

Foraging behaviour of *Myotis mystacinus* and *M. brandtii* in relation to a big road and railway in south-central Sweden

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Abstract

Roads and railways are increasing worldwide. One taxa that is affected by this is bats. Bats are directly killed by vehicles and experience behavioural changes around roads and railways. There are different mitigation measurements to help bats cross roads safely, including different over- and underpasses. One genus of bats that has been shown negatively affected by roads and railways in many areas is *Myotis*.

In this study we examined the behaviour of two *Myotis* species: *M. mystacinus* and *M. brandtii*. We tested the hypothesis that large roads and railways crossing a forest dominated area act as barriers for these species and that they would use over- and underpasses to cross the road and railway safely. To study this, we conducted an auto-box survey and radio-tracked individual bats. We had no recordings or direct observations where bats crossed the road or railway directly. We did, however, observe bats using both over- and underpasses. Our results suggest that large roads and railways act as barriers for *M. mystacinus* and *M. brandtii*, in the sense that they avoid crossing the road and railway. We can conclude that these species use mitigation measurements, such as over- and underpasses, in areas where they are available. It is therefore important to include these mitigation measurements when constructing new roads and railways.

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Introduction

Worldwide, the increase of large roads and railways is causing a decline in biodiversity. These large roads and railways are fragmenting landscapes, which in turn can lead to reduced population size and lowered reproductive success for many animals (Shepard *et al.*, 2008; Boonman, 2011; Medinas *et al.*, 2012; Vandeveld *et al.*, 2014). Previous studies have shown that roads act as barriers for many taxa, such as salamanders, hedgehogs, bears and snails (Shepard *et al.*, 2008). Another taxa that is affected by these barriers is bats (*Chiroptera*; Bach *et al.*, 2004; Kerth & Melber, 2009; Medinas *et al.*, 2013). Since many bat species use forests for roosting and foraging, they are declining worldwide partly due to fragmentation of forests (Kerth & Melber, 2009). Decrease in bat population sizes can be caused by direct mortality from vehicles and reduction of quality of their habitats. The roads also cause behavioural changes in the bats where they avoid the roads and therefore change their foraging and commuting behaviour (Bach *et al.*, 2004; Shepard *et al.*, 2008; Berthinussen & Altringham, 2012a; Medinas *et al.*, 2013).

Other than fragmentation, light pollution is one effect that can cause behavioural changes in bats (Stone *et al.*, 2009; Hölker *et al.*, 2010; Stone *et al.*, 2012). Stone *et al.* (2009) found in an experiment that traditional high-pressure sodium lights have a negative impact on the activity of lesser horseshoe bats (*Rhinolophus hipposideros*). The same team also found that the activity of *R. hipposideros* and *Myotis* species is reduced by LED street lights. On the other hand, they did not see any negative effects on the activity of *Pipistrellus pipistrellus*, *P. pygmaeus* and species of *Nyctalus* and *Eptesicus*. These species are more fast-flying than *R. hipposideros* and *Myotis* species. Their different foraging behaviour can therefore be related to the different effects of artificial lighting (Stone *et al.*, 2012).

As these results indicate, different bat species are affected differently by roads. Kerth and Melber (2009) found that bat species that hunt close to the vegetation (e.g. *Myotis bechsteinii*) are more negatively affected by motorways than species that hunt in more open spaces (e.g. *Barbastella barbastellus*). Schaub *et al.* (2008) found in an experiment about noise pollution that *Myotis myotis*, a species that uses passive listening when foraging (i.e. listens for sounds made by the prey), was negatively affected by traffic noise. Railways are also affecting different species differently. Vandeveld *et al.* (2014) found that railway verges act as positive habitats for *P. pipistrellus* and *N. leislerii* in areas where agriculture was dominant, while they have negative effects on the foraging behaviour of *Myotis* species.

Some measurements to aid bats to cross roads and railways safely are considered when constructing new infrastructure. These aids can include adding underpasses, tunnels, overpasses and different bat bridges (Bach *et al.*, 2004; Berthinussen & Altringham, 2012b). In this study, the bats had access to a wildlife passage (overpass), an underpass with water and vegetation, a car tunnel and a car bridge.

The aim of this study was to observe the foraging behaviour of *Myotis mystacinus* and *M. brandtii* bats in connection to a large road and a railway in a forest dominated area in south-central Sweden and to map the bat activity. We tested the hypothesis that larger roads and railways crossing forest dominated areas act as barriers for *Myotis mystacinus* and *M. brandtii* and consequently no, or very few bats, would cross the road. We also tested the hypothesis that underpasses and

overpasses are being used as alternatives to crossing the road and railway directly. To study this, we conducted an auto-box survey and radio-tracked individual bats.

We chose to work with *Myotis* species because they generally avoid foraging in open places (Ekman & de Jong, 1996) and therefore, we predict that the road will act as a barrier for these species. *Eptesicus* and *Nyctalus* species tend to forage and commute in more open places (Boughey *et al.*, 2011), we therefore believe that these species will cross the road more easily. As shown in previous studies (e.g. Kerth & Melber, 2009; Vandeveld *et al.*, 2014), roads and railways do have negative impacts on *Myotis* species. We chose specifically to work with *M. mystacinus* and *M. brandtii* because they are forest-living species and relatively common in the area.

Materials and methods

Study object

Myotis mystacinus and *M. brandtii* are two very similar species (Fig. 1), previously combined to the same species (Baagøe, 1973). They are both small bats with an adult weight of between four and seven grams. *M. mystacinus* is generally slightly smaller than *M. brandtii* (Dietz *et al.*, 2009). *M. mystacinus* and *M. brandtii* most frequently forage in forest habitats, along edges and above lakes and streams (Wermundsen & Siivonen, 2008; Dietz *et al.*, 2009).



Fig. 1. To the left: *Myotis mystacinus* with radio transmitter on the back and reflective ring on the forearm. To the right: *Myotis brandtii*. (Photo: Johnny de Jong)

Study area

The study was conducted between late June and early August 2015 in the area around Ullbro, 5 km west of the city of Enköping in south-central Sweden. The area was dominated by agriculture and coniferous forest. The area is crossed by a railway and motorway, which run parallel to each other west of the wildlife passage. Crossing the road and railway was possible via an underpass with water and vegetation, a wildlife passage (overpass), a car tunnel or a car bridge (Fig. 2-4).

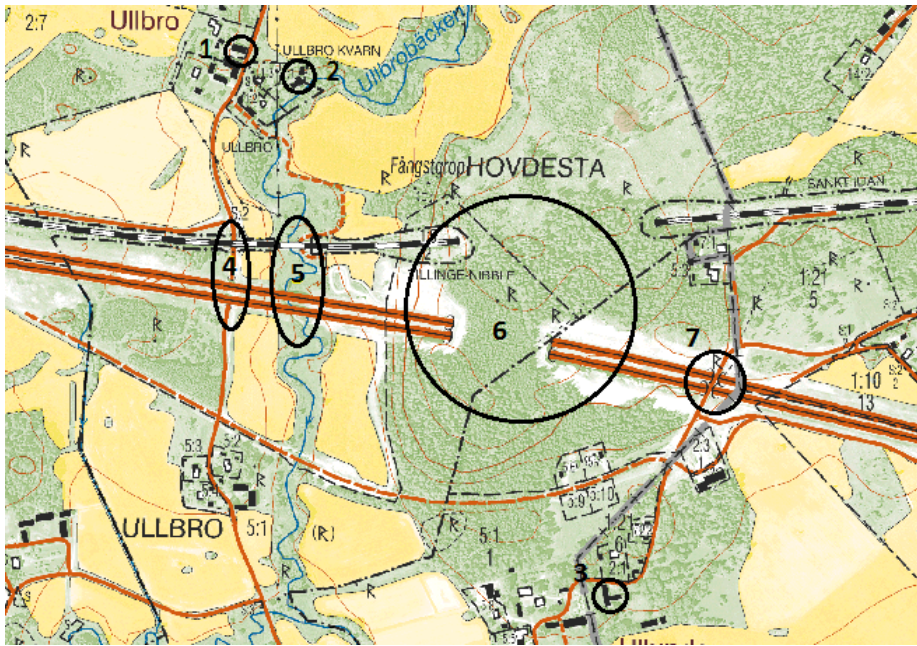


Fig. 2. Map over the area around colonies and motorway and railway. 1 & 2) *M. mystacinus* colony. 3) *M. brandtii* colony. 4) Car tunnel. 5) Underpass with water and vegetation. 6) Wildlife passage (overpass). 7) Car bridge.



Fig. 3. The underpass with water and vegetation west of the wildlife passage. (Photo: Johnny de Jong)



Fig. 4. The wildlife passage seen from the east side. (Photo: Johnny de Jong)

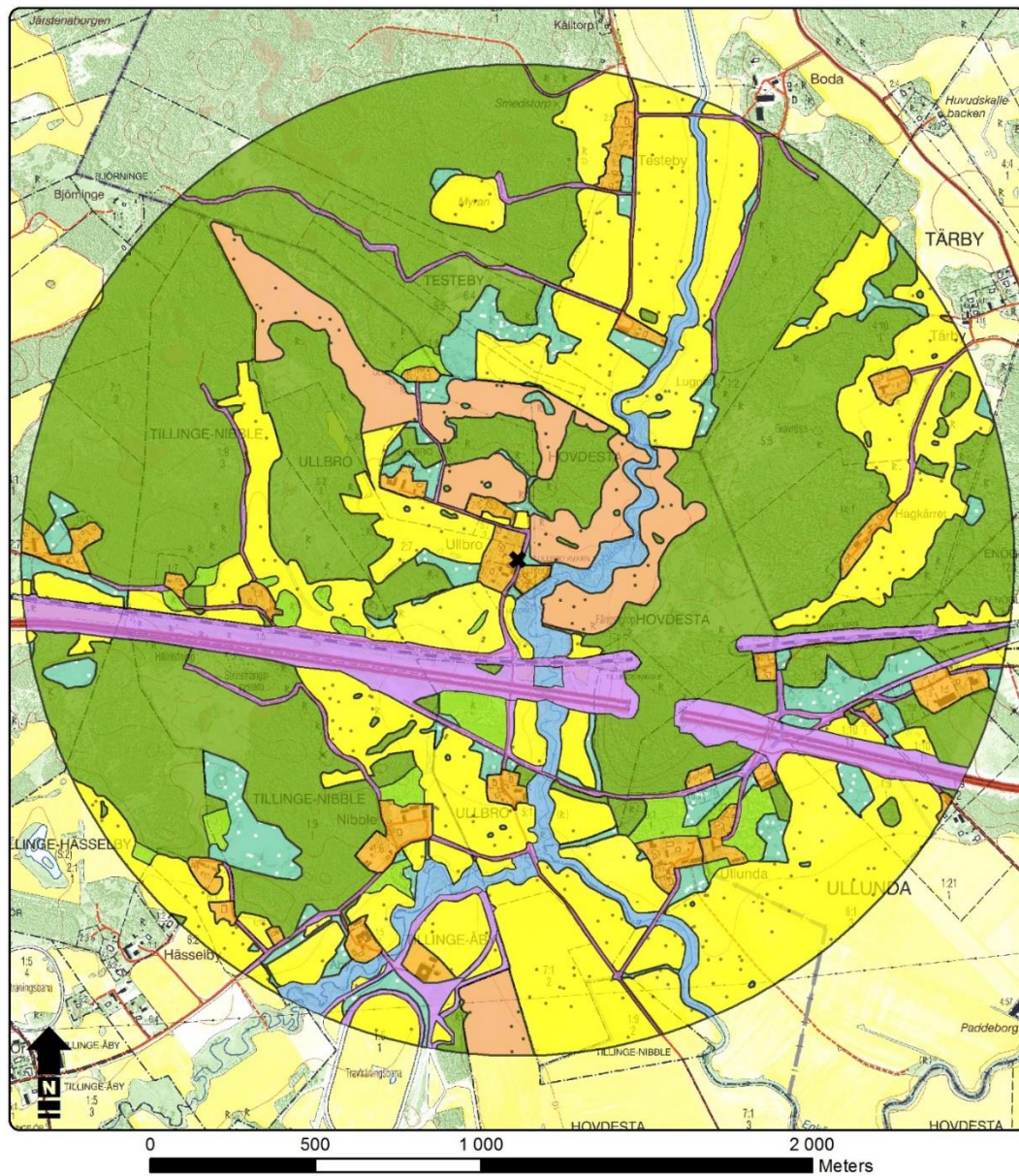
The different habitats of the landscape were mapped using ArcGis (Fig. 5, 6), and classified as: deciduous forest, field, forest, open grassland, road, *Salix* plantation, stream and small village. Stream included the whole corridor with surrounding vegetation. Village was defined as areas with buildings, such as houses and barns, and the area between these buildings. If the property had a big lawn but no more buildings, the area was categorized as open grassland. The forest was dominated by coniferous forest, but may have had some deciduous trees as well. Where possible to distinguish deciduous forest from coniferous, the area was categorized as deciduous forest.

The *M. mystacinus* colony was located 250 m north of the railway and motorway. The landscape within a 1,5 km radius from the colony was dominated by coniferous forest, 44,5 %. The second largest biotope was agricultural fields, 29,0 % (Fig. 5). The *M. brandtii* colony was located 350 m south of motorway. The landscape on the south side was dominated by agricultural fields, 49,3 %, while coniferous forests only made up 23,2 % of the area (Fig. 6).

Auto-boxes

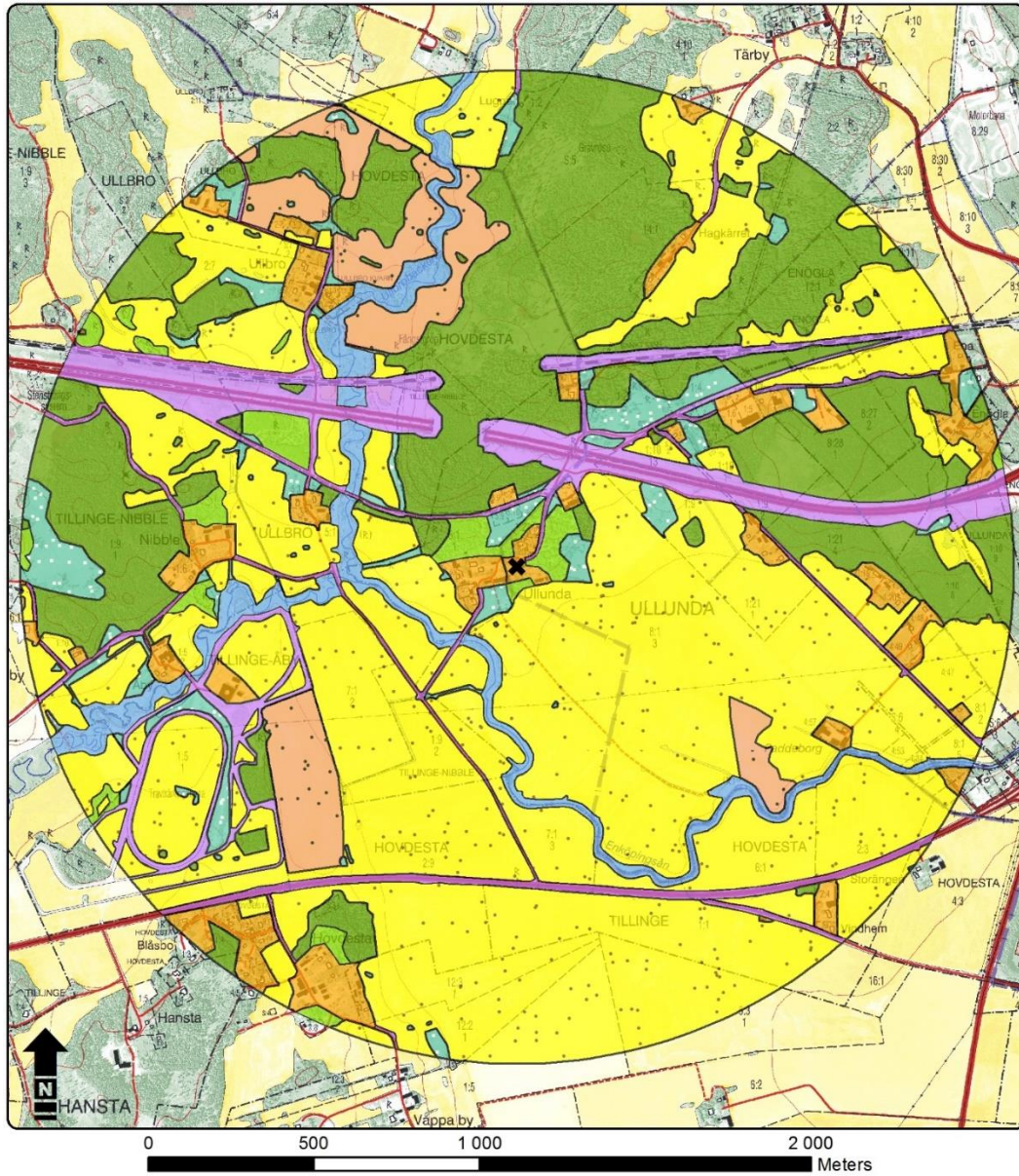
In order to measure bat activity near the road, we used auto-boxes to record ultrasounds (Pettersson Elektronik AB, model D500 X). We put two auto-boxes on top of the wildlife passage, two in a forest nearby as controls, one right by the motorway and one on top of a car bridge (pointing down to the motorway). We also put eight boxes along a small stream leading from the *M. mystacinus* colony area and under the motorway and railway (Fig. 7). These boxes were programmed to automatically record ultrasonic sounds emitted from bats between 22:15 and 04:00. The following settings were used: recording sensitivity (very high), sample frequency (500), pretrig (off), rec-length (3), HP-filter (y), autorec (y), input gain (60), trigger lvl (30) and interval (5). The data was analysed both automatically and manually with Omnibat (Ecom AB). When analysing the data, we only focused on *Myotis* species. The only *Myotis* species observed in the area were *M. mystacinus* and *M. brandtii*. Since these species have similar foraging behaviour and sound, we did not separate the recorded species. The boxes at the wildlife passage with

controls were recording during one night every week for six weeks, the boxes by the motorway and along the stream were recording one night for two weeks.



Biotope		
■ Deciduous forest (1.8 %)	■ Village (3.8 %)	✱ <i>Myotis mystacinus</i> colony
■ Forest (44.5 %)	■ Field (29.0 %)	
■ Open grassland (5.4 %)	■ Salix plantation (4.6 %)	
■ Stream (3.0 %)	■ Road (7.5 %)	

Fig. 5. *Myotis mystacinus* colony with mapped landscape of 1,5 km radius from the colony.












Biotope		
 Deciduous forest (1.7 %)	 Village (5.4 %)	 <i>Myotis brandtii</i> colony
 Forest (23.2 %)	 Field (49.3 %)	
 Open grassland (3.9 %)	 Salix plantation (4.6 %)	
 Stream (3.7 %)	 Road (8.3 %)	

Fig. 6. *Myotis brandtii* colony with mapped landscape of 1,5 km radius from the colony.

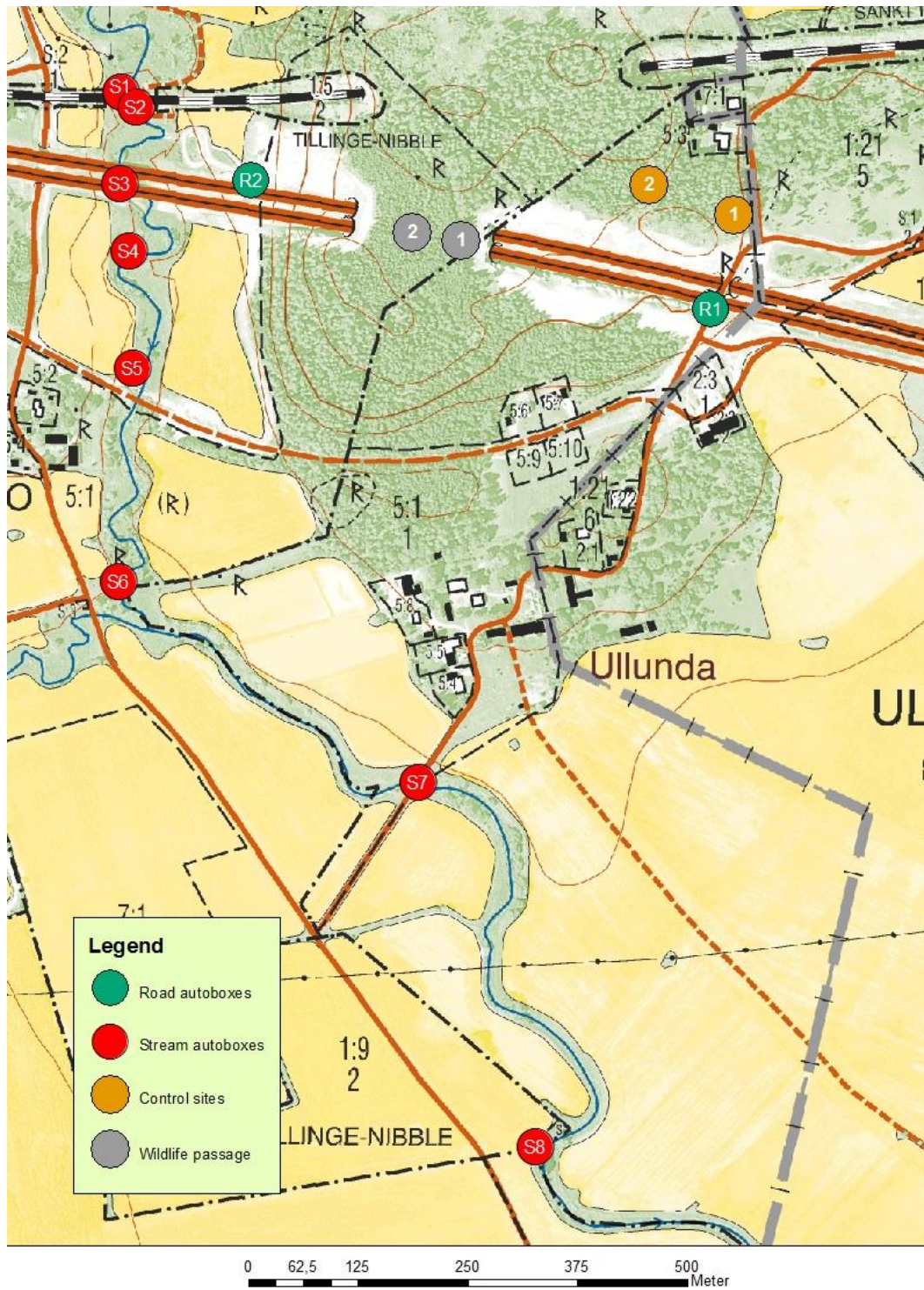


Fig. 7. Map over the auto-box locations. The boxes by the motorway are marked with green; along the stream are red; the wildlife passage control are orange; the wildlife passage are grey.

Radio-tracking

Bats were caught with mist nets outside the colonies or at known hunting grounds close to the colonies. When caught, the bats were gently removed from the net and species, gender and age were determined. Rings with reflective tape were put on the forearms for visual identification. A radio transmitter (Holohil systems Ltd) was then glued (Sikema AB) to the bat between the shoulder blades. The bats were allowed to adapt to the transmitters for at least one night. We listened for the bats with radio receivers and external antennas (FollowIt, model RX 98). From fixed listening points, which we reached either by foot or car, we noted the degrees of direction in which we heard the bat, an estimate of how far away the bat was (visual, close, middle, middle far or far) and the activity of the bat (resting or flying). We noted the bat's position every 15 minutes unless it flew away. We tracked in total 15 bat individuals (11 *M. mystacinus* and four *M. brandtii*) over the course of 37 nights (for five nights we did not track at all due to heavy rain). We focused on one or two bats per night, when we had time we also listened for other bats. As a mean, we tracked the bats for four nights. Two bats were only tracked for one night while one was tracked for eight nights.

When analysing the data, I divided every hour in periods of 15 minutes. I then compared every bat's notes for each night with the mapped landscape. Every biotope used in a 15 minute period was counted as individual observations. The same biotope was only counted once per 15 minutes. All observations of *M. mystacinus* was then added together, the same procedure was used for *M. brandtii*. The results were then tested with a χ^2 test (Neu *et al.*, 1974) to see differences between observations and expectations in the usage of biotopes. This was made to analyse where the bats foraged in relation to the different biotopes. I paid special attention to open biotopes (fields, open grassland and roads) to see if the bats avoided open areas in general or specifically the road. I then focused on where the bats foraged close to the road, and how they behaved close to it.

Results

Auto-boxes

Auto-boxes S1-S8 were placed along the stream, with S1 just north of the railway and S8 1,3 km from S1 to the south (Fig. 7). Boxes S2 and S3 had the most recorded activity, in total 374 and 556 observations respectively. Box S6 also had high recorded activity, 256 observations. Boxes R1 and R2 were placed on top of a car bridge (pointing down to the motorway) east of the passage and down by the motorway west of the passage respectively. Neither of these boxes had recordings of *Myotis* bats (Table 1).

On the wildlife passage, the box with most recordings of *Myotis* bats was WP1 (total 106 observations), except for week 4. WP1 was placed at the edge of the wildlife passage, on the east side. WP2 was placed in the middle of the passage and had a total of 62 observations (Fig. 7, Table 2).

Table 1. Number of *Myotis* observed along the stream and by the road. Boxes S1-S8 were placed along the stream. Box R1 and R2 were placed on top of a car bridge over the motorway and down by the motorway respectively. S = stream; R = road.

Box	S1	S2	S3	S4	S5	S6	S7	S8	R1	R2	Total
Period 1	124	273	248	23	3	152	1	1	0	0	825
Period 2	6	101	308	11	1	104	33	49	0	0	613
Total	130	374	556	34	4	256	34	50	0	0	1438

Table 2. Number of observed *Myotis* on top of the wildlife passage and the control sites. WP = wildlife passage. WPC = wildlife passage control.

Box	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Total
WP1	12	20	21	24	20	9	106
WP2	4	3	7	40	8	0	62
WPC1	0	5	0	11	5	0	21
WPC2	4	18	0	7	4	1	34
Total							
WP	16	23	28	64	28	9	168
WPC	4	23	0	18	9	1	55

Radio-tracking

The data was analysed by χ^2 according to Neu *et al.* (1974). We used a significance level of 5 %, but alpha was changed to 0,1 % with Bonferroni correction (Dunn, 1961). The results from the radio-tracking show that both *M. mystacinus* and *M. brandtii* most frequently forage in village habitat (37,2 % and 72,2 % respectively), despite the relatively low abundance of this habitat (3,8 % and 5,4 % respectively; Fig. 8, 9). The usage of village habitat was more than expected for both species (Table 3, 4). The second most used habitat by *M. mystacinus* was forest, 29,5 %, which was the most abundant habitat, 44,5 %. Forest habitat was, however, used less than expected. The third most used habitat was streams, with 19,6 % of all *M. mystacinus* observations and a landscape coverage of 3,0 %. Streams were used more than expected by *M. mystacinus*. Deciduous forest was used as expected (2,5 % usage, 1,8 % coverage). *Salix* plantation was also used as expected (9,7 % usage, 5,0 % coverage). The *M. mystacinus* individuals foraged less than expected in all open habitats: field (0,2 % usage, 29,0 % coverage), open grassland (1,4 % usage, 5,4 % coverage) and road (0,0 % usage, 7,5 % coverage).

The *M. brandtii* individuals used deciduous forest second most frequently, 12,2 %, while the landscape coverage was 1,7 %. The usage of deciduous forest was as expected. The third and fourth most frequently used habitats were forest, 7,8 %, and stream, 6,7 %. Forest covered 23,2 % of the landscape and was used less than expected. Stream covered 3,7 % and was used as expected. Open grassland was also used as expected (1,1 % usage, 3,9 % coverage). The remaining open habitats were used less than expected: field (0,0 % usage, 49,3 % coverage) and road (0,0 % usage, 8,3 % coverage). The *Salix* plantation was also used less than expected (0,0 % usage, 4,6 % coverage). In total, 444 observations were made of *M. mystacinus* and 90 of *M. brandtii*.

No observations were made where the bats crossed the road or railway directly. Four *M. mystacinus* individuals foraged along the stream close to the road and

railway, whereof two passed under the railway and the motorway to forage further south. No *M. brandtii* bat was observed foraging along the stream close to the road or railway. Four bat individuals (three *M. mystacinus*, one *M. brandtii*) was foraging on the wildlife passage, whereof only one *M. mystacinus* crossed both railway and motorway to forage on the other side of the passage. Two *M. mystacinus* individuals flew 1 and 1,3 km west from their colony, where they foraged in a deciduous forest 100 m north of the railway and in a small village 150 m north of the railway respectively. *M. brandtii* generally foraged in a small area, mostly close to the colony barn 350 m south of the motorway. One *M. brandtii* individual moved 500 m west to another small village and hunted by the stream behind a barn, where it also roosted.

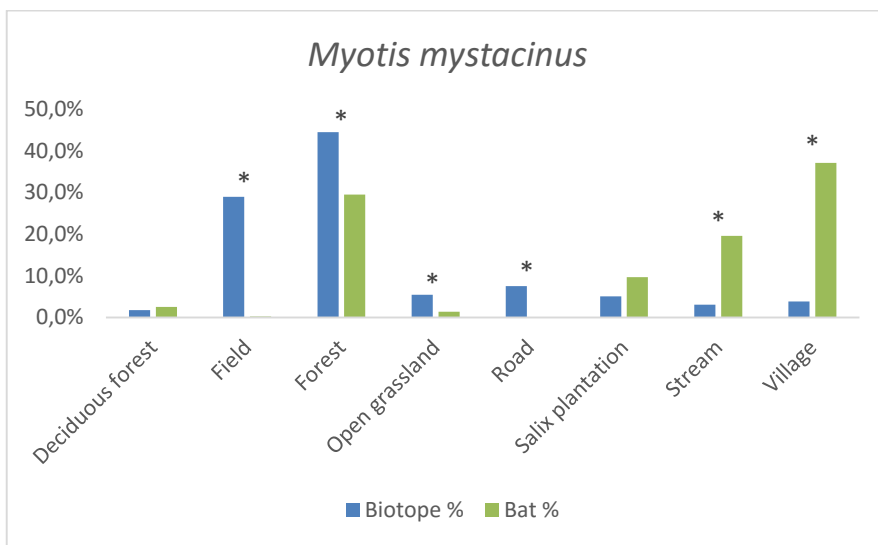


Fig. 8. Percentage of *M. mystacinus* observations in relation to the amount of different biotopes. Blue columns = percentage biotope; green columns = percentage bat observations. Asterisk (*) indicates significant difference in usage of biotope, compared to expected. Total 444 observations of *M. mystacinus*.

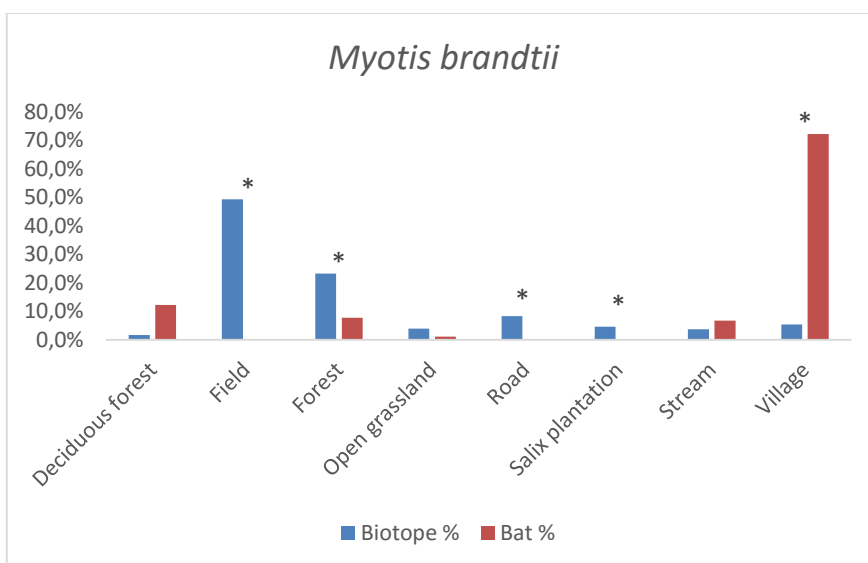


Fig. 9. Percentage of *M. brandtii* observations in relation to the amount of different biotopes. Blue columns = percentage biotope; red columns = percentage bat observations. Asterisk (*) indicates significant difference in usage of biotope, compared to expected. Total 90 observations of *M. brandtii*.

Table 3. Habitat selection of *Myotis mystacinus*. Analysed by χ^2 according to Neu *et al.* (1974). We used significance level 5 %, but with Bonferroni correction alpha was changed to 0,1 % (Dunn, 1961).

Biotope	Area prop. (p _n)	Obs.	Prop. of obs.	Exp.	Conf. interval	p
Deciduous forest	0,018	11	0,025	8	-0,002 <p ₁ < 0,052	ns
Field	0,29	1	0,002	129	-0,006 <p ₂ < 0,010	<0,001
Forest	0,445	131	0,295	198	0,216 <p ₃ < 0,374	<0,001
Open grassland	0,054	6	0,014	24	0,008 <p ₄ < 0,020	<0,001
Road	0,075	0	0	33	0 <p ₅ < 0	<0,001
<i>Salix</i> plantation	0,05	43	0,097	22	0,045 <p ₆ < 0,149	ns
Stream	0,03	87	0,196	13	0,127 <p ₇ < 0,265	<0,001
Village	0,038	165	0,372	17	0,288 <p ₈ < 0,456	<0,001
Total	1	444	1,001	444		

Table 4. Habitat selection of *Myotis brandtii*. Analysed by χ^2 according to Neu *et al.* (1974). We used significance level 5 %, but with Bonferroni correction alpha was changed to 0,1 % (Dunn, 1961).

Biotope	Area prop. (p _n)	Obs.	Prop. of obs.	Exp.	Conf. interval	p
Deciduous forest	0,017	11	0,122	2	-0,005 <p ₁ < 0,249	ns
Field	0,493	0	0	44	0 <p ₂ < 0	<0,001
Forest	0,232	7	0,078	21	-0,026 <p ₃ < 0,182	<0,001
Open grassland	0,039	1	0,011	4	-0,029 <p ₄ < 0,051	ns
Road	0,083	0	0	7	0 <p ₅ < 0	<0,001
<i>Salix</i> plantation	0,046	0	0	4	0 <p ₆ < 0	<0,001
Stream	0,037	6	0,067	3	-0,030 <p ₇ < 0,164	ns
Village	0,054	65	0,722	5	0,549 <p ₈ < 0,896	<0,001
Total	1,001	90	1	90		

Discussion

Our study shows that motorways and railways act as barriers for *Myotis mystacinus* and *M. brandtii* in a forest dominated area. The bats did not cross the road or railway directly, but used either the wildlife passage or the underpass with water and vegetation. By radio-tracking, we could, however, only observe two bats (*M. mystacinus*) that used the underpass to cross both the road and railway. They even used the area directly under the road and railway as a hunting ground. Three *M. mystacinus* and one *M. brandtii* were flying on the passage above the road, whereof only one *M. mystacinus* crossed both the railway and motorway to hunt on the other side. Our result from the auto-boxes show, however, high activity under the railway and the motorway. The results from the auto-boxes on the wildlife passage also suggest more activity than we observed from the radio-tracking. This indicates that the wildlife passage and the underpass are being used more frequently than we observed via radio-tracking of 15 individuals. The reason for the low use of the passage and the stream by the *M. brandtii* colony could be explained by the fact that they had enough resources close to their colony and did not have to fly far away to forage. The *M. mystacinus* colony roosted right by the stream and had no problem finding it and to follow it. In total, seven bats (six *M. mystacinus*, one *M. brandtii*) followed the stream both south and north and foraged by it. The *M. mystacinus* bats could also easily follow a path between the stream and the *Salix* plantation leading

them to the wildlife passage where they could forage. The use of the underpass by *M. mystacinus* bats in our study agrees with the results from Berthinussen and Altringham (2012b). They found that underpasses work as safe alternatives to cross the road directly if they are located at already existing commuting routes. This allows the bats to find and use the underpass without changing their direction or flight height. Since the *M. mystacinus* bats in our study roosted right by the stream, it is safe to say that the underpass was located in their natural commuting route.

Auto-boxes

The results from the auto-boxes along the stream (Table 1) show that *Myotis* bats most frequently foraged by boxes S2 and S3, which were located just south of the railway and just under the motorway respectively (Fig. 7). We also recorded high activity at box S6, which was located south of the small village where a *M. brandtii* bat chose to forage and roost. There was almost no activity at box S5, even though it was located north of that village. This could be because it was located north of a small forest road that crossed the stream, so the natural linear elements of the stream were disrupted. Further north was low activity at box S4, this clearly demonstrates that the bats did not forage much further south than box S3, right under the motorway. Boxes R1 and R2 were located on top of a car bridge east of the passage and down by the motorway west of the passage respectively. There were no recordings of *Myotis* bats at these boxes, which supports our hypothesis that *Myotis* species do not cross the road directly.

Most recordings on the wildlife passage occurred at the east edge of the passage. This can be related to the fact that the middle of the passage was very cluttered, with no proper path leading the bats from one side to the other. The bats in our study seemed to prefer to fly and forage along the edge of the passage forest instead of in the middle of it. The wildlife passage in our study acted as a natural hunting ground for the bats, and not only as a commuting route. The recordings on the wildlife passage and by the underpass suggests that these are being used more than we observed via only radio-tracking.

Radio-tracking

The landscape surrounding the *M. mystacinus* colony (Fig. 5) was dominated by forest, 44,5 %, and agricultural fields, 29,0 %. Forest was the second most frequently used habitat with 29,5 % of all *M. mystacinus* observations, while fields were only used in 0,2 % of the cases. Forest was, however, used less than expected. All open biotopes (field, open grassland and road) were used less than expected, which confirms that *M. mystacinus* avoid open areas. The most frequently used habitat, however, was village, with 37,2 % of all observations and only 3,8 % of the landscape. Village habitat was clearly used more than expected. One obvious explanation of this pattern is that the colonies were located in village habitat, but probably also that insect abundance was high in this habitat due to varied vegetation structure, deciduous-rich forest and the stream nearby. Streams were used more than expected. We observed in total six out of 11 *M. mystacinus* bats foraging along the stream. The usage of *Salix* plantation was as expected. We observed the bats

foraging by the edge of the *Salix* plantation, mostly close to the stream corridor. Deciduous forest was used as expected by *M. mystacinus*, with a coverage of 1,8 % and usage of 2,5 %.

Fig. 6 shows that the landscape around the *M. brandtii* colony was dominated by agricultural fields, 49,3 % of the area. We did, however, not observe any bat activity in these areas, and the usage of fields was obviously less than expected. The usage of roads was also less than expected. The usage of open grassland, however, was as expected, with low landscape coverage (3,9 %) and low use (1,1 %). This confirms that *M. brandtii* avoids foraging in open areas. The second most common biotope, forest 23,2 %, was only used 7,8 % of the observations, which was less than expected. This can be explained by the fact that these coniferous forests were located further away from the colony than deciduous forests. The deciduous forests only make up 1,7 % of the area, but were used 12,2 % of the observations, as expected with corrected alpha. This was the second most used biotope by the *M. brandtii* bats in our study. The relatively high usage of deciduous forest can be related to their close location to the colony village, which was the most frequently used biotope, with 72,2 % of the observations (more than expected). The village biotope only makes up 5,4 % of the area, it is therefore clear that our *M. brandtii* individuals preferred to forage close to the colony in the village, rather than to fly to the coniferous forests to hunt. The use of such a small area by the *M. brandtii* colony can be explained by the fact that they had enough suitable habitat and food close to the colony and therefore did not have to fly further away to forage. The *M. brandtii* colony was also quite small, so the competition over food was presumably low.

The *M. brandtii* bats had their colony south of the wildlife passage, they could therefore quite easily have used it to cross the motorway and access more hunting grounds on the other side. We did, however, only observe one *M. brandtii* individual, and one night, that was on top of the passage, but it never crossed the motorway. We could also observe one bat that had moved from the ordinary colony barn to another barn in a small village 500 m west of the colony. There it foraged over the stream area behind the barn and made a trip to the forest edge below the passage and even visited the ordinary colony. The next night it was back at the new barn and hunted over the stream the whole night.

We made no observations of bats crossing the railway or motorway directly. When we observed bats close to the railway or road, they were foraging under these or on top of the wildlife passage. We did not observe any bats that flew along the railway or road or in any other way flew close to the road in height with it. The *M. mystacinus* individual that moved 1 km west from the colony to forage in a deciduous forest followed, as far as we could observe, the forest edge by the fields to get there, and not close to the railway. The same behaviour was observed for the *M. mystacinus* individual that flew 1,3 km west from the colony. Our results suggest that the bats avoid crossing large open areas, such as the railway and motorway and large fields. We know, however, that the bats crossed small roads and small areas of fields when commuting, but they never stayed in these areas to forage.

Conclusion

Through our study, we can conclude that motorways and railways that cross forest dominated areas act as barriers to *Myotis mystacinus* and *M. brandtii*. They act as barriers in the sense that the bats avoid the roads and railways and preferably do not cross these. With our results, we can also confirm that *Myotis* bats use underpasses and overpasses to safely cross the road. We do not have any observations where the bats crossed the railway and motorway directly. This is, however, not strong enough evidence to say they never do. From this study we can conclude that when the bats have the possibility to cross the road and railway without being exposed in the open landscape, they use that possibility. If the bats have no other choice than to cross the road directly in order to access good hunting grounds, they might do so. The results from our study show that *Myotis* bats follow streams with vegetation and that these kinds of underpasses work just as well as overpasses. We therefore propose that natural vegetation in connection to streams should be conserved and that these areas become underpasses to wildlife when new roads and railways are constructed. When adding underpasses and overpasses to roads and railways, it is important that these are located at already existing bat commuting routes. To be able to conserve bat species it is necessary to consider potential barrier effects when planning constructions of roads and railways. By adding underpasses and overpasses to the roads and railways, humans can mitigate the dangers posed to bats.

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