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Abstract

During the recent years studies of bat activity are predominantly based on ultrasound detection. However this method suffers from several biases such as different species call ranges, temporal and habitat-related variability. In order to test the bias linked to the detection of whispering gleaning bats in temperate lowland forests, we equipped several individuals of *Myotis bechsteinii* and *Plecotus auritus* with transmitters and followed them on their foraging grounds where we simultaneously recorded echolocation calls. Our results highlight the very low detectability of these species whose presence was ascertained at the recording station. On the other hand, we detected the presence of many other species. We suggest methodological recommendations for ultrasound detection whenever gleaning bat species are concerned.

Keywords: Acoustic; Detection; Habitat; *Myotis bechsteinii*; *Plecotus auritus*.

Most inventories or monitoring to identify habitat selection by species are based on presence / absence data. Yet it is often difficult to associate the absence of data with the absence of the target taxon (Brotons *et al*., 2004; Mackenzie, 2005; Royle, Nichols & Kery, 2005). A gap in a set of presence-absence data may have important implications for the production of high-quality analysis, and thus for understanding ecological phenomena, or even for implementing conservation strategies. Such analysis can have various biases that depend on the method used, the observers, the environment in which the observations take place, the viewing conditions (such as the weather), the detectability of species, the behaviour of animals at specific and/or individual levels, or the modelling approach (Nichols *et al*., 2000; Kéry & Schmid, 2004; Pellet & Schmidt, 2005; Gooch *et al*., 2006). The identification of the most appropriate method to answer questions relating to the distribution of a taxon, or the selection of habitats by that taxon, is a priority before beginning a study (Tyre *et al*., 2003; Mackenzie, 2005; Mackenzie & Royle, 2005; Lobo *et al*., 2010; Tanadini & Schmidt, 2011).

For bats most recent studies use ultrasonic detection, a method which overcomes the ethics of capturing protected species and has the advantage of recording all bat species emitting a signal likely to be caught by a suitable device (Parsons & Szewczak, 2009; Britzke, Gillam, & Murray, 2013; Fenton, 2013). In addition, this method can be implemented by an observer or by passive recording (Adams *et al*., 2012, Skalak, Sherwin & Brigham, 2012; Stahlschmidt & Brühl, 2012; Froidevaux *et al.*, 2014; Kubista and Bruckner, 2017). However, studies based on ultrasonic detection have many methodological biases due to the equipment, the sampling effort, the acoustic identification and the measurement of activity, which amplify observer effects (Archaux *et al*., 2013), making it difficult to compare results (Britzke *et al*., 2013). In addition, the probability of detection may be relatively low and very different from one species to another (Barataud, 2012). For example, specific detection probabilities obtained over large areas by combining capture and ultrasonic detection, from the historical data of presence and pseudo-absence varied from 0.239 for *Myotis evotis* to 0.532 for *M. californicus* (Weller, 2008). Detectability is even more different among groups of species (e.g. *Eptesicus/Nyctalus*, *Pipistrellus* and *Myotis*, Barataud, 2012; Archaux *et al*., 2013; Froidevaux *et al*., 2016), especially in forests where the signals are generally low due to attenuation by the foliage, so detection is better in open stands than in dense stands (Patriquin *et al*., 2003; Barataud, 2012). Given the different ecology of taxa and their foraging behaviour which are closely linked to their echolocation strategies (e.g. Aldridge and Rautenbach, 1987; Barataud, 2012; Müller *et al*., 2012), we can assume that the ultrasound detection of each bat will depend on the species, the habitat where the study takes place and the animals' activity (related to the season and the weather). Moreover, the information is limited to the number of contacts per species at each recording station, and does not provide any estimate of the actual number of individuals (Barataud, 2012). Therefore ultrasonic detection requires an evaluation of the probability of species detection to assess its usefulness as a survey method for investigating habitat selection (Gorresen *et al*., 2008; Weller, 2008; Hayes, Ober & Sherwin, 2009; Archaux *et al*., 2013; Pauli *et al*., 2017).

Therefore, we investigated the probability of detecting echolocation calls of two whispering bats (emitting weak echolocation calls detected at 5-20 m according to Barataud, 2012 and Skiba, 2009) in forest whilst being sure of their presence by radio telemetry. We detected simultaneously other species or other individuals of the same species for checking the efficiency of the bat detector.

Recording stations were located in the forest of Tronçais (Auvergne, France; 46°39'N, 02°41'E). This forest is dominated by sessile oak (*Quercus petraea*), which is regularly accompanied by beech (*Fagus sylvatica*). The understory is dominated by oak (*Quercus petraea*), beech (*Fagus sylvatica*) and holly (*Ilex aquifolium*), more rarely by hornbeam

(*Carpinus betulus*) and hazel (*Corylus avellana*). Forest stands can reach more than 40m high, a height which can therefore limit the detection of bats from the ground when they are hunting in the canopy. The canopy is closed, and scattered bushes cover between 20% and 50% of the understory.

We were able to capture three adult females of *M. bechsteinii* (8.5, 9.1 and 11.5 g), and six adult females of *P. auritus* (7.9 ± 0.3 g) using mist nets across pathways and streams in July 2008 (good weather conditions, temperatures more than 10°C). Following Aldridge & Brigham (1988), we equipped them with BD2N transmitters (0.43 g, manufactured by Holohil Systems Ltd., Carp, Ontario, Canada for a total weight, including surgical glue, less than 6% of each bat weight). This study is part of a broader project licensed by the Ministry of Environment. These bats were then monitored by radio telemetry (using a Regall 2000 receiver and a three element Yagi antenna manufactured by Titley Inc., Australia), and located using the "homing in" technique, a close approach method (see Amelon *et al.*, 2009). When the animal's position was ascertained, a second grounded operator stood underneath the foraging animal recorded all bat activity at this station for ten minutes using a Pettersson D1000x bat detector. Bats were identified using both heterodyne and time expansion (with Batsound 3.3 software) proposed by Barataud (2012). For *M. bechsteinii* identification was ascertained by the context of sequences following Barataud (2005). One contact was counted every five seconds for all bat species (maximum 120 contacts). Two individuals of one species recorded at the same time were therefore counted as two contacts.
Using a binomial distribution [B (N, 0.5), where N is the number of radiotracked individuals] we calculated the probability of acoustic detection of both species p ($x \leq n$), where n is the number of times the species should be detected given that at least one individual with a

transmitter was present at the recording station (number of possible contacts) and x the number of times it was detected (number of recorded contacts).

M. bechsteinii was recorded during 10 minutes at 25 different stations (8.3 ± 4.7 per individual), four in young plots (trees under 12m), 17 in old-growth plots and four at a border of old-growth plots. *P. auritus* was recorded at 35 stations (5.8 ± 1.6 per individual), all in old-growth plots.

During the twenty-five recording periods conducted in presence of radiotracked *Myotis bechsteinii*, this species was contacted four times for a total of 108 contacts (Poisson distribution mean: 0.036), with 97 contacts in one old-growth plot (Table 1); for the latter we could not discriminate the presence of one or several individuals. During the thirty-five recording periods of *Plecotus auritus* this species was contacted two times with only one contact (Poisson distribution mean: 4.76^{E-4}). These detection scores are very low. The probability that *Myotis bechsteinii* was detected four times is $p\ (x \leq 4) = 4.55^{E-4}$. The probability that *Plecotus auritus* was detected two times is $p\ (x \leq 2) = 1.84^{E-8}$. Moreover, we detected bats of these two species but not necessarily the radiotracked individuals and always one animal per contact.

At the sixty recording stations we also recorded at least 12 additional bat species and some *Myotis* sp. that were not identified (16 contacts at 8 stations). The most detected species was *Pipistrellus pipistrellus* (595 contacts at 47 stations), followed by *Myotis myotis* (32 contacts at seven stations), and *Nyctalus* sp. (18 contacts at only two stations). *M. nattereri* and *M. mystacinus* were detected in ten and nine periods respectively for 14 contacts each. Other taxa were occasionally detected (Table 1).

Our results show that gleaning bats were only slightly detected in forest whilst they were present. In addition, the number of contacts remained very low, except for one recording station of *Myotis bechsteinii* with ninety-seven contacts in ten minutes, which means almost a permanent detection of the likely radiotracked individual. The ultrasound detection of these gleaner bats is reported to be difficult. Over 38,371 call sequences obtained with ground automatic detectors, Müller *et al*. (2012) recorded *M. bechsteinii* sixty-nine times (for 11530 call sequences only identified as medium-sized *Myotis*) and *Plecotus* only three times. Other tests provided similar results on North American gleaning bats (Skalak *et al*., 2012; Kennedy, Sillett & Szewczak, 2014; Luszcz *et al.*, 2016). This possibly explains why Kubista and Bruckner (2017) found a perplexingly high variability with batcorders placed ca. 10m apart. Most often we did not detect the target species, even if we detected other taxa, sometimes with a high number of contacts for *P. pipistrellus*, but most of the time with only a small number of contacts, including loud calling species such as *E. serotinus*. Thus, an absence of ultrasonic detection or a very small number of contacts does not necessarily mean the absence or the scarcity of the target species. It is indeed common to confuse the lack of contact with the absence of species (Mackenzie, 2005; Albert & Thuiller, 2008). We know for example that populations are often poorly estimated and are even considered absent when the detectability of species is low, as with some amphibians and birds (Mackenzie & Royle, 2005; Tanadini & Schmidt, 2011). Integrating detection bias is nevertheless an essential component for any ecological study, and generally requires methodological adjustments during field observations (Kéry & Schmid, 2004; Pellet & Schmidt, 2005), in particular for bats (Hayes, 2000; Weller, 2008; Barataud, 2012; Archaux *et al*., 2013).

Our results are mainly explained by the echolocation parameters and hunting behaviour of the two studied species. These gleaning bats emit weak echolocation calls and forage in cluttered habitats. *Myotis bechsteinii* and *Plecotus auritus* are two species of forest bats which forage in the foliage (Plank *et al.*, 2012). The range of their echolocation calls is low: ten meters for *Myotis bechsteinii* and five meters for *Plecotus auritus* in forest understory (Barataud, 2012). Moreover, *M. bechsteinii* can hunt insects directly on foliage without emitting ultrasounds (Siemers & Swift, 2006), and could prey on insects in the canopy (Plank *et al.*, 2012), becoming inaudible to a ground observer. *P. auritus* catch moths flying or resting in the foliage (Robinson, 1990; Anderson & Racey, 1991; Shiel *et al.*, 1991; Swift, 1998; Ashrafi *et al.*, 2011), emitting then ultrasonic signals with very low range (Swift, 1998; Barataud, 2012) or hunt by passive listening (Anderson & Racey, 1993). In most situations, the two studied bats are poorly audible by a ground observer unless one animal flies near the bat detector. Our results demonstrate that it is not possible to use acoustic detection from the ground only to survey and monitor forest bats. The use of automatic detectors could be particularly relevant as they afford the installation of microphones in the canopy, as suggested by Rieger & Nagel (2007), Collins & Jones (2009) and Müller *et al.* (2012). However, while automatic recorders offer very long listening periods (Skalak *et al.*, 2012; Stahlschmidt & Brühl, 2012), active detection by an observer can maximize the number of recording stations (Tyre *et al.*, 2003). Moreover, the analysis of recorded calls requires differentiating groups of species, between those which emit their signals over long distances and therefore are easily detectable, and those with a very limited signal range (a few meters) (Hayes *et al.*, 2009; Barataud, 2012). Some studies only provide generic results due to the difficulty in obtaining correct species identification of several groups of taxa, including medium-sized *Myotis* species (Rieger & Nagel, 2007; Collins & Jones, 2009, Barataud 2012). This has little consequence regarding *Plecotus* whose species share quite similar ecology (Ashrafi *et al.*, 2011; Swift, 1998). This is detrimental for *Myotis* species which have very different niches and population trends (Meschede & Heller, 2003; Müller *et al.*, 2012).

In addition, we can issue two types of recommendations, according to the purpose of the study. For inventories, as detection rate is locally low for several bat species we recommend increasing the number of ground recording stations per night in the same forest. For studies of habitat selection, we support using several automatic detectors recording simultaneously bats from the ground to the canopy overnight or, depending on target species, ground observers with microphones also from the ground to the canopy for a long time (one hour or more). One limit then could be the huge amount of files to analyze and timetable and financial trade-offs will drive survey protocols depending on the objectives.

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Table 1: Number of contacts of bat species at recording stations performed in the presence of *Myotis bechsteinii* (3 adult females, 25 stations, left – 1-4: young plots, 5-21: old-growth plots, 22-25: border of old-growth plots) and *Plecotus auritus* (6 adult females, 35 stations in old-growth plots, right) in temperate lowland forests.

Station	*P. pipistrellus*	*P. kuhlii*	*E. serotinus*	*Plecotus sp.*	*B. barbastellus*	***M. bechsteinii***	*M. emarginatus*	*M. nattereri*	*M. mystacinus*	*M. myotis*	*Myotis sp*
1	1							1			
2	3					**5**	1				4
3	3					**1**		1		23	
4			1								
5	72							1			
6	4									1	1
7	5										
8	21										
9	35		3								
10			1			**97**					
11					1						4
12			1					1			
13	1							1			
14	1						1			1	
15	4		1					1		3	
16	37			3							
17											
18	4	3			1						
19	4										
20	9	1				**5**					
21	7								1		
22	6	1			1						
23	8			2				3			
24	31	3	4								1
25	7										
a	263	8	11	5	3	**108**	2	9	1	28	6
b	20	4	6	2	3	**4**	2	7	1	4	3

Station	*R. ferrumequinum*	*P. pipistrellus*	*P. kuhlii*	*E. serotinus*	*Nyctalus sp.*	***Plecotus sp.***	*B. barbastellus*	*M. daubentonii*	*M. nattereri*	*M. mystacinus*	*M. myotis*	*Myotis sp*
1		5						21				
2									2			
3		1								4		4
4							2					
5		1					5		1			3
6												
7												1
8				1						1	2	
9		9								1		
10		6										
11		3	2									
12		6										
13		26				**1**						
14						**1**						
15		1								1	1	1
16	1	1										
17		23										
18		18										
19										2		
20		3							2			
21		19								2		
22		8										
23		3										
24		5										
25		17	4									
26		11	1								1	
27		23			6							
28		3			12							1
29		5										
30		42								1		
31		72						2				
32		16										
33		2										
34		3		2								
35										1		
a	1	332	7	3	18	**2**	7	23	5	13	4	10
b	1	27	3	2	2	**2**	2	2	3	8	3	5