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RESEARCH ARTICLE

Fine-tuned echolocation and capture-flight of *Myotis capaccinii* when facing different-sized insect and fish prey

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ABSTRACT

Formerly thought to be a strictly insectivorous trawling bat, recent studies have shown that *Myotis capaccinii* also preys on fish. To determine whether differences exist in bat flight behaviour, prey handling and echolocation characteristics when catching fish and insects of different size, we conducted a field experiment focused on the last stage of prey capture. We used synchronized video and ultrasound recordings to measure several flight and dip features as well as echolocation characteristics, focusing on terminal buzz phase I, characterized by a call rate exceeding 100 Hz, and buzz phase II, characterized by a drop in the fundamental well below 20 kHz and a repetition rate exceeding 150 Hz. When capturing insects, bats used both parts of the terminal phase to the same extent, and performed short and superficial drags on the water surface. In contrast, when preying on fish, buzz I was longer and buzz II shorter, and the bats made longer and deeper dips. These variations suggest that lengthening buzz I and shortening buzz II when fishing is beneficial, probably because buzz I gives better discrimination ability and the broader sonar beam provided by buzz II is useless when no evasive flight of the prey is expected. Additionally, bats continued emitting calls beyond the theoretical signal-overlap zone, suggesting that they might obtain information even when they have surpassed that threshold, at least initially. This study shows that *M. capaccinii* can regulate the temporal components of its feeding buzzes and modify prey capture technique according to the target.

KEY WORDS: Long-fingered bat, Feedback control, Feeding buzz, Field experiment, Fishing behaviour, Signal-overlap zone

INTRODUCTION

The fundamental trophic niche of an organism is shaped by morphological and physiological constraints (Mayr, 1976). Yet even within these constraints, organisms rarely exploit the full trophic spectrum they are morphophysiologically able to consume because of limitations imposed by local forces extrinsic to the individual, such as food availability, competition and cost/efficiency trade-offs (Hamel et al., 2013; e.g. Heg and van der Velde, 2001; Salsamendi et al., 2012). The appearance of novel resources, however, can trigger an expansion of the trophic niche. Such changes can occur, for instance, with the introduction of alien species (Maerz et al., 2005; Pearson et al., 2000), or with the expansion of populations into new environments with different available food resources (Cucherousset et al., 2012; Michaud et al., 2008). Exploiting new resources commonly requires modifications to enhance capture or consumption efficiency, and behavioural variations in foraging or handling methods can result in a more efficient search, capture or acquisition of novel prey (e.g. Beissinger et al., 1994).

Even though the diet of bats that forage in aquatic environments is usually based on insects (Almenar et al., 2008), a few species are known to have made a further step towards the consumption of fish. Fishing is thought to have begun as a modification of the insect hunting technique known as trawling, which consists of gaffing floating or emerging insects from the water surface using the hind feet. *Noctilio leporinus* and *Myotis vivesi* are primarily piscivorous (e.g. Blood and Clark, 1998; Bordignon, 2006), while several other species consume fish as an occasional dietary component (e.g. Fenton, 1990; Gudger, 1943; Law and Urquhart, 2000; Ma et al., 2003; Whitaker and Findley, 1980). The long-fingered bat [*Myotis capaccinii* (Bonaparte 1837)] is the only European species known to fish, as despite being primarily insectivorous (Almenar et al., 2008), fishing behaviour has been reported for three isolated colonies in the Mediterranean basin (Aihartza et al., 2003; Biscardi et al., 2007; Levin et al., 2006).

There is a substantial leap from insect hunting to fishing. The net energetic profit obtained from fish is much higher compared with that obtained from insects, but also fishing requires considerable modifications to detect, identify, capture and handle the vertebrate prey (Schnitzler et al., 1994). Because insects are captured above water, trawling bats rely on echolocation for obtaining all the information they need to catch them (Siemers et al., 2001). Fish, in contrast, move under or at the water surface, where they may only be partially detectable by echolocation. The size and mass difference between insects and fish is also considerable. For example, chironomids, the staple food of *M. capaccinii* (Almenar et al., 2008), rarely exceed 10 mm in size and 5 mg in mass, while the average size and mass of the fish consumed by this bat has been estimated to be nearly three times longer and 50-fold heavier (Aizpurua et al., 2013). These extreme differences clearly affect the energetics of both hunting and prey consumption.

Considering the differences between different-sized insects and fish, we hypothesized that bats must use different detection, capture and handling techniques, which could entail changes in the echolocation and the attack-flight patterns. In this study we performed a field experiment with wild bats to test: (1) whether adjustments in the echolocation calls occur because of the different detectability and behaviour of the two prey types, (2) whether fishing elicits a different type of dip to catch fish due to the location uncertainty that entails a submerged prey, and (3) whether flight speed varies depending on the intensity of the drag to compensate the expected loss of kinetic energy produced by the friction with water. The results of this field experiment extend our understanding of the echolocating bat's sensorial and motor plasticity, providing insights into the processes of cognitive and behavioural adaptation to novel resources allowing the expansion of the ecological niche.

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RESULTS

Long-fingered bats began their activity after dusk and showed variable activity peaks. Despite the large size of the ponds, bats were commonly observed hunting close to shore (1–4 m) and making long series of large circular flights skimming the water surface, dipping regularly into the water during each flight circle.

Echolocation

Myotis capaccinii produced downward frequency-modulated signals, the first harmonic being much more prominent than the second. When hunting insects, all recordings showed a terminal phase (large insects n=29; small insects n=53), which included the two typical parts, buzz I and buzz II (Fig. 1A–C). In contrast, 5.7% of the fishing recordings lacked buzz II (fish n=53; Fig. 1D).

The flight speed during the terminal phase of the attack was different with the three prey types (see below), producing variation in the time–distance relationship of attacks. The terminal phase's start and end times in relation to the moment of prey contact also differed among prey types (start: ANOVA, Welch's $F_{2,134}$=30.68, P≤0.001; end: ANOVA, Welch's $F_{2,134}$=38.20, P≤0.001). The terminal phase started the earliest during attacks on large insects (−241.06±32.46 ms), 26 and 52 ms earlier than for fish and small insects, respectively (Table 1). However, the end of the phase was similar when aiming for fish (−40.10±9.40 ms) and large insects (−42.15±14.29 ms), but delayed when attacking small insects (−28.84±4.71 ms; Table 1). When the start and end times were calculated in relation to the moment of feet insertion into the water, the differences between small insects and fish disappeared (start: Games–Howell, P=0.101; end: Mann–Whitney, P=1.000), and the terminal phase when attacking both prey types began and finished later than when targeting large insects (start for both prey: Games–Howell, P<0.001; end for both prey: Mann–Whitney, P<0.001; Table 1, Fig. 2). In all cases, bats finished emitting echolocation calls before inserting their feet into the water.

Regarding distance covered during an attack, the beginning of the terminal phase could not be analysed because it was out of the frame. Depending on the reference point used, the terminal phase ended at different distances among targets (Fig. 2). The end occurred farther away from the prey contact moment when fishing (12.33±5.56 cm) than when hunting insects, regardless of their size (small: 6.40±2.43 cm; large: 7.72±2.36 cm; Games–Howell, P≤0.05). In contrast, bats ended their terminal phase farther away from the feet insertion point when capturing large insects (5.46±2.63 cm) than when capturing small insects (2.82±2.46 cm; Games–Howell, P=0.044), while the distance was intermediate and not statistically different when fishing (3.24±4.34 cm; Games–Howell, P>0.05). The duration of the last pulse was 0.99 ms with fish, 1.0 ms with large insects and 1.1 ms with small insects, which allowed us to calculate that the echo overlap zone started at 17, 17.1 and 18.9 cm from the prey, respectively. Thus, bats kept echolocating in the signal-overlap zone for 13.3 ms when fishing, 28.2 ms when hunting small insects and 25.8 ms when hunting large insects.

The total duration and number of pulses emitted during the terminal phase were higher when capturing large insects than when attacking other targets (total duration: Games–Howell, P≤0.01; total number of pulses: Tukey, P≤0.01; Table 1, Fig. 3A,B). Both parts of the terminal phase were also statistically different between targets (buzz I: ANOVA, Welch's $F_{2,134}$=13.38, P≤0.001; buzz II: ANOVA, $F_{2,134}$=53.85, P≤0.001): longer buzz I calls were recorded with large insects and fish compared with small insects (Games–Howell, P≤0.01), while the longest buzz II calls were produced when preying on large insects (65.3±14.1 ms), followed by small insects (50.8±11.9 ms) and fish (33.8±14.7 ms; Tukey, P≤0.001; Table 1). The percentage of pulses in buzz I and II differed between insects (small and large) and fish (Welch's F=35.76, P≤0.001, for both buzz parts),

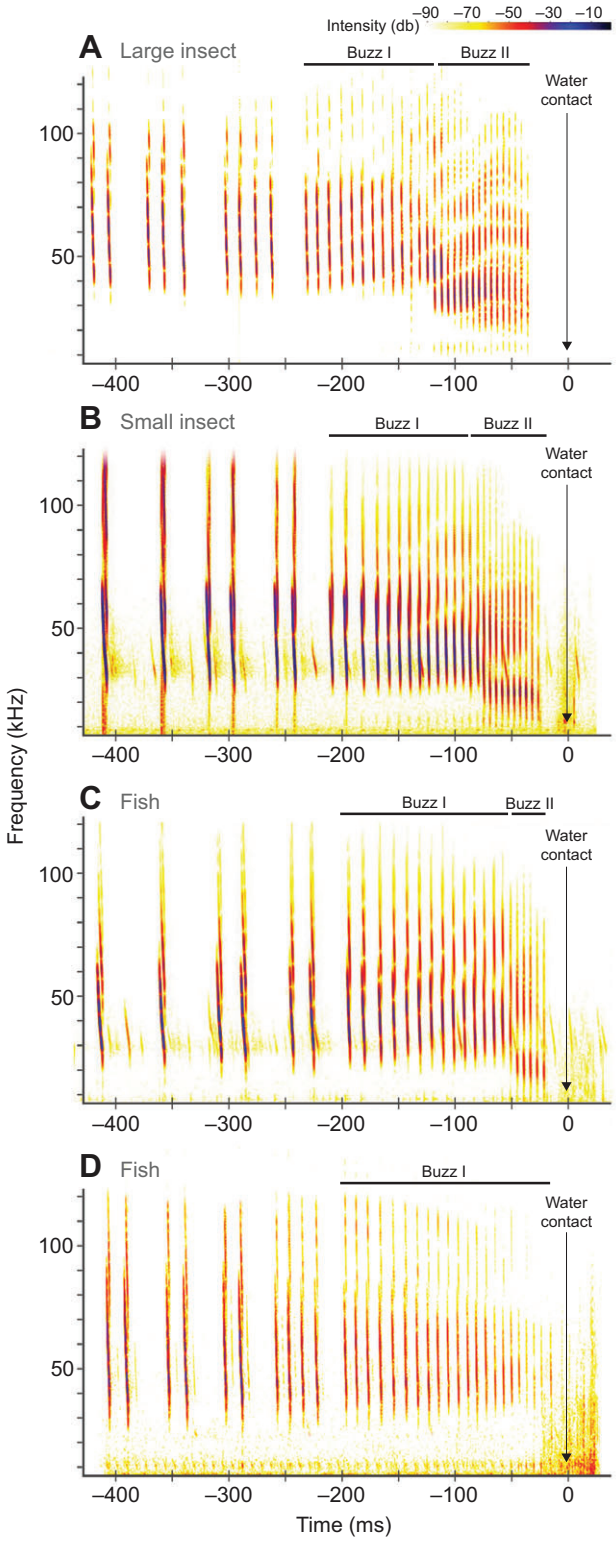

Fig. 1. Sonograms of feeding buzzes emitted by *Myotis capaccinii* during hunting attempts upon three prey types. (A) Large insects; (B) small insects; (C,D) fish. Sonograms are aligned using the water contact moment as reference. Note that in the last sonogram (D) the second buzz is missing.

The Journal of Experimental Biology (2014) doi:10.1242/jeb.104992

Table 1. Measurements of terminal phase, dip and flight speed features of capture attempts by *Myotis capaccinii* according to prey type (large insects, small insects and fish)

	Large insects	Small insects	Fish
Terminal phase			
Total number of pulses	27±3.22[a]	22±3.04[b]	22±4.24[b]
Total duration (ms)	198.72±33.63[a]	160.57±25.87[b]	173.05±39.12[b]
Ref. 1 start (ms)	–241.06±32.46[a]	–189.01±23.35[b]	–215.00±41.20[c]
End (ms)	–42.15±14.29[a]	–28.84±4.71[b]	–40.10±9.40[a]
End (cm)	7.72±2.36[a]	6.40±2.43[a]	12.33±5.56[b]
Ref. 2 start (ms)	–237.64±32.65[a]	–180.62±24.32[b]	–194.16±41.50[b]
End (ms)	–37.44±13.42[a]	–20.46±5.90[b]	–19.63±10.76[b]
End (cm)	5.46±2.63[a]	2.82±2.46[b]	3.24±4.34[b]
Buzz I number of pulses	14±3.30[a]	12±1.84[b]	15±3.34[a]
Buzz II number of pulses	13±2.49[a]	10±2.14[b]	7±2.94[c]
Buzz I duration (ms)	128.63±35.43[a]	105.72±22.60[b]	135.29±39.74[a]
Buzz II duration (ms)	65.34±14.09[a]	50.79±11.88[b]	33.82±14.71[c]
Buzz I number of pulses (%)	51.70±9.04[a]	54.16±5.71[a]	69.76±12.68[b]
Buzz II number of pulses (%)	48.30±9.04[a]	45.84±5.71[a]	30.24±12.68[b]
Dip			
Total duration (ms)	10.67±11.06[a]	19.29±6.25[a]	40.82±20.28[b]
Before prey contact (ms)	5.21±7.20[a]	8.47±4.75[a]	21.10±13.64[b]
After prey contact (ms)	5.46±5.38[a]	10.82±3.94[b]	19.72±9.98[c]
Total distance (cm)	2.59±2.57[a]	7.31±2.13[b]	16.52±5.83[c]
Before prey contact (cm)	2.06±2.01[a]	3.58±2.11[a]	8.52±3.54[b]
After prey contact (cm)	0.50±0.62[a]	3.73±1.26[b]	8.00±3.49[c]
Flight speed (m s^{-1})			
Pre-dip	3.32±0.39[a]	4.11±0.54[b]	4.91±0.99[c]
Post-dip	2.92±0.29[a]	3.95±0.52[b]	3.92±0.72[b]
Speed loss	0.39±0.34[a]	0.16±0.24[a]	0.99±0.90[b]

Ref. 1, the reference point is the moment of contact between the bats' feet and the prey; Ref. 2, the reference point is the moment of contact between the bats' feet and the water. Different superscripted letters following the values indicate statistically significant differences between prey types (see Results). Results are presented as means ± s.d.

being more balanced in both classes of insects and higher in buzz I (70%) than in buzz II (30%) in fish (Fig. 1). Moreover, a short buzz II phase – with eight or fewer pulses (mode value in fishing) – was used in 73.3% of the fishing attempts, while this percentage was reduced to 20 and 0% with small and large insects, respectively.

Dip and flight

Bats touched the water with their hind feet in 55% of the attacks on large insects (n=29), whereas when targeting small insects and fish, 100% of the attempts involved contact with the water in some way (small insects n=53; fish n=53). The mean duration (40.8±20.3 ms) and distance of the dip (8.5±3.5 cm) when fishing was twice that used for catching insects (Kruskal–Wallis, $H_{2,134}$=43.54, P≤0.001; Table 1, Fig. 2, Fig. 3C,D; see supplementary material Movie 1). Although dip duration was not significantly different between different-sized insects (Mann–Whitney, P>0.017), distance of the dip was greater in small insects (Games–Howell, P≤0.001). Moreover, the duration and distance of before-prey-contact dip were similar between insects (duration: Games–Howell, P=0.086; distance: Games–Howell, P=0.164), whereas the after-prey-contact dip duration and distance were different (duration, Mann–Whitney, P<0.017; distance, Games–Howell, P≤0.001). The duration of the before-prey-contact dip was correlated with the end time of the feeding buzz (Pearson, r=–0.56, P≤0.001), so that the sooner the dip began the sooner the feeding buzz ended.

Additionally, bats inserted their feet into the water deeper when fishing than when capturing insects (χ^2_2=26.04, P≤0.01), whereby the uropatagium entered the water only during fishing attempts (33% of attempts). Bats aiming at fish submerged more than half of their hind feet in 66.9% of events and their entire hind feet in 16.7% of cases. In these attempts, bats submerged their hind feet into the water in a rough manner and the splashes produced were audible to the human ear. In contrast, when capturing large and small insects, bats only inserted their feet in 6.2 and 22.9% of the cases, respectively, and never submerged more than half of the foot (see supplementary material Movie 1). Moreover, splashes were rarely heard.

The pre-dip flight speed was different depending on prey type (ANOVA, Welch's $F_{2,47}$=21.37, P≤0.001). The fastest flight speed was recorded when catching fish (4.91±0.99 m s^{-1}), followed by small insects (4.11±0.54 m s^{-1}) and large insects (3.32±0.39 m s^{-1}; Table 1, Fig. 3E). Flight speed slowed down after performing the dip when catching fish (Student's t-test, $t_{1,28}$=3.52, P=0.001) and large insects (Student's t-test, $t_{1,18}$=2.54, P=0.021), but it did not change when hunting small insects (Student's t-test, $t_{1,28}$=0.92, P=0.364). Fish-catching was the action producing the greatest speed losses (0.99±0.90 m s^{-1}). Post-dip speed was similar between small insect and fish captures (Games–Howell, P=0.989), while attempts to catch large insects had lower post-dip speed values (Games–Howell, P≤0.001; Table 1).

After capture, bats transferred the prey to their mouth. During this movement, the uropatagium was spread out, spanning the gap between the feet. In some cases, when bats failed in their fishing attempt, they performed figure-eight flights [similar to those described in captivity by Aihartza et al. (Aihartza et al., 2008)], aiming for the same target over and over again. Different individuals were also observed subsequently trying to capture, sometimes frantically, the same target using figure-eight flights.

DISCUSSION

Myotis capaccinii attacks on different prey items differed significantly in terms of flight speed, prey localisation by

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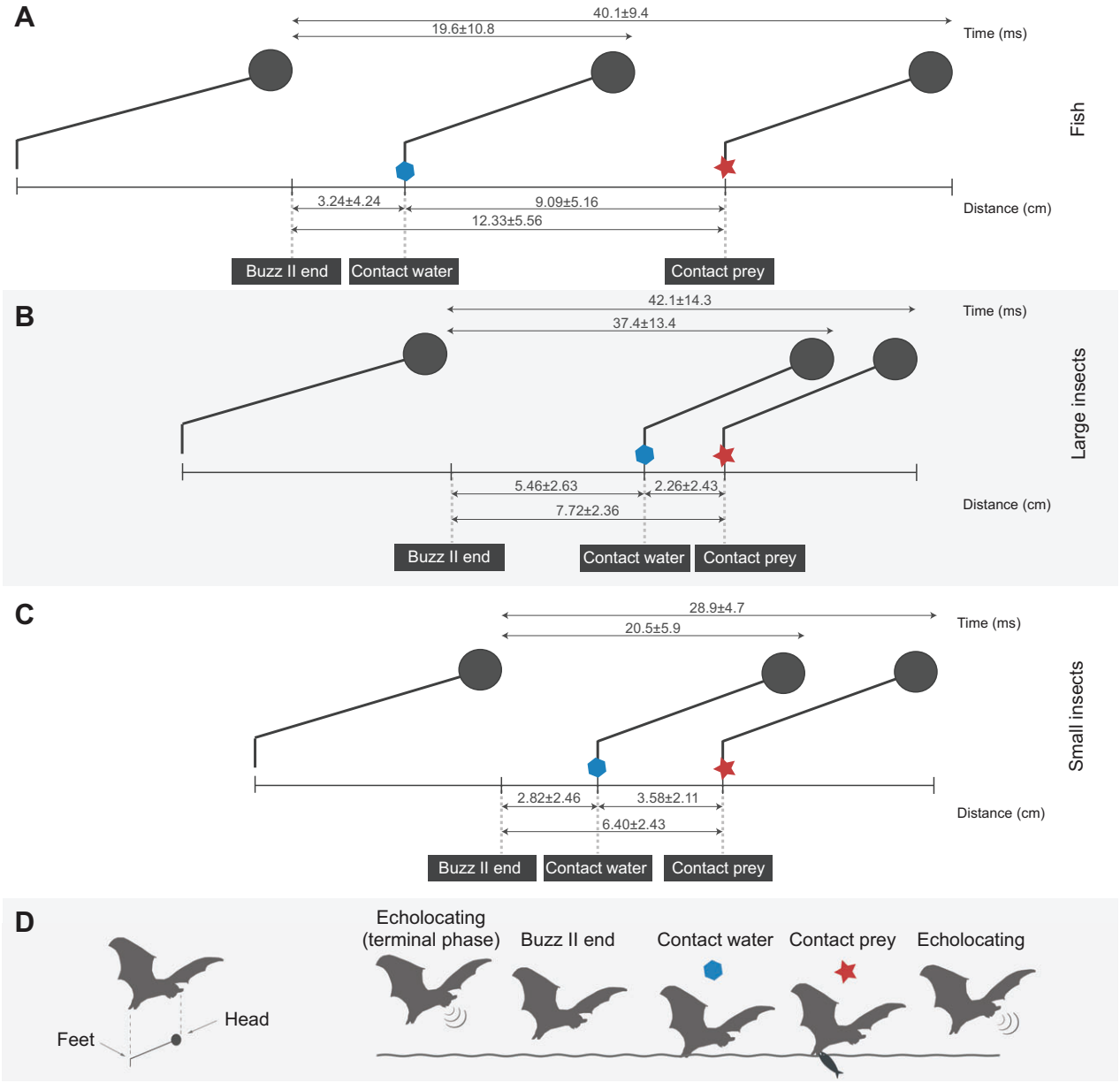

Fig. 2. Distance and time lapse from the end of the terminal phase to the moment of feet insertion (blue hexagon) and the moment of prey contact (red star) in the three target-type capture events. (A) Fish; (B) large insects; (C) small insects. The schematic reconstruction of an attack sequence (D) is also reproduced where the key moments of the capture action are stressed. Values are presented as means ± s.d.

echolocation and dip performance, confirming our hypothesis that the sensory-motor strategy varies depending on target type and size. As with different-sized insects, bats also detected and discerned fish from other prey types, and triggered a specific and seemingly stereotyped behaviour for capturing them.

Echolocation adjustments

The beginning of the terminal phase produced by *M. capaccinii* and its duration differed between different-sized prey, as bats emitted longer buzzes starting at a farther distance from the prey when capturing large insects compared with small insects and fish. The earlier start time of the terminal phase probably relates to the ability to detect large prey from farther distances because of their greater echo reflection (Schnitzler and Kalko, 1998). Even though fish are substantially larger than insects, only part of their mouth usually comes into view above the water (Pyke, 2005), and as bats cannot detect underwater objects (Suthers, 1965), fish detectability may be relatively low.

The end of the terminal phase was also variable and seemed to be determined by two factors, namely, pulse–echo overlap and feet insertion into the water. The feeding buzz ended after the theoretical pulse–echo overlap threshold in the three treatments, which contrasts with several previous studies (Denzinger and Schnitzler, 1994; Kalko and Schnitzler, 1993; e.g. Schnitzler et al., 1987; Wilson and Moss, 2004). Our data suggest that, as in other studies (Schmieder et al., 2012; Siemers and Schnitzler, 2000; Siemers and Schnitzler, 2004), the use of some information from pulses in the so-called signal-overlap zone, at least initially, cannot be ruled out. The signal emission within the echo-overlap zone lasted 25–28 ms when hunting insects, while it was reduced to half (13 ms) when fishing,

The Journal of Experimental Biology (2014) doi:10.1242/jeb.104992

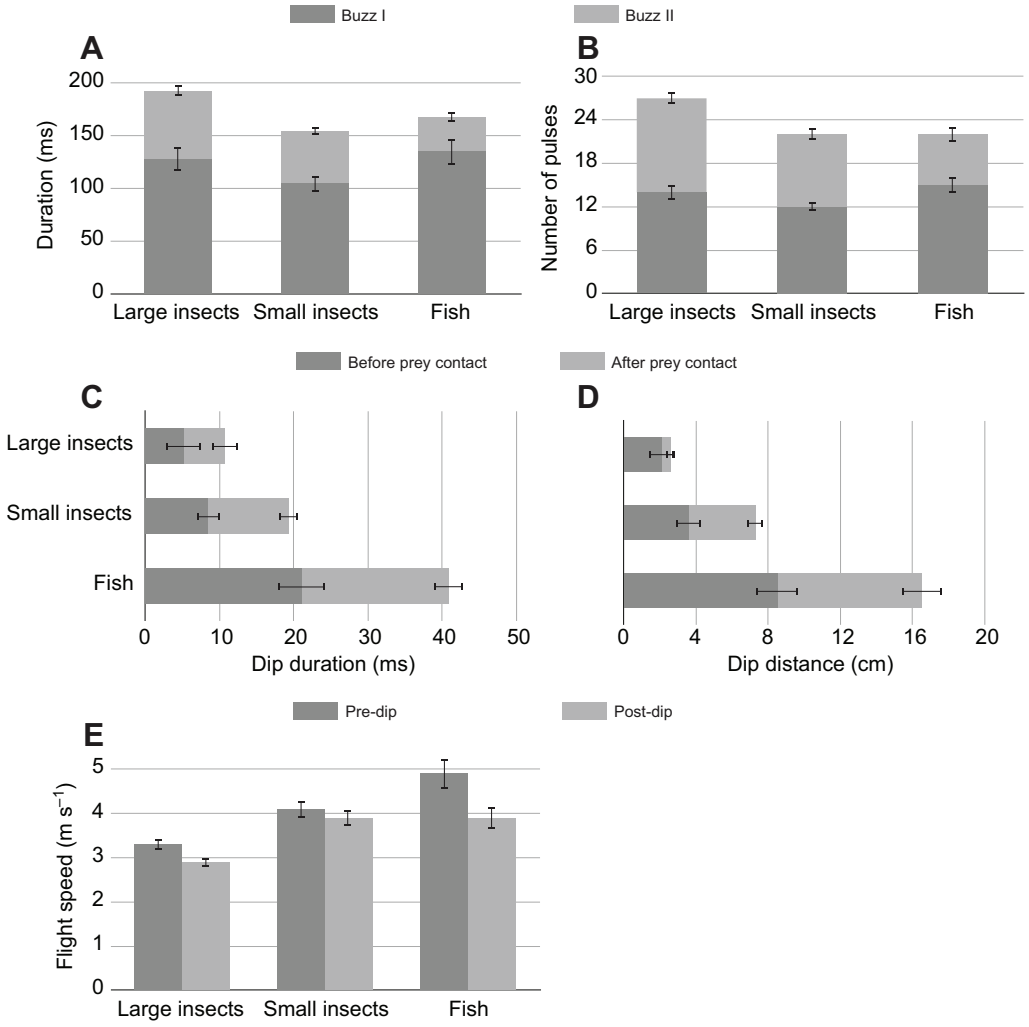

Fig. 3. Characteristics of the terminal phase of echolocation and flight during hunting attempts upon three prey types. (A) Duration and (B) number of pulses of the terminal phase produced by long-fingered bats just prior to capture of large insects, small insects and fish. (C) Duration and (D) distance of the dip bats performed during the captures of the three prey types. (E) Flight speed of long-fingered bats before performing the dip (pre-dip) and after the dip (post-dip). Error bars indicate the standard error.

suggesting that another feature limited the emission of echolocation calls.

The feeding buzz produced during insect capture ended at a similar distance from the prey (6–7 cm), presumably when the signal overlap effectively limits information gathering. Fishing bats ended their feeding buzz at farther distances from the prey compared with when hunting insects, most likely because they were limited by a second factor, namely, feet insertion into the water. No bat was observed emitting echolocation calls once the feet were inserted into the water, presumably because bats cannot perform modifications of flight once they introduce their feet into the water, so they have to acquire all the necessary information beforehand and choose the attack type before introducing their feet into the water. Additionally, there was a strong correlation between the feeding-buzz end time and the duration of the before-prey-contact dip: the longer the dip, the earlier the feeding buzz ends. The uncertainty of prey location because of fish disappearance under the water probably drove bats to perform longer and deeper dips when fishing, inserting their feet into the water farther away from the prey than when capturing insects, so bats ended their feeding buzz at farther distances from the prey.

Although the use of the feeding buzz has been discussed in several studies (e.g. Arlettaz et al., 2001; Faure and Barclay, 1994; Russo et al., 2007) and a large variability has been reported (Schnitzler et al., 1987), the importance of the duration of each part of the terminal phase with different targets has to date not been addressed. We observed that the number of pulses in buzzes I and II varied depending on the target type, resulting in changes of the buzz I/buzz II ratio. This ratio was ~1:1 in captures of both insect types, while in fishing attempts it increased to nearly 2:1. Even though the total duration of the terminal phase was similar when capturing small insects and when fishing, the length of buzz I was substantially longer and the length of buzz II shorter in fishing attempts compared with capturing small insects.

Lengthening buzz I and shortening buzz II is likely beneficial when attacking fish. A sustained buzz I means maintaining higher peak frequency and broader pulse bandwidth compared with buzz II, which may give enhanced information for prey discrimination and localisation and the detection of tiny surface disturbances produced by the fish. In contrast, emitting a long buzz II entails broadening the acoustic field, which may be useful to anticipate the evasive movements of flying insects (Jakobsen and Surlykke, 2010; Matsuta et al., 2013). However, buzz II seems useless when fishing, as fish would in most cases simply vanish underwater, and bats would not obtain benefits from broadening their acoustic field. As a result, the importance of buzz II is reduced during fishing, and accordingly in 5.7% of the fishing attempts buzz II was entirely omitted. Contrary to what had been suggested, we did not observe that such events were related to aborted attempts (Britton and Jones, 1999) and neither did they appear to be a feature that characterises fishing (Aihartza et al., 2008; Aihartza et al., 2013).

The Journal of Experimental Biology (2014) doi:10.1242/jeb.104992

Dip and flight adjustments

The capture mode also changed considerably with prey type. Bats dragged their feet through the water surface and relied on their uropatagium for catching evasive small insects because it provides a much larger capture surface. In contrast, large insects lying on the water surface – often incapable of flying, and therefore less evasive and more easily traceable – were captured using the hind feet. This explains why bats did not touch the water in half of the large insect hunting attempts, instead going directly to the target. Fish were also caught using the hind feet, though they were usually gaffed from the operculum using one or several toes from a single foot. Drag was considerably longer and deeper when fishing, and bats inserted their hind limbs up to the ankle. Fish, in contrast to flying insects, can disappear underwater before they can be reached, and bats likely inserted their feet deeper and along a longer path to increase the probability of capture. In most fishing events the uropatagium was also submerged into the water as reported for *M. vivesi* (Altenbach, 1989). Unlike *N. leporinus* (Schnitzler et al., 1994), the two small *Myotis* bats are unable to fold the interfemoral membrane up and forward, which entails an increased friction with water and could be one of the main features constraining catchable fish size (Aizpurua et al., 2013). In fact, we observed that the longer and deeper dip performed when fishing entailed a greater loss of flight-speed than when hunting insects. Nevertheless, bats compensated for the loss of kinetic energy by increasing their initial flight speed depending on the intensity of the drag, as the longer the drag the higher the flight speed. Therefore, the highest initial flight speed was observed when fishing, followed by hunting small insects and then large insects.

Conclusions

Long-fingered bats distinguish between different types of prey, including fish, and adjust their prey capture behaviour accordingly. This is true not only of the actual capture movements, but also of the acoustic behaviour. Both buzz-I and buzz-II calls were modified depending on the type of prey, which demonstrates the ongoing dynamic feedback control between emission and echo detection to adapt both types of calls to the prey at hand. Thus, the results suggest that bats coordinate modifications in their sensorial and motor systems to adjust the hunting technique to specific target types in a very short time, which indicates that bats identify the type of prey before or during the approach phase, adjusting the flight pattern and fine-tuning the terminal phase of echolocation calls for the type of capture they are about to carry out.

Long-fingered bats exploit a wider trophic spectrum than other insectivorous trawling bats, introducing slight modifications in their hunting behaviour, suggesting that small fish are within the potential niche of this species. Further studies will be needed, however, to understand which stimulus features allow bats to discern different types of prey and whether the ability to perform the modifications we observed in this study is innate or the result of a learning process.

MATERIALS AND METHODS

Study area

The field experiment was carried out in April and June 2010, 2011 and 2012, in two different locations: a pool at ‘La Sella’ golf course (Dénia) and a pond at Vernissa River (Rótova), both located 32 km apart in the Community of Valencia (Eastern Iberian Peninsula). Both sites are foraging grounds of *M. capaccinii* but the pool at the golf course is the only site where fishing has been reported in the area so far (Aizpurua et al., 2013). This is a 192×42 m artificial pool with high fish density and almost constant water level throughout the year. Vernissa River runs over 29 km before flowing into the Mediterranean Sea, and its course is modified by several small-sized artisan ponds used as water supply for irrigation. It is regulated by a Mediterranean-type regime, with minimal flow in summer, when pools and ponds are almost the only areas retaining water.

Experimental setup

The experiment was carried out over 42 nights in high activity spots identified using ultrasound detectors and a night-vision viewer (Night Owel Optics, New York) within natural feeding sites previously located by radio-tracking (Aizpurua et al., 2013). Species identification in the field was based on visual observations and echolocation call analysis (D1000X and BatSound 4.12 software; Pettersson Elektronik, Uppsala, Sweden). *Myotis capaccinii* can be easily identified in the field by its characteristic echolocation calls [frequency-modulated calls with a peak frequency around 47–50 kHz (Almenar et al., 2007)] and flight pattern, foraging near the water surface. The other trawling bat present in the Iberian Peninsula, *M. daubentonii*, shares the mentioned characteristics but it is absent from this area (Boyero, 2007).

We presented three types of prey commonly consumed by *M. capaccinii* (Aizpurua et al., 2013; Almenar et al., 2008): large insects (Lepidoptera; >15 mm), small insects (Culicidae, Chironomidae; <10 mm) and fish (*Gambusia holbrooki*; 10–30 mm). We considered that the noteworthy size difference of both types of insects could affect in the hunting technique of *M. capaccinii*, so they could provide us useful information to understand how and why the fishing technique differs from the insect-hunting technique. Moths were captured using light traps near the water source and small dipterans were collected from the edge of the pond. Fish were caught in the same pond with a hand net.

Insects (moths and dipterans) were tethered from the abdomen with small tweezers, fixed to the submerged tray and placed on the water surface with their legs on the surface and the body completely out of water, allowing them to flutter their wings. In the case of fish, 10 fish were placed on the submerged tray in different ways, including both dead and live individuals of different sizes. Because the stimuli bats rely on to discern fish from other types of prey were unknown, we tried to reproduce as many stimuli as possible that fish could create under natural conditions. Some of the fish were tied underwater with the upper lip protruding from the water surface, resembling the natural position when capturing insects from the surface, while others protruded part of their back or just kept moving their caudal fin under the water. The surroundings of the experimental spot were also full of free-swimming fish, as they included the rest of the pond.

We started sampling after dusk (approximately 22:30 h local time) and stopped 4 h later when bat activity levels dropped. No recordings were made on nights with bad weather conditions (rainy or windy nights) because the number of individuals and the activity of bats dramatically decreased (O.A., personal observation).

Sound and video recordings

We recorded hunting attempts upon the experimental targets combining a low-light high-speed video camera (HiSpec, Fastec Imaging Corporation, USA) capable of recording near-infrared light and an ultrasound detector (D1000X, Petterson Elektronik). The high-speed videos were recorded at 500 frames s^{-1} onto a laptop using Fastec software, and aided by infrared lighting (IREL-45). Audio recordings were made at a high sampling rate (350 kHz) in real-time mode at 16 bits, and stored as WAV files onto Compact Flash memory cards.

We built a trigger to simultaneously launch the download of images and sound, including the 3 s before and the 1 s after triggering. As a first step to synchronise video and sound recordings, the trigger activated an electronic clapper, a device that simultaneously switched on an LED (video reference) and a whistle (sound reference). Both the light and whistling devices were mounted attached to the microphone of the ultrasound detector, which was always set within the video camera’s field of vision, and connected to the detector by a 5 m extension cord. Synchronisation of video and sound recordings by the electronic clapper was calibrated in the laboratory using a Newton’s pendulum for reference, and a delay of 2 ms in the sound signal relative to the video signal was corrected. As a second step, to ensure synchronisation of video and sound, we set a 30×30 cm tray submerged just

below the water surface in the field, on which different prey were presented to bats. Every night we measured the distance from the tray to the ultrasound microphone to calculate the delay of the echolocation calls emitted by bats with respect to the light and sound signals of the electronic clapper. The tray also served as the reference point for setting the video camera and focusing its lens. All of this allowed the fine-grained temporal correlation of visual and acoustic information needed to accurately link the start and end times of the terminal phase of the echolocation sequences with the corresponding distances to the target (Moss and Surlykke, 2001).

Analysis of recordings

We used 135 high-quality ultrasound and high-speed video recordings to analyse the general hunting features (large insects n=29, small insects n=53, fish n=53). However, a smaller subset limited to the perpendicular passes with respect to the recording angle was used for measuring the distances and flight speeds (large insects n=10, small insects n=19, fish n=19).

We used two different temporal reference points to align the capture sequences: (1) the moment of contact between the bats' feet and the water and (2) the moment of contact between the bats' feet and the prey. We present all data relative to these points of reference, which were defined as time zero. Hence, events occurring before these points scored negative time values.

Sound analysis was performed with the software BatSound v 4.12 (Pettersson Elektronik). We analysed only the terminal phase of the echolocation call (feeding buzz), which is defined as a continuous sequence of calls emitted by the bat just before a capture attempt, and is produced after a pre-buzz pause (Aihartza et al., 2008; Britton et al., 1997; Ma et al., 2010). Despite the high activity of bats in the foraging grounds, individual terminal phases were clearly differentiated. They were subsequently divided into two parts: buzz I and buzz II. Sound duration and pulse interval are continuously reduced throughout buzz I, but the peak frequency is kept more or less constant (Melcón et al., 2009). A distinct drop in frequency characterises buzz II (Kalko and Schnitzler, 1998). We measured the total duration and number of pulses of the terminal phase, as well as buzz I and II duration and the number of pulses and their percentage in each buzz.

Video recordings were analysed using Fastec software. Based on the recordings, we measured the total dip duration of each attack (lapse between when the feet first contacted the water and the moment when the feet lost contact with it) and discriminated two parts: before-prey-contact dip duration (lapse between the first contact with the water and prey contact) and after-prey-contact dip duration (lapse between the prey contact and the moment when the feet lost contact with the water). Flight speeds in the 50 ms prior (pre-dip) and subsequent (post-dip) to the dip were also estimated. Additionally, we classified the feet insertion depth (the extent to which the hind feet were inserted into the water) into four categories: (1) no contact with water, (2) touching the water with the toes, (3) insertion of half of the foot into the water and (4) submersion of more than half of the foot into the water.

Being aware that it is impossible to entirely avoid pseudo-replication under natural conditions, we only carried out recordings when more than 15 long-fingered bats were foraging in each pond, and a normal-speed digital camcorder (Sony HDR550, Sony Corporation, Tokyo, Japan) was used to ensure that consecutive capture attempts were performed by different individuals.

Statistical analyses

All statistical analyses were performed using SPSS 20.0 statistical software (SPSS Inc., Chicago, IL, USA). Ultrasound and video recordings used for the analysis were randomly selected from the entire recording dataset to equalise sample sizes. We tested whether data were normally distributed with the Kolmogorov–Smirnov test and determined homogeneity of variance using Levene's test. Parameters that fulfilled both assumptions were analysed using one-way ANOVA and Student's t-test (α=0.050), and the Tukey method was used for *post hoc* multiple test comparisons. When variances were not homogeneous, we used Welch's F as an alternative to the F-ratio test, and the Games–Howell method was used for *post hoc* multiple comparisons. For parameters that did not fulfil the assumption of normality we used the non-parametric Kruskal–Wallis test and when a significant main effect was found, we used the Mann–Whitney test for *post hoc* comparisons with Bonferroni correction to adjust the alpha level and keep the Type I error low [P=(α=0.050)/k, where k is the number of paired comparisons; e.g. where k=3, P<0.017] (Field, 2009). Frequencies were compared using Pearson's chi-squared test.

Ethics statement

Prey capture and handling protocols met the guidelines for treatment of animals in research and teaching (Animal Behaviour Society, 2006) and Spanish legal requirements, and were approved by the Regional Government of Valencia (2010/20964) and *a posteriori* validated by the Ethics Committee for Animal Welfare of the University of the Basque Country (Refs. CEBA/220/2012/AIHARTZA and CEBA/221/2012/AIHARTZA). This study was performed with the permission of the Valencian Government.

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Competing interests

The authors declare no competing financial interests.

Author contributions

O.A., I.G. and J.A. conceived and designed the experiments. O.A., A.A., I.G., J.A. and H.J.B. carried out the fieldwork. O.A. and A.A. performed the experiments. O.A. analysed the data. I.G. and J.A. contributed reagents/materials/analysis tools. O.A., A.A., I.G., J.A. and H.J.B. wrote the paper.

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Supplementary material

Supplementary material available online at
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Movie 1. Long-fingered bats hunting insects (small and large) and fishing. Notice the feet insertion depth and dip duration in each attack.