How we studied the horseshoe bat craziness - (FORMAL TITLE to be agreed on)

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Echocating bats emit loud calls and listen for returning echoes to detect their surroundings. When echolocating in groups, the returning echoes may not be detected due to the loud calls coming from each bat in a group. Bats a show a variety of echolocation responses in the presence of such acoustically challenging conditions. To date however, most studies have been performed on low duty-cycle FM bats. High duty-cycle CF-FM bats have been understudied to date despite their higher chances of call-echo overlap. Studying high duty-cycle CF-FM bats has also been hindered by absence of methods to analyse overlapping calls and a predominance of flightroom and playback studies. We present methods to analyse and extract call parameters from audio with overlapping calls, and study the echolocation of a multiple CF-FM bats in the field. Our results show that bats do not seem to alter their call parameters even in the presence of conspecifics. This lack of response is in contradiction to a previous study. We contextualise our results in the light of the fact that it is an observational study done in the field.

## Potential titles

1. CF bats do not show changes in echolocation calls in the presence of conspecifics
2. Echolocation remains unaltered in the presence of conspecifics in CF bats
3. Echolocation calls in the field remain unaltered even in groups of CF bats
4. Echolocation calls in the field remain unaltered in the presence of CF bats
5. Conspecifics do not affect echolocation call structure in CF bats

## 1.0 Introduction

Active sensing animals like echolocating bats face a sensory challenge when engaging in social behaviours like group emergences or foraging with conspecifics (Ulanovsky and Moss 2008; Gillam et al. 2010). Echolocating bats emit loud ultrasonic calls and detect their surroundings by listening for the echoes reflecting off objects around them[Griffing;FentonBrock]. In groups however, a bat’s returning echoes can be overlapped by calls and echoes from other bats, preventing detection of its surroundings. [(**???**);;Ulanovsky and Moss (2008);maskingstudies]. A combination of laboratory and field studies have shown the diverse responses bats show in groups and similar situations with loud sounds like noise or playbacks. Bats increase their call emission levels, alter temporal features such as call rate, duration and duty cycle (Amichai, Blumrosen, and Yovel 2015; Jarvis, Jackson, and Smotherman 2013, @lu2020echolocating; Hage et al. 2013; Lin, Abaid, and Müller 2016), along with spectral properties such as bandwidth and terminal frequency (Hase et al. 2018; Cvikel et al. 2015; Götze et al. 2016; Fawcett and Ratcliffe 2015). Each of these responses however seem to be species specific, with different species showing seemingly opposite responses to similar situations (Ulanovsky et al. 2004; Amichai, Blumrosen, and Yovel 2015; Jarvis, Jackson, and Smotherman 2013; Adams, Davis, and Smotherman 2017). A wider variety of species need to be studied, to understand the myriad of echolocation responses in context of their ecology and auditory systems.

There are two broad groups of echolocating bats (Fenton, Faure, and Ratcliffe 2012) characterised by their duty cycle, or the fraction of time spent emitting calls. The first and major group of bats are low-duty cycle bats, which typically emit frequency-modulated (FM) calls. The second group is the high-duty cycle bats which typically emit calls with both FM and constant-frequency (CF) components in them, also called CF-FM calls. From a temporal perspective, low-duty cycle bats typically emit calls of a few milliseconds (1 to 20ms) duration at duty cycles between 5-20%. In contrast, high-duty cycle bats emit relatively longer calls (10 to 50ms), at much higher duty cycles of 25% upwards. Echolocation duty cycle is directly proportional to the probability of calls overlapping echoes (Beleyur and Goerlitz 2019). High-duty cycle bats such as rhinolophids and hipposiderids are thus likely to be more affected in group echolocation than low-duty cycle bats, making them a particularly valuable study system to understand the sensory strategies echolocators use in challenging conditions.

A typical CF-FM call may consist of upto three call components (sensu Tian and Schnitzler (1997)) consisting of an initial upwards FM sweep (iFM), leading to the CF segment (CF), and a terminal downward FM sweep (tFM). The CF component is used for the flutter detection of prey wingbeats [@??], and the auditory system of high-duty cycle bats shows a pronounced sensitivity to a narrow band of frequencies around the frequency component (Neuweiler 2000) called the acoustic fovea. Bats show inter-individual variation in the frequency tuning of their acoustic foveas (Schnitzler, Suga, and Simmons 1976). Bats compensate for flight-induced Doppler shifts to maintain the frequency of the CF component’s returning echo within the acoustic foveal range (Schnitzler 1973; Schoeppler, Schnitzler, and Denzinger 2018). Previous investigations (Jones, Sripathi, and Waters 1994; Jones et al. 1993; Furusawa et al. 2012) of group echolocation in CF-FM bats centred around whether CF-FM bat showed ‘jamming avoidance’ type responses (sensu Ulanovsky et al. 2004), where individual bats may shift the spectral ranges of their calls to reduce overlap. Previous studies have not found support (Jones, Sripathi, and Waters 1994; Jones et al. 1993; Fawcett et al. 2015) for changes in CF frequencies. While the CF component is involved in prey detection, the FM components are thought to be involved in distance ranging (Tian and Schnitzler 1997; Neuweiler et al. 1987). The role of the iFM is relatively ambiguous, while the tFM has been strongly linked with distance ranging. CF-FM bats show rapid alterations in tFM bandwidth and duration based on the behavioural context at hand, eg. resting, landing or prey capture (Neuweiler et al. 1987; Schoeppler, Schnitzler, and Denzinger 2018; Tian and Schnitzler 1997). Due to the historical focus on the CF call component, alterations in the FM components in group flight have not been quantified in most studies. There has been only one study (Fawcett et al. 2015) to our knowledge that has quantified FM components, and found an increased tFM duration and bandwidth in the presence of conspecifics. Given the tFM’s malleability and role in ranging , there is a strong need for its explicit quantification in multi-bat contexts. The tFM may show the same kinds of changes in multi-bat contexts as shown in low-duty cycle bats, which also use their FM calls primarily for ranging (Fawcett et al. 2015; Amichai, Blumrosen, and Yovel 2015; Hase et al. 2018).

A majority of studies on group echolocation have so far investigated low-duty cycle bats (Lin, Abaid, and Müller 2016; Fawcett and Ratcliffe 2015; Götze et al. 2016), likely due to their speciosity (most bats except the ~160/1428 species are high-duty cycle species (Fenton, Faure, and Ratcliffe 2012; Database 2020)) and ease of call analysis. Studying group echolocation in high-duty cycle bats entails analysing audio with overlapping calls. The analysis of recordings with overlapping calls is a nascent field (but see Izadi, Stevenson, and Kloepper 2019), and call parameter estimation has not been attempted before to the best of our knowledge. Even studies with multiple high-duty cycle bats have been limited to 2-3 bats in flightroom conditions [Fawcett et al. (2015);Jones, Sripathi, and Waters (1994);Jones et al. (1993);]. Flightrooms provide controlled conditions that can allow direct interpretation of results. However the echolocation of animals is also affected by familiarity (Merten and Siemers 2020; Chen et al. 2015; Yamada et al. 2020), and properties of the experimental room (Surlykke, Boel Pedersen, and Jakobsen 2009). Studying multi-bat echolocation in the wild provides a direct glimpse into the acoustic challenge animals face in a familiar environment and how they respond to changes in group size.

In our study we fill the gaps by 1) shedding light on the group echolocation in the understudied high-duty cycle bats 2) presenting a methodology to quantify echolocation parameters in the presence of overlapping calls and 3) studying group echolocation in the field.

## 2.0 Methods

### 2.1 Study species and site

Two species of rhinolophid bats *Rhinolophus mehelyi* and *R. euryale* were recorded in their natural environment. Both study species emit CF-FM calls (sensu Tian and Schnitzler 1997) with peak frequencies between 102-112 kHz, and are not acoustically distinguishable due to the overlap in their call characteristics (Dietz and Kiefer 2016). While phylogenetically different species, for the purposes of this study we treat them as a single group of bats that may face the problem of acoustic jamming due to the similarity in spectro-temporal call structure.

We used an audio-video recording setup positioned in a dome-shaped cave (Figure 1) next to the main entrance of the Orlova Chuka cave system, Bulgaria. The cave was 5x3x1.6m (lxbxh) in dimensions. The cave had only one opening with roosting sites on the inside where most bats flew in and out of through the night.

### 2.2 Experimental setup

The experimental setup consisted of an audio-video recording system made of three microphones and two infrared cameras. The audio and video feeds were synchronised using the low-cost method described in Laurijssen et al. 2018. ON-OFF signals between 0.08-0.5 s were generated by a portable computer (Raspberry Pi 3). The ON-ON-OFF voltage signal was directly fed into one of the channels of the soundcard. The same voltage signal was also used to drive the blinking of an LED that was recorded by the two cameras. (See Supplementary Information 1.0 for signal generation script, electronic circuit and associated notes).

For the video recording, two consumer grade CCTV cameras with infrared lamps were connected to a digital video recorder to record the flight of bats as they flew in and out of the cave. The system recorded video mostly at 22 Hz, however there was frame rate variation between 18-27 Hz. The video stream of the two cameras was not frame-synchronised, however, we were still able to view the same bat flight on both cameras due to the common timestamps burnt-in on each frame. The two cameras were placed in approximately the same position on every recording night. The cameras were so placed to maximise the total cave volume recorded while also capturing the blinking LED light. Only sections of the video that had stable camera positions were analysed. Video was recorded continuously through the night and resulted in two video files (one per camera) for each recorded hour.

Three Avisoft CM16 microphones (Avisoft Bioacoustics, Germany) were connected to an Avisoft 416H (Avisoft Bioacoustics, Germany) soundcard running at 250kHz sampling rate. Horseshoe bat calls are known to be directional (Matsuta et al. 2013), and the three microphones were placed at different positions in the cave to increase the number of on-axis calls captured. Microphones were placed in the same location with an estimated +/- 10cm error in the cave across multiple nights. The fourth channel of the Avisoft 416H was used to record the audio sync signal. Audio was recorded continuously through the night in the form of consecutive multichannel files of 1 minute length. Our audio recordings showed that the dome was visited by multiple species of bats aside from our two main study species, and we only analysed audio data with *R. euryale* and/or *R. mehelyi* calls in it.

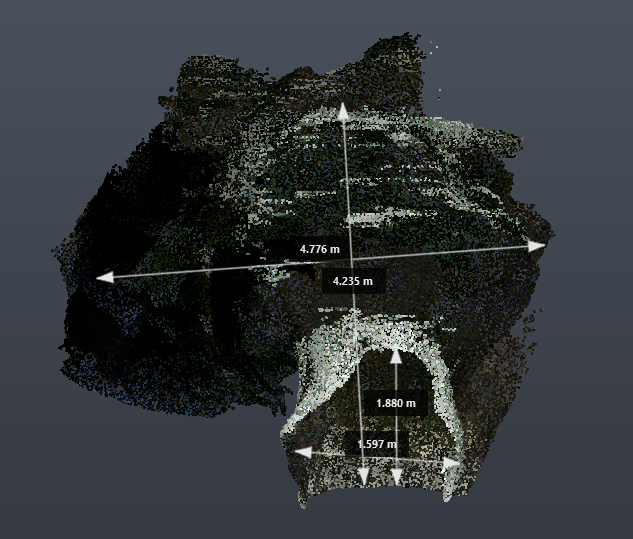


Figure 1: Schematic of how the three microphones and two cameras were placed in the recording volume/Klaus will send a more processed version OR Neetash to make a better one? 3D scanning by Klaus Hochradel, UMIT Tirol

## 3. Data analysis

### 3.1 Video analysis to determine group sizes

Bat activity in the cave was recorded for a total of about 12 hours across four nights in the cave. Bats flew in and out of the cave often showing different flight behaviors such as circling, approaching and following. Sometimes however, bats spent time (few seconds to several minutes) in the roosting sites within the cave and then only exited. The start and end of all bat flight bouts in the video was manually annotated following a series of pre-defined criteria to ensure repeatability (SI 2.0). Each flight bout annotation recorded the number of flying bats as seen in the two camera views.

### 3.2 Matching video annotation to obtain synchronised audio

For each video annotation of a bat flight bout, we attempted to match the corresponding region of recorded audio. For each successfully matched video annotation, we created a separate audio file. Audio matching was done by cross-correlating the blinking ON/OFF LED signal over the video annotation with the recorded ON/OFF voltage signal in the audio. We managed to successfully find audio matches for 1181 video annotations, ( i.e., 55% of all video annotations). The low match rate is primarily due to the fluctuating camera frame rates, and because many of the matched audio files had non-target bat species calls. Multiple species were seen in the audio files as during video annotation it was not possible to distinguish bat species by their flight behaviour. The other observed bat species were from *R. ferrumequinum*, vespertilionid and miniopterid FM bats, all of which are known to occur in the Orlova Chuka cave system (Ivanova 2005). For the acoustic analysis we chose matched audio files that only had *R. euryale* and/or *R. meheyli* calls. The audio files varied in duration between 0.08-62.08 seconds (, median duration: 1.04s , 95%ile range: 0.5-8.54s).

## 4.0 Acoustic parameter analysis

All matched audio files were first forward-backward high-pass filtered at 70 kHz. For the analysis we used recordings from the first microphone, as it appeared to have consistently captured calls with the least reverberance of the three channels. The first microphone was located facing the cave opening, perhaps therefore capturing calls of both entering and exiting bats well.

We quantified frequency, duration and amplitude across different parts of the echolocation call (iFM,CF and tFM) using two complementary acoustic analyses. The first analysis is the ‘individual call’ analysis, where we measured parameters of one horseshoe bat call from each matched audio. The second analysis is the ‘window’ analysis.Each matched audio was split into 50ms windows. We then measured the acoustic parameters from all windows of a matched audio file. In recordings with multiple bats, the 50 ms windows could have overlapping calls.

The advantage of the individual call analysis is that the measurements made on the calls are directly interpretable as call component alterations reveal the sensory decisions of the bats. On the other hand, the disadvantage of the individual call analysis is that especially in multi-bat contexts, it can be difficult to find a non-overlapped call. The window analysis complements individual call analysis by enabling measurements even on audio with overlapping calls. Window analysis also allows a kind of null-hypothesis testing where the observed multi-bat audio can be compared with 1) single bat audio and 2) ‘virtual’ multi-bat audio files created by adding multiple single bat audio files. These ‘virtual’ multi-bat audio files recreate a scenario where two bats echolocate in the same volume without actively responding to each other’s presence. The disadvantage with window analysis is the lack of call-level measurements. Ultimately, using the two approaches simultaneously strengthens the interpretation of results.

### 4.1 Individual call measurements

Individual calls which were not overlapped by other calls and with sufficient signal-to-noise ratio (Figure 2) were manually chosen through a random search protocol (SI 3.0). Briefly, an experimenter began searching from a randomly chosen time point in an audio file for a non-overlapped horseshoe bat call. From this point in the file, the experimenter randomly began searching to the left or right until a suitable horseshoebat call could be identified. The start and end time of this call in the audio file were noted.

We were able to find a total of 226 individual calls (: 177, : 49) across all the synchronised audio files. Calls were automatically segmented into their corresponding parts iFM, tFM or CF (Tian and Schnitzler 1997) (Figure 2) using the *itsfm* package [Beleyur (2020);*biorxivpaper*]. Most approaches to date focus on indirectly segmenting CF-FM calls into their components by high/low pass filtering around the peak frequency of the call (Siemers et al. 2005; Schuchmann, Puechmaille, and Siemers 2012; Tian and Schnitzler 1997; Lu, Zhang, and Luo 2020; Schoeppler, Schnitzler, and Denzinger 2018). For an accurate estimate of the peak frequency, this approach requires an on-axis recording of the call with a prominent CF component. While suitable for laboratory studies, filtering around the peak frequency fails in the analysis of CF-FM calls recorded in the field under a variety of conditions eg. calls with loud FM and faint CF components. *itsfm* overcomes these limitations by tracking the *change* in frequency over the call to segment it into FM and CF components.

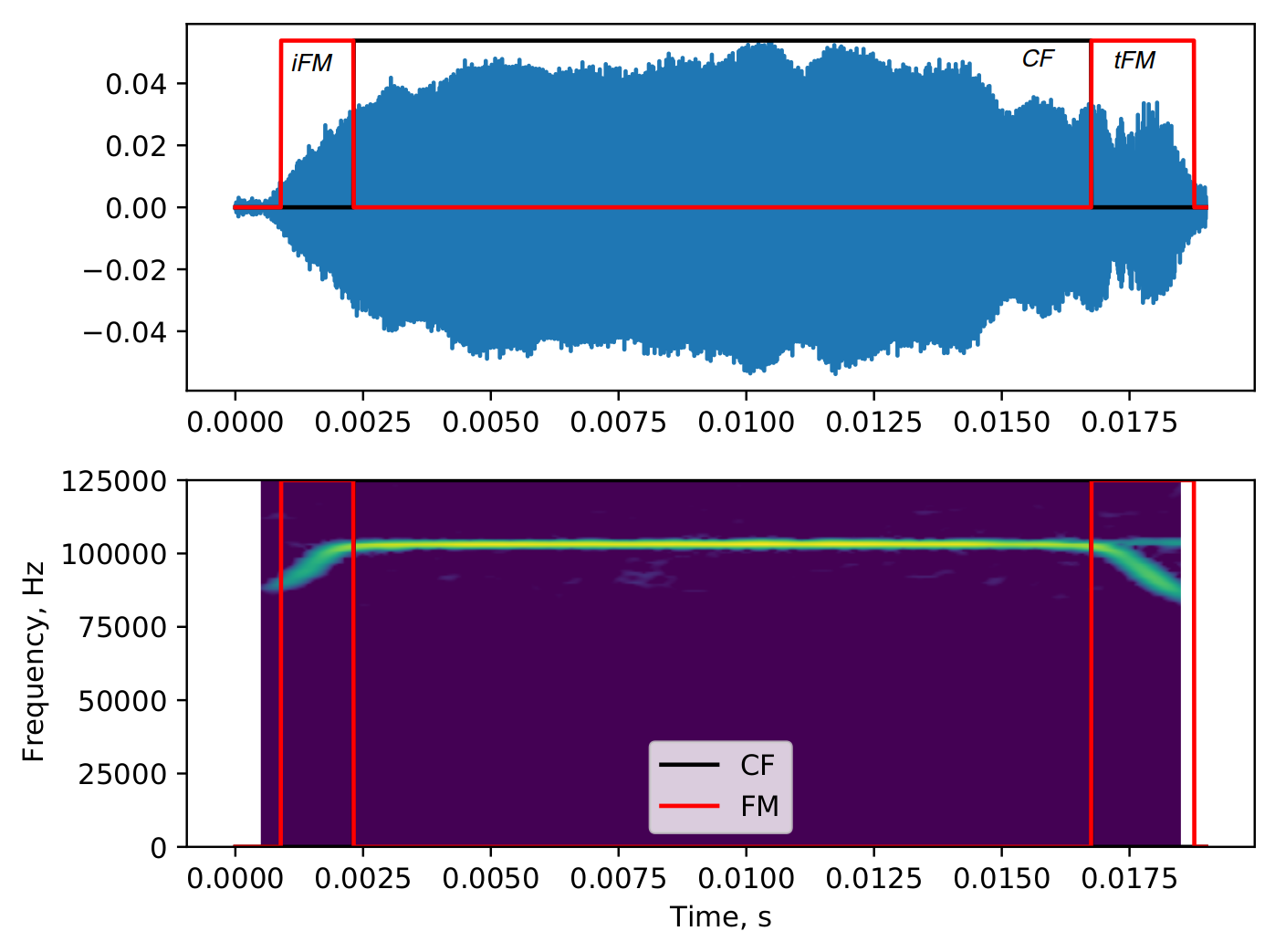


Figure 2: Example of a single CF-FM call with automatically segmented iFM, CF and tFM regions. The raw data is a manually selected audio segment, which is then automatically segmented by the *itsfm* package based on frequency modulation across the call. The *itsfm* package allows accurate segmentation into call parts under challenging recording conditions

From the segmented CF and FM components we measured the following parameters. In the CF component, we measured the peak frequency, RMS amplitude and duration. The CF peak frequency was quantified as bats may shift their CF frequencies in the presence of conspecifics. ‘Jamming avoidance’ type reponses have been a parameter of investigation in previous studies (Habersetzer 1981; Jones, Sripathi, and Waters 1994; Jones et al. 1993; Fawcett et al. 2015; Lu, Zhang, and Luo 2020). From the FM components, we measured the lower frequency (-10 dB peak frequency of the FM audio segment), bandwidth (defined as difference between the CF peak frequency and the lower frequency of the FM segment), RMS amplitude and duration. The bandwidth is important for target ranging in CF-FM bats. An increase in bandwidth in the presence of conspecifics has been shown in at least one previous study (Fawcett et al. 2015). We also calculated the relative i/tFM-CF amplitude ratios (in dB). The relative call component measures were calculated as CF-FM bats are known to independently vary the level of call components in a context specific manner (Tian and Schnitzler 1997; Lu, Zhang, and Luo 2020).

### 4.2 Window measurements

Each audio file was split into consecutive windows of 50ms (SI 4.0). The time window of 50ms was chosen as it provided high spectral resolution (20Hz for 12500 samples at 250kHz sampling rate) to distinguish multiple CF components that may be in it. Often in the multi bat contexts, the 50 ms windows consisted of one to many horseshoe bat calls. Initial observations showed that 50 ms was about the longest observed duration of a bat call in our data, and was also about twice the length of typical calls. This allowed a 50 ms window to contain one long call or multiple short calls.

Over the course of an audio file, there may be multiple windows without calls or very faint calls in them. These ‘silent’ windows were removed from the analysis, and only the ‘non-silent’ windows with bat calls in them were analysed. Non-silent windows were defined as those that were 20 dB RMS more than manually annotated silent audio segments (SI 4.1). On each non-silent window, the following acoustic measurements were performed: 1) received level (dB RMS), 2) dominant frequencies and 3) FM terminal frequencies. Dominant frequencies here are defined as frequency peaks in the smoothed power spectrum that are within 14 dB of the window’s peak frequency. Dominant frequencies are extracted from the various peaks of the power spectrum and are thus a proxy measurement for the CF component frequencies of calls in a window (SI 4.2). FM terminal frequencies were determined by a spectrogram based method which identified FM regions and chose the lowest frequency in each FM region of a given audio window (SI 4.3). There may be multiple terminal frequencies and dominant frequencies values for a single window, however only one received level measurement per window. All measurements chosen in the whole audio analysis were done in an attempt to be analogous to the measurements performed in the individual call analyses. For instance, the dominant frequencies in the whole audio analysis complements the CF peak frequency measuremeents in the individual call analysis. The FM terminal frequencies and RMS measurements are again analogous to the bandwidth and RMS of the individual call analysis.

### 4.3 Virtual multi-bat audio

The advantage of the window analysis is the possibility to make ‘virtual multi-bat’ data (Fawcett et al. 2015; Ratcliffe et al. 2004) by combining observed single bat call sequences. We created virtual multi-bat audio files by combining single bat files that were of similar file lengths (SI 4.4). This allowed us to create a ‘null’ dataset where multiple bats were echolocating, but not responding to each other’s presence. The virtual multi-bat audio was handled using the window analysis as described above.

## 5.0 Statistical analysis

We observed upto 1-4 bats flying in the cave at the same time. However, the sample sizes in group sizes $\geq$2 bats were low (SI 5.0), and we thus decided to combine all annotations with $\geq$2 into a ‘multi’ bat class. We thus performed comparisons of ‘single’ and ‘multi’ bat calls in the individual call analysis. To maintain consistency with individual call analysis we also performed comparisons of ‘single’,‘multi’ and ‘virtual-multi’ windows.

### 5.1 Individual call measurements

We calculated the median difference () between multi and single bat call parameters for all parameters except CF peak frequency. For CF peak frequencies, we calculated the range (max-min) of CF peak frequencies in single and multi bat data. The range was calculated as ‘jamming avoidance’ type responses imply an alteration in the frequencies. Bats may decide to shift their call frequencies, leading to an increased range, or as has been experimentally observed, paradoxically converge (Furusawa et al. 2012). Permutation tests were performed to assess the significance of the observed difference between the groups.

Our dataset has calls from a group of resident wild bats that may have visited the cave site multiple times over the course of a night. Moreover, bat activity over the course of a single night was relatively clustered in time, and flight annotations were thus fairly close to each other at <= 1 minute (median inter-annotation times was 36 s for annotations used in individual calls and 54 s for annotations used in window analysis). Due to the clustered nature of activity, our dataset may have had a disproportionate representation of a few individuals, leading to pseudo replication, and thus a lowering of variation in the data.

To account for the temporal pseudo-replication in our data, we repeated the median/range difference analysis by creating two subsets from our full dataset. We created the ‘clustered’ subset, where all calls from annotations that were less than or equal to 1 min from each other were taken together. The ‘isolated’ subset included all calls from annotations that were at least 1 minute away from other annotations. The ‘isolated’ and ‘clustered’ subsets had unique datapoints, with no overlap in the calls used. One minute was chosen as it was slightly larger than the observed median inter-annotation interval. Broadly speaking, we expect that if the results of our analysis are comparable across the isolated and clustered subsets, there is a common underlying effect that is independent of temporal clustering. However, if the results of the subset analysis do not corroborate each other, it hints at an effect due to temporal clustering/isolation in the dataset.

### 5.2 Window measurements

In analogy to CF peak frequency range in the individual call analysis, we calculated the dominant frequency range () across an audio file. Even in single bat audio files, we expect variation in recorded dominant frequency due to the combined effect of the bat’s active Doppler shift compensation and Doppler shift due to its flight past the microphone. Multi-bat and virtual-multi bat audio files are expected to have an increased range due to presence of multiple bats. We performed a comparison of the median dominant frequency range difference between multi-single and multi-virtual multi audio. A permutation test was performed to assess the significance of the observed difference.

Unlike dominant frequency range, there are multiple, potentially correlated received level and terminal frequency measurements from a single file. We accounted for this potential file-level pseudo-replication by resorting to repeated random subsampling and median difference calculation. To estimate the median difference across groups we randomly chose one measurement value per file for the single, multi and virtual-multi bat groups. The median difference between multi-single and multi-virtual multi group was calculated and followed by the next subsampling round. We performed 10,000 such subsampling iterations, and report the 95 percentile range of median difference for received level and terminal frequency. No tests were run on the median difference estimates obtained for received level and terminal frequency.

To account for temporal pseudo-replication in our study, we also repeated the entire window analysis using clustered and isolated subsets as described in section 5.1. A threshold of 1 minute was used to create the clustered and isolated subsets.

### Software packages used in this paper

This paper was enabled by a series of open-source packages. Signal analysis, data manipulation and visualisation were done in Python (Van Rossum and Drake Jr 1995) through its scientific ecosystem: the scipy, numpy, matplotlib, soundfile and pandas packages (Virtanen et al. 2020; Oliphant 2006; Hunter 2007; Bechtold and Geier 2019; McKinney and others 2010). Median difference and confidence interval estimation was performed with the dabest (Ho et al. 2019) and scikits-bootstrap packages (Evans 2019). Reproducible analysis, documentation and presentation were enabled by the Jupyter Notebook and rmarkdown projects(Kluyver et al. 2016; Xie, Allaire, and Grolemund 2018). Audio visualisation, preliminary measurements and single call annotations were done with Audacity (Audacity-Team 2019).

## 6.0 Results

### 6.1 Individual call analysis

The raw data of all acoustic parameters measured are shown in Figure 3.

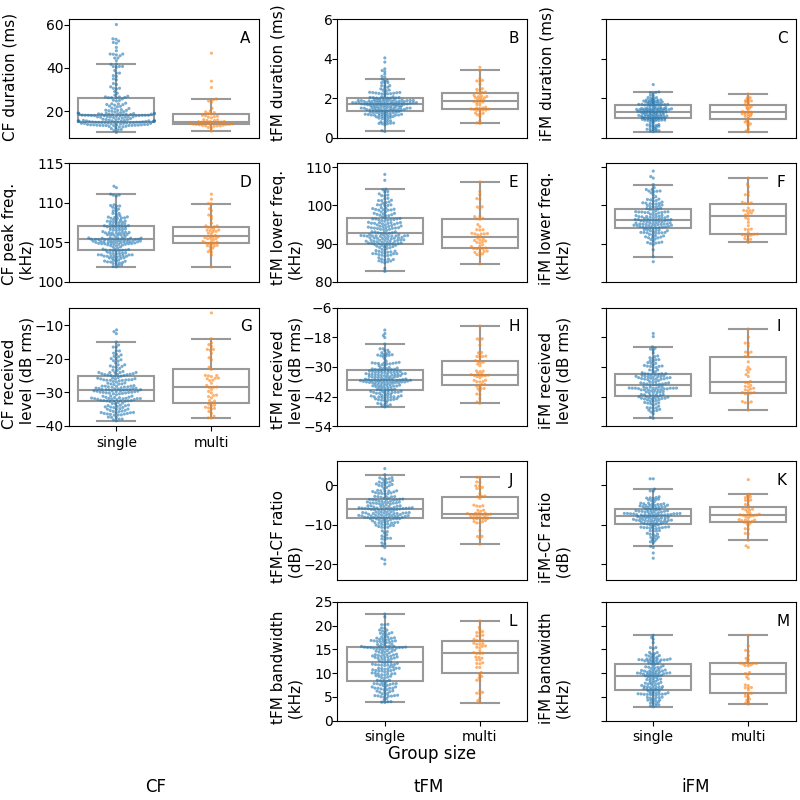


Figure 3: Measured acoustic parameters for the CF, iFM and tFM components of individual calls emitted under single and multi bat conditions. Each column shows the measurements on a call component, while each row shows a group of related measurements A-C) duration D-F) spectral measurements G-I) received level J-K) relative FM-CF ratios L-M) FM component bandwidths

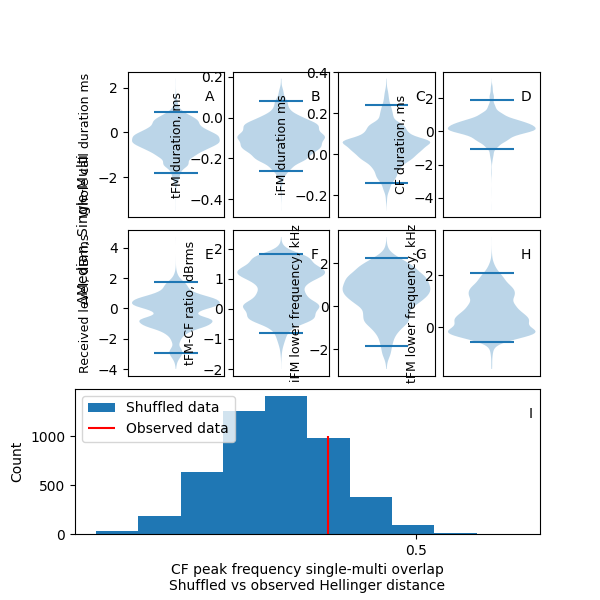


Figure 4: Comparison of various call parameters between single and multi bat context calls. A-H: The bootstrapped median difference of call parameters in calls recorded in single and multi bat contexts. The 95 percentile interval of the bootstrapped median for all variables contains 0, supporting the conclusion that call parameters from both groups belong to the same distribution. I: The observed Hellinger distance for CF peak frequency between single and multi bat contexts, in comparison to the distances generated from shuffled datasets. The observed Hellinger distance is placed at 93.3 percentile of the shuffled distribution, and is thus within the 95 percentile range of the shuffled data. This supports the possibility that the observed Hellinger distance is within the range of what would be expected if single and multi bat calls were from the same distribution

All measured call parameters (Figure 3) appear to be very similar across single and multi bat contexts. The 95 percentile range of the bootstrapped difference of medians for all measured parameters (Figure 4 A-H) include zero, which strongly suggests that the call parameters originate from one distribution. The observed Hellinger distance between single and multi bat calls is positioned in the 93rd of the shuffled Hellinger distances (Figure 4 I). The observed overlap’s location is within the 95 percentile range (2.5-97.5), indicating that the observed overlap is indistinguishable from a scenario where there is no change in CF peak distribution across single and multi bat contexts.

### Whole audio analysis

All measured whole audio parameters (received level, dominant frequencies, terminal frequencies) for both single-multi and multi-virtual multi bootstrapped median difference (Figure 5) included zero in the 95 percentile interval. The bootstrapped median difference 95 percentile intervals for all comparisons and parameters contained zero in them. The inclusion of zero in the 95 percentile hints

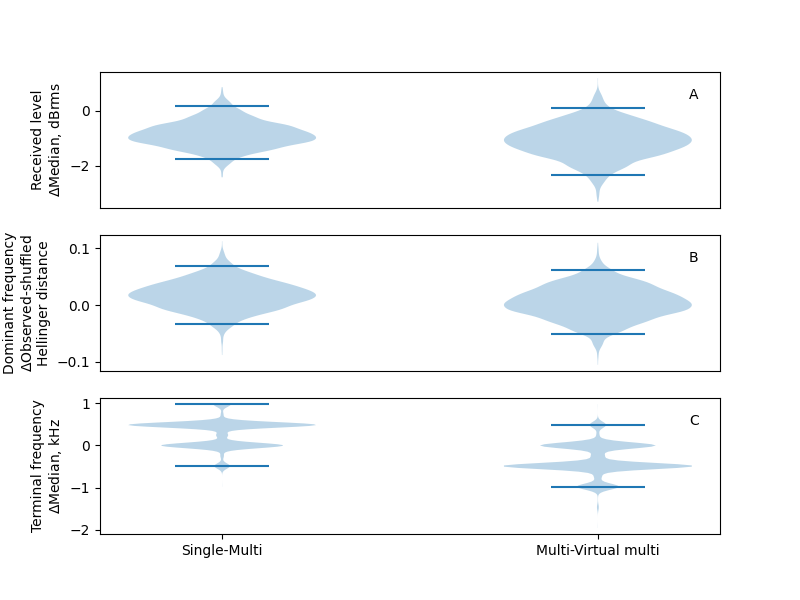


Figure 5: Comparisons of 50ms audio windows across audio with single and multi bat flights.

## Discussion

We quantified the difference in horseshoe bat echolocation calls when alone and with a conspecific under field conditions. Our results suggest no difference in echolocation calls with reference to group size for all of the call parameters measured using two different approaches. This may seem somewhat unexpected, especially considering the fact that bats in our field site were flying in an enclosed reverberant volume - which would only amplify the problem of masking in multi-bat echolocation. We interpret our results below in more detail.

The idea that bats may alter their call frequencies to reduce spectral overlap in groups has been investigated with mixed results over many studies (Jones et al. 1993; Jones, Sripathi, and Waters 1994; Ulanovsky et al. 2004). Specifically in CF-FM bats (Jones et al. 1993; Jones, Sripathi, and Waters 1994; Fawcett and Ratcliffe 2015; Pye 1972) the results do not support the idea that bats may alter their CF frequencies. (Habersetzer 1981) seems to suggest CF frequency shifting in a quasi-CF bat, *Rhinopoma hardwickei* (but see Cvikel et al. (2015)). However, the echolocation of *R. hardwickei* (Simmons, Kick, and Lawrence 1984) is not entirely comparable with those of the hipposiderids and rhinolophids in previous studies. Hipposiderids and rhinolophids are more constrained in their echolocation as they show a marked acoustic fovea that does not vary over short periods of time[REFS]. CF-FM bats are thus constrained to emit calls so that the Doppler-shifted echoes arrive within the acoustic fovea. Our data (Figures 4*I*, 5*B*) also supports previous studies showing an absence of CF frequency shifting in the presence of conspecifics. Our CF peak frequency and dominant frequency measurements are estimated to be Doppler shifted away from the emission frequency by 200-600 Hz (Supplementary Information). However, if bats were showing a systematic alteration of their CF components we would have been able to detect it with our distribution similarity analyses.

Bats are known to increase their call levels in the presence of experimental playbacks [Hage et al. (2013);Hage et al. (2014);Lu, Zhang, and Luo (2020);]. In our study, we did not have access to the source level of the calls as we did not track the bats’ position in 3D. We only had access to the *received* level at the microphone, which is modulated by 1) the bat-microphone distance, and 2) source level of the call. We found no major difference ($\pm$2dB, Figures 4, 5) between the received levels of individual calls or audio segments compared between single and multi bat contexts. Why was there no major difference in received levels even in the audio-segment analysis, despite that overlapping sounds are expected to have a higher received level? The similarity in received levels of audio-segments can be explained by the dominance of the call from the nearest bat to the microphone. Due to the spherical spreading of sound, the received level at the microphone primarily reflects that of the bat calling closest to the microphone. Thus, even when there are multiple bats - the nearest bats’ call dominates the received level measurements. This nearest-bat dominance means we effectively measure the received level of one bat’s call even when the audio has overlapping calls. Single and multi bat audio-segments having similar received levels thus indirectly suggests there is no change in source level even in the presence of another bat.

We introduce two automated analyses that can be performed on audio recordings of multiple CF-FM bats. Automated individual call analyses using the open-source itsfm package allows call component segmentation in a sound agnostic manner according to the actual rate of frequency modulation. The frequency-modulation based segmentation method in our experience is also more robust to field recordings with fainter CF components, and thus performs better than approaches used in previous studies. To analyse recordings with overlapping calls in them we resorted to measuring broad properties of audio segments without assigning the measurements to individual calls. While coarser than the individual call analysis, the audio-segment based approach still provides important supporting evidence to the individual call analyses.

There are a set of parameters that we were not able to measure and thus excluded in our analyses. We did not measure call-sequence related parameters such as inter-call-intervals or duty-cycle. Bats in acoustically difficult situations are known to alter their call rate [Amichai, Blumrosen, and Yovel (2015);Jarvis, Jackson, and Smotherman (2013);], and thus their duty cycle. Measuring inter-call-intervals is possible in single bat contexts, but extremely challenging in multi-bat recordings with overlaps and reverberation. The study of call-sequence related measures in multi-bat contexts is hindered by the difficulty in call identity assignment. In our individual call analyses we specifically avoided audio recordings with only overlapping calls, and this represents an unavoidable observational bias in our dataset. The use of a full microphone array with at least four microphones to assign calls to the emitting bats is the next step in the investigation of group echolocation in CF-FM bats. With the acoustically localised positions of each bat in the group, estimates of flight speed, and thus Doppler shift could also be directly made from the audio recordings.

What are the possible explanations for the absence of a response? The main explanation we posit is that situations with 2-3 bats may not be very challenging echolocation-wise for multiple reasons. CF-FM bats rely on the tFM component to detect the distance of objects around them (Tian and Schnitzler 1997). The tFM components were short (3.4ms, 95 percentile value), and likely emitted every 40-50 ms (including call duration and inter-pulse interval), which is equivalent to a tFM duty cycle between 6.3-7.8%. At these duty cycles, the probability of one echo being overlapped by a conspecific call is relatively low and between 0.4-0.6%. Even if a single echo is overlapped, the bat may still be able to detect it if the signal-to-noise ratio is sufficient. Bats in small groups are unlikely to face major detriments to their echolocation (Beleyur and Goerlitz 2019), and thus not show specific alterations from solitary echolocation. Secondly, Fawcett et al. 2015 observed an increased tFM duration and bandwidth in *R. capensis* flying in pairs in a flight room. This result highlighs the importance of the tFM component in CF-FM echolocation. Their experiments were done in a novel flight room setting. The combination of flight room characteristics (**???**) and species differences, may perhaps have led to the responses seen in this studies. Bats show long-term spatial memory (Barchi, Knowles, and Simmons 2013) and familiarity with the cave’s structure may have allowed them to easily recognise their location over time. Bats also use echoes across multiple calls and are thus resistant to occasional disruptions in echo arrival (Salles, Diebold, and Moss 2020). The combination of spatial memory and multi-echo integration may have allowed our bats to continue echolocating with conspecifics without altering their calls drastically.

Previous manipulative studies [Hage et al. (2013);Hage et al. (2014);Lu, Zhang, and Luo (2020);] have revealed the exquisite control bats have over each call component. However, these call alterations are typically elicited in response to artifical stimuli with little resemblance to their natural auditory scenes. Our results suggest that bats may not regularly be employing strategies observed from playback and flightroom studies to enhance their echo detection while flying with conspecifics. We highlight the importance of observational studies in field settings to understand the frequency with which sensory strategies are actually employed in ecological contexts.

## Data and code availability

All data and code used process data and generate the results and figures in the paper are available in the Zenodo repository [INSERT DOI HERE].

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## Author Contributions

Study design and conception: TB,NMR Data collection: NMR, TB, AK, Audio and video annotation: AK,NM Audio-video synchronisation: TB Analysis: TB,NMR,HRG Interpretation of results: NMR, TB, HRG Manuscript preparation: NMR, TB, HRG. All authors read and approved the final draft of this paper

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