RESEARCH ARTICLE

Adaptive beam-width control of echolocation sounds by CF–FM bats, *Rhinolophus ferrumequinum nippon*, during prey-capture flight

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SUMMARY

The echolocation sounds of Japanese CF–FM bats (*Rhinolophus ferrumequinum nippon*) were measured while the bats pursued a moth (*Goniocraspidum pryeri*) in a flight chamber. Using a 31-channel microphone array system, we investigated how CF–FM bats adjust pulse direction and beam width according to prey position. During the search and approach phases, the horizontal and vertical beam widths were ±22±5 and ±13±5 deg, respectively. When bats entered the terminal phase approximately 1 m from a moth, distinctive evasive flight by *G. pryeri* was sometimes observed. Simultaneously, the bats broadened the beam widths of some emissions in both the horizontal (44% of emitted echolocation pulses) and vertical planes (71%). The expanded beam widths were ±36±7 deg (horizontal) and ±30±9 deg (vertical). When moths began evasive flight, the tracking accuracy decreased compared with that during the approach phase. However, in 97% of emissions during the terminal phase, the beam width was wider than the misalignment (the angular difference between the pulse and target directions). These findings indicate that bats actively adjust their beam width to retain the moving target within a spatial echolocation window during the final capture stages.

INTRODUCTION

Echolocating bats emit ultrasounds and analyze the returning echoes to search for food and/or avoid obstacles while moving from one place to another (Griffin, 1958; Schnitzler et al., 2003). As an adaptation to their ecological conditions within natural habitats, bats show a variety of foraging strategies, as well as species-specific signal types of echolocation sounds (Simmons and Stein, 1980; Neuweiler, 1984; Schnitzler and Kalko, 2001). Specifically, the detection and tracking of small and moving targets by insectivorous aerial-hawking bats requires sophisticated active sensing techniques (Kalko, 1995; Moss and Surlykke, 2001; Simmons, 2005), which provide various insights from the perspective of sonar engineering.

Echolocating bats can be identified by the frequency structure of their broadcasts. For example, Rhinolophidae, Hipposideridae and Mormoopidae emit pulses consisting of a long constant frequency (CF) component [i.e. *Rhinolophus ferrumequinum*: 50–60 ms (Tian and Schnitzler, 1997), *Hipposideros terasensis*: 5–10 ms (Hiryu et al., 2005), *Pteronotus parnellii*: 10–15 ms (Gaioni et al., 1990)], combined with a short (1–2 ms) initial upward and terminal downward frequency modulated (FM) component. These species are called CF–FM bats, whereas bats producing an FM sonar ultrasound are referred to as FM bats (e.g. vespertilionid bats). Some distinct echolocation behaviors are commonly observed in both CF–FM and FM bat species, including decreases in the duration and sound pressure level of emitted pulses with a decrease in the distance to a target (Simmons et al., 1979). In addition, when bats approach prey, they increase the pulse repetition rate, which reaches a maximum (100–200

times per second) during the terminal phase (Vogler and Neuweiler, 1983; Schnitzler et al., 1985; Surlykke et al., 2003). These extremely fast sequences of short sounds (final buzz) typically last for approximately 200–300 ms just prior to prey capture, and start within 1 m from the capture point (Vogler and Neuweiler, 1983; Fujioka et al., 2011). Recently, some hypotheses have been proposed concerning the function of the final buzz. Specifically, FM bats may decrease the frequency range of the buzz so that they can expand the beam width to achieve a wider detection angle during the pursuit of target prey (Jakobsen and Surlykke, 2010). The beam width is an important feature of echolocation behavior because the spatial characteristics of the sonar beam influence the echoes that are received by the bat (Ghose and Moss, 2003), and broadening the echolocation beam during the final stage of prey pursuit may be an effective feature of aerial hawking bat species (Jakobsen and Surlykke, 2010). However, no behavioral study on the beam-width control during prey capture flight has been conducted in CF–FM bats.

Here, we examined the echolocation sounds produced by a species of Japanese CF–FM bats (*Rhinolophus ferrumequinum nippon*) as they approached a moving moth (*Goniocraspidum pryeri*). *Goniocraspidum pryeri* have ears but do not produce clicks to jam bat sonar ultrasounds. The sounds were recorded and analyzed using a telemetry microphone (Telemike) that was mounted on the bats. Horizontal and vertical microphone arrays were used to measure changes in beam direction and width during moth-capture flight. By monitoring the flight trajectories of both the bats and the moths, we investigated how CF–FM bats adjust beam direction and width according to prey position.

MATERIALS AND METHODS
Subjects
Seven adult Japanese horseshoe bats (*Rhinolophus ferrumequinum nippon*, Temminck 1835, body length: 6.0–8.0 cm, body mass: 20–30 g) were used (6 females and 1 male). The bats were captured from natural caves in Hyogo and Osaka Prefectures in Japan under licence and in compliance with current Japanese laws. The animals were housed in a temperature- and humidity-controlled colony room (4 m long, 3 m wide, 2 m high) at Doshisha University in Kyoto, Japan. The bats were free to fly and access water and food (mealworms). The day and night cycle of the room was set to 12 h:12 h dark:light. During the experimental period, the amount of food provided to the bats was reduced by 75% to ensure proper motivation for capturing moths during the experiments. Fig. 1A shows representative echolocation pulses of *R. ferrumequinum nippon*. *Rhinolophus ferrumequinum nippon* uses compound echolocation signals, each consisting of a CF component with a second harmonic (CF_2) around 68–70 kHz being strongest, plus an accompanying initial short upward FM sweep (2–8 kHz, ending at 68–70 kHz for the second harmonic: iFM_2) and a terminal short downward FM sweep (beginning at 68–70 kHz and extending 8–12 kHz lower for the second harmonic: tFM_2) (Hiryu et al., 2008).

Noctuid moths (*G. pryeri*, body length: approximately 20 mm, wingspan: 40–44 mm) were used as target prey. The moths were caught as adults in a cave in Osaka Prefecture, Japan. The moths were stored in a plastic rearing cage (50 cm long, 35 cm wide, 30 cm high) in the bat room under the same light cycle: 12 h:12 h dark:light. *Goniocraspidum pryeri* are widely distributed in Japan, and they diapause from summer to the next spring in caves that are used by *R. ferrumequinum nippon* as a day roost (Sano, 2006). *Goniocraspidum pryeri* have ears; however, their auditory characteristics with respect to the echolocation sounds produced by bats have not been investigated.

These experiments complied with the Principles of Animal Care, publication no. 86-23, revised 1985, of the National Institutes of Health, and with current Japanese laws. All experiments were approved by the Animal Experiment Committee at Doshisha University.

Video recordings
Fig. 1B shows a schematic diagram of the measurement system used in this study. The experiments were conducted in a flight chamber (8 m long, 3 m wide, 2 m high) under long wavelength lighting with filters (>650 nm) to avoid any visual effects. The experimenter released an individual bat from one end of the flight chamber. The flight chamber was constructed of steel plates to minimize interference from external electromagnetic noises and waves used by commercial FM radio stations. A thin polyester string (Shappespun; Fujix, Kyoto, Japan; diameter: 0.1–0.2 mm, length: approximately 1 m) was carefully attached to the dorsal portion of the moth's body using a drop of beeswax (Tree of Life, Tokyo, Japan). As a result, the moth was tethered from the ceiling and was able to move freely along the length of the string during the experiment (dashed ellipse area in Fig. 1B). In this regard, the moth could move freely within 1 m of the tether during recordings. The moth was placed 2 m from the front wall.

Flight behaviors of the bats and moths were recorded using two digital high-speed video cameras (MotionPro X3; IDT Japan, Tokyo, Japan) located on the left and right corners of the flight chamber, behind the bat so as not to interfere with the bat's flight path. The video cameras recorded 125 frames per second, and three-dimensional (3D) coordinates of the bat and moth flight paths were reconstructed from the video images using motion analysis software (DIPPMotionPro v. 2.2.1.0, Ditect Corporation, Tokyo, Japan). Prior to recording the bat flights, a 3D reference frame with known coordinates was positioned in the center of the flight chamber and was briefly recorded by the two video cameras. The analysis software calibrated the 3D flight path reconstruction system using the cameras' stereo view of the reference frame. Based on a direct linear transformation technique from the coordinates of the reference frame, successive positions of the flying bat as well as the locations of other objects were reconstructed from video-scene coordinates measured from the pair of 2D video images. Using the 3D coordinate data, the flight trajectories of the bat and the moth were determined in conjunction with the acoustic characteristics of the bat's echolocation sounds. The target distance was defined as the distance between the positions of the bat and the moth.

Telemike recordings
Echolocation sounds emitted by the flying bat were recorded using a custom-made telemetry microphone (Telemike) that was mounted on the bat. The recording procedure was the same as that used in a previous study (Hiryu et al., 2008). The Telemike consisted of a 1/8-inch omni-directional condenser microphone (model FG-3329, Knowles, Itasca, IL, USA), a miniature custom-designed FM transmitter unit, a 1.5 V hearing aid battery (type SR521SW, Sony, Tokyo, Japan), and a transmitting antenna. The total weight of the Telemike was approximately 0.6 g. The Telemike was attached to the back of the bat with a piece of double-sided adhesive tape. The microphone pointed forward and was positioned approximately 1 cm above the noseleaf, in the center of the right and left pinnae of the bat. The transmitter of the Telemike produced radio signals that were received by an FM antenna (model 15-1859, RadioShack Corporation, TX, USA) that was tethered to the ceiling of the flight chamber. The received signals were demodulated to recover the ultrasonic broadcasts of the bat using a custom-made FM receiver. The total frequency response of the Telemike system was flat within ±4 dB between 20 and 100 kHz. The signals from the receiver were then band-pass filtered from 20 to 150 kHz (model 3625, NF Corporation, Yokohama, Japan), and digitized by a DAT recorder (16-bit, 384 kHz; model SIR-1000W, Sony, Tokyo, Japan) with the control signal that triggered video recordings. All digitized data were stored as files on the hard disk of a personal computer so that the sound recordings could then be synchronized with flight coordinates.

Microphone array recordings
To measure the 3D direction and beam width of pulses emitted by the bat during moth-capture flight, microphone arrays consisting of 31-channel microphones were set up in the chamber (Fig. 1B). A U-shaped 17-channel horizontal microphone array was arranged on the X–Y plane of the chamber, 1.2 m above the floor to measure the horizontal direction and beam width of the emitted pulse (blue frame in Fig. 1B). For vertical parameters, we arranged a U-shaped 7-channel microphone array on the Z–X plane (red frame), combined with an O-shaped 12-channel microphone array on the Y–Z plane at a distance of 2 m from the front wall (green frame). Each microphone of the arrays was spaced at 0.8 m intervals on the X-axis, 0.5 m intervals on the Y-axis and 0.45 m intervals on the Z-axis of the flight chamber (Fig. 1B). The microphone circuit board consisted of a 1/8-inch omni-directional condenser microphone (model FG-3329, Knowles), a custom-designed differential amplifier circuit (+46 dB) and a custom-designed band-pass filter (10–250 kHz). A urethane acoustic absorption material (20×20 cm) was attached to the rear of each condenser microphone to reduce
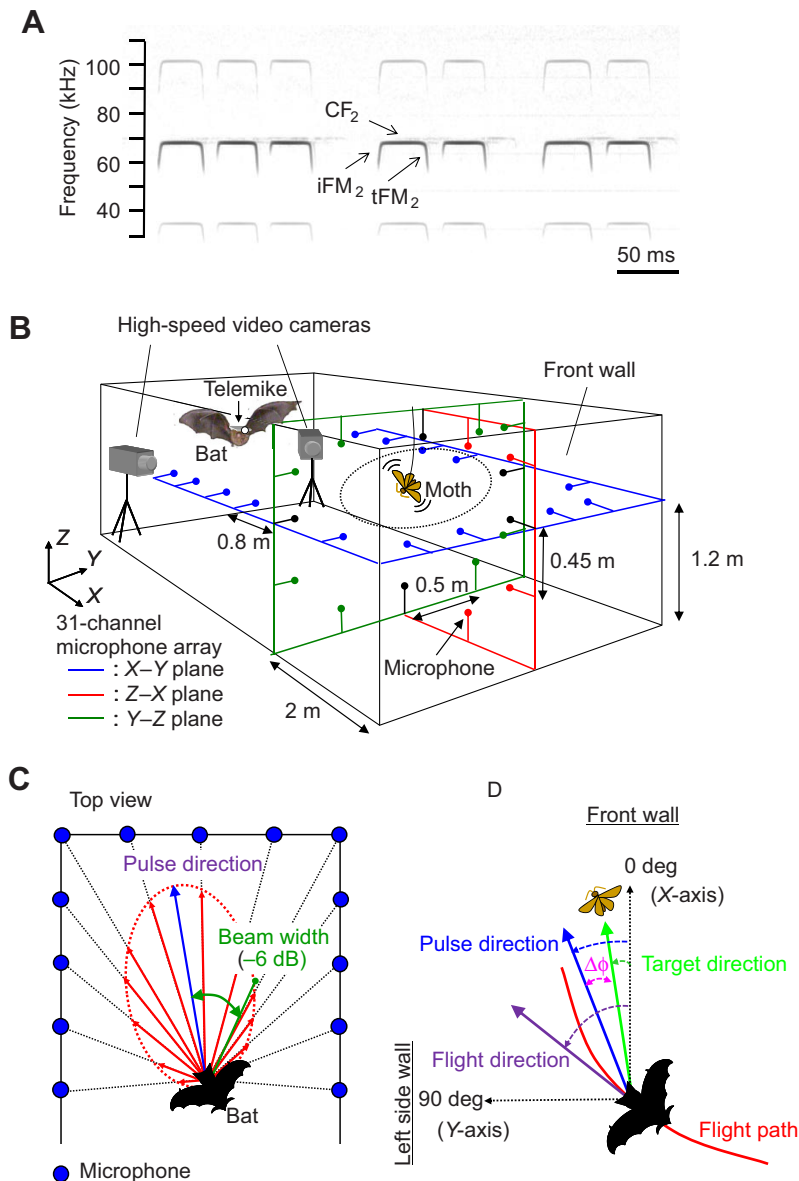
A

B

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Fig. 1. (A) Representative echolocation sounds of *Rhinolophus ferrumequinum nippon* during flight, recorded with the Telemike. (B) Schematic diagram of the microphone array system in a flight chamber for measuring the 3D direction and beam width of pulses emitted by the bats during moth-capture flight. The microphone array consisted of 31-channel microphones. Seventeen microphones were arranged in a U-shape on the X–Y plane 1.2 m from the floor (blue frame). Seven microphones were vertically arranged on the Z–X plane (red frame), combined with 12-channel microphones in an O-shape on the Y–Z plane at a 2 m distance from the front wall (green frame). Each microphone was spaced with a 0.5 m interval in the X-axis, 0.8 m interval in the Y-axis and 0.45 m interval in the Z-axis, respectively. (C) Top view of the horizontal pulse direction and beam width, which were determined based on sounds received with the microphones. The pulse direction was calculated by adding up vectors, which were proportional to the sound pressure level of each microphone. The beam width was calculated by –6 dB off-axis angle from the pulse direction on the pulse directivity pattern. (D) Definitions of the direction components; the X- and Y-axes were defined as 0 and 90 deg in the horizontal plane, respectively. The target direction was determined using the 3D coordinates of the bat and moth. The flight direction of the bat was calculated as the time derivative of the coordinates on the bat's flight trajectory. The angular difference between the pulse direction and target direction (misalignment) was defined as $\Delta\phi$.

echoes from the walls of the chamber. All of the signals recorded by the microphone array system were digitized using a high-speed data acquisition card (16 bit, sampling frequency=200 kHz; model NI PXI-6250, National Instruments, Tokyo, Japan). The digitized signals of all channels were stored as files on the hard disk of a personal computer using a custom program in LabVIEW (LabVIEW 8.0, National Instruments) with the control signal of the video recording so that the microphone array data were synchronized both with flight coordinates and the sound recordings obtained by the Telemike.

Sound analysis

The acoustic characteristics of the broadcast sounds of the flying bat (excluding the direction and beam width of the sound) were analyzed from spectrograms made through the Telemike recordings using custom MATLAB routines on a personal computer. Each pulse was extracted from the recording and then the second harmonic component of the pulse was analyzed for pulse duration, interpulse interval (IPI) and bandwidth of the tFM (tFM_2) (see Fig. 1A). In this study, IPI was defined as the time from the beginning of one

pulse to the beginning of the subsequent pulse. The pulse duration and the bandwidth of tFM_2 were determined from the spectrogram at –25 dB relative to the peak intensity of the pulse.

To analyze the direction and beam width of pulses emitted by the bat during moth-capture flight, tFM_2 components were used because the CF_2 components of the pulses recorded by the microphone array were temporally overlapped with echoes from the surrounding walls of the chamber. The energy maximum in the tFM_2 component spectrogram was measured to quantify changes in the sound pressure levels to reconstruct the directivity pattern of the emitted pulse.

The sound pressure levels of the pulses were corrected for both sound propagation losses between the bat and each microphone in the air, and sensitivity differences among microphones in the array. For propagation loss, a spreading loss was calculated based on the distance between the bat and each microphone. The time at which each bat emitted a pulse was determined from the Telemike recording. Sound absorption was calculated based on a measured absorption coefficient at 65 kHz (α=2.4 dB m^{-1}), which corresponded to the average frequency at the peak energy in the tFM_2 component
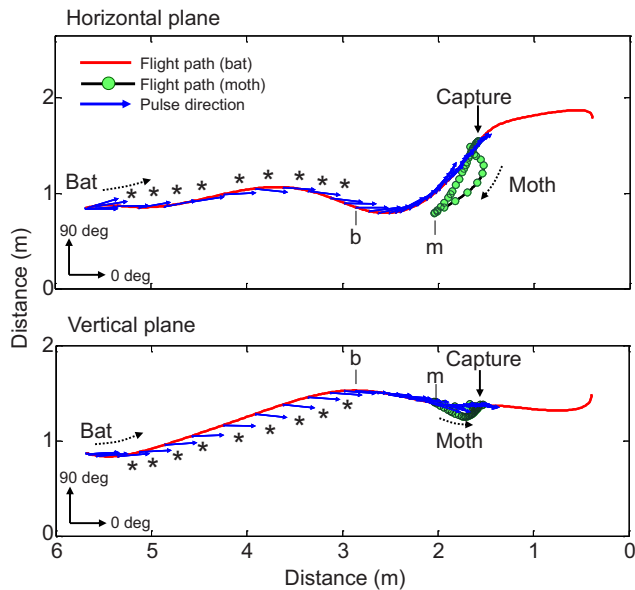
Fig. 2. Representative flight paths of the bat (red line) and moth during moth-capture flight (black line with green plot) in the horizontal (top panel) and vertical planes (bottom panel). The blue arrows indicate the direction of each pulse. 'b' and 'm' indicate the positions of the bat and moth when the moth started evasive flight. *Long CF duration pulse (see Materials and methods).

of the pulse emitted by *R. ferrumequinum nippon*. Sensitivity of each microphone in the array system was measured by presenting a tone burst at 65 kHz using an ultrasonic loudspeaker (PT-R7 III, Pioneer, Kanagawa, Japan) to compensate for sensitivity differences among microphones in the array.

The corrected sound pressure levels of each microphone in the array were converted to vectors (red arrows in Fig. 1C) so that the horizontal and vertical pulse directions were computed by adding up these vectors (blue arrow for horizontal pulse direction, Fig. 1C) (Ghose and Moss, 2003). Fig. 1D shows the definitions of the angular components in this study (only horizontal components are shown in Fig. 1D). We defined the directions of the *X*- and *Y*-axes as 0 and 90 deg, respectively, in the horizontal plane (Fig. 1D). The *Z*-axis direction was defined as 90 deg in the vertical plane. The direction of the moth from the bat (the target direction) was determined using the 3D coordinates of each animal. The flight direction of the bat was calculated as the time derivative of the coordinates on the flight trajectory of the bat. The angular difference between the pulse and target directions was defined as misalignment $\Delta\phi$ (Fig. 1D).

The beam width was defined by –6 dB off-axis angles from the direction of the emitted pulse on the pulse directivity pattern (Fig. 1C, green double-headed arrow).

Prior to the experiments, the measurement errors in pulse direction and beam width created by our microphone array system were investigated when the ultrasound (tone burst at 65 kHz with 3 ms duration; 107 dB SPL at 1 m from the loudspeaker) was presented from the loudspeaker (PT-R7 III, Pioneer) that was set up in the chamber. Pulse direction and beam width were measured while moving the distance of the loudspeaker between 0.5 and 6 m from the front wall. The measurement errors in pulse direction and beam width were less than approximately ±3 and ±5 deg, respectively, and did not change with the distance of 1 to 6 m from the front wall. Hence we placed the target moth at a distance greater than 2 m from the front wall to maintain measurement accuracy.

For statistical comparisons, a Student's *t*-test, one-way factorial ANOVA or *F*-test was used, when appropriate, to test for significant differences in call parameters between data sets.

RESULTS
General features of echolocation sounds during hunting behavior

A total of 93 flight recordings of successful moth captures were taken from seven bats. When the bat was released by the experimenter from one end of the flight chamber, the bat approached and then attacked moths, grasping them by their wings To conduct further detailed analyses of pulse direction and beam width using the microphone arrays, we used 21 flight recordings from seven bats (2–4 flights from each individual) in which the bats flew in the positive X-axis direction for moth capture (the bat was directed towards the microphone array). All of these sound recordings showed good signal-to-noise ratio.

During the recordings, *G. pryeri* sometimes showed distinct evasive flight patterns, such as a sudden change in its flight direction with an increase in its flight velocity in response to the bat's approach. Fig. 2 demonstrates a representative moth-capture flight, showing flight paths of a bat and a moth with the pulse direction of the bat in the horizontal (top panel) and vertical planes (bottom panel). Evasive actions were observed when the bat approached within approximately 1 m of the moth (the positions of the bat and the moth are marked with 'b' and 'm', respectively, in Fig. 2). In the case of the flight shown in Fig. 2, the bat initiated emitting long pulses with a duration of approximately 70–80 ms approximately 4 m from the moth (asterisks in Fig. 2), indicating that the bat started the approach phase for moth capture (Mantani et al., 2012) (we defined the approach phase as consecutive emissions of a long pulse; Fig. 3A). The bat consecutively emitted a single long duration pulse per respiration, which resulted in an almost constant IPI of approximately 100–120 ms (Fig. 3B). When the target distance was within 1 m, the bat started shortening the IPI, accompanied by a decrease in the pulse duration to approximately 10 ms. Therefore, such distinct changes in the acoustical features of the echolocation pulses were observed within 1 m of the target distance (referred to as the terminal phase of echolocation), which corresponded to the target range where the moth began evasive flight behaviors.

Fig. 3C shows a change in the bandwidth of the tFM_2 component of the pulse as a function of the target distance. Unlike pulse duration and IPI, there were no distinct trends in tFM_2 bandwidth changes observed between three echolocation phases throughout entire recordings. The means of the tFM_2 bandwidths were 16.3 ± 2.7 kHz for the search phase (mean ± s.d., $N=72$), 11.9 ± 2.9 kHz for the approach phase ($N=197$) and 12.5 ± 3.5 kHz ($N=233$) for the terminal phase. The tFM_2 bandwidths varied statistically between the three phases (ANOVA, $F_{2,499}=3.01$, $P<0.001$) and there was also a significant difference in the tFM_2 bandwidths between the approach and terminal phases (Student's *t*-test; $P=0.05$). However, the extent of the change in bandwidth was exceedingly small between phases (<5 kHz). Fig. 3D shows a change in the peak frequency of the tFM_2 component that was used to measure the direction and beam width of pulses. Throughout all flight sessions, variation in the peak frequencies of the tFM_2 component was less than 1–2 kHz.

Changes in pulse direction and beam width

Fig. 4 shows changes in the flight direction of the bat (red line), target direction (green line) and pulse direction (blue plot) during
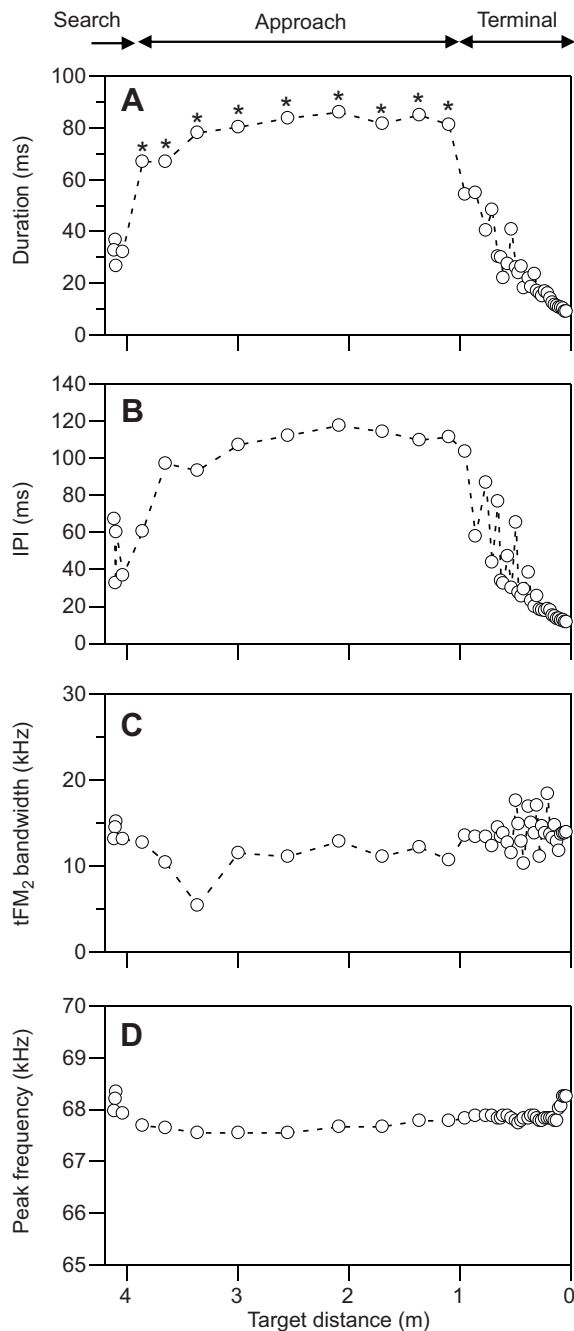
Fig. 3. Changes in acoustical characteristics of emitted pulses as a function of the target distance during intercepting flight, as shown in Fig. 2. (A) Pulse duration. (B) Interpulse interval (IPI). (C) Bandwidth of the tFM$_2$ components. (D) Peak frequency of the tFM$_2$ components. *Long CF duration pulse.
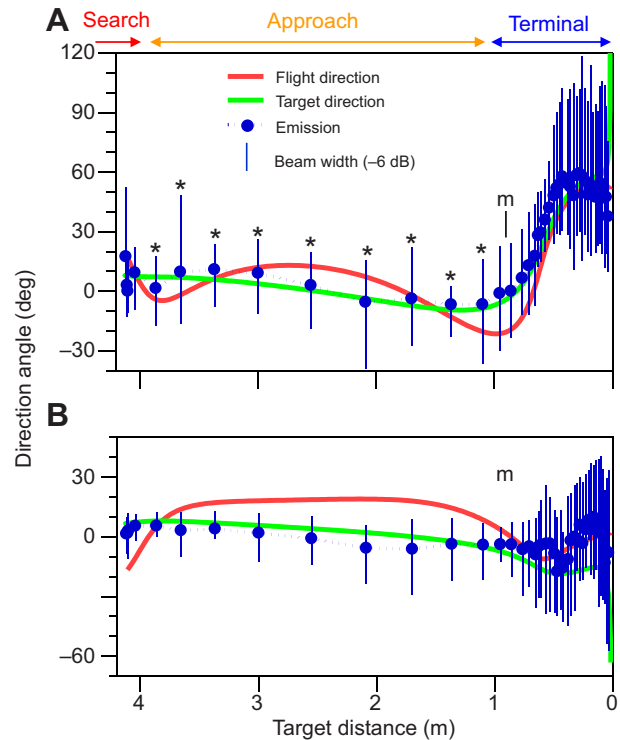

Fig. 4. Changes in the pulse (blue plots), target (green line) and flight (red line) directions as a function of the target distance in the horizontal (A) and vertical (B) planes. Data were taken from the flight shown in Fig. 2. *Long CF duration pulse. The length of the vertical lines corresponds to the beam width (–6 dB) of the pulse. 'm' indicates the timing of the beginning of the evasive flight of the moth.

the moth-capture flight shown in Fig. 2. During the approach phase, the bat tracked the moving moth with an accuracy of less than ±5 deg (Δφ; the angular difference between the pulse and target directions) in both the horizontal and vertical planes. However, when the distance to the moth was within 1 m (terminal phase), the moth started evasive flight, suddenly changing its flight direction (marked 'm' in Fig. 4), i.e. the horizontal target direction changed by approximately 60 deg. As a result, the horizontal and vertical Δφ increased up to 20 deg.

The beam patterns of all sounds are shown in Fig. 5A (horizontal plane) and Fig. 5C (vertical plane). The beam patterns have a bilaterally symmetrical lobe without any side lobe in both the horizontal and vertical planes. During the search and approach phases, the horizontal beam width (–6 dB off-axis angle from the pulse direction; see Fig. 1C) was 22±5 deg (mean±s.d., N=297; red circles in Fig. 5A; green circles greater than 1 m of the target distance in Fig. 5B). When the bat began the terminal phase, the beam width of some emissions (44% of pulses; 249 of 565 pulses) was expanded compared with that during the search and approach phases (we defined the border at 30 deg; see yellow area in Fig. 5B). The maximum horizontal beam width was approximately 50 deg, and the mean expanded beam width (>30 deg) was 36±7 deg (N=249). Expanded beam patterns were also observed in the vertical plane (Fig. 5C,D). The mean of the expanded beam width (>20 deg, yellow area in Fig. 5D) was 30±9 deg (71%, 401 of 565 pulses), whereas that of the search and approach phases was 13±5 deg (N=276). The expanded beam width during the terminal phase was observed in 20 of the 21 analyzed flight sessions (the one case in which a bat did not expand its beam width during the terminal phase is shown in Fig. 8; see Discussion).

Fig. 6 shows the relationship between the target distance and Δφ of each emission (purple plots). During the search phase, the absolute means of Δφ were 7±5 deg in the horizontal plane and 4±2 deg in vertical plane (N=80), which were almost identical to those of the approach phase (6±6 deg horizontal, 4±3 deg vertical, N=196). In Fig. 6, the mean echolocation pulse beam widths are
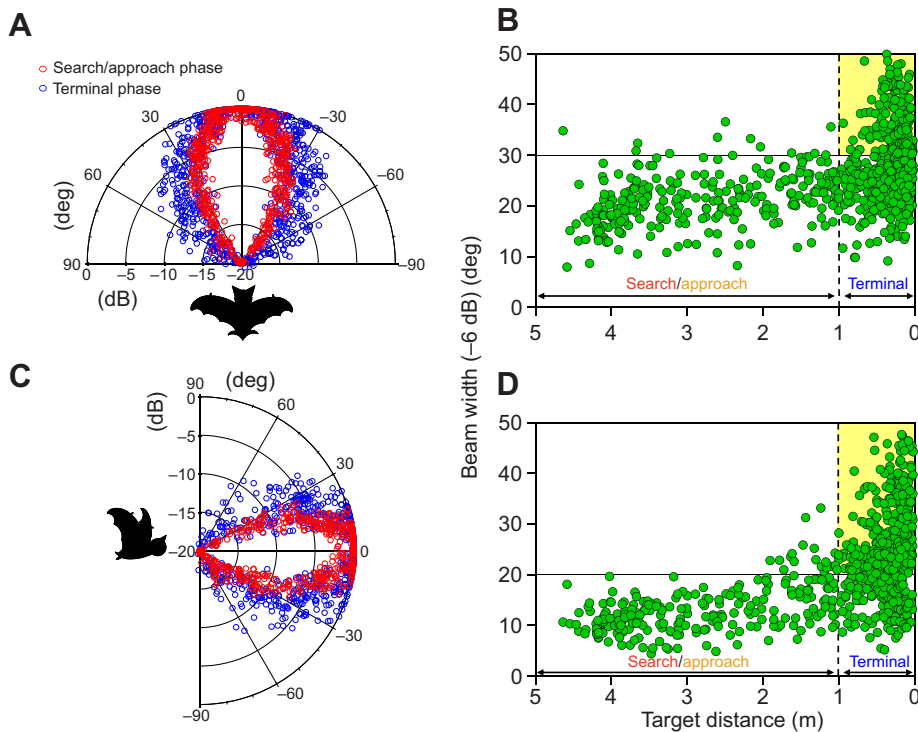
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Fig. 5. Directivity patterns of bat echolocation pulses of the bats during moth capture flight in the horizontal (A) and vertical (C) planes. Data were taken from 21 flight sessions of seven bats during the search/approach (red plots) and terminal (blue plots) phases. (B,D) Changes in the beam width of the pulses (–6 dB) as a function of the target distance in the horizontal (B) and vertical planes (D). The dashed vertical line indicates the beginning of the terminal phase (1 m of target distance). The yellow area indicates the beam width expansion of the pulse during the terminal phase (>30 and >20 deg in the horizontal and vertical planes, respectively).

also shown by red and blue horizontal lines with the standard deviation (shaded area). The beam width of the pulse was wider than the misalignment $\Delta\phi$ in 98% of all emitted pulses during the search and approach phases (271 of 276 pulses). However, because the target direction changed dynamically during the terminal phase due to the evasive flight of the moth, the absolute maximum of $\Delta\phi$ increased up to approximately 50 deg in the horizontal and vertical planes (absolute means: 12±12 deg in the horizontal and 11±10 deg in the vertical plane, respectively; N=565). The bats broadened the beam width to cover the increase in $\Delta\phi$ during the terminal phase, and the beam width of 97% of pulses (544 of 565 pulses) resulted in wider than $\Delta\phi$.

DISCUSSION
Beam expansion during the terminal phase
Some moth species have evolved the ability to detect and respond to the frequency range of ultrasounds produced by bats. These adaptations appear to be the result of selective pressure from bats (Surlykke and Filskov, 1999; Miller and Surlykke, 2001). When the moths detect bat ultrasounds, they exhibit evasive behaviors, such as stopping flight or erratic flight maneuvers, so that chances of being caught by the bat are reduced (Fenton and Fullard, 1979). In addition, some tiger moths are known to produce trains of ultrasonic clicks to jam bat sonar (Corcoran et al., 2009). In the present study, *G. pryeri*, which have ears but do not produce ultrasounds, showed evasive flight patterns; i.e. Fig. 2 shows that the moth suddenly changed its flight direction away from the bat with an increase in its flight speed when the bat approached within approximately 1 m of the target distance. These results suggest that *R. ferrumequinum nippon* appear to adapt their echolocation strategy to overcome moth behavioral strategies, such as evasive flight action during the terminal phase.

In general, misalignment ($\Delta\phi$) is caused by movements of prey between successive pulse emissions. In the present study, during

search and approach phases that have a relatively long target distance (1–5 m), changes in the target direction appeared to be small even when the moths moved, which resulted in a small $\Delta\phi$ of 6±6 deg in the horizontal plane and 4±3 deg in the vertical plane during the approach phase (Fig. 6). The $\Delta\phi$ during the terminal phase (short target distance) becomes larger (left-hand panel in Fig. 7B) even though the moving distance of prey (red arrow) was identical with that during the approach phase (Fig. 7A). In addition, when the moth showed evasive flight, the misalignment increased to a greater extent (i.e. up to 50 deg in Fig. 6; see right-hand panel in Fig. 7B), which means that the decrease in the tracking accuracy of the bat echolocation during the terminal phase compared with that during the approach phases. However, our measurement of the relationship between prey movement and changes in beam width demonstrated that in 97% of pulse emissions during the terminal phase, the beam width of the pulse was wider than the misalignment (Fig. 6). These findings indicate that the bats actively adjusted their beam width to retain the moving target within the spatial window of echolocation during the final stages of capture.

Fig. 8 shows one exception in which the beam width was not changed between the search/approach and terminal phases. In this flight, the moth remained in almost the same place and fluttered (Fig. 8A). Therefore, the target direction did not change in the horizontal or vertical plane throughout the flight (green line in Fig. 8B for the horizontal plane). Consequently, the misalignment $\Delta\phi$ was less than 8 deg and did not increase even in the terminal phase (Fig. 8C). The horizontal and vertical beam widths during the approach phase were approximately ±20 and ±10 deg, respectively, and were not broadened during the terminal phase (Fig. 8D,E). These results also suggest that the broadened beam width is associated with target prey movement. The present study is the first to demonstrate that adjustment of active beam width is a common feature of echolocation behavior not only in FM bats, but also in CF–FM bat species.
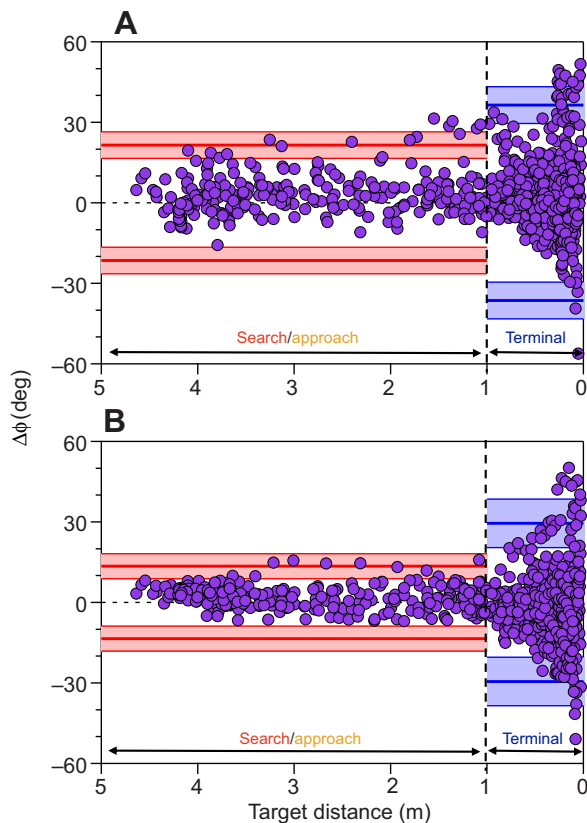
Fig. 6. Changes in Δφ in the horizontal (A) and vertical (B) planes as a function of the target distance. Data were taken from 21 flight sessions of seven bats. Red and blue lines indicate the mean beam widths of the pulses with the standard deviation (shaded area) during the search/approach and terminal phases, respectively. The dashed vertical line indicates the beginning of the terminal phase (1 m of target distance).

How do the bats change their beam pattern?

Echolocation sounds are directional, and the beam patterns of emitted pulses have been measured in several bat species [e.g. –6 dB point in horizontal plane; ±25–35 deg at 90 kHz of the most intense frequency in *Carollia perspicillata* (Hartley and Suthers, 1987), ±70 deg at 25 kHz and ±30 deg at 80 kHz in *Eptesicus fuscus* (Hartley and Suthers, 1989), ±40 deg at 55 kHz in Myotis bats (Shimozawa et al., 1974), and ±23 deg at 84 kHz of CF_2 frequency

in *Rhinolophus ferrumequinum* (Schnitzler and Grinnell, 1977)]. Because the directivity of sounds depends on frequency, echolocation sounds with multiple harmonics show different beam patterns among harmonics (Hartley and Suthers, 1989). All of these previous measurements, however, were conducted on bats that were restrained, i.e. the bat was suspended in a holder, with the head fixed in place by a rod cemented to the skull. In addition, some of these studies used electrical brain stimulation to elicit vocalizations of conscious animals (Shimozawa et al., 1974; Hartley and Suthers, 1987; Hartley and Suthers, 1989). If the bats adeptly change their beam patterns depending on the particular situation as reported by Jakobsen and Surlykke (Jakobsen and Surlykke, 2010) and the present study, the beam patterns of natural vocalizations by bats during free flight will provide interesting novel insights into echolocation behavior.

How do the bats change the beam width? In *Myotis daubentonii*, broadening of the beam width of the echolocation pulse is achieved by lowering the frequency range of FM sounds, i.e. the peak frequency (the frequency with the highest energy in the spectrum of the FM sound) was lowered from 55 to 27.5 kHz during the terminal phase, which is approximately one octave (Jakobsen and Surlykke, 2010). In the present study, variation in peak frequencies of the tFM_2 component in *R. ferrumequinum nippon* was normally 1–2 kHz throughout the flight, which is likely to be due to Doppler-shift compensation. In addition, the mean band width change between the approach and terminal phases was small (<5 kHz). Such small variation in frequency is unlikely to affect the beam width of the tFM_2 component, and the CF–FM bats may broaden their beam width using a different mechanism than lowering frequency range adopted by *M. daubentonii*.

From an acoustical point of view, the beam pattern of sounds emitted from two closely spaced point sources changes depending on the ratio of the wavelength of the sound to a distance between the two sound sources (Strother and Mogus, 1970). Nasal emitters such as Rhinolophidae, Hipposideridae and Phyllostomidae show nostril separation with a half-wavelength of the CF_2 frequency (Möhres, 1953; Schnitzler and Grinnell, 1977; Hartley and Suthers, 1987; Hiryu et al., 2006); i.e. the nostril separation of *R. ferrumequinum nippon* is approximately 2.3–2.4 mm, which is almost half-wavelength of the CF_2 frequency, 68–70 kHz. The half-wavelength separation of nostrils results in interference between the two sound sources in the horizontal plane so that the main beam of the emitted pulse can be directed in the forward direction with less side lobes in the CF_2 frequency (Strother and Mogus, 1970; Schnitzler and Grinnell, 1977). However, when the upper portion
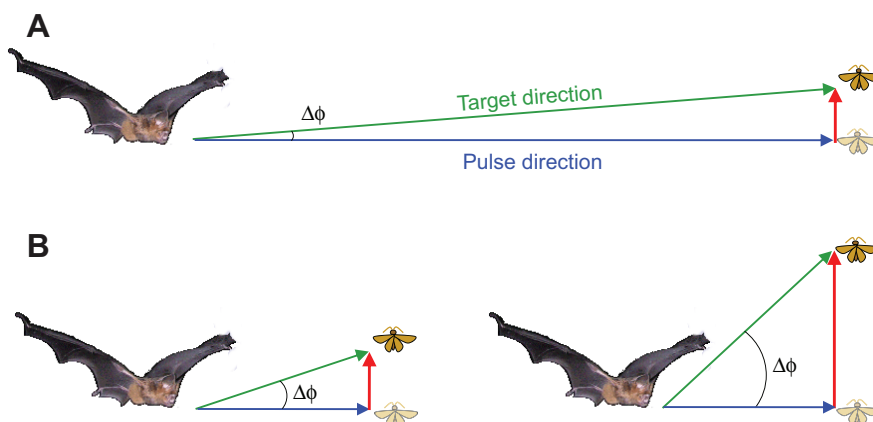

Fig. 7. Relationships between Δφ and target distance in the search/approach (A) and terminal (B) phases. The red arrow indicates the moving distance of a moth between pulse emissions by the bat. Compared with search/approach phases (long target distance), Δφ becomes larger during the terminal phase (left-hand panel in B). When the moth increases its flight velocity as an evasive maneuver in response to the approach of the bat, Δφ increases to a greater extent (right-hand panel in B).
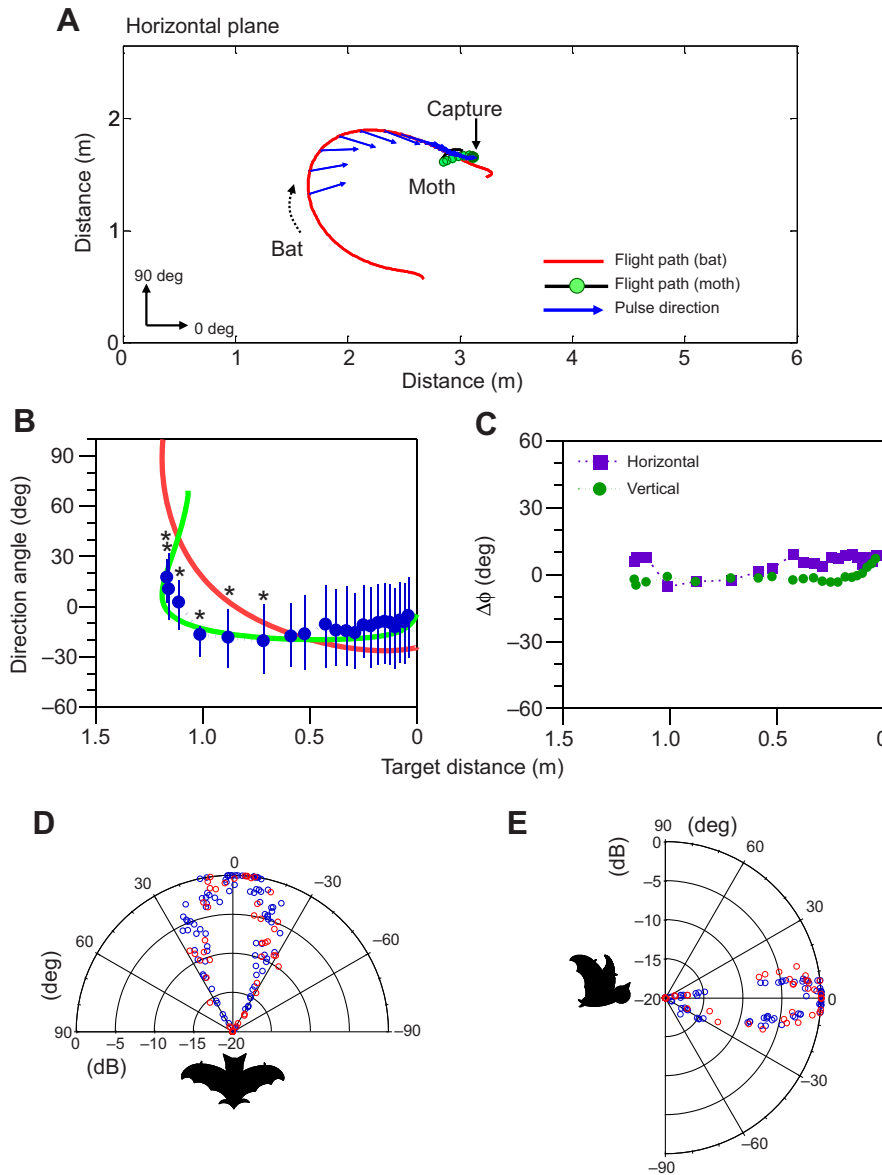
Fig. 8. Flight case in which the bat did not expand its beam width during the terminal phase. (A) Flight paths of the bat (red line) and moth (black line with green plot) in the horizontal plane. The moth remained in almost the same place and fluttered. (B) Changes in the pulse (blue plots), target (green line) and flight (red line) directions as a function of the target distance in the horizontal plane. *Long CF duration pulse. The length of the vertical lines corresponds to the beam width (–6 dB) of the pulse. (C) Changes in Δφ in the horizontal (purple squares) and vertical (green circles) planes as a function of the target distance. (D,E) Directivity patterns of these pulses in the horizontal (D) and vertical (E) planes. Red and blue plots indicate the search/approach and terminal phases, respectively.

of a noseleaf of a horseshoe bat was covered with Vaseline, the smoothness of the beam pattern was destroyed (Schnitzler and Grinnell, 1977). Furthermore, when the entire noseleaf was bent back (the tip of the noseleaf was cemented to the head of the bat), the beam width was approximately doubled in the vertical dimension in *Carollia persplicillata* (Hartley and Suthers, 1987). Strother and Mogus (Strother and Mogus, 1970) suggested that the calculated horizontal beam width of *R. ferrumequinum* at CF_2 frequency based on two closely spaced sources without the lancet was 30 deg, which was wider than previously reported measurements of 21–23 deg (Mohres, 1966; Schnitzler, 1968). These results imply that the noseleaf is likely to focus the beam pattern of nasal emitters. In addition, numerical computations based on the 3D structure of the emitter portion of bats have demonstrated that the noseleaf affects the directivity pattern of the emitted pulse (Zhuang and Müller, 2006; Zhuang and Müller, 2007; Vanderelst et al., 2010; Vanderelst et al., 2011). In the present study, horizontal beam width of *R. ferrumequinum nippon* was ±22±5 deg, and was expanded to ±36±7 deg in the terminal phase. We suggest that changing the noseleaf structure may allow *R. ferrumequinum nippon* to manipulate the beam pattern without changing the frequency of the emitted pulse. Further investigations will be required to determine the detailed mechanisms by which CF–FM bat species broaden the beam pattern during the terminal phase.

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