Differentiating the echolocation calls of Daubenton's bats, pond bats and long-fingered bats in natural flight conditions

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Abstract: Echolocation calls of the three European species of trawling bats were studied in their natural habitats, during hunting, commuting and swarming activities. Daubenton's bats (*Myotis daubentonii*) used pulse durations below 8 ms in most cases, however on rare occasions they used some longer search phase calls up to 13 ms. Pond bats (*Myotis dasycneme*) used a wide variety of pulse durations of up to 26 ms, with signals of >15 ms regularly recorded. The longest pulse durations recorded from the long-fingered (*Myotis capaccinii*) bat were 8 ms. The three species usually emitted starting frequencies below 100 kHz, however some recordings made at very close range revealed starting frequencies up to circa 120 kHz in Daubenton's bat and the long-fingered bat and circa 110 kHz in the pond bat. Although the peak frequencies were variable in all species, the pond bat generally used lower peak frequencies (median 41 kHz for durations of 1-4 ms and median 35 kHz for durations of >14 ms) compared to Daubenton's bat (median 49 kHz for durations of 1-4 ms and median 37 kHz for durations of 8-14 ms) and the long-fingered bat (median 45 kHz for durations of 1-4 kHz). End frequencies were significantly higher in the long-fingered bat (median 32 kHz, for durations of 1-4 ms) than in the other two species (pond bat median 25 kHz, Daubenton's bat median 26 kHz, for durations of 1-4 ms). Quasi-constant frequency parts in the middle of the signal were only found in the pond bat. Long-fingered bats sometimes used pulse series with alternating end frequencies.

Keywords: Daubenton's bat, pond bat, long-fingered bat, *Myotis daubentonii*, *Myotis dasycneme*, *Myotis capaccinii*, echolocation, trawling.

Introduction

Myotis is the largest bat genus with more than 100 species globally and 17 of the 51 currently known European species belong to this genus (Eurobats 2004). They occupy territories all over the world, with the exception of areas where climates are too extreme (such as Antarctica). The extensive spread of the genus is thought to have happened during the late Miocene, when the global climate cooled. Fossil records prove that during this time the original bat fauna disappeared from large land masses at medium latitudes where climates changed from tropical and non-seasonal to temperate and seasonal (Horáček et al. 2000). In Europe, where other Vespertilionid genera (*Pipistrellus*, *Nyctalus*, *Vespertilio, Eptesicus*) hunt prey in open or semi open environments, many *Myotis* species are well adapted for efficient hunting in confined spaces. In order to navigate and detect prey in environments with large amounts of echo-producing obstacles, they use brief frequency modulated echolocation calls with a large bandwidth (Schnitzler & Kalko 2001, Schnitzler et al. 2003). Because of ecological conditions often being similar and the large number of species that have evolved in this genus, identification of *Myotis* species by their echolocation calls is generally considered to be

tricky (Ahlén 1990, Barataud 1996). This issue has challenged bat researchers across Europe. In a review of echolocation calls of wild bats communicated via the Dutch mailing list Zoogmail, Boonman (2007) pointed out that there are differences in pulse shapes, starting and end frequencies among European *Myotis*. In France, Barataud (2004) developed a system of identification of *Myotis* species based on subtle differences in audible sounds of 1/10 time expanded echolocation calls, allowing identification of species that are considered 'very difficult to identify'. Recent advances in portable and lightweight bat detector technology, (i.e. 16 bit analogue-to-digital resolution and better ultrasonic microphones) increase the capability of detecting the very high frequencies that some *Myotis* species use (L. Pettersson & Y. Tupinier, personal communication). This manuscript describes the echolocation calls of the three trawling species of Europe, Daubenton's bat (*Myotis daubentonii*), the pond bat (*Myotis dasycneme*) and the long-fingered bat (*Myotis capaccinii*) that were recorded with a 16 bit detector.

Trawling behaviour, i.e. gleaning the water surface and capturing either floating or submerged prey, is thought to be a derived hunting behaviour in the genus *Myotis*, and aerial hawking probably represents the ancestral state (Fenton & Bogdanowicz 2002). Trawling has appeared through convergent evolution in many parts of the world. Some of the most specialised forms are found in the Neotropical genus *Noctilio* (Hood & Jones 1984, Schnitzler et al. 1994, Kalko et al. 1998) although trawling behaviour has been reported to mainly occur in the genus *Myotis* (Jones & Rayner 1988, Kalko & Schnitzler 1988, Britton et al. 1997, Blood & Clark 1998, Jones & Rayner 1991, Fenton & Bogdanowicz 2002). Trawling bats share a number of key morphological adaptations, such as enlarged hind feet, enlarged claws, modifications to the calcar and a specific wing morphology (long and often broad wings) to allow for slow, manoeuvrable and economical flight, low over water surfaces (Norberg & Rayner 1987, Schober & Grimmberger 1998). Daubenton's bat occupies large parts of the Palaearctic territory, ranging from the boreal to the Mediterranean zone. The two other species have a more restricted distribution area, and are either limited to the temperate humid and boreal zones (pond bat) or the Mediterranean (long-fingered bat) (Horáček & Hanák 1989, Mitchell-Jones et al. 1999).

The abundance of insect prey over freshwater bodies probably triggered the evolution of trawling insectivores. Several insect families have aquatic larval and terrestrial adult stages, including those within Diptera, Ephemeroptera, Trichoptera and Plecoptera. Larvae, pupae and adults of these taxa are commonly found in the diet of bats hunting over water (Brack & Laval 2006, Todd & Waters 2007). Dietary studies confirmed that the three European trawling bats consume a lot of Chironomidae, which are abundant in a wide variety of freshwater habitats (Britton et al. 1997, Sommer & Sommer 1997, Flavin et al. 2001, Levin et al. 2006, Almenar et al. 2007, Biscardi et al. 2007, Krüger et al. 2010). Terrestrial insects and spiders can accidentally fall into the water or are drawn to the water surface by blustery winds and are commonly found in the stomachs of surface dwelling fish, such as trout, as well as in the faeces of Daubenton's bat (Flavin et al. 2001). Large terrestrial insects can also be preyed upon by trawling bats in the air above still water surfaces or around the riparian vegetation. Terrestrial insects can be rewarding prey for trawling bats, not only because they are often larger in size, but terrestrial taxa are also more nutritional per unit of dry weight compared to insects with aquatic subadult stages (Brack & Laval 2006).

Daubenton's bats can spend more than 90% of their nightly foraging time over water surfaces (Encarnação et al. 2004, Encarnação et al. 2005, Dietz & Kalko 2006). In the open habitats of the Netherlands, where large water surfaces are abundant, pond bats spent 75% of

their time hunting over water, 20% over meadows (often in the vicinity of canals) and 5% in other habitats (Haarsma et al. 2006). Hunting behaviour over land, in villages, along linear vegetation and at the edge of small woodlands has been reported by Kapteyn (1995) and Limpens et al. (1997). Long-fingered bats spend the night almost exclusively flying over water, sometimes up to 100% of their foraging activity (Almenar et al. 2006). Observations of individuals hunting over land are rare (Biscardi et al. 2007, Davy et al. 2007, Némoz & Brisorgueil 2008).

The echolocation and flight behaviour of European bats has been studied quite intensively since the 1980s, following pioneer work by Ingemar Ahlén in Sweden (Ahlén 1990). Since the requirements for trawling are not much different than those of hawking in the air, the trawling insectivorous bats navigate and detect prey using intense frequency modulated (FM) calls of a short duration (a few milliseconds) and large bandwidth, similar to aerial hawking bats close to vegetation (Thompson & Fenton, 1982, Kalko & Schnitzler 1988, Britton et al. 1997, Jones et al. 1988, Jones et al 1991, Kalko & Schnitzler 1998). The behaviour of trawling insectivores in the buzz phase is remarkably similar to that of aerial hawking bats and all trawling insectivores are also capable of catching flying insects in air. The fine details and the advantages of echolocating over smooth water surfaces have been illustrated in experiments in flight tents (Siemers et al. 2001, Siemers et al. 2005) and in field studies (Boonman et al. 1998). The smooth surface does not act as an echo producing background for trawling bats flying at low height. The pond bat is the only one out of the three European trawling bats known to use echolocation calls of long duration with a quasi-constant fequency (QCF) part (Ahlén 1990, Kapteyn 1995, Limpens et al. 1995, Limpens et al. 1997). Over water they often hunt in a very similar way to Daubenton's bats, focussing on the large numbers of small prey items that can be found in freshwater habitats. In these flight conditions they use either short FM calls or mixed FM-QCF calls of medium duration. When using high intensity long duration FM-QCF-FM signals (ca 20 ms), pond bats either commute or hunt over large waterways, often chasing large insects in the air above the water surface up to a few metres, and over the adjacent reeds and willows (Ahlén 1990, Kapteyn 1995, Limpens et al. 1997, Van De Sijpe & Holsbeek 2007). A recent study of the diet of long-fingered bats revealed a relatively large amount of terrestrial Lepidoptera, which may have been caught in the air above water surfaces (Almenar et al. 2008).

Compared to insectivorous trawling bats, piscivorous species have larger feet, longer claws and tend to have a larger body size (Fenton & Bogdanowicz 2002). Although Daubenton's bat is able to catch small fish in laboratory conditions (Siemers et al. 2001) piscivorous behaviour in the natural conditions in Europe has only been reported for the long-fingered bat (Levin et al. 2006, Aihartza et al. 2003, Biscardi et al. 2007). *Myotis capaccini* is probably mainly insectivorous, and its piscivorous behaviour seems to be opportunistic, unevenly distributed in space and time. In some diet studies no fish remains at all were found (Almenar et al. 2008), but in others a high occurrence of fish remains (up to 100%) and high volume percentages (up to 82%) were detected (Almenar et al. 2003). In a flight tent the bats used a technique of random dips to catch fish, but only did so only if fish were present in high density (Aihartza et al. 2008). During search flight the bats emitted short FM types and did not use complete buzz phases while dipping in the water, suggesting that they don't rely on echolocation to track individual fish. Short broadband FM calls have also been reported in other (occasional) piscivores, including *Myotis adversus* in the Oceanic zone, *Myotis ricketti* in China and *Myotis vivesi* in Central America (Robson 1984, Blood & Clark 1998, Ma et al. 2003). Whereas *Myotis* species use FM signals (or

sometimes FM-QCF-FM types), *Noctilio* species emit CF-FM calls of medium duration (ca 10 ms) when trawling over water surfaces (Hook & Jones 1984, Schnitzler et al. 1994, Kalko et al. 1998). *Noctilio leporinus,* maybe the most specialised fisher among bat species, uses various techniques to catch fish. Directed pointed dips have been observed, in which the bats use the CF part in the signal to detect and track movements of individual fish on the surface. An alternative random rake technique has also been described, during which the bats can fly up to 10 metres with their claws continuously submerged in the water (Schnitzler et al. 1994).

Methods

Recording sites

Daubenton's bat

Field recordings of Daubenton's bats in natural flight conditions were made in various locations in Belgium (Province of West-Vlaanderen; table 1). Daubenton's bats and pond bats are the only two trawling bat species known to occur in Belgium and the Netherlands. Hunting bats were recorded over water (a rampart moat and a number of small ponds) and over land (in a forest near a roost tree). Commuting bats were recorded over a forest path, in a tunnel and at the edge of a large pond. Swarming bats were recorded in a forest and a castle park where roost sites are known. The forest roosts of Daubenton's bats had been known for about ten years and the bats were identified either by following commuting bats to nearby water surfaces, where they start to hunt in the typical trawling manner, or by sighting the bats in the roost using a Sandpiper tree camera (B. Vandendriessche & F. Verhaeghe, personal communication). One recording of a bat released from captivity was made in a marlow pit (Zichen, Province of Limburg) This bat was identified in the hand prior to release. Commuting activity was identified if a large number of individuals were observed passing by for a brief period of time, with all bats flying in the same direction and not returning. Hunting activity was identified if a bat was found to fly around for a long period of time in a small space, regularly emitting feeding buzzes (Limpens et al. 1997).

Pond bat

Pond bats are rare in Belgium. The only known roost, in a brewery in the Province of West-Vlaanderen, disappeared sometime ago, although since then recordings have been made of pond bats hunting over water (a large canal) south of Mechelen (Muizen, Province of Antwerpen; table 1). The presence of the pond bat around Mechelen was discovered about 10 years ago (S. Verkem, personal communication). Additional recordings of hunting behaviour over water, as well as commuting, swarming and hunting over land were recorded in the Friesian village of Tjerkwerd (the Netherlands; table 1) where a large roost has been known to exist for many years.

Long-fingered bat

A limited number of recordings of this species was made on three consecutive April nights, during a visit to Spain (Province of Valéncia; table 1). The bats were recorded in the vicinity of a known large maternity roost where the main hunting areas were found by radio telemetry (Almenar et al. 2006). The recordings of long-fingered bats were limited to hunting behaviour over smooth river surfaces by trawling. No recordings were made of commuting and swarming behaviour.

Visual observations

Visual observations of the bats' flight behaviour and size were made at dusk, against the bright evening sky, or where light sources in the vicinity provided sufficient illumination to see the bats. In situations too dark for the unaided eye, a monocular image intensifier

Table 1. Details of recording situations of Daubenton's bat (A1-A14), pond bat (B1-B15) and long-fingered bat (C1-C7). SP = search phase, AP = approach phase.

Recording number	Date and time	Location	Habitat		Flight activity over		Flight height	Hunting style
A1	12/08/2007 22:47	Kemmel, BE	small pond	Hunting	water	SP	< 0.5 m	Trawling (*)
A2	25/08/2007 00:11	Loppem, BE	castle pond	Hunting	water	SP	< 0.5 m	Trawling (*)
A3	12/08/2007 22:59	Kemmel, BE	small pond	Hunting	water	SP	< 0.5 m	Trawling (*)
A4	17/07/2010 01:36	Ieper, BE	rampart moat 50 m wide	Hunting	water	SP	< 0.5 m	Trawling (**)
A5	17/07/2010 01:07	Ieper, BE	rampart moat 50 m wide	Hunting	water	SP	< 0.5 m	Trawling (**)
A6	04/04/2008 22:30	Ieper, BE	rampart moat 50 m wide	Hunting	water	AP	< 0.5 m	Trawling (*)
A7	12/07/2010 22:46	Zillebeke, BE	in the forest	Hunting	land	SP	ca 3 m	Hawking
A8	12/07/2010 22:47	Zillebeke, BE	in the forest	Around roost	land	AP	ca 3 m	-
A9	08/09/2007 22:10	Loppem, BE	tunnel under motorway	Commuting	land	SP	ca 2 m	-
A10	08/09/2007 22:12	Loppem, BE	tunnel under motorway	Commuting	land	SP	ca 2 m	-
A11	27/06/2007 22:21	Zillebeke, BE	edge of large pond	Commuting	land	SP	ca 2 m	-
A12	12/05/2008 22:11	Zedelgem, BE	forest path	Commuting	land	SP	ca 3 m	-
A13	25/04/2008 05:53	Woumen, BE	castle park	Swarming	land	-	ca 3 m	-
A14	29/09/2007 00:09	Zichen, BE	marlow pit	Released	land	-	ca 2 m	-
B1	30/07/2009 23:50	Muizen, BE	canal 30 m wide	Hunting	water	SP	< 0.5 m	Trawling (*)
B2	10/06/2008 23:27	Tjerkwerd, NL	canal 13 m wide	Hunting	water	SP	< 0.5 m	Trawling (*)
B3	30/07/2009 23:56	Muizen, BE	canal 30 m wide	Hunting	water	SP	< 0.5 m	Trawling (**)
B4	03/07/2010 23:59	Muizen, BE	canal 30 m wide	Hunting	water	SP	< 0.5 m	Trawling (**)
B5	24/07/2009 00:35	Muizen, BE	canal 30 m wide	Hunting	water	SP	< 0.5 m	Trawling (**)
B6	25/07/2009 23:45	Muizen, BE	canal 30 m wide	Hunting	water	SP	< 0.5 m	Trawling (**)
B7	30/07/2009 00:06	Muizen, BE	canal 30 m wide	Hunting	water	AP	< 0.5 m	Trawling (*)
B8	03/08/2009 22:41	Muizen, BE	over a field of bulrush	Hunting	water	SP	ca 3 m	Hawking
B9	13/07/2007 23:23	Tjerkwerd, NL	church square	Hunting	land	SP	ca 3 m	Hawking
B10	13/07/2007 22:50	Tjerkwerd, NL	village road	Commuting	land	SP	< 0.5 m	-
B11	10/06/2008 23:00	Tjerkwerd, NL	church square	Commuting	land	SP	ca 3 m	-
B12	10/06/2008 23:01	Tjerkwerd, NL	church square	Commuting	land	SP	ca 3 m	-
B13	10/06/2008 23:27	Tjerkwerd, NL	church square	Commuting	land	SP	ca 3 m	-
B14	10/06/2008 23:23	Tjerkwerd, NL	canal 13 m wide	Commuting	water	SP	< 0.5 m	-
B15	11/06/2008 04:21	Tjerkwerd, NL	church square	Swarming	land	-	-	-
C1	29/04/2010 22:55	Prov. Valéncia, ES	river ca 15 m wide	Hunting	water	SP	< 0.5 m	Trawling (*)
C2	29/04/2010 22:48	Prov. Valéncia, ES	river ca 15 m wide	Hunting	water	SP	< 0.5 m	Trawling (*)
C3	28/04/2010 23:04	Prov. Valéncia, ES	river ca 30 m wide	Hunting	water	SP	< 0.5 m	Trawling (**)
C4	28/04/2010 22:53:	Prov. Valéncia, ES	river ca 30 m wide	Hunting	water	SP	< 0.5 m	Trawling (**)
C5	27/04/2010 22:04	Prov. Valéncia, ES	small pool in a river	Hunting	water	SP	< 0.5 m	Trawling (*)
C6	29/04/2010 22:31	Prov. Valéncia, ES	river ca 15 m wide	Hunting	water	SP	< 0.5 m	Trawling (*)
C7	28/04/2010 23:13	Prov. Valéncia, ES	river ca 30 m wide	Hunting	water	SP	ca 2 m	Hawking

* Flight height during recording was <0.5 m over the water surface. During this night the bats were flying continuously low over the water surface and did not make any excursions higher in the air. The bats only attacked prey items floating on the water surface or flying in the lower air <0.5 m.

** Flight height during recording was <0.5 m over the water surface. During this hunting behaviour the low search flights were now and then interrupted by sudden rises whereby the bat chased a prey item after an excursion higher up in the air.

was used (ITT Night Mariner 150, ITT Industries, Roanoke, Virginia, USA). A circuit of 49 GaAs infrared leds with a wavelength of 880 nm (Vision Nachtzicht techniek, Born, the Netherlands) was mounted on the Night Mariner to improve the contrast of the images. When observed flying at close range, pond bats are visibly larger in body size than Daubenton's bats.

Sound recordings

Recordings were made between 2007 and 2010, with a Pettersson D1000X detector (Pettersson Elektronik AB, Uppsala, Sweden), in the direct ultrasonic recording mode, using a sampling rate of 500 kHz (aliasing-free up to 200 kHz; Pettersson Elektronik AB 2005-2007). Input gain was adjusted close to minimum, to avoid clipping when the bats are flying very close to the microphone. The detector has an ADC resolution of 16 bit and the microphone has a flat frequency response between 5 and 170 kHz. Ultrasonic recordings were stored directly on a Compact Flash card for later analysis.

Definitions of the parameters used for sound analysis

The analysis in this paper includes the basic parameters that describe echolocation calls: pulse duration, interpulse interval, starting frequency, end frequency, peak frequency (frequency corresponding to the maximum amplitude) and QCF frequency (see definition below). Pulse duration was defined as the time between the start and the end of the echolocation call, the interpulse interval (IPI) as the time between the start of the call (n) and the start of the next call (n+1). The starting and end frequencies were taken from the fundamental (or first harmonic), excluding higher harmonics. In this paper the QCF part of a signal was defined as the signal part with a minimum duration of 1 ms and a maximum modulation rate of 1 kHz/ms.

Sound analysis methods

The BatSound 4.03 programme (Pettersson Elektronik AB, Uppsala, Sweden) was used for sound analysis. Time related parameters (pulse duration and IPI) were measured using the combined oscillogram-spectrogram. Time measurements are more accurate when measured in the oscillogram, however errors can occur if the signals have a very weak start or end, or are followed by strong echoes. The combined oscillogram-spectrogram allows one to use the spectrogram window to check if a signal has a weak high frequency start and/or echoes are masking the signal end.

Frequency related parameters were measured in the spectrogram. Starting and end frequencies were measured using the highest visible value in the spectrogram (FFT size 1024, Hanning window). Peak frequency was computed automatically using the Pulse Characteristics Analysis function in BatSound. This algorithm automatically searches for the frequency with the maximum amplitude (in the spectrum, not the oscillogram). In a linear signal, typical of *Myotis* bats in highly cluttered conditions, there is not really a discernable 'peak frequency'. When the power spectrum is examined in detail, the amplitude appears quite constant over a wide range of frequencies. Although the algorithm will still find one 'peak frequency' where the energy is a bit higher than elsewhere, the amplitude will be little less at many other frequencies. The longer the signal becomes, the more likely there will be a distinct zone somewhere in the middle where the slope becomes less steep and where the energy is concentrated. In those signals, typical of *Myotis* bats flying in semi-open or open spaces, the peak frequency will most often be located somewhere in the zone of the smallest slope. The modulation rate was checked for signal parts with small

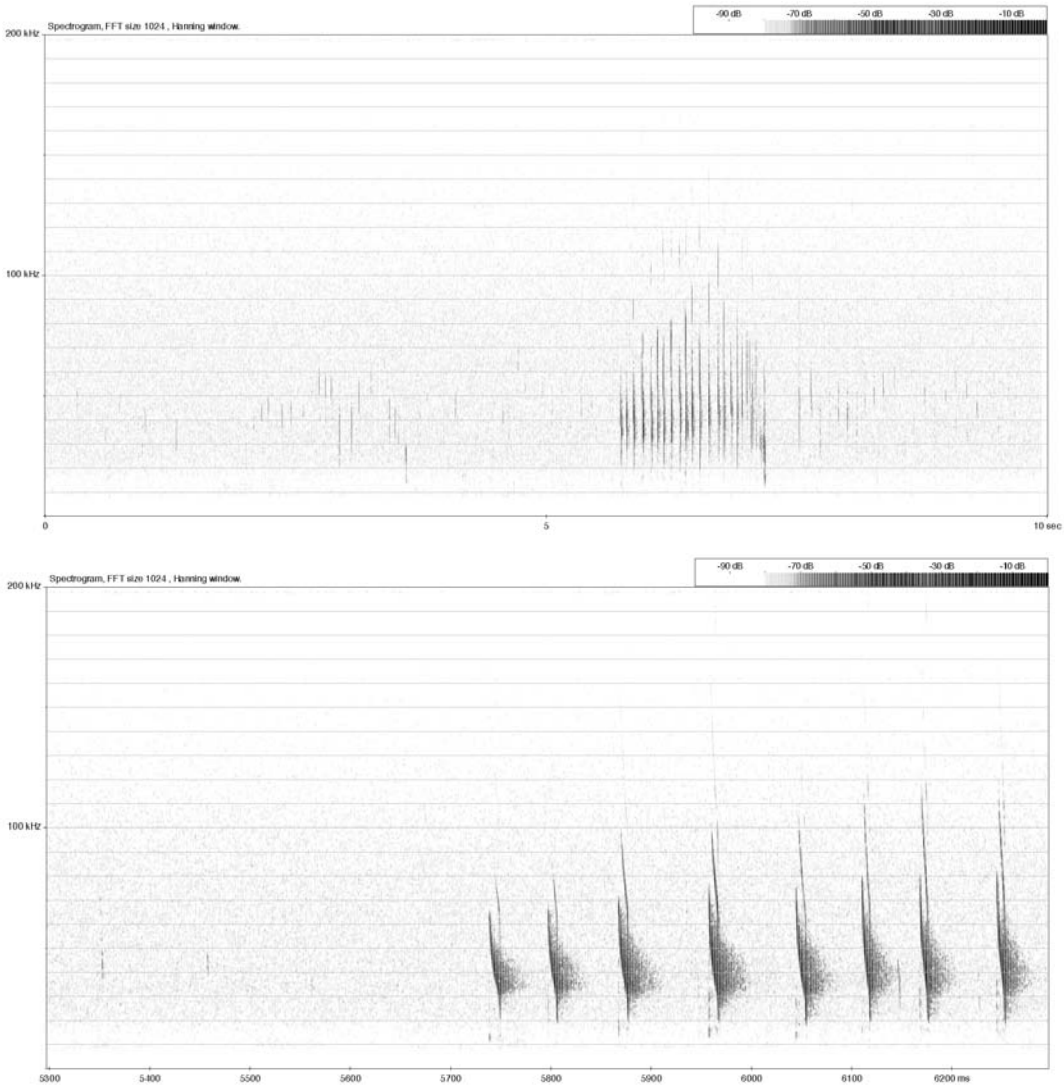

Figure 1. Daubenton's bat hunting low over water, while using an unusual discontinuous echolocation pattern. Flying parallel to and at a distance of approximately 3 m from the bank, the bat was first observed passing by closely while emitting only very weak signals. All of a sudden the bat started to emit a series of very loud echolocation calls (starting at 5730 ms in the spectrogram). Above: 10 s interval. Below: detail of the start of a series of loud calls after a period of weak calls.

slopes. If the frequency modulation rate was less than 1 kHz/ms, the QCF frequency was measured using a power spectrum of a time interval of 1 ms preceding the steep final FM part. In the pond bat the QCF part always precedes the final FM. The other trawling *Myotis* do not use QCF.

Selection criteria

Analysis of echolocation calls was limited to search and approach phase calls. The very short calls of final buzz phases were not included. When selecting the best recordings for analysis, preference was given to those

Table 2. Median (in brackets: minimum - maximum) values of pulse interval, starting, end and peak frequency of echolocation calls of Daubenton's bat (Mdau), pond bat (Mdas) and long-fingered bat (Mcap), all behaviours combined.

		Mdau	Mdas	Mcap
Pulse durations 1-4 ms	Pulse interval (ms)	46.7 (11.6 - 72.5)	67.1 (29.8 - 110.0)	13.5 (9.0 - 95.8)
	Starting frequency (kHz)	101 (86 - 118)	87 (79 - 94)	93 (70 - 117)
	End frequency (kHz)	26 (22 - 38)	25 (24 - 28)	32 (24 - 40)
	Peak frequency (kHz)	49 (42 - 63)	41 (39 - 46)	45 (42 - 59)
Pulse durations 4-8 ms	Pulse interval (ms)	67.0 (41.5 - 161.2)	71.5 (15.6 - 116.6)	64.8 (12.6 - 129.3)
	Starting frequency (kHz)	99 (66 - 114)	95 (62 - 110)	95 (72 - 123)
	End frequency (kHz)	26 (18 - 32)	26 (23 -31)	30 (24 -39)
	Peak frequency (kHz)	46 (38 - 62)	39 (35 - 49)	47 (40 - 57)
Pulse durations 8-14 ms	Pulse interval (ms)	81.5 (57.9 - 191.9)	111.4 (62.2 - 211.4)	-
	Starting frequency (kHz)	72 (61 - 87)	84 (52 - 100)	-
	End frequency (kHz)	20 (18 - 25)	28 (23 - 33)	-
	Peak frequency (kHz)	37 (33 - 40)	37 (35 - 41)	-
Pulse durations >14 ms	Pulse interval (ms)	-	131.3 (111.2 - 227.3)	-
	Starting frequency (kHz)	-	46 (40 - 56)	-
	End frequency (kHz)	-	28 (24 - 30)	-
	Peak frequency (kHz)	-	35 (33 - 38)	-

recordings with the best signal-to-noise ratios, when the bat was flying close to the microphone (<2 m if possible). This made it possible to pick up the high frequencies at the start of the signal. The direction of the bats in relation to the microphone varied, some recordings include bats heading towards the microphone and emitting the sound beam directly towards it (see below). Small bat-to-microphone distances were not always possible due to technical limitations (hand held detector), especially when bats were flying over open water far from the banks or higher in the air. Some signal parameters, especially starting frequency and duration, might be biased in recordings with lower signal-to-noise ratios.

Statistics

Because the starting, end and peak frequencies of pulses within a species may strongly vary with pulse duration, it is advisable to compare echolocation calls of similar durations. For this purpose the respective data sets were split into three sub-sets, based on pulse durations: 1-4 ms (short duration), 4-8 ms (medium duration) and 8-14 ms (long duration). Only the pond bat used very long durations (>14 ms). In several data sets these subsets were normally distributed. In others they were not (Shapiro-Wilk W-tests, $P<0.05$), even after removing the outliers. Non-parametric Mann-Whitney tests were used to analyse the data (Analyse-it Software Ltd, Leeds, UK).

Results

Description of the recording conditions

Details of the selected recordings of Daubenton's bats (A), pond bats (B) and long-fingered bats (C) are shown in table 1. The spectrum of recording conditions is more complete for Daubenton's bat and the pond bat than for the long-fingered bat.

Daubenton's bat

Recordings of Daubenton's bat include hunting flight low over water (A1 to A6), hunting flight over land (A7), commuting flight

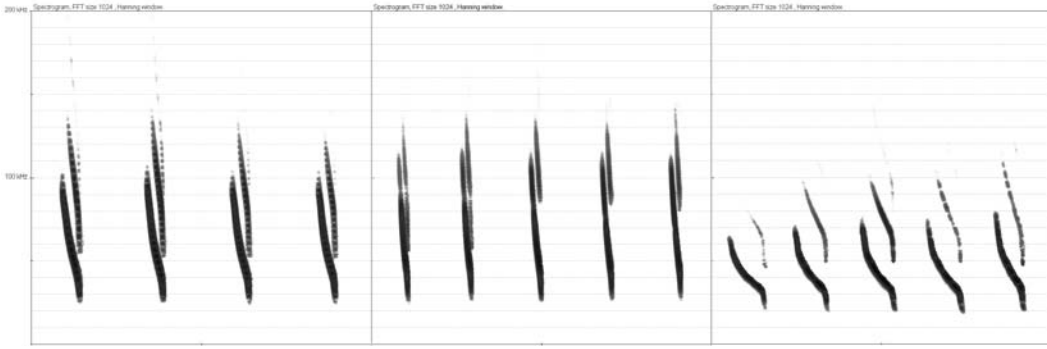

Figure 2. Echolocation calls of Daubenton's bats. Left: bat hunting over a small pond (recording A1). Middle: bat hunting in the forest near the roost (recording A7). Right: bat hunting over a large water surface close to the bank (recording A4).

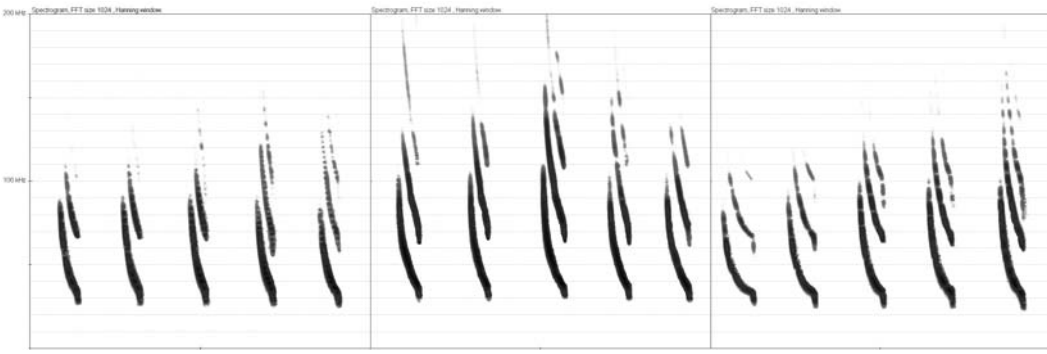

Figure 3. Echolocation calls of pond bats. Left: bat hunting over a large canal close and parallel to the reed margin (recording B1). Middle: bat swarming around a church roof (recording B15). Right: bat commuting low over a small canal (recording B14).

Figure 4. Echolocation calls of pond bats. Left: bat hunting over a large canal, approaching the canal bank (recording B7). Middle: bat hunting over a large canal far from the banks, change from search to approach phase (recording B3). Right: bat hunting over a large canal far from the banks, search phase (recording B6).

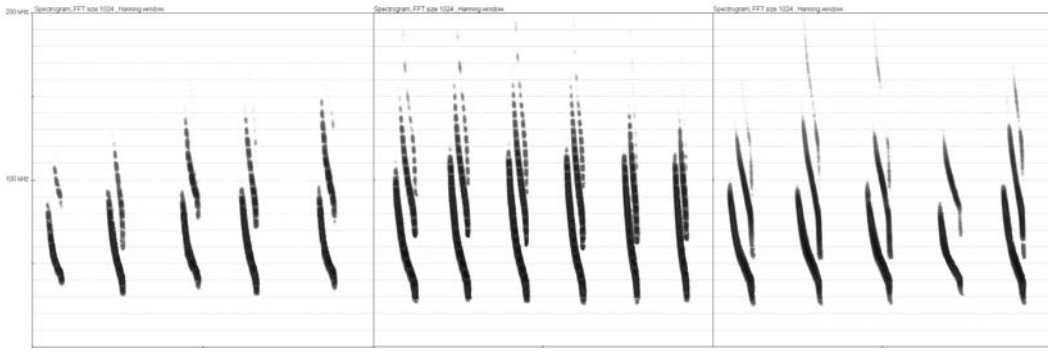

Figure 5. Echolocation calls of long-fingered bats. Left: bat hunting over a small meandering river (recording C1). Middle: bat hunting over a zone of tranquil water in a small river (recording C5). Right: bat hunting over a large river upstream of a waterfall (recording C7).

over land (A9 to A12), swarming flight (A13) and flight after release from captivity (A14). Recording A1 was made shortly after dusk when a few Daubenton's bats arrived at a small pond. A lot of feeding buzzes occurred, indicative of active hunting and an abundance of prey. These pulse durations were fairly short. Recording A3 was made a bit later over the same pond when all the bats except one had disappeared and pulse durations were longer. Recordings A4-A5 represent a rare hunting behaviour for Daubenton's bat. This behaviour took place during a few midsummer nights when the bats were patrolling at a distance of two to three metres from the banks of a large moat. Visual observations, using an image intensifier and infrared illumination, of bats passing at very close range revealed that these were Daubenton's bats (small body size) rather than pond bats. Their flight was linear and a bit faster than usual. A single bat alternated groups of loud pulses with periods of weak pulses, the longest durations were emitted immediately after each silence period (figure 1). Sometimes the bats would suddenly rise and chase an insect in the air. This behaviour resembles a particular hunting mode associated more with the pond bat, although in the latter, the pulse durations and flight speed are considerably larger (Van De Sijpe & Holsbeek 2007). Recording A6 includes an approach phase of a Daubenton's bat, passing very close to the microphone (<1 m), held on the tip of a fisherman's quay protruding over the moat. In A7 a Daubenton's bat was hunting in the forest shortly after emerging from a nearby roost tree. The bat returned to the roost tree (recording A8), maybe to feed its young. A9 and A10 were made in a tunnel under a motorway, used by Daubenton's bats at dusk, as they were travelling from roost to their hunting site. In A13 a group of Daubenton's bats were swarming in front of their tree roost in a park, just before dawn. The bat recorded in A14 was identified in the hand after capture at the entrance of a marlow pit in the late summer swarming season. It was then placed onto a wall in a dead end gallery inside the marlow pit. After a short period of resting it took off spontaneously and flew through the gallery towards the exit where the recording was made. At the exit the bat was flying steadily and the echolocation pattern resembled that of a natural flight condition, though the pulse durations were very short (short distances between the bat and the walls and ceiling).

Pond bat

The selection of pond bat recordings includes hunting flight low over water (B1 to B7), hunting flight over land (B9), commuting flight over water (B14), commuting flight over land (B10 to B13) and swarming flight (B15). In recording B1 a pond bat was flying parallel and close to a reed

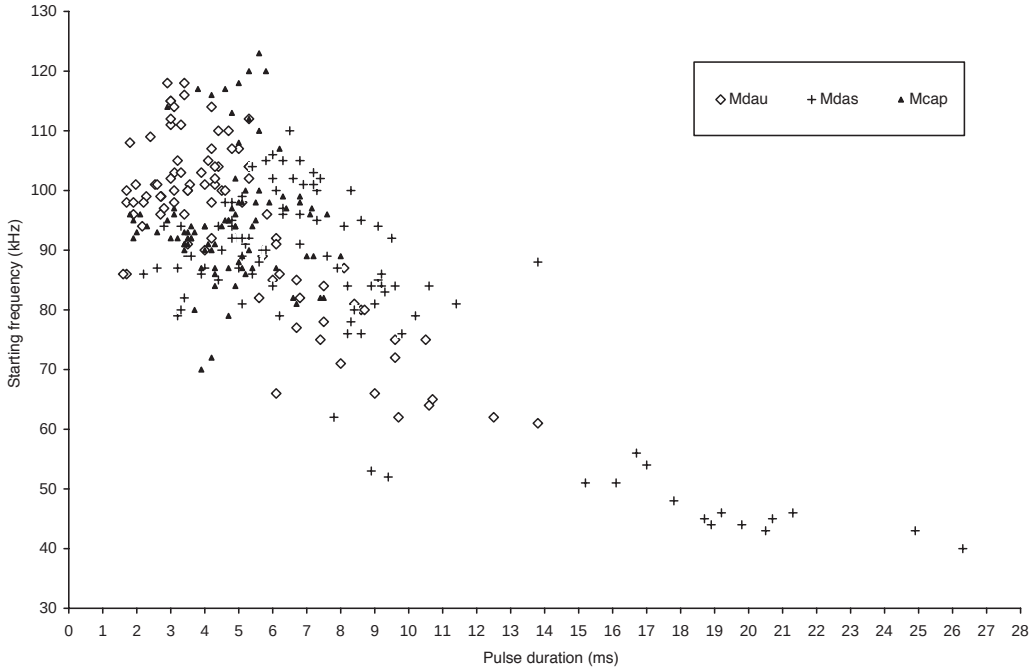

Figure 6. Scatter plot of starting frequencies vs. pulse durations in Daubenton's bat (Mdau), pond bat (Mdas) and long-fingered bat (Mcap), all behaviours combined.

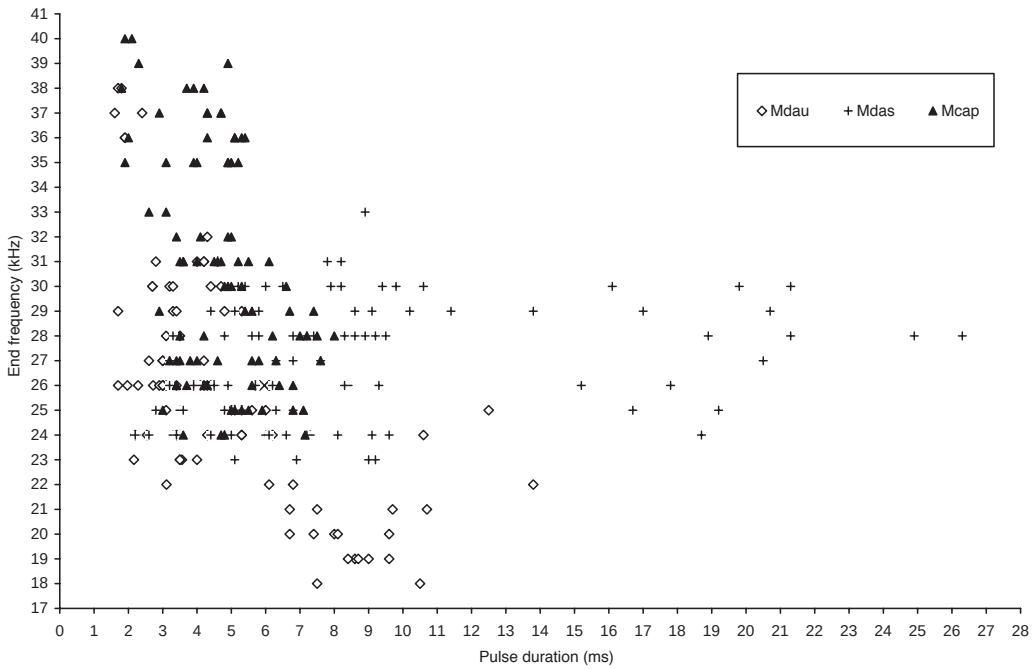

Figure 7. Scatter plot of end frequencies vs. pulse durations in Daubenton's bat (Mdau), pond bat (Mdas) and long-fingered bat (Mcap), all behaviours combined.

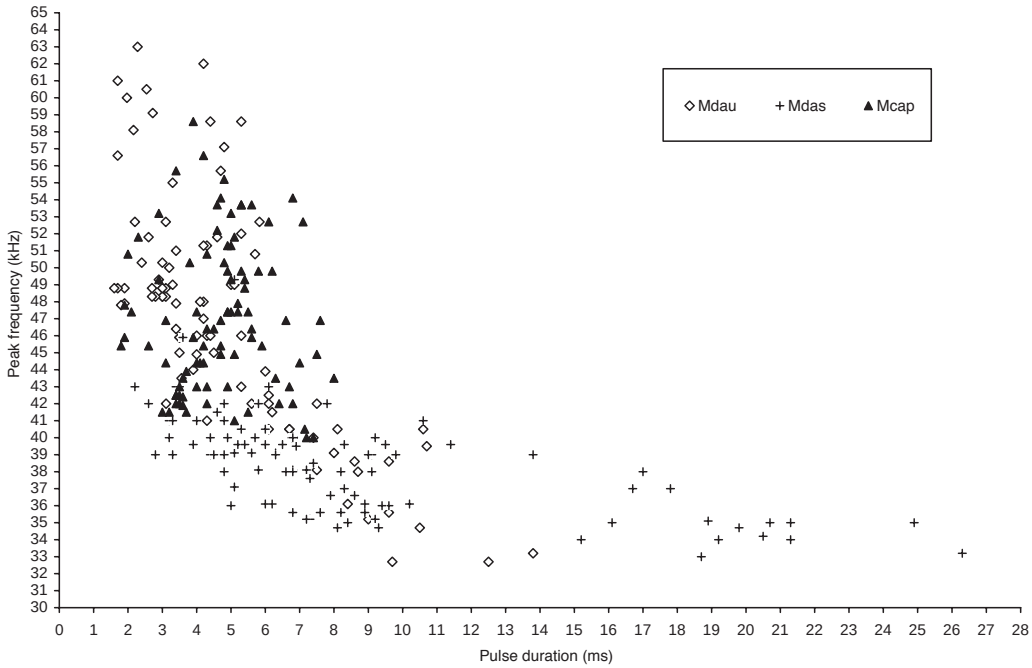

Figure 8. Scatter plot of peak frequencies vs. pulse durations in Daubenton's bat (Mdau), pond bat (Mdas) and long-fingered bat (Mcap), all behaviours combined.

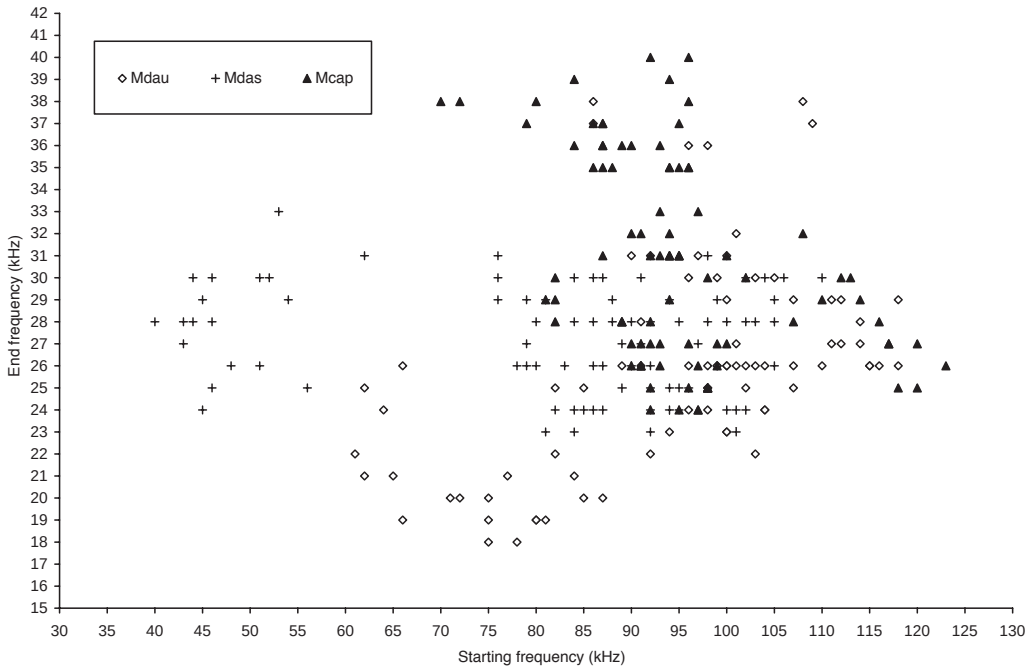

Figure 9. Scatter plot of end frequencies vs. starting frequencies in Daubenton's bat (Mdau), pond bat (Mdas) and long-fingered bat (Mcap), all behaviours combined.

margin (distance to the reeds ca 1 m) where it used FM signals of short duration, before flying more in the middle of the canal where the calls changed to the species-specific long duration FM-QCF-FM types. B2 was made over a rather small canal and includes pulses of medium duration with QCF. B3 to B6 were made during a couple of midsummer nights where pond bats were hunting over a large canal, using fast, linear search flights at a low height along the midline. They often made excursions higher up in the air (ca. 3 m) to chase large insects. In B7 a pond bat was first flying fast along the midline, then suddenly changed flight direction (almost 90°) and flew towards the bank, where it caught an insect on the water surface (ca 2 m from the bank). Recording B8 included short search phase calls of a pond bat flying just above the tops of bulrush (*Typha latifolia*), after an attack on a large insect higher in the air (2 to 3 m). In B9 a pond bat was hunting for a few minutes over a church square after emergence at dusk. The bat continued flying in large circles at a height of approximately 3 m. Several feeding buzzes revealed that this pond bat was hunting by means of aerial hawking. B10 is a recording made from a pond bat commuting very low over a village road (<0.5 m). The durations of B11 to B13 are longer. Here the bats were flying higher over a church square. In B14 a pond bat was flying low over a canal, and passed close to the microphone on the bank (ca. 2 m). These signals have high signal to noise ratios, a QCF part and the durations are of medium size.

Long-fingered bat

Recordings of long-fingered bats were limited to hunting behaviour low over water (C1 to C6) and higher in the air (C7). Recordings C1, C2 and C6 were made at the banks of a small meandering river with alternating zones of smooth and turbulent water surfaces. C5 was made of a bat trawling over a small sized river pool, surrounded by zones with rippling water, steep rocks and reed formations. The long-fingered bat continued to hunt over the pool and passed the bank and microphone at a distance of one metre, just before capturing a prey item. C3, C4 and C7 were made at a larger river (30 m) upstream of a waterfall where the surface was smooth. In the beginning of the night a large group of bats were hunting together. Later on, when the number of hunting bats decreased, one of the remaining individuals changed behaviour and started to make long linear flights low over the surface at a higher speed. Every now and then the bat soared upwards to ca 2 m to attack an insect. C7 was recorded when this bat was flying higher in the air. The recording includes search and approach phase calls preceding a feeding buzz.

Sound analysis data and spectrogram representations

A summary of sound analysis data can be found in table 2. Echolocation calls are presented in spectrograms (frequency versus time plot) in figure 2 (Daubenton's bat), figures 3 and 4 (pond bat) and figure 5 (long-fingered bats). The intervals are partially cut away to show more signals in the same window. Echoes and background noise were removed from the spectrograms for aesthetic reasons.

The ranges of pulse durations

Pulse durations varied between 1.6 and 13.8 ms in Daubenton's bat, between 2.2 and 26.3 ms in the pond bat and between 1.8 and 8.0 ms in the long-fingered bat. Daubenton's bats flying over water usually had durations of below 8 ms, except in recordings A4 (durations up to 10.7 ms) and A5 (durations up to 13.8 ms). The longest duration recorded over land was 6.2 ms (commuting over a broad forest path). Pond bats regularly used signals of a long duration both over water (up to 26.3 ms) and over land (up to 10.2 ms). As the surroundings become more and more confined, the durations decreased. In confined spaces

Table 3. Statistical comparison of datasets of echolocation call parameters between Daubenton's bat (Mdau), pond bat (Mdas) and long-fingered bat (Mcap), all behaviours combined. Significant results ($P<0.01$) in bold.

		Mdau vs. Mdas	Mdau vs. Mcap	Mdas vs. Mcap
Pulse durations 1-4 ms	Pulse interval (ms)	P=0.0117	**P<0.0001**	**P=0.0004**
	Highest frequency (kHz)	**P<0.0001**	**P<0.0001**	**P=0.0025**
	Lowest frequency (kHz)	P=0.0360	**P=0.0017** *	**P=0.0001**
	Peak frequency (kHz)	**P<0.0001**	**P=0.0002**	**P=0.0017**
Pulse durations 4-8 ms	Pulse interval (ms)	P=0.7908	P=0.1309	P=0.4206
	Highest frequency (kHz)	P=0.4239	P=0.5021	P=0.8422
	Lowest frequency (kHz)	P=0.1721	**P<0.0001**	**P<0.0001**
	Peak frequency (kHz)	**P=0.0001**	P=0.3822	**P<0.0001**
Pulse durations 8-14 ms	Pulse interval (ms)	P=0.0426	-	-
	Highest frequency (kHz)	**P=0.0013**	-	-
	Lowest frequency (kHz)	**P<0.0001**	-	-
	Peak frequency (kHz)	P=0.5145	-	-

* $P<0.0001$ if data A8 removed

they used very brief FM signals (2 to 3 ms). Long-fingered bats did not use durations above 8 ms, however the number of surveys was limited to only three nights in the same period of the season.

Starting, end and peak frequencies

General trends

Scatter plots of starting, end and peak frequency are plotted against pulse duration in figures 6, 7 and 8 respectively.

Starting frequencies were highest in signals of about 2 to 5 ms, they gradually decrease with increasing pulse duration (>5 ms) (figure 6). In the range of 2 to 5 ms durations, there is substantial overlap in the starting frequencies between the three species (figure 6). The lowest starting frequencies were found in the longest signals of the pond bat (figures 6 and 9). The end frequencies of pond bats do not change much between short to long duration calls (figure 7). The end frequency of the calls of Daubenton's bat decreases with increasing duration (figure 7). The lowest end frequencies of the three species were found in the longer signals of Daubenton's bat (figures 7 and 9). Long-fingered bats used high end frequencies (30 to 40 kHz) and medium end frequencies (25 to 30 kHz) (figure 7). In the long-fingered bat, end frequencies were lower in longer signals (25 to 30 kHz, durations 7 to 8 ms), nevertheless they are still higher than in signals of a similar duration of Daubenton's bat (17 to 22 kHz) (figures 7 and 9). Long-fingered bats sometimes used a regular alternation of two types of FM signals with a different end frequency (35 versus 30 kHz, e.g. recording C1, figure 5 left), a feature that was not found among Daubenton's bat or the pond bat.

Peak frequencies generally decreased with increasing pulse duration (figure 8). The pond bat generally used the lowest peak frequencies, those of Daubenton's bat and long-fingered bat largely overlapped (figure 8).

Specific patterns in the short duration pulse range (1 to 4 ms)

In this range of durations, the starting frequencies of Daubenton's bats were higher than those of the other two species (tables 2 and 3) and long-fingered bats used higher starting frequencies than pond bats (figure 6). These differences were statistically significant. End frequencies did not differ significantly between Daubenton's bats and pond bats ($P>0.01$) but were significantly higher in long-

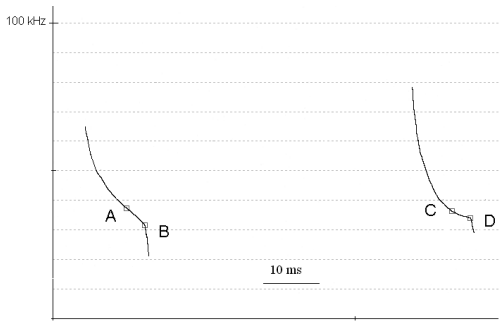

Figure 10. Difference in modulation rates in the last two milliseconds before the final FM part. Left: a 10.5 ms long signal of a Daubenton's bat, modulation rate between A and B: 2.5 kHz/ms. Right: a 10.3 ms long signal of a pond bat, modulation rate between C and D: 0.7 kHz/ms.

fingered bats (tables 2 and 3, figure 7). When recording A8 (Daubenton's bat approaching tree roost and using exceptionally high end frequencies) was excluded from the data set, the difference in end frequencies between Daubenton's bat and long-fingered bat became even more pronounced ($P<0.0001$). Peak frequencies were highest in Daubenton's bats and lowest in pond bats (figure 8).

Specific patterns in the medium duration pulse range (4 to 8 ms)

In the range of medium durations there were not any differences in the starting frequencies of the three trawling species (tables 2 and 3, figure 6). For end frequencies there was a significant difference between the long-fingered bat and the other two species (tables 2 and 3), with the long-fingered bats' being higher (see figure 7). For peak frequencies, the pond bats' had a significantly lower frequency than the other two species (table 3 and figure 8).

Specific patterns in the long duration pulse range (8 to 14 ms)

Starting frequency and end frequency in the long duration pulse range differed significantly between pond bat and Daubenton's bat (tables 2 and 3, figures 6 and 7), although their peak frequencies were not significantly different (table 3, figure 8). Above 8 ms almost all pond bat signals have a QCF part before the final downward FM sweep, Daubenton's bats did not use QCF.

The QCF part

Pond bats start to introduce a QCF part in the middle of the signal as soon as its duration exceeds ca 8 ms. The QCF frequency varies between 31 and 37 kHz (most often 33-35 kHz). The longest pond bat signal without QCF was 9.5 ms, the shortest signal with QCF 7.6 ms. Daubenton's bat do not use QCF, not even in their longest signals (13.8 ms). Instead of introducing a QCF part in the zone just before the final FM, Daubenton's bats gradually decrease the modulation rate of a much wider range of frequencies in the middle of the signal (60 to 30 kHz). Figure 10 shows the difference in modulation rate in the zone of 2 ms just before the final FM between signals of similar duration (around 10 ms) of a Daubenton's bat and a pond bat. In heterodyne, the pulse series of Daubenton's bat, including 10 to 13 ms durations, can be perceived as slightly 'wet' sounds, however when scanning along the frequency band there is no detectable null frequency. When pond bats use signals of 10 ms there is a well marked null frequency (often between 33 and 35 kHz) where the pitch of the wet sound becomes very low.

Discussion

Daubenton's bat

Daubenton's bats only used FM calls, which is in accordance with the literature (Kalko & Schnitzler 1988, Rydell et al. 1999, Siemers et al. 2001, Skiba 2003, Papadatou et al. 2008, Arthur & Lemaire 2009). Most sources mention maximum pulse durations of 7 or 8 ms in Daubenton's bat (Skiba 2003, Arthur & Lemaire 2009). During this study some surprisingly long duration signals in Daubenton's bat

were recorded, including durations between 8 and 13 ms. The peak frequency of these pulses (33-40 kHz) is similar to the pond bat calls of a similar duration, however the pulse shapes are different. The long calls of Daubenton's bat do not have QCF, whereas a QCF-part was always present in signals >10 ms in the pond bat. The flight and echolocation patterns associated with Daubenton's bats emitting long pulses were similar to patterns observed in the pond bat (discontinuous sounds, bats passing at close range without loud sonar emissions, sudden bursts of loud calls, Van De Sijpe & Holsbeek 2007). Some hawking bats are known to skip several consecutive calls in order to wait for the arrival of echoes from distant objects (Holderied & von Helversen 2003). However the periods without high intensity calls that precede periods of loud calls are longer than just a few skipped calls. Therefore this may be a strategy to avoid sonar jamming with nearby conspecifics, as described by Chiu et al. (2008) or maybe a stealth strategy.

When small aquatic insects are abundant on or over the water surface, Daubenton's bats usually emit short FM calls with durations of 4-5 ms. The short durations indicate that the bats are focussing on 'short' distances (Schnitzler & Kalko 2001, Schnitzler et al. 2003). In good hunting conditions there is probably no need for a Daubenton's bat to focus on more distant points. In less favourable conditions, e.g. when the density of small insects is low (i.e. on cool spring nights) one more often hears longer signals, with durations in the range of 6-8 ms. This is also observed in aerial hawking bats, e.g. in pipistrelles (Barataud 1996). By using these longer durations Daubenton's bats are probably trying to increase the detection distance to improve the chances of detecting the few small sized insects available. The small size of the prey probably sets a maximum to the durations that Daubenton's bats can use, because when duration increases the forward masking zone increases in direct proportion (Schnitzler et al. 2003). The smaller the size of the prey, the smaller its 'target strength' and the smaller the detection distance (Waters et al. 1995). The longest signals (8-13 ms) were used during rare behaviour on warm summer nights, when bats also made excursions higher in the air, and suggest that the bats were focussed on larger sized prey items occasionally crossing the air above the water body. Larger prey items have stronger target strengths and allow for larger forward masking zones and hence larger pulse durations (Waters et al. 1995). On warm summer nights many larger insects are on the wing (e.g. Lepidoptera) flying over bodies of water at heights below 3 to 5 m, where they can be detected by trawling bats. Because of the low density of larger insects in the air over open water, long range echolocation will certainly be more appropriate than short range echolocation for efficiently hunting this type of prey.

Although the vast majority of starting frequencies in this study were below or around 100 kHz, in a number of cases they appeared to be higher, up to 118 kHz. Some individuals within a given species have been observed to use exceptionally high frequencies (Markmann & Ronkel 2010). This may be the explanation for the higher frequencies recorded in this study (using a D1000X detector). Most recordings made from Daubenton's bats at very close distance, either with a D1000X detector (own data) or with a high quality Bruel & Kjaer microphone (anonymous referee, personal communication) reveal starting frequencies up to 100 kHz.

The end frequencies recorded match with data in the literature. Kalko and Schnitzler (1998) suggest that each individual may have a preferred end frequency when trawling over the water surface (in their study some individuals used end frequencies of 30 kHz, others of 25 kHz). Similar intra-specific differences between end frequencies were also found here (e.g. recordings A1 versus A2).

In the usual range of durations (1-8 ms) the peak frequencies of Daubenton's bats are between 38 and 50 kHz, generally higher than pond bats. However in the rare long pulses (>8

ms) the peak frequency decreases to between 35 and 40 kHz, creating an overlap of peak frequencies with pond bats. The absence of QCF in Daubenton's bat, even in calls of medium duration, can still be used to separate Daubenton's bats and pond bats in these conditions.

Pond bat

Pond bat echolocation has been studied in detail in the Netherlands, not surprisingly since a large part of the European population of this rare species is concentrated in the country's low-altitude provinces. The signal types (FM and FM-QCF-FM), pulse durations, end frequencies, peak frequencies and QCF frequencies described here are in accordance with the available literature data (Kapteyn 1993, Britton et al. 1997, Limpens et al. 1997, Verboom et al. 1999, Siemers & Schnitzler 2001, Skiba 2003).

Whereas most literature sources mention maximum starting frequencies of 80 to 90 kHz, some starting frequencies in this study exceeded 100 kHz (with a maximum of 110 kHz). Starting frequencies above 100 kHz were found in short duration echolocation signals recorded from pond bats flying close to the microphone, as well as some signals of longer duration (8 ms). However, frequencies above 100 kHz were quite rare and a lot of short signals recorded at close range had starting frequencies around or below 100 kHz. It is not easy to obtain field recordings from this species very close to the microphone, because of its preference for hunting over large open expanses of water, where it is technically difficult to get close to the bat without influencing the flight and echolocation behaviour.

Long-fingered bat

Even though the field surveys of long-fingered bats were limited in this study, they did produce a wide range of pulse durations (1.8 to 8.0 ms). This range corresponds well with data in published literature (Skiba 2003, Arthur & Lemaire 2009). Almenar et al. (2008) found that long-fingered bats not only preyed upon aquatic insect taxa, such as Nematocera and Trichoptera, but also consume terrestrial taxa including Lepidoptera. The authors suggest that long-fingered bats may be able to take Lepidopterans higher in the air. Sudden excursions in the air over water bodies were observed in the long-fingered bat in this study, and are also reported in Daubenton's bat (e.g. in this paper) and in the pond bat (Ahlén 1990, Kapteyn 1995, Van De Sijpe & Holsbeek 2007). Long-fingered bats might possibly use echolocation calls longer than 8 ms when they are focussing on large prey in the air above water surfaces. It can take a lot of time and survey effort, before one comes across rare hunting behaviours that involve echolocation patterns that substantially differ from the 'normal' behaviour (e.g. the observation of Daubenton's bats in this study).

On most occasions the starting frequencies were around or below 100 kHz, although in some recordings substantially higher starting frequencies were detected. Exceptionally high signal-to-noise ratios were recorded from a long-fingered bat that passed very close to the microphone on the bank of a river. The starting frequencies of his recording exceeded 120 kHz. Skiba (2003) previously listed maximum starting frequencies of 90 kHz. Papadatou et al. (2008) and Boonman et al. (2009) mention 100 kHz as a maximum. The few high starting frequencies detected in long-fingered bats suggest these are quite rare situations and may originate from a few individuals in a population who use exceptionally high frequencies. The majority of starting frequencies (even short signals with high signal to noise ratios from short distances) are not higher than 100 kHz, which is in accordance with the literature sources.

The end frequencies correspond well with data listed in Skiba (2003), Papadatou et al. (2008) and Arthur and Lemaire (2009). In Greece, Papadatou et al. (2008) found that the

end frequencies of long-fingered bats (ca 35 kHz) were significantly higher than Daubenton's bats (ca 30 kHz). In France, Arthur and Lemaire (2009) also found that long-fingered bats use higher end frequencies than Daubenton's bat.

Echolocation and flight behaviour

The three European trawling bats use a wide variety of echolocation signals, reflecting their variable flight and hunting behaviours both low over water (their specialised and preferred foraging niche) and over land (where they can exploit habitats in an opportunistic way). All the species can use FM calls of short duration and large bandwidth. This signal type provides the bat with accurate information about distances and angles to objects within their flight path at short ranges. However, the trawling bats appear not to use the very high starting frequencies and bandwidths that are observed in a number of other *Myotis* species, including Natterer's bats (*Myotis nattereri*) and Geoffroy's bats (*Myotis emarginatus*) (Siemers & Schnitzler 2004). The latter are specialised in hunting for prey in confined terrestrial habitats. Increased bandwidths and modulation rates increase the ability to spatially distinguish between two nearby objects (Schnitzler & Kalko 2001, Schnitzler et al. 2003). When occasionally hunting over land, Daubenton's bats and pond bats prefer edge habitats rather than very confined spaces.

The small size of aquatic insects in combination with the large numbers in which they occur explains the short broadband FM calls of high intensity that are typical for trawling insectivorous bats (Rydell et al. 1999). Longer calls would make these prey virtually inaudible, since the forward masking zone created by the long signals is likely to overlap with the small detection range provided by the small target strength of a tiny insect. Long calls are sometimes used in an alternative hunting mode, where the bats focus on larger insects present on the water surface or in the air above the water body. The piscivorous *Myotis* species also use short broadband FM when hunting for fish over water surfaces. They may rely on other sensory systems to detect prey or use random dipping techniques at sites with a high density of fish (Aihartza et al. 2008).

Conclusions

This study of echolocation calls of trawling *Myotis* species in natural flight conditions using a 16 bit detector revealed that some individuals can use higher starting frequencies than currently described in most literature sources. However the majority of starting frequencies did not exceed 100 kHz. High starting frequencies in *Myotis* signals can more easily be recorded using a D1000X detector than other detectors, because of the flat frequency response up to very high frequencies (170 kHz at least), the high sensitivity of the ultrasonic microphone and the large dynamic range inherent to the 16 bit resolution. However even with this detector a lot of short FM calls recorded at very close range did not start at frequencies higher than 100 kHz. The highest frequencies were found only in those sequences captured from the bats flying very close to the microphone and/or pointing the sound beam towards it. Although there is considerable overlap in some of the basic signal parameters, it does seem to be possible to identify the three trawling *Myotis* species in good recording conditions. Pond bats generally use lower peak frequencies and insert a QCF part in signals of medium and long duration, which makes confusion impossible. Daubenton's bats use higher peak frequencies than pond bats and lower end frequencies than long-fingered bats. Pond bats and Daubenton's bats flying over land in confined spaces can emit rather similar short broadband FM calls. In such conditions it can be tricky to identify these species. In short FM signals, the peak frequency

of the pond bat will increase to 40 kHz or even higher. The repertoire of echolocation calls of long-fingered bats presented in this study may be incomplete, although some interesting patterns were found, including sequences in which the bat regularly alternates between two types of FM signals with different end frequency. Finally some sequences of Daubenton's bat revealed medium durations, around 10 ms or even a bit longer.

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Samenvatting

Echolocatiegeluiden van watervleermuizen, meervleermuizen en Capaccini's vleermuizen in natuurlijke omstandigheden

Een studie van echolocatiegeluiden van de drie Europese harkende *Myotis*-soorten met de hulp van een 16 bit bat detector leverde iets hogere startfrequenties op dan doorgaans vermeld in de literatuur. De hoogste frequenties zijn wellicht afkomstig van een beperkt aantal individuen die exceptioneel hoge frequenties gebruikten. De meerderheid van de dieren hadden startfrequenties die beperkt waren tot 100 kHz of lager, zoals ook in de literatuur beschreven. De hoogste frequenties werden enkel gevonden in pulsreeksen van vleermuizen die erg dicht bij de microfoon vlogen en/of die hun sonarbundel naar de microfoon richtten. Alhoewel er veel overlap bestaat in start-, eind- en piekfrequenties lijkt identificatie van de drie

harkende *Myotis*-soorten haalbaar in een aantal omstandigheden. Meervleermuizen (*Myotis dasycneme*) gebruikten in het algemeen lagere piekfrequenties en hebben een QCF-gedeelte in signalen van middellange en lange pulsduur. Dit laatste sluit verwarring uit met de andere twee harkende soorten. Watervleermuizen (*M. daubentonii*) gebruikten doorgaans hogere piekfrequenties dan meervleermuizen en lagere eindfrequenties dan Capaccini's vleermuizen (*M. capaccinii*). Startfrequenties zijn erg variabel, wellicht deels omwille van verschillende afstanden van de vleermuis tot de microfoon tijdens de diverse opnamen (atmosferische uitdoving van hoge frequenties). De drie soorten kunnen vrij hoge startfrequenties gebruiken met maxima tot 110 à 120 kHz wanneer ze dicht bij de microfoon vliegen. Meervleermuizen en watervleermuizen die in gesloten habitat over land vliegen gebruiken korte breedbandige FM-signalen. Onderscheid tussen deze twee soorten wordt dan moeilijk. Bij korte signalen van meervleermuizen kunnen de piekfrequenties rond 40 kHz liggen. Het scala echolocatiegeluiden van de Capaccini's vleermuis is allicht nog onvolledig wegens het beperkt aantal veldexcursies waarbij deze soort bestudeerd werd. Desalniettemin kon interessant materiaal verzameld worden, waaronder pulsreeksen met alternerende eindfrequenties, een echolocatiegedrag dat niet bij water- en meervleermuizen werd waargenomen. Tenslotte werden ook pulsreeksen met middellange echolocatiesignalen opgenomen bij watervleermuizen, waarbij de pulsduur soms meer dan 10 ms bedraagt, maar dergelijke lange pulsen zijn wel heel uitzonderlijk.

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