

**Examining the behavioural and acoustic adaptations of free-flying horseshoe bats  
in response to jamming.**

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*A dissertation submitted for the partial fulfilment of  
BS-MS dual degree in Science*



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## **Certificate of Examination**

This is to certify that the dissertation titled “Examining the behavioural and acoustic adaptations of free-flying horseshoe bats in response to jamming” submitted by Mr. Aditya Krishna (Reg. No. MS15055) for the partial fulfilment of BS-MS dual degree program of the Institute, has been examined by the thesis committee duly appointed by the Institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

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## **Declaration**

The work presented in this dissertation has been carried out by me under the guidance of Dr. Manjari Jain at the Institute of Science Education and Research Mohali and Dr Holger R. Goerlitz at the Max Planck Institute for Ornithology, Seewiesen.

This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any other university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgement of collaborative research and discussions. This thesis is a bonafide record of original work done by me and all sources listed within have been detailed in the bibliography.

Aditya Krishna

Dated: April 24, 2020

In my capacity as the supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge.

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## Abstract

Echolocating bats are often found to fly in dense groups of conspecifics or heterospecifics and the multiple sounds produced create a complex auditory scene where an individual bat receives not only the echoes of their calls but also echoes from the calls of other bats. However, while several mechanisms have been proposed on how bats producing frequency modulated calls deal with this perceptual problem, studies in horseshoe bats who produce calls of long duration and constant frequency have yielded contrasting results. Here we investigated the effect of group size on the echolocation call parameters in mixed-species groups of *Rhinolophus Euryale* and *Rhinolophus mehelyi*. We quantified how often bats experience masking by conspecifics and heterospecifics while flying in their natural habitat. We found that although bats fly alone most of the time, they may still experience jamming significantly. We also investigated the variation in flight parameters with group size as bats navigated in the constrained environment. Passing and trailing are the most common behaviours exhibited during multiple- bat situations. Preliminary analysis indicates that they do not change their call parameters as they fly in groups. The analysis is limited by the inability to extract reliable call parameters. An improved parameter extraction workflow has been built, which will enable us to conclude whether there is variation in call parameters while flying in groups.



## **Chapter 1: Introduction**

### **1.1 Echolocation in bats**

Animals with active sensory systems utilize self-generated signals to sense their surroundings and navigate in environments where light levels are low or absent (Nelson and MacIver, 2006). Echolocation or biosonar is an active sensory system in which objects in the environment are detected by comparing the nature of the emitted calls with the returning echoes (Griffin et al., 1960). Bats employ echolocation not only for navigation but also for detecting and capturing insect prey (Schnitzler and Kalko, 2001).

Bats (Order Chiroptera) represent the second most species-rich order of mammals and are among the most successful species on earth (Tudge, 2000). They are also among the most diverse order with diets ranging from pollen, nectar, flowers to insects, crustaceans, birds, reptiles, fishes, small mammals as well the blood of bats and other mammals (Kunz and Pierson 1994). They are unique in having evolved flight and navigation in complete darkness utilizing sophisticated laryngeal echolocation (Simmons et al, 2008). These characteristics enabled them to radiate to a new niche, previously inaccessible to animals, the night sky, with about 70% of the bat species feeding on insects in all inhabitable locations of the world (Jones and Rydell, 2003).

### **1.2 Diversity in call structure**

The echolocation calls vary considerably in their structure between bat species. A particular clade of bats, most of whom belonging to the family Pteropodidae (the Old World bats) either do not echolocate (Jones and Teeling, 2006) or exhibit a form of echolocation involving either the wing membrane or the tongue (Boonman et al, 2014). Laryngeal echolocating bats can be broadly categorized into two groups based on the structure of their calls (reviewed in Schnitzler and Kalko, 2001). Some species produce short frequency modulated (FM) signals of large bandwidth, generally sweeping through an octave. These species are called FM bats. Their calls last for several milliseconds and are generally emitted 2-12 times while searching for prey. These bats adaptively change call parameters such as interpulse intervals (IPI), frequency bandwidth, duration, and intensity depending on the nature of the environment as well as the

distance to the object. When a bat detects an object of interest, it reduces the IPI and the call duration stereotypically, culminating with a call repetition rate of 100-200 times per second. The echolocation calls while capturing airborne prey are categorized into three distinct phases: search, approach, and terminal buzz (Figure 1.1) (Ratcliffe et al, 2013). Bats determine the distance of an object by the time elapsed between the emitted signal and the returning echoes. Since broadband signals activate the underlying neuronal filters only for a short instant, they help in the precise determination of range and are thus well adapted for target localization.

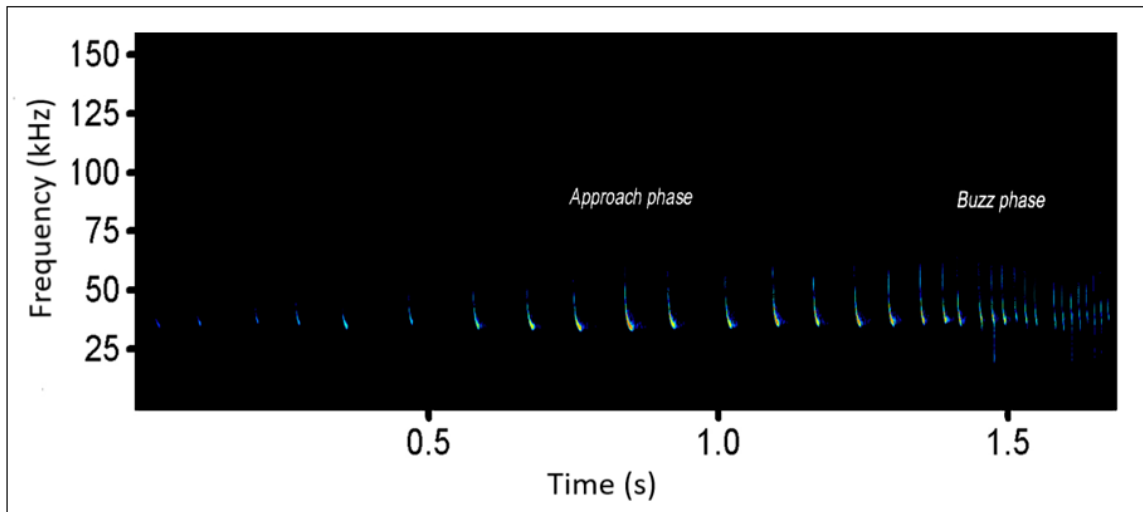


Figure 1.1:  
Echolocation sequence of a free flying Pipistrelle species : During search phase, bats emit calls with shallower sweeps (Not shown in the figure). It moves into approach phase as it tracks an insect and emits steeper calls at shorter inter pulse intervals. The terminal buzz phase is characterised by very small interpulse intervals and occurs right before the bat captures the insect.  
(Calls were recorded using Petterson D1000X at IISER Mohali campus)

Some species of bats produce long narrowband signals (up to 100ms) with a high duty cycle. Their calls consist of a long constant frequency (CF) component either sandwiched between an upward and downward FM component or followed by a downward FM component. These species are called the CF-FM bats. A major section of their inner ear, as well as the underlying neurons, are tuned maximally to a narrow bandwidth of frequencies present in the call of the bat. This is referred to as acoustic fovea and is a result of a disproportionate number of cochlear receptors sensitive to a narrow range of frequencies (Neuweiler, 2003). Narrowband signals are well adapted for the detection of echoes since the neuronal filters tuned to the corresponding frequency band are activated for a long time. CF-FM bats also adjust for the

Doppler shifts, caused as a result of their flight by lowering the emission call frequency, while approaching a target. This ensures that the returning echoes always arrive at the narrow frequency range of the acoustic fovea and are thus not masked by the outgoing calls. (Schnitzler, H.U, 1968, Hiryu et al, 2008).

### **1.3 Diversity in Call frequency**

Although most bat species call in the ultrasonic range (>20 kHz) inaudible to the human ear, the dominant frequencies vary considerably from 9 kHz (*Euderma maculatum*, Vespertilionidae) to 211 kHz (*Cloeotis percivali*, Hipposideridae) (Fullard and Dawson, 1997; Fenton and Bell, 1981). Sound is generally reflected from objects of size greater than its wavelength. Hence calls of higher frequencies (smaller wavelength) will generate stronger echoes from smaller objects and are well adapted for the detection of insects and other prey (Houston et al, 2004). However, higher frequencies experience a greater degree of atmospheric attenuation and are thus not suited for target detection at large distances (Lawrence & Simmons 1982). Hence dominant frequencies between 20-60 kHz are found in most bat species (Fenton et al, 1998).

### **1.4 Echolocation Jamming**

Echolocation, similar to other forms of active sensing, is also prone to the detrimental effect of other signals on the processing of the target signal, known as echolocation or sonar jamming. Jamming can occur passively as a result of any intense sound in the environment such as the calls of the focal bat itself, ambient noise or the echolocation calls of conspecific or heterospecific bat species while foraging together. Active jamming occurs due to echolocation calls produced by heterospecific and conspecific bats to disrupt the competitor's senses or by sonar jamming moths (Corcoran et al, 2009, Corcoran and Conner, 2014)

Bat calls represent one of the loudest sounds in nature (Surlykke and Kalko, 2008). However, the returning echoes can be up to  $10^5$  times fainter than the emitted call and thus would potentially be masked if the bat is still producing calls as the echoes return. This is solved by the production of short sounds of 3-50 ms while the bat searches for the prey. As it starts approaching the prey, they start producing progressively shorter sounds, culminating with calls

of 0.5 ms during the terminal buzz phase (Thomas et al, 2004). This prevents the temporal overlap of faint echoes with the emitted calls. Another source of masking is the ambient noise in the environment including rustling of leaves, flowing of wind, water, and anthropogenic noise. In response to increasing background noise levels, bats increase the amplitude levels of their echolocation calls, a phenomenon known as the Lombard effect, to facilitate signal detection (Luo et al, 2015). In addition to exhibiting the Lombard effect, bats can also decide to forage at a different location.

The similarity in call structure with those of conspecific, as well as heterospecific species, make echolocation extremely susceptible to masking. Eavesdropping on the terminal buzzes of other bats is ubiquitous among insectivorous bats (Lewanzik et al, 2019). Hence, foraging at a different location will not deter masking by conspecific or heterospecific species, since the production of terminal buzzes can attract additional individuals. This is further aggravated by bats foraging together in conspecific and heterospecific groups. (Cvikel et al, 2015; Dechmann et al, 2009). The perceptual challenge of masking affects bats producing FM signals and CF signals differently. CF echolocation calls are extremely susceptible to masking due to their high duty cycle and long call duration. The presence of acoustic fovea constrains the echoes to return at a narrow frequency band and this is achieved by bats actively compensating for their flight induced Doppler shifts (Schintzler, 1968). However, this restricts the freedom to shift their call frequency in response to calls of conspecific bats, as the echoes might fall outside the acoustic fovea and thus the bats would potentially become insensitive to their echoes. FM bats are relatively more resistant to masking due to their low duty cycle and short call duration. Further, they are not constrained to produce calls in a narrow frequency range.

Some inherent properties of bat echolocation can aid in providing resistance to jamming. First, bats create a mental map of the environment analyzing multiple temporally patterned call sequences (Moss and Surlykke, 2001). This high redundancy indicates that masking of a few calls will not have a drastic impact on orientation and other vital tasks. Second, the high directionality of calls (Surlykke et al, 2009) combined with the reduced sensitivity of the ears to sound coming from the sides (Schnitzler and Grinnell, 1977), ensures that not all echoes or conspecific calls will cause masking. Third, the presence of time-sensitive neurons responding

only to a limited range of pulse-echo delay provides the bat with a time window in which they are most sensitive to the returning echoes. (Feng et al., 1978; O'Neill and Suga, 1979).

However, there are situations such as bats emerging from caves in dense groups, where these characteristics of echolocation will not mitigate the effects of interference. In such instances, several bat species alter their echolocation call parameters rapidly in a phenomenon referred to as Jamming Avoidance Response (Ulanovsky et al, 2004), although this was not reported in other studies (Cvikel et al, 2015)

### **1.5 Jamming Avoidance Response**

Jamming Avoidance Response (JAR) was first described in weakly electric fishes who use self-generated electric fields to sense their surroundings (electrolocation). The electrolocation accuracy is diminished in the presence of signals of similar frequencies, such as that of a conspecific. By altering the signal frequencies, weakly electric fishes maximise the difference in frequency between their signals and that of the neighbour. This enables them to overcome the detrimental effects of jamming. (von der Emde et al, 1998 & von der Emde G et al, 1999).

Traditionally, JAR has been defined as a rapid shift in the signal frequency away from the masking signals of a conspecific, to minimize signal similarity (Bullock et al, 1972). However, masking studies in echolocating bats report great variability of vocal responses based on species, context, and the environment. Jones and Conner (2018) argue that JAR in bats should include all-vocal responses aiming to facilitate signal detection in the presence of a masking sound.

The most common adaptation employed by FM bats in response to another conspecific nearby is a shift in call frequency. Big Brown bats (*Eptesicus fuscus*) have been reported to decrease their lowest frequency of the FM component and alter the timing of their calls. (Obrist 1995, Surlykke and Moss, 2000).Ulanovsky et al (2004) describe two types of responses: Symmetric and Asymmetric. Symmetric JAR occurs when both the upper and lower frequency bats would each shift their frequencies upward and downward, respectively, away from the average value to maximize the difference in call frequency. In an asymmetric JAR, only the bat calling at the

higher frequency would shift its call frequency in the upward direction. Asymmetric JAR is exhibited more often and an overall upward or downward shift in the call frequencies in response to conspecifics has been reported in multiple species (Habersetzer, 1981; Ibáñez et al., 2004; Kössl et al., 1999; Miller and Degn, 1981, Hase et al, 2018). In a controlled field experiment with playback stimuli at different frequencies, *Tadarida brasiliensis* demonstrated a rapid upward shift in their call frequency only when the playback stimulus was close to the bat's own emitted frequency (Giliam et al, 2007).

Some bats also alter their call duration while flying with a conspecific to reduce the extent of overlap between calls. Field recordings of *E.fuscus*, *Euderma maculatum*, *Laurisius borealis*, and *L.cinerus* showed that call duration was decreased with an increase with the inter-pulse interval (IPI) while flying with a conspecific (Obirst, 1995). However, a recent study by Amichai et al (2015), report that *Pipistrelle kuhli*, contrary to previous studies, increase their call duration and intensity on playback of bat echolocation calls. The authors argue that this would increase the Signal to Noise Ratio (SNR) of the returning echoes and the long calls would be easier to detect.

Chiu et al (2008) reported a surprising strategy employed by big brown bats to avoid jamming. One bat in the pair would stop vocalizing for long durations (~1 second) depending on the inter-bat distance and the direction of flight. The authors hypothesize that bats navigate by passively listening to the echoes of the other bat during this 'silent interval'. Although many bat species alter some call parameters in response to jamming signals, recent work by Cvikel et al (2015) and Gotze et al (2016) report no jamming avoidance response in *Rhinopoma microphyllum* and *Pipistrellus pipistrellus*. Theoretical studies suggest that a focal bat can detect its neighbour up to group sizes of 100 (Beleyur and Goerlitz, 2019). The variability in the vocal responses is related to the species, jamming stimulus, whether it happens in the field or lab and even the parameter measured.

*Rhinopoma microphyllum*, a bat that emits CF-like calls, alters the intensity but does not shift the frequency of their calls in the presence of a conspecific (Schmidt and Joermann, 1986). *Rhinolophus capensis* decreased the terminal frequency of the downward FM component while

flying with a conspecific or *Miniopterus natalensis*, producing FM calls (Fawcett et al, 2015). Jones et al (1993) flew *Asella tridens* individually and then with conspecifics to determine if they altered their calls in response to jamming. However, they did not significantly shift call frequencies while flying in groups and the authors propose that each bat has a personal frequency at which they are most sensitive. Echoes falling outside this frequency are ignored. This is further supported by studies on *Rhinolophus ferrumequinum nippon*. The echoes of the CF part arriving at the microphone on the bat during paired flights did not vary significantly from when flying alone. Furthermore, the mean inter-individual difference in resting frequency (CF), when arranged in ascending order, varies from 0.08 to 0.19 kHz (Furasawa et al, 2011). This small difference in frequency could be sufficient for the bats to distinguish their echoes from that of other bats.

### **1.6 Behavioural adaptations in response to jamming**

Bats emit a highly directional beam in the field (Surlykke et al, 2009). It has been thus proposed that bats can point their sonar beam away from conspecifics to prevent jamming (Simmons, 1995). While paired big brown bats (*Eptesicus fuscus*) competed for a single food item in a laboratory environment, they often remained in classical pursuit, where one bat follows the other (Chiu et al, 2010). A trailing bat can anticipate the movement of the leading bat and is also protected from the jamming signals of the other bat. The trailing bat also often pointed its beam towards the leading one. However, when they flew past each other, they pointed their beams away from one another, potentially avoiding jamming. However, a follow-up study on behavioural adaptations while paired bats fly in a cluttered environment reported significantly larger time spent in converging and diverging flight (Warnecke et al, 2015).

### **1.7 Objectives**

Bats producing FM calls have received most of the attention in interference studies. Almost all of the experiments have dealt with situations with 2 bats flying together. However, this is a much simpler situation compared with what is seen in the wild. The only exceptions are studies with artificial (non-bat) signals and playback of bat calls under laboratory conditions (Griffin et al, 1963; Bates et al, 2008; Cvikel et al, 2015). To our knowledge, there have not been studies aiming to understand how CF-FM bats respond to jamming signals, while they fly along with

more than 2 bats in the wild. Similarly, no studies have investigated changes in flight behaviour in CF-FM bats while flying in groups. In the light of this recent discussion, we aim to explore various aspects of jamming in mixed-species groups of horseshoe bats:

- a) To quantify how often horseshoe bats experience masking sounds by conspecifics and heterospecifics in their natural habitat.
- b) To examine whether bats alter their flight behaviour while flying in groups to reduce the effects of jamming.
- c) To investigate whether bats alter their acoustic call parameters in response to jamming by conspecifics and heterospecifics.

### **1.8 Model System**

To investigate these questions, echolocation calls of free-flying mixed-species groups of *Rhinolophus euryale* and *Rhinolophus mehelyi* were recorded. The two species are similar in their morphology and their distributions overlap extensively (Mitchell-Jones et al, 1999). They diverged from a common ancestor only 3 mya (Gullen et al, 2003). The peak frequencies of both species overlap with calls of *R. euryale* ranging from 102-109 kHz and those of *R. mehelyi* ranging from 104-112 kHz (Russo et al. 2001). The overlap in the peak frequencies, as well as the similarity in call structure, results in a great degree of temporal and spectral overlap of calls, making them an excellent system to study the effects of jamming.



Figure 1.3: a) Portrait of *Rhinolophus euryale*



b) Portrait of *Rhinolophus mehelyi*



## **Bibliography**

Tudge, C. (2000). *The variety of life: a survey and a celebration of all the creatures that have ever lived*. Oxford: Oxford University Press.

Kunz, T. H., & Pierson, E. D. (1994). *Bats of the world: an introduction*. pp1-46 in.

Simmons, N. B., Seymour, K. L., Habersetzer, J., & Gunnell, G. F. (2008). Primitive Early Eocene bat from Wyoming and the evolution of flight and echolocation. *Nature*, 451(7180), 818-821.

Jones, G., & Rydell, J. (2003). *Bat ecology*.

Nelson, M. E., & MacIver, M. A. (2006). Sensory acquisition in active sensing systems. *Journal of Comparative Physiology A*, 192(6), 573-586.

Griffin, D. R., Webster, F. A., & Michael, C. R. (1960). The echolocation of flying insects by bats. *Readings in the Psychology of Perception*, 21.

Schnitzler, H. U., & Kalko, E. K. (2001). Echolocation by insect-eating bats. *Bioscience*, 51(7), 557-569.

Jones, Gareth, and Emma C. Teeling. "The evolution of echolocation in bats." *Trends in Ecology & Evolution* 21, no. 3 (2006): 149-156.

Boonman, A., Bumrungsri, S., & Yovel, Y. (2014). Nonecholocating fruit bats produce biosonar clicks with their wings. *Current Biology*, 24(24), 2962-2967.

Ratcliffe, J. M., Elemans, C. P., Jakobsen, L., & Surlykke, A. (2013). How the bat got its buzz. *Biology Letters*, 9(2), 20121031.

Neuweiler, G. (2003). Evolutionary aspects of bat echolocation. *Journal of Comparative Physiology A*, 189(4), 245-256.

Schnitzler, H. U. (1968). Die ultraschall-ortungslaute der hufeisen-fledermäuse (Chiroptera-Rhinolophidae) in verschiedenen orientierungssituationen. *Zeitschrift für vergleichende Physiologie*, 57(4), 376-408.

Hiryu, S., Shiori, Y., Hosokawa, T., Riquimaroux, H., & Watanabe, Y. (2008). On-board telemetry of emitted sounds from free-flying bats: compensation for velocity and distance stabilizes echo frequency and amplitude. *Journal of Comparative Physiology A*, 194(9), 841-851.

Fullard, J., & Dawson, J. (1997). The echolocation calls of the spotted bat *Euderma maculatum* are relatively inaudible to moths. *Journal of Experimental Biology*, 200(1), 129-137.

Woodsworth, G. C., Bell, G. P., & Fenton, M. B. (1981). Observations of the echolocation, feeding behaviour, and habitat use of *Euderma maculatum* (Chiroptera: Vespertilionidae) in south central British Columbia. *Canadian Journal of Zoology*, 59(6), 1099-1102.

Houston, R. D., Boonman, A. M., & Jones, G. (2004). Do echolocation signal parameters restrict bats' choice of prey. *Echolocation in bats and dolphins*, 339, 345.

Lawrence, B. D., & Simmons, J. A. (1982). Measurements of atmospheric attenuation at ultrasonic frequencies and the significance for echolocation by bats. *The Journal of the Acoustical Society of America*, 71(3), 585-590.

Fenton, M. B., Portfors, C. V., Rautenbach, I. L., & Waterman, J. M. (1998). Compromises: sound frequencies used in echolocation by aerial-feeding bats. *Canadian Journal of Zoology*, 76(6), 1174-1182.

Corcoran, Aaron J., Jesse R. Barber, and William E. Conner. "Tiger moth jams bat sonar." *Science* 325, no. 5938 (2009): 325-327.

Corcoran, A. J., & Conner, W. E. (2014). Bats jamming bats: food competition through sonar interference. *Science*, 346(6210), 745-747.

- Surlykke, A., & Kalko, E. K. (2008). Echolocating bats cry out loud to detect their prey. *PLoS one*, 3(4).
- Thomas, J. A., Moss, C. F., & Vater, M. (Eds.). (2004). *Echolocation in bats and dolphins*. University of Chicago Press.
- Luo, J., Goerlitz, H. R., Brumm, H., & Wiegrebe, L. (2015). Linking the sender to the receiver: vocal adjustments by bats to maintain signal detection in noise. *Scientific reports*, 5, 18556.
- Lewanzik, D., Sundaramurthy, A. K., & Goerlitz, H. R. (2019). Insectivorous bats integrate social information about species identity, conspecific activity and prey abundance to estimate cost–benefit ratio of interactions. *Journal of Animal Ecology*, 88(10), 1462-1473.
- Dechmann, D. K., Heucke, S. L., Giuggioli, L., Safi, K., Voigt, C. C., & Wikelski, M. (2009). Experimental evidence for group hunting via eavesdropping in echolocating bats. *Proceedings of the Royal Society B: Biological Sciences*, 276(1668), 2721-2728.
- Cvikel, N., Berg, K. E., Levin, E., Hurme, E., Borissov, I., Boonman, A., & Yovel, Y. (2015). Bats aggregate to improve prey search but might be impaired when their density becomes too high. *Current Biology*, 25(2), 206-211.
- Moss, C. F., & Surlykke, A. (2001). Auditory scene analysis by echolocation in bats. *The Journal of the Acoustical Society of America*, 110(4), 2207-2226.
- Surlykke, A., Boel Pedersen, S., & Jakobsen, L. (2009). Echolocating bats emit a highly directional sonar sound beam in the field. *Proceedings of the Royal Society B: Biological Sciences*, 276(1658), 853-860.
- Schnitzler, H. U., & Grinnell, A. D. (1977). Directional sensitivity of echolocation in the horseshoe bat, *Rhinolophus ferrumequinum*. *Journal of comparative physiology*, 116(1), 51-61.
- Feng, A. S., Simmons, J. A., & Kick, S. A. (1978). Echo detection and target-ranging neurons in the auditory system of the bat *Eptesicus fuscus*. *Science*, 202(4368), 645-648.

- Suga, N., & O'Neill, W. E. (1979). Neural axis representing target range in the auditory cortex of the mustache bat. *Science*, 206(4416), 351-353.
- Ulanovsky, N., Fenton, M. B., Tsoar, A., & Korine, C. (2004). Dynamics of jamming avoidance in echolocating bats. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 271(1547), 1467-1475.
- Von Der Emde, G., Schwarz, S., Gomez, L., Budelli, R., & Grant, K. (1998). Electric fish measure distance in the dark. *Nature*, 395(6705), 890-894.
- Von der Emde, G. (1999). Active electrolocation of objects in weakly electric fish. *Journal of experimental biology*, 202(10), 1205-1215.
- Watanabe, A., & Takeda, K. (1963). The change of discharge frequency by AC stimulus in a weak electric fish. *Journal of Experimental Biology*, 40(1), 57-66.
- Jones, T. K., & Conner, W. E. (2019). The jamming avoidance response in echolocating bats. *Communicative & integrative biology*, 12(1), 10-13.
- Obrist, M. K. (1995). Flexible bat echolocation: the influence of individual, habitat and conspecifics on sonar signal design. *Behavioral ecology and sociobiology*, 36(3), 207-219.
- Surlykke, A., & Moss, C. F. (2000). Echolocation behavior of big brown bats, *Eptesicus fuscus*, in the field and the laboratory. *The Journal of the Acoustical Society of America*, 108(5), 2419-2429.
- Habersetzer, J. (1981). Adaptive echolocation sounds in the bat *rhinopoma hardwickii*. *Journal of Comparative Physiology*, 144(4), 559-566.
- Ibáñez, C., Juste, J., López-Wilchis, R., & Núñez-Garduño, A. (2004). Habitat variation and jamming avoidance in echolocation calls of the sac-winged bat (*Balantiopteryx plicata*). *Journal of Mammalogy*, 85(1), 38-42.
- Kössl, M., Mora, E., Coro, F., & Vater, M. (1999). Two-toned echolocation calls from *Molossus molossus* in Cuba. *Journal of Mammalogy*, 80(3), 929-932.

Miller, L. A., & Degn, H. J. (1981). The acoustic behavior of four species of vespertilionid bats studied in the field. *Journal of comparative physiology*, 142(1), 67-74.

Hase, K., Kadoya, Y., Maitani, Y., Miyamoto, T., Kobayasi, K. I., & Hiryu, S. (2018). Bats enhance their call identities to solve the cocktail party problem. *Communications biology*, 1(1), 1-8.

Gillam, E. H., Ulanovsky, N., & McCracken, G. F. (2007). Rapid jamming avoidance in biosonar. *Proceedings of the Royal Society B: Biological Sciences*, 274(1610), 651-660.

Amichai, E., Blumrosen, G., & Yovel, Y. (2015). Calling louder and longer: how bats use biosonar under severe acoustic interference from other bats. *Proceedings of the Royal Society B: Biological Sciences*, 282(1821), 20152064.

Chiu, C., Xian, W., & Moss, C. F. (2008). Flying in silence: echolocating bats cease vocalizing to avoid sonar jamming. *Proceedings of the National Academy of Sciences*, 105(35), 13116-13121.

Schmidt, U., & Joermann, G. (1986). The influence of acoustical interferences on echolocation in bats. *Mammalia*, 50(3), 379-390.

Fawcett, K., Jacobs, D. S., Surlykke, A., & Ratcliffe, J. M. (2015). Echolocation in the bat, *Rhinolophus capensis*: the influence of clutter, conspecifics and prey on call design and intensity. *Biology open*, 4(6), 693-701.

Jones, G., Morton, M., Hughes, P. M., & Budden, R. M. (1993). Echolocation, flight morphology and foraging strategies of some West African hipposiderid bats. *Journal of Zoology*, 230(3), 385-400.

Furusawa, Y., Hiryu, S., Kobayasi, K. I., & Riquimaroux, H. (2012). Convergence of reference frequencies by multiple CF-FM bats (*Rhinolophus ferrumequinum nippon*) during paired flights evaluated with onboard microphones. *Journal of Comparative Physiology A*, 198(9), 683-693.

Cvikel, N., Levin, E., Hurme, E., Borissov, I., Boonman, A., Amichai, E. and Yovel, Y., 2015. On-board recordings reveal no jamming avoidance in wild bats. *Proceedings of the Royal Society B: Biological Sciences*, 282(1798), p.20142274.

Götze, S., Koblitz, J. C., Denzinger, A., & Schnitzler, H. U. (2016). No evidence for spectral jamming avoidance in echolocation behavior of foraging pipistrelle bats. *Scientific reports*, 6(1), 1-13.

Beleyur, T., & Goerlitz, H. R. (2019). Modeling active sensing reveals echo detection even in large groups of bats. *Proceedings of the National Academy of Sciences*, 116(52), 26662-26668.

Simmons, J. A., Ferragamo, M. J., Saillant, P. A., Haresign, T., Wotton, J. M., Dear, S. P., & Lee, D. N. (1995). Auditory dimensions of acoustic images in echolocation. In *Hearing by bats* (pp. 146-190). Springer, New York, NY.

Chiu, C., Reddy, P. V., Xian, W., Krishnaprasad, P. S., & Moss, C. F. (2010). Effects of competitive prey capture on flight behavior and sonar beam pattern in paired big brown bats, *Eptesicus fuscus*. *Journal of Experimental Biology*, 213(19), 3348-3356.

Warnecke, M., Chiu, C., Engelberg, J., & Moss, C. F. (2015). Active listening in a bat cocktail party: adaptive echolocation and flight behaviors of big brown bats, *Eptesicus fuscus*, foraging in a cluttered acoustic environment. *Brain, behavior and evolution*, 86(1), 6-16.

Griffin, D. R., McCue, J. J. G., & Grinnell, A. D. (1962). *The resistance of bats to jamming* (No. TR-285). HARVARD UNIV CAMBRIDGE MA BIOLOGICAL LABS.

Bates, M. E., Stamper, S. A., & Simmons, J. A. (2008). Jamming avoidance response of big brown bats in target detection. *Journal of Experimental Biology*, 211(1), 106-113.

Mitchell-Jones, A. J., Amori, G., Bogdanowicz, W., Krystufek, B., Reijnders, P. J. H., Spitzenberger, F., Stubbe, M., Thissen, J.B.M., Vohralik, V. & Zima, J. (1999). *The atlas of European mammals* (Vol. 3). London: Academic Press.

Guillén-Servent, A., Francis, C. M., & Ricklefs, R. E. (2003). Phylogeny and biogeography of the horseshoe bats. *Horseshoe bats of the world*. Exeter: Pelagic Publishing Ltd.

Russo, D., Jones, G., & Mucedda, M. (2001). Influence of age, sex and body size on echolocation calls of Mediterranean and Mehely's horseshoe bats, *Rhinolophus euryale* and *R. mehelyi* (Chiroptera: Rhinolophidae). *Mammalia*, 65(4), 429-436

## Chapter 2: General Materials and Methods

### 2.1 Field Site

Field experiments were performed for five nights between 14 to 19 August 2018 in a small natural cave next to the entrance of Orlova Chuka cave close to Ruse, Bulgaria (Fig 2.1). Orlova Chuka is home to multiple species of bats (Ivanovo et al, unpublished work). Bats visited this natural cave during emergence at dusk and while returning to the roost at dawn.



Figure 2.1 : Front view of the field site

### 2.2 Observation Setup and Data collection

All observations were performed in a natural cave (3.5m wide and 1.6m high). Two consumer-grade CCTV cameras, recording at 22 frames per second, attached to a Digital Video Recorder (DVR), were installed at two corners of the cave (Figure 2.2). Three ultrasound sensitive microphones (CM-16 condenser microphones, Avisoft Bioacoustics, Glienicke, Germany) were placed on the floor to record the echolocation calls of the bats flying inside the cave. The audio recordings were digitized at a sampling rate of 250 kHz and 16-bit resolution with Ultrasound 416H (Avisoft Bioacoustics). Audio and video data were recorded throughout the night.



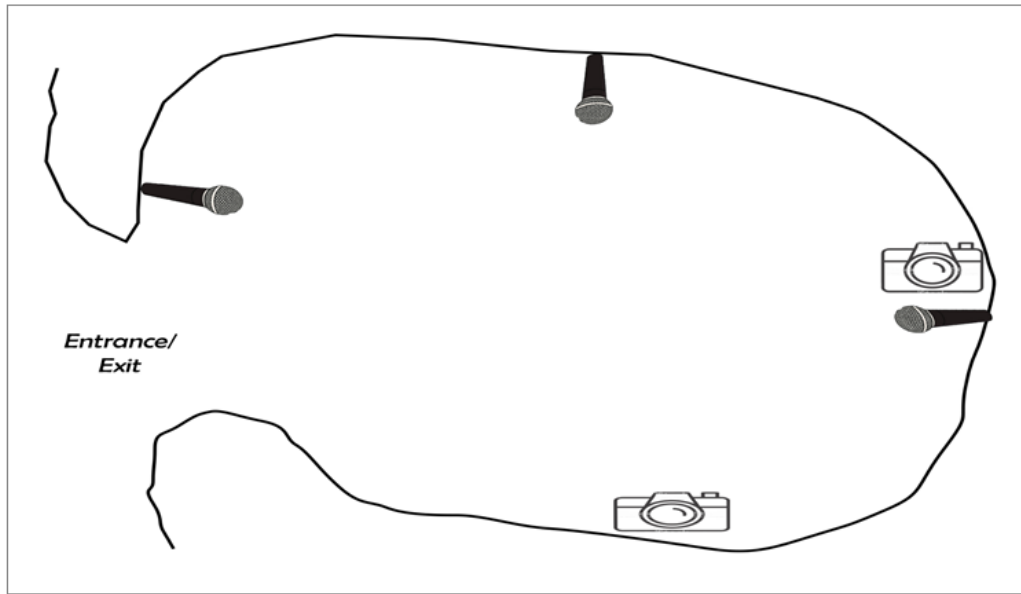


Figure 2.2: Cross section of the cave

The audio and video files were synchronized using a Raspberry Pi employing a method proposed by Laurijssen et al (2018). A random ON/OFF signal was generated from the Raspberry Pi (script by Thejasvi Beleyur, Max Planck Institute for Ornithology, Germany). This signal was used to light an LED, which was recorded by the video cameras, and a copy of the voltage signal was recorded by connecting the Raspberry pi to one of the channels in Avisoft 416H. The cross-correlation of these sequences recorded on the audio and video systems were calculated to determine the time shift and thus to synchronize the audio and video recordings.

I was involved only in the pilot trials of the data collection, conducted during June 2018. However, most of the data were collected in the field by Thejasvi Beleyur and Neetash Mysuru (Max Planck Institute of Ornithology, Germany).

## **Bibliography**

Ivanova T., I. Borissov, H. Stomanyakova, S. Popov. 2005. Biodiversity of Orlova Chuka cave (Municipality Dve Mogili, Ruse Distr.). unpublished report, Project “The wonders of Orlova Chuka cave”, Ruse Municipality, 37 p. (in Bulgarian).

Laurijssen, D., Verreycken, E., Geipel, I., Daems, W., Peremans, H., & Steckel, J. (2018). Low-cost synchronization of high-speed audio and video recordings in bio-acoustic experiments. *Journal of Experimental Biology*, 221(4).

## **Chapter 3: Quantifying group flight in a natural cave habitat.**

### **3.1 Background**

Caves are utilized as roosting sites by many bat species since they provide sites for reproduction, rearing young, hibernation and other social activities (Kunz, 1982). It is also common for many caves to be inhabited by multiple species of bats (Arita, 1993). When multiple bats fly together in close proximity, the loud calls of conspecifics can mask the faint echoes, thereby hindering navigation (Ulanovsky and Moss, 2008). The probability of occurrence of such ‘multiple bat situations’ is higher in caves since they house large aggregations of bats (McCracken, 2003). The emergence timings and activity patterns drastically differ in different bat species. This is related to the foraging technique, diet of the species as well as the flight speed (Jones and Rydell, 1994). The difference in emergence timings could potentially reduce the extent of overlap of calls, at least between heterospecific species. As the first part of the objectives, we focus on investigating how often multiple bat situations involving *Rhinolophus euryale* and *Rhinolophus mehelyi* occur in their natural habitat.

### **3.2 Methodology**

#### **3.2.1 Video Analysis**

All of the videos were analysed on Shotcut (Version 19.04.30). Videos were captured using two CCTV cameras and contained on an average, 22 frames per second. The videos from both the cameras were displayed simultaneously on Shotcut and were subsequently analysed frame-by-frame. Video annotation criteria were formulated and executed in collaboration with Neetash Mysuru.

A bat event is described as the time interval during which the number of bats flying inside the cave is constant. A new event starts when the number of bats flying in the cave changes. This occurs either when a new bat enters or a bat flying inside exits the cave. For instance, in a situation involving the entry of two bats into the cave separated by a short-time-interval, the

interval where the first bat flies inside the cave before the second bat enters is a single bat event.

Videos were analysed to screen for the occurrence for bat events and the number of bats flying inside the cave during each bat event was counted. The frame at which the bat is visible in the camera inside the cave was considered as the start timestamp and the one in which it leaves the cave was taken as the exit timestamp. Camera 1 and Camera 2 were found to be not in sync and possess independent variation in their frame rate. However, the video from both the cameras could be synced with audio sync signal and thus the timestamps visible from both the cameras were used for synchronisation. It was ensured that the start timestamp, as well as the end timestamp of a particular bat event, were chosen from the same camera.

Video footage from both the cameras covered the entirety of the cave except a small region (roosting area) behind both of the inner walls. Bats were found to fly into this region and disappear from the camera view for extended periods. In such cases, the time at which the bat disappeared from the view was considered as the end timestamp of the bat event. However, if the bat flew back into the camera view within a short interval (2 seconds), this period was considered as a part of the bat event. A period of 6 frames was kept as a buffer zone between consecutive events to ensure proper separation. The total number of single bat and multiple situations were quantified using these criteria.

### **3.2.2 Acoustic Analysis**

Species identification was not achievable from the video files owing to the poor resolution of the camera. Since the field site is home to multiple species of bats (Ivanova et al, 2005, Unpublished work), the audio files matched to the video annotations were analysed to quantify the number of multiple bat situations involving only the focal species, *Rhinolophus euryale* and *Rhinolophus mehelyi*.

After the audio-synchronisation was complete, the audio files matched with the video annotations were extracted using a Python code by Thejasvi Beleyur. However, the audio files of all the bat events could not be extracted due to the presence of segments of the video where

the blinking of LED could not be reliably tracked or having frame rates less than 22 Hz. Hence, only a subset of the total number of bat events could be analysed for quantifying the bat events involving only the focal species. Over 1000 audio files from 12 hours across 3 nights were extracted. The duration of the audio files varied significantly ranging from a few milliseconds to over 10 seconds. All of the audio files were manually analysed in Audacity(R) recording and editing software (Version 2.3.3) after employing a high pass filter with 12 dB roll-off/octave at 80 kHz. The focal species can be reliably distinguished from other species by their peak frequencies (100-110 kHz) and hence, spectrograms were constructed to identify the events involving only the focal species. These were subsequently analysed separately.

### **3.2.3 Statistical Analysis**

For each analysis, Chi-squared test was performed in Statistica Version 12.

### **3.3 Results**

In the 13 hours of video data analysed, 2021 bat events occurred. In over 76% of the events (n=1537), bats flew alone (Figure 3.1). This was significantly greater than the number of bat events with multiple bats flying inside the cave (Chi-square (df=1) = 1097.29,  $p < 0.01$ ). Bat events with multiple bats accounted for 24% of all events. In these, 2-bat events occurred the most frequently (n= 392), followed by 3-bat events (n=77) and 4-bat events occurred the least (n=15). 2 bat events occurred significantly greater than 3 bat events (Chi-square (df=1) = 239.34,  $p < 0.01$ ). Similarly, the occurrence of 3 bat events was significantly greater than that of 4 bat events (Chi-square (df=1) = 8.73,  $p < 0.01$ ).

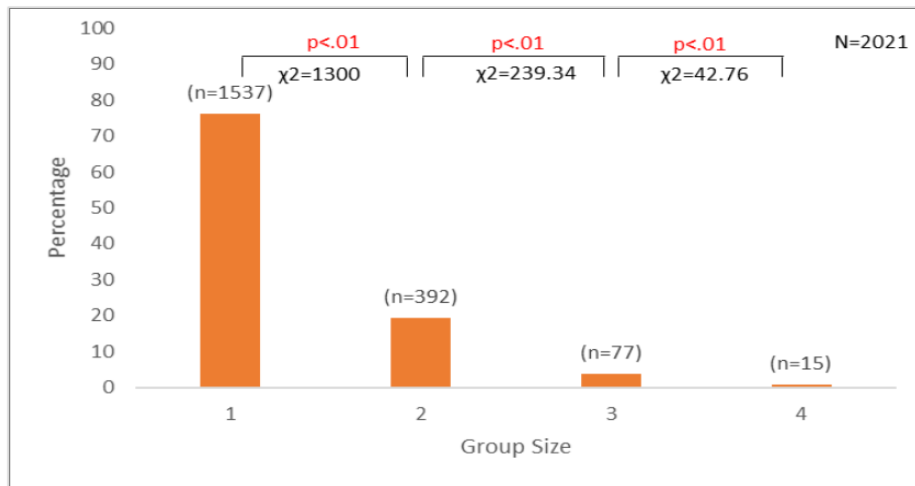


Figure 3.1: Bar Plot of Percentage of occurrence vs Group Size

In the 1000 audio files manually analysed, 652 involved calls of only the focal species, *R euryale* and *R. mehelyi*. Bats flew alone in 73% of these events (n=480). Single-bat events occurred significantly greater than events involving multiple bats (Chi-square (df=1) = 290.99, p < 0.01). Multiple bat events constituted 27% of all events with 2 bat events occurring most-frequently (n =142) (Chi-square (df=1) = 98.26, p < 0.01). 3 bats events (n=23) occurred significantly greater than 4 bat events (Chi-square (df=1) = 8.73, p = .031)

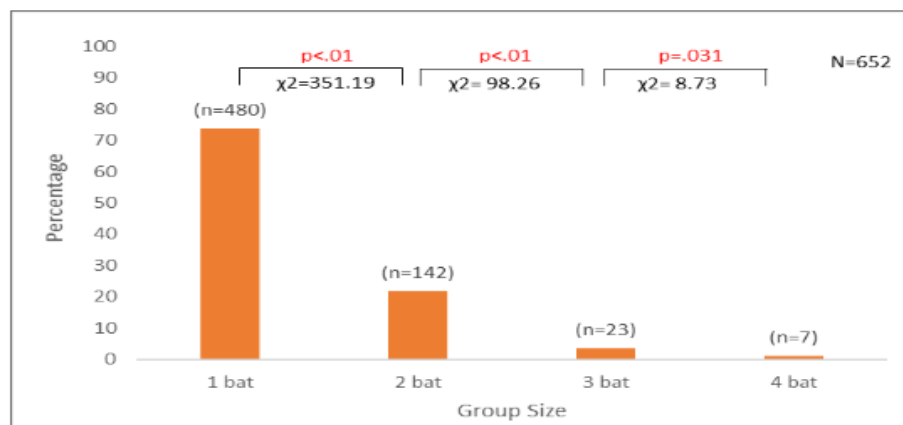


Figure 3.2: Bar plot of Percentage of Occurrence vs Group Size (Focal species only)

### **3.4 Conclusion**

Although bats fly alone in most of the observational time, they may still experience jamming regularly from conspecifics and heterospecific species in their natural habitat.

## Bibliography

Kunz T. H. 1982. Roosting ecology of bats. Pp. 1–55 in Ecology of bats (T. H. Kunz, ed.). Plenum Press, New York

Arita H. T. 1993. Conservation biology of the cave bats of Mexico. *Journal of Mammalogy* 74:693–702.

N. Ulanovsky, C. F. Moss, What the bat's voice tells the bat's brain. *Proc. Natl. Acad. Sci. U.S.A.* 105, 8491–8498 (2008).

McCracken GF (2003) Estimates of population sizes in summer colonies of Brazilian free-tailed bats (*Tadarida brasiliensis*). In: O'Shea TJ, Bogan MA (eds) Monitoring trends in bat populations in the United States and territories: problems and prospects. Geological Survey, US, pp 21–30

Foraging strategy and predation risk as factors influencing emergence time in echolocating bats. (1994). *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 346(1318), 445–455. doi: 10.1098/rstb.1994.0161

Ivanova T., I. Borissov, H. Stomanyakova, S. Popov. 2005. Biodiversity of Orlova Chuka cave (Municipality Dve Mogili, Ruse Distr.). unpublished report, Project “The wonders of Orlova Chuka cave”, Ruse Municipality, 37 p. (in Bulgarian).

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## **Chapter 4: Quantification of flight behaviours exhibited during multiple bat situations**

### **4.1 Background**

While flying in a group, animals coordinate their trajectories with other individuals. The beamwidth is an important aspect of echolocation behaviour since it spatially limits the area from which echoes originate and are received by the bat (Ghose and Moss, 2003). Bats emit highly directional beams in the field and adaptively change their echolocation beamwidth in response to the environment as well as rapid changes in the perceived-echo scene (Surlykke et al., 2009; Jakobsen and Surlykke, 2010; Matsuta et al., 2010). Since the echolocation calls are highly directional, the relative position of bats will influence the extent of overlap of calls while flying with a conspecific or heterospecific species. Hence, the detrimental effects of jamming can be reduced by exhibiting flight behaviours, where minimal overlap with calls of the other bat occurs. Previous studies on paired FM bats in the lab have found that they exhibited ‘Following/ Trailing’ behaviour 75% of the time (Chiu et al., 2010). However, there have been no studies on flight behaviour changes in response to conspecifics in CF-FM bats and for multiple bat situations involving more than two bats. We hypothesise that CF-FM bats will also exhibit flight behaviours that reduce the extent of overlap of their calls.

### **4.2 Methodology**

#### **4.2.1 Video Analysis**

The number of bats was counted according to the criteria mentioned in chapter 3. Furthermore, the video timestamps with multiple bats flying inside the cave were analysed to identify prominent behaviours exhibited based on their relative positions with respect to each other. They exhibited three prominent behaviours while flying alongside a heterospecific/ conspecific:

a) Trailing Behaviour.

The bats exhibited trailing behaviour where one bat flew along the same general trajectory of the other bat.

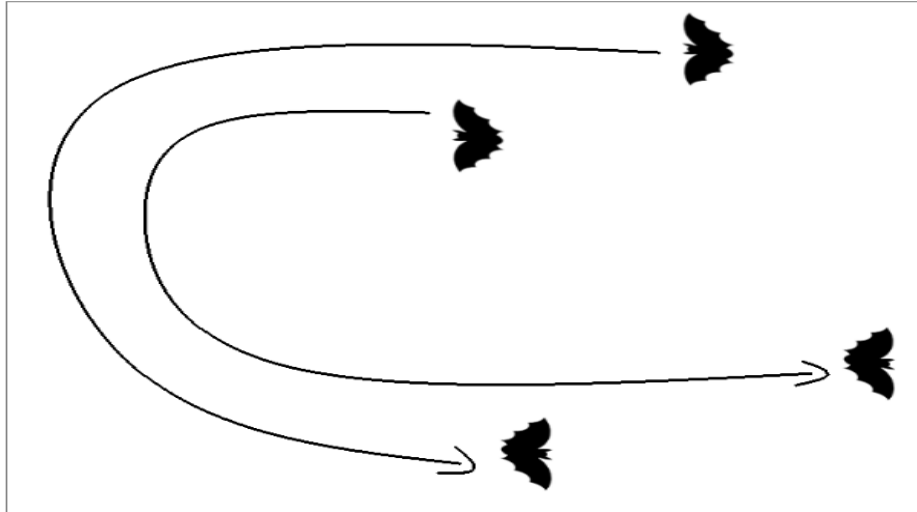


Figure 4.1: Pictorial representation of trailing behaviour

b) Passing Behaviour

The bats flew past each other in opposite directions inside the cave, generally for a brief duration.

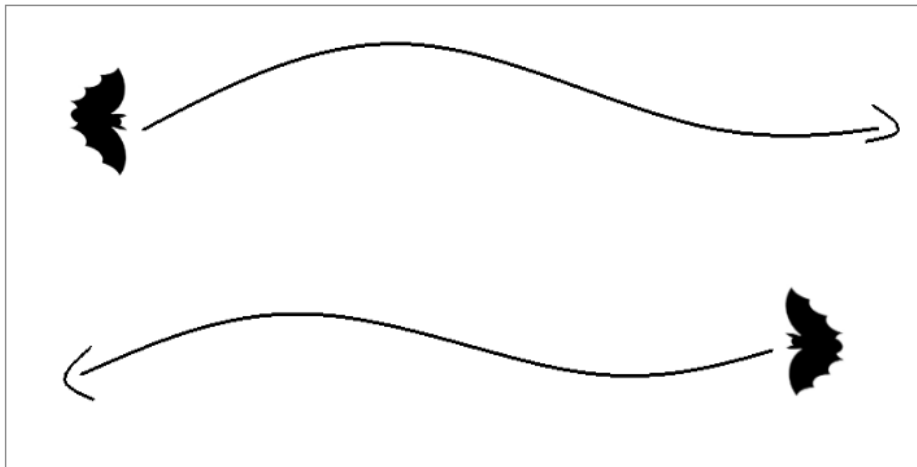


Figure 4.2: Pictorial representation of passing behaviour

c) Heads-On Behaviour

The bats flew towards each other in the same imaginary line from approximately opposite directions, and either one or both the bats rapidly altered their flight to fly in near- opposite directions relative to the original trajectory.

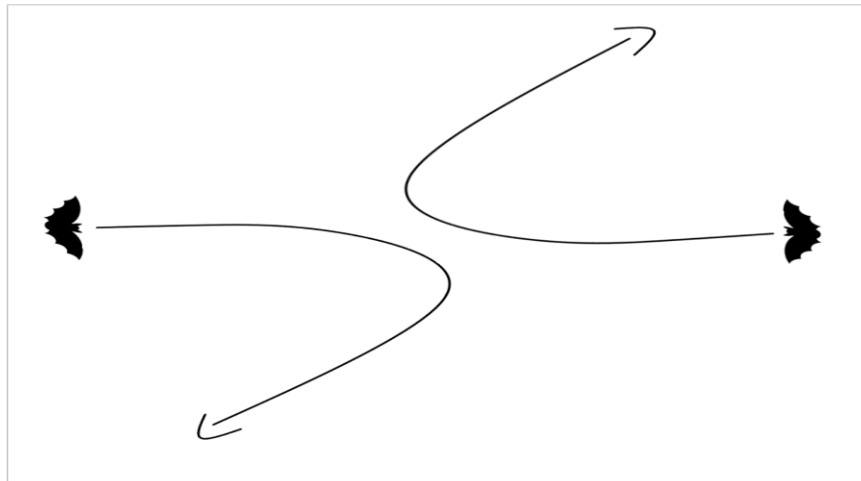


Figure 4.3: Picorial representation of Heads-on behaviour

Bats often exhibited more than one behaviour during a single ‘multiple-bat situation’. The situations where bats exhibited none of the above-mentioned behaviours was annotated as “NA”.

After the identification of prominent behaviours, the videos were screened to quantify the occurrence of each behaviour and how it varies with group size. The events involving only the focal species have been determined (according to criteria in Chapter 3). The flight behaviours exhibited during these events were separately analysed.

#### **4.2.2 Statistical Analysis**

For each analysis, Chi-squared test was performed in Statistica Version 12.

### **4.3 Results**

#### **4.3.1 All bat events**

In the 392 events involving two bats flying inside the cave (Fig 4.4 (a)), both trailing and passing are exhibited equally likely ( $n=200$ ) (Chi-square ( $df=1$ ) = 0,  $p=1$ ). Both of these behaviours are exhibited significantly more than heads-on behaviour (Chi-square ( $df=1$ ) = 111.95). In 60 events, bat did not exhibit any of these behaviours. Since bats often exhibit

multiple behaviours during one event, the total count of behaviours exceeds the number of multiple-bat events.

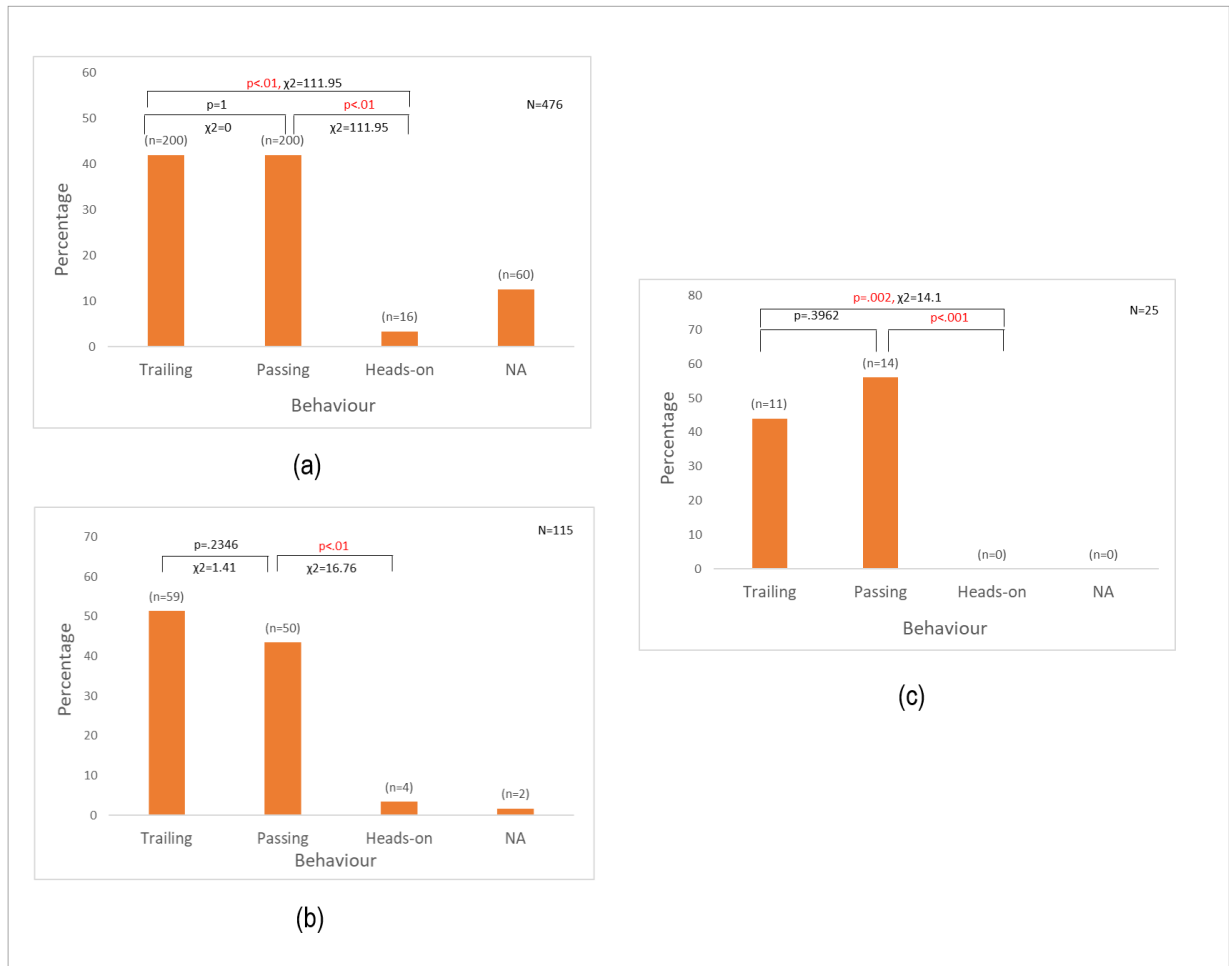
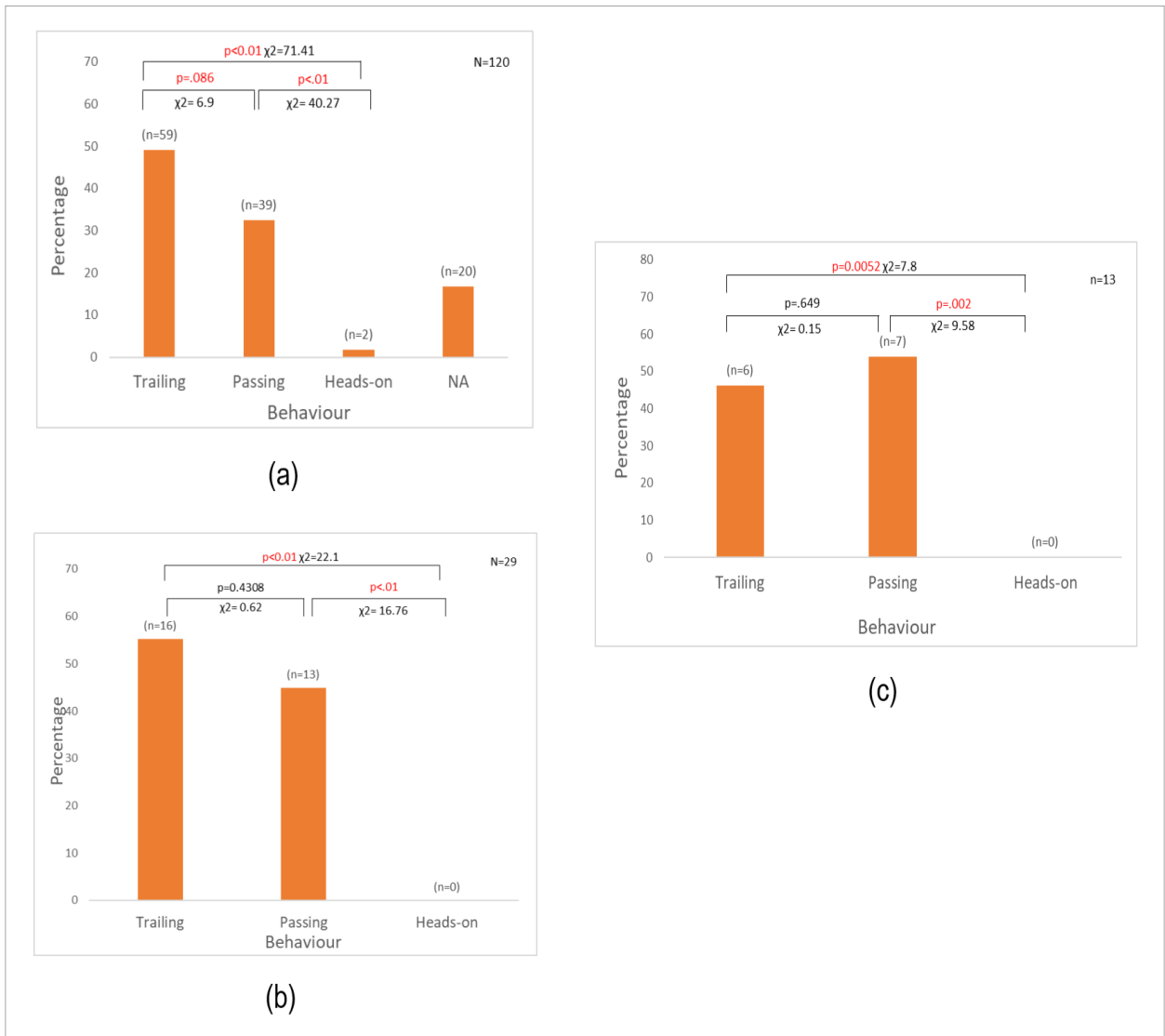


Figure 4.4: Bar plots of Percentage of Occurrence versus Behaviour exhibited during a) 2 bat event b) 3 bat event d) 4 bat event

Trailing behaviour constituted 51% (n=59) of all behaviours exhibited during a 3 bat event, whereas passing accounts for 42% (n=50) (Fig. 4.4 (b)). They do not show any statistical difference in their occurrence (Chi-square (df=1) = 1.41, p=0.2346). Both passing and trailing occur significantly more than heads-on behaviour (Chi-square (df=1) = 16.76, p<.01). None of these behaviours was exhibited by bats in two events. Trailing and passing are the only

behaviours exhibited during a 4-bat event. There is no statistical difference in their occurrence (Chi-square (df=1) =0.72, p=0.3962).

### 4.3.2 Bat events involving only the focal species.



**Figure 4.5:**  
Bar plots of Percentage of Occurrence vs Behaviour exhibited in a) 2 bat event b) 3 bat event c) 4 bat event (involving focal species only)

Figure 4.5 (a) shows the behaviours exhibited by the focal species during a 2 bat situation. Trailing is the most commonly-occurring behaviour (50% of all exhibited behaviours) and is found to significantly occur more than passing (Chi-square (df=1) = 6.9,  $p = .086$ ) and heads-on (Chi-square (df=1) = 71.41,  $p < .0001$ ). Heads-on behaviour is exhibited the least.

In events involving 3 and 4 bats inside the cave, trailing and passing are the only behaviours exhibited. They do not occur significantly different in either of the situations (Chi-square (df=1) = .62,  $p = .4308$ , Chi-square (df=1) = .15,  $p = .649$ ).

#### **4.4 Conclusion**

The results indicate that whereas heads-on is the least exhibited behaviour across all group sizes, trailing and passing are most commonly exhibited during a multiple bat situation, both while considering all bat events as well as the ones involving only the focal *Rhinolophus* species.

## Bibliography

Ghose, K., & Moss, C. F. (2003). The sonar beam pattern of a flying bat as it tracks tethered insects. *The Journal of the Acoustical Society of America*, *114*(2), 1120–1131. doi: 10.1121/1.1589754

Surlykke, A., Pedersen, S. B., & Jakobsen, L. (2008). Echolocating bats emit a highly directional sonar sound beam in the field. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1658), 853–860. doi: 10.1098/rspb.2008.1505

Jakobsen, L., & Surlykke, A. (2010). Vespertilionid bats control the width of their biosonar sound beam dynamically during prey pursuit. *Proceedings of the National Academy of Sciences*, *107*(31), 13930–13935. doi: 10.1073/pnas.1006630107

Matsuta, N., Hiryu, S., Fujioka, E., Yamada, Y., Riquimaroux, H., & Watanabe, Y. (2013). Adaptive beam-width control of echolocation sounds by CF-FM bats, *Rhinolophus ferrumequinum nippon*, during prey-capture flight. *Journal of Experimental Biology*, *216*(7), 1210–1218. doi: 10.1242/jeb.081398

Chiu, C., Xian, W., & Moss, C. F. (2008). Flying in silence: Echolocating bats cease vocalizing to avoid sonar jamming. *Proceedings of the National Academy of Sciences*, *105*(35), 13116–13121. doi: 10.1073/pnas.0804408105

## **Chapter 5: Quantifying the acoustic call parameters of echolocation calls in free-flying horseshoe bat aggregations.**

### **5.1 Background**

Lab studies in Horseshoe bats have examined how they deal with the challenging problem of masking in group sizes up to 2, which is much simpler than what is found in the wild and also show contrasting results: one finding indicating no change and the other one found convergence to a narrow range of frequencies (Fawcett et al, 2015, Furasawa et al, 2011). Furthermore, there have been no field studies on how CF-FM bats overcome the detrimental effects of masking. This study aims to fill the gap by recording echolocation calls of mixed-species groups of horseshoe bats in their natural habitat, where they are known to fly along with conspecifics in group sizes greater than two.

The dominant frequency (the frequency with the highest energy in the spectrum) of horseshoe bat calls is the frequency of its CF component. However, CF- FM bats utilize their FM components for ranging since broadband signals are well suited for the precise localization of targets. When multiple bats vocalise simultaneously, they have to keep track of the movement of conspecifics to avoid colliding with each other. Since FM-components are suited for target estimation (Schnitzler and Kalko, 2001), we hypothesize that horseshoe bats will alter their acoustic call parameters by increasing the energy content in the FM component while flying in groups.

### **5.2 Methodology**

#### **5.2.1 Acoustic Analysis**

After the audio files were synchronized with the video recordings, the audio files corresponding to the individual bat events were extracted (using a code by Thejasvi Beleyur). These recordings were manually analysed in version 2.3.3 of Audacity(R) editing and recording software.

#### **5.2.2 Isolation of calls**



Since the setup consisted of three microphones, each audio file consisted of recordings in 3 channels. However, the audio recordings from channel 1 were only considered for the analysis since microphone 1 faced the entrance of the cave and is expected to record the maximum number of on-axis calls. A highpass filter with 12 dB roll-off/ octave at 80 kHz cut off frequency was applied after the audio files were loaded in Audacity. Audio files consisting of calls of only the focal species, *Rhinolophus mehelyi* and *Rhinolophus euryale*, were used for the analysis. These audio files were determined, by visualising the spectrograms after applying the high pass filter and checking if only the calls of the focal species were present. The calls of the focal species could be reliably distinguished from the calls of other CF- FM and FM bats since the peak frequencies ranged from 100 to 110 kHz. A random call was selected in each of the files using the following procedure:

- a) A random number between 0-1 was generated. The fraction of time corresponding to the length of the annotation file was chosen as the start time. For instance, if the number generated was 0.1 and the file duration was 1s, then  $0.1 * 1 = 0.1$  second was the start time.
- b) Another random number between 0-1 was generated to determine the search direction. If the number generated was smaller than 0.5, calls were searched to the left to the start point and vice versa.
- c) The start and end timestamp of a horseshoe bat call with no overlap and no interference pattern in either the CF or FM component with Signal to Noise Ratio (SNR)  $>20$  were selected. The SNR was calculated by comparing the RMS amplitude of the call with that of a 'silent period' of the audio file as the background.
- d) If there were no suitable calls from the start point until the end of the audio file, calls were searched in the opposite direction from the start point.
- e) If a suitable call was still not found, the audio file was not selected for the analysis.

### **5.2.3 Extraction of call parameters**

After the start and end timestamps of the suitable calls were selected, the CF and FM components were separated and the call parameters were extracted using a script developed by Thejasvi Beleyur, as follows:

- The peak frequency of the whole call is calculated and this is assigned as the peak frequency of the CF component.
- The CF component is then removed by using a notch filter centered at the peak frequency.
- The remaining portions of the call represent the upward and downward FM component.
- The following call parameters were calculated: whole call energy and duration, upward FM duration and energy, Downward FM duration and energy, Terminal frequency of upward and downward FM components. Further, the RMS amplitude ratio of CF to the downward FM component was computed to understand whether bats focus more energy in the FM component while flying in groups.

### **5.3 Results**

#### **5.3.1 Single call measurements**

Although the script is successful in separating the CF and FM call components and the subsequent extraction of the call parameters (Figure 5.2), the signal gets altered after notch filtering

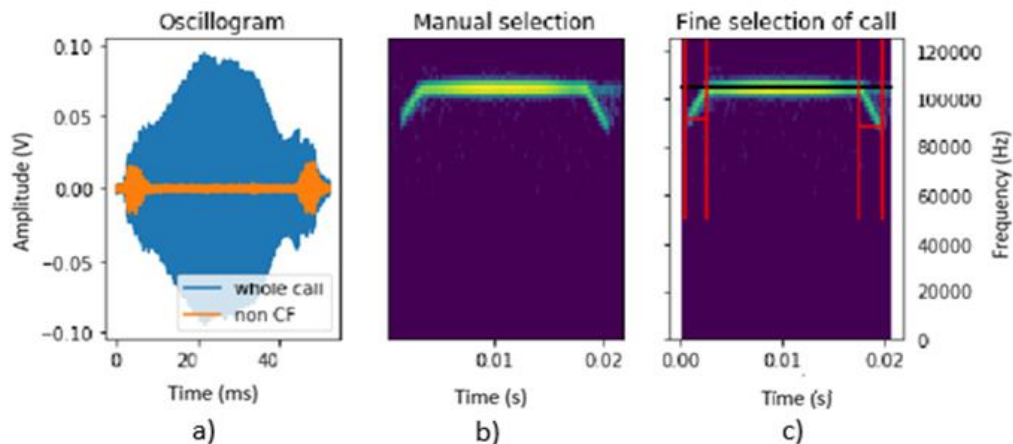


Figure 5.1: a) The oscillogram of the call before and after applying the notch filter. b) Spectrogram of a manually selected call c) Fine selection of the call: Black line represents the peak frequency, horizontal red line represents the terminal frequency and vertical red line represents the start and end of FM segments.

In Figure 5.1a), the blue waveform represents the whole call, whereas the orange waveform represents the FM components obtained after filtering. Although the exact start timestamp of the FM component cannot be determined from the spectrogram, it seems that the amplitude of the FM component gets reduced substantially after signal processing. This process is streamlined based on the assumption that the CF component is a pure tone and only this gets removed after the notch filter is applied. However, bat calls represent a biological sound. The call recorded at the microphone also includes reverberated sound and is not a pure tone. Hence, the filter removes not only the target frequency but also the nearby frequencies resulting in the loss of parts of the signal. This will affect the calculated RMS amplitude values. Similarly, the workflow fails in extracting reliable parameters of calls with interference patterns from the echoes.

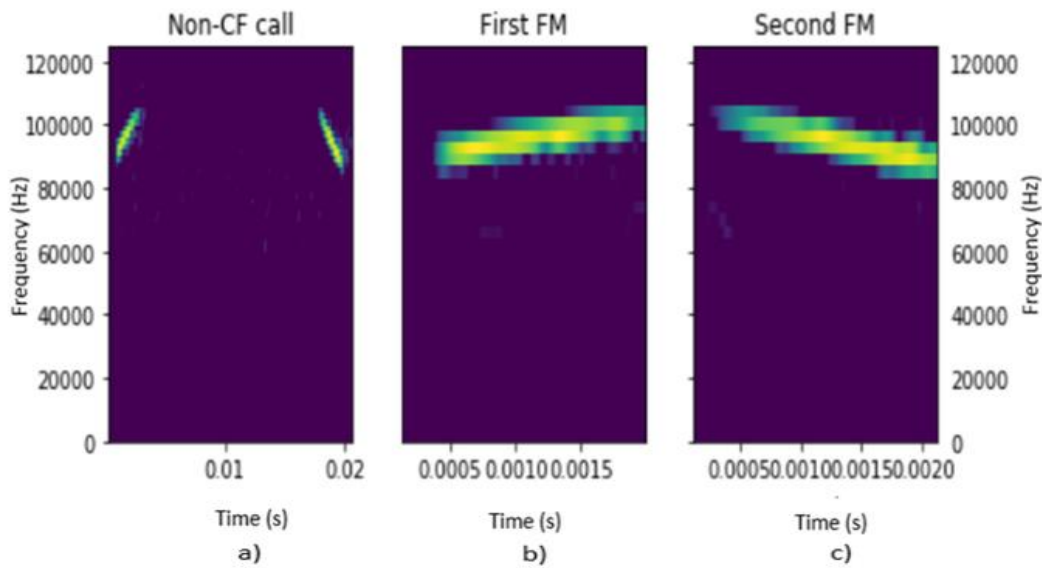


Figure 5.2: a) Spectrogram of the call after the CF component is removed  
 b) Spectrogram of the upward FM component  
 c) Spectrogram of the downward FM component

### 5.3.2 Variation in call parameters across group sizes

Figure 5.3 depicts the box plots of the variation in acoustic call parameters with changes in group size. Since the extraction of call parameters could not be efficiently carried out, only a

preliminary analysis with a limited sample size was performed and statistical tests have not been performed.

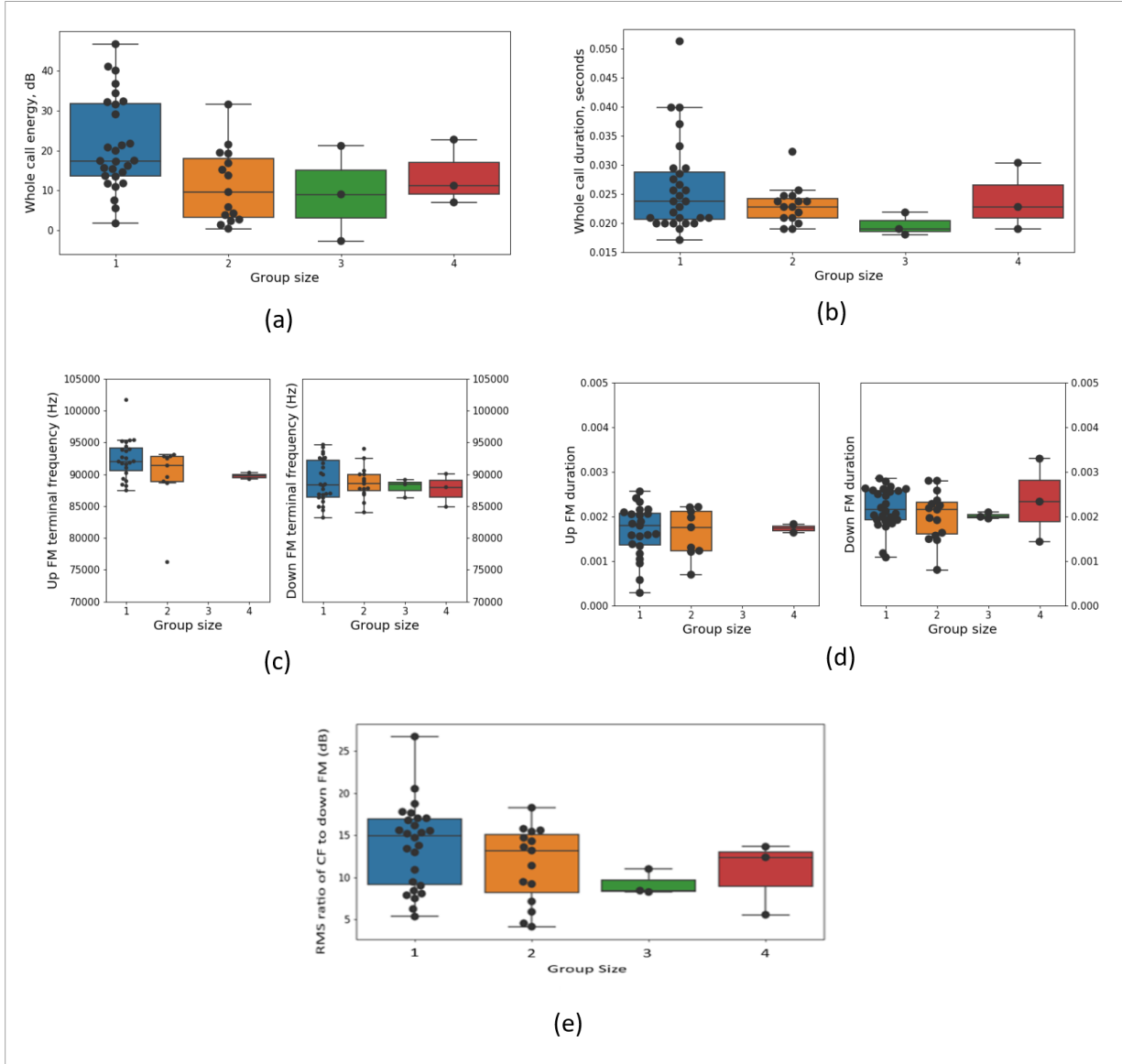


Figure 5.3: Box Plots a) Whole Call Energy vs Group Size b) Whole call duration vs Group Size c) Terminal frequency vs Group size d) FM durations vs Group size e) CF-FM RMS amplitude ratio vs Group Size

## **5.4 Conclusion**

Although preliminary results indicate that horseshoe bats do not change their call parameters while flying in groups, the sample size has to be increased to conclude whether they do so.

## **Bibliography**

Fawcett, K., Jacobs, D. S., Surlykke, A., & Ratcliffe, J. M. (2015). Echolocation in the bat, *Rhinolophus capensis*: the influence of clutter, conspecifics and prey on call design and intensity. *Biology Open*, *4*(6), 693–701. doi: 10.1242/bio.201511908

Furusawa, Y., Hiryu, S., Kobayasi, K. I., & Riquimaroux, H. (2012). Convergence of reference frequencies by multiple CF–FM bats (*Rhinolophus ferrumequinum nippon*) during paired flights evaluated with onboard microphones. *Journal of Comparative Physiology A*, *198*(9), 683-693.

Schnitzler, H. U., & Kalko, E. K. (2001). Echolocation by insect-eating bats: we define four distinct functional groups of bats and find differences in signal structure that correlate with the typical echolocation tasks faced by each group. *Bioscience*, *51*(7), 557-569.

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## Chapter 6: Discussions

- **Objective 1:** Our results indicate that although bats fly alone most of the time, they still may regularly experience jamming from conspecific or heterospecific species in their natural habitats. Multiple bat situations occurred ~25% of the observation time while bats flew alone ~75% of the time, both for all bat events, as well as only our focal *Rhinolophus* species. This can be explained by the presence of large aggregations of bats in caves (Furey and Racey, 2016) and the prevalence of foraging in groups (Dechmann et al, 2009). The present study looks at the occurrence of multiple bat situations during their emergence from roosting sites. Resource partitioning is prevalent in sympatric bat species and is achieved by differences in diet, echolocation frequency, and foraging area (Emrich et al, 2014). The focal species of horseshoe bats have similar dietary preferences and overlapping echolocation calls. Thus, it would be interesting to look at how often bats experience jamming in their foraging sites. This represents a more complex scenario, where bats have to deal with jamming signals while hunting for prey in cluttered environments.
- **Objective 2:** Our findings show that trailing and passing are the most commonly occurring behaviours during a multiple-bat situation in this constrained space, apart from two bat events involving only the focal species where trailing is the most prevalent. The variation in flight behaviour is linked to the extent of overlap of calls that the bat experiences during each behaviour. The trailing bat is at an advantageous position since it experiences reduced overlap of calls of the leading bat, as well as can track the movement of the leading bat by directing its sonar beam towards it. Both the bats experience a significant overlap of calls as they converge in flight during passing behaviour. The extent of the overlap of calls is dependent on the inter-bat distance as they fly past each other and is greatest when they converge on each other from approximately opposite directions during heads-on behaviour. The rapid change in the flight direction could indicate the inability of bats to navigate in the presence of jamming signals. The absence of collisions and the rarity of heads-on behaviour

indicate that bats can navigate efficiently in the small cave, even in the presence of jamming. Trailing behaviour has been associated with naive bats learning a foraging task while following an experienced individual in *Eptesicus fuscus* (Wright et.al, 2014). Similarly, paired bats exhibit following behaviour most often while competing for a single food item under laboratory conditions and the bat in the following position had more success in capturing the prey (Chiu et al, 2010). However, territorial behaviour cannot explain the prevalence of trailing in the present study since neither naive bats were present nor do they compete for food. Trailing bats are less prone to jamming signals of the leading bat since the calls are frontally directed, whereas both the bats experience jamming signals as they converge in flight. Further, trailing likely allows the bats to use the same space most efficiently with minimal interference and no collisions. This could explain why bats fly inside the cave for long intervals exhibiting trailing behaviour rather than exiting immediately on the entrance of another bat, during which they would have to pass each other. Although bats can direct their sonar beams away from each other to reduce jamming as they converge, the small dimensions of the cave suggest that bats may still experience a significant overlap of calls as a result of reverberations of the walls. My results quantify the occurrence of each behaviour, but not the duration. Further analysis needs to be performed quantifying the time spent by bats performing each behaviour. Preliminary analysis suggests that bats often exhibit following behaviour, which supports previous studies in FM bats.

- **Objective 3:** Preliminary results suggest that there are no differences in the echolocation call parameters of horseshoe bats with varying group sizes. However, this is limited by the sample size and the inability to perform reliable single call measurements. CF bats appear to be more susceptible to jamming due to the presence of long calls, high duty cycle and the lack of freedom to shift their echolocation frequency. But the narrowband tuning of the auditory neurons to the peak frequency of the bat itself (Neuweiler J,2003) and ‘personal frequencies’ could help deter the occurrence of spectral interference. Habersetzer (1998) reports that CF-FM bats utilize FM calls while flying in groups as they leave their roosting sites and employ CF calls while they fly alone. This can be explained by FM calls being well suited for precise



localization and range estimation. Similarly, horseshoe bats could alter their call structure and produce more intense FM calls while flying in groups. Currently, an improved parameter extraction workflow has been built by Thejasvi Beleyur. This will enable us to extract the acoustic parameters of the calls already selected and compare them across different group sizes. Paired bats experience varying degrees of Doppler shift based on the relative direction of flight. Further analysis can be performed comparing the call parameters while bats exhibit converging flight, diverging flight and fly along with each other in the same direction.

## **Bibliography**

Furey, N. M., & Racey, P. A. (2016). Conservation ecology of cave bats. In *Bats in the Anthropocene: Conservation of bats in a changing world* (pp. 463-500). Springer, Cham.

Dechmann, D. K., Heucke, S. L., Giuggioli, L., Safi, K., Voigt, C. C., & Wikelski, M. (2009). Experimental evidence for group hunting via eavesdropping in echolocating bats. *Proceedings of the Royal Society B: Biological Sciences*, 276(1668), 2721-2728.

Emrich, M. A., Clare, E. L., Symondson, W. O., Koenig, S. E., & Fenton, M. B. (2014). Resource partitioning by insectivorous bats in Jamaica. *Molecular Ecology*, 23(15), 3648-3656.

Wright, G. S., Wilkinson, G. S., & Moss, C. F. (2011). Social learning of a novel foraging task by big brown bats, *Eptesicus fuscus*. *Animal Behaviour*, 82(5), 1075-1083.

Chiu, C., Reddy, P. V., Xian, W., Krishnaprasad, P. S., & Moss, C. F. (2010). Effects of competitive prey capture on flight behavior and sonar beam pattern in paired big brown bats, *Eptesicus fuscus*. *Journal of Experimental Biology*, 213(19), 3348-3356.

Neuweiler, G. (2003). Evolutionary aspects of bat echolocation. *Journal of Comparative Physiology A*, 189(4), 245-256.

Habersetzer, J. (1981). Adaptive echolocation sounds in the bat *Rhinopoma hardwickei*. *J. Comp. Physiol. (A)* 144, 559-566.