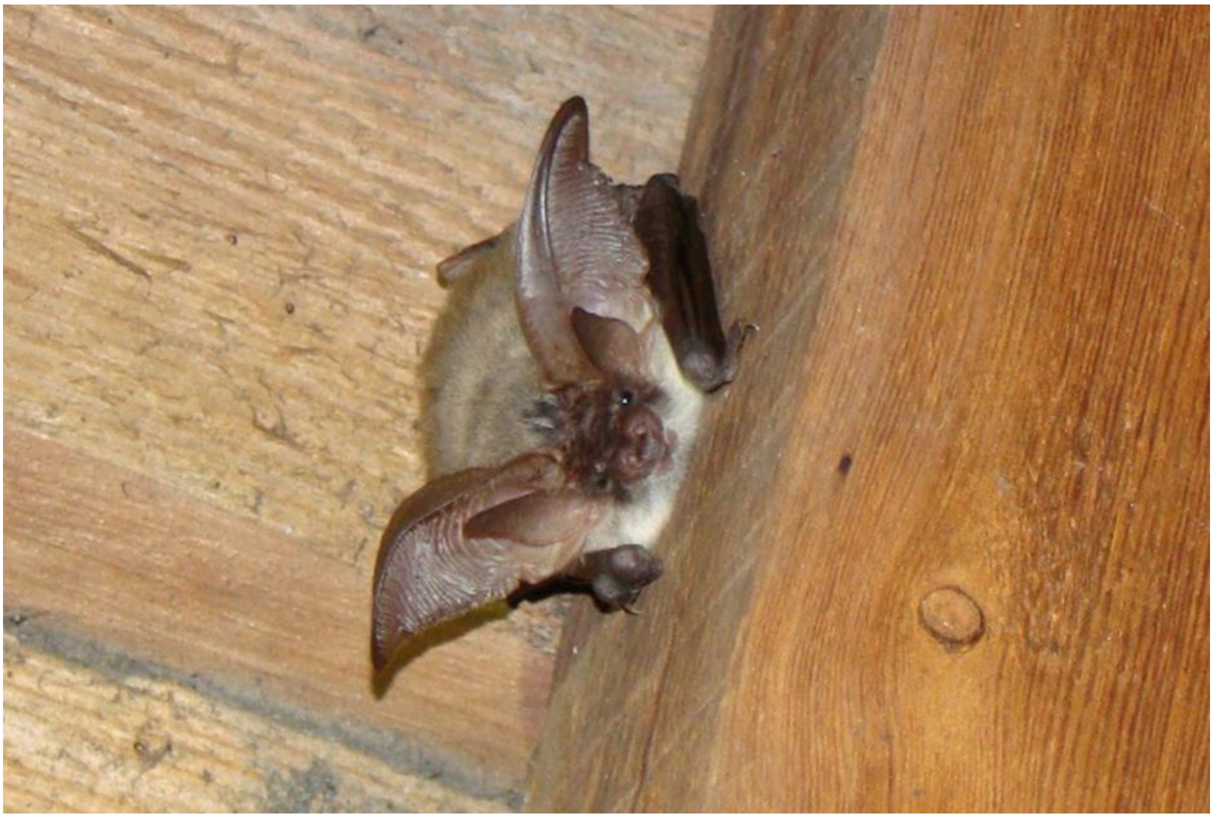
Shining a light on bats: a study into the effects of artificial light sources on grey long-eared bats (*Plecotus austriacus*)



Research report

25th January 2024

T. de Munck

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Preface

This research was conducted under commission for Stichting Landschapsbeheer Zeeland, as part of their three-year project to restore the local grey long-eared bat population in Zeelandic Flanders, together with the Zoogdiervereniging. I conducted this research during my internship of my Master Biology at Wageningen University and Research.

I am extremely grateful to Sam Janse and Lucien Calle of Stichting Landschapsbeheer Zeeland in particular for supervising me and providing me with knowledge and feedback.

I want to express my deepest gratitude to Kevin Matson of Wageningen University and Research for supervising me and helping me with issues related to administration and practical problems related to the research project.

I want to thank friend and fellow student Towi van der Putten for providing me with feedback on my research project report.

The completion of my research would not have been possible without Alex Wieland of Ecologisch Adviesbureau Wieland and Mark Dobbelaar of Ecomark Advies en Beheer who allowed me to use their unpublished data for publication in my research and lent me the required equipment to perform my research. Many thanks to them!

Many thanks to Kamiel Spoelstra of the Netherlands Institute of Ecology (NIOO-KNAW) for providing me with knowledge and new information and insights concerning the topic of my research project.

I would like to acknowledge René Janssen of Bionet Natuuronderzoek for providing the flight tracking data of two grey long-eared bat individuals from the metapopulation in Sint Jansteen.

Samenvatting

Kunstmatige verlichting en lichtvervuiling zijn de oorzaak voor de ontregeling van de levenscycli van vele organismen. Dit kan hun ecologie, gedrag en evolutie beïnvloeden. Vleermuizen zijn een diersoortgroep die significante beïnvloeding van kunstmatige verlichting op hun gedrag ervaren. Van vleermuizen van de genus *Plecotus* is bekend dat individuen licht vermijden gedurende de nacht. Echter is van één soort, de grijze grootoorvleermuis (*P. austriacus*), weinig bekend met betrekking tot dit onderwerp. De soort vertoont een afnemende populatiegrootte in Zeeland, en meer kennis is nodig om de oorzaak te achterhalen. In dit onderzoek is onderzocht of lichtintensiteit, afstand tot lichtbron en lichtkleur een significante invloed hebben op de aanwezigheid en passeringsfrequentie van *P. austriacus* op 78 locaties in het oosten van Zeeuws-Vlaanderen in de provincie Zeeland. Daarnaast is er onderzocht of er een verschil in respons op licht is tussen *P. austriacus* en de andere soort uit het genus *Plecotus* die inheems is in Nederland, de gewone grootoorvleermuis (*P. auritus*), en of het juist is om deze twee soorten als soortgroep gelijkend te behandelen in het kader van lichtgevoeligheid. Geluidsfragmenten van vleermuizen zijn opgenomen op elke locatie, van januari tot oktober 2023. Deze zijn vervolgens geanalyseerd en gebruikt om de aanwezigheid en passeringsfrequentie van *P. austriacus* en *P. auritus* te bepalen. Lichtintensiteit is gemeten met een digitale lichtmeter. De relaties tussen lichtintensiteit, afstand tot licht en lichtkleur op *P. austriacus*, *P. auritus* en *Plecotus spp.* zijn gemodelleerd. Er is geen effect van lichtintensiteit, afstand tot licht of lichtkleur op de aanwezigheid en passeringsfrequentie van beide soorten apart of gecombineerd gevonden. Deze resultaten indiceren dat individuen mogelijk dichtbij, maar niet door, lichtbronnen vliegen richting hun foerageerhabitat. Vervolgend onderzoek is nodig om de invloed van licht op deze soorten verder te analyseren. Er kan bijvoorbeeld een veldexperiment worden opgezet, op locaties met contoleerbare afstanden van lichtbronnen en lichtkleuren, gecombineerd met radiotelemetrie om vliegroutes en barrières in het landschap te achterhalen.

Abstract

Artificial lighting and light pollution is assumed to be the cause for disruption of the life cycles of many organisms, which may influence their ecology, behaviour and evolution. Bats are a group of animals that experience significant influence of artificial lighting on their behaviour. The bat genus *Plecotus* is known for avoiding light during the night. However, one species, *P. austriacus*, remains understudied in regards to this subject. The species shows a decrease in population size in the province of Zeeland, the Netherlands, and more research is needed to determine the cause. In this research, it was assessed whether lighting intensity, distance to light and light colour significantly affected *P. austriacus* presence and passing rate in 78 locations throughout the east of Zeelandic Flanders in Zeeland. Additionally, it was studied whether there was a difference in response to light between *P. austriacus* and the other species in this genus indigenous to the Netherlands, *P. auritus*, and whether it is justified to consolidate the two into one species group when studying the effect of light. Bat sound fragments were recorded on each location between January and October 2023, which were analysed and used to determine *P. austriacus* and *P. auritus* presence and passing rates on each location. Light intensity was measured using a digital light meter. The relationships among lighting intensity, distance to light and light colour on *P. austriacus*, *P. auritus*, and *Plecotus spp.* was modelled. No effect of lighting intensity, distance to light, or light colour on the presence and passing rate of both species separately or combined was found. These results may indicate that individuals do commute close to light sources, explaining that there is no significant effect. It is however speculated that individuals avoid flying directly through light beams. Additional research is recommended to further analyse the influence of light sources on these species. For example, a field experiment using locations with controllable distances from light sources and light colours, combined with a radio tracking research to establish commuting routes and barriers may be performed.

Contents

Introduction

The effect of anthropogenic influences on nature has been widely studied and discussed (e.g. Lande, 1998 and Terborgh, 1999). Humans influence their environment through a wide range of interferences, such as habitat destruction and fragmentation, overexploitation, pollution, overpopulation, introduction of invasive species and anthropogenic climate change (Prakash, 2017; Kumar and Verma, 2017; Prakash and Verma, 2022). These interferences are assumed to have a large role in the decrease of global biodiversity (Prakash & Verma, 2022). One of these interferences, urbanization, also introduces another consequence, namely light pollution (Burt et al., 2023). The effect of the decrease in darkness on biodiversity, ecology and ecosystems are thus far largely understudied (Burt et al., 2023). However, it is known that the cycles of light and darkness and the seasonal fluctuations therein have a significant role in the life cycles of many organisms (Helm et al., 2013). Thus, it is reasonable to assume that disturbance of this natural life cycle may influence ecology, behaviour and evolution of many species. In fact, studies have shown that artificial light may disturb natural behaviours such as reproduction, foraging, sleep and migration of a variety of both wild and captive animal species (Navara and Nelson, 2007). However, studies on the effects of artificial light on wildlife are scarce (Raap et al., 2015).

An additional effect of artificial light sources is fragmentation of the habitats of nocturnal animals (Barré et al., 2023). For example, research has shown Columbia black-tailed deer (*Odocoileus hemionus columbianus*), deer mouse (*Peromyscus maniculatus*) and opossum (*Didelphis virginiana*) avoiding lit passageways, causing reduced habitat connectivity (Bliss-Ketchum et al., 2016). Another species group that is influenced by artificial lighting is bats. Artificial light at night and its different characteristics can influence bat species in different ways. All European bats are nocturnal insectivores (Dietz and Kiefer, 2018). Additionally, their prey is often found near lights (Juddin et al., 2023). Thus, the ecology of bats suggests that artificial lighting may have a significant influence on most bat species, either in a positive or negative manner. For example, some species, such as the common pipistrelle (*Pipistrellus pipistrellus*), tolerate artificial light and may benefit from foraging near them (Barré et al., 2023; Juddin et al., 2023), while other species, like the mouse-eared bats (*Myotis spp.*), the common long-eared bat (*Plecotus auritus*) and the lesser horseshoe bat (*Rhinolophus hipposideros*) are known to avoid artificial light sources while flying and foraging (Valanne, 2023). This may lead to a competitive advantage for bat species that tolerate artificial light sources over species that do not (Arlettaz et al., 2000). This may eventually lead to the decline of species with an intolerance for artificial light (Arlettaz et al., 2000). Additionally, the variance in artificial light, for instance in terms of type and intensity, influences the prey species that are attracted to it (Van Langevelde et al., 2011), as well as the bat species that may or may not forage near it, depending on their respective tolerances for artificial light (Pauwels et al., 2021, but see Stanley et al., 2023). Studies have shown that bats of the *Plecotus* genus tend to be aversive to artificial light, although these studies focussed either on common long-eared bats or the genus *Plecotus* (i.e. a mix of common long-eared bats and grey long-eared bats (*P. austriacus*)) (see Rydell et al., 2017 and Barré et al., 2023). Thus, the effect of artificial light on the grey long-eared bat as a species remains understudied.

The grey long-eared bat is a European bat with a large distribution throughout the European continent, except for Scandinavia (Dietz and Kiefer, 2018). Despite being currently listed as near-threatened by the IUCN, its total population size has declined by at least 25% over the last three generations (Gazaryan and Godlevska, 2021). In the Netherlands, *P. austriacus* is considered to be rare (Van Norren et al., 2021). It only occurs in the southernmost provinces, particularly in the province of Limburg, in the province of North Brabant along the border with Belgium, and Zeelandic Flanders, the southernmost region of the province of Zeeland (Van Norren et al., 2021). The species has been increasing in number in the Netherlands, while it has been decreasing in most of its geographical range throughout Great-Britain and Europe (Van Norren et al., 2021). Despite increases in the Netherlands, *P. austriacus* has been disappearing from its winter habitats such as the attics of old buildings, mainly churches (Van Norren et al., 2021), in Zeelandic Flanders. It is assumed that exterior artificial lighting of such buildings may play a significant role in its disappearance (Van Norren et al., 2021). However, the influence of artificial lighting at night on the behaviour aspects of *P. austriacus*, including foraging and commuting, are understudied.

With this study, I analysed the effects of artificial lighting around roosts, foraging habitats and commuting routes on the behaviour of *P. austriacus* metapopulations that roost in churches in the east of Zeelandic Flanders and across the border with Belgium. I tested the hypothesis that the presence and amount of passings of *P. austriacus* depend on the intensity (amount of lux) of artificial lighting sources, the distance of the location to the nearest light source, and light colour. I predicted that individuals would avoid locations with white lights and high lux levels compared to locations with yellow or amber lights with low lux levels and compared to locations with no artificial lighting source. Additionally, I tested the correlation between *P. austriacus* and *P. auritus* presence to determine if studying effects of light on long-eared bats at the genus level is representative. Lastly, I tested the effect of ALAN characteristics (light intensity, distance to light, and light colour) on the presence and amount of passings of *P. austriacus* and *P. auritus* as individual species, and combined at the genus level (*Plecotus spp.*).

Materials and methods

Description of species

The grey long-eared bat, which range in size from 4 to 6 centimetres, occurs throughout Europe (Dietz and Kiefer, 2018; Gazaryan and Godlevska, 2021) (Figure 1). In western Europe, *P. austriacus* mainly lives in the roofs of old buildings in both summer and winter (Dietz and Kiefer, 2018). It feeds mainly on flying insects and is usually relatively sedentary, with distances between resting and foraging habitats usually not exceeding 5.5 kilometres (Dietz and Kiefer, 2018).

It is extremely difficult to distinguish between *P. austriacus* and *P. auritus*. The best method to distinguish *P. austriacus* from *P. auritus* is by physical characteristics that are best determined by capturing individuals. For example, *P. austriacus* has a broader tragus, shorter thumbs, shorter thumb claws and shorter feet than *P. auritus* (Dietz and Kiefer, 2018). Additionally, *P. austriacus* tends to forage in open landscape habitats while *P. auritus* forages in forested landscape habitats (Dietz and Kiefer, 2018). Lastly, there is a slight difference in echolocation characteristics between both species, which aids in identifying them using automatic bat identifying software.

Figure 1: The geographical range of the grey long-eared bat (Plecotus austriacus) (Gazaryan and Godlevska, 2021).

Study sites

This study focused on metapopulations of *P. austriacus* that roost in church attics in Zeelandic Flanders and across the border with Belgium. The churches are artificially illuminated, which may influence the behaviour of individuals that use these churches as roosting spots. The surrounding rural areas consist of open cultural landscape, with mostly agricultural land use, which *P. austriacus* uses for foraging (Dietz and Kiefer, 2018). Some individuals have been reported to be foraging in nearby livestock stall barns in addition to the open areas. Individuals here mainly use dykes with canopy vegetation as commuting routes (Dietz and Kiefer, 2018). Sound fragments of bat species were recorded by bat experts, preceding this study, at 78 locations throughout the east of Zeelandic Flanders (i.e. in and around Hulst, Clinge, Sint Jansteen, Heikant, Koewacht, Zuiddorpe and Axel) between the 6th of January and the 5th of October 2023 (Figure 2). Locations were chosen based on suitability as commuting routes or foraging habitats for the western barbastelle bat (*Barbastella barbastellus*) (straight landscape elements such as dykes with canopy vegetation, and open areas).

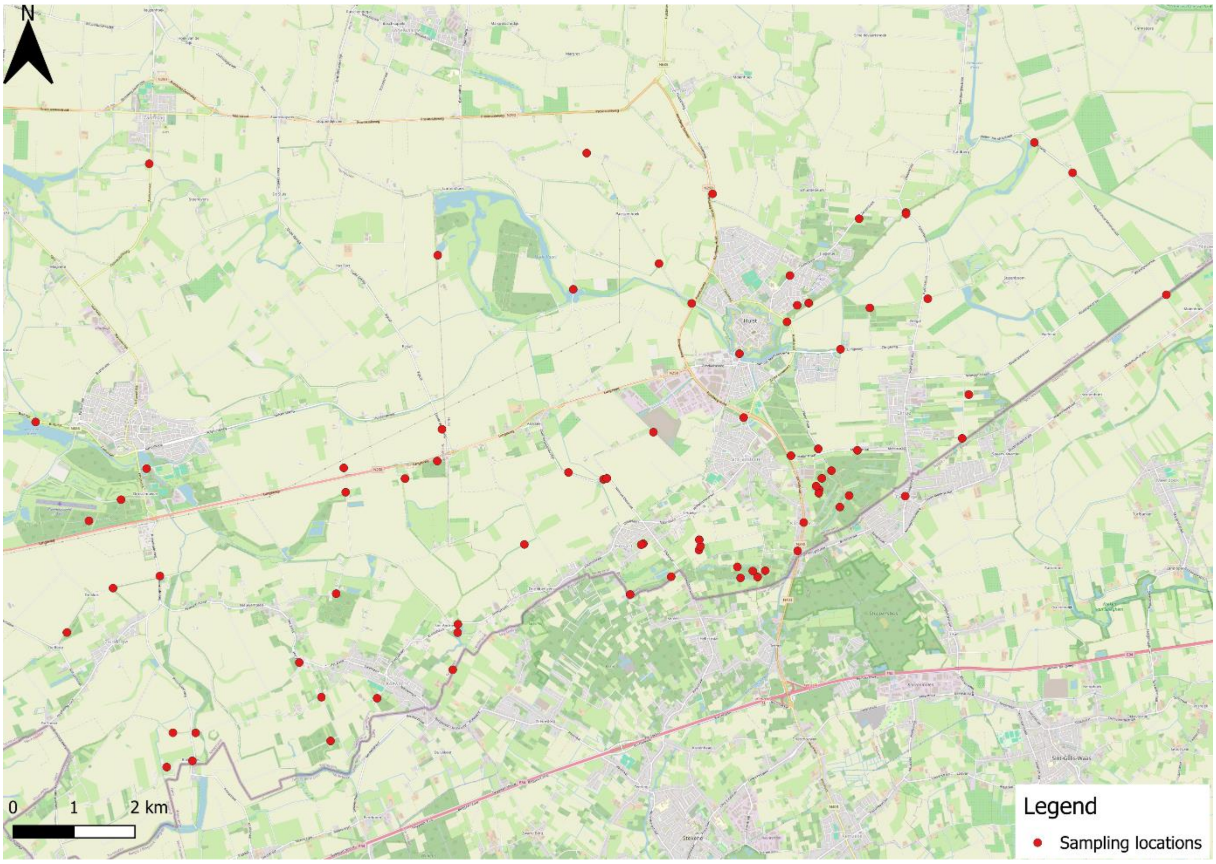

Figure 2: Map of all locations where bat sound fragments have been recorded.

Research methods

Analysis of sound fragments

The sound fragments of bats were used to determine the presence or absence of *P. austriacus* and *P. auritus* at each location, as well as the amount of passings during the survey. The sound fragments have been recorded using a Song Meter SM4BAT FS Ultrasonic Recorder (Wildlife Acoustics, Inc., USA). Sound fragments were analysed using Kaleidoscope Pro version 5.6.3 Analysis Software (Wildlife Acoustics, Inc., USA). The program was used in Bat Analysis Mode, with the option Auto ID for Bats enabled. Bats of Europe 5.4.0 was used as classifier for automatic identification of species in sound fragments. Identification sensitivity was set to balanced (neutral). Sound fragments with species identifications were saved as output, while noise files (sound fragments with no or poor quality bat calls) were not saved. For each location, *P. austriacus* and *P. auritus* presence was recorded (defined as binary data, either yes or no), as well as the number of passings (defined as the number of sound fragments of *P. austriacus* and *P. auritus*), if applicable. *Plecotus spp*. presence was defined as present when one or both species were recorded on a location. The amount of passings were calculated by summing the amount of passings of both species on a location. Since sampling effort was not equal for each location, the passing rate (average number of passings per hour) was calculated per location by dividing the total amount of passings for each location by the total survey duration.

Light measurements

The amount of lux was measured on each location where bat sounds were recorded and the distance (meters) from each location to the nearest artificial lighting source was estimated. Additionally, if applicable, the colour of the nearest artificial lighting source was recorded. The measurements were taken by using a Voltcraft MS-200LED digital light meter (Conrad Electronic SE, Germany). Lux values were measured by directing the light meter sensor horizontally, approximately 2 meters from the ground level, on the coordinates of each sound recording location. Measurements were taken at each cardinal direction, after which an average was calculated. Measurements were taken from October to December of 2023, during nights with clear skies.

Analysis

I calculated correlations between *P. austriacus* and *P. auritus* presence (phi coefficient) and passing rate (Pearson coefficient). Then, using R (version 4.3.1) (R Core Team, 2024), I tested the relationship between the presence/absence (with binomial logistic regressions) and the passing rate (with multiple linear regressions) of *P. austriacus*, *P. auritus and Plecotus spp.* (as response variables) and artificial lighting intensity, distance to light, and colour (as response variables). Lux is defined as amount of lux on a location, distance to light is divided in classes; 1 = 0-25m, 2 = 25-50m, 3 = >50m, and colour is nominal; either white, yellow/amber or dark. The statistical significance for each complete model was calculated by using the Chi-Square score. For the multiple linear regressions, *P. austriacus, P. auritus,* and *Plecotus spp.* passings are the dependent variable. The independent variables again are lighting intensity, distance to light, and colour. In this analysis, only non-zero passings were included.

Results

According to the sound fragment analysis, grey long-eared bats have passed 27 of the 78 surveyed locations during this study, while common long-eared bats have passed 35 locations. On 22 locations, both species passed. In total, 40 locations have been passed by at least one the long-eared bat species (Figure 3).

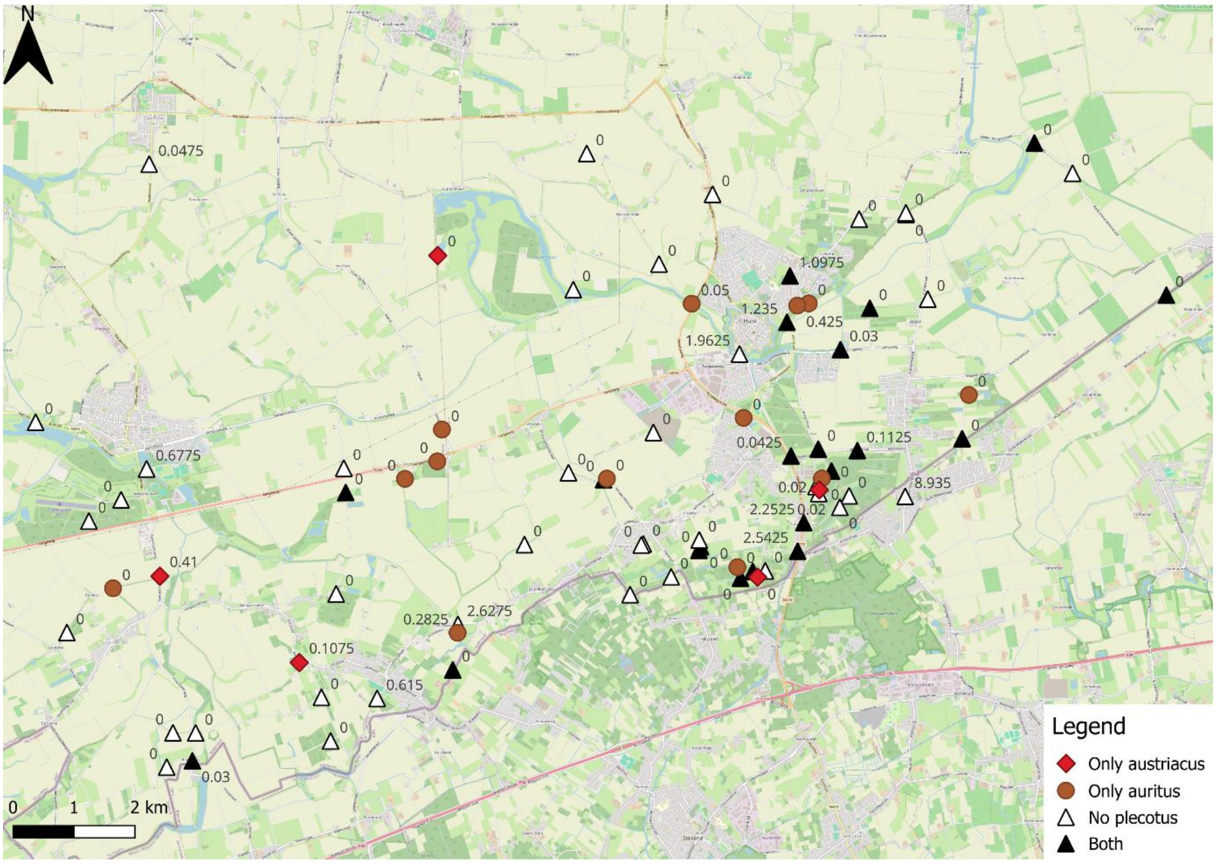

Figure 3: Map of all sampled locations. The colours of the locations indicate which species has been found at each location; only P. austriacus (grey), only P. auritus (brown), neither (white), or both (black). The numbers indicate the amount of lux measured at each location.

Overall, there was a lack of natural variation on the surveyed locations. On 56 of the locations, no amount of lux was measured in any direction. Furthermore, 61 locations were more than 50 meters removed from the closest artificial lighting source.

When testing for correlation between presence of both species at each location, a strong positive correlation was found (r = 0.54). A weak positive correlation was found between the number of passings of both species at each location (r = 0.30).

No significant effect of lighting intensity was found on *P. austriacus* (*P* > .05), *P. auritus* (*P* > .05), or *Plecotus spp.* presence (*P* > .05) (Figure 4). Additionally, no effect of distance to light was found on *P. austriacus* (*P* > .05), *P. auritus* (*P.* > .05) or *Plectotus spp.* presence (*P* > .05) (Table 1). Furthermore, no effect of light colour was found on *P. austriacus* (*P* > .05), *P. auritus* (*P* > .05), or *Plecotus spp.* presence (*P* > .05) (Table 2). Lastly, each model does not significantly explain the variance in the dataset (N = 78; *P* > .05; Table 3).

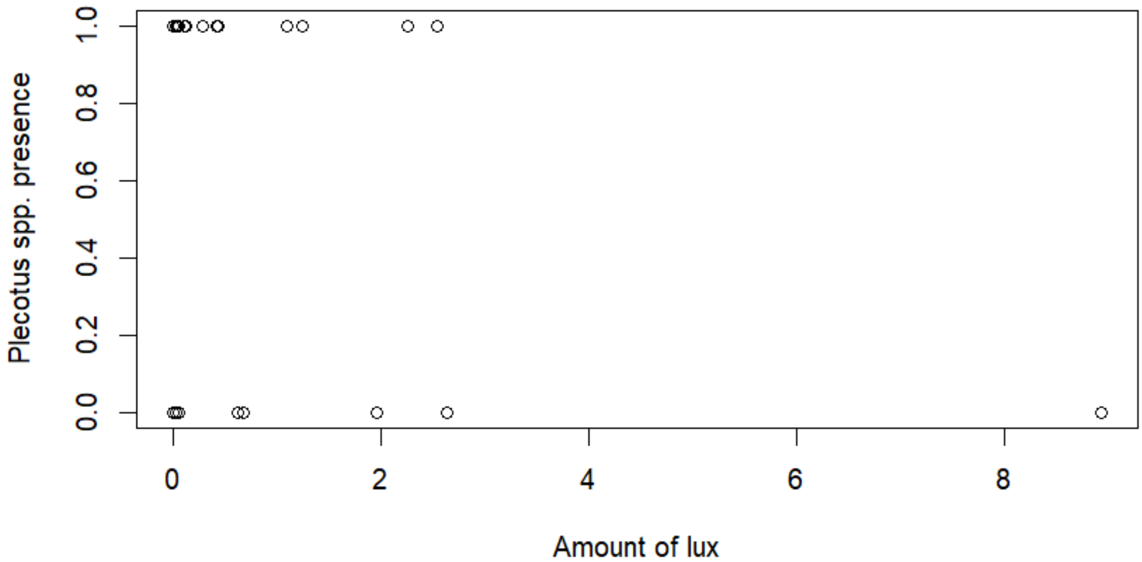

Figure 4: Scatterplot showing the spread of the presence of Plecotus spp. compared to the amount of lux. No relationship between presence and amount of lux was found for each species (group) (N = 78; P > .05). This graph only shows combined Plecotus spp. data since species presence was significantly positively correlated with each other.

Table 1: Table showing the number of locations in each distance to nearest light source category, and the percentage of locations where only P. austriacus, only P. auritus, or at least one of the two species was present. No relationship was found between presence and absence of species or species group and the distance to the nearest light source (N = 78; P > .05).

Distance to nearest light	N locations	% P. austriacus present	% P. auritus present	% Plecotus spp. present
<25 m	11	36.4%	36.4%	54.5%
25 – 50 m	6	83.3%	83.3%	83.3%
>50 m	61	29.5%	42.6%	47.5%

Table 2: Table showing the number of locations in each light colour category, and the percentage of locations where only P. austriacus, only P. auritus, or at least one of the two species was present. No relationship was found between presence and absence of the species or species group and the light colour of the nearest light source (if applicable) (N = 78; P > .05).

Light colour	N locations	% P. austriacus present	% P. auritus present	% Plecotus spp. present
White	10	70%	40%	70%
Yellow/Amber	10	30%	50%	50%
Dark	58	29.3%	44.8%	48.3%

Table 3: Table showing the binomial logistic regression models for P. austriacus, P. auritus, and Plecotus spp. presence. P-values show there is no significant effect of the independent variables in any of the three models. The total model P-values are shown in the P-value column in the same row as the model names. These show that these models do not accurately explain the variance of the dependent variables (presence of species) (N = 78).

Model	Estimate	Std. error	z value	P-value	Null deviance	Residual deviance
P. austriacus presence				.14	100.63 on 77 df	95.12 on 74 df
Intercept	-2.1809	1.9657	-1.109	.27		
Average lux	-0.1607	0.3086	-0.521	.60		
Distance to light	0.1244	0.5348	0.233	.82		
Light colour	0.8959	0.4849	1.848	.06		
P. auritus presence				.87	107.31 on 77 df	106.60 on 74 df
Intercept	1.0353	1.9077	0.543	.59		
Average lux	-0.2025	0.2943	-0.688	.49		
Distance to light	-0.3445	0.5192	-0.664	.51		
Light colour	-0.1979	0.4662	-0.424	.67		
Plecotus spp. presence				.35	108.08 on 77 df	104.77 on 74 df
Intercept	0.8266	1.9982	0.414	.68		
Average lux	-0.4359	0.4121	-1.058	.29		
Distance to light	-0.4093	0.5570	-0.735	.46		
Light colour	0.3126	0.4708	0.664	.51		

No significant effect of lighting intensity was found on *P. austriacus* (N = 27; $P > .05$), *P. auritus* (N = 35; $P > .05$) or *Plecotus spp.* passings (N = 40) ($P > .05$) (Figure 5). No significant effect of distance to light on *P. austriacus* (N = 27; $P > .05$), *P. auritus* (N = 35; $P > .05$) or *Plecotus spp.* passings (N = 40; $P > .05$) was found either (Figure 6). Additionally, yellow/amber lighting seems to not influence passing rate for *P. auritus* and *Plecotus spp.* However, no effect of light colour on *P. austriacus* (N = 27; $P > .05$), *P. auritus* (N = 35; $P > .05$) or *Plecotus spp.* passings (N = 40; $P > .05$) was found either (Figure 7). Similar to the presence models, these models do not significantly explain the dataset variance ($P > .05$) (Table 4).

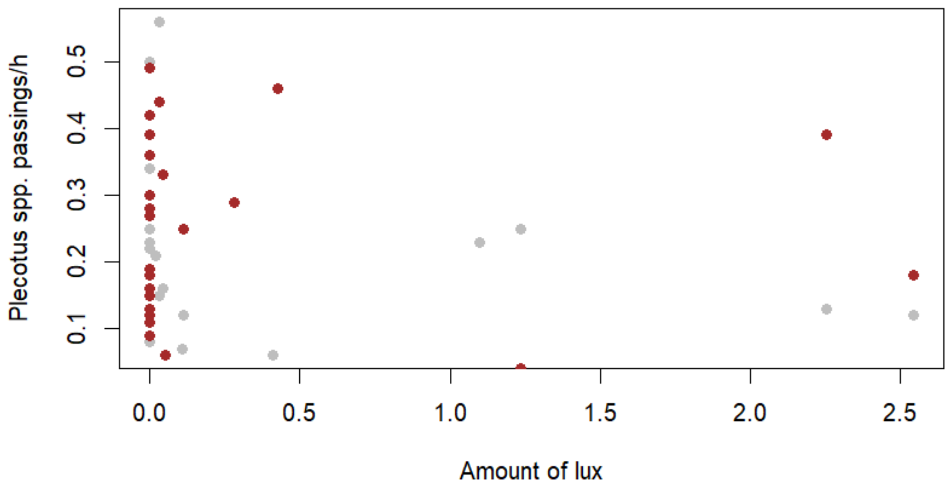

Figure 5: Scatterplot showing the passing rate per hour of Plecotus spp. when compared to the amount of lux (N = 40). In grey are locations where P. austriacus was present, in brown locations where P. auritus was present. One data point of a P. auritus individual passing more than 8 times per hour on a location with 0.0 lux has been left out for visualisation purposes. No relationship between passing rate and amount of lux was found for each species (group) (P > .05).

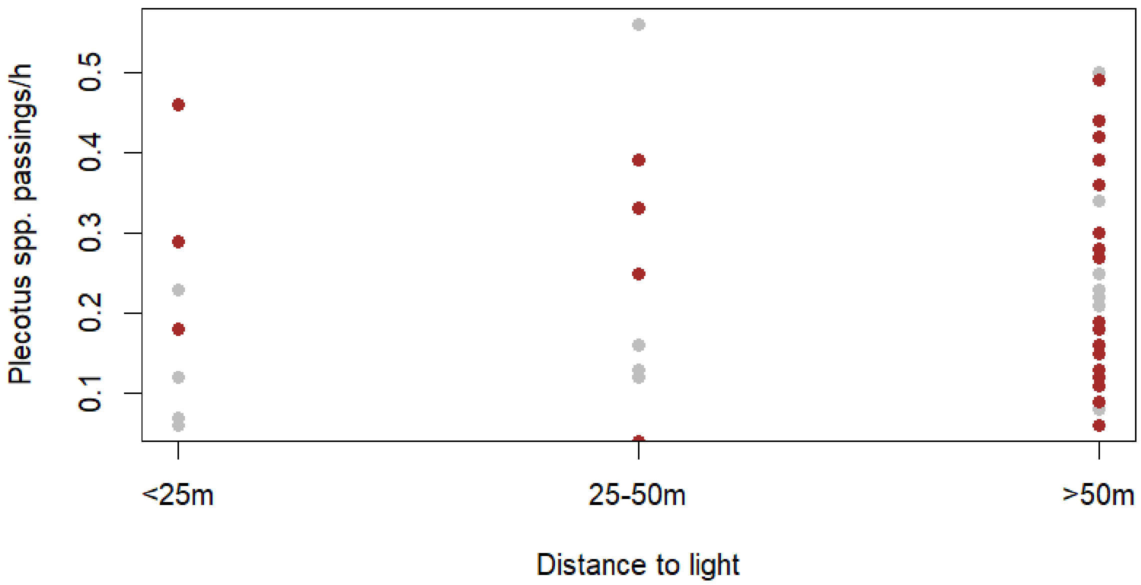

Figure 6: Scatterplot showing the spread of the passing rate per hour of Plecotus spp. when compared to the distance to the nearest light source (N = 40). In grey are locations where P. austriacus was present, in brown locations where P. auritus was present. One data point of a P. auritus individual passing more than 8 times per hour on a location more than 50 m removed from light has been left out for visualisation purposes. No relationship between passing rate and distance to light was found for each species (group) (P > .05).

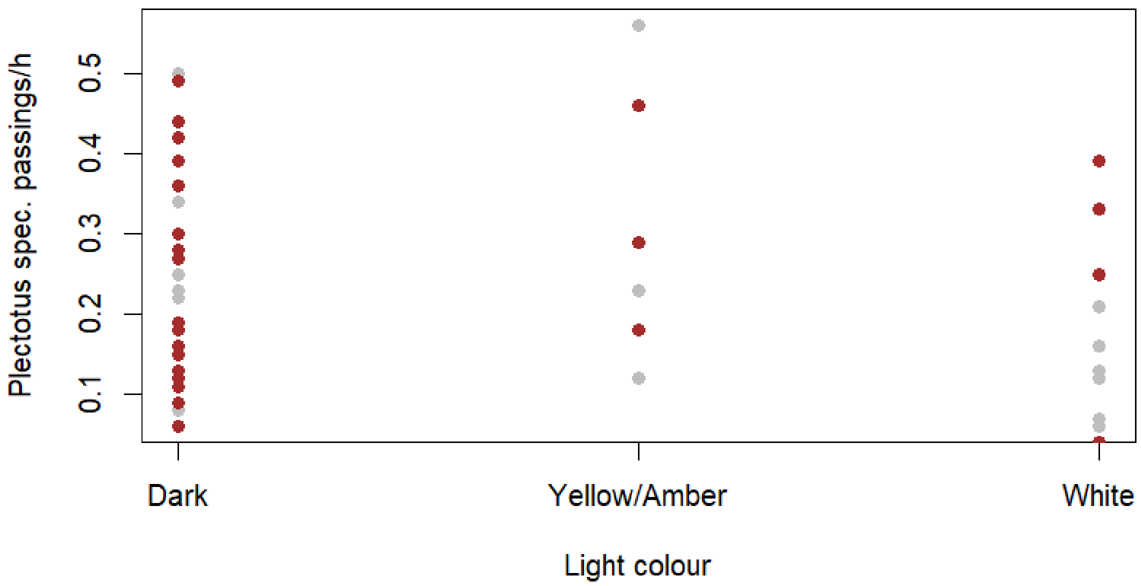

Figure 7: Scatterplot showing the spread of the passing rate per hour of Plecotus spp. when compared to the light colours (N = 40). In grey are locations where P. austriacus was present, in brown locations where P. auritus was present. One data point of a P. auritus individual passing more than 8 times per hour on a location where it was dark has been left out for visualisation purposes. No relationship between passing rate and light colour was found for each species (group) (P > .05).

Table 4: the variables used in the multiple linear regression models for P. austriacus (N = 27), P. auritus (N = 35) and Plecotus spp. (N = 40) passings, with their respective p-values, adjusted R^2 of the model, the degrees of freedom, the F-value and the p-value of the whole model. The adjusted R^2, the F-values, and the total model p-values show that these models do not accurately explain the variance of the dependent variable.

	Independent variable p-values						
Dependent variable	**Average lux**	**Distance to light**	**Light colour**	**Adjusted R^2**	**Degrees of freedom (df)**	**F-value**	**Total model p-value**
P. austriacus passings	.76	.85	.68	-0.06	3, 23	0.44	.73
P. auritus passings	.86	.87	.76	-0.09	3, 31	0.07	.97
Plecotus spp. passings	.99	.94	.64	-0.07	3, 36	0.13	.94

Discussion

This research shows that *P. austriacus* and *P. auritus* are often present in the same location, and therefore, it may be justified to research the effect of artificial lighting on the genus *Plecotus* or *P. auritus* instead of the rarer *P. austriacus*. However, no effect of artificial lighting on presence or passing rate of *P. austriacus, P. auritus* or *Plecotus spp.* has been found in this research. This may be explained by the setup of the experiment, species ecology, or both.

Strong positive correlations were found between presence of *P. austriacus* and *P. auritus*. This overlap result might suggest that the species have significant overlap in habitat preferences. However, it has been reported that both species have distinct habitat preferences (Ashrafi et al., 2013; Starik et al., 2021). Another explanation could be that both species use the same landscape structures for commuting. This could be a possibility, since individuals from both species are reported to sometimes roost together in the same location (Dietz et al., 2009). It could also be a byproduct of the difficulty of distinguishing the species on sound only, despite the accuracy of the program used. It should be noted that echolocation calls of species from the *Plecotus* genus have a small range of ca. 5 m (Dietz and Kiefer, 2018). This means that there is a possibility that presence of one or both species on a location have been missed, due to their calls not being recorded because of the distance between the individual and the recorder. This could mean one or both species being present on locations when noted as absent. For a better understanding of the possible overlap in habitat use, a combination of radio tracking a number of individuals per colony, and placing multiple recorders on different heights and distances from known commuting and foraging 'hotspots' should be used. Radio tracking research has been done on individuals of these metapopulations before this research took place (Janssen et al., 2024), which could be intensified. Nonetheless, these results may serve as preliminary evidence to support the generalization of *P. austriacus* and *P. auritus* into the *Plecotus* genus in (future) studies on the effect of light.

The weak correlation between passing rates for both species may be explained by the habitat characteristics of the sample locations. It is assumed that locations with suitable habitat characteristics for *P. auritus* (forested (non-agricultural) areas (Starik et al., 2021)) were overrepresented in the dataset, although no analysis was done on the habitat characteristics to confirm this. In future studies, differences in passing rates in different types of habitat should be studied in more detail.

No relationship between *P. austriacus*, *P. auritus*, or *Plecotus spp.* presence and the lighting intensity, distance to nearest light source and light colour was uncovered. However, in other studies, it was found that colonies of *P. auritus* disappeared from churches after light was installed (Rydell et al., 2017), and that *Plecotus spp.* were less active on lit sites (Barré et al., 2023). Differences in the current study and previous studies might be due to the experimental setup of the natural experiment performed in this study. In the current study, sampling locations were based on habitat suitability for bats, which may introduce a bias in the variation in the data. As observed, locations with artificial lighting were underrepresented in the dataset. Thus, it may be that the unequal sampling sizes are not able to reliably determine the effect of artificial lighting on the presence of the studied species. A more insightful approach could be to set up a field experiment, where sound fragments will be recorded on fixed distances from light sources at a number of locations, and comparing the presence data on these locations between the different distances, as well as with locations that do not have any artificial lighting in the vicinity. This approach allows for more control in the hands of the researcher, and may create a more even distribution of independent variable data among response variables (Diamond, 1986). Additionally, it should be noted that many bat species are able to perceive ultraviolet light. Thus, lights that produce ultraviolet light could have the same lux value as lights that do not, but have a greater effect on bats and their prey (Longcore and Rich, 2004). However, since lux is the unit used commonly by lighting engineers, designers and environmental regulators, it may influence comprehension of research results if another, more representative unit is used (Longcore and Rich, 2004).

The second possible explanation for no relationship between artificial light an species presence and passing rate being found relates to species ecology. It could be that the individuals from the metapopulations of both *P. austriacus* and *P. auritus* in the study area have had to adapt to the changing circumstances in artificial lighting in their habitat in order to survive. Although only a few bat species are reported to change their behaviour in relation to light (Rydell, 2006), and consequentially adaptation to light is rare in all but a few species of bats (Hormes, 2021), a *P. austriacus* individual has been reported foraging near hanging lamps (Borg and Sammut, 2002). Yet that report only constitutes one individual. The (foraging) flight height of *P. austriacus* has been reported to average between 2 and 5 m (Bauerova, 1982), while street lights in the Netherlands are between 2 and 10 m, thus eliminating the possibility of a strategy of flying on a higher altitude than the street lights. Thus, another, more plausible, explanation is that individuals fly around them, avoiding flying through the light beam entirely, while still being in range of the recording devices. It is the known behaviour observed in research on *Plectotus spp.* or *P. auritus* (Rydell et al., 2017; Barré et al., 2023; Valanne, 2023). In the current study, distances only varied from less than 25 m to more than 50 m. Consequently, it may be of value to research the average distance that individuals keep to light sources when commuting or foraging. This could be included in a research study following up from Janssen et al. (2024). Another research question to consider is the influence of the contrast in brightness. For example, there is a probability of bright lights in an otherwise dark environment negatively influencing bats to a greater extent than the same light in an environment lit by sky glow or moonlight (Mathews et al. (2015). This could also aid in conservation programmes, if a minimum distance to light sources needed for these species to commute and forage can be found. For example, governmental organisations could be advised on the placement and density of street lights, as well as the brightness.

Overall, no effect of light intensity, distance to light or light colour on the presence/absence or passing rates of *P. austriacus*, *P. auritus*, or *Plecotus spp.* as a whole was found. These current results contrast with results from previous studies on *P. auritus* or *Plecotus spp.*. This may have implications for bat research and conservation, as this might be caused by a greater adaptational capacity of these species than anticipated. Therefore, it would be worthwhile to further study the effects of light on bats, through methods that are more accurate in recording species presence *in situ*, such as radio tracking. This method has been successfully used to determine foraging and roosting behaviour in bats (e.g. Kalko et al., 1999; Bontadina et al., 2002; Jones et al., 2017). Despite not finding an effect of light on *Plecotus spp.*, light pollution is worsening habitat loss and fragmentation, as one of the consequences of human-altered landscapes (Falchi et al., 2016). Increasing the knowledge on the effects of light pollution on bats, insects and other nocturnal animals is therefore essential in the conservation of wildlife. Additionally, this research relates to the goals of Stichting Landschapsbeheer Zeeland as the future research I recommend will aid to the goal to continue conservation of *P. austriacus*. Furthermore, this and further research will contribute to the knowledge gap concerning the effect of light on the species.

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