species use the Kalkberg cave: *M. nattereri*, *M. daubentonii*, *M. brandtii/mystacinus*, *M. bechsteinii*, *M. dasycneme*, *M. myotis*, and *Plecotus auritus* (sorted from common to rare; MELUND, 2019). Prior to hibernation, between August and November, the vicinity of the cave is extensively used for autumn swarming ([Video 1](#)). In addition, we recorded swarming bats at another site in Northern Germany (Lüneburg) while simultaneously capturing bats with mist nets in the direct vicinity of their swarming site.

2.2 | Acoustic recordings and data preparation

We employed a total of three data sets consisting of echolocation call sequences for the analyses. The first data set (test data A and B) contained recordings of overlapping echolocation call sequences of swarming bats in front of the Kalkberg cave (A) and the second site in Northern Germany (B).

We wanted to identify the predominantly echolocating species in these recordings with the help of a second data set (reference data), which contained echolocation call sequences of *M. daubentonii* or *M. nattereri* in a single flight, assigned to species level via photos from synchronized camera trap images. A third data set (control data) contained additional previously identified echolocation call sequences of both focal species from single flights. We used the reference data as training data in a discriminant function analysis (DFA) to classify recordings from the test data and the control data as either *M. daubentonii* or *M. nattereri*. Due to the difference in recording

quality, recordings from the three data sets were in part prepared differently for subsequent analyses (for details, see below).

2.2.1 | Test data: unidentified echolocation call sequences of swarming *Myotis* bats

Recordings were conducted during 45 nights in two consecutive swarming seasons (August to November 2018 and August to October 2019) at various times between sunset and sunrise, mainly during the highest swarming activity (2 h after sunset until 2 am) at both entrances of the Kalkberg cave (test data set A). Recording sessions were initiated based on the local weather at the beginning of the night (no rainfall, mild temperatures, little wind, i.e., Beaufort Force 0–3). As weather conditions sometimes changed drastically during the night, the recording nights were not always the nights with the highest swarming activity. During the recording nights in 2018, the maximum activity (sum of arrivals and departures counted via light beam interruptions) was 10,415 and the minimum activity was 1182 (Figure 1). During the recording nights of 2019, the maximum activity was 11,678 and the minimum activity was 2162.

Acoustic recordings were made whenever a high number of individuals was swarming simultaneously (observed with a thermal video camera; FLIR E95, Teledyne FLIR LLC, Wilsonville, USA). We are aware that this selection of specific recording situations may cause a bias in our data set (e.g., rarer species may only swarm when

it is less crowded) but our first priority was to test whether our approach works during high swarming activity with many overlapping calls (proof of concept). Echolocation call sequences were recorded (sampling rate 500kHz, 16-bit depth resolution) using a high-quality ultrasonic microphone (Avisoft USG 116Hm with condenser microphone CM16; frequency range 1–200kHz) connected to a small computer (Dell Venue 8) running the software Avisoft Recorder (v4.2.05, R. Specht, Avisoft Bioacoustics, Glienicke, Germany). For the subsequent acoustic analysis, 564 echolocation call sequences (mean: 11.3 sequences per night; range: 1–29) with a length of 4 s each were selected based on the quality of the sound recordings and the presence of a high number of echolocation calls without interfering social vocalizations. Again, this choice may have caused a bias in our data set (e.g. some species may produce many social calls during swarming and would thus be less represented in our data set) but it was unavoidable because we did not have a training set of social calls identified to species level to complement our training set of echolocation calls. The selected call sequences were band-pass filtered (15–150kHz) and amplified digitally by 6 dB in Avisoft-SASLab Pro (v5.2.13, R. Specht, Glienicke, Germany) prior to further analysis.

We also recorded swarming bats during one night (22.09.2021) at another site in Northern Germany (Lüneburg) and simultaneously captured swarming bats with mist nets located 2 meters away from the microphone (test data set B). Recordings were made, selected, and subsequently processed as described above. Data set B was much smaller than data set A, comprising only 30 echolocation call sequences with a length of 4 s each but valuable because the

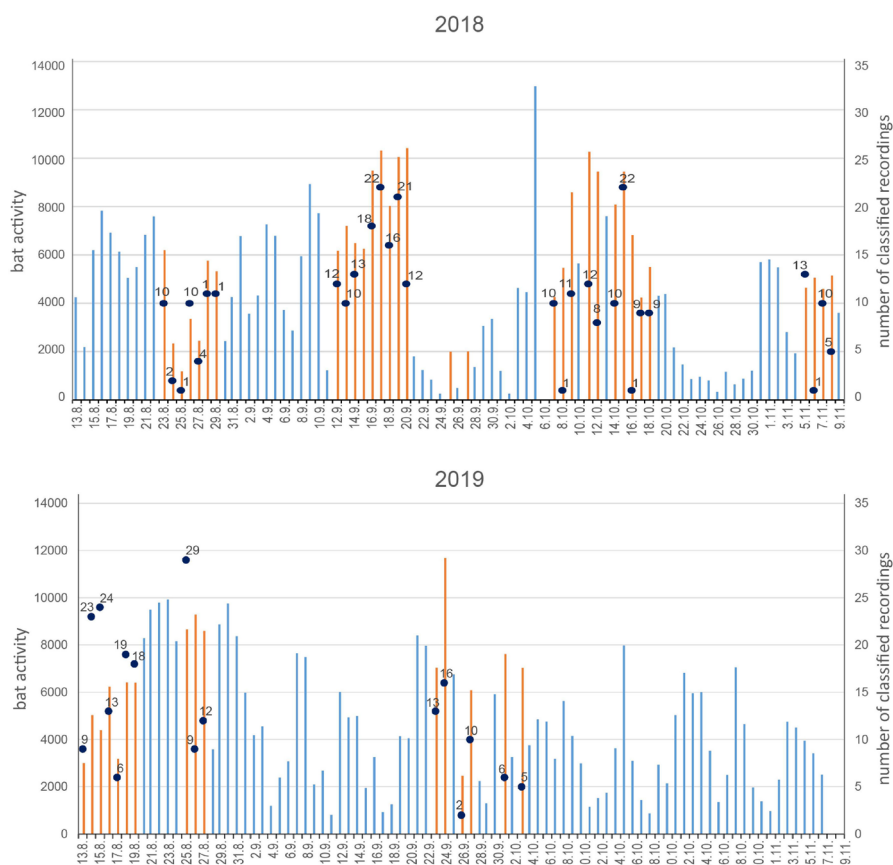


FIGURE 1 Total activity (sum of arrivals and departures counted via light beam interruptions) of bats at the hibernaculum per night during the swarming seasons of 2018 and 2019. The nights during which sound recordings were conducted are highlighted in orange. Recordings started in mid-August and continued until mid-November. Numbers indicate the amount of analyzed echolocation call sequences per recording night, which were classified as *Myotis daubentonii* or *Myotis nattereri* with a classification probability of 90% or higher.

dominant species of the swarming bats was confirmed by simultaneous capture (87% of captured bats were *M. nattereri*).

2.2.2 | Reference data

To classify the recorded echolocation call sequences from the swarming situation, identified echolocation call sequences of *M. daubentonii* and *M. nattereri* were used as a reference (i.e. as training set in a discriminant function analysis). These echolocation call sequences came from singly flying individuals and were recorded at 10 underground sites with a Batcorder (ecoObs GmbH, Nürnberg, Germany) using a sampling rate of 500kHz and a trigger threshold of -36dB (quality 26–28). The calling species were identified via photos from synchronized camera trap images (Wimmer & Kugelschafter, 2015): Whenever bats were flying through a narrow underground passage, a light barrier was interrupted, which triggered a sound recording and a corresponding photo from a camera trap (ChiroTEC, Lohra, Germany). If it was possible to identify the species of the calling bat based on the photo, the respective recording was saved in a database. From these recordings, we selected 60 sequences of five high-quality echolocation calls per species, *M. daubentonii* and *M. nattereri*, for further analysis (Figure 2). Selected sequences were 0.1–0.3 seconds long. Prior to acoustic analyses, the

noise was reduced by 50 dB, high-pass filtering was applied (25 kHz) and the volume of the calls was raised by 6 dB in Avisoft-SASLab Pro. To avoid treating the background noise like a signal during the feature extraction (details below), it had to be eliminated prior to further analysis. For this purpose, we deleted all temporal gaps between echolocation calls in the reference data. Even though in the reference data echolocation calls of *M. daubentonii* were often multi-harmonic in structure (due to the very small distance of bats to the microphone at the underground sites), only the first harmonic (fundamental frequency) was used for acoustic analyses. The second harmonic is not recorded when *M. daubentonii* is echolocating at a distance (Britton & Jones, 1999; Schaub & Schnitzler, 2007), as it was the case for our recordings from the Kalkberg cave (test data set A) and Northern Germany (test data set B).

2.2.3 | Control data

To validate our statistical classification of the test data sets A and B, we classified an additional data set as a control using the same reference data. For this control data set, the species identity of the calling bats was also deduced unequivocally, e.g., when bats were recorded flying near their roost and the species composition of the roost was fully known. The echolocation call sequences in the

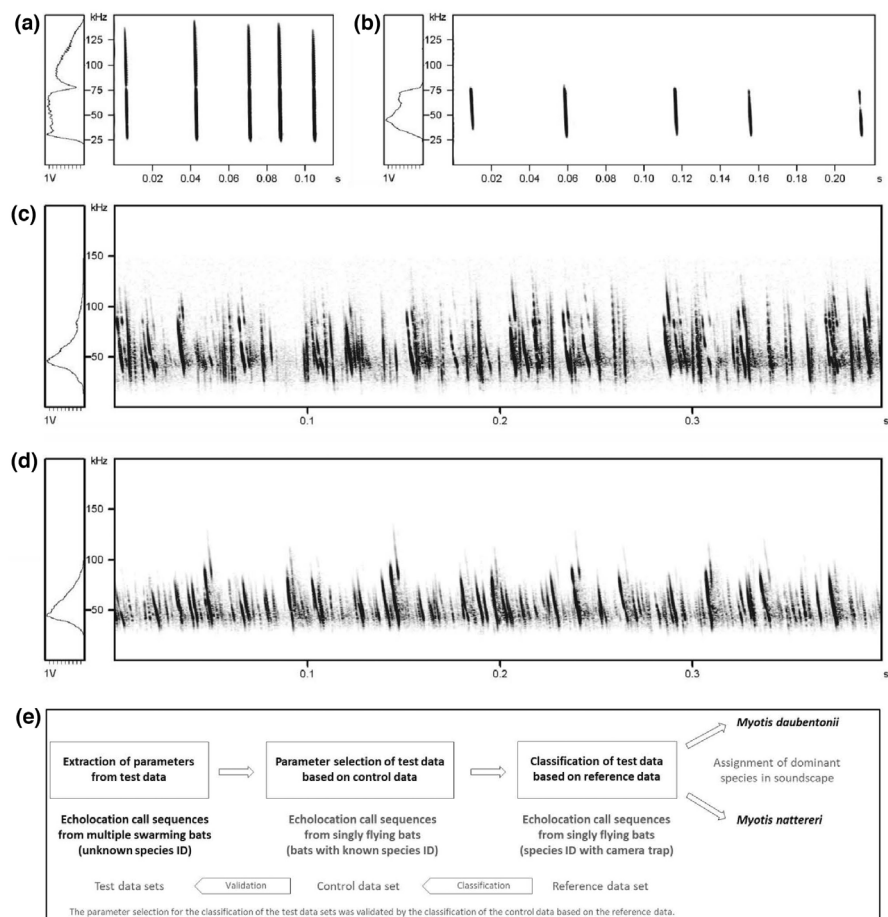


FIGURE 2 Reference calls of *Myotis nattereri* (a) and *Myotis daubentonii* (b) were used to classify recordings with unknown echolocation calls (c and d). The employed sequences consisted of five consecutive echolocation calls without background noise. Based on the reference data set, recordings with overlapping echolocation calls from a swarming situation were classified as predominantly *M. nattereri* (c) or *M. daubentonii* (d). The bottom panel (e) illustrates our classification procedure and how reference, control and test data sets are connected. Spectrograms were created using a 1024 FFT and a Hamming window with 87.5% overlap. See Figure A1 for a visualization of the “swarming soundscape” analysis.

control data set were recorded using a Petterson D980 (Petterson Elektronik AB, Sweden) in time expansion mode (Skiba, 2009). All sound recordings were stored on magnetic tape and digitized at the Museum for Natural History, Berlin (300kHz, 16 bit). We selected 16 high-quality echolocation call sequences of *M. daubentonii* and *M. nattereri*, respectively. These sequences consisted of 4–7 consecutive echolocation calls each and were 0.4 s long; they were slightly longer than the sequences in the reference data set because of larger inter-call intervals. The control data were used to validate our statistical classification (i.e., the selection of acoustic parameters to discriminate between *M. daubentonii* and *M. nattereri*) and treated in the same way as the reference data (noise reduction, high-pass filtering, volume change, gap removal) prior to acoustic analyses. It was not necessary to remove the second harmonic for echolocation calls of *M. daubentonii* in the control data set because only the first harmonic was recorded (as it is normally the case for field recordings).

2.3 | Acoustic analysis

In total, we extracted 14 acoustic parameters for a general description of calls and subsequent statistical analysis, four spectral parameters (start, end and peak frequency, spectral centroid) and 10 derived acoustic parameters (mean and standard deviation for LFCC 1 to 5).

Spectral parameters: Start, end, and peak frequency for the test data sets A and B were calculated with a custom-made MATLAB routine over the entire file in 10 ms frames using the *meanfreq* function from the Signal Processing toolbox. The analysis of single calls was not possible in the test data sets A and B (swarming bats) because there was much overlap between calls in the sequences. For the reference and the control data sets, start, end, and peak frequency of all echolocation calls in a sequence were measured in Avisoft-SASLab Pro (threshold of –24 dB relative to the peak amplitude; values averaged over the entire call). In contrast to the test data, single calls were measured in the reference and control data sets. For all data sets, we also calculated the spectral centroid of each echolocation call sequence in Avisoft-SASLab Pro (threshold: –28 dB relative to peak amplitude).

Linear-frequency cepstral coefficients (LFCCs): We additionally used an acoustic feature extraction technique based on LFCCs for all data sets. As spectral-based representations of entire signals, LFCCs capture the most important features of a signal in a compact form. For all data sets the feature analysis was run with a custom-made routine in the speech processing toolbox “voicebox” in MATLAB (v. R2018b). In total, five LFCCs were extracted (Hamming window; test data: 100ms frame; reference and control data: 3 ms frame). Subsequently, values for each frame were summarized by calculating the mean and standard deviation for each of the five features for every analyzed echolocation call sequence.

2.4 | Statistical analysis

To test for species identity (i.e., the identity of the dominant species in each recording; see Figure A1), we performed stepwise discriminant function analyses (DFA) with subset validation, in which the reference data (with known species ID, 120 sequences) functioned as the training set. In the first DFA, the control data (also with known species ID, 32 sequences) was used to validate our statistical approach and select the acoustic parameters most important for the correct species identification. Resulting from this, the second DFA was applied to classify the test data set A (564 sequences with unknown species ID) using the parameters spectral centroid, start frequency, peak frequency, and mean and standard deviation of the LFCCs 1 and 3. We selected those parameters because they were the most important ones for correctly classifying the control data, as indicated by an initial stepwise DFA (end frequency, LFCC 2, 4, and 5 were excluded by the analysis). A third DFA was conducted with the same selection of acoustic parameters to classify the test data set B associated with simultaneous bat capture (30 sequences with unknown species ID). Prior to the analyses, we checked our data for multivariate normality and homogeneity of variances/covariances. Statistical tests were conducted using SPSS (version 20, SPSS Inc., Chicago, IL, USA).

3 | RESULTS

3.1 | Control data were classified correctly

Using seven acoustic parameters (start and peak frequency, spectral centroid, mean and standard deviation of LFCC 1 and 3) in a stepwise DFA with subset validation, the species ID of all 32 echolocation call sequences in the control data set could be classified correctly with a minimum classification probability of 94% (DFA: Training $N = 120$, Test $N = 32$, Eigenvalue = 12.225, explained variation = 100%, Wilk's $\lambda = 0.076$, $\chi^2 = 295.648$, $p < .0001$). The same parameters were afterward employed in the second and third DFA with unidentified echolocation call sequences from swarming bats as test data sets.

3.2 | Most call sequences from swarming bats could be classified to species level

The test data set A contained 564 sequences (4 s each) of overlapping echolocation calls of multiple swarming bats. With the selected seven parameters described above, we could classify the vast majority of the recordings (509 sequences, 90.2%). Out of the 564 call sequences, 184 were classified as *Myotis daubentonii* and 325 as *Myotis nattereri* with a classification probability of 90% or higher (DFA: Training $N = 120$, Test $N = 564$, Eigenvalue = 12.225, explained variation = 100%, Wilk's $\lambda = 0.076$, $\chi^2 = 295.648$, $p < .0001$, see also Table A1 and A2). The other 55 echolocation call sequences had a lower classification probability and were thus discarded from further analysis.

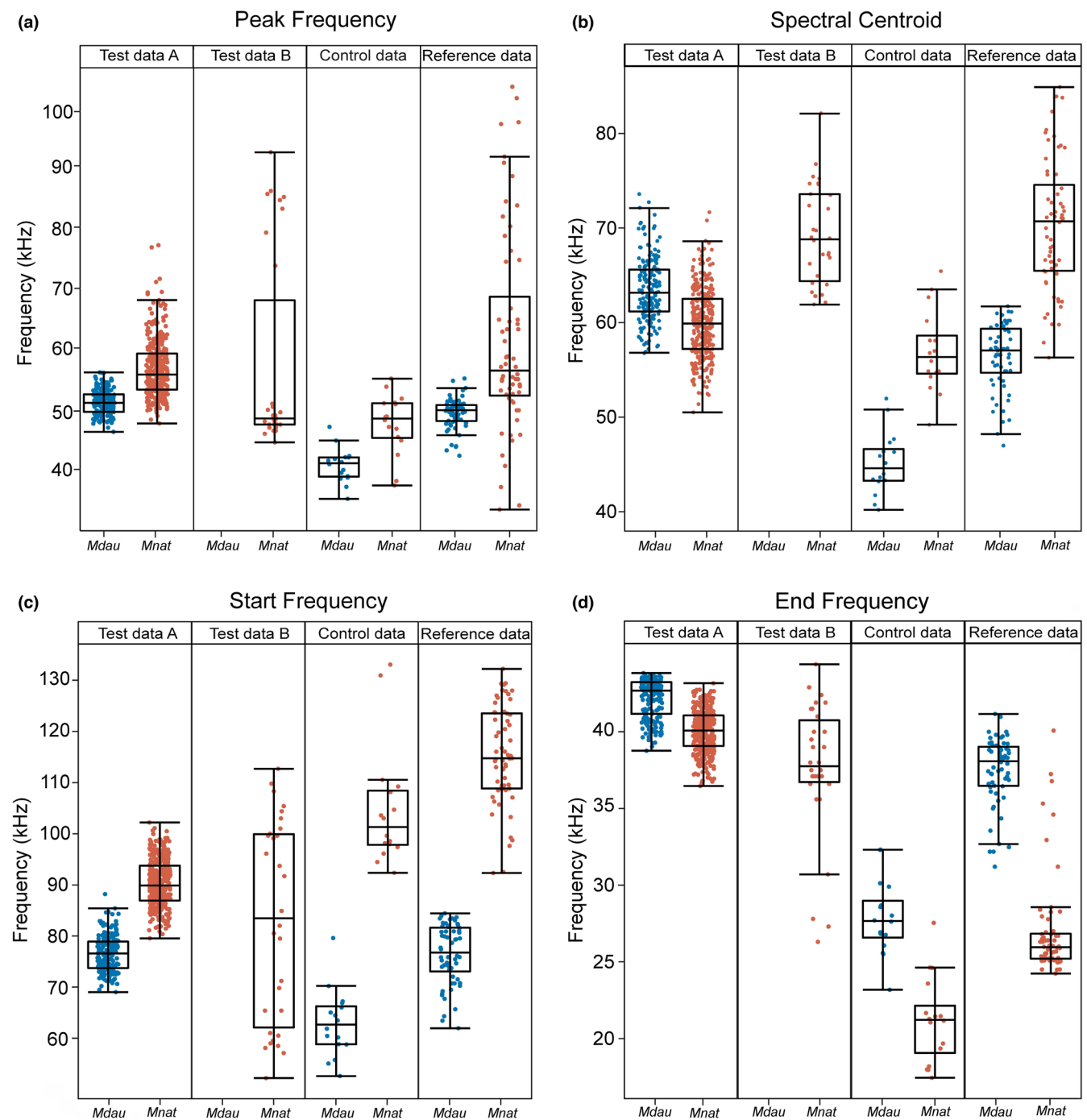


FIGURE 3 To classify unknown echolocation call sequences, the classical acoustic parameters peak frequency (a), spectral centroid (b) and start frequency (c) were used in addition to linear frequency cepstral coefficients (see Figure A2). The end frequency (d) was excluded from further analysis because this parameter was not crucial for discriminating the control data based on the reference data. For the control and reference data the species identity was known before. The species identification of the test data was based on our analysis. All recordings from test data set B were classified as *M. nattereri*. Mdau = *Myotis daubentonii*; Mnat = *Myotis nattereri*.

3.3 | Differences in the parameter distribution of all data sets

The distribution of the extracted classical acoustic parameters differed between all data sets (Figure 3). The largest differences between species were visible in the start frequency. For all data sets

containing both species, the start frequency of *M. nattereri* was higher than that of *M. daubentonii*. However, in the test data set A, the differences were more subtle and both species' start frequencies overlapped between 80 and 85 kHz. The peak frequency of *M. nattereri* varied considerably in the reference data and test data set B, while the ranges were lower in the other data sets and also for

M. daubentonii. Nevertheless, in all data sets the median of the peak frequency of *M. nattereri* was higher than that of *M. daubentonii*. For peak and start frequency all data sets displayed the same relation. Also, in the excluded end frequency the relation between species was the same in all data sets, even though the difference between species in the test data set A was the smallest. By contrast, the spectral centroid was the only parameter for which the distribution of the test data set A, and both the reference and the control data were opposed: for *M. daubentonii* it was higher in the test data set A but lower in the reference and control data. The inconsistent pattern for the spectral centroids may have been caused by the fact that the test data set constituted a much more chaotic acoustic situation than the other data sets (many bats from two different species echolocating simultaneously). The distribution of the extracted acoustic features (LFCCs) can be found in the Figure A2; those values were much less scattered than the original acoustic parameters.

For all three classic acoustic parameters (start, peak, and end frequency), the frequency values of the two focal species differed less during swarming (test data) than during single flight close to clutter (control and reference data). As the test data sets A and B were recorded in very crowded swarming situations, the probability is high that in each sequence of 4 s, more than one species was present. Thus, both species' echolocation calls influence the frequency distributions while our classification results emphasize only the predominant species.

3.4 | Classification results reflect known swarming phenology

Previous studies and intense monitoring and mist netting over several years indicate that at the Kalkberg cave *M. daubentonii* swarm from August onwards and immigrate into the hibernaculum from mid-September to the end of October. In September multiple nights are clearly dominated by swarming *M. nattereri*, which immigrate into the hibernaculum from mid-October to the end of November (Kugelschafter, 1999, 2000, 2001). This well-documented swarming phenology is also reflected in our classification results, thus further validating them. In August, the echolocation call sequences were equally classified as *M. daubentonii* and *M. nattereri*. In the subsequent months, the proportion shifted in favor of *M. nattereri* (62% in September, 85% in October) until they made up for 100% in November (Figure 4).

3.5 | Classification results correspond to the species ID of bats captured while recording

As an additional validation, we used the reference data to classify the test data set B (30 sequences, 4 s each), which was obtained while simultaneously capturing bats with mist nets in direct vicinity of the swarming site (mist nets were placed 2 m away from the microphone). This data set was not recorded at the Kalkberg

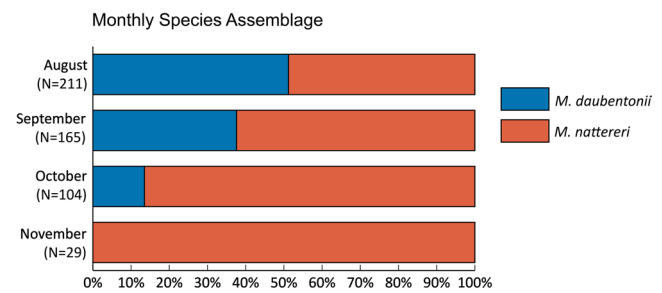


FIGURE 4 Monthly species assemblage in the course of the swarming season, based on the analysis of the echolocation calls of swarming bats from 2018 and 2019. The known phenology of both species is reflected in the species assemblage. With a classification probability of 90% or higher 184 sequences were classified as *Myotis daubentonii* and 325 as *Myotis nattereri*, 55 call sequences were discarded due to a lower classification probability.

but at a different swarming site in Northern Germany. In total, we captured 349 bats, 304 *M. nattereri* (87%) and 42 *M. daubentonii* (12%); the remaining bats (1%) were 2 *M. myotis* and 1 *M. bechsteinii*. Correspondingly, all recordings were classified as *M. nattereri* (DFA: Training $N = 120$, Test $N = 30$, Eigenvalue = 12.225, explained variation = 100%, Wilk's $\lambda = 0.076$, $\chi^2 = 295.648$, $p < .0001$, see also Table A1 and A2). For details on the acoustic parameters of the recordings, please see Figure 3 and Figure A2.

4 | DISCUSSION

Identifying swarming bats noninvasively is challenging, but we were able to assign echolocation call sequences of swarming *Myotis* bats to species level based on a combination of classical acoustic parameters and linear frequency cepstral coefficients (LFCCs), thereby indicating the predominant species. The combined use made analyzing single calls obsolete – which often is impossible during swarming, anyway – and enabled us to distinguish between two *Myotis* species in a swarming context. Some *Myotis* species in Germany employ rather similar echolocation calls, thus making them difficult to distinguish acoustically (Rydell et al., 2017), even in otherwise ideal recording situations (Wimmer & Kugelschafter, 2015). However, we focused on two species with a more distinct call design than others in the genus *Myotis*, which probably explains our satisfactory classification results. Future studies are needed to investigate how well our approach would work for other, acoustically more similar *Myotis* species.

Our statistical classification of echolocation call sequences corresponds well to the known swarming phenology of *M. nattereri* and *M. daubentonii* at our study site (Kugelschafter, 1999, 2000, 2001; MELUND, 2019). In contrast to hibernacula located in the UK (Parsons, Jones, et al., 2003; Rivers et al., 2006), the Netherlands and Belgium (van Schaik et al., 2015), a high proportion of *M. nattereri* were already present in August and September. It is unclear whether this is a regional difference in swarming phenology or caused by the

fact that the Kalkberg cave is one of the largest hibernacula for *M. nattereri* in Central Europe and is also used as a summer roost for males (MELUND, 2019). Our statistical classification of echolocation call sequences also corresponds to the species ID of swarming bats captured while recording at another site. This indicates that acoustic monitoring is a suitable alternative or valuable addition to more invasive methods for species identification during swarming.

Combining classical acoustic parameters and LFCCs can enhance the success of bat species identification in situations that have been challenging in the past and can help to make the most of acoustic monitoring. The combination of classical acoustic parameters and cepstral coefficients has led to convincing classification results for other species such as fish (Noda et al., 2016) and insects (Noda et al., 2019) and it has also been used to discriminate between individuals or contexts (giant otters: Mumm & Knörnschild, 2017; bats: Araya-Salas et al., 2020; Fernandez & Knörnschild, 2020; Knörnschild et al., 2017). In contrast to our test data, all those studies are based on sound recordings containing vocalizations of one individual or one particular species. As we could prove by identifying the control data correctly, our approach also works for the analysis of single calls. However, the necessary amount of postprocessing is higher for the analysis of single calls, because the gaps between calls have to be removed to minimize the influence of background noise. In comparison, sequences of multiple calls of swarming bats in the test data made the influence of background noise neglectable for LFCC extraction. Thus, less postprocessing is required for recordings of swarming bats, making our method best applicable to recordings of multiple overlapping calls. As the amount of postprocessing and analyzing time hardly increases with a higher number of recordings, monitoring over several nights or the whole season is easily feasible, making our approach suitable for long-term monitoring of large bat groups. For future studies, it would be best to use an automatic recording device that is permanently installed at the swarming site and randomly select a fixed number of recordings per night for subsequent analysis. This fine-scaled approach may enhance our understanding of the species-specific phenology at swarming sites.

The main benefit of our approach is minimizing disturbances of hibernating bats by applying noninvasive acoustic monitoring techniques prior to hibernation. Mist netting at mass hibernacula during swarming can impact the animals and lead to disturbances of the natural behavior. Also, in demanding environments such as cliffs mist netting of bats often is not an option for species identification. In such scenarios, noninvasive acoustic monitoring shows its major advantages, as the effort of acoustic recording is comparatively low and it can be conducted over several nights during the season near hibernacula without affecting the animals. However, our approach has caveats as well: it is currently not possible to gain information on the presence of swarming species that occur in low numbers (because focusing on soundscapes will only identify the most dominant species in a recording), and even if a species is abundant enough to be detected, it is difficult to determine the species-specific onset and cessation of activity for the species that are not dominating the

recordings. This severely limits our ability to understand swarming patterns (size, species assemblage, annual occurrence, etc.), which are crucially needed to improve species conservation in the long term. Our approach currently represents a proof of concept, showing that it is possible to classify recordings made during swarming based on the soundscape that the predominantly echolocating species creates.

Another application possibility is the identification of so far understudied social calls emitted by bats on the wing during autumn swarming. Species information about in-flight social calls of European *Myotis* bats is scarce, especially in a swarming context (Pfalzer, 2002; Pfalzer & Kusch, 2003), and the same is true for North American bats (Bohn & Gillam, 2018). We assume that it should be possible to identify social calls to the species level based on the surrounding echolocation calls.

Overall, our introduced noninvasive approach simplifies species identification especially in demanding environments and in situations with many calling individuals such as swarming. So far, we are able to acoustically separate two swarming *Myotis* species based on a set of reference data containing identified call sequences of both species. With additional high-quality reference data sets for other species, our approach should be easily adaptable to identify more than two species, which is especially important for hibernacula with a more diverse species assemblage. Ultimately, we aspire to the application of our approach at swarming sites with so far unknown attendees to gain information about new autumn swarming sites and thus hibernacula. The more we know about species assemblage, phenology, and overall behavior at swarming sites, the better we will be able to protect endangered bat species and their hibernacula in the future.

AUTHOR CONTRIBUTIONS

Anja Bergmann: Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); visualization (equal); writing – original draft (lead). **Lara Sophie Burchardt:** Formal analysis (equal); software (equal); writing – review and editing (equal). **Bernadette Wimmer:** Investigation (equal); writing – review and editing (equal). **Karl Kugelschaffer:** Investigation (equal); writing – review and editing (equal). **Florian Gloza-Rausch:** Conceptualization (equal); investigation (equal); supervision (equal); writing – review and editing (equal). **Mirjam Knoernschild:** Conceptualization (equal); formal analysis (equal); investigation (equal); methodology (equal); software (equal); supervision (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

FUNDING INFORMATION AND PERMITS

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DATA AVAILABILITY STATEMENT

The sound recordings used in this study are available on Dryad <https://doi.org/10.5061/dryad.wdbrv15s8>.

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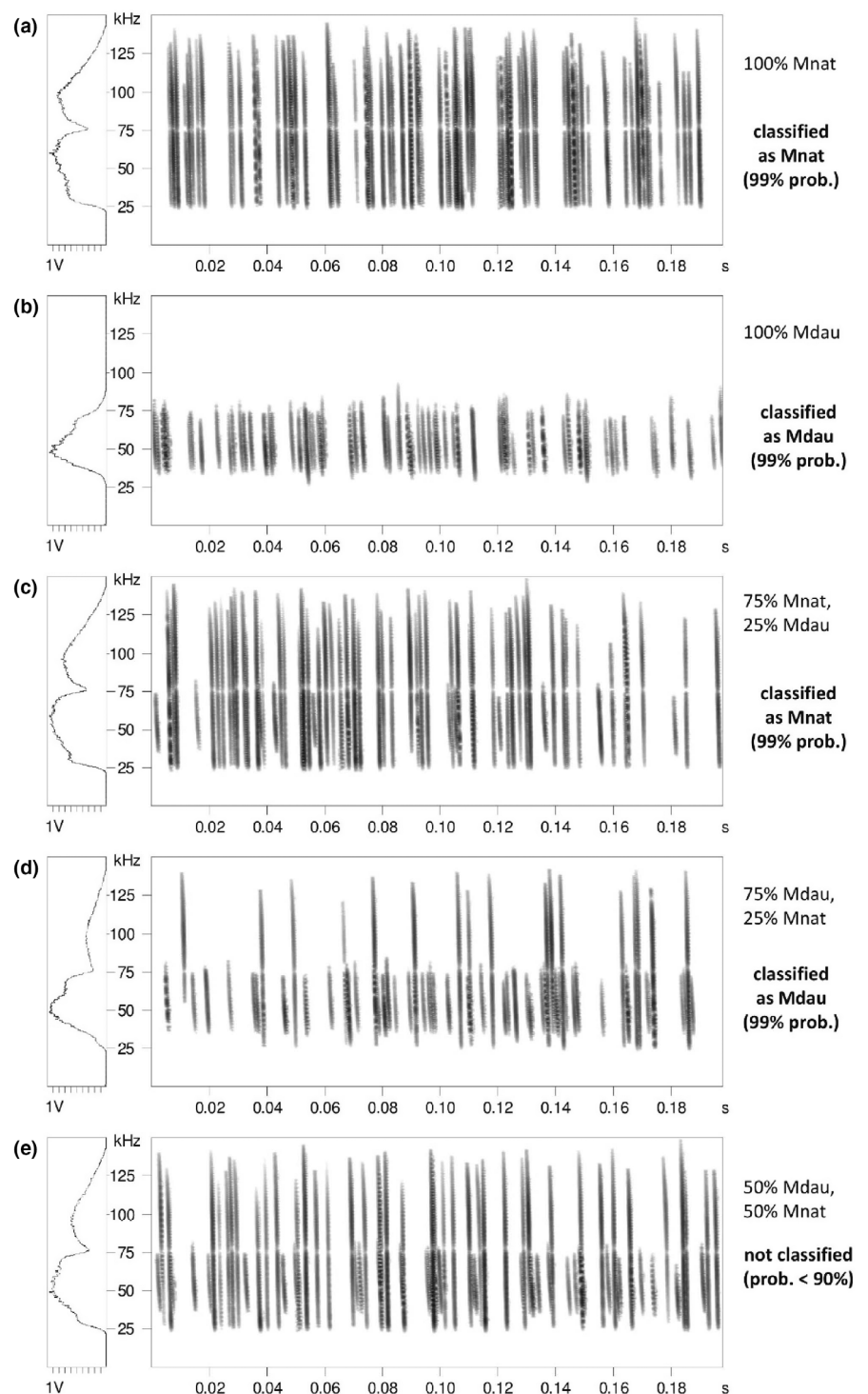
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APPENDIX A

FIGURE A1 Visualization of the “swarming soundscape” analysis. Artificial “swarming” recordings were created by mixing 20 passes with known species ID (Mnat = *M. nattereri*, Mdau = *M. daubentonii*) in five different frequency ratios (a: 100% Mnat; b: 100% Mdau; c: 75% Mnat and 25% Mdau; d: 75% Mdau and 25% Mnat; e: 50% Mdau and 50% Mnat) and subsequently classifying their dominant species based on our reference data. Recordings could be classified correctly with the exception of the balanced mix (e). The classification probability of the balanced mix did not exceed our threshold of >90%, indicating that—as expected—the dominant species in this soundscape cannot be correctly identified.



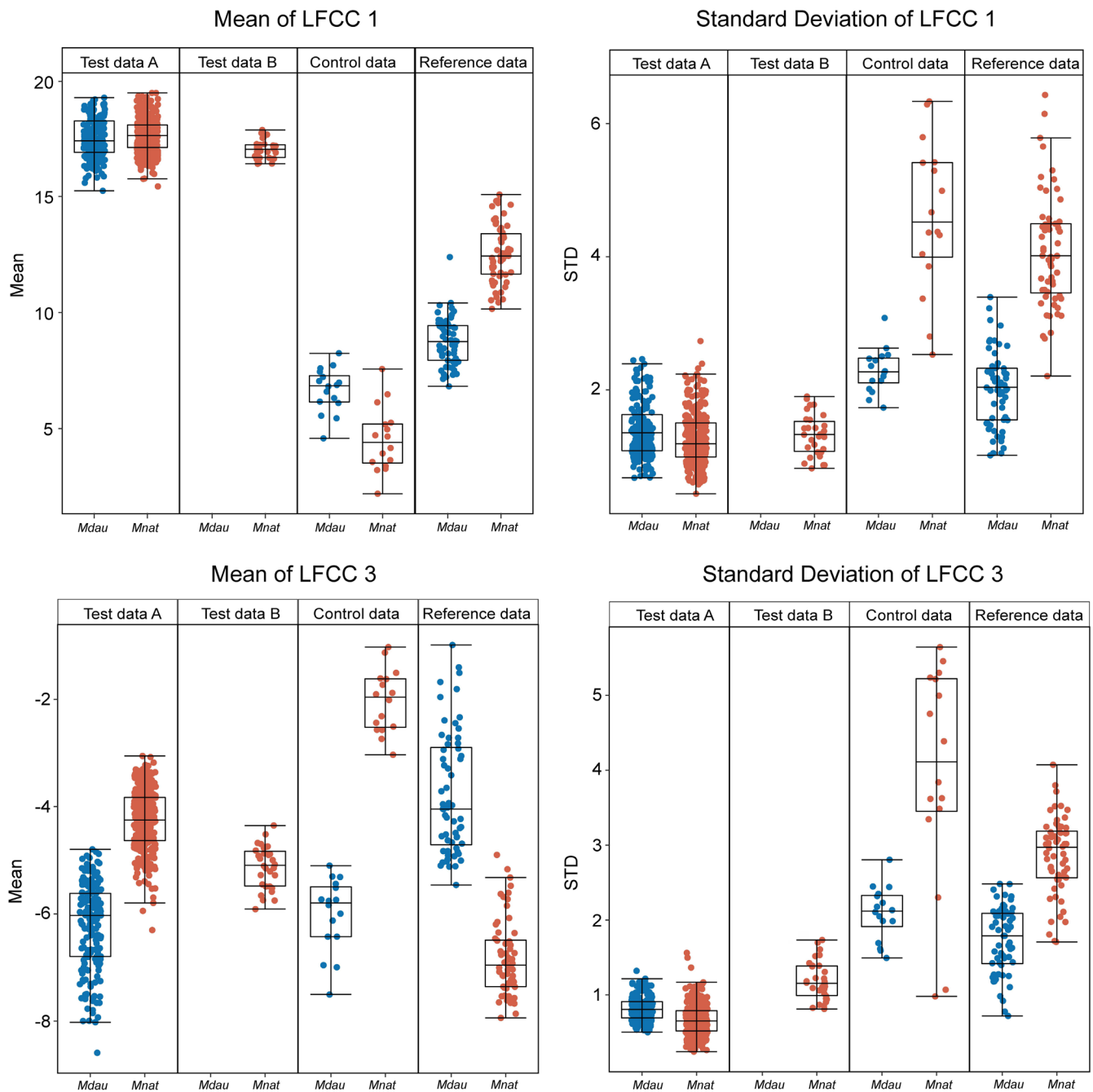


FIGURE A2 Distribution of mean and standard deviation of LFCC 1 and 3 for all data sets. For the control and reference data, the species identity was known before. The species identification of the test data sets A and B was based on our analysis (and, for set B, validated by simultaneous bat capture). Mdau = *Myotis daubentonii*, Mnat = *Myotis nattereri*.

TABLE A1 Assessment of model fit of all discriminant function analyses

Function	Eigenvalue	% of variance	Canonical Correlation	Test of function	Wilkins λ	χ^2	df	p
1	12.225	100	0,961	1	0,076	295,648	7	<.0001

TABLE A2 Structure matrix showing the canonical loading (indicating the contribution of different acoustic parameters to the discriminant function) of the discriminant function for the seven acoustic parameters included in the stepwise discriminant function analyses.

Acoustic parameter	df 1
peak frequency	-0.019
start frequency	1.515
spectral centroid	-0.739
LFCC_MEAN_1	0.524
LFCC_STD_1	0.238
LFCC_MEAN_3	0.423
LFCC_STD_3	0.126

Chapter Two



Similarities in social calls during autumn swarming may facilitate interspecific communication between *Myotis* bat species

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Cover of Chapter Two: Two Natterer's bats (*Myotis nattereri*) in front of the speaker during playback experiment.



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Similarities in social calls during autumn swarming may facilitate interspecific communication between *Myotis* bat species

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Bats employ a variety of social calls for communication purposes. However, for most species, social calls are far less studied than echolocation calls and their specific function often remains unclear. We investigated the function of in-flight social calls during autumn swarming in front of a large hibernaculum in Northern Germany, whose main inhabitants are two species of *Myotis* bats, Natterer's bats (*Myotis nattereri*) and Daubenton's bats (*Myotis daubentonii*). We recorded social calls in nights of high swarming activity and grouped the calls based on their spectro-temporal structure into ten types and verified our visual classification by a discriminant function analysis. Whenever possible, we subsequently assigned social calls to either *M. daubentonii* or *M. nattereri* by analyzing the echolocation calls surrounding them. As many bats echolocate at the same time during swarming, we did not analyze single echolocation calls but the "soundscape" surrounding each social call instead, encompassing not only spectral parameters but also the timbre (vocal "color") of echolocation calls. Both species employ comparatively similar social call types in a swarming context, even though there are subtle differences in call parameters between species. To additionally gain information about the general function of social calls produced in a swarming context, we performed playback experiments with free-flying bats in the vicinity of the roost, using three different call types from both species, respectively. In three out of six treatments, bat activity (approximated as echolocation call rate) increased during and after stimulus presentation, indicating that bats inspected or approached the playback site. Using a camera trap, we

were sometimes able to identify the species of approaching bats. Based on the photos taken during playbacks, we assume one call type to support interspecific communication while another call type works for intraspecific group cohesion.

KEYWORDS

bats (Chiroptera), autumn swarming, Natterer's bat, Daubenton's bat, social calls, interspecific communication, vocalization, *Myotis*

Introduction

Information is transmitted from a signaler to a receiver not only between individuals of one species (conspecifics), but often also between individuals belonging to different species (heterospecifics). This information transfer, referred to as communication, incorporates different sensory modalities, allowing animals to communicate *via* olfactory, visual, tactile, and acoustic signals (Bradbury and Vehrencamp, 2011). Acoustic stimuli provide a variety of information about an individual at a distance, such as individual identity (Carlson et al., 2020), emotional state (Briefer, 2012) and population affiliation (Podos and Warren, 2007). Correspondingly, the functions of intraspecific communication are diverse and can be essential for fitness, survival or reproductive success (Maynard Smith and Harper, 2003; Wilkins et al., 2013). For obvious reasons, animals should respond stronger to conspecific than to heterospecific communication signals (Ord and Stamps, 2009) but interspecific communication occurs as well. In the majority of cases, this form of communication is most accurately described as eavesdropping, where individuals gain information by listening in on the communication signals between heterospecifics. Across many vertebrates, eavesdropping is commonly used and the increased information uptake can provide benefits to the listener such as increased foraging opportunities or earlier detection of predators (Oda and Masataka, 1996; Mönkkönen and Forsman, 2002; Lea et al., 2008; Magrath et al., 2015).

For bats, acoustic signals are highly developed and not only important for communication but also for orientation in a predominantly dark environment. As nocturnal, fast-moving animals, bats rely mainly on echolocation calls as acoustic cues to perceive their surroundings (Fenton, 1984). Such calls are often species-specific, adapted to prey preferences or foraging technique (Schnitzler and Kalko, 2001; Neuweiler, 2003; Siemers and Schnitzler, 2004) and enable the bats to extraordinary spatial discrimination (Simmons et al., 1983). Although in echolocation the sender is also the receiver of the signal, the calls may encode information on individual identity (Kazial et al., 2008; Yovel et al., 2009; Voigt-Heucke

et al., 2010), colony membership (Masters et al., 1995; Jameson and Hare, 2009), sex (Jones et al., 1992; Siemers et al., 2005; Knörnschild et al., 2012), or age (Jones et al., 1992; Masters et al., 1995) which can be processed by conspecifics. Echolocation calls may also facilitate species recognition interspecifically and have a communicative potential allowing interspecific eavesdropping in the wild (Schuchmann and Siemers, 2010; Dorado-Correa et al., 2013).

In contrast to echolocation, social vocalizations have purely communicative purposes and thus the goal to elicit a behavioral response from other individuals (Rendall et al., 2009; Bradbury and Vehrencamp, 2011). With their lower frequency, longer duration and more variable structure than echolocation calls, social vocalizations are better suited for information transfer and detection over longer distances and many bat species exhibit a diverse repertoire of social vocalizations serving a variety of behavioral functions (Pfalzer and Kusch, 2003; Middleton et al., 2014; Chaverri et al., 2018). Agonistic calls are emitted to defend foraging sites (Barlow and Jones, 1997), contact calls facilitate group cohesion (Chaverri et al., 2010; Arnold and Wilkinson, 2011), isolation calls are essential for females to identify their pups (Bohn et al., 2007; Knörnschild et al., 2013) and some bat species are known to employ multi-syllabic songs for male advertisement (Behr and von Helversen, 2004; Sachtleben and von Helversen, 2006) while others rely on shorter courtship calls (Barclay and Thomas, 1979; Knörnschild et al., 2014).

In bat social calls, more personal information is transmitted than in echolocation calls. Therefore, social vocalizations should be of higher diversity and more species-specific to reach the intended receiver than echolocation calls (Fenton, 1994; Chaverri et al., 2018). Nevertheless, social vocalizations can be used in interspecific eavesdropping, e.g., distress calls may attract heterospecifics who approach the calls to investigate the situation by themselves (Carter et al., 2015) and thus increase the chance of repelling predators (Russ et al., 2004) or even deter predators directly (Ancillotto et al., 2022). This interspecific communication might be further facilitated, especially in the case of distress calls, by shared acoustic call features (Hechavarría et al., 2020). Several studies have directly compared the effect of conspecific and heterospecific social

calls on bats, with the overall effect that heterospecific social calls elicit a weaker response, if any, than conspecific social calls (Fenton et al., 1976; Schöner et al., 2010; Furmankiewicz et al., 2011; Hörmann et al., 2021). Despite this fact, bat vocalizations produced in the same general social context often have a noticeable similar spectro-temporal structure, especially in closely related species (Knörnschild et al., 2010; Bosia et al., 2022), which should facilitate interspecific eavesdropping at the very least.

Social vocalizations with known functions can provide valuable information about a species' biology (Bohn and Gillam, 2018; Chaverri et al., 2018). However, for most bats, social vocalizations are far less studied than echolocation calls and their specific function often remains unclear. This is particularly the case for social calls that are not produced in the bats' roost but on the wing. A behavior highly associated with a large amount of in-flight social calls and social group interactions is autumn swarming. Prior to hibernation many temperate zone bat species that hibernate in underground sites are engaged in such interaction, characterized by intense flight activity, chase flights and circling in and around the entrances of the roost without entering, accompanied by a large amount of both echolocation calls and social vocalizations (Fenton, 1969; Parsons et al., 2003). Various, not mutually exclusive functions of swarming are suggested, such as the finding and assessment of suitable hibernacula (van Schaik et al., 2015; Stumpf et al., 2017) or the facilitation of gene flow between otherwise isolated colonies and promiscuous mating behavior (Kerth et al., 2003; Veith et al., 2004; Rivers et al., 2005; Burns and Broders, 2015). Although bats produce high numbers of social calls during autumn swarming, comprehensive studies on their function are scarce (Furmankiewicz et al., 2013; Schmidbauer and Denzinger, 2019).

To expand our knowledge on social calls produced on the wing, we described the social call repertoire during autumn swarming at a large German hibernaculum. During winter, the hibernaculum is mainly inhabited by two Vespertilionid bats, Natterer's bats (*Myotis nattereri*) and Daubenton's bats (*Myotis daubentonii*), both of which also predominate the swarming population. We expected to record a variety of social calls due to the various functions of swarming. In addition, we conducted playback experiments with three social calls of both species, respectively, to get insights into the function and species-specificity of those social calls. If calls were used for group cohesion, we would expect a higher bat activity (indicated by a higher echolocation call rate) or even phonotaxis in response to our playbacks. If calls were used to keep other individuals at bay, we would expect the opposite effect. Photos taken of bats entering the playback area helped us to identify some reacting bats to species level and provided evidence whether calls served an intraspecific or interspecific communicative function.

Materials and methods

Study site and sound recordings

We observed swarming bats during 45 nights in two consecutive swarming seasons (August to November 2018 and August to October 2019) at both entrances of the Kalkberg cave (Bad Segeberg, Northern Germany, 10°18'57"E, 53°56'09"N) and conducted sound recordings on nights with very high swarming activity at various times between sunset and sunrise. Among the 30,000 hibernating bats in the natural cave are various *Myotis* species, with Natterer's bat (*M. nattereri*) and Daubenton's bats (*M. daubentonii*) making up for about 90% of the winter population at the hibernaculum (winter roost). Further inhabitants are pond bat (*Myotis dasycneme*), Bechstein's bat (*Myotis bechsteinii*), greater mouse-eared bat (*Myotis myotis*), Brandt's bat (*Myotis brandtii*) and brown long-eared bat (*Plecotus auritus*).

Prior to hibernation, between August and November, the vicinity of the cave is extensively used for autumn swarming. We recorded the social calls of swarming bats using a high-quality ultrasonic microphone (Avisoft USG 116 Hm with condenser microphone CM16; frequency range 1–200 kHz, sampling rate 500 kHz, 16-bit depth resolution) connected to a small computer (Dell Venue 8) running the software Avisoft Recorder (v4.2.05, R. Specht, Avisoft Bioacoustics, Glienicke, Germany). Both entrances of the cave have been monitored with light barriers since 1991 (ChiroTEC, Lohra, Germany). During the recording nights in 2018, the maximum activity (sum of individual arrivals and departures counted *via* light beam interruptions) was 10,415 and the minimum activity was 1,182. During the recording nights of 2019, the maximum activity was 11,678 and the minimum activity was 2,162. This means that we were recording during periods of high swarming activity, with dozens to hundreds individuals in the air at the same time (see [Supplementary Video 1](#) for a video of swarming bats).

To complement our in-flight recordings, we recorded the social calls of several *M. daubentonii* and *M. nattereri* individuals roosting together in small crevices at another large German hibernaculum (Spandau Citadel; 13°12'46"E 52°32'28"N). Species identity of bats was achieved visually because the crevices were accessible and allowed us to see the bats' faces clearly.

Moreover, we searched an already existing data set (Wimmer and Kugelschafter, 2015) for social calls emitted by single bats while they were flying in ten different underground hibernacula across Bavaria and Baden-Württemberg (Germany). In this data set, species identity was confirmed *via* photos taken from a camera connected to a light barrier. A bat passing through the light beam triggered both a photo and a sound recording, thus assigning species identity to each recording (see Wimmer and Kugelschafter (2015) for details on recording equipment). We used this data set to check whether the social call types found in

single bats with clear species ID correspond to the call types we recorded from swarming bats.

Acoustic analysis of social vocalization and grouping into call types

Social calls were detected visually from the recordings and analyzed in Avisoft SASLabPro (v5.2.13, R. Specht, Glienicke, Germany). Based on their spectro-temporal structure we grouped the calls into ten distinct types. Eight call types were monosyllabic and two call types were multisyllabic: one consisted of the same syllable repeated several times, the other consisted of two different syllable types. We selected high-quality social calls without interfering echolocation calls to measure their parameters. Start and end of calls were determined manually based on the oscillograms. Even though some calls were multiharmonic, we measured only the fundamental frequency (first harmonic) because it contained most of the sound energy. Measurements were taken from oscillograms and spectrograms generated using a 1,024-point fast Fourier transformation, a frame size of 100% and a Hamming window with 93.75% overlap. We measured one waveform parameter (energy), two temporal parameters (duration, time to maximum amplitude) and five spectral parameters (peak frequency, minimum frequency, maximum frequency, bandwidth and entropy) in Avisoft SASLabPro. Entropy is a measure of the width and uniformity of the power spectrum (on a scale of 0–1, white noise has an entropy value of 1 and a pure tone has an entropy value of 0). Spectral parameters were measured at start, center and end of the call and also averaged over the entire call. Additionally, we measured the above-mentioned five spectral parameters at ten locations evenly distributed over the entire call to estimate the frequency and entropy curvature of the call. Derived curvature parameters combined various frequency (or entropy) measurements, thus reducing multicollinearity between original acoustic parameters. We performed principal component analyses (PCAs) with varimax rotation separately for frequency parameters and entropy parameters. For the frequency curvature, we extracted five principal components (with eigenvalues >1) which explained 92.16% of the total variance. For the entropy curvature, we extracted three principal components (with eigenvalues >1) which explained 72.44% of the total variance. Both PCAs fulfilled Kaiser–Meyer–Olkin (KMO) and Bartlett's test criteria. In total, we measured 266 social calls.

To confirm our preliminary visual classification of social calls based on their spectro-temporal appearance, we performed a discriminant function analysis (DFA). Even though we had only ten different social call types, our DFA had twelve groups (one call type, the squawk, was recorded separately from both roosting *M. daubentonii* and *M. nattereri*, and another call

type, the combined UI-shape call, consisted of two different syllables). In total, we included measurements for 266 social calls (6–49 calls per type; mean: 22.17 calls). We selected 18 acoustic parameters, checked them for multicollinearity and included them simultaneously into the DFA: energy, duration, time to maximum amplitude, peak frequency (start), peak frequency (end), peak frequency (center), peak frequency (mean), minimum frequency (mean), maximum frequency (mean), entropy (mean), frequency curvature 1–5 and entropy curvature 1–3. We used a cross-validation procedure to estimate the correct classification success, which classified each call based on discriminant functions established with all calls except the call being classified (n-1 cross-validation procedure). The DFA was adjusted to the unequal number of analyzed calls per type by computing group sizes based on prior probabilities. We subsequently checked for each group in our DFA whether the obtained classification success was better than a random classification (8.33%).

Species identification *via* feature analysis

Although some social calls have a similar spectro-temporal structure, they might be emitted by different species. To assign the social calls to species level we performed an analysis of the surrounding echolocation calls, focusing on the total soundscape rather than single calls. To do so we analyzed 1s-echolocation-snippets surrounding the social call (test data, [Figure 1](#)) to identify the predominantly swarming species (*M. daubentonii* or *M. nattereri*) directly before and after the social call was produced. Naturally, it is not a guarantee that the social call in question was produced by the species who was predominantly swarming at the time of social call production but it is an approximation at the very least (and currently the only method available to assign social calls of multiple swarming bat species to species level). Echolocation calls were assigned to species level (*M. daubentonii* or *M. nattereri*) based on a set of reference data [recorded by [Wimmer and Kugelschafter \(2015\)](#)] consisting of identified call sequences of single individuals from both species. This reference data set was used as a training set in a DFA and the echolocation snippets surrounding the social calls were used as a test data set. We only considered a species identification to be reliable if both echolocation snippets surrounding a social call were assigned to the same species by the DFA with a probability higher than 90%.

Echolocation snippets consisted of many overlapping echolocation calls (the “swarming soundscape”) which we analyzed as a whole instead of focusing on single echolocation calls. For the test data set, start, end and peak frequency of the echolocation snippets were calculated with a custom-made MATLAB routine over the entire file in 10 ms frames using the meanfreq function from the Signal Processing toolbox.

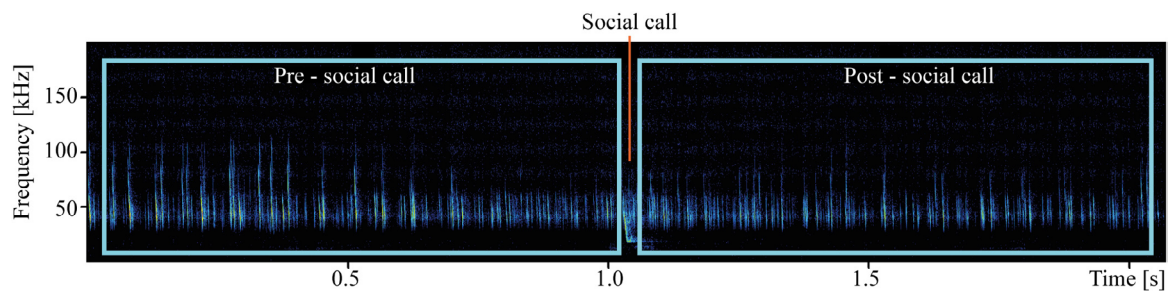


FIGURE 1

One second of overlapping echolocation calls from multiple swarming bats (“swarming soundscape”) before and after a social call was produced. The swarming soundscape was analyzed to extract acoustic parameters and assign the social call to a predominantly calling species based on a set of identified reference data.

For the reference data set, start, end, and peak frequency of single echolocation calls were measured in Avisoft SASLab Pro (threshold of -24 dB relative to the peak amplitude; values averaged over the entire call). For both the test and the reference data set, the spectral centroid was calculated in Avisoft SASLab Pro (threshold: -28 dB relative to peak amplitude) and an acoustic feature extraction technique was used to extract five linear frequency cepstral coefficients (Hamming window; test data: 100 ms frame; reference data: 3 ms frame) with a custom-made routine in the speech processing toolbox “voicebox” in MATLAB (v. R2018b). Linear frequency cepstral coefficients (LFCCs) are spectral-based representations of entire signals and incorporate timbre (vocal “color”) as well as classical spectral parameters (Zhou et al., 2011). For details on feature extraction, please see Bergmann et al. (2022). Due to the different requirements for recording quality, another subset of social calls was used for the species identification described above than for the parameter measurements of social calls described in the previous section.

To assess the species identity of echolocation snippets surrounding social calls (i.e., the identity of the predominantly echolocating species in each recording), we performed a DFA in which the reference data set (with known species ID, 120 echolocation call sequences) functioned as training set and the echolocation snippets surrounding the social calls functioned as a test data set (854 echolocation snippets with unknown species ID) using the parameters spectral centroid, start frequency, peak frequency and mean and standard deviation of the LFCCs 1 and 3. For details on parameter selection, please see Bergmann et al. (2022).

Subsequently, we tested for species-specific differences in acoustic parameters of selected social calls by calculating a MANOVA with selected acoustic parameters as dependent variables and species ID, call type and their interaction as independent variables. The data set consisted of 57 social calls with sufficient quality for acoustic measurements (26 from *M. daubentonii* and 31 from *M. nattereri*) which had been previously classified to species level based on their surrounding

echolocation calls. The social calls belonged to four different call types (FM pulses, U-shape, L-shape, inverted N-shape). We included eight acoustic parameters, namely duration, peak frequency at start, center and end of a call, and peak, minimum and maximum frequency as well as entropy averaged over the entire call.

Playback stimuli

For the playbacks, we selected three commonly used social calls of *M. daubentonii* and *M. nattereri*, respectively. The calls were recorded from swarming bats at the Kalkberg cave in 2018 and 2019 (inverted N-shape and U-shape call) or at the Spandau Citadel (squawks) from visually identified bats roosting in crevices. U-shape and inverted N-shape calls were classified to species level as described above. We trimmed the recordings close to the social calls and eliminated background noise or contemporaneously emitted echolocation calls. For the inverted N-shape call, the noise was reduced in Cool Edit 2000 (Syntrillium Software Corporation, Phoenix, AZ, USA) and silence was inserted around syllables until a total file length of 100 ms. After that, another noise reduction was conducted in Avisoft SASLabPro (FFT 1024; precision 4; removed noise below -70 dB; reduced noise by 80 dB) and remaining artifacts were erased manually, whenever necessary. For the U-shape calls the noise was reduced in Avisoft SASLabPro (FFT 1024; precision 4; removed noise below -60 dB; reduced noise by 30 dB), residual noise was erased manually and a second noise reduction was applied whenever necessary. The squawks did not require noise reduction, as they were recorded from bats in crevices and not in a swarming context. Thus, neither noise nor echolocation calls were present in those recordings, and the files were trimmed close to the social calls. For each final playback file of 30 s length, 15 calls, randomly drawn from the library of playback stimuli, were compiled in random order intermitted by silence in Cool Edit 2000. For the inverted N-shape calls, the library of high-quality playback stimuli consisted of 14 *M. daubentonii*

and 11 *M. nattereri* calls, the U-shape of 9 *M. daubentonii* and 11 *M. nattereri* calls and the squawk files assembled 55 *M. daubentonii* and 42 *M. nattereri* calls.

Playback set up and analysis

We conducted the playbacks in 14 nights between 30.08.2020 and 15.09.2020 at three locations close to the entrances of the Kalkberg cave (**Supplementary Figure 1**). At each location, we observed swarming bats in former years. Each playback trial had a total duration of 90 s and consisted of a silent pre-observation, stimulus presentation and silent post-observation phase. Each phase was 30 s long, as social calls are often emitted in sequence and during this time passing bats have the chance to change their course and show phonotaxis behavior (**Figure 2**). To broadcast the stimuli we used a BatLure Ultrasound Speaker (Pettersson Elektronik AB, Uppsala, Sweden) mounted on a tripod and directed upward (**Supplementary Figure 1** location B and C) or hanging at a wall and directed forward (**Supplementary Figure 1** location A). Sound pressure levels of stimuli were kept constant (100 dB SPL at 1 m) during the playbacks and were lower than what is reported for echolocation calls of our focal species (Melcón et al., 2007; Jakobsen et al., 2013). In each night, playbacks of all call types were conducted at all locations in a randomized order between 10 p.m. and 2 a.m. In total, we conducted 40 playbacks consisting of six trials each (i.e., broadcasting three different call types from both species). All playbacks were started manually after three bats passed the video-recorded sector and ended automatically after 90 s.

We recorded the vocal response of bats during playbacks using an ultrasonic microphone (similar set up as for sound recordings) pointed in the same direction as the speaker such that bat calls directed to the speaker would be highest in amplitude, facilitating discrimination between playback and corresponding bat activity at the playback site. The audio files were analyzed semi-automatically using a pulse train analysis in Avisoft SASLabPro (all echolocation calls that exceeded a threshold of -24 dB relative to the oscillogram's maximum amplitude were counted). To interpret the bats' behavior in response to the playbacks, we compared the number of emitted echolocation calls between the pre-observation phase and the mean number of calls emitted in the playback and post-observation phase. The number of emitted echolocation calls depend on both the number of bats and their calling rate. We used a binomial test, separately for each of the six combinations of stimulus type and species ID. All statistical tests were conducted using SPSS (version 28, SPSS Inc., Chicago, IL, U.S.A.).

In addition, we observed bat flight behavior using a thermal camera (FLIR E95, FLIR Systems, Wilsonville, OR, US) directed at the speaker at a distance of approximately 6 m. Whenever

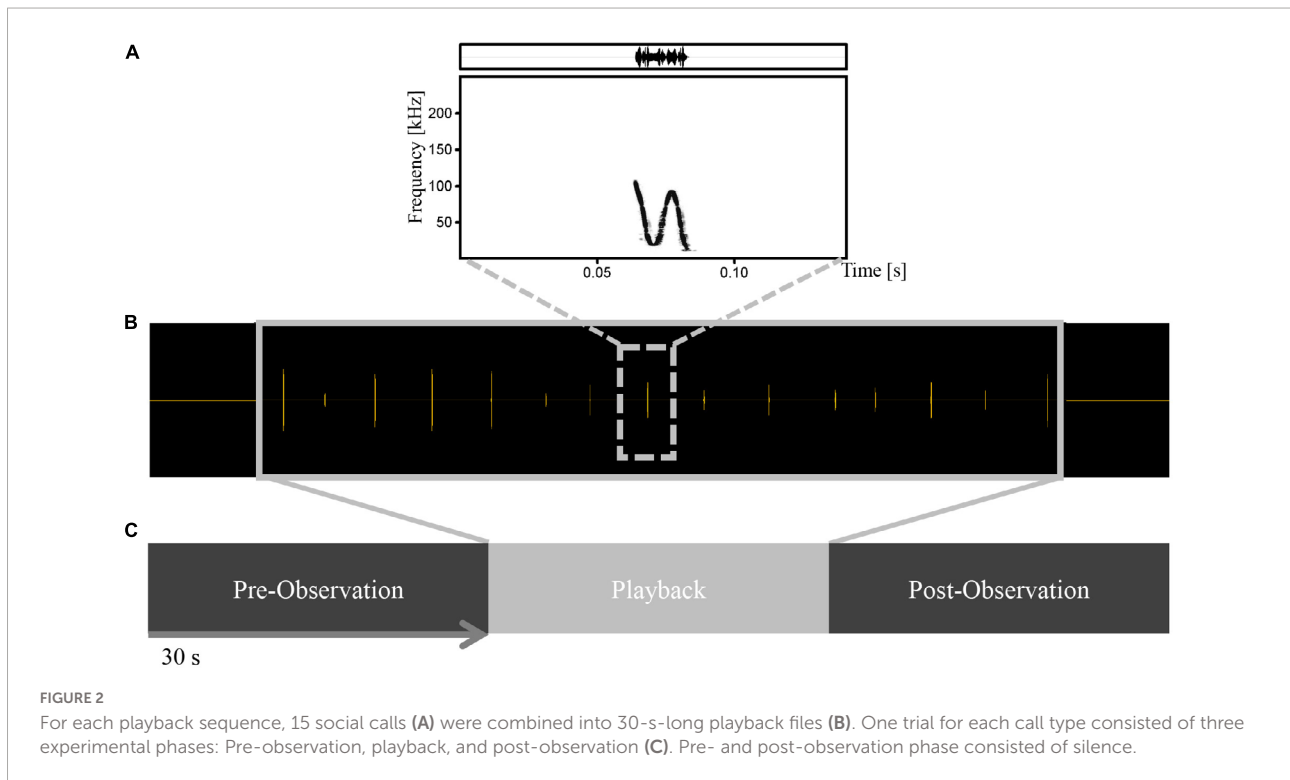
an approaching bat was visible on the thermal camera's screen, we took a photo by triggering a remote-control release, which operated a camera (Nikon D3S, Nikon Corporation, Tokyo, Japan). A set of seven flashlights (Yongnuo YN560, Shenzhen Yong Nuo Photographic Equipment, Shenzhen, China) was assembled around the speaker and triggered *via* slave function from another flash light on top of the camera to illuminate approaching bats. We identified the photographed bats to species level based on the characteristics of their wings, ears and/or tails. However, not all approaching and passing bats could be photographed and not all of the photographed bats could be identified to species level. Nevertheless, the photo set-up is a good non-invasive approach to complement other playback results as it allowed us to get a rough estimate of the species-specificity of social calls without having to catch approaching individuals.

Results

Ten call types were produced by swarming bats

We analyzed 2,135 recordings containing one or more social calls and identified ten call types emitted during swarming (**Figure 3**, for original recordings see **Supplementary Audio 1**). We grouped the calls into types based on their spectro-temporal structure (**Table 1**) and confirmed our grouping by a DFA, which classified 77.4% of all calls to the correct type (**Figure 4**, for detailed DFA results see **Table 2**).

The longest social calls produced at the swarming site were squawks, atonal harsh screeches of constant low frequency, which were emitted frequently not only in-flight but also from individuals roosting in crevices in close vicinity of the swarming bats (**Figure 3**). Squawks recorded from roosting *M. daubentonii* and *M. nattereri* had clear species-specific acoustic differences (**Figure 4**) but these differences could not be analyzed for squawks produced by bats in a swarming context; squawks were often emitted from crevices near the swarming bats and as roosting bats do not echolocate the classification of echolocation calls would be misleading. Furthermore, we recorded a variety of frequency-modulated tonal calls which we assigned to nine distinct call types based on their spectro-temporal structure. Four call types (FM downsweep, modulated FM downsweep, L-shape and U-shape) were comparatively similar but could nevertheless be grouped into distinct call types by the DFA. Four other call types (inverted N-shape, combined UI-shape, hook, FM pulses) differed in their spectro-temporal structure to a greater degree (**Figure 3**) and were thus classified better by the DFA (**Figure 4**). Combined UI-shape and FM pulses were the only two multisyllabic calls produced by swarming bats. Inverted N-shape and hook were easy to recognize call types because they showed very little variation. The remaining call



type (variable tonal call) was a broad call type category that encompassed all tonal calls with a high variability in frequency modulation and a wider range of start and end frequencies. Even though this was a very common call type, the different calls were too variable to group into meaningful subtypes. Variable tonal calls and inverted N-shape calls were easy to differentiate despite their similarity at first sight because inverted N-shape calls had very regular frequency modulations. Due to the high activity at the swarming site, we were rarely able to make a connection between a call and the associated behavior of the caller. Nevertheless, on some occasions, we could observe the FM pulses being emitted when swarming bats collided or got very close to each other. It is therefore possible that FM pulses are used to maintain or negotiate the distance between swarming individuals.

Both species employed comparatively similar calls

Based on the surrounding echolocation calls we assigned a total of 305 social calls to either *M. daubentonii* or *M. nattereri*. Out of 854 analyzed echolocation snippets surrounding a social call, we could classify 760 to species level with a classification probability of more than 90% (DFA: Training $N = 120$, Test $N = 854$, Eigenvalue = 12.225, explained variation = 100%, Wilk's $\lambda = 0.076$, $\chi^2 = 295.648$, $p < 0.0001$). A total of 150 of 760 snippets were discarded because the two snippets surrounding a

social call were not assigned to the same species, thus making the classification ambiguous. The remaining 610 echolocation snippets allowed us to classify 305 social calls to species level (*M. daubentonii* or *M. nattereri*). Further, some social calls were discarded from analysis because they could not be assigned unequivocally to one of the ten formerly defined call types (51 calls). Only few FM downsweeps (6 calls), modulated FM downsweeps (5 calls) and hooks (1 call) were classified due to low number of recordings, unsuitable echolocation snippets or/and based on the classification constraints. Additionally, we discarded 34 variable tonal calls from further analysis as they were often emitted in long sequences and frequently interrupted the surrounding echolocation snippets. Also the 49 squawks were discarded because their species ID could not be reliably established based on the surrounding echolocation call soundscape (squawks were often emitted by roosting bats near the swarming area which did not echolocate). Of the analyzed inverted N-shape (29 calls) and L-shape (36 calls) calls, around half was classified as *M. daubentonii*, respectively (Figure 5). Two-thirds of the U-shape calls (65 calls in total) and around 80% of FM pulses (29 calls in total) were classified as *M. daubentonii*.

In the data set of Wimmer and Kugelschafter (2015) we found FM Pulses, variable tonal calls and FM downsweeps emitted by both species in correspondence with the calls recorded during swarming (Supplementary Table 1). Furthermore, they recorded hooks of a lower frequency than ours from both species and squawks and L-shape

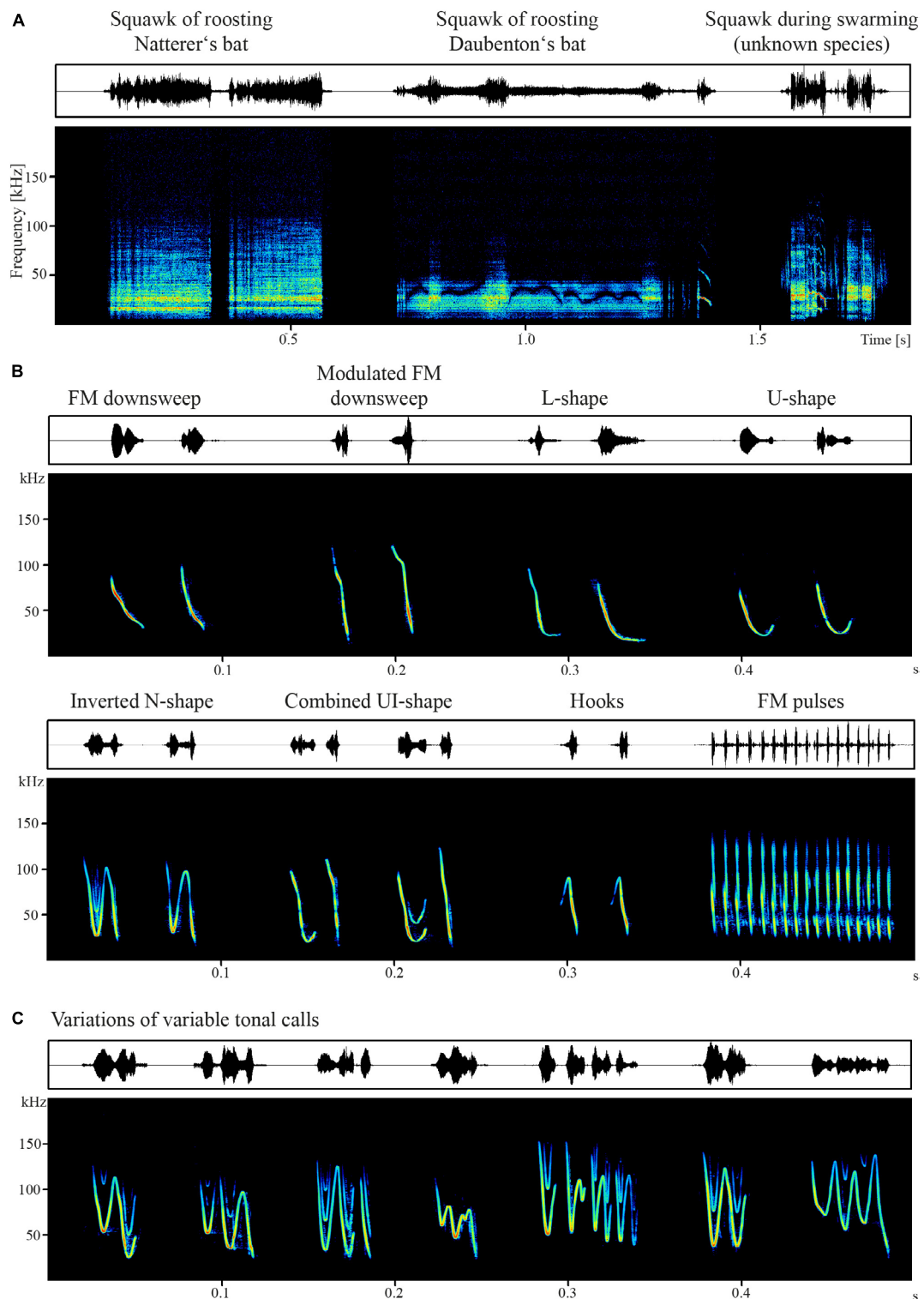


FIGURE 3

Spectrograms of all call types we found during autumn swarming. The calls were grouped based on their spectro-temporal structure and confirmed through a discriminant function analysis with temporal and spectral parameters. **(A)** Squawks recorded at the Spandau Citadel from identified bats in crevices (left and middle) and from an unidentified bat during autumn swarming at the Kalkberg cave (right). **(B)** Short tonal calls emitted singly or in sequence. **(C)** Variable tonal calls grouped into one group, usually longer than other tonal calls and often emitted in sequence. Spectrograms were created using Avisoft SASLabPro with a Hamming window, 100% frame size and an overlap of 87.5% **(B,C)** or 50% **(A)**.

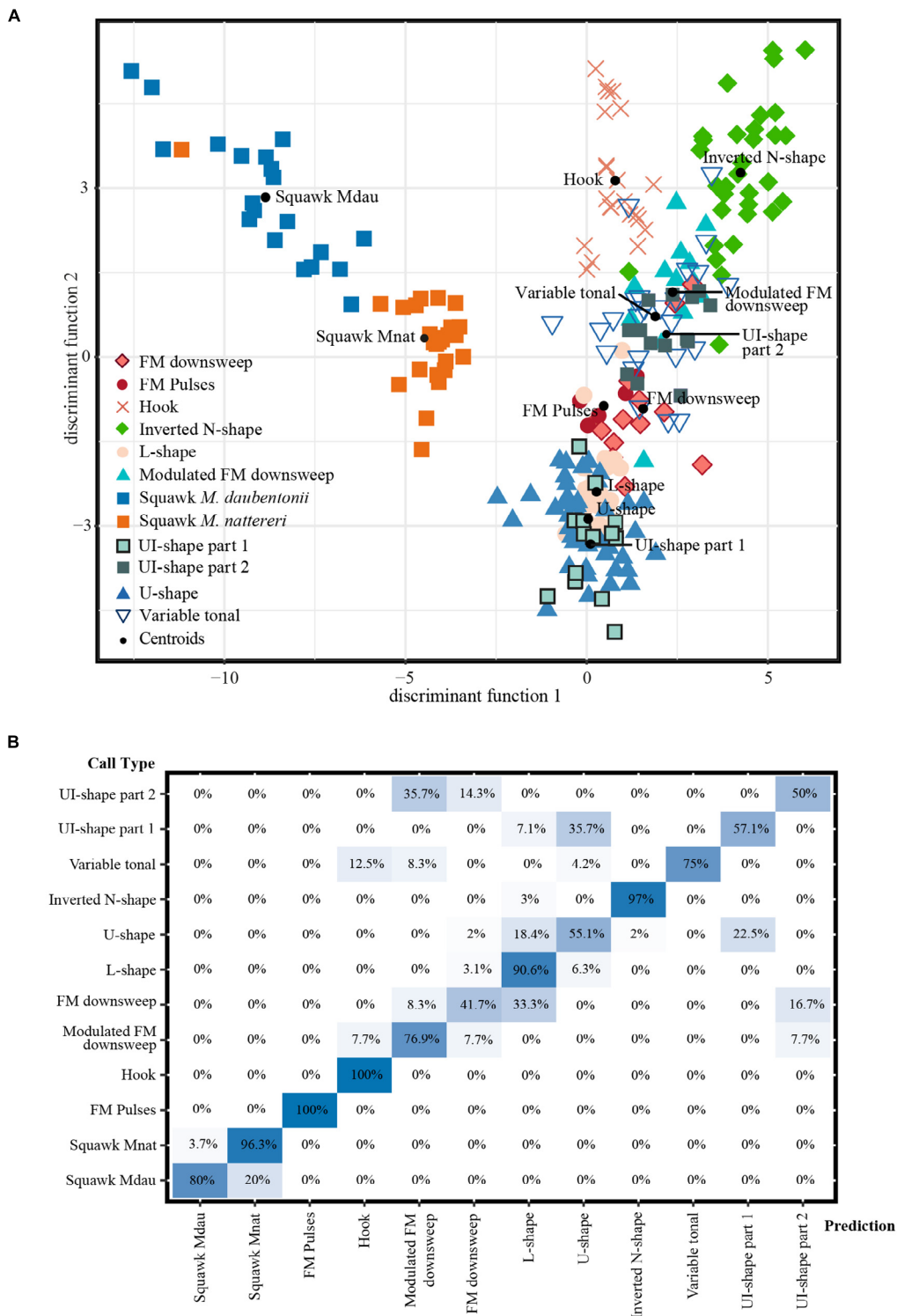


FIGURE 4

(A) Relative position of social calls produced by swarming *Myotis* bats based on their spectral and temporal parameters. The two-dimensional signal space is defined by the first two discriminant functions, which were most important for call type discrimination. The ten call types are represented by different symbols; black circles depict centroids and are labeled with the respective call type. One call type, the squawk, was further discriminated by species because squawks were recorded from identified bats in crevices. All other calls were recorded from bats on the wing. One of those in-flight social calls, the combined UI-shape consisted of two different parts which were entered separately into the DFA. (B) Confusion Matrix indicating the call types to which analyzed calls were assigned. 77.4% of cross-validated cases could be classified correctly. Mdaui, *Myotis daubentonii*; Mnat, *Myotis nattereri*.

TABLE 1 Mean and standard deviation of social call parameters per call type (range is given in parentheses).

Call type	N	Call duration (ms)	Peak frequency (kHz)	Start frequency (kHz)	End frequency (kHz)	Peak to peak (mV)	Entropy	Abundance
Squawk	20	750.72 ± 237.86 (351.2–1298.9)	32 ± 7 (21.7–44.9)	25.47 ± 6.44 (16.6–40.5)	23.51 ± 5.15 (19–42.9)	0.44 ± 0.08 (0.24–0.5)	0.51 ± 0.06 (0.38–0.62)	Very common (18.3%)
<i>M. daubentonii</i>								
Squawk <i>M. nattereri</i>	27	292.9 ± 188.81 (195–1224)	26.01 ± 3.65 (17.1–31.6)	24.18 ± 4.74 (14.6–28.8)	25.14 ± 8.31 (13.1–43.9)	0.38 ± 0.09 (0.19–0.5)	0.49 ± 0.05 (0.42–0.6)	
FM downsweep	12	12.46 ± 3.37 (6.08–17.9)	52.01 ± 10.92 (38.1–75.5)	93.53 ± 19.69 (66.8–127.9)	24.08 ± 5.64 (14.1–32.2)	0.98 ± 0.58 (0.2–1.92)	0.32 ± 0.07 (0.19–0.42)	Rare (2.8%)
L-shape	32	20.15 ± 3.66 (14.8–28.5)	37.6 ± 6.01 (27.7–50.3)	75.96 ± 6.67 (61.5–93.7)	26.19 ± 9.04 (15.6–55.6)	0.9 ± 0.62 (0.11–1.98)	0.35 ± 0.1 (0.2–0.52)	Common (6.4%)
U-shape	49	17.41 ± 5.03 (8.51–30.4)	40.87 ± 7.8 (28.1–57.6)	85.31 ± 19.28 (43.4–124.5)	38.64 ± 10.09 (22.9–58.5)	0.65 ± 0.49 (0.14–2)	0.43 ± 0.1 (0.22–0.62)	Common (8.4%)
Inverted N-shape	33	19.37 ± 2.84 (12.73–24.76)	61.37 ± 9.46 (38.7–84.9)	105.65 ± 13.87 (60–136.7)	28.38 ± 7.92 (12.6–38)	0.61 ± 0.43 (0.09–1.98)	0.44 ± 0.06 (0.32–0.55)	Very common (15%)
Modulated FM downsweep	13	9.76 ± 1.43 (8.1–12.35)	69.52 ± 8.92 (50.4–83.7)	105.33 ± 8.24 (94.7–119.1)	26.54 ± 5.79 (14.6–39)	0.89 ± 0.6 (0.23–1.98)	0.4 ± 0.08 (0.26–0.53)	Very rare (1.7%)
Combined UI-shape part 1	14	14.96 ± 3.01 (10.81–20.6)	47.81 ± 8.46 (30.4–62.2)	98.27 ± 14.38 (73.7–118.1)	43.56 ± 13.52 (24.4–64.9)	1.07 ± 0.54 (0.24–1.98)	0.38 ± 0.08 (0.29–0.64)	Very rare (1.9%)
Combined UI-shape part 2	14	5.94 ± 1.38 (3.2–7.93)	64.96 ± 9.6 (50.5–81.3)	105.01 ± 18.83 (71.7–136.2)	29.44 ± 3.4 (23.9–36.6)	0.95 ± 0.55 (0.21–1.99)	0.42 ± 0.09 (0.27–0.59)	
Hook	22	9.31 ± 2.03 (5.95–12.8)	71.86 ± 5.3 (65.4–82.8)	69.95 ± 11.34 (55.1–94.2)	39.5 ± 3.82 (32.2–46.3)	1.26 ± 0.61 (0.23–1.98)	0.45 ± 0.08 (0.34–0.58)	Very rare (1.4%)
FM pulses	6	1.84 ± 0.12 (1.62–1.94)	43.92 ± 5.45 (38.56–51.09)	54.07 ± 7.31 (46.47–63.59)	36.3 ± 3.44 (32.94–40.44)	1.07 ± 0.34 (0.62–1.42)	0.42 ± 0.07 (0.34–0.52)	Very common (12%)
Mean of single pulses per call								
FM pulses	6	79.25 ± 18.65 (48.38–103.23)	54.68 ± 6.3 (46.7–63.6)	68.95 ± 12.55 (47.3–80)	34.12 ± 5.18 (29.2–43.9)	1.34 ± 0.34 (0.88–1.75)	0.57 ± 0.07 (0.49–0.65)	
Total call								
Variable tonal	24	29.53 ± 8.12 (19.77–57.08)	60.33 ± 12.28 (42–85.4)	103.36 ± 23.67 (39–150.8)	39.7 ± 15.93 (21.4–91.3)	0.99 ± 0.64 (0.34–1.98)	0.4 ± 0.08 (0.27–0.54)	Very common (15.3%)

The abundance is calculated from the number of recordings containing the focal call type from a total of 2,135 analyzed recordings (note that recordings often contained more than one social call).

TABLE 2 Assessment of model fit of the discriminant function analyses on social calls.

Function	Eigenvalue	% of variance	Test of function	Wilks' Lambda	Chi-square	df	p
1	11.745	42.1	1–11	0.000	2384.78	187	<0.0001
2	6.003	21.5	2–11	0.001	1747.21	160	<0.0001
3	5.001	17.9	3–11	0.007	1259.64	135	<0.0001
4	2.538	9.1	4–11	0.039	810.75	112	<0.0001
5	1.077	3.9	5–11	0.139	494.24	91	<0.0001
6	0.740	2.7	6–11	0.289	311.09	72	<0.0001
7	0.526	1.9	7–11	0.503	172.38	55	<0.0001
8	0.156	0.6	8–11	0.767	66.54	40	0.005
9	0.075	0.3	9–11	0.886	30.26	27	0.303
10	0.035	0.1	10–11	0.953	12.08	16	0.738
11	0.014	0	11	0.986	3.42	7	0.844

calls of *M. daubentonii* and inverted N-shape calls of *M. nattereri* only.

Additionally, the acoustic properties of four social call types (inverted N-shape, U-shape, L-shape, FM pulses; only calls with sufficient quality for acoustic measurements were included) differed significantly between species and between

call types (MANOVA; species ID: F8, 42 = 4.686, $p < 0.001$, partial $\eta^2 = 0.472$; call type: F24, 122.4 = 21.694, $p < 0.001$, partial $\eta^2 = 0.799$; species ID*call type: F24, 122.4 = 1.570, $p = 0.059$, partial $\eta^2 = 0.229$). Two acoustic parameters differed significantly between species (between-subjects effects; peak frequency at the start of a call: $p = 0.017$; maximum frequency

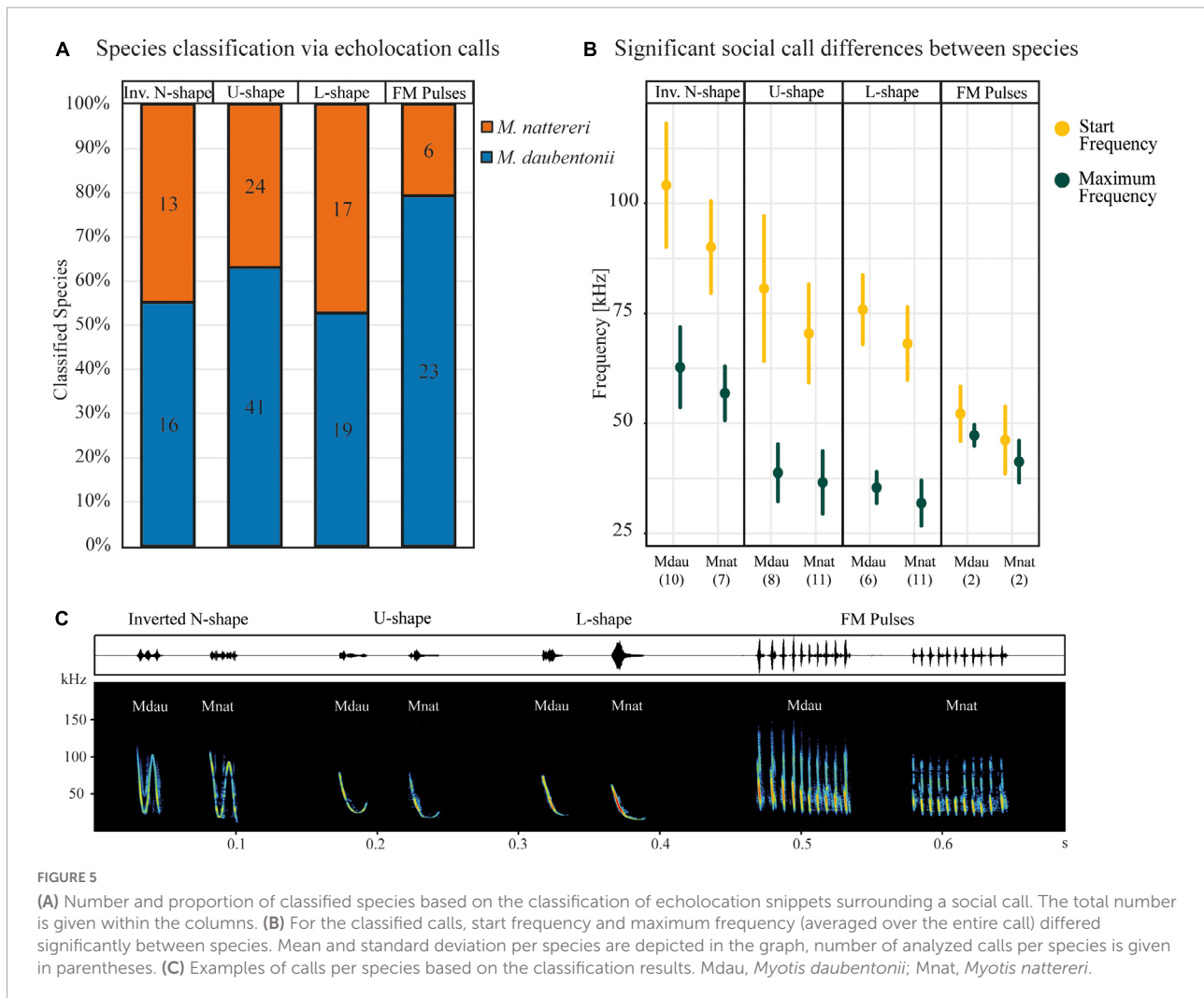


FIGURE 5

(A) Number and proportion of classified species based on the classification of echolocation snippets surrounding a social call. The total number is given within the columns. (B) For the classified calls, start frequency and maximum frequency (averaged over the entire call) differed significantly between species. Mean and standard deviation per species are depicted in the graph, number of analyzed calls per species is given in parentheses. (C) Examples of calls per species based on the classification results. Mdau, *Myotis daubentonii*; Mnat, *Myotis nattereri*.

averaged over the entire call: $p = 0.47$) and seven acoustic parameters differed significantly between call types (between-subjects effects; all $p < 0.001$, except for entropy averaged over the entire call). When comparing species-specific properties within the same call type, calls classified as *M. daubentonii* had higher frequencies than calls classified as *M. nattereri* (Figure 5), even though it is the opposite for the species' echolocation calls. These results indicate that both species employ social calls that are rather similar in their spectro-temporal structure.

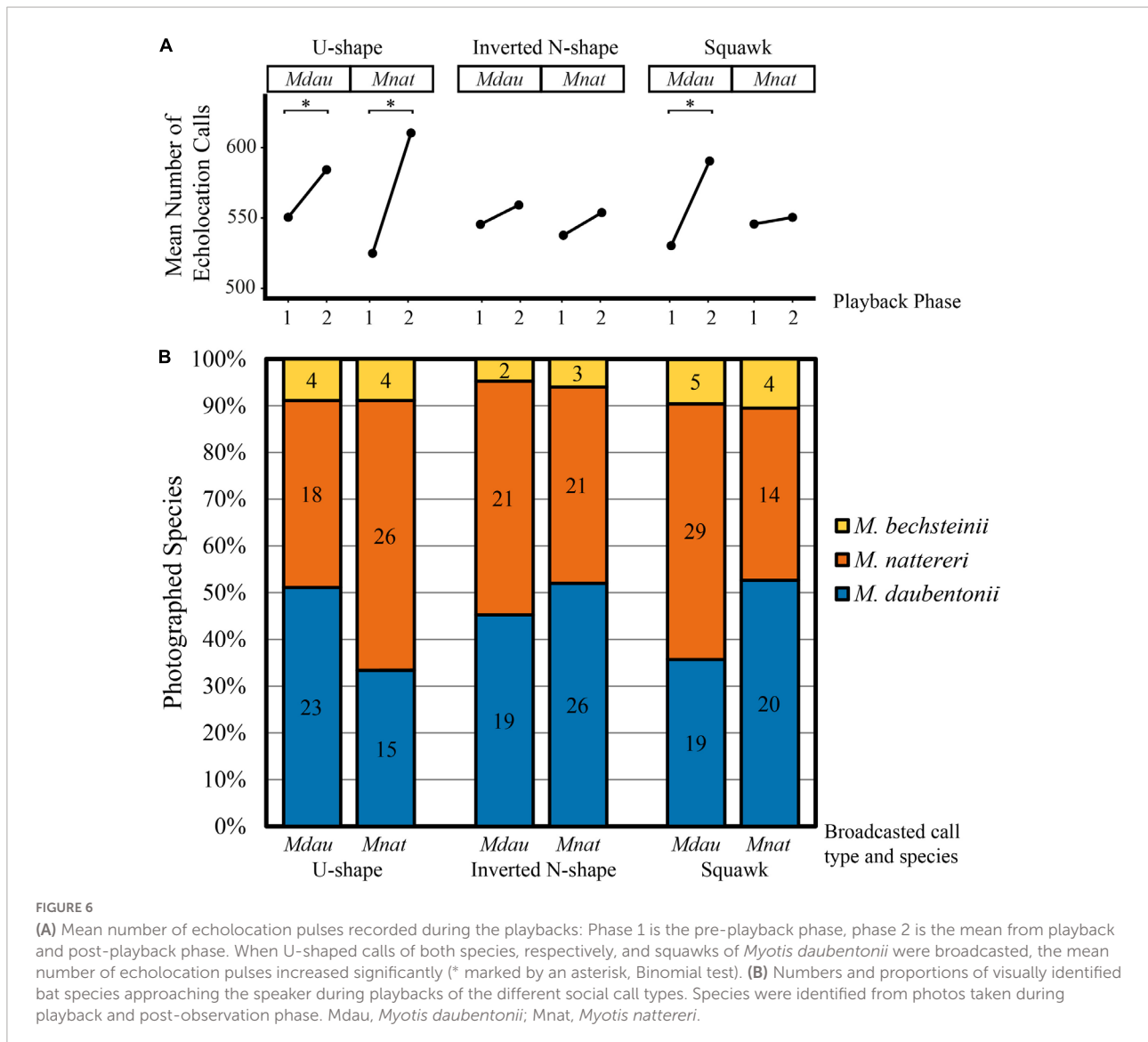
The reaction to playbacks differed between call types

To investigate the reaction of free ranging bats to different social call types, we conducted playbacks (40 playbacks with six trials each) and broadcasted the inverted N-shape, the U-shape and the squawk call of *M. daubentonii* and *M. nattereri*, respectively. We tested whether bats reacted

to the broadcasted calls with a higher rate of echolocation calls, which would suggest increased interest in the playback location, or even with phonotaxis. To do so, we analyzed all echolocation calls we recorded during the playbacks regardless of species specificity.

When U-shape calls were broadcasted, the echolocation call rate was significantly higher during playback and post-playback phase in comparison to the pre-playback for both species (Figure 6A, Binomial test; *M. daubentonii*: $p = 0.04$, *M. nattereri*: $p = 0.019$). For the inverted N-shape calls, calls of neither species led to an increased echolocation call rate (Binomial test; *M. daubentonii*: $p = 0.215$, *M. nattereri*: $p = 0.563$). The squawks from *M. daubentonii* triggered an increased echolocation call rate but the squawks of *M. nattereri* did not (Binomial test; *M. daubentonii*: $p = 0.003$, *M. nattereri*: $p = 0.563$).

During the playback and post-playback phase, we additionally photographed the passing or approaching bats and could identify individuals to species level in 273



photos (see [Figure 7](#) for details of species identification). We were able to not only identify *M. daubentonii* and *M. nattereri* on the photos but also a much rarer bat species, *M. bechsteinii* ([Figure 6B](#)). While the bats' reaction to broadcasted U-shape calls was slightly species-specific but far from exclusive, the bats' reaction to broadcasted inverted N-shaped calls was not species-specific at all. Interestingly, when we broadcasted squawks, we took more photos of approaching heterospecific bats than of conspecifics. Based on these findings and with regard to former descriptions of the calls we suggest U-shape calls to assist in group cohesion, while low frequency squawks emitted in an aggressive context might relay roost location to passing bats and the N-shape calls (which are often emitted in combination with more complex variable tonal calls) could play a role in context of mating.

Discussion

By observing a shared swarming site over a period of many nights in two consecutive swarming seasons we could document a broader variety of social calls from two species of *Myotis* bats than described in a swarming context before. Based on their spectro-temporal structure we grouped the calls into ten distinct types and found evidence that some call types are produced by both observed species, *M. daubentonii* and *M. nattereri*. With regard to the playback results we assume that some of the calls facilitate interspecific communication while others are employed for intraspecific communication.

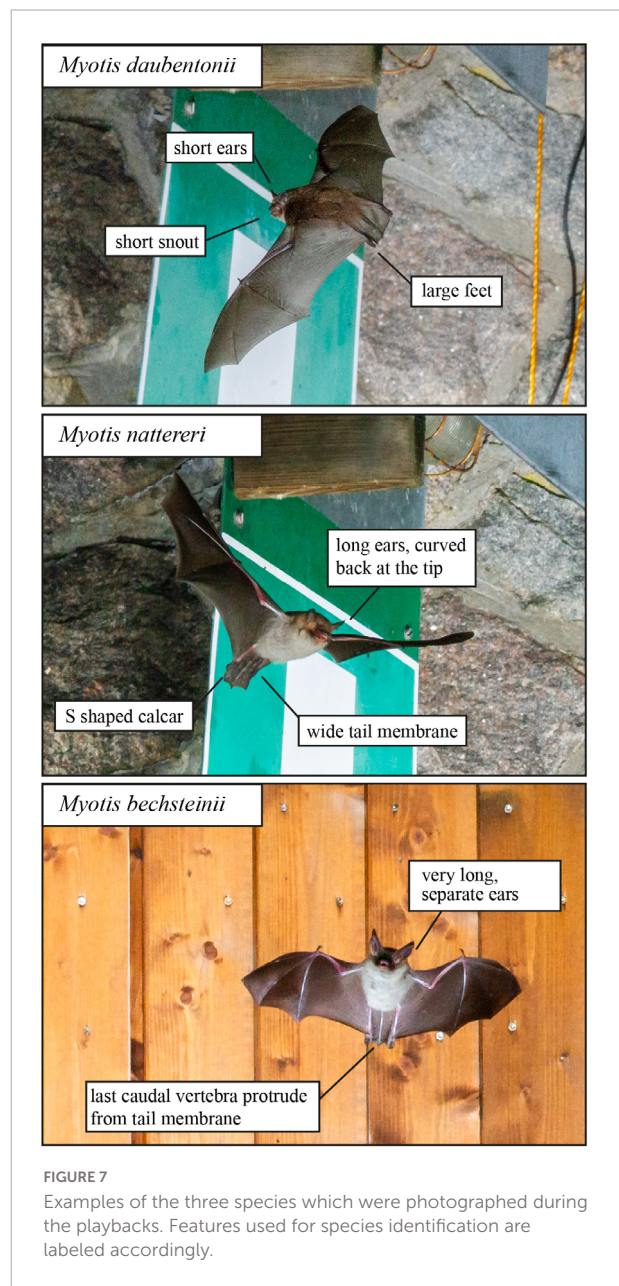
[Pfalzer and Kusch \(2003\)](#) described a variety of social calls from Vespertilionid bats in different contexts and organized them into four groups based on structure and function. Squawk-like, noisy calls were mostly observed in agonistic contexts (type

A), repetitive trills were produced under distress (type B), cheep-like or curve-structured single pulses were used for mother-pup interaction or group cohesion (type C) and song-like, complex structure and multiple frequency modulated elements were produced for mate attraction or in a territorial context (type D). We observed all such call types during autumn swarming, indicating various functions of swarming (see **Supplementary Table 1** for former classification of described call types).

We recorded a relatively high number of squawks, not only from crevices near the entrances to the hibernaculum, but also from swarming bats. Such calls are thought to be used aggressively or in a threatening context and were frequently recorded from captured bats or in association with roosting situations before (Middleton et al., 2014). Due to their long duration and low frequency, squawks have the potential to be audible over long distances. During the playback experiments, the squawks of Daubenton's bats but not Natterer's bats led to a significantly higher calling rate of passing bats, which might be caused by the longer duration of the Daubenton's bats' broadcasted squawks. However, the photos made during the experiment suggest that the reaction might not be species-specific as individuals of both species, *M. daubentonii* and *M. nattereri*, were approaching the speaker during and after the playback phase. Emitted during swarming, squawks should be well audible to passing bats and may serve to relay the location of the swarming site and thus the hibernaculum. Thus, we assume the calls to function as a cue for hibernacula and both heterospecifics and conspecifics tend to approach emitted squawk calls. As both species have comparatively similar hibernacula preferences and often hibernate in mixed-species groups, it is conceivable that squawks can facilitate interspecific eavesdropping to find suitable crevices.

Another commonly observed call type were FM pulses, which consist of a series of frequency-modulated, downward-sweeping elements of short duration and were also assigned to both species. Such calls are often observed in situations of distress (Middleton et al., 2014) and might work for intraspecific (Russ et al., 1998) but also interspecific communication during which heterospecifics can be attracted by distress calls to elicit a mobbing response to repel predators (Russ et al., 2004). During autumn swarming, we observed such calls being emitted in flight when bats were almost or actually colliding with other swarming bats. Such situations do not require species-specificity as both con- and heterospecifics might be the receiver of the call.

The high number of variable tonal calls we recorded corresponds well to the calls Pfalzer and Kusch (2003) described as Type D. Also Schmidbauer and Denzinger (2019) found such highly variable calls and assumed that those longer trills are closely linked to mating behavior as they were emitted in high numbers at an autumn swarming site but not at a maternity roost and both species are known to mate at autumn swarming sites (Encarnação et al., 2004; Pfeiffer and Mayer, 2013). Furthermore such comparatively long calls



potentially enhance the signal efficacy and detectability in contrast to shorter calls (Morton, 1986). In consideration of their high variability and frequent repetition, we concur with Schmidbauer and Denzinger (2019) that the variable tonal calls may be produced in the context of courtship and mating.

Even though structural similarities can be seen between some variable tonal calls and the inverted N-shape calls, the second are characterized by very regular frequency modulations. Our inverted N-shape calls coincide with call type C Schmidbauer and Denzinger (2019) recorded from Natterer's bats and probably also with the V-shaped call Pfalzer (2002) described, although he thought them to consist of two elements. We analyzed the surrounding echolocation calls and

our results strongly suggest that the inverted N-shape calls, like various call types, are employed by both *M. daubentonii* and *M. nattereri*. To our knowledge, the inverted N-shape call was so far only ascribed to Natterer's bats. However, to avoid errors, species identification solely based on the appearance of inverted N-shape calls will need some further investigation in the future. Although the calls did not lead to a change in echolocation calling rate during playback experiments, with regard to the structural similarities of the variable tonal calls we suggest them to be also mating related and maybe work in combination with variable tonal calls, as such calls were often emitted in rows.

Another common call type were U-shape calls, which are apparently also produced by both *M. daubentonii* and *M. nattereri*. Similar calls have been described in various situations so far and might be associated with tandem flights or group cohesion and coordination (Middleton et al., 2014). When broadcasted in playback experiments, these calls caused an increased echolocation call rate, suggesting phonotaxis or heightened interest in the playback area which corresponds well with the assumption of group cohesion as a function for U-shape calls. It is important to note that we recorded a high number of calls which could be placed on a continuum between the L- and U-shape calls. It was nevertheless possible to group them into two call types based on the differences at the calls' end but there was large acoustic overlap. Large overlaps were furthermore present between the U-shape and the first part of the combined UI-shape calls, which is not surprising as the first part is also U-shaped. However, the combined UI-shape call is characterized by the combination of two parts and has an additional second part which is rather similar to the modulated FM downsweep. Schmidbauer and Denzinger (2019) were the first who described such calls frequently at an autumn swarming site and a maternity roost of Natterer's bats and suggested them to function as contact calls.

Some further call types were recorded rather rarely, among them a very low number of modulated FM downsweeps, which coincides with the observation of Schmidbauer and Denzinger (2019) that those calls were much more abundant at a summer roost than at an autumn swarming site. Furthermore low numbers of FM downsweeps without modulation, and one call type (hook) that has not been described before. However, these call types were very rare and we have too little information to make assumptions about their function.

We are aware of the constraint of the species identification *via* surrounding echolocation calls. The social calls are often louder and audible over broader distances, while echolocation calls are stronger attenuated (Pfalzer and Kusch, 2003; Middleton et al., 2014; Chaverri et al., 2018), making the parallel recording of echolocation and social call difficult, especially in such a crowded swarming situation. Furthermore,

an individual not belonging to the predominant species at time of recording might emit the social call and thus lead to ascribing the social call to the wrong species based on the classification result. Nevertheless, significant differences in call parameters between the assigned species were present indicating that both species employ calls of a very similar structure. Based on this, classification *via* social calls alone should be conducted with great care and other parameters for species identification should be taken into account. This is especially the case for Natterer's bats which are often identified solely based on abundance of the inverted N-shape call even though our results indicate that *M. daubentonii* can also produce inverted N-shape calls.

Overall, the observed *Myotis* bats emitted a broad variety of social calls during autumn swarming. Noisy squawk calls seem to have an interspecifically attracting function to passing bats, while U-shape calls might facilitate group cohesion intraspecifically. For other calls we could not elucidate their function during swarming and given the great variety of social calls we could not cover the full repertoire in our playback experiments. Thus, recordings and more playbacks of various call types on and near swarming sites (ideally those used by only one bat species at a time) will be necessary to get further insights regarding species-specificity and call function. Nevertheless, with our work we provide a comprehensive description of the call repertoire at a shared autumn swarming site and thus make an important contribution to the knowledge about swarming and especially the use of social calls in free ranging *Myotis* bats.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This animal study was reviewed and approved by the Internal Ethics Committee of the Landesamt für Landwirtschaft, Umwelt und ländliche Räume Schleswig-Holstein under license LLUR_521_20180703.

Author contributions

AB, FG-R, and MK conceived and planned the study and analyzed the data. AB, BW, and KK collected the data. MK

extracted acoustic parameters. AB and MK carried out the statistical analyses. AB wrote the first draft. All authors provided the feedback and guidance.

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Conflict of interest

Author KK was employed by ChiroTEC.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2022.950951/full#supplementary-material>

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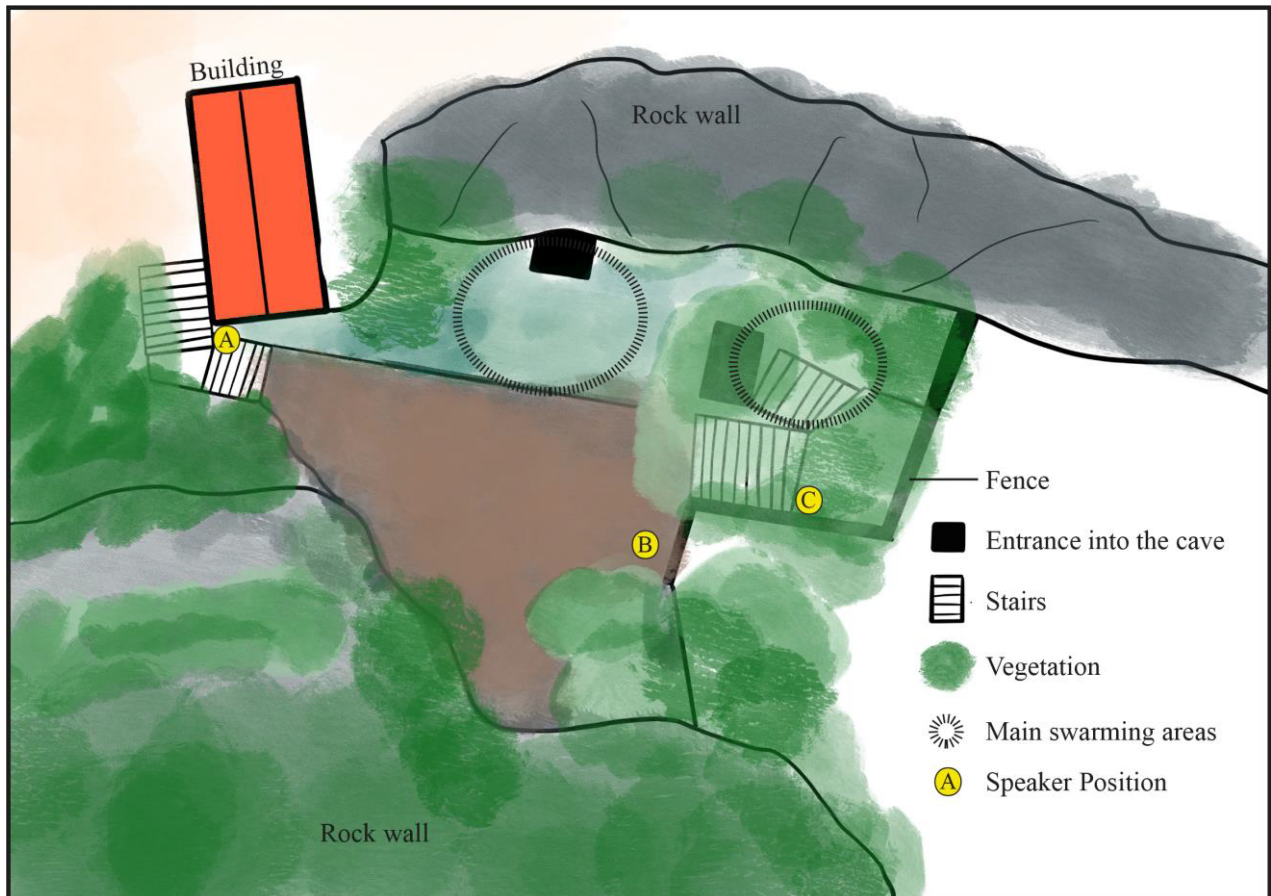
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Supplementary Material

Supplementary Video 1. Video of swarming bats in front of one of the two entrances of the Kalkberg cave filmed with a thermal camera (FLIR E95, Teledyne FLIR LLC, Wilsonville, USA).

Supplementary Audio 1. Audio files of the social call recordings shown in Figure 3.

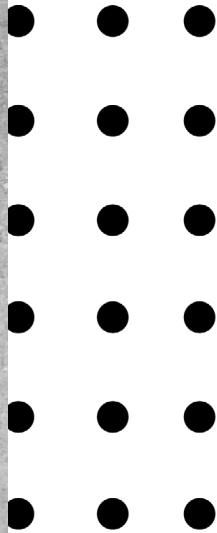


Supplementary Figure 1. Locations of playback experiments in the vicinity of the swarming areas. The speaker was mounted on a tripod directing upwards at playback location B and C and hanging from a wall, directing forward at location A. Bats swarmed above the low vegetation in the swarming area on the left, while larger trees covered the swarming area on the right.

Supplementary Table 1. Classification of observed call types in former publications and information whether the respective call types were recorded by Wimmer and Kugelschafter (2015) from free ranging bats identified via a photo trap. The last columns indicates whether the respective call type was assigned to both species in our analysis of surrounding echolocation call snippets.

Call type	Pfalzer and Kusch 2003	Schmidbauer and Denzinger 2019	Wimmer and Kugelschafter 2015		Classified to both species in our analysis
			<i>M. daubentonii</i>	<i>M. nattereri</i>	
Squawks	A (squawk)	(E) Squawk with noisy signal	X		X
Modulated FM downsweep	C (curved or cheep)	(A) Short cheep-like call with steep frequency modulation and shallowly modulated middle part			
FM downsweep	C (curved or cheep)		X	X	
L-shape	C (curved or cheep)		X		X
U-shape	C (curved or cheep)				X
UI-shape		(B) Two element call with an upward hooked element followed by a steep frequency modulated (FM) element			
Inverted N-shape	D (complex or song)	(C) Fast modulated call with by a rapid downward-upward-downward frequency modulation		X	X
Hook					
Hook of lower frequency	C (curved or cheep)		X	X	
FM pulses	B (repeated or trill)	(F) Churring-like call consisting of short FM pulses	X	X	X
Variable tonal	D (complex or song)	(D) Long broadband trill	X	X	

Chapter Three



Prior familiarity plays a bigger role for detecting newly installed bat boxes than improved echo-reflective box properties

Cover of Chapter Three: Pipistrelle bats inside an artificial roost box during visual controls.

Prior familiarity plays a bigger role in detecting newly installed bat boxes than improved echo-reflective box properties

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Abstract

Habitat loss in Europe is severely affecting bats, particularly tree-roosting species due to the decreasing availability of tree cavities. One common conservation strategy is the installation of artificial roost boxes. However, the occupation of newly installed roost boxes can take up to several years, and the underlying mechanisms for successful roost detection in bats are still poorly understood. This study proposes enhancing the visibility of roost boxes to echolocating bats by incorporating hollow hemispheres that provide highly conspicuous echoes. The hemispheres strongly reflect the echolocation calls of passing bats and are thus well-detectable over a broad range of angles. We hypothesized that roost boxes equipped with these hemispheres would attract more bats and exhibit greater bat activity compared to standard unmodified boxes. To evaluate this, we placed 30 modified boxes and 30 unmodified boxes across three forest areas in Northern Germany, each differing in proximity to known bat hibernation sites and the prior presence of artificial roosts. We monitored bat activity by measuring light beam interruptions at each box and found that the activity of bats at the boxes varied considerably. Contrary to our hypothesis, our findings indicate that bat activity was more significantly influenced by the bats' previous experience with artificial roosts rather than by the added detectability from hollow hemispheres. Furthermore, our study revealed that light beam interruptions indicated bat presence at the boxes earlier than visual checks for bats or feces, showcasing the benefits of non-invasive monitoring techniques. Conservation efforts are complex, and these results imply that for effective bat conservation, increasing bats' familiarity with artificial roosts may be more important than merely enhancing the detectability of these structures.

Introduction

Bats represent one of Earth's most ecologically diverse and successful mammalian orders. They exhibit remarkable adaptations to various habitats, can actively fly, and orientate via a sophisticated echolocation system. These adaptations are accompanied by specializations in a broad variety of food sources and roosting opportunities. However, in the Anthropocene era, bat populations globally face severe threats due to habitat degradation, fragmentation, and loss. Bats' specialized life history strategy, comprising large aggregations for breeding and hibernation, coupled with low reproduction rates, renders them particularly susceptible to environmental changes (Altringham, 2011).

In Germany, more than 50 % of the occurring bat species are classified as endangered (Meining et al., 2020) and all are protected under European and National law (BNatschG, 1992). Among the main drivers of their decline is the loss of natural habitats. Reasons for habitat loss may vary and include, but are not limited to, deforestation or the early removal of old and dead trees, which leads to the critical loss of suitable tree cavities and thus roosting opportunities for tree-dwelling species (Mickleburgh et al., 2002; Davidson-Watts et al., 2006). While suitable trees are in decline worldwide (Lindenmayer & Laurance, 2017), the development of cavities through natural processes is slow (Vesk et al., 2008). In response, artificial bat boxes have been installed, especially across Europe and North America to mitigate the loss of natural roosting sites and to enlarge the habitats for endangered bat species (Kunz & Lumsden, 2003). Initially used to establish bat populations for pest control (as reported in Kiser & Kiser, 2002 for instance), the use of artificial roosts turned clearly towards conserving endangered species in the past years (as reviewed in Rueegger, 2016).

Despite the potential benefits of artificial roosts, their effectiveness is often unpredictable due to an incomplete understanding of the specific requirements of target bat species, which can vary widely between different species and even among individuals. Factors affecting the success of roost boxes include box design (Baranauskas, 2009; Dodds & Bilston, 2013; Pschonny et al., 2022), the number and deployment of boxes (Brittingham & Williams, 2000; Zahn & Hammer, 2017), the time since installation (Agnelli et al., 2010), and microclimate characteristics (Kerth et al., 2001; Goldingay & Stevens, 2009). Moreover, the availability of other artificial roosting opportunities, such as bird boxes, also impacts bats' decisions regarding alternative habitats (Zahn & Hammer, 2017). Consequently, while some roost boxes are in regular use after only a few days, other boxes will never be colonized.

Furthermore, how bats search for and find new roost sites is still poorly understood. For certain species, listening to the echolocation or social calls of conspecifics within the roost can reduce search time and increase detection rates (Ruczyński et al., 2009; Schöner et al., 2010; Furmankiewicz et al., 2011). Yet, additional olfactory cues such as guano or urine do not play a significant role in roost location (Ruczyński et al., 2007, 2009; Brown & Carter, 2022). Enhancing the visibility of bat boxes to passing bats could improve roost occupation, as locating roost entrances through echolocation presents a significant challenge.

One way could be to enhance the echo-reflective properties of newly installed boxes by incorporating an acoustic reflector. In the neotropics, certain tropical plants have evolved echo-reflective leaves or petals to attract nectar-feeding bats as pollinators (von Helversen & von Helversen, 1999; Simon et al., 2011). These leaves or petals produce a distinctive, consistently strong echo across a broad range, serving as an echo-reflective cue. Such cues can be highly conspicuous to bats in an otherwise constantly changing environment, aiding them in locating plants and guiding them to nectar sources, thereby increasing the likelihood of successful pollination (von Helversen & von Helversen, 1999). Hollow hemispheres hold the potential to replicate similar characteristic echo patterns, resembling the bell-shaped concave form of bat-pollinated flower petals or leaves. Nectar-feeding bats can distinguish between hemispheres of different sizes (Simon et al., 2006), and in experimental settings, temperate zone bats can learn to associate them with suitable roosts (Hernández-Montero et al., 2020). This approach holds the potential for improving the visibility of artificial roosts, potentially facilitating their quicker and more successful adoption by bats.

We aimed to assess the effectiveness of hollow hemispheres as cues to reduce the time bats need to locate newly installed bat boxes and thereby enhance occupancy rates. Gable boxes were equipped with hollow hemispheres (modified boxes) and installed alongside unmodified boxes in three distinct forest sides that are known summer habitats of various *Myotis* species and near popular hibernacula (winter roosts). Anticipating higher detectability, we hypothesized increased activity rates at modified boxes compared to unmodified boxes.

Two of the study sites are known to be inhabited by bats from the Kalkberg Cave, one of the largest hibernacula in Northern Germany, hosting more than 30,000 hibernating bats each winter. To habituate bats to the hemispheres prior to installation in the forests, we placed hemispheres at both entrances of the hibernaculum. Based on the pre-exposure, we assumed that bats in the forests near the Kalkberg Cave would be more inclined to settle down in modified boxes than the bats at the third study site, as the former already were familiar with the echo-reflective cue and associated the hemispheres with a suitable roost. Subsequently, we investigated the varying activity rates of bats at boxes with and without hollow hemispheres across all three study sites.

Methods

Pre-observation

To ensure that bats were not deterred by the hollow hemispheres, we conducted a pre-observation at the Kalkberg Cave, a large hibernaculum in Northern Germany (Bad Segeberg, 10°18'57"E, 53°56'09"N). The vicinity of the cave is extensively used for late summer and autumn swarming prior to hibernation.

We tested a configuration of five hollow stainless-steel hemispheres, each with a diameter of 10 cm, arranged in a row and mounted on a wooden plank at a blind side entrance without a connection to the cave in the swarming season of 2018. Using a night-vision camera, bat activity at the entrance was

observed for 10 minutes, counting the number of bats within a 50 cm radius around the hemispheres. We compared observations with and without hollow hemispheres using a Wilcoxon test (SPSS, version 20, SPSS Inc., Chicago, IL, USA). Subsequently, in autumn 2019, we installed hollow hemispheres at the two main entrances of the cave to habituate incoming and departing bats to the specific echoes of the hemispheres and allow the bats to associate the hemispheres with a suitable hibernaculum. The hemispheres remained at the entrances until spring 2021.

Box set up and equipment

We utilized gable boxes of the type FLH14 with a 14 mm opening ($27 \times 18 \times 25$ cm, Hasselfeldt GmbH, Aukrug, Germany) and attached the hemispheres with a diameter of 10 cm to the front part of the boxes' roof with a screw (modified boxes, Figure 1). All boxes then were equipped with self-made light barrier systems, based on an Arduino circuitry. Sender and receiver units were affixed on both sides of the box entrance. When the connection between both LEDs was interrupted, the event was recorded on an SD card. The conductor board with the computer and the electrical power supply were stored in a waterproof case attached to the sidewall of the bat box. Regular maintenance involved replacing batteries every three weeks and visually inspecting boxes for bats and/or feces.



Figure 1. Gable boxes without and with an attached hollow hemisphere as an echo-reflective cue. The waterproof box located on the side houses the electrical components and power supply for the light beam system. Meanwhile, the diodes that measure interruptions, and thus bat activity, are affixed to both sides of the box entrance.

Study Sites

The study was carried out across three forest sites in Schleswig-Holstein, Germany. In each forest, we installed ten modified (with hollow hemispheres) and ten unmodified boxes (without hollow hemispheres). Installation was conducted in March (Forest A and C) and April 2021 (Forest B), with observations continuing until September 2021.

Forest A is located about 8 km near Kalkberg Cave, Forest B is located 21.5 km further north (Figure 2). Bats hibernating in the Kalkberg Cave roosted in both forests during the summer and were already familiar with bat boxes. Forest C is about 66 km away from the Kalkberg Cave and was selected because it had not yet been equipped with bat boxes and is located in the vicinity of another mass hibernaculum

of *Myotis* bats (MUNA-Kropp; 9°52'47''E, 54°39'75'') where bats were not habituated to the hemispheres (for forest characterizations, see also Table 1).

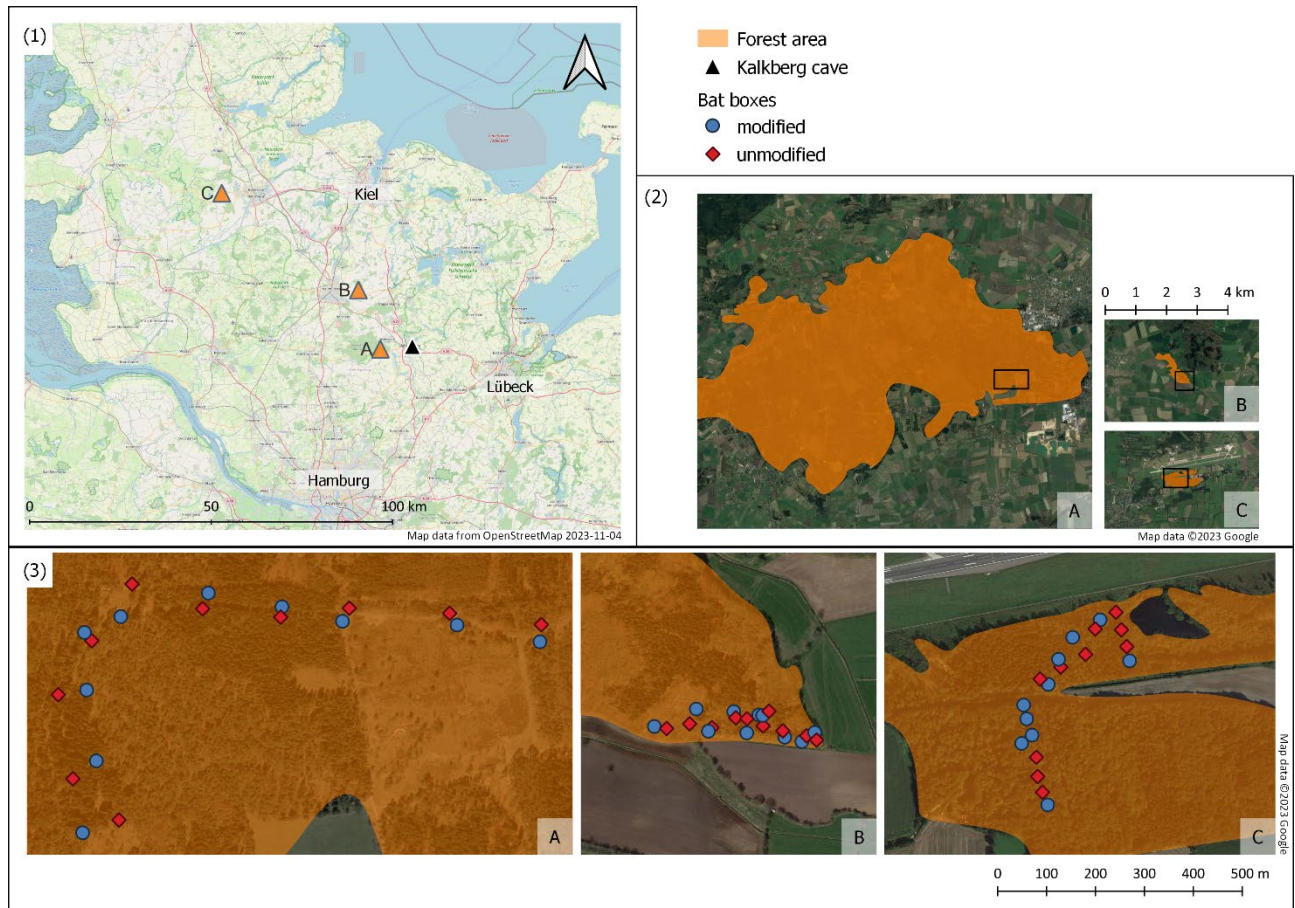


Figure 2. (1) Overview of the three study sites in Schleswig-Holstein, Germany. (2) Shape and relative size of the sites. Rectangles mark the sections in (3) with detailed bat box positions within the three forests. Modified bat boxes had a hollow hemisphere attached to the front roof as acoustic reflectors. Scales: (1) 1:2.000.000, (2) 1:240.000, (3) 1:15.000.

Data analysis

To determine whether the activity at boxes with an echo-reflective cue differed from those without such a cue, we conducted a comparison based on the number of light-beam interruptions observed on modified and unmodified boxes. We specifically focused on interruptions between 7 pm and 7 am, as these were most likely associated with bat activity at the box. For the analysis, interruptions with a duration ranging from 1 second to 15 seconds were considered, while those exceeding 15 seconds were excluded. This exclusion was necessary as it was not possible to determine whether such interruptions were caused by bats blocking the entrance, any technical issues, or other animals.

Especially at the beginning of the observation period, we encountered some technical issues related to light beam or SD card failure. These issues may have arisen due to extremely cold nights during March and April. As the season progressed, certain boxes were occupied by species other than bats, especially hornets, rendering them uninhabitable for bats. To account for the fluctuating number of operational

boxes over time, *interruptions per box-night* (I/BN) were calculated as a relative measure of activity. Box nights were determined by counting nights during which the light beams were operational and boxes were confirmed to be unoccupied by species other than bats. The total number of interruptions was then divided by box-nights for each box individually. The total number of observable nights per study site was 193 at both sites A and C, and 171 at site B, resulting in a maximum total of 3,860 box-nights for sites A and C, and 3,420 for site B, respectively. The actual box-nights varied due to the aforementioned occupancy by other species or technical problems, amounting to 2,368 box-nights for site A, 2,087 for site B, and 1,975 for site C (Table 1).

To assess the impact of modification, prior familiarity with boxes, and pre-exposure to hollow hemispheres on the activity rates at the boxes (measured as I/BN), we employed a generalized linear mixed model (GLMM, gamma distribution with log link) with Wald Chi-squared test and subsequent Tukey's post hoc tests (R v. 4.3.0, The R Foundation for Statistical Computing). The study site was included as a random factor in the model.

Table 1. Characterizations of the three study sites.

Study Site	Summer roost of bats hibernating in the Kalkberg Cave	Prior familiarity with boxes	Maximum possible number of		Actual number of box-nights
			Observable nights per box	Box-nights per forest	
A	Yes	Yes	193	3,860	2,368
B	Yes (Potentially)	Yes	171	3,420	2,087
C	No	No	193	3,860	1,975

Results

Hemispheres do not deter bats

The pre-observation at the blind side entrances of the Kalkberg Cave did not reveal any difference in bat activity when the hollow hemispheres were present or absent (Wilcoxon-Test: $Z = -0.560$, $N = 8$, exact $p = 0.641$). Consequently, we concluded that the hollow hemispheres did not have any deterring impact on the swarming bats and proceeded with attaching hemispheres to the main entrances of the Kalkberg Cave and modifying artificial roosts.

Significant variation in bat activity at boxes

The bat activity at the boxes varied considerably, with an overall mean of 9.03 interruptions per box-night (I/BN) for unmodified boxes and a mean of 4.87 I/BN for modified boxes (Table 2).

The activity level was greater at boxes at sites A and B, where bats were already using artificial roosts. Unmodified boxes in Forests A and B displayed the highest bat activity, while the lowest activity was observed at the modified boxes in Forests B and C. However, in Forest C, the activity was comparatively similar between treatments (Figure 3).

Table 2. Number of interruptions and box nights per forest for modified and unmodified boxes, I/BN = number of interruptions per box night, calculated as the mean I/BN over all boxes per study site.

Study site		Unmodified boxes			Modified boxes		
		Interruptions	Box nights	I/BN	Interruptions	Box nights	I/BN
Study site	A	15,488	1,490	9.52	8,877	1,108	6.95
	B	13,308	1,142	13.33	4,150	993	4.32
	C	3,554	811	4.19	4,094	1,066	3.49
	total	32,350	3,443	9.03	17,121	3,167	4.87

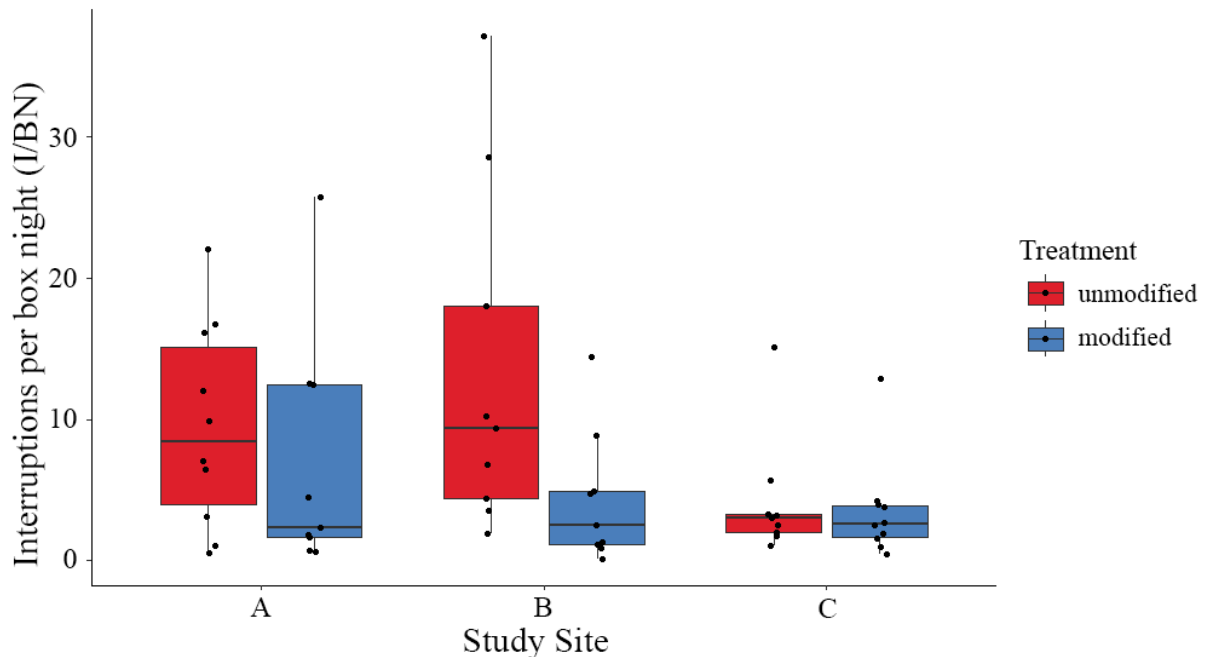


Figure 3. Distribution of interruptions per box night (I/BN) by treatment and location. The interruptions per box night differed depending on the modification of boxes with hollow hemispheres and the familiarity of bats with bat boxes before. Prior familiarity of bats with boxes was given on study sites A and B.

A Generalized Linear Mixed Model (GLMM) was conducted with I/BN as dependent variable, modification and pre-exposure as fixed factors, location as random factor, and Gamma family with log link. The analysis revealed that interruptions per box night significantly decreased with modification when observed across all sites (GLMM with Gamma distribution and log link, estimate = 0.5252, t-value = 2.068, $p = 0.0386$), but increased with familiarity (GLMM with Gamma distribution and log link; estimate = 0.7198, t-value = 2.684, $p = 0.00727$).

At sites A and B, unmodified boxes exhibited higher activity rates when compared to modified boxes, although the difference was not statistically significant (Tukey's post hoc test, estimate = -0.525, $z = -2.068$, $p = 0.1635$). Conversely, at site C, there was no observable difference between treatments (Tukey's post hoc test, estimate = -0.525, $z = -2.068$, $p = 0.163$), but the overall activity was notably lower than at sites A and B. When comparing activity rates for sites A and B (prior familiarity) to C (no boxes before) significant differences could be observed at both modified and unmodified boxes, respectively (Tukey's post hoc test, estimate = -0.72, $z = -2.684$, $p = 0.0365$). This suggests that the

primary driver for activity differences was the familiarity of bats with boxes as artificial roost opportunities rather than the box modification with hollow hemispheres.

Light beams measured bat activity earlier than visual controls did

In addition to the automated monitoring via light beams, the boxes were visually controlled every three weeks between April and September. On average the first light beam interruptions could be observed after about a month, while the first sighting of individuals or feces took more than 100 days on average (Table 3, Figure 4).

Table 3. Mean number of days after which the first light beam interruption, feces, or bats were observed in the boxes (bold); given in parentheses is the total number of boxes in which the according observation was made (with a maximum of 10 boxes per treatment), the second row contains the minimum and maximum number of days of first observation per box.

		Unmodified boxes			Modified boxes		
		Light beam	Feces	Bats	Light beam	Feces	Bats
Study Site	A	36.4 (10) 7-68	136.33 (6) 104-193	141 (6) 104-173	43.33 (9) 16-68	148.5 (4) 104-193	156.67 (3) 104-193
	B	40.2 (10) 26-82	122.75 (8) 81-171	125.33 (3) 82-171	36.75 (8) 28-91	107.25 (4) 81-123	127.33 (3) 82-150
	C	45.11 (9) 6-86	145 (1)	(0)	45.6 (10) 3-87	78.5 (2) 33-124	124 (1)

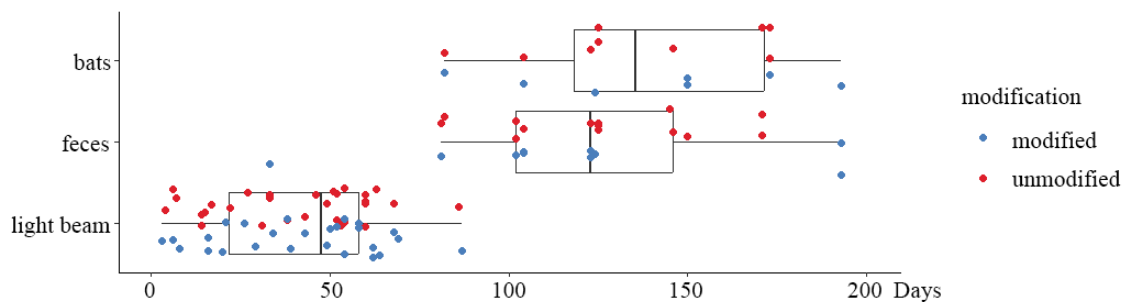


Figure 4. At both modified and unmodified boxes light beam interruptions indicated bat activity several days or weeks before feces or bats were encountered during visual controls.

Discussion

We aimed to investigate whether hollow hemispheres, as highly conspicuous echo-reflective cues, influence bat activity at newly installed bat boxes. By comparing the mean number of light beam interruptions per night, we found that the activity of bats at the boxes varied considerably. However, the primary factor influencing activity was not the presence of hollow hemispheres on boxes but rather the bats' familiarity with boxes as artificial roosting opportunities within the observed forest sites.

At site C, where bats had no prior familiarity with boxes, both overall activity and the difference between modified and unmodified boxes were lower. Finding new roosts can be very time-consuming for bats, especially if they are not already using similar structures nearby. The detection of newly installed boxes becomes more likely if bats are familiar with boxes (Zahn & Hammer, 2017), and as there were no artificial roosts at site C prior to our study, bats may need additional time to detect, habitually occupy, and use them. The similar activity rates between modified and unmodified boxes at site C suggest that the presence of hemispheres did not influence activity rates significantly.

At sites A and B, where bats were already familiar with artificial roosts, overall bat activity at boxes was higher. However, while the activity was higher at the unmodified boxes, no significant differences were based on modification for these sites. The trend might result from bats familiar with artificial roosts having a predefined “search pattern” for identifying roost characteristics. With the modification by hemispheres this pattern would be interrupted, and bats already familiar with artificial roosts before may have inspected new structures less often.

Site B, being a smaller forest with high “roost saturation”, exhibited higher activity rates at unmodified boxes. So, as the bats have no problems finding new roosts they might be even less interested in new structures, such as the modified boxes. Increased box density in an area might reduce occupancy rates, as the same number of bats spreads over a higher number of boxes, and the overall box occupancy only starts to increase slowly when bats begin to reproduce (Dietz & Kiefer, 2018; Pschonny et al., 2022). Given this perspective, the activity at modified boxes could potentially increase over the years. When comparing sites A and B, bat activity at unmodified boxes was comparable, while the activity at modified boxes was higher at site A. The lower overall roost saturation at site A and a more widespread setup across the forest may mitigate the effects described above at site B.

Taken together, the hollow hemispheres did not lead to an increase in bat activity at modified boxes. Considering the higher activity at unmodified boxes, bats seem to prefer them. Factors contributing to this preference could include neophobia, where bats are hesitant or uninterested in unfamiliar structures. Bats accustomed to artificial roosts and regularly using them already recognize what to seek in a roost. Hence, they might not need to adjust to the altered appearance of the modified boxes, especially if sufficient unmodified boxes or other roosting opportunities exist in the habitat. Interestingly, the activity was nearly the same between treatments in Forest C, where bats were unfamiliar with boxes, further supporting the effect of such search patterns.

We assumed that attaching hemispheres to boxes would enhance their detectability. Unfortunately, due to technical issues arising after the installation of the boxes, we cannot confidently determine whether the modified boxes were located earlier than the unmodified ones. Consequently, we are unable to conclude the efficacy of the hemispheres in improving box detectability. However, a comparable approach (Hernández-Montero et al., 2021) found that echo-reflective cues did not consistently reduce search time, and contrary to their expectations, the reflectors did not significantly enhance detectability

on a measurable level. In summary, despite reflecting a conspicuous echo, the hollow hemispheres might have altered the boxes so that bats no longer recognize them as suitable roosts.

The influence of pre-exposure to hemispheres appears to be negligible. Hernández-Montero et al. (2020) demonstrated that, despite bats distinguishing between suitable and unsuitable roosts based on size differences in attached hollow hemispheres, they revisited all boxes in the subsequent season. This behavior could be attributed to a lack of memory retention during hibernation or the need to reevaluate all possibilities that might have changed over time. Unfortunately, our data cannot contribute to this observation as the bats pre-exposed to hemispheres at the Kalkberg Cave were also already familiar with artificial roosts, thus making the influence of pre-exposure impossible to disentangle from the effect of familiarity with bat boxes in general.

Additionally, we would like to highlight our monitoring technique. Besides initial technical challenges, the light beams operated reliably, revealing high levels of activity at some boxes even though we never visually observed a single bat during our controls. Some bat species change their roosts on a nightly basis, making it challenging to conclude the use of a specific roost box during such monitoring. By checking boxes once per season, Pschonny et al. (2022) found 40 % of newly installed gable boxes being occupied within the first year. However, different observation methods can yield varying results, and feces may naturally be found in more boxes than bat individuals (Tajek & Tajkova, 2016). Our light beam systems registered interruptions, indicating bat activity at over 90 % of the boxes in total. The majority of interruptions were recorded long before individuals or feces could be observed inside boxes, making it a suitable method to measure bat activity.

In conclusion, automated monitoring systems, such as light beams, offer a valuable solution to assess bat activity in detail. These low-cost systems enable non-invasive monitoring over an extended period. However, we recommend a trial of the final set-up on a limited number of systems for at least one season to address potential technical issues proactively. We observed bat activity at over 90 % of the newly installed boxes at the end of the study period. It appears that in this area, bats did not encounter significant challenges in locating new roosts. Therefore, providing a substantial number of artificial roost opportunities could be a beneficial complement to the ongoing efforts to preserve natural habitats.

Author contributions

AB, FG-R, and MK conceived and planned the study. AB and FG-R carried out the field work and collected the data. AB and MK carried out the statistical analyses. AB wrote the first draft, and all authors provided feedback and guidance.

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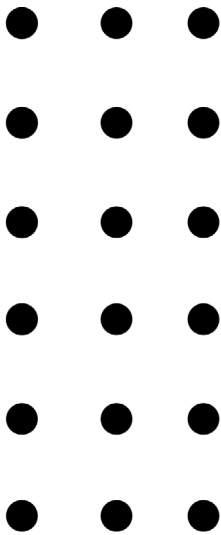
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General Discussion



The need for comprehensive monitoring of bats

Bats account for about a fifth of all mammalian species worldwide, representing a very diverse group economically and taxonomically. With over 1,450 species recognized today, they are the most widely distributed terrestrial mammals (Burgin et al., 2018). According to the International Union for Conservation of Nature (IUCN), 18 % of all bat species are classified as threatened and for another 15 %, there is not even enough data for a meaningful classification – a significantly higher number of species classified as data deficient than among other mammals or birds (Frick et al., 2019). In addition, over half of the bat species are either ranked with unknown or decreasing population trends, equaling 988 species (80 % of the bats assessed by the IUCN) requiring conservation or at the very least research attention (Frick et al., 2019).

The challenges bats face are as diverse as the various bat species. Thereby, comparable to many other species, bats predominantly suffer from anthropogenic impacts, such as the loss of natural habitats caused by extensive agriculture, woodland management, and human population growth (Mickleburgh et al., 2002). Based on species-specific traits and lifestyles, bat species are unequally affected by environmental changes. For instance, among insectivorous European temperate zone bats diverse foraging strategies coupled with dietary preferences can be observed, ranging from aerial insect hunting to gleaning prey from vegetation or open ground (Fenton, 1990; Schnitzler & Kalko, 2001). Besides, roost selection varies from man-made structures, such as attics and cellars to more natural settings such as tree cavities or caves (Dietz et al., 2007). Furthermore, sometimes preferences undergo seasonal changes, leading to variations in bat behavior throughout the year. This diversity introduces a diverse array of threats requiring adjusted conservation strategies and comprehensive insights into both species-specific behaviors as well as seasonal changes to enable the implementation of more effective mitigation strategies.

Bats are highly vocal animals, enabling non-invasive acoustic monitoring and species identification, as the echolocation calls differ in certain acoustic parameters such as duration or frequencies (Fenton & Bell, 1981; Jones et al., 2000). However, with similar ecological niches similar calls evolved (Schnitzler & Kalko, 2001), and thus large similarities and overlaps are visible in echolocation calls of several genera, for instance, the genus *Myotis*, thus hindering species identification in the field (Wimmer & Kugelschafter, 2015). This makes thoughtfully planned and executed experiments essential for information acquisition, especially within this genus.

Application of new methods for species identification

Large numbers of Daubenton's bats and Natterer's bats (*Myotis daubentonii* and *Myotis nattereri*) gather during autumn swarming at the Kalkberg Cave (Bad Segeberg, Germany). In this situation, the analysis of single calls proves impractical as both *Myotis* species predominantly present at the study site exhibit rather similar echolocation calls. Those calls become even less distinguishable in a crowded swarming

scenario. In **Chapter One**, I tested the possibility of species identification from overlapping echolocation call sequences recorded in a crowded swarming situation.

By employing a set of unequivocally identified reference data and based on a combination of classical acoustic parameters and Linear Frequency Cepstral Coefficients (LFCCs), the majority of sequences could be assigned to one of the two predominant *Myotis* species at the Kalkberg Cave. These results demonstrate that LFCCs can facilitate species recognition in challenging environments. The species classification, based on a combined approach using LFCCs and classic acoustic parameters, not only reflected the known phenology at the investigated swarming site but also proved effective at another swarming site. At the second site, simultaneous mist netting revealed a predominance of Natterer's bats, and accordingly, all acoustic recordings were assigned to Natterer's bats. This is a great improvement when compared to the necessity of analyzing single calls. Although comparable approaches have been employed to discriminate between species (Noda et al., 2016, 2019), individuals (Araya-Salas et al., 2020), or contexts of vocalizations (Knörnschild et al., 2017; Fernandez & Knörnschild, 2020), those studies typically utilized sound recordings of only one vocalizing animal, in contrast to multiple animals considered here.

Analyzing soundscape instead of individual calls has the potential to make long-term monitoring of swarming sites easier in the future. Usually, the two major challenges in analyzing passively recorded data are the detection of calls and the classification of species (Brinklöv et al., 2023). As the analysis of soundscape utilizes overlapping calls, the need for call detection is mostly eliminated. However, the application of algorithms for species identification and classification often remains the main challenge and despite the potential advancement, the proposed approach has only been validated for two species so far, *Myotis daubentonii* and *Myotis nattereri*. These two species indeed showcase the most distinct call designs among the *Myotis* species, potentially contributing to the successful classification. To extend the applicability, a more comprehensive strategy necessitates gathering a larger number of unequivocally assigned reference calls for further species. Ideally, these reference calls should be collected across diverse situations and be rigorously tested, such as during the swarming of a single known species, or at sites with two species and known phenology, or validated through simultaneous bat captures. Additionally, so far prior knowledge about the expected species is essential for the analysis and only the predominant species at the time of recording can be reliably assigned by focusing on the soundscape. Alternative methods are necessary to account for swarming species occurring in lower numbers. As of now, there is no fully automated method for the required postprocessing of the recordings, but this remains a major constraint of many classification approaches (Brinklöv et al., 2023). Sequences for analysis were manually selected, prioritizing those with the highest number of overlapping echolocation calls while minimizing interference from social calls. Although this approach is applicable for single, non-overlapping calls, the extensive postprocessing time to eliminate the background noise between calls might render other classification approaches more practical.

With the classification of the predominant species, valuable information about species assemblage and species-specific phenology throughout the swarming season can be provided. The more we know about species assemblage during autumn swarming, the better we can infer the winter inhabitants of a cave. Rivers et. al (2006) proposed that for cave-dwelling bats such as Natterer's bats, conclusions about the winter inhabitants of a hibernaculum can be drawn from surveys of the species assemblage during swarming. While some studies found no clear relation between swarming and hibernation assemblage (Hall & Brenner, 1968; Parsons et al., 2003), van Schaik et. al (2015) supported this concept by demonstrating that the swarming assemblage serves as an informative proxy for estimating relative population size and assemblage during hibernation. They conducted bat captures throughout the entire swarming season, recognizing the changes in the assemblage and linking it to the winter population. The documented change in species assemblage at the Kalkberg Cave is reflected in the classification results and thus makes this approach applicable for gaining information about the winter inhabitants of the cave. Consequently, the broader utilization of non-invasive long-term monitoring holds the potential to provide support and, to some extent, reduce the need for labor-intensive and disruptive winter inspections in large hibernacula.

In summary, despite the existing limitations, this work establishes a foundation for alternative future classification methodologies and simplifies species identification in scenarios where multiple individuals are calling simultaneously, and single-call analysis renders impractical. This gets especially helpful in demanding environments where mist-netting might not be feasible.

The variety of social calls during autumn swarming

When analyzing overlapping echolocation snippets, interference from social calls can pose challenges. But what if we could utilize those social calls for species identification? Social calls primarily aim to elicit behavioral responses from the intended receiver, typically a conspecific. While various bat species employ social calls that often display similarities in sonographic structure, most calls nevertheless exhibit species-specific features, facilitating species recognition. This can be important for individual recognition (Pfalzer & Kusch, 2003).

In **Chapter Two**, I investigated the social calls of *Myotis* bats during autumn swarming. Out of a total of 2,135 recordings, each containing one to several social calls, I identified ten distinct call types, some of which were previously undocumented in a swarming context. These calls were categorized based on their spectro-temporal structure, with grouping confirmed through a Discriminant Function Analysis (DFA). Utilizing the echolocation call sequences surrounding the social calls, some of the calls could be assigned to species level by employing the combination of classical acoustic parameters and LFCCs (see Chapter One). This analysis was applied to four of the observed call types (inverted N-shape, L-shape, U-shape calls, and FM pulses). All call types were assigned to both Natterer's bats and Daubenton's bats respectively, suggesting that both species utilize rather similar calls in a swarming

context. This suggestion was further supported by the analysis of classical acoustic parameters of the social calls. Despite their overall similarity in spectro-temporal structure, significant differences in both the start and maximum frequency of all calls were revealed between both species.

To gain further insights into call functions, I conducted playback experiments in the vicinity of the swarming site with three of the call types assigned to both species respectively (inverted N-shape calls, U-shape calls, squawks). Bats' reactions were observed through a thermal camera, and detailed analyses were performed by taking photos and recording the calls emitted in response to the broadcasted calls as a proxy for activity rates. No change in echolocation call rate was noted for the inverted N-shape calls. In contrast, the U-shape calls of both species led to a significant increase in echolocation call rate. While testing squawks, the sole call type unequivocally assigned to one species based on visual observation, only squawks of Daubenton's bats led to an increase in bat activity, while Natterer's bats' squawks did not affect echolocation call rates.

Both observed species are believed to mate at autumn swarming sites (Encarnação et al., 2004; Pfeiffer & Mayer, 2013), a behavior that correlates with the emission of a variety of tonal calls assumed to be closely linked to mating behavior (Pfalzer & Kusch, 2003; Schmidbauer & Denzinger, 2019). The variability in these calls, however, poses a significant challenge for their integration into playback experiments with free-ranging bats, as of now the syntax of such vocalizations is not fully deciphered. During swarming, the inverted N-shape calls were frequently observed in conjunction with variable tonal calls, suggesting a potentially related function. Encoding of meaningful sequences from complex animal vocalizations is still an ongoing challenge in bioacoustics (as reviewed in Kershenbaum et al., 2016), and in the playback experiments, the solitary emission of inverted N-shape calls did not alter the echolocation call rate of approaching bats. This observation suggests that the critical communicative content of these calls may be diminished or lost when they are emitted in isolation, rather than in conjunction with variable tonal calls, underscoring the complexity of bat communication and the importance of contextual factors in interpreting animal vocalizations.

The U-shape calls have not only been produced by both species during the observations but also led to a significant increase in the echolocation call rate in the playback experiments. Simultaneously taken photos revealed the tendency for the calls to be species-specific. In line with former studies, these calls might be most important for intraspecific communication such as group cohesion or mother-pup communication (Pfalzer & Kusch, 2003; Middleton et al., 2022). With the swarming behavior being highly social, this is not surprising. In a crowded situation, the bats need to communicate with their conspecifics and maintain information transfer directly to the intended receiver. Thus, these results fit well into the known functions of swarming.

Furthermore, the playback results suggest that interspecific communication is taking place besides intraspecific communication. The noisy, broad-band squawks which were typically of longer duration than the other calls were emitted mainly from crevices near the swarming area, but also from bats on the

wing. During the playback experiments, they led to higher echolocation call rates from approaching bats, and slightly higher numbers of heterospecifics reacted in comparison to the other call types. Due to their low frequency, they are well audible over long distances and have the potential to work as a cue to passing bats for both the swarming site and a potential roost. To find suitable roosts and hibernacula it makes sense to follow the calls of heterospecifics when they exhibit similar habitat preferences.

Although significant differences in the structure of calls between species were present, more work is needed for an unambiguous species identification via social calls. The ultimate goal would be to identify bats on the wing from their social calls alone. To accomplish this, a more comprehensive collection of social calls, distinctly and reliably identified to species level, is essential. Nevertheless, the findings from the playback experiments highlight the multiple roles of social calls and autumn swarming: information regarding suitable roosts, mating opportunities, or group cohesion is transferred not only between conspecifics but sometimes also between individuals of different species.

Utilization of swarming sites for information transfer

As highlighted in Chapter Two, the autumn swarming site is a place of communication and information transfer. The swarming bats also intensively perceive their surroundings via large amounts of echolocation calls. Beyond the intra-, or even interspecific communication, it might be possible to present new information, namely echo-reflective cues, that consequently trigger a positive connection between the cue and the roost. To test this potential connection the main objective in **Chapter Three** was to guide bats towards newly installed roosting boxes, as the occupation of such often takes up to several years. With the combination of a highly conspicuous echo-reflective cue (hollow hemisphere) and its establishment in a roosting context beforehand, new roosts should be more conspicuous, and thus activity rates at such should increase.

However, contrary to the initial hypothesis, the study revealed that activity rates at boxes equipped with hollow hemispheres were lower than at those without these features when observed across all three observed study sites. Several factors potentially played a role in these results. First of all, the Kalkberg Cave, a place for mating and hibernation, might not have triggered the desired connection and was maybe not perceived in relation to foraging and summer roosting habitats by the bats. While at least Bechstein's bats are known to quickly learn to distinguish between suitable and unsuitable roosts based on external echo-reflective cues through associative learning (Hernández-Montero et al., 2020a), the connection between the hibernaculum and a suitable roost might have been too abstract for bats to make. However, even bats that verifiably learned such connection during one season tend to check out all roosting opportunities again in the next season, as during hibernation, a state of inactivity, and metabolic depression in bats, the retention of memory regarding specific cues might be diminished or even lost (Hernández-Montero et al., 2020b). This potential memory impairment may prevent bats from recognizing or responding to these cues upon emerging from hibernation. Therefore, introducing such

cues in locations more directly associated with their daily living and foraging activities might be more effective, as bats could be more likely to form and retain associations with new roosting opportunities in these areas.

But which other possibilities do we have to support bats in their roost selection? Roost boxes are a primary mitigation measure, and with the decline of natural habitats, they are especially vital for forest-dwelling bats that frequently change their communal day roosts. For those species, it is crucial to preserve a large pool of suitable roosts to ensure the viability of populations, evade parasites, and maintain optimal roosting temperature (O'Donnell & Sedgely, 1999; Kerth et al., 2001; Reckardt & Kerth, 2007; Olson & Barclay, 2013). We know that bats are more inclined to occupy new roosts if they have previous experience with roost boxes (see results in Chapter Three, and Zahn & Hammer, 2017). Leveraging this tendency could facilitate habitat expansion by gradually introducing new boxes, thereby incrementally integrating new forest areas into their habitat. However, while promising for long-term habitat expansion, this approach is time-intensive and not ideal for immediate mitigation.

Besides, in the face of increasing habitat fragmentation and loss, another objective of this experiment was to find a possibility of guiding bats to wildlife overpasses or underpasses near roads, as these are crucial, especially for low-flying species. Species that depend on landscape features like hedgerows and trees for navigation are particularly affected by road expansion and construction. They not only lose familiar navigational landmarks but also face a heightened risk of collisions due to their low-flying habits (Lesiński et al., 2011; Claireau et al., 2019). As the approach did not yield the expected results there seems to be no one-for-all solution. Thus, it remains important to explore alternative methods for guiding bats, and additional mitigation strategies are essential. Effective bat protection thereby could include measures like implementing speed limits (Oliveira & Bueno, 2022), establishing new commuting routes (Bennett & Zurcher, 2013), and thoughtfully designing underpasses (Altringham & Kerth, 2016). These measures, either individually or in combination, can offer protection without relying on artificial structures for guidance.

How can bat identification be facilitated in the future?

A key limitation of the methodology established in Chapter One is its tendency to overlook rare species, as it primarily identifies the most prevalent species present at the time of recording. To tackle this, integrating social calls into the analysis emerges as a promising solution to detect rarer species. However, despite notable variations in call structures among different species, the current research indicates that more work is needed for precise species identification through social calls alone. The long-term goal would still be to identify bats on the wing from their social calls, but therefore more unequivocally assigned social calls as references would be indispensable.

In parallel, the field of automated bat identification is evolving fast and machine learning as a branch of artificial intelligence offers powerful algorithms for analyzing patterns in data, but there are still several

challenges to be overcome. While artificial neural networks (ANN) can accelerate species identification, their accuracy often does not significantly exceed that of human observers (Jennings et al., 2008), and sometimes automated bat call classifiers are prone to false identifications (Rydell et al., 2017). Even though some models achieve over 90 % accuracy, they often only identify bats at the genus level, which is particularly true for *Myotis* species (Walters et al., 2012; Alipek et al., 2023) limiting the effectiveness of species identification in locations with diverse *Myotis* populations, such as the Kalkberg Cave. However, deep learning models are continually improving in speed and efficiency, and the use of convolutional neural networks (CNN) as image classifiers represents a significant advancement (Krizhevsky et al., 2012; Khan et al., 2020). The first deep-learning model for distinguishing bat echolocation from ambient noise (Mac Aodha et al., 2018) was recently enhanced by an algorithm for species identification (Mac Aodha et al., 2022). Among the most promising approaches at the moment is a classification based on spectrogram images as graphical representations of acoustic signals: A deep CNN that has recently entered the field of automated bat call classification reached an accuracy of up to 96 % across 18 included species, with an accuracy between 93.21 % and 100 % for the included *Myotis* species (Schwab et al., 2023).

However, although the models are continually improving, trained models, which form the cornerstone of traditional machine-learning approaches, require extensive datasets that are carefully annotated by humans for training (Alhazmi et al., 2021). This becomes particularly challenging in ecological studies where species are numerous, their calls vary, and the environmental conditions are diverse. On the other hand, models that operate without explicit human training, often referred to as unsupervised learning models, can identify patterns and make inferences from data without pre-labeled examples. However, since they are not guided by human-annotated examples, they might find patterns that are not biologically or ecologically relevant, and interpreting the results of unsupervised models can get challenging, as the criteria they use for classification are not always clear or aligned with human (or animal) understanding (Kershenbaum et al., 2016). Thus, to comprehensively understand animal behavior, especially in relation to their vocalizations, it remains crucial to further complement advancements in artificial intelligence and big data with direct fieldwork. Observing and listening to animals in their natural habitats is still essential to draw informed conclusions and avoid being misled by apparent patterns that may not hold significance in the real world.

Advantages and constraints of non-invasive monitoring

In studying the natural behavior of animals, the application of non-invasive monitoring techniques offers invaluable insights, particularly crucial for studying endangered species like bats where stress and disturbance must be minimized to protect their welfare. Such techniques allow for observation and data collection without direct animal interference. All the former results were generated mostly non-invasive, showcasing advantages but also limitations depending on the context of observations.

In **Chapter One**, swarming soundscape rather than single echolocation calls enabled species identification in a very challenging acoustic situation. Thereby, a comprehensive overview of the phenology throughout the swarming season is possible for two species that show a change in abundance over time. One main constraint is the potential to overlook rarer species as only the predominant species at the time of recording can be identified. Also, the time-consuming post-processing of data is a common problem, especially in passive acoustic monitoring (Brinkløv et al., 2023), which might be potentially tackled by further automation of both post-processing and classification in the future. However, this method has the potential to facilitate species identification, e.g. on so far understudied swarming sites. By gathering information on swarming phenology and species assemblage, conclusions about winter populations in hibernacula can be drawn, and help to reduce disruptive winter controls to a minimum.

Chapter Two explored the role of social calls during autumn swarming through playback experiments. Non-invasive playback experiments are not that easy to obtain and in comparison, to classic mist-netting, the species identification via camera trap images was not consistently achievable. Furthermore, it did not provide insights into individual features, and consequently, distinctions in swarming behavior based on sex, or even age, remained unaddressed. To comprehensively tackle these open questions, playbacks in conjunction with simultaneous mist-netting would be indispensable. However, the great advantage was, that through the non-invasive approach, I was able to conduct playbacks in such close proximity to a significant hibernaculum without causing undue stress for the animals in focus. In conclusion, those non-invasive playback experiments can be conducted nearly anywhere to gain first insights into call function and behavior, informing the need for further experiments.

In **Chapter Three**, I complemented visual controls of bat boxes by non-invasive monitoring via light beams to track box use. Traditional visual inspections, typically conducted once per season, are labor-intensive and need preparation and manpower. By comparing the results of my visual controls to the activity rates measured via light beams, I revealed that most boxes were actively used weeks or even months before any signs of occupancy, such as droppings or individuals, were visible. The application of low-maintenance light beam systems has the potential to provide us with comprehensive knowledge of bats' willingness to occupy newly installed boxes. And while light beam systems efficiently track bat activity and box colonization, they can give us insights, we would not gain from visual controls, e.g. about habitat use or population dynamics. However, the applied system does not provide exact numbers of individuals or detailed information about them. Implementing bidirectional light beams could enhance the system by differentiating incoming and outgoing activity, indirectly estimating occupancy. Moreover, maintenance demands, namely battery replacement, could be reduced to a minimum by adding solar panels together with rechargeable battery systems. For further insights, tagging bats with RFID chips would be another option, although clearly not non-invasive and rather impractical for a widespread application.

Overall, when deploying new techniques and technologies, it is crucial to find a balanced approach that considers the costs and benefits for both human observers and the focal animals. Particularly in the context of conservation, we must question the necessity of extensive details, and non-invasive monitoring is thereby often proving the best opportunity. It contains the potential to provide broad insights and comprehensive overviews, thus laying a basis for making informed conservation decisions.

Conclusion

Human activities constantly alter the natural environment, leading to a significant man-made decline in biodiversity worldwide. Large numbers of mammals are facing threats of extinction due to climate change, habitat destruction, or poaching. Many bat species worldwide are considered endangered due to severe declines in populations, with inadequate data for many others. Half of the bat species present in Germany are listed as endangered, while all of them are protected under national law. However, bats are highly vocal animals, and even though we do not fully understand their language yet, their vocalizations already provide us with meaningful insights into caller identity in terms of species, sex or age, and specific behaviors. Utilizing these signals for information acquisition in non-invasive monitoring is an important tool for the conservation of bats. In the end, humans, like all species, depend on a certain biodiversity and intact ecosystems, underlining the necessity for comprehensive conservation efforts. Incorporating both soundscape and social calls into monitoring enhances information acquisition and yields further insights into habitat use, species assemblage, phenology, and seasonal changes in bat populations.

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LIST OF PUBLICATIONS

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