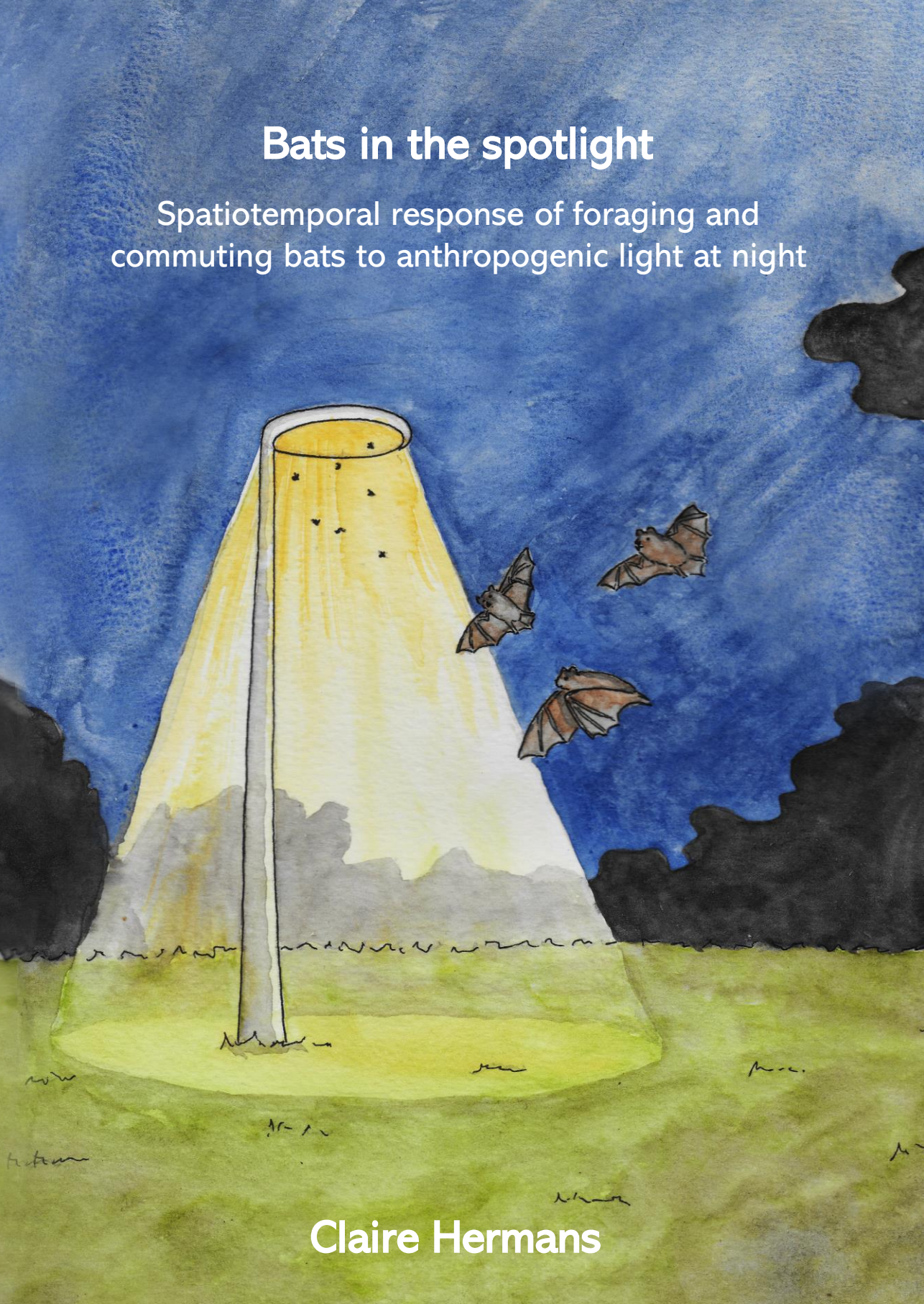# Bats in the spotlight

Spatiotemporal response of foraging and commuting bats to anthropogenic light at night

Claire Hermans

Propositions

1. Effectiveness of simple measures to mitigate the effect of anthropogenic light on ecosystems is difficult to assess.
   (this thesis)

2. Anthropogenic light is leading to winner-loser species replacements in bat assemblages.
   (this thesis)

3. Researchers working in nature have to be resourceful, as field situations are challenging.

4. Constant comparison of researchers is pointless, we all follow our own path.

5. Scientists need to follow the example of opportunistic bats: being in the spotlight pays off.

6. To make things happen at higher hierarchical levels, actions first need to be taken in small collectives.

7. Appreciation is essential to encourage people to persevere in their sustainable actions.

Propositions belonging to the thesis, entitled

Bats in the spotlight: Spatiotemporal response of foraging and commuting bats to anthropogenic light at night

Claire Hermans
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Bats in the spotlight:

Spatiotemporal response of foraging and commuting bats to anthropogenic light at night

Claire Hermans

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Bats in the spotlight:

Spatiotemporal response of foraging and commuting bats to anthropogenic light at night

Claire Hermans

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"De l'ombre ou de la lumière,
Lequel des deux nous éclaire ? "

Grand Corps Malade et Calogero

Chapter 1

General Introduction

Light pollution and its ecological consequences

Artificial light at night (ALAN) is an anthropogenic disturbance, which is mainly the result of urbanisation. It causes light pollution that we can define at different spatiotemporal scales. The first one is astronomical light pollution, where celestial bodies are not visible anymore because of upward directed light reflected back in the atmosphere. The second one is ecological light pollution, for which artificial light modifies the natural patterns of light and dark (Longcore and Rich, 2004). Light pollution has become a major concern in ecology due to a drastic continuous growth of lit outdoor areas over the past few decades (Falchi et al., 2016; Falchi and Bará, 2023; Kyba et al., 2023, 2017a). A recent study showed an increase in sky brightness of 10% per year (Kyba et al., 2023), while artificially lit outdoor area growth was previously estimated around 2% per year between 2012 and 2016 (Kyba et al., 2017a).

This increase has been mainly accelerated by the introduction of light-emitting diodes (LEDs), replacing other light types in outdoor lighting (Pagden et al., 2020). Nowadays, the LED sales represent about half of the global illumination market (46.5 % in 2019, Zissis et al., 2021). LEDs have major advantages, for instance they are cost- and energy-efficient and long-lasting, the spectrum is adaptable as well as the intensity, and they generate directional emissions. LEDs also emit less in the ultra-violet (UV) part of the spectrum than metal halide lamps for example, reducing the potential effect on insects and birds that are sensitive to these wavelengths (Donners et al., 2018; Gaston et al., 2012). However, the impact of this transition to LEDs on ecosystems is unclear.

The increase of artificial light at night has many ecological impacts and is likely to affect nocturnal communities, which is a main concern since 30% of vertebrates and more than 60% of invertebrates are nocturnal (Gaston et al., 2013; Hölker et al., 2010). These impacts may be experienced at different scales and thus having a cascading effect on the ecosystems. At the individual level, effects have notably been observed on behaviour, physiology, circadian rhythm, activity pattern, reproduction and communication of many group species including insects, birds, fish, amphibians and mammals (Sanders et al., 2021). Some of those alterations may have direct effects on population ecology. Others impacts have been observed on the population and community levels, especially on abundance, diversity, competition, and predation (Cravens and Boyles, 2019; Davies et al., 2012; Spoelstra et al., 2017). As artificial light alters natural day-night rhythms, its effects are not limited to night and affect diurnal communities as well (Giavi et al., 2021; Sanders et al., 2022). Some diurnal species may even take advantage of artificial light at night to extent their activity and use the night-time niche (Gaston et al., 2017).

Artificial light is also responsible of habitat loss, which is the reduction of the spatial range of natural habitat (Liu et al., 2016). Artificial light at night is considered as a non-structural landscape factor by making a greater or lesser area unsuitable for animal species depending on their sensitivity to light (Laforge et al., 2019; LaPoint et al., 2015). Some species may be attracted for example by prey concentration around light sources, while artificial light reduces habitat suitability for light-averse species. In natural conditions, darkness provides safety against visually oriented predators. The trade-off between food and safety shapes the "landscape of fear" of a species, which is the "variation in an animal's perception of predation risk" (Kohl et al., 2018). This suggests that prey exhibits variation in its antipredator behaviour according to the risk it is facing in time and space (Moll et al., 2017). For many nocturnal animals, such as small rodents and bats, the presence of light is a hazard in the form of predation risk: it negates the safety of darkness, the essence of their temporal niche in the ecosystem. Artificial light at night may therefore reshape the landscape of fear both spatially (Shier et al., 2020; Spoelstra et al., 2015) and temporally (Henke et al., 2022; Mariton et al., 2022). It also generates asynchronies inducing mismatches in prey-predator interactions if the two groups respond differently to light (Sanders et al., 2023) and thus reduces the quality of food patches.

In addition to that, illumination can cause a barrier effect in the landscape, and therefore cause habitat fragmentation, which can be defined as "the discontinuity, resulting from a given set of mechanisms, in the spatial distribution of resources and conditions present in an area at a given scale that affects occupancy, reproduction, or survival in a particular species" (Franklin et al., 2002). For many species, dark corridors are essential to connect suitable habitat patches. For instance, migrating birds avoid urban lit areas, and stopovers are located in dark, rural areas (Korpach et al., 2022). Common toads (*Bufo bufo)* avoid roads illuminated with white or green light during spring migration (van Grunsven et al., 2017). Artificial light also creates resistance to bat movements in urban areas (Hale et al., 2015; Laforge et al., 2019). While tunnels or underpasses may be used as corridors, bats and other mammals, such as deer and rodents, cross less frequently lit under-road passage structures (Bhardwaj et al., 2020; Bliss-Ketchum et al., 2016).

Thus, in order to overcome the effects of outdoor lighting on ecosystems, several mitigation measures have been explored (Gaston et al., 2012). The first recommendation is to protect or create natural unlit areas, as implemented by the Dark Ecological Network ("Trame noire") in France (Challéat et al., 2021; Sordello, 2017). Secondly, controlling light directionality is important to restrict the light trespass. This can be achieved by changing light design, for instance adapting the light shape, orientation and height in order to protect light-sensitive species (Azam et al., 2018; Bolliger et al., 2022). The third guideline is to limit the lighting duration by implementing novel lighting concepts such as adaptive (traffic-

dependent) and restricted lighting (predetermined nightly schedule). However, these concepts might not coincide with peak activity of many nocturnal organisms (Azam et al., 2015; Day et al., 2015; Gaston et al., 2012). The fourth approach is to adapt the spectrum of the light source. Reducing the short waves of the spectrum (blue in particular) would limit the impact of artificial light on several nocturnal species including bats (Longcore et al., 2018; Spoelstra et al., 2015, 2017; Straka et al., 2020). The last recommendation is to change the lighting intensity, but the response to light intensity may also vary with the spectral composition of light (Spoelstra et al., 2017; Zeale et al., 2018). In this thesis, I will focus on the last two recommendations to better understand how the response of nocturnal species such as bats depends on light spectrum and light intensity to reduce the impact of artificial light and prevent habitat loss and fragmentation.

Ecology of bats

Nocturnality

Bats are considered as particularly vulnerable to light pollution because this animal order consists of almost exclusively nocturnal species (Rydell and Speakman, 1995). Bats avoid light at night because of the risk of predation by visually-oriented predators like raptors (Lesiński et al., 2009; Rosina and Shokhrin, 2011; Speakman, 1991a). The safety of darkness is the essence of their temporal niche (Duvergé et al., 2000; Jones and Rydell, 1994).The predation hypothesis is supported by the fact that slow-flying bats emerge later from their roosts compared to fast and agile bats (Jones and Rydell, 1994). A comparable response has been observed within a species, with heavier pregnant females emerging relatively late (Duvergé et al., 2000; Speakman, 1991a). The singular diurnal activity of the Azorean bat (*Nyctalus azoreum*) also corroborates the anti-predation theory as no diurnal avian predators are known to inhabit the Azores archipelago (Irwin and Speakman, 2003). Other bat species tend to line up their activity with sunset and sunrise times (Erkert, 1982), even if it implies shorter and restricted foraging periods during summer, especially at the northern limit of their distribution in the Northern hemisphere (Frafjord, 2021; Speakman et al., 2000). Evolution therefore shaped their sensory system in order to navigate and forage in the darkness of the night.

Orientation & navigation

Animals rely on multiple sensory systems, in which light plays an essential role for many species. A good example of multisensory integration can be found in bats, as they integrate bimodal information through echolocation and vision.

Echolocation is a biological sonar used to navigate and to capture prey items. Lazzaro Spallanzani first showed in the XVIIIth century that bats were capable of orientation when blinded. This acoustic orienting behaviour has then been named as echolocation by Donald R. Griffin (Griffin and Galambos, 1941). Bats emit ultrasonic sound pulses and they listen for the returning echoes from prey and obstacles to create a sound-picture of their environment. An echolocation sequence can be divided in three stages (Russ et al., 2012). The first phase is the search flight, where echolocation calls are loud and regular with a slow repetition rate. When a prey is detected, the bat starts the second phase, the approach. As the bat closes in on its prey, the echo takes less time to return, therefore the echolocation rate gets faster. The last phase is the terminal or feeding buzz: in order to avoid an overlap between the pulses and the echoes when the prey is even closer, the bat decreases the duration of its calls and the frequency drops, preceding the prey capture (Figure 1.1).
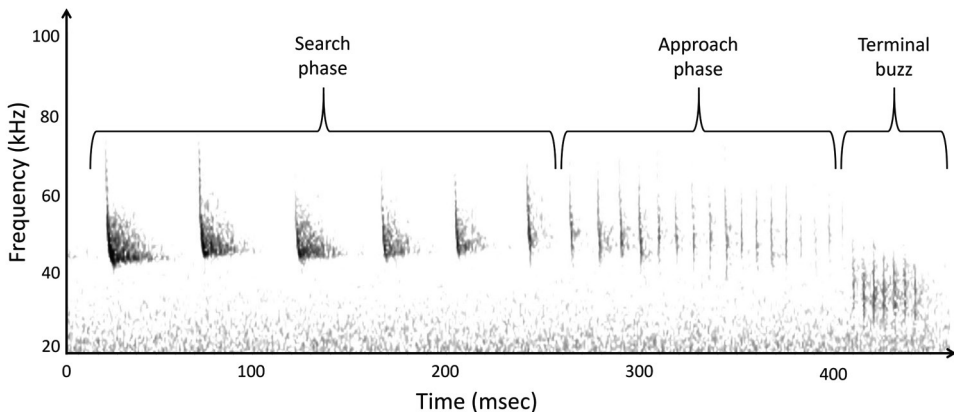
Figure 1.1: Echolocation sequence of a common pipistrelle (*Pipistrellus pipistrellus*) ending up with a feeding buzz.

However, bats can also rely on their visual cues for orientation. Vision is especially important for tropical fruit-eating species that generally have larger eyes for higher spatial acuity and sensitivity (Danilovich and Yovel, 2019). Although insectivorous bats have smaller eyes and rely heavily on echolocation, vision seems to also play a role in their short-range orientation and navigation (Céchetto et al., 2023; Jones and Moss, 2021; Orbach and Fenton, 2010). Other studies revealed the presence of functional opsin genes, allowing for colour vision (Müller et al., 2009; Zhao et al., 2009). This correlates with previous results showing stronger electrical response of the retinas of four bat species to light flashes between 540 and 620 nm (Hope and Bhatnagar, 1979). Those results are consistent with the hypothesis that echolocating bats may also rely on visual cues. Bats may even adapt their echolocation according to the available visual information (Danilovich et al., 2015; Danilovich and Yovel, 2019; Orbach and

Fenton, 2010). A recent study also suggests that echolocating bats use vision to orient themselves rather than hunt (Céchetto et al., 2023). Therefore, insectivorous bats rely primarily on echolocation to capture prey.

Since echolocation is a major cue to orientate and forage for many bat species, a variety of echolocation calls has evolved in bats according to their flight morphology and their habitat use in order to optimise foraging efficiency (Denzinger and Schnitzler, 2013; Fenton, 1990). The first example among insectivorous bats present in the Netherlands is the aerial hawkers, such as *Pipistrellus* spp., *Nyctalus* spp. and *Eptesicus* spp.. In this case, bats catch their prey on the wing in the open air. This strategy involves low frequency, narrow bandwidth signals that are better adapted for mid- to long-range detection (Figure 1.2). On the other hand, gleaners like some *Myotis* species or *Plecotus* spp. catch prey from surfaces like vegetation or from the ground. Similarly, trawling bats capture their prey on water surface. The last two strategies are associated to high frequency, broadband calls (Figure 1.2) in order to provide more accurate information on objects' structure (Neuweiler, 1989).

Habitat use

Even in darkness, only a limited number of species ventures out in relatively open space when foraging, and these are generally fast-flying and agile species (Figure 1.2) (Fenton, 1990; Neuweiler, 1989). Many other species seek extra protection against predation by flying in cluttered environment; these are typically slow-flying species (Figure 1.2) (Fenton, 1990; Neuweiler, 1989).
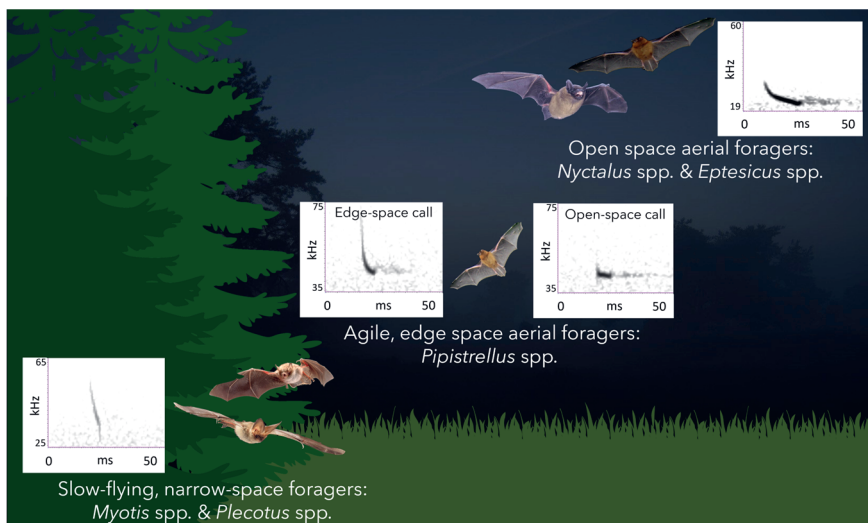
Figure 1.2: Bats foraging habitat with spectrograms of echolocation calls in representative species groups of different foraging guilds. Intraspecific variation in echolocation signals in the transition zone between open- and edge-space is shown for *Pipistrellus* spp. as an example.

In addition to the interspecific differences in echolocation calls, there is also a tremendous intraspecific variety when flying in different environments as the structure of the natural vegetation is a strong driver of call characteristics. Generally, calls change from open-space to edge-space in response to distance to background objects, such as the ground and vegetation (Denzinger and Schnitzler, 2013). Such changes include increasing bandwidth and shortening interval and call duration when approaching vegetation (Figure 1.2) (Siemers and Schnitzler, 2000). However, the transition zone between open- and edge-space calls has been studied with limited spatial resolution (Kalko and Schnitzler, 1993; Schaub and Schnitzler, 2007).

Due to their flight and echolocation abilities, bats are well adapted to move between habitats, making them a very mobile taxon. They particularly make use of linear structures to fly through the landscape. Many European insectivorous bat species use forest edge habitat as foraging patches (Schnitzler et al., 2003). Treelines and hedgerows are also employed as commuting routes (Barré et al., 2023; Stone et al., 2009; Verboom and Huitema, 2010). Trawling bats can also use waterways as commuting routes (Downs and Racey, 2006; Haarsma and Siepel, 2014) as they are adapted to fly and catch prey above the water surface (Britton et al., 1997; Kalko and Schnitzler, 1989). Bats are able to fly dozens of kilometres to commute to their foraging areas (Haarsma and Siepel, 2014) or even thousands of kilometres during migration (Petersons, 2004). This high capacity for mobility throughout the landscape makes them very sensitive and vulnerable to habitat loss and fragmentation caused by multiple anthropogenic pressures, such as urbanisation (Avila-Flores and Fenton, 2005; Lintott et al., 2016; Russo and Ancillotto, 2015; Stidsholt et al., 2024), intensive farming (Frey-Ehrenbold et al., 2013; Park, 2015), wind turbines (Ellerbrok et al., 2022; Gaultier et al., 2023; McKay et al., 2024), solar farms (Barré et al., 2023a; Tinsley et al., 2023) and artificial light at night (Laforge et al., 2019; Stone et al., 2015; Voigt et al., 2021). Thus, bats provide a very good study case of the effect of artificial light on nocturnal species in terms of habitat loss and fragmentation.

Effect of light in bats

There is accumulating evidence that bats' behaviour is affected by ambient light. In line with nocturnality in general, the response of bats to low light levels at night is widely recognized as related to predator avoidance (Rydell and Speakman, 1995; Speakman, 1991b, 1991a). The avoidance of light may cause bats to seek shelter in cluttered environment and as a result become less conspicuous. Indeed, several studies reported lower activity of bats with moonlight, mainly in tropical bat species (Appel et al., 2017; Ciechanowski et al., 2007; Saldaña-Vázquez and Munguía-Rosas, 2013; Vásquez et al., 2020), or change in activity within vegetation layers (Hecker and Brigham, 1999; Lang et al., 2006). Flying closer to

vegetation may hamper prey capture success, as background echoes may interfere with prey echo when flying closer to vegetation, especially for open-space and edge-space foragers (Schnitzler and Kalko, 2001). The potential benefit of extra safety against predation by flying close to the vegetation is therefore a trade-off between predator avoidance and foraging efficiency, which may be influenced by ambient light and altered by artificial light.

Besides foraging (Lewanzik and Voigt, 2014; Polak et al., 2011), other behaviours are also altered due to artificial light, such as commuting (Hale et al., 2015; Kuijper et al., 2008; Stone et al., 2009; Zeale et al., 2018), roosting (Boldogh et al., 2007; Straka et al., 2020), drinking (Russo et al., 2017) and migrating (Voigt et al., 2018). In addition to the effects on the individual level, artificial light has an impact at the community level (Cravens and Boyles, 2019) and in food webs (Sanders et al., 2023). Different bat species might respond differently, light-tolerant or synanthropic bats showing positive phototaxis while light-averse species avoid lit areas (Azam et al., 2018; Lewanzik and Voigt, 2017; Spoelstra et al., 2017). Artificial light alters prey-predator interactions by changing prey availability. Insects are attracted to light, especially the UV and the blue component of the light spectrum (Donners et al., 2018), thus creating a "vacuum effect" in unlit areas. Therefore, artificial light at night may also lead to bat species redistribution and alter ecological networks (Sanders et al., 2023).

Moreover, bats respond in a different way to dissimilar light types and spectra. Replacement of old light types is often a good opportunity to study the effects of light types transition in outdoor lighting. Stone et al. showed that the activity of *Pipistrellus pipistrellus*, *P. pygmaeus* and *Nyctalus/Eptesicus* spp. was significantly higher when low-pressure sodium (LPS) lights were replaced by white metal halide (Stone et al., 2015). Zeale et al. compared bat activity along hedgerows illuminated by lights with different spectra: high-pressure sodium (HPS) with orange hue, neutral white LED and two induction lamps emitting mainly green and red hue, respectively (Zeale et al., 2018). The response of bats was species-dependent: activity of light-averse species such as *Rhinolophus hipposideros* decreased under all light types but red light had no effect on *Myotis* spp. activity. On the other hand, agile species like *Pipistrellus* spp. were more active when hedgerows were illuminated with orange, green or white light. Similarly, Spoelstra et al. (2017) studied the effect of light spectrum on bat activity in forest-edge habitat using only LEDs with different spectral composition (green, white, red). This study demonstrated similar results: *Myotis* spp. and *Plecotus* spp. avoided green and white light, while *Pipistrellus* spp. were more abundant around these lights (Spoelstra et al., 2017). These results suggest that red light may be used to mitigate the impact of artificial light on light-averse bat species. However, bat response is context-specific. Bats might be for instance less light-sensitive when commuting compared to foraging, likely due to their shorter exposure to illumination (Hooker et al., 2022; Kuijper et al., 2008; Spoelstra et al., 2018).

On the other hand, very little is known about the effect of light intensity on bat behavioural response, despite the fact that reducing light intensity is one of the most recommended mitigation measures. So far, most studies have focused on the effect of artificial light compared to no light at all. However, the presence of light cannot be reduced to a presence/absence event, it is a continuum from bright light in the vicinity of the light source to very low light levels further away. It is nevertheless notoriously difficult to establish dose-response curves of bat activity depending on light intensity, and therefore estimate the intensity threshold at which bats avoid the lit parts of their habitat or stop using commuting routes. The dose-dependent responses are usually tested in controlled laboratory conditions (De Jong et al., 2016; Dominoni et al., 2018; Jägerbrand et al., 2023; Quintanilla-Ahumada et al., 2022) or determined by using distance to light source as a proxy (Azam et al., 2018; Shier et al., 2020). Yet, LEDs are a perfect model to study the effect of light intensity thanks to the capacity of dimming their light emission. Some studies already demonstrated than even low light levels reduce the activity of light-averse species (Azam et al., 2018; Stone et al., 2012). However, little is known about how light tolerance thresholds vary with the spectral composition. Therefore, the behavioural response of bats to light intensity, in synergy with the light spectrum, requires deeper investigation to prevent habitat loss and fragmentation in bats.

Aim and outline of the thesis

The overall aim of this thesis was to investigate the spatiotemporal response of bats to light intensity under different light spectra. This study would help to draw guidelines to prevent negative impact of nightly illumination on bats, especially in terms of habitat loss and habitat fragmentation. This is of great importance since bats provide many ecosystem services, for example by consuming large amounts of pest insects. All European species are legally protected, but there are still many anthropogenic disturbances that could be avoided or at least diminished, such as the continuous increase of artificial light at night. Therefore, an outright demand for mitigation measures arises to protect bats. In order to establish effective mitigation measures, for instance by keeping illumination intensity below the disturbance threshold, it is essential to know how the response of bats to light depends on light intensity.

In **Chapter 2**, I describe a combined methodology to reconstruct the three-dimensional spatial data of vegetation and map bat behaviour using fine-scale measurements, using LiDAR technology and acoustic tracking. This approach provides insights to study bat-habitat relationship *in situ*.

In **Chapter 3 and 4**, I focus on the spatiotemporal response to artificial light of synanthropic bats that are known to take advantage of concentrated prey resources around light sources. Based on the methodology presented in **Chapter**

2, I provide novel information on how the presence of artificial light and vegetation spatially affect foraging activity of synanthropic bats in **Chapter 3**. I further show in **Chapter 4** how light spectrum alters habitat quality of bats in terms of prey availability. I study how bat foraging activity is temporally driven by light spectrum and insect abundance by measuring insect and bat activity throughout the night.

Chapters 5 and 6 put the emphasis on the response of light-averse bat species to artificial light. In **Chapter 5**, I investigate how light-averse bats spatially alter their foraging behaviour in their natural environment in response to artificial light with different spectra to determine their light tolerance threshold. In **Chapter 6**, I focus on how the impact of artificial light on the flight behaviour of commuting trawling bats varies for different light spectra and light intensities.

Finally, in **Chapter 7**, I summarize my findings and discuss how these different studies together improve our understanding of the effects of artificial light at night on the ecology of bats. I also discuss possible implications of our findings for lighting policy in order to prevent habitat loss and fragmentation caused by artificial light. I end the general discussion with several suggestions for future research.

Chapter 2

Combining acoustic tracking and LiDAR to study bat flight behaviour in three-dimensional space

Claire Hermans, Jens C. Koblitz, Harm Bartholomeus, Peter Stilz, Marcel E. Visser, Kamiel Spoelstra

Abstract

Habitat structure strongly influences niche differentiation, facilitates predator avoidance, and drives species-specific foraging strategies of bats. Vegetation structure is also a strong driver of echolocation call characteristics. The fine-scale assessment of how bats utilise such structures in their natural habitat is instrumental in understanding how habitat composition shapes flight- and acoustic behaviour. However, it is notoriously difficult to study their species-habitat relationship *in situ*. Here, we describe a methodology combining Light Detection and Ranging (LiDAR) to characterise three-dimensional vegetation structure and acoustic tracking to map bat behaviour. This makes it possible to study fine-scale use of habitat by bats, which is essential to understand spatial niche segregation in bats. Bats were acoustically tracked with microphone arrays and bat calls were classified to bat guild using automated identification. We did this in multiple LiDAR scanned vegetation plots in forest edge habitat. The datasets were spatially aligned to calculate the distance between bats' positions and vegetation structures. Our results are a proof of concept of combining LiDAR with acoustic tracking. Although it entails challenges with combining mass-volumes of fine-scale bat movements and vegetation information, we show the feasibility and potential of combining those two methods through two case studies. The first one shows stereotyped flight patterns of pipistrelles around tree trunks, while the second one presents the distance that bats keep to the vegetation in the presence of artificial light. By combining bat guild specific spatial behaviour with precise information on vegetation structure, the bat guild specific response to habitat characteristics can be studied in great detail. This opens up the possibility to address yet unanswered questions on bat behaviour, such as niche segregation or response to abiotic factors in interaction with natural vegetation. This combination of techniques can also pave the way for other applications linking movement patterns of other vocalizing animals and 3D space reconstruction.

Introduction

Vegetation structure is a key biotic factor that affects animal movement (Davies and Asner, 2014). Vegetation influences, among others, prey-predator interactions as it offers shelter from predators (Cassini and Galante, 1992; Fisher, 2000). While some nocturnal species are night active to reduce predation risk (Rydell and Speakman, 1995; Speakman, 1991b), they often make use of the vegetation cover to find additional shelter (Prugh and Golden, 2014). Bats in particular depend on nocturnal darkness (Duvergé et al., 2000; Jones and Rydell, 1994). Even in darkness, only a limited number of species ventures out in relatively open space when foraging, and these are generally fast-flying and agile species (Fenton, 1990; Neuweiler, 1989). Many other species stay in cluttered environments when foraging; these are typically slow-flying species (Fenton, 1990; Neuweiler, 1989). Therefore, vegetation structure strongly influences bat activity and niche segregation between bat guilds (Adams et al., 2009; Schnitzler and Kalko, 2001). Studying the effect of vegetation structure on the behaviour and ecology of bats is often done at the landscape level (Downs and Racey, 2006; Ferreira et al., 2022; Laforge et al., 2021, 2019). Detailed information on how bats from different bat guilds adjust their small-scale spatial behaviour to vegetation structure is highly important to understand species-habitat relationships in forest environment (Adams et al., 2009; Froidevaux et al., 2016; Schnitzler and Kalko, 2001).

Bat guilds may not only be differentiated by their flight behaviour, but also by their echolocation calls (Denzinger and Schnitzler, 2013; Fenton, 1990). Bats rely on echolocation to navigate, avoid obstacles and locate prey (Griffin et al., 1960; Griffin and Galambos, 1941). As bats adjust their calls to the task and environment they are faced with (Schnitzler and Kalko, 2001), the structure of natural vegetation is a strong driver of call characteristics. Generally, calls differ with distance to background objects, such as the ground and vegetation. Such changes include increasing bandwidth and shortening interval and call duration when approaching vegetation (Siemers and Schnitzler, 2000). However, the transition zone between open- and edge-space calls has been studied with limited spatial resolution (Kalko and Schnitzler, 1993; Schaub and Schnitzler, 2007). Precise measures of call parameter adjustment in response to the background can only be estimated by combining the position of a bat at the time of the call emission with the distance to the vegetation and the ground.

Knowing the precise positions of bats relative to vegetation structures is essential to understand the response of bats to different environmental factors. For example, there is accumulating evidence that bats' behaviour is affected by ambient light. In line with nocturnality in general, the response of bats to low light levels at night is widely recognized as related to predator avoidance (Rydell and Speakman, 1995; Speakman, 1991b). This hypothesis is supported by the fact

that slow-flying bats emerge later from their roosts compared to fast and agile bats (Jones and Rydell, 1994). Higher ambient light levels may cause bats to fly closer to vegetation structures in order to be less conspicuous to potential predators. This may reduce the possibility to observe bats and indeed, several studies reported lower activity of bats with moonlight, mainly in tropical bat species (Appel et al., 2017; Ciechanowski et al., 2007; Saldaña-Vázquez and Munguía-Rosas, 2013; Vásquez et al., 2020). Flying closer to vegetation may hamper prey capture success, as background echoes may interfere with prey echo, especially for open-space and edge-space foragers (Schnitzler and Kalko, 2001). The potential benefit of extra safety by flying close to the vegetation is therefore a trade-off between predator avoidance and foraging efficiency, which may be influenced by light.

In order to study the interaction between vegetation and flight behaviour, high resolution information on both flight pattern and vegetation structure is essential. Very few studies have explored the three-dimensional (3D) spatial data of vegetation to study bat behaviour. Some laboratory-based evidence demonstrates that bats use visual and auditory cues to navigate (Jones and Moss, 2021) and fly in stereotyped flight paths (Hulgard et al., 2016), but studies in real-life environments are sparse, most likely because of the labour-intensiveness of 3D flight path assessment. Few studies have studied bats in their natural habitat using remote sensing methods such as Light Detection and Ranging (LiDAR) (Blakey et al., 2017; Froidevaux et al., 2016; Jung et al., 2012; Müller et al., 2013; Rauchenstein et al., 2022). While aerial laser scan (ALS) was preferred to cover larger areas (Froidevaux et al., 2016; Jung et al., 2012; Müller et al., 2013; Rauchenstein et al., 2022; Roeleke et al., 2018b), very fine-scale vegetation information can be obtained at the plot level with terrestrial laser scanning (TLS), especially below the canopy, where bats potentially fly to avoid predators or search for prey (Gomes et al., 2020; Hecker and Brigham, 1999; Lang et al., 2006). TLS has become the method of choice for precise 3D scanning of vegetation relevant for bat habitat, as it is the best approach for scanning the canopy and the vegetation below (Jones and Holderied, 2007). In previous studies, spatial distribution of bats was assessed using ultrasonic bat detectors, thermal imaging (Yang et al., 2013), GPS (Global Positioning System) tags (Roeleke et al., 2018b) or mist nets (Ciechanowski et al., 2007). While bat detectors or mist nets provide limited detail on vertical stratification of bats (Adams et al., 2009; Ciechanowski et al., 2007), thermal imaging can mainly be used in open areas, as it is difficult to combine data from multiple cameras in dense forest and thus to reconstruct bats' flight patterns. Several cameras are needed and must be carefully positioned with greater but precisely known distance and exact angle in order to calculate precise positions of bats, and this has to be done again every single time they are set up in a new environment. Therefore, these techniques are much less suitable to study the fine-scale species-habitat relationship of bats.

Nowadays, acoustic localisation can overcome some disadvantages of these methods. It offers great opportunities to precisely study animal movements on a fine but limited spatial scale. Acoustic localisation is done by calculating the time-of-arrival-difference (TOAD) of each signal between several microphones (Ing et al., 2016; Koblitz, 2018). Acoustic tracking using microphone arrays is easy to deploy: it is limited to mounting a frame with microphones at fixed positions on a tripod. As long as one knows the position and the angle of the frame relative to the ground plane, bats' positions can be calculated relative to the array (Koblitz, 2018). As echolocating bats produce numerous echolocation calls per second, the technique allows for tracking with a high spatial and temporal resolution, and echolocation calls can be simultaneously used for species identification. The method is thereby a great complementation to GPS tracking, which provides much less precise spatial data, but at a much larger scale and specific for each individual. Another benefit of acoustic localisation is that animals do not need to be captured and carry a logger or transmitter, so their behaviour is not altered by this technique and even very small bat species can be tracked.

Here, we show that the difficulty of accurate assessment of the interaction between bat flight behaviour and spatial structures can be resolved with combining acoustic bat tracking and LiDAR vegetation scans. This opens up the possibility to acquire knowledge on the fundamental mechanisms on how bats interact with their environment. Recent developments in portability and ease of deployment of both techniques facilitate quick collection of spatial data on vegetation structure and bat flight behaviour in the field. Although it entails challenges with combining mass-volumes of fine-scale bat movements and vegetation information, here we show the feasibility and potential of combining those two methods for future studies on bats. This combined method could be applied as well to other systems linking movement patterns and measurements of 3D space.

Material and methods

Field sites

Acoustic bat tracking was combined with LiDAR scans in forest edge habitat at seven experimental sites set up to study the effect of artificial light at night on the forest-edge ecosystem (Spoelstra et al., 2015). The sites are located in the Netherlands and consist of either coniferous forest with Scots pine (*Pinus sylvestris*) or Douglas fir (*Pseudotsuga menziesii*), or mixed forest with Scots pine and common oak (*Quercus robur*) and birch (*Betula* sp.). At each site we collected data at three plots (Figure 2.1). The distance between the centre of two plots varied between 88 and 386 m (average 204 m; standard error, s.e. 17).
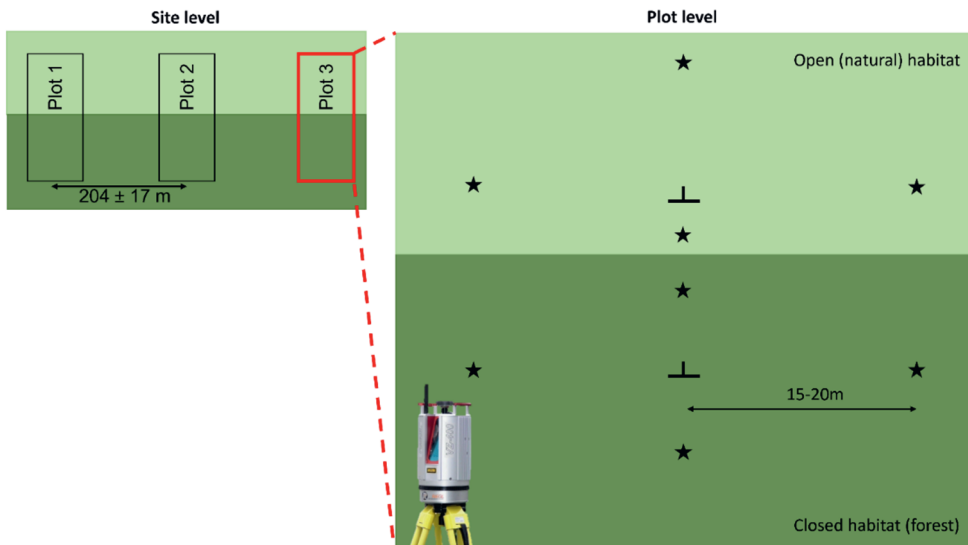
Figure 2.1: Set-up to collect LiDAR data at one plot within a site with a RIEGL VZ-400 terrestrial laser scanner. To include both open and closed vegetation structures, we deployed microphone arrays (T-shaped) at the forest edge and in the forest and we scanned the plot up to 15-20 m from the array. Each star represents an individual scan location at which both an upright and tilted scan were done.
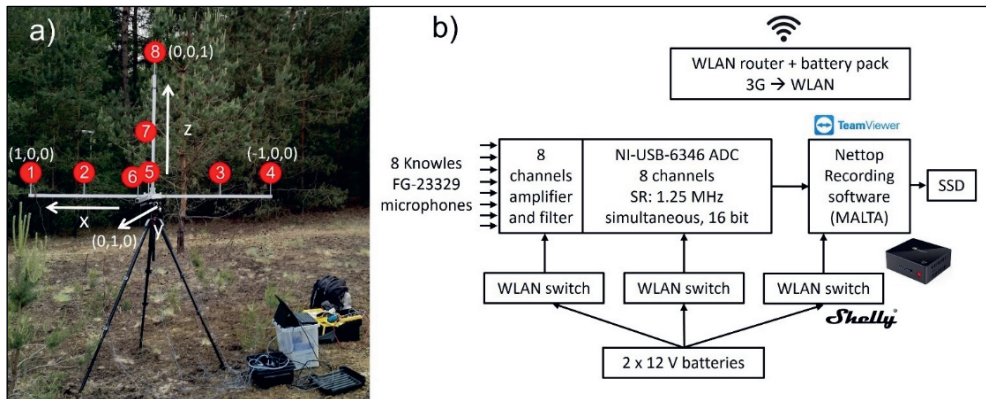
Acoustic localisation

Data collection

Bats were acoustically recorded from 15 minutes before sunset to 15 minutes after sunrise for a total of 27 nights between May 8th 2020 and August 9th 2020. Up to seven microphone arrays were deployed at one site per evening in the open area, at the forest edge and in the forest to account for different vegetation structures (three plots per site, two to three arrays per plot).

Technical description of the system

Each array consisted of eight microphones (omnidirectional microphones FG-23329 Knowles Electronics, Itasca, IL, USA), fitted on an aluminum frame with arms in x, y and z directions (see Figure 2.2A for precise layout). The array frame could be disassembled for easy transport in remote field sites only accessible by foot. The microphones were connected to a custom-made amplifier and filter unit (Figure 2.2B). Sound recordings were digitised with an Analog-Digital-Converter USB-6346 (DAQ) (National Instruments, TEX, USA) at a sampling rate of 300 kHz and 16 bit resolution. MALTA Software (Microphone Array Localisation Tool for Animals, version 3.6, CAE Software & Systems, Germany) allowed real-time visualization of time series of all channels and computation of real time spectrogram of one channel at a time. All recording parameters were controlled

and set in the MALTA Software, and all sound recordings were stored on Mini PCs (Gemini X, Beelink). Recording systems were remotely controlled and monitored using WLAN routers (TP-Link M7200 MiFi) and TeamViewer (TeamViewer GmbH, Germany).

Each system was battery-powered by one 12 V 20 Ah battery for the Mini PC and the DAQ and one 12 V 5 Ah battery for the amplifier, adding up to 25 Ah in total. The power requirements of the entire system were 1.3 A at 12 V, i.e. approximately 15 W, allowing for 20 hours of recording. The recordings were stored on an external 1 TB SSD. All the recording equipment including batteries easily fits in a 20 L box (Supplementary data, Figure S2.1).

Figure 2.2: Acoustic localisation setup. A) array set-up in the field, red dots indicate the positions of the eight microphones (also numbered), B) schematic of the set-up.

Calculating 3D positions

Each echolocation call reaches each of the eight microphones at a different time because of the distance between the microphones. The time-of-arrival-difference (TOAD) between the signal of the reference microphone (in this case the top microphone) and the signal of each of the other microphones was determined by cross correlation using a custom-built software (TOADSuite, P. Stilz, J.C. Koblitz and H.R. Goerlitz) (Goerlitz, 2019) in MATLAB R2020a (The MathWorks, Inc., Natick, MA, USA). The bat's position at the moment of signal emission was calculated based on these TOADs (Götze et al., 2020).

For the analysis two approaches are possible: 1) the 3D position of every call localised with sufficient precision is considered as an individual data point. 2) subsequently, flight paths based on the spatial temporal pattern of successive localised echolocation calls can be computed. Note that the spatial coverage of the array is limited to a hemisphere with a radius of 5-20 m depending on bat species. Animals frequently leave and re-enter this hemisphere and it is impossible to determine whether the same or a different bat is recorded.

Localisation error assessment

Technically, only four microphones are needed for 3D localisation. In this study we added four extra microphones, resulting in an overdetermined array with eight microphones (Figure 2.2). The use of an overdetermined array allows assessment of localisation error by comparing the theoretical TOADs based on isotropic spherical sound spreading from the localised sound source position with the real TOADs of the incident sound at the multiple microphone positions. Two types of localisation errors can be assessed, namely the radial error and the tangential error. The radial error defines the difference between the actual and calculated 3D position of the sound source in a direct line to the centre of the array. The tangential error defines the difference between the actual and calculated 3D position of the sound source in the plane perpendicular to the axis between the centre of the array and the calculated position (see Supplementary data, Appendix S2.1).

Large localisation errors may occur for short and faint calls with short inter-pulse interval during the feeding buzz phase (the moment bats capture an insect; (Griffin et al., 1960)), or when calls from two individuals are recorded simultaneously, leading to cross correlation mismatch between the microphones. As a rule of thumb, an accurate localisation can be achieved in a distance of one to ten times the array dimension (in this case 2 to 20 m with an array aperture of 2 m). 3D positions based on recordings of pulses very close to the array are the least precise localisations due to reflection and shading artefacts from the array frame, relatively large TOADs because of the array geometry and microphones receiving highly different signal shapes of different emission directions. Therefore, positions within two meters of the centre of the microphone array were excluded (Supplementary data, Figure S2.4). Positions located more than 20 m away were kept, as it is possible to detect and localise very loud calls emitted in the open area. Moreover, as the aim of this method is to combine bats' positions with fine-scale vegetation data, positions were excluded if one of the two localisation errors was greater than 0.5 m.

Species identification

Sound files were analysed with the Tadarida software ((Bas et al., 2017), online repository: https://github.com/YvesBas, January 2021 version) to detect and classify sound events. As the identification of bats to the species level is difficult based on their echolocation parameters, we limited identification to the following bat guilds: the ENV group including *Eptesicus* spp., *Nyctalus* spp. and *Vespertilio* spp. that are open space aerial foragers, the *Myotis* group including *Myotis* spp. that forage close to and within foliage or over water surfaces, and the *Pipistrellus* group including *Pipistrellus* spp. that are edge space aerial foragers (Denzinger and Schnitzler, 2013; Neuweiler, 1989). Lastly, we linked each microphone array

derived 3D position to the species group identified by Tadarida, using the detection time of the bat calls.

LiDAR

Multiple returns Terrestrial LiDAR data were collected from June 2020 to April 2021 with a RIEGL VZ-400 terrestrial laser scanner (RIEGL LaserMeasurement Systems, Horn, Austria). Scans were done under leaf-on conditions (presence of foliage on deciduous trees), but plots with almost exclusively coniferous species were scanned later in the season. For each plot in which we linked acoustic localisation with LiDAR, scans were done at 7-16 locations (dependent on the number of arrays and density of the understorey vegetation), following the setup shown in Figure 2.1. One scan was always done close to the microphone array, and additional scans were done at a distance of ~15-20 m from the array, to ensure that we captured the vegetation structure in the whole plot. At each scan location, two scans were done; the first scan with the scanner straight up, covering zenith angles between 30º and 130º off nadir. The second scan was acquired with the instrument tilted at 90º from the vertical to sample the full hemisphere. Scans were done with an angular resolution of 0.06 degrees, resulting in a cloud density with a mean Euclidean nearest neighbour distance of <2 cm within the plot. Reflective targets were used to co-register and align the individual scan locations using RIEGL's RiSCAN Pro Software version 2.8.0 (Wilkes et al., 2017). Finally, the co-registration was optimised using the Multi-Station Adjustment (MSA) algorithm, within RiSCAN Pro. MSA modifies the position and orientation of individual scan locations in several iterations to calculate the best overall alignment. The resulting point clouds were filtered based on the deviation of the returned LiDAR signal. Returns with a higher pulse deviation often represent semi-returns, softer targets or noise which can hinder further analysis (Pfennigbauer and Ullrich, 2010). Therefore, all points with a pulse deviation higher than 15 were filtered out. Per plot this resulted in point clouds containing between 17 to 206 million points, depending on the size of the area scanned and the vegetation density.

Combining LiDAR and acoustic localisation

Microphone arrays were set up during the LiDAR scans in exactly the same position and angle as when tracking bats (Supplementary data, Appendix S2.2). The array is thus present in both 3D datasets. The array coordinates (the four ends and the centre of the frame) in LiDAR scans were subsequently used as a reference to apply a rigid body transformation (translation and rotation, [see Supplementary data, Appendix S2.2]) on bats' positions to align them with the coordinate system of the vegetation scans in CloudCompare (version 2.12 beta, 2022).

Distance to the vegetation can be directly assessed by calculating the distance for each bat's position to the closest vegetation point using the Cloud-to-Cloud

Distance computation tool in CloudCompare. However, isolated vegetation points may interfere with the calculation. Therefore, the point clouds are 'voxelised' by converting data into a 3D volume of data values. This yields a 3D grid with the number of points per voxel indicating voxel specific vegetation density (Vosselman et al., 2004). Unlike point cloud data, voxels have a defined length, width, area and volume, which can contain quantitative information on the space occupied by vegetation. These parameters depend on voxel size; small voxels result in data redundancy, while large voxels overestimate the space occupied by objects (Ross et al., 2022; Vosselman et al., 2004). According to Ross et al. (Ross et al., 2022), a 10-25 cm resolution is the optimal size for estimating canopy gaps in forest plots, which is an important factor to take into account when studying bat flight behaviour. This also corresponds to the closest distance between target and background clutter required for bats such as *Myotis nattereri* to detect prey (Siemers and Schnitzler, 2000). Lasvoxel tool in LAStools (version 210418, rapidlasso GmbH, Gilching, Germany) was used to build a 3D grid of 20x20x20 cm cubic voxels. For each voxel we thus obtain an occurrence value of vegetation points, and we can define voxels with a vegetation count value below a specific threshold as background voxels.

Results

Overall activity

Out of 138 recorded array nights (one array night is a full night of recording for one microphone array), 6822 (s.e. 1465) 3D positions per night were calculated in average. By defining a bat pass as a 10 seconds file containing at least one position, this resulted in 326 (s.e. 38.3) passes per night in average. 93.52% of the 3D positions were assigned to the *Pipistrellus* group, 6.29% to the ENV group (*Eptesicus* spp., *Nyctalus* spp. and *Vespertilio* spp.) and 0.19% to the *Myotis* group. This is consistent with previous studies carried out at the same sites using different acoustic monitoring devices (Barré et al., 2021; Spoelstra et al., 2017).

Individual tracks

Individual tracks can be constructed using subsequent 3D positions, which has the advantage that two individuals recorded at the same time can be spatially separated. This can be done in some cases using just one spatial dimension (i.e. x, y or z coordinate values; see Figure 2.3 and 2.4). In Figure 2.3, one bat flies back and forth from 4 m at one side of the array to 2 m at the other side of the array with a very regular movement pattern (see Supplementary data, Figure S2.2 for the track in 3D). On the other hand, Figure 2.4 shows two to four distinctive flight tracks. As individuals leave and enter the recorded hemisphere, it is however impossible to assess whether the same or different bats are recorded over time.

The maximal recorded distances for the ENV, *Pipistrellus* and *Myotis* groups are respectively 37.25 m, 34.27 m and 20.70 m from the array.
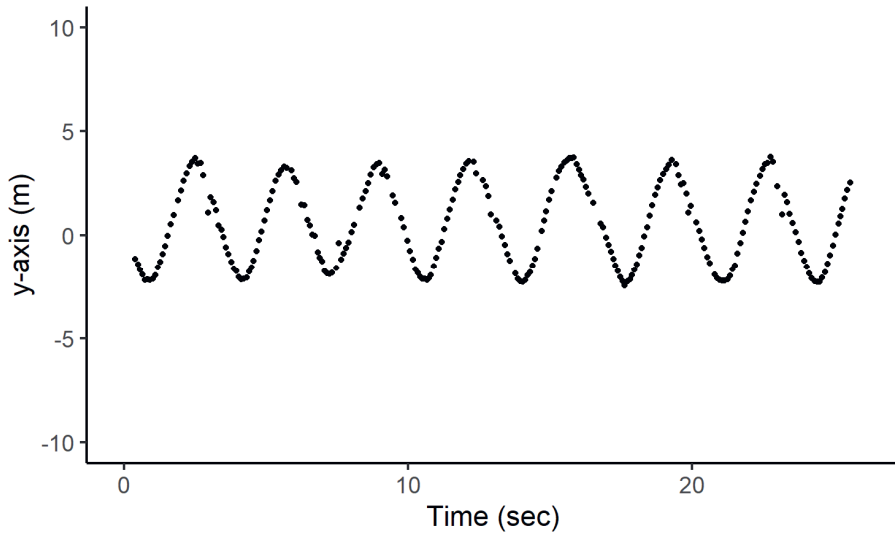
Figure 2.3: Individual flight track of bat in one dimension (Y coordinate over time). Y = 0 m corresponds to the centre of the microphone array (positions are more than 2 m away from the array centre in three-dimensional space).
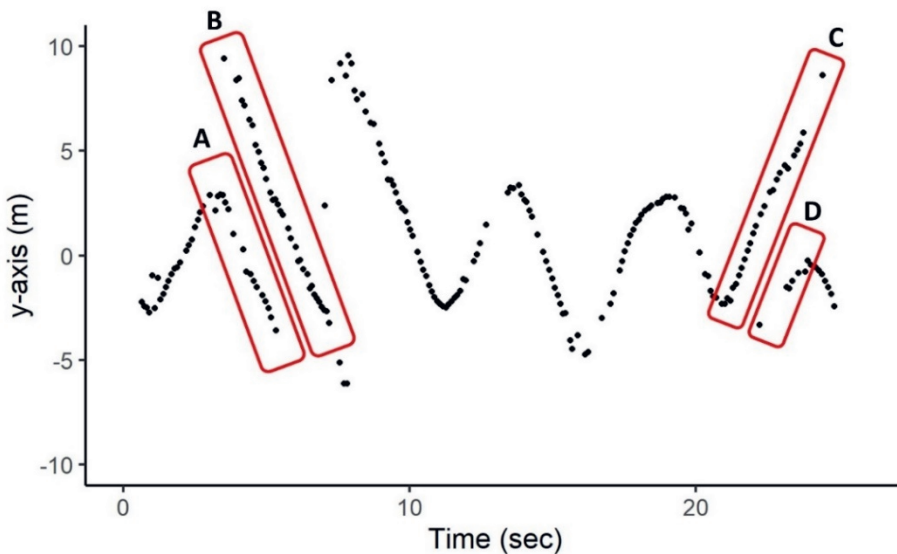
Figure 2.4: Multiple flight tracks of bats in one dimension (Y coordinate over time). Y = 0 m corresponds to the centre of the microphone array (positions are more than 2 m away from the array centre in three-dimensional space). Red boxes highlight the presence of two individuals at the same time. A and B correspond to two individuals with distinctive flight tracks. C and D are either tracks of the same individuals or from other two individuals.

Integration of LIDAR and acoustic localisation

Case study 1: Stereotyped flight paths

Spatial alignment of vegetation scans and bats' positions shows that bats' positions can be well aligned with the vegetation, as represented in Figure 2.5. Obstacles like tree trunks obstruct sound propagation of bat calls when in between the bat and the microphone array, thus the echolocation calls cannot reach the microphones and cannot be localised. These acoustic shadows validate the alignment of the two 3D datasets by matching the missing bats' positions with the obstacles in LiDAR scans. Figure 2.5 shows data from three nights of recordings at the same plot. Pipistrelles use stereotyped flight paths in cluttered environment by circling around the trees each night in each vegetation layer (subfigures of Figure 2.5). However, it is not feasible to estimate how many individuals were flying in these stereotyped flight paths based on acoustic recordings. This first case study is one of the few pieces of evidence of stereotyped flight paths of pipistrelles in their natural habitat (Fujioka et al., 2014).
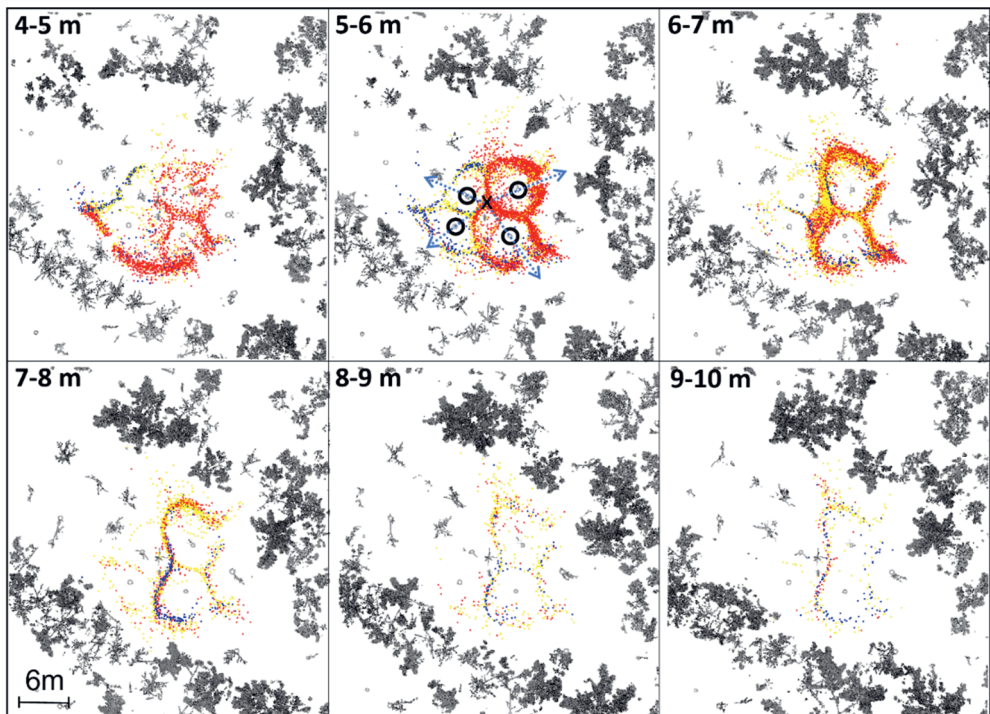
Figure 2.5: Top view of *Pipistrellus* spp. positions (coloured dots) integrated to vegetation scan (black dots) at heights that included most of the calculated bats' positions (from 4 to 10 m above the ground). Each colour corresponds to a different recorded night (blue for June 9th 2020, red for July 28th 2020 and yellow for July 30th 2020). In the second plot (from 5 to 6 m high), the cross indicates the microphone array's position. The tree trunks (circled) produce acoustic shadows beyond them (represented by the blue arrows).

Case study 2: Distance to vegetation

The second case study describes the distance that bats keep to the vegetation structure and obstacles. Figure 2.6 depicts the distance pipistrelles keep to the vegetation in forest-edge habitat. The flight path of one individual along the forest edge is also reconstructed as an example (Figure 2.6). Spatial data are structured in 20x20x20 cm voxels. In this case study, pipistrelles fly around a lamppost, while keeping their distance both from the vegetation and the lamppost, as shown in Figures 2.6 and 2.7. When flying in a wider corridor, bats stay further away both from the vegetation (Figure 2.7a) and the light source (Figure 2.7b). In the wide corridor (7.6 m wide), bats fly in average at 4.07 m from the lamppost and 3.99 m from the vegetation (Welch t-test = 7.06, p < 0.001). In the narrow corridor (4.8 m wide), bats fly in average at 3.14 m from the lamppost and 2.84 m from the vegetation (Welch t-test = 12.4, p < 0.001). Thus, pipistrelles fly closer to the vegetation than the lamppost, but they also keep a certain distance to the vegetation to avoid clutter.
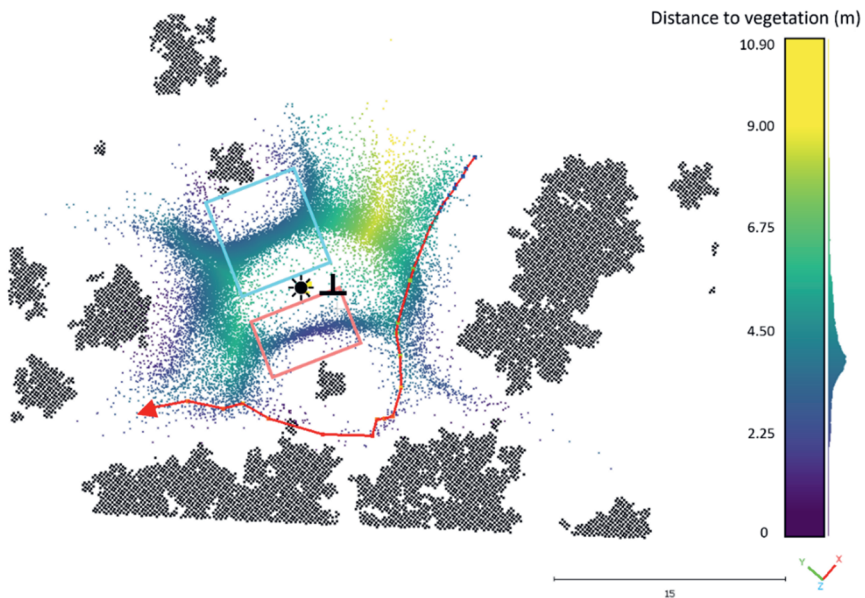
Figure 2.6: Distance to the vegetation. Top view of *Pipistrellus* spp. positions (coloured dots) integrated with the voxelised vegetation scan (black dots) within the horizontal plane between 3.5 and 4.5 meters above the ground. The T-shape represents the array. The red arrow shows an example of a flight trajectory. Voxels have a size of 20x20x20 cm. Only voxels with at least ten vegetation points are marked as 'vegetation voxels' as this preserves a fine-scale resolution in vegetation while filtering out background voxels containing isolated vegetation points. The distance to the vegetation corresponds to the absolute distance (in meters) of each bat position to the closest vegetation voxel containing at least ten vegetation points. The black icon next to the microphone array shows the position of the lamppost (height of 4 m), which is part of the experimental setup of the 'Light on Nature' sites. The highlighted sections indicate the narrow (light red) and the wide (light blue) corridors described in Figure 2.7.
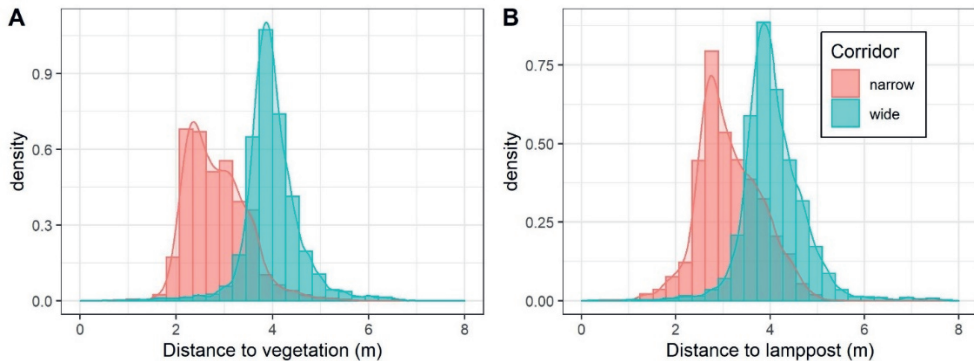
Figure 2.7: Space use of pipistrelles in response to artificial light in a narrow (4.8 m wide, n=1342 positions) and a wide (7.6 m wide, n=6463 positions) corridor shown in Figure 2.6. A) Distribution of bats' distance to the closest vegetation voxel containing at least ten vegetation points. B) Distribution of bats' distance to the lamppost.

Myotis and ENV groups data from the same plot are available in Supplementary data, Figure S2.3. *Pipistrellus* and ENV groups exhibit a different use of space (*Myotis* group was not compared, as only two tracks were recorded, see Figure S2.3C). While most pipistrelles fly up to 4 to 5 m from the vegetation, ENV species have a wider distribution of distance to the vegetation (Figure 2.8A; the two distributions are significantly different according to the Kolmogorov-Smirnov test, D = 0.22, p < 0.001). Moreover, pipistrelles generally fly closer to the lamppost than ENV species (Figure 2.8B; the two distributions are significantly different according to the Kolmogorov-Smirnov test, D = 0.46, p < 0.001).
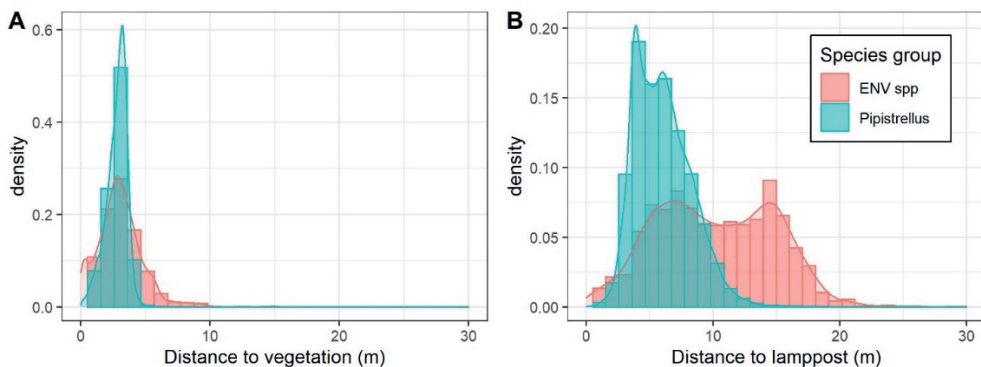
Figure 2.8: Space use of the ENV group (n=1790 positions) and the *Pipistrellus* group (n=62336 positions) in forest edge habitat. A) Distribution of bats' distance to the closest vegetation voxel containing at least ten vegetation points. B) Distribution of bats' distance to the lamppost.

Discussion

In this study, we show that the combination of acoustic localisation and LiDAR vegetation scanning is a method of great potential to study the interaction of bats with their immediate surroundings. The ability to study bat behaviour in relation to fine-scale structures is of relevance as many bat species strongly rely on these for nightly foraging and commuting routines (Brigham et al., 1997; Verboom and Huitema, 2010, 1997). They may change this interaction depending on momentary and local weather (Verboom and Spoelstra, 1999), light conditions (Hale et al., 2015) or prey availability (Hecker and Brigham, 1999; Lang et al., 2006). By combining acoustic localisation and LiDAR, these interactions can be precisely quantified (Aschoff et al., 2006). With the improvements on the acoustic tracking system, microphone arrays have become easy to deploy and recordings can now be remotely controlled and monitored for two full nights. The ability to use the microphone array system remotely controlled for consecutive nights allows for additional assessment of temporal changes in addition to spatial data.

Acoustic tracking data have a very high spatial accuracy, with spatial resolution of few centimetres. This is very valuable to study the fine-scale interaction of bat flight with habitat structure, which would not be possible with GPS or radio tags. It also provides very high temporal resolution data, as bats emit numerous echolocation calls per second. As we used an overdetermined array, it is possible to use differences in predicted and observed TOADs to estimate localisation errors.

The limitations of acoustic localisation depend on a complex relation of a multitude of parameters, for example shading artefacts from the array frame, array geometry, call directionality and shape, or inter-pulse intervals. Interfering calls and low signal-to-noise ratio can limit the localisation precision. Therefore, the selection of an optimal array setup depends on the task to solve and the recording conditions. More accurate localisation can be achieved with larger TOADs using an array with a larger aperture. However, if the targeted sound is highly directional, it may not reach some of the microphones. Here, an array with a smaller aperture may remain a better option for good localisation results.

The range for spatial detection is species-dependent, as acoustic parameters of echolocation pulses vary across bat species (Russ et al., 2012). ENV calls are often louder and at lower frequencies, and therefore can be detected and localised further away. *Myotis* species tend to reduce their call amplitude when flying in cluttered environment and approaching prey (Boonman and Jones, 2002), thus they will be less detectable. However, the error variance in 3D positions calculation follows the same patterns between the three species groups (Supplementary data, Figure S2.4).

Combining 3D data obtained by the two different techniques requires precise alignment, but we show this is well feasible with the use of reference locations, in

our case the array position itself (but if necessary, more reference locations can be added). As shown in the first case study, hard objects, such as tree trunks, create acoustic shadows and impair the ability to localise bats when in between the bat and the microphone array. Although this can be solved with the construction of flight paths, LiDAR data may further be helpful to solve potential issues with acoustic shadows produced by obstacles as these data reveal the location of such obstacles. Strictly speaking, the LiDAR data do not validate the 3D bats' positions, but precisely explain the acoustic shadows produced by trees in case of proper alignment.

This first case study is also one of the few pieces of evidence of stereotyped flight paths of pipistrelles in their natural habitat (Fujioka et al., 2014). As already suggested by Hulgard et al. (2016) (Hulgard et al., 2016) in the big brown bat (*Eptesicus fuscus*), pipistrelles may also reduce their sensory processing load for navigation in known area in order to ameliorate their foraging efficiency. Combining the bats' positions with vegetation scans provides an additional layer of information in our understanding of the acoustic field of view in echolocating bats. The ability of prey detection depends on the distance to clutter of a foraging bat. Therefore, it is highly relevant to get high-detailed information on vegetation through LiDAR scans and combine this with acoustic localisation data to study how vegetation affects echolocation behaviour of bats in cluttered habitat. This would allow to investigate the plasticity in echolocation signals at a fine-scale spatial resolution in the field.

The second case study shows the potential of unravelling the interaction of bat flight behaviour and the vegetation structure. Different bat guilds keep distinct distances to obstacles; in our example, open-space foragers such as ENV species stay further away from the vegetation and from a lamppost than opportunistic species like pipistrelles. Therefore, measuring the distance of bats to the vegetation is particularly relevant to understand niche segregation of bat guilds in relation to the density of habitat clutter.

How bats respond to abiotic factors, such ambient light by the moon or artificial light sources and how these interact with vegetation (Barré et al., 2021) can be studied in much more detail using acoustic tracking and LiDAR. The deterrent effect of light could also be studied in greater detail by mapping the light level around lampposts and link this with the vegetation structure around the light source and bat 3D activity.

This example also shows the potential of this method to precisely look at behaviours such as the use of corridors by bats and their flight characteristics via the analysis of their trajectories, which has important implications for bat protection measures at the landscape level. Lastly, parameters such as flight

speed or straightness of the flight trajectories can also be computed to evaluate bat responses to obstacles such as vegetation.

Bats are appropriate model organisms to validate our combined method, as their flight behaviour is shaped by habitat characteristics and their echolocation signals are excellent for high-resolution acoustic tracking. However, combining LiDAR with acoustic tracking could be applied for other vocalizing organisms (i.e., nocturnal species for which visual survey methods are ineffective such as crickets, katydids (Jain and Balakrishnan, 2012) and frogs (Gerhardt and Bee, 2006; Page and Ryan, 2008)). Combining the two techniques could also help to better understand acoustic behavioural changes in shrews in response to habitat clutter (Siemers et al., 2009), or map song posts and territories of songbirds (Collier et al., 2010; Kirschel et al., 2011; Wilson and Bayne, 2018). The main criterion to combine LiDAR with acoustic tracking is to use one or more common objects in both datasets (in our case, the array) as reference for co-registration. At least three reference points (here we used five points) are needed to apply a rotation and translation matrix on one 3D dataset to align it with the other one. LiDAR could also help to precisely map microphones that are separated from each other on larger distances and synchronized by radio-transmission or GPS signal in thick vegetation.

Conclusions

Combining techniques as acoustic localisation and LiDAR allows to precisely map bat flight movements in response to spatial structure, opening up the possibility to address open and novel questions on fine-scale bat behaviour, such as niche segregation between different bat guilds, and responses to artificial light at night. While it is important to consider wider landscape composition to study forest management and bat conservation, studies on the local, fine scale may prove highly important to provide bats high-quality foraging habitat. There are other vocalizing animal species, such as songbirds, of which novel information can be collected to exploit the potential of the combination of the methods, making the methodology outlined in this paper relevant for a wide range of study systems.

Acknowledgments

Supplementary data to Chapter 2

Figure S2.1: Recording equipment for acoustic localisation of bats.
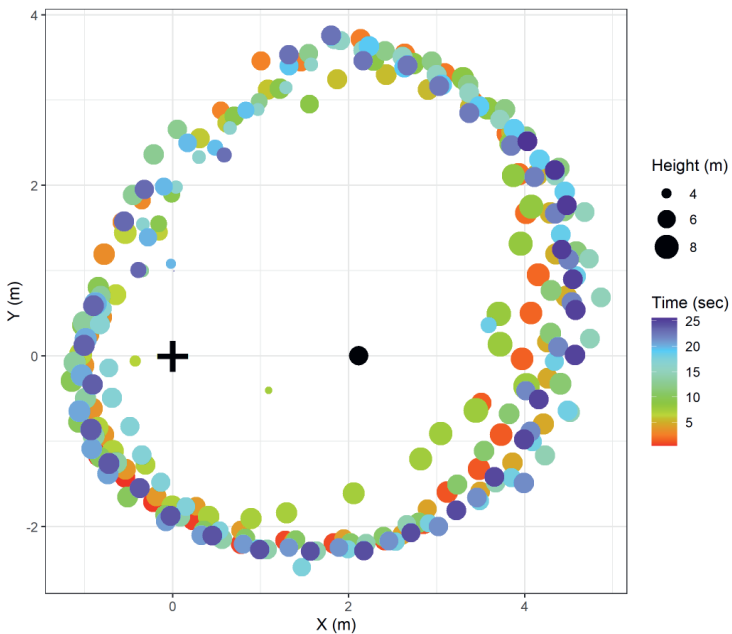
Figure S2.2: Individual flight track of bat, top view. The dot size and the colour scale indicate respectively the bat's height and the time of the call emission. The black cross shows the array's position. The black dot indicates the position of a lamppost, which is part of the experimental setup of the 'Light on Nature' sites.
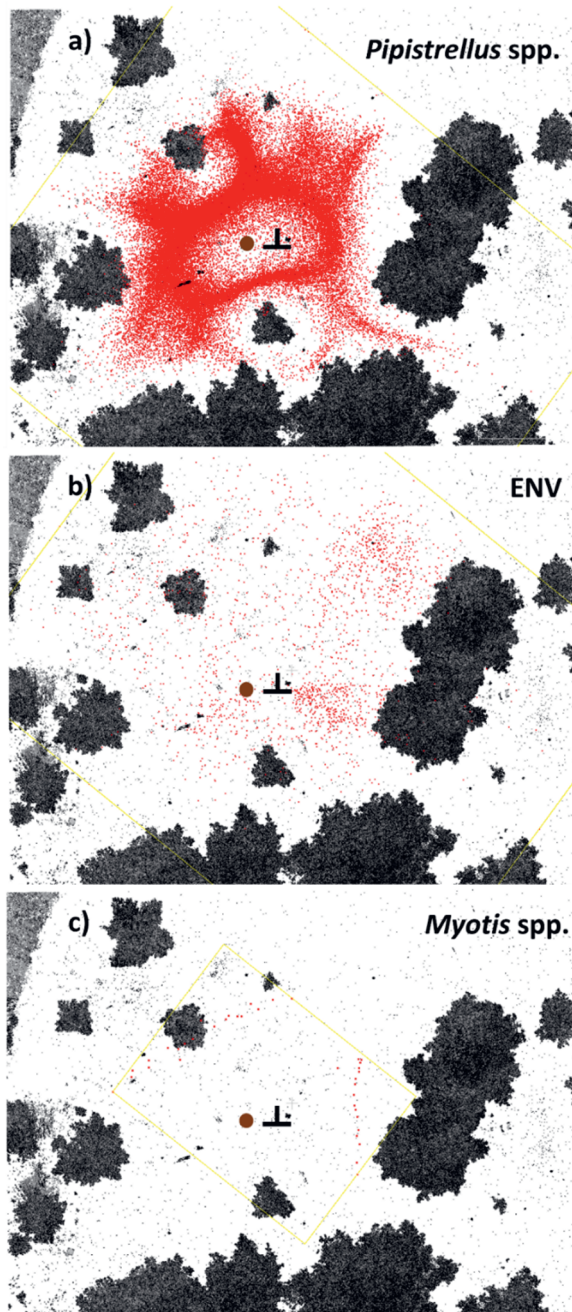
Figure S2.3: Top view of bat positions (red dots) integrated to vegetation scan (raw data). A) *Pipistrellus* spp., B) ENV group (*Eptesicus* spp., *Nyctalus* spp. and *Vespertilio* spp.) and C) *Myotis* spp.. The T-shape represents the array. The brown dot next to the microphone array shows the position of a lamppost (height of 4 m), which is part of the experimental setup of the 'Light on Nature' sites.

Appendix S2.1: Localisation error assessment

Technically, only four microphones are needed for 3D localisation. In this study the array has four extra microphones, resulting in an overdetermined array with eight microphones.

The use of an overdetermined array allows assessment of the localisation error: based on the arrival time at all microphones, the location of the sound source is calculated such that the sum of all squared differences between recorded and predicted time of arrival at each microphone has the lowest value. Subsequently, the largest difference measured between predicted and recorded arrival time of a sound pulse at one of the eight microphones is defined as the "maximum TOAD error" (TOAD = Time-of-arrival-difference). This error is multiplied with the speed of sound to obtain the corresponding distance error, defined as "maximum TOAD distance error".

Using the maximum TOAD distance error, two types of localisation errors can be estimated, namely the radial and the tangential error. The radial error defines the difference between the actual and calculated location of the sound source in a direct line to the centre of the array. The tangential error defines the difference between the actual and calculated location of the sound source in the plane perpendicular to the axis between the centre of the array and the calculated location. The location reconstruction error in radial direction is approximated by:

$$radial\ error = 2 \times max\ TOAD\ distance\ error \times \left(\frac{source\ distance}{array\ aperture}\right)^2$$

On the other hand, the position reconstruction error in tangential direction is approximated by:

$$tangential\ error = max\ TOAD\ distance\ error \times \frac{source\ distance}{array\ aperture}$$

As a rule of thumb, an accurate localisation can generally be achieved within a distance of one to ten times the array aperture (in this case 2 to 20 m with an array aperture of 2 m). Figure S2.4 shows indeed that within 2 m of the array, the three types of errors increase drastically. Therefore, we excluded positions within two meters of the centre of the microphone array.

As the aim here is to combine bat positions with fine-scale vegetation data, we excluded positions if one of the two localisation errors was greater than 0.5 m.
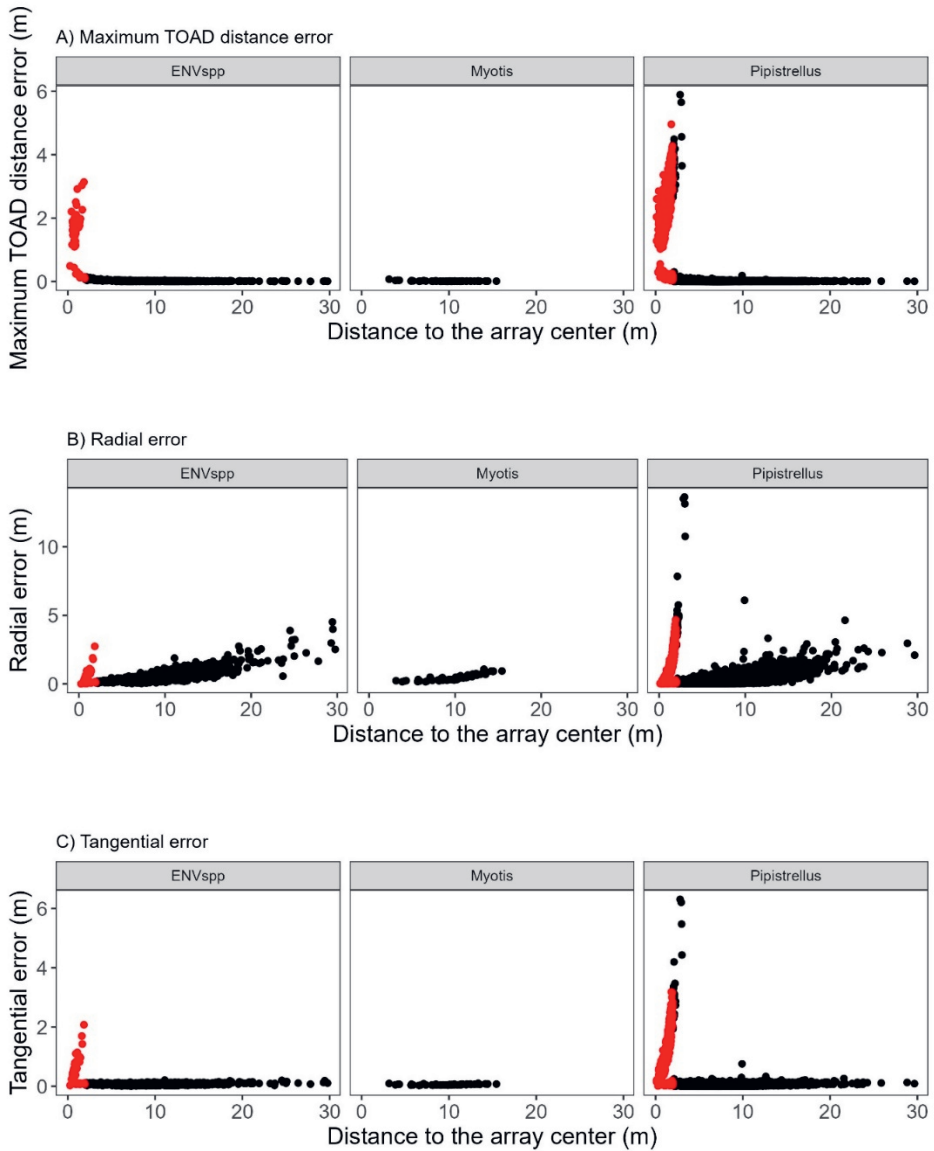
Figure S2.4: Errors assessment over the distance to the array for positions calculated over one night of recording with substantial bat activity. A) Max TOAD distance error. B) Radial error. C) Tangential error. The red dots indicate the positions within two meters of the centre of the microphone array, where localisation can generally not be achieved precisely.

Appendix S2.2: Procedure to align two 3D datasets (bats' positions and vegetation scan)

1) We placed two metal tubes in the ground in which we could place poles (see the picture below, with one of the metal tubes visible on the right side of the picture). We placed them such that they were aligned just behind microphones #1 and #4. Poles were removed before the start of the array recordings to avoid sound reflection or obstruction, and placed back to use later for the microphone setup at the start of the LiDAR scanning. These poles allow us to keep exactly the same array position between nights with the same angle as well, which is essential to properly combine this with the LiDAR data.
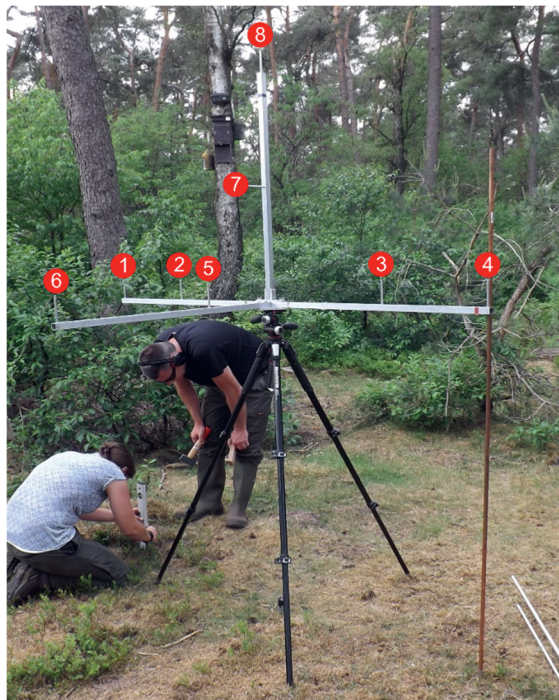
2) Create a text file with array coordinates:
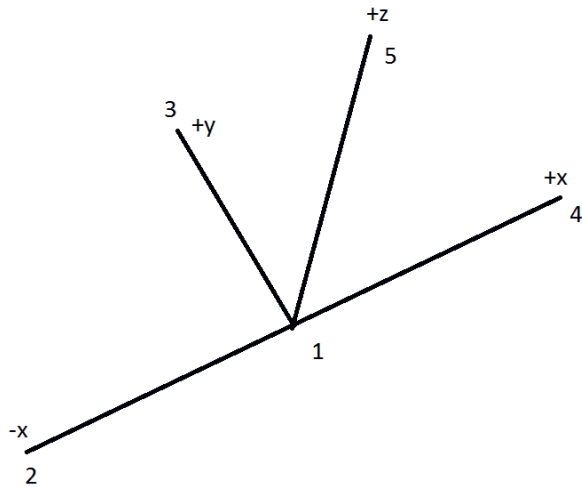
array.txt - Notepad

File   Edit   Format   View   Help

1	0	0	0
2	-1	0	0
3	0	1	0
4	1	0	0
5	0	0	1

3) Import it in CloudCompare
4) Import the LiDAR scan – Do not apply any shift on x,y,z coordinates
5) Create a cross section to localise the array
6) Select both the array data and the cross section data and click on "Aligns two clouds by picking (at least 4) equivalent point pairs
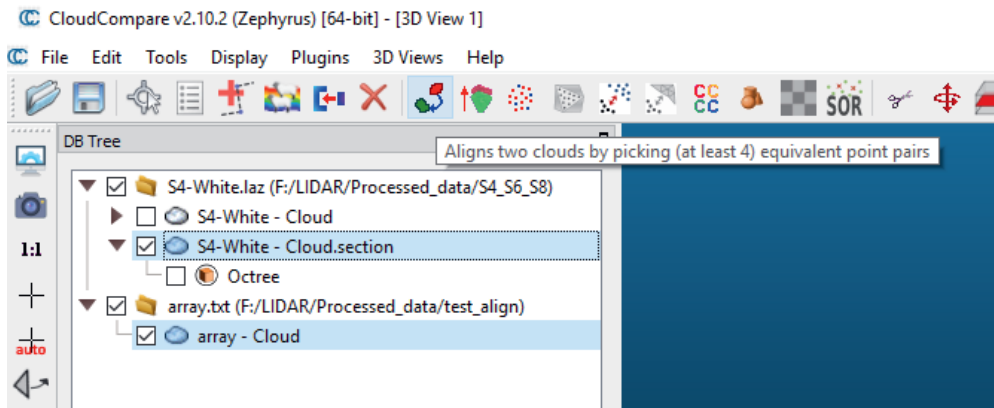
7) Use the cross section as the Reference

8) Write down the coordinates of the first array's point from the text file, then select the same point on the scan. Do the same with all the 5 points.
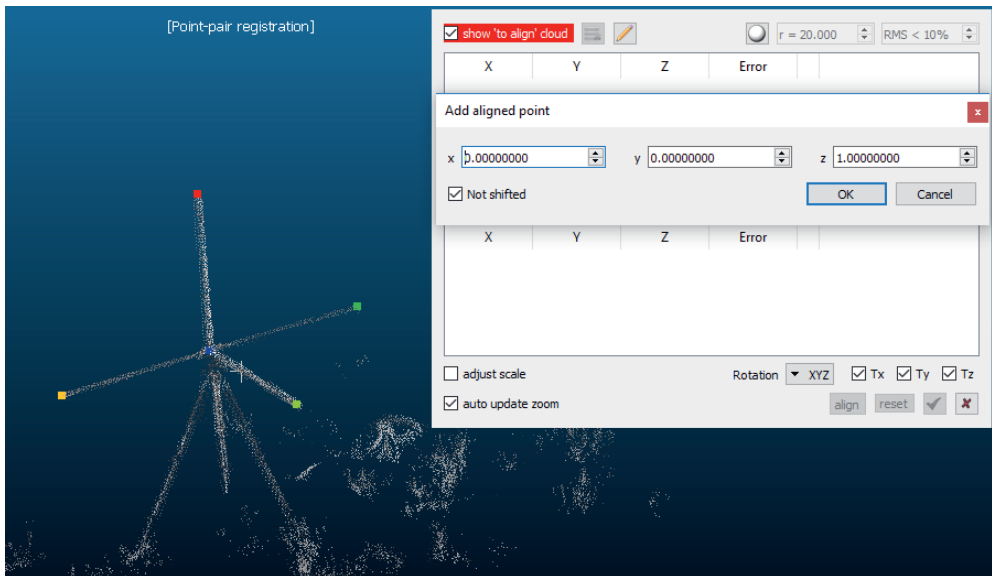
9) In the console, copy the RMS and the Rotation matrix and save it to align bats positions to TLS.
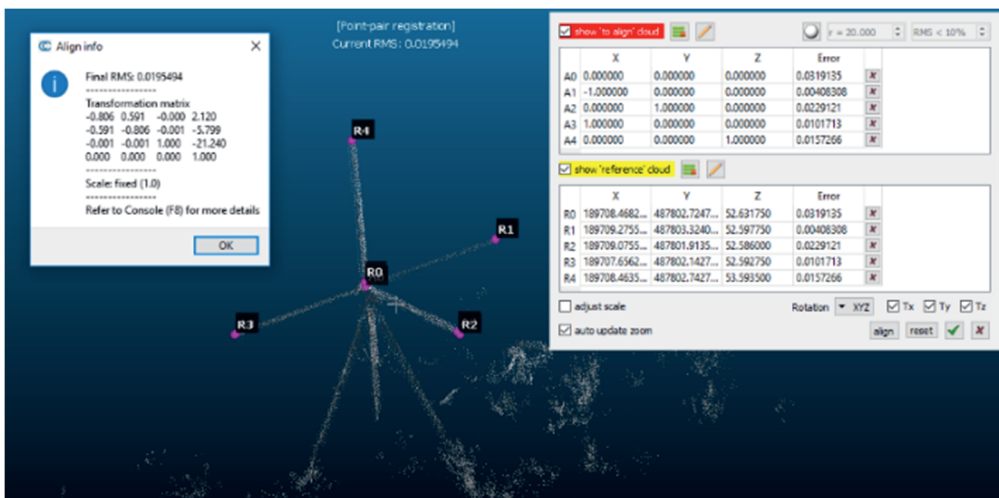
In CloudCompare, import bats positions. Select the bats cloud – Edit – Apply transformation – Copy the rotation matrix

Equation 1: Rigid body transformation (translation and rotation) applied on raw data of 3D bats positions to align them with the corresponding LiDAR scan. Data are shown in Figure 2.5.

$$\begin{bmatrix} Xnew \\ Ynew \\ Znew \\ 1 \end{bmatrix} = \begin{bmatrix} 0.598 & 0.802 & 0.010 & 0.794 \\ -0.802 & 0.598 & -0.001 & -6.566 \\ -0.006 & -0.008 & 1.000 & -16.219 \\ 0 & 0 & 0 & 1 \end{bmatrix} \times \begin{bmatrix} Xraw \\ Yraw \\ Zraw \\ 1 \end{bmatrix}$$

Equation 2: Rigid body transformation (translation and rotation) applied on raw data of 3D bats positions to align them with the corresponding LiDAR scan. Data are shown in Figure 2.6.

$$\begin{bmatrix} Xnew \\ Ynew \\ Znew \\ 1 \end{bmatrix} = \begin{bmatrix} 0.570 & 0.821 & 0.005 & 2.959 \\ -0.821 & 0.570 & 0.013 & 12.628 \\ 0.014 & -0.004 & 1.000 & -19.132 \\ 0 & 0 & 0 & 1 \end{bmatrix} \times \begin{bmatrix} Xraw \\ Yraw \\ Zraw \\ 1 \end{bmatrix}$$

Chapter 3

Bouncing synanthropic bats: the interaction between light spectrum, insect availability and environmental structure determines the distance that bats keep to light sources

Claire Hermans, Harm Bartholomeus, Jens C. Koblitz, Marcel E. Visser, Kamiel Spoelstra

Abstract

Artificial light at night (ALAN) is a major threat to biodiversity, especially for nocturnal species, as it alters habitat availability and quality. Among these, insectivorous bats are strongly affected as their prey accumulates near light sources. Some synanthropic bat species take advantage of these concentrated prey resources, but in order to get these, they need to trade-off food reward against a perceived increased predation risk at higher light levels. Little is known on the fine-scale spatial response of synanthropic bats to light intensity in their natural environment. What factors determine the light levels that bats are prepared to expose themselves to when foraging around lampposts, and how do these depend on the vegetation density around these? Here we investigated how synanthropic bats such as *Pipistrellus* species spatially alter their foraging behaviour in their natural environment in response to ALAN with different spectra. We used acoustic localisation to precisely study bat movement patterns around experimental lampposts, and LiDAR to assess the vegetation structure directly around these. We hypothesized that bats avoid exposure to high light intensities, and only fly into the light to catch an insect. As clutter impairs prey detection in pipistrelles, we hypothesized that the vegetation density directly around the lights interacts with this response. We found that pipistrelles kept less distance from experimental lampposts when searching for insects compared to natural unlit conditions, and flew closer to light posts emitting white light compared to red light. Bats got significantly closer to the light when catching an insect, thereby exposing themselves to higher light intensities. The presence of clutter directly around light sources caused bats to stay further away from the lights. These results indicate that, although synanthropic pipistrelle bats are seemingly attracted to light, they avoid high light levels and only do so if there is a direct food reward.

To be submitted

Introduction

The use of artificial light at night (ALAN) has drastically increased over the last decades (Falchi et al., 2016; Falchi and Bará, 2023; Kyba et al., 2017a, 2023), mainly due to urbanisation and the introduction of light-emitting diode (LED) lamps in outdoor lighting. Artificial light disrupts natural night-time cycles and its impact on ecological communities depends on its scale and its spatial variation (Kehoe et al., 2022). Nocturnal taxa are the most affected by ALAN, which is a main concern as 30% of vertebrates and more than 60% of invertebrates are nocturnal (Gaston et al., 2013; Hölker et al., 2010). The safety of darkness is the essence of the temporal niche of many nocturnal prey species to avoid predation by avoiding overlap of their activity patterns with those of their predators (Brook et al., 2012; Cunningham et al., 2019; Erkert, 1982). The spatiotemporal distribution of nocturnal animals also varies according to the natural light conditions due to moonlight exposure (Appel et al., 2017; Hedenström et al., 2022; Lang et al., 2006; Prugh and Golden, 2014; Roeleke et al., 2018b; Wereszczuk and Zalewski, 2023). However, artificial light is a very different kind of exposure that alters these natural spatiotemporal patterns (Evens et al., 2023; Mariton et al., 2022; Shier et al., 2020).

Bats are well represented among nocturnal species affected by ALAN. Bats are also assumed to be nocturnal to avoid predation by visually-oriented predators like raptors (Lesiński et al., 2009; Mikula et al., 2016; Rosina and Shokhrin, 2011; Speakman, 1991a, 1991b). Bats shift their spatial use in response to higher ambient light levels due to moonlight exposure, which is potentially related to predator avoidance and prey distribution (Hecker and Brigham, 1999; Lang et al., 2006; Roeleke et al., 2018b; Saldaña-Vázquez and Munguía-Rosas, 2013). When exposed to artificial light, slow-flying species are generally light-averse, while some fast-flying species are light-opportunistic and take advantage of concentrated prey resources around streetlights. This is mainly the case of agile species such as several *Pipistrellus* species (Bolliger et al., 2020b; Spoelstra et al., 2017) and *Nyctalus* and *Eptesicus* species that dive in the light cone of streetlights to catch moths (Rydell, 1992, 1991).

However, the response of synanthropic bats to light is context-specific and may be stronger or reverse according to the context (Voigt et al., 2021). Their response depends for instance on their behaviour. As an example, *P. kuhlii* reduces its drinking activity but increases its foraging activity in response to artificial light (Russo et al., 2017). Bats also respond differently to various light spectra. While pipistrelles are attracted to white and green light to forage but not to red light (Spoelstra et al., 2017), migrating species such as *P. pygmaeus* and *P. nathusii* show a positive phototactic response to red and green light (Voigt et al., 2018, 2017). Light intensity is also a major factor influencing bat response to artificial light (Azam et al., 2018; Bolliger et al., 2020b; Hale et al., 2015). Synanthropic

bat species such as several *Pipistrellus* species generally tolerate higher light levels than light-averse bats (Azam et al., 2018; Lacoeuilhe et al., 2014; Pauwels et al., 2021), probably to take advantage of the greater number of insects being attracted by higher light levels (Bolliger et al., 2020b; Rowse et al., 2018). Finally, their response to light also varies across habitats. While pipistrelles commonly forage around streetlights (Bolliger et al., 2020b; Rowse et al., 2018; Spoelstra et al., 2017), their activity is negatively affected by ALAN in riparian habitat (Hooker et al., 2022) and in urban areas (Barré et al., 2020; Hale et al., 2015; Laforge et al., 2019; Pauwels et al., 2019).

There is also a strong link between flight and echolocation behaviour of bats and their surroundings. On a wider scale, bats adapt their spatiotemporal activity pattern according to the vegetation structure of their environment (Adams et al., 2009; Beilke et al., 2021; Blakey et al., 2017; Brigham et al., 1997). On a fine spatial scale, they also have to adjust their call parameters according to clutter level in order to orientate and capture prey, as clutter echoes hamper prey detection (Brinkløv et al., 2010; Kalko and Schnitzler, 1993; Schnitzler and Kalko, 2001). While vegetation may impede prey capture success (Arlettaz et al., 2001; Rainho et al., 2010), flying close to the vegetation may provide a potential benefit of extra safety against predators. Therefore, bats face a trade-off between predation risk and food reward. Moreover, several studies showed that the introduction of artificial light in their habitat interferes with the effect of vegetation structure on bat behaviour (Barré et al., 2023b, 2021; Hermans et al., 2023; Jung and Kalko, 2010; Mathews et al., 2015) and higher light intensity levels increase bats conspicuousness. Therefore, light intensity probably influences the trade-off between the benefits of prey aggregation and increased predation risk around light sources. However, little is known on the factors determining the light levels that bats are prepared to expose themselves to, and how these depend on the spatial complexity of their habitat.

Here we investigate how synanthropic bats such as *Pipistrellus* spp. spatially alter their foraging behaviour in their natural environment in response to artificial light with different spectra. We used acoustic localisation to precisely study bat movement patterns around experimental lampposts, and Light Detection and Ranging (LiDAR) to assess the vegetation structure around these. We hypothesized that bats only fly closer to light sources when they catch a prey item to avoid exposure to high light intensity levels. We hypothesized that the amount of clutter directly around the lights interacts with this response, as vegetation clutter impairs prey detection by bats and hence it is less profitable to forage under lit but highly cluttered areas.

Material and methods

Field sites

Data were collected in forest edge habitat at seven experimentally illuminated sites in the Netherlands (Spoelstra et al., 2015). Each site consists of four rows perpendicular to the forest edge, each with five 4 m tall lampposts separated by 25 m (except two sites with rows of three lampposts), the central one being at the forest edge. Rows were randomly assigned to either white (Philips Fortimo White), green (Philips Fortimo ClearSky) or red (Philips Fortimo ClearField) light, and the last transect was permanently kept dark. The light illuminance at ground level is 7.6 ± 1.2 Lux (1 s.e.m.) beneath each lamppost (Spoelstra et al., 2015), which is comparable to the illumination levels of countryside roads (Gaston et al., 2013). As previous studies carried out at these sites showed that green light does not reduce the impact of ALAN of the activity of light-averse bat species (Spoelstra et al., 2017), this treatment was excluded from this study.

LiDAR

Each transect was scanned using a RIEGL VZ-400 terrestrial laser scanner (RIEGL LaserMeasurement Systems, Horn, Austria) to map the vegetation around the lampposts. Data were collected from June 2020 to April 2021 under leaf-on conditions (presence of foliage on deciduous trees), but plots with almost exclusively coniferous species were scanned later in the season. Technical details on data collection and data processing are provided in Hermans et al. (2023).

The point clouds were voxelised (pixelization in 3D) using Lasvoxel tool in LAStools (version 210418, rapidlasso GmbH, Gilching, Germany) to build a 3D grid of 20x20x20 cm cubic voxels. Only voxels containing at least ten LIDAR returns are considered as 'vegetation voxels', as this preserves a fine-scale resolution in vegetation while filtering out background voxels containing isolated vegetation points (Cifuentes et al., 2014).

The vegetation density was estimated around each lamppost. The number of vegetation voxels was calculated in a square rectangular cuboid of 6x6x3 m (length, width, height) around the lamppost, with the lamppost situated in the top-centre location (Figure 3.1). The voxels between ground level and 1 m above ground level were ignored to exclude ground voxels and only consider vegetation voxels. Here we made the assumption that bats do not fly in this low area, because of tall grass and obstacles such as tree trunks present in most sites.
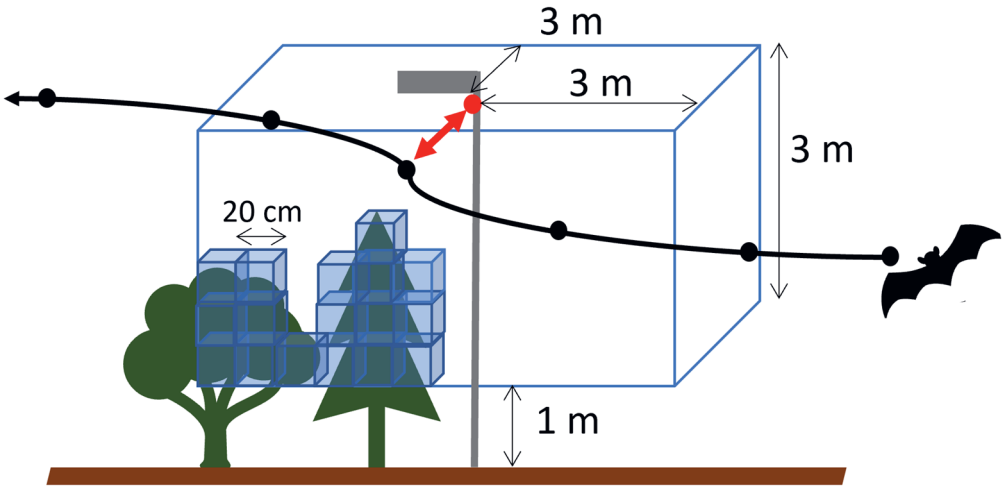
Figure 3.1: Schematic of the assessment of the vegetation density around the lamppost (in grey) and the minimum distance of bats to the light. The number of vegetation voxels (blue cubes) was assessed in a square rectangular cuboid of 6 m x 6 m x 3 m around the lamppost. The red dot is the reference point on the lamppost. Black dots represent 3D positions of one bat pass. For each bat pass, the closest position to the light source was determined to measure the minimum Euclidian distance (calculated in 2D rasterized data) of the bat to the lamp.

Acoustic localisation

Bats were acoustically tracked with microphone arrays from 15 min before sunset to 15 min after sunrise for 27 nights (two to four nights per site) between May 8th and August 9th 2020 on nights without rainfall. Sensors connected to data loggers (BL30 Climate-Data Logger, Trotec, Germany) were programmed to record air temperature and relative humidity every 20 seconds to monitor weather conditions during the recordings. For each microphone array, eight microphones (omnidirectional microphones FG-23329 Knowles Electronics, Itasca, IL, USA) were fitted on an aluminium frame and mounted on a tripod. The technical description of the recording system is provided in Hermans et al. (2023). To account for different levels of vegetation density, six microphone arrays were deployed at one site per night. One microphone array was placed in front of the lamppost at the forest edge and another one in front of the first lamppost inside the forest for the three light treatments. The centre of the array was placed at 1.07 to 1.79 m (average 1.35 m; standard deviation, s.d. 0.11 m) above the ground and 1.56 to 6.61 m (average 3.11 m; s.d. 1.1) from the lamppost depending on the vegetation surrounding the lamppost.

Recordings were stored in 10-second files. 3D positions of bats were reconstructed by measuring the time lag of the arrival time of the calls between the top microphone (reference channel) and the other seven microphones. This time-of-arrival-difference (TOAD) was calculated with a cross-correlation function using a

custom-built software (TOADSuite, P. Stilz, J.C. Koblitz and H.R. Goerlitz) (Goerlitz, 2019) in MATLAB R2020a (The MathWorks, Inc., Natick, MA, USA). Bats' 3D positions were then integrated with the vegetation scans following the method described in Hermans et al. (2023).

Species identification of bat calls were performed using the Tadarida software ((Bas et al., 2017), online repository: https://github.com/YvesBas, January 2021 version). As identification of echolocation calls to the species level is difficult, bat calls were assigned to the following species groups: the ENV group including *Eptesicus* spp., *Nyctalus* spp. and *Vespertilio* spp. (open space aerial foragers), the *Myotis* group including *Myotis* spp. (narrow space foragers), and the *Pipistrellus* group including *Pipistrellus* spp. (edge space forager). Each 3D position was then assigned to a species group using the detection time of the bat call. Although we identified the three species groups, 93% of the calls were attributed to the synanthropic pipistrelle species.

Bat feeding activity was determined by examining the feeding buzzes, which correspond to insect capture attempts and consist of rapid sequences of short, linear pulses with pulse intervals gradually decreasing (Griffin et al., 1960; Schnitzler and Kalko, 2001). Buzzes were detected using the bat sonotype classifier (online repository: https://github.com/YvesBas/Tadarida-C/tree/master/Sonotypes, November 2020 version) built by Roemer et al. (2021). 93% of the buzzes were attributed to the synanthropic pipistrelle species. It is challenging to reconstruct 3D positions of calls during the feeding buzz phase, as the pulse intervals are very short and the call amplitude is much lower (Holderied et al., 2005). Therefore, we made a distinction between call sequences in which bats are searching for insects (absence of feeding buzz) and call sequences in which bats attempt to catch a prey (presence of a feeding buzz) for further analyses (Figure 3.2).

In order to compare the light treatments to the dark control, we used the distance to the light source as a proxy for light intensity. The light source is directed towards the ground and the light does not spillover above the luminaire. Here we used the 2D Euclidean distance to the light source (in x and y axis, using the anchor point of the light fixture to the pole as the reference point for the lamppost) for bat positions below the lamp where light is actually emitted (between 0 and 4 m above the ground). For each file containing echolocation calls, the closest bat position to the light source was determined to measure the willingness of bats to fly around the lamp (Figure 3.1), assuming that every bat call in a 10-second file belongs to the echolocation sequence of one single individual. Each echolocation sequence is hereafter referred as a bat pass.

Statistical analysis

Analyses were carried out using the statistical software R (version 4.1.2, R Core Team, 2021). Data were summarised by light treatment (dark, red, white), site, date of recording, bat pass, and buzz (presence or absence during the bat pass).

We first ran a linear mixed model for the data collected at the dark treatment as a null model. The response variable of this model was the minimum distance to the lamppost per bat pass. The distance to the lamppost was square root transformed, and we ensured normal distribution of the residuals by visually checking quantile-quantile plots of the models. We applied a linear mixed model using the *lmer* function from lme4 (Bates et al., 2015). We included vegetation density around the lamppost (transformed into a z-score by substracting the mean and dividing the difference by the standard deviation), and presence of buzz or not as well as their interaction as fixed factors. We also included the moon phase (using the moonlit package, Śmielak, 2023) and the temperature at the time of the bat pass as covariates. We accounted for the hierarchical structure of the data by adding nested random effects of site and night to the model intercept. The results of this model can be found in the supplementary data (Figure S3.1 and Table S3.1).

The predicted values of the null model were extracted for the values of vegetation density around the lampposts at the red and white light treatments. The difference between the square-root of the minimal distance to the lamppost per bat pass and the predicted value under dark condition for the same vegetation density value was then calculated for the data collected at the two light treatments. A negative value therefore indicates a positive phototactic response to the light treatment, while a positive value indicates a negative phototactic response. We ran a second model in which the dark treatment is therefore considered as the baseline, using the calculated difference as the response variable. We included the same variables as in the first model and we added the light treatment (red or white light), as well as the three-way interaction of light, vegetation density and the presence of buzz as fixed factors. The *emtrends*, *emmeans* and *contrast* functions from the emmeans package (Lenth, 2023) were used to perform post hoc tests with a Bonferroni correction. We also ran the model with the three light treatments (dark, red and white) as explanatory variables. The results of this model can be found in the supplementary data (Figure S3.2 and Table S3.2).

Results

The mean number of pipistrelle bat passes per night which include 3D positions below the luminaire's height varies greatly between light treatments, with more activity under white light than red light and the dark control (Table S3.3). Some examples of bat positions integrated in the LiDAR scans are provided in Figure 3.2.

We found a significant three-way interaction between the light treatment (white vs red), the vegetation density and the presence of a feeding buzz, using the model for the dark control as a baseline (Table S3.3). The distance to the lamp is positively correlated with the vegetation density around the lamp under both light treatments, irrespective whether bats attempt to catch insects or not (Figure 3.3). On average, bats fly closer to white light than red light, both if they emit a buzz (Z ratio = 6.274, p < 0.0001) or not (Z ratio = 21.294, p < 0.0001). The positive phototaxis is stronger when bats are actively foraging (Figure 3.3A, Z ratio = -6.686, p < 0.0001 under white light and Z ratio = -2.934, p = 0.0134 under red light when comparing passes with a feeding buzz to passes without any buzz), especially when the lamppost is surrounded by less vegetation. When bats are searching for insects (and not actually catching these), they get closer to the lamppost when the vegetation density is lower. On the other hand, when the vegetation is denser around the lamppost, bats stay further away from the lamp compared to the natural unlit situation (Figure 3.3B).
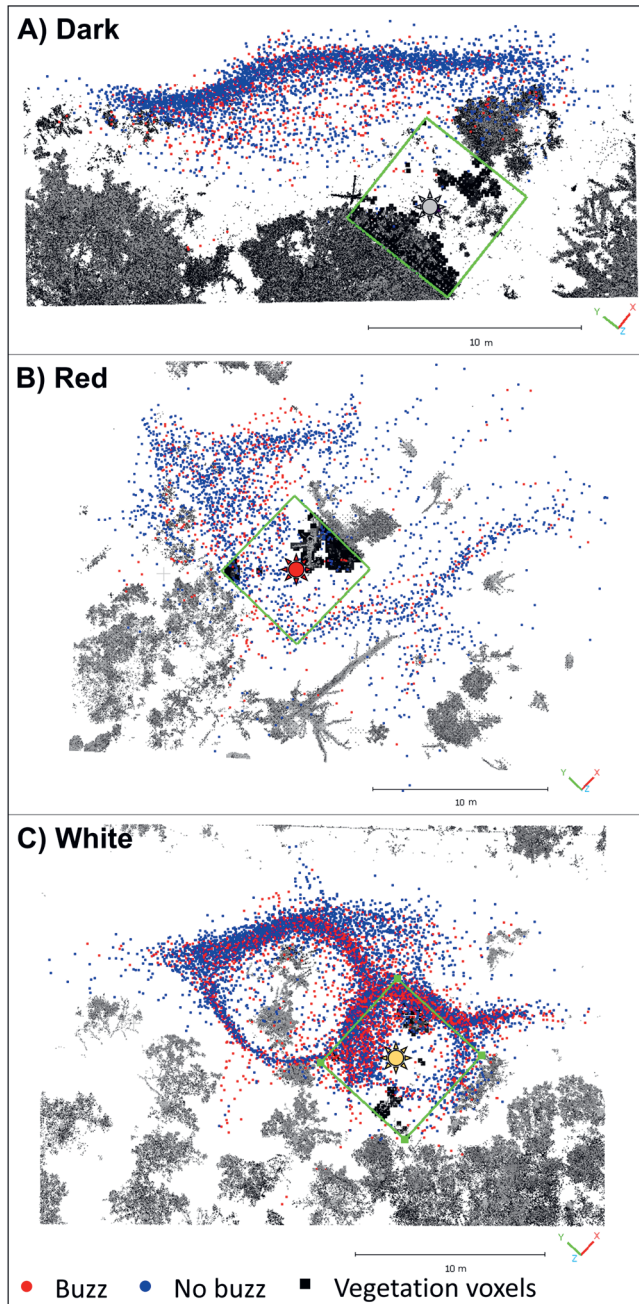
Figure 3.2: Three examples of bat positions around lampposts. Top view of bat positions integrated in LiDAR scans between 1 m above the ground and the top of the 4 m tall lampposts for one night of recording. A) Dark control on 23/06/2020, B) Red treatment on 17/07/2020, C) White treatment on 30/07/2020. Red and blue dots are the positions from bat passes with a feeding buzz and without any feeding buzz, respectively. Black squares represent the vegetation voxels in a square rectangular cuboid of 6 m x 6 m x 3 m (green square) to assess vegetation density around the lamppost (see Figure 3.1).
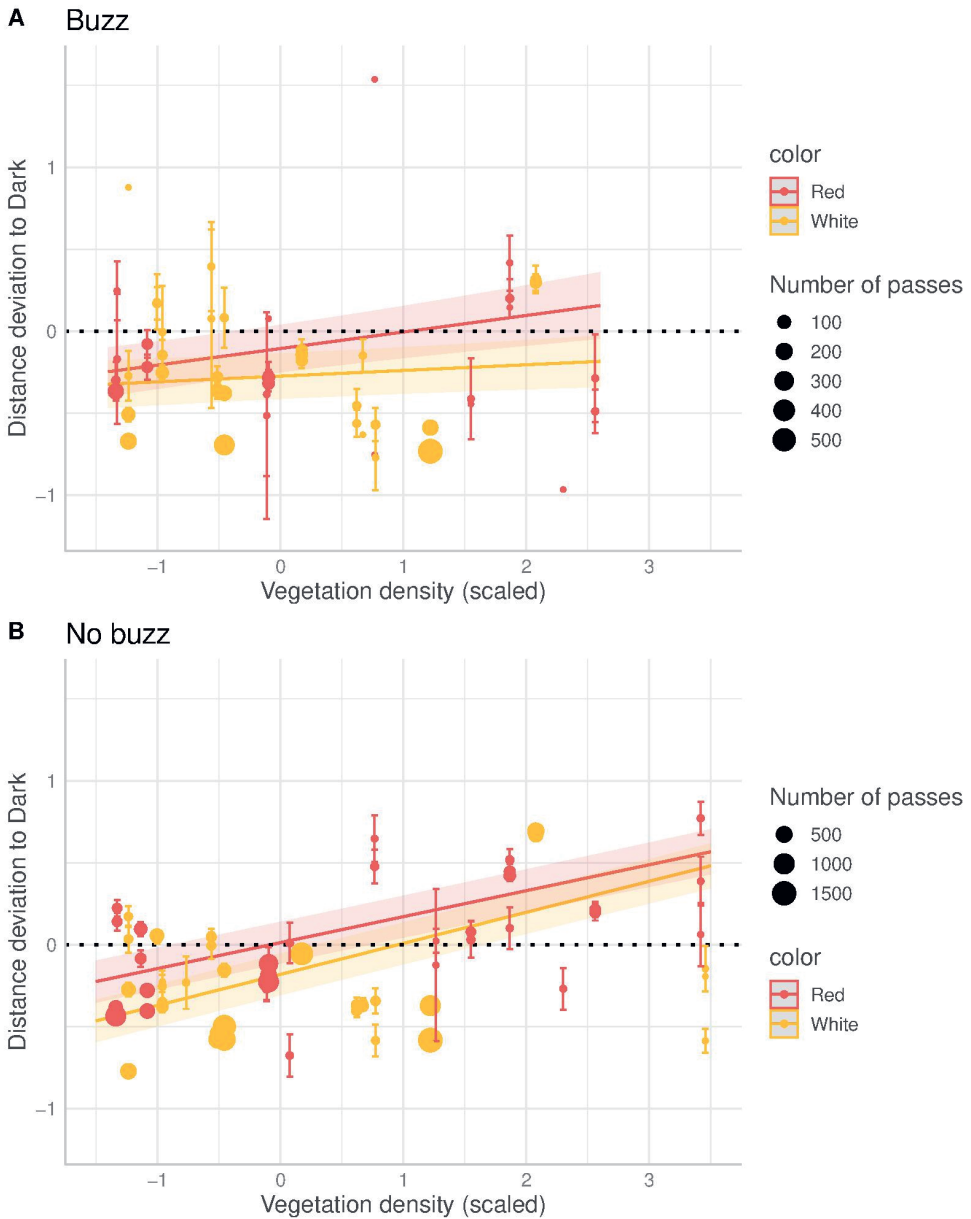
Figure 3.3: Model predictions (with 95% confidence intervals) of the effect of light treatment, presence of feeding buzz and vegetation density around the lamppost (z-score) on the minimum distance to the light per bat pass. In this model, the distance deviation is the distance to the lamppost (square root transformed) relative to this distance for the dark control, predicted from the null model at the same level of vegetation density. A) Bat passes in which a feeding buzz is emitted. B) Bat passes without any feeding buzz. Data have been averaged over nights of recording (± standard error) for graphical purposes only.

Discussion

Our results show that pipistrelle bats stay out of bright light whilst still being attracted by insects that have been lured by the light, in order to quickly snatch these out of the bright area. This positive phototactic response of pipistrelles to light, especially when actively foraging, supports the trade-off hypothesis between risk to fly in lit areas and food reward around light sources. However, if the lamppost is directly surrounded by more vegetation, bats fly further away from the light source. Unlike gleaner bats such as *Myotis* or *Plecotus* species that take prey from surfaces, pipistrelles only hunt airborne prey (Fenton, 1990) and cannot detect insects very close to vegetation due to their echolocation signals. Kalko and Schnitzler (1993) described the "overlap free window", which corresponds to the distance range in which insect detection is neither impaired by the emitted echolocation call nor by clutter echoes. Thus, it is more difficult for pipistrelles to forage around lampposts surrounded by dense vegetation. They need to keep their distance to the vegetation so the echo from their prey does not overlap with the clutter echo, and therefore they need to keep their distance to the lamp as well.

We also showed that the effect of artificial light is spectrum-dependent, as bats flew closer to lampposts emitting white light compared to red light. Nocturnal insects are attracted by artificial light, especially by the UV and the blue component of the light spectrum (Donners et al., 2018; Wilson et al., 2021), while red light is considered as a less attractive light source (Spoelstra et al., 2017; Wilson et al., 2021, **Chapter 4** of this thesis). Therefore, the stronger positive phototaxis response of bats under white light is likely attributable to the attraction of aerial insects around the light source. These results are consistent with previous studies corroborating the attraction-by-insects hypothesis instead of the attraction-by-artificial-light hypothesis (Rowse et al., 2018; Spoelstra et al., 2017). Moreover, artificial light reduces the efficiency of antipredator flight in moths (Hügel and Goerlitz, 2020; Wakefield et al., 2015). Pipistrelles may take advantage of the fact that moths are not able to escape around light sources and therefore increase the predation pressure, as suggested by the stronger positive phototaxis when emitting a feeding buzz.

A previous study carried out at these experimental sites concluded that the probability of pipistrelles to fly inside the forest increases when flying closer to the light, especially under the red and white treatment (Barré et al., 2021). However, in that study vegetation structure was not precisely assessed and bats were only recorded around the lampposts at the forest edge. The probability of flying into the forest was measured by the proportion of positions on the side of the lamp towards the forest compared to the side towards the open area. Our results suggest that pipistrelles do not fly inside the forest but circle the light source along the forest edge in lit conditions. In a dark natural situation (Figure 3.2A),

pipistrelles venture out in the open area further away from the forest edge and therefore from the lamppost, thus reducing the probability of pipistrelles to fly inside the forest as measured by Barré et al. (2021). In lit conditions, especially under white light, pipistrelles have been observed to circle the lamppost (Figure 3.2C as an example, but this behaviour has been also recorded during other nights of recording at other sites). Therefore, bats are not flying inside the forest to seek refuge as suggested by Barré et al. (2021), but they take advantage of the lamp to forage around it.

To conclude, being exposed to light is a necessary evil for synanthropic pipistrelle bats as it enables them to catch insects but they avoid exposing themselves to high light levels, especially if the vegetation is too dense to detect the insects flying around the light source. Therefore, even though pipistrelles are usually considered as light-tolerant bats, they do not expose themselves to higher light levels but just forage on the concentrated prey resources around the lamp.

Acknowledgments

Supplementary data to Chapter 3

Table S3.1: Summary of the null model (generalized linear mixed model for the dark treatment only). Results are presented in Figure S3.1.

Predictors	Minimum distance per bat pass (square root transformation)		
	Estimates	**CI**	**p**
Intercept	2.08	1-82 – 2.33	**<0.001**
Buzz (no buzz)	0.18	0.09 – 0.26	**<0.001**
Vegetation density (scaled)	0.00	-0.10 – 0.10	0.969
Moon phase	0.12	-0.13 – 0.38	0.348
Temperature	-0.01	-0.02 – 0.01	0.274
Buzz (no buzz) * Vegetation density (scaled)	-0.14	-0.24 – -0.03	**0.011**
Random effects			
σ^2	0.28		
$\tau_{00\ site:date}$	0.04		
ICC	0.12		
N_{site}	7		
N_{date}	19		
Observations	2467		
Marginal R^2 / Conditional R^2	0.043 / 0.159		
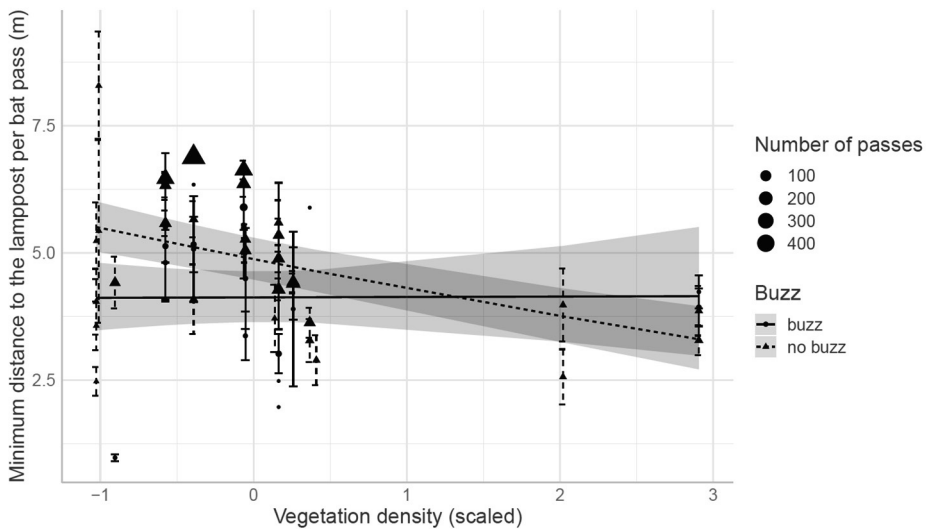
Figure S3.1: Predictions (back-transformed, with 95% confidence intervals) of the null model with only the dark control as light treatment for further comparison with the other two light treatments. Data have been averaged over nights of recording (± standard error) for graphical purposes only.

Table S3.2: Summary of the generalized linear mixed model with the three light treatments as explanatory variable. Results are presented in Figure S3.2.

Predictors	Minimum distance per bat pass (square root transformation)		
	Estimates	**CI**	**p**
Intercept	1.88	1-68 – 2.07	**<0.001**
Color (red)	-0.10	-0.20 – 0.00	**0.041**
Color (white)	-0.29	-0.38 – -0.21	**<0.001**
Buzz (no buzz)	0.22	0.13 – 0.30	**<0.001**
Vegetation density (scaled)	-0.07	-0.16 – 0.03	0.179
Moon phase	0.07	-0.20 – 0.35	0.606
Temperature	0.01	0.00 – 0.01	**0.001**
Color (red) * Buzz (no buzz)	0.05	-0.06 – 0.15	0.365
Color (white) * Buzz (no buzz)	0.05	-0.04 – 0.14	0.319
Color (red) * Vegetation density (scaled)	0.17	0.06 – 0.28	**0.002**
Color (white) * Vegetation density (scaled)	0.11	0.01 – 0.21	**0.031**
Buzz (no buzz) * Vegetation density (scaled)	-0.16	-0.26 – -0.06	**0.003**
Color (red) * Buzz (no buzz) * Vegetation density (scaled)	0.09	-0.03 – 0.21	0.125
Color (white) * Buzz (no buzz) * Vegetation density (scaled)	0.16	0.06 – 0.27	**0.003**
Random effects			
σ^2	0.28		
$\tau_{00 \text{ site:date}}$	0.05		
ICC	0.16		
N_{site}	7		
N_{date}	19		
Observations	22633		
Marginal R^2 / Conditional R^2	0.067 / 0.214		
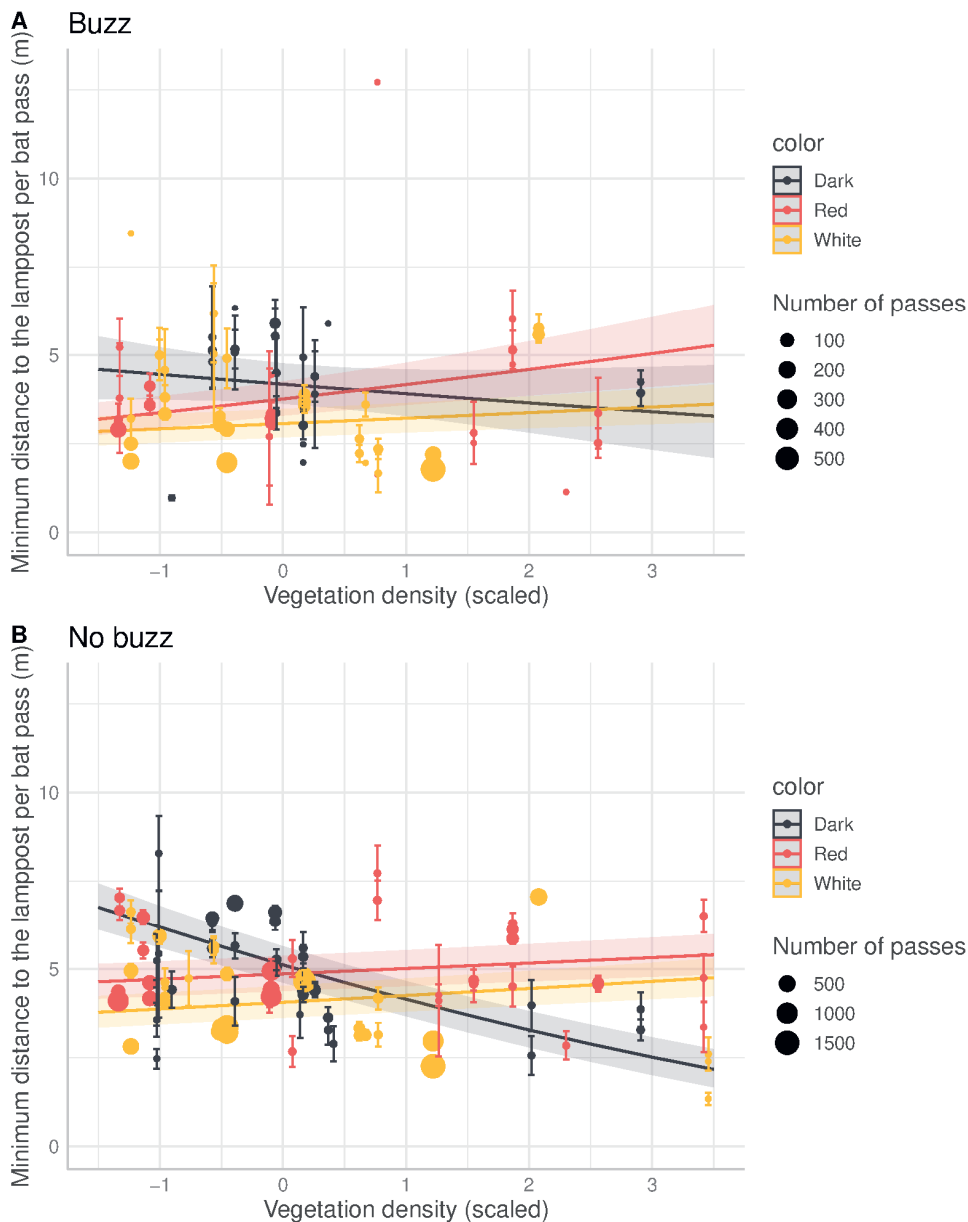
Figure S3.2: Model predictions (back-transformed, with 95% confidence intervals) of the effect of vegetation density around the lamppost (z-score) on the minimum distance to the light per bat pass under the three light treatments. A) Bat passes in which a feeding buzz is emitted. B) Bat passes without any feeding buzz. Data have been averaged over nights of recording (± standard error) for graphical purposes only.

Table S3.3: Number bat passes per night that include 3D positions below the luminaire's height per light treatment.

Light treatment	Presence of buzz	Mean number of bat passes	Standard error
Dark	No	72	17.40
	Yes	8	1.95
Red	No	178	44.99
	Yes	23	8.00
White	No	340	73.18
	Yes	76	22.91

Table S3.4: Summary of the generalized linear mixed model with the distance to the lamppost under white and red light (square root transformed) relative to this distance for the dark control (predicted from the null model at the same level of vegetation density). Results are presented in Figure 3.3 of the manuscript.

Predictors	Estimates	CI	p
Intercept	-0.25	-0.46 – -0.04	**0.020**
Color (white)	-0.19	-0.25 – -0.13	**<0.001**
Buzz (no buzz)	0.08	0.03 – 0.14	**0.003**
Vegetation density (scaled)	0.09	0.04 – 0.14	**<0.001**
Moon phase	0.01	-0.30 – 0.32	0.941
Temperature	0.01	0.01 – 0.02	**<0.001**
Color (white) * Buzz (no buzz)	-0.00	-0.06 – 0.06	0.974
Color (white) * Vegetation density (scaled)	-0.04	-0.10 – 0.01	0.125
Buzz (no buzz) * Vegetation density (scaled)	0.07	0.02 – 0.12	**0.005**
Color (white) * Buzz (no buzz) * Vegetation density (scaled)	0.07	0.01 – 0.13	**0.017**
Random effects			
σ^2	0.28		
$\tau_{00\ site:date}$	0.07		
ICC	0.19		
N_{site}	7		
N_{date}	19		
Observations	20166		
Marginal R^2 / Conditional R^2	0.094 / 0.270		

Chapter 4

Artificial light at night drives diel activity patterns of synanthropic pipistrelle bats and their prey

Claire Hermans, Iryna Litovska, Mélyssa de Pastors, Marcel E. Visser, Kamiel Spoelstra

Abstract

The use of artificial light at night (ALAN) has increased drastically worldwide over the last decades. ALAN can have major effects on nocturnal communities, including insects and bats. Insects are attracted to street lights and few bat species take advantage of this by foraging on the attracted insects. ALAN potentially affects the temporal patterns of insect abundance and thereby bat foraging behaviour. In a natural dark environment, these patterns are usually bimodal, with an activity peak in the early evening and the morning. Little is known about how ALAN affects insect presence throughout the night, and whether the light spectrum plays a role. This is important, as these temporal changes may be a key driver of disturbances in bat-insect interactions. Here, we studied how white and red light affect insects' and bats' nightly activity patterns. The activity of insects and bats (*Pipistrellus* spp.) was recorded throughout the night at seven experimentally illuminated sites in a forest-edge ecosystem. ALAN disrupted activity patterns, with both insects and bats being more active throughout the night. ALAN facilitated all-night foraging in bats especially near white light, but these effects were attenuated near red light. The ability to forage throughout the night may be a key advantage causing synanthropic bats to dominate in illuminated environments, but this could also prove detrimental in the long term. As red light reduced disturbing effects of ALAN on insects and bats diel activity pattern, it opens the possibility of using spectral composition as a mitigation measure.

Introduction

The use of artificial light at night (ALAN) has shown a drastic increase over the past decades (Falchi et al., 2016; Falchi and Bará, 2023; Kyba et al., 2017a, 2023), mainly accelerated by the introduction of light-emitting diode (LED) lamps in outdoor lighting. ALAN disrupts natural light cycles in time and space and effects are wavelength dependent (Gaston et al., 2013). Therefore, natural patterns of resource use by organisms exposed to ALAN may be altered, affecting a wide range of taxa, especially nocturnal animals (Sanders et al., 2021), including bats and insects.

Nocturnal insects are attracted by ALAN, especially by the UV and the blue component of the light spectrum (Donners et al., 2018). This can have negative impacts on their foraging (van Langevelde et al., 2017), reproduction (Owens and Lewis, 2022) and cause exhaustion or death (Eisenbeis, 2006), which can lead to population decline (van Grunsven et al., 2020). The attraction of insects from unlit to lit areas, also called the 'vacuum effect', subsequently alters the foraging opportunities of their predators such as insectivorous bats (Russo et al., 2019; Rydell, 1992).

Bats need to exploit spatial heterogeneity in food availability, and light sources offer predictable foraging locations with higher insect densities. While most bat species are light averse, a few others take advantage of insects' aggregation around light sources. Several *Pipistrellus* species are typical examples of synanthropic bats, as they commonly forage around street lights, where aerial insects aggregate (Bolliger et al., 2020b; Russo et al., 2019; Rydell, 1992; Spoelstra et al., 2017). However, bats' response is context-dependent, as Hooker et al. (2022) showed that the feeding activity of pipistrelles can decrease under lit treatments along waterways.

Under natural, dark conditions, flying insects often show a temporal pattern with a large activity peak around dusk and a smaller peak around dawn (Racey and Swift, 1985; Rydell et al., 1996; Swift, 1980). This is especially the case for dipterans, on which synanthropic bats like pipistrelles mainly prey upon (Rydell et al., 1996; Swift et al., 1985). Bats generally show the same bimodal pattern (Ciechanowski et al., 2009; Kunz, 1973; Mariton et al., 2023; Rachwald, 1992), so that their activity pattern is closely related to the temporal activity pattern of their prey (Erkert, 1982; Racey and Swift, 1985; Rydell et al., 1996; Swift, 1980). The lack of foraging activity in the middle of the night coincides with the lowest insect abundance (Racey and Swift, 1985). Bats may stop foraging with lower insect activity, and lactating bats can feed their young while waiting for the morning insect peak (Racey and Swift, 1985; Swift, 1980). The activity patterns of flying insects and bats have mainly been studied under natural conditions (Hayes, 1997; Mariton et al., 2023; Ruczyński et al., 2020; Russo et al., 2011;

Rydell et al., 1996; Speakman et al., 2000; Swift, 1980), and little is known about the changes in their temporal patterns in response to ALAN (Azam et al., 2015; Hooker et al., 2022; Mariton et al., 2022). If insects shift their activity within the night, bats likely adjust their temporal activity to this.

To answer this question, a simultaneous and continuous assessment of bat and insect activity throughout the night is essential. Bats can effectively be monitored by recording bats' echolocation calls, but the measurement of insect activity is a challenge because most methods use light, which obviously will interfere with the light treatments (Froidevaux et al., 2018). Suction traps or flight-interception traps can be programmed for interval collection of insects (Bolliger et al., 2020a; Johnson, 1950), but this is logistically challenging, and collected insects are no longer available for bats. A solution is the use of infra-red (IR) cameras as these can capture insects in low light levels conditions (Rowse et al., 2018), but it enables a less specific census (Ruczyński et al., 2020).

In order to assess the impact of artificial light on the temporal activity pattern of light-tolerant bats and their prey, we simultaneously assessed nocturnal insect abundance, bat foraging and feeding activity in response to different light spectra using camera traps and passive acoustic monitoring near experimental light posts in forest edge habitat. The activity of both aerial insects and bats was continuously recorded throughout the night to measure changes in their activity pattern in response to ALAN. We hypothesized that natural activity patterns are disrupted by artificial light. We predicted that the insect activity would be constantly high throughout the night in lit conditions, compared to a bimodal activity pattern in dark natural conditions. We predicted that these temporal changes in insect activity would drive alterations in foraging activity of bats.

Material and methods

Field sites and data collection

Insect abundance and bat activity were assessed in forest edge habitat at seven experimental sites in the Netherlands set up to study the effect of ALAN on the forest edge ecosystem. The forest edges border mostly heathland; the sites and the area around these are located inside nature reserves. Sites are described in more detail in Spoelstra et al. (2015). At each site insect abundance and bat activity were estimated under three different light treatments: white light (Philips Fortimo White), red light (Philips Fortimo Clearfield) as well as a dark control (wooden poles), around a 4 m tall lamppost placed at the forest edge. The distance between light treatments varied between 88 and 386 m (average 204 m; standard error, s.e. 17). All lampposts are programmed to be on from sunset to sunrise since spring 2012. The illuminance at ground level is 7.6 ± 1.2 Lux (1 s.e.m.) beneath each lamppost. Sensors connected to data loggers (BL30 Climate-Data

Logger, Trotec, Germany) were programmed to record air temperature and relative humidity every 20 seconds to monitor weather conditions. The sensors were attached to the tripod on which we installed the microphones, at about 1 to 1.5 m above the ground. The study was carried out during 27 nights without rainfall and a minimum temperature (during the night) of 8ºC, between May 8th 2020 and August 9th 2020. Bat and insect activity were measured simultaneously at the three light treatments at one site per night. Data were included for analysis only if the bat recordings and the corresponding insect data from camera traps were complete for a full night. This resulted in 19, 19 and 18 nights of data for the control, red and white treatments respectively, with two to four nights per site (Supplementary data, Table S4.1).

Insect activity using camera traps

Insect abundance was continuously measured throughout the night with infrared interval photography, using Reconyx HC500 HyperFire Semi-Covert IR camera traps. Two camera traps were placed at 30 cm below the luminaire of each lamppost, one facing the direction of the light post (front) and the second one the opposite direction (back) (Figure 4.1A), in order to detect insects at both sides of the lamppost. There was no vegetation in the close vicinity of the lamppost. Therefore, the camera traps' field of view was not obstructed and the insect detection was not impaired by vegetation. Cameras were set to take pictures every minute between 20:00 and 08:00 the next morning. Insects can be seen as white dots or short lines on the dark background (Figure 4.1B), so species identification was not possible. Insects were counted manually using MapView Professional software (Reconyx, 2016).

Validating camera trap data using sticky sheet traps

Sticky sheets were used to validate the total insect abundance per night recorded by infrared interval photography. Here we made the assumption that the capture of insects by the sticky sheets did not interfere with the assessment of insect abundance by camera traps. Sticky sheets traps (Fentini Biocontrol, The Netherlands) were cut (18.5*25cm) to fit in a frame to prevent bats accidentally touching it. Traps were placed on the lampposts at 30 cm below the luminaire (Figure 4.1A) between 20:00 and 22:00 and were collected between 07:00 and 09:00 the next morning, immediately placed in a plastic cover and frozen. All insects at both sides of the sheet were counted, with usage of a magnifying glass when needed.
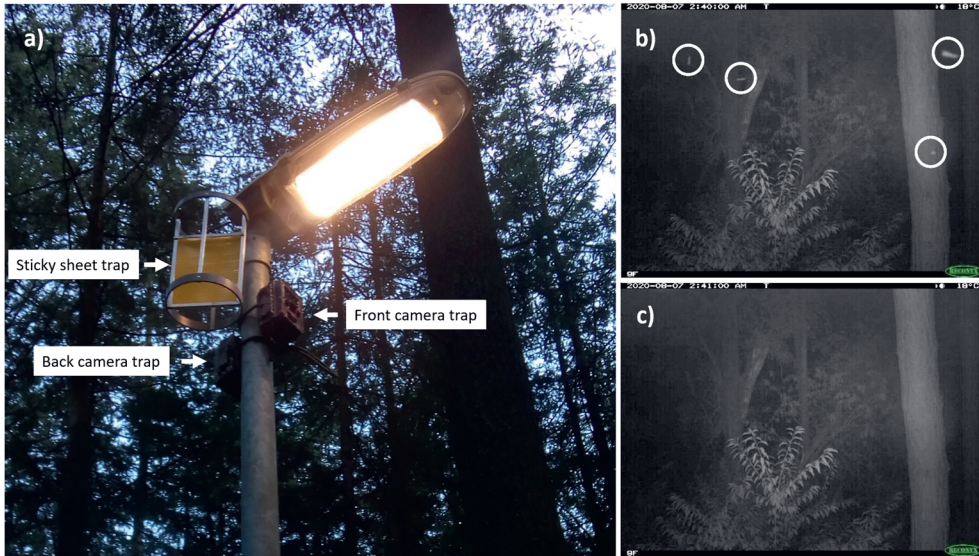
Figure 4.1: Method to monitor insect abundance around experimental lampposts. A) Sticky sheet trap, front and back camera traps on a lamppost. B-C) Pictures captured by a camera trap at one-minute interval. B) Four insects are highlighted with a white circle. C) No insect was present.

Bat activity

Bat activity was assessed with omnidirectional microphones FG-23329 (Knowles Electronics, Itasca, IL, USA) powered with 12 V batteries and connected to a custom-made amplifier and filter unit. Microphones were placed at 2.07 to 2.52 m (average 2.35 m; standard deviation, s.d. 0.11) above the ground and 1.87 to 4.40 m (average 2.92 m; s.d. 0.70) from the lamppost depending on the vegetation surrounding the lamppost. Sound recordings were digitised with an Analog-Digital-Converter USB-6346 (DAQ) (National Instruments, TEX, USA) at a sampling rate of 300 kHz and 16-bit resolution. All recording parameters were controlled and set with the MALTA software (Microphone Array Localisation Tool for Animals, version 3.6, CAE Software & Systems, Germany), and all sound recordings were stored on Mini PCs (Gemini X, Beelink). Recordings are stored in 10-second files. Bat activity was continuously recorded from 15 minutes before sunset to 15 minutes after sunrise.

Species identification was performed using the Tadarida software (Bas et al., 2017, online repository: https://github.com/YvesBas, January 2021 version). As 93% of the calls were attributable to synanthropic pipistrelle species, we limited our analyses to these species. A bat pass was defined as the occurrence of two or more echolocation calls of a pipistrelle during a 10-second file.

Bat feeding activity was measured by counting the number of feeding buzzes per night, which correspond to insect capture attempts and consist of rapid sequences

of short, linear pulses with pulse intervals gradually decreasing (Griffin et al., 1960; Schnitzler and Kalko, 2001). Buzzes were detected using the bat sonotype classifier (online repository: https://github.com/YvesBas/Tadarida-C/tree/master/Sonotypes, November 2020 version) built by Roemer et al. (2021). Spectrograms of all detected buzzes were visually inspected to avoid false positives. A subsample was taken as well to check for false negatives. We used the 'buzz ratio' of feeding buzzes relative to the number of bat passes as a measure of bat feeding activity. A buzz ratio of one indicates that every bat pass contains a feeding buzz (Vaughan et al., 1997).

Statistical analysis

Analyses were carried out using the statistical software R (version 4.1.2, R Core Team, 2021).

Insect abundance per night

To test for the effect of the light treatment on the insect abundance per night (using camera traps data), a generalised mixed model (GLMM) with a negative binomial distribution was applied, using the *glmer.nb* function from lmer package (Bates et al., 2015). We included light treatment and mean air temperature per night as fixed factors in the model. We accounted for the hierarchical structure of the data by adding nested random effects of site and night to the model intercept. The *emmeans* function from the emmeans package (Lenth, 2023) was used to perform post hoc tests with a Bonferroni correction.

Foraging activity per night

As the buzz ratio is a proportion, we fitted a zero-inflated beta model (using *glmmTMB* from glmmTMB package, Brooks et al., 2017) with a beta-distribution (link = logit), and light treatment and log-transformed insect abundance as well as their interaction as explanatory variables. We accounted for the hierarchical structure of the data by adding nested random effects of site and night to the model intercept. The *emtrends* function from the emmeans package (Lenth, 2023) was used to estimate the slopes of the covariate trend for each light treatment.

Temporal activity patterns

As sunset and sunrise times changed throughout the data collection and as bat activity tends to line up with sunset and sunrise times (Erkert, 1982), the time of observations were centred around the astronomical midnight of each recorded night using the *sunTime* function of the overlap package (Ridout and Linkie, 2009). We estimated temporal activity patterns using negative binomial generalized additive mixed-effect models (GAMMs) (*gam* function in the mgcv package (Wood, 2011)). The number of insect observations, bat passes and feeding buzzes per hour after sunset (relative to astronomical midnight) was used

as the response variable. The light treatment and the hours after sunset were used as the predictor variables and the date as a random effect on the intercept to account for night-to-night variation in activity. Activity patterns were compared using plots of GAMM-predicted relative activity for each variable (insect abundance, number of bat passes and number of feeding buzzes).

Results

Insect abundance

Insect abundance as measured with sticky sheets and camera traps was positively correlated under red and white light (R=0.75; p<0.001 and R=0.77; p<0.001 respectively). However, the data from sticky sheets and camera traps were not correlated under the control dark treatment, with camera traps capturing more insects than sticky sheets (R=0.31; p=0.245).

Insect abundance as recorded by camera traps was significantly higher under the white treatment than under the other two light treatments and temperature did not have a significant effect on insect abundance (Figure 4.2 and Supplementary data, Tables S4.2 and S4.3).
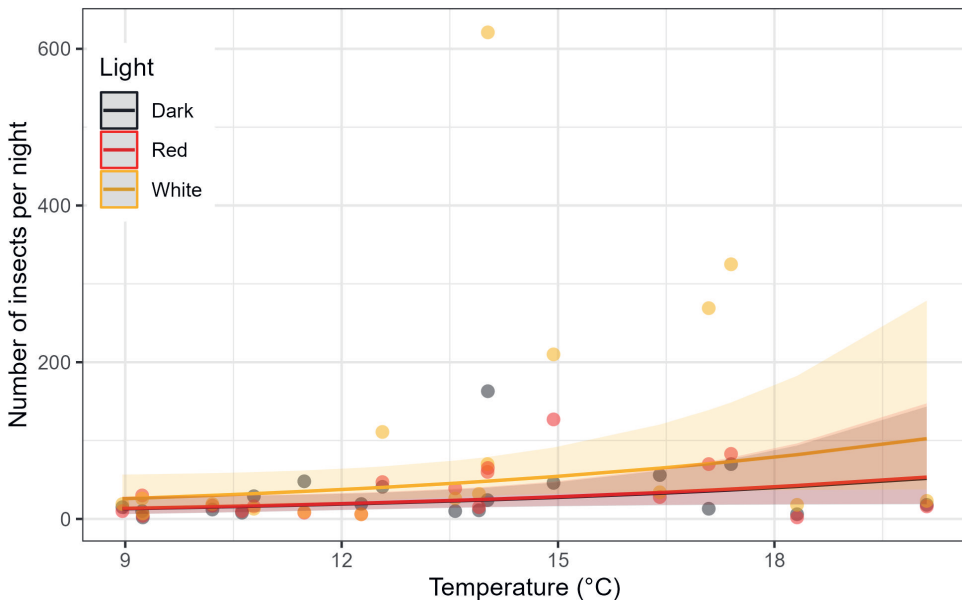
Figure 4.2: Insect abundance (predicted lines from a statistical model with 95% confidence intervals) in response to light treatment and air temperature for camera trap data. Dots represent the raw data.

Bat foraging activity

The number of bat passes and feeding buzzes per night was positively correlated for all light treatments (R=0.69; p=0.001 for dark, R=0.9; p<0.001 for red and R=0.78; p<0.001 for white).

When the insect abundance was low, the buzz ratio was low for all the light treatments, with about one feeding buzz for every 40 passes (Figure 4.3). When insect abundance increased, the buzz ratio slightly increased under red light and at the control treatment, but it only increased significantly under white light (Supplementary data, Tables S4.4 and S4.5). Thus, when prey availability was high, up to one pass out of four contained a feeding buzz at the white treatment, while bats tried to catch an insect every ten passes at the control and the red treatment (Figure 4.3).
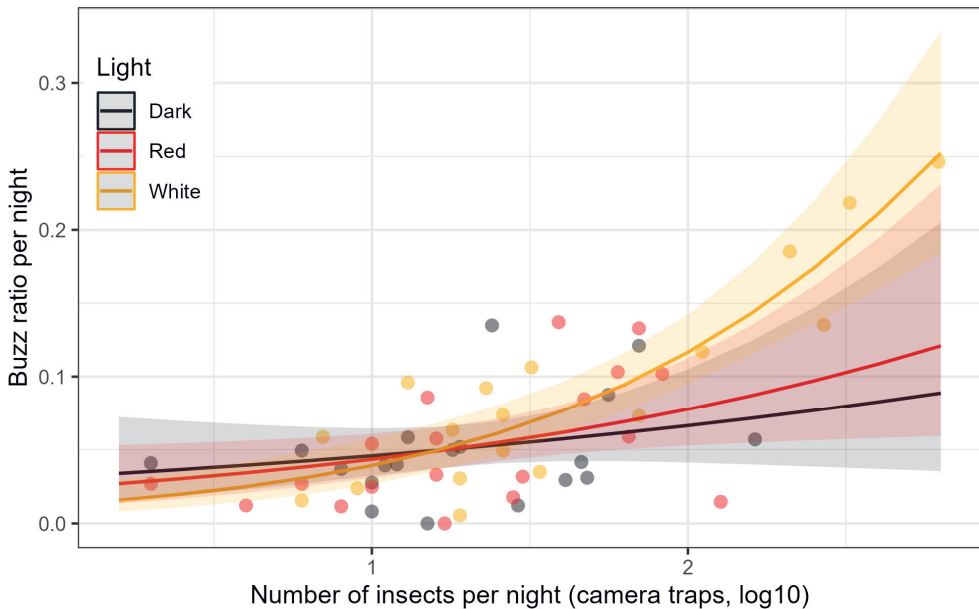
Figure 4.3: Buzz ratio per night (predicted lines from a statistical model with 95% confidence intervals) in response to the insect abundance per night for each light treatment. Dots represent the raw data.

Activity patterns

Nightly insect abundance was greater only under white light (Z=6.039, p<0.0001 see Supplementary data, Table S4.6). GAMMs showed a significant effect of time for each light treatment (Supplementary data, Table S4.6). Under dark natural conditions, insect abundance decreased throughout the night and did not show a bimodal activity pattern. Under red and white light, the number of insects per hour reached a maximum around four hours after sunset before gradually decreasing until sunrise (Figure 4.4A).

The number of bat passes and feeding buzzes followed similar temporal patterns under the same light conditions. In unlit conditions, pipistrelles exhibited activity peaks after sunset and before sunrise (Figure 4.4B and 4.4C). The hourly number of bat passes also showed a slight increase around midnight (Figure 4.4B). Under red light, the activity remained constant throughout the night (passes: χ^2=2.606, p=0.107; feeding buzzes: χ^2=2.736, p=0.101, see Supplementary data, Tables S4.7 and S4.8). Under white light, the hourly number of bat passes and feeding buzzes reached a plateau between three hours and eight hours after sunset, before decreasing until sunrise (Figure 4.4B and 4.4C).

4
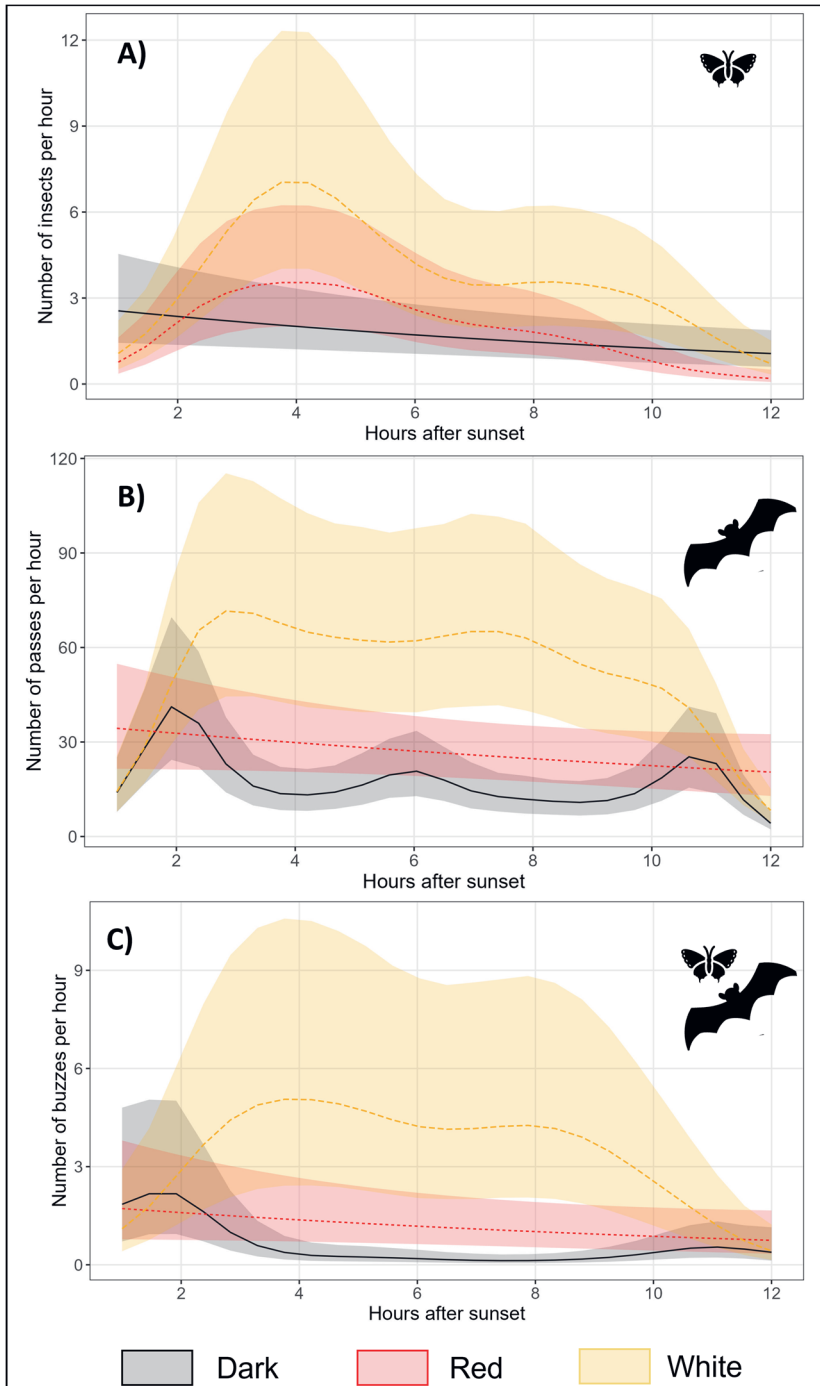
Figure 4.4: Temporal distribution of A) insect abundance, B) bat passes and C) bat feeding buzzes throughout the night with 95% confidence intervals. The time of each observation was centered to the astronomical midnight. 6 and 12 hours after sunset correspond to midnight and sunrise, respectively.

Discussion

We showed that the insect abundance is higher in lit conditions and that their natural activity pattern is disrupted by ALAN. Similarly, light-tolerant pipistrelles are more active throughout the night in lit conditions and their foraging activity pattern is also altered compared to dark natural conditions. Overall, natural activity patterns are less impaired by red light than with light.

Insect abundance

Our results showed that ALAN increases insect abundance in forest edge habitat, but the effect varies according to the light spectrum. Shorter wavelengths of the white light treatment are more attractive for aerial insects than red light (Donners et al., 2018). Here we found a similar pattern per night in insect abundance using sticky sheets and camera traps, except at the dark control. ALAN may alter the insects' spatial distribution with more insects getting closer to lampposts (Russo et al., 2019) and thus getting captured both by camera traps and sticky sheets, while insects at the dark control are evenly spatially distributed and thus being more captured by camera traps than sticky sheets.

The insect size and the distance to the camera also introduce a bias in insect detection (Ruczyński et al., 2020); the IR interval photography works well for larger insects such as macro moth species, where small dipterans may go undetected except when flying very close to the camera. This bias is important to consider when studying food availability for bats, as different bat species prey upon other types of insects. For example, *P. pipistrellus* generally feeds mostly on flies (Diptera) with wingspans of 5 mm or more (Swift et al., 1985). However, some studies reported already changes in diet preferences in several bat species, including *P. pipistrellus* which also feed on Lepidoptera (Arlettaz et al., 2000; Rydell, 1992). As ALAN alters the assemblage compositions of invertebrates (Grubisic and van Grunsven, 2021; Hakbong et al., 2021), this could lead to cascading effects on the diet preferences of bats.

As we cannot identify individuals, both for bats and insects, only the global activity for both taxa can be estimated. Furthermore, it is not possible to clearly identify insects on camera traps images as it would be with sticky sheets. Thus, we only took into account the number of insects and not the species groups to keep a global quantitative approach for both methods.

Bat foraging activity

White light attracts more insects and thus provides predictable foraging opportunities for bats (Prat and Yovel, 2020). However, bats optimize foraging rate according to a cost/benefit trade-off, which depends on the bat's ability to detect and catch prey and the risk of predation due to light (Jones and Rydell, 1994). In this study the buzz ratio drastically increased under white light

compared to the dark situation or under red light when insects were present in larger numbers. This means that pipistrelles pipistrelles have more feeding opportunities in lit conditions with higher insect densities, with foraging benefits outweighing the potential increase in predation risk by visually-oriented predators like owls (Lesiński et al., 2009; Rosina and Shokhrin, 2011; Speakman, 1991b). However, in previous studies, pipistrelles did not produce more buzzes in lit conditions along waterways or with increased moth availability (Charbonnier et al., 2014; Hooker et al., 2022). Therefore, bats' foraging response to ALAN and insect abundance is context-dependent. Some previous studies used bat passes as an estimator of foraging activity, as the number of feeding buzzes is often correlated with the number of bat passes (Mariton et al., 2022). In this study we however showed an interaction effect of ALAN and insect abundance on the buzz ratio, indicating that other parameters might alter the correlation between bat passes and feeding buzzes.

Temporal activity patterns

Both insects and bats (passes and feeding buzzes) displayed comparable temporal patterns under natural dark conditions with more activity after sunset, which is consistent with previous observations (Erkert, 1982; Mariton et al., 2023; O'Farrell and Bradley, 1970; Racey and Swift, 1985; Rydell et al., 1996; Swift, 1980). While bats exhibited a bimodal activity pattern in unlit conditions, the second peak before sunrise was not detectable for insects in our study. In lit conditions, the insect activity peak shifted to later in the night although it stayed before midnight. The nightly activity of pipistrelles increased under red light compared to unlit conditions, but remained constant throughout the night. This effect was stronger under white light, especially between three and eight hours after sunset. Therefore, bat activity is closely related to the diel activity pattern of their prey, but they also continue to forage during the second half of the night while the insect abundance gradually decreases under lit conditions, thus taking advantage of food availability as much as possible.

The activity expansion throughout the night in lit conditions may have short-term benefits for the few light-tolerant bat species by providing a selective advantage of matching their activity pattern to that of their prey (Erkert, 1982). Artificial light could then alter community dynamics, for example in competition patterns, even between potentially competing pipistrelle species (Salinas-Ramos et al., 2021). This time expansion could also lead in overexploitation of their food resources, or they could be exposed to new predators (Tougeron and Sanders, 2023). Thus, this could lead to population decline in the long term for pipistrelles, but also lead to cascading effects across their food web. These changes might also be detrimental for light-averse species (i.e. *Myotis* spp.), as pipistrelles are dominant, and dark areas are lacking food because insects agglomerate around the lights. Our results also show that natural diel activity pattern of insects and

bats is less disrupted under red light than white light, thus opening the possibility of using spectral composition as a mitigation measure. Some previous studies already showed that red light is less harmful than other light spectra, especially for light-averse species (Spoelstra et al., 2017; Zeale et al., 2018). Using red light reduces the local benefit for opportunistic bats to forage on insects around light sources, and leaves the possibility for light-averse bats to forage there as well.

Conclusion

ALAN drives diel activity pattern of nocturnal aerial insects, and insect presence is a key driver for bat activity. Therefore, the advantage of the presence of light for synanthropic bats may be strongly determined by the continuous provision of insects throughout the night. Although light may be detrimental on a wider scale for both synanthropic and light-shy bats, the temporal effects of insect availability are an important factor behind the local advantage in foraging opportunities for synanthropic bat species.

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Acknowledgments

Supplementary data to Chapter 4

Date collection

Table S4.1: Number of nights used for the subsequent analyses per site and light treatment.

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Total
Dark	4	3	2	2	4	2	2	**19**
Red	4	3	2	2	4	2	2	**19**
White	3	3	2	2	4	2	2	**18**

Insect abundance

Table S4.2: Output of statistical analysis on the insect abundance using camera traps data. Significant effects are indicated in bold. The prediction plot from this model is shown in Figure 4.2.

Camera traps			
Predictors	Incidence Rate Ratios	CI	p
(Intercept)	4.29	0.67 – 27.37	0.123
Light [Red]	1.03	0.68 – 1.56	0.883
Light [White]	1.98	1.30 – 3.02	**0.002**
Temperature	1.13	0.99 – 1.29	0.069
Random Effects			
σ^2	0.32		
$\tau_{00\ Site\_n:date\_start}$	0.76		
ICC	0.71		
N_{Site_n}	7		
N_{date_start}	19		
Observations	56		
Marginal R^2 / Conditional R^2	0.199 / 0.764		
AIC	521.6		

Table S4.3: Results of the (Bonferroni corrected) post-hoc pairwise comparisons of the model on insect abundance using camera traps data. Significant differences between pairs are indicated in bold.

	estimate	SE	df	Z ratio	P value
Dark – Red	-0.0313	0.212	Inf	-0.148	1.0000
Dark – White	-0.6828	0.216	Inf	-3.163	**0.0047**
Red – White	-0.6515	0.209	Inf	-3.116	**0.0055**

Foraging activity

Table S4.4: Output of statistical analysis on the buzz ratio. Significant effects are indicated in bold. The prediction plot from this model is shown in Figure 4.3.

Buzz ratio			
Predictors	Estimates	CI	p
Count Model			
(Intercept)	0.03	0.01 – 0.08	**<0.001**
Light [Red]	0.76	0.22 – 2.54	0.650
Light [White]	0.39	0.12 – 1.25	0.113
Insects (log)	1.48	0.78 – 2.83	0.234
Insects (log) x Light [Red]	1.25	0.54 – 2.89	0.599
Insects (log) x Light [White]	2.18	1.04 – 4.59	**0.039**
Zero-Inflated Model			
(Intercept)	0.04	1.04 – 4.59	**<0.001**
Random Effects			
σ^2	0.24		
$\tau_{00 \ Site\_n:date\_start}$	0.00		
$N_{\ Site\_n}$	7		
$N_{\ date\_start}$	19		
Observations	56		
Marginal R^2 / Conditional R^2	0.485 / NA		
AIC	-212		

Table S4.5: Estimates of slopes of the covariate trend for each light treatment based on the buzz ratio model.

Light	Insects (log) trend	SE	df	Lower confidence limit	Upper confidence limit
Dark	0.393	0.330	47	-0.2713	1.06
Red	0.617	0.271	47	0.0719	1.16
White	1.173	0.188	47	0.7959	1.55

Temporal activity patterns

Table S4.6: Generalized additive model to explain variation in hourly insect abundance under different light treatments.

Independent variables	Category	Df/edf	Parameter estimate	SE	Test statistic	p
Parametric terms					Z	
Intercept		1	0.50253	0.24619	2.041	**0.0412**
Light (reference category = Dark)	Red	1	-0.16204	0.10881	-1.489	0.1364
	White	1	0.59680	0.09883	6.039	**<.0001**
Smoothed terms					χ^2	
	Hour x Light Dark	1.003			7.819	**0.00522**
	Hour x Light Red	5.371			67.727	**<.0001**
	Hour x Light White	5.767			64.379	**<.0001**
	Hour x Date	45.184			617.955	**<.0001**

The model used a negative binomial error family and a log-link function. Max k = 12. Overdispersion statistic= 1.07, deviance explained= 67.2%. The prediction plot from this model is shown in Figure 4.4A. Abbreviation: edf, effective degrees of freedom.

Table S4.7: Generalized additive model to explain variation in hourly number of bat passes under different light treatments.

Independent variables	Category	Df/edf	Parameter estimate	SE	Test statistic	p
Parametric terms					Z	
Intercept		1	2.73041	0.17508	15.595	**<.0001**
Light (reference category = Dark)	Red	1	0.54862	0.07696	7.129	**<.0001**
	White	1	1.03792	0.07807	13.295	**<.0001**
Smoothed terms					χ^2	
	Hour x Light Dark	9.274			77.889	**<.0001**
	Hour x Light Red	1.004			2.606	0.107
	Hour x Light White	7.334			91.241	**<.0001**
	Hour x Date	66.304			675.400	**<.0001**

The model used a negative binomial error family and a log-link function. Max k = 12. Overdispersion statistic= 1.16, deviance explained= 61.8%. The prediction plot from this model is shown in Figure 4.4B. Abbreviation: edf, effective degrees of freedom.

Table S4.8: Generalized additive model to explain variation in hourly number of bat feeding buzzes under different light treatments.

Independent variables	Category	Df/edf	Parameter estimate	SE	Test statistic	p
Parametric terms					Z	
Intercept		1	-0.9441	0.3273	-2.885	**0.00392**
Light (reference category = Dark)	Red	1	1. 0737	0.1559	6.886	**<.0001**
	White	1	1.9445	0.1559	12.477	**<.0001**
Smoothed terms					χ^2	
	Hour x Light Dark	5.914			45.759	**<.0001**
	Hour x Light Red	1.006			2.736	0.101
	Hour x Light White	5.128			33.458	**<.0001**
	Hour x Date	54.695			407.621	**<.0001**

The model used a negative binomial error family and a log-link function. Max k = 12. Overdispersion statistic= 0.88, deviance explained= 64.2%. The prediction plot from this model is shown in Figure 4.4C. Abbreviation: edf, effective degrees of freedom.

4

Chapter 5

Light-averse *Myotis* bats avoid streetlights from afar

Claire Hermans, Marcel E. Visser, Kamiel Spoelstra

Abstract

Artificial light at night (ALAN) is a driver of habitat loss, especially for nocturnal species. This is of major concern, especially for light-averse insectivorous bat species, as ALAN causes loss of foraging opportunities by altering the bats' habitat availability and quality. There is a crucial need for information on the light tolerance threshold of light-averse bat species to mitigate the impact of artificial light on bat communities. Here we investigated how light-averse bats such as *Myotis* species spatially alter their foraging behaviour in their natural environment in response to ALAN by lamps with different spectra. We used acoustic localisation to precisely study bat activity around experimental lampposts of which we mapped the light distribution. Our results show that *Myotis* bats avoid lit areas from afar, and even avoid illuminance levels below 0.1 lux, both for white and for red lights. They completely avoid the areas with light levels above 20 lux under white light and 40 lux under red light conditions. Thus, this suggests that red light is less harmful than white light, even though both light spectra impede bat activity in a significant amount of otherwise suitable foraging habitat. We therefore recommend keeping key habitats for light-averse bat species as dark as possible to limit habitat loss caused by artificial light.

Introduction

Ecosystems and biodiversity face a threat from the drastic increase of artificial light at night over the last decades (Falchi et al., 2016; Falchi and Bará, 2023; Gaston et al., 2013; Hölker et al., 2021; Sanders et al., 2023). Artificial light is a main driver of habitat loss and fragmentation (Barré et al., 2020; Bhardwaj et al., 2020; Gaston et al., 2014; Hale et al., 2015; Lewanzik and Voigt, 2014; van Grunsven et al., 2017). Insectivorous bats are particularly vulnerable to this threat, as they are mainly nocturnal and fly across the landscape to forage at specific habitat patches (Haarsma and Siepel, 2014; Verboom and Huitema, 2010; Zeale et al., 2018). Moreover, artificial light alters foraging opportunities, as insects accumulate near light sources, creating a vacuum effect from unlit to lit areas (Russo et al., 2019; Rydell, 1992).

Unlike synanthropic bats such as pipistrelles that take advantage of concentrated prey resources around light sources, species like *Myotis* spp or *Plecotus* spp are light-averse (Azam et al., 2018; Bolliger et al., 2020b; Lacoeuilhe et al., 2014; Spoelstra et al., 2017; Stone et al., 2012). Light may therefore drastically reduce habitat availability for species that avoid lit foraging areas (Azam et al., 2018; Jägerbrand and Spoelstra, 2023; Polak et al., 2011; Sanders et al., 2021; Shier et al., 2020; Spoelstra et al., 2017).

If the light tolerance threshold under natural conditions is known, this enables to design lighting setups that minimise the infringement of habitat of light-averse species. However, it is notoriously difficult to establish dose-response curves of bat activity depending on light intensity, especially in free-flying animals. The dose-dependent responses are usually tested in controlled laboratory conditions (De Jong et al., 2016; Dominoni et al., 2018; Jägerbrand et al., 2023; Quintanilla-Ahumada et al., 2022) or determined by using distance to light source as a proxy (Azam et al., 2018; Shier et al., 2020).

Here we investigated the activity of light-averse bat species in response to illuminance levels of two light spectra in order to establish a dose-response curve of activity of light-averse bat species. We collected data at seven experimentally illuminated sites in forest-edge habitat and compared the response of *Myotis* bats to white and red light using a control treatment as a null model for the distribution of bat activity around the light posts. We calculated the light distribution for this dark control as if there was artificial light and did so for both white and red light, based on the fixture specific light distribution. We compared the null model to each light spectrum, in order to determine the light intensity threshold at which bats avoid the lit parts of their habitat. We hypothesized that the disturbance threshold is higher under red light than white light.

Material and methods

Field sites

Data were collected in forest-edge habitat at seven experimentally illuminated sites in the Netherlands (Spoelstra et al., 2015). Each site consists of four rows perpendicular to the forest edge, each with five 4 m tall lampposts separated by 25 m (except two sites with rows of three lampposts), the central one being at the forest edge. Rows were randomly assigned to either white (Philips Fortimo White), green (Philips Fortimo ClearSky) or red (Philips Fortimo ClearField) light, and the last transect was permanently kept dark. The illuminance at ground level is 7.6 ± 1.2 Lux (1 s.e.m.) beneath each lamppost (Spoelstra et al., 2015), which is comparable to the illumination levels of countryside roads (Gaston et al., 2013). As previous studies carried out at these sites showed that green light does not reduce the impact of ALAN of the activity of light-averse bat species (Spoelstra et al., 2017), this treatment was excluded from this study.

Acoustic localisation

Bats were acoustically tracked with microphone arrays from 15 min before sunset to 15 min after sunrise for 27 nights (two to four nights per site) between May 8th and August 9th 2020 on nights without rainfall. Six microphone arrays were deployed at one site per night. One microphone array was placed in front of the lamppost at the forest edge and another one in front of the first lamppost inside the forest for the three light treatments. The centre of the array was placed at 1.07 to 1.79 m (average 1.35 m; standard deviation, s.d. 0.11 m) above the ground and 1.56 to 6.61 m (average 3.11 m; s.d. 1.1) from the lamppost depending on the vegetation surrounding the lamppost. For each microphone array, eight microphones (omnidirectional microphones FG-23329 Knowles Electronics, Itasca, IL, USA) were fitted on an aluminium frame and mounted on a tripod. The technical description of the recording system is provided in Hermans et al. (2023). Recordings were stored in 10-second files. 3D positions of bats were reconstructed by measuring the time lag of the arrival time of the calls between the top microphone (reference channel) and the other seven microphones. This time-of-arrival-difference (TOAD) was calculated with a cross-correlation function using a custom-built software (TOADSuite, P. Stilz, J.C. Koblitz and H.R. Goerlitz) (Goerlitz, 2019) in MATLAB R2020a (The MathWorks, Inc., Natick, MA, USA).

Species identification of bat calls were performed using the Tadarida software (Bas et al., 2017, online repository: https://github.com/YvesBas, January 2021 version). As identification of echolocation calls to the species level is difficult, bat calls were assigned to the following species groups: the ENV group including *Eptesicus* spp., *Nyctalus* spp. and *Vespertilio* spp. (open space aerial foragers), the *Myotis* group including *Myotis* spp. (narrow space foragers), and the *Pipistrellus* group including *Pipistrellus* spp. (edge space forager). Each 3D position

was then linked to a species group using the detection time of the bat call. In order to investigate the activity of light-averse bat species in response to illuminance and light spectrum, we only selected *Myotis* 3D positions and we manually checked these recordings to verify the species group identification.

Light distribution

We used the illuminance (in lux) per 20 cm x 20 cm x 20 cm voxel in a square rectangular cuboid of 25 m x 25 m x 10 m, with the lamppost at the centre (Figure 5.1). Direct incidence at the centre of each voxel was calculated with calibrated fixture-specific goniophotometric data in a straight line towards the light; hence no cosine correction was applied.

We assigned each *Myotis* position to the illuminance of the closest voxel based on the Euclidean distance between the bat position and the centre of the voxel (Figure 5.1). Bat positions were classified into categories of illuminance up to the maximum illuminance measured for bat positions at the two light treatments or for their calculated light illuminance at the dark control, as explained in the 'Statistical analyses' section ([0;1[, [1;5[, [5;10[, [10;20[, [20;30[, [30;40[, [40;50[, [50;60[, [60;70[, [70;80[, [80;90[and [90;110[for white light and [0;1[, [1;5[, [5;10[, [10;20[, [20;30[, [30;40[, [40;50[and [50;60[for red light). Based on the fixture-specific goniophotometric data and light measurements with a lux meter (B 360, LMT Lichtmesstechnik GmbH, Berlin, Germany), the two light spectra do not have the same luminous flux. Therefore, the illuminance values under white light are approximately twice the values under red light.

To correct for the different number of voxels per light category (the number of voxels decreases with increasing illuminance), we divided the number of bat positions per light category by the number of available voxels within each category present in the entire grid.
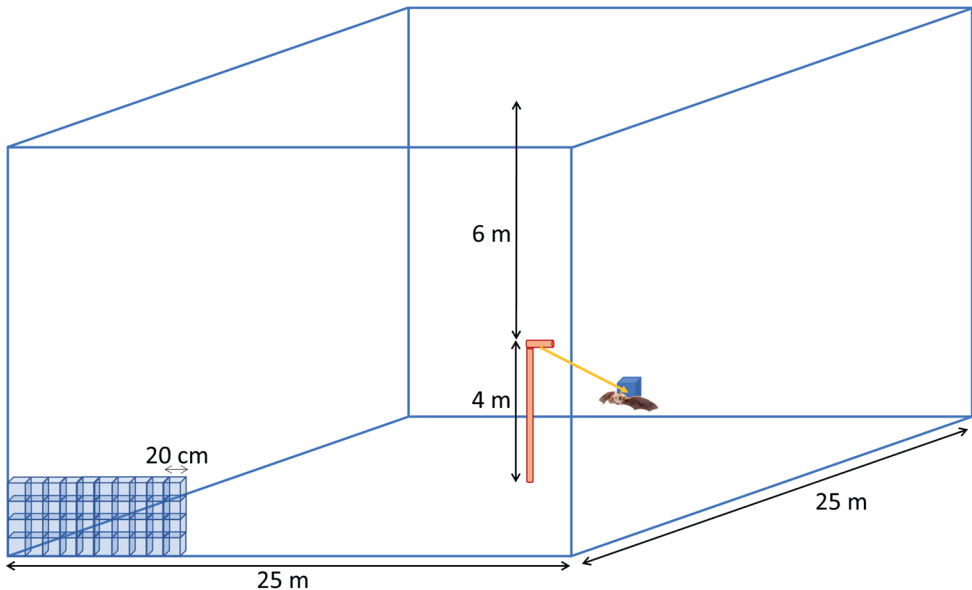
Figure 5.1: Schematic of the assessment of the illuminance encountered by *Myotis* bats around the lamppost. A square rectangular cuboid of 25 m x 25 m x 10 m with lamppost at the centre was divided into voxels (blue cubes, only a small number of all 781,250 voxels depicted) of 20 cm x 20 cm x 20 cm in which the illuminance (lux) was assessed. Each bat position was assigned to the illuminance of the closest voxel (here is dark blue) based on the 3D Euclidean distance between the bat position and the voxel.

Statistical analysis

Analyses were carried out using the statistical software R (version 4.1.2, R Core Team, 2021). As most bat positions were in the light category below 1 lux, and because a potential illuminance threshold appeared to be below 1 lux, we zoomed in on this category and performed a logistic regression, using bat activity in voxels as a binomial response variable (where one corresponded to voxels containing a bat position and zero corresponded to voxels without any bat position). Note that voxels with more than one position were extremely rare (seven voxels with two positions out of 656,303 voxels for white light and 693,050 voxels for red light).

We fitted a generalized mixed model (using the *glm* function from the R package stats) with binomial distribution and with illuminance, light treatment and their interaction as explanatory variables. However, illumination restriction due to vegetation cover and the fact that some voxels could not be used by bats because there was vegetation in there were not incorporated in the models. We used the dark control treatment as a null model for the distribution of bat activity around the light posts. As the two light spectra did not exactly have the same luminous flux, we calculated the light distribution for dark control as if there was red light, to compare the dark control voxel use by bats with the voxel use under the red light posts. Similarly, we calculated the light distribution for dark control as if there

was white light to compare the bat voxel use between dark control and white light. We ran a first model to compare the white treatment with the dark control and another model to compare the red treatment with the dark control.

Results

In total (all sites combined), 146 positions were calculated under white light, 327 under red light and 749 at the dark control. The maximum light levels measured for bat positions were 20.1 lux at white light, and 40.5 lux at red light. In comparison, bats were detected closer to the dark control 'lamps', at distances that would have had 51.4 or 102.7 lux if we simulate red and white light respectively at the dark control sites (Figures 5.2 and 5.3).
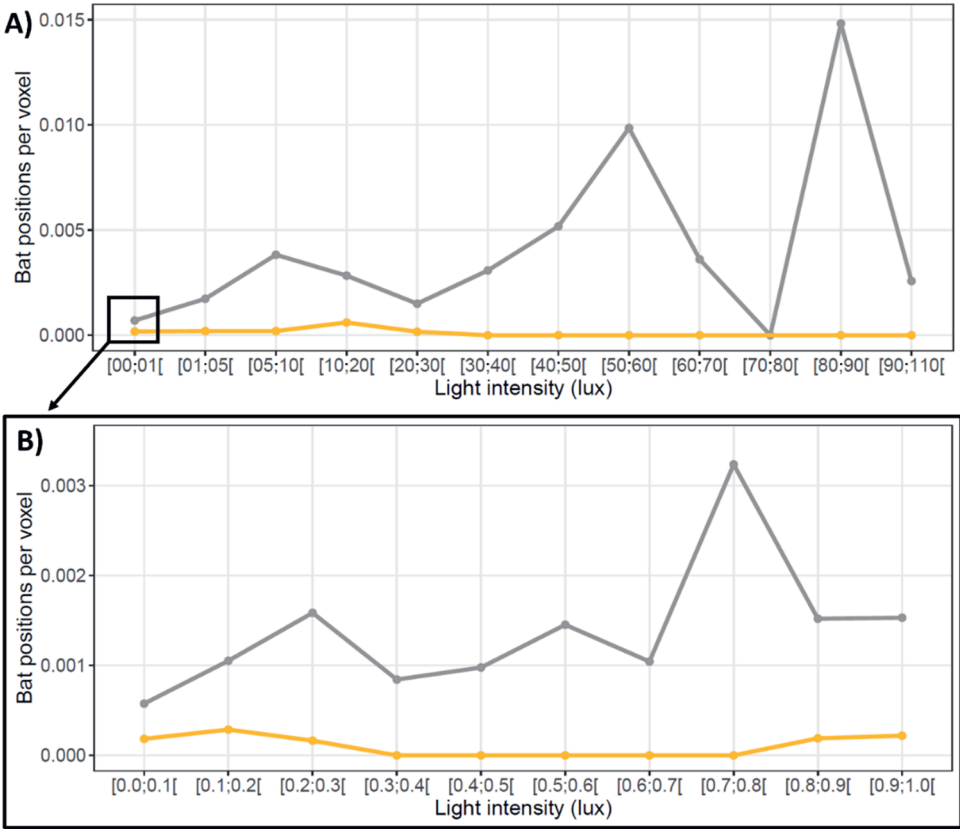
Figure 5.2: A) Bat activity (number of positions) per voxel in each illuminance category under white light. B) Zoom on bat activity per voxel below 1 lux. Yellow: data at the white treatment. Grey: data at the dark control treatment with illuminance calculated as if there was a white light at the lamppost.
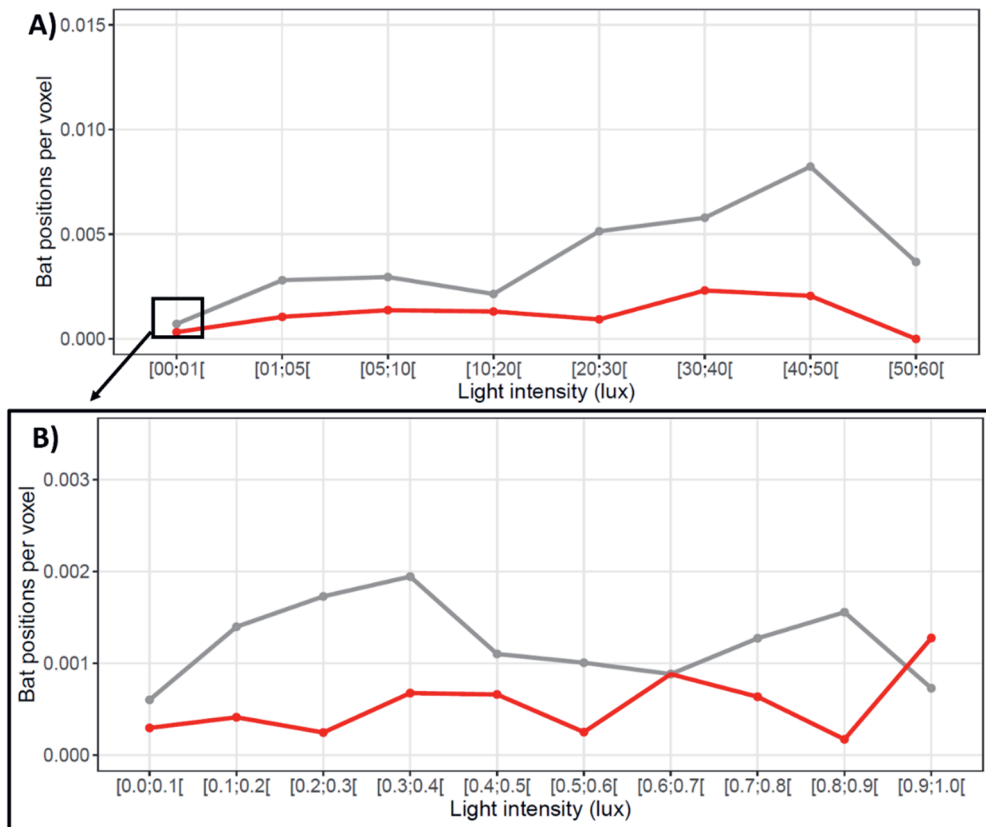
Figure 5.3: A) Bat activity (number of positions) per voxel in each illuminance category under red light. B) Zoom on bat activity per voxel below 1 lux. Red: data at the red treatment. Grey: data at the dark control treatment with illuminance calculated as if there was a red light at the lamppost.

In the light category below 1 lux, 117 positions were calculated under white light, 227 under red light and 458 and 497 at the dark control for the white and red light, respectively. The logistic regression model comparing white light with the dark control showed that the probability of recording bat activity increases with illuminance under dark conditions, while it decreases under white light (Table 5.1). On the other hand, the logistic regression model comparing red light with the dark control indicated that the probability of recording bat activity increases with illuminance under both light treatments, but the overall probability is lower under red light than at the dark control (Table 5.2).

Table 5.1: Estimates, standard errors and p-values of the effect of the illuminance and the light treatment on the probability of bat activity in a voxel derived from the logistic regression, comparing the white treatment and the dark treatment with simulated white light.

	Estimate	Standard error	z-value	p-value
Intercept (Dark)	-7.46	0.05	-136.02	**< 0.001**
Illuminance	1.60	0.19	8.57	**< 0.001**
Colour (White)	-1.11	0.12	-9.50	**< 0.001**
Light illuminance:Colour (White)	-2.54	0.75	-3.41	**< 0.001**

Table 5.2: Estimates, standard errors and p-values of the effect of the illuminance and the light treatment on the probability of bat activity in a voxel derived from the logistic regression comparing the red treatment and the dark treatment with simulated red light.

	Estimate	Standard error	z-value	p-value
Intercept (Dark)	-7.36	0.05	-145.19	**< 0.001**
Illuminance	1.19	0.19	6.21	**< 0.001**
Colour (Red)	-0.78	0.09	-8.60	**< 0.001**
Light illuminance: Colour (Red)	0.05	0.34	0.14	0.89

Discussion

The fine-scale measurements of bat movement around lampposts show that light-averse *Myotis* bats avoid streetlights from afar. Even at the lowest illuminance level at which we measured bat activity, the probability to detect a bat was lower in lit conditions than at the dark control. Hence, we cannot establish a precise illuminance threshold under which these bats are not disturbed, both for red and white light. The threshold must however be below 0.1 lux. Moreover, *Myotis* bats never forage on insects trapped in the brighter illuminated space close to the lampposts, completely avoiding the areas with light levels above 20 lux or 40 lux under white and red light conditions, respectively. Thus, this suggests that red light is less harmful in disturbing foraging activity than white light, as it reduces bat activity to a lesser extent. Both light spectra however impede bat activity in a significant amount of otherwise suitable foraging habitat.

The disturbance threshold was apparently at such low light levels (below 0.1 lux) that these fell outside of the microphone array detection range. *Myotis* species are detected up to 20 m (Hermans et al., 2023), but their calls are fainter and at higher frequencies than noctules for example, therefore localisation becomes harder at larger distances. Our results are consistent with a previous study that shows streetlight avoidance of *Myotis* species from at least 25 m and that the negative effect of illuminance on their activity was detected below 1 lux (Azam et

al., 2018). Maximum illuminance during full moon only reaches 0.3 lux in extreme cases (Kyba et al., 2017b), but it elicits lunar phobia and change in bat activity, especially in tropical bat species (Appel et al., 2017; Ciechanowski et al., 2007; Saldaña-Vázquez and Munguía-Rosas, 2013; Vásquez et al., 2020). It is therefore not surprising that even low light levels of anthropogenic light can affect *Myotis* species (Seewagen et al., 2023). Besides, moonlight intensity is relatively high only during a minor number of nights per lunar cycle, whilst artificial light is virtually continuously present. Finally, the glare produced by the light posts can be seen from a large distance in the unlit surroundings and could deter bats from afar.

Conclusion

This study aimed at investigating the activity of light-averse bat species in response to illuminance and light spectrum in order to establish a dose-response curve of bat activity versus light level. Our results show that *Myotis* bats avoid streetlights from afar, and negative effect of illuminance on their activity is already detected below 0.1 lux. We hence recommend keeping key habitats for light-averse bat species as dark as possible and reducing light trespass into areas that are unintended to be lit in order to limit habitat loss caused by artificial light for light-sensitive bats.

Acknowledgments

5

Chapter 6

No effect on flight behaviour of commuting pond bats (*Myotis dasycneme*) by artificial light of realistic intensity

Claire Hermans, Laura Kijm, Marieke Paardekooper, Jens C. Koblitz, Peter Stilz, Anne-Jifke Haarsma, Marcel E. Visser, Kamiel Spoelstra

Abstract

Artificial light at night may act as a barrier and cause habitat fragmentation, especially for bat species that are generally considered to be light-averse. Bats use linear structures in the landscape to commute from their roost to their foraging areas. Trawling bats such as the pond bat (*Myotis dasycneme*) and the Daubenton's bat (*M. daubentonii*) forage predominantly above water bodies and use waterways as commuting routes. Artificial light on bridges potentially leads to interruptions of commuting routes, or changes flight behaviour of trawling bats, but impact of light may vary for different light spectra and light intensities. Here, we tested the flight response of commuting pond bats to four light spectra at two light intensities by placing an experimental lamppost along waterways known as commuting routes. A microphone array was placed next to the light to reconstruct flight paths of bats. Flight parameters such as flight speed and straightness were calculated for the trajectory of each passing bat. Flight behaviour of pond bats was unaffected by the presence of light, hence the short presence on a bridge of a light with realistic intensity does not pose a barrier effect for bats commuting along waterways. A similar light installation on the long term may however impair landscape connectivity.

To be submitted

Introduction

The drastic increase of artificial light at night over the last decades (Falchi et al., 2016; Falchi and Bará, 2023; Kyba et al., 2023, 2017a) represents a threat to ecosystems and biodiversity (Gaston et al., 2013; Hölker et al., 2021; Sanders et al., 2023). As artificial light is now omnipresent in almost all populated areas worldwide, light pollution impedes movement of light-sensitive animal species across the landscape. If artificial light is placed along infrastructures, it fragments the habitat for many different species (Gaston et al., 2014). Artificial light especially alters landscape connectivity for mobile taxa such as birds, bats or toads during migration or while commuting to foraging areas (Hale et al., 2015; Korpach et al., 2022; Laforge et al., 2019; Stone et al., 2009; van Grunsven et al., 2017). A way to mitigate the effect of artificial light at night on the ecosystems is to change the light spectrum. As many taxa are highly sensitive to the blue part of the light spectrum, reducing the light emission in these wavelengths would minimize the adverse effects of lighting (Longcore et al., 2018). Therefore, adapting the light spectrum of lighting in key habitats could facilitate landscape connectivity.

Bats are particularly vulnerable to this threat, as they are mainly nocturnal and fly across the landscape to forage at specific habitat patches (Haarsma and Siepel, 2014; Verboom and Huitema, 2010; Zeale et al., 2018). Bats display a specific echolocation and flight behaviour when commuting or migrating, as they generally fly faster and straighter than in foraging situations (Barré et al., 2023a; Britton et al., 1997; Grodzinski et al., 2009; Troxell et al., 2019; Van De Sijpe and Holsbeek, 2007; Verboom et al., 1999). Slow-flying bats such as *Myotis* species are generally considered as light-shy species and avoid illumination to potentially reduce predation risk (Rowse et al., 2018; Rydell, 1992; Spoelstra et al., 2017). Among this genus, trawling bats like the pond bat (*Myotis dasycneme*) and the Daubenton's bat (*Myotis daubentonii*) commonly hunt insects low over water. These species are known to fly to their foraging sites up to 15 kilometers from their roost by using waterways as commuting routes (Haarsma, 2023). As these structures are essential in terms of landscape connectivity for those species (Britton et al., 1997; Haarsma and Siepel, 2014), illumination along these could create a barrier effect and induce habitat fragmentation. Moreover, previous studies suggested that echolocating bats may use vision to orient themselves (Céchetto et al., 2023). Therefore, artificial light may have a disrupting effect on bat orientation and flight behaviour when installed on their usual commuting route, especially along obstacles like bridges.

However, previous studies found little to no effect of experimental lighting on commuting trawling bats. Spoelstra et al. (2018) found no effect of the presence of light, or light spectrum, on the number of passes of *M. daubentonii* in a choice experiment using two identical culverts along their commuting route. In Kuijper

et al. (2008), a strong 1000 W halogen lamp was placed on the banks of canals used as commuting routes by pond bats. They did not find an effect of experimental light on the number of passes nor did more bats use a different commuting flyway. However, light did disturb the flight patterns of pond bats as many bats turned around in response to the light, most of them already before reaching the light beam. Passing bats tried to evade the light beam. Hooker et al. (2022) suggested that *Myotis* species are less light-sensitive when commuting compared to foraging, likely due to their shorter exposure to illumination. Despite the fact that the number of passes along the commuting route is generally not altered by artificial light in these previous studies, the fine-scale flight behaviour of pond bats when exposed to artificial light of variable light intensity and light spectrum while commuting has not been investigated yet.

Here we studied how artificial light affects flight behaviour of pond bats along waterways while commuting to their foraging grounds. We exposed pond bats to four light spectra at different light intensities using an experimental lighting setup placed on bridges crossing their commuting routes. We used microphone arrays to record bats and reconstruct their trajectories to thereby calculate parameters such as flight speed and straightness. We hypothesized that pond bats fly faster and straighter when exposed to light, with a lower response to light with reduced blue light emission.

Material and methods

Study sites and data collection

The highest population densities of pond bats in the Netherlands in summer are found in the Provinces of South- and North Holland, Friesland and Overijssel (Broekhuizen et al., 2016). Male colonies usually contain 10 to 65 individuals while maternity colonies contain on average 165 individuals with exceptions of up to 750 individuals (Haarsma, 2011). Pond bats mainly forage in areas along the IJsselmeer lake and use linear waterbodies (i.e. canals or rivers) and routes over land (i.e. treelines, roads or slope of a dyke) to commute from their roost site (Haarsma, 2023). Five bridges (hereafter called sites) along waterways known as commuting routes were selected for the experimental setup (Figure 6.1). These sites were located 500 m to 5.3 km from the roost location. Some sites had ambient lighting (i.e. Makkum – red boat traffic lights, see Figure 6.2, Joure – one streetlight along the commuting route, and Balk – many streetlights along the canal, see Figure S6.1). We assumed that bats were habituated to these situations as these lights were permanently present and we did not alter the ambient lighting conditions.

Data were collected between June 16th 2021 and July 7th 2021, only during dry circumstances (for the equipment) and wind speed with a maximum of 3 Bft to maximize the signal-to-noise ratio.
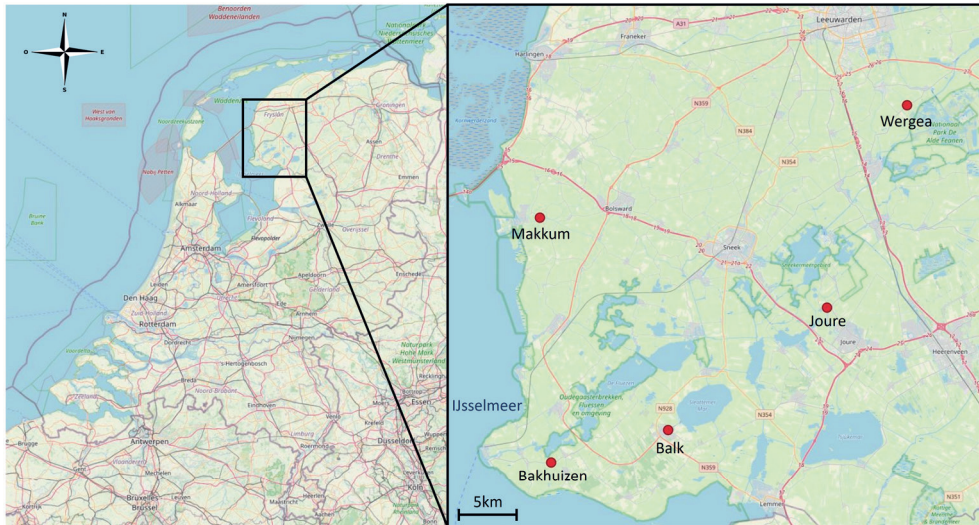
Figure 6.1: Map of the study area (Friesland, The Netherlands) with the sampled sites (map made in QGIS, 2023).

Experimental lighting setup

An experimental lamppost was placed at the centre of the bridge against the railing, pointing towards the waterway on the side where bats were approaching when flying from their roost site (Figure 6.2). The custom-built luminaire (Signify, Eindhoven, The Netherlands) was connected to a laptop via a Philips Smart-Jack Pro converter to control the lighting installation. Light intensity and light spectrum of the luminaire were adjusted via a predetermined sequence set up in DMX-DALI Controller (version 7.4). The sequence was launched as soon as the first pond bat of the evening passed the bridge.

A two-factorial in-situ experiment was carried out in which bats were exposed to four different light spectra at two light intensities. The experimental luminaire was fitted with light emitting diodes (LEDs) emitting white light either with a colour temperature of 2200 K or 3000 K (hereafter 'White 2200 K' and 'White 3000 K'), red light or phosphor-converted amber light (Philips, Amsterdam, The Netherlands). The spectral composition of each light type was measured with a QE Pro-Vis-NIR spectrometer (Ocean Insight, Orlando, FL, US). The spectral compositions are provided in Figure 6.3. We used two illuminance levels at the water surface level, namely 5 lux and 20 lux, measured with a lux meter (B 360, LMT Lichtmesstechnik GmbH, Berlin, Germany). Those intensities are in the range

of commonly used street light intensities in the Netherlands as included in the guidelines for the European Union (Austrian Energy Agency, 2017). As the ambient light level along the waterway at one of the sites (Balk) exceeded 5 lux at street level (but less than 0.1 lux in front of the bridge at the water surface level, Figure S6.1), this site was only sampled one night at 20 lux.

Based on the five light treatments (four light types and dark control) and two light intensities, pond bats were exposed to nine possible treatment combinations. To allocate treatment combinations, a randomized complete block design was applied (Figure 6.4). Each site was sampled two non-consecutive nights. During a sampling night, only one light intensity level was used because of time limitations (most commuting bats from one colony passed within one to two hours, Figure S6.2). The order of intensity levels was randomly assigned for each site. Each sampling night consisted of three blocks with the aim of applying each light treatment equally often, as bats do not pass at regular intervals but follow a skewed normal distribution (Figure S6.2) (Haarsma and Siepel, 2014). One block included all five light spectra in a random order. We never applied the same light spectrum twice in a row and never applied the same order in the three blocks within one evening. Each treatment lasted five minutes, followed by a two-minutes break of no light as a washout-period, except for the control treatment (no light) which had no washout-period afterwards. Lights were switched on and off automatically without a fading phase. In case pond bats would be withheld from passing the illuminated spot on the waterway during light treatments, breaks between treatments allowed them to pass, thereby we minimized possible disturbance effects and eliminated possible confounding factors from previous treatments. We did not include bats passing during these washout-periods in our analyses. We waited for at least five days between two recording nights at the same site to avoid habituation or confounding effect of the previous light intensity level.

6

Figure 6.2: Pictures of the four light spectra (A-B: Red Clearfield, C-D: Amber, E-F: White 2200 K and G-H: White 3000 K) at two 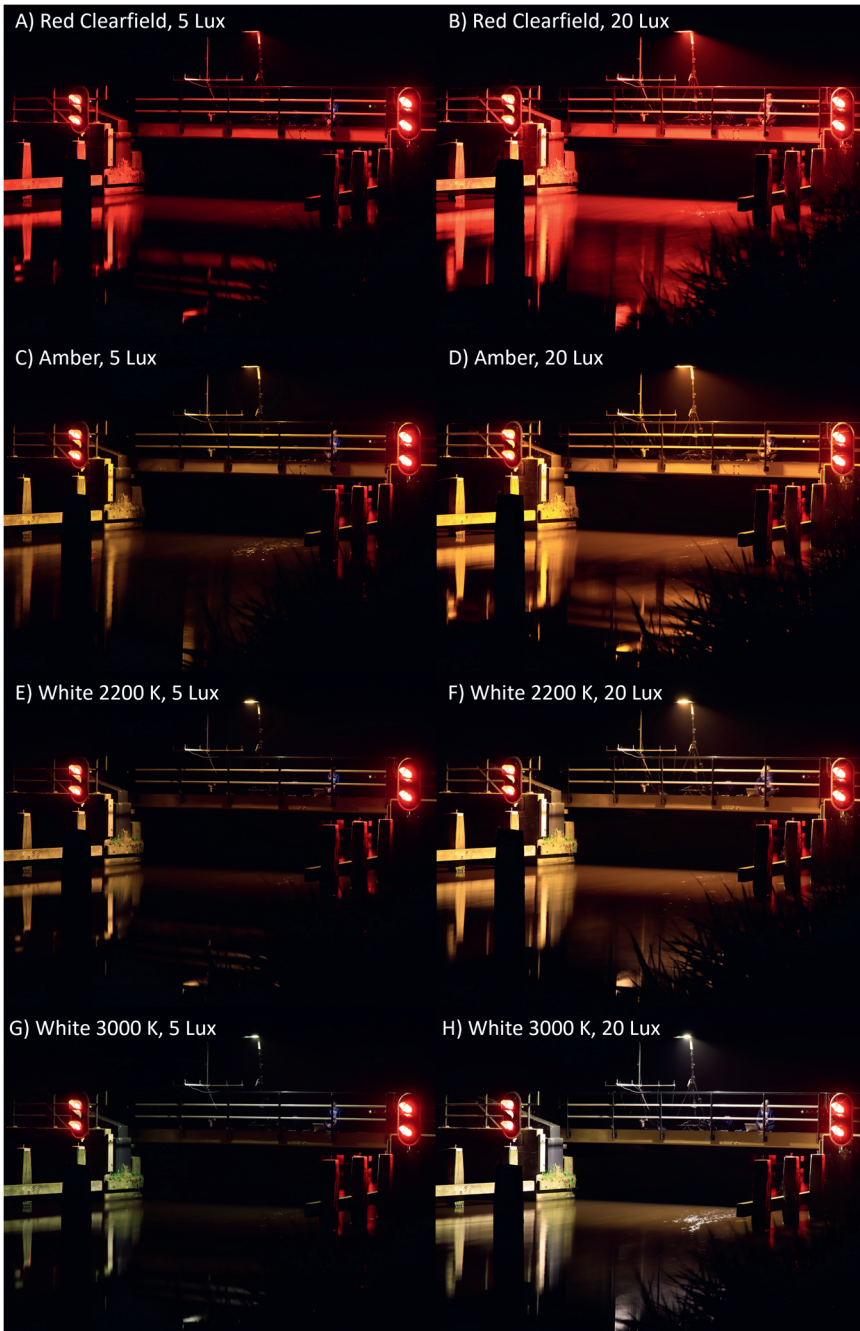light intensity levels (A,C,E,G: 5 lux and B,D,F,H: 20 lux) at one of the sites (Makkum). The experimental lamppost was placed at the centre of the bridge against the railing, pointing towards the waterway on the side where bats were approaching when flying away from their roost site in the evening. The microphone array was placed next to the lamppost facing the same direction.
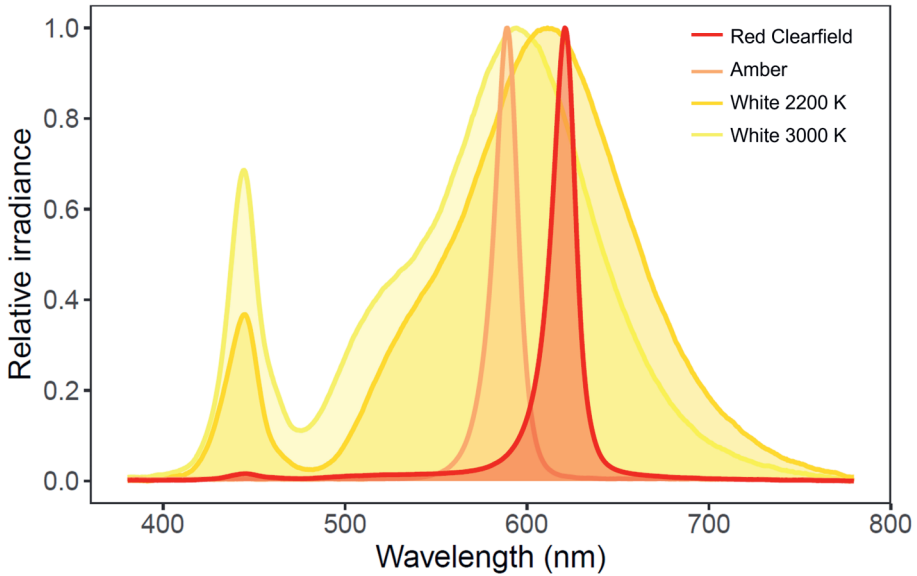
Figure 6.3: Spectral composition of the four light treatments.
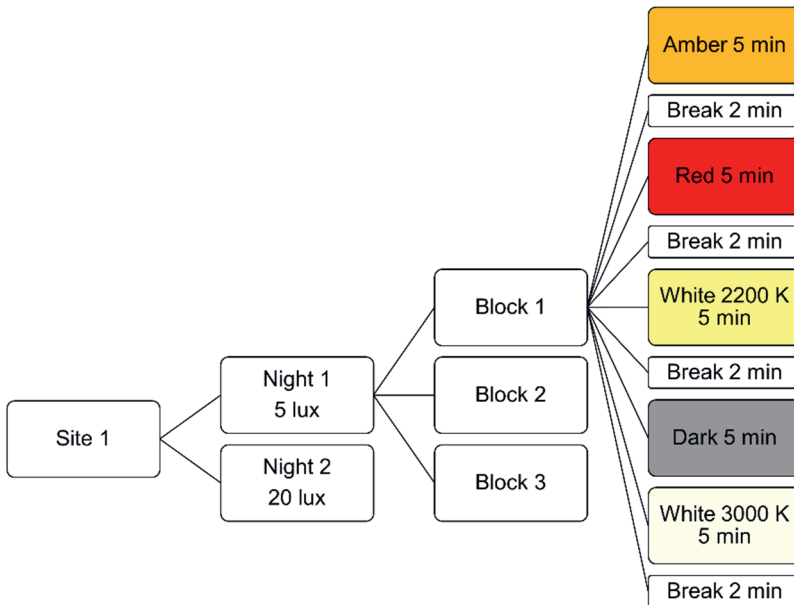
Figure 6.4: Schematic overview of the experimental design for each site, where night was randomly assigned to one light intensity, either 5 or 20 lux. Each sampling night consisted of three blocks and each block consists of all spectra emitted once with washout-periods ("breaks") in between. The light treatments were never applied in the same order in the three blocks within one evening. The sequence of light treatments is only shown for one of the three blocks during one of the two nights of recording for graphical purposes.

Acoustic tracking and flight parameters

Bats were acoustically tracked with a microphone array placed next to the lamppost on the bridge (Figure 6.2). Eight microphones (omnidirectional microphones FG-23329 Knowles Electronics, Itasca, IL, USA) were fitted on an aluminium frame and mounted on a tripod. The technical description of the recording system is provided in Hermans et al. (2023).

Recordings were stored in 10-second files. As pond bats mainly flew low over water, echolocation calls are reflected on the water surface, thus creating interferences that impair acoustic localisation. We mathematically removed the reflections from the water surface using a custom-built script in MATLAB2020a (Appendix S6.1). 3D positions of bats were then reconstructed by measuring the time lag of the arrival time of the calls between the reference channel and the other seven microphones. This time-of-arrival-difference (TOAD) was calculated with a cross-correlation function using custom-built software (TOADSuite, P. Stilz, J.C. Koblitz and H.R. Goerlitz) (Goerlitz, 2019) in MATLAB R2015a (The MathWorks, Inc., Natick, MA, USA). The flight tracks were manually checked to remove physically implausible outliers, such as positions far outside the bats' trajectory or positions resulting in rapid jumps within their flight trajectory.

We considered individual flight paths as independent since it was highly unlikely that the same bats repeatedly passed and returned to the roost in the early evening. The few bats that were coming from the other side of the bridge, and thus not experiencing the light in the same way, were not taken into account.

We calculated flight speed (m/s) between two consecutive positions following the R script available in Barré et al. (2021). We used horizontal speed, as pond bats mainly flew low over water, except for a few bats that flew over the bridge. Moreover, horizontal and 3D flight speed were highly correlated (correlation coefficient = 0.96). The mean trajectory speed is the mean of all segment speeds within the trajectory (Figure 6.5). Trajectories with mean speed above 20 m/s are unlikely to be accurate (Barré et al., 2023a; O'Mara et al., 2021) and were considered as outliers and removed for further analyses (four trajectories were removed).

The straightness index was defined as the ratio between the length of the trajectory, which is the sum of call-to-call distances, and the displacement (D_{total}), which is the straight spatial distance from the start to the end of the trajectory (Figure 6.5). The minimum straightness index of 1 therefore indicates a straight line and the index value increases with sinuosity.
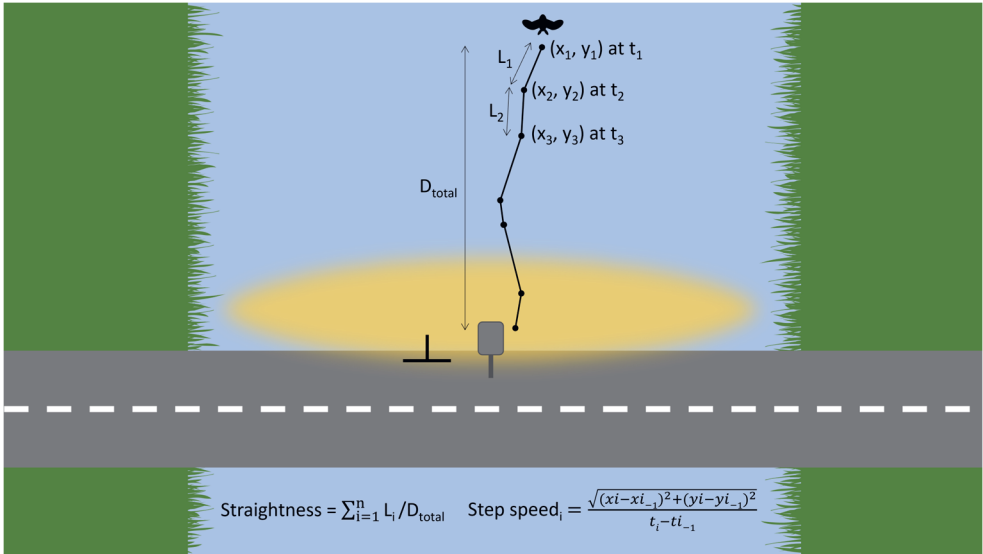
Figure 6.5: Schematic description of the setup and the flight parameters used for the analysis. The T-shape represents the microphone array. The lamppost was placed at the centre of the bridge against the railing, pointing towards the waterway on the side where bats were approaching when flying away from their roost site in the evening.

Statistical analysis

Analyses were carried out using the statistical software R (version 4.1.2, R Core Team, 2021). For flight speed, we fitted a linear model with log-transformed mean horizontal speed per trajectory as response variable. For flight straightness, we fitted a generalized linear model with Gamma distribution with identity-link. The shape parameter of the gamma distribution was estimated using the *gamma.shape* function of the MASS package (Venables and Ripley, 2002). In both models, the site, the time from the start of the lighting sequence (transformed into a z-score by subtracting the mean and dividing the difference by the standard deviation for each site), the interaction of the latter, as well as the light intensity, the light spectrum and the interaction of these were included as explanatory variables. We subsequently compared models with and without the different fixed effects using the *compare_performance* function of the performance package (Lüdecke et al., 2021).

Results

We recorded between 20 and 450 commuting pond bats per night (mean=114.89 bats per night, SD=132.26). In total 698 trajectories were included in the analysis (Tables S6.1 and S6.2), of which only eight bats turned around and/or flew over the bridge.

We found no significant effect of light intensity and light spectrum on the flight behaviour of pond bats, neither the flight speed (Table 6.3) nor the flight straightness (Table 6.4). Flight speed varied across sites and decreased throughout the measurement period (Table 6.1 and S6.3, Figure S6.3). The interaction effect between site and time significantly affected flight straightness (Table 6.2 and S6.4, Figure S6.4).

Table 6.1: Result of the linear models' comparison with flight speed (log transformed) as response variable. The most parsimonious model is highlighted in grey.

Explanatory variables	Df	R^2	AIC	Performance score (%)
Null model		0	2966.52	0.006
Site	6	0.037	2948.45	23.22
Time	3	0.026	2950.39	19.17
Site + Time	**7**	**0.067**	**2928.25**	**85.79**
Site x Time	11	0.076	2929.33	63.37
Site + Time + Light intensity	8	0.07	2928.16	75.34
Site + Time + Light colour	11	0.071	2933.15	46.79
Site + Time + Light intensity + Light colour	12	0.074	2932.96	48.49
Site + Time + Light intensity x Light colour	16	0.086	2931.65	56.91
Site x Time + Light intensity x Light colour	20	0.096	2932.37	59.91

Table 6.2: Result of the generalized linear models' comparison with straightness as response variable. The most parsimonious model is highlighted in grey.

Explanatory variables	Df	Nagelkerke's R^2	AIC	Performance score (%)
Null model		0	-1015.34	0.40
Site	6	0.003	-1009.536	0.81
Time	3	0.006	-1017.768	2.76
Site + Time	7	0.01	-1011.928	3.15
Site x Time	**11**	**0.105**	**-1074.111**	**94.63**
Site + Time + Light intensity	8	0.01	-1010.206	3.09
Site + Time + Light colour	11	0.02	-1011.433	6.73
Site + Time + Light intensity + Light colour	12	0.021	-1009.521	6.58
Site + Time + Light intensity x Light colour	16	0.031	-1008.128	9.67
Site x Time + Light intensity x Light colour	20	0.122	-1068.187	52.18

Discussion

Our results show that commuting pond bats did not respond to experimental light of realistic intensity installed on a bridge along their commuting route during a single evening. These results align with previous studies, which showed no response or little effect of artificial light on commuting behaviour of trawling bats (Kuijper et al., 2008; Spoelstra et al., 2018). On the other hand, other studies showed light-averse responses of *Myotis* species to artificial light in different situations, i.e. in foraging habitat (Rowse et al., 2018; Rydell, 1992; Spoelstra et al., 2017) or during roost emergence (Boldogh et al., 2007). As suggested by Hooker et al. (2022), *Myotis* species may be less light-sensitive when commuting compared to foraging, likely due to their shorter exposure to illumination. The absence of a reaction to our experimental light treatments may be because we used realistic light levels, i.e. in the range of commonly used street light intensities in the Netherlands. Nonetheless, there is anecdotal evidence of bats turning around when exposed to much brighter light levels (Kuijper et al., 2008 and personal observation when conducting a pilot study in summer 2020 in which bats were exposed to white light with a colour temperature of 3000 K at 180 lux at the water surface level). One could assume that the lighting setup on bridges can affect commuting pond bats from afar, outside of the microphone array's detection range. To test this hypothesis, we also monitored their commuting behaviour upstream (35 m to 1.6 km from the bridge, depending on the site) and we did not observe bats going back to the roost direction. Moreover, we could easily observe

pond bats flying towards the experimental setup from afar in the early evening when the ambient light was sufficient, and we did not observe any light-averse reaction to the lighting setup with the realistic light levels.

We additionally performed a power analysis, which reveals that based on our dataset, we have a likelihood 37% and 33% of detecting a true effect of light (interaction of spectrum and intensity) on flight speed and straightness, respectively. In order to obtain a statistical power of 80% as commonly accepted, the number of trajectories needs to be increased to almost double (1198 and 1277 instead of 698 trajectories for flight speed and straightness, respectively).

The difference in flight speed and straightness between sites can be explained by differences in bridges and waterways configuration. For instance, at Bakhuizen and Wergea, bats have to take a turn to follow the waterway about 200 m and 70 m before the bridge, respectively, therefore probably affecting their flight speed compared to a situation with a straight waterway. Previous studies also showed that flight speed differs across sites (Britton et al., 1997), probably because of the differences in acoustic landmarks perceived by pond bats (Van De Sijpe and Holsbeek, 2007). This can also be due to the distance from the roost and/or the foraging areas they need to reach, which varies between sites. Bats may also not respond to artificial light if their flight behaviour is limited by other parameters, for instance the energy cost or the risk of hitting an obstacle around the bridge.

Further research is needed to determine if artificial light impairs landscape connectivity for pond bats on the long term. Our experimental setup only allows us to study the short-term effects of artificial light, but it may create a barrier effect on the longer term and bats may abandon these routes or adapt their flight pattern differently. However, long-term monitoring is quite difficult to carry out and entails additional risks such as local population decline as a result of route abandonment. Acoustic tracking is a powerful tool to study fine-scale flight response of bats to artificial light, but its deployment and the manual check of trajectories are labour intensive. Pond bats may respond differently on their way back to the roost in the early morning. In the evening, they have a stronger urge to reach the foraging ground after fasting during daytime. On the way back, they are replete and might afford for example to do a detour to avoid lit areas.

In conclusion, this study shows no alteration of flight behaviour of pond bats along their commuting route in response to our experimental lighting setup, suggesting that realistic intensity levels do not pose a barrier effect of artificial light along waterways. However, we urge for caution to illuminate bridges as this study was conducted to quantify a short-term response, and it can be that a similar light installation on the long term will impair landscape connectivity. As bats generally depend on darkness, it is advisable that light spill onto the water surface is kept to minimum levels. Different spectra, albeit below threshold in our experiment,

may also have different impact. Further research on potential long-term effects is needed for good outdoor lighting design along bat commuting routes in order to facilitate bat movement across landscapes.

Acknowledgments

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Supplementary data to Chapter 6

Figure S6.1: Experimental configuration at the site in Balk with ambient light level along the waterway exceeding 5 lux at street level due to streetlights along the canal. The experimental lamppost and the microphone array were placed on the bridge in the background of the picture.
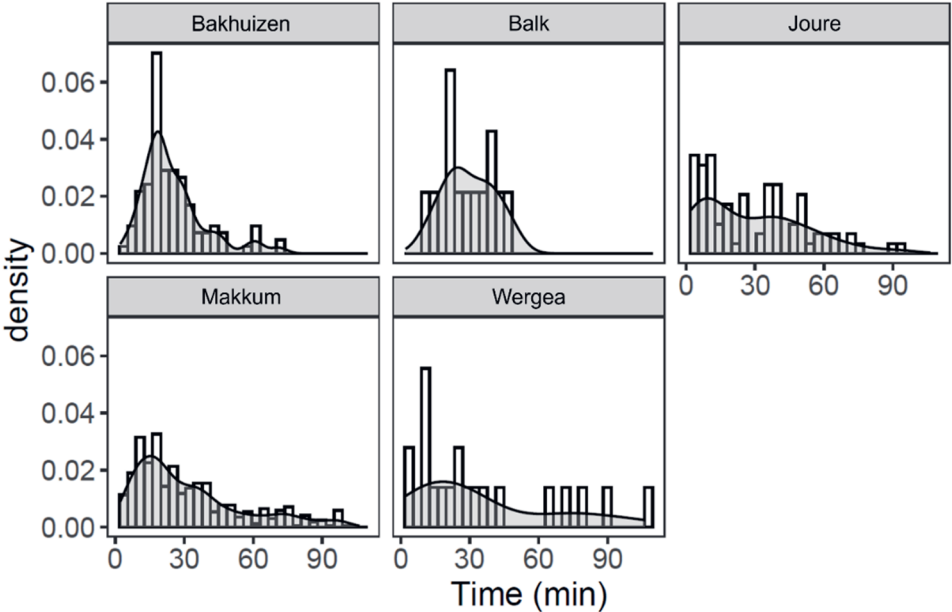
Figure S6.2: Distribution of the number of pond bats passing the bridge over time after the start of the lighting sequence (min) at each site.

Table S6.1: Number of trajectories and flight parameters (± standard deviation) per recording night at each site.

Site	Dates	Light intensity (lux)	Number of trajectories	Mean flight speed ± SD (m/s)	Straightness index ± SD
Bakhuizen	16 June 2021	5	61	7.87 ± 1.63	1.04 ± 0.05
	07 July 2021	20	54	7.46 ± 2.17	1.10 ± 0.47
Balk	20 June 2021	20	13	7.18 ± 1.39	1.06 ± 0.05
Joure	21 June 2021	20	35	6.51 ± 1.86	1.05 ± 0.05
	26 June 2021	5	46	7.27 ± 2.40	1.07 ± 0.07
Makkum	23 June 2021	20	246	7.96 ± 2.33	1.06 ± 0.09
	01 July 2021	5	223	7.33 ± 2.10	1.08 ± 0.12
Wergea	17 June 2021	5	8	10.43 ± 2.69	1.07 ± 0.10
	22 June 2021	20	12	8.94 ± 2.78	1.13 ± 0.34

Table S6.2: Number of trajectories and flight parameters (± standard deviation) per light treatment (combination of light spectrum and intensity).

Light spectrum	Light intensity (lux)	Number of trajectories	Mean flight speed ± SD (m/s)	Straightness index ± SD
Dark	0 (5)	62	7.85 ± 2.41	1.05 ± 0.05
	0 (20)	101	7.52 ± 2.22	1.05 ± 0.05
Amber	5	82	7.74 ± 2.04	1.07 ± 0.13
	20	74	8.03 ± 2.51	1.04 ± 0.04
Red	5	74	7.17 ± 1.84	1.07 ± 0.12
	20	57	7.61 ± 2.36	1.07 ± 0.15
White 2200 K	5	90	7.59 ± 2.23	1.06 ± 0.10
	20	79	7.97 ± 2.35	1.10 ± 0.39
White 3000 K	5	30	6.61 ± 1.93	1.10 ± 0.10
	20	49	7.59 ± 1.94	1.08 ± 0.17

Table S6.3: Estimates, standard errors and p-values of the effect of site and time after the start of the lighting sequence on flight speed of commuting pond bats (log-transformed).

Parameter	Estimate	Standard error	p-value
Site (Bakhuizen)	0.872	0.011	$p < 0.001$
Site (Balk)	0.849	0.032	$p < 0.001$
Site (Joure)	0.820	0.013	$p < 0.001$
Site (Makkum)	0.868	0.005	$p < 0.001$
Site (Wergea)	0.963	0.026	$p < 0.001$
Time (scaled)	-0.021	0.004	$p < 0.001$
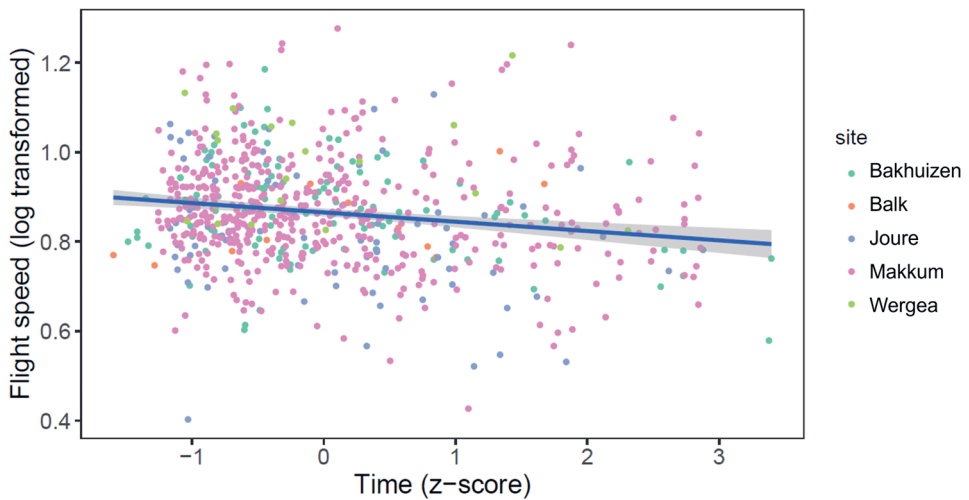
Figure S6.3: Relation between the time after the start of the lighting sequence (transformed into a z-score) and flight speed at each site.

Table S6.4: Estimates, standard errors and p-values of the effect of site and time after the start of the lighting sequence on flight straightness of commuting pond.

Parameter	Estimate	Standard error	p-value
Site (Bakhuizen)	0.918	0.028	p < 0.001
Site (Balk)	0.929	0.016	p < 0.001
Site (Joure)	1.042	0.025	p < 0.001
Site (Makkum)	1.069	0.011	p < 0.001
Site (Wergea)	1.157	0.051	p < 0.001
Time (scaled)	0.0001	0.00002	p < 0.001
Site (Balk) * Time	-0.00006	0.00006	0.325
Site (Joure) * Time	-0.00009	0.00002	p < 0.001
Site (Makkum) * Time	-0.0001	0.00002	p < 0.001
Site (Wergea) * Time	-0.0001	0.00002	p < 0.001
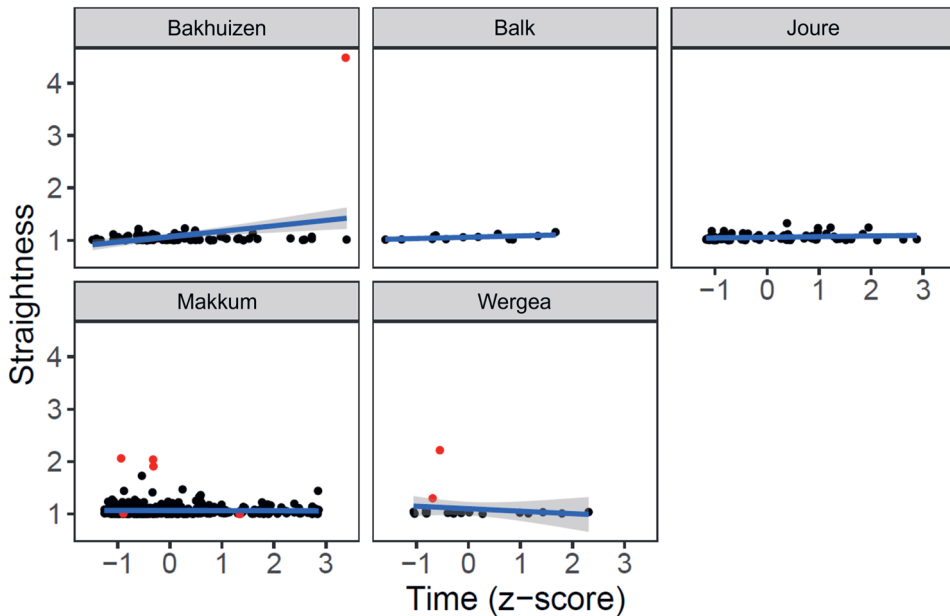
Figure S6.4: Relation between the time after the start of the lighting sequence (transformed into a z-score) and flight straightness at each site. Bats that turned around or passed over the bridge are shown in red.

Appendix S6.1: Echo suppression in pond bat calls

When recording echolocating bats flying close to a reflective surface, strong echoes overlap with the emitted call. With short frequency-modulated (FM) calls, the first arrival will always be the direct call and subsequent received signals that origin from the same call are echoes. However, once FM calls are less steep and slightly longer in duration (which pond bats regularly emit), the echoes overlap with the call impairing the ability to establish accurate arrival times and hence acoustic localisation of pond bats flying close to water.

By applying a number of post-processing steps to all recordings using a custom-built script in MATLAB2020a, we were able to localise the bats by masking the echoes.

These steps were performed on all recordings:

1) A bandpass filter (30-80 kHz) was applied to eliminate acoustic energy outside of the call range of pond bats.
2) A short artificially created FM-signal was cross correlated to the whole recording for pulse compression and time separation of calls and echoes.
3) A matching function was derived from the envelope of the cross-correlation.
4) A suppression function was created by applying a delayed leaky integrator on the matching function.
5) A masking function was created by nonlinear inverse exponential scaling of the suppression function.
6) The initial cross-correlation was scaled by multiplication of the initial cross-correlation with the masking function. This suppressed any potential signal for a short time (~ 0.5 ms) after a good pattern correlation with the original pulse and thus suppressed echoes, as well as occasional calls from conspecifics, shortly arriving after the initial call.
7) The output of the scaled cross-correlation function was convoluted back to represent an echo-suppressed recording of pond bat calls, thereby preventing the TOAD routine to include call echoes in the localisation of the original call (Figure S6.5).
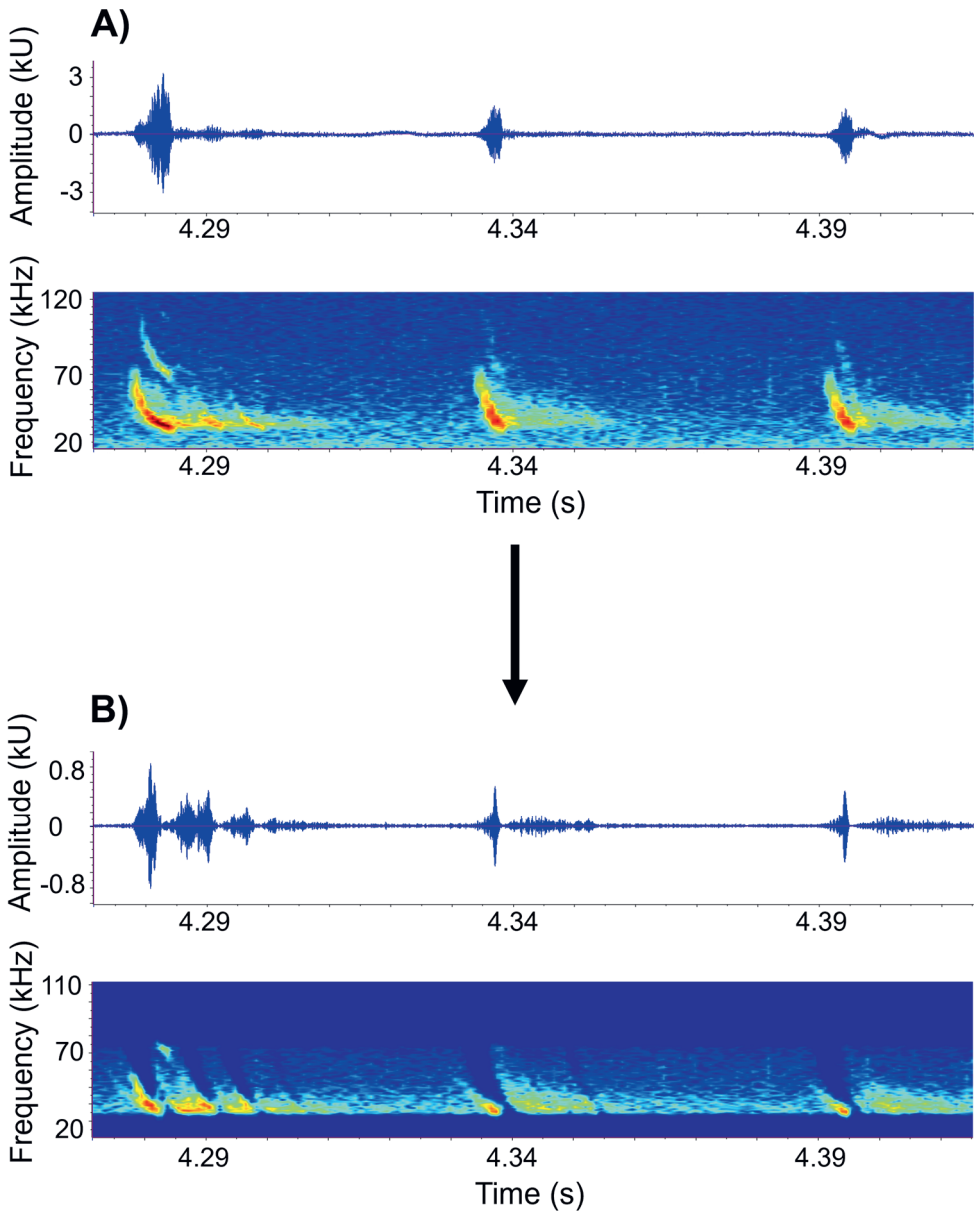
Figure S6.5: Echo suppression in echolocation calls of pond bats flying close to water. A) Oscillogram and spectrogram of a raw recording. B) Oscillogram and spectrogram of the echo-suppressed recording.

"Entendre, c'est écouter le temps qui passe."
Jérôme Sueur, "Histoire naturelle du silence"

Chapter 7

General discussion

Thesis summary and main findings

Artificial light is a major threat for ecological communities, especially for nocturnal species like bats. Our knowledge on how bats alter their fine-scale behaviour in lit environment is however limited (**Chapter 1**). In order to establish effective mitigation measures, for instance by keeping illumination intensity below the disturbance threshold, it is essential to know how the response of bats to light depends on light intensity. In this thesis, I investigated the spatiotemporal response of bats to light intensity under different light spectra with the intention of preventing negative impact of nightly illumination on bats, especially in term of habitat loss and habitat fragmentation.

To first understand how bats make use of their habitat, I analysed the bat-habitat relationship by combining LiDAR to characterize 3D vegetation structure and acoustic tracking to map bat behaviour (**Chapter 2**). This combined methodology paves the way for important but yet unanswered questions on bat habitat use. I applied this method at seven experimentally lit forest-edge sites to provide novel information on how the presence of artificial light and vegetation spatially affect foraging activity. These sites are part of the 'Light on Nature' project designed to assess the long-term effects of artificial light, especially light spectrum, on the ecosystem. In my thesis, I distinguished the response of synanthropic bats such as *Pipistrellus* species that are considered as light-opportunistic bats, and light-averse bats such as *Myotis* species.

In **Chapters 3 and 4**, I focused on the spatiotemporal response of synanthropic bats. I found that pipistrelles are mainly attracted to white light due to the high abundance of prey around the light source. Artificial light temporally affects insect abundance, therefore bats alter their temporal activity pattern to take advantage of concentrated prey resources (**Chapter 4**). However, spatial data revealed that pipistrelles stay out of bright light whilst still being attracted by insects to snatch these out of these bright areas (**Chapter 3**). Therefore, being exposed to light is a necessary evil for synanthropic pipistrelle bats as it enables them to catch insects, but it also exposes themselves to high light levels.

Secondly, I investigated the response of light-averse bats to artificial light in **Chapters 5 and 6**. I found that *Myotis* species avoid lit areas from afar where illuminance is below 0.1 lux, effectively excluding a significant amount of suitable foraging habitat at the 'Light on Nature' sites (**Chapter 5**). However, an additional experiment revealed that flight behaviour of pond bats (*Myotis dasycneme*) is unaffected by the presence of light of realistic intensity along waterways used as commuting routes (**Chapter 6**). These results suggest that *Myotis* species may have a context-specific tolerance threshold. In forest-edge habitat commonly used to forage, bats were exposed to light levels above their tolerance threshold

(**Chapter 5**), while the light levels were below the threshold in **Chapter 6**, as pond bats behaved normally while commuting.

The findings of this thesis thus provide novel insights into the habitat loss and fragmentation caused by artificial light for bats. Below, I will further discuss my main results on the spatiotemporal response to artificial light of synanthropic and light-averse bats in the context of habitat loss and fragmentation. Moreover, I will tackle some strengths and weaknesses of our methodology. Finally, I will consider the implications of the findings for policy and conservation and I will provide some recommendations for future research.

Bat habitat use

Vegetation structure is a key biotic factor that affects bat behaviour; their flight behaviour is shaped by habitat characteristics (Fenton, 1990; Neuweiler, 1989) and the structure of natural vegetation is a strong driver of echolocation call characteristics (Denzinger and Schnitzler, 2013; Fenton, 1990). We showed the potential of combining LiDAR to characterize 3D vegetation structure and acoustic tracking to map bat behaviour in order to provide new insights into the bat-relationship *in situ* with very fine resolution (**Chapter 2**). The detection of bats is limited to 15-20 m around the microphone array, but the acoustic data provide very detailed information on how bats adjust their small-scale spatial behaviour to vegetation structure. These data are very valuable to understand their species-habitat relationship in forest environment (Adams et al., 2009; Froidevaux et al., 2016; Schnitzler and Kalko, 2001). For other purposes, for instance studying the effect of habitat composition on the ecology of bats at the landscape level, I would recommend to combine other methods, such as GPS loggers with aerial laser scanner (ALS) (Roeleke et al., 2018b).

Our results show that bats keep their distance to the vegetation, especially pipistrelles (Figure 2.6 and 2.7). This confirms that pipistrelles avoid clutter spaces as their echolocation calls are less adapted to detect insects in such environment (Kalko and Schnitzler, 1993). There is also a tremendous intraspecific variety in echolocation signals when flying in different environments (Kalko and Schnitzler, 1993; Siemers and Schnitzler, 2000). The acoustic transition zone between open-space and edge-space calls could be studied in great details if one knows the distance that bats keep to the vegetation, but this would require the bats to fly towards the microphone array due to the high directionality of their echolocation calls.

Moreover, the example provided in Figure 2.5 is one of the few pieces of evidence of stereotyped flight paths of pipistrelles in their natural habitat. Few studies have previously showed similar flight patterns in laboratory conditions (Fujioka et al., 2014) or in a more open natural environment (Barchi et al., 2013; Hulgard et al.,

2016). Here we show that bats are able to fly in more cluttered environment with obstacles such as tree trunks. Bats probably make use of their spatial memory to reduce their sensory load for navigation and ameliorate their foraging efficiency by flying in very specific flight patterns (Barchi et al., 2013).

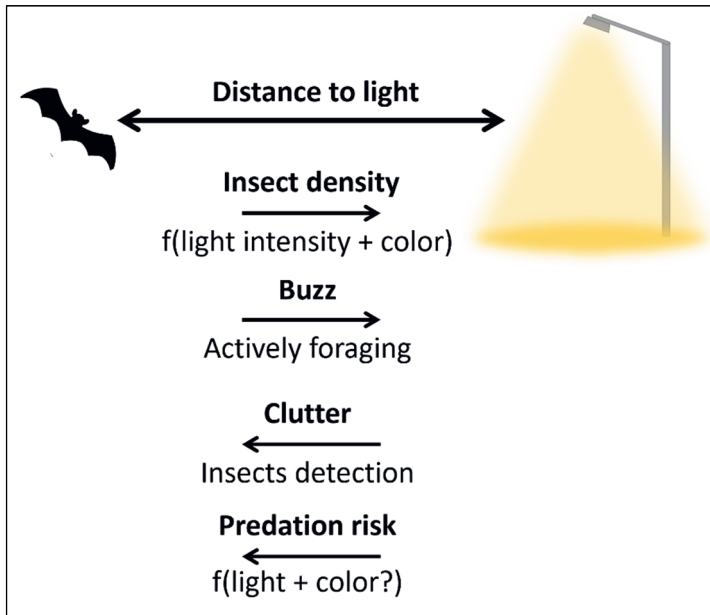## Response of synanthropic bats to anthropogenic light

It is common knowledge that pipistrelles are light-opportunistic, as they frequently forage around streetlights (Bolliger et al., 2020b; Russo et al., 2019; Spoelstra et al., 2017). However, their fine-scale behaviour around those lights has not been investigated yet. The vegetation structure around those light sources is also an important factor that influences bat behaviour. While vegetation may impede prey capture success (Arlettaz et al., 2001; Rainho et al., 2010), flying close to the vegetation may provide a potential benefit of extra safety against predators. Therefore, bats face a trade-off between predation risk and food reward.

Our results show that pipistrelles fly closer to light sources than open-space foragers such as noctules and serotines (**Chapter 2**, Figure 2.8B). The positive phototactic response is also stronger under white light than red light, which is consistent with previous research at the 'Light on Nature' sites showing that pipistrelles are more active under white light than red light and the control transects (Spoelstra et al., 2017). However, pipistrelles fly closer to the light source mostly to snatch insects out of the bright areas but stay further away otherwise (**Chapter 3**, Figure 3.3). In **Chapter 4**, we showed a positive correlation between the insect abundance collected with sticky sheets and camera traps at white and red light but not at the control treatment. This suggests that artificial light alters the insects' spatial distribution with more insects getting closer to the lampposts. Pipistrelles then shift their spatial behaviour as well to take advantage of concentrated prey resources around the light source. However, pipistrelles avoid exposing themselves to high light levels, especially if the vegetation is too dense to detect the insects flying around the light source (**Chapter 3**). Pipistrelles are indeed less adapted to forage in clutter environment and therefore cannot take advantage of concentrated prey resources around the light if the vegetation is too dense. Therefore, being exposed to light is a necessary evil for synanthropic pipistrelle bats to take advantage of concentrated prey resources around the light source, but vegetation also plays a role in this conundrum.

In order to fully exploit prey resources around streetlights, bats need not only to follow their prey's spatial pattern, but also their temporal activity pattern. As insects shift their activity within the night in lit conditions, we also observed a temporal shift in pipistrelles' activity. Insects and bats are more active throughout the night near white light, but these effects are attenuated near red light (**Chapter**

4, Figure 4.4). Therefore, our results indicate that synanthropic bats are not attracted by light *per se* but by the insects that are themselves attracted to light. This is consistent with previous studies corroborating the 'attraction-by-insects' hypothesis instead of the 'attraction-by-artificial-light' hypothesis (Rowse et al., 2018; Spoelstra et al., 2017). Light sources offer predictable foraging locations with higher insect densities during longer periods throughout the night. The ability to forage throughout the night may give a key advantage to synanthropic bats to dominate in lit environments. This chapter also highlights the relevance of recording insects and bats activity simultaneously, as bats follow the temporal pattern of their prey.

Artificial light alters natural activity patterns of diurnal, nocturnal and crepuscular animals, both spatially and temporally (De Jong et al., 2016; Evens et al., 2023; Jägerbrand and Spoelstra, 2023; Mariton et al., 2022; Sanders et al., 2023; Shier et al., 2020). As a result, food webs are being reshaped by changing the communities' composition in different trophic levels (Giavi et al., 2021), in this case insects and bats (Grubisic and van Grunsven, 2021; Lacoeuilhe et al., 2014). Some bat species take advantage of accumulated insects in lit areas, other do not (Cravens and Boyles, 2019). For pipistrelles, the habitat quality, which can be defined as "the ability of the environment to provide conditions appropriate for individual and population persistence" (Hall et al., 1997), would increase with the presence of anthropogenic light as it increases food availability. However, a study experimentally showed the contribution of artificial light to the decline of local population of moths over years (van Grunsven et al., 2020). Even though lights concentrate insects and facilitate foraging in bats, the total food availability in an area decreases in the long run. The habitat quality is also influenced by the configuration of vegetation or the predation pressure that may result from more conspicuousness in lit environment (Figure 7.1). It should be noted that predation risk is notoriously difficult to estimate for bats, even though it is a commonly assumed hypothesis to explain light avoidance in bats (Duvergé et al., 2000; Lesiński et al., 2009; Rosina and Shokhrin, 2011; Speakman, 1991a). Other theories have been raised to explain nocturnality in bats, for instance bats could suffer from hyperthermia in daytime, or they face less competition at night (Rydell and Speakman, 1995). However, the fact that even synanthropic bats stay away from high light levels except to catch insects (**Chapter 3**) supports the predation hypothesis. On the other hand, altering their spatiotemporal pattern could also expose them to new predators (Tougeron and Sanders, 2023), as visual predators also adjust their foraging behaviour and space use in response to artificial light (Bolton et al., 2017; Miller et al., 2017; Rodríguez et al., 2021).

Figure 7.1: Graphic summary of factors influencing the distance that synanthropic bats keep to anthropogenic light sources, based on the results of Chapters 3 and 4.

Synanthropic bats take advantage of accumulated prey resources around light sources, which gives them a selective advantage to dominate in lit environment, both spatially and temporally. This may lead to changes in bat communities which would result in 'winner-loser species replacements', where disturbance-tolerant species like light-opportunistic bats proliferate, while disturbance-sensitive species such as light-averse bats are disadvantaged and decline (Arlettaz et al., 2000; Filgueiras et al., 2021). The same paradigm may occur for bats' prey. Urban moths exposed to light pollution for many generations can show less flight-to-light behaviour than individuals from dark-sky populations, increasing their survival (Altermatt and Ebert, 2016). Smaller moth species and indivuals with reduced mobility due to smaller wings are also less attracted to light (Van de Schoot et al., 2024; van Langevelde et al., 2011). Besides, moths display less antipredator behaviours when exposed to light (Hügel and Goerlitz, 2020; Wakefield et al., 2015), adding to the accessibility of insects as prey for bats. Catching larger and more vulnerable prey around light sources would be energetically beneficial for bats, but this could also results in morphometric adaptation in bats (Tomassini et al., 2014). This could also lead to changes in diet composition for light-averse ones, as artificial light alters prey species richness and abundance (van Langevelde et al., 2011). It is challenging to determine if the changes in spatiotemporal behaviour of insects and bats will translate into fitness effects. According to our results, pipistrelles may get a direct benefit of concentrated prey

resources around light sources. However, an overall insect decline in the surroundings of light posts due to anthropogenic light (van Grunsven et al., 2020) may reduce their fitness on the long-term. Light may be a driver of the population decline of *Pipistrellus pipistrellus* observed in France in the last decades (Bas et al., 2020; Kerbiriou et al., 2015). Indeed, a negative association of its distribution with lighting has been shown on a landscape scale (Laforge et al., 2019; Mathews et al., 2015). In case light-averse bats are able to find sufficient prey insect species that are unaffected by light, whilst light-attracted insect populations decline and are hence not available to light-tolerant bats, 'winner and loser replacement' paradigm will tip in favour of the light-shy bats.

To conclude, our results do not suggest that artificial light causes habitat loss for pipistrelles. However, as they avoid exposing themselves to high light levels, it is advisable that illuminance is kept to minimum levels. Reducing light pollution would also reduce the alteration of natural niche segregation. Future species assemblages will certainly differ from current ones, but not necessarily only because of artificial light. Artificial light may be a factor that exacerbates the effect of factors like climate change (Russo et al., 2024; Tomassini et al., 2014), but not the main factor *per se* of the 'winner and loser replacement' in bat communities.

Response of light-averse bats to anthropogenic light

In contrast to synanthropic bats, anthropogenic light drastically reduces habitat availability for light-averse bats as these stay away from lit foraging areas. Our results indicate that *Myotis* species, which are commonly considered as light-averse, avoid lit areas from afar where illuminance is below 0.1 lux, both for red and white light. In the work reported in **Chapter 5**, we took efforts to establish a dose-response curve of bat activity depending on light intensity, but the disturbance threshold was apparently at such low light levels (below 0.1 lux) that these fell outside of the microphone array detection range. Maximum illuminance during full moon only reaches 0.3 lux in extreme cases (Kyba et al., 2017b), but it elicits lunar phobia and change in bat activity, especially in tropical bat species (Appel et al., 2017; Ciechanowski et al., 2007; Saldaña-Vázquez and Munguía-Rosas, 2013; Vásquez et al., 2020). It is therefore not surprising that even low light levels of anthropogenic light can affect *Myotis* species (Seewagen et al., 2023).

In order to estimate habitat loss, it is important to take into account the lamppost specifications (fixture, lamp type, shielding, etc). For example, if we consider a grid of 25 m by 25 m (for the sake of comparison with **Chapter 5**) around the lampposts at the 'Light on Nature' sites that are 4 m high, the illuminance is above 0.1 lux for the entire surface at ground level under white light, while only 582.8 m^2 out of 625 m^2 are illuminated above 0.1 lux under red light. In terms of volume, this represents 2584.9 m^3 and 1470.4 m^3 under white and red light respectively,

which is equivalent to 98.5% and 56% of the volume around the lampposts. Moreover, *Myotis* species never forage on insects trapped in the brighter illuminated space close to the lampposts, completely avoiding the areas with light levels above 20 lux or 40 lux under white and red light conditions, respectively (**Chapter 5**). Therefore, this suggests that red light is less harmful than white light, even though both light spectra impede bat activity in a significant amount of otherwise suitable foraging habitat. Unlit areas are lacking food due to this vacuum effect, by insects being attracted to the lit areas (Eisenbeis, 2006; van Grunsven et al., 2020). Thus, the habitat quality of the remaining dark patches is also reduced for light-averse species. If one needs to estimate the habitat loss at a larger scale, this requires large-scale data such as satellite data (Ditmer et al., 2021). However, these data only show the light reflected upward and are not representative of the light levels that bats or other organisms are actually exposed to.

One might question whether their light avoidance is directly due to the effect of light or caused by interspecific competition. Do 'light-averse' bats forage elsewhere because of synanthropic bats that dominate in lit environment? Previous studies suggested that open-space foragers alter their spatiotemporal activity to avoid intra- and interspecific competition for food throughout summer (Roeleke et al., 2018a). Thus, light-averse bats may select different spatiotemporal ecological niches than synanthropic bats to avoid interspecific competition in lit areas and exploit more distant but less rich foraging patches (Arlettaz et al., 2000; Cravens and Boyles, 2019; Salinas-Ramos et al., 2021).

To reach the foraging patches, bats need to commute from their roosting site to the foraging area. Linear structures are essential in terms of landscape connectivity for bats. Waterways are particularly important for trawling bats like the pond bat (*Myotis dasycneme*) and the Daubenton's bat (*Myotis daubentonii*) that commonly hunt insects low over water (Britton et al., 1997; Haarsma and Siepel, 2014). Illumination along these could create a barrier effect and induce habitat fragmentation. In **Chapter 6**, we exposed pond bats to four light spectra at different light intensities using an experimental lighting setup placed on bridges crossing their commuting routes and we studied their flight response via acoustic localisation. Both flight speed and straightness were unaffected by the presence of light of realistic intensity, regardless of the light spectrum. These results align with previous studies, which showed no response or little effect of artificial light on commuting behaviour of trawling bats (Kuijper et al., 2008; Spoelstra et al., 2018). As suggested by Hooker et al. (2022), *Myotis* species may be less light-sensitive when commuting compared to foraging, likely due to the shorter exposure to illumination. The absence of a reaction to our experimental light treatments may be because we used realistic light levels, that do not pose a barrier effect along waterways. Nonetheless, there is anecdotal evidence of bats turning around when exposed to much brighter light levels (Kuijper et al., 2008).

We also conducted a pilot study in summer 2020 in Makkum, one of the sites used for the experiment in **Chapter 6**. During this trial, pond bats were exposed to 180 lux for white light at 3000 K and 70 lux for red light at the water surface level and we observed bats turning around when exposed to white light. Thus, we urge for caution to illuminate bridges to prevent a barrier effect, not only on pond bats, but also on aquatic species that would also be exposed (Vega et al., 2023).

Moreover, as this study was conducted to quantify a short-term response only, it can be that a similar light installation on the long-term will impair landscape connectivity. The study presented in **Chapter 5** took place at experimental sites that are illuminated since 2012, while commuting pond bats were "naive" to the lighting setup in **Chapter 6**. Artificial light might have different effects or consequences, depending on the context and the spatiotemporal level of interest in the study. Effects of light may only surface after prolonged exposure to artificial light. Therefore, we recommend long-term monitoring along commuting routes, however we are aware that this is quite difficult to carry out and it entails additional risks such as route abandonment.

To conclude, we tried to establish a dose-response curve of bat activity depending on light intensity in free flying *Myotis* species. However, the disturbance threshold is so low for these light-averse bats in foraging habitat that it is hard to assess. As *Myotis* species avoid lit areas from afar where illuminance is below 0.1 lux, this effectively excludes a significant amount of suitable foraging habitat. Our experiment along commuting routes did not show an effect of artificial light on the flight behaviour of pond bats (**Chapter 6**), but we cannot exclude that artificial light creates a barrier effect as well in the light of **Chapter 5**.

Some notes on the methodology

The combination of LiDAR and acoustic localisation provides much more detailed information on how bats adjust their small-scale spatial behaviour to vegetation structure than other methods, as described in **Chapter 2**. Thus, it appeared to be the best choice to assess bat response to artificial light and study how it interacts with their immediate surroundings. However, the main drawback of acoustic localisation, as any acoustic monitoring method, is that we cannot estimate how many individuals were flying around the microphone array throughout the night. However, two individuals of the same species flying at the same time could be identified based on their derived 3D-positions (Figure 2.4). During our experiment in Friesland, at least two observers were continuously present on site and they manually counted bats passing and observed their behaviour to note any unusual behaviour or bats turning around before reaching the bridge. However, this laborious work could not be carried out in a long-term monitoring as we did at the 'Light on Nature' sites.

Moreover, despite the fact that acoustic localisation provides very high temporal resolution data, it is quite challenging to accurately localise calls during the feeding buzz phase, as the pulse intervals are very short and the call amplitude is much lower than search calls (Holderied et al., 2005). We only made a distinction between call sequences in which bats were searching for insects (absence of feeding buzz) and call sequences in which bats attempted to catch a prey (presence of a feeding buzz) (**Chapter 3**). Knowing exactly where bats catch an insect in the light beam would provide better information on the precise light levels to which pipistrelles expose themselves to get food. However, as pipistrelles stop emitting signals 3.5 to 10 cm from their prey (Kalko, 1995), one could not determine the exact position of capture based on acoustic localisation.

This thesis also proved the difficulties to quantify habitat loss in free-flying bats, and especially to establish dose-response curve of bat activity depending on light intensity. It is easier to control for light intensity in laboratory conditions but here we can see that bats avoid lit areas from far away in natural conditions. This is particularly the case for light-averse *Myotis* species, for which the disturbance threshold must be below 0.1 lux (**Chapter 5**, Azam et al., 2018; Seewagen et al., 2023). One could tag bats with GPS loggers to obtain space use data on a larger scale but with lower spatial resolution. A better solution would be to place microphone arrays along a gradient from the lamppost to areas of near darkness to obtain fine-scale and continuous flight data along this gradient. This method would provide very detailed data but would be very laborious, both in the field and for data processing.

In **Chapters 3 and 5**, I used two different approaches to investigate the effect of light intensity on bats, using either the distance of bats to the light source as a proxy (**Chapter 3**) or the illuminance *per se* (**Chapter 5**). The illuminance (in lux) is a metric weighted by the human spectral sensitivity, and hence biased by human vision, which does not represent light perception in bats. Nonetheless, it is an appropriate unit to convey scientific results to the lighting industry that formally uses this unit for outdoor lighting. As previously mentioned, we cannot precisely localise echolocation calls during the feeding buzz phase, therefore we cannot precisely estimate the light levels that bats encounter while catching an insect. We also only took into account positions of pipistrelles below the light source where light is actually emitted. These are the reasons why the distance seemed to us a better proxy in **Chapter 3**. On the other hand, we also included *Myotis* positions above the lampposts in **Chapter 5**, as these bats were generally flying higher where light levels are close to zero. In this case, the distance to the light source appeared to be less relevant than the illuminance.

Suggestions for policy and conservation

All European species are legally protected (Council of the European Communities, 1992), but there are still many anthropogenic disturbances that could be avoided or at least diminished, such as the continuous increase of artificial light at night. Some of the findings in this thesis can be used as recommendations for policy and conservation. One of the main and important results is the disturbance threshold lower than 0.1 lux for light-averse bats like *Myotis* species, irrespective of the light spectrum (**Chapter 5**). We hence highly recommend keeping key habitats for light-averse bat species as dark as possible and reducing light trespass into areas that are unintended to be lit in order to limit habitat loss caused by artificial light for light-sensitive bats. However, this is hardly reachable near cities, as artificial light is amplified by skyglow, which is the light reflection in the atmosphere. Skyglow therefore exposes adjacent lands by increasing light levels around urbanised areas (Ditmer et al., 2021; Secondi et al., 2017). This results in drastic fragmentation of numerous mammal ranges, including in bats, and isolation of dark refuges (Ditmer et al., 2021). In order to reduce light emissions in unwanted directions and thus reduce skyglow, we recommend to adapt lampposts' height and use effective shielding fixtures.

Spectral composition can also be used as a mitigation measure. Even though they respond to light from far away, *Myotis* bats dare to fly under higher light levels of red light than white light (**Chapter 5**). The effect of artificial light on the temporal pattern of insect abundance and bat activity is also attenuated under red light (**Chapter 4**). This is one of the few pieces of evidence that the spectral composition of artificial light at night plays a role in the disturbance of temporal activity patterns of organisms (De Jong et al., 2017). However, we did not find responses in flight behaviour of commuting pond bats when illuminating their flying route with various light spectra at realistic intensity (**Chapter 6**). In this study, we also included amber light, as this light spectrum is commonly considered as eco- and bat-friendly, despite very few and anecdotal evidence. Different spectra, albeit below threshold in our experiment, may also have different impact, but we cannot conclude that based on our short-term experiment. Adapting the light spectrum should also be carried out cautiously as this can create an ecological trap (Durmus et al., 2024; Jägerbrand and Spoelstra, 2023). Spectral sensitivity of bats is higher in the blue part of the spectrum; therefore, they sense less red or amber light than white light. Using longer wavelength light potentially increases their vulnerability to visually-oriented predators, which, in turn, have a good visual acuity in this part of the light spectrum.

In the framework of my thesis, I only focus my research on the effect of anthropogenic light *per se*, and more specifically on light intensity under different light spectra. This is why a large part of the research took place at the 'Light on Nature' site, to avoid other confounding factors. However, bats may respond

differently in more urbanised landscapes, where other factors such as noise pollution also interfere with light pollution (Halfwerk and Jerem, 2021). Our findings show that artificial light provides predictable food resources in time and space for synanthropic bats (**Chapters 3 and 4**). In urban settings, insects are less numerous and distributed over greater distances, providing less food for bats (Stidsholt et al., 2024). Studying the response of bats to artificial light along a gradient from rural to urban habitat would help to disentangle the effect of light and urbanisation in order to adapt outdoor lighting according to the habitat composition.

There is an urgent need from the society and lighting producers to establish clear light thresholds for conservation policies. Illuminance is used to define environmental zones that specify the level of protection of the area of interest (Institution of Lighting Professionals, 2021). Knowing the species-specific disturbance threshold could be used in terms of practical solutions by municipalities and environmental consultancy. For instance, *Myotis* species are less present in urban than rural settings, therefore reducing light levels in the latter is especially of importance to preserve important areas for these bat species. In the future, a collaborative approach is needed between stakeholders such as local institutions, lighting designers and scientists to minimise the impact of artificial light on biodiversity. As we have seen with the rapid development and use of LEDs in outdoor lighting, new lighting technologies may swiftly arise. Scientists must study these innovations and investigate their potential impacts before they are installed.

Recommendations for future research

Based on the combined methodology described in **Chapter 2**, this thesis opens up the possibility to address open and novel questions on fine-scale bat behaviour. For instance, the transition zone between open- and edge-space calls has been studied with limited spatial resolution so far (Kalko and Schnitzler, 1993; Schaub and Schnitzler, 2007). With our methodology, plasticity in echolocation signals can be investigated at a fine-scale spatial resolution. We also showed stereotypic flight patterns in pipistrelles (**Chapter 2**), which is evidence of spatial memory that potentially reduces their sensory load for navigation and ameliorates their foraging efficiency. Their spatial memory could be studied by placing, for example, new obstacles along their usual flight path and by observing how long it would take them to adapt their flight path to stereotypic patterns again.

Long-term studies are essential to provide novel insights into evolutionary consequences of artificial light. So far, we have little perspective on how species adapt to artificial light as light pollution only started to raise more attention in the last two decades (Rodrigo-Comino et al., 2023). For example, moths coming from populations exposed to long-term urban light pollution are less attracted to light

than moths from dark-sky populations, which may directly reduce their mortality risk and therefore act as an agent of selection (Altermatt and Ebert, 2016). The 'Light on Nature' sites are a unique set up to study the effect of different spectra on the forest-edge ecosystem without any other confounding factors such as noise pollution. This experiment is one of the few long-term experiments set up to assess ecosystem-wide consequences of light pollution (Bennie et al., 2015; Hölker et al., 2015; Knop et al., 2017). Additional long-term studies are necessary to better comprehend ecological and evolutionary consequences of light pollution. This is especially the case for understudied ecosystems such as aquatic habitats where organisms are also exposed to artificial light (Secondi et al., 2017).

Our short-term experiment along the commuting routes of pond bats indicates that their flight behaviour is unaffected by the presence of light of realistic intensity, regardless of the light spectrum (**Chapter 6**). Further research on potential long-term effect of artificial light on commuting bats is needed for good outdoor lighting design along commuting routes in order to facilitate bat movement across the landscape. Pipistrelles are also known to commute along linear structures such as treelines that provide wind protection (Verboom and Huitema, 2010; Verboom and Spoelstra, 1999). Despite the fact they are light-opportunistic (**Chapters 3 and 4**), synanthropic bats seem to use the darkest spaces while commuting and gap crossing (M. Boonman, pers. comm., Hale et al., 2015). For that reason, they might show opposite reaction to artificial light in this situation, or no response as pond bats as shown in **Chapter 6**.

Another mitigation measure to overcome the effects of outdoor lighting on the ecosystems is to limit the lighting duration by implementing novel lighting concepts such as adaptive (traffic-dependent) and restricted lighting (predetermined nightly schedule) (Gaston et al., 2012). Pipistrelles, as well as their prey, show a direct response when lights are switched off at the 'Light on Nature' site for a single night, with the disappearance of the effects of light treatment on their activity (Spoelstra et al., 2017). Using acoustic localisation when turning off the lights would also give more information on how part-time lighting affects their spatial behaviour. Moreover, LEDs at the 'Light on Nature' sites will be replaced in the near future to implement adaptive or restricted lighting for future research.

7

Conclusions

The overall aim of this thesis was to investigate the spatiotemporal response of bats to light intensity under different light spectra in order to prevent habitat loss and habitat fragmentation caused by anthropogenic light. Through my thesis, I showed that technological innovation allows us to shed light on bat ecology, even though many aspects of these fascinating creatures remain obscure. It is evident that bat response varies according to the bat guild (light-averse vs light-opportunistic bats) and the context (foraging vs commuting behaviour). While synanthropic bats take advantage of concentrated prey resources in lit areas, light-averse species suffers from great habitat loss caused by artificial light. Besides, the quality of their remaining foraging patches is reduced, as food resources are depleted in unlit areas. As negative effect of illuminance on bat activity is already detected below 0.1 lux for light-averse species, we recommend keeping key habitats as dark as possible and reducing light trespass into areas that are unintended to be lit in order to limit habitat loss caused by artificial light for light-sensitive bats.

References

Adams, M.D., Law, B.S., French, K.O., 2009. Vegetation structure influences the vertical stratification of open- and edge-space aerial-foraging bats in harvested forests. Forest Ecology and Management 258, 2090–2100. https://doi.org/10.1016/j.foreco.2009.08.002

Altermatt, F., Ebert, D., 2016. Reduced flight-to-light behaviour of moth populations exposed to long-term urban light pollution. Biology Letters 12, 20160111. https://doi.org/10.1098/rsbl.2016.0111

Appel, G., López-Baucells, A., Magnusson, W.E., Bobrowiec, P.E.D., 2017. Aerial insectivorous bat activity in relation to moonlight intensity. Mammalian Biology 85, 37–46. https://doi.org/10.1016/j.mambio.2016.11.005

Arlettaz, R., Godat, S., Meyer, H., 2000. Competition for food by expanding pipistrelle bat populations (*Pipistrellus pipistrellus*) might contribute to the decline of lesser horseshoe bats (*Rhinolophus hipposideros*). Biological Conservation 93, 55–60. https://doi.org/10.1016/S0006-3207(99)00112-3

Arlettaz, R., Jones, G., Racey, P.A., 2001. Effect of acoustic clutter on prey detection by bats. Nature 414, 742–745. https://doi.org/10.1038/414742a

Aschoff, T., Holderied, M., Marckmann, U., Runkel, V., 2006. Forstliche Maßnahmen zur Verbesserung von Jagdlebensräumen von Fledermäusen (No. 22437), Abschlussbericht für die Vorlage bei der Deutschen Bundesstiftung Umwelt.

Austrian Energy Agency, 2017. LED Street Lighting Procurement & Design Guidelines (No. Ares(2017)587406). European Union.

Avila-Flores, R., Fenton, M.B., 2005. Use of spatial features by foraging insectivorous bats in a large urban landscape. Journal of Mammalogy 86, 1193–1204. https://doi.org/10.1644/04-MAMM-A-085R1.1

Azam, C., Kerbiriou, C., Vernet, A., Julien, J.-F., Bas, Y., Plichard, L., Maratrat, J., Le Viol, I., 2015. Is part-night lighting an effective measure to limit the impacts of artificial lighting on bats? Global Change Biology 21, 4333–4341. https://doi.org/10.1111/gcb.13036

Azam, C., Le Viol, I., Bas, Y., Zissis, G., Vernet, A., Julien, J.-F., Kerbiriou, C., 2018. Evidence for distance and illuminance thresholds in the effects of artificial lighting on bat activity. Landscape and Urban Planning 175, 123–135. https://doi.org/10.1016/j.landurbplan.2018.02.011

Barchi, J.R., Knowles, J.M., Simmons, J.A., 2013. Spatial memory and stereotypy of flight paths by big brown bats in cluttered surroundings. The Journal of Experimental Biology 216, 1053–1063. https://doi.org/10.1242/jeb.073197

Barré, K., Baudouin, A., Froidevaux, J.S.P., Chartendrault, V., Kerbiriou, C., 2023a. Insectivorous bats alter their flight and feeding behaviour at ground-mounted solar farms. Journal of Applied Ecology 61, 328–339. https://doi.org/10.1111/1365-2664.14555

Barré, K., Kerbiriou, C., Ing, R.-K., Bas, Y., Azam, C., Le Viol, I., Spoelstra, K., 2021. Bats seek refuge in cluttered environment when exposed to white and red lights at night. Movement Ecology 9, 3. https://doi.org/10.1186/s40462-020-00238-2

Barré, K., Spoelstra, K., Bas, Y., Challe, S., Lapostolle, D., Kerbiriou, C., 2020. Artificial light may change flight patterns of bats near bridges along urban waterways. Animal Conservation 24, 259–267. https://doi.org/10.1111/acv.12635

Barré, K., Thomas, I., Le Viol, I., Spoelstra, K., Kerbiriou, C., 2023b. Manipulating spectra of artificial light affects movement patterns of bats along ecological corridors. Animal Conservation 26, 865–875. https://doi.org/10.1111/acv.12875

Bas, Y., Bas, D., Julien, J.-F., 2017. Tadarida: A Toolbox for Animal Detection on Acoustic Recordings. Journal of Open Research Software 5, 6. https://doi.org/10.5334/jors.154

Bas, Y., Kerbiriou, C., Roemer, C., Julien, J.-F., 2020. Bat population trends. Muséum national d'Histoire naturelle. [WWW Document]. URL https://croemer3.wixsite.com/teamchiro/population-trends (accessed 2.20.24).

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67, 1–48. https://doi.org/10.18637/jss.v067.i01

Beilke, E.A., Blakey, R.V., O'Keefe, J.M., 2021. Bats partition activity in space and time in a large, heterogeneous landscape. Ecology and Evolution 11, 6513–6526. https://doi.org/10.1002/ece3.7504

Bennie, J., Davies, T.W., Cruse, D., Inger, R., Gaston, K.J., 2015. Cascading effects of artificial light at night: resource-mediated control of herbivores in a grassland ecosystem. Philosophical Transactions of the Royal Society B: Biological Sciences 370, 20140131. https://doi.org/10.1098/rstb.2014.0131

Bhardwaj, M., Soanes, K., Lahoz-Monfort, J.J., Lumsden, L.F., van der Ree, R., 2020. Artificial lighting reduces the effectiveness of wildlife-crossing structures for insectivorous bats. Journal of Environmental Management 262, 110313. https://doi.org/10.1016/j.jenvman.2020.110313

Blakey, R.V., Law, B.S., Kingsford, R.T., Stoklosa, J., 2017. Terrestrial laser scanning reveals below-canopy bat trait relationships with forest structure. Remote Sensing of Environment 198, 40–51. https://doi.org/10.1016/j.rse.2017.05.038

Bliss-Ketchum, L.L., De Rivera, C.E., Turner, B.C., Weisbaum, D.M., 2016. The effect of artificial light on wildlife use of a passage structure. Biological Conservation 199, 25–28. https://doi.org/10.1016/j.biocon.2016.04.025

Boldogh, S., Dobrosi, D., Samu, P., 2007. The effects of the illumination of buildings on house-dwelling bats and its conservation consequences. Acta Chiropterologica 9, 527–534. https://doi.org/10.3161/1733-5329(2007)9[527:TEOTIO]2.0.CO;2

Bolliger, J., Collet, M., Hohl, M., Obrist, M.K., 2020a. Automated flight-interception traps for interval sampling of insects. PLoS ONE 15, e0229476. https://doi.org/10.1371/journal.pone.0229476

Bolliger, J., Haller, J., Wermelinger, B., Blum, S., Obrist, M.K., 2022. Contrasting impacts of street light shapes and LED color temperatures on nocturnal insects and bats. Basic and Applied Ecology 64, 1–12. https://doi.org/10.1016/j.baae.2022.07.002

Bolliger, J., Hennet, T., Wermelinger, B., Bösch, R., Pazur, R., Blum, S., Haller, J., Obrist, M.K., 2020b. Effects of traffic-regulated street lighting on nocturnal insect abundance and bat activity. Basic and Applied Ecology 47, 44–56. https://doi.org/10.1016/j.baae.2020.06.003

Bolton, D., Mayer-Pinto, M., Clark, G.F., Dafforn, K.A., Brassil, W.A., Becker, A., Johnston, E.L., 2017. Coastal urban lighting has ecological consequences for multiple trophic levels under the sea. Science of The Total Environment 576, 1–9. https://doi.org/10.1016/j.scitotenv.2016.10.037

Boonman, A., Jones, G., 2002. Intensity control during target approach in echolocating bats; stereotypical sensori-motor behaviour in Daubenton's bats, *Myotis daubentonii*. The Journal of Experimental Biology 205, 2865–2874.

Brigham, R.M., Grindal, S.D., Firman, M.C., Morissette, J.L., 1997. The influence of structural clutter on activity patterns of insectivorous bats. Canadian Journal of Zoology 75, 131–136. https://doi.org/10.1139/z97-017

Brinkløv, S., Kalko, E.K.V., Surlykke, A., 2010. Dynamic adjustment of biosonar intensity to habitat clutter in the bat *Macrophyllum macrophyllum* (Phyllostomidae). Behavioral Ecology and Sociobiology 64, 1867–1874. https://doi.org/10.1007/s00265-010-0998-9

Britton, A.R.C., Jones, G., Rayner, J.M.V., Boonman, A.M., Verboom, B., 1997. Flight performance, echolocation and foraging behaviour in pond bats, *Myotis dasycneme* (Chiroptera: Vespertilionidae). Journal of Zoology 241, 503–522. https://doi.org/10.1111/j.1469-7998.1997.tb04842.x

Broekhuizen, S., Spoelstra, K., Thissen, J.B.M., Canters, K.J., Buys, J.C., 2016. Atlas van de Nederlandse zoogdieren, Natuur van Nederland. Naturalis, Leiden.

Brook, L.A., Johnson, C.N., Ritchie, E.G., 2012. Effects of predator control on behaviour of an apex predator and indirect consequences for mesopredator suppression. Journal of Applied Ecology 49, 1278–1286. https://doi.org/10.1111/j.1365-2664.2012.02207.x

Brooks, M., E., Kristensen, K., Benthem, K., J. ,van, Magnusson, A., Berg, C., W., Nielsen, A., Skaug, H., J., Mächler, M., Bolker, B., M., 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. The R Journal 9, 378–400. https://doi.org/10.32614/RJ-2017-066

Cassini, M.H., Galante, M.L., 1992. Foraging under predation risk in the wild guinea pig: the effect of vegetation height on habitat utilization. Annales Zoologici Fennici 29, 289–294.

Céchetto, C., Jakobsen, L., Warrant, E.J., 2023. Visual detection threshold in the echolocating Daubenton's bat *(Myotis daubentonii)*. Journal of Experimental Biology 226, jeb.244451. https://doi.org/10.1242/jeb.244451

Challéat, S., Barré, K., Laforge, A., Lapostolle, D., Franchomme, M., Sirami, C., Le Viol, I., Milian, J., Kerbiriou, C., 2021. Grasping darkness: the dark ecological network as a social-ecological framework to limit the impacts of light pollution on biodiversity. Ecology and Society 26, 15. https://doi.org/10.5751/ES-12156-260115

Charbonnier, Y., Barbaro, L., Theillout, A., Jactel, H., 2014. Numerical and functional responses of forest bats to a major insect pest in pine plantations. PLoS ONE 9, e109488. https://doi.org/10.1371/journal.pone.0109488

Ciechanowski, M., Zając, T., Biłas, A., Dunajski, R., 2009. Nathusius' pipistrelles *Pipistrellus nathusii* (Chiroptera) reveal different temporal activity patterns in wooded and open riparian sites. Mammalia 73, 105–109. https://doi.org/10.1515/MAMM.2009.027

Ciechanowski, M., Zając, T., Biłas, A., Dunajski, R., 2007. Spatiotemporal variation in activity of bat species differing in hunting tactics: effects of weather, moonlight, food abundance, and structural clutter. Canadian Journal of Zoology 85, 1249–1263. https://doi.org/10.1139/Z07-090

Cifuentes, R., Van der Zande, D., Farifteh, J., Salas, C., Coppin, P., 2014. Effects of voxel size and sampling setup on the estimation of forest canopy gap fraction from terrestrial laser scanning data. Agricultural and Forest Meteorology 194, 230–240. https://doi.org/10.1016/j.agrformet.2014.04.013

Collier, T.C., Kirschel, A.N.G., Taylor, C.E., 2010. Acoustic localization of antbirds in a Mexican rainforest using a wireless sensor network. The Journal of the Acoustical Society of America 128, 182–189. https://doi.org/10.1121/1.3425729

Council of the European Communities, 1992. Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. Official Journal of the European Communities L 206.

Cravens, Z.M., Boyles, J.G., 2019. Illuminating the physiological implications of artificial light on an insectivorous bat community. Oecologia 189, 69–77. https://doi.org/10.1007/s00442-018-4300-6

Cunningham, C.X., Scoleri, V., Johnson, C.N., Barmuta, L.A., Jones, M.E., 2019. Temporal partitioning of activity: rising and falling top-predator abundance triggers community-wide shifts in diel activity. Ecography 42, 2157–2168. https://doi.org/10.1111/ecog.04485

Danilovich, S., Krishnan, A., Lee, W.-J., Borrisov, I., Eitan, O., Kosa, G., Moss, C.F., Yovel, Y., 2015. Bats regulate biosonar based on the availability of visual information. Current Biology 25, R1124–R1125. https://doi.org/10.1016/j.cub.2015.11.003

Danilovich, S., Yovel, Y., 2019. Integrating vision and echolocation for navigation and perception in bats. Science Advances 5, eaaw6503. https://doi.org/10.1126/sciadv.aaw6503

Davies, A.B., Asner, G.P., 2014. Advances in animal ecology from 3D-LiDAR ecosystem mapping. Trends in Ecology & Evolution 29, 681–691. https://doi.org/10.1016/j.tree.2014.10.005

Davies, T.W., Bennie, J., Gaston, K.J., 2012. Street lighting changes the composition of invertebrate communities. Biology Letters 8, 764–767. https://doi.org/10.1098/rsbl.2012.0216

Day, J., Baker, J., Schofield, H., Mathews, F., Gaston, K.J., 2015. Part-night lighting: implications for bat conservation. Animal Conservation 18, 512–516. https://doi.org/10.1111/acv.12200

De Jong, M., Caro, S.P., Gienapp, P., Spoelstra, K., Visser, M.E., 2017. Early birds by light at night: effects of light color and intensity on daily activity patterns in blue tits.

Journal of Biological Rhythms 32, 323–333. https://doi.org/10.1177/0748730417719168

De Jong, M., Jeninga, L., Ouyang, J.Q., Van Oers, K., Spoelstra, K., Visser, M.E., 2016. Dose-dependent responses of avian daily rhythms to artificial light at night. Physiology & Behavior 155, 172–179. https://doi.org/10.1016/j.physbeh.2015.12.012

Denzinger, A., Schnitzler, H.U., 2013. Bat guilds, a concept to classify the highly diverse foraging and echolocation behaviors of microchiropteran bats. Frontiers in Physiology 4, 164. https://doi.org/10.3389/fphys.2013.00164

Ditmer, M.A., Stoner, D.C., Carter, N.H., 2021. Estimating the loss and fragmentation of dark environments in mammal ranges from light pollution. Biological Conservation 257, 109135. https://doi.org/10.1016/j.biocon.2021.109135

Dominoni, D.M., De Jong, M., Bellingham, M., O'Shaughnessy, P., Van Oers, K., Robinson, J., Smith, B., Visser, M.E., Helm, B., 2018. Dose-response effects of light at night on the reproductive physiology of great tits (*Parus major*): Integrating morphological analyses with candidate gene expression. Journal of Experimental Zoology Part A: Ecological and Integrative Physiology 329, 473–487. https://doi.org/10.1002/jez.2214

Donners, M., van Grunsven, R.H.A., Groenendijk, D., van Langevelde, F., Bikker, J.W., Longcore, T., Veenendaal, E., 2018. Colors of attraction: Modeling insect flight to light behavior. Journal of Experimental Zoology Part A: Ecological and Integrative Physiology 329, 434–440. https://doi.org/10.1002/jez.2188

Downs, N.C., Racey, P.A., 2006. The use by bats of habitat features in mixed farmland in Scotland. Acta Chiropterologica 8, 169–185. https://doi.org/10.3161/1733-5329(2006)8[169:TUBBOH]2.0.CO;2

Durmus, D., Jägerbrand, A., Tengelin, M., 2024. Research Note: Red light to mitigate light pollution: Is it possible to balance functionality and ecological impact? Lighting Research & Technology. https://doi.org/10.1177/14771535231225362

Duvergé, P.L., Jones, G., Rydell, J., Ransome, R.D., 2000. Functional significance of emergence timing in bats. Ecography 23, 32–40. https://doi.org/10.1111/j.1600-0587.2000.tb00258.x

Eisenbeis, G., 2006. Artificial night lighting and insects: attraction of insects to streetlamps in a rural setting in Germany, in: Rich, C., Longcore, T. (Eds.), Ecological Consequences of Artificial Night Lighting. Island Press, Washington DC, USA, pp. 191–198.

Ellerbrok, J.S., Delius, A., Peter, F., Farwig, N., Voigt, C.C., 2022. Activity of forest specialist bats decreases towards wind turbines at forest sites. Journal of Applied Ecology 59, 2497–2506. https://doi.org/10.1111/1365-2664.14249

Erkert, H.G., 1982. Ecological aspects of bat activity rhythms, in: Kunz, T.H. (Ed.), Ecology of Bats. Springer US, Boston, MA, pp. 201–242. https://doi.org/10.1007/978-1-4613-3421-7_5

Evens, R., Lathouwers, M., Pradervand, J.-N., Jechow, A., Kyba, C.C.M., Shatwell, T., Jacot, A., Ulenaers, E., Kempenaers, B., Eens, M., 2023. Skyglow relieves a crepuscular bird from visual constraints on being active. Science of The Total Environment 900, 165760. https://doi.org/10.1016/j.scitotenv.2023.165760

Falchi, F., Bará, S., 2023. Light pollution is skyrocketing. Science 379, 234–235. https://doi.org/10.1126/science.adf4952

Falchi, F., Cinzano, P., Duriscoe, D., Kyba, C.C.M., Elvidge, C.D., Baugh, K., Portnov, B.A., Rybnikova, N.A., Furgoni, R., 2016. The new world atlas of artificial night sky brightness. Science Advances 2, e1600377. https://doi.org/10.1126/sciadv.1600377

Fenton, M.B., 1990. The foraging behaviour and ecology of animal-eating bats. Canadian Journal of Zoology 68, 411–422. https://doi.org/10.1139/z90-061

Ferreira, D.F., Gibb, R., López-Baucells, A., Nunes, N.J., Jones, K.E., Rocha, R., 2022. Species-specific responses to land-use change in island insectivorous bats. Journal for Nature Conservation 67, 126177. https://doi.org/10.1016/j.jnc.2022.126177

Filgueiras, B.K.C., Peres, C.A., Melo, F.P.L., Leal, I.R., Tabarelli, M., 2021. Winner–Loser species replacements in human-modified landscapes. Trends in Ecology & Evolution 36, 545–555. https://doi.org/10.1016/j.tree.2021.02.006

Fisher, D.O., 2000. Effects of vegetation structure, food and shelter on the home range and habitat use of an endangered wallaby. Journal of Applied Ecology 37, 660–671. https://doi.org/10.1046/j.1365-2664.2000.00518.x

Frafjord, K., 2021. The influence of night length: Activity of the northern bat *Eptesicus nilssonii* under conditions of continuous light in midnight sun compared to a southern population. BMC Zoology 6, 34. https://doi.org/10.1186/s40850-021-00099-1

Franklin, A.B., Noon, B.R., George, T.L., 2002. What is habitat fragmentation? Studies in Avian Biology 25, 20–29.

Frey-Ehrenbold, A., Bontadina, F., Arlettaz, R., Obrist, M.K., 2013. Landscape connectivity, habitat structure and activity of bat guilds in farmland-dominated matrices. Journal of Applied Ecology 50, 252–261. https://doi.org/10.1111/1365-2664.12034

Froidevaux, J.S.P., Fialas, P.C., Jones, G., 2018. Catching insects while recording bats: impacts of light trapping on acoustic sampling. Remote Sensing in Ecology and Conservation 4, 240–247. https://doi.org/10.1002/rse2.71

Froidevaux, J.S.P., Zellweger, F., Bollmann, K., Jones, G., Obrist, M.K., 2016. From field surveys to LiDAR: Shining a light on how bats respond to forest structure. Remote Sensing of Environment 175, 242–250. https://doi.org/10.1016/j.rse.2015.12.038

Fujioka, E., Aihara, I., Watanabe, S., Sumiya, M., Hiryu, S., Simmons, J.A., Riquimaroux, H., Watanabe, Y., 2014. Rapid shifts of sonar attention by *Pipistrellus abramus* during natural hunting for multiple prey. The Journal of the Acoustical Society of America 136, 3389–3400. https://doi.org/10.1121/1.4898428

Gaston, K.J., Bennie, J., Davies, T.W., Hopkins, J., 2013. The ecological impacts of nighttime light pollution: a mechanistic appraisal: Nighttime light pollution. Biological Reviews 88, 912–927. https://doi.org/10.1111/brv.12036

Gaston, K.J., Davies, T.W., Bennie, J., Hopkins, J., 2012. Reducing the ecological consequences of night-time light pollution: options and developments. Journal of Applied Ecology 49, 1256–1266. https://doi.org/10.1111/j.1365-2664.2012.02212.x

Gaston, K.J., Davies, T.W., Nedelec, S.L., Holt, L.A., 2017. Impacts of artificial light at night on biological timings. Annual Review of Ecology, Evolution, and Systematics 48, 49–68. https://doi.org/10.1146/annurev-ecolsys-110316-022745

Gaston, K.J., Duffy, J.P., Gaston, S., Bennie, J., Davies, T.W., 2014. Human alteration of natural light cycles: causes and ecological consequences. Oecologia 176, 917–931. https://doi.org/10.1007/s00442-014-3088-2

Gaultier, S.P., Lilley, T.M., Vesterinen, E.J., Brommer, J.E., 2023. The presence of wind turbines repels bats in boreal forests. Landscape and Urban Planning 231, 104636. https://doi.org/10.1016/j.landurbplan.2022.104636

Gerhardt, H.C., Bee, M.A., 2006. Recognition and localization of acoustic signals, in: Narins, P.M., Feng, A.S., Fay, R.R., Popper, A.N. (Eds.), Hearing and Sound Communication in Amphibians, Springer Handbook of Auditory Research. Springer New York, pp. 113–146. https://doi.org/10.1007/978-0-387-47796-1_5

Giavi, S., Fontaine, C., Knop, E., 2021. Impact of artificial light at night on diurnal plant-pollinator interactions. Nature Communications 12, 1690. https://doi.org/10.1038/s41467-021-22011-8

Goerlitz, H.R., 2019. TOADSuite Manual - a software manual for acoustic 3D-tracking of vocalizing moving animals. ZENODO. https://doi.org/10.5281/zenodo.3518761

Gomes, D.G.E., Appel, G., Barber, J.R., 2020. Time of night and moonlight structure vertical space use by insectivorous bats in a Neotropical rainforest: an acoustic monitoring study. PeerJ 8, e10591. https://doi.org/10.7717/peerj.10591

Götze, S., Denzinger, A., Schnitzler, H.-U., 2020. High frequency social calls indicate food source defense in foraging Common pipistrelle bats. Scientific Reports 10, 5764. https://doi.org/10.1038/s41598-020-62743-z

Griffin, D.R., Galambos, R., 1941. The sensory basis of obstacle avoidance by flying bats. Journal of Experimental Zoology 86, 481–506. https://doi.org/10.1002/jez.1400860310

Griffin, D.R., Webster, F.A., Michael, C.R., 1960. The echolocation of flying insects by bats. Animal Behaviour 8, 141–154. https://doi.org/10.1016/0003-3472(60)90022-1

Grodzinski, U., Spiegel, O., Korine, C., Holderied, M.W., 2009. Context-dependent flight speed: evidence for energetically optimal flight speed in the bat *Pipistrellus kuhlii*? Journal of Animal Ecology 78, 540–548. https://doi.org/10.1111/j.1365-2656.2009.01526.x

Grubisic, M., van Grunsven, R.H., 2021. Artificial light at night disrupts species interactions and changes insect communities. Current Opinion in Insect Science 47, 136–141. https://doi.org/10.1016/j.cois.2021.06.007

Haarsma, A.-J., 2023. Pond Bat *Myotis dasycneme* (Boie, 1825), in: Hackländer, K., Zachos, F.E. (Eds.), Handbook of the Mammals of Europe, Handbook of the Mammals of Europe. Springer International Publishing, Cham, pp. 1–32. https://doi.org/10.1007/978-3-319-65038-8_51-1

Haarsma, A.-J., 2011. De meervleermuis in Nederland (Rapport van de Zoogdiervereniging In opdracht van het ministerie van Economische Zaken, Landbouw, en Innovatie No. 2011.40). Zoogdiervereniging.

Haarsma, A.-J., Siepel, H., 2014. Group size and dispersal ploys: an analysis of commuting behaviour of the pond bat (*Myotis dasycneme*). Canadian Journal of Zoology 92, 57–65. https://doi.org/10.1139/cjz-2013-0052

Hakbong, L., Yong-Chan, C., Sang-Woo, J., Yoon-Ho, K., Seung-Gyu, L., 2021. Changes in nocturnal insect communities in forest-dominated landscape relevant to artificial light intensity. Journal of Ecology and Environment 45, 24. https://doi.org/10.1186/s41610-021-00207-9

Hale, J.D., Fairbrass, A.J., Matthews, T.J., Davies, G., Sadler, J.P., 2015. The ecological impact of city lighting scenarios: exploring gap crossing thresholds for urban bats. Global Change Biology 21, 2467–2478. https://doi.org/10.1111/gcb.12884

Halfwerk, W., Jerem, P., 2021. A systematic review of research investigating the combined ecological impact of anthropogenic noise and artificial light at night. Frontiers in Ecology and Evolution 9, 765950. https://doi.org/10.3389/fevo.2021.765950

Hall, L.S., Krausman, P.R., Morrison, M.L., 1997. The habitat concept and a plea for standard terminology. Wildlife Society Bulletin 25, 173–182.

Hayes, J.P., 1997. Temporal variation in activity of bats and the design of echolocation-monitoring studies. Journal of Mammalogy 78, 514–524. https://doi.org/10.2307/1382902

Hecker, K.R., Brigham, R.M., 1999. Does moonlight change vertical stratification of activity by forest-dwelling insectivorous bats? Journal of Mammalogy 80, 1196–1201. https://doi.org/10.2307/1383170

Hedenström, A., Sparks, R.A., Norevik, G., Woolley, C., Levandoski, G.J., Åkesson, S., 2022. Moonlight drives nocturnal vertical flight dynamics in black swifts. Current Biology 32, 1–7. https://doi.org/10.1016/j.cub.2022.03.006

Henke, S.E., Wester, D.B., Eversole, C.B., 2022. Holiday lights create light pollution and become ecological trap for eastern fox squirrels: case study on a university campus. Human–Wildlife Interactions 16, 12. https://doi.org/10.26077/00d4-03eb

Hermans, C., Koblitz, J.C., Bartholomeus, H., Stilz, P., Visser, M.E., Spoelstra, K., 2023. Combining acoustic tracking and LiDAR to study bat flight behaviour in three-dimensional space. Movement Ecology 11, 25. https://doi.org/10.1186/s40462-023-00387-0

Holderied, M.W., Korine, C., Fenton, M.B., Parsons, S., Robson, S., Jones, G., 2005. Echolocation call intensity in the aerial hawking bat *Eptesicus bottae* (Vespertilionidae) studied using stereo videogrammetry. Journal of Experimental Biology 208, 1321–1327. https://doi.org/10.1242/jeb.01528

Hölker, F., Bolliger, J., Davies, T.W., Giavi, S., Jechow, A., Kalinkat, G., Longcore, T., Spoelstra, K., Tidau, S., Visser, M.E., Knop, E., 2021. 11 Pressing research questions on how light pollution affects biodiversity. Frontiers in Ecology and Evolution 9, 767177. https://doi.org/10.3389/fevo.2021.767177

Hölker, F., Wolter, C., Perkin, E.K., Tockner, K., 2010. Light pollution as a biodiversity threat. Trends in Ecology & Evolution 25, 681–682. https://doi.org/10.1016/j.tree.2010.09.007

Hölker, F., Wurzbacher, C., Weißenborn, C., Monaghan, M.T., Holzhauer, S.I.J., Premke, K., 2015. Microbial diversity and community respiration in freshwater sediments influenced by artificial light at night. Philosophical Transactions of the Royal Society B: Biological Sciences 370, 20140130. https://doi.org/10.1098/rstb.2014.0130

Hooker, J., Lintott, P., Stone, E., 2022. Lighting up our waterways: Impacts of a current mitigation strategy on riparian bats. Environmental Pollution 307, 119552. https://doi.org/10.1016/j.envpol.2022.119552

Hope, G.M., Bhatnagar, K.P., 1979. Electrical response of bat retina to spectral stimulation: comparison of four microchiropteran species. Experientia 35, 1189–1191. https://doi.org/10.1007/BF01963279

Hügel, T., Goerlitz, H.R., 2020. Light might suppress both types of sound-evoked antipredator flight in moths. Ecology and Evolution 10, 12635–13571. https://doi.org/10.1002/ece3.6904

Hulgard, K., Moss, C.F., Jakobsen, L., Surlykke, A., 2016. Big brown bats (*Eptesicus fuscus*) emit intense search calls and fly in stereotyped flight paths as they forage in the wild. Journal of Experimental Biology 334–340. https://doi.org/10.1242/jeb.128983

Ing, R.K., Colombo, R., Gembu, G.-C., Bas, Y., Julien, J.-F., Gager, Y., Hassanin, A., 2016. Echolocation calls and flight behaviour of the elusive pied butterfly bat (*Glauconycteris superba*), and new data on its morphology and ecology. Acta Chiropterologica 18, 477–488. https://doi.org/10.3161/15081109ACC2016.18.2.014

Institution of Lighting Professionals, 2021. Guidance note: The reduction of obtrusive light (No. GN01/21).

Irwin, N.R., Speakman, J.R., 2003. Azorean bats *Nyctalus azoreum*, cluster as they emerge from roosts, despite the lack of avian predators. Acta Chiropterologica 5, 185. https://doi.org/10.3161/001.005.0203

Jägerbrand, A., Andersson, P., Nilsson Tengelin, M., 2023. Dose–effects in behavioural responses of moths to light in a controlled lab experiment. Scientific Reports 13, 10339. https://doi.org/10.1038/s41598-023-37256-0

Jägerbrand, A.K., Spoelstra, K., 2023. Effects of anthropogenic light on species and ecosystems. Science 380, 1125–1130. https://doi.org/10.1126/science.adg3173

Jain, M., Balakrishnan, R., 2012. Does acoustic adaptation drive vertical stratification? A test in a tropical cricket assemblage. Behavioral Ecology 23, 343–354. https://doi.org/10.1093/beheco/arr191

Johnson, C.G., 1950. A suction trap for small airborne insects which automatically segregates the catch into hourly samples. Annals of Applied Biology 37, 80–91. https://doi.org/10.1111/j.1744-7348.1950.tb00952.x

Jones, G., Holderied, M.W., 2007. Bat echolocation calls: adaptation and convergent evolution. Proceedings of the Royal Society B: Biological Sciences 274, 905–912. https://doi.org/10.1098/rspb.2006.0200

Jones, G., Rydell, J., 1994. Foraging strategy and predation risk as factors influencing emergence time in echolocating bats. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 346, 445–455. https://doi.org/10.1098/rstb.1994.0161

Jones, T.K., Moss, C.F., 2021. Visual cues enhance obstacle avoidance in echolocating bats. Journal of Experimental Biology 224, jeb241968. https://doi.org/10.1242/jeb.241968

Jung, K., Kaiser, S., Böhm, S., Nieschulze, J., Kalko, E.K.V., 2012. Moving in three dimensions: effects of structural complexity on occurrence and activity of insectivorous bats in managed forest stands. Journal of Applied Ecology 49, 523–531. https://doi.org/10.1111/j.1365-2664.2012.02116.x

Jung, K., Kalko, E.K.V., 2010. Where forest meets urbanization: foraging plasticity of aerial insectivorous bats in an anthropogenically altered environment. Journal of Mammalogy 91, 144–153. https://doi.org/10.1644/08-MAMM-A-313R.1

Kalko, E.K.V., 1995. Insect pursuit, prey capture and echolocationin pipistrelle bats (Microchiroptera). Animal Behaviour 50, 861–880. https://doi.org/10.1016/0003-3472(95)80090-5

Kalko, E.K.V., Schnitzler, H.U., 1993. Plasticity in echolocation signals of European pipistrelle bats in search flight: implications for habitat use and prey detection. Behavioral Ecology and Sociobiology 33, 415–428. https://doi.org/10.1007/BF00170257

Kalko, E.K.V., Schnitzler, H.U., 1989. The echolocation and hunting behavior of Daubenton's bat, *Myotis daubentoni*. Behavioral Ecology and Sociobiology 24, 225–238. https://doi.org/10.1007/BF00295202

Kehoe, R., Sanders, D., Van Veen, F.J.F., 2022. Towards a mechanistic understanding of the effects of artificial light at night on insect populations and communities. Current Opinion in Insect Science 100950. https://doi.org/10.1016/j.cois.2022.100950

Kerbiriou, C., Julien, J.F., Bas, Y., Marmet, J., Lorrilliere, R., Azam, C., Gasc, A., Lois, G., 2015. Vigie-Chiro : 9 ans de suivi des tendances des espèces communes. Symbioses 34–35.

Kirschel, A.N.G., Cody, M.L., Harlow, Z.T., Promponas, V.J., Vallejo, E.E., Taylor, C.E., 2011. Territorial dynamics of Mexican Ant-thrushes *Formicarius moniliger* revealed by individual recognition of their songs. Ibis 153, 255–268. https://doi.org/10.1111/j.1474-919X.2011.01102.x

Knop, E., Zoller, L., Ryser, R., Gerpe, C., Hörler, M., Fontaine, C., 2017. Artificial light at night as a new threat to pollination. Nature 548, 206–209. https://doi.org/10.1038/nature23288

Koblitz, J.C., 2018. Arrayvolution: using microphone arrays to study bats in the field. Canadian Journal of Zoology 96, 933–938. https://doi.org/10.1139/cjz-2017-0187

Kohl, M.T., Stahler, D.R., Metz, M.C., Forester, J.D., Kauffman, M.J., Varley, N., White, P.J., Smith, D.W., MacNulty, D.R., 2018. Diel predator activity drives a dynamic landscape of fear. Ecological Monographs 88, 638–652. https://doi.org/10.1002/ecm.1313

Korpach, A.M., Garroway, C.J., Mills, A.M., Von Zuben, V., Davy, C.M., Fraser, K.C., 2022. Urbanization and artificial light at night reduce the functional connectivity of migratory aerial habitat. Ecography 2022, e05581. https://doi.org/10.1111/ecog.05581

Kuijper, D.P.J., Schut, J., van Dullemen, D., Toorman, H., Goossens, N., Ouwehand, J., Limpens, Herman J G A, 2008. Experimental evidence of light disturbance along the commuting routes of pond bats (*Myotis dasycneme*). Lutra 51, 37–49.

Kunz, T.H., 1973. Resource Utilization: Temporal and Spatial Components of Bat Activity in Central Iowa. Journal of Mammalogy 54, 14–32. https://doi.org/10.2307/1378869

Kyba, C.C.M., Altıntaş, Y.Ö., Walker, C.E., Newhouse, M., 2023. Citizen scientists report global rapid reductions in the visibility of stars from 2011 to 2022. Science 379, 265–268. https://doi.org/10.1126/science.abq7781

Kyba, C.C.M., Kuester, T., Sánchez de Miguel, A., Baugh, K., Jechow, A., Hölker, F., Bennie, J., Elvidge, C.D., Gaston, K.J., Guanter, L., 2017a. Artificially lit surface of Earth at night increasing in radiance and extent. Science Advances 3, e1701528. https://doi.org/10.1126/sciadv.1701528

Kyba, C.C.M., Mohar, A., Posch, T., 2017b. How bright is moonlight? Astronomy & Geophysics 58, 1.31-1.32. https://doi.org/10.1093/astrogeo/atx025

Lacoeuilhe, A., Machon, N., Julien, J.-F., Le Bocq, A., Kerbiriou, C., 2014. The influence of low intensities of light pollution on bat communities in a semi-natural context. PLoS ONE 9, e103042. https://doi.org/10.1371/journal.pone.0103042

Laforge, A., Archaux, F., Coulon, A., Sirami, C., Froidevaux, J., Gouix, N., Ladet, S., Martin, H., Barré, K., Roemer, C., Claireau, F., Kerbiriou, C., Barbaro, L., Algar, A., 2021. Landscape composition and life-history traits influence bat movement and space use: Analysis of 30 years of published telemetry data. Global Ecology and Biogeography geb.13397. https://doi.org/10.1111/geb.13397

Laforge, A., Pauwels, J., Faure, B., Bas, Y., Kerbiriou, C., Fonderflick, J., Besnard, A., 2019. Reducing light pollution improves connectivity for bats in urban landscapes. Landscape Ecology 34, 793–809. https://doi.org/10.1007/s10980-019-00803-0

Lang, A.B., Kalko, E.K.V., Römer, H., Bockholdt, C., Dechmann, D.K.N., 2006. Activity levels of bats and katydids in relation to the lunar cycle. Oecologia 146, 659–666. https://doi.org/10.1007/s00442-005-0131-3

LaPoint, S., Balkenhol, N., Hale, J., Sadler, J., Ree, R., 2015. Ecological connectivity research in urban areas. Functional Ecology 29, 868–878. https://doi.org/10.1111/1365-2435.12489

Lenth, R.V., 2023. emmeans: Estimated Marginal Means, aka Least-Squares Means.

Lesiński, G., Gryz, J., Kowalski, M., 2009. Bat predation by tawny owls *Strix aluco* in differently human-transformed habitats. Italian Journal of Zoology 76, 415–421. https://doi.org/10.1080/11250000802589535

Lewanzik, D., Voigt, C.C., 2017. Transition from conventional to light-emitting diode street lighting changes activity of urban bats. Journal of Applied Ecology 54, 264–271. https://doi.org/10.1111/1365-2664.12758

Lewanzik, D., Voigt, C.C., 2014. Artificial light puts ecosystem services of frugivorous bats at risk. Journal of Applied Ecology 51, 388–394. https://doi.org/10.1111/1365-2664.12206

Lintott, P.R., Barlow, K., Bunnefeld, N., Briggs, P., Gajas Roig, C., Park, K.J., 2016. Differential responses of cryptic bat species to the urban landscape. Ecology and Evolution 6, 2044–2052. https://doi.org/10.1002/ece3.1996

Liu, Z., He, C., Wu, J., 2016. The relationship between habitat loss and fragmentation during urbanization: an empirical evaluation from 16 world cities. PLoS ONE 11, e0154613. https://doi.org/10.1371/journal.pone.0154613

Longcore, T., Rich, C., 2004. Ecological light pollution. Frontiers in Ecology and the Environment 2, 191–198. https://doi.org/10.1890/1540-9295(2004)002[0191:ELP]2.0.CO;2

Longcore, T., Rodríguez, A., Witherington, B., Penniman, J.F., Herf, L., Herf, M., 2018. Rapid assessment of lamp spectrum to quantify ecological effects of light at night. Journal of Experimental Zoology Part A: Ecological and Integrative Physiology 329, 511–521. https://doi.org/10.1002/jez.2184

Lüdecke, D., Ben-Shachar, M.S., Patil, I., Waggoner, P., Makowski, D., 2021. performance: An R package for assessment, comparison and testing of statistical models. Journal of Open Source Software 6, 3139. https://doi.org/10.21105/joss.03139

Mariton, L., Kerbiriou, C., Bas, Y., Zanda, B., Le Viol, I., 2022. Even low light pollution levels affect the spatial distribution and timing of activity of a "light tolerant" bat species. Environmental Pollution 305, 119267. https://doi.org/10.1016/j.envpol.2022.119267

Mariton, L., Le Viol, I., Bas, Y., Kerbiriou, C., 2023. Characterising diel activity patterns to design conservation measures: Case study of European bat species. Biological Conservation 277, 109852. https://doi.org/10.1016/j.biocon.2022.109852

Mathews, F., Roche, N., Aughney, T., Jones, N., Day, J., Baker, J., Langton, S., 2015. Barriers and benefits: implications of artificial night-lighting for the distribution of common bats in Britain and Ireland. Philosophical Transactions of the Royal Society B: Biological Sciences 370, 20140124. https://doi.org/10.1098/rstb.2014.0124

McKay, R.A., Johns, S.E., Bischof, R., Matthews, F., Van Der Kooij, J., Yoh, N., Eldegard, K., 2024. Wind energy development can lead to guild-specific habitat loss in boreal forest bats. Wildlife Biology e01168. https://doi.org/10.1002/wlb3.01168

Mikula, P., Morelli, F., Lučan, R.K., Jones, D.N., Tryjanowski, P., 2016. Bats as prey of diurnal birds: a global perspective: Predation of bats by diurnal birds. Mammal Review 46, 160–174. https://doi.org/10.1111/mam.12060

Miller, C.R., Barton, B.T., Zhu, L., Radeloff, V.C., Oliver, K.M., Harmon, J.P., Ives, A.R., 2017. Combined effects of night warming and light pollution on predator–prey interactions. Proceedings of the Royal Society B: Biological Sciences 284, 20171195. https://doi.org/10.1098/rspb.2017.1195

Moll, R.J., Redilla, K.M., Mudumba, T., Muneza, A.B., Gray, S.M., Abade, L., Hayward, M.W., Millspaugh, J.J., Montgomery, R.A., 2017. The many faces of fear: a synthesis of the methodological variation in characterizing predation risk. Journal of Animal Ecology 86, 749–765. https://doi.org/10.1111/1365-2656.12680

Müller, B., Glösmann, M., Peichl, L., Knop, G.C., Hagemann, C., Ammermüller, J., 2009. Bat eyes have ultraviolet-sensitive cone photoreceptors. PLoS ONE 4, e6390. https://doi.org/10.1371/journal.pone.0006390

Müller, J., Brandl, R., Buchner, J., Pretzsch, H., Seifert, S., Strätz, C., Veith, M., Fenton, B., 2013. From ground to above canopy—Bat activity in mature forests is driven by vegetation density and height. Forest Ecology and Management 306, 179–184. https://doi.org/10.1016/j.foreco.2013.06.043

Neuweiler, G., 1989. Foraging ecology and audition in echolocating bats. TREE 4, 160–166. https://doi.org/10.1016/0169-5347(89)90120-1

O'Farrell, M.J., Bradley, W.G., 1970. Activity patterns of bats over a desert spring. Journal of Mammalogy 51, 18–26. https://doi.org/10.2307/1378527

O'Mara, M.T., Amorim, F., Scacco, M., McCracken, G.F., Safi, K., Mata, V., Tomé, R., Swartz, S., Wikelski, M., Beja, P., Rebelo, H., Dechmann, D.K.N., 2021. Bats use topography and nocturnal updrafts to fly high and fast. Current Biology 31, 1311-1316.e4. https://doi.org/10.1016/j.cub.2020.12.042

Orbach, D.N., Fenton, B., 2010. Vision impairs the abilities of bats to avoid colliding with stationary obstacles. PLoS ONE 5, e13912. https://doi.org/10.1371/journal.pone.0013912

Owens, A.C.S., Lewis, S.M., 2022. Artificial light impacts the mate success of female fireflies. Royal Society Open Science 9, 220468. https://doi.org/10.1098/rsos.220468

Pagden, M., Ngahane, K., Amin, Md.S.R., 2020. Changing the colour of night on urban streets - LED vs. part-night lighting system. Socio-Economic Planning Sciences 69, 100692. https://doi.org/10.1016/j.seps.2019.02.007

Page, R.A., Ryan, M.J., 2008. The effect of signal complexity on localization performance in bats that localize frog calls. Animal Behaviour 76, 761–769. https://doi.org/10.1016/j.anbehav.2008.05.006

Park, K.J., 2015. Mitigating the impacts of agriculture on biodiversity: bats and their potential role as bioindicators. Mammalian Biology 80, 191–204. https://doi.org/10.1016/j.mambio.2014.10.004

Pauwels, J., Le Viol, I., Azam, C., Valet, N., Julien, J.-F., Bas, Y., Lemarchand, C., Sanchez de Miguel, A., Kerbiriou, C., 2019. Accounting for artificial light impact on bat activity for a biodiversity-friendly urban planning. Landscape and Urban Planning 183, 12–25. https://doi.org/10.1016/j.landurbplan.2018.08.030

Pauwels, J., Le Viol, I., Bas, Y., Valet, N., Kerbiriou, C., 2021. Adapting street lighting to limit light pollution's impacts on bats. Global Ecology and Conservation 28, e01648. https://doi.org/10.1016/j.gecco.2021.e01648

Petersons, G., 2004. Seasonal migrations of north-eastern populations of Nathusius' bat *Pipistrellus nathusii* (Chiroptera). Myotis 41–42, 29–56.

Pfennigbauer, M., Ullrich, A., 2010. Improving quality of laser scanning data acquisition through calibrated amplitude and pulse deviation measurement, in: Turner, M.D., Kamerman, G.W. (Eds.), . Presented at the SPIE Defense, Security, and Sensing, Proceedings Volume 7684, Laser Radar Technology and Applications XV, p. 76841F. https://doi.org/10.1117/12.849641

Polak, T., Korine, C., Yair, S., Holderied, M.W., 2011. Differential effects of artificial lighting on flight and foraging behaviour of two sympatric bat species in a desert: Light pollution in deserts and bat foraging. Journal of Zoology 285, 21–27. https://doi.org/10.1111/j.1469-7998.2011.00808.x

Prat, Y., Yovel, Y., 2020. Decision making in foraging bats. Current Opinion in Neurobiology 60, 169–175. https://doi.org/10.1016/j.conb.2019.12.006

Prugh, L.R., Golden, C.D., 2014. Does moonlight increase predation risk? Meta-analysis reveals divergent responses of nocturnal mammals to lunar cycles. Journal of Animal Ecology 83, 504–514. https://doi.org/10.1111/1365-2656.12148

QGIS.org, 2023. QGIS Geographic Information System. Open Source Geospatial Foundation Project.

Quintanilla-Ahumada, D., Quijón, P.A., Manríquez, P.H., Pulgar, J., García-Huidobro, M.R., Miranda, C., Molina, A., Zuloaga, R., Duarte, C., 2022. Artificial light at night (ALAN) causes variable dose-responses in a sandy beach isopod. Environmental Science

and Pollution Research 29, 35977–35985. https://doi.org/10.1007/s11356-021-17344-2

R Core Team, 2021. R: A language and environment for statistical computing.

Racey, P.A., Swift, S.M., 1985. Feeding Ecology of *Pipistrellus pipistrellus* (Chiroptera: Vespertilionidae) during pregnancy and lactation. I. Foraging Behaviour. The Journal of Animal Ecology 54, 205–211. https://doi.org/10.2307/4631

Rachwald, A., 1992. Habitat preference and activity of the noctule bat *Nyctalus noctula* in the Białowieża Primeval Forest. Acta Theriologica 37, 413–422. https://doi.org/10.4098/AT.arch.92-42

Rainho, A., Augusto, A.M., Palmeirim, J.M., 2010. Influence of vegetation clutter on the capacity of ground foraging bats to capture prey: Vegetation clutter and ground foraging bats. Journal of Applied Ecology 47, 850–858. https://doi.org/10.1111/j.1365-2664.2010.01820.x

Rauchenstein, K., Ecker, K., Bader, E., Ginzler, C., Düggelin, C., Bontadina, F., Obrist, M.K., 2022. LiDAR metrics predict suitable forest foraging areas of endangered Mouse-eared bats (*Myotis myotis*). Forest Ecology and Management 515, 120210. https://doi.org/10.1016/j.foreco.2022.120210

Reconyx, I., 2016. Reconyx MapView professional software.

Ridout, M.S., Linkie, M., 2009. Estimating overlap of daily activity patterns from camera trap data. Journal of Agricultural, Biological, and Environmental Statistics 14, 322–337. https://doi.org/10.1198/jabes.2009.08038

Rodrigo-Comino, J., Seeling, S., Seeger, M.K., Ries, J.B., 2023. Light pollution: A review of the scientific literature. The Anthropocene Review 10, 367–392. https://doi.org/10.1177/20530196211051209

Rodríguez, A., Orozco-Valor, P.M., Sarasola, J.H., 2021. Artificial light at night as a driver of urban colonization by an avian predator. Landscape Ecology 36, 17–27. https://doi.org/10.1007/s10980-020-01132-3

Roeleke, M., Johannsen, L., Voigt, C.C., 2018a. How bats escape the competitive exclusion principle - seasonal shift from intraspecific to interspecific competition drives space use in a bat ensemble. Frontiers in Ecology and Evolution 6, 101. https://doi.org/10.3389/fevo.2018.00101

Roeleke, M., Teige, T., Hoffmeister, U., Klingler, F., Voigt, C.C., 2018b. Aerial-hawking bats adjust their use of space to the lunar cycle. Movement Ecology 6, 11. https://doi.org/10.1186/s40462-018-0131-7

Roemer, C., Julien, J.-F., Bas, Y., 2021. An automatic classifier of bat sonotypes around the world. Methods in Ecology and Evolution 12, 2432–2444. https://doi.org/10.1111/2041-210X.13721

Rosina, V.V., Shokhrin, V.P., 2011. Bats in the diet of owls from the Russian Far East, southern Sikhote Alin. Hystrix, the Italian Journal of Mammalogy 22, 205–213. https://doi.org/10.4404/hystrix-22.1-4503

Ross, C.W., Loudermilk, E.L., Skowronski, N., Pokswinski, S., Hiers, J.K., O'Brien, J., 2022. LiDAR voxel-size optimization for canopy gap estimation. Remote Sensing 14, 1054. https://doi.org/10.3390/rs14051054

Rowse, E.G., Harris, S., Jones, G., 2018. Effects of dimming light-emitting diode street lights on light-opportunistic and light-averse bats in suburban habitats. Royal Society Open Science 5, 180205. https://doi.org/10.1098/rsos.180205

Ruczyński, I., Hałat, Z., Zegarek, M., Borowik, T., Dechmann, D.K.N., 2020. Camera transects as a method to monitor high temporal and spatial ephemerality of flying nocturnal insects. Methods in Ecology and Evolution 11, 294–302. https://doi.org/10.1111/2041-210X.13339

Russ, J., Barlow, K., Briggs, P., Sowler, S., 2012. British bat calls: a guide to species identification. Pelagic Publishing, Exeter.

Russo, D., Ancillotto, L., 2015. Sensitivity of bats to urbanization: a review. Mammalian Biology 80, 205–212. https://doi.org/10.1016/j.mambio.2014.10.003

Russo, D., Cistrone, L., Garonna, A.P., Jones, G., 2011. The early bat catches the fly: Daylight foraging in soprano pipistrelles. Mammalian Biology 76, 87–89. https://doi.org/10.1016/j.mambio.2009.08.002

Russo, D., Cistrone, L., Libralato, N., Korine, C., Jones, G., Ancillotto, L., 2017. Adverse effects of artificial illumination on bat drinking activity. Animal Conservation 20, 492–501. https://doi.org/10.1111/acv.12340

Russo, D., Cosentino, F., Festa, F., De Benedetta, F., Pejic, B., Cerretti, P., Ancillotto, L., 2019. Artificial illumination near rivers may alter bat-insect trophic interactions. Environmental Pollution 252, 1671–1677. https://doi.org/10.1016/j.envpol.2019.06.105

Russo, D., Jones, G., Martinoli, A., Preatoni, D.G., Spada, M., Pereswiet-Soltan, A., Cistrone, L., 2024. Climate is changing, are European bats too? A multispecies analysis of trends in body size. Ecology and Evolution 14, e10872. https://doi.org/10.1002/ece3.10872

Rydell, J., 1992. Exploitation of insects around streetlamps by bats in Sweden. Functional Ecology 6, 744–750. https://doi.org/10.2307/2389972

Rydell, J., 1991. Seasonal use of illuminated areas by foraging northern bats *Eptesicus nilssoni*. Ecography 14, 203–207. https://doi.org/10.1111/j.1600-0587.1991.tb00653.x

Rydell, J., Entwistle, A., Racey, P.A., 1996. Timing of foraging flights of three species of bats in relation to insect activity and predation risk. Oikos 76, 243–252. https://doi.org/10.2307/3546196

Rydell, J., Speakman, J.R., 1995. Evolution of nocturnality in bats: Potential competitors and predators during their early history. Biological Journal of the Linnean Society 54, 183–191. https://doi.org/10.1006/bijl.1995.0011

Saldaña-Vázquez, R.A., Munguía-Rosas, M.A., 2013. Lunar phobia in bats and its ecological correlates: A meta-analysis. Mammalian Biology 78, 216–219. https://doi.org/10.1016/j.mambio.2012.08.004

Salinas-Ramos, V.B., Ancillotto, L., Cistrone, L., Nastasi, C., Bosso, L., Smeraldo, S., Sánchez Cordero, V., Russo, D., 2021. Artificial illumination influences niche segregation in bats. Environmental Pollution 284, 117187. https://doi.org/10.1016/j.envpol.2021.117187

Sanders, D., Baker, D.J., Cruse, D., Bell, F., Van Veen, F.J.F., Gaston, K.J., 2022. Spectrum of artificial light at night drives impact of a diurnal species in insect food web. Science of The Total Environment 831, 154893. https://doi.org/10.1016/j.scitotenv.2022.154893

Sanders, D., Frago, E., Kehoe, R., Patterson, C., Gaston, K.J., 2021. A meta-analysis of biological impacts of artificial light at night. Nature Ecology & Evolution 5, 74–81. https://doi.org/10.1038/s41559-020-01322-x

Sanders, D., Hirt, M.R., Brose, U., Evans, D.M., Gaston, K.J., Gauzens, B., Ryser, R., 2023. How artificial light at night may rewire ecological networks: concepts and models. Philosophical Transactions of the Royal Society B: Biological Sciences 378, 20220368. https://doi.org/10.1098/rstb.2022.0368

Schaub, A., Schnitzler, H.U., 2007. Echolocation behavior of the bat *Vespertilio murinus* reveals the border between the habitat types "edge" and "open space." Behavioral Ecology and Sociobiology 61, 513–523.

Schnitzler, H.U., Kalko, E.K.V., 2001. Echolocation by insect-eating bats. BioScience 51, 557–569. https://doi.org/10.1641/0006-3568(2001)051[0557:EBIEB]2.0.CO;2

Schnitzler, H.U., Moss, C.F., Denzinger, A., 2003. From spatial orientation to food acquisition in echolocating bats. Trends in Ecology & Evolution 18, 386–394. https://doi.org/10.1016/S0169-5347(03)00185-X

Secondi, J., Dupont, V., Davranche, A., Mondy, N., Lengagne, T., Théry, M., 2017. Variability of surface and underwater nocturnal spectral irradiance with the presence of clouds in urban and peri-urban wetlands. PLoS ONE 12, e0186808. https://doi.org/10.1371/journal.pone.0186808

Seewagen, C.L., Nadeau-Gneckow, J., Adams, A.M., 2023. Far-reaching displacement effects of artificial light at night in a North American bat community. Global Ecology and Conservation 48, e02729. https://doi.org/10.1016/j.gecco.2023.e02729

Shier, D.M., Bird, A.K., Wang, T.B., 2020. Effects of artificial light at night on the foraging behavior of an endangered nocturnal mammal. Environmental Pollution 263, 114566. https://doi.org/10.1016/j.envpol.2020.114566

Siemers, B.M., Schauermann, G., Turni, H., von Merten, S., 2009. Why do shrews twitter? Communication or simple echo-based orientation. Biology Letters 5, 593–596. https://doi.org/10.1098/rsbl.2009.0378

Siemers, B.M., Schnitzler, H.U., 2000. Natterer's bat (*Myotis nattereri* Kuhl, 1818) hawks for prey close to vegetation using echolocation signals of very broad bandwidth. Behavioral Ecology and Sociobiology 47, 400–412. https://doi.org/10.1007/s002650050683

Śmielak, M.K., 2023. Biologically meaningful moonlight measures and their application in ecological research. Behavioral Ecology and Sociobiology 77, 21. https://doi.org/10.1007/s00265-022-03287-2

Sordello, R., 2017. Pollution lumineuse et trame verte et bleue : vers une trame noire en France ? Territoire en mouvement 35. https://doi.org/10.4000/tem.4381

Speakman, J.R., 1991a. Why do insectivorous bats in Britain not fly in daylight more frequently? Functional Ecology 5, 518–524. https://doi.org/10.2307/2389634

Speakman, J.R., 1991b. The impact of predation by birds on bat populations in the British Isles. Mammal Review 21, 123–142. https://doi.org/10.1111/j.1365-2907.1991.tb00114.x

Speakman, J.R., Rydell, J., Webb, P.I., Hayes, J.P., Hays, G.C., Hulbert, I.A.R., McDevitt, R.M., 2000. Activity patterns of insectivorous bats and birds in northern Scandinavia (69° N), during continuous midsummer daylight. Oikos 88, 75–86. https://doi.org/10.1034/j.1600-0706.2000.880109.x

Spoelstra, K., Ramakers, J.J.C., van Dis, N.E., Visser, M.E., 2018. No effect of artificial light of different colors on commuting Daubenton's bats (*Myotis daubentonii*) in a choice experiment. Journal of Experimental Zoology Part A: Ecological and Integrative Physiology 329, 506–510. https://doi.org/10.1002/jez.2178

Spoelstra, K., van Grunsven, R.H.A., Donners, M., Gienapp, P., Huigens, M.E., Slaterus, R., Berendse, F., Visser, M.E., Veenendaal, E., 2015. Experimental illumination of natural habitat--an experimental set-up to assess the direct and indirect ecological consequences of artificial light of different spectral composition. Philosophical Transactions of the Royal Society B: Biological Sciences 370, 20140129. https://doi.org/10.1098/rstb.2014.0129

Spoelstra, K., van Grunsven, R.H.A., Ramakers, J.J.C., Ferguson, K.B., Raap, T., Donners, M., Veenendaal, E.M., Visser, M.E., 2017. Response of bats to light with different spectra: light-shy and agile bat presence is affected by white and green, but not red light. Proceedings of the Royal Society B: Biological Sciences 284, 20170075. https://doi.org/10.1098/rspb.2017.0075

Stidsholt, L., Scholz, C., Hermanns, U., Teige, T., Post, M., Stapelfeldt, B., Reusch, C., Voigt, C.C., 2024. Low foraging rates drive large insectivorous bats away from urban areas. Global Change Biology 30, e17063. https://doi.org/10.1111/gcb.17063

Stone, E.L., Harris, S., Jones, G., 2015. Impacts of artificial lighting on bats: a review of challenges and solutions. Mammalian Biology 80, 213–219. https://doi.org/10.1016/j.mambio.2015.02.004

Stone, E.L., Jones, G., Harris, S., 2012. Conserving energy at a cost to biodiversity? Impacts of LED lighting on bats. Global Change Biology 18, 2458–2465. https://doi.org/10.1111/j.1365-2486.2012.02705.x

Stone, E.L., Jones, G., Harris, S., 2009. Street lighting disturbs commuting bats. Current Biology 19, 1123–1127. https://doi.org/10.1016/j.cub.2009.05.058

Straka, T.M., Greif, S., Schultz, S., Goerlitz, H.R., Voigt, C.C., 2020. The effect of cave illumination on bats. Global Ecology and Conservation 21, e00808. https://doi.org/10.1016/j.gecco.2019.e00808

Swift, S.M., 1980. Activity patterns of Pipistrelle bats (*Pipistrellus pipistrellus*) in north-east Scotland. Journal of Zoology 190, 285–295. https://doi.org/10.1111/j.1469-7998.1980.tb01428.x

Swift, S.M., Racey, P.A., Avery, M.I., 1985. Feeding ecology of *Pipistrellus pipistrellus* (Chiroptera: Vespertilionidae) during pregnancy and lactation. II. Diet. The Journal of Animal Ecology 54, 217–225. https://doi.org/10.2307/4632

Tinsley, E., Froidevaux, J.S.P., Zsebők, S., Szabadi, K.L., Jones, G., 2023. Renewable energies and biodiversity: Impact of ground-mounted solar photovoltaic sites on

bat activity. Journal of Applied Ecology 1365-2664.14474. https://doi.org/10.1111/1365-2664.14474

Tomassini, A., Colangelo, P., Agnelli, P., Jones, G., Russo, D., 2014. Cranial size has increased over 133 years in a common bat, *Pipistrellus kuhlii*: a response to changing climate or urbanization? Journal of Biogeography 41, 944–953. https://doi.org/10.1111/jbi.12248

Tougeron, K., Sanders, D., 2023. Combined light pollution and night warming as a novel threat to ecosystems. Trends in Ecology & Evolution 38, 701–704. https://doi.org/10.1016/j.tree.2023.05.012

Troxell, S.A., Holderied, M.W., Pētersons, G., Voigt, C.C., 2019. Nathusius' bats optimize long-distance migration by flying at maximum range speed. Journal of Experimental Biology 222, jeb176396. https://doi.org/10.1242/jeb.176396

Van de Schoot, E., Merckx, T., Ebert, D., Wesselingh, R.A., Altermatt, F., Van Dyck, H., 2024. Evolutionary change in flight-to-light response in urban moths comes with changes in wing morphology. Biology Letters 20, 20230486. https://doi.org/10.1098/rsbl.2023.0486

Van De Sijpe, M., Holsbeek, L., 2007. Hunting strategy and tympanate moth predation by the pond bat (*Myotis dasycneme*). Lutra 50, 91–106.

van Grunsven, R.H.A., Creemers, R., Joosten, K., Donners, M., Veenendaal, E.M., 2017. Behaviour of migrating toads under artificial lights differs from other phases of their life cycle. Amphibia Reptilia 38, 49–55. https://doi.org/10.1163/15685381-00003081

van Grunsven, R.H.A., van Deijk, J.R., Donners, M., Berendse, F., Visser, M.E., Veenendaal, E., Spoelstra, K., 2020. Experimental light at night has a negative long-term impact on macro-moth populations. Current Biology 30, R694–R695. https://doi.org/10.1016/j.cub.2020.04.083

van Langevelde, F., Ettema, J.A., Donners, M., WallisDeVries, M.F., Groenendijk, D., 2011. Effect of spectral composition of artificial light on the attraction of moths. Biological Conservation 144, 2274–2281. https://doi.org/10.1016/j.biocon.2011.06.004

van Langevelde, F., van Grunsven, R.H.A., Veenendaal, E.M., Fijen, T.P.M., 2017. Artificial night lighting inhibits feeding in moths. Biology Letters 13, 20160874. https://doi.org/10.1098/rsbl.2016.0874

Vásquez, D.A., Grez, A.A., Rodríguez-San Pedro, A., 2020. Species-specific effects of moonlight on insectivorous bat activity in central Chile. Journal of Mammalogy 101, 1356–1363. https://doi.org/10.1093/jmammal/gyaa095

Vaughan, N., Jones, G., Harris, S., 1997. Habitat use by bats (Chiroptera) assessed by means of a broad-band acoustic method. The Journal of Applied Ecology 34, 716–730. https://doi.org/10.2307/2404918

Vega, C.P., Jechow, A., Campbell, J.A., Zielinska-Dabkowska, K.M., Hölker, F., 2023. Light pollution from illuminated bridges as a potential barrier for migrating fish - linking measurements with a proposal for a conceptual model. Basic and Applied Ecology S1439179123000658. https://doi.org/10.1016/j.baae.2023.11.001

Venables, W.N., Ripley, B.D., 2002. Modern Applied Statistics with S, Fourth Edition. ed. Springer, New York.

Verboom, B., Boonman, A.M., Limpens, H.J.G.A., 1999. Acoustic perception of landscape elements by the pond bat (*Myotis dasycneme*). Journal of Zoology 248, 59–66. https://doi.org/10.1111/j.1469-7998.1999.tb01022.x

Verboom, B., Huitema, H., 2010. The influence of treeline structure and wind protection on commuting and foraging common pipistrelles (*Pipistrellus pipistrellus*). Lutra 53, 63–80.

Verboom, B., Huitema, H., 1997. The importance of linear landscape elements for the pipistrelle *Pipistrellus pipistrellus* and the serotine bat *Eptesicus serotinus*. Landscape Ecology 12, 117–125. https://doi.org/10.1007/BF02698211

Verboom, B., Spoelstra, K., 1999. Effects of food abundance and wind on the use of tree lines by an insectivorous bat, *Pipistrellus pipistrellus*. Canadian Journal of Zoology 77, 1393–1401.

Voigt, C.C., Dekker, J., Fritze, M., Gazaryan, S., Hölker, F., Jones, G., Lewanzik, D., Limpens, H.J.G.A., Mathews, F., Rydell, J., Spoelstra, K., Zagmajster, M., 2021.

The impact of light pollution on bats varies according to foraging guild and habitat context. BioScience biab087. https://doi.org/10.1093/biosci/biab087

Voigt, C.C., Rehnig, K., Lindecke, O., Pētersons, G., 2018. Migratory bats are attracted by red light but not by warm-white light: Implications for the protection of nocturnal migrants. Ecology and Evolution 8, 9353–9361. https://doi.org/10.1002/ece3.4400

Voigt, C.C., Roeleke, M., Marggraf, L., Pētersons, G., Voigt-Heucke, S.L., 2017. Migratory bats respond to artificial green light with positive phototaxis. PLoS ONE 12, e0177748. https://doi.org/10.1371/journal.pone.0177748

Vosselman, G., Gorte, B.G.H., Sithole, G., Rabbani, T., 2004. Recognising structure in laser scanner point clouds. ISPRS 2004 : proceedings of the ISPRS working group VIII/2 : laser scanning for forest and landscape assessment, Freiburg, October 3-6, 2004 33–38.

Wakefield, A., Stone, E.L., Jones, G., Harris, S., 2015. Light-emitting diode street lights reduce last-ditch evasive manoeuvres by moths to bat echolocation calls. Royal Society Open Science 2, 150291. https://doi.org/10.1098/rsos.150291

Wereszczuk, A., Zalewski, A., 2023. An anthropogenic landscape reduces the influence of climate conditions and moonlight on carnivore activity. Behavioral Ecology and Sociobiology 77, 55. https://doi.org/10.1007/s00265-023-03331-9

Wilkes, P., Lau, A., Disney, M., Calders, K., Burt, A., Gonzalez de Tanago, J., Bartholomeus, H., Brede, B., Herold, M., 2017. Data acquisition considerations for Terrestrial Laser Scanning of forest plots. Remote Sensing of Environment 196, 140–153. https://doi.org/10.1016/j.rse.2017.04.030

Wilson, R., Wakefield, A., Roberts, N., Jones, G., 2021. Artificial light and biting flies: the parallel development of attractive light traps and unattractive domestic lights. Parasites Vectors 14, 28. https://doi.org/10.1186/s13071-020-04530-3

Wilson, S.J., Bayne, E.M., 2018. Use of an acoustic location system to understand how presence of conspecifics and canopy cover influence Ovenbird (*Seiurus aurocapilla*) space use near reclaimed wellsites in the boreal forest of Alberta. Avian Conservation and Ecology 13, 4. https://doi.org/10.5751/ACE-01248-130204

Wood, S.N., 2011. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models: estimation of semiparametric generalized linear models. Journal of the Royal Statistical Society: Series B (Statistical Methodology) 73, 3–36. https://doi.org/10.1111/j.1467-9868.2010.00749.x

Yang, X., Schaaf, C., Strahler, A., Kunz, T., Fuller, N., Betke, M., Wu, Z., Wang, Z., Theriault, D., Culvenor, D., Jupp, D., Newnham, G., Lovell, J., 2013. Study of bat flight behavior by combining thermal image analysis with a LiDAR forest reconstruction. Canadian Journal of Remote Sensing 15.

Zeale, M.R.K., Stone, E.L., Zeale, E., Browne, W.J., Harris, S., Jones, G., 2018. Experimentally manipulating light spectra reveals the importance of dark corridors for commuting bats. Global Change Biology 24, 5909–5918. https://doi.org/10.1111/gcb.14462

Zhao, H., Xu, D., Zhou, Y., Flanders, J., Zhang, S., 2009. Evolution of opsin genes reveals a functional role of vision in the echolocating little brown bat (*Myotis lucifugus*). Biochemical Systematics and Ecology 37, 154–161. https://doi.org/10.1016/j.bse.2009.03.001

Zissis, G., Bertoldi, P., Serrenho, T., 2021. Update on the status of LED-lighting world market since 2018., EUR 30500 EN. Publications Office of the European Union, LU.

Summary

Urbanisation is a major threat for biodiversity due to various sources of pollution, including artificial light at night, which lead to disturbance of the natural habitat. Lit outdoor areas grow drastically and continuously worldwide, especially because of the replacement of older lighting systems by controllable and energy-efficient light emitting diodes (LEDs). This increase has many ecological impacts and is likely to affect nocturnal ecological communities, which is a main concern since 30% of vertebrates and more than 60% of invertebrates are nocturnal. Bats are almost exclusively nocturnal; the safety of darkness is the essence of their temporal niche in the ecosystem. This makes them particularly vulnerable to light pollution. Therefore, an outright demand for mitigation measures arises to protect bats. In order to establish effective mitigation measures, for instance by keeping illumination intensity below the disturbance threshold, it is essential to know how the response of bats to light depends on light intensity. The overall aim of this thesis was to investigate the spatiotemporal response of bats to light intensity under different light spectra. This study would help to draw guidelines to prevent negative impact of nightly illumination on bats, especially in term of habitat loss and habitat fragmentation.

It is possible to record bat flight movements very quickly and accurately in three dimensions with a microphone array. This technique, called acoustic tracking, can now be deployed relatively quickly and is extremely suitable for answering various questions about the spatial behavior of bats. I first developed a methodology to map the space use of bats by combining acoustic tracking data with the three-dimensional vegetation structure from Light Detection and Ranging (LiDAR) scans (**Chapter 2**). This method enables us to study how bats make use of their habitat at a very fine-scale resolution and paves the way for important but yet unanswered questions on bat habitat use. In order to provide novel information on how the presence of artificial light and vegetation spatially affect foraging activity, I used this method at seven sites of the 'Light on Nature' project designed to assess the long-term effects of artificial light, especially light spectrum, on the ecosystem.

In **Chapters 3 and 4**, I showed that synanthropic bats, such as *Pipistrellus* species, alter their spatiotemporal activity pattern to take advantage of concentrated prey resources around light sources, especially under white light. In **Chapter 3**, our findings reveal that pipistrelles fly closer to the light source mostly to snatch insects out of the bright areas but stay further away otherwise. Therefore, being exposed to artificial light is a necessary evil for synanthropic pipistrelles, as it enables them to catch insects. They avoid exposing themselves to high light levels unless they can catch an insect, probably to reduce predation risk. Thus, artificial light alters the trade-off between predator avoidance and foraging efficiency in bats. In **Chapter 4**, I compared bat activity and insect abundance under different light treatments using the acoustic tracking data and camera traps data. I showed that white light temporally affects insect abundance, and bats alter their temporal activity pattern to take advantage of concentrated prey resources. Under red light conditions, this effect is attenuated.

Slow-flying bats such as *Myotis* species are commonly considered as light-averse. In **Chapter 5**, I tried to establish a dose-response curve of bat activity depending on light intensity in free flying *Myotis* bats. Our findings indicate that their disturbance threshold is below 0.1 lux, both under red and white light conditions, excluding a considerable amount of suitable foraging habitat. In **Chapter 6**, I investigated the response of the pond bat (*Myotis dasycneme*) to artificial light along their commuting routes to their foraging ground to assess the potential barrier effect of light resulting in habitat fragmentation. Waterways are essential linear structures for pond bats to move across the landscape. Therefore, I tested the impact of light with an experimental lamppost on bridges that illuminated the waterway with various light spectra and light intensities. Flight behaviour of pond bats was unaffected by the presence of light within the range of common road lighting intensities. Hence, the short presence of a light with realistic intensity does not pose a barrier effect for bats commuting along waterways. These results suggest that *Myotis* species may have context-specific tolerance threshold. In forest-edge habitat commonly used to forage, bats were exposed to light levels above their tolerance threshold (**Chapter 5**), while the light levels were below the threshold in **Chapter 6**, as pond bats behaved normally while commuting.

Altogether, the results of my thesis have brought new information about bat response to light intensity under different light spectra. Synanthropic bats take advantage of accumulated prey resource around light sources, which gives them a selective advantage to dominate in lit environment, both spatially and temporally. On the other hand, light-averse bats have a disturbance threshold at very low light levels in the same environmental conditions. This considerably reduces the amount of suitable foraging habitat due to the widespread occurrence of outdoor lighting. Conversely, pond bats did not respond to artificial light of realistic intensity on the short term. However, we urge for caution to illuminate bridges, as it can be that a similar, permanently present light installation will impair landscape connectivity. The same goes for foraging patches, we recommend keeping them as dark as possible and reducing light trespass into areas that are unintended to be lit, in order to limit habitat loss caused by artificial light for light-sensitive bats. Moreover, as pipistrelles also avoid exposing themselves to high light levels, it is advisable that illuminance is kept to minimum levels for synanthropic bats as well. Adapting the light spectrum is also a possible mitigation measure, but it should be carried out cautiously as this can create an ecological trap for bats.

Unfortunately, our night sky keeps getting brighter, which makes bats' future dim. Keeping natural habitat suitable for bats and other organisms should encourage scientists to continue to explore the effect of anthropogenic light on ecosystems, and urge stakeholders to apply scientific knowledge to minimise the impact of artificial light on biodiversity.

Samenvatting

Translated by Kamiel Spoelstra

Urbanisatie is een serieuze bedreiging voor onze biodiversiteit. Een van de oorzaken hiervan is de verstoring van onze natuurlijke omgeving door lichtvervuiling. Het areaal wat 's nachts wordt verlicht bereidt zich steeds verder uit. Dit komt onder andere door het vervangen van oudere soorten verlichting door energie-efficiënte en regelbare Ledverlichting. Deze toename in verlichting heeft grote ecologische impact en heeft zeer waarschijnlijk gevolgen voor de nachtactieve soortgemeenschappen. Dat is zorgwekkend omdat 30% van alle gewervelden, en meer dan 60% van alle ongewervelden nachtactief zijn. Vleermuizen zijn vrijwel exclusief nachtactief; de veiligheid van de duisternis is bepalend voor hun temporele niche in het ecosysteem. Dit maakt ze in het bijzonder kwetsbaar voor lichtvervuiling, en daarom is er een grote behoefte aan mitigerende maatregelen voor vleermuizen. Voor het vaststellen van effectieve mitigerende maatregelen, bijvoorbeeld door verlichtingsniveaus zo te verlagen dat vleermuizen niet langer verstoord worden, is het essentieel dat we ook weten waar de drempelwaarde in intensiteit van verstorend licht zich bevindt. Het algemene doel van het werk in deze thesis was om het ruimtelijk gedrag van vleermuizen in reactie op de intensiteit van licht met verschillende spectra te onderzoeken. Dit onderzoek kan daarmee helpen om richtlijnen op te stellen om de negatieve impact van nachtelijke verlichting op vleermuizen te voorkomen, in het bijzonder als het gaat om habitat fragmentatie.

Met een 'microphone array' is het mogelijk om heel snel en nauwkeurig vliegbewegingen van vleermuizen in drie dimensies vast te leggen. Deze techniek, die acoustic tracking heet, is tegenwoordig relatief snel in te zetten en is uitermate geschikt voor het beantwoorden van verschillende vragen over het ruimtelijk gedrag van vleermuizen. Ik ontwikkelde eerst een methode om het ruimtegebruik van vleermuizen goed in kaart te brengen, door de data van akoestische tracking van vleermuizen te combineren met de driedimensionale vegetatie structuur van Light Detection and Ranging (LiDAR) scans (**Hoofdstuk 2**). Deze combinatie maakt het mogelijk om heel precies te kijken naar het habitatgebruik van vleermuizen, en lang bestaande vragen hierover te kunnen beantwoorden. Teneinde nieuwe informatie te verzamelen over het effect van licht en de vegetatie structuur op het ruimtelijk gedrag van vleermuizen heb ik deze methode toegepast op de zeven experimentele onderzoek sites van het 'Licht op Natuur' project, wat is opgezet om lange-termen effecten te meten van nachtelijk kunstlicht (en verschillende licht spectra) op het ecosysteem.

In **Hoofdstuk 3 en 4** liet ik zien dat synantropische vleermuizen, zoals *Pipistrellus* soorten, hun ruimtelijk gedrag veranderen om gebruik te maken van de door lampen aangetrokken insecten; dit gold vooral voor witte lampen. In **Hoofdstuk 3** laat ik zien dat dwergvleermuizen rond lichtbronnen evengoed afstand houden, en er alleen heel dichtbij vliegen wanneer ze daar een insect kunnen vangen. Blootstelling aan licht is voor dwergvleermuizen een noodzakelijk kwaad bij het vangen van insecten vlakbij lichtbronnen, wat ze anderszins vermijden – meest waarschijnlijk vanwege een verhoogd predatie risico. De aanwezigheid van licht verandert derhalve de afweging

die dwergvleermuizen maken tussen efficiënt foerageren en het vermijden van predatie risico. In **Hoogdstuk 4** vergeleek ik hoe de vleermuisactiviteit en insecten dichtheid rondom lampen verschilt tussen verschillende kleuren verlichting. Ik liet zien dat wit licht een effect heeft op het patroon in de nachtelijke beschikbaarheid van insecten, en dat vleermuizen hun temporele activiteitenpatroon veranderen vanwege het voordeel van de hogere prooidichtheid. Dit effect is minder sterk rond rode verlichting.

Langzaam vliegende soorten, zoals die van het geslacht *Myotis* staan bekend als lichtschuw. In **Hoofdstuk 5** heb ik getracht een dosis-response curve vast te stellen voor het effect van licht intensiteit op vliegende *Myotis* soorten. Onze bevindingen laten zien dat de drempelwaarde voor verstoring lager ligt dan 0.1 Lux, voor zowel wit als rood licht. Dit betekent dat de aanwezigheid van het licht van een lantaarnpaal leidt tot een aanzienlijk verlies van geschikt foerageergebied. In **Hoofdstuk 6** onderzocht ik de reactie van de meervleermuis (*Myotis dasycneme*) op nachtelijk kunstlicht bij hun vliegroutes naar hun jaaggebieden. Voor meervleermuizen zijn waterwegen belangrijk om zich te verspreiden in het landschap. Nachtelijk kunstlicht langs waterwegen is mogelijk een barrière voor meervleermuizen, en mogelijk leidt dit tot habitat fragmentatie. Ik testte daarom de impact van licht met verschillende spectra en intensiteit op het vlieggedrag van meervleermuizen, met behulp van een experimentele lantaarnpaal die het water op verschillende locaties vanaf bruggen verlichtte. Het vlieggedrag van meervleermuizen werd echter niet beïnvloed door de aanwezigheid van licht met een intensiteit die gangbaar is voor wegverlichting. De korte aanwezigheid van licht met een realistische intensiteit vormde geen barrière voor vleermuizen die heen- en weer vlogen langs de verlichte waterwegen. De resultaten wijzen erop dat de drempelwaarden in licht tolerantie van *Myotis* soorten waarschijnlijk context-afhankelijk zijn: bij de vleermuizen die joegen langs bosranden was het experimentele licht al snel boven de drempelwaarde (**Hoofdstuk** 5), terwijl het bij de waterwegen (**Hoofstuk** 6) altijd onder de drempelwaarde bleef aangezien de meervleermuizen op hun route van de kolonie naar het jachtgebied hun gedrag niet veranderden.

De resultaten van mijn thesis hebben gezorgd voor nieuwe informatie over hoe de reactie van vleermuizen verband houdt met de intensiteit van nachtelijk kunstlicht, en hoe dit afhangt van verschillende licht spectra. Synantropische vleermuizen profiteren van de accumulatie van prooidieren rond lichtpunten, en door dat voordeel kunnen ze dominant zijn in een verlichte omgeving, in zowel ruimte als tijd. Omdat ook dwergvleermuizen hoge lichtintensiteiten vermijden, is het ook voor deze soorten verstandig om lichtniveaus tot een minimum te beperken. Voor lichtschuwe vleermuissoorten wordt het aandeel geschikt jaaggebied juist kleiner vanwege de lage drempelwaarde in lichtintensiteit waarbij deze soorten al verstoord worden. Dit verlies aan habitat is aanzienlijk door de wijdverspreide aanwezigheid van openbare verlichting. We adviseren om deze zo donker mogelijk te houden en te zorgen dat er geen strooilicht terechtkomt op plaatsen waar het niet gewenst is, teneinde habitatverlies te beperken. Al reageerden meervleermuizen niet op de kortstondige aanwezigheid van licht van een realistische intensiteit, het blijft echter belangrijk om voorzichtig te zijn met het verlichten van bruggen, omdat het niet uitgesloten is dat

de permanente aanwezigheid van vergelijkbare verlichting uiteindelijk toch kan zorgen voor habitat fragmentatie. Hetzelfde geldt voor foerageergebied van lichtschuwe vleermuissoorten: we adviseren om deze zo donker mogelijk te houden en te zorgen dat er geen strooilicht terechtkomt op plaatsen waar het niet gewenst is, teneinde habitatverlies te beperken. Omdat ook dwergvleermuizen hoge lichtintensiteiten vermijden, is het ook voor deze soorten verstandig om lichtniveaus tot een minimum te beperken. De aanpassing van het licht spectrum is ook een optie, maar dit moet met beleid worden gedaan omdat dit 'ecological trap' kan vormen voor vleermuizen.

Onze nachtelijke hemel wordt helaas steeds lichter, wat een duistere toekomst betekent voor vleermuizen. Het belang van bescherming van de habitat van vleermuizen en andere soorten zou een belangrijke stimulans moeten zijn voor wetenschappers om door te gaan met onderzoek naar de effecten van nachtelijk kunstlicht op ecosystemen, en een aansporing voor beheerders van openbare verlichting om wetenschappelijke kennis toe te passen om het effect van licht op biodiversiteit te minimaliseren.

Résumé

L'urbanisation est une menace majeure pour la biodiversité en raison des nombreuses sources de pollution, notamment la lumière artificielle nocturne, qui induisent une perturbation des habitats naturels. L'éclairage des zones extérieures prend de l'ampleur dans le monde entier de manière drastique et continue, notamment en raison du remplacement des anciens systèmes d'éclairage par des diodes électroluminescentes (LED) maniables et économes en énergie. Cette augmentation a de nombreux impacts écologiques et affecte probablement les communautés écologiques nocturnes, ce qui constitue une préoccupation majeure puisque 30 % des vertébrés et plus de 60 % des invertébrés sont nocturnes. Les chauves-souris sont presque exclusivement nocturnes ; la protection de l'obscurité est l'essence même de leur niche temporelle au sein de l'écosystème. Cela les rend particulièrement vulnérables à la pollution lumineuse. Par conséquent, des mesures d'atténuation sont vivement exigées pour les chauves-souris. Afin d'établir des mesures d'atténuation efficaces, par exemple en maintenant l'intensité lumineuse en dessous du seuil de perturbation, il est essentiel de savoir comment la réponse des chauves-souris à la lumière varie en fonction de l'intensité lumineuse. L'objectif général de cette thèse est d'étudier la réponse spatio-temporelle des chauves-souris à l'intensité lumineuse sous différents spectres lumineux. Cette étude vise à élaborer des recommendations afin de limiter l'impact négatif de l'éclairage nocturne sur les chauves-souris, notamment en termes de perte et de fragmentation d'habitat.

Il est possible de déterminer les mouvements de vol des chauves-souris en trois dimensions de façon rapide et précise avec un 'microphone array'. Cette technique, aussi appelée suivi acoustique, peut désormais être déployée relativement vite et est extrêmement appropriée pour répondre à de nombreuses questions concernant le comportement spatial des chauves-souris. J'ai d'abord développé une méthode combinant les données de suivi acoustique avec la structure tridimensionnelle de la végétation obtenue par détection et l'estimation de la distance par la lumière (Light Detection and Ranging, « LiDAR »), afin de cartographier le comportement des chauves-souris (**Chapitre 2**). Cette méthode nous permet d'étudier l'utilisation de l'habitat par les chauves-souris à une échelle de résolution très précise et ouvre la voie à des questions importantes, mais encore sans réponse, sur leur utilisation de l'habitat. Afin de fournir de nouvelles informations sur la façon dont la lumière artificielle et la végétation affectent spatialement leur recherche de nourriture, j'ai utilisé cette méthode sur sept sites du projet 'Light on Nature' conçu pour évaluer les effets à long terme de la lumière artificielle sur l'écosystème, en particulier le spectre lumineux.

Dans les **Chapitres 3 et 4**, j'ai montré que les chauves-souris synanthropes, telles que les espèces du genre *Pipistrellus*, modifient leur rythme d'activité spatio-temporelle afin de profiter des proies agrégées autour des sources lumineuses, en particulier sous lumière blanche. Dans le **Chapitre 3**, nos résultats révèlent que les pipistrelles volent plus près des sources lumineuses pour capturer les insectes qui se trouvent dans les zones fortement éclairées, mais restent en retrait dans le cas contraire. L'exposition à la lumière artificielle est donc un mal nécessaire pour les

pipistrelles synanthropes, car elle leur permet d'attraper des insectes. Elles évitent de s'exposer à des niveaux de lumière élevés, à moins de pouvoir attraper un insecte, probablement pour réduire le risque de prédation. Ainsi, la lumière artificielle modifie l'équilibre à respecter entre réduire le risque de prédation et améliorer l'efficacité de la recherche de nourriture chez les chauves-souris. Dans le **Chapitre 4**, j'ai comparé l'activité des chauves-souris et l'abondance d'insectes sous différents traitements lumineux à l'aide des données de suivi acoustique et de pièges photographiques. J'ai montré que la lumière blanche affecte temporellement l'abondance d'insectes et que les chauves-souris modifient leur rythme d'activité pour profiter de l'abondance de proies. Sous lumière rouge, cet effet est atténué.

Les chauves-souris volant lentement, telles que les espèces du genre *Myotis*, sont généralement considérées comme lucifuges. Dans le **Chapitre 5**, j'ai essayé d'établir une relation dose-réponse de l'activité des espèces du genre *Myotis* en fonction de l'intensité lumineuse en milieu naturel. Nos résultats indiquent que leur seuil de perturbation est inférieur à 0,1 lux, tant sous lumière rouge que blanche, excluant une quantité considérable d'habitat propice à la recherche de nourriture. Dans le **Chapitre 6**, j'ai étudié la réponse du murin des marais (*Myotis dasycneme*) à l'éclairage artificiel le long de routes de vol vers ses zones de chasse afin d'évaluer l'effet barrière potentiel de la lumière entraînant une fragmentation de leur habitat. Les cours d'eau sont des structures linéaires essentielles permettant aux murins des marais de se déplacer à travers le paysage. J'ai donc testé l'impact de la lumière avec un lampadaire expérimental placé sur des ponts et qui éclairait le cours d'eau avec différents spectres lumineux et intensités lumineuses. Le comportement de vol des murins des marais n'a pas été affecté par la présence de lumière dans la gamme d'intensités couramment utilisées pour l'éclairage routier. Ainsi, la présence éphémère d'une lumière d'intensité réaliste ne constitue pas un effet barrière pour les chauves-souris se déplaçant le long des cours d'eau. Ces résultats suggèrent que le seuil de tolérance des espèces du genre *Myotis* pourrait varier en fonction du contexte. En effet, dans les habitats en lisière de forêt couramment utilisés comme zone de chasse, les chauves-souris ont été exposées à des niveaux lumineux supérieurs à leur seuil de tolérance (**Chapitre 5**), tandis que les niveaux lumineux étaient inférieurs au seuil de tolérance dans le **Chapitre 6**, étant donné que les murins des marais se comportaient normalement le long de leur route de vol.

En résumé, les résultats de ma thèse ont apporté de nouvelles informations sur la réaction des chauves-souris à l'intensité lumineuse sous différents spectres lumineux. Les chauves-souris synanthropes profitent des proies agglomérées autour des sources lumineuses, ce qui leur donne un avantage sélectif pour dominer dans un environnement éclairé, à la fois de façon spatiale et temporelle. En revanche, les chauves-souris lucifuges ont un seuil de perturbation à des niveaux de lumière très faibles dans les mêmes conditions environnementales. Cela réduit considérablement la quantité de zones de chasse en raison de la présence généralisée d'éclairage extérieur. À l'inverse, les murins des marais ne réagissaient pas à une lumière artificielle d'intensité réaliste, à court terme. Cependant, nous invitons à la prudence lorsqu'il faut illuminer des ponts, car il se peut qu'un éclairage similaire, présent en permanence,

nuise à la connectivité écologique. Il en va de même pour les zones de chasse ; nous recommandons de les garder aussi sombres que possible et de réduire l'intrusion de la lumière dans les espaces qui ne sont pas destinés à être éclairés, afin de limiter la perte d'habitat causée par la lumière artificielle pour les chauves-souris lucifuges. De plus, comme les pipistrelles évitent aussi de s'exposer à des niveaux lumineux élevés, il est conseillé que l'éclairage soit également maintenu à des niveaux lumineux faibles pour les chauves-souris synanthropes. L'adaptation du spectre lumineux est également une mesure d'atténuation possible, mais elle doit être effectuée avec prudence car cela peut créer un piège écologique pour les chauves-souris.

Malheureusement, notre ciel nocturne continue de s'illuminer, ce qui assombrit l'avenir des chauves-souris. Maintenir un habitat naturel adapté aux chauves-souris et à d'autres organismes devrait encourager les scientifiques à continuer d'explorer l'effet de la lumière anthropique sur les écosystèmes, et inciter les parties prenantes à appliquer les connaissances scientifiques pour minimiser l'impact de la lumière artificielle sur la biodiversité.

Curriculum vitae

Claire Hermans was born on April 22, 1994 in Anderlecht, Belgium. At the age of 1 she moved to Luxembourg, where she grew up in the countryside. Driven by curiosity about the nature around her, she studied Biology of Organisms and Ecology at UCLouvain University (Louvain-la-Neuve, Belgium) from 2012 to 2017. During her last year of Master, she carried out a research internship in Magnus Wahlberg's laboratory at the University of Southern Denmark (Kerteminde, Denmark), which focuses on bioacoustics in aquatic animals. Her deep interest in acoustics gave her the desire to continue to learn and to work in this field. Finding a position in marine bioacoustics was not an easy task. Fortunately, bats are fascinating creatures that use echolocation, just like cetaceans. In 2018 she worked five months as a research assistant in the Acoustic and Functional Ecology research group led by Dr Holger Goerlitz at the Max Planck Institute of Ornithology (Seewiesen, Germany). This project allowed her to learn acoustic tracking methods to track bats in particular. She also completed a certificate in statistics and data science at UCLouvain University in 2019.

All those earlier experiences led her to start her PhD in July 2019 at the Animal Ecology Department of the NIOO-KNAW under the supervision of Prof. Dr Marcel Visser and Dr Kamiel Spoelstra. This PhD project is part of the 'Light on Landscape' project, the results of which are presented in this thesis. She is now ready to crawl out of her bat cave and explore the world. In April 2024, she started as a postdoctoral researcher with Prof. Dr Nicolas Schtickzelle at UCLouvain University and she is looking forward to seeing what the future holds.

List of publications

Peer-reviewed

Hermans C., Koblitz J.C., Bartholomeus H., Stilz P., Visser M.E., Spoelstra K. (2023) Combining acoustic tracking and LiDAR to study bat flight behaviour in three-dimensional space. *Movement Ecology*, 11, 25, DOI: 10.1186/s40462-023-00387-0 (**Chapter 2**)

Mäkelin S., Wahlberg M., Osiecka A.N., **Hermans C.** & Balsby T.J.S. (2021) Vocal behaviour of the Great Cormorant *Phalacrocorax carbo sinensis* during the breeding season, *Bird Study*, 68:2, 211-219, DOI: 10.1080/00063657.2021.1987383

Mallefet J., Duchatelet L., **Hermans C.**, Baguet F. (2019) Luminescence control of Stomiidae photophores. *Acta Histochemica*, 121(1): 7-15, DOI: 10.1016/J.ACTHIS.2018.10.001

Duchatelet L., **Hermans C.**, Duhamel G., Cherel Y., Guinet C., Mallefet J. (2019) Coelenterazine detection in five myctophid species from the Kerguelen Plateau. Proceedings of the second symposium on the Kerguelen plateau: marine ecosystem and fisheries. Eds. Welsford D., Dell J., Duhamel G. (Kingston, Tasmania, Australia: Australian Antarctic Division), 31–41.

Submitted for publication

Hermans C., Litovska I., de Pastors M., Visser M.E., Spoelstra K. (*under review*) Artificial light at night drives diel activity patterns of synanthropic pipistrelle bats and their prey. *Science of the Total Environment* (**Chapter 4**)

Hermans C., Visser M.E., Spoelstra K. (*submitted*) Light-averse *Myotis* bats avoid streetlights from afar. *Journal of Applied Ecology* (**Chapter 5**)

In preparation

Hermans C., Bartholomeus H., Koblitz J.C., Visser M.E., Spoelstra K. Bouncing synanthropic bats: the interaction between light spectrum, insect availability and environmental structure determines the distance that bats keep to light sources (**Chapter 3**)

Hermans C., Kijm L., Paardekooper M., Koblitz J.C., Stilz P., Haarsma A.-J., Visser M.E., Spoelstra K. No effect on flight behaviour of commuting pond bats (*Myotis dasycneme*) by artificial light of realistic intensity (**Chapter 6**)

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My academic career actually started few years ago. My previous experiences led me to this PhD project. Thanks to **Magnus** and **Holger**, who guided my path into bioacoustics, first in marine mammals, then in bats. I could not have dreamed of much better start with those two teams! During my stay in Holger's lab, I also met **Jens**, who suggested me to apply to this PhD position. I am very happy that we could collaborate on this project before you decided to follow another career path. I had a very good time with you in the field "all night long". I was also glad to have a partner in crime to sneeze and cry after fieldwork because of hay fever.

Harm and **Alvaro** also joined the project along the way, as the LiDAR part was not planned from the start. However, your support and goodwill helped us to put this in place fairly easily. I really appreciated the added value of this work in my project and I enjoyed discovering how it works. I was so amazed the first time I combined LiDAR and acoustic tracking data and saw how nicely it fitted together. Thanks for bringing me this childlike sense of wonder!

I could not have collected all these data without a lot of help throughout the field seasons, especially from my students **Iryna**, **Roel**, **Laura**, **Marieke**, **Fäbe**, and **Mélyssa** who have been of great help! Carrying all the equipment in the field was a nice workout, also for **Miriam**, **Gabriel**, **Sander** and **Tim**. Thanks also to **Martijn**, **Peter**, **Kamiel**, **Harm** and **Alvaro** for helping me with the LiDAR, aka the ping-pong machine. I also want to thank **Anne-Jifke** and her team who helped us to find suitable sites for our experiment in Friesland. We also had a lot of support from many Frisians, offering power supply, tea, strawberries and good company during our recordings. Thanks also to **Marc** who provided many labeled bat recordings that were very helpful to build the reference database for species

identification. Many thanks also to the users committee's members of the Light on Landscape project for the fruitful discussions over the years and their contribution to this project.

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And speaking about friends, I especially want to thank my paranymphs **Eva** and **Natalie**. Thank you for supporting me throughout my PhD. **Natalie**, I had a twinge in my heart when I succeeded you as the queen of the office. But feeling your support even from far North in the last stretch warmed my heart! **Eva**, I always enjoyed our discussions during a walk, around a coffee or with tons of Chinese food. You are always attentive to others and I really value that. I also want to thank my friends from Luxembourg and Belgium, in particular **Audrey**, **Eve**, **Maëlle**, **Aurélie**, **Gabrielle** and **Manon**. Even if we take different paths in our lives, I know we can count on each other. **Anne-Sophie**, I am very glad that we found each other again in the Netherlands. **Anti**, thanks for following me in mysterious cavities for bat counting. **Fleur**, thank you for drawing the cover of my thesis, your artistic skills have always impressed me! I also want to thank my roommates **Justine** and **Youri** who were/are also part of the AnE team and with whom I enjoyed sharing moments and beers when coming home from work.

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PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review/project proposal (4.5 ECTS)
- Behavioural response of bats to light intensity variation and its consequences in terms of habitat loss and fragmentation

Post-graduate courses (5.4 ECTS)
- Full-stack bioacoustics: field kit to AI to action; Lorentz Center, Leiden (2022)
- R and Big data; PE&RC, WUR (2019)
- Intermediate R course; PE&RC, WUR (2022)
- Tidy data transformation and visualization with R; PE&RC, WUR (2020)
- Data carpentry with Python workshop; eScience Center; eScience Center (2021)
- Reproducible research with R packages (2023)

Invited review of journal manuscripts (1 ECTS)
- Journal of animal ecology: effect of light pollution on nocturnal migrating birds (2021)

Competence, skills and career-oriented activities (2.1 ECTS)
- Start to supervise BSc & MSc thesis students; WUR (2019)
- Efficient writing strategies; WUR & WGS (2020)
- Workshop: communication with non-expert; KNAW (2020)
- KNAW PhD & postdoc career day; KNAW (2022)

Scientific integrity/ethics in science activities (0.7 ECTS)
- Scientific integrity; WIAS, WUR
- Workshop good academic practice; NIOO-KNAW

PE&RC Annual meetings, seminars and PE&RC weekend/retreat (1.8 ECTS)
- PE&RC Weekend for first years (2019)
- PE&RC Day (2021)
- PE&RC Last year retreat (2023)

Discussion groups/local seminars or scientific meetings (9.9 ECTS)
- Symposium on bat migrations; Ostende, Belgium (2019)
- WEES Seminars (2019-2023)
- Journal club NIOO Animal Ecology Department (2019-2023)
- Science lunch NIOO Animal Ecology Department (2019-2023)
- Nederlandse vleermuizen-studiedag (2019, 2020, 2023)
- BatLab; Finland (2020)
- Bat symposium natuurpunt & Natagora Plecotus; Brussels, Belgium (2022)
- NIOO Creative team transformative change (2022-2023)
- Naturalist conference Natagora; Namen, Belgium (2023)
- NWO Teknowlogy festival (2023)
- Evolving urban communities; Amsterdam, the Netherlands (2024)

International symposia, workshops and conferences (14.2 ECTS)
- German bat research meeting; poster presentation; Frauenchiemsee, Germany (2020)
- NAEM poster presentation; Lunteren, the Netherlands (2020)
- The society for integrative & comparative biology meeting; oral presentation; Washington, DC, USA (2021)
- European bat research symposium; oral presentation; Turku, Finland (2021)
- NAEM 2022; oral presentation; Lunteren, the Netherlands (2022)
- SEB Centenary conference; oral presentation; Edinburgh, UK (2023)
- ALAN Conference; oral presentation; Calgary, Canada (2023)

Societally relevant exposure (0.3 ECTS)
- Zoogdier: vleermuizen volgen met hun eigen geluid (2023)

BSc/MSc thesis supervision (6 ECTS)
- Effects of experimental light on activity patterns of insects and bats
- What happens after dark: the effect of artificial light on the activity patterns of bats
- Artificial lights do not affect the flight speed of commuting pond bats (*Myotis dasycneme*)
- The light-opportunistic common pipistrelle (*Pipistrellus pipistrellus*) avoids high light intensities
- Effects of artificial light on foraging activity of pipistrelles (*Pipistrellus* spp.)