First recording of the soprano pipistrelle (*Pipistrellus pygmaeus*) in the Netherlands

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Abstract: In the early 1990s, the soprano pipistrelle (*Pipistrellus pygmaeus*) was recognised as a separate (cryptic) species. It is now considered to be widespread in Europe, but was unknown in the Benelux countries until 1998, when the first bioacoustic recording was reported in Belgium. This paper reports the first confirmed record of *Pipistrellus pygmaeus* in the Netherlands verified using bioacoustics. Intermediate pulse intervals of echolocation calls preceding songflight calls and the bandwidth of songflight calls are proposed as new characteristics that can be used to discriminate between *Pipistrellus pygmaeus* and *Pipistrellus pipistrellus*.

Keywords: *Pipistrellus pygmaeus*, soprano pipistrelle, first recording, the Netherlands.

Introduction

Amongst the bat species known to be present in the Netherlands are the two pipistrelle species: Nathusius' pipistrelle (*Pipistrellus nathusii* (Keyserling & Blasius 1839)) and common pipistrelle (*Pipistrellus pipistrellus* (Schreber 1774)). The sibling species of the latter, the soprano pipistrelle (*Pipistrellus pygmaeus* (Leach 1825)), is known to be a rather common species in the countries bordering the Benelux, but had not yet been recorded within the Netherlands (Jones & van Parijs 1993, Sattler 2003) (although Dietz et al. (2007) includes the Netherlands in the species' distribution area). *Pipistrellus pygmaeus* was first reported to be present in Belgium in 1998 (Kapfer et al. 2007, Dekeukeleire 2010).

It seemed just a matter of time before *Pipistrellus pygmaeus* would be found in the Netherlands and several unconfirmed observations have been reported. One of the author's own recordings made at the end of 2007 in a forest lane near Leersum in the Langbroekerwetering seemed to contain faint traces of *Pipistrellus pygmaeus* calls, from a bat probably flying at some distance. In 2008 and the following years, I was able to make closer, distinctive recordings on many occasions at the same location in Leersum, confirming the presence of this species in the Netherlands.

Since then there have been a number of confirmed recordings of *Pipistrellus pygmaeus* all over the Netherlands, in Groningen, Den Haag, and near Utrecht and Zeewolde. In Zundert, near the border with Belgium, a bat captured in July 2011 was morphologically identified as *Pipistrellus pygmaeus*. Last but not least, I recorded two *Pipistrellus pygmaeus* bats calling at the same time near Leersum in September 2011.

This paper, however, will focus on the bat that was recorded in Leersum in 2007.

As they have evolved, European microchiropteran bats have developed different kinds of echolocation calls, with frequencies generally between 10 and 180 kHz. Their calls can be of almost constant frequency (CF), frequency modulated (FM), or a combination of both; pulse lengths of regular echolocation calls range from a few microseconds to 80 ms. Bat species use the pulse characteristics that most suit their needs. Aerial hawkers, flying fast in open spaces, tend to use low, long quasi-constant frequency (qCF) calls allowing them to detect prey and obstacles at long ranges. By contrast gleaners such as *Myotis* and *Plecotus* species that scan foliage for prey at a slow pace, tend to use short, steep FM calls. Pulse characteristics such as maximum and minimum frequency, pulse duration and inter pulse interval, usually differ between species, although some overlap is possible (Barataud 1996, Boonman et al. 2008, Skiba 2009).

Bats also produce sounds for intra and interspecies communication, to attract or repel other bats. The pulses of these types of calls are often lower in frequency, broad banded, longer in duration, more varied and are often repeated quickly in succession. Lower frequencies are used because these are less attenuated by air and thus travel further. These social calls can be emitted when the bat is stationary (e.g. from a tree hole) or whilst flying (songflight calls). The characteristics of these social calls are also often species dependent, although overlaps can also occur (Barataud 1996, Pfalzer 2002, Skiba 2009).

Depending on the quality of recording and the openness of the environment, several European species can be successfully identified on the basis of the characteristics of their echolocation pulses, songflight or social calls, using proper analysis software (Barataud 1996, Pfalzer 2002, Skiba 2009). These characteristics were heavily utilised within this survey (see below).

The two pipistrelle species present in the Netherlands are, in general, easily distinguished on the basis of the frequency of maximum energy of their qCF pulses: for *Pipistrellus nathusii* this is around 38 kHz and for *Pipistrellus pipistrellus* it is around 46 kHz (Jones & van Parijs 1993, Barataud 1996, Skiba 2009). Deviations from these values are possible, resulting in some overlap of the frequencies of these species, which makes discrimination more problematic. *Pipistrellus pipistrellus* calls can also occasionally end above 50 kHz, creating a possible overlap with the calls of *Pipistrellus pygmaeus*, which has a frequency of maximum energy that is commonly between 53 and 57 kHz (Jones & van Parijs 1993).

The study was carried out in the Langbroekerwetering area, located in the centre of the Netherlands, between the Kromme Rijn river and the Utrechtse Heuvelrug (figure 1). It contains several estates and a mosaic of orchards, wooded banks, watercourses, woodlands and pastures. On the instigation of the Utrecht provincial government a three-year research survey, led by Eric Jansen, was started in 2007 by the Dutch Mammal Society and a local nature conservation group called the 'Vereniging Natuur en Milieu Wijk bij Duurstede'. Many volunteers, including the author, took part in this project which aimed to investigate the use of this unique landscape by bats. One of the survey's first goals was to determine the bat species present in the area.

On 23 August 2007 I made a recording of songflight calls, from a *Pipistrellus nathusii* and from what was assumed to be a *Pipistrellus pipistrellus*. Only later that year in October, closer examination of the spectrogram showed a couple of very faint pipistrelle-like qCF pulses of 6 ms long, ending at 56 kHz, with an intermediate pulse interval (IPI) of around 80 ms. The bandwidth of one set of songflight calls seemed to be wider and higher in frequencies than what one would normally expect of *Pipistrellus pipistrellus*. This finding was discussed with two bat experts, Herman Limpens and John Mulder, but it was concluded that there was not enough data to positively identify the calling bat as *Pipistrellus pygmaeus*.

In 2008, my first visit to the area was at 02:00 a.m. on July 5, and after a few minutes I recorded new qCF calls ending at around 55 kHz. On further visits, similar pulse sequences were recorded, many of which were as loud as the typical echolocation calls from *Pipistrellus pipistrellus* flying nearby. Other bat workers working in the field were able to confirm these observations.

On 16 July and 6 September 2008 an attempt was made to capture the calling bat with mistnets, but without success. During the remainder of 2008, I recorded qCF pulse sequences ending above 55 kHz during all my visits to the area except for one, when the weather was bad. In the following years, I recorded similar sequences, culminating in the recording of simultaneous songflight calls with similar characteristics from two bats near Leersum in September 2011.

For comparison purposes, personal recordings of *Pipistrellus pipistrellus* bats near Leersum as well as of *Pipistrellus pygmaeus* bats in the UK are used in this article.

Materials and methods

This survey used heterodyne bat detectors (D100 and D200; Pettersson Elektronik AB, Sweden) which allow for some bat species producing qCF calls to be readily identified in the field. For species that produce FM pulses, e.g. *Myotis* species, time expansion (TE) bat detectors (D240x, Pettersson Elektronik AB, Sweden) were used. On one occasion an Anabat detector (Titley Scientific, Australia) was used with a PDA attached, showing real-time Anabat division graphs. The use of mistnets provided additional data when TE recordings were inconclusive or when other information was desired.

A D240x TE bat detector (307 kHz sample rate, 8 bit resolution) was used to make 3.4 second long recordings. During replay, these recordings were slowed down 10 times by the detector and recorded as TE recordings onto a solid state Transcend T.sonic 520 wav recorder, with a sample rate of 32 kHz and 16 bit resolution. An Edirol R09-HR recorder was also used, with a sample rate of 44.1 kHz and 16 bit resolution. Both recorders stored information in lossless format (i.e as wav files, not as compressed MP3s). The Edirol allowed more tuning of the input signal level and had better recording characteristics than the T.sonic. However, this was barely noticeable during analysis of the recordings since the resulting performance of the D240x, during replay, was lower than those of both recorders.

Two software programs, Cool Edit 96 (Syntrillium Software Corporation, USA) and BatSound (Pettersson Elektronik AB, Sweden) were used for spectrogram and power spectrum analysis. Characteristics such as start and end frequency, maximum and minimum frequency, frequency of maximum energy, pulse duration, IPI and pulse type were measured in an attempt to identify the species that were the source of the recordings.

Results

Songflight calls

Most recordings made in Leersum after mid-July 2007 contained songflight calls interspersed with echolocation calls. Songflight activity was especially high around midnight, gradually shifting forward in the evening as a year progressed. These calls consisted of either three (*n*=9), but more often four syllables (*n*=29), with increasing intensity and duration. The first syllable often had a smaller bandwidth (mean=17.1 kHz, sd=4.3) and all but the last one had a hook-like appearance in the spectrograms, with the last syllable ending in a downward sweep (figure 2). The pitch of the syllables in the calls decreased slightly[1].

The songflight calls covered frequencies from 48.4 (mean=41.2 kHz, sd=3.9) down to 18.0 kHz (mean=21.1 kHz, sd=1.8) (table 1). The median frequency of maximum intensity was 22.2 kHz (*n*=38); the median bandwidth was 23.8 kHz (figure 3). Songflight calls were in general preceded and followed by an echolocation pulse with median IPI lengths of 40.0 ms and 92 ms, respectively (figures 4 and 5).

Similar measurements were taken from

[1] A comparison of the songflight calls of the two bats in Leersum in 2011 showed that the syllables in the calls of the second bat were slightly increasing instead of decreasing, thus making it possible to distinguish the bats from each other.

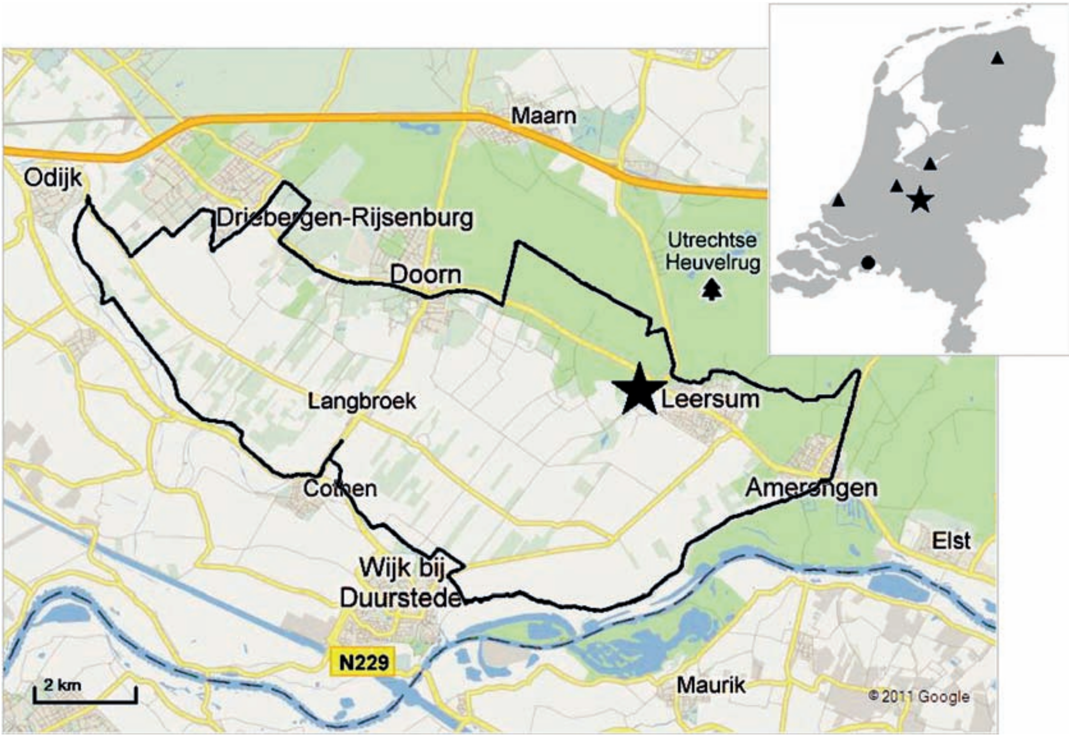

Figure 1. Location of the first confirmed record of the soprano pipistrelle (*Pipistrellus pygmaeus*) (asterisk) in 2007 in the Langbroekerwetering (bounded area) in the centre of the Netherlands. The insert shows other locations of recorded *Pipistrellus pygmaeus* until September 2011 in the Netherlands (triangles: bioacoustic identification; circle: morphological identification).

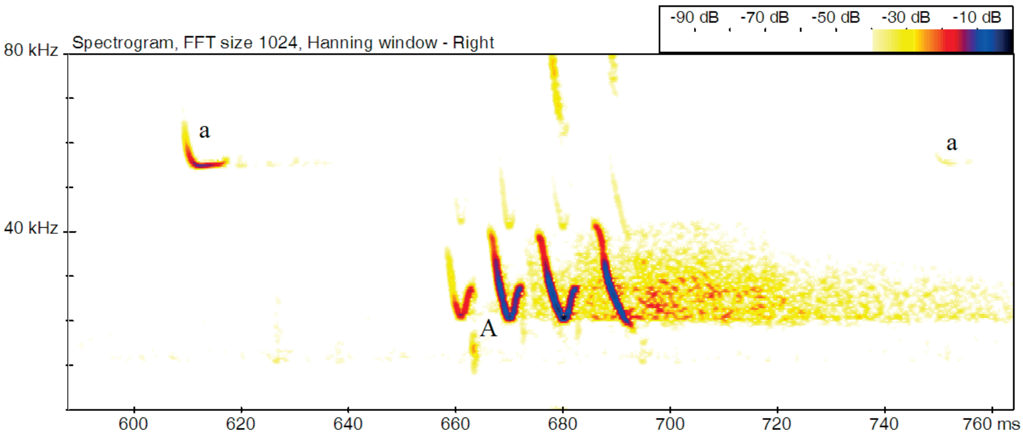

Figure 2. Spectrogram of an echolocation call (a: duration: 8.7 ms, minimum frequency: 54.5 kHz) followed, after 48 ms, by a songflight call (A: four syllables) of the Leersum bat; the recording was made on 22 August 2008, 00:41 a.m., Leersum.

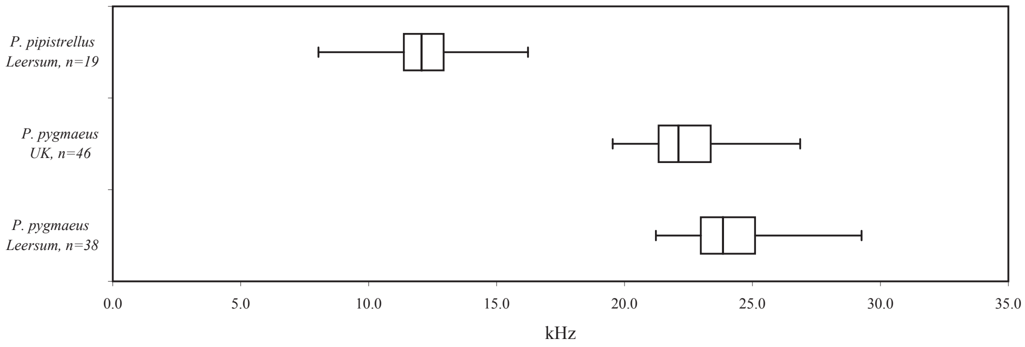

Figure 3. Boxplot showing the bandwidth (median, 25% and 75% percentiles min and max) of songflight calls.

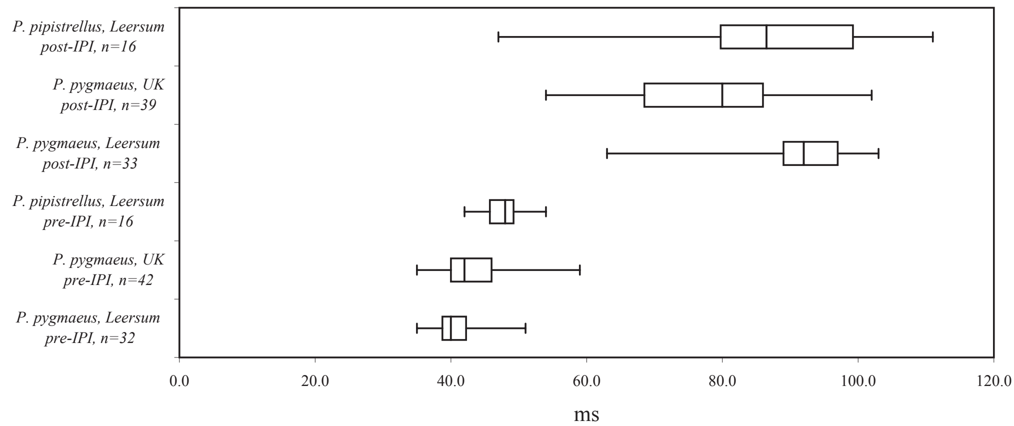

Figure 4. Intermediate pulse intervals (median, 25% and 75% percentiles, min and max) of echolocation calls immediately preceding (pre-IPI) and following (post-IPI) songflight calls.

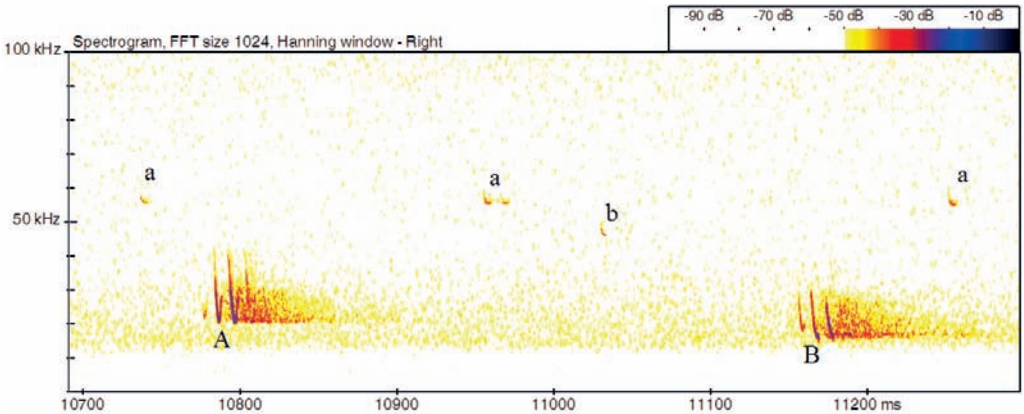

Figure 5. Spectrogram of songflight calls A, B (three syllables) and echolocation calls a, b of the Leersum bat (A, a) and a *Pipistrellus pipistrellus* (B, b); 15 August 2008, 00:13 a.m., Leersum.

Table 1. Number of songflight calls containing 2, 3 or 4 syllables, and the start and minimum frequencies (median, min, max) of complete songflight calls. Authors recordings: Llanthony, Powys, May 2008; Trentishoe, Devon and Ebbesbourne, near Salisbury, August 2009.

	Number of calls *n* syllables long			Starting frequency (kHz) Median (Min-Max)	Minimum frequency (kHz) Median (Min-Max)
	2	3	4		
Pipistrellus pygmaeus near Leersum	0	9	29	41.9 (28.4-48.4)	20.8 (18.0-27.4)
Pipistrellus pygmaeus in the UK[1]	1	31	14	40.0 (28.5-44.7)	19.8 (16.4-22.8)
Pipistrellus pipistrellus near Leersum	1	9	9	26.5 (19.5-33.6)	16.3 (13.6-21.4)

recordings of *Pipistrellus pipistrellus* bats flying near Leersum and *Pipistrellus pygmaeus* bats in the UK (Llanthony, Powys, May 2008; Trentishoe, Devon and Ebbesbourne, near Salisbury, August 2009).

Echolocation pulses

The echolocation pulses from the Leersum bat for sequences without songflight calls showed a bimodal distribution of IPIs. The main peak of IPIs occurred around 82 ms (*n*=48), with a minor second maximum occurring at around 157 ms (*n*=6) (figure 6). The median frequency of the maximum energy of echolocation pulses in sequences without songflight calls was 56.5 kHz, the corresponding median minimum frequency was 56.0 kHz (*n*=60) (figure 7). The mean pulse duration was 5.0 ms (*n*=60, sd=1.0). Echolocation pulse durations tended to be longer in sequences containing songflight calls than in sequences without, but this difference was not further investigated. Similar measurements were obtained from recordings made of *Pipistrellus pipistrellus* bats flying near Leersum.

Discussion

As the Leersum bat has not been captured, a morphological determination (Dietz & von Helversen 2004) could not be performed, hence identification was based solely on the characteristics of the recordings. Measured values for the frequency with the maximum energy, minimum frequency and pulse duration of the Leersum bat are all typical of *Pipistrellus pygmaeus*, especially as the minimum frequency was never observed to be lower than 52 kHz and always maintained normal pulse durations of at least 5 ms (cf. Boonman et al. 2008, Pfalzer 2008, Skiba 2009). *Pipistrellus pipistrellus* is also known to be able to issue qCF calls ending above 52 kHz which means that misidentification is possible (Wicht et al. 2003). On these results alone it is possible that the Leersum bat could be a *Pipistrellus pipistrellus*. However, one would expect the pulse duration to be much shorter than 5 ms and the call intensity to be lower. Unfortunately, Wicht et al. (2003) did not report on exact pulse durations and intensity for the two reported *Pipistrellus pipistrellus* bats. These could have been misidentified as *Pipistrellus pygmaeus* solely on the basis of their echolocation calls above 56 kHz.. The authors also did not report whether these two bats were also able to produce normal *Pipistrellus pipistrellus* echolocation calls, ending at around 46 kHz. Thirdly, the bats were released 'from the hand', which could have influenced call characteristics in several ways, compared to a bat recorded in its natural habitat during normal flight behaviour.

The Leersum bat in this study is clearly distinct from *Pipistrellus pipistrellus* by virtue of several characteristics, although for two char-

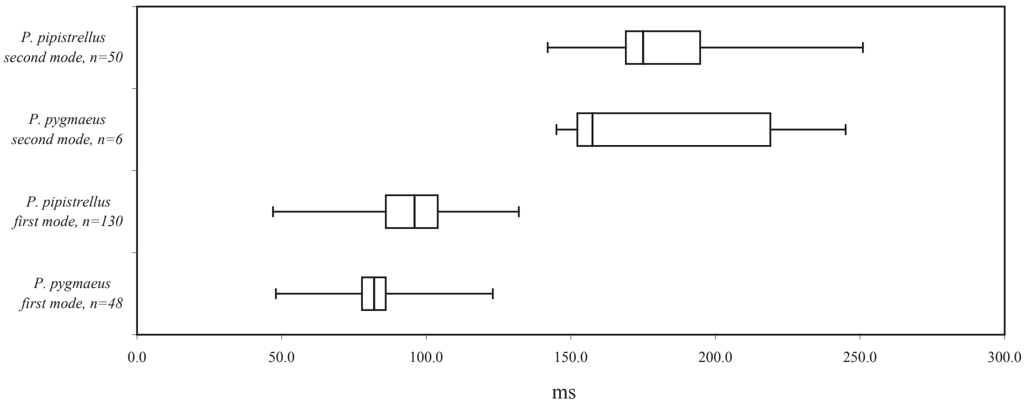

Figure 6. Bimodal distribution of intermediate pulse intervals (median, 25% and 75% percentiles min and max) of echolocation pulses in recordings without songflight calls of bats in Leersum.

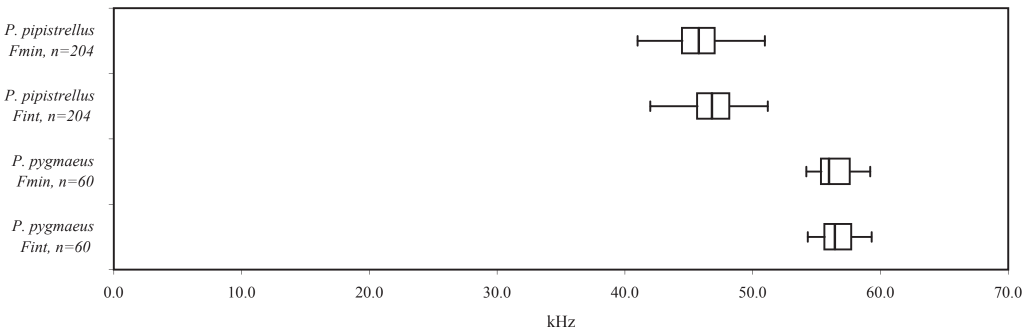

Figure 7. Minimum frequency (Fmin) and frequency of maximum intensity (Fint) (median, 25% and 75% percentiles, min and max) of echolocation pulses in recordings without songflight calls of bats in Leersum.

acteristics there are similarities (figures 3, 4, 6 and 7). Firstly, the overlaps in the plots for the IPIs of an echolocation pulse immediately following a songflight call (post-IPIs) indicate that this characteristic should not be used for differentiation (figure 4). Secondly, the overlap in the box plots for the second peak of IPIs of echolocation pulses in recordings without songflight calls makes identification seem inconclusive (figure 6). However, the overlap, or less well pronounced difference between the two species in the typical values for this second mode of IPIs of echolocation pulses, is well known (Boonman et al. 2008, Skiba 2009).

By contrast the plots for the IPIs of an echolocation pulse preceding a songflight call strongly indicate that these pre-IPIs can very well be used as a differentiation characteristic (figure 4). Wingbeats are strongly synchronised with the emission of echolocation calls (Wong & Waters 2001). This might also be true for songflight calls, which are more intense and have a much longer duration than echolocation calls, and need to be integrated into a bat's call behaviour. Before a bat can issue a songflight call, it first issues an echolocation pulse and listens for echoes to ascertain that it will not encounter any obstacles during its flight. An IPI of 40 ms will cover about seven metres. The bat then issues a songflight call of roughly 30 ms and starts to listen for responses from any other bats, while trying to postpone the issuing of the next echolocation pulse for as long as possible. This could explain the large range of measured post-IPI values. The small range of pre-IPI values seems to suggest that bats have a preferred wing position and stroke direction for making a songflight call and its preceding echolocation pulse. Wong and Waters (2001) report that intermediate pulse intervals of 40-50 ms were the result of echolocation pulses issued at the beginning of the downstroke and at the end of the upstroke in the same wingbeat. The pre-IPI values measured in this article seem to suggest that this is also the case for the songflight call and its preceding echolocation call.

The recorded songflight calls of the Leersum bat had a wide range of bandwidths and the frequency of maximum energy was above 20 kHz, which is typical of *Pipistrellus pygmaeus*. Those of *Pipistrellus pipistrellus* are less wide and lie below 20 kHz (Barlow & Jones 1997, Pfalzer 2008, Skiba 2009). On the other hand, the majority of observed numbers of syllables (four) in the songflight calls of the Leersum bat is more typical of *Pipistrellus pipistrellus*; although some overlap does occur between the two species (Barlow & Jones 1997, Pfalzer 2008, Skiba 2009, personal recordings in the UK) (table 1).

Unfortunately, measurements of other characteristics of the songflight calls from the Leersum bat cannot be compared to the values reported by Pfalzer (2008) for the two species. For *Pipistrellus pygmaeus* Pfalzer only reports on calls consisting of three syllables, whereas the bat in my study mainly uses four. For *Pipistrellus pipistrellus*, Pfalzer made no differentiation between the numbers of syllables in a call, which makes statistical evaluation rather dependent on the distribution of the number of syllables in his set of calls. I suspect pipistrelle songflight call characteristics are highly dependent on the number of syllables in the call; e.g., the total call duration largely depends on the number of syllables.

None of the recordings (until September 2011) showed two bats calling at the same time with qCF calls ending at around 55 kHz. This suggests that all the call sequences were probably obtained from a single, isolated bat resident near Leersum. Furthermore, these calls were only recorded within a relatively small area around Leersum. This means that is unlikely that there is a nearby *Pipistrellus pygmaeus* colony. The large number of songflight calls issued after mid-July indicates that this bat is probably a male. For the same reason, the second bat observed in September 2011 also appears to be male.

It is not yet known why no other recordings had been made of *Pipistrellus pygmaeus* in the Netherlands, nor how this first bat came to be resident near Leersum. Studies have shown that *Pipistrellus pygmaeus* prefers riparian habitats, but there is no valid explanation why the species is so rare in the Netherlands. The increasing number of observations seems to indicate that *Pipistrellus pygmaeus* is finally starting to colonise the Netherlands. My very recent observation of a second male near Leersum could be an indication of that.

Conclusion

The bandwidth of songflight calls and the inter pulse intervals of echolocation calls preceding songflight calls seem to be useful characteristics for identifying *Pipistrellus pygmaeus*. Taken together with other echolocation and songflight call characteristics, the first recorded bat near Leersum in 1997 can be identified as *Pipistrellus pygmaeus*, which has been confirmed by others (H. Limpens, G. Jones, personal communication). Hence, an accidental recording of a bat on 23 August 2007 finally resulted in the recording of *Pipistrellus pygmaeus* as a new species for the Netherlands, an unforeseen spin-off of the Langbroekerwetering bat project.

Acknowledgements: I would like to thank all other volunteers of the Langbroekerwetering survey and specifically its enthusiastic project leader Eric Jansen for the many inspiring events spent together on this survey. I am also grateful to Herman Limpens (Dutch Mammal Society), John Mulder (Ecologisch Adviesbureau Mulder) and Gareth Jones (University of Bristol) for their help and critical comments. I would also like to thank two anonymous referees, Jasja Dekker, and former colleague Douggie Parsons for their reviews, and my wife Liesbeth Dirks who supported my hobby of 'going bats' in numerous ways.

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Samenvatting

De eerste waarneming van de kleine dwergvleermuis (*Pipistrellus pygmaeus*) in Nederland

Na afloop van een vleermuisinventarisatie op 23 augustus 2007 op het landgoed Broekhuizen bij Leersum in de Langbroekerwetering maakte ik in de omgeving extra Time Expansion opnames met een batdetector. Tijdens een spectrogramanalyse in oktober 2007 ontdekte ik enkele zeer zwakke, maar lange *Pipistrellus*-echolocatiepulsen met een eindfrequentie van boven de 55 kHz in een van de opnames, met daarnaast enkele sociale geluiden die typisch zijn voor *Pipistrellus*. In 2008 werden tijdens aanvullende bezoeken aan het gebied opnieuw opnames van pulsreeksen gemaakt met eindfrequenties van boven de 55 kHz, ook door andere vleermuisonderzoekers. Ook in de jaren daarna, tot aan het maken van de definitieve versie van dit artikel in oktober 2011, konden in Leersum dergelijke opnames gemaakt worden. Aan de hand van kenmerken zoals (minimum, maximum, piek-) frequentie, pulslengte en pulsintervallengte kon voor het eerst de aanwezigheid van een kleine dwergvleermuis (*Pipistrellus pygmaeus*) in Nederland worden bevestigd.

Received: 31 March 2009
Accepted: 10 October 2011