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Acoustic and Flight Behaviour of Emerging Greater Horseshoe Bats in Paired Flight

Jacob Bollinger

A dissertation submitted to the University of Bristol in accordance with the requirements for award of the degree of Master of Science in the Faculty of Life Science.

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Abstract

Emergence can be particularly complicated for bats as they orientate in an environment with a higher chance of possibly jamming due to conspecifics. Yet jamming avoidance in bats is understudied in emergence, especially in high duty cycle bats. Here I examined the high duty cycle bat *Rhinolophus ferrumequinum* for evidence of jamming avoidance responses while emerging.

Bats were recorded from a field site in Wales via acoustic tracking. Solo and paired individuals were identified and subsequently analysed for flight and acoustic jamming avoidance behaviours. I concluded that while the paths of paired flights were statistically different there was not enough evidence to truly suggest that paired flight paths were different from solo flight paths. The bat experience for each paired bat was calculated, allowing for the investigation of differences in the frequency and the timing of the calls. Here, I present the first indications of the intentional alternation of calls in a high duty cycle bat, a jamming avoidance strategy in use by low duty cycle bats, and the use of a synchronization strategy as well. In frequency, pairs were found to be expanding the bandwidth of the terminal FM sweep, confirming other reports. However, I concluded that this was not a clutter response, as previously suggested. Some pairs were found to be separating their echo frequencies more than expected but more interestingly one pair was found to have frequencies closer than expected. Considering that this pair was also synchronising calls it would appear that this pair was was intentionally jamming one another. I suggest that this is actually a jamming avoidance response in which echoes are matched so that there is no jamming, termed 'allo-echoic interception.' This study provides some evidence for the possibility of acoustic jamming avoidance responses in high-duty cycle bats during emergence, both theorized and novel.

Acknowledgments

I must start off by thanking Professor Marc W. Holderied for your support, guidance, and expertise both in this work and in my personal life. Taking on an American who hadn’t worked in biology for five years was certainly a risk and I will be forever grateful for the opportunity you provided me. It has been a crazy two years and without your guiding hand none of this would have been possible. Without a doubt your support during lockdown was a bright spot for me.

Another large amount of thanks needs to go to the fine folks in the BASELab. You are a lovely, incredibly supportive group that has helped me keep my sanity many times by listening to my bat ramblings that I could tell you only partially understood while waiting to get back to moth work. The friendship you have provided me helped me through a very difficult time.

A special thanks goes to my brother Adam Bollinger and my best friend Brandon Hughes who both helped me keep in touch with home and provided a necessary distraction on the weekends to help me reset. Long live the super awesome wolf squad!

I also need to acknowledge my family for their support. I’m sure you thought I had lost my mind when I said I was pursuing a postgraduate degree in a foreign country, but you have had my back every step of the way. I always looked forward to the biweekly calls and the quarterly care packages in particular were always a godsend.

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COVID-19 Impact Statement

This MSc(R) project started in mid-August 2019 about eight months before the worldwide outbreak of COVID-19. Fortunately, I was able to get a night of data collection in before the winter and had data to work with and examine through the winter months. That night ultimately provided me with enough pairs of bats to begin analysis. I originally planned to return to the field site in May of 2020 to complete another round of data collection. It is likely that this would have doubled or even tripled the amount of pairs that could be analysed. Per University policy, all fieldwork was cancelled during the lockdown and subsequently that plan was cancelled. Due to the analysis time required and the uncertainty of undertaking field work in the autumn of 2020, I declined to attempt another night of data collection in the autumn. Ultimately, this left me with only 17 pairs to examine. The results of this study show evidence of interesting trends in paired flight behaviour but with so few pairs to analyse it was difficult to get a full understanding on the behaviours at play. While COVID-19 did limit this work, I feel that I adapted well to the circumstances and was able to complete the project within the original time frame (Aug 2019 – July 2021) without requiring an extension.

Author’s declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED: .....*Jacob Bollinger*....

DATE: .....2/8/2021...

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Glossary and Terms

Acoustic Timing Characteristics (: All the characteristics that are involved in the timing of a call (e.g. duration, IPI, etc.).

Allo-call: The call (echo or emission) of a conspecific as received by the focal bat.

Alt: The call timing classification of alternating.

Array 1 (A1): The microphone array closest to the building wall.

Array 2 (A2): The microphone array further from the building wall.

Attenuation: The weakening of an acoustic signal.

Auditory Fovea: A modified cochlea in which a section of the basilar membrane consists of disproportionate amount of finely tuned foveal neurons for a narrow range of frequencies

Auto-call to allo-call Groups (AAG): The auto-call to allo-call groups: auto-emission to allo-emission, auto-emission to allo-echo, and auto-echo to allo-echo.

Auto-call: The focal bat's own call (echo or emission).

Auto-echo to allo-echo (OO): The focal echo compared to the secondary echo.

Auto-emission to allo-echo (EO): The focal emission compared to the secondary echo.

Auto-emission to allo-emission (EE): The focal emission frequency compare to the secondary emission.

Backward masking: Masking caused by a sound that occurs after the target signal has been received.

Bandwidth (BW): Range of frequencies.

Call Structure: The form of an echolocation call in both time and frequency

Call Timing Classification (CTC): The classification of timing for a single call as either synchronizing, alternating, or undefined.

Constant Frequency (CF): The sustained portion of a high duty cycle call that is focused on a particular frequency.

Doppler Shift Compensation (DSC): The act of shifting emission frequency in order to compensate for movement so that the echo returns at the reference frequency.

Doublet call: A type of call that incorporates multiple calls, usually two, that are very close together in time. The first call is generally a shortened version of a solitary call followed by a short IPI and another call that is generally much shorter.

Echo Frequency: The frequency of the echo received by the receiver.

Echo: The sound received by a receiver after the sound has bounced off another object.

Flight path: The reconstructed path taken by a bat.

FMi: Initial FM portion of a high duty cycle call, preceding the constant frequency portion.

Forward masking: Masking caused by a sound that occurs before the target signal has been received.

Frequency difference: Difference in the echo, or emission, frequencies of two bats.

Frequency: The rate at which a sound pressure wave replicates per second.

Hertz (Hz): The amount of cycles per second.

High Duty Cycle (HDC) bat: A bat with a regular duty cycle $\geq$ 25%.

Initial FM (FMi): The frequency sweep that precedes the constant frequency of a high duty cycle call.

Interference: In acoustics, any extraneous signal that results that impairs the reception of the signal in some manner.

Interpulse interval (IPI): The time between the end of a call to the start of the following call.

Jamming Avoidance Response (JAR): A change in the behaviour of an organism with the intent to avoid jamming.

Jamming: Masking caused via signal from another sound producer (i.e. another bat).

Low Duty Cycle (LDC) bat: A bat with a normal duty cycle $<$ 25%.

LF (LF): The bat flying in front in a Paired Flight.

LF Corridor (LC): The corridor of the Lead Flights.

Loudness: The perception of the sound strength.

Masking: The action of another (masking) sound obscuring the target signal.

Paired Flight (PF): Flight paths of two bats that have been classified as flying as pair.

Parent portion: The first portion of a doublet call.

Path Timing Classification (PTC): The classification of a specific path as either synchronization, alternation, undefined, or random based on whichever type was the most dominant call in call timing classification.

Path Velocity (PV): The median velocity of a flight path.

PF Corridor (PT): The corridor of the Paired Flights.

Pulse rate repetition (PRR): The rate at which a bat calls per second, reported in Hz.

Reference Frequency: The echo frequency consistently received by a DSC bat through DSC.

SF Corridor (SC): The corridor of Solo Flights.

Simultaneous masking: Masking caused by a sound during the reception of the target signal.

Solitary call: The standard call of a high duty cycle bat.

Solo Flight (SF): Flight path of a singular bat that has not been classified as being flying with a conspecific.

Sound intensity: Sound power per unit area.

Spectral shifting: The shifting of frequencies as a response to jamming.

Strobe Groups (SG): Clusters of vocalizations by utilized by bat such as doublets and buzzes.

Subsidiary portion: The portion(s) that come after a parent portion. Generally only one but sometimes more.

Syn: The call timing classification of synchronizing.

Temporal shifting: The shifting of call timing as a response to jamming.

Terminal FM (FMt): The frequency sweep that follows the CF portion of a HDC call.

TF (TF): The bat flying last in a Paired Flight.

TF Corridor (TC): The corridor of Trail Flights.

Undefined (und): The call timing classification of undefined.

Velocity: The distance travelled per second by an object in a particular direction.

1 Introduction

For centuries the process of how bats perceive their world has intrigued scientists. It was not until the 1940s that the idea of echolocation was confirmed to be the primary sensory modality for most bats (Griffin, 1944; Griffin and Galambos, 1941). In the 70 years since, biologists' understanding of bat echolocation has grown, leading to revelations such as a bat's call structure changes dependent on the aim of the bat (e.g., foraging or commuting; Kalko and Schnitzler, 1993; Schnitzler and Ostwald, 1983)**,** calls are so specialized that a single call can be used for species identification (Vaughan *et al*., 1997), and echolocation can be so highly adapted that some bat species can identify insect species via echoes (von der Emde and Schnitzler, 1990).

1.1 Bat Perception

Bats rely on three main acoustic features of echoes for echolocation: time delay, sound intensity, and the amplitude spectrum of frequency. Time delay is the time it takes for the call to return after emission. It provides the target range of an object (Simmons, 1971). Sound intensity and time delay provide the cues for localization and size of the object (Simmons and Vernon, 1971). The interaural cues from the sound intensity, as well as time delay, enable the bat to localize the object both in azimuth and elevation (Chiu and Moss, 2007; Grinnell and Grinnell, 1965; Schnitzler, 1968 as cited by Schnitzler, 1973). The amplitude spectrum of frequencies provides the shape of the object (Simmons *et al.*, 1974). This spectrum can also provide the texture of the object, which can be used by bats to memorize guiding structures (Kugler *et al*., 2016). It has been hypothesized that as successive calls are made, the bat organizes the echoes into what has been termed as 'echo streams' (Moss and Surlykke, 2001). The timing of each distinct auditory source is unique and can be tracked as a stream, allowing bats to track each detectable object and create an 'auditory scene' (Moss and Surlykke, 2001).

Depending on the aim of the bat, the acoustic characteristics of the calls will be adjusted to fit specific needs. Foraging bats will emit long narrowband calls to search for prey. In pursuit of prey, bats will shorten the duration of calls and increase both call rate and bandwidth in order to focus on localization. Whilst flying close to clutter, bats will change their calls to allow for better localization. By changing the duration of the call, bats can alter the signal overlap zone, the timing area where emission and returned echoes occur at the same time (Kalko and Schnitzler, 1993). In cluttered environments, the duration must be shortened in order to avoid 'hiding' the echoes of nearby objects. The interpulse interval (IPI; time from the end of a call to the star of the next) is maintained so that during the 'search phase' the next call is not produced until the furthest detectable echo of a flying target would have returned (Holderied and von Helversen, 2003). Fast-flying bats in open space will occasionally skip calls, extending the range and IPI, allowing for further echoes to be returned from objects such as walls and hedges (Holderied and von Helversen, 2003). Skipping calls decreases the call rate and therefore the amount of acoustic images received, but some species alternate the call frequency between calls to separate echoes from further objects while still receiving updates on possible nearby objects (Jung *et al.*, 2007; Kingston *et al.*, 2003). Advancements in acoustic tracking technology in the field and analysis approaches have opened the door for detailed analysis of spatiotemporal movement and echolocation strategies, particularly when it comes to echolocating in the presence of conspecifics.

1.2 Flying with conspecifics

1.2.1 The Cocktail Party Nightmare

In flight, bats are actively sensing their environment through their echoes. The number of echoes returned to a bat is tied to the number of detectable objects and number of bats using biosonar concurrently. The number of echoes grows with the number of conspecifics. In order to orientate safely the bat must identify its own echoes amongst the conspecific echo clutter, which has been termed the 'cocktail party nightmare' (Ulanovsky and Moss, 2008). Emergence can involve as few as one to thousands of bats, like *Tadarida brasiliensis,* leaving the roost at the same time (Gillam *et al*., 2007). Most bat species do not congregate in such number as *T. brasiliensis*, but it does not take many conspecifics to create a 'cocktail party nightmare.' It has been suggested that even the presence of one conspecific negatively affects perception (Ulanovsky *et al.*, 2004).

1.2.2 Acoustic Interference

To orientate and navigate, each of the co-flying bats must extract its own signal from the acoustic interference caused by other individuals. Acoustic interference can be any extraneous audio signal that negatively affects the reception, or quality, of the target signal. One form of acoustic interference is masking, defined here as any obscuration of a target signal. There are three kinds of masking: forward, backward, and simultaneous**.** Forward masking is any masking signal that occurs before the target signal is received, such as a bat's own emission call covering up the returning echo (Schnitzler and Kalko, 2001). Backward masking is any masking signal received very soon after the target signal is received (Schnitzler and Kalko, 2001). Simultaneous masking is when both the target signal and masking signal are received at the same time. Masking by another individual's biosonar is referred to as jamming. Naturally, the risk of jamming increases in echolocators as the number of conspecifics increases. When flying in increased clutter, bat calls will change reducing the chances of masking and similar changes occur when bats fly close to conspecifics (Chiu *et al.*, 2009; Cvikel *et al.*, 2015; Holderied *et al.*, 2006; Kalko and Schnitzler, 1993). Any change in an echolocation call that reduces the effects of acoustic interference is defined as a jamming avoidance response (JAR; Jones & Conner, 2019).

1.2.3 Jamming Avoidance

JARs often involve adaptive changes in frequency and timing. Frequency shifting, also known as spectral shifting, is one of the more common JARs. Spectral shifting has been shown in recorded call frequency in *Pipistrellus pipistrellus* (Miller and Degn, 1981)*, Pipistrellus pygmaeus* (Bartonička *et al.*, 2007), *Pipistrellus nathusii* (Necknig and Zahn, 2011), *Tadarida teniotis* (Ulanovsky *et al.*, 2004), *Balantiopteryx plicata* (Ibáñez *et al.*, 2004), *Eptesicus fuscus, Lasiurus borealis, Lasiurus cinereus,* and *Euderma macalutum* (Obrist, 1995), and in the emitted frequency of *T. brasiliensis* (Gillam *et al.*, 2007). Separating frequencies from a conspecific call (in this text referred to as an allo-call) ensures that the bat can identify its own call (auto-call). However, some species, like *Taphozous perforatus* do not shift frequency in the presence of conspecifics as a JAR(Ulanovsky et al., 2004). A similar process was found to be in use by the frequency modulated (FM) bat *E. fuscus* while flying alone in clutter where calls were alternated in frequency (Hiryu *et al.*, 2010).

The alternation in call frequency 'tags' each call and allows the bat to accurately localize a previous echo even if another call has been produced (Hiryu *et al.*, 2010). In another study on the same species, bats flying near one another displayed recorded frequency separation relative to distance; the closer the bats were the larger the spectral separation (Chiu *et al.*, 2009). This same study also determined that the bats were timing their calls to avoid temporal overlap (Chiu *et al.*, 2009). Other species have also reported exhibiting similar temporal changes (Obrist, 1995). A study on *Noctilio albiventris* demonstrated that acoustic interference was ineffective at reducing ranging ability if the interfering source was outside the neural timing window, the window at which signals can be processed. (Roverud and Grinnell, 1985). In a study on *T. brasiliensis* JAR, the calling characteristics of the call were different in the presence of conspecifics, particularly in calling rate, duration, and IPI (Gillam *et al.*, 2007). Much like spectral avoidance, all these changes in call structure and timing can be used to tag specific calls and therefore avoid jamming. These and most other JAR studies have focused on low duty cycle (LDC) bats and not on high duty cycle (HDC) bats.

1.3 HDC Bats

Duty cycle (DC) refers to the percentage of time that a bat's signal is on compared to the pulse interval (the amount of time from the start of one call to the start of the next). Previous work has defined HDC bats as bats that exhibit search calls with a DC of ≥ 25% and generally use constant-frequency (CF) calls (Fenton *et al.*, 2012). In some HDC bats, such as *Rhinolophus ferrumequinum*, DCs are around 57% (Fenton *et al.*, 2012).

1.3.1 Call Structure and Role

LDC bats typically use broadband calls, which are distinct from the typical narrowband calls of HDC bats. The differences between the two calls are quite obvious (Figure 1). First, broadband calls are shorter and sweep over a larger range of frequencies (usually 1-20 ms; Fenton *et al.*, 2012), the narrowband calls are much longer and cover a smaller range of frequencies (usually >30 ms; Jones and Teeling, 2006). The structure of narrowband calls and broadband calls are typically quite different from one another.

The narrowband calls of HDC bats usually consist of three parts: an initial FM (FMi) sweep upwards, a long narrowband portion at a CF, and finally a terminal FM (FMt) sweep downwards. This creates a staple-like looking call. Prior research has determined that the FMt portion of the call is used primarily for target localization (Tian and Schnitzler, 1997) and clutter orientation (Jones and Rayner, 1989). The FMi portion is not always present in HDC calls and is sometimes shortened both in bandwidth and duration; there is still some discussion on its role. Jones and Rayner (1989) suggested that FMi plays a role in target identification and target ranging in clutter scenarios. The CF portion is extremely narrowband, the longest portion of the call, and plays a role in prey identification (von der Emde and Schnitzler, 1990) and localization (Müller and Schnitzler, 2000; Vanderelst *et al.*, 2011b, 2011a).

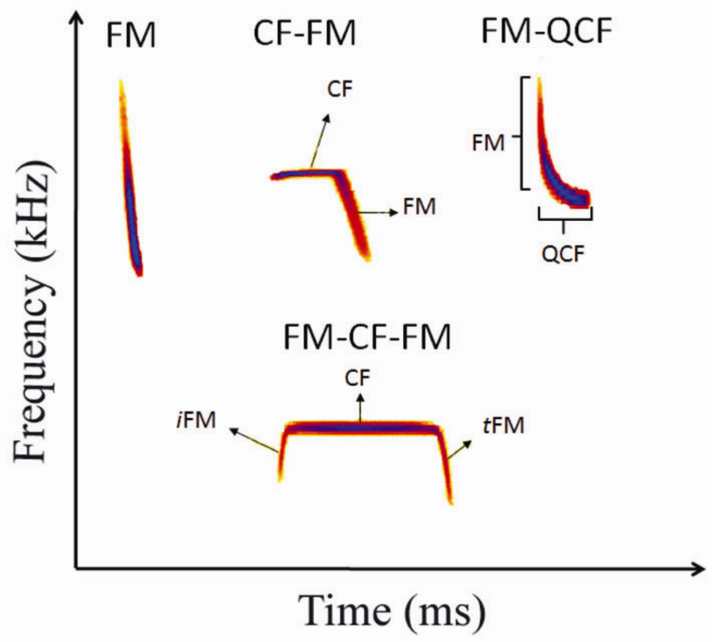

Figure 1: *Examples of the different types of echolocation calls used by bats. FM, CF-FM, and FM-quasiCF are common calls of LDC bats. FM-CF-FM is the standard call for HDC bats. Adapted from Raghuram et al. (2014).*

1.3.2 Localization via Pinna

The pinnae of HDC bats move continuously during the reception of echoes, while perched or in flight (Griffin *et al.*, 1962; Pye *et al.*, 1962; Pye and Roberts, 1970). This movement alters the echo's interaural intensity, allowing for greater localization than if the pinnae were stationary (Qiu and Müller, 2020; Schnitzler, 1973). Experiments that limited the ability of pinna movements confirmed its role in localization, particularly in the vertical plane (Mogdans *et al.*, 1988; Schneider and Möhres, 1960 as cited in Yin and Müller, 2019). Other studies on the pinna have shown that it plays a role in acquiring target angle (Gorlinsky and Konstantinov, 1978 as cited in Mogdans *et al.*, 1988) filtering out other echoes (Mogdans *et al.*, 1988), and pinna movement allows for the localization of the CF component (Vanderelst *et al.*, 2011b, 2011a).

1.3.3 Auditory Fovea and Doppler Shift Compensation

HDC bats possess an auditory fovea: a modified cochlea where a disproportionate section of its length is dedicated to a narrow range of frequencies**.** The auditory fovea provides a frequency band of high sensitivity and high frequency resolution at the cost of being less sensitive at frequencies outside of that range. For example, audiogram studies have shown that *R. ferrumequinum* is highly tuned to frequencies in the 82-85 kHz range, losing sensitivity outside of that range (Neuweiler *et al.*, 1971**;** Figure 2). The frequencies of maximum sensitivity are referred to as the reference frequency. Research has shown that HDC bats actively change the emission frequency of their calls in order to ensure that the echoes of their target are received at their personal reference frequency (Hiryu *et al.*, 2008; Schnitzler, 1970 as cited in Schnitzler and Denzinger, 2011; Trappe and Schnitzler, 1982). This changing of frequency is known as Doppler shift compensation (DSC). This DSC occurs mostly in flight, where the emission frequency is shifted to compensate for the speed of the bat (Schnitzler, 1973). This DSC also allows HDC bat to avoid forward masking via their own calls. The long duration of a HDC call results in echoes returning before the emission is completed. To accomplish DSC the emitted frequency has to be lowered (due to the physics of Doppler shifts) in order for the echo frequency (the frequency received by the bat) to be returned near the reference frequency (Schnitzler, 1973). The separation in frequency required to compensate for the flight speed is large enough that the bat avoids forward masking (Schnitzler, 1973; Schuller, 1977, 1974).

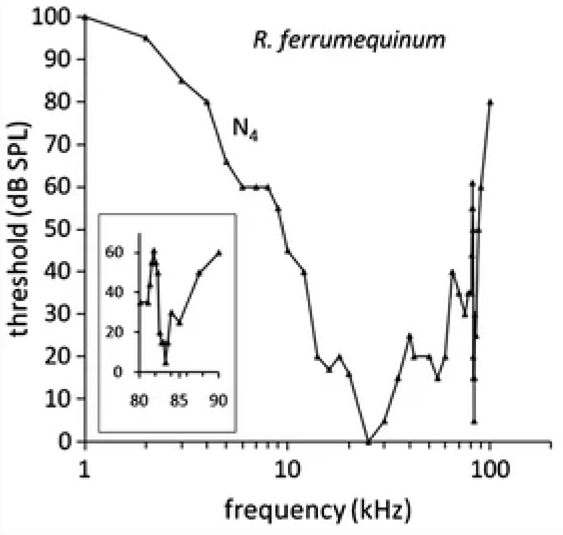

Figure 2: *Audiogram of R. ferrumequinum with an inset that shows the frequency range of highest sensitivity (80-90 kHz; adapted from Schnitzler and Denzinger, 2011).*

1.3.4 JAR in HDC bats

Jamming avoidance is more difficult for HDC bats than LDC bats. The auditory fovea limits the amount of bandwidth that HDC bats can effectively echolocate (limiting spectral shifts) and the long duration of the calls limits the amount of possible temporal separation. Without a JAR, temporal and spectral jamming could be quite common in HDC bats. Despite this apparent propensity for jamming, JARs in HDC bats are understudied. This is likely due to the fact that only three families are known to regularly use HDC calls: Rhinolophidae, Hipposideridae, and *Pteronotus parnelli* within Mormoopidae. It has been argued that HDC bats cannot use spectral JAR due to the narrowband range of effective frequencies dictated by the auditory fovea (Jones *et al.*, 1994; Jones and Rayner, 1989). In a JAR study on *Rhinolophus capensis,* researchers did not find any spectral shifting in the CF component but they did observe changes in the FMt portion of the call (Fawcett *et al.*, 2015). However, these researchers suggested that it was not an actual JAR but rather a change more akin to that of reacting to an obstacle rather than a conspecific. A few other studies have also argued that previous JAR claims (in both LDC and HDC bats) are actually call changes in response to proximity to a new 'obstacle' (the conspecific) and should not be counted as a JAR (Cvikel *et al.*, 2015; Götze *et al.*, 2016). Another study attempted to determine if *R. ferrumequinum nippon* changed reference frequency in the presence of conspecifics (Furusawa *et al.*, 2012). They were unable to find evidence of spectral separation, although they found some evidence of shifting reference frequency closer together, which would appear to increase the chances for jamming (Furusawa *et al.*, 2012).

Reference frequencies for HDC species are very narrowband and stay relatively consistent over the bat's lifetime (Jones and Ransome, 1993). Studies have shown that multiple Rhinolophid adults emit a higher frequency than juveniles(Chen et al., 2009; Jones and Ransome, 1993; Russo et al., 2001; Siemers et al., 2005; Yoshino et al., 2006). Jones and Ransome demonstrated that *R. ferrumequinum* juveniles increase frequency over their first two years and then stabilize until the effects of old age force a change (1993). That same study saw changes in call variation as the seasons changed but other research has shown that frequency is correlated with body temperature (Huffman and Henson, 1993; Jones and Ransome, 1993). In 1967, Möhres suggested that *R. ferrumequinum* could identify conspecifics via calls (Möhres, 1967 as cited in Jones and Siemers, 2011). This has never been confirmed for R. ferrumequinum but there is evidence for conspecific identification in *M. lucifugus* (Kazial et al., 2008) and *M. myotis* (Yovel et al., 2009). Such consistency in reference frequency, and the ability to pick out individuals, could minimise the chance of jamming. If each bat kept itself to a 'personal' reference frequency and is aware of the conspecific reference frequencies of its roost mates, those they are the most likely to run into jamming situations with, than spectral separation could possibly be maintained between each bat. This use of a 'personal' frequency would likely remove the need for another form of JAR.

1.3.5 Guiding Structures

Bats may be flying in close proximity to one another for a variety of reasons, including foraging in a particularly cluttered feeding site, being attracted to the foraging site by the sound of feeding buzzes and joining conspecifics (Bastian and Schmidt, 2008; Boughman and Wilkinson, 1998; Dechmann *et al.*, 2009; Dorado-Correa *et al.*, 2013; Wilkinson and Boughman, 1998), emergence, or even as an emergence tactic for avoiding predators (Fenton *et al.*, 2012). Naturally, emergence comes with the highest chance of encountering a conspecific. Bats tend to take consistent emergence paths night after night (Racey and Swift, 1985). The paths and distance from clutter are influenced by the bat's speed and prey preference (Denzinger and Schnitzler, 2013). *R. ferrumequinum* has been classified as a 'narrow space flutter detecting forager', which means that this particular bat travels near clutter (Denzinger and Schnitzler, 2013). Although HDC are considered 'narrow-space' bats they rely on edge space, like many other bats, for navigation and orientation (Denzinger and Schnitzler, 2013; Hein *et al.*, 2009). Bats that rely on guiding structures for navigation and orientation do so even if it does not appear energetically efficient (Mostert and van Winden, 1989 as cited in Limpens and Kapteyn, 1991; Murray and Kurta, 2004). One such bat, *Myotis sodalis*, has been described as increasing the distance travelled by around 55% by only using edge space (Murray and Kurta, 2004). Another study observed bats taking a long route via a corridor when flying in open space would have surely been a quicker route **(**Mostert and van Winden, 1989 as cited in Limpens and Kapteyn, 1991).

These guiding structures can range from objects like tree lines to hedges and canal banks to walls (Britton *et al.*, 1997; Krull *et al.*, 1991). The distance from guiding structures is generally kept constant, even from year to year (Schaub and Schnitzler, 2007). Research on why these animals prefer edge-space suggests that it may be due to reduce the chances of predation, reduce the effects of wind, create easier spatial mapping, and a chance to encounter prey (Limpens and Kapteyn, 1991; Racey and Swift, 1985; Rodriguez-Duran and Lewis, 1985).

1.4 Aims

Emerging *R. ferrumequinum* were studied to characterize their JAR strategies during commute. Any JAR that might exist in this species should fall under one of two categories, which I have termed: (1) Spatial JAR and (2) Acoustic JAR.

1.4.1 Spatial JAR

A natural reaction to a source creating interference is to move away from that source (e.g., moving away from a crying child while having a phone conversation). The spacing between bats, however, is not usually discussed as a particular type of JAR even though the differences in distance and position from one another can influence the separation between auto- and allo-echoes.

Due to the high attenuation of sound in air, particularly at high ultrasonic frequencies, changes in distance could provide enough difference in amplitude to avoid jamming. Chiu *et al.* (2009) have already demonstrated that the distance between bats plays a role in the amount of spectral shifting required for a JAR. In *P. pipistrellus*, the maximum detectable range of prey echoes is about 3.5 m (Holderied and von Helversen, 2003). Beyond that echoes become too attenuated to be detected (Holderied and von Helversen, 2003). The maximum detectable range for structures along a path is larger due to the size of the object but the principle remains the same, at a certain distance the echo is no longer detectable. Naturally, two bats flying at a distance larger than the maximum detectable range will never experience any jamming. It is those that are within that range that run the risk of jamming. If a bat finds itself being jammed, it could increase the distance between itself and the conspecific, thereby altering the respective sound intensities. At a certain distance the echo strength of allo- and auto-calls will be so large that they can be separated.

The relative position of the bats could also provide a JAR. Bates *et al.* (2011) demonstrated that echoes returning from off-axis can be separated from on-axis echoes via amplitude discrimination. The LDC bat *E. fuscus* has been shown capable of being resilient to masking if echoes were returned at an angle greater than 23˚ from the original beam direction (Sümer *et al.*, 2009). Therefore, the relative positions of conspecifics could act as a JAR without the need for changes to either call, even if they were ensonifying the same target as the echoes may be received at an angle different from the auto-call.

Flight paths could be more variable in the presence of conspecifics. Flight paths exiting the roost tend to be repeated night after night (Racey and Swift, 1985). Orientation along these paths may become more difficult in the presence of conspecifics. Cluttered emergence paths, or those with obstacles, could become especially dangerous as jamming can cause ranging errors possibly leading to collisions with these ‘obstacles’ (Roverud and Grinnell, 1985). Bats have been found to build spatial maps (Jensen *et al.*, 2005; Neuweiler and Möhres, 1967 as cited in Ulanovsky and Moss, 2008; Winter and Stich, 2005)**.** In this scenario, the bat remembers the emergence path and knows an obstacle is approaching. However, due to the localization error caused by masking the bat cannot actively localize the object as accurately as normal. Without an acoustic JAR, the bat could theoretically rely on its memory and give the obstacle a wider berth than normal (i.e., take a wider turn) thereby decreasing the risk of collision.

1.4.2 Acoustic JAR

As discussed earlier, temporal and spectral shifting has been described in other bat species and could be a tool for *R. ferrumequinum* as well. Call structures changes are extremely common in LDC bats (e.g., changing bandwidth) and they have been described in HDC bats, although not to the same extent as LDC bats. Like LDC bats, it is well established that *R. ferrumequinum* will change their calls in the pursuit of food, or in landing, although the general staple structure of the call remains mostly consistent (Tian and Schnitzler, 1997). This indicates that HDC bats have the capacity to possibly adjust temporal components of their calls, making it possible to better space out their calls amongst one another. The full alternation of calls is unlikely (i.e., each bat taking 'turns' calling), and would be easily observed, due to the DC of these bats.

Previous research has argued that spectral shifts are unlikely in HDC bats (Fawcett and Ratcliffe, 2015; Jones *et al.*, 1994), however, spectral changes have been observed in FMt (Fawcett and Ratcliffe, 2015). A lab study on *R. ferrumequinum nippon* has also suggested that auto-reference frequency is shifted closer to the conspecific's auto-reference frequency (Furusawa *et al.*, 2012). The same study suggested that this species is capable of separating echoes that were different by 0.1% (0.07 kHz) of the resting frequency based on the previous work on *P. parnelli* (Furusawa et al., 2012; Riquimaroux et al., 1991, 1992). *R. ferrumequinum* likely has a similar discrimination threshold and may display spectral shifts of a similar amount (~0.08 kHz). To the best of my knowledge, no study has attempted to compare the auto-reference frequency and the allo-reference frequency of HDC bats to confirm the absence of spectral shifting in the presence of conspecifics.

1.4.3 Hypothesis

In this study, I found it useful to discriminate and investigate four separate JAR categories:

1. Flight path alteration
2. Change in calling characteristics (duration, DC, etc.)
3. Temporal (call timing)
4. Spectral (frequency shifts or separation)

Path alterations, calling characteristics, and temporal changes are the most likely avenue of JAR for *R. ferrumequinum*. Path alteration could help make auditory JAR more effective or could be effective enough on its own that acoustic JAR is unneeded. Temporal changes are likely going to be tied to calling characteristic changes, as that would be the most efficient way to separate conspecific calls. Alternating calls would require the shortening of call duration and specific timing of calls. Spectral JAR is perhaps unlikely because of the fixed frequencies of the auditory fovea, but not entirely out of the question. Other species have shown effectiveness in the use of spectral JAR. The auditory fovea of *R. ferrumequinum* is extremely sensitive and may be capable of sorting out conspecific echoes that are only marginally shifted from the bats' own echoes. To the best of my knowledge, this is one of the few studies to examine HDC bats for JAR during emergence and the first to track and record emerging HDC bats.

2 Methods

2.1 Fieldwork

2.1.1 Study Site

The study was conducted at Stackpole Estate, Pembrokeshire, Wales, on 31 August 2019 from 1900 to 2100. A roost of *R. ferrumequinum* was identified within the north end of a building located on the estate (Figure 3). The bats would exit the roost and fly alongside the building wall running towards the west. The west end of the building was met with a hedge protruding at a right angle from the building and was followed by bats in emergence flight.

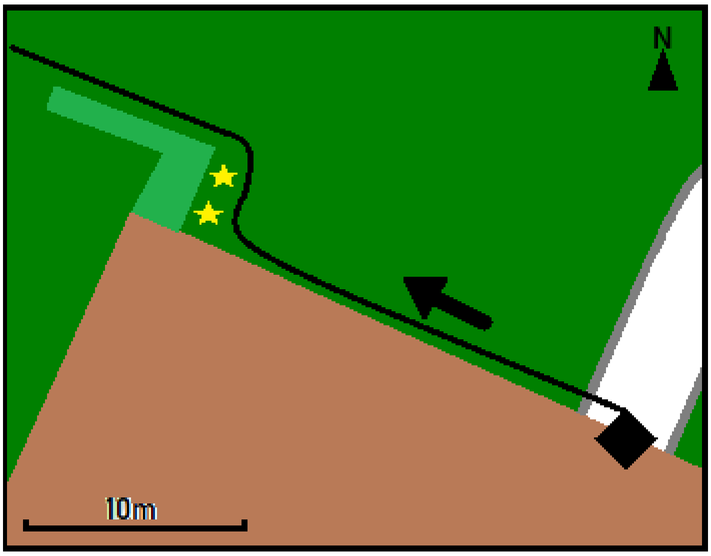

Figure 3*: Top-down view of the field site in Stackpole, Wales. Stars denotes placement of the microphone arrays. The green polygon represents a hedge that influenced the flight path of the bats. The diamond indicates the roost emergence point and the black line indicates the generic flight path of the bats as visually observed during the recording session. The arrow indicates flight direction of emerging bats. The brown portion indicates the roost building, and a small road is denoted by the white path.*

2.1.2 Acoustic Flight Path Tracking

2.1.2.1 Microphone Array

Two microphone arrays, each comprised of four Knowles FG-O microphones (USG Omnidirectional Electret Ultrasound Microphone**,** Avisoft Bioacoustics, Glienicke/Nordbahn, Germany) were arranged in a star-shaped pattern. Each microphone was 0.58 m from the central microphone [identified as Ch.1 in Array 1 (A1) and Ch.5 in Array 2 (A2)]. These microphone arrays allow for the capability to determine the position at the time of emission and therefore recreate a recorded bats flight path (see 2.2.2 for more detail; Holderied & Von Helversen, 2003).

2.1.2.2 Recording Equipment and Operational Parameters

Using an Avisoft UltraSoundGate 1216H (Avisoft Bioacoustics, Glienicke/Nordbahn, Germany), sound files were recorded at a 16-bit resolution and sampled at 500 kHz. The sound files were recorded as .WAV files. Sound file creation was automatic and initiated when a bat call was detected by one of the microphones at 1% of the max level. The sound recording lasted until a bat call had not been detected for 2 s**.** The recording system was set up with a 2 s ring buffer. Sound was continuously recorded in this ring buffer. Once the 1% threshold had been met, a new sound file was created and the recording of the ring buffer was added to the start of the sound file. This buffer did not start rerecording until the sound file was completed. The buffer had to be filled before the next file could be recorded, leading to a minimum of 2 s between each sound file.

Recording was to start with the emergence of the first bat and end at the cessation of emergence activity. Previous studies have reported that the median emergence times of *R. ferrumequinum* in England is 31 minutes after sunset (Jones and Rydell, 1994) and that *R. ferrumequinum* emergence typically lasts 30 minutes from start to finish (Funakoshi and Maeda, 2003). For this study, emergence activity was considered completed once 30 minutes had passed since the first bat and no other bats had been observed emerging for five minutes.

2.1.3 Acoustic Recording

On the night of recording, the arrays were placed 1.64 m apart, A1 was angled 5˚ towards A2 and A2 was angled 6˚ towards A1. The central microphone of both arrays was 0.91 m from the ground and A1 was 1.15 m from the building wall. Arrays were placed in front of the hedge (Figure 3) so that the bats would fly directly towards and pass in front of the microphones. Recording was initiated with the emergence of the first bat, which occurred at 2023, 15 minutes after sunset. At the time of recording start, temperature was measured at 15˚C with a relative humidity of 65%. Emergence activity, and therefore recording, ceased at 2100, 52 minutes after sunset. A cursory emergence count was conducted during the recording with two observers. This count was intended as a general sense check on the numbers of bats recorded and was not treated as a proper emergence count and will not be used in any further analysis.

2.2 Data Analysis

2.2.1 Recording Analysis

Every sound file spectrogram was visually checked through the Sound Analysis and Synthesis Laboratory (SASLab) v5.2.14 (Avisoft Bioacoustics, Glienicke/Nordbahn, Germany). Each file was manually inspected for total number of bats, overlapping calls, presence of non-target species, and any technical or recording glitches. Any files with technical errors (defined as a sound file created but no bat present or random sound ‘clicks’ within a file) were excluded (*n* = 11). Files only containing one bat were classified as a ‘Solo Flight’ (SF; *n* = 54). Some files contained multiple bats but lacked any overlap in time between individuals, meaning that the calls of the first had ceased being recorded before the calls of the second had started. As long as there was at least 0.3 s between the last call of the first bat and first call of a second bat, both flights were deemed as a SF. Files with any overlapping bat calls were defined as a pair, hereafter referred to as a ‘Paired Flight’ (PF; *n* = 45). Due to time and technical constraints, files with more than two overlapping bats were excluded from further processing (*n* = 36).

2.2.2 Path Creation

Reconstruction of bat flight paths was based on previous acoustic flight tracking methods by Holderied and von Helversen (2003). All path creation work was done through a combination of scripts in MATLAB v9.4.01 (The MathWorks, Natick, MA, USA). Each microphone recorded arrival times for every call. The difference in arrival time between the microphones was then used to localize the bat. However, the study that this technique was based on localized LDC bats rather than HDC bats and the sound files and analysis methods required some adjustment.

The MATLAB script 'removeCF.m' (de Bij and Holderied, 2016)was used to automatically identify and remove the horizontal portions (i.e., the CF portion of the call) of the spectrogram. An adjusted .WAV with only the vertical (i.e., FM) portions of the call were outputted by this script. By utilizing only the FM portions of the call, localizations occurred at the start and end of the HDC call. These files were then converted into .ORT files via the 'WAV2ORT.m' script (Holderied, 2015a).This script automatically converts the acoustic sound information of the adjusted .WAV into geometric points. This is done by taking the recorded time of each call and determining the time delay between each microphone. A localized point was created based on the time delays of each array. The official localized point was determined by taking the angle from each array to its respective localized point, the intersection of those angles would be the localized point. Calls that did not occur in every microphone within a specific array could not be localized by this process. Localizations did have a margin of error of 0.2-2% of the distance from the array.

Localized points from 'WAV2ORT.m' were then visually analysed via the script 'Batsonar2p4.m' (Holderied, 2015b) to confirm localizations and turn individual points into a path. 'Batsonar2p4.m' provided a visual representation of the localized points that could then be manually manipulated. Outlier points, obvious triangulation errors, or calls that belonged to another bat, were manually removed before any other steps were taken. An automated path function within the script connected the points based on the limitations of flight velocity. The maximum recorded speed of *R. ferrumequinum* is 8.3 ms^{-1} while foraging (Ransome, 1991). Studies have shown that bats fly faster when commuting versus foraging (Britton *et al.*, 1997; Grodzinski *et al.*, 2009; Jones & Rayner, 1989). A study comparing *P. kuhlii* foraging and commuting speed recorded a difference of around 2.5 ms^{-1} (Grodzinski *et al.*, 2009). Since emergence flight is commuting flight, path construction was not restricted to the recorded maximum speed. For this study, the maximum possible flight speed 'allowed' was 15 ms^{-1}, nearly double that of the record. *R. ferrumequinum* were not expected to fly at these speeds, rather any paths that came close to this limitation (>12 ms^{-1}) were inspected manually for localization errors. If the velocities of the surrounding points were very dissimilar or preceding points did not indicate that the bat was in the process of consistently changing speeds (i.e., slowing down or speeding up) the point was removed. The point was also removed if it did not ‘fit’ into the general structure of the path.

These completed paths would then be visually inspected for the smoothness of their trajectories and the smoothness of the velocity profile. Due to the nature of the ‘connecting-the-points' of the path function, occasionally paths would have jagged, or abrupt, portions that would be impossible in actual flight. Velocities of the bats were also subject to this error. The ‘BatSonar2p4.m’ script provided the capability of analysing both the path and velocity changes over time. Velocity was manually inspected for spikes that did not match the rest of the path. Paths were manually inspected for the aforementioned jagged portions. Any point that was deemed unfit was removed to create a more realistic flight and velocity profile, while preserving the general shape of the path.

Some of the erroneous points were due to the triangulation error of a particular array. The times of the removed points were noted and the paths were manually rechecked. If one of the arrays provided a point that fit and the other array provided a clear error, then the non-error point would be manually incorporated into the flight path. However, if the triangulated point based solely on either array still did not fit the velocity or path profile then it was not reinstated.

Not every call from the bats could be localized by the scripts, for example calls that did not make the amplitude threshold or did not appear in every microphone. The script ‘BatCall_V10.m’ (Holderied, 2019a) was used to interpolate the missing points and create a full reconstruction of the flight path. The spectrogram of each file and the corresponding flight path were automatically linked together. Through the spectrogram, un-localized calls and error calls removed from earlier in the path creation process were visually identified through the spectrogram and then interpolated on the flight path based on the time of the call and the structure of the flight path. To avoid over-interpolation, paths were required to have at least five actual triangulated points to construct the flight path.

2.2.3 Path Creation: adjustments for PFs

PFs underwent the same process as SFs with a few differences. First, the automated system for connecting the flight path created impossible zigzag paths and points which had to be connected manually. The PF paths were created by using ‘flight logic’ (i.e., the bats were always flying in the same general direction and were not going to initiate any jack-knife turns as the automated path suggested) and by referring to the timing of the calls in the spectrogram. The interpolation process was generally similar, except the non-adjusted spectrogram was consistently cross-consulted to confirm which call belonged with which bat call sequence. The extra time, effort, and uncertainty that the addition of a third bat would have caused is what led to the decision to analyse only pairs of bats but not larger groups. The first bat that was recorded in a PF was termed the Lead Flight (LF); the second bat was termed the Trail Flight (TF).

Only PF when the time delay between the LF and TF was less than 1.2 s (temporal separation) were analysed (*n* = 17), which ensured sufficient overlap in flight paths with an adequate number of calls to analyse. Temporal separation between the bats in each PF was calculated by determining the amount of time it took for the TF to reach a specific number of meters from the array in the Y direction, after LF passed that same point. In this particular study, 5 m was selected as the point due to every path being tracked at 5 m and usually not yet starting its turn in front of the arrays. By using this measure, the temporal separation value would be relatively unaffected by any changes in speed that may be caused by the bat adjusting for the turn.

2.2.4 Path Restrictions

I analysed paths 8-2 m from the arrays in the Y direction unless otherwise stated. Localization error increased beyond 8 m, and at less than 2 m the target focus likely changed.

2.2.4.1 Localization error

The first localizations of a path have higher localization errors as error increased with distance from the arrays. Most starting localization calls occurred in the 10-8 m range but the velocities in this range varied wildly. By 8 m velocity had usually steadied and localizations were more reliable. The 2 m restriction was partially used due to a different kind of localization error. Nearly half of PFs (*n* = 8) had at least one path that was not completely localized as it crossed the arrays. This was likely due to overlapping calls that affected the ability to localize. Recording quality of the FM portions also influenced the ability to localize and would cut off some paths. This error was present occasionally in SF (*n* = 4), but to keep the two datasets comparable in analysis the 2 m cut-off was applied.

2.2.4.2 Target Focus

The 2 m restriction was also used due to the likely change in target focus of the bats. Past the 2 m mark nearly all of the bats were either beginning to cross or already crossing in front of the arrays. As *R. ferrumequinum* are flying towards the hedge, they need to be constantly updated on the locations of the building wall and the hedge. The echo from the hedge is being returned at the maximum Doppler shift; as the bat is flying directly at the hedge it is the likely focus target. The wall is the guiding structure and the Doppler shift of the wall echo is at zero because it is parallel to the bat. Once the bat begins its turn, Doppler shift of the hedge echo changes and is no longer the main target of interest as the bat looks forward to the next portion of its path.

The 8-2 m restriction was also used due to increased likelihood of jamming. In this section, the bats are moving in a generally direct path towards the hedge. If both bats are ensonifying the hedge, then the chances of hearing similar frequencies increases thereby increasing the likelihood of jamming. If the LF has turned and TF has not, it is next to impossible for TF to jam LF with its echoes as the Doppler shift of that echo would be shifted below the LF's auditory fovea (a more detailed discussion is found at 4.2.2).

2.2.4.3 Other restrictions

Only flight paths that followed the general emergence path (Figure 3) were used. Paths that flew over the hedge (*n* = 5), flew the opposite direction (*n* = 1), or flew in a 'complicated' path (*n* = 3; e.g., turning around) were excluded from analysis.

2.2.5 Path Analysis

The output files from 'BatCall_V10.m' calculated the velocity, Doppler shift, the time, and geometric positioning of each localization. Each localization point was either a start or end of a call. Measurements were taken for those points as the long duration of the CF portion makes it impossible to localize. By taking each start and end point, measurements for velocity and Doppler shift can be averaged to find a Doppler shift and velocity for the call rather than just localizations. Position to position velocity was calculated automatically through 'Batcall_V10.m' by subtracting the distance between previous localization and the next localization, divided by difference in time between the two. The relative velocity towards Ch. 1 was used to calculate the Doppler shift for each localization. The Doppler shifts for each start and end of a call were averaged out to deduce the Doppler shift of the CF portion of the call. Path velocity (PV) was calculated by finding the median velocity of each path.

The point where the bat began its turn (i.e., turning point) was found by developing a MATLAB script 'Trajectory.Workshop.m' (Holderied, 2019b). The turning point was defined as the first localization where the bat would have had to turn more than 10˚ to arrive at the next localization point. Turning points for every bat were identified and then averaged to deduce the average turning point based on the flight path type (SF, PF, LF, or TF).

Paths were divided into three 2 m phases: initial (8-6 m), mid (6-4 m), and turn (4-2 m). The initial phase covers the earliest localizations and are generally characterized by a straight approach. Paths in the mid phase were mostly heading straight towards the hedge but some bats began to turn. The turn phase was characterized by nearly every bat either starting or already turning. Based on these divisions the median height (Z) and lateral (X) position were calculated per path. These medians were then averaged to find the average height and lateral position of SFs, PFs, LFs, and TFs.

The paths of each flight path type were grouped and a standardized 'corridor' was created. Corridors were created by cutting the paths into half meter sections and calculating the kernel density. This provided a density plot at each half meter increment. These plots were converted into a polygonal slice and then joined together creating a corridor that the ‘average’ bat of that path type would fly in. Corridors were cut off at 2 m as the PF did not provide enough points to continue the corridor towards the array. This was completed by using the newly custom MATLAB script ‘plot_corridors.m’ (Holderied, 2021). Corridors were overlapped one another to identify differences in height, width, and shape.

PFs were placed within the SF corridor (SC) to determine similarity in flight path. To determine if PFs truly had a different corridor from SF, the percentage of paths contained within the SC was calculated. This was done by creating paths that had points at 0.5 m intervals and placing the SC over the path via ‘ST_comp_percentage_of_points.m’(Bollinger and Holderied, 2021). The amount of points outside of the SC were compared to the total amount of points present to provide a rough estimation of the percentage of the path contained by the SC (referred to as SC percentage; Figure 4).

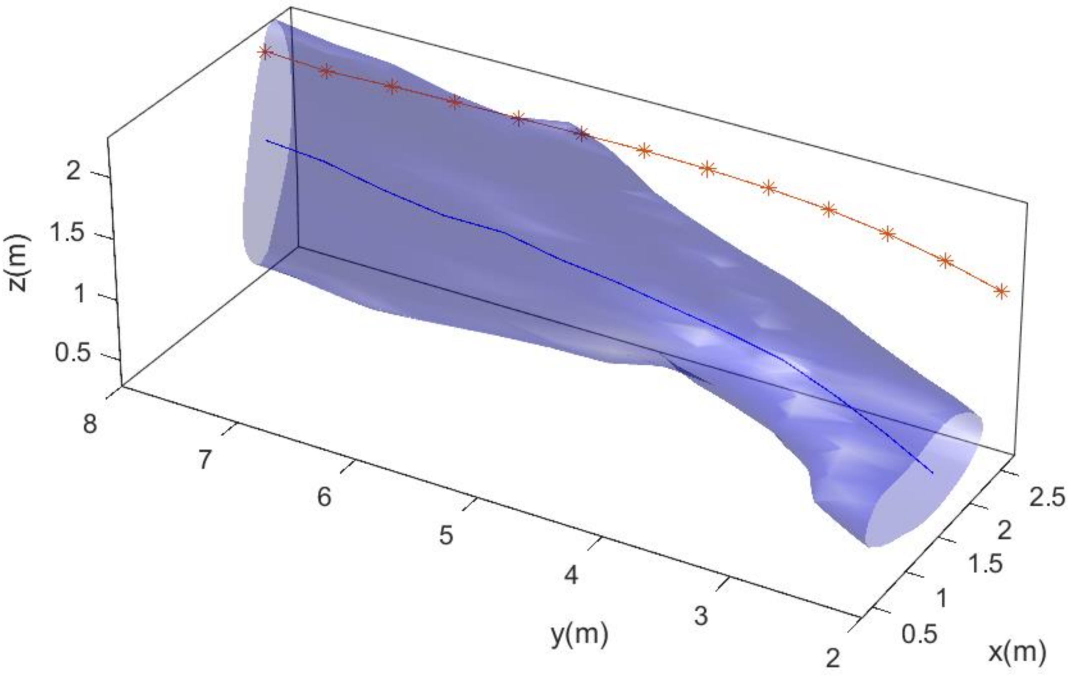

Figure 4: *An example of the comparison figures used to determine path containment. Each asterisk is the location of the bat in 0.5 m increments. A point was considered as contained if it was within the SC (the blue 3D structure).*

2.2.6 Statistics

Statistical analysis of the data were performed using Rstudio v1.2 (RStudio Inc., Boston, MA, USA.). For this study, the alpha level was set to 0.05. The PVs of SF and PF were compared via the Student's T-test. PVs of SF, LF, and TF were compared via ANOVA and subsequently underwent a Tukey test to determine the level of difference amongst the three groups. Individual Pearson's Product-Moment Correlations were run to determine if PV had any relationship to emergence time after sunset (SF only), LF and TF PV was related, LF (and TF separately) PV was related to temporal separation, and if the difference in PF PV was related to the temporal separation. A chi-square test was used to determine if PF SC percentage was different from SF SC percentage.

2.3 Acoustic Data

2.3.1 Acoustic measurements

Each path's original .WAV file was manually turned into a spectrogram for manual manipulation based on Ch. 1. Ch.1 was used as it was in the position deemed the best for receiving calls as the bats directly approached the array and gave high quality calls of the bats even as they turned away from the microphone. Microphones on A2 were good for picking up calls as the bat turned but had a much lower quality of calls before then. Acoustic data were extracted for all SF and PF through Avisoft-SASLab Pro version 5. The spectrograms were analysed via a 256-FFT window with 75% overlap. Any echoes were manually removed via the erasing tool.

2.3.1.1 Adjustments for PF

The acoustic measurement processes for PFs were similar with a few key differences to SFs. To create a spectrogram for each path in a PF, the file had to be analysed twice. The first time, all TF calls were removed, providing a LF spectrogram. The second, with all LF calls removed, creating a TF spectrogram. When there was uncertainty about which call belonged to which path, the path figures were referred to as they could provide positional information at the time of the uncertain call. For example, if two calls were recorded very close to one another with so much overlap that it was impossible to tell which FM portion belonged to whom, then the path figure would be referred to. The path would provide the localization point of both calls and the time of the calls, clearly showing which call belonged to which. Ultimately this process created two spectrograms (one for LF and the other for TF) and both were analysed separately from one another.

2.3.2 Strobe Group Identification

At times, bats will call quickly in succession outside of their normal calling rate. This usually occurs during feeding buzzes but does happen as a reaction to clutter and helps in localization (Tian and Schnitzler, 1997). Previous work has separated bat calls into call, or strobe, groups (SGs) for LDC bats

(Kothari *et al*., 2014; Moss *et al*., 2006; Moss and Surlykke, 2001). In this study, calls were defined as belonging to one of four categories: solitary, doublet, buzz, or unknown. Solitary calls were defined as a standard *R. ferrumequinum* call, usually lasting around 40 ms with an IPI $\geq$ 15 ms. These calls demonstrated the standard *R. ferrumequinum* calls. Doublet calls were any calls that constituted a train of calls that usually had shorter than normal IPI (approximately < 15 ms; Figure 5) and duration. Despite the name, doublets could involve more than two calls but were typically only two. The first call in a doublet was termed the parent call and any call after that was termed the subsidiary call**.** A buzz would have been any group of calls that occurred in rapid succession, giving the appearance of a feeding buzz, but no such calls were ever observed in this study. Unknown calls only occurred in PFs and were any call that could not be confidently identified as a solitary or doublet, usually due to a missing start or end to either that call or the calls around it.

Unknown calls only occurred in portions of heavy overlap in calls. The IPI between an unknown call and its preceding and following call was calculated to determine if the call was an unidentified doublet call. The duration of combining the preceding and following calls was also used. If the duration was found to be longer than 100 ms from the preceding IPI to the FMi of the unknown or the IPI from the unknown FMt to the following call was longer than 15 ms**,** then the call was identified as solitary. Otherwise, it would be classified as a doublet call and placed appropriately as either the primary or subsidiary call. There were some instances where it was impossible to make a determination. These remained classified as unknown calls (*n* = 13).

SGs were manually identified by visual inspection of each path spectrogram. Automatic measurements (based on the duration of 40 ms and IPI of 15 ms) were then used to confirm classifications. In scenarios of disagreement between automatic and visual classification, the call was visually re-examined to determine classification.

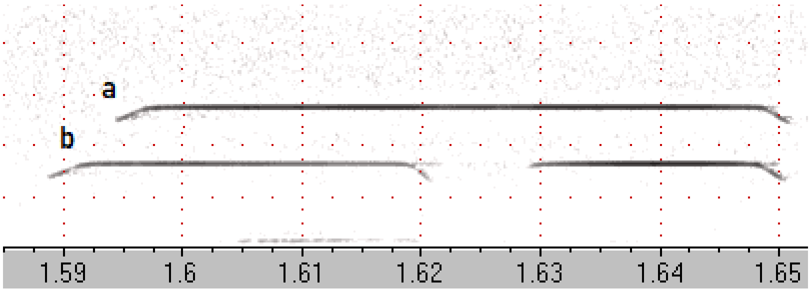

Figure 5: *Spectrogram examples of a defined solitary (a) and doublet (b) call. The first part of the doublet (parent call) looks extremely similar to that of a solitary call albeit shorter. The second part of the doublet (subsidiary call) was quite often present with a non-existent or extremely short FMi sweep. Frequencies are not representative.*

2.3.3 Acoustic Parameters

An automated spectral parameter (Automatic Parameter Measurements**,** settings in Appendix 4) tool in SASLab provided call duration, IPI, recorded start and end times, peak and minimum FMi frequency, peak and minimum FMt frequency, and peak and minimum for mean frequency (i.e. the recorded CF) for every call.

When PF calls overlapped, CF could not be automatically measured. If two calls were overlapping, only the most intense CF would be provided by the automated system. When required, CF were manually extracted by using a fast Fourier Transform (FFT) power spectrum tool (Hamming window). This tool provided the energy spectrum of frequencies. If a portion of a call was clearly not overlapped, that section would be measured by the FFT tool and that frequency would then be assigned to that particular call. When calls overlapped, two distinct peaks were observed in the FFT tool and then recorded. In these overlapped calls, the frequency was assigned based on the trend. For example, if frequency of the focal bat was continually dropping as it approached the array, then the frequency that was closest, and lower than, that focal bat's previous frequency would be assigned to that call. Only 2.2% of calls were not assigned a CF (n = 33).

These steps were not put into use for FMi and FMt frequencies; they were only ever recorded via the automated tool as emission and echo of CFs was the main focus of this project in regards to spectral shifting. FM portions were successfully measured 83% of the time. In general, FM portions were more likely to be too quiet to measure and shortened FMis were likely to be lost in overlapping calls when the small spectral sweep was covered by a conspecific CF. The bandwidth of FMi and FMt was then determined by subtracting the minimum FM frequency from CF. Any FMi that was not lower than 76.0 kHz was not used in this analysis as these were considered shortened bandwidths.

DC of each call was calculated by dividing the call duration by the full time of the pulse (duration + IPI) and reported as a percentage. Pulse Rate Repetition (PRR) was calculated by dividing one by the pulse duration and reported in Hz. Calls were separated by SG (solitary, doublet, and all calls) and path medians were calculated for duration, IPI, FMi bandwidth, FMt bandwidth, peak CF, DC, and PRR. Doublets were treated as a single sound event (i.e., no matter how many subsidiary calls there were they only ever counted as one total call) due to the role of doublets being tied together to create an auditory picture (Moss and Surlykke, 2001). Doublet timing characteristics of duration, IPI, DC, and PRR were calculated by treating the start of the parent call as the start of the call and the end portion of the final subsidiary call as the end of the call. Frequencies were not found to be significantly different between parent and subsidiary calls and therefore each frequency of the doublet portions was used in frequency analysis.

Pulse rate per path (PRP) was calculated by subtracting the time of the last (or last call before leaving passing 2 m) and the first (after passing 8 m) recorded call and then dividing by the number of calls within the 8-2 m zone. Spatial calling density was calculated by dividing the number of calls by the tracked distance of the bat within the 8-2 m zone. Solitary call percentage was calculated by dividing the number of solitary calls by the number of total calls (while still treating parent and subsidiary calls of a doublet call as one call).

All data calculations were restricted to data from 8-2 m (as previously stated in 2.2.4) and completed via a newly created R script 'descrip_stats_fig_creation.R'. Spatial calling density was the lone exception as it was calculated via a newly developed R script 'call.spatial.density.R'.

Non-parametric tests were run on all the variables due to the non-normality of the data. Wilcoxon tests were run on all the parameters, except solitary call percentage, to compare the differences between SF and PF. The same parameters also underwent Kruskal-Wallis tests to determine if there was any difference among SF, LF, or TF. Parameters with significance in that test were then analysed via the post-hoc Dunn's tests to determine the level of difference amongst the groups. A Bonferroni correction was applied to the alpha level of Dunn's test (0.025). A chi-square test was run on solitary call percentage to determine if there was a difference among flight types: SF vs PF, SF vs LF, SF vs TF, and LF vs TF. All tests were run within the respective scripts that calculated their median data.

2.3.4 Bat Experience Charts

The timing of calls and frequency differences were all calculated based on recorded times, but this fails to describe at what times, and at what frequencies, the bat is receiving these calls. The tracking data provides the location and velocities of the bats, making it possible to calculate the times and frequencies that the bat itself is experiencing without the need for on-board microphones.

2.3.4.1 Timing

The R script, 'emit and heard times calc_new.R', was developed to automatically calculate call emission start and end times. This was done by subtracting the recorded time from the amount of time it would take the call to travel to the microphone at the speed of sound (c; 340 ms^{-1} under the conditions of the recording night). The returning time of the echo from the hedge was calculated by determining the distance the bat was from the arrays at the time of the call and the amount of time it would take for the call to emit and bounce back. The speed of sound is about 40 times quicker than the bats in this study, so even at 8 m the difference in the bat's position by the time the echo was received was within the margin of error for localization. Therefore, the change in the bat's position from emission to echo reception was not accounted for.

2.3.4.2 Frequency and Doppler Shift

Emitted frequency (F_{emit}) was manually calculated by applying Schnitzler's equation (1973) for emission with the recorded frequency (F_{rec}) and the calculated velocity of the call ($Velo_{bat}$):

$$F_{emit} = F_{rec} * [(c - Velo_{bat})/c]$$

To determine the echo frequency (F_{echo}), the Doppler shift needs to be adjusted for the target of the call. The methods used in this study do not provide enough information to determine the beam direction of the call and therefore the target of the call cannot be confirmed. However, the hedge was a large landscape feature that was directly in front of the emerging bats, regardless of height or lateral position. The hedge is the likely point of acoustic interest for the *R. ferrumequinum* (see 2.2.4.2 for full explanation). Even if the hedge was not the target of the call, it is a prominent enough feature that a portion of the hedge will always be directly in the beam direction of the bat and therefore, the maximum returning echo frequency can be calculated with a high degree of certainty.

To calculate the echo frequency, the Doppler shift of the bat at the echo time and the frequency bouncing off the hedge (F_{Hedge}) is required. To create hedge frequency, the Doppler shift at emission is multiplied by the emitted frequency. At the time of emission, the velocity in the Y direction was used to calculate the Doppler shift from the hedge point directly in front of the bat. This same velocity towards the hedge was used to calculate the Doppler shift of the echo. The echo Doppler shift is applied to the hedge frequency providing the echo frequency. The echo frequency calculations were done manually through formulas in Excel. The equation for calculating echo frequency was adapted from Schnitzler (1973)**:**

$$F_{echo}= F_{Hedge} * [(c + Velo_{bat})/(c - Velo_{bat})]$$

2.3.4.3 Experience for Paired Flight

The above provides the experience of SF but does not completely describe PF. PFs are not just experiencing their own emission and echoes but also the echoes and the emissions of the conspecific. The bat whose 'experience' is being calculated was termed the focal, or primary, bat. The emissions and echoes of the focal bat are referred to as the auto-emission and auto-echo respectively. The calls received by the focal bat that are from the conspecific (also referred to as the secondary bat) are referred to as allo-calls (allo-emission and allo-echo).

The scripts 'emitted_heard.R' and 'other_echo_heard.R', were developed in R to automatically calculate:

1. the allo-emission and allo-echo times

2. the Doppler shift of the focal bat at those times
3. the allo-emission and allo-echo frequencies

Start and end times for both allo-emission and allo-echo were calculated by finding the XYZ position of both the focal and secondary bats. For allo-emission, the distance between the two bats was calculated and the time it would take for the call to travel to the focal bat was added to the secondary bat's emission time. To calculate allo-echo times, the time it would take for an emitted call to travel from the secondary bat to the hedge and then reflect back to the focal bat was measured (i.e. allo-echo travel time). The allo-echo travel time was then added to the secondary bat's emission time to provide the allo-echo reception time. The movement of the focal bat was taken into account to calculate the XYZ position when the hedge allo-echo was received.

The velocity of the focal bat, at each allo-call time, was calculated by finding the rate of change in velocity between the preceding and following focal call to each allo-call time. The two velocities were subtracted and divided by the amount of time between each, providing the rate of change in velocity. This rate of change was then multiplied by the time difference of the closest call to the allo-time and allo-time, providing the velocities of the focal bat upon reception of allo-echoes and allo-emissions.

To calculate the allo-emission frequency, the distance between the bats was determined at the time of emission. The newly calculated velocity for the focal bat determined if the focal bat was getting closer or farther away from the secondary bat, velocity difference (V_{Diff}). The Doppler shift was calculated by:

$$DS = 1 / (1 - V_{Diff}/c)$$

Allo-echo frequency was calculated by first determining the focal bat's Doppler shift via the focal bat's velocity toward the hedge at the time of allo-echo reception. Much like echo frequency (see 2.3.4.2), the Doppler shift was then applied to hedge frequency of that allo-call.

2.3.4.4 Extrapolation of Data

One in every four calls was missing either a start or end portion of the call (*n* = 790). To fill in these gaps, geographic data were extrapolated. First, spectrograms were visually inspected to determine a manual best estimation of the start, or end, of the call. The estimation was based on the last possible time call was confirmed to be present**.** This was done primarily through spectral analysis via the FFT tool, confirming that a second CF was present as missing calls were almost always due to the overlapping of calls.

To extrapolate velocity data, one R script ('rel.vel.calc.r') was developed to automatically calculate the rate of change between the previous call and the next call and multiply that by the time difference to the missing call. This was done for the velocities in the X, Y, and Z directions. The actual velocity of the bat was then manually calculated. In another R script, ‘new.extrap.XYZ.r’, the directional velocities were applied to the corresponding geometric points of the closest call, providing the extrapolated position.

Extrapolation only occurred between the first tracked call and last tracked call of the focal path, even if there were recorded times present before or after. This restriction was put into place due to the variability in velocity past 8 m and the uncertainty of the path taken by the bats as they turned. Extrapolating paths during the turn would have created paths that would take a direct path based on the last known heading and velocity, which would have been in stark contrast to the paths observed (most bats took a curved path in turns). Attempting to extrapolate paths to earlier times could have been done in one of two ways, both resulting in likely localization error. Either the first recorded velocity is assumed to be the velocity of the bat until that point or the rate of velocity change for the first few calls is assumed to be a consistent rate of velocity change. In the first approach, new localizations would likely be too close to the array. At no point was velocity every truly constant for bats. Using the second approach ultimately ends with the bats flying much more quickly than they truly were. Some bats slowed down considerably as they flew through the initial phase (8-6 m). If the second approach was applied to these then extrapolation would have suggested a massive spike in velocity in the early stages, likely putting some bats way over the 15 ms^{-1} threshold that was originally used as velocity limit for these bats. Both of these errors were avoided by not extrapolating past points where there was not any tracking data.

2.3.4.5 Spectrogram creation

All of the timings and frequencies for auto-calls and allo-calls were compiled and inserted into a MATLAB script (‘BatAuditoryExperience.m’) created by Marc Holderied (2021). This script automatically converted the data into a spectrogram that visualized the ‘experience’ of each bat in PF (Figure 13). The frequencies for FMi and FMt were not calculated into emission, or echo, Instead the FMi portions of these charts are based off a randomly selected observed solitary call time (3.0 ms) and bandwidth (14.00 kHz) of FMi; this was also done for FMt (2.2 ms, 16.90 kHz). The FM portions were used to make a standard looking HDC call and to provide clear indicators for start and end. They were not in used in any analysis.

2.3.4.6 Analysis and Statistics

The medians per path were calculated for auto-emitted and auto-echo CF of SF, PF, LF, and TF. These CFs were compared via the same statistical method as recorded CFs in 2.3.3.

2.3.5 JAR

2.3.5.1 Temporal Call Spacing

Using the same data that created the bat experience charts, temporal call timings were calculated by determining the time delay between the target call and the closest preceding or following allo-call. Call timings were calculated based on three different auto-call to allo-call groups (AAGs): the timing of focal emission based on the allo-emission (EE), timing of focal emission based on the allo-echo (EO), and the timing of focal echo based on the allo-echo (OO). If bats were timing their emission calls to the allo-emission they would need to account for the amount of time it would take for the echoes to return to ensure that echoes were timed as they preferred. If the timing was based on EO then the focal bat would only have to account for the time for the auto-echo to travel back. The simplest call timing would be to directly use OO; bats in this scenario would just ensure that auto- and allo-echoes were timed to their preference. Echo to allo-emission (OE) was calculated but likely does not provide any benefit over any of the other three AAGs and was excluded from further analysis. Timing auto-echo to allo-emission would require bats to calculate the expected auto-echo time whilst being aware of an allo-emission that is occurring in a frequency range that is likely below their sensitive range. The other timing options are simpler, and likely more effective, options.

2.3.5.1.1 Call Timing Classification

Through the creation of 'call_decision_analysis_graph.R' bat calls were classified into one of three timing categories: synchronizing (syn), alternating (alt), or undefined (und). Call timing classifications (CTCs) were run with two different methods: preceding and following. Preceding based all the timing off the allo-call that occurred just before the target call. Following is exactly the same except it is based off of the following allo-call. The start time of the target call (the call being measured) was subtracted from the start time of allo-call, determined by the method in use, to provide the time difference.

In a previous analysis step (see 2.2.5), each call was labelled as being a solitary or doublet call. In this analysis, the two calls in a doublet call were treated as a single sound event. Per previous research, doublets are used in order to quickly update information on the surrounding area (Kothari *et al.*, 2014; Moss *et al.*, 2006; Moss and Surlykke, 2001). While a bat could reasonably predict the calling rate of a conspecific using solitary calls, it cannot predict the usage of a doublet. Temporally aligning calls can only work if both parties are engaging with constant calling rates. Due to these assumptions, subsidiary calls were never used as a target call, and doublets were treated as a singular acoustic event**.** Some calls did not receive a designation of solitary or doublet call due to the inability to determine the start or end portion of that call, or the calls around it. These were termed unknown calls (*n* = 13). Any call that could not be classified as solitary or doublet was not used in this analysis for the purpose of determining call timing.

Average pulse interval of the SF solitary call was 97 ms. For ease of calculation, pulse interval (time from start of the call to the start of the next call) was rounded to 100 ms, which is within the margin of time delay error of our microphones, and divided equally into three sections. A perfect synchronization call would be at 0 ms. Any call that was +16.67 ms to -16.67 ms was termed a syn. Any call that occurred between +/- 83.33 ms and +/-116.67 ms would also be syn. Occurring in this range indicates that the preceding (+), or following (-), call would be synchronized with allo-call, provided that the bat calls with the standard IPI. A perfect alternating call should be around +/-50 ms so any call occurring in +/-33.33 ms to +/-66.67 ms was classified as alt. Undefined calls were any calls that did not fit into either of these categories. Any call that started +/- 116.68 ms was disregarded. These calls are so far away from the target call that they are more likely to influence the timing of a focal preceding (-), or following(+), call. Percentage charts for each CTC category were created to determine how frequent each CTC was. Bats with fewer than three calls were not used in call percentage analysis to avoid skewing the data.

2.3.5.1.2 Path Timing Classification

For each bat, charts visualizing the CTC were made for EE, EO, and OO. These charts were then used to determine path timing classification (PTC), the type of call timing that occurred at least 50% of the time. The four possible classifications were synchronization, alternation, undefined, and random. Any path that did not have one type of CTC reach the 50% threshold, or had two call types tied for 50%, was classified as random. The one exception to this was if one of the tied CTCs was und. In that scenario, the other call type would be used as the classification.

Und calls are likely an indicator for randomly placed calls than a particular strategy. Additionally, many und calls were extremely close to being classified differently. Biologically speaking an und call is a poor, and possibly ineffective, alt or syn (whichever it is closest to). Under this reasoning, undefined should be considered as either evidence of random timing, or unidentified alt, or syn, calls due to the time parameters in use. When an und was just outside the time window for alt or syn classification, the other und calls typically exhibited a similar amount of time separation from the focal call. At times this led to timing charts that were classified as undefined even though it should have been a different PTC. For example, if 3 out of 5 calls were classified as und and unds just missed out on being defined as alt and the other 2 calls were alt, then this would be classified as undefined PTC. If this occurs over multiple paths the amount of alt calls would steadily increase, so when analysis is run the undefined strategy appears significant as the proportion of alt calls would be higher than random. Under this reasoning, paths that were classified as undefined were not considered an actual strategy in use by *R. ferrumequinum*.

Any bat with one calculated call, or less, was removed from this classification process (*n* = 5). For each bat a total of six charts were made: two charts based on the call reference method (preceding or following) for each AAG.

2.3.5.2 Spectral Shifting

Similar to call timing, frequency difference was calculated by using the information created in the bat experience charts. The aim was to determine whether there was any separation in emitted or echo frequency between the two bats. Two approaches were available to determine the extent of frequency difference between the pairs. The first was comparing all the frequencies within each pair to one another. This provided a full range of the calls by each bat and their median difference, but it did not provide a direct comparison in calls. To do a direct comparison, frequency difference was calculated by taking the preceding allo-frequency from the auto-frequency of the focal call. This did result in a smaller number of calls to analyse but provided a more direct comparison of frequencies. The first approach was deemed unfit as it did not directly compare the frequency difference and was therefore not used in any further analysis. As discussed earlier(see 1.3.4), CF is unlikely to shift (Fawcett *et al.*, 2015; Jones *et al.*, 1994; Jones and Ransome, 1993). If there is spectral shifting, it would seem unlikely that the shifting would occur mid-call. Due to the long nature of HDC calls, it makes more sense for a focal bat to receive an allo-echo frequency and adjust its next auto-call accordingly so it is sufficiently far enough to avoid spectral overlap.

Frequency differences were calculated for EE and OO. The frequency difference was calculated by finding the target call frequency and subtracting it from the allo-frequency of the call that preceded it. Absolute and actual frequency differences were calculated for both LFs and TFs. Frequency differences were automatically calculated by developing a new R script ‘allo_auto_freq_dif_calc.R’.

2.3.5.3 Virtual Assignment

In order to compare the observed values of call timing and frequency difference, bats were virtually paired up to create random data. PFs were used in this process because they provided realistic calling characteristics of bats flying in pairs. The 34 bats in the PF were virtually paired against one another and themselves. Each virtual pair was given one of the observed temporal separations (*n* = 17) and the two paths were subsequently adjusted to match that difference. Each pair was run until it had been run under every observed separation. When virtual pairs matched observed pairs, in the correct order (i.e., LF was placed in the LF spot) with their observed difference (e.g., LF#1 and TF#1 with temporal separation #1) that datum was removed. In total 9,798 virtual pairings were created, 576 for each temporal separation. These pairs were put through the same process that created the bat experience charts. Each of these pairs also underwent the same process as the observed to determine CTC, including removal if there was not enough overlap. Random pairs were not given PTCs, only the calls themselves were classified. These virtual pairs were also placed through the same process as observed to determine frequency difference. The entire virtual pair process was done automatically by creating the R script 'rand.freq.analysis.R'.

2.3.5.4 Statistics

Chi-square tests were run comparing the call timing ratios for each call reference method. Four different chi-square tests were run, each comparing the observed ratios to the virtual pair ratios. LFs and TFs were treated as separate groups rather than together. The first test compared the ratios at a specific PF temporal separation. Unfortunately, none of the paths provided enough data points to run this test and it was ultimately removed. The second test grouped the pairs into temporally separated groups of close proximity (*n* = 4; ≤ 0.4 s), mid proximity (*n* = 6; between > 0.4 and ≤0.65s), far (*n* = 4 >0.65 s), close half proximity (*n* = 6, < 0.5 s), and further half proximity (*n* = 7, ≥0.5 s). These timings were based on distribution of the temporal separation. There was a distinct gap between 0.4 and 0.5 s, which is what led to the classification of the 'close proximity' group. There was another distinct gap at around 0.65 which was the cut off for the 'mid proximity' group. The three furthest paths could not be analysed here due to the lack of overlapping calls which left 14 PFs to analyse. The spread of these PFs showed a gap in temporal separation that split the data nearly in half at 0.5s. Any PF with temporal separation smaller than 0.5s was grouped in the closer 'half', if greater than the further 'half.'

The third test was run by grouping the paths via PTC, while still maintaining the separation of LF and TF. This test was done to determine if the PTC was an intentional strategy. This third test was repeated three times, one for each AAG. Chi-square was also run to clarify if the classification in EE effected the proportion of OO CTC. This test was not used in analysis as the classifications were not different between EE and OO because echoes will return almost immediately after emission due to the speed of sound. A distance large enough to experience a different classification between EE and OO would be too separated to be considered a PF.

The absolute and actual frequency differences for observed paths were compared to their corresponding virtual temporal separation data via Wilcoxon tests (i.e., absolute TF #1 frequency difference data were compared to the absolute virtual TF data at temporal separation #1).

3 Results

3.1 Flight Characteristics

3.1.1 Flight Path

3.1.1.1 Solo Flight

Among the 54 SF paths, only 46 were acceptable for analysis (Figure 6). Maximum and minimum flight height recorded were 3.74 m and 0.226 m respectively. During the initial phase of flight (8-6 m), SFs averaged 2.37 ±0.03 m from A1 and 1.20 ±0.09 m from the ground. As the bats turned (4-2 m), they tended to descend resulting in an average distance from the ground of 0.90 ±0.05 m.

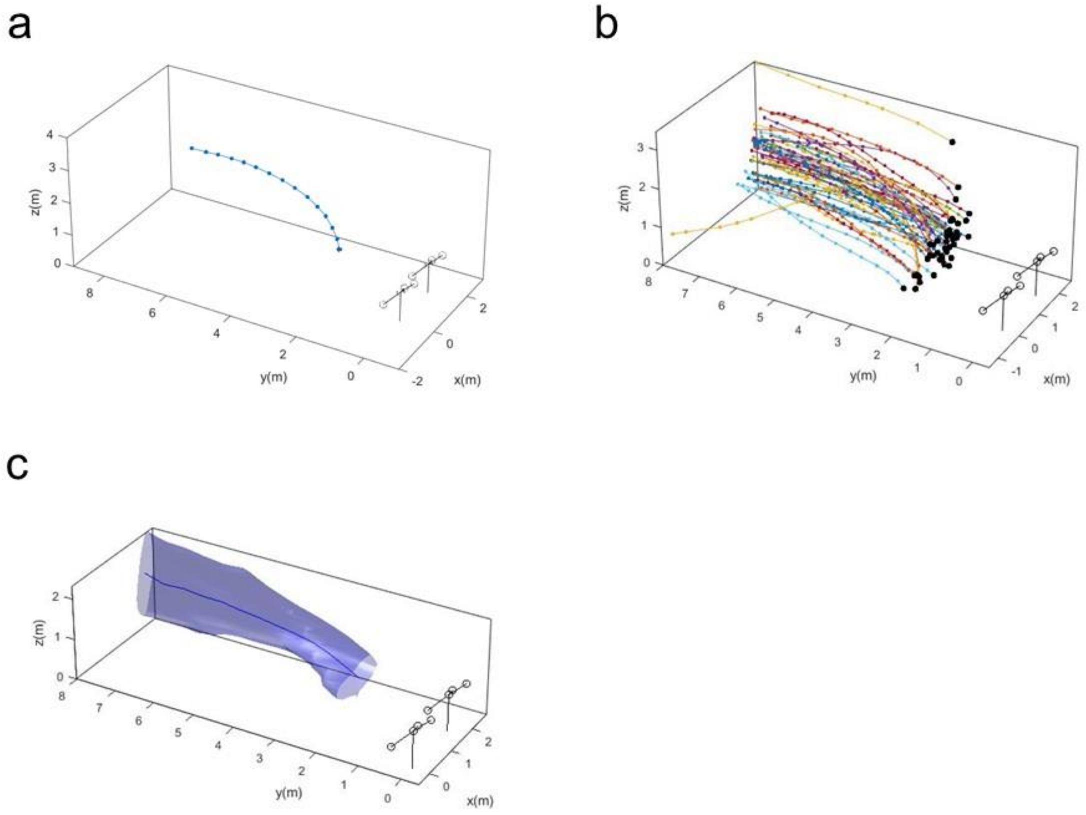

Figure 6: *Flight path reconstructions of Solo Flights (SF), restricted to 8-2 m for analysis. Flight direction was towards the microphone arrays (circles at Y = 0 m). Each point represents the start, or end, of each call. The black points indicate the last tracked point before either being unable to track the bat or the bat passed the 2.0 m cut-off. (a) An example of a standard SF, mostly straight flight until around 4.0 m where a turn, and drop in height, was initiated. (b) Every analysed SF plotted together. (c) The flight corridor of the SFs (SF tunnel). The dark mid-line within the SF tunnel represents the median path of SFs.*

Bats flew in a relatively tight corridor, in the X plane, close to the roost building wall (Figure 6c). The SF corridor (SC) was found to have a volume of 8.925 m^3. Before the turn, flight height ranges about 1.5 m and the range of distances from the building wall is much narrower, ~0.5 m. As the bats turned, the flight height range shrank considerably and became about 0.5 m.

The average SF turning point was 3.88 ±2.69 m from the arrays. The automated process for measuring turning points provided a high amount of variation for all flight types (PF: 5.13 ±4.21, LF:4.13 ±2.43 m, and TF: 6.07 ±4.21 m). The use of a defined angle proved to be problematic for defining the turn. Even when using other angle definitions, some bats were defined as never taking a turn due to extremely shallow angles or consecutive calls that lessened the call-to-call angle. Due to these errors, turning point data were removed from further analysis.

3.1.1.2 Solo Flight vs Paired Flight

Only 17 PFs ($n = 34$ bats) met the criteria for analysis (figures of each PF can be found in Appendix 1; *Figure* 7a). Pairs averaged 1.08 ±0.08 m from the ground and 2.37 ±0.03m from A1 during the initial phase (8-6 m) of flight (*Figure* 7b). Height minimally decreased to 1.00 ±.03 m during the turn phase (4-2 m).

The corridor of PFs (PC) was found to have a volume of 7.88 m^3 (*Figure* 7c), which is slightly larger than the SC at 6.99 m^3 (*Figure* 7d). The PC maintained a similar shape to the SC, following the building wall and crossing in front of the arrays. In the approach phase, the two corridors are very similar. Corridor width is about 0.5 m and corridor height is about 2 m with PC only marginally larger than SC. Both corridors constrict through the mid phase particularly in height. Max height in SC declines much more quickly than in PC in this section. During the turn phase SC narrows even more in height, ultimately resulting in a corridor height of ~0.5 m. In comparison, the PC slightly constricts and is larger in corridor height at ~1.5 m. The corridor width of the two is similar, although SC is slightly larger (~1.75 vs ~1.5 m). While the height of the two corridors is different, when viewing the paths from above the PC is almost entirely contained within the SC**.**

Median containment percentage within the SC for PFs was 61.5% (38.5.2-76.9). For SFs it was 84.6% (58.7-100). Only four of the 34 individuals in PFs were never contained within the SC; three were completely encapsulated. Of the SFs, seven of the 46 were completely outside the SC and 21 were contained. The PF ratios of containment were significantly different from SF ($p < 0.001$).

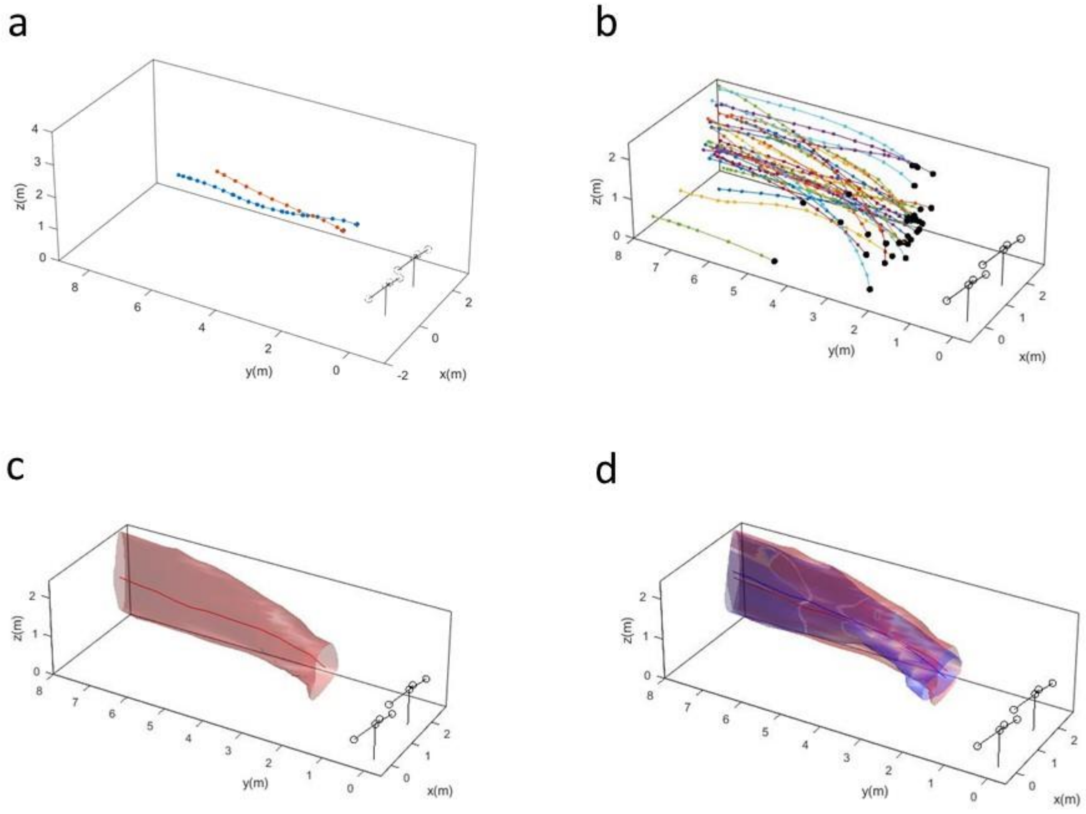

Figure 7: Flight path reconstructions of Paired flights (PF), restricted to 8.0-2.0 m for analysis. Flight direction was towards the microphone arrays (circles at Y = 0). Each point represents the start, or end, of each call. The black points indicate the last tracked point before either being unable to track the bat or the bat passed the 2 m cut-off. (a) An example of a standard PF, the lead bat was denoted by the blue path, trail by the orange. (b) Every analysed PF (n = 34) grouped together. (c) The flight corridor of the PFs (PC). The dark mid-line within the PC represents the median path of PFs. (d) PC (red) overlain with SF corridor (SC; blue). The PF corridor mostly matched the SF corridor although it had a larger volume (7.88 m^3 vs 6.99 m^3). The largest amount of difference between the two corridors occurred in the turn phase (4-2 m) where the SC narrowed corridor height to 0.5 m, whereas PC did not narrow and maintained a corridor height of 1.5 m.

3.1.1.3 Lead Flight and Trail Flight

LFs and TFs displayed similar average lateral position (2.35 ±.04 m, 2.42 ±.05) during the initial phase but differed by about 0.5 m in height (0.98 ±.04 m and 1.51 ±.12 m respectively; Figure 8a,c). By the turn phase, both flight types had lowered their average height from the ground, LFs at 0.92 ±.08 m and TFs at 1.07 ±.01 m.

TC was larger than LC (8.58 vs 6.12 m^3), especially in the initial and mid phases (Figure 8e). TC's corridor height was ~2.25 m while LC's was ~1.5m. TC shrank in height during the turn phase while LC actually expanded slightly. TF median path was always higher than LF until the turn phase where TF drops and they converge. LC is larger in both turn phase corridor height (~1.5 m to ~1.0 m) and width (~1.5 m to ~1.0 m).

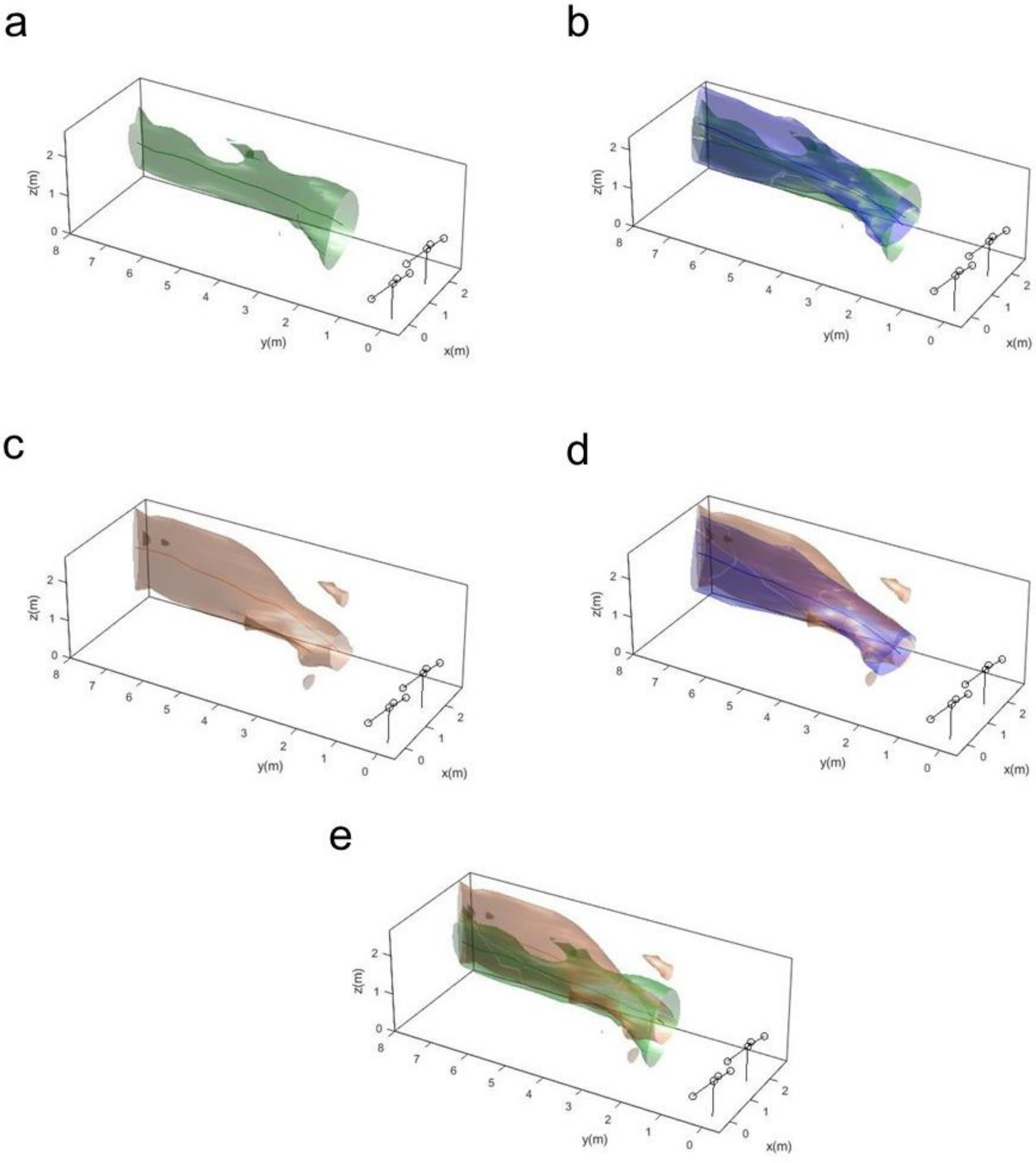

Figure 8: *Flight corridors of Lead Flight (LF; a), LF compared to Solo flight (SF; b), Trail Flight (TF; c), TF compared to SF (d), and LF compared to TF (e). The darker coloured midline represents the median flight path of that particular flight type. Flight direction was towards the microphone arrays (circles at Y = 0). Analysis on paths was restricted to 8-2 m and corridors were subsequently restricted. (b) LF corridor was smaller than SF (6.12m^3 vs 6.99m^3). LF corridor was more constricted in the initial phase (8-6 m) and generally maintained that shape throughout, unlike SF. (d) TF corridor was quite similar to the SF corridor, although it was larger (8.58m^3 vs 6.99 m^3) particularly in height. TF corridor did exhibit a similar constriction of the corridor in the turn phase (4-2 m). (e) TF corridor was considerably larger than LF corridor (8.58 m^3 vs 6.12 m^3). LFs appeared to prefer to stay lower to the ground, whereas TFs were much more variable. The two were particularly different in the turn phase where the corridor width and height of LF was larger than TF.*

3.1.1.4 Solo Flight vs Lead Flight/Trail Flight

The start of LC is very similar and contained within the SC, although the bottom of LC corridor is always lower than that of SC and LC does not show the same variance in height (Figure 8b). In the mid phase, corridor width is essentially the same at ~0.25 m. As the two corridors go into the turn phase, LC changes from SC. Unlike SC, LC expands corridor height in both directions instead of constricting corridor height. At 2 m, corridor height for SC is about 0.5 m whereas LC is about 1.5 m and corridor width is ~1.75 m and ~1.5 m respectively. It appears that the actual passing of the arrays of LFs is happening later as the median LF path and corridor are both shifted slightly to the right of the SC.

Throughout the entire path, the median flight path of TC was always slightly higher than SC (Figure 8d). The TC also had a larger corridor height than SC through most of the flight. This was especially pronounced in the mid phase, ~2.25 m vs ~2 m (6-4 m). The TC was slightly shifted to the right, closer to the wall, in comparison to SC but the general shape of the corridors was similar, especially in the turn phase, although the corridor height of SC (~0.5 m) was narrower than TC (~1.0 m).

3.1.2 Velocity

3.1.2.1 Path Velocities

SFs averaged a PV of 8.48 ±1.08 ms^{-1} (Figure 9). The slowest PV was measured at 5.99 ms^{-1}, the fastest at 11.16 ms^{-1}. SFs were only marginally faster than PF on average, 8.48 ±1.1 to 8.41 ±1.3 ms^{-1}. LFs averaged a velocity of 8.3 ±1.2 ms^{-1} and TFs 8.5 ±1.4 ms^{-1}. The fastest bat was a SF that averaged 11.03 ±1.16 ms, the slowest bat was a LF that averaged 5.98 ±.99 ms^{-1}.

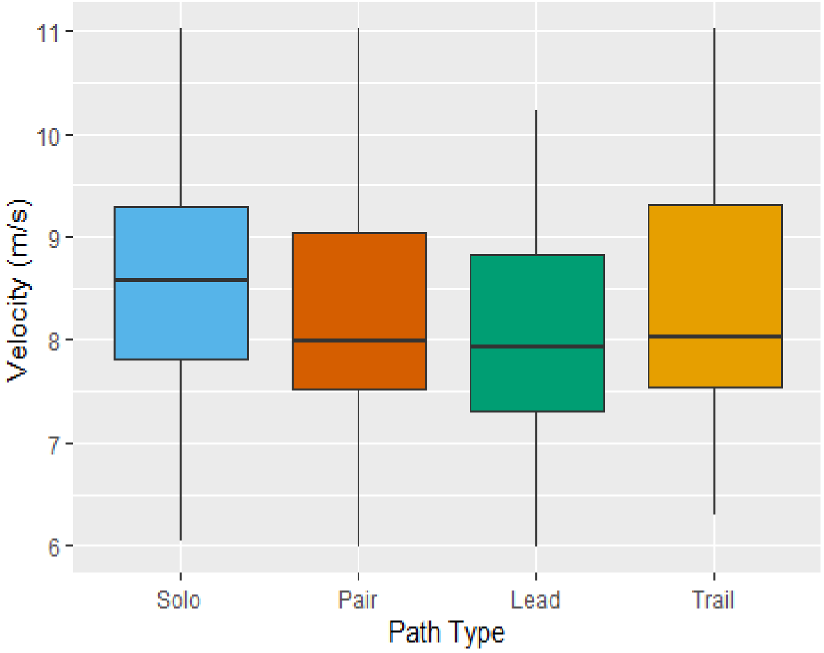

Figure 9: Boxplots *(median, 25% CI, and range) of path velocity of Solo (n = 46), Pair (n = 34), Lead (n = 17), and Trail (n = 17) flights.*

3.1.2.2 Path Velocity and Emergence Time

As the night wore on, flight speeds steadily decreased ($p < 0.001$, R = -0.69, Figure 10). Forty SFs emerged during civil twilight (0-37 minutes after sunset), 6 during nautical twilight (37-86 minutes after sunset).

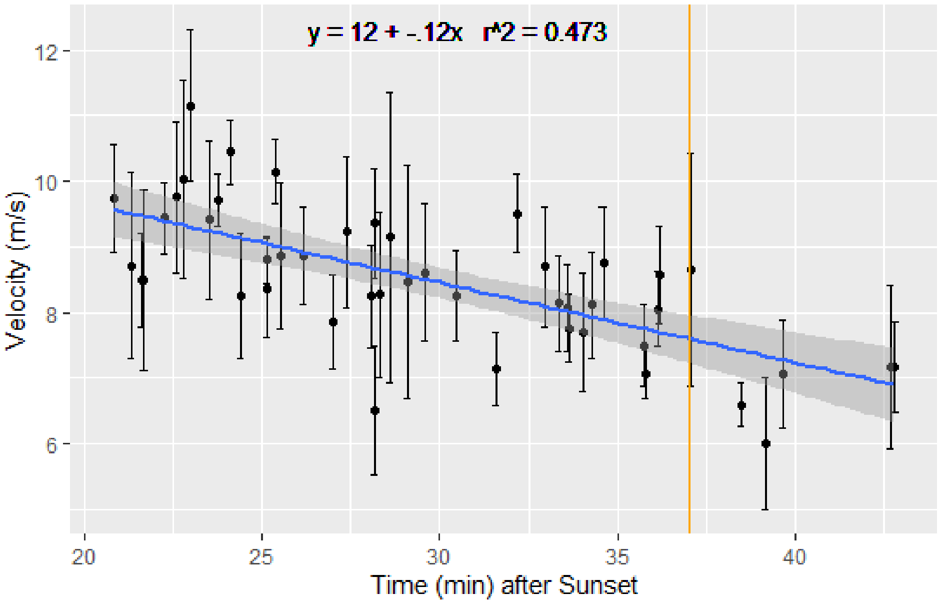

Figure 10: *Negative linear relationship between Median SF path velocity (n = 46) and emergence time after sunset (R = -0.69, p < 0.001). Median path velocities are denoted by the black dot, error bars indicate the standard deviation. The orange vertical line denotes the change from civil to nautical twilight. The blue line denotes the regression line, the grey area represents the 95% confidence interval.*

3.1.2.3 Temporal Separation and Path Velocity

The average absolute difference in PV between bats in PF was 1.00 ±0.39 ms^{-1} (Figure 11a). The largest observed difference in PV was 2.27 ms^{-1}, the smallest was 0.15 ms^{-1}. There was no relationship between PF PV difference and temporal separation. Neither PF PV difference, LF, nor TF PVs were related to temporal separation. There was a slight positive correlation between LF and TF PV ($p < 0.05$, R = 0.55; Figure 11b).

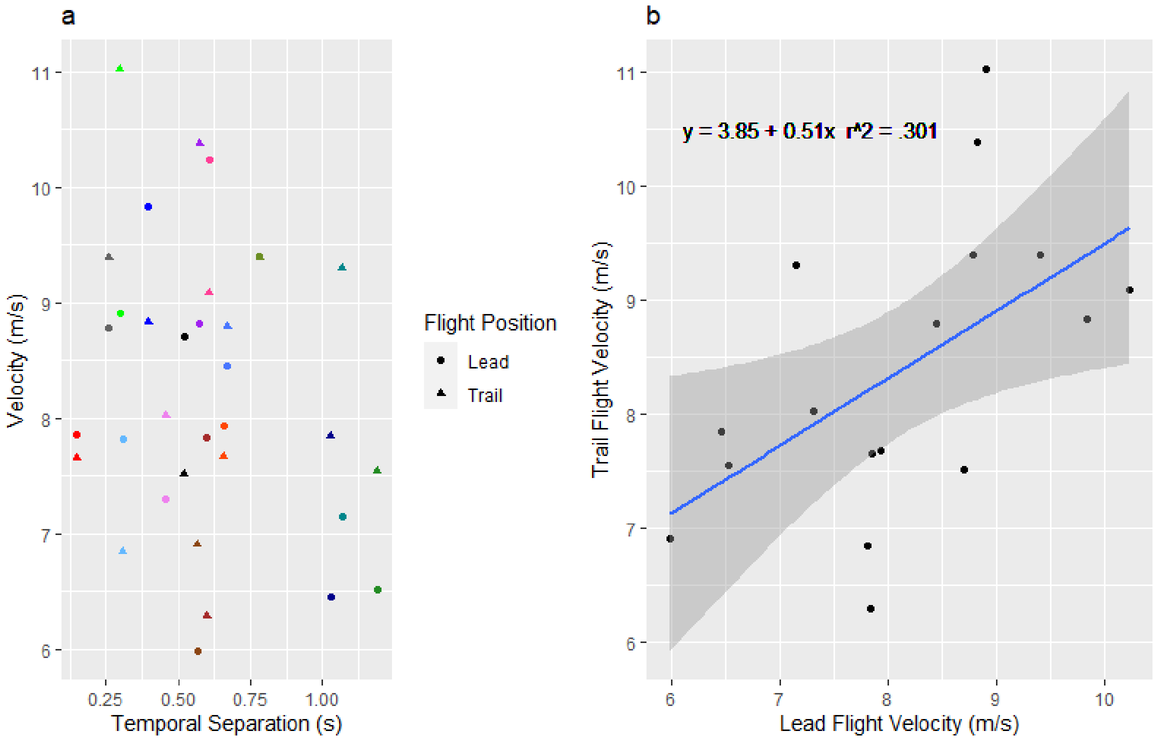

Figure 11: *Relationship of path velocity for Lead (n = 17) and Trail (n = 17) flights to temporal separation (a) and each other (b). There was not a relationship between Lead (circle) and Trail (triangle) path velocity and temporal separation. Leads and Trails belonging to the same pair were coloured similarly (a). There was a positive linear relationship between Lead path velocity and Trail path velocity (R = 0.55, p < 0.05). The blue line denotes the regression line, the grey area represents the 95% confidence interval (b).*

3.2 Acoustic Characteristics

3.2.1 Timing characteristics

Over 1,500 calls (n = 1526) were recorded but only 586 calls occurred within the analysis zone (8-2 m) and were subsequently analysed (Table 1). Duration, IPI, DC, and PRR were within the standard observations of *R. ferrumequinum*. Solitary call duration was the only characteristic that was different between SF and PF, 59.8 (57.4-61.1) ms and 55.9 (54.3-58.9) ms respectively ($p < 0.05$). As expected, timing characteristics of doublet calls changed as expected from solitary calls (e.g. duration got longer) but there was not any difference in SF and PF doublet call characteristics. Median PF solitary call percentage was 78.9% (62.5-90.6) and 84.5% (68.8-88.4) for SF (Figure 12). The call percentage ranged from 100 to 33.3% (30.0 in PF) in both types. Solitary calls were used as the only call in 26% of both SFs (*n* = 12) and PFs (*n* = 9). The average spatially calling density was found to be 1.43 (1.39-1.59) calls m^{-1} in SF and 1.55 (1.35-2.00) calls m^{-1} in PF**.**

Table 1: *Medians of the timing characteristics of SF (N = 46 bats), PF (N = 34), LF (N = 17), TF (N = 17) paths based on strobe group classification of solitary, doublet, and every call. Reported sample size (n) is the number of calls present, excluding subsidiary calls of doublet calls. Asterisk denotes that solitary call duration was different between SF and PF (p < 0.05). The 95% confidence interval is denoted by brackets.*

Call Type/Class (n)	Duration (ms)	IPI (ms)	DC (%)	PRR (Hz)	Calls m-1
Solitary Calls					
SF (271)	59.8 (57.4-61.1)*	39.6 (36.9-41.0)	60.2 (58.7-61.9)	9.93 (9.87-10.38)	NA
PF (193)	55.9 (54.3-58.9)*	38.7 (37.5-42.5)	58.7 (57.3-59.8)	10.29 (9.89-10.70)	NA
LF (91)	56.1(53.2-61.3)	38.4 (34.8-42.5)	58.7 (56.6-63.7)	10.50 (9.62-10.96)	NA
TF (102)	55.8 (54.3-58.9)	39.9 (37.4-43.1)	59.2 (56.1-60.1)	10.12 (9.63-10.62)	NA
Doublet Calls					
SF (67)	74.6 (68.3-76.5)	36.0 (31.5-39.9)	67.5 (65.4-70.0)	18.74 (18.16-19.81)	NA
PF (55)	68.1 (62.0-74.5)	31.3 (23.8-39.2)	68.2 (64.9-76.2)	18.56 (17.76-19.65)	NA
LF (38)	68.1 (62.0-74.5)	32.0 (10.0-38.1)	70.9 (64.9-86.1)	19.08(17.85-20.20)	NA
TF (17)	69.7 (57.5-77.9)	35.8 (24.1-41.4)	67.2 (60.9-74.7)	18.54 (17.37-19.57)	NA
All Calls					
SF (339)	55.6 (45.6-58.7)	36.6 (35.5-38.9)	60.2 (58.3-61.8)	10.71 (10.28-12.35)	1.43 (1.39-1.59)
PF (257)	48.2 (42.0-55.1)	36.9 (34.5-40.5)	58.5 (57.2-59.7)	11.78 (10.07-13.70)	1.55 (1.35-2.00)
LF (133)	41.5 (36.4-54.8)	35.4 (30.0-41.5)	58.4 (57.1-63.7)	13.56 (10.04-16.56)	1.94 (1.30 – 2.21)
TF (124)	51.2(45.8-58.6)	38.8 (36.3-41.9)	59.2 (56.2-60.1)	10.28 (9.89-12.14)	1.45 (1.31 – 1.63)

Timing characteristics were similar across all the measured parameters between LF (*n* = 133 calls) and TF (*n* = 124), save for solitary call percentage**.** LF solitary call percentage was different from TF (*p* < 0.001), 62.5% (50.0-90.0) against 85.7% (70.0-100). LF solitary call percentage was also lower than the SF percentage (p < 0.001). Spatial calling density of LF was 1.94 (1.30-2.21) calls m^{-1} and 1.44 (1.31-1.63) calls m^{-1} for TF.

3.2.2 Bat Experience

Each bat in a pair had their respective ‘experience’ calculated and made into a spectrogram (Figure 13). Some of these charts show very clear examples of synchronizing calls (Appendix 3a), alternation of calls (Figure 13), spectral overlap in echo (Appendix 3m), and spectral separation in echo (Appendix 3c). Every PF ‘experience’ can be seen in Appendix 3.

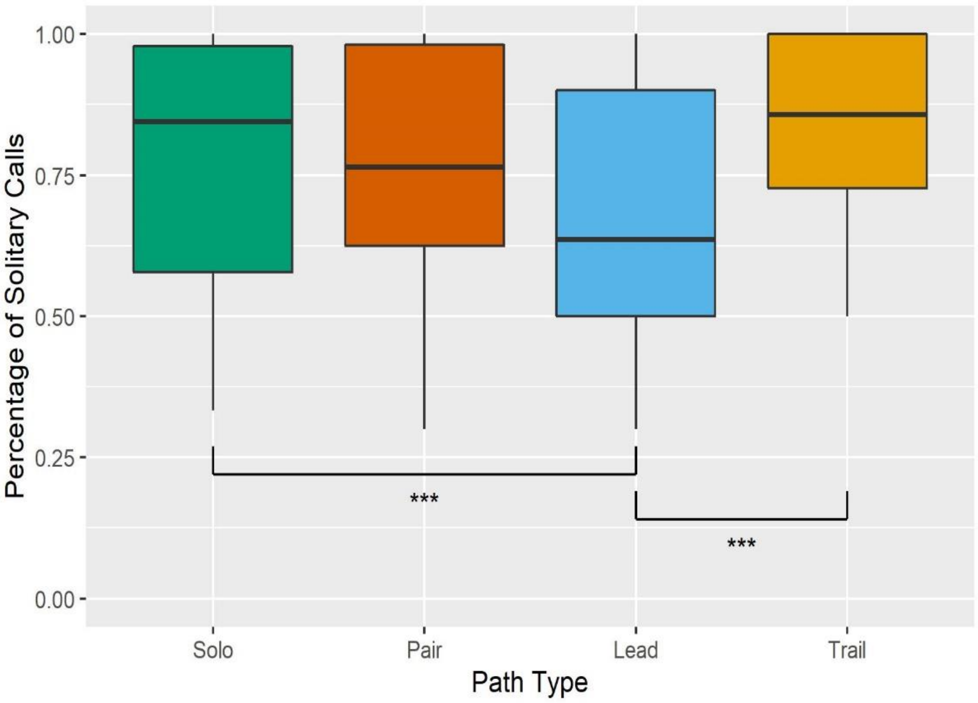

Figure 12: *Boxplots (median, 25% CI, and range) of the solitary call percentage of Solo (n = 46), Pair (n = 34), Lead (n = 17), and Trail (n = 17) flights. Lead Flight solitary call percentage was different from both Solo and Trail (p < 0.001).*

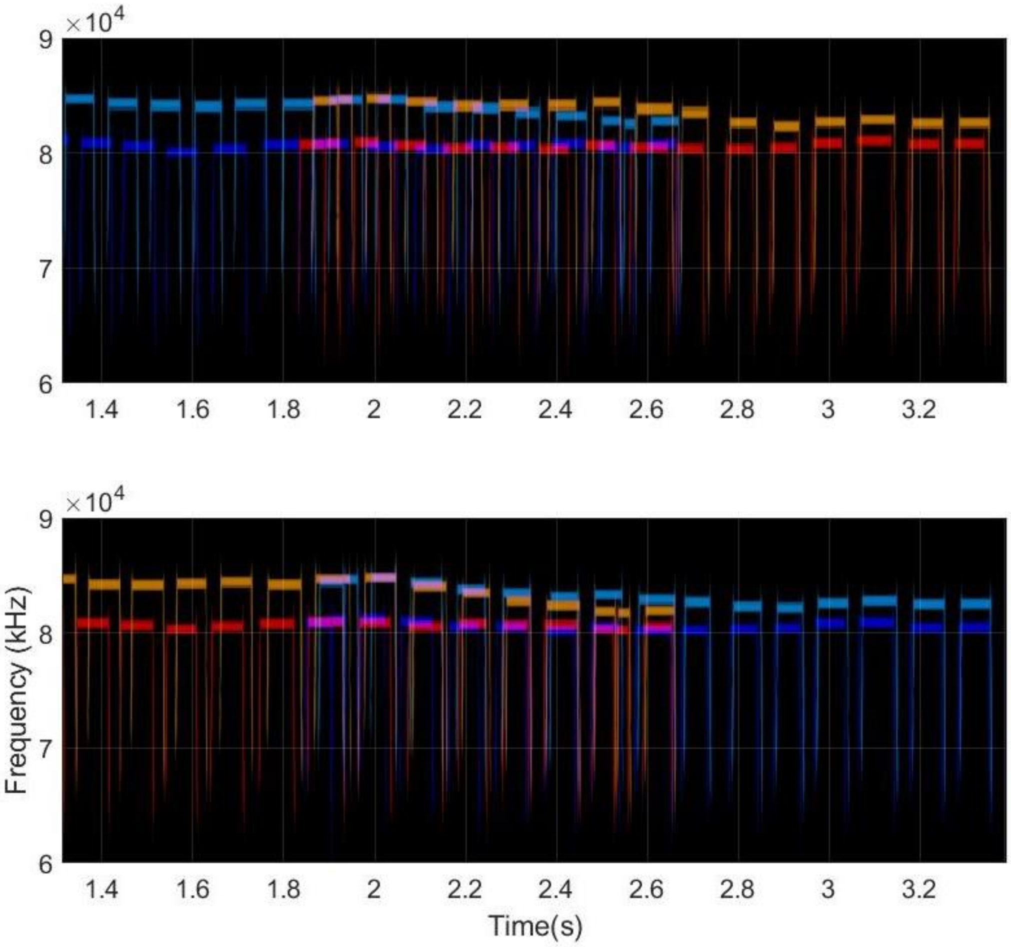

Figure 13: *An example of a bat experience chart. The top box represents the experience of the Trail, bottom the experience of Lead. Emission calls of the focal bat are represented by the red calls, the corresponding maximum returnable frequency of the hedge echo. The emission calls of the secondary bat, as experienced by the focal bat, are represented by the dark blue calls. The subsequent echo is denoted by the light blue calls. FM portions are representative of the standard FMi and FMt frequency bandwidth and duration.*

3.2.3 Temporal Call Spacing

3.2.3.1 Focal Emission to Secondary Emission

3.2.3.1.1 Preceding Call

Alt calls constituted of least 50% of calls in 7 paths. Four paths did not exhibit any alt calls (Figure 14a). Seven paths were found to produce syn calls at least 50% of the time. Three of which presented syn calls more than 75% of the time. Only 4 paths did not exhibit any syn calls (Figure 14b). Und calls were the majority call in 30% of paths ($n = 6$). Like alt and syn, 4 paths did not exhibit any und calls (Figure 14c). Fourteen of the 34 paths did not provide enough overlapped calls to run CTC. When basing the timing of EE calls on the closest preceding call, synchronization was the most common P ($n = 12$; Figure 15), alternation and undefined were equally common ($n = 7$), and random was the rarest ($n = 5$). Only 5 paths could not have PTC determined due to low call overlap. When calls were grouped together based on their PTC, synchronization was found to have a higher proportion of syn, alternation had a higher proportion of alt, and undefined had a higher proportion of und than the virtual pairs ($p < 0.05$). The close proximity TFs (< 0.4 s apart) were using more syn calls than expected ($p < 0.05$).

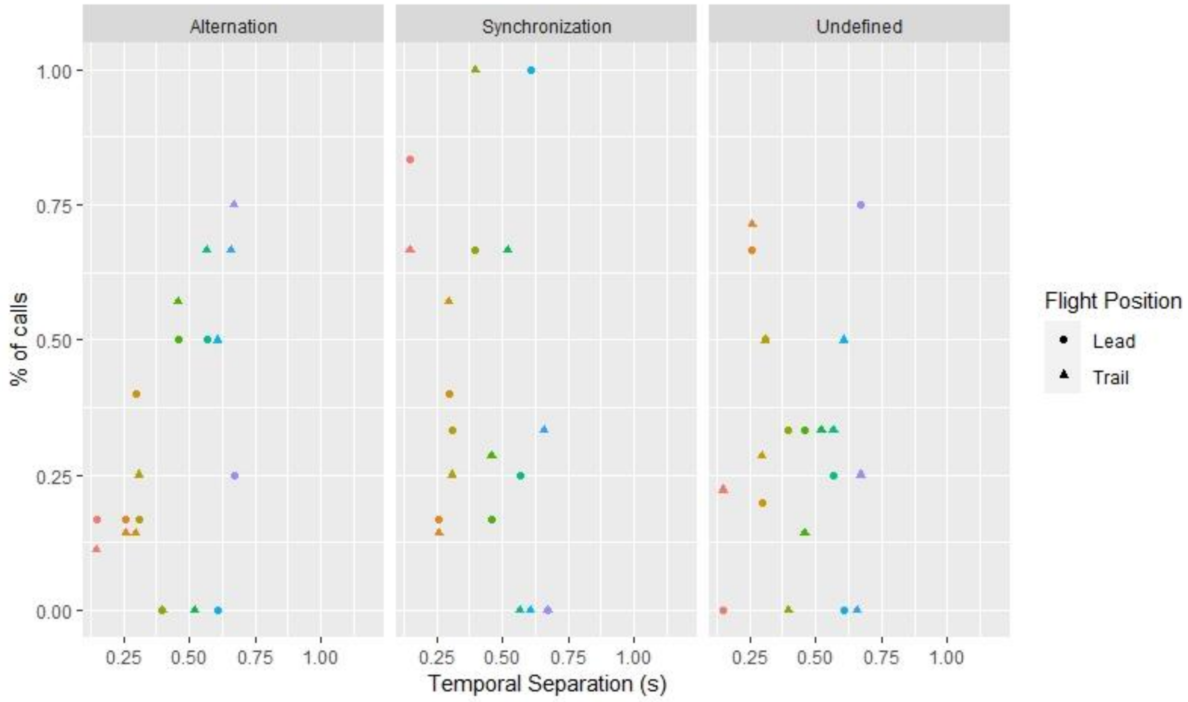

Figure 14: *Percentage of auto-emission to allo-emission calls that are alternating (a), synchronizing (b), or undefined (c) when timing is based on the preceding call. Each point represents one bat (n = 20), the flight position of the bat is denoted by shape. Paths of the same Paired Flight share the same colour and are placed accordingly on the x-axis displaying the amount of temporal separation between the Trail and Lead bats.*

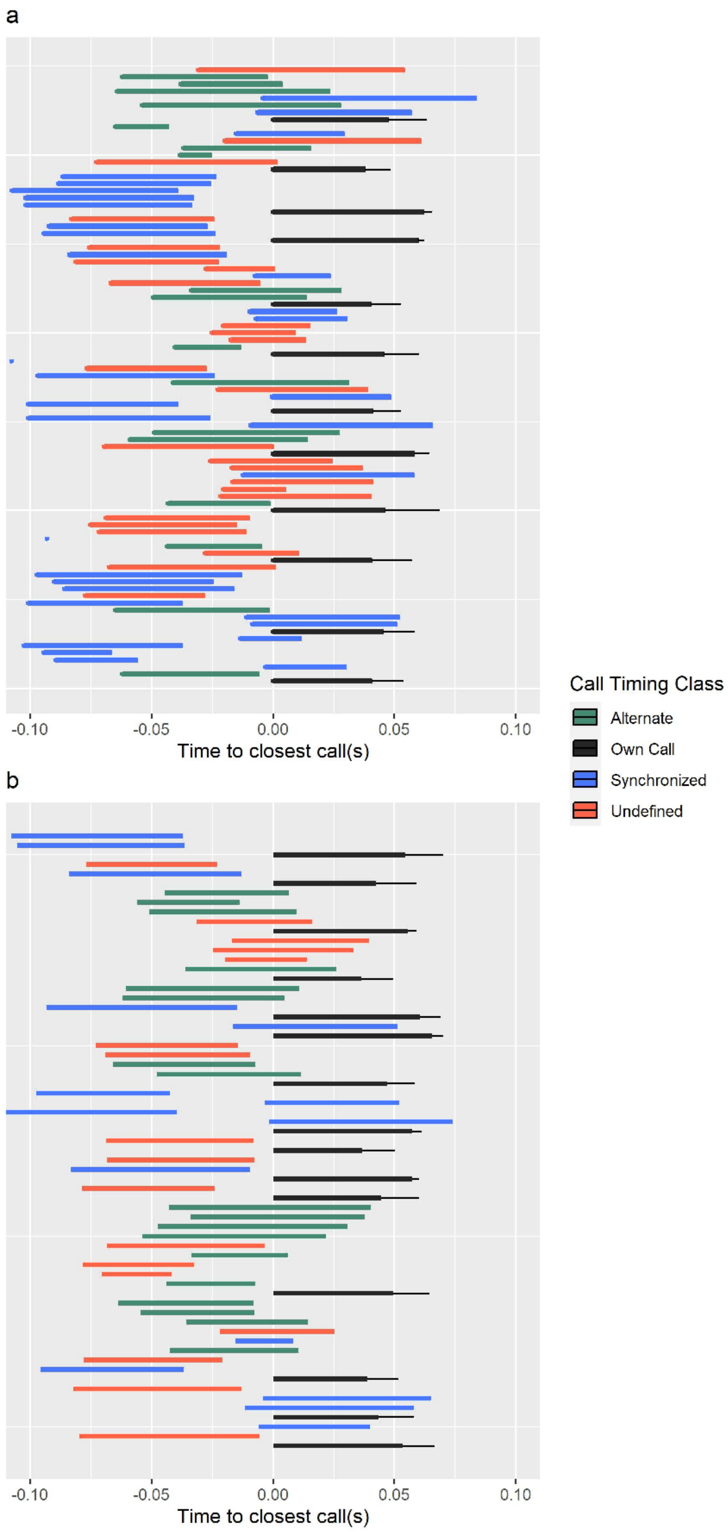

Figure 15: *The call timing of every preceding call that could be analysed for emission to allo-emission timing. Every black bar denotes a different bat (n = 29). The black bar also provides the average duration of that particular bat's call and the tiny bar extending beyond that is the standard deviation. The differently colored bars above the black bar are the allo-calls for that particular bat. Colors denote the call timing classification of each allo-call: synchronizing (-0.167 to 0 s or -1.117 to -0.83 s), alternating (-0.667 to - 0.333 s), or undefined. Duration of the allo-call was displayed when possible. Figure split into a and b to improve readability.*

3.2.3.1.2 Following Call

Five paths exhibited alt calls as the most common call, only 1 path presented alt 100% of the time. Almost a third of the analysed paths (n = 7) did not exhibit alt calls (Figure 16a). Another third of paths (n = 7) did not show any syn calls. Eight paths exhibited syn calls as their most frequent call, 2 of which only had syn calls (Figure 16b). Und calls were present in all but 3 paths. One path only exhibited und calls, while 5 others were found to have und calls at least 50% of the time (Figure 16c). Eleven paths did not meet the criteria for CTC. Ten paths were classified as using a synchronization timing strategy (Figure 17). The next most common strategies were undefined (*n* = 6), random (*n* = 6), and alternation (*n* = 5). Five PFs did not have enough overlap for PTC. The proportions of synchronization, alternation, undefined were all different from the proportions of virtual pairs ($p < 0.001$). Naturally PTC followed CTC type (e.g., synchronization had more syn).

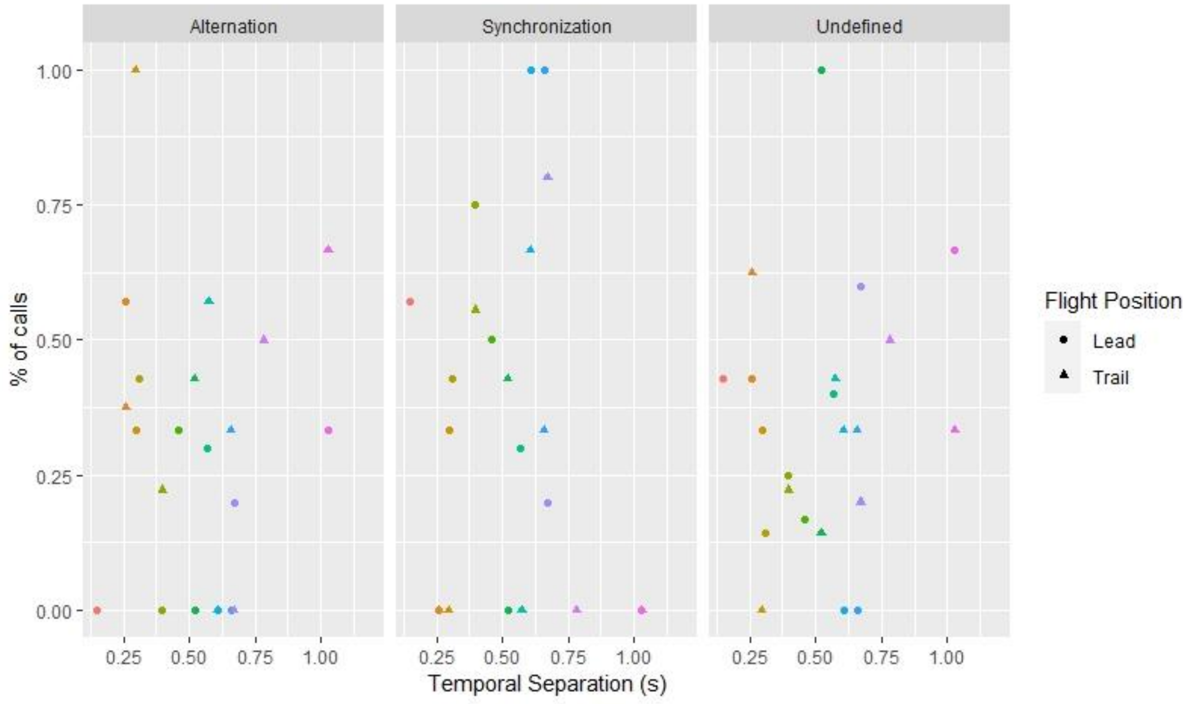

Figure 16: *Percentage of auto-emission to allo-emission calls that are alternating (a), synchronizing (b), or undefined (c) when timing is based on the following call. Each point represents one bat (n = 23), the flight position of the bat is denoted by shape. Paths of the same Paired Flight share the same colour and are placed accordingly on the x-axis displaying the amount of temporal separation between the Trail and Lead bats.*

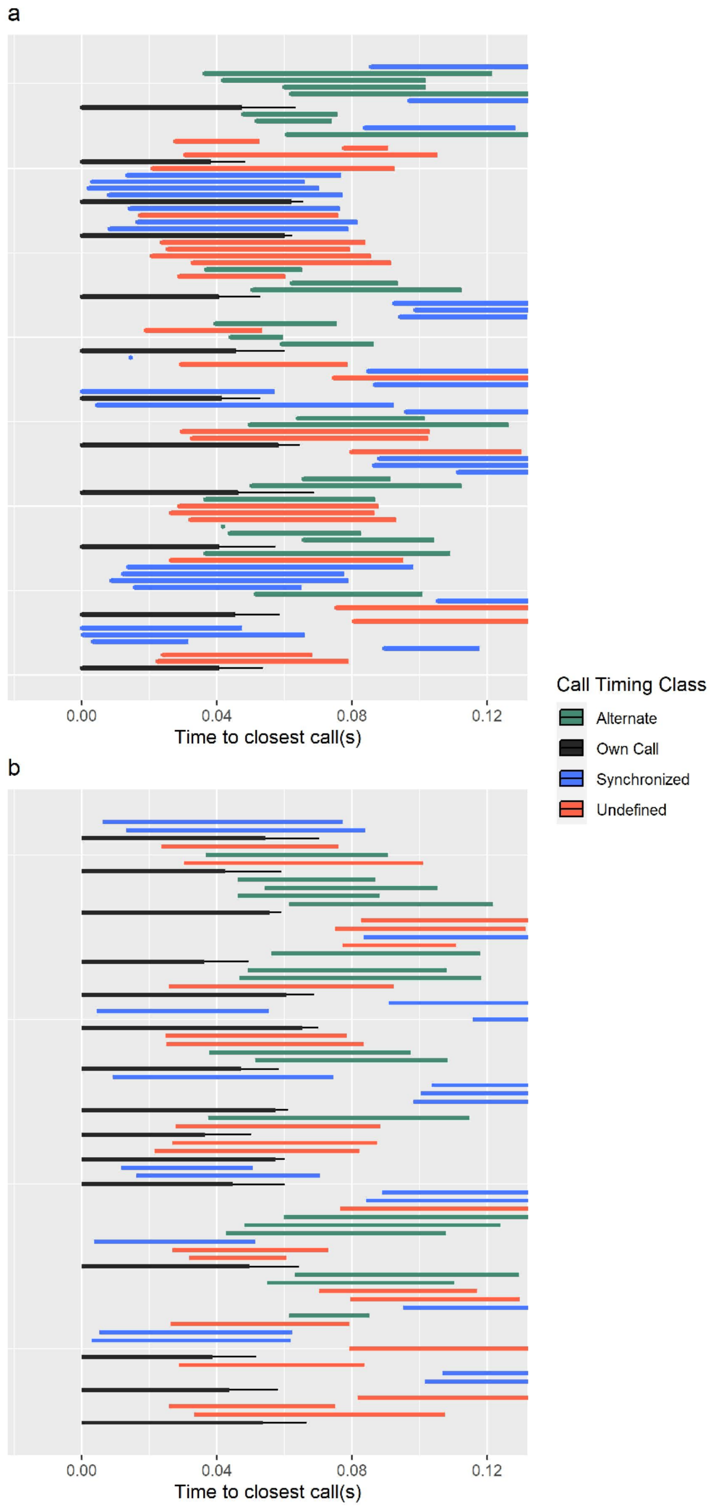

Figure 17: *The call timing of every following call that could be analysed for emission to allo-emission timing. Every black bar denotes a different bat (n = 29). The black bar also provides the average duration of that particular bat's call and the tiny bar extending beyond that is the standard deviation. The differently coloured bars above the black bar are the allo-calls for that particular bat. Colours denote the call timing classification of each allo-call: (0 to 0.167 s or 0.83 to 1.117 s), alternating (0.333 to 0.667 s), or undefined. Duration of the allo-call was displayed when possible. Figure split into a and b to improve readability.*

3.2.3.2 Focal Emission to Secondary Echo

3.2.3.2.1 Preceding Call

Alt calls were common, appearing in all but 2 of the paths (*n* = 19). Alt calls were at least half the calls in a third of the paths (*n* = 7), 3 of which used only alt calls (Figure 18a). Three paths presented syn as the most common call type (Figure 18b). Over a quarter of the paths (n = 5) did not exhibit syn at all. A similar proportion did not show any und calls (*n* = 5). Und calls were only present more than half the time in 2 paths, both of which occurred more than 75% of the time (Figure 18c). Fifteen paths were removed from CTC analysis per the criteria. Most paths adopted an alternating strategy (*n* = 12) in their timing of EO (Figure 19). Undefined (*n* = 4), synchronization (*n* = 3), and random (*n* = 8) strategies were also present. Seven paths could not complete PTC. The call proportion of alternation was found to be different from the virtual pairs (p < 0.01). Both synchronization and undefined were too small to run the chi-square test. TFs of the close proximity group (temporal separation < 0.4s) were found to more likely have alt calls ($p < 0.01$). The TFs of the close half proximity group (< 0.5 s) were also found to have more alt calls ($p < 0.05$).

3.2.3.2.2 Following Call

Similar to preceding call, only 3 paths did not show any alt calls. Alt calls were at least tied for the majority call in 9 paths. Three paths relied solely on alt calls (Figure 20a). There was 1 path that showed syn calls 100% of the time and 3 others that had syn constitute at least 50% of their calls (Figure 20b). Six paths did not use syn, 6 other did not use und. Und was the most common call type found in 5 paths (Figure 20c). Twelve of the 34 paths had to be removed from CTC analysis. Alternation (*n* = 14) timing was utilized the most in EO (Figure 21). Undefined (*n* = 6) was the second most common, followed by synchronization (*n* = 5) and random (*n* = 2). Seven individual PF could not be analysed for PTC. When calls were grouped by PTC, synchronization ($p < 0.001$), alternation ($p < 0.001$), and undefined ($p < 0.05$) were each found to have a different proportion of CTC than virtual pairs. PTC followed CTC type (e.g. synchronization had more syn).

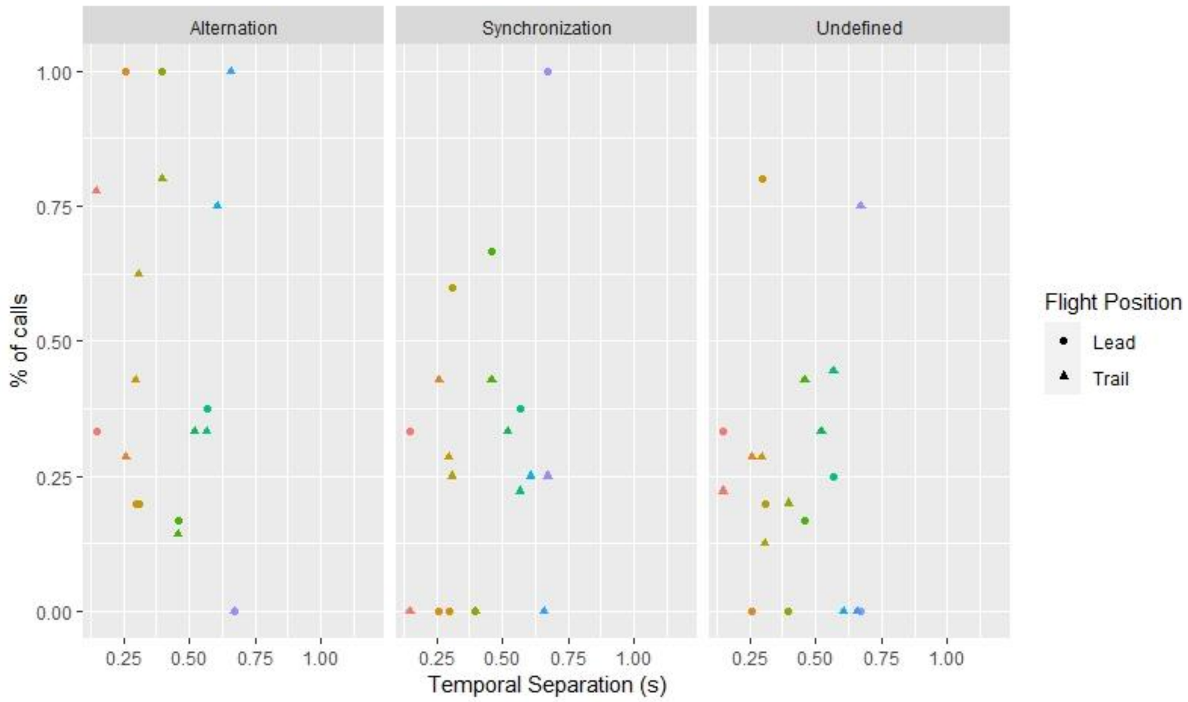

Figure 18: *Percentage of auto-emission to allo-echo calls that are alternating (a), synchronizing (b), or undefined (c) when timing is based on the preceding call. Each point represents one bat (n = 19), the flight position of the bat is denoted by shape. Paths of the same Paired Flight share the same colour and are placed accordingly on the x-axis displaying the amount of temporal separation between the Trail and Lead bat.*

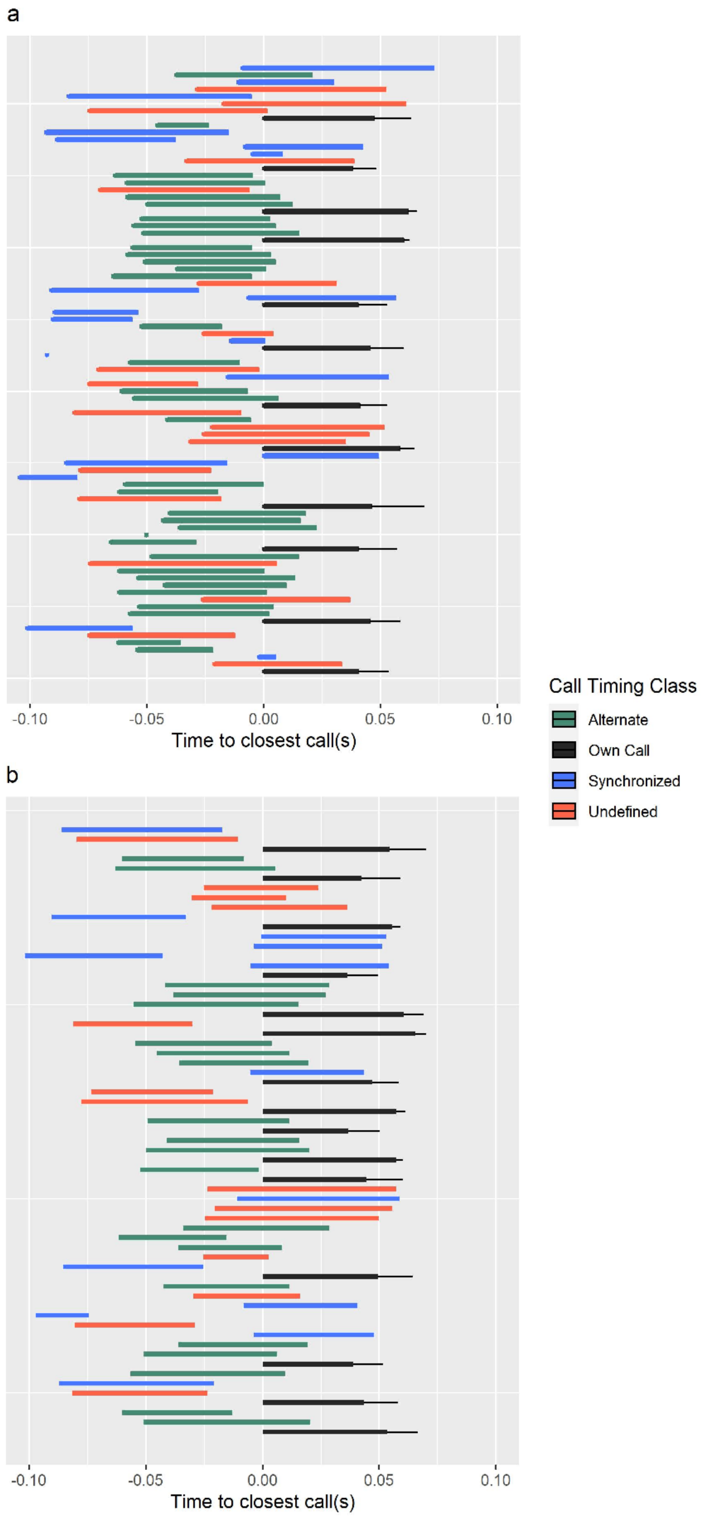

Figure 19: *The call timing of every preceding call that could be analysed for emission to allo-echo timing. Every black bar denotes a different bat (n = 27). The black bar also provides the average duration of that particular bat's call and the tiny bar extending beyond that is the standard deviation. The differently coloured bars above the black bar are the allo-calls for that particular bat. Colours denote the call timing classification of each allo-call: synchronizing (-0.167 to 0 s or -1.117 to -0.83 s), alternating (-0.667 to - 0.333 s), or undefined. Duration of the allo-call was displayed when possible. Figure split into a and b to improve readability.*

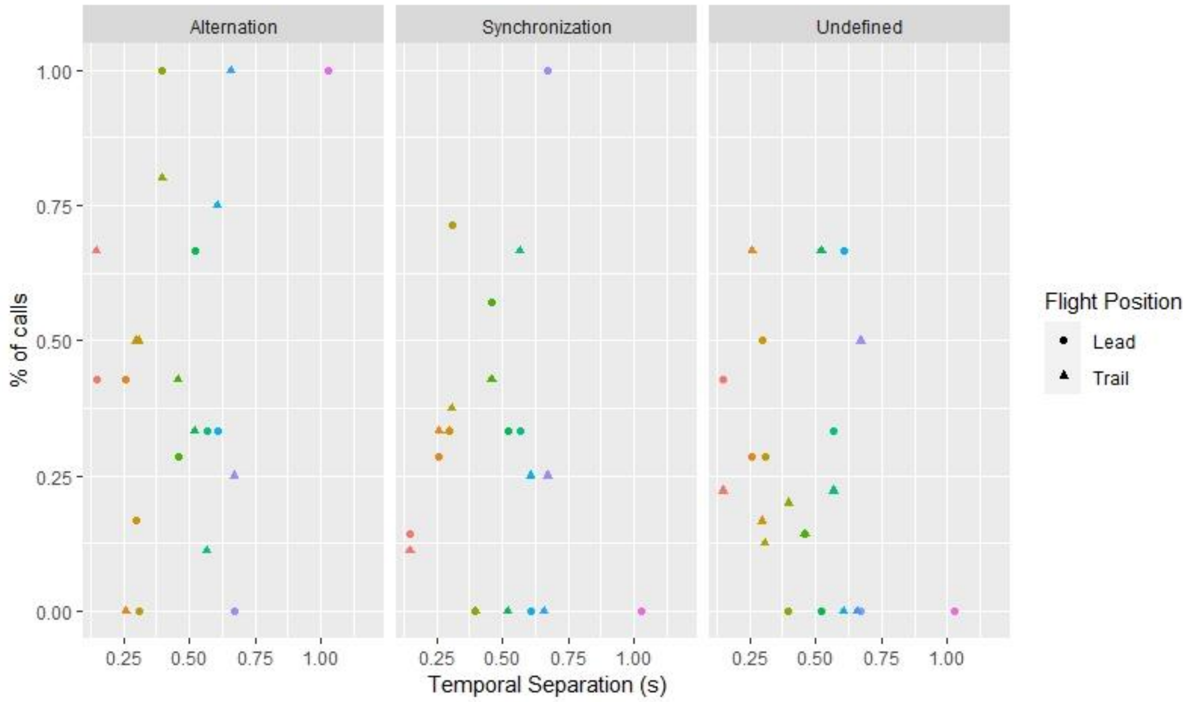

Figure 20: *Percentage of auto-emission to allo-echo calls that are alternating (a), synchronizing (b), or undefined (c) when timing is based on the following call. Each point represents one bat (n = 22), the flight position of the bat is denoted by shape. Paths of the same Paired Flight share the same colour and are placed accordingly on the x-axis displaying the amount of temporal separation between the Trail and Lead bat.*

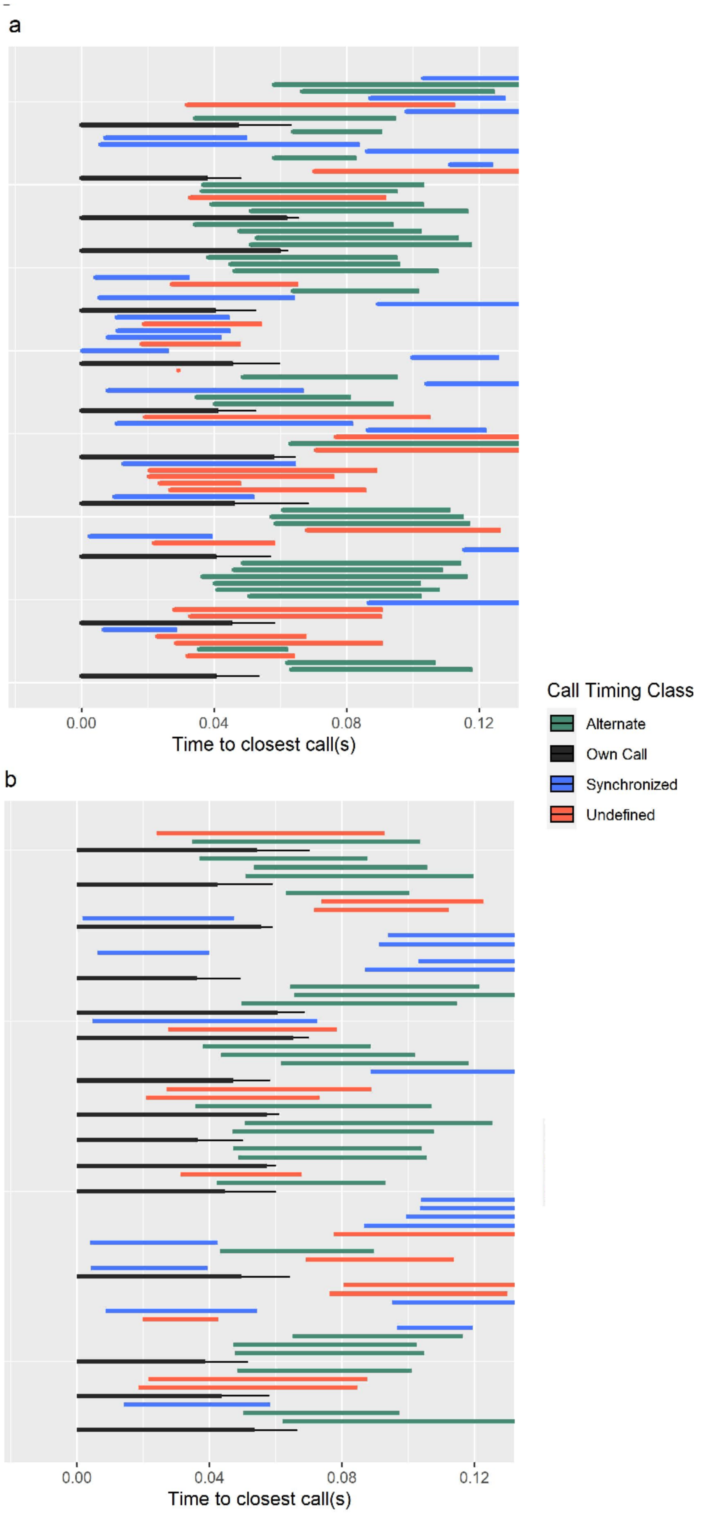

Figure 21: *The call timing of every following call that could be analysed for emission to allo-echo timing. Every black bar denotes a different bat (n = 27). The black bar also provides the average duration of that particular bat's call and the tiny bar extending beyond that is the standard deviation. The differently coloured bars above the black bar are the allo-calls for that particular bat. Colours denote the call timing classification of each allo-call: synchronizing (0 to 0.167 s or 0.83 to 1.117 s), alternating (0.333 to 0.667 s), or undefined. Duration of the allo-call was displayed when possible. Figure split into a and b to improve readability.*

3.2.3.3 *Focal Echo to Secondary Echo*

3.2.3.3.1 Preceding Call

Syn calls were the majority call for 6 paths but were non-existent in 5 others (Figure 22b). The number of paths showing alt calls were similar, 7 and 5 respectively (Figure 22a). Undefined calls were not present in 1 path and only constituted 50% or more of calls in 4 paths (Figure 22c). Fifteen paths were excluded from CTC analysis. Alternation (n = 10) was once again the most common strategy in use with OO timing; synchronization was a very close second (n = 9; Figure 23). Four paths were classified as undefined, 4 others random. Seven paths could not undergo PTC. The CTC proportions of alternation and synchronization were both found to be different from the virtual pair proportions ($p < 0.01$). Alternation had a higher proportion of alts, synchronization more syns. There were not enough calls for undefined to be run in the chi-square test. TF in the close proximity group were found to use alt calls more than expected ($p < 0.05$).

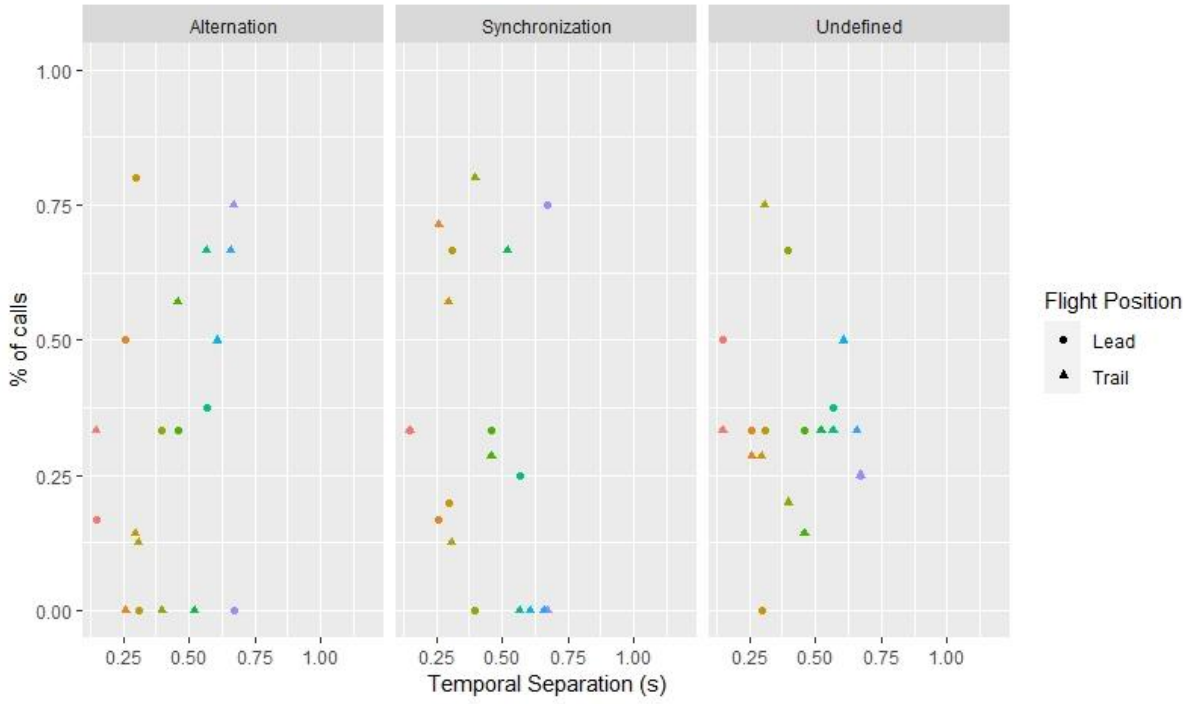

Figure 22: *Percentage of auto-echo to allo-echo calls that are alternating (a), synchronizing (b), or undefined (c) when timing is based on the preceding call. Each point represents one bat (n = 19), the flight position of the bat is denoted by shape. Paths of the same Paired Flight share the same colour and are placed accordingly on the x-axis displaying the amount of temporal separation between the Trail and Lead bat.*

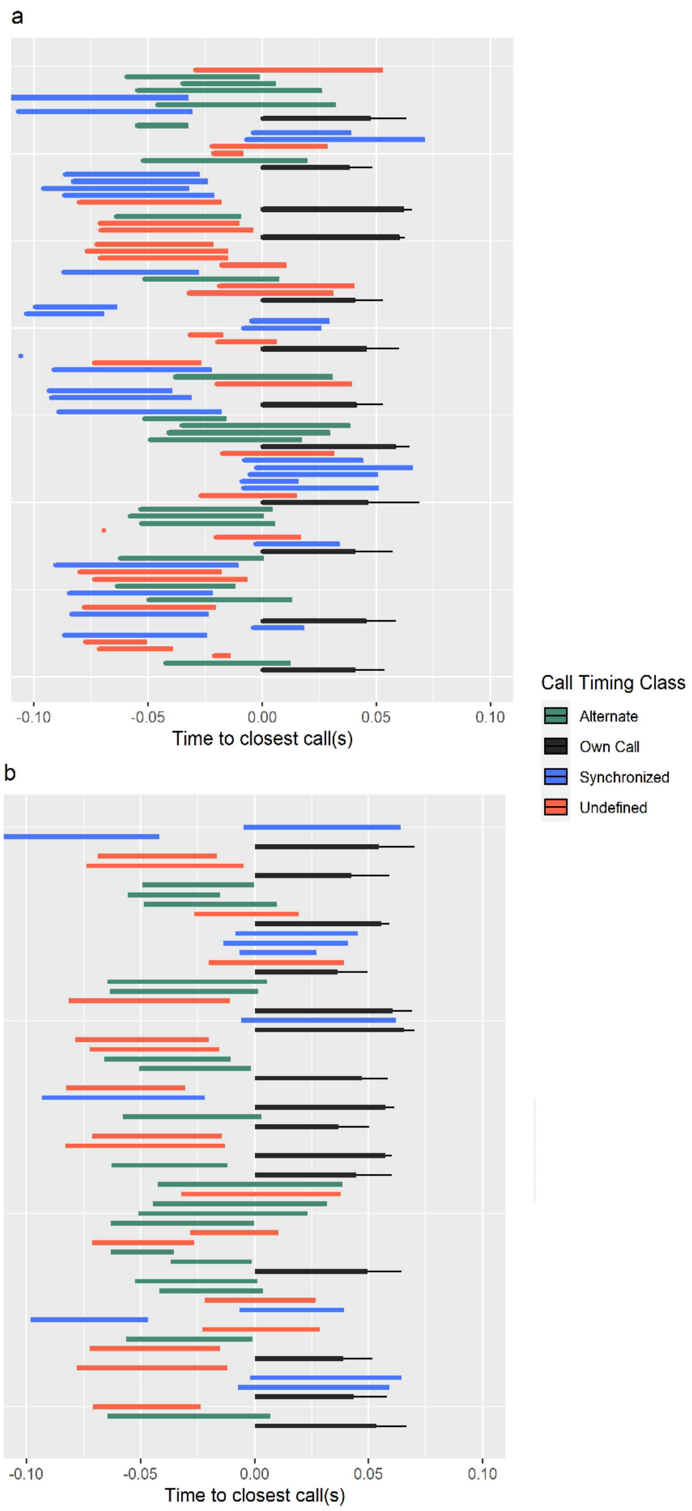

Figure 23: *The call timing of every preceding call that could be analysed for echo to allo-echo timing. Every black bar denotes a different bat (n = 27). The black bar also provides the average duration of that particular bat's call and the tiny bar extending beyond that is the standard deviation. The differently coloured bars above the black bar are the allo-calls for that particular bat. Colours denote the call timing classification of each allo-call: synchronizing (-0.167 to 0 s or -1.117 to -0.83 s), alternating (-0.667 to - 0.333 s), or undefined. Duration of the allo-call was displayed when possible. Figure split into a and b to improve readability.*

3.2.3.3.2 Following Call

Over half of the paths (n = 11) presented syn calls more than half the time, 5 of which only had syn calls (Figure 24b). Almost a third of the paths (n = 7) used alt calls for the majority of the time (Figure 24a). Alt was the sole call of 1 path. Five paths did not show any alt calls at all. Undefined calls were at least tied for the majority call of 5 paths and non-existent in 3 (Figure 24c). Twelve paths did not contain enough overlapping calls to undergo CTC. In OO timing, synchronizing (n = 11) and alternating (n = 8) were the most common classifications (Figure 25). Undefined (n = 5) and random (n = 3) were also present. PTC was not run on 7 paths. Synchronization, alternation, and undefined (*p* < 0.01) all occurred more frequently than expected. When calls were grouped together based on their PTC, synchronization, alternation, and undefined were all found to have proportions different from the virtual pairs (*p* < 0.05). The three PTCs each had more calls in their corresponding CTC (e.g., more syn in synchronization).

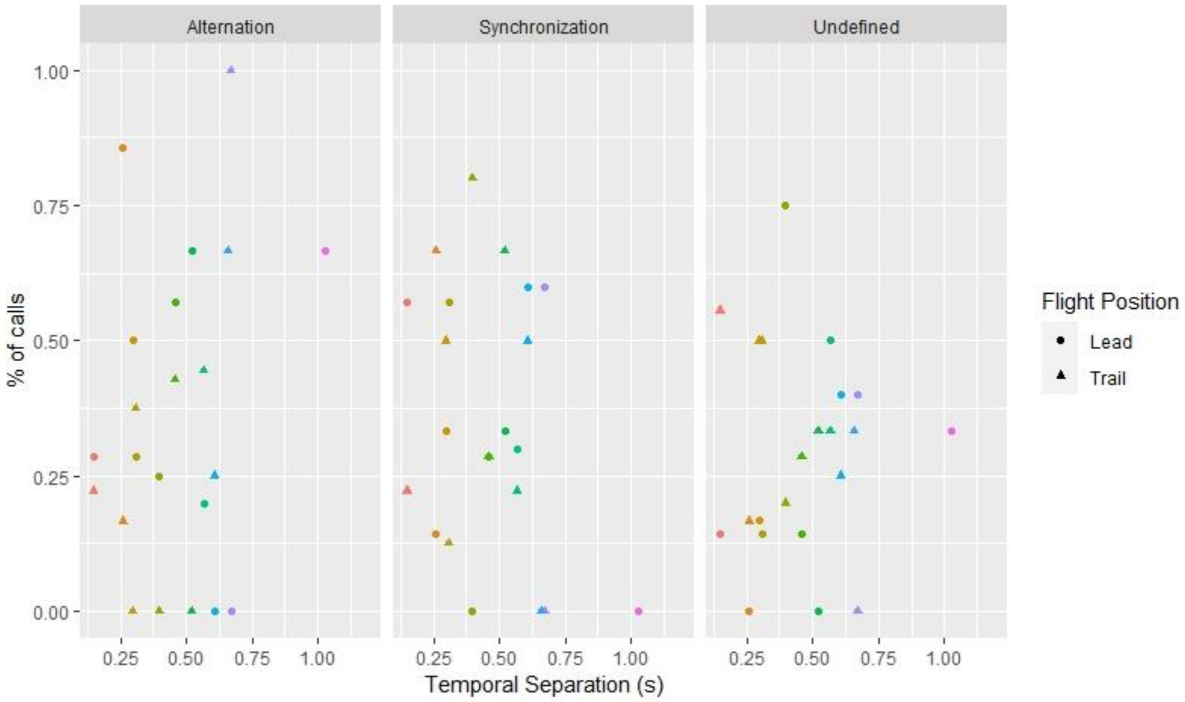

Figure 24: *Percentage of auto-echo to allo-echo calls that are alternating (a), synchronizing (b), or undefined (c) when timing is based off of the following call. Each point represents one bat (n = 22), the flight position of the bat is denoted by shape. Paths of the same Paired Flight share the same colour and are placed accordingly on the x-axis displaying the amount of temporal separation between the Trail and Lead bat.*

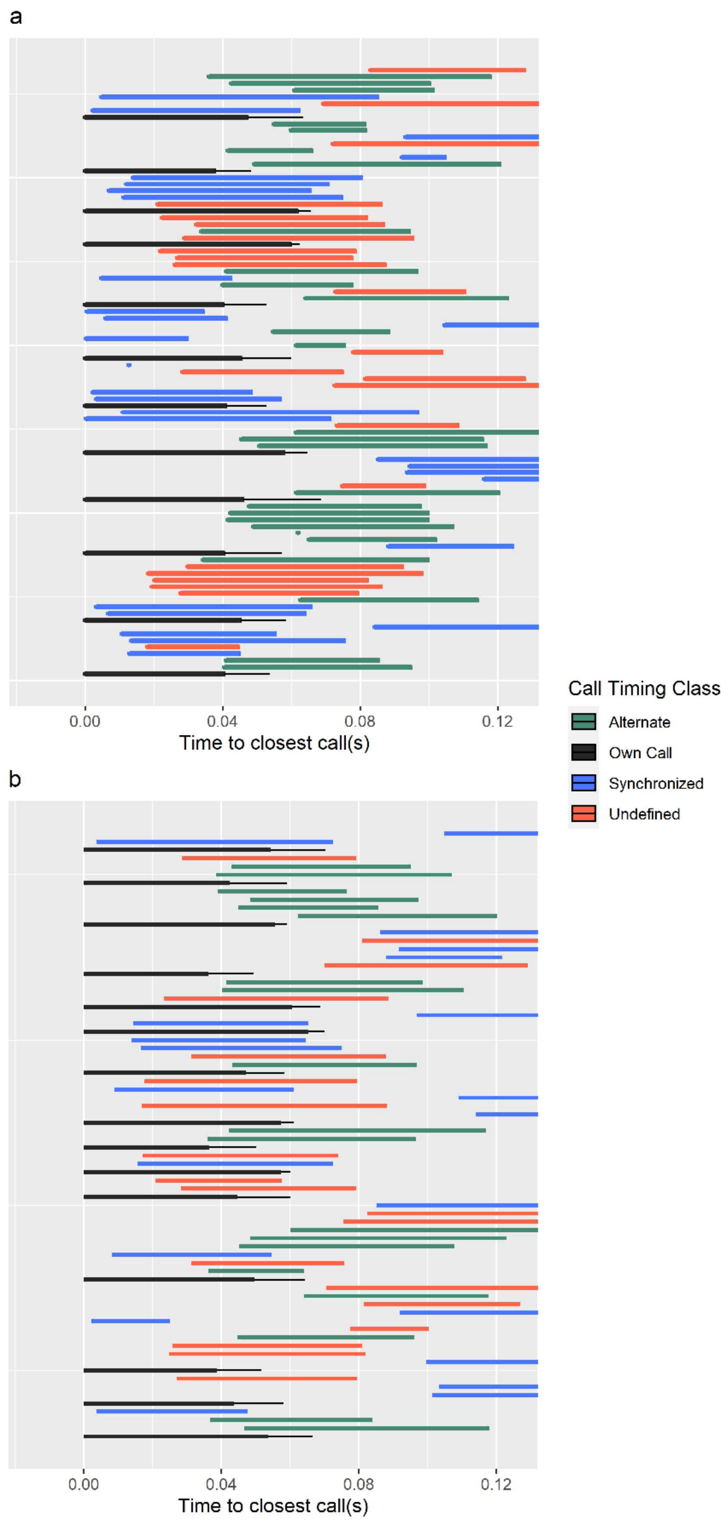

Figure 25: *The call timing of every following call that could be analysed for echo to allo-echo timing. Every black bar denotes a different bat (n = 27). The black bar also provides the average duration of that particular bat's call and the tiny bar extending beyond that is the standard deviation. The differently coloured bars above the black bar are the allo-calls for that particular bat. Colours denote the call timing classification of each allo-call: synchronizing (0 to 0.167 s or 0.83 to 1.117 s), alternating (0.333 to0.667 s), or undefined. Duration of the allo-call was displayed when possible. Figure split into a and b to improve readability.*

3.2.4 Frequency Characteristics

3.2.4.1 FMi and FMt

The median FMi bandwidth for SF was 12.05 (11.85 – 12.56) kHz, PF FMi bandwidth was 12.12 (11.75-12.47) kHz (*Figure 26*a). LF median FMi bandwidth was 12.29 (11.49-13.09) kHz and 12.10 (11.69-12.46) kHz for TF. PF FMt bandwidth was found to be larger 13.49 (12.46-14.47) kHz than SF, 12.16 (11.69-12.96) kHz (p <0.001; *Figure 26*b). Median FMt of LF and TF were not as similar as their FMi, 13.32 (12.32-14.66) kHz and 13.62 (12.02-14.71) kHz respectively. Both LF ($p < 0.001$) and TF ($p < 0.05$) FMt bandwidths were larger than SF FMt.

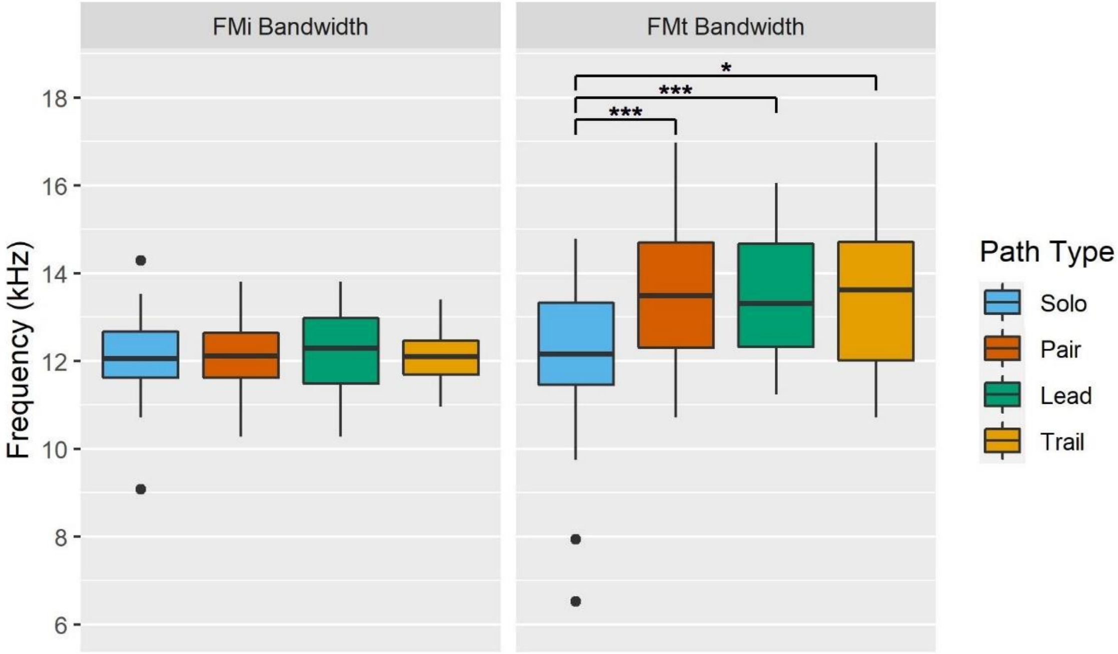

Figure 26: Boxplots (median, 25% CI, and range) of the median path bandwidth of initial FM (FMi; a) and terminal FM (FMt; b) for the various path types: Solo (n = 46), Pair (n = 34), Lead (n = 17), Trail (n = 17. Black dots represent outliers. The FMt bandwidth of Pair (p < 0.001), Lead (p< 0.001), and Trail (p < 0.05) were all larger than Solo CF.

3.2.4.2 Recorded, Emitted, and Echo CF

The median path CF for recorded frequency was 82.11 (81.91 - 82.22) kHz for SF and 82.32 (82.06-82.49) kHz for PF (Figure 27). The recorded CF for PF was found to be significantly different than SF ($p < 0.05$). LF median recorded CF was 82.34 (82.02-82.52) kHz, TF median recorded CF of 82.27 4(81.97-82.71) kHz. LF and TF were not significantly different from one another but LF was different from SF ($p < 0.025$), TF did approach significance from SF ($p = 0.026$).

The median emitted and echo CF of SF was 80.14 (79.84-80.34) kHz and 84.07 (83.95-84.21) kHz respectively (Figure 27). In comparison, PFs were 80.47 (80.23-80.71) kHz in emission and 84.29 (83.94-84.64) kHz in echo CF. The maximum (84.99 kHz) and minimum (82.71 kHz) heard CFs were both observed in PFs**.** LF and TF median emission CFs were 80.56 (80.04-80.73) kHz and 80.44 (79.93-80.91) kHz respectively. Median LF echo CF was 84.31 (83.79-84.67) kHz. The TF median echo CF was 84.19 (83.96-84.65) kHz. The maximum recorded (83.06 kHz), emitted (81.46 kHz), and echo (84.99 kHz) path medians were observed in different PFs but always belonged to a LF. The TFs provided the lowest emitted (79.09 kHz), and echo (82.71 kHz) CF path medians.

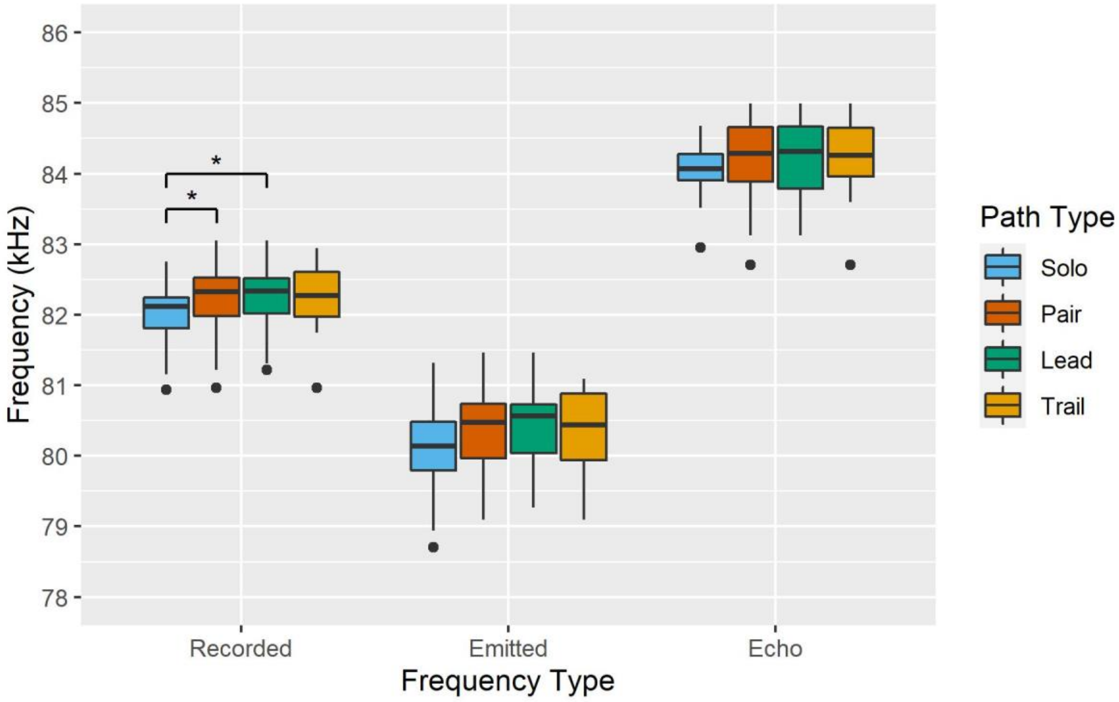

Figure 27: *Boxplots (median, 25% CI, and range) of each path type [Solo (n = 46), Pair (n = 34), Lead (n = 17), and Trail(n = 17)] by the recorded constant frequency (CF), emitted CF, and echo CF. Black dots represent outliers Pair, Lead, and Trail Recorded CF were all significantly different from Solo recorded CF ($p < 0.05$).*

3.2.4.3 Spectral Shifting

3.2.4.3.1 Emission vs Secondary Emission

3.2.4.3.1.1 Lead Flight Frequency Difference

Actual frequency difference ranged from –2.24 to 3.22 kHz (Figure 28a). LF frequency difference in EE ranged from 0.03 to 3.22 kHz in absolute difference (Figure 28b). Four of the 5 closest PFs, and 4 others, were significantly different in actual difference ($p < 0.001$). Of the 5 closest PFs, the 1 path that did not display a significant difference did not have enough overlapping calls to be analysed. All the significant paths exhibited frequency differences that were positively shifted from the expected frequency difference based on the virtual pairs (1.77 ±1.19 kHz). Seven of the 10 PFs that were significant in actual difference also exhibited absolute frequency differences that were smaller than the virtual pair frequency differences (1.81 ±1.12 kHz; $p < 0.01$). Only 1 of the closest 7 PFs, that were measurable, did not show an absolute difference that was lower than expected.

3.2.4.3.1.2 *Trail Flight Frequency Difference*

Trail EE frequency difference ranged from -3.96 to 2.89 kHz in actual difference and 0.003 to 3.9 kHz in absolute difference (Figure 29). In both absolute and actual frequency difference, 4 of the 5 closest PFs and 3 others were significant ($p < 0.05$). Two paths exhibited an actual difference more negative than expected based on the virtual pairs (-1.47 ±1.18 kHz); the other significant paths were more positive. All of the significant paths in absolute EE were smaller than the expected frequency difference of 1.59 ±1.161 kHz ($p < 0.05$).

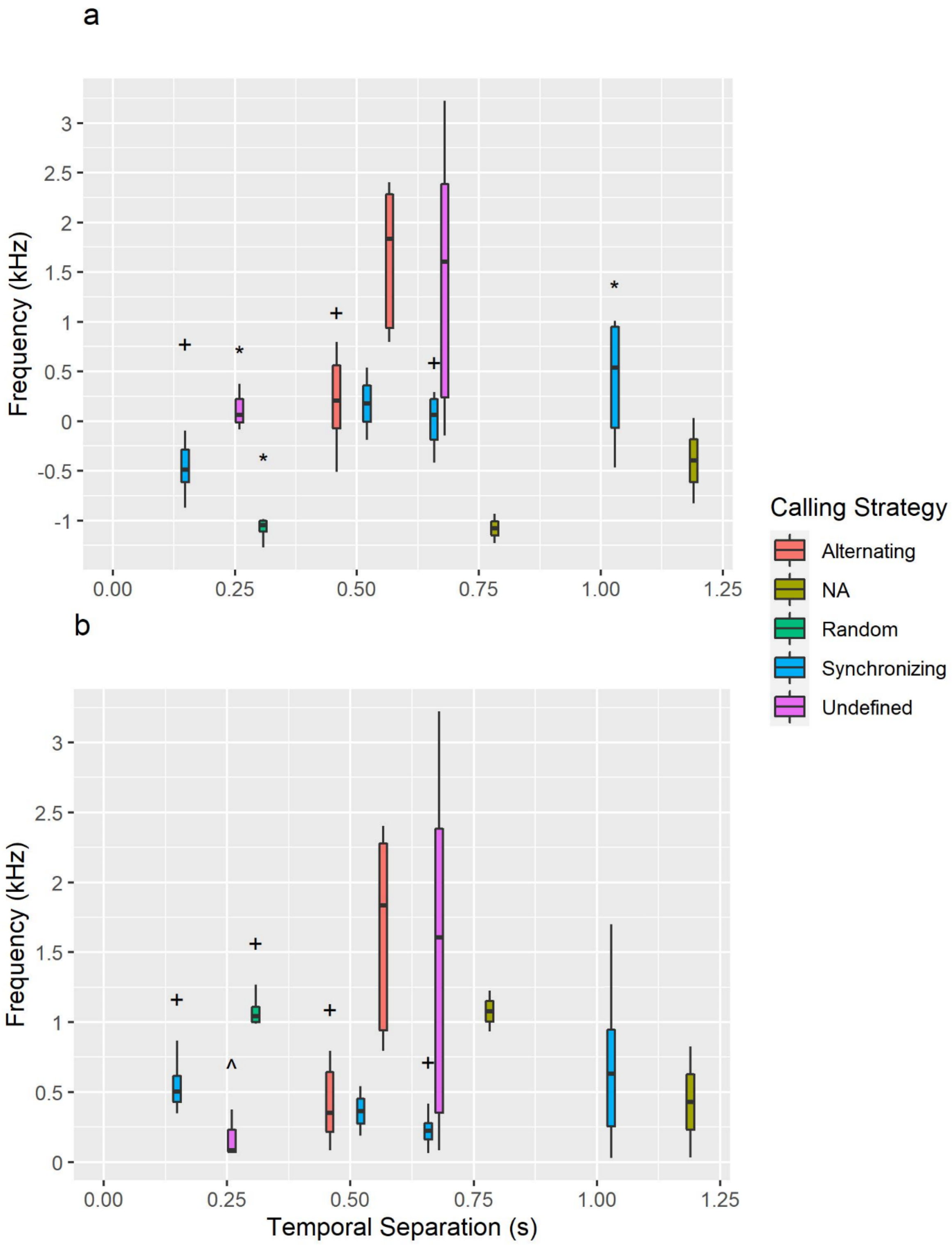

Figure 28: *Boxplots (median, 25% CI, and range) of each Lead Flight frequency difference in emission to allo-emission (n = 11). Black dots indicate outliers. Figures are split into actual difference (a) and absolute difference (b). Lead Flights are ordered by time separation between the Lead and Trail Flights. Multiple Lead Flights displayed frequency differences larger and smaller than the frequency differences calculated via virtual pairs (* = p < .05, ^ = p < .01, + = p < .001). The color of each boxplot corresponds to the most common call timing strategy in use when basing call timing off of the preceding call.*

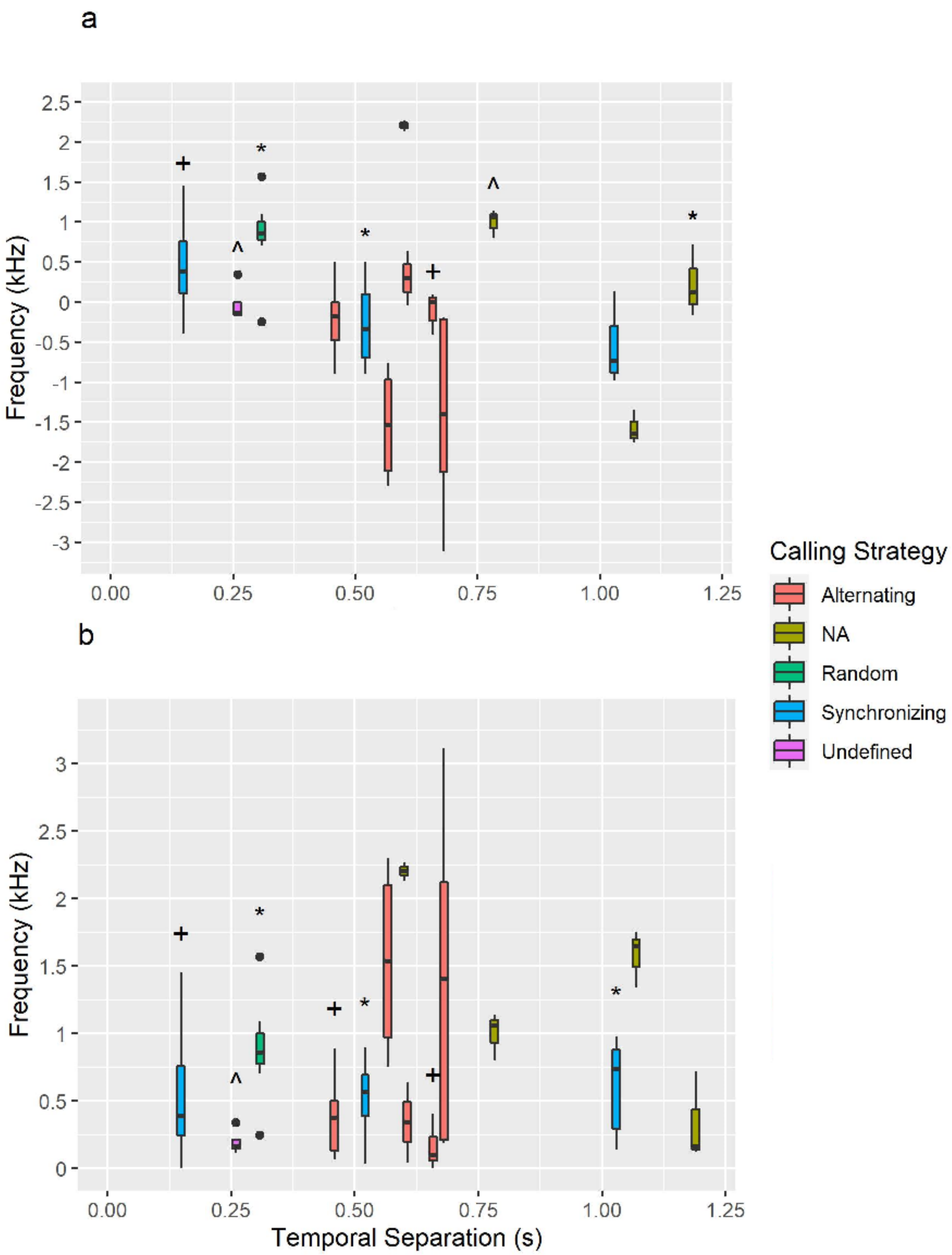

Figure 29: *Boxplots (median, 25% CI, and range) of each Trail Flight frequency difference in emission to allo-emission (n = 14). Black dots indicate outliers. Figures are split into actual difference (a) and absolute difference (b). Lead Flights are ordered by time separation between the Lead and Trail Flights. Multiple Lead Flights displayed frequency differences larger and smaller than the frequency differences calculated via virtual pairs (* = p < .05, ^ = p < .01, + = p < .001). The colour of each boxplot corresponds to the most common call timing strategy in use when basing call timing off of the preceding call.*

3.2.4.3.2 Echo vs Secondary Echo

3.2.4.3.2.1 Lead Flight Frequency Difference

Absolute echo to secondary echo frequency difference for LFs spanned from 0.02 to 3.33 kHz (Figure 30a). Actual difference for the same data were -3.33 to 1.59 kHz (Figure 30b). In actual difference 3 of the 6 significantly different frequency differences were lower than expected (-0.57 ±0.89 kHz; $p < .05$), the other 3 higher ($p < 0.01$). Two of the 5 PFs separated by less than 0.5 s had absolute frequency differences different from the expected frequency difference calculated via virtual pairs (0.85 ±0.63 kHz; $p < 0.01$). Three of the 4 significantly different frequency differences were higher than expected ($p < 0.05$).

3.2.4.3.2.2 Trail Flight Frequency Difference

TF absolute OO frequency difference was from as low as 0.02 kHz to as high as 3.22 kHz (Figure 31a). TF actual difference was as low as –1.98 to 3.22 kHz (Figure 31b). Seven TF OO actual frequency differences were significantly different from the frequency difference of the virtual pairs (0.28 ±0.87 kHz; $p < .05$). Two of those 7 frequency differences were lower than expected. Three of the 6 closest analysable paths showed significance in actual frequency difference; only 2 did in absolute frequency difference ($p < 0.05$). In total, 6 absolute frequency differences were different from the virtual pair absolute frequency difference (0.73 ±0.57 kHz). Only 1 frequency difference was lower than expected.

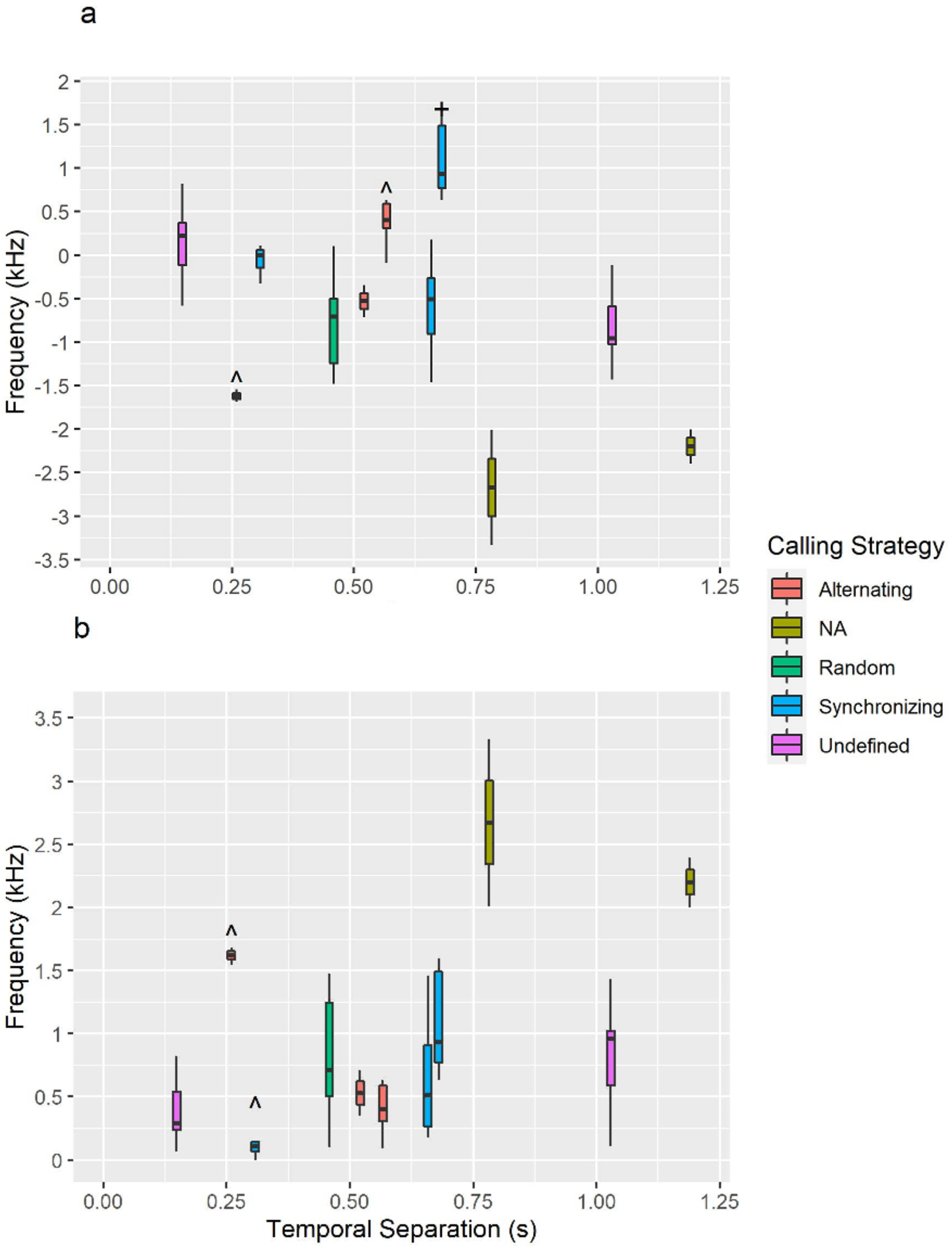

Figure 30: *Boxplots (median, 25% CI, and range) of each Lead Flight frequency difference in echo to allo-echo (n = 11). Black dots indicate outliers. Figures are split into actual difference (a) and absolute difference (b). Lead Flights are ordered by time separation between the Lead and Trail Flights. Multiple Lead Flights displayed frequency differences larger, and smaller, than the frequency differences calculated via virtual pairs (* = p < .05, ^ = p < .01, + = p < .001). The colour of each boxplot corresponds to the most common call timing strategy in use when basing call timing off of the preceding call.*

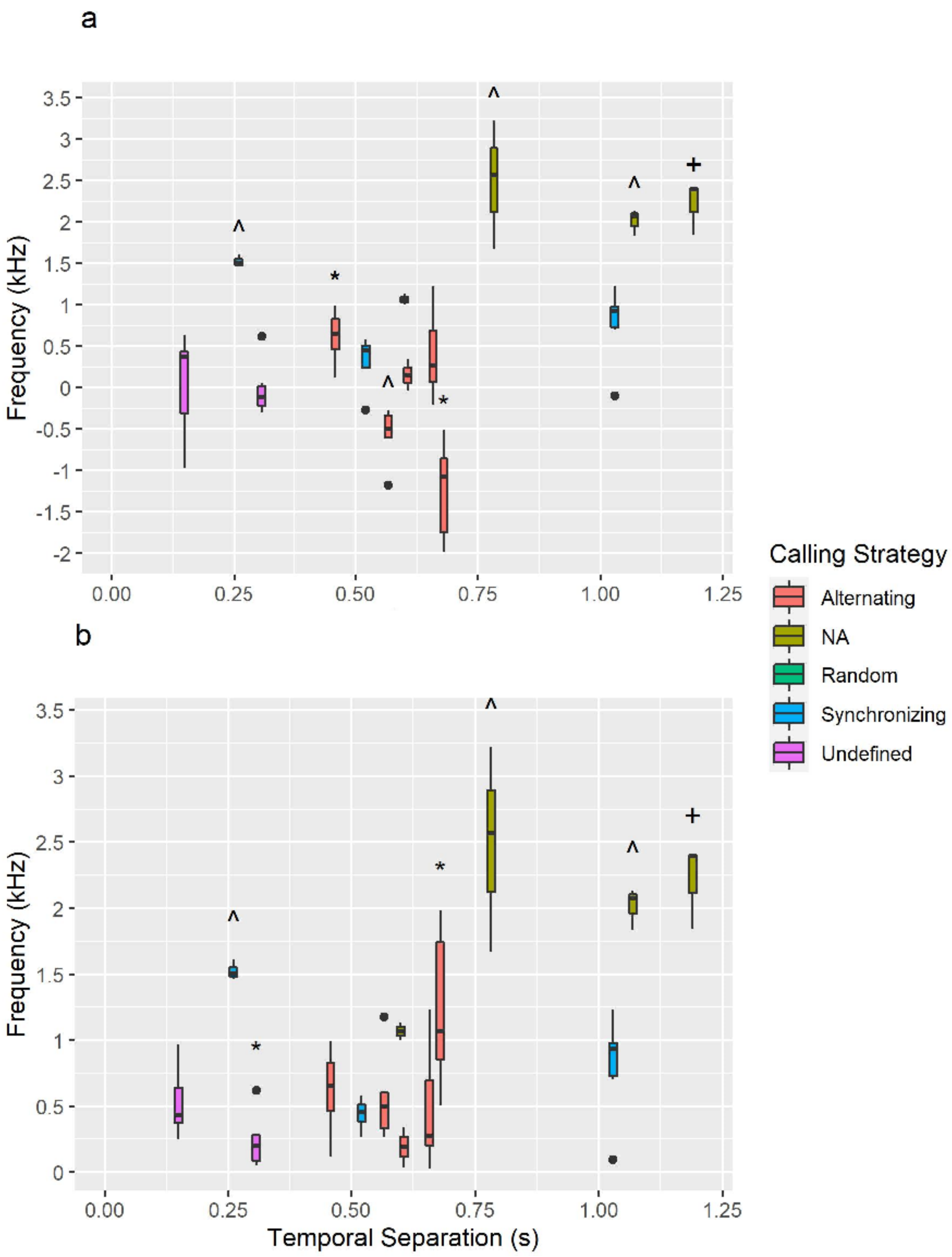

Figure 31: *Boxplots (median, 25% CI, and range) of each Trail Flight frequency difference in echo to allo-echo (n = 14). Black dots indicate outliers. Figures are split into actual difference (a) and absolute difference (b). Lead Flights are ordered by time separation between the Lead and Trail Flight. Multiple Lead Flights displayed frequency differences larger and smaller than the frequency differences calculated via virtual pairs (* = p < .05, ^ = p < .01, + = p < .001). The colour of each boxplot corresponds to the most common call timing strategy in use when basing call timing off of the preceding call.*

4 Discussion

4.1 Methods Considerations

A few aspects of the methods did influence this study. It was originally planned to have a second night of data collection in May 2020. The COVID-19 outbreak and subsequent lockdown effectively eliminated that plan. By the time fieldwork of that kind could be completed there would not have been enough time to run the new data through all the analysis steps. This unfortunately resulted in only 17 PFs to analyse which undoubtedly led to the reliance on non-parametric tests and the inability to reach clear conclusions, particularly in paths and frequency.

It should be noted that at no point have I calculated the actual received echo frequency of the bats. The calculated echo frequency is the highest possible frequency that could be received by the bat from the hedge. The beam direction of the bat may very well not have been directly in front, thereby changing the actual received echo frequency. However, the difference from the actual received echo frequency should be minimal as the hedge is the likely target of focus as explained in the methods (see 2.2.4.2). Additionally, the triangulation of each calling point comes with its own margin of error, which also means that velocity calculations have a similar margin of error. These errors could have influenced the calculated Doppler shift and therefore the calculated frequencies (i.e., emitted and echo). These margin of errors are quite small and would be expected to shift frequencies by only about 0.02 kHz when the margin of error was the highest (8 m from the arrays).

Finally, I was partially limited by both the equipment and the nature of HDC bats. The high CF of *R. ferrumequinum* attenuates very quickly in the air and the quality of the CF was generally not very good until around 9 m. The furthest localized point in the entire study was only 11 m from the arrays. This naturally restricted the area in which I could run analysis. Additionally, the HDC of the bats also meant that there were not many calls to analyse in each path. With a spatial calling density of 1.54 m^{-1} and analysis being restricted in a 6 m range (8-2 m), a best case scenario of standard bats would provide only ~9 calls to analyse. The number of calls shrank when comparing pairs. Comparison for call timing and frequency difference could not start until the TF reached 8 m and would stop once the LF passed 2 m. This created a narrow window with which to compare calls. The maximum number of calls compared in paired analysis was 10 but more commonly there was only around 4. Due to lost calls some of the PFs could not be used in call timing or frequency difference analysis. These limitations in overlapping calls made investigating call timing and spectral shifting particularly difficult.

4.2 Path Characteristics

4.2.1 Flight Corridor

The general flight corridor closely following the building and then approaching the hedge was not unexpected as multiple studies have reported on bats' tendencies to follow large structures when commuting (Britton *et al.*, 1997; Krull *et al.*, 1991). With a few exceptions (*n* = 8), SFs followed a very similar flight corridor as they exited the roost. The SC indicated that variation in height becomes much more restricted as SFs turn in front of the arrays. Interestingly, a similar amount of variation was also present during initial tracking (8-6 m) although it was in width rather than height. The increase in width variation during the turn was expected due to both the differences in turn angles and turning points. The relatively small corridor height in the turn phase, however, was unexpected and the corridor height's similarity to initial corridor width was peculiar. Indeed, the turn phase portion of the corridor almost looks like the initial phase, just rotated 90°.

The observed choice of flight corridor could be an attempt to mitigate predation risk. It has been hypothesized that bats prefer to fly closer to structures that provide more visual cover from predators (Schaub and Schnitzler, 2007)**.** Higher flying bats relying on these structures for predation protection would need to lower flight height as the hedge was only half as high as the building. However, this idea of predation mitigation fails to provide an adequate answer for those bats that were initially tracked flying lower than average but eventually increased their height.

R. ferrumequinum is considered a landscape-hugging flyer that is using guiding structures. As the bats turned, they were without a lateral guiding structure (the wall), apart from the ground. The ground is a constant feature and can be used as a guide until the next vertical structure is approached. Research regarding guiding structures has suggested that the distance between the guiding structure and the bat remains relatively consistent (Schaub and Schnitzler, 2007)**.** The corridor widths from 8-6 m and heights from 4-2 m are both the same at ~0.5 m, possibly indicating that the guiding structure was used (i.e., the lateral wall at Y = 8-6 m and the ground at Y < 4 m). Another study on *R. ferrumequinum* reported that bats were flying lower than normal when in open space (Duvergé and Jones, 2003). It may be that the bats observed here, and in previous studies, are using the ground as a guiding structure when no adequate vertical guiding structure is available.

Bats have been shown to rely on spatial maps to navigate familiar environments (Jensen *et al.*, 2005; Neuweiler and Möhres, 1967 as cited in Ulanovsky and Moss, 2008). In areas of open space bats do not have a structure to use as a landmark, however research has described bats capable of utilizing acoustic-flow and path integration (Aharon *et al.*, 2017; Müller and Schnitzler, 1999, 2000). The bats can use the hedge and the building wall as landmarks to return to the roost but the turn portion of the path may not be able to be memorized via a landmark. I suggest that the consistency in corridor variability during the turn is due to *R. ferrumequinum* using the ground for acoustic-flow or path integration to memorize the path back home.

4.2.2 Flight Corridor Comparison

I had predicted that there would be very clear and obvious path differences between PFs; that was hardly the case. Some PF flights showed path differences, such as a bat turning much earlier than average while the other maintained the standard course, but this was not common (*n* = 4). Only one pair was found to have the one bat turn early and the other turn very late (Appendix 2d) although this specific behaviour did occur with the bats with one of the smallest amount of separation. The general structure of the SC and TC are the same: relatively close to the wall and similar turns towards the hedge at a similar Y-position. While SC constituted 84.6% of SFs, SC was found to contain only 61.5% of PFs ($p < 0.001$). This suggests that the presence of conspecifics does influence the paths taken by the bats. The level of influence though is still to be determined. SC may not have contained as much PF path, but many of those uncontained points were only just outside the SC. The PC confirms that PF paths were more variable than SFs but not by a large margin. The calculated flight corridor of PFs was only slightly larger than that of SFs. PFs did not fly in such a narrow corridor as the SFs during the turn phase and surprisingly had a smaller width in this phase as well. Upon visual inspection, PC only seems marginally larger than SC and does not suggest that the general path taken by PFs was any different from SFs. However, LC and TC tell a different story.

Beam direction and the angle at which echoes are returned play an important role in the separation of echoes *(*Bates *et al., 2011;* Sümer *et al.*, 2009). If the bat has found a path that provides a JAR then the bat is likely to maintain course rather than take a chance that a change in course creates a jamming scenario. This may explain the consistency of LFs flying so low so consistently and TFs using a corridor similar to SC. If a LF has found a path where it is not being interfered with then it should maintain that course until the landscape forces it to change (i.e., the hedge). The use of this strategy may explain why LC corridor height was generally narrower than SC and why TC corridor height was higher than SC. Under the assumption that paths were being maintained due to the risks of jamming, both LFs and TFs would be unlikely to change flight position until absolutely necessary. This would reduce the amount of variation in flight path movement. For LFs, this resulted in a corridor that was mostly straight and generally without change in corridor height. For TFs, the lack of path adjustment may explain why the TC drop off in corridor height happens later than SC. SFs included bats flying higher than the maximum corridor height of TC but nearly all of those paths quickly adjusted their flight as they approached the arrays. Admittedly, the sample size may also play a role here as those higher SFs were more likely to be excluded from the SC. Only paths with excessive differences from the average TF would be excluded from TC due to the low sample size. The lack of path adjustment in TFs would create a corridor that maintains its height rather than the steady decline observed in SC. TC does eventually drop and somewhat resembles SC by the end of tracking. This may be due to differences between being a LF and a TF.

The TF will likely incorporate the same strategy of avoiding flight changes but when the LF has become parallel to the hedge, the risk of TF being jammed is reduced. This is because the echoes from the LF are coming at a drastically different angle than before and can easily be separated from auto-echoes. The Doppler shift will also change by a large margin, likely moving the allo-echo frequency out of the auditory fovea. By the time TFs reached the turn phase, LFs were already past or parallel with the hedge. Interestingly, the turn phase portion of TC looks much more like SC. The flight behaviour of TFs in the turn phase resembles SFs, who are certainly not being jammed. TF path behaviour is consistent with the idea that TF is not being jammed once a LF has turned.

The corridors of LC and TC may have been unduly influenced by sample size. For example, the steep drop-off of TC was partially a result of one of the higher paths no longer being trackable. Corridor construction was prone to add in flight paths at later stages that were initially deemed as not belonging to the average flight corridor which formed the disconnected ‘bubbles’ from the corridor. These flight paths had not changed in any significant way, rather as some paths could no longer be tracked the sample size to create the corridor became smaller and these ‘bubble’ paths were now considered part of the average flight corridor. These bubbles should not, and are not, considered part of the standard corridor. The loss of paths may have affected the corridors in a multitude of ways. TF only lost 2 paths by the end but LF lost 5 (losing 3 by 3 m). This may have negatively impacted the ability to determine the extent in turning variation. PFs were expected to have a wider corridor during the turn phase yet SC had the widest corridor. The majority of path loss occurred during turns which could have put an unintentional limit on the range of X values. My personal visual analysis of all PFs within the SC did not suggest that the missing trajectories would significantly widen PC corridor width at any point. While it was unfortunate to lose portions of the path, I do not believe that these missing portions concealed the true nature of the corridors. The presence of conspecific does appear to change the paths taken by bats but the use of paths as a Flight JAR remains unclear.

4.2.3 Path Velocity

The fastest recorded *R. ferrumequinum* was reported by Ransome (1981) at 8.3 ms^{-1} while foraging. In this study the average recorded PV of SF was 8.4 ms^{-1}. Over a quarter of SFs (*N* = 13) were recorded flying faster than 9 ms^{-1}, four of which were faster than 10 ms^{-1}. It is unsurprising to observe that *R. ferrumequinum* emergence flight speeds are faster than foraging speeds. Emerging flight's main aim is to leave the roost and arrive at a foraging site to begin feeding. Per aerodynamic theory, the speed of foraging flight is somewhere between the V_{mp} (the speed at minimum power expenditure) and V_{mr}. While commuting flight is expected to be close to V_{mr}, the most energetically efficient velocity based on how far the bat will travel during the commute. While studies comparing foraging and commuting flights in the same species are uncommon, those that have been done have consistently shown that commuting flight is faster (Britton *et al.*, 1997; Grodzinski *et al.*, 2009; Jones and Rayner, 1989). A study on *P. kuhlii* found that the commuting flight speeds were considerably higher, about 1.5 times faster, than foraging flight speeds (Grodzinski *et al., 2009)*. The method in use in this study was extremely similar to the tracking method in Grodzinski *et al.* and found that *R. ferrumequinum* flew faster than previously reported, which follows the expected trend of commuting flight speeds being quicker than foraging flight speed. The PVs reported here would be just about 1.8 times faster than recorded foraging flight speeds in labs (maximum of 4.8 m/s; Aldridge, 1986)**.** Flight speeds in lab environments are assumed to be slower than in the field, which indicates that the PVs reported here are in line with what would be expected of *R. ferrumequinum* commuting flight speed.

PV had a significant negative relationship with time after sunset ($R^2 = 0.47$, Figure 10). This relationship may be explained by predation avoidance and prey acquisition. Bat species that fly faster are known to emerge earlier than slow flying bats due to both the lower predation risk of flying fast and the peak abundance times of prey preference (Jones and Rydell, 1994). There is a higher predation risk in early emergence from raptors (Fenton *et al.*, 1994). The visual ability of raptors declines quickly as the sun sets, creating a safer environment for bat flight as it gets darker (Fox *et al.*, 1976). Under this pressure bats should be expected to emerge at much later times. However, one of the peak times for prey abundance is at dusk (Rydell *et al.*, 1996). Bats must balance the risk of predation with the ease of prey access.

At the time of first emergence, 20 minutes after sunset, bats were easily seen by the surveyors and likely also visible to potential predators. *R. ferrumequinum* at this site were not tracked past the arrays and therefore the actual foraging sites and prey preference are unknown. However, previous work by Jones (1990) found that *R. ferrumequinum* forage primarily on Lepidopterans (moths) and Coleoptera (of the genus *Aphodius*) in late August. Peak abundance of moths tends to be during darkness (more than two hours after sunset) rather than in the twilight hours (Acharya, 1995; Rydell *et al.*, 1996). Therefore, the idea that *R. ferrumequinum* needs to fly faster to reach foraging sites to arrive at peak prey abundance is unlikely. Flying faster to avoid predation appears to be the more likely driving force in this particular case.

4.2.4 Path Velocity Comparison

The PVs of PFs were no different from SFs, however an interesting observation lay within PFs. Other research in bats flying in pairs have reported pairs flying at the same velocity and the same was expected to occur in this study (Kong *et al.*, 2014). The difference in velocity between LF and TF may raise the risk of jamming or collision. If the TF is faster than the LF then eventually the TF will overtake LF. The LF cannot determine the location of the TF as it is approaching, which means it cannot track the TF and adjust its path accordingly to avoid TF. Bats likely want to avoid overtaking another while commuting as the chances of jamming and collision could rise, although differences in the Doppler shift caused by the difference in velocity may provide a natural spectral separation for HDC bats. Only 4 PFs were found to have PV differences less than 0.5 ms^{-1} (ranging from 0.1-4.2% difference). The largest PV difference (2.16 ms^{-1}, +30.2% from LF), where TF was faster, was the second largest temporal separation (and largest separation in actual distance). The distance between the two individuals in a pair allows for some variation in PV but an extreme positive PV difference for a TF would eventually result in an overtaking scenario. However, no relationship between PV difference and temporal separation was found.

The PV of LFs was weakly correlated with the PV of TF ($R^2 = 0.30$; $p < 0.01$). The regression slope (0.51) was shallow, further indicating only a partial correlation between LF and TF PV. Due to the following nature, it must be assumed that the PV of a LF is influencing the PV of a TF bat as a LF bat cannot track, and therefore be aware of, a TF's PV (see 4.2.2). PV differences between LF sand TFs were large enough that the Doppler shifts experienced by each would be different, thereby experiencing both emissions and echoes at different frequencies, necessitating the calculation of auto- and allo-calls.

4.3 Acoustic Characteristics

4.3.1 Timing Characteristics

4.3.1.1 Solitary Call Duration

One of the possible JAR strategies for *R. ferrumequinum*, would have been to adjust call timing so that the temporal alternation of emission calls was possible, as observed in emission calls in LDC species (Chiu *et al.*, 2009; Obrist, 1995). The alternation of calls would also result in the alternation of echoes as the speed of sound is so fast that the distance required to receive echoes at a different timing (e.g., alternating emission vs synchronizing echo) would be so large that the two bats would not be considered a PF in this study and would be highly unlikely to jam each other. Solitary call duration and solitary call percentage were the only timing parameters that changed in the presence of conspecifics. A jamming study on *R. capensis* did not show any change in call duration while in the presence of conspecifics but rather when in the presence of another HDC species, *Rhinolophus clivosus* (Fawcett *et al.*, 2015). Decreasing call duration has been suggested to be a reaction to increase the number of FMts and thereby provide more localizations of the surrounding conspecifics, which has been attributed as treating other bats as clutter (Fawcett *et al.*, 2015). However, that study focused on bats as they flew around in flight rooms and rarely observed bats flying in pairs (Fawcett *et al.*, 2015**)**. This is an important distinction because while TFs in this study could have shortened call duration to better track LFs, the same reasoning cannot be applied to LFs tracking TFs. The effective beam direction of bats is limited to the general flight direction of the bat, making LF incapable of tracking the TF (Ghose and Moss, 2003; Seibert *et al.*, 2013). Similar to the conclusions of Obrist (1995), the reduction in duration in this study cannot be attributed to attempts to track the conspecific because there were more FMts in LFs, the bat unable to track the conspecific, than TFs which could track the conspecific.

Previous work has observed duration changes in both LDC and HDC calls in the presence of other bats (Fawcett *et al.*, 2015; Gillam *et al.*, 2007). For the bats in this study, PF median solitary call was 3.7 ms shorter than SF, which is only a 6% decrease in duration. In comparison, Fawcett *et al.* (2015) observed a nearly 25% decrease in duration. The decrease observed in this study is hardly large enough change to allow for the perfect alternation of HDC calls. While the DC of a standard *R. ferrumequinum* solitary call makes it impossible to perfectly alternate calls, the shortening of the call duration may provide more space with which to alternate calls; this will be discussed in more detail later (see 4.3.2).

PF solitary duration was different from SF ($p < 0.05$) but when comparing TF and LF to SF solitary duration no difference was detected, although they were approaching significance (Kruskal-Wallis $p = 0.07$). The inability to find a difference in either LF (n = 17) or TF (n = 17) from SF was likely tied to the sample size. Both LF and TF solitary call durations are more similar to PF solitary call duration than SF (Table 1). A larger sample size may have confirmed that both TF and LF solitary durations were shorter than SF. I can conclude that duration is slightly smaller in PFs and that there was not a difference in the durations of LF and TF, indicating that the shortening of call duration is a general response to the presence of a conspecific and not dependent on the flight position (i.e., LF or TF).

4.3.1.2 *Solitary Call Percentage*

Solitary call percentage was smaller in LF than in SF or TF ($p < 0.001$). The increased amount of doublet calls in LF may be a JAR. A study by Roverud and Grinnell (1985) illustrated that in the presence of white noise jamming, *N. albiventris,* an LDC bat, required successive CF-FM calls to accurately discriminate distance. Additionally, SGs of solo bats have been suggested to improve echo reception and help create clearer echo streams in situations with clutter (Kothari *et al*., 2014; Moss *et al*., 2006). SGs are observed just before and after attacking a prey item and are thought to be a reaction to obtain the highest amount of detail before proceeding with the flight (Moss and Surlykke, 2001). By using more doublet calls, LFs could be creating a clearer auditory scene for themselves in these situations of potential jamming.

SGs are known to increase in complex and cluttered pathways (Moss *et al.*, 2006; Moss and Surlykke, 2001) ergo the flight path taken could have influenced the amount of doublets. However, the relatively small differences in flight paths makes flight path an unlikely influence on the solitary call percentage. Within the analysed range (8-2m), TC was generally closer to clutter (i.e., the wall) but did not show any change in solitary call ratio from SF. It could be argued that the slightly smaller width in LC and TC suggests that these bats are waiting longer to start their turns. They would therefore be turning closer to clutter and could be using SGs as they approached. If this was the case, then the solitary call percentage of both LF and TF should be different from SF as both LC and TC are narrower in turn phase width. Instead, solitary call percentage is only different for LF. Additionally, doublet calls were not found to be more frequent in the turn phase (4-2 m) where they would be expected to deal with approaching clutter. The difference in LF solitary call percentage is therefore unlikely to be a response to the path taken and is more likely to be a response to the presence of a conspecific.

The lack of difference between SF and TF solitary call percentage is peculiar, given that TF is flying in the same acoustic environment as LF. I propose two reasons that may be in use simultaneously for the difference in solitary call percentage between LFs and TFs. First, as mentioned previously (section 4.2.2), once a LF turns the chances of jamming declines for the TF. The LF turn may create a less complicated auditory scene that no longer requires SGs from the TF to successfully orientate which means at a certain point TFs can use SF calling strategies to navigate their environment. The second possible explanation is that TFs are actively avoiding doublet calls to avoid creating more acoustic clutter (i.e., keeping the cocktail party nightmare from getting worse). If both bats call more frequently the amount of acoustic clutter will rise, thereby increasing the chances of jamming for all the bats. If TF does not call more, then LF has a simpler auditory scene to decipher. However, this may increase the chances of jamming for TF. This would not be the first time that a seemingly altruistic behaviour was observed in bats in regards to creating a simpler acoustic scene. A couple of studies have found species that decrease the sound intensity while in the presence of others thereby reducing interference (Brinkløv *et al.*, 2009; Fawcett *et al.*, 2015). Another study found that *E. fuscus* will at times completely stop calling in the presence of a conspecific, although researchers suggested that these bats were orienting off the conspecific's echoes (Chiu *et al.*, 2008). Regardless of whether or not the TF is intentionally simplifying the acoustic scene, TFs are apparently not using doublet calls as a way of separating echoes and are likely using a different JAR.

4.3.2 Temporal Call Spacing

One of the predicted JAR hypotheses for HDC bats was temporally spacing calls. Prior research has described temporal call spacing in some LDC bats (Chiu *et al.*, 2009; Obrist, 1995) but temporal call spacing has never been observed in HDC bats. The call timing results of this study suggest that there are two very distinctive temporal call spacing strategies in use by *R. ferrumequinum*. The classifications of synchronization and alternation were significant in every tested approach, except where the number of synchronization classified paths (*n* = 3**,** preceding EO) was too small to determine significance. The evidence here suggests that adopting an alternation or synchronization call strategy was intentional. The use of these strategies can be visually seen via the Bat Experience charts (e.g. Appendix 3c is an alternation, 3a is a synchronization).

Temporal alternation of emitted calls is an effective form of JAR in many LDC bats (Chiu *et al.*, 2009; Obrist, 1995)**.** However, the long duration of calls and short IPI in HDC bats does not provide a lot of silence for conspecifics to call in uninterrupted. Alternating would allow *R. ferrumequinum* to temporally separate their call from conspecifics, albeit with some call overlap as perfect alternation cannot occur without significantly changing the duration, IPI, and DC of calls. To the best of my knowledge timing discrimination of HDC bats in the presence of a jamming sound has not been investigated. However, respective research on the LDC bat *N. albiventri*s, whose CF-FM narrowband calls are arguably much shorter than HDC CF calls but share structural similarities, offers some opportunities for comparison. This research on CF-FM calls revealed a gating mechanism that allowed *N. albiventris* to avoid interference of an artificial CF-FM call (Roverud and Grinnell, 1985). Any artificial call that occurred 30 ms after the actual call did not interfere with the range discrimination task (Roverud and Grinnell, 1985). Allo-calls occurring within this time window were found to interfere with calls, however the sound intensity required to interfere was sizable and it was argued that sound intensity like that would only be present when both bats were nearby and attacking the same food item (Roverud and Grinnell, 1985). This study exhibits evidence of the first examples of intentional temporal call alternation in HDC bats.

Overall, alternation was the most common method of PTC, constituting the PTC of a third of analysed paths (n = 56, including all AAGs and call reference methods). When CTC was based on the preceding call, regardless of the AAG, those paths with PTC of alternation always reported a higher proportion of alt calls than the virtual pairs (*p* < 0.01). This suggests that alternation of calls was intentional. Although this is the first time that a HDC bat has been described as intentionally alternating its calls, alternation could be a viable way of segregating echoes for HDC bats. Moss and Surlykke have argued that temporal shifts could be enough to separate echoes of allo- and auto-calls (2001). Researchers have also found possible evidence that bats could create an 'efference copy' of its signal, which could then be used to 'tag' its own call and allow it to ignore allo-echoes (Sinha and Moss, 2007). The observation in this study of alternating calls suggests that alternating one's calls in the presence of a conspecific provides some benefit and it is likely to be jamming-related. However, alternation was not the only PTC found to be intentional.

One of the most unexpected results of this study was the finding that the PTC of synchronization was quite common. Synchronization was the second most common PTC across all AAGs and call reference methods (30%, *n* = 50), some of which relied solely on syn (*n* = 6). Paths classified as synchronizers were synching the preceding call of both EE and OO more than expected (*p* < 0.01) suggesting that the bats are intentionally using a synchronization strategy in EE and OO. Stranger still, TFs with the smallest temporal separation (< 0.4 s; *n* = 4), were found more likely to be using syn calls in EE and OO, when timing was based off of the preceding call, than virtual pairs (*p* < 0.01).

Overall, the synchronization of calls is quite peculiar as it should increase the risks of jamming of any bat species and especially for HDC bats that rely on such a small range of frequencies. The closer the bats are, the higher the chances for jamming and synchronizing calls. This behavior likely exacerbates the issue as the temporal component of separating calls no longer becomes viable. Interestingly, research on the ranging discrimination of CF-FM calls by LDC bats showed that artificial calls were not effective at jamming if they occurred at less than 8 ms after the call (Roverud and Grinnell, 1985). Outside of this 8 ms window the success rate of the bats quickly declined, although the allo- and auto-echo frequency needed to match (Roverud and Grinnell, 1985). Provided that OO frequencies match, *R. ferrumequinum* may be intentionally synchronizing echoes in order to avoid jamming and will be discussed in more detail later (see section 4.3.4.1).

While not as common as synchronization or alternation, random and undefined PTCs were observed. As explained previously (see 2.3.5.1.2), the PTC of undefined was not treated as an intentional strategy, although some of the chi-square tests for undefined did come back as significant. The presence of und and the PTC classifications of undefined and random could be argued as evidence that call spacing is not intentional. After all, if there was a benefit to timing calls then it would make sense to maintain that timing throughout rather than to change. There may be a few explanations for the random and undefined PTC. Firstly, und calls could have been a result of observing the bat transition from synchronization to alternation PTC, or vice versa. At no point was a bat observed 'skipping' calls, so to switch between strategies would require at least one transitional call. Secondly, und calls also may have been misidentified. The classification system in use assumed that calls were of a standard solitary call duration. Deviation from that length, or the use of doublets, may have resulted in an identified und call which was actually a syn call. The random classifications may have been a result of the small amount of calls used to make classifications. For example, two syn, one alt, and two und (that may have been misidentified syn) calls would create a random PTC. Correctly identifying one of those unds would have changed random to synchronization. The undefined and random PTCs were most likely a result of either not having enough calls to truly get a sense of the timing strategies at play due to the limited snapshot of calls available (particularly with random) or that classification was limited by the method.

4.3.3 Frequency

4.3.3.1 FMt Bandwidth

The PFs in this study exhibited a couple differences in frequency measurements from SF. First, the FMt bandwidths of both LF and TF were larger than SF. A previous study, on the HDC bat *R. capensis*, showed that FMt bandwidth was also larger while in the presence of conspecifics (Fawcett *et al.*, 2015). The researchers in that study concluded that the bandwidth elongation, both in time and in bandwidth, was part of a response by focal bats to 'track' conspecifics (Fawcett *et al.*, 2015). Multiple studies have stated that the FMt bandwidth of a HDC call provides the ranging component for both obstacles and moving objects (Fawcett *et al.*, 2015; Jones and Rayner, 1989; Neuweiler *et al.*, 1987; Schnitzler, 1968; Schnitzler *et al.*, 1985; Schnitzler and Denzinger, 2011; Tian and Schnitzler, 1997). The elongation of FM bandwidths in LDC bats is suggested to encode more detailed information on the environment and target focus (Simmons and Stein, 1980). Fawcett *et al.* (2015) have proposed that the same is likely true for FM portions of HDC calls. The elongation of FM bandwidths in the presence of conspecifics has been observed in LDC bats but has also been attributed to be a ranging response rather than a jamming response (Cvikel *et al.*, 2015; Götze *et al.*, 2016). Those studies do not quite accurately reflect the parameters of the present study, as the LDC bats observed were foraging rather than commuting (Cvikel *et al.*, 2015; Götze *et al.*, 2016). The logic of reacting to a conspecific as clutter is only supported when the conspecifics can detect the other, which is not occurring in this study as previously stated (4.2.2). If bats were reacting to conspecifics as clutter, then only TFs should have exhibited a change in FMt bandwidth. Therefore, the increase in bandwidth must be due to some other factor.

The elongation of FMt bandwidth may in fact be a JAR. In a study on the LDC bat *E. fuscus,* researchers showed that call structure was changed in order to differentiate its calls from others (Chiu *et al.*, 2009). Other studies on LDC bats have shown allo-calls that are more similar to the auto-call structure have a larger interference effect on ranging discrimination (Masters and Raver, 1996; Miller, 1991; Roverud and Grinnell, 1985; Surlykke and Miller, 1985). For HDC bats, the FMt could be adjusted to avoid the interference effects caused by conspecifics. If the presence of a conspecific is affecting the ranging ability of the standard FMt then the FMt must change in structure to become effective again. Changing the original structure of a call due to the presence of a conspecific very squarely fits the definition of a JAR. The results of this study suggest that in the presence of conspecifics, *R. ferrumequinum* elongates the FMt bandwidth as a JAR rather than a clutter response.

4.3.3.2 CF

Another possible, although unlikely, JAR was the spectral shifting of the CF component. Within recorded CF a difference in CF was found between SFs and PFs, as well as between SFs and both TFs and LFs. Previous studies have argued against, and at times shown, a lack of spectral shifting in the CF component of HDC calls (Fawcett *et al.*, 2015; Jones *et al.*, 1994; Jones and Rayner, 1989). Yet it appears this study may have evidence of spectral shifting. This would not be the first evidence of an HDC bat changing CF in the presence of conspecifics, as a study by Furusawa *et al.* (2012) interestingly reported that CFs were closer while flying with conspecifics than if the two bats had maintained the same CF used while flying solo. However, emitted and echo CF were not significant amongst any of the path types of this study, which is much more biologically relevant than recorded CF. The lack of difference in emitted or echo CF would suggest that spectral shifting is not present here. This may have been an effect of the sample size of this study. The CF of emit and echo PFs is approaching significance ($p = 0.06$) and the range and inter-quartile range are both larger than SF. It could be argued that the calculations of echo and emitted frequency introduced the variability but the same calculations were used for SF and SF clearly has a narrower set of echo CF values.

From this data, it is unclear if these bats spectrally shifted their CFs. This study did not involve playback recordings and as such was measuring for static, rather than dynamic JAR. The PFs had at least a full 10 m to fly with the conspecific and adjust to allo-calls before ever being recorded by the arrays which means by the time the bats enter the analysis zone (8-2 m) the spectral shifting would have already occurred and both bats would be consistently emitting at the frequencies that provided a JAR. While the frequency discrimination ability of *R. ferrumequinum* is currently unknown, previous research has shown that LDC bats can separate conspecific calls that are different by at least 0.50 kHz (Cvikel *et al.*, 2015). Some research on HDC bats has confirmed that the frequency discrimination ability is much more sensitive than LDC. *P. parnelli* was found to have a discrimination ability of 0.08% of its resting frequency (Riquimaroux et al., 1992, 1991). A previous study has suggested that *R. ferrumequinum nippon* has a discrimination threshold of at least 0.1% (Furusawa *et al.*, 2012). Applying that threshold to the bats of this study would mean that *R. ferrumequinum* could separate echoes that were separated as small as 0.08 kHz. Such a small spectral shift would be quite hard to detect and may explain why previous researchers, and this study, have yet to confirm the presence of spectral shifts in HDC bats.

4.3.4 Spectral Shifting

While I was unable to confirm spectral shifting based on the auto-CFs of the different path types, the comparisons of LF and TF OO and EE provided some interesting insight into HDC bat jamming. Twelve of 25 EE absolute frequency differences were different from the expected values calculated by the virtual pairs. While interesting to note that more than half of the paths were significantly different, difference in EE does not necessarily translate into OO due to the varying Doppler shifts experienced by the bats. To truly determine spectral overlap and the presence of spectral shifting, OO frequency difference must be explored.

The spectral shift of frequencies has been described before but not in echo frequency nor in HDC bats (Bartonička *et al.*, 2007; Gillam *et al.*, 2007; Ibáñez *et al.*, 2004; Jones *et al.*, 1994; Jones and Rayner, 1989; Miller and Degn, 1981; Necknig and Zahn, 2011; Obrist, 1995; Ulanovsky *et al.*, 2004)**.** HDC bats are more adept echolocators than LDC bats and appear to be capable of discriminating frequencies as small as 0.05 kHz (Riquimaroux *et al.*, 1992, 1991). While *R. ferrumequinum* may not be able to discriminate changes as small as 0.05 kHz, discrimination of changes as small as 0.08 kHz are possible (Furusawa *et al.*, 2012). Using this assumption then the results of this study in OO suggest that spectral shifting may be a very uncommon strategy for HDC bats, not because they are incapable but rather because the DSC required during flight already provides a large enough difference in frequency to separate echoes. Virtual pairing data suggested that if PFs were placed together at random then the OO absolute frequency difference would likely be between 0.73-0.85 ±0.57 kHz, a frequency difference considerably larger than the expected difference required, even a larger difference than what is utilized by LDC bats (Cvikel *et al.*, 2015). This could be an indication of why spectral shifting has never been observed in HDC bats. The differing velocities of the bats result in different Doppler shifts, and small differences in velocities can lead to sizable changes in frequency. For example, if two bats call at the same emission frequency of 80 kHz but the bats are separated in speed by 0.5 ms^{-1} then the OO frequency difference experience by the two is 0.12 kHz. Such a small difference in speed can lead to a large enough gap in OO frequency that a JAR is not needed. Therefore, spectral jamming in HDC bats may actually be rare as it appears quite difficult to be close enough to create jamming.

Although 12 paths were separated in EE frequency, the effects of differing PV ultimately meant that only eight paths (2 LF, 6 TF) exhibited significant differences in OO absolute frequency difference ($p < 0.05$). All but two (from the same PF) had frequencies separated by more than what was expected. In absolute frequency difference of OO, only 2 of the closest 4 PFs were significant. Interestingly, the two are drastically different. The first (Appendix 3a) has an extremely clear separation in echo frequency, 1.62 (1.54-1.68) kHz difference between LF and TF. It could be argued that the frequency difference observed here is a byproduct of two bats that just happen to have dissimilar reference frequencies. This pairing does possess the second largest difference in median echo frequency (1.17 kHz) and the fifth largest difference in PV (TF was faster by 1.27 ms^{-1}). With this velocity difference, the PV alone would have shifted the frequency by 0.31 kHz (e.g., LF OO absolute difference would be 1.31 kHz instead of 1.62 kHz) provided that the measured echo frequencies were not adjusted to the presence of a conspecific. The conclusions of previous research suggests that HDC bats are not capable of spectral shifting (Fawcett *et al.*, 2015; Jones *et al*., 1994; Jones and Rayner, 1989)**.** If that is the case, then it makes the results reported here quite strange. Assuming that the echo frequency is the reference frequency for these bats, why would they bother maintaining an OO difference of 1.62 (1.54-1.68) kHz? As stated previously, *R. ferrumequinum* is assumed able to discriminate frequencies as small as 0.08 kHz. An OO difference of 1.3 kHz is therefore more than large enough to separate echoes, and 1.6 kHz is larger still. The high sensitivity of the auditory fovea makes such a great difference in echoes excessive. I argue that in the case of spectral shifting, a bat attempting to avoid jamming will aim to separate frequency as much as possible, without approaching frequencies that they are not sensitive to, rather than separate frequency just past the separation 'determination' point and run the risk of the conspecific changing the frequency slightly and forcing another spectral adjustment. Some researchers have observed spectral separation of LDC bats at, or near, the 'determination' point (Cvikel *et al.*, 2015; Gillam *et al.*, 2007) while others have observed large shifts more similar to what was observed in this study (Ulanovsky *et al.*, 2004).

The other PF of note exhibited a much different behavior of keeping the respective frequencies close together. These two bats were separated in OO frequency by only 0.11 (0.01-0.33) kHz. The two bats' median echo frequencies were squarely in the middle of the distribution of PF reference frequency at 84.46 (LF) and 84.15 (TF) kHz and were ranked 9th of 17 for PF difference in reference frequencies. The PV difference (LF was ~0.82 ms^{-1} faster) between the two would have changed the allo-echo frequency ~0.18 kHz (i.e., allo-echo frequencies would be different by 0.18 kHz if the auto-echo frequencies matched). Therefore, accounting for velocity and the difference between assumed reference frequencies of the two bats, these bats should have experienced a difference of 0.12 kHz when flying near one another, which is fairly similar to the observed difference. The bats in this particular pairing may very well be jamming one another at times. Two of the 6 calls that could be analyzed were below 0.08 kHz (the predicted frequency discrimination ability of this species). One call overlap in particular was as close as 0.56 Hz. Such intense spectral overlap is surely to be avoided and if *R. ferrumequinum* could truly shift frequency than a shift should have been observed. Drawing conclusions from this particular pairing would corroborate the results and theories that HDC bats do not spectrally shift (Fawcett *et al.*, 2015; Jones *et al*., 1994; Jones and Rayner, 1989)**.** Even so, to match frequencies so closely while in such close proximity, seems like a detrimental strategy for safe orientation. It becomes especially peculiar considering that the LF of this pair was also syncing OO calls. As discussed previously, another study has also observed 'anti-JAR' behavior in a subspecies of *R. ferrumequinum* in a lab environment (Furusawa *et al.*, 2012). Perhaps there is some benefit to moving OO closer together.

4.3.4.1 Allo-echo Interception

Multiple studies across several species have shown that bats have the capability to eavesdrop on others, even on other species (Bastian and Schmidt, 2008; Boughman and Wilkinson, 1998; Dechmann *et al.*, 2009; Dorado-Correa *et al.*, 2013; Jones and Siemers, 2011; Pfalzer and Kusch, 2003; Wilkinson and Boughman, 1998)**.** Some of these studies illustrate that bats can determine the general direction of the calls and react to them (Boughman and Wilkinson, 1998; Dechmann *et al.*, 2009; Wilkinson and Boughman, 1998). One study in particular has shown that *E. fuscus* is capable of using another's echo to obtain information about a prey item and successfully attack (Chiu *et al.*, 2009)**.** Another study by the same research group observed a bat in parallel flight go silent for several hundred milliseconds and was thought to be orienting based off the conspecific's echoes (Chiu *et al.*, 2008). The concept of orienting from allo-echoes has been theorized in echolocating animals (especially dolphins) but has never been proved (Götz *et al.*, 2006; Gregg *et al.*, 2007; Xitco and Roitblat, 1996). There has been some lab evidence similar to the spectral shifting of frequencies being closer together observed in this study. In 2012, researchers reported that the reference frequencies of paired *R. ferrumequinum nippon* were closer than expected (Furusawa *et al.*, 2012). That study looked at the auto-echo frequency only in a lab environment but they did find 60% of bats were moving their auto-frequencies so that they were closer to the conspecific auto-frequency than if they had relied on their auto-frequency while flying solo (Furusawa *et al.*, 2012). The peculiar trend of *R. ferrumequinum* shifting their frequencies closer together and calling in a synchronizing manner, observed in this study, may be the first field evidence of 'echoic eavesdropping' in bats. Here I have termed it allo-echo interception.

Allo-echo interception would be the intentional change to match auto-echoes to allo-echoes both temporally and spectrally. In HDC bats, by shifting frequency so that auto- and allo-echo match, the focal bat ensures that both echoes of the target focus are returned at the expected auto-echo frequency. This should ensure that *R. ferrumequinum* obtains accurate information on how quickly it is approaching the target structure (e.g., the hedge). The timing synchronization of the echoes would mean that both echoes are providing the same, or extremely similar, ranging information. Work by Roverud and Grinnel (1985) demonstrated that a LDC bat using a CF-FM call could correctly complete a ranging task, whilst being jammed, if the jamming call occurred 8 ms or less after emission. The interception of allo-echoes may also allow for greater localization. *R. ferrumequinum* utilizes rapid pinna movements in order to localize objects via the CF echo (Vanderelst *et al.*, 2011b, 2011a). The reception of the allo-echo, at a similar frequency as the auto-echo, may provide different additional sound intensity cues that can be received and provide a higher resolution of localization than is normally possible. There is likely not much margin for error as previous research has shown that CF-FM calls that occurred 8 ms after the original call had a sizable negative effect on ranging ability (Roverud and Grinnell, 1985). Such a small margin for error in eavesdropping, and the lack of spectral separation, could mean that this helpful JAR could quickly become a very disruptive jamming signal. For now, allo-echoic eavesdropping is only a theoretical explanation for the peculiar observation that one PF in this study matched both call timing and OO frequency, a change that seems more likely to cause jamming than avoid it.

4.4 Future Work

The findings of this study have demonstrated that there is still much to learn about HDC JAR, especially during emergence. The observation of a bat apparently synchronizing the timing of calls and receiving auto-echo frequencies that are similar to allo-echo is of particular interest. To the best of my knowledge, this is the second time that *R. ferrumequinum* has been described as changing calls in a fashion that would likely cause jamming rather than avoiding it. The only other evidence of this involved *R. ferrumequinum nippon* in a lab study (Furusawa *et al.*, 2012). This study measured reference frequency from when the bats were flying solo and in pairs, defined as two bats flying in the same flight room (Furusawa *et al.*, 2012). They subsequently observed that 60% of the bats had a paired reference frequency difference smaller than the difference that would have been present if both bats used their solo reference frequency (Furusawa *et al.*, 2012). While Furusawa *et al.* did not calculate the allo-frequency, the behaviour of shifting frequency closer together is peculiar. The results of the Furusawa *et al.* (2012) and this study appear to suggest that *R. ferrumequinum* is moving frequencies closer together. However, current research is unable to sufficiently explained why this would be the case. The idea of allo-echoic interception may be one explanation but the current understanding of HDC JAR limits such research.

Unfortunately, the scientific understanding of HDC JAR is quite limited compared to that of LDC bats. Only one HDC bat has had its frequency discrimination ability measured (Riquimaroux *et al.*, 1992, 1991). This has served as the basis for Rhinolophidae frequency discrimination in this study and in Furusawa *et al.* (2012) even though the HDC bat of the discrimination experiments belonged to another family, Mormoopidae (Riquimaroux *et al.*, 1992, 1991). Additionally, the closest understanding of HDC call jamming, in regards to timing, is a study of a LDC bat using a CF-FM call (Roverud and Grinnell, 1985). Naturally, those particular findings do not take into the account the role an auditory fovea may play nor how the extended duration of HDC calls may affect a bat's ability to 'tag' its own call. Without a better understanding of the call discrimination ability of HDC bats, JAR studies will be limited in their ability to confirm JARs in the field.

Work should also continue to attempt to understand the effect of conspecifics on calling characteristics and path. I have shown in this study that both flight paths and calling characteristics change in the presence of conspecifics. The finding of an expanded FMt bandwidth confirms the results of previous HDC conspecific bat studies but the purpose of the expansion is now in question. Fawcett *et al.* (2015) argued that FMt elongation was a tracking response, a response that carries very little weight in this study (see 4.3.3.1 for discussion). It may be that both conclusions are correct, as these two studies occurred in different flight scenarios (foraging in flight rooms, commuting in the field). This highlights the need for more JAR research in commuting rather than the common focus of foraging. These are two unique flight scenarios and they may require very different JAR strategies.

Additionally, this work has highlighted the need to undertake JAR research with some form of audio analysis that can calculate the allo- and auto-calls experienced by a bat, whether through on-board microphones or via tracking methods as in this study. Attempting JAR research without it may lead to incorrect assumptions, such as that those bats 'paired' in flight are traveling at the same flight speeds and therefore experiencing the same Doppler shift. This study shows that this is clearly not the case and the difference of Doppler shifts experienced by the pairs can have quite an effect on the frequencies experienced, providing essential insight into the auditory scene of the bat.

4.5 Conclusion

The aim of this work was to find evidence of JAR in flight or acoustic behaviour in the HDC bat *R. ferrumequinum.* For the first time, the emergence behaviours of *R. ferrumequinum* were tracked via acoustic tracking. The paths taken amongst the different flight groups were not overly different although it does appear that PFs were more variable. However, no conclusions could be made regarding a flight-based JAR. I found that bats flying close enough together to jam one another are not matching flight velocity, as commonly thought, and are therefore experiencing different Doppler shifts. I developed a process that creates the acoustic experience of a bat, providing both the time and maximal returnable frequency of auto and allo-echoes. Analysis of this data led to the finding of specific call timing strategies of alternation and synchronization. The OO frequency difference of most of the PFs was more than large enough to separate auto- and allo-echoes but two interesting approaches emerged. A few bats, appeared to shift their OO frequencies farther apart than expected, even though they were surely at a low risk of spectral jamming. More interesting though was the PF that shifted OO frequencies closer together and synced call timing, a possible anti-JAR. Previous research has observed a similar anti-JAR (Furusawa *et al.*, 2012). I suggest that this observation may be intentional as a way of making allo-calls more like auto-calls so that both may be used to navigate, which I have termed allo-echoic interception. I have confirmed that in the presence of conspecifics *R. ferrumequinum* elongates the bandwidth of the FMt portion, much like another member of Rhinolophidae (Fawcett *et al.*, 2015). Based on the results of my study, I refute the idea that the bandwidth elongation is a 'clutter' response. The findings here are the first to suggest that a HDC bat is intentionally timing calls, elongating FMt bandwidth, and possibly shifting frequency in the presence of a conspecific during emergence. Research on bat JAR has been limited to foraging studies, and LDC bats, and this study illuminates that there can be much to learn and discover in the JAR of emerging HDC bats.

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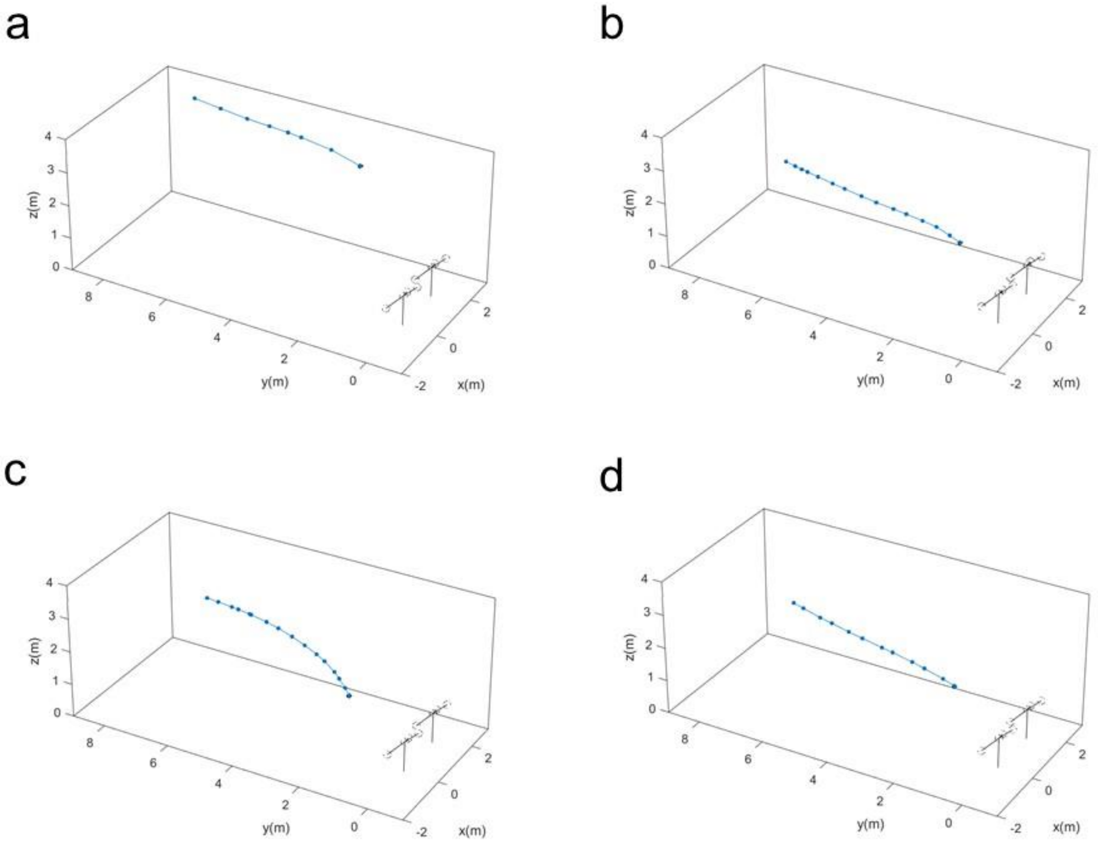
Appendices

Appendix 1 (a-oo): *Flight path reconstructions of each Solo flight (SFs), restricted to 8-2 m for analysis. Flight direction was towards the microphone arrays (circles at Y = 0 m). Each point represents the start or end of each call. The black points indicate the last tracked point before either being unable to track the bat or the bat passed the 2 m cut-off. Flight paths are usually in blue. Orange paths (l, s, gg, ii) display the second SF that was present in the same sound file but sufficiently different in time to be considered an SF.*



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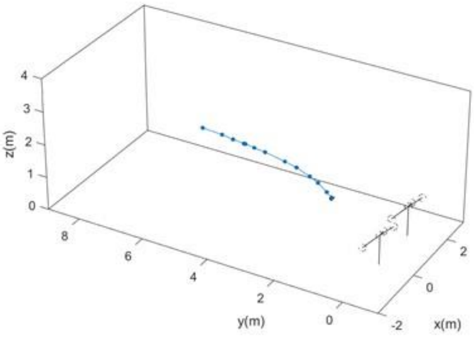
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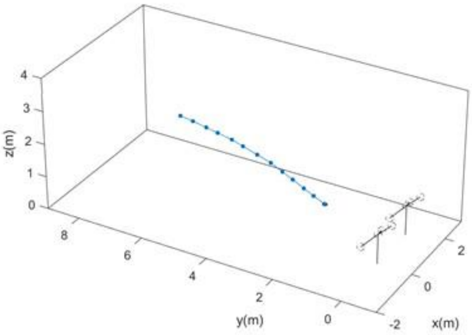
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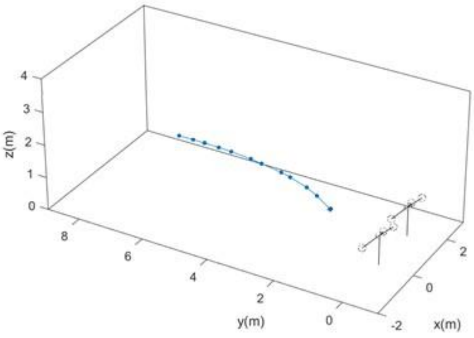
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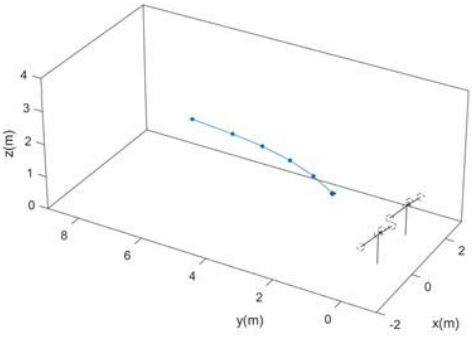
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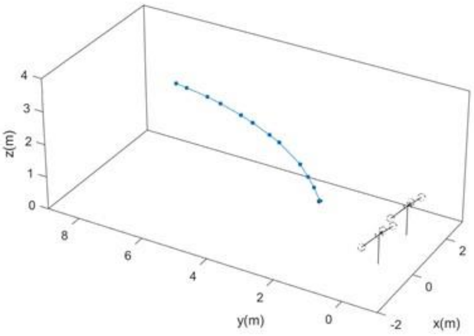
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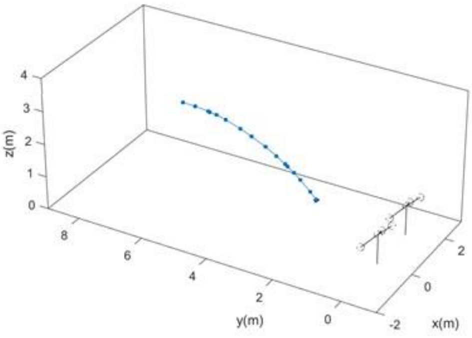
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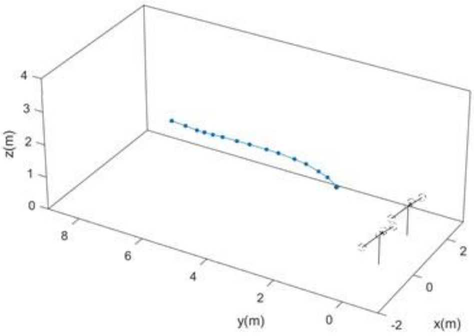
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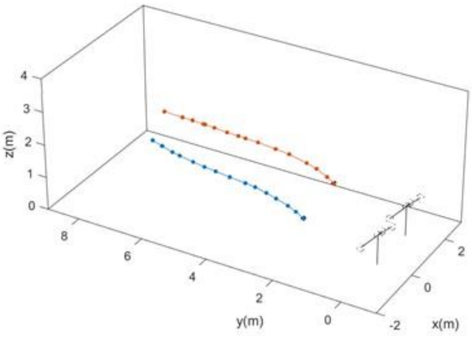
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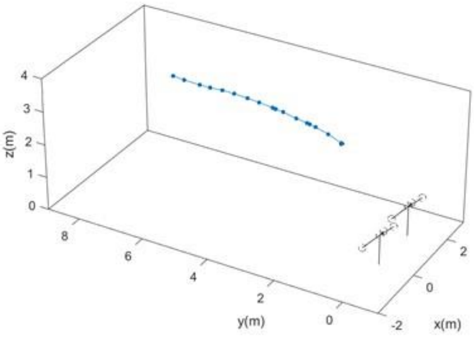
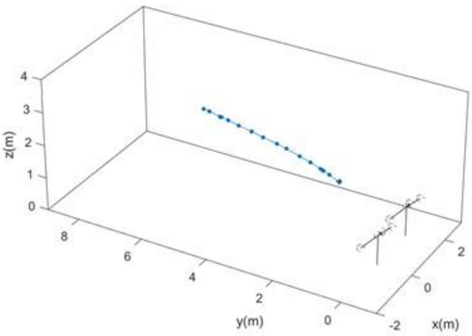
6
4
2
0
y(m)
2
0
-2
x(m)

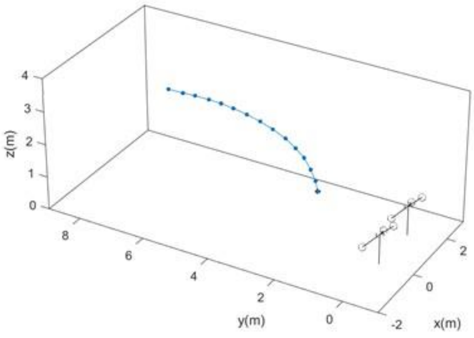
m


z(m)
4
3
2
1
0
8
6
4
2
0
y(m)
-2
0
2
x(m)

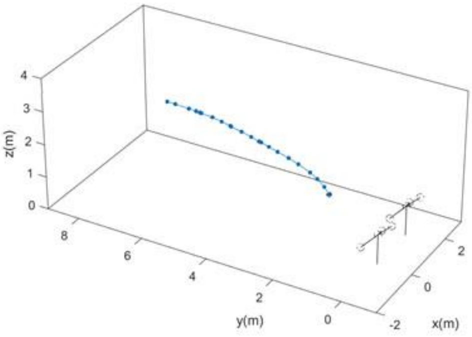
n


z(m)
4
3
2
1
0
8
6
4
2
0
y(m)
-2
0
2
x(m)

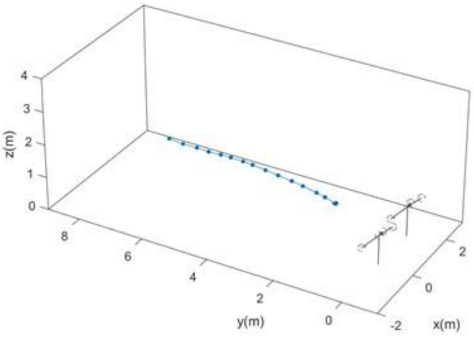
o


z(m)
4
3
2
1
0
8
6
4
2
0
y(m)
-2
0
2
x(m)

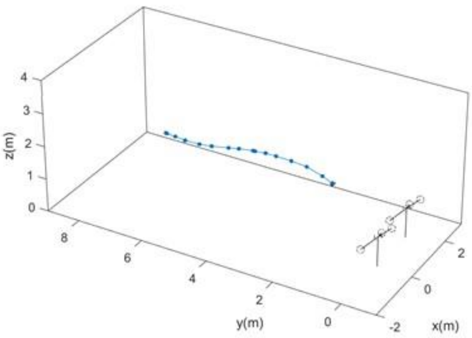
p


z(m)
4
3
2
1
0
8
6
4
2
0
y(m)
-2
0
2
x(m)

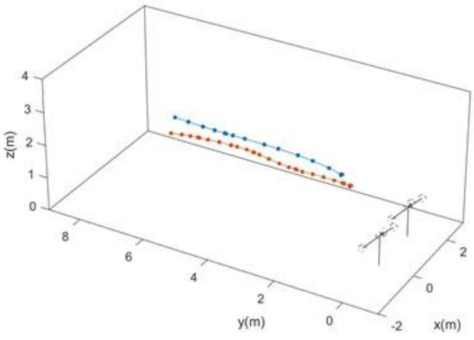
q


z(m)
4
3
2
1
0
8
6
4
2
0
y(m)
-2
0
2
x(m)

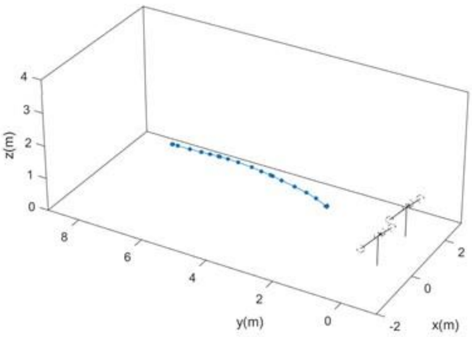
r


z(m)
4
3
2
1
0
8
6
4
2
0
y(m)
-2
0
2
x(m)

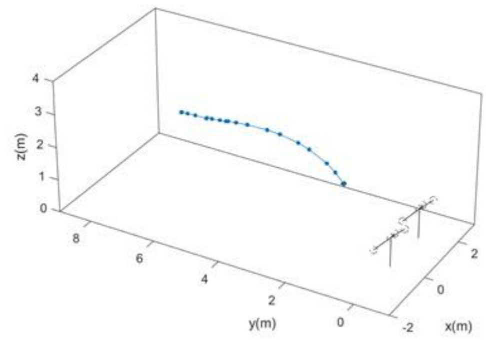
s


z(m)
4
3
2
1
0
8
6
4
2
0
y(m)
-2
0
2
x(m)

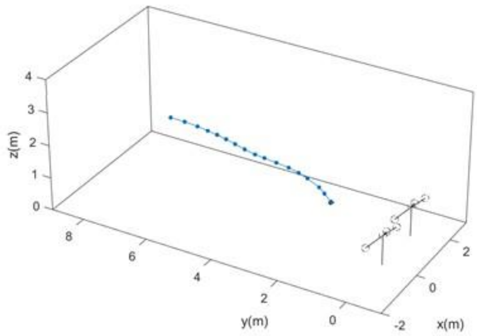
t


z(m)
4
3
2
1
0
8
6
4
2
0
y(m)
-2
0
2
x(m)

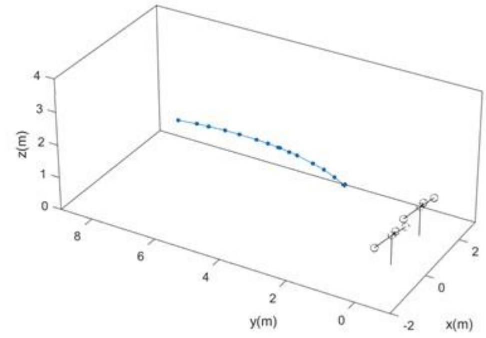
u


z(m)
4
3
2
1
0
8
6
4
2
0
y(m)
-2
0
2
x(m)

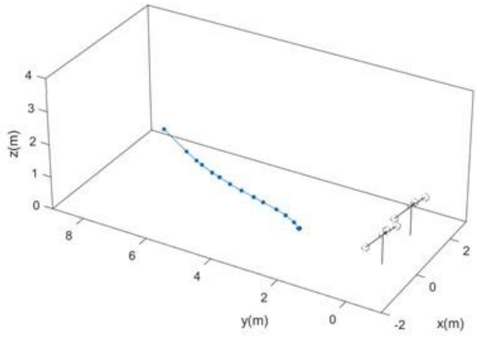
v


z(m)
4
3
2
1
0
8
6
4
2
0
y(m)
-2
0
2
x(m)

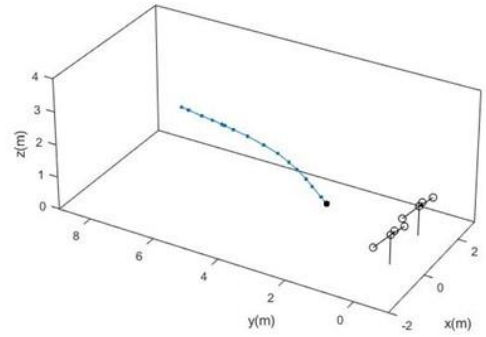
w


z(m)
4
3
2
1
0
8
6
4
2
0
y(m)
-2
0
2
x(m)

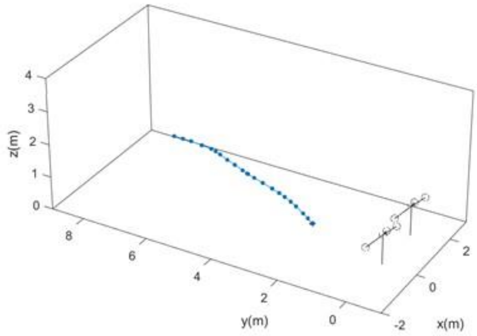
x


z(m)
4
3
2
1
0
8
6
4
2
0
y(m)
-2
0
2
x(m)

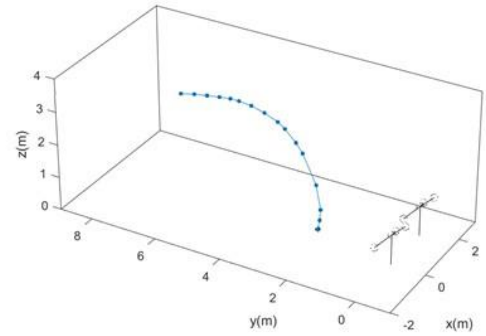
y


z(m)
4
3
2
1
0
8
6
4
2
0
y(m)
-2
0
2
x(m)

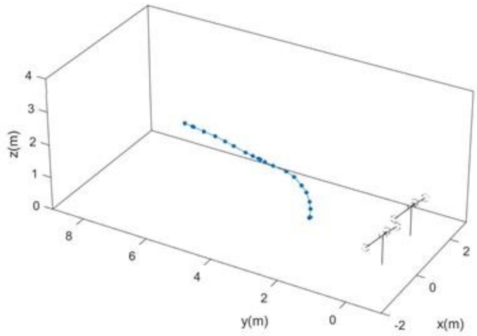
z


z(m)
4
3
2
1
0
8
6
4
2
0
y(m)
-2
0
2
x(m)

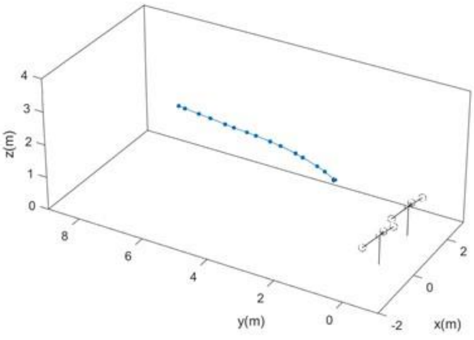
aa


z(m)
4
3
2
1
0
8
6
4
2
0
y(m)
-2
0
2
x(m)

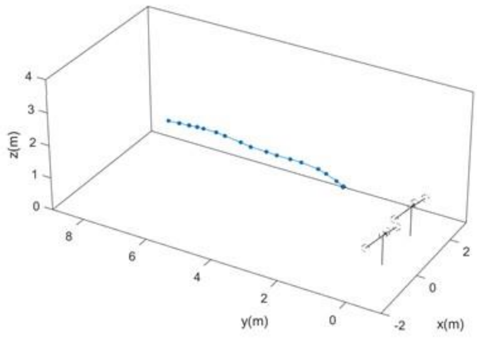
bb


z(m)
4
3
2
1
0
8
6
4
2
0
y(m)
-2
0
2
x(m)

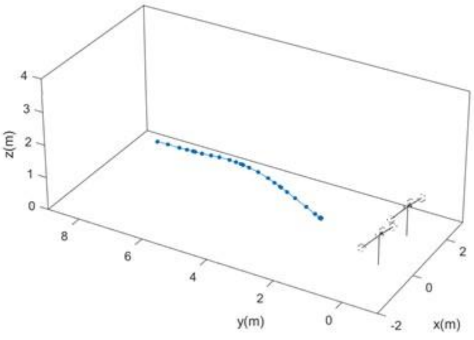
cc



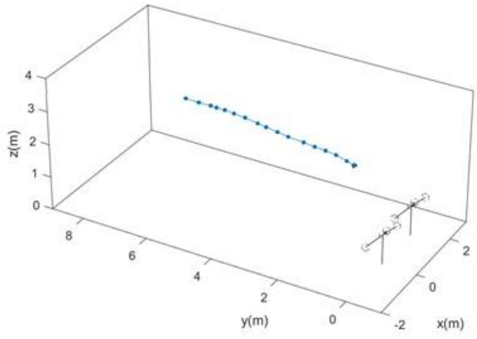
dd



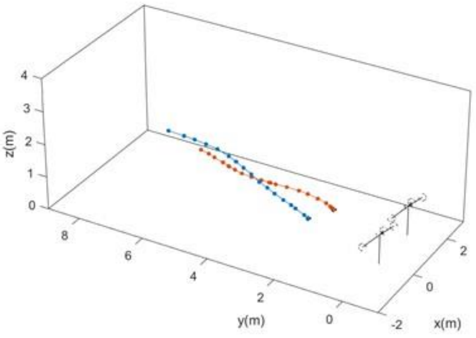
ee



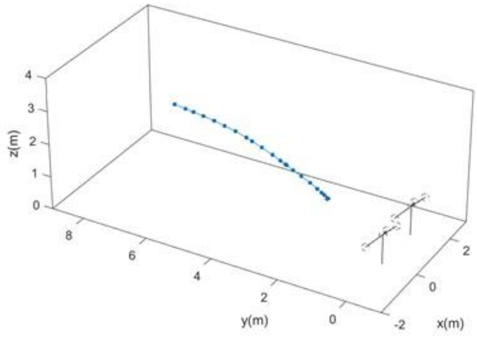
ff



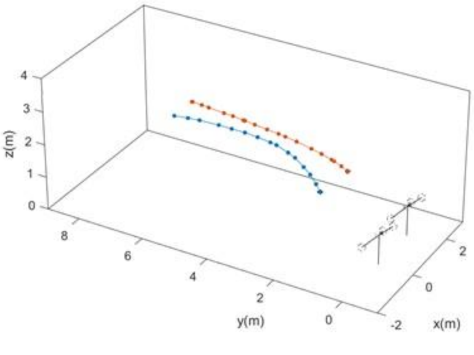
gg



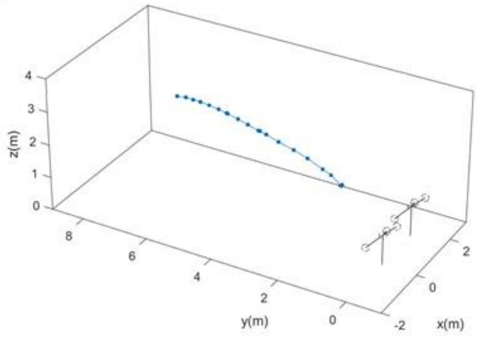
hh

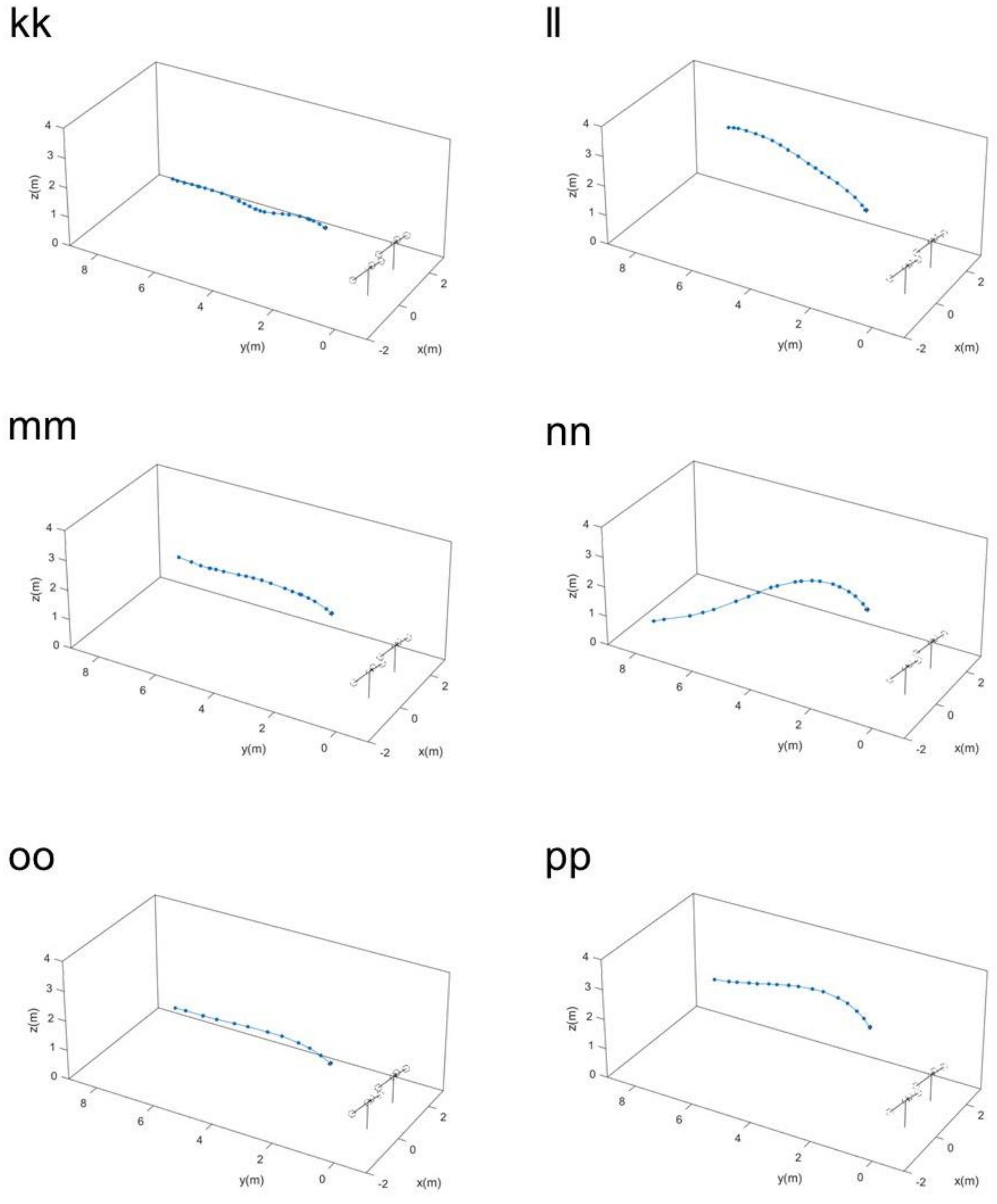


ii

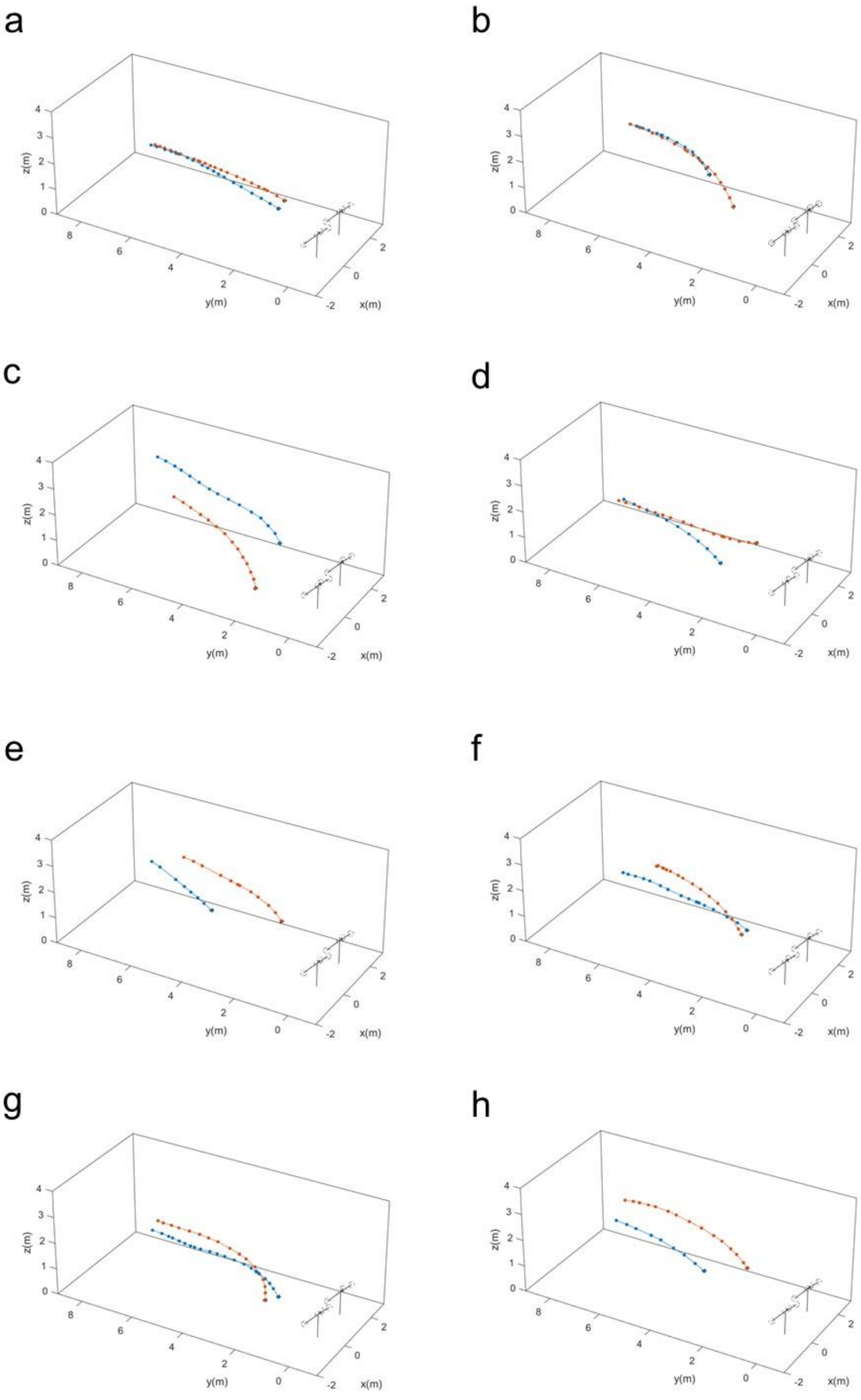


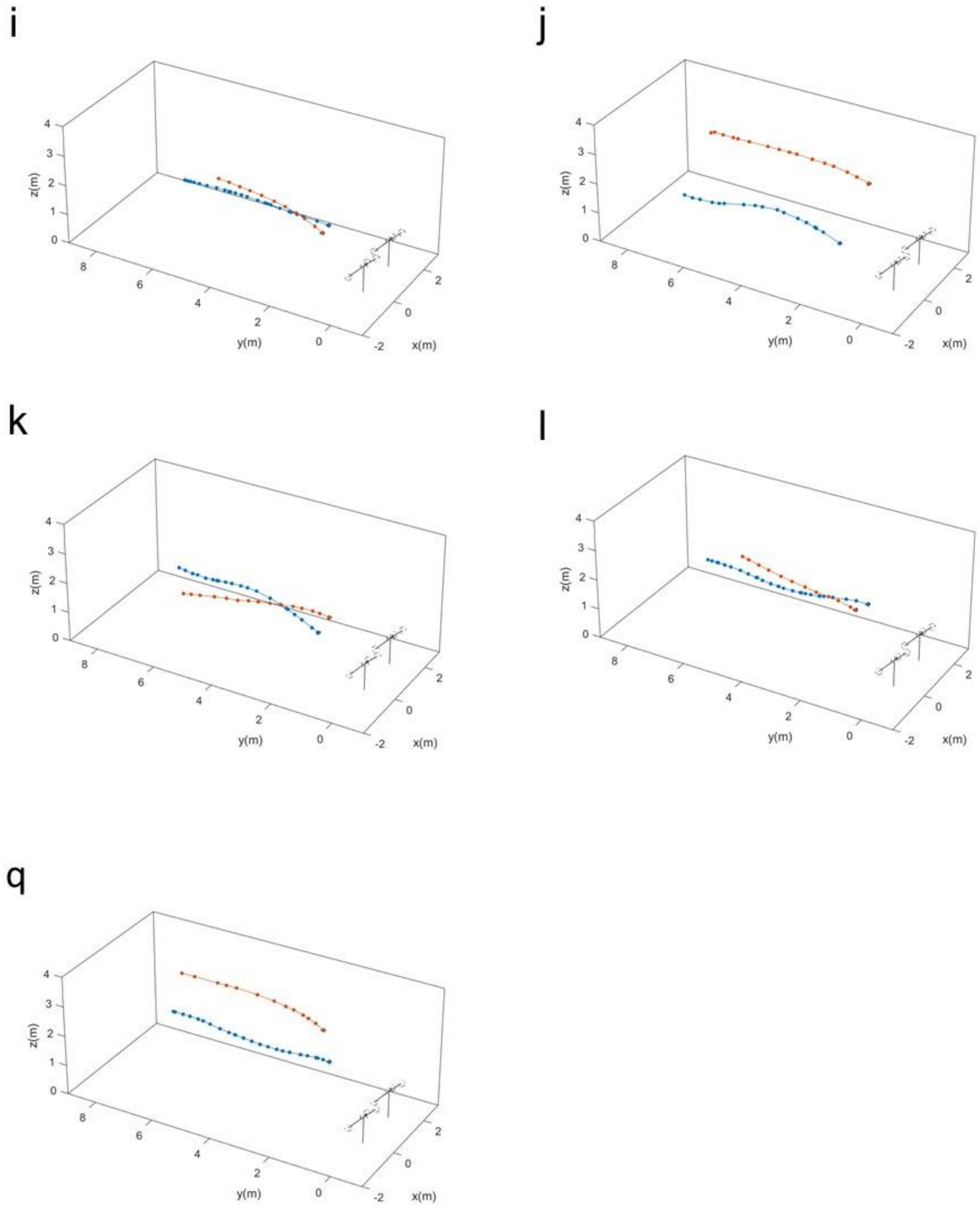
jj



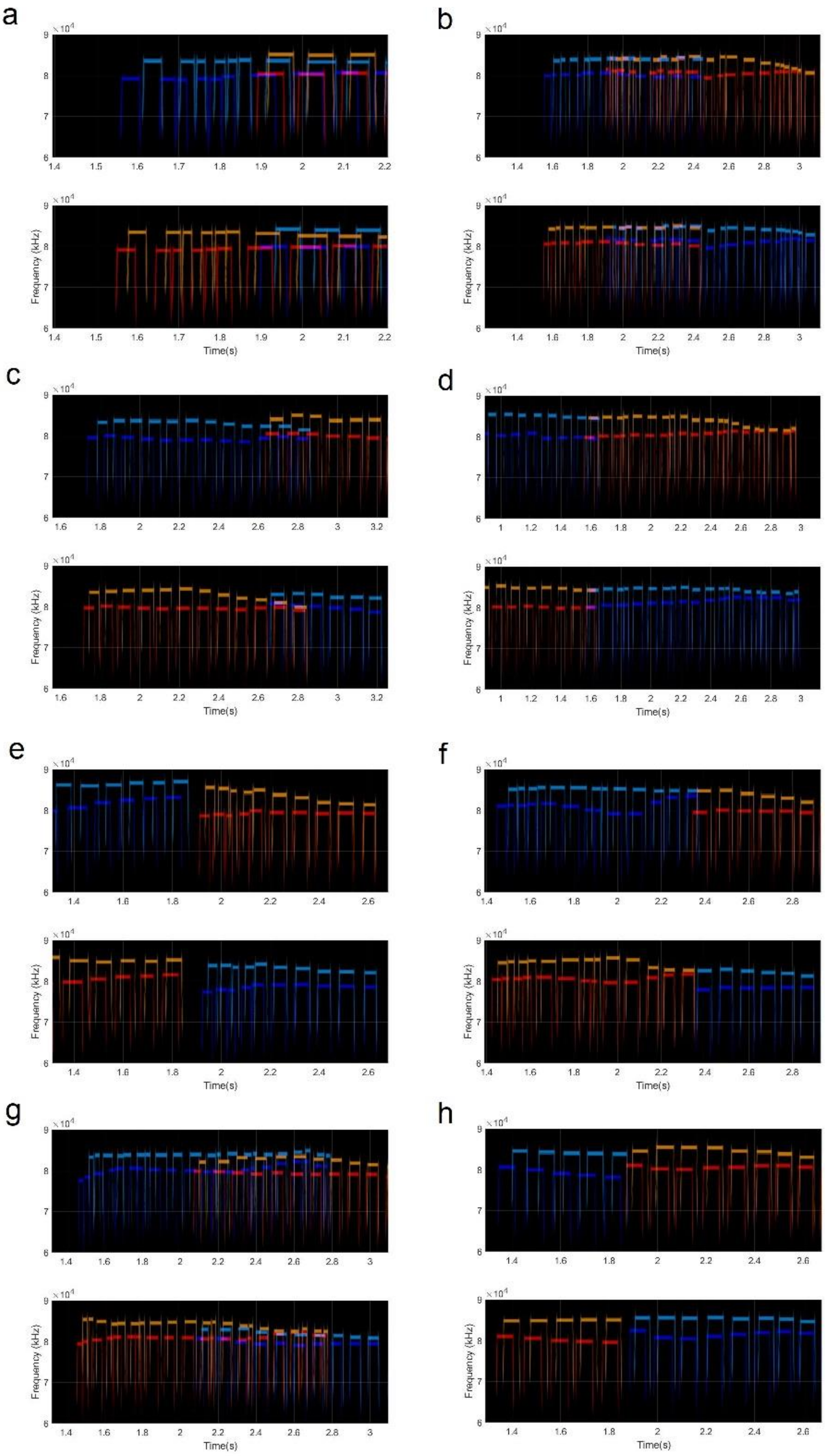


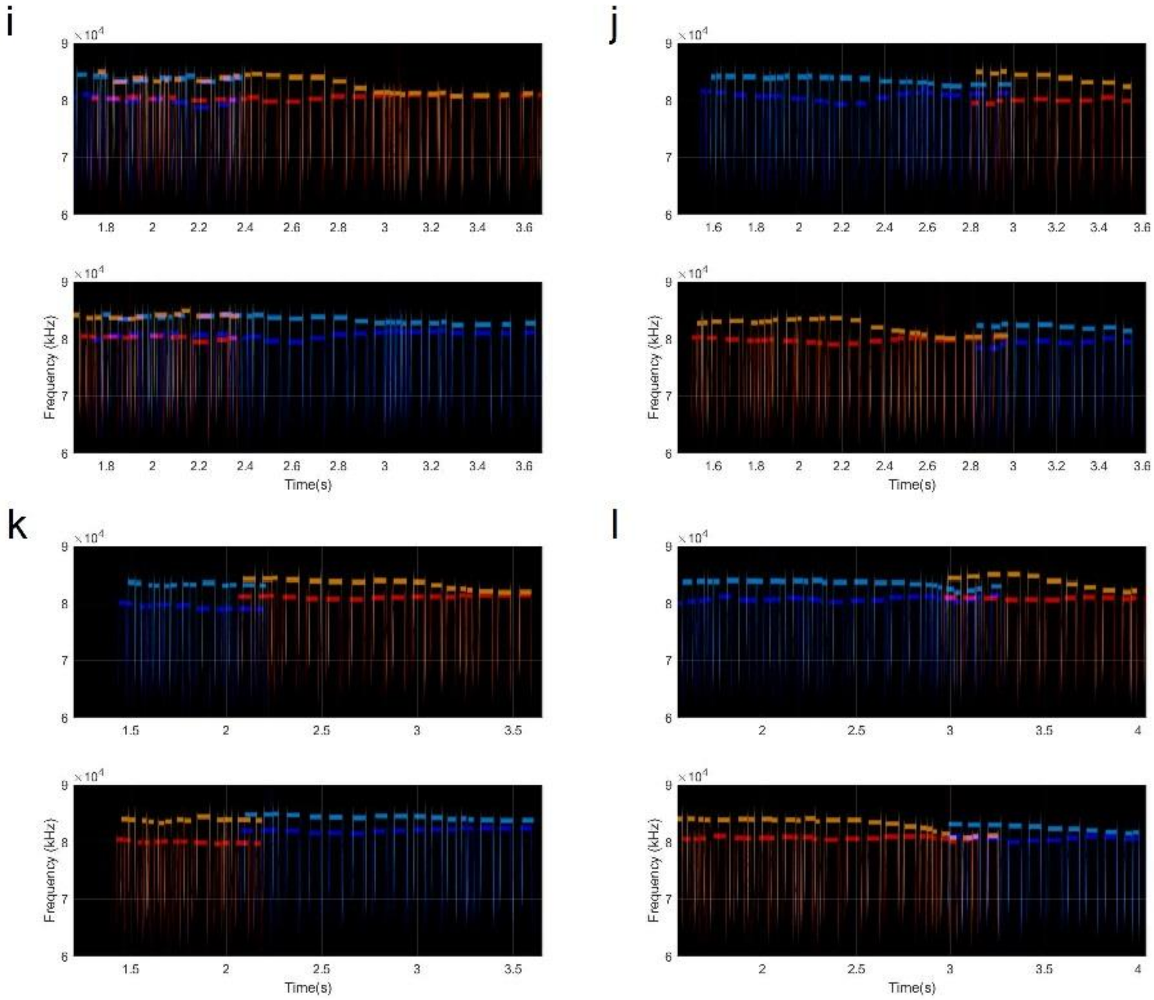
Appendix 2 (a-q): *Flight path reconstructions of Paired flights (PFs), restricted to 8-2 m for analysis. Flight direction was towards the microphone arrays (circles at Y = 0). Each point represents the start or end of each call. The black points indicate the last tracked point before either being unable to track the bat or the bat passed the 2 m cut-off. The Lead bat was denoted by the blue path, Trail by the orange.*


a
b
c
d
e
f
g
h
z(m)
0
1
2
3
4
y(m)
8
6
4
2
0
x(m)
-2
0
2

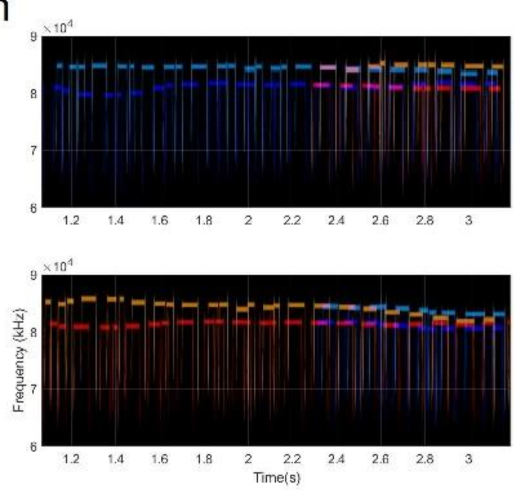


Appendix 3 (a-q): *Bat experience spectrograms for Lead and Trail bats. The top box represents the experience of the Trail, bottom the experience of Lead. The warm colors are the calls of the focal bat, the cooler colors of the secondary bat as experienced by the focal bat. The darker calls are the emitted calls. The lighter colors are the maximum returnable frequency of the hedge echo. FM portions are representative of the standard FMi and FMt frequency bandwidth and duration.*

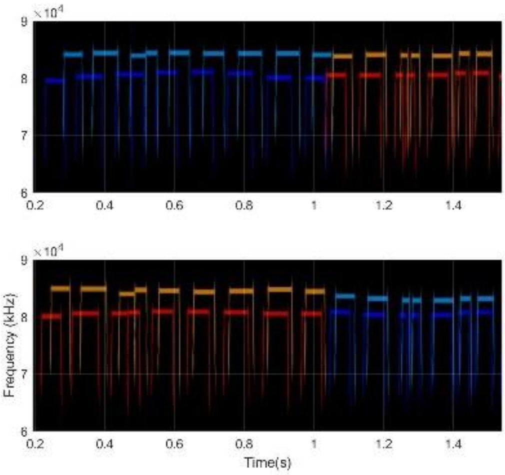

a
b
c
d
e
f
g
h
×10^4
Frequency (kHz)
Time(s)
6
7
8
9
1.4 1.5 1.6 1.7 1.8 1.9 2 2.1 2.2
1.4 1.6 1.8 2 2.2 2.4 2.6 2.8 3
1.6 1.8 2 2.2 2.4 2.6 2.8 3 3.2
1 1.2 1.4 1.6 1.8 2 2.2 2.4 2.6 2.8 3
1.4 1.6 1.8 2 2.2 2.4 2.6
1.4 1.6 1.8 2 2.2 2.4 2.6 2.8
1.4 1.6 1.8 2 2.2 2.4 2.6 2.8 3
1.4 1.6 1.8 2 2.2 2.4 2.6


i
j
k
l
×10^4
Frequency (kHz)
Time(s)
9
8
7
6
1.8
2
2.2
2.4
2.6
2.8
3
3.2
3.4
3.6
1.6
1.5
2.5
3.5
4

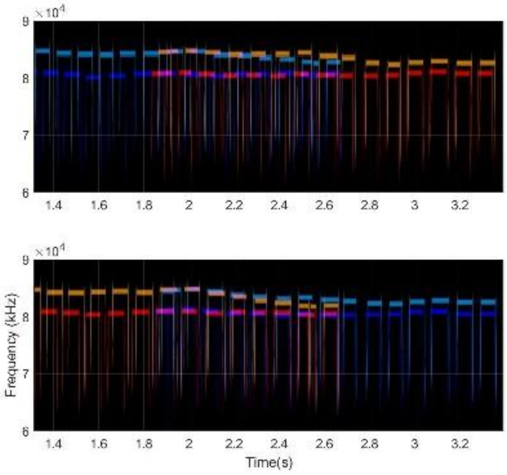
m



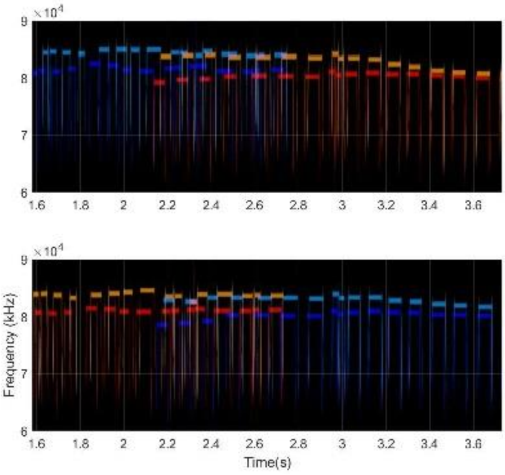
n



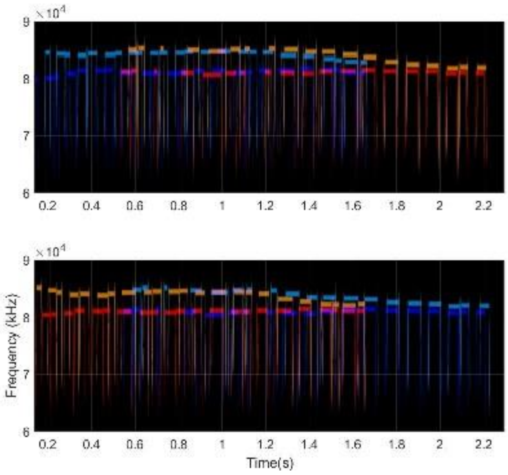
o



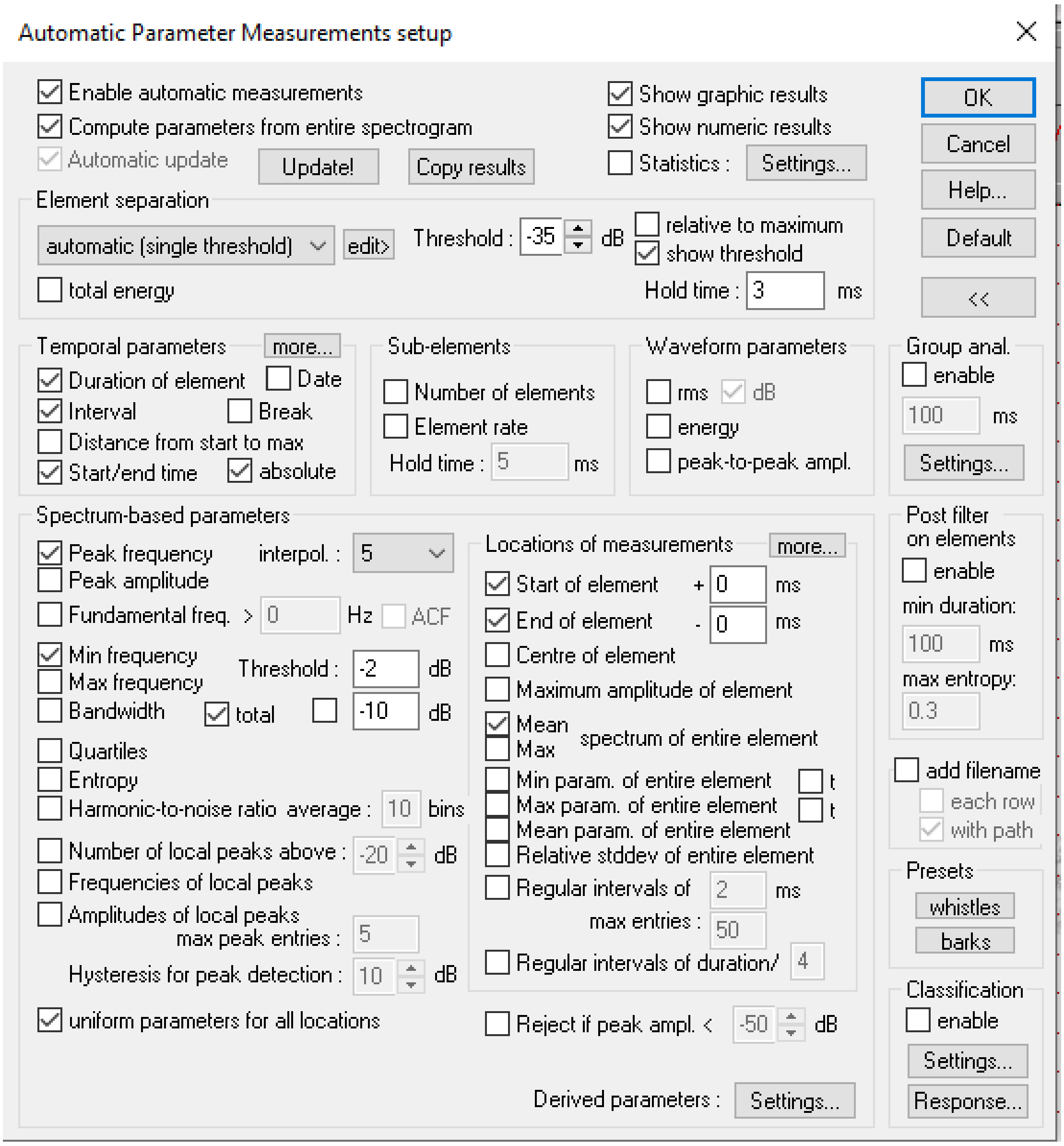
p



q



Appendix 4: Settings for SASLAB automatic parameter measurements.



Automatic Parameter Measurements setup

☑ Enable automatic measurements
☑ Compute parameters from entire spectrogram
☑ Automatic update
Update!
Copy results
☑ Show graphic results
☑ Show numeric results
☐ Statistics : Settings...
OK
Cancel
Help...
Default
<<

Element separation
automatic (single threshold)
edit>
Threshold : -35 dB
☐ relative to maximum
☑ show threshold
☐ total energy
Hold time : 3 ms

Temporal parameters more...
☑ Duration of element
☐ Date
☑ Interval
☐ Break
☐ Distance from start to max
☑ Start/end time
☑ absolute

Sub-elements
☐ Number of elements
☐ Element rate
Hold time : 5 ms

Waveform parameters
☐ rms ☑ dB
☐ energy
☐ peak-to-peak ampl.

Group anal.
☐ enable
100 ms
Settings...

Spectrum-based parameters
☑ Peak frequency
interpol. : 5
☐ Peak amplitude
☐ Fundamental freq. > 0 Hz ☐ ACF
☑ Min frequency
Threshold : -2 dB
☐ Max frequency
☐ Bandwidth
☑ total ☐ -10 dB
☐ Quartiles
☐ Entropy
☐ Harmonic-to-noise ratio average : 10 bins
☐ Number of local peaks above : -20 dB
☐ Frequencies of local peaks
☐ Amplitudes of local peaks
max peak entries : 5
Hysteresis for peak detection : 10 dB
☑ uniform parameters for all locations

Locations of measurements more...
☑ Start of element + 0 ms
☑ End of element - 0 ms
☐ Centre of element
☐ Maximum amplitude of element
☑ Mean
☐ Max
spectrum of entire element
☐ Min param. of entire element ☐ t
☐ Max param. of entire element ☐ t
☐ Mean param. of entire element
☐ Relative stddev of entire element
☐ Regular intervals of 2 ms
max entries : 50
☐ Regular intervals of duration/ 4
☐ Reject if peak ampl. < -50 dB

Derived parameters : Settings...

Post filter
on elements
☐ enable
min duration:
100 ms
max entropy:
0.3

☐ add filename
☐ each row
☑ with path

Presets
whistles
barks

Classification
☐ enable
Settings...
Response...