The methods adopted by BaTML for recording the echolocation calls of *Pipistrellus* spp. using Time Expansion Detectors and the analysis thereafter

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Abstract

The BATS & The Millennium Link (BaTML) project undertook Time Expansion bat detector surveys during the period 2001 to 2005 in order to record the distribution of *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus* along the canal corridors within the Central Belt of Scotland. This paper reports on our methods for data collection and subsequent analysis.

Key Words: Bats, Millennium Link, surveys, *pygmaeus,* canal, Central Belt, Scotland

Introduction

BATS & The Millennium Link (BaTML) was launched in 2000 to study the bat populations along and adjacent to the canal network in the Central Belt of Scotland (Middleton *et al.*, 2004). During our activities we conducted a survey programme, using Time Expansion bat detectors (TED), to map the distribution of *Pipistrellus pipistrellus* (Common or Bandit pipistrelle) and *Pipistrellus pygmaeus* (Soprano pipistrelle). Due to the recent separation of these species (Barratt *et al.*, 1997) most existing local bat records fell short, in that they could, since the split, only now be described as *Pipistrellus* spp. We therefore undertook to contribute more precise data in order to assist in establishing the local geographical distribution of each species.

In order to allocate the bats we encountered to species level, our analysis methods used the recorded frequencies of maximum energy for these species (Russ, 1999).

An overview of the species being studied

Pipistrellus pipistrellus (Common pipistrelle) was thought to be a single species, however research during the 1990s (as summarised in the following paragraphs) began to suggest otherwise and culminated with the support for its reclassification, after DNA analysis, as two different species (Barratt *et al.*, 1997). These two biologically separate species are now referred to as *P. pipistrellus* (Schreber, 1774) and *P. pygmaeus* (Leach, 1825) (Jones & Barratt, 1999).

Each of these species occupy their own separate maternity and mating roosts (Park *et al.*, 1996) and occur throughout the UK and many parts of Europe, although in some parts of Europe there are areas where either of the two species appear to be the more dominant (Jones & van Parijs, 1993).

Subtle differences in their physical characteristics, which can be difficult to ascertain (Dietz & von Helversen, 2004), have also been documented. *P. pipistrellus* is, on average, slightly larger and also appears to have a dark face giving the impression of a bandits mask, hence one of its commonly referred to names, Bandit pipistrelle. A subtle difference occurs in the pattern of the venation in the wing membranes of each species. A difference is also often apparent when comparing the ratios of the lengths relating to the 2^{nd} phalanx to the 3^{rd} phalanx, on the third finger.

Habitat preferences have also been described. *P. pygmaeus* appears to show a stronger association with habitat featuring water (Vaughan *et al.*, 1997a; Bartonicka & Rehak, 2004) and analysis of its diet (Barlow, 1997) has supported this. *P. pipistrellus* appears to be more of a generalist occurring in a far wider range of habitats (Vaughan *et al.*, 1997a; Russo & Jones, 2003; Davidson-Watts & Jones, 2005), albeit including habitat associated with water features.

Pipistrellus pipistrellus typically produces frequency modulated echolocation calls with a frequency of maximum energy (FmaxE) averaging at circa 45/46 kHz, whilst *P. pygmaeus* produces a similar looking call but at higher frequencies with a FmaxE usually between 52 kHz and 56 kHz (Jones & van Parijs, 1993; Vaughan *et al*., 1997b; Russ, 1999; Parsons

& Jones, 2000). Figure 1 shows a comparison of echolocation calls from each species after recording from a TED and displayed as a spectrogram (i.e. frequency compared against duration/time) using sound analysis software (Pettersson Elektronik AB, BatSound, V3.0). Figure 4 shows a Power Spectrum (the tool used to establish the FmaxE) of a typical *P. pygmaeus* call. The Power Spectrum analysis allows the power of a signal to be compared across the range of its frequency components.

It should be noted that the possible extremities of range of the FmaxE emitted by each species is wide enough to produce an overlap in the frequencies between 48.8 kHz and 51.8 kHz (Russ, 1999). This means that bats emitting a FmaxE within these parameters cannot be safely allocated to species level on the grounds of FmaxE alone. However, when considered in association with call length and end frequency it may be possible to be conclusive (Mayer & von Helversen, 2001).

In addition to their echolocation differences, each species has been shown to have their own characteristic social calls (Barlow & Jones, 1997).

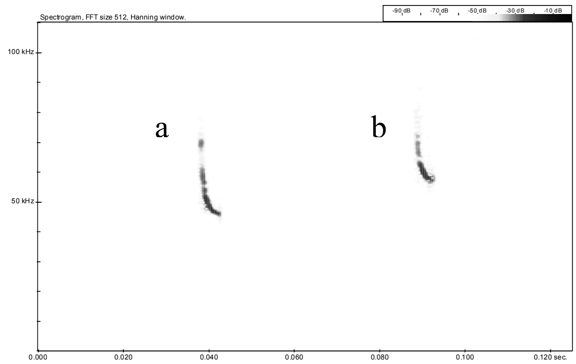


Figure 1: Spectrogram showing typical echolocation calls of *Pipistrellus pipistrellus* (a) and *Pipistrellus pygmaeus* (b)

Methods

Time Expansion - A Summary

This system allows sequences of echolocation to be temporarily captured by a bat detector in real time before being played back for recording purposes at a slower speed (e.g. 10 times). This means that the ultrasonic echolocation (i.e. occurring above the frequencies of normal human hearing) is presented to a listener at a lower frequency and over a longer period of time. For example, if a bat is echolocating at frequencies of 50 kHz over a period of 0.6 sec (beyond human comprehension), this can be slowed down by 10 times to become an audible sequence at 5 kHz and lasting 6 sec. The recorded sound, played back by the detector, is exactly the same as the original, only 10 times slower. This means that it can be captured and retained on a recording device for review using one of a number of computer software tools available for sound analysis. The software allows sound to be analysed through spectrogram (frequency against time), oscillogram (amplitude against time) and power spectrum (power against frequency) features. Figures 2, 3 and 4 give examples of these formats (Pettersson Elektronik AB, BatSound, V3.0) using a *Pipistrellus pygmaeus* recording taken during one of our surveys (Fawnspark, Union Canal, 04.07.2005).

A TED system, used in conjunction with sound analysis software, is in many respects ideal for interpreting bat echolocation. The quality of the results obtained is superior to anything else that currently exists. However, unlike other systems where sound analysis is possible (i.e. Frequency Division), a weakness when using a TED system is the fact that whilst the detector is playing back the results of a captured sequence, it is no longer active as a surveying tool (Parsons *et al*., 2000; Ahlen, 2004). Therefore it must be borne in mind that bat passes will be missed as a result. If quantity, as opposed to quality, is more crucial to a proposed survey method then other systems, or combinations of systems, should be considered.

We wished to focus on identification and distribution, therefore we accepted that we would potentially miss bat passes and when describing any results relating to activity and abundance we would need to make clear the limitations of the methods we adopted.

Data Collection Methods

Figure 5 shows the typical set up of the TED system used by BaTML during our surveys. The TED (Courtpan, EcoTranquility) records all echolocation, regardless of frequency, within its range (15 kHz to 120 kHz). It is set to a time expansion factor of ten times and a signal storage time of 0.640 sec. This signal storage time allows a more than adequate length of recorded time to enable identification relating to *Pipistrellus* spp.

The TED is mounted onto a tripod which is extended to 150 cm in height. A stereo lead is connected from the Record Line Out of the TED to the Microphone Line In on the recorder (Sony Professional Walkman, WM D6C) which is attached to the tripod beneath the detector.

The TED is positioned facing directly across the canal at a 45 degree angle towards the sky. This is in order to maximise the chances of picking up *Pipistrellus* spp. that pass by over the canal corridor. The TED is switched on and the Record/Play buttons on the tape recorder are engaged, quickly followed by the Pause button. A test signal is then generated using the TED in order

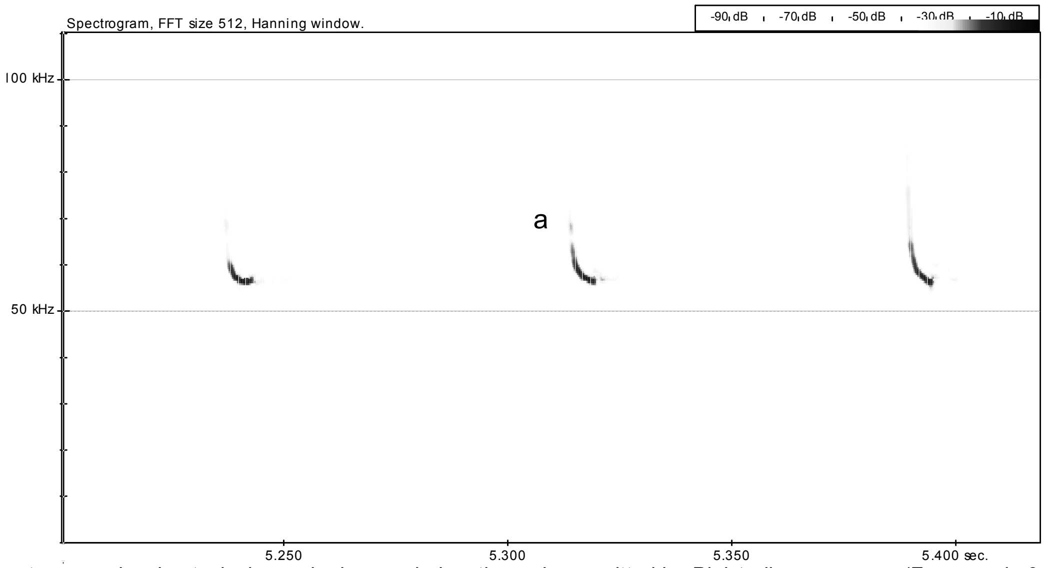


Figure 2: Spectrogram showing typical search phase echolocation pulses emitted by *Pipistrellus pygmaeus* (Fawnspark, 04.07.2005)

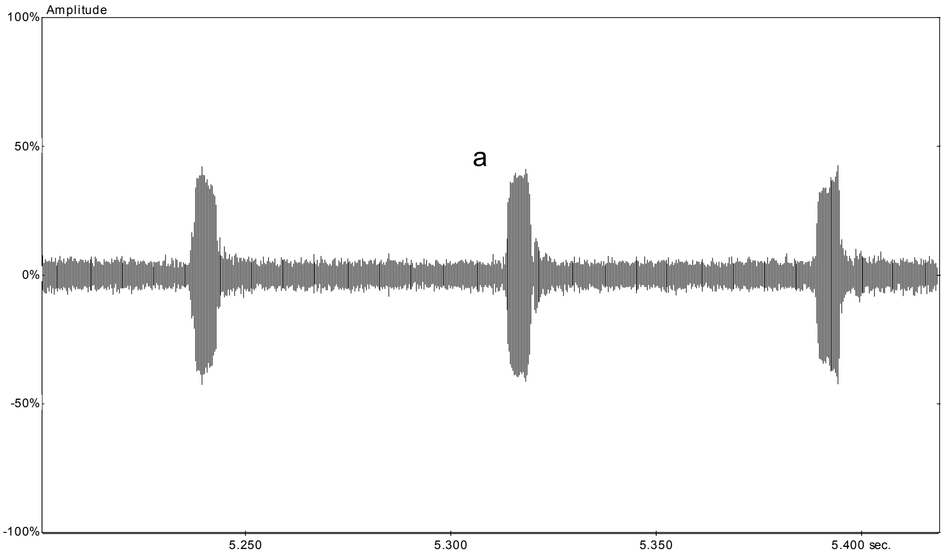


Figure 3: Oscillogram for the same echolocation sequence shown in Figure 1

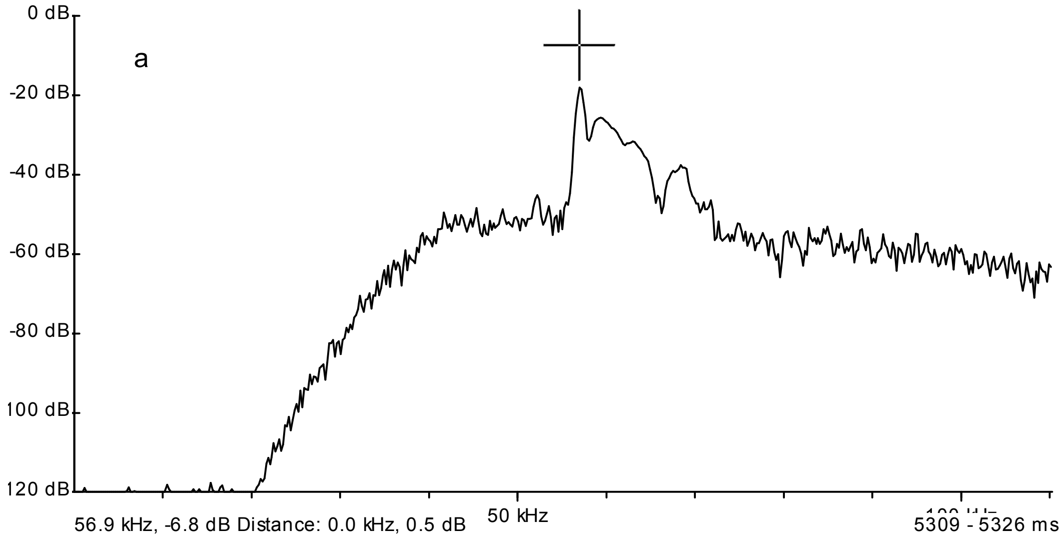
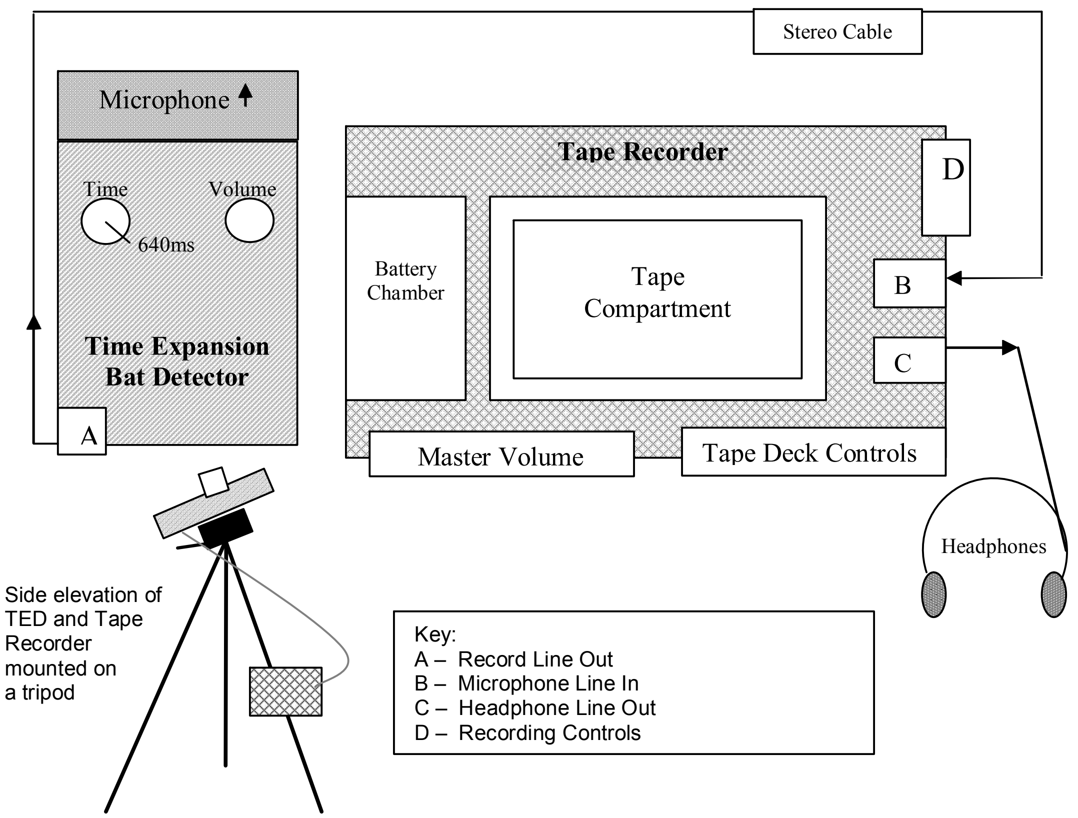


Figure 4: Power Spectrum relating to pulse 'a' shown in Figures 1 and 2. Frequency of maximum energy (FmaxE) = 56.9 kHz

to set the manual recording volume level on the tape recorder. A level that allows a strong signal to be received from the TED without potential clipping/distortion occurring to the received recordings is selected (note that during analysis recordings can be checked to ensure that no clipping has occurred using the oscillogram feature). Headphones are connected from the tape recorder so that surveyors can check what is being recorded during the evening. The Master Volume on the Tape Recorder allows the headphone level to be set independently from the recording volume. Noise coming directly from the speaker of the TED is reduced by turning the volume control of the TED to zero.

At 30 minutes after sunset the Pause button on the tape recorder is disengaged, surveying begins and activity is recorded for 90 minutes thereafter. All data is timed and recorded onto two appropriately numbered C90 tapes (TDK FE, IEC I /TYPE I, Normal Position). Tape 1 is inserted into the recorder at the beginning of the survey and whilst testing the equipment. During the survey, after 45 minutes, Tape 1 is removed and replaced with Tape 2 which upon its completion (another 45 minutes later) concludes the survey. The survey methodology also allows for the collection of weather conditions (Middleton, 2004).

Figure 5: Time Expansion Recording System. Typical set up flow chart (items not to scale)



The BaTML project has successfully used this method to record *Pipistrellus* spp. in Scotland between 2001 and 2005. Over this period, 117 surveys were completed and the equipment and protocol proved to be robust and consistent. Over 5,000 bat passes were recorded and analysed.

Sound Analysis Methods

The tapes from each survey are analysed using sound analysis software (Pettersson Elektronik AB, BatSound, V3.0). The tape recorder is set up and the two tapes relating to the survey are rewound to their starting positions. The tapes are numbered according to the order in which they were used during the survey evening. During the analysis four 23 minute time slots are created so that any changes in behaviour relative to sunset can be considered. All data is recorded onto paper survey forms (Middleton, 2004) and later stored in a digital format (MS Excel and MS Access, 2003).

In order to carry out the actual analysis the tape recorder is connected to a PC via a stereo audio cable. This cable is connected to the Line Out of the tape recorder and a Line In on the PC. Analysis is then undertaken using the spectrogram, oscillogram and power spectrum features within BatSound V3.0. We used a sampling rate of 22.05 kHz, with 16 bits/sample and a 512 pt FFT with a Hanning window for analysis (Pettersson Elektronik AB, 2000; Catto & Agate, 2003; Russ, 1999).

Every sequence of echolocation producing a recording of adequate quality is measured using the Power Spectrum feature (Figure 4). To establish an average FmaxE for each sequence we select three calls within the pass and calculate an average measurement, rounded off to one decimal place. Individual call frequencies are then logged onto a spreadsheet (Microsoft Excel) and the results summarised on our paper survey forms (Middleton, 2004) and MS Access database files. All sound files are labelled and stored on disc in case further analysis is required at a later date.

The analysis adopted was consistent during the five years, and in all but a handful of occasions the same equipment was used throughout. The equipment was regularly checked for under/over performance as we were able to audit actual recording timescales against the 640 ms time frame selected during surveys. In order to further check the equipment/processes, collection and analysis relating to other non-BaTML sites was also introduced. On two occasions we used other TED machines and a minidisk recorder side by side with the equipment used in our main study in order to compare results from the same groups of bats. The analysis compared favourably during these exercises and provided reassurance that our equipment performance and analysis thereafter was robust.

Summary

The survey and analysis methods adopted appear to have supported the aims for this study well and we are now in the process of interpreting the results which will be published separately in the near future.

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