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Department of Ecology

# Bat activity at a major road in Sweden

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## Abstract

Humans have changed ecosystems steadily over the years, leading to habitat fragmentation and loss. Road- and railroad networks are rapidly expanding around the world and disintegrating natural landscapes. Habitat loss, habitat fragmentation and habitat degradation are three factors why bat species are threatened, and infrastructure such as roads has the potential to contribute to all of these factors. This is the first study of bats and the impact roads have on bats in Sweden. In this study we tested whether the major road acts as a barrier to movement of four different taxa of bats. Especially we focused on the activity of *Myotis* species at various environments in the landscape in relation to a major road. We placed auto boxes that automatically record bat ultra sounds in 34 study sites around Enköping for seven weeks. We had 8 sites along a major road, 8 sites in open gaps between forests without any roads, 4 sites at wildlife passages and 14 control sites within the forest. The results of this study show avoidance behaviour in *Myotis*, and *Eptesicus* species. For *Nyctalus* the road does not reveal to be any obstacle. Observations of *Pipistrellus* species were too few to draw any conclusions. An important conclusion in this study is that there is not a difference in *Myotis*- activity between the road and open gaps in the forest. Both types of openings are avoided. However, wildlife passages are used for foraging and commuting. It is important to do further studies to improve the mitigation measures for bats in infrastructure projects in Sweden. More knowledge about the different taxa's behaviour near major roads and an understanding of the thresholds, such as limit distances for *Myotis* in open gaps are necessary to draw conclusions about mitigation measures and alternatives for ecoducts.

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## 1. Introduction

Humans have changed ecosystems steadily over the years, leading to habitat fragmentation and loss. Road- and railroad networks are rapidly expanding around the world and disintegrating natural landscapes. They play an important role for humans by, among others, improving communication, but they also have a serious impact on biodiversity and ecological functions. Road ecology attracts increasing interest among ecologists, but the effects of roads on wildlife need further research (Berthinussen & Altringham 2012). Lack of knowledge typically comprises ecosystems and populations and various groups of animals such as birds and bats. Roads can affect wildlife negatively and produce direct and indirect effects on the ecosystem. Some of these effects, besides from the direct mortality, are habitat degradation and destruction, noise- and light disturbance, pollution, genetic isolation, animal avoidance and also mortality in roadside areas and near the roads (Ogden 2012). Habitat fragmentation is one of the main reasons why many species are endangered nowadays. It can lead to decreasing population size, increased mortality during dispersion, reduced reproduction potential and reduced gene flow. Factors that in long term can increase the risk of population extinction and reduce biodiversity (Soule 1986). Studies prove that roads are a strong barrier for some species where high mortality while crossing the roads and behavioural avoidance being two of the most important factors. Though, studies also have shown that roads can be a dispersal corridor for some species (Shepard et al 2008)

Habitat loss, habitat fragmentation and habitat degradation are three factors why bat species are threatened, and infrastructure such as roads and railroads has the potential to contribute to all of these factors. Bats capacity to fly does not confer immunity to major roads, because most bat species are normally foraging close to the ground and in connection to forest, tree rows etc. (Altringham & Berthinussen 2014).

As models for investigating species specific effects of habitat fragmentation, bats are excellent object to study. Bats developed different morphological adaptations for different types of specific habitats, and several variations of wing morphology emulate different strategies for foraging. Conservation biology is important for bats because many of the bat species are endangered. Many of them rely on forests for roosting and foraging and fragmentation of forest are an important impact, caused by e. g. road- and railway networks spreading around the world (Kerth & Melber 2009). All 19 species of bats in Sweden are protected by Species Protection Ordinance and by legislation. Bats are also conserved by the European convention EUROBAT which also includes protection of important hunting areas and settlements. It is forbidden to hunt, kill or move bats and to destroy their home areas (Naturvårdsverket 2015-06-08).

Several studies on bats and roads reveal that major roads can have large negative impacts on some species of bats and that road- and railroads can create barriers. Hunting areas can be separated from roosts (Berthinussen & Altringham 2012, Ogden 2012, Kitzes & Merenlender 2014) and in fact, that the effect on bat diversity and abundance are profound and mitigation and impact assessment are important (Altringham & Berthinussen 2014). Traffic noise and lightening of the streets has been proven to reduce foraging activity and success, also avoidance behaviour near roads have been contemplated in commuting bats (Berthinussen & Altringham 2012). Road mortality turns

conservation more difficult for bats due to their late maturation and low fecundity. Bat species can be differently affected by these components according to their behavioural variations, such as the foraging strategies and flight height. Smaller bat species, which often fly lower to the ground, have higher risk of mortality from collisions with vehicles (Medinas et al 2013). According to Kitzes & Merenlender (2014), the activity of four common bat species and total bat activity are frequently depressed near three big roads in California, compared to controls sites close to the road. Previous data shows that younger individuals and bat species flying lower to the ground are more frequently killed (Lesiński et al 2011). It has also been proven that the activity of the species, *Pipistrellus pygmaeus* is positively correlated with the distance from the road (Berthinussen & Altringham 2012). The effect roads have on bat species can also depend on the surroundings and habitat suitability areas around the roads where the bats hunt. Light pollution, such as street lights, is an increasing global problem as well. Previous studies have shown that it also may have a negative impact on the choice of flight route for bats (Stone et al 2009). Whether bats use wildlife passages or bridges to cross the roads is little known but some studies have been done, for example if bats use underpasses or gantries to cross roads. Results from one study show that bats use underpasses if they are allowed to pass without changing flight direction or flight height. Thought this study also showed that, if bats had to change their original commuting routes, they crossed the road at the same height as vehicles pass. (Berthinussen & Altringham 2012).

To avoid obstacles and to commute between places, when searching for food, microchiropteran bats use echolocation. Wing shape determines in which habitat a species can forage (Wermundsen & Siivonen 2008). Previous studies of foraging preferences of bats, show that *Myotis* spp. avoid roads in coniferous and mixed forests, tree lines and built up areas (Ciechanowski 2015) or for example the mouse-eared bat (*Myotis myotis*) relies rustling sounds to find food. This makes them vulnerable for disturbing noise in the environment and traffic can be a factor of that (Schaub et al 2008). A study made by Bennett et al. (2013) in Indiana, showed that roads can change the permeability of the landscape for foraging *Myotis sodalist*. The effect is associated to the traffic volume and the number of lanes. As the traffic volume and the number of lanes increased the incidence of bats exhibiting road-related avoidance behaviors did as well.

This is the first study of bats and the impact of roads in Sweden. One special condition in Sweden are the light summer nights in the beginning of summer, until mid-July. During this period bats are more exposed for predators and this might affect their behaviour. The aim with this project was to study how different species of bats behave near big roads and from the results draw conclusions about compensation or mitigation measures for infrastructure projects. We focused on the activity of *Myotis*-species at various environments in the landscape in relation to a major road, since the *Myotis*-species are known to avoid open areas (Ekman & de Jong 1996). The main questions we asked were: Are roads barrier for bats in a forest dominated landscape, and if so, for which species? Are wildlife passages a solution?

In order to test the effect of the roads as potential barriers, we designed the study to compare bat activity between: a) roads and control sites, b) roads and forest gaps, c) wildlife passage and control sites, d) different parts of the season.

## **2. Material and methods**

### **2.1 Study area 1 - major road**

The study site was located close to the city Enköping, in south- central Sweden. The road E18 in the study (59°38'01.18"N, 17°17'21.19"E) connects Oslo and Stockholm and is a double lane motorway with a central barrier consisting of trees, shrubs, grass, stones etc. The surroundings are mostly a mix of forest and open areas with farmlands and some small rivers. Traffic volume on the motorway varies a great deal. An average taking from 2011-2014 is 10898 vehicles per day a year (Trafikverket 2012). Eight sites were selected for bat studies with 1 km distance from each other along the road, between Ekolsund in the east and Enköping in the west. However, one important criteria was to have forests on both sides of the road, thus when the road passed farmland there was a longer distance between the sites. The boxes were placed in trees in the middle of the road, with the purpose to record all bat individuals passing the road. Control- sites were used in the forest about 50-100 meters from the road in order to be able to relate road passage to the general abundance of bats in the area.

### **2.2 Study area 2 – gaps between forests**

To be able to investigate if bats avoid open areas in general, gaps between forest patches with about the same size as the road were investigated. Eight forest sites around Enköping with open gaps were selected for the study. At each site, one box was placed in trees in the middle of the gap, to record if the bats flew over the open area. Five control sites were chosen in the forest near the gaps to record general abundance of bats in the area.

### **2.3 Study area 3- wildlife passages**

Two tunnels with roof of natural vegetation that can be used as wildlife passages over the E18 were included in the study. The width of the passages are about 100 meters. One passage (passage A) has dense forest. The surroundings adjacent to the passage are dense forest, but get sparser further away. A forest plantation (wood for energy purpose, *Salix spp.*) also exists close to the passage. A small river flows on the west side close to the passage. The second passage (passage B) has more sparse forest and is more accessible for mammals, humans and birds and bats. The east side of this passage is dominated by sparse conifer forests and the west side is dominated by farmlands. A small river also flows on the west side under the road close to the passage. On both wildlife passages one auto box was placed in the middle of the passage and one near the edge. 2 auto boxes were placed as controls in the connecting forest.

The sampling at all sites (in total 34 sites) was repeated once a week for seven weeks (in total 238 samples). However, data from the last week from the wildlife passages is missing (All the sites locations are shown in Fig 1).

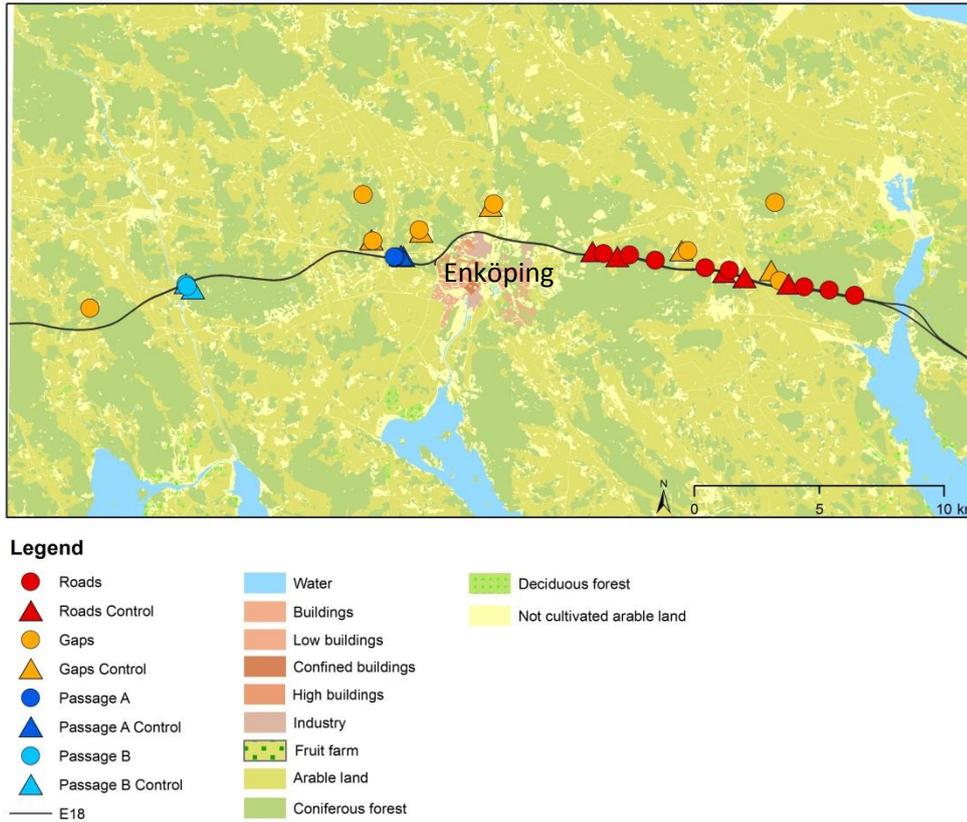


Figure 1. Locations of the 36 sites in the study along E18 motorway.

## 2.4 Auto boxes

For the automatic recording of the ultrasound of the bats, we used the auto box type D500x (Pettersson Elektronik AB). The sounds were recorded from 22.00 until 04.00 each night. The following settings for auto boxes were: Recording sensitivity (very high), sample frequency (500), pretrig (off), rec-length (3), HP-filter (y), auto rec (y), input gain (60), trigger lvl (30) and interval (5). The used settings have a high sensitivity, which means that the probability that a passing bat is recorded is very high.

## 2.5 Data analysis

The recordings from the auto boxes were analysed in the program Omnibat, (Ecom AB), that sorts out junk recordings and other animal sounds from bat sounds. It also sorts out different groups and species of bats. The sorted files were then more carefully, manually sorted into groups of bats. We sorted the files into four different taxa of bats; *Myotis*, *Pipistrellus*, *Nyctalus* and *Eptesicus* and used the amount of observations of each taxa. Since the data were not normally distributed, the Mann Whitney U- test was the most suitable test to compare the activity between different sites.

For all of the tests we used all the collected data from 7 weeks (22<sup>nd</sup> of June 2015 until 8<sup>th</sup> of August 2015) and also week 1 to 3 (22<sup>nd</sup> of June until 11<sup>th</sup> of August) and week 4 to 7 (13<sup>th</sup> of June until 8<sup>th</sup> of August) to see if there was any difference in the activity for the first and the second part of the season. We tested the different parts of the seasons for *Myotis*. We used a significant level of  $\alpha = 0.05$ .

### 3. Results

#### 3.1 Activity of different taxa of bats along the road

In total we had 7240 observations of bats (*Myotis*, *Eptesicus*, *Nyctalus* and *Pipistrellus*), 2395 of the observations were on the wildlife passages (33 % of all the recordings), 1056 on the wildlife passage controls (thus, wildlife passage and wildlife passage control contain 47, 6% of all recordings), 728 on the road, 1591 on the gaps, and 1470 on the control sites (gap- and road controls Fig. 2, table 1). This means that the wildlife passages and the wildlife passage controls have the highest number of observations of bats within overall observations in the studied site. Of all 7240 observations, 2399 were *Myotis*, 3408 were *Eptesicus*, 1212 were *Nyctalus*, and 221 were *Pipistrellus* (Fig. 2, table 1).

From the road sites it was a total of 452 observations of *Myotis* and on the controls 437 observations. When comparing roads and controls (by using Mann Whitney U- test) during the whole study period (week 1-7), we found a significant difference ( $p < 0.05$ ). The mean value of *Myotis* observations was higher at the road than at the controls. When comparing roads and road control during one part of the season (week 1-3 and week 4-7) there was no significant difference. (Fig.4, table 1 & 2).

In *Eptesicus*, 134 observations were made at the road sites and 853 observations at the control sites. For the first season (week 1 to 3) the Mann Whitney- U test was significant ( $p < 0.05$ ). The mean value for the control sites was a great deal higher than the mean value of the road. For the second part of the season (week 4-7) and for the whole study period (week 1 to 7) there were no significant differences (Fig. 4, table 1, 2).

In *Nyctalus*, a total of 120 observations were made at the road sites and 115 observations at the control sites. For *Pipistrellus*, there were a total of 22 observations on the road sites and 65 on the control sites. For *Nyctalus* and *Pipistrellus*' species there is no significant difference in their activity between the road and the control sites. (Fig. 3 Table 1, 2).

Table 1. Sample size (n), total number of observations ( $\Sigma$ obs), mean values, max and min observations and standard deviations for all observed taxa during the whole study period and in different part of study period.

<b>Weeks</b>	<b>Sites/species</b>	<b>n</b>	<b><math>\Sigma</math>obs</b>	<b>Mean value</b>	<b>Max</b>	<b>Min</b>	<b>SD</b>
<b>Road</b>							
W1-3	<i>Myotis</i>	24	126	5,3	80	0	16,9
	<i>Eptesicus</i>	24	25	1,04	5	0	1,33
	<i>Nyctalus</i>	24	77	3,21	13	0	3,87
	<i>Pipistrellus</i>	24	7	0,75	4	0	1,67
W4-7	<i>Myotis</i>	32	326	10,3	143	0	29,4
	<i>Eptesicus</i>	32	109	3,41	19	0	3,8
	<i>Nyctalus</i>	32	43	1,47	7	0	2,11
	<i>Pipistrellus</i>	32	15	0,72	7	0	1,44
W1-7	<i>Myotis</i>	56	452	8,1	143	0	24,8
	<i>Eptesicus</i>	56	134	2,39	19	0	3,21
	<i>Nyctalus</i>	56	120	2,21	13	0	3,08
	<i>Pipistrellus</i>	56	22	0,73	7	0	1,53
<b>Gaps</b>							
W1-3	<i>Myotis</i>	24	18	0,75	3	0	0,944
	<i>Eptesicus</i>	24	164	6,8	65	0	13,35
	<i>Nyctalus</i>	24	538	22,41	212	0	58,0
	<i>Pipistrellus</i>	24	18	0,75	7	0	1,67
W4-7	<i>Myotis</i>	32	163	5,09	23	0	6,36
	<i>Eptesicus</i>	32	250	7,81	43	0	9,9
	<i>Nyctalus</i>	32	417	13,03	159	0	35,7
	<i>Pipistrellus</i>	32	23	0,71	7	0	1,44
W1-7	<i>Myotis</i>	56	181	3,23	23	0	5,28
	<i>Eptesicus</i>	56	414	7,39	65	0	11,40
	<i>Nyctalus</i>	56	955	17,05	212	0	46,4
	<i>Pipistrellus</i>	56	41	0,73	7	0	1,53
<b>Controls</b>							
W1-3	<i>Myotis</i>	30	68	2,27	12	0	3,18
	<i>Eptesicus</i>	30	477	15,9	291	0	53,3
	<i>Nyctalus</i>	30	50	1,67	14	0	3,06
	<i>Pipistrellus</i>	30	40	1,33	16	0	3,15
W4-7	<i>Myotis</i>	40	369	9,2	88	0	18,7
	<i>Eptesicus</i>	40	376	9,4	215	0	33,9
	<i>Nyctalus</i>	40	65	1,63	16	0	2,96
	<i>Pipistrellus</i>	40	25	0,63	7	0	1,53
W1-7	<i>Myotis</i>	70	437	6,2	88	0	14,6
	<i>Eptesicus</i>	70	853	12,2	291	0	43
	<i>Nyctalus</i>	70	115	1,64	16	0	2,98
	<i>Pipistrellus</i>	70	65	0,93	16	0	2,37

Table 1. Continued

Weeks	Sites/species	n	$\Sigma$ obs	Mean value	Max	Min	SD
<b>Wildlife passages</b>							
W1-3	<i>Myotis</i>	12	202	16,8	90	0	24,6
	<i>Eptesicus</i>	12	1118	93,2	557	1	164,7
	<i>Nyctalus</i>	12	7	0,58	2	0	0,79
	<i>Pipistrellus</i>	12	43	3,58	14	0	5,14
W4-7	<i>Myotis</i>	12	244	20,3	47	0	16,5
	<i>Eptesicus</i>	12	753	62,75	250	0	88,12
	<i>Nyctalus</i>	12	5	0,42	2	0	0,79
	<i>Pipistrellus</i>	12	23	1,35	16	0	4,66
W1-7	<i>Myotis</i>	24	446	18,6	90	0	20,6
	<i>Eptesicus</i>	24	1871	78	557	0	130,12
	<i>Nyctalus</i>	24	12	0,5	2	0	0,78
	<i>Pipistrellus</i>	24	66	2,75	16	0	4,87
<b>Wildlife passage controls</b>							
W1-3	<i>Myotis</i>	12	455	37,9	184	0	53,9
	<i>Eptesicus</i>	12	64	5,3	37	0	10,76
	<i>Nyctalus</i>	12	1	0,08	1	0	0,29
	<i>Pipistrellus</i>	12	14	1,7	5	0	1,53
W4-7	<i>Myotis</i>	12	428	26,8	111	0	32,9
	<i>Eptesicus</i>	12	72	6	21	0	6,68
	<i>Nyctalus</i>	12	9	0,75	3	0	0,81
	<i>Pipistrellus</i>	12	13	1,08	5	0	1,27
W1-7	<i>Myotis</i>	28	883	31,5	111	0	42,6
	<i>Eptesicus</i>	28	136	4,86	37	0	8,5
	<i>Nyctalus</i>	28	10	0,36	3	0	0,68
	<i>Pipistrellus</i>	28	27	0,96	5	0	1,37

Table 2. P-values (Mann Whitney U- test) for roads and controls from the first part of the season (week 1 to 3), the second part of the season (week 4 to 7) and for the whole study period (week 1 to 7) for *Myotis*, *Nyctalus*, *Eptesicus* and *Pipistrellus* species.

Weeks	<i>Myotis</i>	<i>Nyctalus</i>	<i>Eptesicus</i>	<i>Pipistrellus</i>
W1-3	0,0735	0,1488	0,0286	0,3226
W4-7	0,0684	0,9709	0,6587	0,3355
W1-7	0,0180	0,3024	0,2143	0,9714

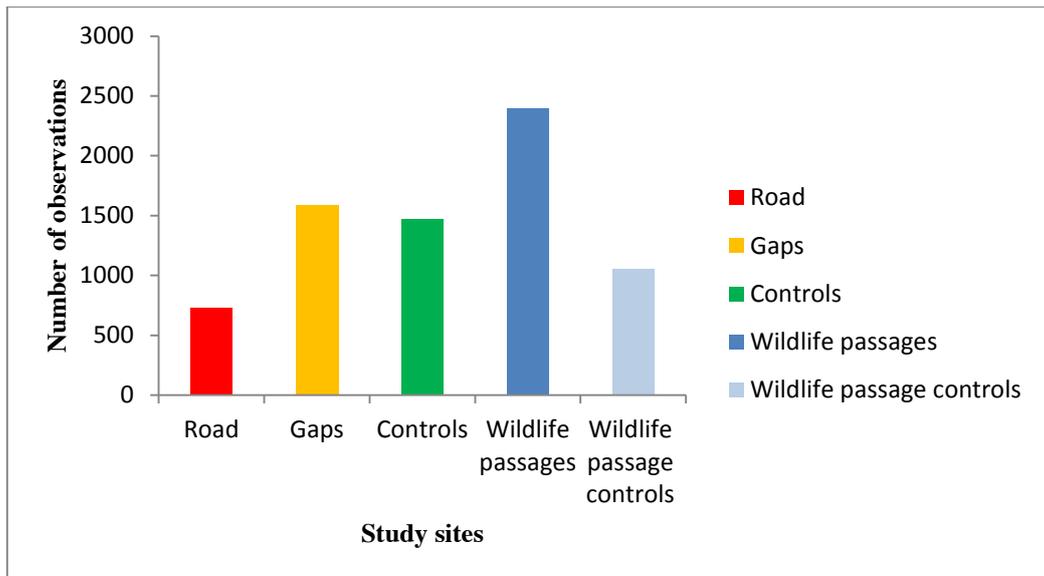


Figure 2. Number of bat observations at different sites in the study (*Myotis*, *Nyctalus*, *Eptesicus* and *Pipistrellus*).  $n_{gaps}=56$ ,  $n_{Road}=56$ ,  $n_{Wildlife\ passages}=24$ ,  $n_{Controls}=70$  and  $n_{Wildlife\ passage\ controls}=28$ . There was no significant difference between gap- and road controls, therefore we combined this controls.

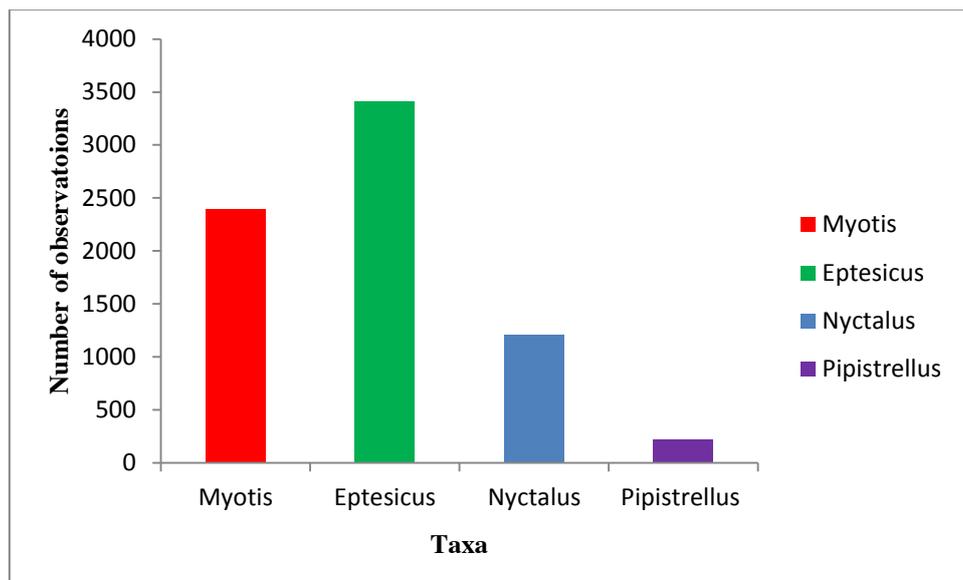


Figure 3. Total observations of the recorded taxa of bats at all sites.  $n_{Myotis} = 238$ ,  $n_{Eptesicus}=238$ ,  $n_{Nyctalus}=238$  and  $n_{Pipistrellus}=238$ .

### 3.2 The difference in activity of *Myotis*

The activity of *Myotis* species varies a lot between different locations in the landscape and between different parts of the study period. The total number of observations of *Myotis* was 2399 (road=452, control sites= 437, gaps= 181, wildlife passages= 446, and the wildlife passage controls= 883).

Between gap- and road sites there was no significant difference in the activity in either season; also there was no significant difference in the activity between gaps and control sites (table 3).

The wildlife passages and the wildlife passage controls have the highest mean values of all the sites in this study for *Myotis* species and the activity is significantly higher on wildlife passages than on the road (table 1). There is also a difference in the activity between the gaps and the wildlife passages. *Myotis* species have been observed more frequently at passages than at the gaps (Fig. 4, table 3).

Our data shows that the relatively high abundance of *Myotis* at the road only due to one site (road site 6). The observations at this site during the three samplings occasions in July are 80, 82 and 143 while most of the observations oscillate between 0 and 5. When analysing the data without this site, we found that the mean values for the controls is higher than the mean values for the roads and the activity is significantly higher on the controls than the road during the second part of the season and during the whole season (Fig. 5 table, 4).

Table 3. *P-values for differences in the number of Myotis observations made at different locations and during different periods. The variables are R1-3 (road week 1 to 3), R4-7 (road week 4 to 7), R1-7 (road week 1 to 7), G1-3 (gap week 1 to 3), G4-7 (gap week 4 to 7), G1-7 (gap week 1 to 7), Cntrl1-3 (controls week 1 to 3), Cntrl4-7 (controls week 4 to 7), Cntrl1-7 (controls week 1-7), WP1-3 (wildlife passages week 1 to 3), WP4-7 (wildlife passages week 4 to 7), WPA1-7 (wildlife passages week 1 to 7), WPC1-3 (wildlife passages week 1 to 3), WPC4-7 (wildlife passages week 4 to 7) and WPC1-7 (wildlife passages week 1 to 7).*

Variables	R1-3	R4-7	R1-7	G1-3	G4-7	G1-7	WP1-3	WP4-7	WP1-7
R1-3							0,0019		
R4-7								0,0053	
R1-7									0
G1-3	0,77						0,0002		
G4-7		0,13						0,0049	
G1-7			0,24						0
Cntrl1-3	0,0735			0,0689			0,0029		
Cntrl4-7		0,0684			0,8772			0,0091	
Cntrl1-7			0,0180			0,1218			0,0002
WPC1-3							0,8159		
WPC4-7								1	
WPC1-7									0,7964

Table 4. P-values for differences in the number of *Myotis* observations made at road and controls during different periods without data from road site 6. The variables are *R1-3* (road week 1 to 3), *R4-7* (road week 4 to 7), *R 1-7* (road week 1 to 7), *Cntrl1-3* (controls week 1 to 3), *Cntrl4-7* (controls week 4 to 7) and *Cntrl1-7* (controls week 1-7).

Variables	R1-3	R4-7	R1-7
Cntrl1-3	0,0679		
Cntrl4-7		0,0222	
Cntrl1-7			0,0052

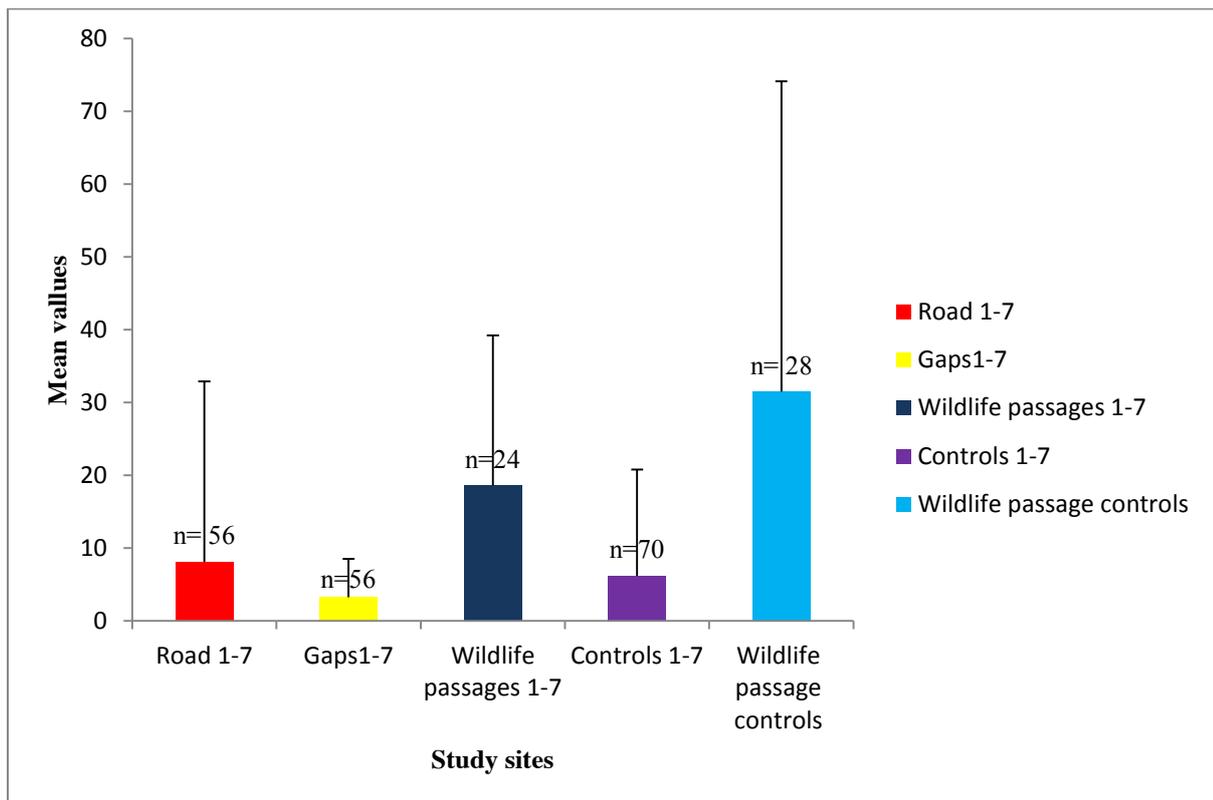


Figure 4. Mean values (Total number of observations of *Myotis*/n) for *Myotis* species in different sites from week 1 to 7 ( $n_{Road}=56$ ,  $n_{Gaps}=56$ ,  $n_{Wildlife\ passage}=24$ ,  $n_{Controls}=70$ ,  $n_{Wildlife\ passage\ controls}=28$ ). ( $SD_{Road}=24,8$ ,  $SD_{Gaps}=5,28$ ,  $SD_{Wildlife\ passages}=20,6$ ,  $SD_{Controls}=14,6$ ,  $SD_{Wildlife\ passage\ controls}=42,6$ ),  $Mean_{Road}=8,1$ ,  $Mean_{Gaps}=3,23$ ,  $Mean_{Wildlife\ passages}=18,6$ ,  $Mean_{Controls}=6,2$ ,  $Mean_{Wildlife\ passage\ controls}=31,5$ ). There was no significant difference between gap- and road controls, therefore we combined this controls.

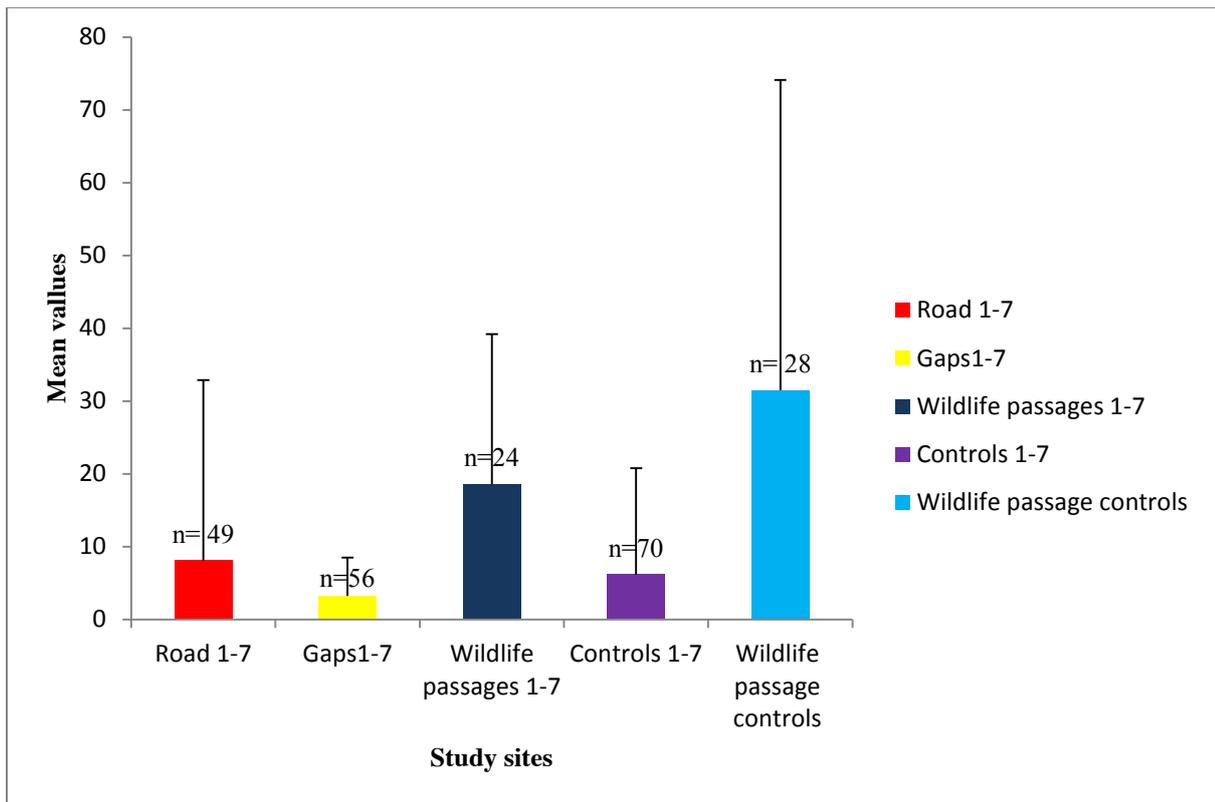


Figure 5. Mean values (Total number of observations of Myotis/n) of Myotis species in different sites from week 1 to 7, without data from road site 6. ( $n_{Road}=49$ ,  $n_{Gaps}=56$ ,  $n_{Wildlife\ passage}=24$ ,  $n_{Controls}=70$ ,  $n_{Wildlife\ passage\ controls}=28$ . ( $SD_{Road}=24,8$ ,  $SD_{Gaps}=5,28$ ,  $SD_{Wildlife\ passages}=20,6$ ,  $SD_{Controls}=14,6$ ,  $SD_{Wildlife\ passage\ controls}=42,6$ ). ( $Mean_{Road}=3$ ,  $Mean_{Gaps}=3,23$ ,  $Mean_{Wildlife\ passages}=18,6$ ,  $Mean_{Controls}=6,2$ ,  $Mean_{Wildlife\ passage\ controls}=31,5$ ). There was no significant difference between gap- and road controls, therefore we combined this controls.

## 4. Discussion

The most important findings in this study were that *Myotis* species seldom crossed the road. In most cases the observations of *Myotis* species at the road were between 0 and 5 at each site during all seven weeks, which is a small number, related to the observations at the control sites. When excluding road site six the *Myotis* activity is significantly lower at the road compared to the control sites.

This result indicates that major roads function as barriers for *Myotis* species. This has also been demonstrated in previous studies (Berthinussen & Altringham 2012, Ciechanowski 2015 Schaub et al. 2008). The reason why one site differs from all other sites is not clear, but we have two different possible explanations: 1. Though *Myotis* species normally avoid open areas, they will cross the road under certain circumstances that we are not able to understand yet. There might be a colony at one side, good hunting sites at the other side and the road is located in the commuting zone. The activities at site 6 were high only during the second part of the study period when the nights got darker. In general, it seems that *Myotis* species more often cross open areas late in the season when the nights get darker. If so, roads are barriers only in the beginning of activity period.

2. An alternative explanation might be that the bats did not cross the road but used tunnels under the road, built for small streams, with openings to the tree lane in the mid-road area, and used the mid road area for foraging. There is one such tunnel about 200 meters from the sampling site no. 6, but not at any other sampling site. This would increase the total number of observations of *Myotis*.

A second important conclusion from this study is that there is not a difference in *Myotis*- activity between the road and open gaps in the forest. They do avoid not only roads but open areas in general, which is also supported by Ekman & de Jong (1996). The reason why *Myotis* avoid open areas is not known but possible explanations include predator avoidance and insect abundance (Verboom 1998).

The results show also that bats do use wildlife passages. A wildlife passage may have two different functions, guiding bats when they are commuting between foraging sites, or work as a foraging site in itself (Abbott et al. (2012)). The use of wildlife passages by *Myotis* and other species is confirmed also in studies by, Berthinussen & Altringham (2012) and Abbott et al. (2012). However, the passage was used only if bats didn't have to change their original commuting routes. This is also confirmed by studies made by Berthinussen & Altringham (2012) and Abbott et al. (2012). If the wildlife passages were not available, the bats would have a more restricted hunting area. On both sides of wildlife passage A there were colonies of *Myotis* species and the high number of observations of *Myotis* species indicates that they use the passage to forage. Although we don't know if there were any colonies near wildlife passage B, the environment and the landscapes around the passages A and B were similar. Both areas around and on the passages provide good habitats for bats to roost and forage.

Though we can't draw any conclusions about the road being a barrier for the other species than *Myotis*, the results indicate that *Eptesicus* species do avoid the road. The mean values for *Eptesicus* are higher for the control sites than for the road. There was a significant difference in the activity in the first part of the season (week 1 to 3) and the activity is significantly higher in the forest on the

control sites than on the road for these species at this period. A previous study by Kitzes & Merenlender (2014) shows that the activity of four common bat species and the total bat activity is consistently depressed near three large highways compared to control points 300 m from these roads. One of these species is *Eptesicus fuscus*, which is very similar to the species in our study area *Eptesicus nilssonii*. For *Nyctalus* species the road does not reveal to be any obstacle in this study. *Nyctalus* is not vulnerable to predators; they are bigger and fast, they have a different foraging behavior and other food preferences compared to the minor species such as *Pipistrellus* and *Myotis*. They also have a higher flight height and are not exposed to the same hazards (Kronwitter 1988). For *Pipistrellus* species the observations were too few to draw any conclusions. Obviously the abundances of these species are too low in the area.

The sampling sites in this study were selected randomly without any information about bat colonies, and the sampling effort is rather big. However, the abundance of the different bat species, especially the abundance of *Myotis* is low in the study area. This makes interpretation of the result more difficult. It's risky to draw general conclusions, because it might be enough with one or a few single exceptions, e.g. if one of the sampling point happens to be close to a colony, to change the whole pattern. Alternative study design could be to increase the sample size even more, to focus only on areas with known distributions of bat colonies, or to use more direct observations, e.g. by radio-tracking. In our study, road site no. 6 was one such exception which was difficult to explain. However, in spite of this exception we are confident that our interpretation is correct. After excluding site six, we received significant differences.

#### **4.1 Conclusions**

This is the first study of bats and the impact roads have on bats in Sweden. The main conclusions we draw from this study are that both roads and gaps in the forest are avoided. The avoidance behaviour is most obvious in the beginning of summer (until mid-July). This also means that major roads can act as barriers for *Myotis* species in a forest dominated landscape. Wildlife passages are used by several species of bats. In our case, they were used as foraging habitats, and also as corridors with higher abundance of bats than all other habitats in the study.

It is important to do further studies to improve conservation, compensation and mitigation measures for bats in infrastructure projects in Sweden. More knowledge about the different taxa's behaviour near major roads and an understanding of the thresholds, such as limit distances for *Myotis* in open gaps are necessary to draw conclusions about mitigation measures and alternatives for ecoducts. It is also important to know how many mitigation measures are needed to hold a viable population in a certain area.

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