

Prey-capture success revealed by echolocation signals in pipistrelle bats (*Pipistrellus pygmaeus*)

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Summary

Three *Pipistrellus pygmaeus* bats were trained to capture prey on the wing while flying in the laboratory. The bats' capture behaviour and capture success were determined and correlated with acoustic analyses of post-buzz echolocation signals. Three acoustic parameters revealed capture success: in case of success, post-buzz pauses (pbP) were longer, interpulse intervals (IPI) of the post-buzz signals were longer and, most notably, the spectra of the echolocation signals showed a number of notches that were absent after unsuccessful attempts. If the bats touched the prey without seizing it, pbP was significantly increased, but by less than was seen following a successful capture. Thus, acoustic recordings can be used to determine the outcome of a capture attempt with

72–75% correct using IPI or pbP, and with 78% correct using notches. Even more trials (>85%) were classified correctly by using the first canonical discriminant factor from principal component analysis combining the three parameters. Four types of prey were used, of 7–22 mm length and 7–240 mg mass. After successful captures, interpulse intervals, post-buzz pause and notches all depended on prey type, but not in a way that was systematically related to size, indicating that acoustic parameters cannot reveal prey size.

Key words: dat, *Pipistrellus pygmaeus*, echolocation, prey capture, post-buzz phase, biosonar signals.

Introduction

Many microchiropteran bats use echolocation or biosonar signals to orient and locate prey. They emit short ultrasonic signals and listen for the echoes reflected from surroundings and prey. During a pursuit the signals change in a typical way: signal duration decreases, while repetition rate increases over the course of the three phases of the pursuit: search, approach and terminal buzz phase. Bandwidth often increases in the approach, but may decrease again in the terminal phase (Griffin, 1958). Aerial hunting bats capture prey either with the wing or the tail membrane. After the buzz, there is a pause in which the bat retracts the prey from the tail pouch before it emits the first post-buzz signals and returns to a new search phase (Kalko, 1995). Despite great variations in search signals between bat families, the pattern of changes during a pursuit sequence by insectivorous bats hunting insects on the wing show many similarities (Simmons et al., 1979; Neuweiler, 1989). Thus, the number of pursuits can be counted simply by listening to the echolocation signals of aerial hunting bats. In ecological studies focusing on energetics, the number of feeding buzzes have been used to estimate the number of insects caught by the bats (e.g. Racey and Swift, 1985; Vaughan et al., 1997).

However, not all capture attempts are successful. Sometimes there is enough light for visual inspection, as when bats hunt

around street lights (Acharya and Fenton, 1999) or at high latitudes (Rydell, 1992). These and other studies revealed that capture success is nearly always considerably less than 100%, e.g. approx. 35% for *Eptesicus nilsonii* attempting to catch ghost moths (Rydell, 1998; Jensen et al., 2001). In many studies of bat–insect interactions, the actual number of captures is more interesting than the number of attempts. One obvious solution has been to look for acoustic parameters in the bats' post-buzz signals that could reveal if the capture attempt was successful.

Schnitzler et al. (1987) suggested that the duration of the pause following the buzz was correlated with success, with longer pauses after catches. Acharya and Fenton (1992) confirmed this for two species of *Lasiurus* bats. Their data varied from year to year but, for example, in 1989 they found average durations of post-buzz pauses of 165 and 265 ms after successes and 121 ms and 167 ms after unsuccessful attempts for *L. cinereus* and *L. borealis*, respectively. Britton and Jones (1999) found a similar correlation for *Myotis daubentonii* hunting in the laboratory but, surprisingly, they found no effect on post-buzz pause in their field data. They did find, however, a correlation with interpulse intervals (IPI) in the post-buzz signals. IPIs were significantly longer after successful than after unsuccessful capture attempts, both in the laboratory and

in the field. In the laboratory Britton and Jones (1999) also found that the minimum frequency, F_{\min} , of the first signal emitted after the post-buzz pause was higher after captures than after misses. However, this difference was not seen in their field data.

The overlap between post-buzz pause distributions after successes and misses and the general lack of consistency in the data make it difficult to identify successful attacks unambiguously. Thus, the purpose of this study was to extend the analysis to include not only temporal parameters, but also frequency parameters in post-buzz signals, in an attempt to obtain a more reliable assessment of bats' capture success. Prey size is another parameter that is of importance for studies of feeding ecology and energetics. Acharya and Fenton (1992) looked for prey-size effects on buzz duration, and Britton and Jones (1999) inspected post-buzz pauses and post-buzz IPI, but none of them found any significant effects of size of the captured prey, although IPI showed a tendency to increase with prey size. We analysed our data for any correlation of both frequency and temporal parameters with prey size. We performed the experiments in the laboratory, exploiting the inherent advantages of high quality sound recordings combined with video monitoring, and we discuss our results in relation to field recordings.

Materials and methods

Bats and prey items

We used three pipistrelle bats, *Pipistrellus pygmaeus* (Leach 1825), (the former 55 kHz phonic type of *Pipistrellus pipistrellus* Jones and Barratt, 1999), two males and one female. The bats were captured from the wild and trained within the first week to fly clockwise in a flight cage and catch prey in the air (Fig. 1). The bats were fed only during sessions. Between sessions the bats were kept in small cylindrical cages (20 cm diameter, 35 cm height) with free access to water containing added vitamins. Stable conditions were 25°C, 45% air humidity and a 12 h:12 h light:dark cycle.

We used four different prey items: microworms (buffalo worms) *Alphitobius diaperinus*, 7–13 mm long and weighing 7–28 mg, mealworm larvae *Tenebrio molitor*, 14–27 mm long and weighing 44–15 mg, mealworm pupae, 13–18 mm long and weighing 64–145 mg, and moth bodies, wild-caught *Orthosia* species with wings and legs removed, 14–22 mm long and weighing 62–238 mg.

Flight cage and video recordings

The flight cage was a net tent (7 m long \times 4.8 m wide \times 2.4 m high) placed in a large room. A curtain partly divided the cage longitudinally, creating an oval flight track for the bats (Fig. 1). Half way up one side a custom-built mealworm catapult was placed on the floor. Two video cameras were focused on the catch volume, which was approximately 45 cm \times 30 cm by 55 cm, centred 180 cm above the floor. One camera (Sony CXC-101P, connected to a Panasonic AG-6200 video recorder) was placed at one end facing the bat, when it

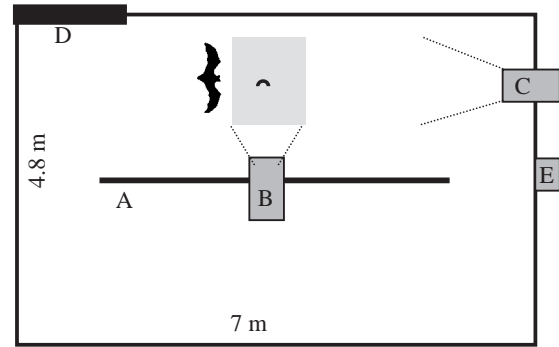


Fig. 1. Diagram of the flight room seen from above. (A) Curtain partly dividing the flight cage. (B) Video camera filming across the track. (C) Video camera filming along the track. Brüel & Kjaer microphone and bat detector were also placed at C. (D) Wooden door into the flight room. (E) Concrete pillar. The mealworm catapult and computer operating devices were placed on the floor right under the capture volume indicated by the grey shading.

approached the prey. We used this video recorder to store comments and the bat detector output (divide-by-ten; Andersen and Miller, 1977). The other video camera (Panasonic NV-M10 camcorder) recorded the bat from the side in the middle of the flight cage, where it captured the prey. This camera was connected to a multiframe system to allow synchronized firing of the flashes with the video recording. Thus, the 'sync' pulse from the video camera elicited one flash 1 ms long per video frame. The 30 μ s synchronised pulses were also recorded in the sound files together with the bats' sonar signals (see below), to allow for synchronization between video and sound recordings.

Sound recordings and analysis

A $\frac{1}{4}$ " microphone (Brüel & Kjaer 4135, grid off) with preamplifier (Brüel & Kjaer 2633) was placed at the end of the flight cage facing the bat when it approached the capture area (Fig. 1). The microphone was 180 cm above ground at approximately the same height as the bat's flight path and a few cm above and in front of the Panasonic video camera. Recording of a trial was started immediately after the bat passed the curtain on the short side furthest away from the microphone and was stopped when the bat had passed the curtain on the opposite short side next to the microphone. The microphone signals were amplified (Brüel & Kjaer type 2607), high-pass filtered (15 kHz) and mixed (custom-made signal mixer) with the synchronization pulses from the flash system. The output from the signal mixer was digitised on-line and stored in one file per trial on a personal computer using a Digital Signal Processing (DSP) board (SPB2, Signal-Data, Copenhagen) and specially developed software (S. Boel Pedersen, Centre for Sound Communication, Odense University of Southern Denmark). The signals were A/D (Analogue-to-Digital) converted (sample rate: 400 kHz) and stored in a ring-buffer (FirstInFirstOut, FIFO). Up- and down-trigger levels were adjusted above the noise floor to detect the

beginning and end of all signals. The system digitised and stored the microphone signal from 750 μ s before the up-trigger to 750 μ s after the down-trigger to ensure that the low-amplitude beginning and end of the sonar signals were included. Each signal was labelled with a time-stamp marking the time of crossing the up-trigger level. Microphone output between bat vocalisations was not stored. This not only saved around 90% data-storage space, but also enabled us to scroll quickly from signal to signal within a trial during analysis using a custom-made sound analysis program: BatViewer (S. Boel Pedersen, Centre for Sound Communication, Odense University of Southern Denmark).

Field recordings were done using the same microphone and amplifiers as above and stored on tape (Racal Store 4D). They were later digitized in one file per capture sequence using the same hard- and software as in the laboratory.

We determined signal duration, interpulse interval (IPI) and post-buzz pause (pbP) duration. The energy and power spectrum of each signal were calculated. The spectra were used to determine the maximum, minimum and peak frequency of the first harmonic. We also determined the BW_{-10dB} (bandwidth measured 10 dB below the peak of the spectrum) and the number of harmonics of the signals.

The number of notches was counted in all signals in all analysed sequences. The spectra always contain notches caused by the frequency overlap between the first and second harmonic (approximately 110 kHz for *P. pygmaeus*), but these were not included in the count. We only counted surplus notches in the first harmonic. Those mainly occurred after captures. We used BatSound™ (Pettersson Electronic) to produce spectrograms (512 point FFT, Hann windows with 80% overlap) to display whole pursuit sequences for fast examination of notches in post-buzz signals. The notches were inspected more closely in power spectra of the signals (2048 point FFT, rectangular (uniform) window. A 2048 point window (5.12 ms) was longer than all signals recorded, and only notches that were at least 5 dB deep were included. If a signal had one or more surplus notches it was scored as 'with notches', independent of the number of surplus notches. NOTCH in the following is the percentage of signals in a sequence (e.g. a post-buzz sequence) containing surplus notches.

Database

A 'trial' was defined as one capture attempt of one prey item. Trials where the bat made no obvious attack, trials where the bat-prey encounter took place out of view of both cameras, and trials where the prey was clearly out of the bat's reach (e.g. too low), were discarded from the analyses. A 'session' was defined as all trials conducted with one bat during a single day. Each trial was classified as a wide (w), a touch (t) or a capture (c), based on both immediate inspection and subsequent control using the video recordings. Trials where the bat caught the prey and carried it away were defined as 'captures'. 'Touches' were trials where the bat touched and deflected the prey from its trajectory without getting hold of it. 'Wides' were

trials where the bat made a clear attempt to capture the prey without touching it. For some analyses touches and wides were pooled in the single category 'fail' (f).

The database included 50 successful capture trials with each bat and each prey type plus the associated number of touches and wides, i.e. a total of around 350 trials for each bat. These trials were used for calculating overall capture success. 20 captures with each bat and each prey item and all associated touches and wides were chosen for analysis of sonar sounds, giving a total of 240 captures, 71 touches and 114 wides analysed for all three bats. All together 4487 sonar signals from captures, and 1998 from fails, were analysed, mainly from the post-buzz signals, but some search-phase signals were also analysed. The search-phase was defined as the signals from the beginning of a trial until the mealworm catapult was triggered, which was itself defined as the start of the approach phase. An abrupt increase in pulse repetition rate (PRR) indicated the transition from approach to buzz. The buzz was subdivided in two phases, Buzz_I and Buzz_{II} (see Griffin, 1958; Surlykke et al., 1993) and was followed by the post-buzz pause, pbP. The signals after the pbP were defined as post-buzz signals.

Using corresponding sound and video recordings we compared the duration of the acoustical post-buzz pause with the duration of the head-down stage seen in the video recordings.

Control without light

In most experiments two neon tubes were used to provide sufficient light for video recordings. To rule out the possible use of visual cues, we performed control trials without light using the two male bats. The outcome of these trials was scored on the basis of sound cues. A trial was registered as a fail if we heard the sound of a prey item falling to the ground, i.e. wides and touches were pooled, because they sounded the same. Captures were indicated by the lack of this sound and confirmed by the bat's chewing sounds. None of the bats showed any hesitation or other signs of disturbance by the lack of light. Further, their success rates were 88% and 90%, and thus not reduced by the lack of light.

Statistical analyses

A number of parameters were analysed. Most distributions of temporal and spectral parameters for all three bats and three capture outcomes (capture, touch, wide) were normally distributed (D'Agostino-Pearson χ^2 test), but a few were not. However, removal of six outlying datapoints from a total of 425 trials restored normality and two-way analysis of variance (ANOVA) analysis was performed on all data to test for differences between capture, touch and wide values in the post-buzz sequences. This was followed by a Tukey's test when significant differences were found. A significance level of 1% was employed (with Bonferroni correction for multiple comparisons). For those parameters where significant differences were found between post-buzz values of capture, touch and wide trials, the search sequences of the particular trials were also tested for differences between these

parameters. Groups were compared pairwise using a Kruskal–Wallis/Mann–Whitney non-parametric test with Bonferroni corrections.

The average pbP, IPI and NOTCH in post-buzz signals differed significantly between successful and non-successful capture attempts, but there were large overlaps between the distributions. To increase discriminability between groups we performed a canonical discriminant analysis (CANDISC, SAS statistical software package) on the data. This analysis constructs a new parameter, the first canonical discriminant function, CD1. CD1 is a linear combination of the original parameters (NOTCH, IPI and pbP) and provides maximal correlation with the capture categories.

Receiver operating characteristics (ROC) curves were constructed using the three original parameters (NOTCH, IPI, pbP), as well as CD1. In each case the data for the post-buzz signals of a trial were used to classify the trial as either capture or fail (touches and wides were pooled). Depending on the actual outcome of the trial the classification as a capture could be scored as either a hit (correct identification of a capture) or a false alarm (incorrect classification as capture, actual outcome a fail, i.e. touch or wide). Similarly, classification as a fail could be a correct rejection (correct) or a miss (incorrect; actual outcome was a capture). The terms hit, miss, false alarm and correct rejection are used to retain consistent terminology with classical signal detection theory (Green and Swets, 1966; Ohl et al., 2001). Ten different criteria were used to produce ten corresponding sets of hit and false alarm rates, which were then plotted in four ROC curves, one for each of the three raw parameters and one for the CD1 parameter (Scheich et al., 1998; Tougaard, 1999). Data from all three bats were pooled, for two reasons. Firstly, the differences observed in the pooled data set were also always consistent for all three bats tested individually. Secondly, pooled data are most often what is experienced in the field, where one cannot be certain that all recordings are from the same individual.

Results

Hunting behaviour in the laboratory

Immediately after release in the flight cage the bats usually flew at rather high

speed. When ready to start feeding they slowed down, and the session started and continued until the bats were satiated. The bats were not caught or handled during a session. The bats flew clockwise in the flight cage. The catapult release was manually triggered at the moment when the bat turned the far corner on the catapult side and flew towards the capture area.

The bats captured the prey either with the tail (interfemoral) membrane, the wing membrane or a combination of both. 81% of all captures were made using only the tail membrane to intercept the prey items. The bat retrieved the prey by bending the head into the tail pouch (Fig. 2, frame 11). A few recordings strongly indicated that the bats could seize prey

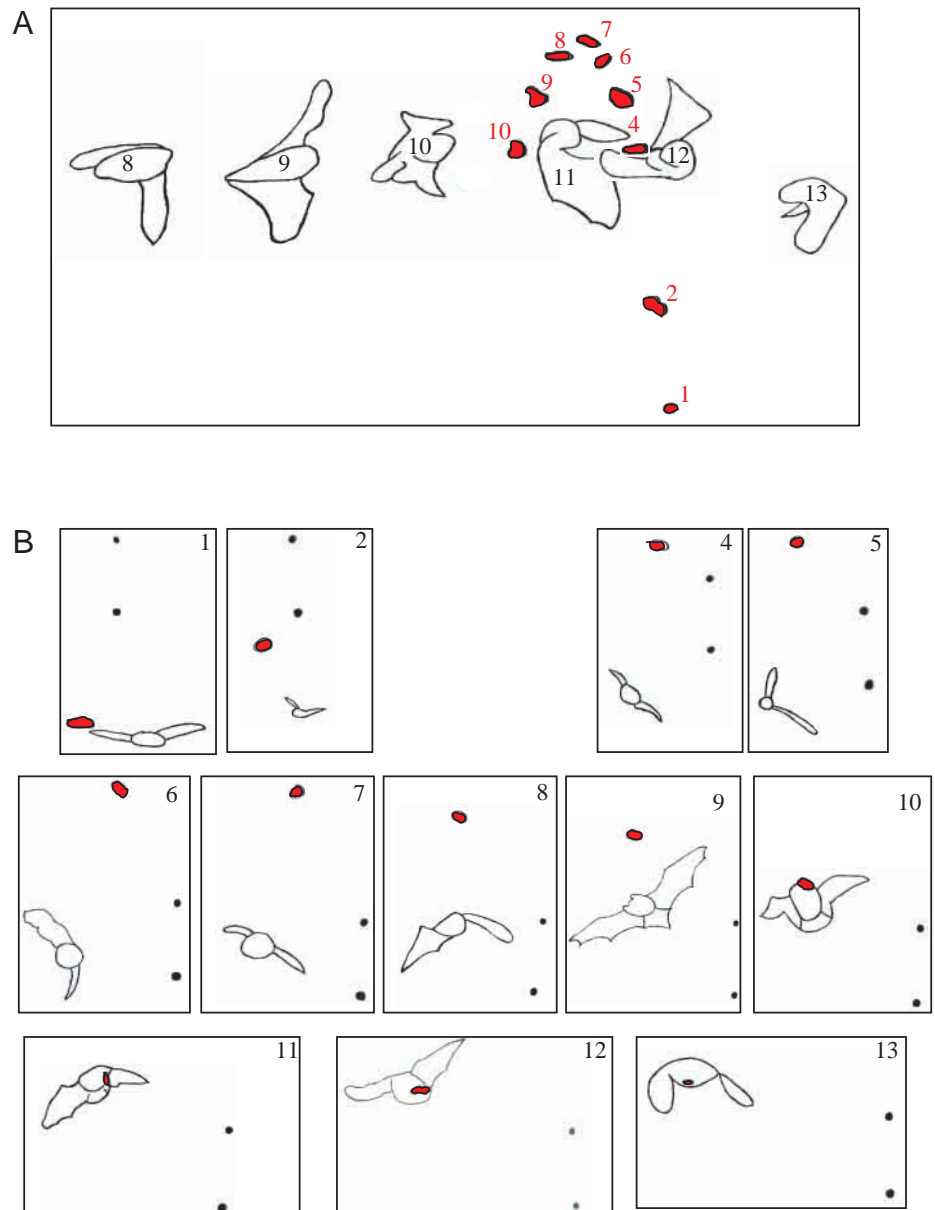


Fig. 2. Outline of bat and mealworm (red) in a successful capture attempt. (A) Superimposed successive frames (40 ms between frames) from camera B, (see Fig. 1) giving a side view. (B) Successive frames along the flight path (camera C, Fig. 1). The two black dots are reference points on the walls opposite the cameras. Flash 3 did not go off in this trial.

directly by the mouth, although the resolution and the frame rate of the video system were not sufficient to establish this unequivocally. In these cases the video showed the bat in a continuous straight flight before, during and after the capture without doing a somersault. There was only 40 ms between video-frames. Kalko (1995) gives 50 ms as the minimum duration of the capture manoeuvre. Hence, it seems unlikely that the normal wing- or tail-membrane capture could occur between two frames.

The sonar signals emitted in the laboratory during pursuit sequences consisted of search, approach, buzz and post-buzz signals, as in the field. In each trial we recorded approx. 1.5 s of sonar signals centred on the catch (Fig. 3). The search-phase signals were 3–5 ms long, of broad bandwidth, with a first harmonic sweeping down from 110 kHz to end in a short tail at 55 kHz. A second harmonic was clearly seen. The IPI was approximately 70 ms, corresponding to a PRR of 14–15 Hz. During the approach, signal duration decreased and PRR increased to approximately 40 Hz, whereas the bandwidth remained unchanged. Signals in Buzz_I and Buzz_{II} of the terminal phase differed spectrally, with Buzz_I signals being

similar to the approach-phase signals and Buzz_{II} signals characterized by a downward shift in frequency. The PRR increased gradually during Buzz_I to reach a plateau of approximately 200 Hz throughout Buzz_{II}. After a post-buzz pause the bat again produced search-like signals (Fig. 3).

Capture success and spectral parameters

The recordings included between 3 and 22 post-buzz signals per trial. We determined the relative number of signals with surplus notches fulfilling the 5 dB criterion in case of captures, touches and wides in search and post-buzz signals, respectively. The search signal had very few notches (Fig. 3). As expected, NOTCH in search signals did not correlate with the outcome of the following capture attempt (NOTCH_c=6%, NOTCH_t=4%, NOTCH_w=7%) (Table 1). Post-buzz signals in general contained more notches than search signals, no matter whether they came from capture, touch, or wide trials ($P < 0.001$, Kruskal–Wallis/Mann–Whitney-test of search signals *versus* post-buzz signals in case of all three outcomes: capture, touch and wide). However, NOTCH in post-buzz signals was much higher following a capture than a touch or

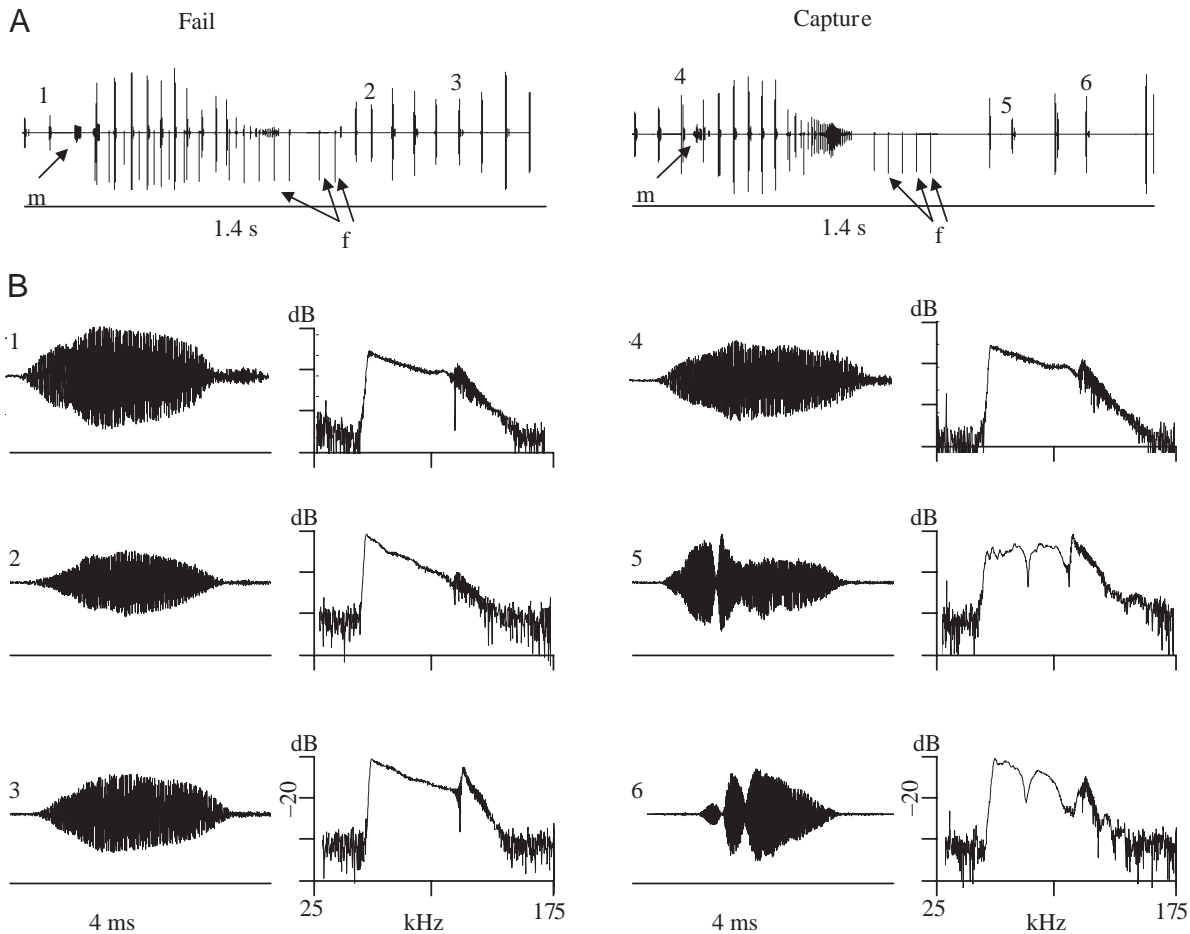


Fig. 3. (A) Overviews of the sonar signals of two trials recorded the same day from bat V. Left: fail, right: capture. (B) High-resolution oscillograms and spectra of the search and two post-buzz signals from each trial, numbered as in the overviews. Spectra of post-buzz signals following successful captures contain notches (right), which are not seen after fails (left). m, the sound of the mealworm catapult; f, some of the negative flash-sync pulses.

Table 1. NOTCH, interpause interval and post-buzz pause for post-buzz signals for all bats pooled, and for the three individual bats

	Outcome	Search (all)	Post-buzz (all)	Bat B	Bat S	Bat V
NOTCH (%)	c	6±12	68±21	66.9±19.7	65.0±20.3	70.8±22.8
	t	4±9	39±18	43.4±20.3	39.1±17.1	34.7±16.0
	w	7±16	34±20	35.7±25.4	36.2±15.5	30.6±21.0
IPI (ms)	c	67.9±8.1	84.4±19.8	83.5±20.1	84.2±17.4	85.7±21.8
	t	70.5±22.2	67.1±10.6	62.3±6.6	67.3±9.5	69.5±13.2
	w	65.8±8.9	66.1±12.8	62.3±11.3	68.2±14.0	66.3±12.1
pbP (ms)	c		273±116	241±86	220±84	357±124
	t		181±78	193±77	149±77	214±66
	w		118±71	113±40	104±84	135±69

IPI, interpause interval; pbP, post-buzz pause.

c, capture; t, touch; w, wide.

Values for search signals are also given for the pooled data.

Values are means ± S.D. *N* for individual bats: Bat B: 80 c, 16 t, 28 w; Bat S: 80 c, 31 t, 44 w; Bat V: 80 c, 24 t, 42 w; *N* for all bats: 240 c, 71 t, 114 w.

wide (NOTCH_c=67%, NOTCH_t=39%, NOTCH_w=34%, two-way ANOVA with bat number and outcome as independents; $P<0.001$) (Fig. 3). The difference was consistent for all three bats and there were no significant differences in NOTCH between bats. The difference between touches and wides was not significant (Fig. 4, Table 1).

Capture success and temporal parameters

Capture success was correlated with significant changes in IPI and pbPs in all three bats (Fig. 4, Table 1). The mean IPI was lengthened in post-buzz signals following captures. The IPI increase was significant, both compared to IPI of search signals ($P<0.001$, Kruskal–Wallis/Mann–Whitney), as well as to IPI of post-buzz signals following fails (IPI_c=84.4 ms, IPI_t=67.1 ms, IPI_w=66.1 ms; two-way ANOVA with bat number and outcome as independents; $P<0.001$). The difference was again consistent for all bats and there was no significant difference in IPI between bats. The difference between touches and wides was not significant. In search sequences (i.e. before a capture) there was, as expected, no significant difference between the three outcomes (IPI_c=67.9 ms, IPI_t=70.5 ms, IPI_w=65.8 ms) (Table 1). The IPI-distributions did not show significant differences between individual bats.

The pbP was longer following touches than wides, and even longer following captures than touches (pbP_c=272.7 ms, pbP_t=181.2 ms, pbP_w=117.9 ms). The differences were significant for the pooled data for

all bats as well as for individual data from the three bats (two-way ANOVA; $P<0.001$) (Fig. 4, Table 1). Thus, for this parameter there was a significant difference between the two unsuccessful outcomes: touch and wide. pbP was the parameter that varied most between the bats. Regardless of the outcome of the capture attempt, Bat V had significantly longer pauses ($P<0.001$) than the other bats (Table 1). However, the relative increase in pbP after touch or capture compared to wide was

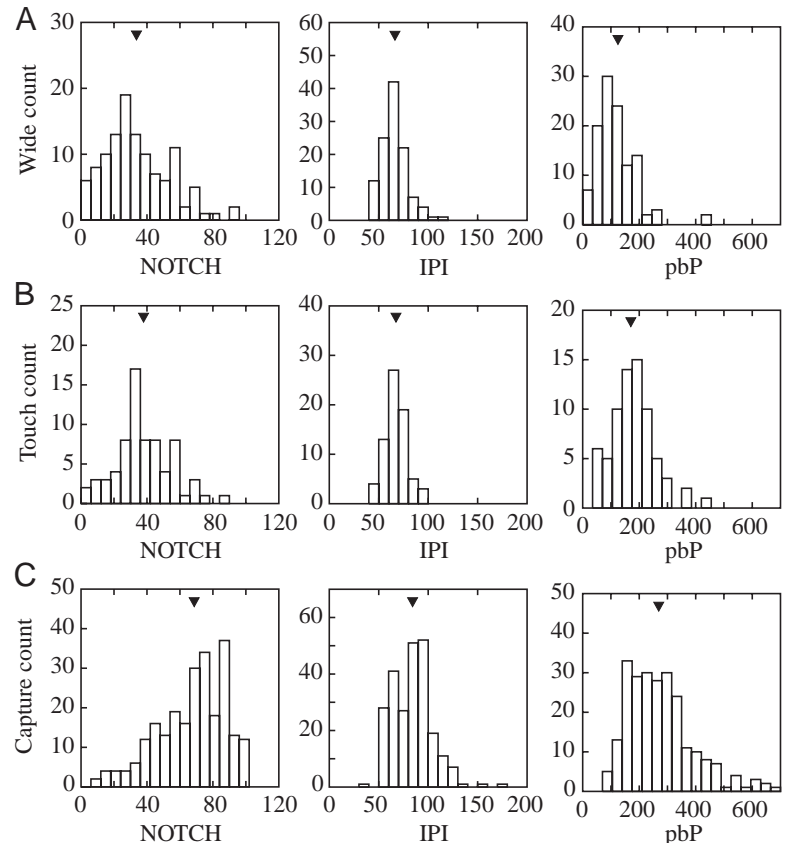


Fig. 4. Distribution of NOTCH, interpause interval (IPI) and post-buzz pause (pbP) in post-buzz signals after capture attempts. (A) Wides, (B) touches, (C) captures. Data are pooled for all bats; black triangles indicate group means.

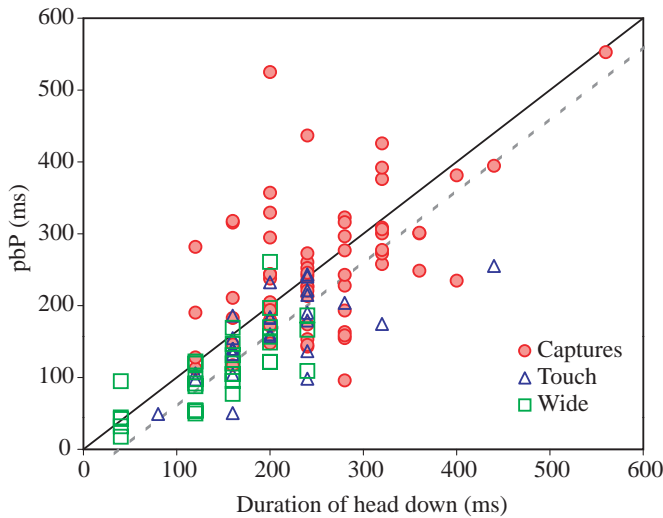


Fig. 5. Post-buzz pause (pbP) plotted as a function of the duration of the head-down stage determined from video recording of the same trial. Points above the solid diagonal line are from trials where pbP was longer than the maximum duration of the head-down stage. The broken line indicates the average duration of the head-down stage (see text for explanation).

about the same for all three bats, approximately 150% and 230%, respectively (Table 1).

We used the best video recordings to determine the length of time that the bat had its head in the tail pouch ('head-down stage'; see Kalko, 1995) by counting the number of video frames. Since each frame lasts 40 ms, three frames would correspond to a head-down stage lasting from a minimum of 80 ms to a maximum of 160 ms. The average duration of the head-down stage was determined from the medium time, i.e. three frames was counted as 120 ms. Average head-down stage in capture trials ($N=62$) lasted 214 ± 81 ms (mean \pm S.D.), and average pbP in the same trials was 255 ± 94 ms. In touches, average head-down ($N=27$) was 166 ± 71 ms, and pbP was 162 ± 56 ms. Head-down in wides ($N=31$) was 107 ± 61 ms on average, and pbP was 113 ± 54 ms.

To be on the safe side, when comparing video and sound recordings of individual trials, we used the maximum time the head-down stage could have lasted (i.e. in the example above: 160 ms for a head-down stage lasting three video frames). In a high proportion ($>40\%$) of the successful capture trials the acoustic post-buzz pauses clearly lasted longer than the maximum time the bat had its head in the tail pouch. In touch and wide trials the proportion of trials where pbP outlasted the head-down stage was smaller, 15% and 20%, respectively (Fig. 5).

Acoustic parameters unaffected by capture success

A number of acoustic characteristics of the post-buzz signals did not correlate significantly with capture success. These included temporal parameters as signal duration, and spectral parameters as maximum (F_{\max}), minimum (F_{\min}) and peak

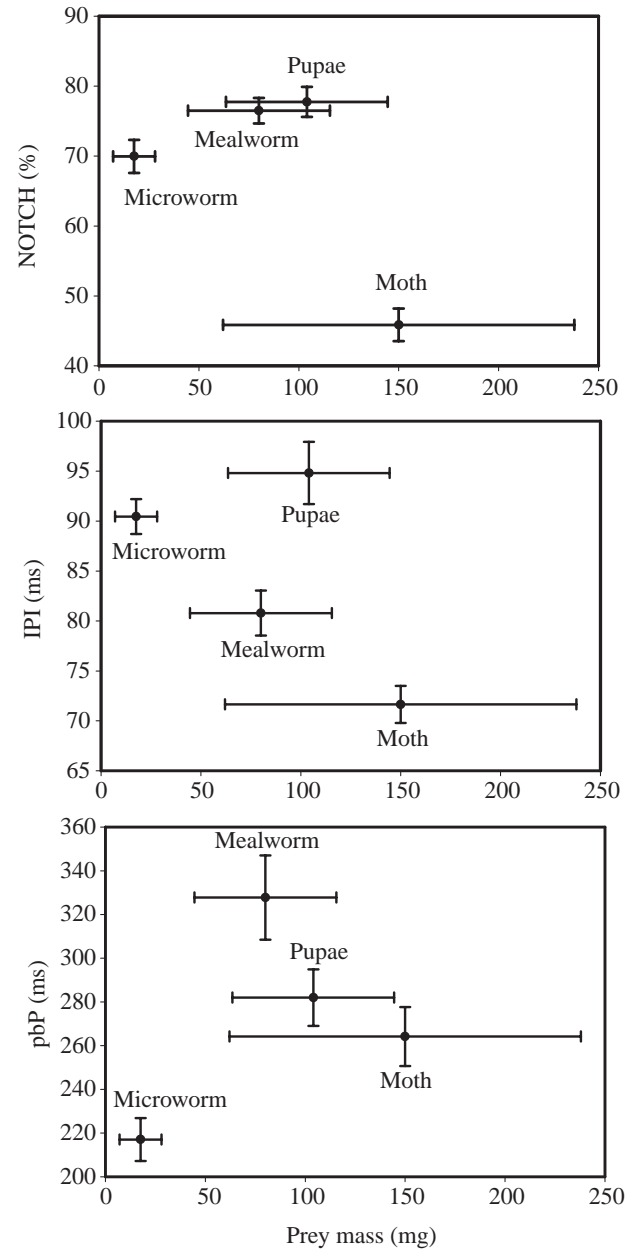


Fig. 6. Prey type effects on NOTCH, interpause interval (IPI) and post-buzz pause (pbP) of post-buzz signals following successful captures. The x-axis gives the mean mass of each prey type. Horizontal error bars indicate the size range for the prey type. Values are means \pm S.E.M. (vertical error bars). $N=60$ for all points.

(F_{peak}) frequencies of the first harmonic, $BW_{-10\text{dB}}$, and the harmonic structure of the signals.

Prey size

The prey type, but apparently not the prey size *per se*, had significant influence on IPI, pbP and NOTCH in post-buzz signals following captures (Fig. 6). After fails there were no differences. The differences were consistent for all three bats. The four types of prey affected NOTCH, IPI and pbP differently. For example, capture of microworms resulted in

long IPI and high NOTCH, but short pbP, whereas capture of mealworms gave high NOTCH and long pbP, but only intermediate IPI (Fig. 6). Neither comparison to mass (Fig. 6) nor to length revealed any obvious correlation with prey size. There was a considerable overlap between sizes of the four prey types. Therefore, in addition to comparing to the average prey size (mass or length) we also used data from 20 capture trials with each bat and each prey type to look for a correlation with size of each individual prey item. Again, no size dependence was found; all r^2 for linear correlations were below 0.3. None of the other acoustic parameters tested (duration, minimum, maximum and peak frequency, bandwidth and harmonic structure) were correlated with overall prey size. Hence, in spite of significant effects of prey type on some acoustic parameters (IPI, pbP, NOTCH), no systematic relation to size (neither mass nor length) was found within the range of prey sizes tested.

Using post-buzz signals to evaluate capture success

To test how reliably the acoustic parameters reflect the capture success, we categorized trials solely on basis of the acoustic recordings. The ease with which the two distributions (capture and no-capture) can be separated is reflected in the area below the ROC curve. The larger the area, the fewer errors will be made in discrimination if a suitable criterion is chosen. Capture success could be inferred with reasonable accuracy based on either a NOTCH, IPI or pbP criterion, as seen in the ROC curves in Fig. 7.

The actual percentage of correctly classified trials depends on the criterion. Fig. 8 shows that all three original parameters can be used in classification, with 72–78% correct classifications at the optimal criteria. The range of NOTCH criteria providing success rates above 70% was broad: from 29% to 71% notches, with a maximum of 78% correct (Fig. 8). The ranges of optimal criteria were almost the same for the three individual bats, 22–64%, 37–72% and 28–79%, respectively. The overall best criterion both for the individual bats as well as the pooled set was 50% notches.

If IPI was used to evaluate capture success, the criterion range 72–82 ms produced success rates above 70%, with a maximum of 72% correct. Thus, IPI was not as reliable a cue as NOTCH and had a more narrow range of useful criteria (Fig. 8). The three individual bats had best criterion ranges of 62–81 ms, 67–81 ms and 74–87 ms, respectively. The overall best criterion was approximately 75–78 ms IPI.

The pbP was better than IPI, but not as reliable as NOTCH. pbP criteria between 99 ms and 245 ms gave above 70% success in determining trial outcome, when used on the pooled data for all three bats (Fig. 8), with a maximum of 75%

correct. Ranges for best criteria for the individual bats were 86–219 ms, 93–192 ms and 132–338 ms, respectively. A best overall criterion is not evident, as this parameter displays the largest variation between bats. However, 160 ms seems a fair compromise.

Although all three parameters correlate well with capture success, they do not correlate with each other, except for a weak, yet significant correlation between NOTCH and IPI for captures (partial correlation: $r=0.22$, Bonferroni-corrected $P=0.002$). We thus used a principal component analysis to combine the information from the three useful parameters, NOTCH, IPI and pbP, into a single new parameter, the first canonical discriminant function, CD1 (Fig. 9). All three parameters contributed significantly, with weights of 0.629, 0.358 and 0.566 for NOTCH, IPI and pbP, respectively. This function could accommodate 98.5% of the total dispersion and is given as:

$$CD1 = 0.031 \times \text{NOTCH} + 0.021 \times \text{IPI} + 0.0055 \times \text{pbP} - 4.52,$$

where IPI and pbP are in ms. A value of CD1 was calculated for each trial and a new ROC curve could be constructed by employing 10 different criteria on the CD1 values (Figs 7, 8).

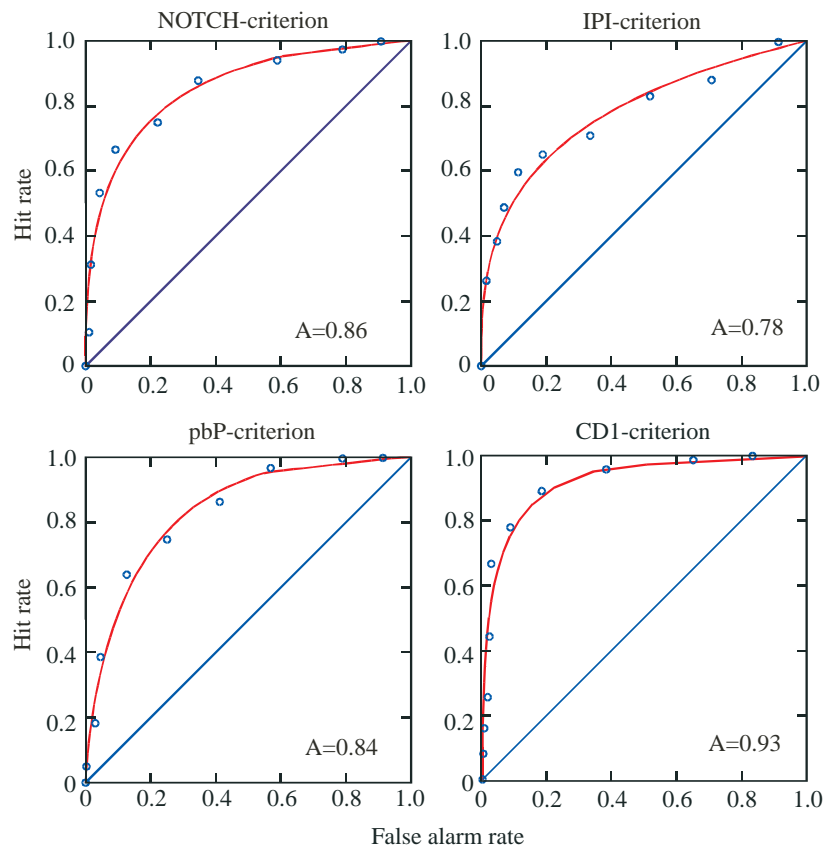


Fig. 7. Receiver operating characteristics (ROC) plots for criteria based on the three 'raw' parameters, NOTCH, IPI and pbP, and for the combined first canonical discriminant factor (CD1). For all parameters, 10 different criteria were chosen to give 10 sets of hits and false alarms to define the ROC-curve. Curves are best-fitting ROC curves assuming Gaussian distribution with unequal variance. The area, A , below the ROC curve is indicated in each panel.

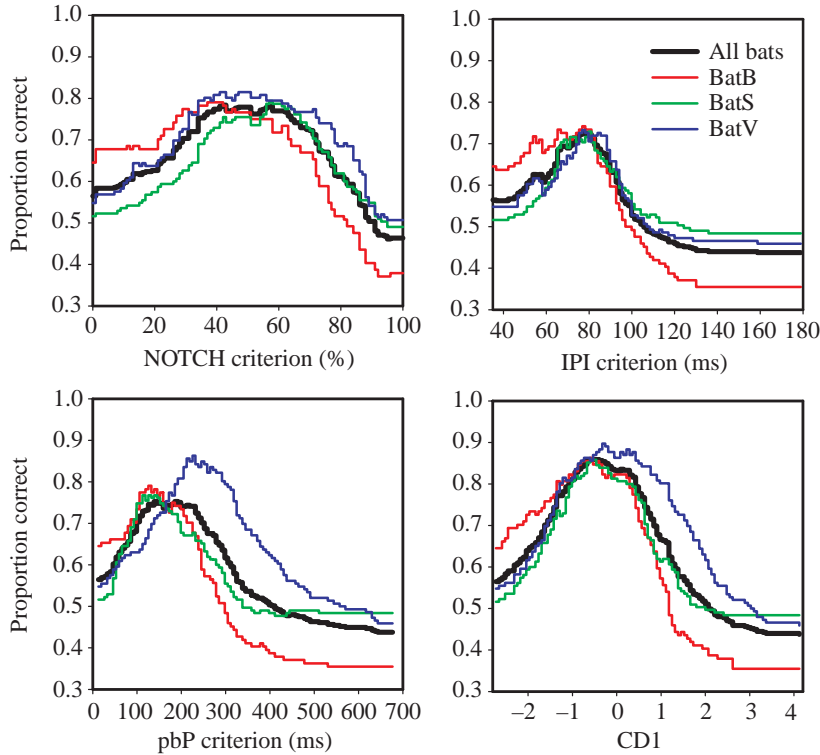


Fig. 8. Success in using sound recordings to determine the actual outcome of capture attempts as a function of criterion for all four parameters: NOTCH, interpause interval (IPI) and post-buzz pause (pbP) and first canonical discriminant function (CD1). CD1 gives a high proportion correct over a broad interval of criteria, >80% correct from approx. -1.1 to $+0.4$. Coloured curves represent individual bats; the thick black curve is the pooled data for all bats.

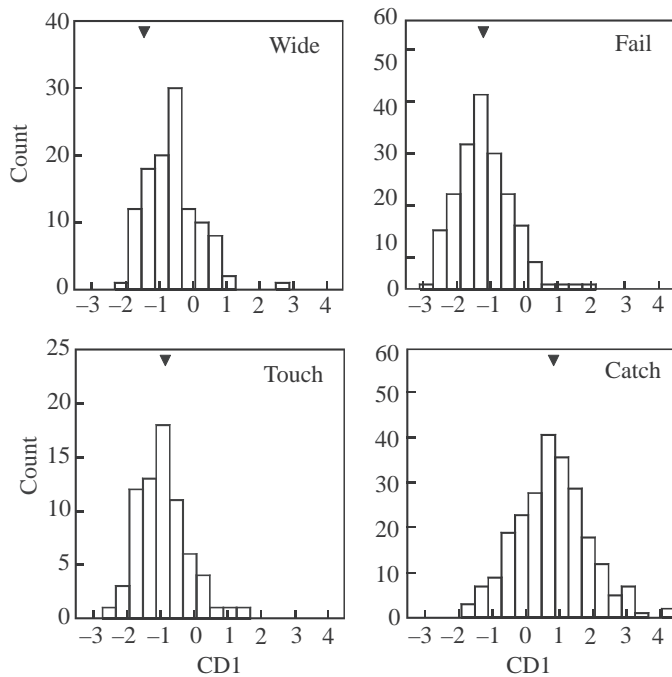


Fig. 9. Distribution of the first canonical discriminant factor (CD1) in cases of capture, touch or wide. 'Fail' shows the distribution for pooled touch and wide data. Data are pooled for all bats; black triangles indicate group means.

A substantial improvement in classification was obtained compared to either of the original parameters alone, reflected in the larger area below the curve in ROC plots (Fig. 7). CD1 criteria between -1.1 and 0.4 gave above 80% correct classification, with a maximum of 86% for the pooled data (Fig. 8). For individual bats, criterion ranges of -1.4 to 0.3 , -1.0 to 0.3 and -1.3 to 1.1 , respectively, gave above 80% correct classification. The overall best criterion was approximately -0.5 .

Field data

We analysed five pursuit sequences from Danish *P. pygmaeus* recorded in an open area where more bats were hunting in a group (courtesy of Marianne E. Jensen) (Table 2). Obviously, the field recordings are of more variable quality than laboratory recordings, not the least because of the unpredictable position of the bats relative to the microphones, and this is reflected in larger variations in the acoustic parameters. The IPI values were generally longer in the field recordings compared to our laboratory recordings, while pbP values were much shorter in the field. Britton and Jones (1999) also reported shorter pbP values in field than laboratory for *Myotis daubentonii*. In four of five sequences there was a clear increase in signals with notches (NOTCH) after the buzz compared to pre-buzz signals.

If we apply the laboratory criteria to the field recordings, we infer that none of the pursuits were successful, since all pbP values were short, as noted above, and below the criteria. However, they fell into two groups: two very short pbP values and three longer ones. Long pbP values were coupled to an increase in NOTCH in the post-buzz signals. Furthermore, the two with the longest pbP values had a large increase in IPI following the buzz, thus suggesting that at least those two were successful captures, indicating a success rate in the field data of $2/5 = 40\%$.

Discussion

Three different acoustic parameters of the post-buzz signals correlated with capture success in pipistrelle bats capturing prey on the wing in the laboratory. In addition to the two temporal parameters, IPI and pbP, we found a new spectral cue, the number of notches, which correlated strongly with capture success.

Capture behaviour

We used a laboratory set-up that allowed the bats to behave as naturally as possible. From beginning to end of a session the bats were not handled by humans. Sessions run without light indicated that the bats were neither disturbed nor assisted by

Table 2. *Acoustic characteristics of search and post-buzz signals for five recordings of P. pygmaeus flying in an open area in Denmark*

	NOTCH (%)		IPI (ms)		pbP (ms)	N	
	S	PB	S	PB		S	PB
pyD-1	0	29	60	96	104	9	7
pyD-2	0	33	74	130	124	4	3
pyD-3	75	0	86	85	21	5	4
pyD-4	0	33	82	48	86	5	6
pyD-5	0	33	95	86	29	5	9

IPI, interpulse interval; pbP, post-buzz pause; S, search; PB, post-buzz.

the light when hunting. The set-up provided some clutter, but probably not much more than a pipistrelle bat might encounter in the field when it hunts close to ground and vegetation (Kalko, 1995; Schnitzler and Kalko, 1998). Prey capture by the bats was probably facilitated by the fact that prey items always occurred at the same general area in the room and were catapulted in a predictable vertical arc rather than the fluttering flight path of most insect prey. Added to this was an acoustic cue from the release of the catapult. In spite of these unnatural circumstances, however, the capture technique closely resembled those described previously from field studies (Kalko, 1995).

It has been speculated that the post-buzz pause could be used as a rough measure of the time taken to retrieve the prey from the tail pouch (Schnitzler et al., 1987). Kalko and Schnitzler (1989a) suggested that the duration of the post-buzz pause corresponded to the 'head-down' stage in *Myotis daubentonii*, and Kalko (1995) proposed the same hypothesis for three species of pipistrelle bats from her field recordings. However, our results showed many examples where pbP was clearly longer than the head-down stage, especially after successful captures, where more than 40% of the trials had pbP values outlasting the head-down stage. The pause thus represents a true pause in signal emission, and not just a 'muffling' of the sounds by the tail membrane.

Temporal cues of post-buzz signals

We found that the pulse interval was significantly longer after captures than after fails, thus corroborating the general result of Britton and Jones (1999). However, Britton and Jones found that *Myotis daubentonii* doubled the interpulse intervals (IPI) after successful captures. They recorded chewing sounds and suggested that chewing replaced every second echolocation signal. Our results do not support a similar explanation for *P. pygmaeus*, since the increase in average IPI was only approximately 25%, and was due to a combination of occasional skipping of a signal and a true elongation of interval between pulses (see Fig. 3). In fact, Britton and Jones' own field data also failed to show as large an increase in IPI as found in the laboratory. Since signal emission seems closely

related to wing beat rate in all bats studied (Kalko, 1994; Wong and Waters, 2001), the implication of the reduced pulse repetition rate is that they also beat their wings at a slower rate. Our video frame-rate was not sufficiently fast to establish whether this was the case, but it was our distinct impression that the flight speed was slower after captures. This should be assessed in future studies. The flexibility of bats in adapting to different habitat conditions, including the laboratory (Surlykke and Moss, 2000; Obrist, 1995), makes it likely that bats have a fairly wide range of wing beat rates and concomitant pulse emission rates.

The post-buzz pause following captures lasted significantly longer than following touches, hence confirming the findings (laboratory, but not field) of Britton and Jones (1999) for *Myotis daubentonii* and of Acharya and Fenton (1992) for *Lasiurus borealis* and *L. cinereus* in the field. However, the variation in pbP values between the bats was quite substantial. Discrimination between captures and fails (including both wides and touches) is further complicated by the significant increase after touches, which probably also partly explains why Britton and Jones (1999) reported high variability of pbP after unsuccessful capture attempts in the laboratory and found no significant difference in the field. Individual variation does not explain the large overlap between capture and fail distributions as suggested by Acharya and Fenton (1992). Even if we restrict our analysis to data from only one bat feeding on one prey type, there is still no unambiguous threshold value. Thus, although the results show that pbP is increased in case of a successful capture, neither our data, nor any of the previous studies, indicate that it is possible to define an absolute threshold value for any bat species that reliably can discriminate captures from fails (as for example the 100 ms suggested for *Pipistrellus kuhli* in the field; Schnitzler et al., 1987).

It is interesting that pbP increases by approximately 50% after touches. This may indicate that the mere touching of a prey initiates a fixed vocal (and behavioural) motor pattern – or parts of it – which the bat goes through even when it does not seize the prey.

Spectral cues of post-buzz signals

We found no differences in F_{\min} , F_{\max} or F_{peak} between captures, touches and wides, and hence could not confirm the increase in F_{\min} found in laboratory recordings of *Myotis daubentonii* (Britton and Jones, 1999). Furthermore, none of these frequency parameters were correlated with the length or mass of the prey, suggesting that even the biggest prey (within the range tested) does not influence the frequency range of the sonar signal in pipistrelle bats.

Although the overall bandwidth, F_{\min} and F_{\max} were not affected by capture success, the occurrence of excess notches after captures shows that a successful catch does affect the spectrum of the signals. Surplus notches are seen in signals recorded from bats hunting close over water (e.g. *Myotis daubentonii*). These notches are due to the interference between the directly recorded signal and the signal reflected

from the water surface, with notch frequency determined by the delay of the reflected signal (Kalko and Schnitzler, 1989b). In our set-up the general increase in number of notches in post-buzz signals compared to search signals can probably also be explained by interference with reflections from objects in the flight room. In the post-buzz phase the bats were beyond the capture area and thus closer to the microphone and video set-up and concrete pillars at the end of the flight track. However, a more interesting change in notch occurrence is seen in post-buzz signals following captures compared to fails (touches and wides). It seems likely that a relatively large prey in the mouth will act as a frequency filter, changing the outgoing signal, either directly or by affecting the sound-producing mechanisms. The NOTCH effect serves as a reliable clue such that the outcome of a trial attempt can be determined with approximately 75% success using a NOTCH criterion alone.

Conclusions

The laboratory recordings revealed that three acoustic parameters in post-buzz signals depended on capture success. Their correlation with success was so strong that evaluations of the bats' success based only on those acoustic parameters gave correct classification of trials in capture/fail in up to 85% of the cases, when all three parameters were combined in a first canonical discriminant factor. However, these are laboratory results and comparative acoustic studies of bats in the laboratory and field do show substantial differences (Britton and Jones, 1999; Surlykke and Moss, 2000). Thus, one should be cautious when applying the laboratory results to field data. Not only are acoustics different and more complicated in the field, but the bats themselves produce different signals in the confined laboratory space compared to the field (Surlykke et al., 1993). Bats can learn to predict the trajectory of catapulted food (Miller and Olesen, 1979). Besides, the prey we offered in the laboratory was of bigger size than most of the natural prey of pipistrelle bats (Swift and Racey, 1985; Barlow, 1997), and none of the laboratory prey items could perform evasive manoeuvres.

The high level of control in the laboratory revealed results that would be very difficult to determine in field studies, for example the increased post-buzz pause after touches or the fact that the post-buzz pause often outlasts the head-down stage substantially. The carefully controlled laboratory situation enabled the formation of the hypothesis, but laboratory recordings alone, and of only three bats, are not sufficient to determine classification criteria for field recordings. Before applying this acoustical method to field studies, it requires a substantial basis of good recordings, preferably from different types of hunting areas, to adjust the criteria to the particular recording situation.

Nevertheless, whilst noting those reservations, our laboratory results are quite clear-cut for the three bats and the types and sizes of prey. Furthermore, the acoustic capture behaviour of the bats in the laboratory resembles that in the field. Thus, we believe it is likely that evaluation of capture

success based on similar acoustic methods will be possible in future field studies.

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References

- Acharya, L. and Fenton, M. B. (1992). Echolocation behaviour of vespertilionid bats (*Lasiurus cinereus* and *Lasiurus borealis*) attacking airborne targets including arctiid moths. *Can. J. Zool.* **70**, 1292-1298.
- Acharya, L. and Fenton, M. B. (1999). Bat attacks and moth defensive behaviour around street lights. *Can. J. Zool.* **77**, 27-33.
- Andersen, B. B. and Miller, L. A. (1977). A portable ultrasonic detection system for recording bat cries in the field. *J. Mamm.* **58**, 226-229.
- Barlow, K. E. (1997). The diets of two phonic types of the bat *Pipistrellus pipistrellus* in Britain. *J. Zool., Lond.* **243**, 597-609.
- Britton, A. R. C. and Jones, G. (1999). Echolocation behaviour and prey-capture success in foraging bats: laboratory and field experiments on *Myotis daubentonii*. *J. Exp. Biol.* **202**, 1793-1801.
- Green, D. M. and Swets, J. A. (1966). *Signal Detection Theory and Psychophysics*. New York: Wiley.
- Griffin, D. R. (1958). *Listening in the Dark*, 2nd edition. New York: Yale University Press; 2nd edition 1986, Cornell University.
- Jensen, M. E., Miller, L. A. and Rydell, J. (2001). Detection of prey in a cluttered environment by the northern bat *Eptesicus nilssonii*. *J. Exp. Biol.* **204**, 199-208.
- Jones, G. and Barratt, E. M. (1999). *Vespertilio pipistrellus* Schreber, 1774 and *V. pygmaeus* Leach, 1825 (currently *Pipistrellus pipistrellus* and *P. pygmaeus*; Mammalia, Chiroptera): proposed designation of neotypes. *Bull. Zool. Nomen.* **56**, 182-186.
- Kalko, E. K. V. (1994). Coupling of sound emission and wingbeat in naturally foraging European pipistrelle bats (Microchiroptera: Vespertilionidae). *Folia Zool.* **43**, 363-376.
- Kalko, E. K. V. (1995). Insect pursuit, prey capture and echolocation in pipistrelle bats (Microchiroptera). *Anim. Behav.* **50**, 861-880.
- Kalko, E. K. V. and Schnitzler, H.-U. (1989a). The echolocation and hunting behavior of Daubenton's bat, *Myotis daubentoni*. *Behav. Ecol. Sociobiol.* **24**, 225-238.
- Kalko, E. K. V. and Schnitzler, H.-U. (1989b). Two-wave-front interference patterns in frequency-modulated echolocation signals of bats flying low over water. *J. Acoust. Soc. Am.* **85**, 961-962.
- Miller, L. A. and Olesen, J. (1979). Avoidance behavior in green lacewings. *J. Comp. Physiol. A* **131**, 113-120.
- Neuweiler, G. (1989). Foraging ecology and audition in echolocating bats. *Trends Ecol. Evol.* **4**, 160-166.
- Obrist, M. K. (1995). Flexible bat echolocation: the influence of individual, habitat and conspecifics on sonar signal design. *Behav. Ecol. Sociobiol.* **36**, 207-219.
- Ohl, F. W., Scheich, H. and Freeman, W. J. (2001). Change in pattern of ongoing cortical activity with auditory category learning. *Nature* **412**, 733-736.
- Racey, P. A. and Swift, S. M. (1985). Feeding ecology of *Pipistrellus pipistrellus* (Chiroptera: Vespertilionidae) during pregnancy and lactation. I. Foraging behaviour. *J. Anim. Ecol.* **54**, 205-215.
- Rydell, J. (1992). Exploitation of insects around streetlamps by bats in Sweden. *Funct. Ecol.* **6**, 744-750.
- Rydell, J. (1998). Bat defence in lekking ghost swifts (*Hepialus humuli*), a moth without ultrasonic hearing. *Proc. R. Soc. Lond. B* **265**, 1373-1376.
- Scheich, H., Baumgart, F., Gaschler-Markefski, B., Tegeler, C., Tempelmann, C., Heinze, H. J., Schindler, F. and Stiller, D. (1998). Functional magnetic resonance imaging of a human auditory cortex area involved in foreground-background decomposition. *Eur. J. Neurosci.* **10**, 803-809.
- Schnitzler, H.-U. and Kalko, E. K. V. (1998). How echolocating bats search and find food. In *Bat. Biology and Conservation* (ed. T. H. Kunz and P. A. Racey), pp. 183-196. Washington, London: Smithsonian Institution Press.

- Schnitzler, H.-U., Kalko, E. K. V., Miller, L. A. and Surlykke, A.** (1987). The echolocation and hunting behavior of the bat, *Pipistrellus kuhli*. *J. Comp. Physiol. A* **161**, 267-274.
- Simmons, J. A., Fenton, M. B. and O'Farrell, M. J.** (1979). Echolocation and pursuit of prey by bats. *Science* **203**, 16-21.
- Surlykke, A., Miller, L. A., Möhl, B., Andersen, B. B., Christensen-Dalsgaard, J. and Jørgensen, M. B.** (1993). Echolocation in two very small bats from Thailand: *Craseonycteris thonglongyai* and *Myotis siligorensis*. *Behav. Ecol. Sociobiol.* **33**, 1-12.
- Surlykke, A. and Moss, C. F.** (2000). Echolocation behavior of big brown bats, *Eptesicus fuscus*, in the field and the laboratory. *J. Acoust. Soc. Am.* **108**, 2419-2429.
- Swift, S. M. and Racey, P. A.** (1985). Feeding ecology of *Pipistrellus pipistrellus* (Chiroptera: Vespertilionidae) during pregnancy and lactation. II. Diet. *J. Anim. Ecol.* **54**, 217-225.
- Tougaard, J.** (1999). Receiver operating characteristics and temporal integration in an insect auditory receptor cell. *J. Acoust. Soc. Am.* **106**, 3711-3718.
- Vaughan, N., Jones, G. and Harris, S.** (1997). Habitat use by bats (Chiroptera) assessed by means of a broad-band acoustic method. *J. Appl. Ecol.* **34**, 716-730.
- Wong, J. G. and Waters, D.** (2001). The synchronisation of signal emission with wingbeat during the approach phase in soprano pipistrelles (*Pipistrellus pygmaeus*). *J. Exp. Biol.* **204**, 575-583.