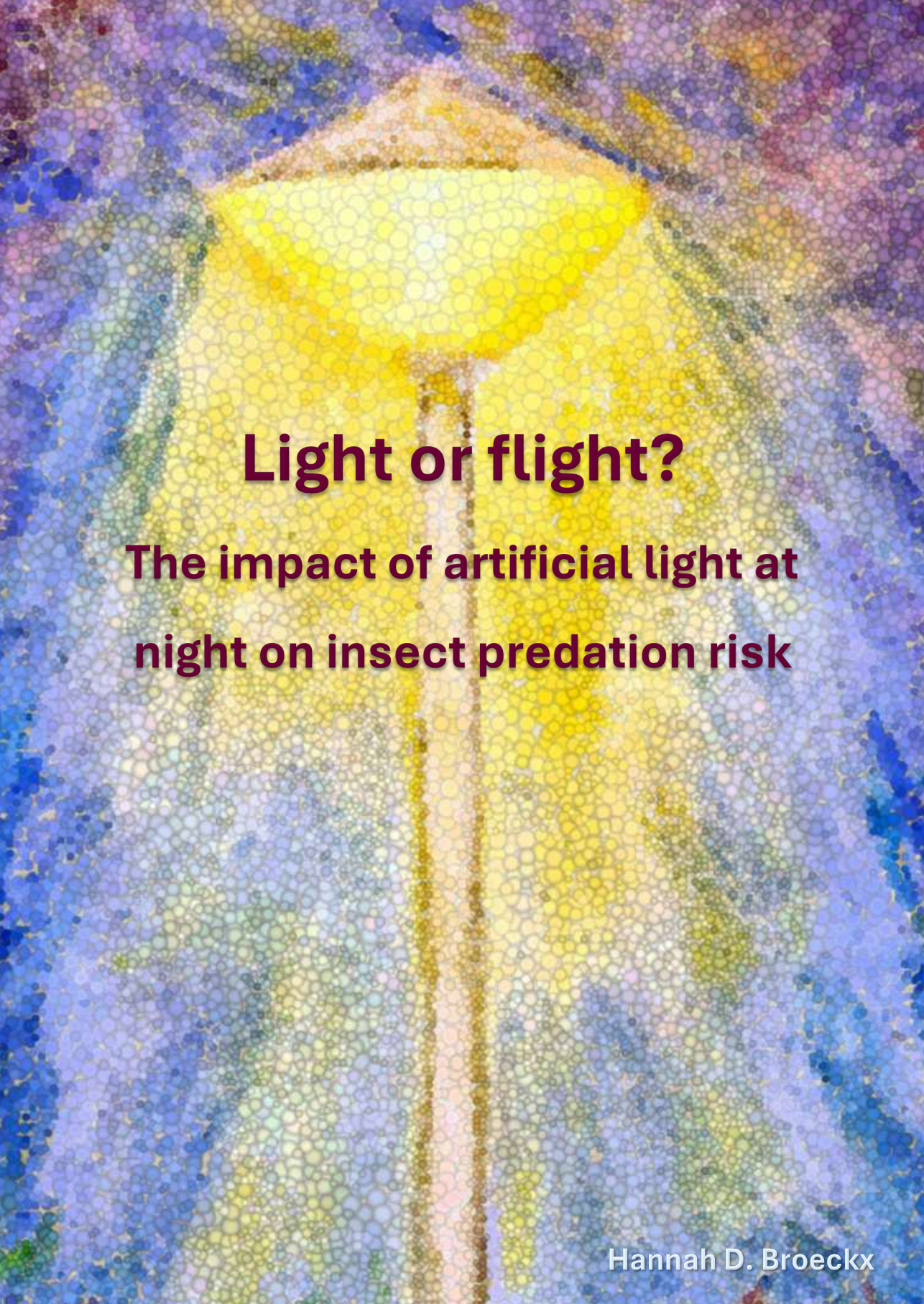


Light or flight?

The impact of artificial light at night on insect predation risk

Hannah D. Broeckx

Propositions

1. Scientists can be activists, especially when it concerns their own research field.

2. Universities can set a leading example by practicing what they teach.

3. A good working atmosphere is essential for good research.

4. Science communication is a vital part of research.

5. Research can profit by more interaction and shared vocabulary between different research fields.

6. Observer bias should be carefully accounted for in every study design.

Light or flight?

The impact of artificial light at night on insect predation risk

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op gezag van de Rector Magnificus
ten overstaan van een door het College voor Promoties ingestelde commissie,
in het openbaar te verdedigen in de Aula der Universiteit
op vrijdag 11 september 2026, te 14.00 uur

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Hannah David Broeckx

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Faculteit der Natuurwetenschappen, Wiskunde en Informatica

Achteraf weet je alles van tevoren.

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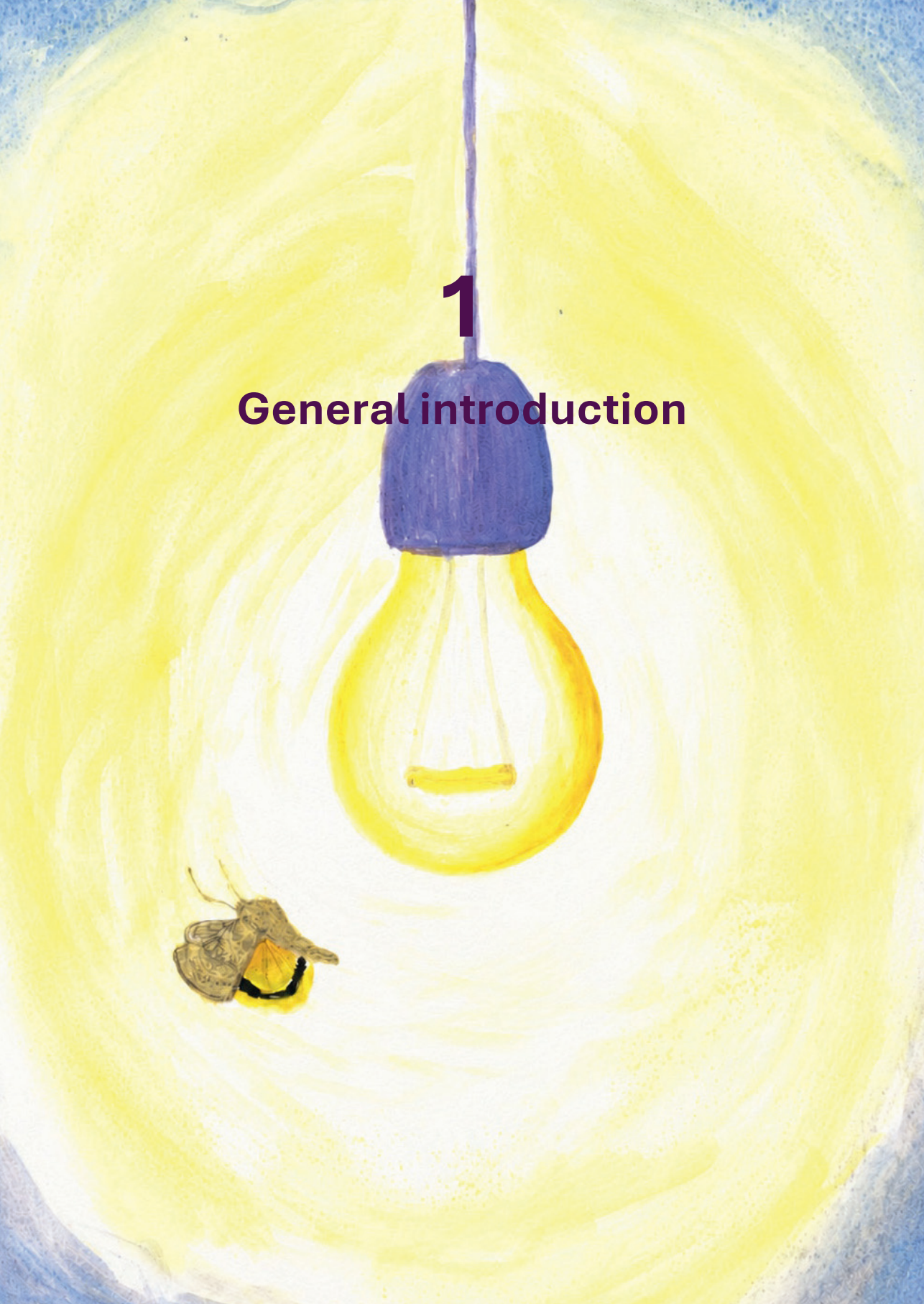
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1

General introduction



This thesis examines the impact of artificial light at night (ALAN) on predator-prey interactions. In this chapter, I begin by introducing the general problem of ALAN, with a particular focus on the ecological consequences of light pollution. I then concentrate on how ALAN alters predator–prey dynamics, outlining the different pathways through which ALAN disrupts these interactions. Finally, I present the structure of this thesis and briefly introduce the objectives and main themes of the subsequent chapters.

1. Artificial light at night (ALAN)

1.1. Description

Artificial light at night, also called anthropogenic light at night (ALAN) (Durmus, 2024), encompasses all the light that has been added to the night sky by human influence. The first human-created light came in the form of fire. Later it developed to gaslight, which slowly spread the human usage of light. Finally, with the invention of electrical light the true proliferation began, fundamentally transforming the nightscape (Kyba et al., 2011).

ALAN consists of direct light from discrete light sources (e.g. streetlights, building lights) and skyglow (Grenis et al., 2023; Van de Schoot et al., 2025). Skyglow is the indirect light that brightens the sky, caused by light scattering through the air and reflecting off atmospheric particles (Kyba et al., 2011). Through the process of air scattering, light can move several kilometers away from its origin, affecting areas without any primary light sources (Jechow et al., 2020; Kyba et al., 2015).

Characteristically, ALAN is very different from natural light. It appears at very unusual times and places (Falchi et al., 2016) and can reach intensities and spectra unprecedented by natural light sources (Dominoni & Nelson, 2018). For example, typical moonlight illumination is estimated to be around 0.05–0.30 lux at ground level, depending on the moon phase (Kyba et al., 2017), whereas a regular streetlight can be up to 800 times brighter, ranging from 10 to 40 lux at ground level (Gaston et al., 2017).

1.2. Dynamics and global extent

Currently, sky brightness increases globally at an annual average rate of 2.2%-9.6% (Hölker et al., 2010; Kyba et al., 2017, 2023). ALAN is closely tied to urban development (Jechow et al., 2020; Walker, 1977). As the human population grows, driving the expansion of human infrastructure and the spread of urban areas, ALAN expands accordingly (Gu et al., 2021). However, the rate at which ALAN emissions increase even surpasses the rate of population growth (Gaston & Miguel, 2022), intensified by the continuous development of more efficient lighting technologies, such as Light Emitting Diodes (LED) (Sánchez de Miguel et al., 2021).

Presently, more than 80% of the human population lives in light polluted areas (Falchi et al., 2016). These bright night skies prevent one-third of the human population from seeing the Milky Way from where they live (Falchi et al., 2016).

1.3. Associated costs

ALAN has enabled people to extend their productivity and social life into the night time niche, and has ameliorated our sense of safety (Himschoot et al., 2024; but not necessarily the actual safety (Gaston et al., 2015)). However, ALAN also comes with a price.

First, there is an enormous economic cost to ALAN. Expenses are estimated to account for 0.72% of the global gross domestic product, as seen in 2010 (Tsao et al., 2010). When factoring in the costs associated with the loss of ecosystem services due to ALAN, this adds an additional 2.87 trillion euros per year (Anderson et al., 2024).

Electric lighting consumes about 17-20% of the globally produced electricity (Zissis, 2016), with public lighting accounting for approximately 2.3% (Lobão et al., 2015; Zissis, 2016). This way, electrical lights contributes a substantial 6% to our carbon dioxide emissions, promoting climate change (United Nations Environment Programme, 2013).

On top of the economic cost and the carbon footprint, light also acts as a sensory pollutant. Generally, we distinguish between two types of light pollution: astronomical light pollution and ecological pollution (Longcore & Rich, 2004). Astronomical light pollution entails the concealment of the night sky and celestial bodies (Longcore & Rich, 2004), which hampers astronomy but is also seen as a cultural loss for humankind (Varela Perez, 2023). Ecological light pollution describes the obscuration of the natural light-dark cycle and celestial cues, which is associated with a variety of ecological disturbances (Longcore & Rich, 2004).

1.4. Ecological light pollution

The spinning movement of planet Earth creates a rhythmic pattern of light and darkness. This roughly 24-hour cycle also shows seasonal variation. On top of that, the moon adds its own illuminance cycle, transitioning from the low-light conditions of the new moon to the high brightness of the full moon. Life that emerged on Earth evolved under these natural light-dark patterns, making light a central part in the functioning of organisms. Light became a key resource in vital processes, such as photosynthesis. However, darkness is equally essential, for example, to recover from oxidative stress or ozone damage (Queval et al., 2007; Vollsnes et al., 2009). Furthermore, the light-dark cycle sustains the circadian and circannual rhythms of species, timing a myriad of

internal and behavioural processes (Johnsson, 2008). The temporal niches provided by these dark and light periods allow for niche partitioning between species, helping to avoid competition and predation (Gutman & Dayan, 2005; Jones & Rydell, 1994). Light also has an important role in the spatial orientation of species (Deutschlander et al., 1999; Fabian et al., 2024; Smolka et al., 2016). Finally, light is an essential component in visual perception (Briolat et al., 2021).

When ALAN emerged, the natural light-dark cycle blurred and new light sources appeared, affecting the temporal, spatial and spectral signature of light (Gaston et al., 2013). Since natural light is at the basis of biological organisation, ecological light pollution has disrupted a myriad of processes. Many genetic, physiological and behavioural consequences have been found on an individual level (e.g. Khan et al. 2018; Elgert et al., 2020; Altermatt and Ebert, 2016), which in turn can influence the distribution and abundances of populations (e.g. Duarte et al., 2019; Van Langevelde et al., 2017) and alter community compositions and food web interactions (e.g. Knop et al., 2017; Davies et al., 2015; McMunn et al., 2019).

Evidence from both terrestrial and aquatic systems shows that ALAN affects a broad diversity of organisms, from microorganisms (e.g. Ayalon et al., 2021; Bari, 2024; Li et al., 2024; Maggi et al., 2020) to plants (e.g. Cieraad et al., 2023; Meng et al., 2022), fungi (e.g. Wang et al., 2024) and animals, invertebrates (e.g. Cai et al., 2025; Gao et al., 2024; Gomes, 2020; Jahnsen-Guzmán et al., 2025; Willmott et al., 2019) and vertebrates (e.g. Dominoni et al., 2020; Forsburg et al., 2021; Hermans et al., 2024; Pulgar et al., 2019; Thawley & Kolbe, 2020). However, the most negative consequences of ALAN are most likely to be found in nocturnal species, as their traits have been tailored to darkness (Ditmer et al., 2021; van Langevelde et al., 2018). This is substantial, as 30% of vertebrates and more than 60% of invertebrates are nocturnal (Hölker et al., 2010).

1.5. Impact on predator-prey interactions

This thesis focusses on the impact of ALAN on predator-prey interactions. Predation is an ecological interaction in which one organism kills and consumes all or a substantial fraction of another organism (Abrams, 2000). Through this interaction, predators and prey strongly influence each other's fitness, as predator-prey dynamics shape individual survival, recruitment, behaviour, habitat choice, foraging, growth, energy allocation, and ultimately species distributions and community interactions (Beauchamp, 2007). Given its direct effects on fitness, the behaviours and morphological traits that determine the outcomes of predator-prey interactions are often subject to strong natural selection (Abrams, 2000).

Because predator-prey interactions are key components of food webs, a good understanding of the effects of ALAN on predator and prey activities and behaviours is important to understand community-level consequences of light pollution. Previous research has shown that ALAN can cause varying outcomes in predator-prey interactions depending on the system at hand. While several studies reported an increase in predation (Mcmunn et al., 2019; Miller et al., 2017; Nuñez et al., 2021), others reported decreased predation under ALAN conditions (Eckhartt & Ruxton, 2022; Jackson et al., 2025). In addition, there were cases where ALAN did not impact the amount of predation (Amoroso, 2024; Grenis et al., 2015). Thus, outcomes of ALAN on these species interactions are context and species specific (Seymoure et al., 2023).

The main mechanisms by which ALAN can directly cause shifts in predator-prey interactions are through its impact on spatio-temporal niche overlap, alterations in visual ecology and behavioural interference.

1.5.1. Shifts in spatial niche overlap

ALAN acts as a spatial organiser. While certain species move towards light (Charvalakis et al., 2025; Eccard et al., 2018; Rebke et al., 2019; Spoelstra et al., 2017; Willmott et al., 2019), others move away from it (Berger et al., 2020; Eccard et al., 2018; Sanna et al., 2024; Spoelstra et al., 2017; Vowles & Kemp, 2021). Thereby, ALAN can greatly influence the spatial niche overlap between species, which is essential for them to interact. Several mechanisms can be at play.

Firstly, ALAN can disorient animals. Many species use light for their spatial orientation, so ALAN can act as a false spatial cue, misguiding species and causing them to inadvertently end up in the light sphere. This mechanism likely at least partly explains the capturing effect of artificial lights, evident in both nocturnally migrating birds and insects congregating around light sources (Charvalakis et al., 2025; Rebke et al., 2019). Insects from different orders tilt their dorsum towards the light (Fabian et al.,2024). Under normal conditions, i.e. under a naturally lit sky, this behaviour helps insects to stay upright. However, near artificial light sources this behaviour leads to continuously steering around the light. For birds, ALAN has the potential to interfere with all their main orientation strategies, including solar, stellar, and magnetic orientation (Adams et al., 2021).

Secondly, light can be associated with positive or negative fitness outcomes, thereby actively attracting or deterring species. For many, often nocturnal species, light is likely associated with an increased predation risk (Prugh & Golden, 2014). Therefore, these prey species prefer darkness and are deterred by ALAN (Spoelstra et al., 2017). Their ALAN-triggered alertness may also make them hide more and avoid open areas

(Dissegna & Chiandetti, 2025; Rotics et al., 2011), adding another layer to this spatial filtering beyond phototaxis.

On the other hand, ALAN can be attractive because of the associated foraging gains. Certain taxa can benefit from the local increases in light-attracted insects around a light source (González-Bernal et al., 2016; Heiling, 1999; Hermans et al., 2024). Others can benefit from the increased search efficiency in a lit foraging patch (Santos et al., 2010).

There are likely many more positive or negative phototactic behaviours that may be affected by ALAN, such as light-associated mating, temperature or moisture regulation, etc. (Kadochová et al., 2019; Seiger & Woodruff, 1987; Togashi et al., 1999). However, throughout this thesis I focus only on the mechanisms described above, as these are the most likely to explain our observations.

1.5.2. Shifts in temporal overlap

Light partitions the day into several temporal niches, creating distinct activity periods: diurnal (day-active), nocturnal (night-active), crepuscular (active during twilight), and cathemeral (active at irregular intervals during both day and night) (Seymoure et al., 2023). Many species have evolved distinct activity patterns that minimise predation risk by exploiting temporal niches in which their predators are less active or less efficient (Cunningham et al., 2019; Helm et al., 2017). Since ALAN affects circadian rhythms (Dominoni et al., 2022; Duarte et al., 2019), shifts in predator-prey interactions are likely to occur.

ALAN has created the so-called night light niche (Longcore & Rich, 2004; Schwartz & Henderson, 1991). For example, predators which are usually limited to daytime activity can extend their foraging into the nighttime with ALAN. Evidence for this night light niche comes from a taxonomically diverse range of predators (Dwyer et al., 2013; Frank, 2009; Lebbin et al., 2007; Maurer et al., 2019; Miller et al., 2017). Diurnal species that exploit the night light niche might also become prey for nocturnal predators that are not deterred by the light (Henke et al., 2022). Conversely, the activity of nocturnal species might be delayed or inhibited, reducing predator-prey encounters (Matsushige & Hibino, 2023; Moyse et al., 2023; Stone et al., 2009).

1.5.3. Impact on visual ecology

ALAN can alter the visual capacity of species and play an important role in the visual ecology of predator and prey, thereby affecting foraging efficiency and prey selection (Briolat et al., 2021; Cole et al., 2019). This is especially true for diurnal species that mainly rely on their well-developed photopic vision. For them, ALAN can be a useful

tool allowing them to extend their activity into the night (Cerveira et al., 2019). However, the prey detection of some nocturnal species may also increase when they use vision as one of their sensing modalities (Amoroso, 2024; Passarotto et al., 2025).

As well as influencing visual acuity, ALAN can affect colour discrimination in predators. Broad-spectrum light in particular can have a profound impact on colour perception (McMahon et al., 2022). As such, ALAN can affect the effectiveness of camouflage (McMahon et al., 2022; Moyse et al., 2023). For example, the relative predation rate by intertidal crabs on two cryptic colour morphs of aquatic snails switched under ALAN compared to control nights (Moyse et al., 2023). Although less studied in the context of ALAN, the effectiveness of aposematic and deflective colour patterns depends on natural light conditions (Nokelainen et al., 2022; Olofsson et al., 2010; Rojas et al., 2014), suggesting that these strategies are also likely impacted by ALAN. Furthermore, alternative colour-based foraging preferences developed under different artificial light environments can become fixed in populations after several generations of exposure (Cole et al., 2019), indicating that ALAN can exert strong selection pressures.

ALAN can also affect selection on colours in prey, which can be especially important for those that use camouflage. To be less conspicuous, cryptic prey benefit from a matching background, either by a proper background choice or by adapting their own colours to the background. However, ALAN can impede this process, making the prey more conspicuous in their environment. For example, the marine isopod *Ligia Oceanica* (Ligiidae) sought out darker backgrounds more often under diffuse ALAN conditions, while they themselves turned lighter (Bullough et al., 2023).

1.5.4. Behavioural interference

Finally, ALAN can interfere with the behaviours of both predator and prey, altering their performance (Minnaar et al., 2015). A well-known example of such behavioural interference is the impairment of evasive flight manoeuvres in eared moths under ALAN conditions (Minnaar et al., 2015; Wakefield et al., 2015). Under normal conditions, tympanate moths initiate last-ditch evasive manoeuvres after detecting the echolocation calls of hunting bats, but under ALAN conditions they often fail to do so. A possible explanation for this interference could be that this defensive reaction is not adaptive during daytime when bats are not active and that this behaviour is therefore limited under ALAN conditions (Svensson et al., 2003). Other forms of behavioural interference are also possible. For example, nocturnal wolf spiders start moving later under ALAN, which can impede their foraging ability (Amoroso, 2024). A similar freezing effect has been found in isopod prey under ALAN, where a postponed escape could increase its vulnerability to predation (Dissegna & Chiandetti, 2025). All these behavioural alterations can greatly impact the efficiency of both predator and prey.

2. Outline of the thesis

The general aim of this thesis was to investigate the impact of ALAN on predator-prey interactions. Specifically, we examined how ALAN affects the spatio-temporal patterns of activity, behaviours, and visual ecology of predators, which can ultimately alter predation pressures.

I aimed to answer following questions:

- Does ALAN affect the activity patterns of predators and prey?
- Does ALAN affect the spatial distributions of prey and predators?
- Does the impact of ALAN depend on the visual ecology of species?
- Does ALAN alter predation pressures on insect prey?

This work focused on insect prey, as insects occupy a central position in the food web. Insects serve both as prey for higher tropic levels, and affect plants through pollination, herbivory and detritivory (Hassell & May, 1986; Mattson & Addy, 1975; Ollerton et al., 2011; Yang & Gratton, 2014). Alterations in insect communities are therefore likely to have a wide impact on the food web. Current severe insect declines (Hallmann et al., 2017) emphasise the need for a more comprehensive understanding of their challenges, and the development of effective conservation strategies. Insects are impacted by a wide range of stressors, including climate change, chemical pollution, alien species, habitat conversion and fragmentation (Sánchez-Bayo & Wyckhuys, 2019). Although light pollution has only recently been recognised as one of these major stressors, it has the potential to greatly affect insect populations by altering their foraging, movement, reproduction, and susceptibility to predation (Boyes et al., 2021; Grubisic et al., 2018; Owens et al., 2020; van Langevelde et al., 2018).

Throughout the different chapters, I focused on taxonomically diverse predator groups, including avian, arthropod, and mammalian predators. This diversity helped to assess which responses are generalisable and which are more species, or taxa, specific.

In **Chapter 2**, I explored potential changes in caterpillar behaviour under dark and ALAN conditions, which could influence predation rates on these prey. To assess whether and how ALAN would affect species with different adult circadian rhythms and caterpillar anti-predator colourations, I observed the behaviours of three different species: the night-active and cryptic *Mamestra brassicae* (Noctuidae), the day-active and aposematic *Pieris brassicae* (Pieridae), and the day-active, cryptic *Pieris napi (*Pieridae). In this way, I determined how these traits affect the response to ALAN exposure.

In **Chapter 3**, I explored predator-prey behaviours and predation pressure under both dark and ALAN conditions. Given that the nocturnally active, cryptic *M. brassicae* was most affected by ALAN, as established in **Chapter 2**, I subjected this species to predation by the avian predator *Parus major* (Paridae) in a controlled laboratory environment under both dark and ALAN conditions. In addition, to further determine the impact of anti-predator colouration, I conducted a field experiment using artificial dummy caterpillars with different colour patterns, which were placed along dark and ALAN transects.

In **Chapter 4**, I examined how ALAN may influence an arthropod predator: the jumping spider *Marpissa muscosa* (Salticidae). I first investigated the impact of different ALAN conditions on the activity periods of this diurnal species in the absence of prey. I then added prey to examine whether altered activity patterns could lead to nocturnal foraging by this diurnal, visually oriented predator. Since I found that increased nocturnal activity in the spiders was triggered at higher light intensities than those typically found in caterpillar habitats, I used fruit flies instead of caterpillars as prey in this experiment.

In **Chapter 5**, I focused on flying insect prey and bats as mammalian predators to examine how ALAN impacts the spatio-temporal activity patterns of both *Pipistrellus spp.* (Vespertilionidae) bats and flying insects. Bats are known to be highly impacted by light. However, some bat species, including pipistrelle bats, are known to take advantage of light-clustered insects. In this field study, Sander Buddendorf and I investigated whether and how much pipistrelle bats exploited light-attracted insects in highly urbanised settings. We also dimmed streetlights to assess whether dimming light could effectively minimise the disturbance caused by ALAN to both bats and insects. For data collection, we employed interval photography to monitor insect activity, complemented by automated bat recording.

In **Chapter 6**, we validated an open-source object detection model that was trained by Gabriel Charvalakis, whereby we used the manually processed insect data and pictures from **Chapter 5** as an independent validation dataset. This way, we investigated the comparability of manual and automated detection methods and assessed the significance of ambient light conditions and temporal detail for the performance of the model.

Finally, in **Chapter 7**, I integrated all findings to place them in a wider scientific context and synthesised the main implications of my results.

With this work, I aim to advance our scientific understanding on the ecological impacts of ALAN and provide evidence-based insights that can guide conservation strategies to mitigate the harmful effects of ALAN on declining insect populations.

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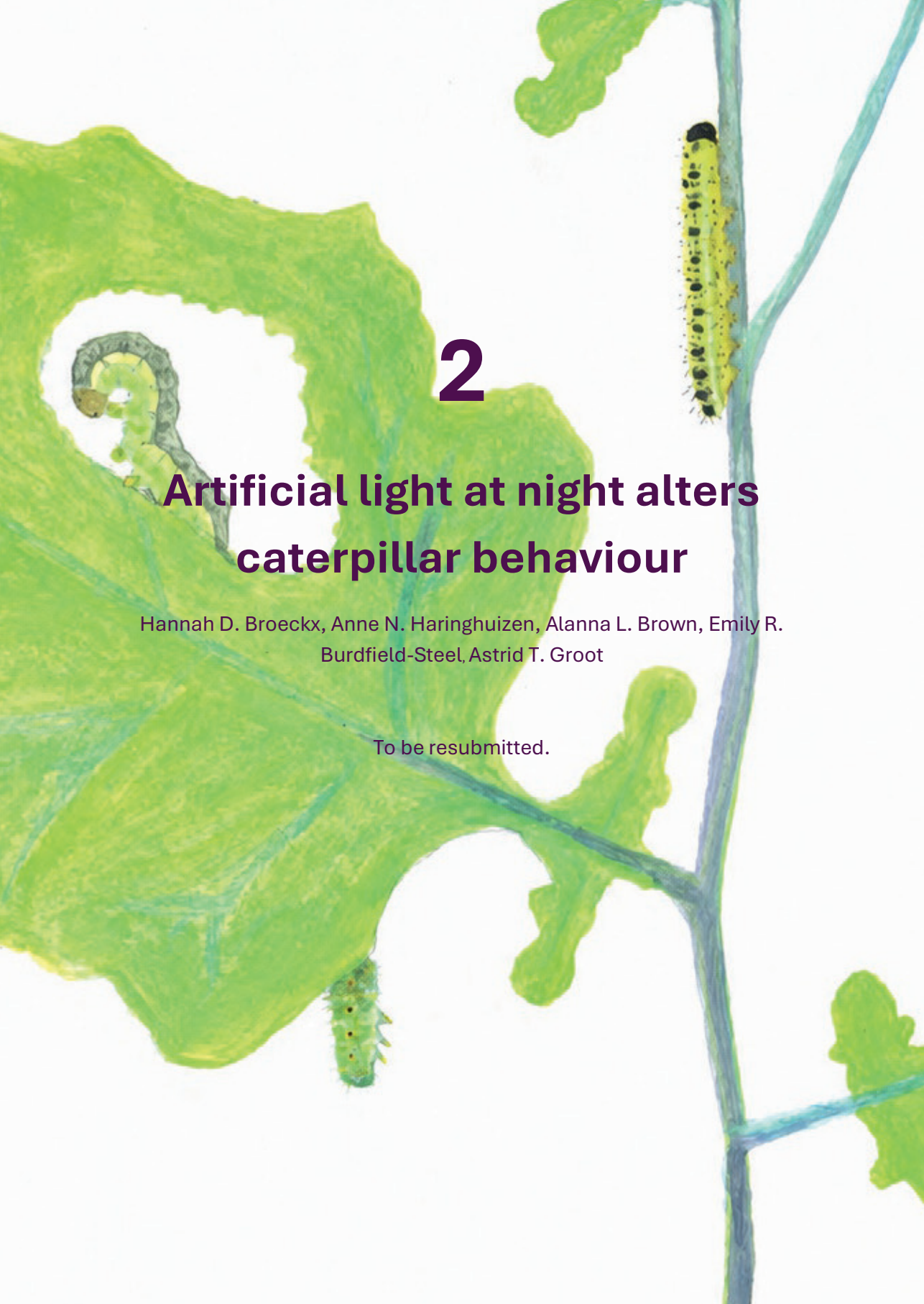
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2

Artificial light at night alters caterpillar behaviour

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1. Abstract

Anthropogenic light at night (ALAN) is increasingly recognised as a driver of arthropod declines. In this study, we tested whether dim light pollution (1.5 lux) affects larval behaviour of three lepidopteran species with differing larval anti-predator colouration and adult activity periods: *Mamestra brassicae* (cryptic, nocturnal), *Pieris napi* (cryptic, diurnal), and *Pieris brassicae* (aposematic, diurnal). Since little is known about the activity periods of larval stages, we first examined whether the larval activity periods align with those of the adult stages. Single caterpillars were observed on *Brassica nigra* plants hourly, noting activity (resting, feeding, moving) and position (side of the leaf, half of the plant). *P. napi* larval activity did not align with adult patterns, unlike for the other two species. Instead, larval activity seemed more closely linked to the colouration of the larvae: cryptic caterpillars were primarily nocturnal, while the aposematic species was diurnal. Furthermore, all three species were affected by ALAN, with differing responses. Under dim light, *P. brassicae* caterpillars were more often on the upper side of leaves compared to dark conditions. *P. napi* caterpillars became more active overall, while *M. brassicae* caterpillars became less active and were found more on leaf undersides and plant tops. Additionally, we tracked feeding activities on differently orientated leaf disks. In these experiments, we found an overall increase in nocturnal feeding by all three species under dim light conditions. This study thus shows that even dim ALAN can alter caterpillar behaviour, which potentially impacts their fitness and exposure to predators.

2. Keywords

Artificial light at night, Caterpillar behaviour, Temporal niches, Larvae, Lepidoptera

3. Introduction

Since life emerged on earth, light has been one of the most stable external cues to synchronise behaviours and physiological processes of organisms (Sharma & Chandrashekaran, 2005). The light cycle has been a strong selective force for a myriad of traits (e.g. visual, morphological, navigational) (Seymoure et al., 2023). With advancing technologies and expanding urbanisation, anthropogenic light at night (ALAN) has become a significant environmental stressor in recent decades, continuing to grow in both surface area and intensity (Kyba et al., 2017, 2023). Anthropogenic light now appears at unusual times and places, with unprecedented intensities and spectra (Falchi et al., 2016). This has distorted the natural light cycle, leading to disruptions on all levels of biological organisation (Boyes et al., 2021a; Grubisic & van Grunsven, 2021; Knop et al., 2017; Sanders et al., 2021). A disruptive effect of ALAN has been found on a wide range of taxa (Sanders & Gaston, 2018).

In arthropods, light pollution is now recognised as one of the main drivers of species declines (Grubisic et al., 2018; Owens et al., 2020). In general, nocturnal and crepuscular species are thought to be most vulnerable to ALAN (Ditmer et al., 2021; van Langevelde et al., 2018). For example, van Langevelde et al. (2018) showed that lepidopteran species with light-sensitive traits, such as positive phototaxis and nocturnality, show stronger population declines than species without these traits. However, negative impacts of ALAN are also expected for diurnal species (Seymoure, 2018). In addition, ALAN can impact temporal niches directly. In some diurnal taxa, such as jumping spiders and parasitoid wasps, ALAN has led to temporal niche expansion (Frank, 2009; Gomes et al., 2024), while the temporal niches of nocturnal taxa, such as parasitic beetles and Geometrid moths, have been contracted (Jiang et al., 2023; van Geffen et al., 2015).

Whether arthropods are classified as nocturnal or diurnal is mostly determined by adult activity patterns (Kawahara et al., 2018). Studies focusing on activity periods of larval stages are still limited (Niepoth et al., 2018). However, caterpillars have a central position in the food web, as they are important herbivores and form a valuable food source for a large range of invertebrate and vertebrate predators (Clark et al., 2016; Perrins, 1991; Seress et al., 2018), as well as parasitoids (Stireman & Shaw, 2022). Therefore, it is important to assess the impact of ALAN across life-history stages, including the larval stage.

So far, ALAN has been shown to negatively influence the larval mass of nocturnal Lepidoptera, either directly or by reducing plant palatability (Grenis & Murphy, 2019; Van Geffen et al., 2014). ALAN can also inhibit larval diapause (Merckx et al., 2023; Van Geffen et al., 2014). In addition to physiological changes, behavioural alterations in

larvae have been reported. For example, caterpillars of the diurnal monarch butterfly (*Danaus Plexippus,* Nymphalidae) increased their feeding rate twofold when exposed to light compared to dark conditions (Haynes et al., 2023). In addition, bright light increased the hiding behaviour of caterpillars of the nocturnal northern armyworm (*Mythimna separata*, Noctuidae), although this was due to a change in emissions of light-dependent plant volatiles (Shiojiri et al., 2006). In the field, negative effects of light on local populations have also been shown, as nocturnal caterpillars were less present in lit sites than in unlit sites (Boyes et al.,2021b).

The effects of ALAN on caterpillars likely depend on how colourful they are. As a protection against natural enemies, caterpillars can have different anti-predator colours, such as cryptic and aposematic colouration. In cryptic species, their colour matches their habitat background (Stevens & Merilaita, 2011). In aposematic species, their colour stands out, and their increased conspicuousness advertises their unpalatability (Mappes et al., 2005). The effectiveness of these anti-predator colours relies on caterpillar behaviours. For example, an increased exposure of aposematic species to predators can benefit their survival, probably because the clear display of the aposematic signal reduces recognition errors by the predators (Nielsen & Mappes, 2020). In contrast, sitting still, hiding or finding a good background are more opportune behavioural strategies for cryptic species (Heinrich, 1979; Ioannou & Krause, 2009; Kang et al., 2015). As an organism's level of conspicuousness is affected by light (Nokelainen et al., 2022; Rojas et al., 2014), ALAN is likely to have a different impact on cryptic and aposematic caterpillars and thus also on their behaviours. Understanding these different effects is crucial given the caterpillars' central role in the food web.

In this study, we investigated the larval activity periods and the effects of ALAN on the activities and position choices in three lepidopteran species which differ in their adult activity periods and in their caterpillar colourations: the night-active, cryptic *Mamestra brassicae*, the day-active, cryptic *Pieris napi*, and the day-active, aposematic *Pieris brassicae*. As these species exhibit key differences in their anti-predator colouration and adult activity period, we expected to find different responses to ALAN. Under the hypothesis that larvae and adults have the same activity periods, ALAN would reduce activity in the nocturnal *M. brassicae* larvae, while increasing activity in the diurnal *P. brassicae* and *P. napi* larvae. However, since it was unclear whether the larvae exhibit activity patterns similar to their adult stages, we first analysed the activity rhythms of the larval stages to determine if they align with those of the adults. Furthermore, we expected that the cryptic species, *M. brassicae* and *P. napi*, would exhibit more frequent hiding behaviour (e.g. staying at the base of the plant or residing on the lower side of the leaf) under ALAN conditions, while the aposematic species, *P. brassicae,* would show itself more when exposed to ALAN.

4. Material and methods

4.1. Study species

To assess the effect of ALAN on the behaviour of caterpillars, we tested three species: *M. brassicae* (Lepidoptera, Noctuidae), *P. brassicae* (Lepidoptera, Pieridae), and *P. napi* (Lepidoptera, Pieridae). All three species feed on *Brassica* plants, so their feeding behaviours could be compared on the same plant species.

Eggs of the three species were obtained from laboratory populations reared at Wageningen University & Research (WUR), the Netherlands. All species had already been reared for several generations at 21°C, 60% humidity, and a 16L:8D light regime. The eggs were transported to the University of Amsterdam, the Netherlands, where they were further reared under constant climate room conditions (21°C, 60% humidity, and a 16L:8D light regime). The eggs were immediately assigned to one of the light treatments, being exposed to either dark (0 lux) or light (1.5 lux) night conditions. For the experiments, both 2nd and 3rd instar caterpillars (6-10 days post hatching) were used.

4.2. Plants

Experiments were conducted with *Brassica nigra* (Brassicaceae) plants. These plants were grown from seeds collected from a field population in Wageningen, in 2012. Plants were cultivated under constant greenhouse conditions (23 degrees, 18L:6D, and 40% humidity) for 5-8 weeks, ensuring similar heights between trials. Initially, the plants used in the first two trials received a 16L:8D regime for three weeks. After that, we switched to 18L:6D to promote plant growth. However, three days prior to the experiment, all plants were placed in the 16L:8D regime, where one group was exposed to dim light at night, the other not, allowing them to acclimate.

4.3. Experimental setup

4.3.1. Caterpillar behaviour on plants

To investigate the larval rhythmicities and the impact of ALAN on the behaviours of the three caterpillar species, we conducted an observational study, whereby caterpillars were allowed to roam freely on *Brassica nigra* plants. While one group experienced a completely dark night, referred to as the dark treatment, the other group was exposed to dim nocturnal light, i.e. 1.5 lux, to which we refer as the light treatment. The light level of 1.5 lux was selected as it represents a more dim light intensity level commonly encountered in vegetation in the broader vicinity of streetlights, where caterpillars

could be found (*personal measurements*; Boyes et al., 2021b). Dim light levels were obtained using Strips of LED lights (RexTech, the Netherlands; 5500K) of which individual LEDs were covered with aluminum tape to obtain 1.5 (± 0.05) lux at shelf height, 50 cm below the light source. Light levels were measured using a LXP-10 luxmeter (Sonel, Poland). Spectral measurements were made with a spectrophotometer (QE Pro High Performance Spectrophotometer; Ocean Insight, United States) (Fig. S1).

Four trials were conducted, two in the summer of 2023 and two in the winter of 2024. In each of these trials, 10 caterpillars of each species were selected for each treatment. We placed one caterpillar on one plant. To avoid escapes, plant pots were placed in trays filled with water. Caterpillars of the three species were randomly ordered on the shelves of the climate room. During the experiment, the climate room conditions were kept to a constant 21 degrees, 60% humidity and a 16:8 light regime, with dark or dim night light conditions. Daytime illumination was provided by MASTER TL-D Super 80 fluorescent tubes (Philips, the Netherlands; 4000 K; suppl. material).

Over the course of a 24-hour trial, hourly observations were made during which caterpillar behaviour and position was recorded. Once a caterpillar was located (search time max. three minutes), it was observed for a few (1-10) seconds. We categorised behaviour into three types: feeding (= moving mouth parts), moving (= body in motion) or resting (= no movement). For the positioning of the caterpillars on and within the plants, we noted their position on the plant, discriminating between the upper and lower half of the plant, and position on the leaf, i.e. upper or lower side of the leaf. In both treatments, caterpillars were checked using red headlamps. Red light was used to minimise disturbance to the caterpillars, as the larval stemmata generally do not contain red receptors (Ichikawa & Tateda, 1982; but see Angersbach, 1975).

Caterpillars that escaped from the plant pot were placed back on the plant (Table S1). When caterpillars were not found anywhere during two consecutive rounds of observation, a new caterpillar was placed on the plant (Table S2). Some *P. napi* and *M. brassicae* caterpillars were found moulting (*P. napi*: N = 4; *M. brassicae*: N = 10) during the experiment. These individuals were not excluded from the analysis, but the possible (interaction) effect was explicitly tested in the models. An overview of the total amount of observations can be found in the supplementary material (Table S3 & S7).

4.3.2. Caterpillar feeding on leaf disks

Feeding is an important larval behaviour, which typically occurs in short feeding bouts (Reynolds et al., 1986). Hourly checks do not fully capture this behaviour. Therefore, we set up an additional experiment to test the effect of ALAN on feeding more thoroughly. Here, we examined the effect of ALAN on the feeding of the three species

using standardised, differently oriented leaf disks, i.e. either the upper side or the lower side of the leaf facing upwards. The orientation of the leaves was altered to test for a preference for one leaf side over the other, under conditions where no side offered differential hiding.

Individual caterpillars were placed in single cups (93 mm diameter, 17 cm high) containing six *Brassica nigra* leaf disks (24 mm diameter). The leaf disks were placed in a circle, alternating the lower or upper side of the leaf. To ensure that the leaves would stay in place and moist, leaf disks were pressed into a semi-solidified agar substratum (Agar-Agar Kobe I, Carl Roth: 3.2 g agar per 100 mL demineralised water). This bottom layer was completely hardened during the experimental period. To prevent escapes, the rims of the cups were covered with a thick layer of Vaseline. If caterpillars did manage to escape, they were placed back in the centre at the next check round.

At the start of the experiment, we placed a single caterpillar in the centre of the cup. In total 10 caterpillars of each species (*P. brassicae*, *M. brassicae* and *P. napi*) were used per treatment. The two light treatments were the same as described above.

The eaten leaf surfaces were monitored over the course of 24 hours, using Technaxx Mini TX-117 cameras (Technaxx, Germany), with infrared night vision. To improve focus, lenses of reading glasses (+3.00) were placed in front of the camera lens. We calculated the surface area of the leaf disks from the pictures using ImageJ software. Surface areas were calculated at four time points: hour 0 (start of the experiment), hour 11 (right before the lights turned off), hour 19 (right after the lights turned back on) and hour 24 (end of the experiment).

4.4. Statistical analyses

All statistical analyses were performed in R, version 4.4.0 (R Core Team, 2024). Models were made, using the glmmTMB package (Brooks et al., 2017). For each model, model assumptions were tested, using the DHARMa package (Hartig et al., 2024). Full and reduced models were compared by looking at the Akaike information criterion (AIC) of each model. Fixed factors were removed from the model if the AIC value of the model including the factor was not significantly better than the model without the factor (< Δ2 AIC). Since the effect of treatment and time of day were our main interest, these factors were always kept in the model. When the interaction term or a fixed effect with more than two levels (species, activity) was significant, a post-hoc pairwise comparison was made, using emmeans (Lenth et al., 2025). Visualisations were made, using ggplot2 (Wickham, 2016).

4.4.1. Caterpillar behaviour on plants

Since we were mainly interested in the individual species responses rather than the direct comparisons between species, all analyses were made for each of the three species separately. All behaviours (movement, resting and feeding, plant position, leaf side preference) were analysed using generalised linear mixed models with binomial distributions. For each activity type model, the full model consisted of treatment, time of day, moulting, the interaction between treatment and time of day and the interaction between treatment and moulting as fixed factors. To assess larval rhythmicity, we again analysed the amount of resting, but only with the data from the dark treatment as this represented the natural situation. Therefore, the model only contained time of day and moulting as fixed factors. For the caterpillar position models, the full model consisted of treatment, time of day, moulting, activity type, the interaction between treatment and time of day, and the interaction between treatment and moulting as fixed factors. First, we checked for problematic multicollinearity between time of day and activity, using the standardised generalised variance inflation factor (GVIF^(1/(2*Df)) < 5). Trial (= one 24-hour observation period) and caterpillar ID were included as random factors in all models. An overview of all tested and final models can be found in the supplementary material (Table S4 & S8-S10).

4.4.2. Caterpillar feeding on leaf disks

To analyse the percentage of eaten leaf surface, a generalised linear mixed model was constructed for day and night separately. Day and night data were analysed separately, since the unequal 16:8 light regime would otherwise cause problems due to the accumulative nature of the percentage of eaten leaf surface. The Tweedie distribution was used to analyse this percentage data, as this distribution gave the model the best fit. The Tweedie distribution is one of the more flexible distributions within the exponential dispersion family, as it is based on three parameters: mean, dispersion, power (Jorgensen, 1997; Miller et al., 2013 (suppl. material)). It is a suitable distribution for a dataset with positive values and zeros (Kurz, 2017). Because of the small sample size, we did not analyse the responses in separate model per species. Therefore, both full models included leaf side, treatment and species as main fixed effects as well as their pairwise interactions. Caterpillar ID was included as random factor. An overview of all tested and final models can be found in the supplementary material (Table S17).

4.5. Ethical note

The lepidopteran species, *P. brassicae*, *P. napi* and *M. brassicae*, used in the experiments are not under ethical regulation. They were all derived from stock cultures from Wageningen University & Research (WUR), the Netherlands. We used noninvasive

handling methods and interference was kept to a minimum. Caterpillars were able to feed at all times and could roam around within the context of the experiment. After completion of the experimental procedure, caterpillars were euthanised by freezing.

5. Results

5.1. Caterpillar behaviour on plants

5.1.1. Activity period of caterpillars (Fig. 1, Table S3-S6)

We found that in the dark treatment, which simulates the natural situation, the aposematic *P. brassicae* caterpillars were more active during the day than during the night ($\chi^2(1, N = 954) = 30.06$, $p < 0.001$), while the cryptic *M. brassicae* and *P. napi* caterpillars were more active at night than during the day ($\chi^2(1, N = 947) = 10.98$, $p < 0.001$; $\chi^2(1, N = 959) = 4.05$, $p < 0.05$, respectively).

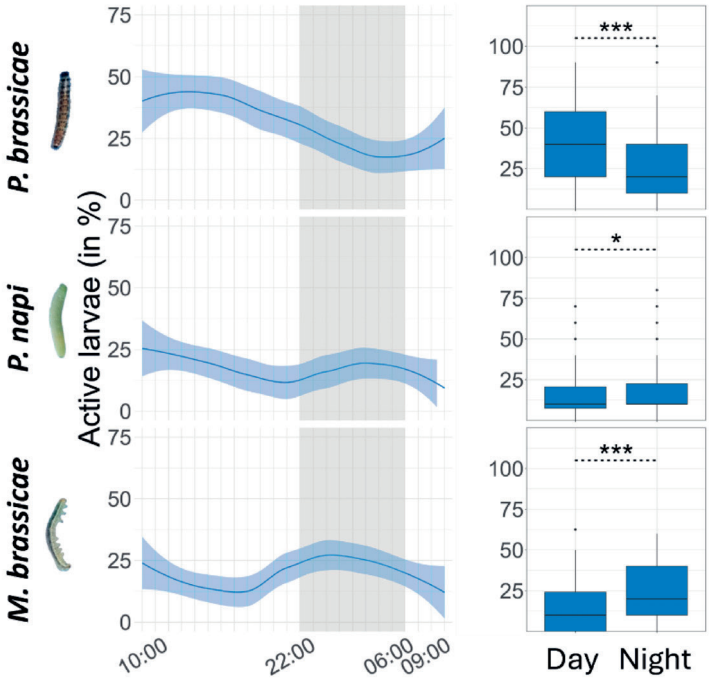


Fig. 1. The activity patterns of aposematic *P. brassicae* (top), cryptic *P. napi* (middle), cryptic *M. brassicae* (bottom) in the dark treatments on complete plants. Left: the percentage of active caterpillars per check round, which was performed at each hour of the day (starting at 10 a.m.). The main line shows the smoothened trend over time, with the coloured 95% confidence interval

around it. Temporal patterns have been added for visualisation, but were not further analysed. Right: Boxplots of the percentages of active larvae, separated between day and night. Only significant differences are shown, *: $p < 0.05$, ***: $p < 0.001$.

5.1.2. Caterpillar activity on plants (Fig. 2, Table S7-S12 & S14-S16)

We found that the day-active, aposematic *P. brassicae* caterpillars did not show any behavioural changes in response to the light treatment (feeding: $\chi^2(1, N = 1854) = 0.15$, $p = 0.69$; moving: $\chi^2(1, N = 1854) = 1.10$, $p = 0.29$; resting: $\chi^2(1, N = 1854) = 0.35$, $p = 0.56$). In both treatments, the caterpillars moved more and generally rested less during the day than during the night (moving: $\chi^2(1, N = 1854) = 4715$, $p < 0.001$; resting: $\chi^2(1, N = 1854) = 44.80$, $p < 0.001$), while feeding was constant over night and day in both treatments ($\chi^2(1, N = 1854) = 1.02$, $p = 0.31$).

The night-active, cryptic *P. napi* caterpillars did show differences in behaviours under the light compared to the dark treatment: at night they moved more ($z = -2.74$, $p < 0.05$), during the day they fed more ($z = -2.58$, $p < 0.05$) in the light than in the dark treatment. Overall resting behaviour was not significantly affected by light ($\chi^2(1, N = 1907) = 3.60$, $p = 0.06$). Within the dark treatment, caterpillars fed more during the night than during the day ($z = -4.23$, $p < 0.001$). In both treatments, they rested less at night than during the day ($\chi^2(1, N = 1907) = 4.50$, $p < 0.05$). Caterpillars that moulted during the experiment moved more ($\chi^2(1, N = 1907) = 4.90$, $p < 0.05$).

The night-active, cryptic *M. brassicae* caterpillars rested more in the light treatment compared to the dark treatment ($\chi^2(1, N = 1867) = 5.72$, $p < 0.05$). However, the light treatment did not have an effect on moving or feeding, as the caterpillars moved and fed similarly in both treatments ($\chi^2(1, N = 1867) = 1.70$, $p = 0.19$; $\chi^2(1, N = 1867) = 3.76$, $p = 0.05$, respectively), which was more during the night than during the day (moving: $\chi^2(1, N = 1867) = 5.65$, $p < 0.05$; feeding: $\chi^2(1, N = 1867) = 6.03$, $p < 0.01$). In both treatments this resulted in less rest during the night than during the day ($\chi^2(1, N = 1867) = 13.02$, $p < 0.001$). Caterpillars that moulted during the experiment fed less ($\chi^2(1, N = 1867) = 10.65$, $p < 0.001$) and rested more ($\chi^2(1, N = 1867) = 10.21$, $p < 0.01$).

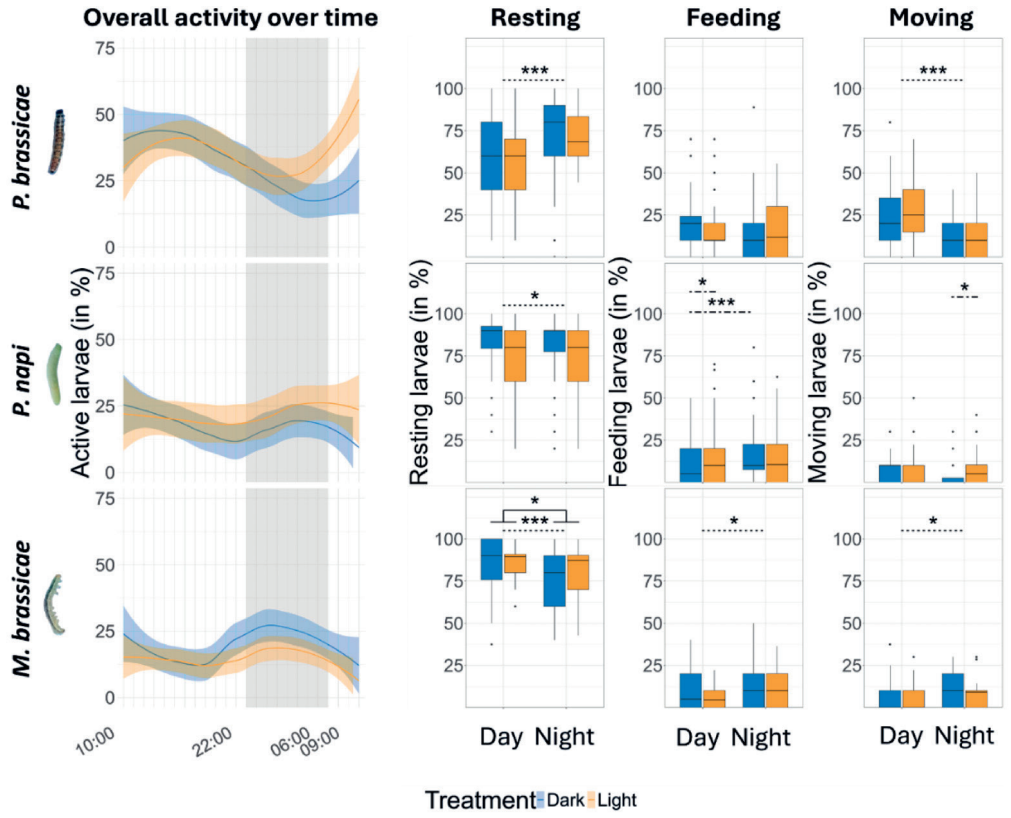


Fig. 2. Behavioural activity patterns of aposematic *P. brassicae* (top), cryptic *P. napi* (middle), cryptic *M. brassicae* (bottom) in the dark and light treatments on complete plants. Left: the percentage of active caterpillars per check round, which was performed at each hour of the day (starting at 10 a.m.). The main line shows the smoothened trend over time with 95% confidence intervals. Temporal patterns have been added for visualisation, but were not further analysed. Right: Boxplots of the percentages of resting, feeding and moving larvae, separated between day and night. Only significant differences are shown, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. Solid lines above the boxplots refer to overall treatment effects. Dotted lines above the boxplots refer to a time of day effect. Double dashed lines above the boxplots refer to an interaction effect between treatment and time of day, showing significant pairwise comparisons.

5.1.3. Caterpillar positioning on plants (Fig. 3, Table S7-S16)

Investigating the position of caterpillars on the plants, we found that ALAN had no effect on the aposematic *P. brassicae* caterpillars ($\chi^2(1, N = 1721) = 0.01$, $p = 0.94$). In both treatments, caterpillars were found more in the upper half of the plant during the night than during the day ($\chi^2(1, N = 1721) = 22.69$, $p < 0.001$). Caterpillars in the top half of the plant were more likely to be resting compared to the other behaviours (feeding: $z = -4.74$, $p < 0.001$; moving: $z = -3.16$, $p < 0.01$). As for their position on the leaves, we

found that caterpillars were more on the upper side of the leaf during the night in the light treatment compared to the dark treatment (z = -5.07, p < 0.001). In the light treatment caterpillars were also found more on top of the leaves during the night compared to the day (z = -2.64, p < 0.05), while the pattern was opposite in the dark treatment where caterpillars were located more on the upper side of the leaves during the day compared to the night (z = 3.65, p < 0.01). Caterpillars found on top of the leaves were found to be resting less compared to the other behaviours (feeding: z = 3.02, p < 0.01; moving: z = 2.35, p < 0.05).

For the cryptic *P. napi* caterpillars the main model indicated a significant interaction between time of day and treatment (χ^2(1, N = 1738) = 4.17, p < 0.05). The trend towards a higher position at night compared to the day that only occurred in the dark treatment (z = -233, p = 0.09) seemed to be driving the initial interaction effect. Caterpillars in the top half were found to be feeding significantly more than that they were resting (z = 2.94, p < 0.01). The interaction between moulting and treatment, as well as the main effect of moulting were kept in the model. However, both terms had a non-significant effect (χ^2(1, N = 1738) = 0.00, p = 1.00, χ^2(1, N = 1738) = 0.00, p = 1.00, respectively). As for their position on the leaves, caterpillars were found on the upper and lower sides of the leaves similarly often in the light and dark treatment (χ^2(1, N = 1719) = 0.00, p = 0.95), both day and night (χ^2(1, N = 1719) = 0.66, p = 0.42).

The cryptic *M. brassicae* caterpillars were positioned in the top half of the plant more often in the light treatment than in the dark treatment (χ^2(1, N = 1843) = 4.96, p < 0.05). Positions within the plant were similar between night and day, in both treatments (χ^2(1, N = 1843) = 0.71, p = 0.40). During nighttime, *M. brassicae* caterpillars were found more on the lower side of the leaf in the light treatment compared to the dark treatment (z = 4.66, p < 0.001). Linked to this, there was only a day-night shift in the light treatment, with more caterpillars on the lower side of the leaves at night compared to the day (z = 4.67, p < 0.001). Caterpillars found on top of the leaves were found to be resting less compared to the other behaviours (feeding: z = 4.08, p < 0.001; moving: z = 4.87, p < 0.001).

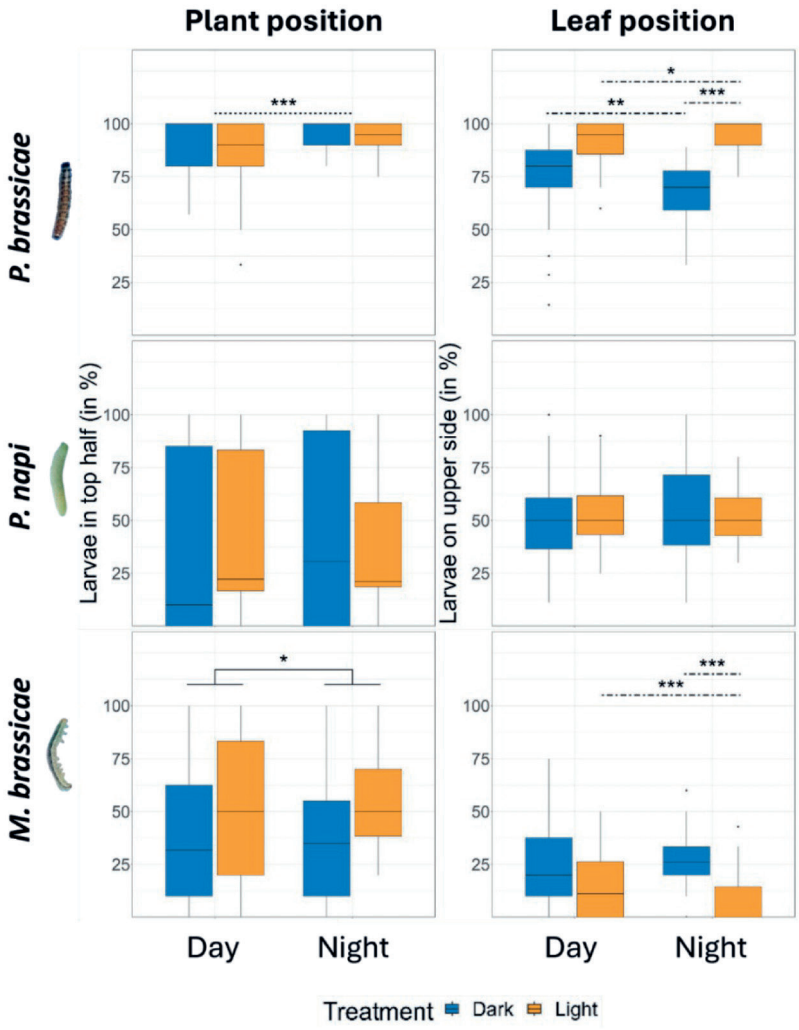


Fig. 3. Positional responses of aposematic *P. brassicae* (top), cryptic *P. napi* (middle), cryptic *M. brassicae* (bottom) in the different light treatments on complete plants, separated by day and night. Plant position refers to the percentage of larvae being in the top half of the plant instead of the bottom half. Leaf position refers to the percentage of larvae being on the upper side of a leaf instead of the lower side. Only significant differences are shown, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. Solid lines above the boxplots refer to overall treatment effects. Dotted lines above the boxplots refer to an overall time of day effect. Double dashed lines above the boxplots refer to an interaction effect between treatment and time of day, showing significant pairwise comparisons.

5.2. Caterpillar feeding on leaf disks

5.2.1. Nighttime feeding (Fig. 4 & 5, Table S17-S20)

In contrast to what we find in the on-plant experiment, we found that all three species of caterpillars observed in the leaf disk experiment fed more in the light treatment compared to the dark treatment ($\chi^2(1, N = 120) = 4.21$, $p < 0.05$). The three species did show differences in feeding ($\chi^2(2, N = 120) = 19.06$, $p < 0.001$), as *M. brassicae* fed significantly less than *P. brassicae* ($z = -4.12$, $p < 0.001$) and *P. napi* ($z = -3.95$, $p < 0.001$), while no significant difference was found between *P. brassicae* and *P. napi* ($z = 0.21$, $p = 0.98$). In addition, all species preferred to eat from the leaves with the upper side facing upwards in both treatments ($\chi^2(1, N = 120) = 9.74$, $p < 0.01$).

5.2.2. Daytime feeding (Fig. 4 & 5, Table S17-S20)

Daytime feeding was not affected by the light treatment ($\chi^2(1, N = 120) = 0.98$, $p = 0.32$). Also during daytime, species differences were found ($\chi^2(2, N = 120) = 32.69$, $p < 0.001$), i.e. *M. brassicae* fed significantly less than *P. brassicae* ($z = -5.67$, $p < 0.001$) and *P. napi* ($z = -4.37$, $p < 0.001$). We found no significant difference between *P. brassicae* and *P. napi* ($z = 1.44$, $p = 0.32$). Similar to the nighttime, all three species preferred to feed from the upper side of the leaves in both treatments ($\chi^2(1, N = 120) = 7.82$, $p < 0.01$).

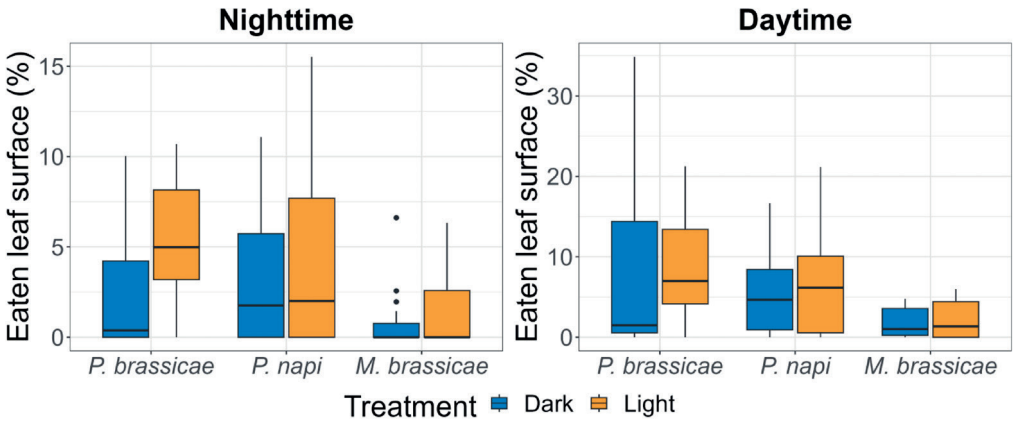


Fig. 4. The effect of the nocturnal light treatment on the percentage of eaten leaf surface during night and daytime, separated per species. Light increased feeding for all species during nighttime ($p < 0.05$), while it had no effect during daytime ($p > 0.05$).

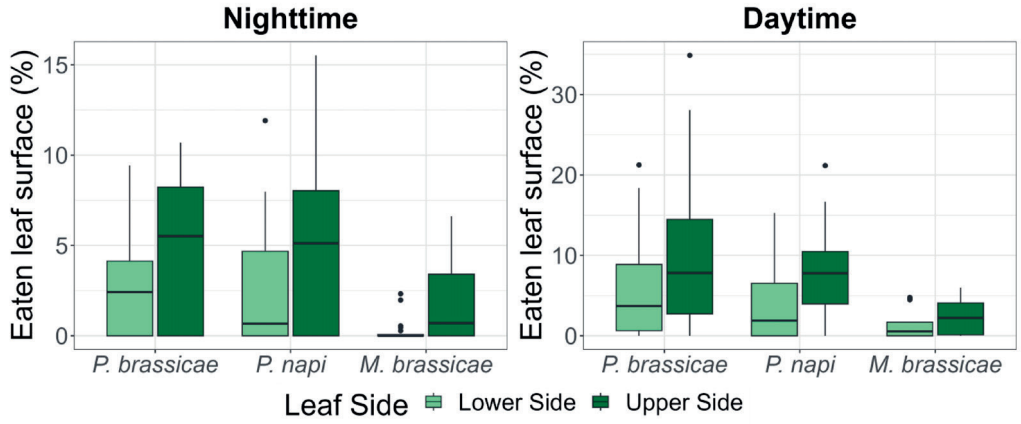


Fig. 5. The chosen leaf side during night and daytime, separated per species. All species preferred to feed from the upper side of the leaves, both during the day and during the night ($p < 0.01$).

6. Discussion

In this study, we found that the caterpillars of all three lepidopteran species had a preference for either day time or night time activity and that caterpillars respond to ALAN by altering their behaviour and positions on the plant. Responses varied per species, likely influenced by temporal niche variation and anti-predator colouration. Below we discuss our results in the light of the three hypotheses we stated in the introduction.

Do caterpillars show the same activity patterns as their adult stages?

While the activity period is known for most adult Lepidoptera, the rhythmicity of caterpillars is largely unknown (Niepoth et al., 2018). As a null hypothesis we postulated that caterpillars would mirror the adults' activity periods. Indeed, the aposematic *Pieris brassicae* caterpillars were mainly day-active and the cryptic *Mamestra brassicae* caterpillars mainly night-active, just like their adults. In contrast, the cryptic *Pieris napi* caterpillars were mainly night-active, while adults are day-active (Kingsolver, 1985).

As both life stages occupy very different niches, differences in activity patterns between caterpillars and adults are actually not surprising. As we found that both cryptic caterpillar species were mainly night-active, while the aposematic species was mainly day-active, caterpillar rhythms may be mostly related to anti-predator colouration. Cryptic species may limit daytime movement to avoid visual enemies (Berger & Gotthard, 2008; Fitzgerald et al., 1988; Heinrich, 1979; Ioannou & Krause,

2009), while aposematic species benefit from being in the light to show their aposematic colours that advertise their unpalatability (Nielsen & Mappes, 2020). However, Ruf et al. (2001) investigated the relationship between caterpillar activity rhythms and larval conspicuousness in three Lasiocampidae species and found no overall correlation. This lack of general association is likely because abiotic factors and life-history traits are also of great importance in shaping the most effective activity period and colouration strategy (Ruf et al., 2001).

Does ALAN affect the activity patterns of day and night active caterpillars?

We found no effect of ALAN on the behaviour of aposematic, day-active *P. brassicae* caterpillars, despite our hypothesis that day-active species would become more active under lit conditions. Light levels might have been too dim to activate the species, or other factors were stabilising their biological clock. In contrast, both nocturnal, cryptic species were affected by ALAN, but in different ways. The cryptic *M. brassicae* larvae were resting more in the light treatment compared to the dark treatment, aligning with our hypothesis that light would inhibit activity of nocturnal species (Jiang et al., 2023; van Geffen et al., 2015). However, the cryptic *P. napi* showed increased nocturnal movement in ALAN compared to the dark treatment, possibly because they attempted to move away from the light source but were unable to in our setup. Notably, we also observed a spill-over effect of nocturnal light during the daytime, as ALAN-exposed *P. napi* caterpillars increased their feeding during the day compared to those in the dark treatment. Since ALAN-treated caterpillars were more active at night, this increased diurnal feeding might have been a compensation for the energy lost due to their increased nocturnal activity. Moulting did influence caterpillar behaviour, but this did not appear to interact with the effect of ALAN, although overall numbers of moulting individuals were low (*P. napi*: N = 4; *M. brassicae*: N = 10). The increased activity of *P. napi* under ALAN could have negative fitness consequences, because it increases their visibility to natural enemies (Ioannou & Krause, 2009). Visual enemies are generally active during the day, but might also extend their foraging activities due to ALAN (Frank, 2009; Gomes et al., 2024).

While the full plant experiment only showed an increased diurnal feeding in *P. napi* and not in the other two species, our leaf disk experiment showed an increase in nocturnal feeding in all three species in the light treatment compared to the dark treatment. Increased feeding in ALAN was expected for the diurnal species, but not for the nocturnal species. Similar to our results, *Danaus plexippus* (Nymphalidae) caterpillars, which are arrhythmic in their larval phase (Niepoth et al., 2017), doubled their feeding under light compared to darkness (Haynes et al., 2023). In contrast, local nocturnal caterpillar communities showed reduced feeding in lit sites (Boyes et al., 2021b). ALAN also inhibited feeding in adult moths (*Mamestra brassicae* (Noctuidae),

Rivula sericealis (Erebidae), *Idaea biselata* and *Dysstroma truncate* (Geometridae))(van Langevelde et al., 2017). Increased feeding may enhance development, although not necessarily (Haynes et al., 2023), but also heightens predator or parasitoid detection risk through released chemical signals during feeding (Paré & Tumlinson, 1996; Vieira et al., 2019). Decreased feeding may delay development but could reduce detection risks by natural enemies.

The differences observed between the leaf disk and on-plant experiments likely stem from the difference in sensitivity of these methods to detect short feeding bursts. However, results might also differ because, in our living plant setup, caterpillar responses might have been mediated by their host plant's response to light – for example through an altered growth-defence balance or through altered chemical cues (Cieraad et al., 2023; Shiojiri et al., 2006). While the leaf disk experiment provided more precise measurements of the total feeding activity, it lacks the ecological realism of a living plant environment. Therefore, we see both experiments as complementary.

Does ALAN affect the hiding behaviour of cryptic and aposematic caterpillars?

Only the cryptic, night-active *M. brassicae* caterpillars altered their position within the plant in response to ALAN. These caterpillars were found more in the top half of the plants in ALAN than caterpillars in the dark treatment, throughout the 24-hour cycle. It is usually assumed that caterpillars hide at the base of the plant (Stamp & Bowers, 1992). However, the high position may still be a hiding response, as there were typically more leaves in the top, offering greater concealment. Closer to the ground there is also a higher risk of predation by arthropod predators (Hansen et al., 1984). Alternatively, ALAN-treated caterpillars might have been in the top because they were directly attracted to the light source (Gillet & Gardner, 2009; Perkins et al., 2008). Another explanation for the higher position of the *M. brassicae* caterpillars could be an indirect effect of ALAN-induced physiological changes within the plants (Cieraad et al., 2023). Light can shift the growth-defense balance, whereby ALAN often increases growth and palatability (Crump et al., 2021; Mondy et al., 2021). As fresh, young top leaves are usually more defended (Papazian et al., 2019), alterations such as increased growth and increased palatability might make these top leaves more favourable. While less specialised species, such as *M. brassicae,* typically prefer older, less defended bottom leaves (Bowers & Stamp, 1993; de Rijk et al., 2016; Gutbrodt et al., 2012), these ALAN-induced changes could allow them to shift to higher positions within the plant to feed. However, we did not observe a similar response in the other two species. For *P. napi,* although we did not detect a positional shift, caterpillars located in the top half were primarily engaged in feeding. *P. brassicae*, on the other hand, showed a strong preference for the top half of the plant across all light conditions, consistent with previous studies (de Rijk et al., 2016; Gutbrodt et al., 2012). For *P. brassicae,* this high

position was not solely associated with feeding, as they were found even more frequently in the top during the night, when they were primarily resting.

While the reasons for changing positions of caterpillars within the plant remain uncertain, their position on the leaves, upper side or lower side, is likely linked to hiding versus exposure. Cryptic *M. brassicae* caterpillars were found more on the lower side of the leaves during nights with light, while aposematic *P. brassicae* caterpillars were found more on the upper side of the leaves. Both responses support our hypothesis: aposematic species use nocturnal light to enhance their warning signals, while cryptic species hide more under lit conditions (Heinrich, 1979; Nielsen & Mappes, 2020). Shiojiri et al. (2006) also found that cryptic, nocturnal *Mythimna separata* (Noctuidae) caterpillars hid more when exposed to lights. In the leaf disk experiment, hiding was not possible and then all species preferred to feed on the upper side. Also during the on-plant experiment, *P. brassicae* caterpillars were commonly active on the upper leaf surface, whereas *M. brassicae* caterpillars were only rarely seen on top of the leaves but also exhibited active feeding or moving behaviour when they were. In general, cryptic caterpillars are more often found on the lower side where they likely hide from predators and parasitoids (Heinrich, 1979; Watanabe et al., 2018; Watts & Kariyat, 2021), despite the upper side being more palatable (Watts & Kariyat, 2021). Aposematic species are thought to benefit from increased exposure to visual predators (Nielsen & Mappes, 2020), and thus can move freely to the preferred upper side of leaves to feed. Still, increased exposure might also affect search efficiency of less visually oriented enemies, such as parasitoids (Watanabe et al., 2018), for which chemical defenses may be less effective (Zvereva & Kozlov, 2016). However, the exact role of caterpillar position in parasitoid search efficiency remains unclear (de Rijk et al., 2016; Watanabe et al., 2018).

During our experiments, red light was used to perform hourly observations with minimal disturbance. This was based on evidence that larval stemmata generally lack red-sensitive photoreceptors (Ichikawa & Tateda, 1982). However, it is worth noting that in *P. brassicae*, light up to 661 nm that is captured by the ocelli is involved in pupal melanisation (Angersbach, 1975). Whether such wavelengths also influence larval behaviour remains uncertain. Nevertheless, no behavioural changes were observed under red light during our study. Overall illumination levels were kept to a minimum to further reduce any potential effects.

While our study offers valuable insights into the behavioural responses of caterpillars to ALAN and provides a general indication of their natural activity periods, we acknowledge that our reliance on 1-hour observations for one 24-hour period per individual limits the temporal resolution and robustness of the behavioural patterns detected. Future studies incorporating longer monitoring periods would help to refine

and validate these findings. Nevertheless, by focusing on an underrepresented life stage and by examining species with different ecologies, our work underscores the broader ecological relevance of ALAN and its potential to influence invertebrate behaviours.

7. Conclusions

Our results show that even dim levels of 1.5 lux light pollution can already alter caterpillar behaviour. This highlights the urgency of protecting our night skies and emphasises the need to include larval stages in future research. Given that ALAN is a key driver of insect declines, it is crucial to investigate whether caterpillar behavioural changes result in negative fitness consequences. Expanding our understanding in this area will be essential for developing effective mitigation strategies against ecological light pollution.

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11. Supplementary material

11.1. Spectral composition

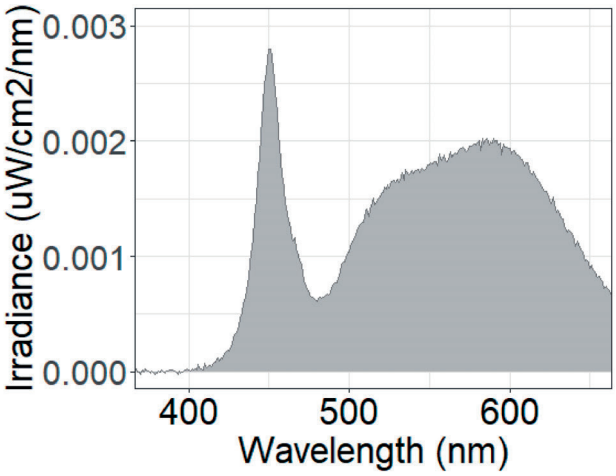


Fig. S1. Spectral composition of the LED light treatment (RexTech, the Netherlands; 1.5lux, 5500K), measured for a representative LED strip. Measurements were taken at shelf height, 50 cm below the LED strip. The detector was oriented perpendicular to the light source. Spectral data were collected using a QE Pro High-Performance Spectrophotometer (Ocean Insight, United States). The spectrophotometer slit was connected to an optical fiber, coupled to a cosine corrector with a collection area of 0.119 cm^2 (OceanOptics, United States).

Information on the spectral composition of the daylights (MASTER TL-D Super 80 58W – 4000K; Philips, the Netherlands) can be found in *Philips product specifications*.

11.2. Caterpillar behaviour on plants

Table S1. An overview of the affirmed escaped caterpillars per species, per trial, per treatment. These caterpillars were placed back upon notice.

	Trial 1		Trial 2		Trial 3		Trial 4	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
P. brassicae	1	0	10	3	1	4	3	22
P. napi	0	0	1	0	2	1	3	10
M. brassicae	0	1	2	0	1	0	1	4
TOTAL	1	1	13	3	4	5	7	36

Table S2. An overview of the missing caterpillars per species, per trial, per treatment. These caterpillars likely escaped, but were never found back. After two missing check rounds, they were replaced with a new individual.

	Trial 1		Trial 2		Trial 3		Trial 4	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
P. brassicae	1	0	1	1	0	1	0	0
P. napi	0	0	0	0	0	0	0	0
M. brassicae	1	0	2	0	1	2	1	2
TOTAL	2	0	3	1	1	3	1	2

11.2.1. Activity period of caterpillars

Table S3. An overview of the number of observations included in the on-plant activity analyses. Excluded activity observations resulted from missing caterpillars.

	Activity observations (N)
P. brassicae	954
P. napi	959
M. brassicae	947

Table S4. Overview of all activity period models, compared during the model simplification process. For each model, the AIC (Akaike Information Criterion) values are given together with the test statistics for each of the included explanatory variables. The model that performed best is indicated in bold. Random factors (Trial and Larva ID) were always kept in the model, but are not shown here.

	Model	AIC	TOD	Moult
P. brassicae	**M1**	/	$\chi2(1, N = 954) = 30.06, p < 0.001$	/
P. napi	M1	832.54	$\chi2(1, N = 959) = 4.05, p = 0.0441$	$\chi2(1, N = 959) = 0.01, p = 0.9071$
	M2	830.55	$\chi2(1, N = 959) = 4.05, p = 0.0441$	
M. brassicae	**M1**	/	$\chi2(1, N = 947) = 10.98, p < 0.001$	$\chi2(1, N = 947) = 3.88, p = 0.0489$

Table S5. Generalised linear mixed model output from the on-plant experiment, looking into larval rhythmicity for each species. Test statistics, including chi-square (χ^2), degrees of freedom (df) and *p*-value *(p)* are given for the fixed effects, time of day and moult. Significant *p*-values are indicated in bold. For the random factors, variance and standard deviation (SD) are given.

P. brassicae		**Resting**		
	Predictors	X^2	*df*	*p*
	Time Of Day (TOD)	30.06	1	**< 0.001**
	Random factors	*Variance*		*SD*
	Trial	0.21		0.4566
	Larva ID	0.23		0.4784
P. napi		**Resting**		
	Predictors	X^2	*df*	*p*
	Time Of Day (TOD)	4.05	1	**0.0441**
	Moult			
	Random factors	*Variance*		*SD*
	Trial	0.61		0.7796
	Larva ID	0.63		0.7910
M. brassicae		**Resting**		
	Predictors	X^2	*df*	*p*
	Time Of Day (TOD)	10.98	1	**< 0.001**
	Moult	3.88	1	**0.0489**
	Random factors	*Variance*		*SD*
	Trial	0.02		0.15
	Larva ID	0.00		0.00

Table S6. Summary outputs for the on-plant analyses, looking into larval rhythmicity of each species. For each analysis, the Log-Odds, confidence interval (CI) and *p*-value (*p*) are given for the fixed predictor effects. Significant *p*-values are indicated in bold.

	Resting *P. brassicae*			**Resting *P. napi***			**Resting *M. brassicae***		
Predictors	*Log-Odds*	*CI*	*p*	*Log-Odds*	*CI*	*p*	*Log-Odds*	*CI*	*p*
(Intercept)	0.30	-0.20 – 0.80	0.234	1.94	1.09 – 2.78	**<0.001**	1.58	1.31 – 1.85	**<0.001**
TOD [Night]	0.87	0.56 – 1.19	**<0.001**	-0.38	-0.75 – -0.01	**0.044**	-0.56	-0.89 – -0.23	**0.001**
Moult							0.60	0.00 – 1.20	**0.049**

11.2.2. Caterpillar activity and positioning

Table S7. An overview of the number of observations included in the on-plant analyses. Excluded behavioural observations resulted from missing caterpillars or caterpillars that were/had been exposed to faulty lights.

	Behavioural observations (N)	Leaf side observations (N)	Plant position observations (N)
P. brassicae	1854	1612	1721
P. napi	1907	1719	1738
M. brassicae	1867	1794	1843

Table S8. Overview of all ***Pi.brassicae*** models, compared during the model simplification process. For each model, the AIC (Akaike Information Criterion) values are given together with the test statistics for each of the included explanatory variables. The model that performed best is indicated in bold. Random factors (Trial and Larva ID) were always kept in the model, but are not shown here.

	Model	AIC	Treatment	TOD	Treatment:TOD	Activity
Resting	M1	2372.68	$\chi2(1, N = 1854) = 0.03, p = 0.8733$	$\chi2(1, N = 1854) = 29.80, p < 0.001$	$\chi2(1, N = 1854) = 1.02$, p = 0.3115	/
	M2	2371.71	$\chi2(1, N = 1854) = 0.35, p = 0.5568$	$\chi2(1, N = 1854) = 44.80, p < 0.001$		
Feeding	M1	1671.20	$\chi2(1, N = 1854) = 0.86, p = 0.3541$	$\chi2(1, N = 1854) = 2.45, p = 0.1172$	$\chi2(1, N = 1854) = 1.53, p = 0.2162$	/
	M2	1670.73	$\chi2(1, N = 1854) = 0.15, p = 0.6947$	$\chi2(1, N = 1854) = 1.02, p = 0.3136$		
Moving	M1	1841.25	$\chi2(1, N = 1854) = 0.87, p = 0.3498$	$\chi2(1, N = 1854) = 24.62, p < 0.001$	$\chi2(1, N = 1854) = 0.02, p = 0.8797$	/
	M2	1839.27	$\chi2(1, N = 1854) = 1.10, p = 0.2935$	$\chi2(1, N = 1854) = 47.15, p < 0.001$		
Plant position	M1	580.69	$\chi2(1, N = 1721) = 0.00, p = 0.9905$	$\chi2(1, N = 1721) = 10.05$, p = 0.0015	$\chi2(1, N = 1721) = 0.28, p = 0.5980$	$\chi2(1, N = 1721) = 23.64, p < 0.001$
	M2	578.97	$\chi2(1, N = 1721) = 0.01, p = 0.9368$	$\chi2(1, N = 1721) = 22.69, p < 0.001$		$\chi2(1, N = 1721) = 23.51$, p < 0.001
Leaf position	**M1**	/	$\chi2(1, N = 1612) = 5.98, p = 0.0144$	$\chi2(1, N = 1612) = 13.28, p < 0.001$	$\chi2(1, N = 1612) = 17.23, p < 0.001$	$\chi2(1, N = 1612) = 11.64, p < 0.001$

Table S9. Overview of all ***Pj.napi.***models, compared during the model simplification process. For each model, the AIC (Akaike Information Criterion) values are given together with the test statistics for each of the included explanatory variables. The model that performed best is indicated in bold. Random factors (Trial and Larva ID) were always kept in the model, but are not shown here.

	Model	AIC	Treatment	TOD	Moult	Treatment:TOD	Treatment:Moult	Activity
Resting	M1	1809.87	$\chi2(1, N = 1907) = 4.03, p = 0.0448$	$\chi2(1, N = 1907) = 3.99, p = 0.0459$	$\chi2(1, N = 1907) = 0.42, p = 0.5147$	$\chi2(1, N = 1907) = 0.58, p = 0.4481$	$\chi2(1, N = 1907) = 0.27, p = 0.6023$	/
	M2	1808.14	$\chi2(1, N = 1907) = 4.54, p = 0.0332$	$\chi2(1, N = 1907) = 3.98, p = 0.0459$	$\chi2(1, N = 1907) = 1.16, p = 0.2814$	$\chi2(1, N = 1907) = 0.57, p = 0.4490$		
	M3	1806.71	$\chi2(1, N = 1907) = 3.99, p = 0.0457$	$\chi2(1, N = 1907) = 4.50, p = 0.0339$	$\chi2(1, N = 1907) = 1.17, p = 0.2804$			
	M4	1805.87	$\chi2(1, N = 1907) = 3.60, p = 0.0577$	$\chi2(1, N = 1907) = 4.47, p = 0.0340$				
Feeding	M1	1386.27	$\chi2(1, N = 1907) = 6.26, p = 0.0124$	$\chi2(1, N = 1907) = 17.85, p < 0.001$	$\chi2(1, N = 1907) = 0.31, p = 0.5791$	$\chi2(1, N = 1907) = 6.95, p = 0.0084$	$\chi2(1, N = 1907) = 0.00, p = 0.9456$	/
	M2	1384.28	$\chi2(1, N = 1907) = 6.42, p = 0.0113$	$\chi2(1, N = 1907) = 17.85, p < 0.001$	$\chi2(1, N = 1907) = 0.37, p = 0.5406$	$\chi2(1, N = 1907) = 6.95, p = 0.0084$		
	M3	1382.66	$\chi2(1, N = 1907) = 6.67, p = 0.0098$	$\chi2(1, N = 1907) = 17.85, p < 0.001$		$\chi2(1, N = 1907) = 6.94, p = 0.0084$		
Moving	M1	950.98	$\chi2(1, N = 1907) = 0.02, p = 0.8873$	$\chi2(1, N = 1907) = 6.03, p = 0.0141$	$\chi2(1, N = 1907) = 2.25, p = 0.1333$	$\chi2(1, N = 1907) = 6.61, p = 0.0178$	$\chi2(1, N = 1907) = 0.52, p = 0.4703$	/
	M2	949.50	$\chi2(1, N = 1907) = 0.09, p = 0.7639$	$\chi2(1, N = 1907) = 6.03, p = 0.0141$	$\chi2(1, N = 1907) = 4.90, p = 0.0268$	$\chi2(1, N = 1907) = 5.62, p = 0.0178$		
Plant position	**M1**	737.21	$\chi2(1, N = 1738) = 0.84, p = 0.3584$	$\chi2(1, N = 1738) = 5.41, p = 0.0200$	$\chi2(1, N = 1738) = 0.00, p = 0.9980$	$\chi2(1, N = 1738) = 4.17, p = 0.0411$	$\chi2(1, N = 1738) = 0.00, p = 0.9969$	$\chi2(2, N = 1738) = 9.14, p = 0.0104$
	M2	742.01	$\chi2(1, N = 1738) = 2.12, p = 0.1458$	$\chi2(1, N = 1738) = 5.42, p = 0.0199$	$\chi2(1, N = 1738) = 4.26, p = 0.0391$	$\chi2(1, N = 1738) = 4.18, p = 0.0408$		$\chi2(2, N = 1738) = 9.19, p = 0.0101$
Leaf position	M1	1575.88	$\chi2(1, N = 1719) = 0.03, p = 0.8522$	$\chi2(1, N = 1719) = 1.65, p = 0.1991$	$\chi2(1, N = 1719) = 0.34, p = 0.5604$	$\chi2(1, N = 1719) = 0.93, p = 0.3354$	$\chi2(1, N = 1719) = 0.00, p = 0.9748$	$\chi2(2, N = 1719) = 0.85, p = 0.6549$
	M2	1573.88	$\chi2(1, N = 1719) = 0.03, p = 0.8543$	$\chi2(1, N = 1719) = 1.65, p = 0.1991$	$\chi2(1, N = 1719) = 0.54, p = 0.4608$	$\chi2(1, N = 1719) = 0.93, p = 0.3354$		$\chi2(2, N = 1719) = 0.85, p = 0.6551$

	M3	1.570.73	$\chi2(1, N = 1719) = 0.03, p = 0.8633$	$\chi2(1, N = 1719) = 1.44, p = 0.2296$	$\chi2(1, N = 1719) = 0.53, p = 0.4672$	$\chi2(1, N = 1719) = 0.79, p = 0.3733$
	M4	1569.27	$\chi2(1, N = 1719) = 0.04, p = 0.8460$	$\chi2(1, N = 1719) = 1.44, p = 0.2295$		$\chi2(1, N = 1719) = 0.79, p = 0.3729$
	M5	1568.06	$\chi2(1, N = 1719) = 0.00, p = 0.9540$	$\chi2(1, N = 1719) = 0.66, p = 0.4170$		

Table S10. Overview of all **M. brassicae.** models, compared during the model simplification process. For each model, the AIC (Akaike Information Criterion) values are given together with the test statistics for each of the included explanatory variables. The model that performed best is indicated in bold. Random factors (Trial and Larva ID) were always kept in the model, but are not shown here.

	Model	AIC	Treatment	TOD	Moult	Treatment:TOD	Treatment:Moult	Activity
Resting	M1	1652.39	$\chi2(1, N = 1867) = 1.28, p = 0.2571$	$\chi2(1, N = 1867) = 11.16, p < 0.001$	$\chi2(1, N = 1867) = 2.55, p = 0.1100$	$\chi2(1, N = 1867) = 0.82, p = 0.3638$	$\chi2(1, N = 1867) = 2.65, p = 0.1034$	/
	M2	1651.22	$\chi2(1, N = 1867) = 3.37, p = 0.6626$	$\chi2(1, N = 1867) = 13.06, p < 0.001$	$\chi2(1, N = 1867) = 2.53, p = 0.1117$		$\chi2(1, N = 1867) = 2.69, p = 0.1009$	
	M3	1652.12	$\chi2(1, N = 1867) = 5.72, p = 0.0168$	$\chi2(1, N = 1867) = 13.02, p < 0.001$	$\chi2(1, N = 1867) = 10.21, p = 0.0014$			
Feeding	M1	1145.68	$\chi2(1, N = 1867) = 2.37, p = 0.1237$	$\chi2(1, N = 1867) = 2.53, p = 0.1118$	$\chi2(1, N = 1867) = 4.06, p = 0.0439$	$\chi2(1, N = 1867) = 0.19, p = 0.6658$	$\chi2(1, N = 1867) = 1.81, p = 0.1783$	/
	M2	1143.86	$\chi2(1, N = 1867) = 2.47, p = 0.1159$	$\chi2(1, N = 1867) = 6.05, p = 0.0139$	$\chi2(1, N = 1867) = 4.07, p = 0.0437$		$\chi2(1, N = 1867) = 1.80, p = 0.1793$	
	M3	1144.18	$\chi2(1, N = 1867) = 3.76, p = 0.0526$	$\chi2(1, N = 1867) = 6.03, p = 0.0140$	$\chi2(1, N = 1867) = 10.65, p = 0.0011$			
Moving	M1	979.59	$\chi2(1, N = 1867) = 0.02, p = 0.8936$	$\chi2(1, N = 1867) = 8.63, p = 0.0033$	$\chi2(1, N = 1867) = 0.06, p = 0.8014$	$\chi2(1, N = 1867) = 2.92, p = 0.0874$	$\chi2(1, N = 1867) = 1.33, p = 0.2485$	/
	M2	978.99	$\chi2(1, N = 1867) = 0.03, p = 0.8558$	$\chi2(1, N = 1867) = 8.65, p = 0.0033$	$\chi2(1, N = 1867) = 1.59, p = 0.2071$	$\chi2(1, N = 1867) = 2.95, p = 0.0857$		

	M3	978.66	$\chi2(1, N = 1867) = 0.03, p = 0.8719$	$\chi2(1, N = 1867) = 8.64, p = 0.0033$		$\chi2(1, N = 1867) = 2.97, p = 0.0849$		
	M4	979.68	$\chi2(1, N = 1867) = 1.70\ p = 0.1922$	$\chi2(1, N = 1867) = 5.65, p = 0.0175$				
Plant position	M1	852.80	$\chi2(1, N = 1843) = 3.84, p = 0.0500$	$\chi2(1, N = 1867) = 0.01, p = 0.9237$	$\chi2(1, N = 1867) = 0.81, p = 0.3683$	$\chi2(1, N = 1867) = 0.28, p = 0.5962$	$\chi2(1, N = 1867) = 0.00, p = 0.9914$	$\chi2(2, N = 1867) = 1.27, p = 0.5301$
	M2	850.80	$\chi2(1, N = 1867) = 4.58, p = 0.0324$	$\chi2(1, N = 1867) = 0.01, p = 0.9237$	$\chi2(1, N = 1867) = 1.48, p = 0.2240$	$\chi2(1, N = 1867) = 0.28, p = 0.5962$		$\chi2(2, N = 1867) = 1.27, p = 0.5302$
	M3	849.08	$\chi2(1, N = 1867) = 4.98, p = 0.0257$	$\chi2(1, N = 1867) = 0.55, p = 0.4571$	$\chi2(1, N = 1867) = 1.48, p = 0.2243$			$\chi2(2, N = 1867) = 1.23, p = 0.5397$
	M4	846.34	$\chi2(1, N = 1867) = 4.94, p = 0.0263$	$\chi2(1, N = 1867) = 0.71, p = 0.3997$	$\chi2(1, N = 1867) = 1.54, p = 0.2145$			
	M5	847.88	$\chi2(1, N = 1867) = 4.96, p = 0.0259$	$\chi2(1, N = 1867) = 0.71, p = 0.3989$				
Leaf position	M1	1347.87	$\chi2(1, N = 1794) = 2.71, p = 0.0998$	$\chi2(1, N = 1794) = 0.11, p = 0.7383$	$\chi2(1, N = 1794) = 0.09, p = 0.7630$	$\chi2(1, N = 1794) = 17.40, p < 0.001$	$\chi2(1, N = 1794) = 0.22, p = 0.6361$	$\chi2(2, N = 1794) = 34.85, p < 0.001$
	M2	1346.09	$\chi2(1, N = 1794) = 3.77, p = 0.0520$	$\chi2(1, N = 1794) = 0.11, p = 0.7391$	$\chi2(1, N = 1794) = 0.00, p = 0.9991$	$\chi2(1, N = 1794) = 17.40, p < 0.001$		$\chi2(2, N = 1794) = 34.87, p < 0.001$
	M3	1344.01	$\chi2(1, N = 1794) = 3.78, p = 0.0520$	$\chi2(1, N = 1794) = 0.11, p = 0.0520$		$\chi2(1, N = 1794) = 17.40, p < 0.001$		$\chi2(2, N = 1794) = 34.90, p < 0.001$

Table S11. Generalised linear mixed model output from the on-plant experiment, looking into the effect of light treatment, time of day and their interaction on behaviour (resting, feeding, moving) and position (leaf side and plant position). Test statistics, including chi-square (χ^2), degrees of freedom (df) and *p*-value *(p)* are given for each fixed predictor effect. The test statistics for the interaction effect are only shown when included in the final model. Significant *p*-values are indicated in bold. For the random factors, variance and standard deviation (SD) are given.

		Resting			***Feeding***			***Moving***			***Leaf side***			***Plant position***		
P. brassicae	*Predictors*	X^2	*df*	*p*	X^2	*df*	*p*	X^2	*df*	*p*	X^2	*df*	*p*	X^2	*df*	*p*
	Treatment	0.35	1	0.5568	0.15	1	0.6947	1.10	1	0.2935	5.98	1	**0.0144**	0.01	1	0.9368
	Time Of Day (TOD)	44.80	1	**< 0.001**	1.02	1	0.3136	47.15	1	**< 0.001**	13.28	1	**< 0.001**	22.69	1	**< 0.001**
	Treatment: TOD										17.23	1	**< 0.001**			
	Activity	/			/			/			11.64	2	**0.0030**	23.51	2	**< 0.001**
	Random factors	*Variance*		*SD*	*Variance*		*SD*	*Variance*		*SD*	*Variance*		*SD*	*Variance*		*SD*
	Trial	0.07		0.27	0.15		0.39	0.01		0.08	0.31		0.56	6.77		2.60
	Larva ID	0.25		0.50	0.15		0.39	0.24		0.49	3.14		1.77	8.42		2.90
		Resting			**Feeding**			**Moving**			**Leaf side**			**Plant position**		
P. napi	*Predictors*	X^2	*df*	*p*	X^2	*df*	*p*	X^2	*df*	*p*	X^2	*df*	*p*	X^2	*df*	*p*
	Treatment	3.60	1	0.0577	6.67	1	**0.0098**	0.09	1	0.7639	0.00	1	0.9540	0.84	1	0.3584
	Time Of Day (TOD)	4.50	1	**0.0340**	17.85	1	**< 0.001**	6.03	1	**0.0141**	0.66	1	0.4170	5.41	1	**0.0200**
	Moult							4.90	1	**0.0268**				0.00	1	0.9980
	Treatment: TOD				6.94	1	**0.0084**	5.62	1	**0.0178**				4.17	1	**0.0411**
	Treatment:Moult													0.00	1	0.9969
	Activity	/			/			/						9.14	2	**0.0104**

	Random factors	*Variance*		*SD*	*Variance*		*SD*	*Variance*		*SD*	*Variance*		*SD*	*Variance*		*SD*
	Trial	0.51		0.72	0.75		0.87	0.34		0.59	0.71		0.84	55.36		7.44
	Larva ID	0.51		0.72	0.74		0.86	0.33		0.58	6.47		2.54	5.40		2.32
		Resting			**Feeding**			**Moving**			**Leaf side**			**Plant position**		
M. brassicae	*Predictors*	X^2	*df*	*p*	X^2	*df*	*p*	X^2	*df*	*p*	X^2	*df*	*p*	X^2	*df*	*p*
	Treatment	5.72	1	**0.0168**	3.76	1	0.0526	1.70	1	0.1922	3.78	1	0.0520	4.96	1	**0.0259**
	Time Of Day (TOD)	120.77	1	**< 0.001**	6.03	1	**0.0140**	5.65	1	**0.0175**	0.11	1	0.7391	0.71	1	0.3989
	Moult	10.21	1	**0.0014**	10.65	1	**0.0011**									
	Treatment: TOD										17.40	1	**< 0.001**			
	Treatment:Moult															
	Activity	/			/			/			34.90	2	**< 0.001**			
	Random factors	*Variance*		*SD*	*Variance*		*SD*	*Variance*		*SD*	*Variance*		*SD*	*Variance*		*SD*
	Trial	0.01		0.10	0.03		0.16	0.00		0.00	0.35		0.59	54.69		7.40
	Larva ID	0.24		0.49	0.18		0.43	0.39		0.62	2.46		1.57	13.83		3.72

Table S12. Results of the post-hoc analyses for the on-plant experiment, showing the pairwise contrasts between different treatment and time of day groups (only performed when the interaction term was significant). For each analysis, the odds ratio, standard error (SE), degrees of freedom (df), *z ratio* and *p*-value (*p*) are given per pairwise contrast. Significant *p*-values are indicated in bold.

	Contrasts	*Odds ratio*	*SE*	*df*	*z ratio*	*p*
P. brassicae **Leaf side**	Day Dark / Day Light	0.30	0.15	Inf	-2.45	0.0687
	Night Dark / Night Light	0.05	0.03	Inf	-5.07	**< 0.001**
	Day Dark / Night Dark	2.19	0.47	Inf	3.65	**0.0015**
	Day Light / Night Light	0.39	0.14	Inf	-2.64	**0.0416**
	Day Dark / Night Light	0.11	0.07	Inf	-3.78	**< 0.001**
	Night Dark / Day Light	0.14	0.07	Inf	-3.94	**< 0.001**
	Contrasts	*Odds ratio*	*SE*	*df*	*z ratio*	*p*
P. napi **Feeding**	Day Dark / Day Light	0.48	0.14	Inf	-2.58	**0.0481**
	Night Dark / Night Light	1.04	0.32	Inf	0.12	0.9993
	Day Dark / Night Dark	0.40	0.09	Inf	-4.23	**< 0.001**
	Day Light / Night Light	0.87	0.17	Inf	-0.70	0.8969
	Day Dark / Night Light	0.41	0.13	Inf	-2.87	**0.0215**
	Night Dark / Day Light	1.20	0.35	Inf	0.62	0.9270
	Contrasts	*Odds ratio*	*SE*	*df*	*z ratio*	*p*
P. napi **Moving**	Day Dark / Day Light	0.92	0.24	Inf	-0.30	0.9906
	Night Dark / Night Light	0.33	0.13	Inf	-2.74	**0.0310**
	Day Dark / Night Dark	2.338	0.809	Inf	2.456	0.0671
	Day Light / Night Light	0.85	0.22	Inf	-0.66	0.9111
	Day Dark / Night Light	0.78	0.230	Inf	-0.84	0.8354
	Night Dark / Day Light	0.40	0.15	Inf	-2.47	0.0652

	Contrasts	*Odds ratio*	*SE*	*df*	*z ratio*	*p*
P. napi **Plant position**	Day Dark / Day Light	0.00	0.01	Inf	-0.00	1.0000
	Night Dark / Night Light	0.00	0.01	Inf	-0.00	1.0000
	Day Dark / Night Dark	0.45	0.15	Inf	-2.33	0.0921
	Day Light / Night Light	1.11	0.32	Inf	0.38	0.9808
	Day Dark / Night Light	0.00	0.01	Inf	-0.00	1.0000
	Night Dark / Day Light	0.00	0.01	Inf	-0.00	1.0000
	Contrasts	*Odds ratio*	*SE*	*df*	*z ratio*	*p*
M. brassicae **Leaf side**	Day Dark / Day Light	2.25	0.94	Inf	1.94	0.2100
	Night Dark / Night Light	10.61	5.38	Inf	4.66	**< 0.001**
	Day Dark / Night Dark	0.94	0.18	Inf	-0.33	0.9873
	Day Light / Night Light	4.42	1.41	Inf	4.67	**< 0.001**
	Day Dark / Night Light	9.94	4.929	Inf	4.63	**< 0.001**
	Night Dark / Day Light	2.40	1.03	Inf	2.03	0.1764

Table S13. Results of the post-hoc analyses for the on-plant experiment, showing the pairwise contrasts between different activities (only performed when activity had a significant effect). For each analysis, the odds ratio, standard error (SE), degrees of freedom (df), *z ratio* and *p*-value (*p*) are given per pairwise contrast. Significant *p*-values are indicated in bold.

	Contrasts	*Odds ratio*	*SE*	*df*	*z ratio*	*p*
P. brassicae **Plant position**	Feeding/Moving	0.61	0.21	Inf	-1.45	0.3169
	Feeding/Resting	0.22	0.07	Inf	-4.74	**< 0.001**
	Moving/Resting	0.35	0.12	Inf	-3.16	**0.0045**
	Contrasts	*Odds ratio*	*SE*	*df*	*z ratio*	*p*
P. brassicae **Leaf side**	Feeding/Moving	1.16	0.32	Inf	0.54	0.8523
	Feeding/Resting	1.99	0.45	Inf	2.35	**0.0498**
	Moving/Resting	1.71	0.39	Inf	2.35	**0.0498**

	Contrasts	*Odds ratio*	*SE*	*df*	*z ratio*	*p*
P. napi **Plant position**	Feeding/Moving	2.15	0.87	Inf	1.88	0.1437
	Feeding/Resting	2.10	0.53	Inf	2.94	**0.0092**
	Moving/Resting	0.98	0.37	Inf	-0.07	0.9975
	Contrasts	*Odds ratio*	*SE*	*df*	*z ratio*	*p*
M. brassicae **Leaf side**	Feeding/Moving	0.70	0.22	Inf	-1.13	0.4942
	Feeding/Resting	2.46	0.54	Inf	4.08	**< 0.001**
	Moving/Resting	3.49	0.89	Inf	4.87	**< 0.001**

Table S14. Summary outputs for all on-plant analyses, looking into the effect of light treatment, time of day and their interaction on ***Pieris brassicae*** behaviour (resting, feeding, moving) and position (leaf side and plant position) for which we also show the effect of activity type. For each analysis, the Log-Odds, confidence interval (CI) and *p*-value (*p*) are given for each main fixed predictor effect. The test statistics for the interaction effect is only shown when included in the final model. Significant *p*-values are indicated in bold.

	Resting			**Feeding**			**Moving**			**LeafPosition**			**PlantPosition**		
Predictors	*Log-Odds*	*CI*	*p*	*Log-Odds*	*CI*	*p*	*Log-Odds*	*CI*	*p*	*Log-Odds*	*CI*	*p*	*Log-Odds*	*CI*	*p*
(Intercept)	0.33	-0.01 – 0.67	0.059	-1.61	-2.05 – -1.16	**<0.001**	-1.21	-1.45 – -0.96	**<0.001**	2.53	1.59 – 3.46	**<0.001**	4.03	0.92 – 7.14	**0.011**
Treatment [Light]	-0.09	-0.38 – 0.21	0.557	-0.06	-0.37 – 0.24	0.695	0.17	-0.15 – 0.49	0.293	1.21	0.24 – 2.18	**0.014**	0.07	-1.61 – 1.75	0.937
TOD [Night]	0.75	0.53 – 0.97	**<0.001**	-0.14	-0.41 – 0.13	0.314	-1.01	-1.30 – -0.72	**<0.001**	-0.78	-1.21 – -0.36	**<0.001**	1.71	1.01 – 2.41	**<0.001**
Activity [M]										-0.15	-0.69 – 0.39	0.590	0.49	-0.17 – 1.15	0.148
Activity [R]										-0.69	-1.13 – -0.24	**0.003**	1.54	0.90 – 2.17	**<0.001**
Treatment [Light] × TOD [Night]										1.74	0.92 – 2.56	**<0.001**			

Table S15. Summary outputs for all on-plant analyses, looking into the effect of light treatment, time of day and their interaction on ***Pi.napi*** behaviour (resting, feeding, moving) and position (leaf side and plant position) for which we also show the effect of activity type. For each analysis, the Log-Odds, confidence interval (CI) and *p*-value (*p*) are given for each main fixed predictor effect. The test statistics for the interaction effect are only shown when included in the final model. Significant *p*-values are indicated in bold.

	Resting			**Feeding**			**Moving**			**LeafPosition**			**PlantPosition**		
Predictors	*Log-Odds*	*CI*	*p*	*Log-Odds*	*CI*	*p*	*Log-Odds*	*CI*	*p*	*Log-Odds*	*CI*	*p*	*Log-Odds*	*CI*	*p*
(Intercept)	1.86	1.09 – 2.63	**<0.001**	-2.86	-3.82 – -1.90	**<0.001**	-2.87	-3.57 – -2.16	**<0.001**	-0.14	-1.33 – 1.06	0.824	0.44	-7.44 – 8.32	0.913
TOD [Night]	-0.27	-0.51 – -0.02	**0.034**	0.92	0.49 – 1.35	**<0.001**	-0.85	-1.53 – -0.17	**0.014**	0.12	-0.17 – 0.40	0.417	0.80	0.13 – 1.48	**0.020**
Treatment [Light]	-0.39	-0.80 – 0.01	0.058	0.74	0.18 – 1.31	**0.010**	0.08	-0.43 – 0.59	0.764	0.04	-1.18 – 1.25	0.954	0.75	-0.85 – 2.36	0.358
TOD [Night] × Treatment [Light]				-0.78	-1.36 – -0.20	**0.008**	1.02	0.18 – 1.86	**0.018**				-0.91	-1.79 – -0.04	**0.041**
Moult							1.29	0.15 – 2.43	**0.027**				-16.56	-12697.24 – 12664.11	0.998
Activity [M]													-0.77	-1.56 – 0.03	0.060
Activity [R]													-0.74	-1.23 – -0.25	**0.003**
Treatment [Light] × Moult													25.22	-12655.45 – 12705.90	0.997

Table S16. Summary outputs for all on-plant analyses, looking into the effect of light treatment, time of day and their interaction on ***Mj brassicae*** behaviour (resting, feeding, moving) and position (leaf side and plant position) for which we also show the effect of activity type. For each analysis, the Log-Odds, confidence interval (CI) and *p*-value (*p*) are given for each main fixed predictor effect. The test statistics for the interaction effect are only shown when included in the final model. Significant *p*-values are indicated in bold.

	Resting			**Feeding**			**Moving**			**LeafPosition**			**PlantPosition**		
Predictors	*Log-Odds*	*CI*	*p*	*Log-Odds*	*CI*	*p*	*Log-Odds*	*CI*	*p*	*Log-Odds*	*CI*	*p*	*Log-Odds*	*CI*	*p*
(Intercept)	1.55	1.27 – 1.83	**<0.001**	-2.20	-2.53 – -1.86	**<0.001**	-2.82	-3.22 – -2.43	**<0.001**	-0.98	-1.88 – -0.09	**0.031**	1.13	-6.98 – 9.25	0.785
TOD [Night]	-0.47	-0.73 – -0.21	**<0.001**	0.40	0.08 – 0.73	**0.014**	0.70	0.23 – 1.17	**0.003**	0.07	-0.32 – 0.45	0.739	0.18	-0.24 – 0.59	0.399
Treatment [Light]	0.41	0.07 – 0.75	**0.017**	-0.37	-0.75 – 0.00	0.053	-0.04	-0.59 – 0.50	0.872	-0.81	-1.63 – 0.01	0.052	2.45	0.29 – 4.60	**0.026**
Moult	1.05	0.41 – 1.69	**0.001**	-1.47	-2.36 – -0.59	**0.001**									
TOD [Night] × Treatment [Light]							-0.65	-1.38 – 0.09	0.085	-1.55	-2.28 – -0.82	**<0.001**			
Activity [M]										0.35	-0.26 – 0.96	0.258			
Activity [R]										-0.90	-1.33 – -0.47	**<0.001**			

11.3. Caterpillar feeding on leaf disks

Table S17. Overview of the leaf disk models, compared during the model simplification process. For each model, the AIC (Akaike Information Criterion) values are given together with the test statistics for each of the included fixed factors. The model that performed best is indicated in bold. The random factor (Larva ID) was always kept in the model, but is not shown here.

	Model	AIC	Treatment	Species	LeafSide	Treatment:Species	LeafSide:Species	LeafSide: Treatment
Daytime	M1	660.72	$\chi2(1, N = 120) = 0.13, p = 0.7177$	$\chi2(2, N = 120) = 9.01, p = 0.0110$	$\chi2(1, N = 120) = 1.36, p = 0.2431$	$\chi2(2, N = 120) = 0.04, p = 0.9803$	$\chi2(2, N = 120) = 0.16, p = 0.9236$	$\chi2(1, N = 120) = 0.08, p = 0.7799$
	M2	656.76	$\chi2(1, N = 120) = 0.71, p = 0.3979$	$\chi2(2, N = 120) = 14.17, p < 0.001$	$\chi2(1, N = 120) = 1.35, p = 0.2455$		$\chi2(2, N = 120) = 0.16, p = 0.9212$	$\chi2(1, N = 120) = 0.08, p = 0.7800$
	M3	652.92	$\chi2(1, N = 120) = 0.74, p = 0.3894$	$\chi2(2, N = 120) = 32.56, p < 0.001$	$\chi2(1, N = 120) = 4.52, p = 0.0335$			$\chi2(1, N = 120) = 0.08, p = 0.7760$
	M4	651.00	$\chi2(1, N = 120) = 0.98, p = 0.3221$	$\chi2(2, N = 120) = 32.69, p < 0.001$	$\chi2(1, N = 120) = 7.82, p = 0.0052$			
Nighttime	M1	502.26	$\chi2(1, N = 120) = 0.98, p = 0.3226$	$\chi2(2, N = 120) = 10.20, p = 0.0061$	$\chi2(1, N = 120) = 6.75, p = 0.0094$	$\chi2(2, N = 120) = 0.71, p = 0.7002$	$\chi2(2, N = 120) = 3.49, p = 0.1746$	$\chi2(1, N = 120) = 0.03, p = 0.8661$
	M2	500.29	$\chi2(1, N = 120) = 1.16, p = 0.2813$	$\chi2(2, N = 120) = 10.27, p = 0.0059$	$\chi2(1, N = 120) = 7.72, p = 0.0055$	$\chi2(2, N = 120) = 0.71, p = 0.7024$	$\chi2(2, N = 120) = 3.48, p = 0.1754$	
	M3	497.00	$\chi2(1, N = 120) = 4.37, p = 0.0365$	$\chi2(2, N = 120) = 13.38, p = 0.0012$	$\chi2(1, N = 120) = 7.67, p = 0.0056$		$\chi2(2, N = 120) = 3.45, p = 0.1783$	
	M4	496.65	$\chi2(1, N = 120) = 4.21, p = 0.0403$	$\chi2(2, N = 120) = 19.06, p < 0.001$	$\chi2(1, N = 120) = 9.74, p = 0.0018$			

Table S18. Generalised linear mixed models output of the leaf disk experiment, looking into the effect of light treatment, leaf side and species on eaten leaf surface. Performed for day and nighttime separately. Test statistics, including chi-square (χ^2), degrees of freedom (df) and *p*-value *(p)* are given for each fixed predictor effect. Significant *p*-values are indicated in bold. For the random factors, variance and standard deviation (SD) are given.

Leaf Disks Nighttime	*Predictors*	X^2	*df*	*p*
	Treatment	4.21	1	**0.0403**
	Leaf side	9.74	1	**0.0018**
	Species	19.06	2	**< 0.001**
	Random factors	*Variance*		*SD*
	Larva ID	4.99e-10		2.23e-05
Leaf Disks Daytime	*Predictors*	X^2	*df*	*p*
	Treatment	0.98	1	0.3221
	Leaf side	7.82	1	**0.0052**
	Species	32.69	2	**< 0.001**
	Random factors	*Variance*		*SD*
	Larva ID	4.00e-09		6.33e-05

Table S19. Results of the post-hoc analyses showing the pairwise differences in eaten leaf surface between the different species. The analysis was separate for night and daytime. For these analyses, the estimate, standard error (SE), *z ratio* and *p*-value (*p*) are given per pairwise contrast. Significant *p*-values are indicated in bold.

	Contrasts	*Estimate*	*SE*	*df*	*z ratio*	*p*
Leaf Disks Nighttime	M. brassicae – P. brassicae	-1.38	0.34	Inf	-4.12	**< 0.001**
	M. brassicae – P. napi	-1.33	0.34	Inf	-3.95	**< 0.001**
	P. brassicae – P. napi	0.05	0.25	Inf	0.21	0.9757
	Contrasts	*Estimate*	*SE*	*df*	*z ratio*	*p*
Leaf Disks Daytime	M. brassicae – P. brassicae	-1.48	0.26	Inf	-5.67	**< 0.001**
	M. brassicae – P. napi	-1.17	0.27	Inf	-4.37	**< 0.001**
	P. brassicae – P. napi	0.31	0.21	Inf	1.44	0.3192

Table S20. Summary output for the night and daytime leaf disk analyses, looking into the effect of species, light treatment, and leaf side on eaten leaf surface. For each analysis, the estimates, confidence interval (CI) and *p*-value (*p*) are given for each main fixed predictor effect. Significant *p*-values are indicated in bold.

	Eaten_Percentage_Night			**Eaten_Percentage_Day**		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	-0.71	-1.41 – -0.01	**0.045**	0.22	-0.30 – 0.73	0.408
SpeciesID [Pb]	1.38	0.73 – 2.04	**<0.001**	1.47	0.96 – 1.98	**<0.001**
SpeciesID [Pn]	1.33	0.67 – 1.99	**<0.001**	1.17	0.64 – 1.69	**<0.001**
Treatment [Light]	0.48	0.02 – 0.94	**0.040**	0.19	-0.18 – 0.56	0.322
LeafSide [Up]	0.75	0.28 – 1.22	**0.002**	0.54	0.16 – 0.91	**0.005**

3

Artificial light at night does not increase caterpillar predation

Evidence across birds and forest communities under short- and long-term exposure

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1. Abstract

Artificial light at night (ALAN) is a widespread ecological pollutant that can disrupt predator-prey interactions by altering species' behaviours, spatial and temporal niches, and visual ecology. Lepidopteran populations are particularly vulnerable to ALAN. However, the effects of ALAN on predation pressure, particularly in relation to predator activity and anti-predator strategies, remain unclear. To address this, we conducted two experiments. In an indoor aviary study, we exposed great tits (*Parus major*) and cabbage moth caterpillars (*Mamestra brassicae*) feeding on brussels sprouts (*Brassica oleracea*) to either darkness or dim ALAN (1.5 lux). We then assessed the behavioural responses of both predator and prey. We found that captive great tits were more restless and became active earlier in the day under ALAN, but predation rates on caterpillars were unaltered. Caterpillar feeding and hiding behaviours did not differ between light treatments either, but caterpillars did hide more in response to predator presence. In the outdoor experiment, we placed cryptic and aposematic caterpillar models along long-term illuminated and dark forest transects and evaluated night- and daytime predation by the broader predator community. Predation rates on both cryptic and aposematic models did not differ between illuminated and dark transects. Overall, our results highlight that changes in predator activity in response to ALAN may not result in changes in predation rates and suggest that long-term light exposure may not increase predation rates as seen in other studies.

2. Keywords

Artificial light at night, Predation, Caterpillars, Great tits, Temporal niches, Anti-predator colouration, Behaviour

3. Introduction

Artificial light at night (ALAN) has profoundly altered the natural nightscape, affecting at least 23% of the land surface between 75°N and 60°S. Furthermore, sky brightness keeps increasing by an average of 2.2-9.6 % per year (Falchi et al., 2016; Hölker et al., 2010; Kyba et al., 2017, 2023). ALAN functions as an ecological pollutant, disrupting the natural light-dark cycles and celestial cues that almost all taxa evolved to rely on (Longcore & Rich, 2004). This disruption can alter the behaviour and physiology of numerous organisms (Galaz-Guajardo et al., 2025; Miner et al., 2021; Taylor et al., 2022; Velasque et al., 2023), impacting their life history and fitness (Gomes et al., 2024; van Geffen et al., 2014; Willmott et al., 2018). These alterations can cascade through the food web, affecting species interactions, and ultimately disrupting entire ecological communities (Bennie et al., 2018; Knop et al., 2017).

One type of species interaction affected by ALAN is the predator-prey relationship. ALAN can alter these interactions by shifting the temporal and spatial niches of organisms, influencing their behaviours, and impacting their visual ecology. Since the temporal niches of species are distributed over the light-dark cycle (Hut et al., 2012), ALAN can affect the temporal niche overlap between predators and prey (Seymoure et al., 2023). In the presence of ALAN, diurnal species can expand their activity and forage into the night (Dwyer et al., 2013; Frank, 2009; Gomes et al., 2024; Henke et al., 2022; Leopold et al., 2010; Leveau, 2020; Maurer et al., 2019; Russ et al., 2015). In contrast, the activity of crepuscular and nocturnal species can be delayed or completely hampered by the presence of ALAN (Boldogh et al., 2007; Jiang et al., 2023; Oosthuizen et al., 2024; Touzot et al., 2020).

ALAN can also alter the spatial distributions of species, affecting the spatial predator-prey niche overlap. On short temporal scales, these spatial shifts often result from the direct or indirect attraction or repulsion of species to light (Duarte et al., 2019; Heiling, 1999; McMunn et al., 2019; Spoelstra et al., 2017). Over longer periods, additional factors come into play, depending on the sensitivities of species to ALAN. Different sensitivities can lead to local population declines or increases (Boyes et al., 2021a; Cremer et al., 2022; van Langevelde et al., 2018), and can exert strong selective pressures that may either reduce or enhance species' attraction to light (Altermatt & Ebert, 2016; Czaczkes et al., 2018).

Community-level studies have shown that ALAN alters the composition of ground-dwelling arthropod communities, changing the temporal and spatial functional diversity over time (Davies et al., 2012, 2017; Spoelstra et al., 2023). For example, predation studies using newly installed light sources frequently report increased local predation pressures on fixed ground-dwelling prey in illuminated areas (Deitsch &

Kaiser, 2023; McMunn et al., 2019; Nuñez et al., 2021). In contrast, predation studies near pre-established light sources often do not observe increased local predation (Eckhartt & Ruxton, 2022; Grenis et al., 2015; Heinen & López Pérez, 2025). These different findings thus suggest differential short- and long-term effects of ALAN on predator-prey interactions for less mobile, ground-dwelling communities.

ALAN can also impact the anti-predator behaviours of prey. For many species, light is associated with an increased perceived predation risk (Prugh & Golden, 2014). Under these perceived high-risk conditions, prey species reduce their risk-taking behaviour, increase vigilance, and show increased sheltering and minimised movement behaviours (Dissegna & Chiandetti, 2025; Velasque et al., 2023; Zhang et al., 2020). These behavioural changes may have implications for the prey's fitness, as there is a trade-off between predator avoidance and performing essential tasks, such as food acquisition (Houston et al., 1993).

Finally, ALAN can impact the visual ecology of species, which can affect predator-prey interactions (Briolat et al., 2021). Prey can exhibit different types of anti-predator colouration, with two common strategies being crypsis and aposematism. While cryptic species match with their habitat background (Stevens & Merilaita, 2011), aposematic species stand out, advertising their unpalatability and increasing predator learning (Mappes et al., 2005; Nielsen & Mappes, 2020). The ambient light condition is an important factor in determining the effectiveness of these strategies (Douglas, 2013; Olofsson et al., 2010; Pegram et al., 2012). ALAN, especially broad-spectrum LED light, can increase the conspicuousness of cryptic prey and thereby increase predation (McMahon et al., 2022; Seymoure et al., 2024). Furthermore, ALAN can shift the relative effectiveness of different colour morphs of both cryptic and aposematic species (Moyse et al., 2023; Nokelainen et al., 2022).

This study focuses on caterpillars with cryptic and aposematic colours. Caterpillars have a central position in the food web, serving as important prey for a myriad of predators and parasitoids, while also being important herbivores of many plants. Multiple studies have documented a decline, especially in nocturnal Lepidoptera (Antão et al., 2020; Bell et al., 2020; Groenendijk & Ellis, 2011; Wagner et al., 2021), which is likely partly due to ALAN (Boyes et al., 2021a; van Langevelde et al., 2018). Light pollution affects all life stages of these insects (Boyes et al., 2021a). However, due to the limited mobility of caterpillars, they may be particularly vulnerable to the local light environment, which is reflected in the observed decrease in caterpillar abundance near light sources (Boyes et al., 2021b).

In this study, we investigated the effects of ALAN on the predation of caterpillars. We first focused on the effect of ALAN on the predation behaviour of great tits (*Parus major*)

in an indoor experiment. The great tit is an insectivorous bird species that heavily depends on caterpillars as a food source during the breeding season (Isaksson & Andersson, 2007; Naef-Daenzer et al., 2000). ALAN advances the onset of activity of this diurnal insectivore (Spoelstra et al., 2018), and even earlier foraging has been reported (Da Silva et al., 2017). However, in that case birds foraged at familiar feeders placed in areas with relatively high illumination (average ambient light intensity: 4 lux), making the task visually much easier than detecting cryptic caterpillars on plants under dim light. To determine whether an earlier onset of activity also results in increased nocturnal foraging under these more challenging conditions, we measured great tit activity and caterpillar predation rates. In the same experiment, we investigated the behavioural response of caterpillars to both predators and light, measuring the eaten leaf surface, caterpillar weight gain, and their positioning on the plant. Because caterpillar responses to light can be indirect via plant-mediated processes (Shiojiri et al., 2006), caterpillars were placed on plants exposed to the same light conditions. We hypothesised that under ALAN the earlier onset of activity of great tits would lead to an increase in nocturnal activity and foraging, compared to dark controls (Leveau, 2020; Spoelstra et al., 2018). In addition, we expected that caterpillars would respond to both ALAN and predators with increased vigilance, increasing their hiding behaviour (residing more underneath a leaf and/or in the plant's bottom half) and decreasing their feeding behaviour on a plant, resulting in a lower caterpillar weight gain and eaten leaf surface (Heinrich, 1979; Shiojiri et al., 2006).

In an additional outdoor experiment, we assessed the impact of long-term light exposure (twelve years) on the attack rates on cryptic and aposematic dummy caterpillars along illuminated transects and dark controls. In this experiment, we tested the hypothesis that ALAN leads to higher levels of predation compared to dark conditions (Sanders et al., 2021), due to extended foraging by diurnal predators (Frank, 2009; Titulaer et al., 2012), increased vision of predators (Kacelnik, 1979) and direct attraction of arthropod predators to illuminated areas (Deitsch & Kaiser, 2023; McMunn et al., 2019). We also predicted that nocturnal predation would be highest for cryptic models in lit conditions, because broad-spectrum white LED light improves colour discrimination, making cryptic prey more conspicuous and therefore more vulnerable (McMahon et al., 2022; Seymoure et al., 2024). Finally, we hypothesised that ALAN would have carry-over effects on daytime predation, as we expected altered communities at the light transects after 12 years of exposure (Davies et al., 2012; Spoelstra et al., 2023).

4. Material and methods

4.1. Indoor bird predation experiment

4.1.1. Plants, insects and birds

The caterpillars that we used in the indoor experiment were from the noctuid cabbage moth, *Mamestra brassicae* (Lepidoptera, Noctuidae), the caterpillars of which are cryptically coloured. Eggs were obtained from a laboratory population, reared at Wageningen University & Research (WUR), Wageningen, the Netherlands. Larvae were cultured in individual rearing cups on a pinto bean-based artificial diet, under controlled conditions (14-16°C, 60 % humidity, 12L:12D). Second and third instar caterpillars were used for the experiment.

The host plant on which *M. brassicae* caterpillars feed is cabbage. We used brussels sprouts, *Brassica oleracea* L. *gemmifera* DC (Brassicaceae) plants (Gebr. Eveleens) that were grown under optimal growing conditions (22°C, 60% humidity, 16L:8D) in the greenhouse of the University of Amsterdam (UvA), Amsterdam, the Netherlands. Plants were grown for 7-8 weeks before being used in the experiment.

Great tits, *Parus major* (Passeriformes, Paridae), used in this study were F2 offspring from a common-garden experiment (Tomotani et al., 2025). The parental (P) generation originated from urban populations in three Dutch cities (Amsterdam, Delft, and Utrecht). Eggs from these urban pairs were collected immediately after laying and fostered by great tits in a nearby forest population (Bennekomse Bos). Ten days after hatching, F1 chicks were transferred to the Netherlands Institute of Ecology (NIOO-KNAW, Wageningen, the Netherlands) and hand-raised following a standardised protocol (Drent et al., 2003). F1 birds from the same source populations were paired to produce the F2 generation; their eggs were again fostered by Bennekomse Bos birds, and F2 chicks were brought to NIOO-KNAW at 10 days of age. All birds were reared in captivity and housed in outdoor aviaries. For this experiment, we used 16 great tits (8 females, 8 males), randomly assigned to form single-sex pairs.

4.1.2. Experimental set-up

The indoor bird predation experiment was conducted in sixteen indoor climate aviaries (2m x 2m x 2.25m) at the NIOO-KNAW, Wageningen, the Netherlands. The aviaries were maintained at a constant temperature of 14°C. The light-dark cycle was fixed at 8L:16D, with an additional half-hour of dim lighting at the start and end of the dark period to simulate dawn and dusk.

Eight aviaries were exposed to nocturnal light (MM03227 night light; MEGAMAN, the Netherlands; 2700 K), at an intensity of 1.5 lux in the centre of the aviary. The other eight experienced fully dark nights. The dark and the lit night treatments each included four aviaries with a single-sex bird pair, which were randomly assigned. In total, this meant that eight aviaries out of sixteen had a pair of great tits and eight did not.

To allow for acclimatisation, all plants, caterpillars and birds were exposed to their assigned light treatment for five days prior to the experiment. For the first four days, plants, caterpillars and birds acclimated separately in different climate rooms.

On the fifth day, plants were spread over the different aviaries, forming three plant patches per aviary. Each plant patch consisted of three potted brussels sprouts plants connected through their leaves, so that each aviary had nine plants in total. Each pot contained one dried and sterilised dead *Acer sp*. leaf that served as an additional hiding spot without feeding options. On each plant patch, 10 cabbage moth caterpillars were placed. During this period all three patches were covered by net cages (40cm x 40cm x 60cm), allowing the plants, caterpillars and birds to sense one another and start behaving accordingly, while still preventing predation.

At midnight on sixth day, two out of three net cages were removed to allow nocturnal great tit foraging on these plant patches, while safeguarding the caterpillars on the third patch for plant and caterpillar measurements. Foraging was permitted between 00:00 and 8:30, encompassing nighttime and dawn. Dawn was included in the experimental period, to not disturb subtle differences in foraging timing. All measurements were taken at the end of this period.

The experiment was conducted in two trials, exposing each bird pair to both the light treatment and the dark control. Plants and caterpillars were replaced between trials. The first trial took place from 2 to 9 December 2022, and the second from 9 to 16 December 2022.

4.1.3. Measuring activity and behaviour

4.1.3.1. Great tit activity

Throughout the experiment, great tit activity was monitored using radar-based detectors (Conrad Electronics) that recorded values of 0, 1, or 2 every minute, depending on the amount of movement detected during that minute.

4.1.3.2. Predation rate on caterpillars

Great tit predation on caterpillars was assessed by counting the missing caterpillars on each of the uncovered plant patches. Counts were conducted for both bird-present and bird-absent treatments to account for caterpillar escape and observer retrieval errors. To standardise the searching effort, the maximum searching time per patch was set to 15 minutes.

4.1.3.3. Caterpillar hiding

To evaluate the hiding behaviour of the caterpillars, their positions on the covered plant patch were recorded immediately at the end of the experiment, i.e. at 8:30. This single timepoint was chosen to minimise disturbance, as searching required a thorough and invasive examination of the plants. The position of each larva was noted, including whether it was on the plant (top or bottom half), on the stalk, on the upper or lower side of a leaf, in the pot, underneath the dead leaf, in the soil, or elsewhere in the cage.

4.1.3.4. Caterpillar feeding

Caterpillar feeding activity was assessed using caterpillar weight gain and the surface area of eaten leaves as proxies. The initial group weight of the ten larvae on the covered plant patch was recorded at 17:00 on day 5, just before they were placed on the plants. Their group weight was measured again at the end of the experiment at 8:30 on day 7. Group weight was used as individual caterpillars could not be distinguished. To quantify the surface area of plant material consumed, photographs were taken of leaves that showed signs of herbivory and analysed using ImageJ software (v1.47; Schneider et al., 2012).

4.1.4. Analysis

All model analyses were conducted using R Statistical Software v4.4.0 (R Core Team, 2024). Except for the onset of activity, which was analysed with a Student's t-test, all variables were analysed, using the glmmTMB package (Brooks et al., 2017). Model selection was performed via stepwise removal of the least significant fixed factor. The importance of each fixed factor was evaluated by comparing the AIC values of the full and reduced models (> Δ2 AIC). The assumptions of each model were validated with the DHARMa package (Hartig et al., 2024). Visualisations were made, using ggplot2 (Wickham, 2016).

4.1.4.1. Great tit activity

Calculations of bird onset based on activity data were performed using Chronoshop v1.1 (Spoelstra, 2018), categorising data as active (1) or non-active (0). Due to limited sample size, only light was included as an explanatory variable to avoid over-parametrisation. Onset data were analysed using a Student's t-test, and activity level was analysed using a generalised linear model with a negative binomial distribution.

4.1.4.2. Predation rate on caterpillars

Predation was analysed using a generalised linear mixed model with a Poisson distribution. The full model included light treatment, predator treatment, their interaction, and trial as fixed factors. Initially we included aviary and observer as random factors, but both factors caused a singular fit problem and were therefore removed from the model.

4.1.4.3. Caterpillar hiding

To examine the positioning of larvae under the different treatments, we specifically analysed if the placement in the top versus bottom half was affected, as well as being underneath a leaf (on the plant, or the added dead leaf) or anywhere else. Caterpillar positions were analysed using a generalised linear mixed model with a binomial distribution (nbinom2 family). The full models included light treatment, predator treatment, their interaction, trial, and checking time as fixed factors, with aviary as a random factor.

4.1.4.4. Caterpillar feeding

Caterpillar weight and eaten leaf surface were analysed using general linear mixed models. The full model included light treatment, predator treatment, their interaction, and trial as fixed factors, with aviary as a random factor.

4.1.5. Ethical considerations

All experimental procedures were carried out under the licenses of the Central Authority for Scientific Procedures on Animals and the Animal Welfare Body of the Royal Netherlands Academy of Sciences. Great tits were raised as part of a separate study (protocol NIOO 21.04 AVD 80100 2019 9005), but their use in this study was approved as a sub-threshold experiment (NIOO 22.17/ IvD 2496a).

4.2. Outdoor model predation experiment

4.2.1. Study sites

The outdoor experiment was conducted at four experimental field sites in forest edge habitat within nature reserves at the Veluwe, the Netherlands. These sites, established in 2012 under the Light on Nature project, introduced light transects to previously dark forest areas (Spoelstra et al., 2015). For this experiment, we used full spectrum white light and dark transects. The light transect comprised three 4 m tall streetlights (Fortimo white; Philips, the Netherlands; 2700K) operating from sunset to sunrise, with a ground-level light intensity of 7.6 ± 0.3 lux, comparable with regular streetlights (CIE, 2010). At the time of our experiment, the lights had been on for 12 years. This experiment was conducted over two weeks: sites 1 and 2 were used from 15 to 20 April and from 4 to 9 June 2024, and sites 3 and 4 were used from 21 to 26 April and from 27 May to 1 June 2024 (Table S1 & Fig. S1).

4.2.2. Experimental set-up

To test the effect of ALAN on caterpillar predation rates, a clay model predation experiment was conducted. This technique has been effectively used to estimate predation rates (Cupitra-Rodríguez et al., 2023; Low et al., 2014; Medina et al., 2025; Seifert et al., 2016). Dummy caterpillar models were made of soft modelling clay (Staedtler FIMO). Both cryptic green (T50 - Pistachio nut) and aposematic (green with three yellow-black dots: 16 – Sunflower; 9 – Black) were created (Fig. 2A and 2B). Aposematic models were designed to generically resemble local aposematic species, such as *Saturnia pavonia* (Saturniidae). Models were 40 mm in length and 5 mm in diameter.

All caterpillar models were attached to blueberry plants (*Vaccinium myrtillus*, Ericaceae) using green iron wire (Sencys, Ø1mm). In the absence of blueberry bushes, other small shrubs were used. Models were placed along two lines parallel to the three forest lampposts, with a second line of models near the lampposts (Fig. 1). Placement positions were approximately 5 m apart. In the dark controls we used reference sticks instead of lampposts. Each position contained both a cryptic and an aposematic model, approximately 30 cm apart, to ensure similar illumination levels. This setup resulted in 30 models per type, per transect.

Models were placed in the field for five days and checked for attacks at dusk and dawn to distinguish between day and night attacks. To not disturb foraging during the twilight periods, twilight was included in the nighttime period. To simulate caterpillar herbivory, and the subsequent release of plant volatiles, a hole was punched into a nearby leaf at

the start of the experiment and at each check (Fig. 2C). This method was used to make the model predation experiment more applicable to more chemically-oriented predators, that locate prey via these herbivory signs (Deitsch & Kaiser, 2023; Vieira et al., 2019).

Attacks were classified as invertebrate, mammalian, avian, or undetermined (Low et al., 2014). Attacked models were either replaced or restored. Every day, two observers alternated between the two transect pairs (light & dark) to minimise observer bias.

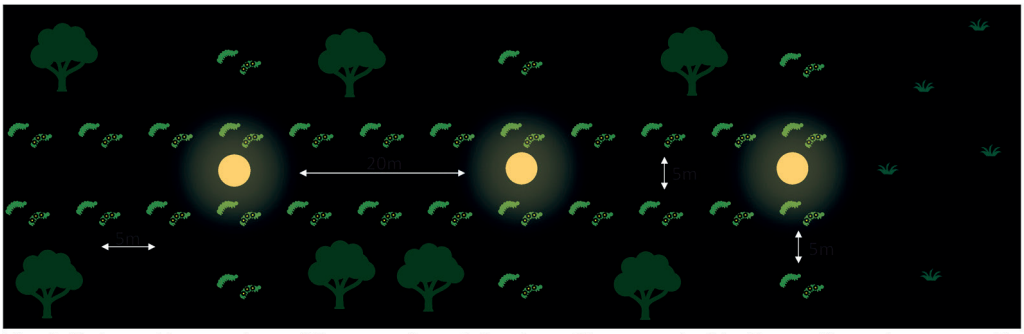


Fig. 1. Schematic overview of the experimental set-up. Placement of both cryptic and aposematic models along the light transect. Same set-up for the dark transects, but without the three lampposts.



Fig. 2. A: Example of a cryptic model in the field. B: Example of an aposematic model in the field. C: Punched hole to mimic herbivory.

4.2.3. Larvae survey

To assess the local abundance of cryptic and aposematic larvae, a sweep-net survey of Lepidoptera and Symphyta was conducted. To avoid disturbing the predation experiment, sweep netting was performed either one week before or after the experiment. Half of each transect (20m) was sampled with a standardised number of 40 sweeps. This method allowed us to sample larvae occurring in herbs and shrubs, where we also placed our dummy caterpillars. Larvae occurring in trees were not sampled.

4.2.4. Light measurements

Light measurements were performed on one clear night per location and sampling week using a LXP-10B luxmeter (Sonel, Poland). Along the light transects, measurements were taken at each model location, in the middle of the cryptic and aposematic models. In the dark transects, three reference measurements were taken along the central line of the transect, spaced twenty meters apart. This simplified approach was used because typical light values ranged between 0.00 lx and 0.01 lx, and the luxmeter was not sensitive enough to discriminate between such low light levels.

4.2.5. Analysis

All statistical analyses were performed in R, version 4.4.0 (R Core Team, 2024). Model selection was performed via stepwise removal of the least significant fixed factor. The importance of each fixed factor was evaluated by comparing the AIC values of the full and reduced models (> Δ2 AIC). Visualisations were made, using ggplot2 (Wickham, 2016).

To test which factors helped to predict the predation risk of the caterpillar models, we performed a mixed effects cox proportional hazard model from the coxme package (Therneau, 2024). The full model included light intensity, model colour, time of day, the interaction between light intensity and model colour, the interaction between time of day and model colour and the three-way interaction between light intensity, model colour and time of day as fixed effects. As random effects, the model included week (expressed as days after the start of the first trial), transect and observer.

To plot the adjusted survival probability, we used the categorical light treatment instead of the continuous light intensity values. Because coxme does not directly provide adjusted survival curves, we fitted a Cox model using coxph from the survival package (Therneau & Grambsch, 2026). In this model, we included a shared frailty term that combined the random effects (week, transect, and observer) to account for clustering. We then used the adjustedsurv function from the adjustedCurves package (Denz et al., 2023) together with predictRisk from the riskRegression package (Gerds et al., 2025) to estimate survival curves by light treatment, model colour and time of day, respectively. These curves are population-average estimates: they show survival probabilities by each of these fixed effects, averaged over the observed covariates and adjusted for the clustering in week, transect, and observer through the frailty term.

5. Results

5.1. Indoor bird predation experiment

In one aviary a great tit succeeded in breaking into a net cage surrounding one of the plant patches. This aviary was therefore excluded from all analyses.

5.1.1. Great tit activity

Birds showed a significant response to light in both their activity onset time and their activity levels. Specifically, bird pairs in the ALAN treatment showed an earlier onset in activity ($t(6) = 3.29$, $p < 0.05$) and a higher level of nocturnal activity ($\chi 2(1, N = 14) = 12.57$, $p < 0.001$) compared to those in the dark control (Fig. 1).

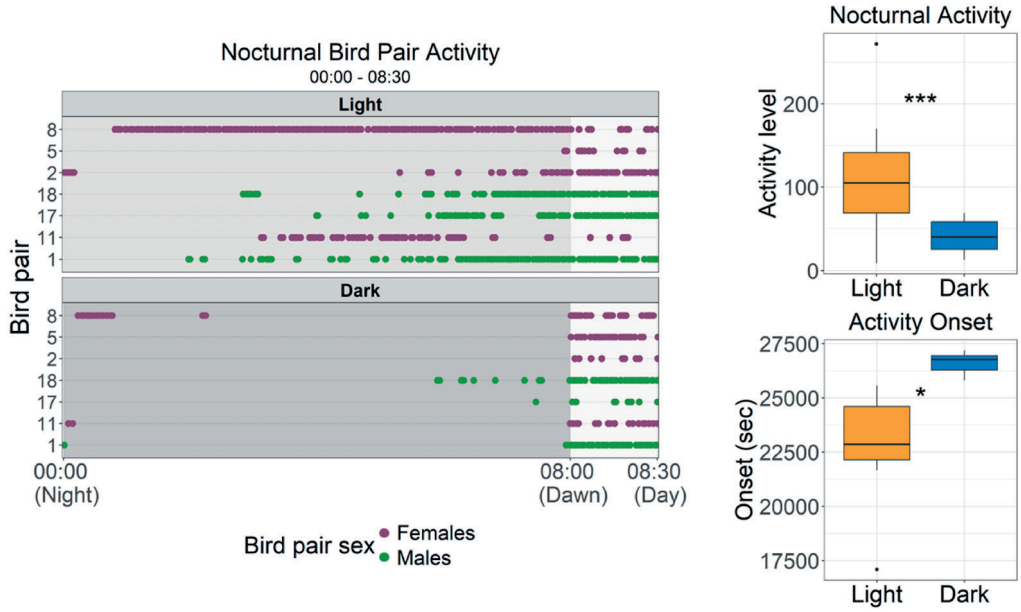


Fig. 1. Left: Nocturnal bird pair activity per aviary over the course of the experiment, which ran from 00:00 till 08:30. Nighttime was between 17:00 and 08:00. Between 16:30-17:00 and 08:00-8:30 twilight periods were mimicked with dim background lights. Top right: The activity level measured per aviary between 00:00 and 08:30. Bottom right: The onset of activity measured per aviary, expressed as seconds after midnight. *: $p < 0.05$, ***: $p < 0.001$.

5.1.2. Predation rate on caterpillars

Light treatment did not help to explain the number of missing caterpillars and was removed from the final model (Fig. 2; Table S3). The best performing model only included predator treatment (Table S3). The presence of birds significantly decreased

the number of caterpillars that could be retrieved ($\chi2(1, N = 34) = 14.24$, $p < 0.001$), suggesting that missing caterpillars were indeed foraged upon by the birds (Fig. 2).

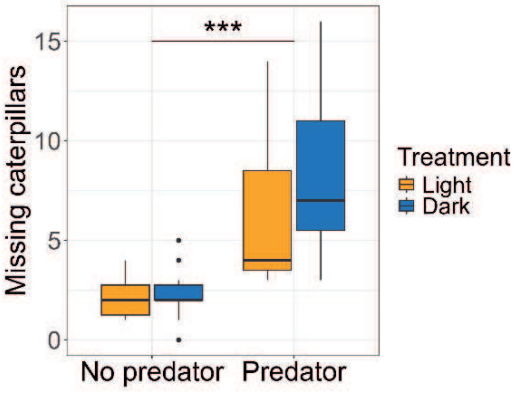


Fig. 2. The number of missing caterpillars per aviary out of a total of 20, per predator and light treatment. ***: $p < 0.001$.

5.1.3. Caterpillar hiding

Light treatment had no predictive value for the position of caterpillars on the plant (Fig. 3, Table S3). The position of caterpillars on the plant was instead explained by predator treatment, trial and checking time, with these factors included in the final model (Table S3). The proportion of caterpillars in the top half of the plant was higher in the presence of predators compared to the predator-free controls ($\chi2(1, N = 184) = 5.44$, $p < 0.05$; Fig. 3). However, this predator effect only became visible when checking time was accounted for in the model (Fig. 3). The proportion of caterpillars in the top half of the plant increased, the later their patch was examined ($\chi2(1, N = 184) = 12.88$, $p < 0.001$; Fig. 3). In the second trial fewer caterpillars were found in the top half compared to the first trial ($\chi2(1, N = 184) = 5.68$, $p < 0.05$).

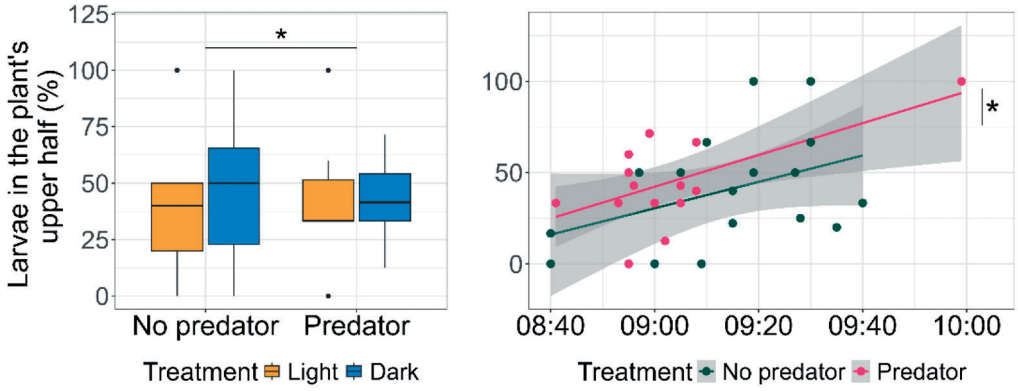


Fig. 3. Left: The portion per aviary of caterpillars found in the upper half of the plant, by predator and light treatment. Right: The portion per aviary of caterpillars found in the upper half of the plant per predator treatment, as recorded at different checking times. *: $p < 0.05$.

Light and predator treatment did not help to explain caterpillar hiding underneath leaves (Fig. 4; Table S3). Hiding underneath a leaf was best explained by only retaining trial as fixed effect in the model (Table S3). In the second trial, more caterpillars were found underneath a leaf than the first trial ($\chi2(1, N = 320) = 4.74$, $p < 0.05$).

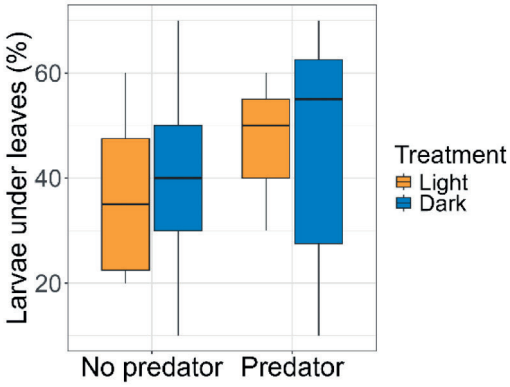


Fig. 4. The portion of caterpillars per aviary hiding underneath a leaf (alive *Brassicae* leaf or dead *Acer* leaf), shown without and with predator per light treatment.

5.1.4. Caterpillar feeding

Caterpillar feeding behaviour was not effectively explained by light or predator treatment (Fig. 5; Table S3). Therefore, the optimal models for weight gain and eaten leaf surface only included trial (Table S3). Weight gain and eaten leaf surface were larger in the second trial than in the first trial ($\chi2(1, N = 34) = 270.98$, $p < 0.001$; $\chi2(1, N = 34) = 58.37$, $p < 0.001$, respectively).

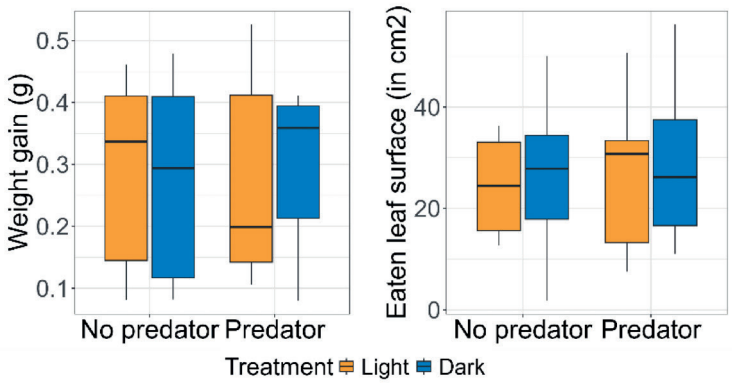


Fig. 5. Left: Caterpillar weight gain per group of 10 caterpillars per aviary (from day 5, 17:00 to day 7, 08:30), shown by predator and light treatment. Right: Total leaf area consumed per aviary, shown by predator and light treatment.

5.2. Outdoor model predation experiment

Over the course of four trials, 9600 observations were made. In total, we recorded 265 attacks of which 200 were classified as invertebrate, 33 as mammal, and 20 as bird attacks (Fig. 6). Twelve attacks could not be classified (Fig. 6). At one observation, we recorded two different predatory marks on one model. Since only a limited amount of the attacks could be categorised into mammal or bird attacks, we analysed all attacks together without discriminating between predators.

The optimal model to explain survival probability of the caterpillar models included model colour and time of day (Table S4). Light intensity did not help to explain survival probability (Fig. 7; Table S4). Cryptic models had significantly lower survival probabilities than aposematic models ($\chi 2(1, N = 9601) = 4.19$, $p < 0.05$). In addition, models had higher chances of attack during the night $\chi 2(1, N = 9601) = 8.72$, $p < 0.01$).

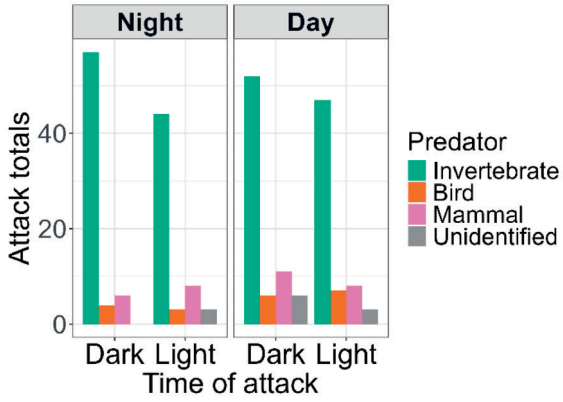


Fig. 6. The total number of attacks during night- and daytime of respectively invertebrate, bird, mammal and unidentified predators, shown per light treatment.

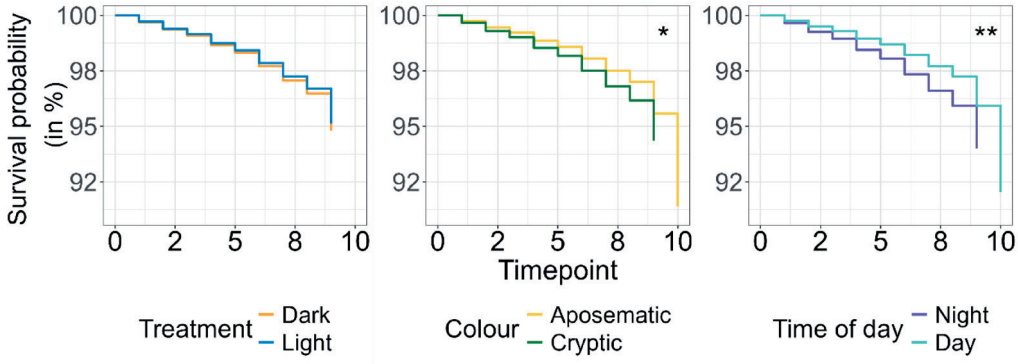


Fig 7. The survival probability of caterpillar models, depending on the light treatment (left), model colouration (centre) and time of day (right). The curves represent the population-average survival, adjusted for the random factors (week, transect and observer). The night also included the twilight periods. Timepoints represent the bi-daily (morning, evening) checkpoints over a period of 5 days. * : $p < 0.05$; ** : $p < 0.01$.

6. Discussion

This study assessed whether artificial light at night (ALAN) affects caterpillar predation by great tits in a controlled indoor experiment and by predator communities exposed to long-term artificial lighting in an outdoor experiment. We did not detect any effect of ALAN on predation in either experiment. While great tits did increase their activity under ALAN, this did not result in higher attack rates on caterpillars. Outdoors, the model

predation experiment did not show any difference between the illuminated and dark transects in survival probability of cryptic and aposematic caterpillar models.

The finding that great tits exhibited an earlier onset of activity when exposed to low levels of ALAN in our indoor experiment corroborates results of other studies (de Jong et al., 2016; Spoelstra et al., 2018). However, despite increased nocturnal bird activity in lit conditions, we did not detect an increase in nocturnal predation on caterpillars, in contrast to our hypothesis. This finding cannot be attributed to changes in behaviour by the prey, because we did not find increased caterpillar hiding behaviour under lit conditions. Instead, the dim light level used in the experiment (1.5 lux at the centre of the aviary) was probably insufficient for birds to visually detect the cryptic *Mamestra brassicae* caterpillars on plants, limiting their foraging to the dawn period. Indeed, great tits forage less efficiently on cryptic prey models below approximately 7 lux (Kacelnik, 1979). We specifically chose the dim light condition that is representative of the light levels encountered in a wider range of their foraging habitats, not only close to the light source (*personal observations*, Dominoni et al., 2013). For example, free-ranging urban European blackbirds (*Turdus merula,* Turdidae) equipped with micro light loggers experienced nocturnal light levels between 0.07 and 2.2 lux (Dominoni et al., 2013).

In addition to not being able to detect the prey, birds may prioritise other behaviours than inefficient foraging during early hours. In natural environments, territorial behaviours such as singing are most effective at dawn (Kacelnik & Krebs, 1983). Since our experimental set-up did not include video monitoring, we cannot confirm which behaviours birds engaged in during their nocturnal activity periods.

Nocturnal foraging under ALAN has been found for several bird species (Da Silva et al., 2017; Lebbin et al., 2007; Leopold et al., 2010; Leveau, 2020; Russ et al., 2015; Santos et al., 2010). Possibly, the birds observed in these studies may have better vision, their food source might have been easier to spot, or the light conditions might have been more favourable for effective foraging.

In the indoor experiment, we could also assess changes in caterpillar behaviour. Unexpectedly, the light treatment did not affect hiding behaviours of caterpillars, as we did not find an increase in caterpillars underneath living *Brassica* or dead *Acer* leaves. The presence of predators did not affect their hiding underneath leaves either. Many caterpillar species, particularly palatable ones, are frequently found on the underside of leaves, a behaviour generally considered protective against natural enemies and unfavourable abiotic conditions (Heinrich, 1979; Watabe et al., 2018; Watts & Kariyat, 2021). Notably, caterpillars exhibit this behaviour even when the underside is less profitable, they do so even in a predator-free laboratory environment (Watts & Kariyat,

2021). This suggests that such sheltering may be evolutionarily fixed and not strongly influenced by immediate predation risk or light conditions.

Caterpillars did shift their position in the plant in response to the presence of predators, regardless of light conditions. Specifically, we found caterpillars more often in the upper half of the plant when great tits were present than when they were absent. This result was contrary to our prediction that caterpillars would be in the lower parts of the plant to hide from the birds, that frequently perched on a high branch or flew above. One possible explanation is that the upper half of the plants contained more leaves, providing more cover and better hiding opportunities for the caterpillars.

We also did not find any caterpillar feeding response to the light treatment. Their feeding behaviour, measured by caterpillar weight gain and leaf consumption, was not affected by ALAN. Previous research did find ALAN effects on caterpillar feeding activity and/or mass (Grenis & Murphy, 2019; Haynes et al., 2023; Van de Schoot et al., 2025). For example, nocturnal *Apamea sordens* (Noctuidae) caterpillars weighed 43% less under ALAN conditions (Grenis & Murphy, 2019), while caterpillars of the diurnal *Danaus plexippus* (Nymphalidae) butterfly doubled their nocturnal feeding, although pupal mass was unchanged (Haynes et al., 2023). For the mainly diurnally active *Arctia caja* (Erebidae) caterpillars, ALAN reduced pupal mass, but only in females (Van de Schoot et al., 2025). Notably, these studies assessed ALAN effects over the entire development, while our study focused on short-term changes over approximately 39 hours.

In contrast to our hypothesis, caterpillar feeding behaviours were also not affected by predator exposure, i.e. presence of the birds. Other researchers found that exposure to predatory stink bugs strongly reduce leaf consumption in caterpillars, such as *Manduca sexta* (Sphingidae) and *Trichoplusia ni* (Noctuidae) (Kaplan et al., 2014). Because stink bugs moved freely on the plants, their presence may have had a stronger influence on caterpillar feeding behaviour than birds in our experiment that were kept at a distance with a net cage. However, caterpillar weight was not affected by predator presence in either experiment. In our study, measuring weight gain at the group level (ten caterpillars per group) may have masked individual-level responses, but as we did not find a difference in leaf consumption between treatments either, our results appear robust.

In the outdoor experiment, predation rates on both anti-predator colour types did not differ between ALAN and dark conditions, contrary to our expectations. This lack of an effect may be explained by the small proportion of visual predators in our system. Only 8% of attacks were attributed to birds, 12% to mammals, and the vast majority (75%) to arthropods. Had we seen a higher number of attacks from visually hunting predators

such as birds, then we might have seen higher attack rates on caterpillars in the illuminated sites, especially on the more vulnerable cryptic ones. With only a limited number of attacks by visual predators, we had limited power to detect such potential patterns.

Still, even with most attacks coming from invertebrates, we would have expected higher predation under ALAN than in darkness. This expectation was based on the idea that diurnal predators might have extended their foraging into the night under artificial light (Frank, 2009), and that invertebrate predators could have been directly attracted to illuminated areas (Deitsch & Kaiser, 2023; McMunn et al., 2019).

Similar studies, using fixed, non-flying prey show mixed effects of ALAN on predation. Some report higher predation under ALAN (Deitsch & Kaiser, 2023; McMunn et al., 2019; Nuñez et al., 2021), whereas others find no effect or even reduced predation (Eckhartt & Ruxton, 2022; Grenis et al., 2015; Heinen & López Pérez, 2025). This inconsistency is also evident when considering only dummy caterpillar experiments (Deitsch & Kaiser, 2023; Heinen & López Pérez, 2025). A notable pattern is that studies reporting increased predation typically introduced new artificial lights for the experiment, whereas studies reporting no or negative effects examined existing light sources. This suggests that any initial increase in predation under newly introduced ALAN may only be short-term or offset by longer-term changes in local predator communities.

Supporting this idea, a community study at our experimental research sites found that the composition of ground-dwelling arthropods changed after several years of light exposure, with for example less ants in the lit areas (Spoelstra et al., 2023). Such shifts in community composition could greatly affect species interactions and predator-prey dynamics (Davies et al., 2012, 2017; Spoelstra et al., 2023). Community alterations could also cause carry-over effects of ALAN on daytime predation (Davies et al., 2012). However, such carry-over effects were not found in our study.

Since we did not perform a similar predation experiment when our previously light-naïve forest edge research areas were first exposed to light, we can only speculate short-term effects of ALAN in these communities. However, this highlights the importance of these long-term studies, as they more accurately mimic the effects of chronic ALAN that now exists across much of the planet.

Irrespective of light exposure, models were mainly attacked during the night. Since our models were mainly attacked by invertebrates, and 60% of invertebrates are nocturnal (Hölker et al., 2010), higher nighttime predation is not unexpected. However, a similar cryptic model predation experiment conducted in a tropical habitat found higher invertebrate predation during the day, supporting the idea that nighttime functions as

an enemy-safe space for caterpillars (Heinrich, 1979; Seifert et al., 2016). Caterpillar models may have been especially vulnerable during twilight periods (Da Silva et al., 2017), which we included as part of the nighttime to avoid disturbing the subtle effects of ALAN on foraging timing. In addition, the day–night ratio was not perfectly balanced throughout the sampling period (period one: ~11 h night, 11 h day; period two: ~10 h night, 12 h day; excl. checking time). However, this imbalance was small, so it is unlikely to explain the observed differences in attack rates.

Furthermore, we found that cryptic models were more frequently attacked than aposematic models, again independent of light exposure. It remains unclear why invertebrates would preferentially attack cryptic models, as these more chemically oriented predators are not expected to make a distinction between colour models (Aslam et al., 2020). One possibility is that different clay types had different chemical profiles, but the main body of both cryptic and aposematic models was formed from the same clay, making this unlikely. The extra clay colours or increased handling for aposematic models might have resulted in slightly different chemical profiles, although again this is expected to be minimal since all models were stored in the same containers. During the experimental period we only found cryptic caterpillars in the area (Table S2), which might indicate that this is the most encountered colour type by predators.

Dummy predation experiments are widely used to compare attack rates (Cupitra-Rodríguez et al., 2023; Low et al., 2014; Seifert et al., 2016). However, this method has its shortcomings (Nagy et al., 2020; Rößler et al., 2018). For example, not all marks are left by species that would or could attack alive caterpillars. Conversely, not all true natural enemies would attack these dummies. Despite these limitations, dummy experiments remain a practical and effective way to compare attacks under different conditions.

Taken our indoor and outdoor experimental results together, our main conclusion is that some predator-prey interactions may be more robust to disturbance by ALAN, especially when light pollution levels are relatively low and when light exposure has lasted already for multiple years. In addition, the results of our study with the great tits show that changes in predator behaviour do not necessarily cascade down to the prey. Since several studies have reported ALAN-induced changes in predation rates (Deitsch & Kaiser, 2023; Eckhartt & Ruxton, 2022; Heinen & López Pérez, 2025; McMunn et al., 2019), the lack of impact observed in our study should not be interpreted as evidence that ALAN has no effect on predation. Instead, it highlights that the effects of ALAN on predator–prey interactions are strongly context-dependent.

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10. Supplementary material

10.1. Site locations

Table S1. An overview of the site locations, names and given numbers, as well as the date since when the lights were on. Adapted from Spoelstra et al. (2015).

Site number	Site name	Light on since	Coordinates	
1	Klaterweg 1	21/04/2012	52 22'36''	5 53'51''
2	Klaterweg 2	21/04/2012	52 22'6''	5 53'11''
3	ASK Doornspijk 1	10/05/2012	52 21'52''	5 51'25''
4	ASK Doornspijk 2	10/05/2012	52 22'2''	5 51'13''

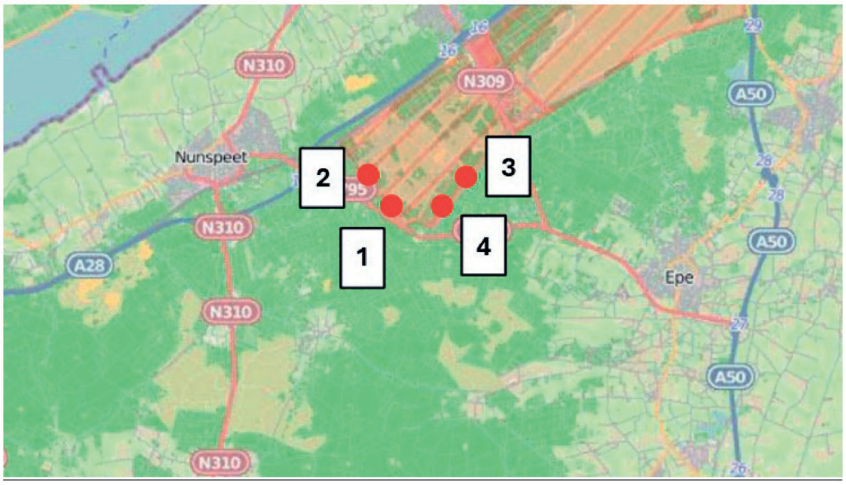


Fig S1. Site locations on the map.

10.2. Attack marks



Fig. S2. Slug feeding marks. Marks from large slugs were especially hard to distinguish from rodent bite marks.

10.3. Larvae survey

To get an idea of the locally occurring anti-predator colourations in the field, we sampled half of each transect by sweep netting. Both Lepidoptera and Symphyta larvae were included, as these larvae have a very similar visual appearance to predators. Larvae were categorised into three colouration types: green cryptic, brown cryptic or aposematic larvae. We also measured the sizes of each larva. On average green cryptic larvae were 13 mm, ranging from 6 mm to 30 mm. Brown cryptic larvae were on average 8 mm, ranging from 4 mm to 13 mm. No aposematic larvae were found during our experiment.

Table S2. Overview of larval colourations found in the locally sampled larval communities (Lepidoptera & Symphyta) at the time of our experiments.

Location	Klaterweg (first period)				ASK (first period)				ASK (second period)				Klaterweg (second period)			
Treatment	Light		Dark		Light		Dark		Light		Dark		Light		Dark	
Transect	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Cryptic larvae green	0	0	0	0	0	0	0	0	0	1	1	1	2	0	4	1
Cryptic larvae brown	0	0	0	0	0	0	0	0	2	3	2	0	0	0	0	0
Aposematic larvae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

10.4. Model selection

Table S3. Overview of all model selection steps, performed for the analysis of the indoor experiment. For each model, the AIC (Akaike Information Criterion) values are given together with the test statistics for each of the included explanatory variables. The model that performed best is indicated in bold. Aviary was included as a random factor in all models except for the predation rate model, where the inclusion of random terms caused a problem with singular fit. For the random factor, variance (S^2) and standard deviation (SD) are given. Random factors were not considered in the model simplification process.

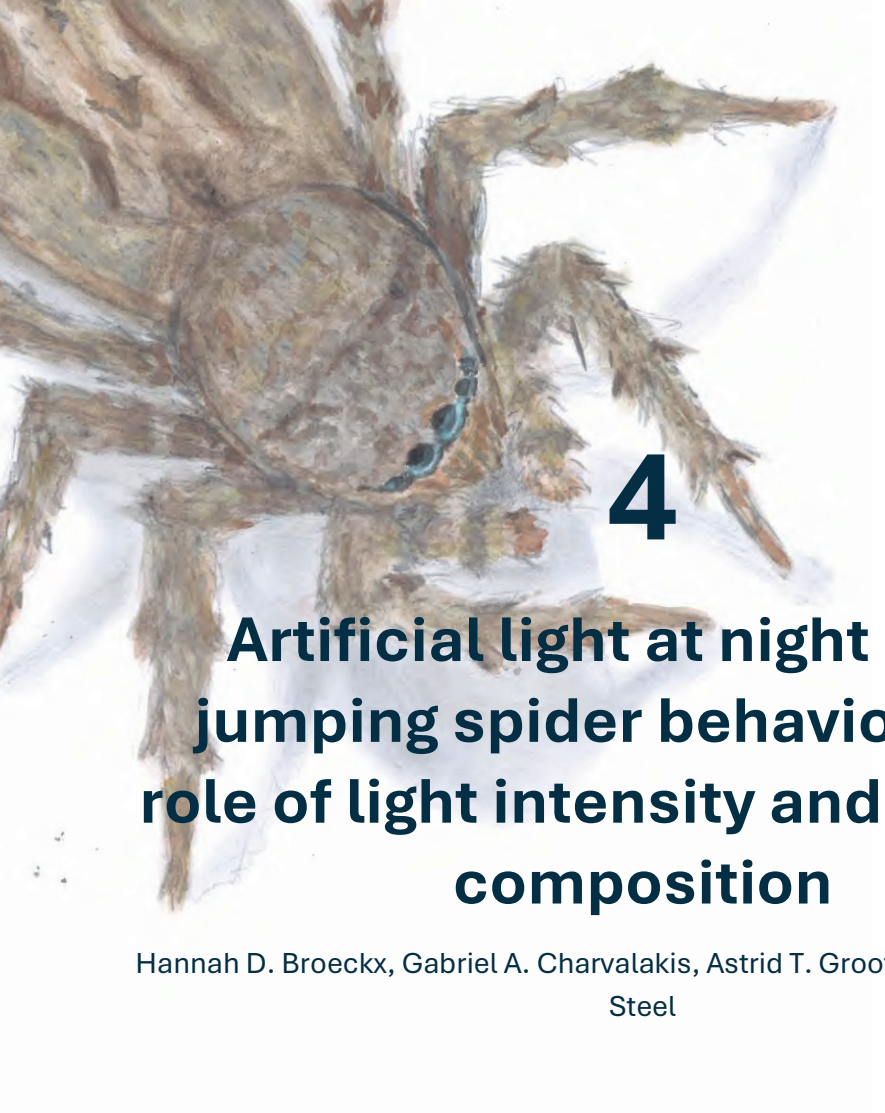
Predation rate on caterpillars						
Model	AIC	Light	Predator	Trial	Light:Predator	/
M1	188.37	$\chi2(1, N = 34) = 0.01$, $p = 0.9156$	$\chi2(1, N = 34) = 4.62$, $p = 0.0317$	$\chi2(1, N = 34) = 0.37$, $p = 0.5426$	$\chi2(1, N = 34) = 0.71$, $p = 0, 4001$	
M2	187.08	$\chi2(1, N = 34) = 0.52$, $p = 0.4722$	$\chi2(1, N = 34) = 14,24$, $p = 0.0002$	$\chi2(1, N = 34) = 0.31$, $p = 0.5798$		
M3	185.39	$\chi2(1, N = 34) = 0.48$, $p = 0.4879$	$\chi2(1, N = 34) = 14.24$, $p = 0.0002$			
M4	183,87		$\chi2$ $(1, N = 34) = 14.24$, $p = 0.0002$			
Caterpillar feeding: weight gain						
Model	AIC	Light	Predator	Trial	Light:Predator	*Aviary*
M1	-91.78	$\chi2(1, N = 34) = 0.42$, $p = 0.5191$	$\chi2(1, N = 34) = 0.19$, $p = 0.6632$	$\chi2(1, N = 34) = 288.46$, $p = 0.0000$	$\chi2(1, N = 34) = 0.05$, $p = 0.8276$	S^2: 0.00 SD: 0.02
M2	-93.73	$\chi2(1, N = 34) = 1.04$, $p = 0.3081$	$\chi2(1, N = 34) = 0.15$, $p = 0.7011$	$\chi2(1, N = 34) = 288.57$, $p = 0.0000$		S^2: 0.00 SD: 0.02

M3	-95.58	$\chi2(1, N = 34) = 1.04$, $p = 0.3081$		$\chi2(1, N = 34) = 288.57$, $p = 0.0000$		S^2: 0.00 SD: 0.02
M4	-96.57			$\chi2(1, N = 34) = 270.98$, $p = 0.0000$		S^2: 0.00 SD: 0.04
Caterpillar feeding: Eaten leaf surface						
Model	AIC	Light	Predator	Trial	Light:Predator	*Aviary*
M1	253.50	$\chi2(1, N = 34) = 0.41$, $p = 0.5242$	$\chi2(1, N = 34) = 0.49$, $p = 0.4834$	$\chi2(1, N = 34) = 59.60$, $p = 0.0000$	$\chi2(1, N = 34) = 0.00$, $p = 0.9659$	S^2: 17.38 SD: 4.17
M2	251.50	$\chi2(1, N = 34) = 0.75$, $p = 0.3873$	$\chi2(1, N = 34) = 0.85$, $p = 0.3575$	$\chi2(1, N = 34) = 59.93$, $p = 0.0000$		S^2: 17.38 SD: 4.17
M3	250.23		$\chi2(1, N = 34) = 0.85$, $p = 0.3575$	$\chi2(1, N = 34) = 58.37$, $p = 0.0000$		S^2: 16.24 SD: 4.03
M4	249.06			$\chi2(1, N = 34) = 58.37$, $p = 0.0000$		S^2: 18.40 SD: 4.29
Caterpillar hiding: on the plant						
Model	AIC	Light	Predator	Trial	Time	Light:Predator	*Aviary*
M1	237.64	$\chi2(1, N = 184) = 0.51$, $p = 0.4767$	$\chi2(1, N = 184) = 3.47$, $p = 0.0625$	$\chi2(1, N = 184) = 6.39$, $p = 0.0114$	$\chi2(1, N = 184) = 13.78$, $p = 0.0002$	$\chi2(1, N = 184) = 0.01$, $p = 0.9074$	S^2: 0.01 SD: 0.08
M2	235.66	$\chi2(1, N = 184) = 0.78$, $p = 0.3767$	$\chi2(1, N = 184) = 5.90$, $p = 0.0151$	$\chi2(1, N = 184) = 6.38$, $p = 0.0115$	$\chi2(1, N = 184) = 13.81$, $p = 0.0002$		S^2: 0.01 SD: 0.10
M3	234.44		$\chi2(1, N = 184) = 5.44$, $p = 0.0197$	$\chi2(1, N = 184) = 5.68$, $p = 0.0171$	$\chi2(1, N = 184) = 12.88$, $p = 0.0003$		S^2: 0.02 SD: 0.16

Caterpillar hiding: underneath a leaf or not							
Model	AIC	Light	Predator	Trial	Time	Light:Predator	*Aviary*
M1	441.07	$\chi2(1, N = 320) = 0.12, p = 0.7316$	$\chi2(1, N = 320) = 3.27, p = 0.0706$	$\chi2(1, N = 320) = 5.72, p = 0.0168$	$\chi2(1, N = 320) =1.73, p = 0.1887$	$\chi2(1, N = 320) = 0.06, p = 0.8055$	S^2: 0.06 SD: 0.24
M2	439.14	$\chi2(1, N = 320) = 0.06, p = 0.8107$	$\chi2(1, N = 320) = 4.51, p = 0.0337$	$\chi2(1, N = 320) = 5.74, p = 0.0166$	$\chi2(1, N = 320) = 1.75, p = 0.1858$		S^2: 0.06 SD: 0.25
M3	437.19		$\chi2(1, N = 320) = 4.58, p = 0.0324$	$\chi2(1, N = 320) = 5.97, p = 0.0145$	$\chi2(1, N = 320) = 1.71, p = 0.1912$		S^2: 0.06 SD: 0.25
M4	436.97		$\chi2(1, N = 320) = 3.42, p = 0.0643$	$\chi2(1, N = 320) = 5.19, p = 0.0227$			S^2: 0.05 SD: 0.23
M5	438.13			$\chi2(1, N = 320) = 4.74, p = 0.0295$			S^2: 0.11 SD: 0.33

Table S4. Overview of all model selection steps, performed for the analysis of the outdoor experiment. For each model, the AIC (Akaike Information Criterion) values are given together with the test statistics for each of the included explanatory variables. The model that performed best is indicated in bold. All models also included random factors: week, transect, observer.

Adjusted survival probability							
Model	AIC	Light intensity	Model colour	Time of day	Light intensity : Model colour	Time of day : Model colour	Light intensity : Time of day : Model colour
M1	4286.94	$\chi2(1, N = 9601) = 2.70, p = 0.1006$	$\chi2(1, N = 9601) = 4.83, p = 0.0279$	$\chi2(1, N = 9601) = 2.29, p = 0.1306$	$\chi2(1, N = 9601) = 0.45, p = 0.5032$	$\chi2(1, N = 9601) = 1.55, p = 0.2126$	$\chi2(2, N = 9601) = 4.14, p = 0.1259$
M2	4288.02	$\chi2(1, N = 9601) = 0.14, p = 0.7073$	$\chi2(1, N = 9601) = 7.94, p = 0.0048$	$\chi2(1, N = 9601) = 0.41, p = 0.5244$	$\chi2(1, N = 9601) = 0.37, p = 0.5412$	$\chi2(1, N = 9601) = 3.70, p = 0.0543$	
M3	4286.39	$\chi2(1, N = 9601) = 1.23, p = 0.2673$	$\chi2(1, N = 9601) = 7.67, p = 0.0056$	$\chi2(1, N = 9601) = 0.41, p = 0.5233$		$\chi2(1, N = 9601) = 3.69, p = 0.0546$	
M4	4285.71		$\chi2(1, N = 9601) = 7.67, p = 0.0056$	$\chi2(1, N = 9601) = 0.41, p = 0.5225$		$\chi2(1, N = 9601) = 3.69, p = 0.0547$	
M5	4287.44		$\chi2(1, N = 9601) = 4.19, p = 0.0406$	$\chi2(1, N = 9601) = 8.72, p = 0.0032$			



4

Artificial light at night alters jumping spider behaviour: the role of light intensity and spectral composition

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1. Abstract

The use of artificial light at night (ALAN) enhances perceived safety and enables humans to extend their activity periods beyond the natural daylight hours. As a result, ALAN continues to increase worldwide. However, humans are not the only diurnal species capable of exploiting this night light niche. Despite its growing prevalence, the extent to which other species utilise ALAN, and the resulting ecological consequences, remain poorly understood. In this study, we examined if nocturnal light with varying intensities and spectra impacted the movement activity and sheltering behaviour of the diurnal jumping spider, *Marpissa muscosa*. We found that bright, cold white LED light (100 lux, 5500 K) significantly increased the nocturnal movement of these jumping spiders, while dimmer and warmer light treatments did not. Furthermore, bright, warm white LED light (100 lux, 2700 K) increased overall sheltering behaviour. We then investigated if altered nocturnal activities of the spiders translated to an increased predation rate on *Drosophila* prey. The increased activity in cold white light also resulted in increased nocturnal predation of *Drosophila*, suggesting that jumping spiders exploit ALAN. While the longer-term physiological and fitness consequences of these altered spider activities need to be further investigated, our results show that intense ALAN can impact predator-prey interactions.

2. Keywords

Jumping spiders, Artificial light at night, Movement, Activity, Sheltering, Nocturnal foraging

3. Introduction

ALAN disrupts life on earth

Artificial light at night (ALAN) is increasingly recognised as a significant environmental pollutant. In just a short time, humans have introduced numerous sources of artificial light into the nightscape, primarily to extend human activity and create a sense of safety (Boyce, 2019). Sky brightness increases by 2.2-9.6% each year (Hölker et al., 2010; Kyba et al., 2017, 2023). Through the process of light scatter, the effects of ALAN can extend far beyond the immediate vicinity of its sources (Jechow et al., 2020). As a result, pristine darkness has become a rare phenomenon (Falchi et al., 2016), leaving almost no taxa untouched by ALAN. Since life on Earth has evolved under stable natural light-dark cycles, the introduction of ALAN at unusual times, places, intensities and wavelengths has far-reaching consequences (Dominoni & Nelson, 2018). These disruptions can alter biological organisation at every level, from genetic, physiological or behavioural processes in individual organisms to the functioning of entire ecosystems (Boyes et al., 2021; Gaston et al., 2015; Hopkins et al., 2018; Sanders & Gaston, 2018).

ALAN disrupts temporal niches

One way in which ALAN impacts organisms is through distortion of the natural daily light cycle, which steers their daily activity patterns (Borges, 2022). For multiple diurnal species, it has been shown that ALAN leads to a temporal niche expansion, where diurnal organisms extend their activities into the night (Alaasam et al., 2018; Frank, 2009; E. Gomes et al., 2021, 2024; Henke et al., 2022; 2020; Maurer et al., 2019; Spoelstra et al., 2018). Simultaneously, ALAN can compress or even inhibit the activity of nocturnal species (Jiang et al., 2023; Rotics et al., 2011; Stone et al., 2009; van Geffen et al., 2015). As a result, ALAN alters species interactions, both increasing or reducing the temporal overlap between different groups of organisms. This can lead to changes in many key ecological processes, including competition and predator-prey dynamics.

Spiders as key predators shaping ecosystems

Spiders are an important group of predators in terrestrial ecosystems. They form one of the most abundant, ubiquitous and diverse group of arthropod predators, with a variety of hunting techniques and temporal activity patterns (Khan & Singh, 2024; Wise, 1995). Spiders occupy a central position in the food web. They act both as predator and prey, regulating invertebrate populations while also serving as food sources (Khan & Singh, 2024).

Most of our knowledge on the impact of ALAN on spiders comes from web-building spiders. Research on two orb-web spider species, *Larinioides sclopetarius* and *Eriophora biapicata* (Araneidae), showed a preference for web building in illuminated sites, where spiders typically have higher prey captures (Heiling, 1999; Willmott et al., 2019). However, when prey density and light are experimentally decoupled, and spiders have the choice between prey dense sites and lit sites, *Agelenopsis pennsylvanica* (Agelenidae) chooses light over prey (Walsh et al., 2025). The attraction to light appears to be an innate response, as lab-reared orb-web spiders (*E. biapicata*), also showed a preference for lit environments, even when they had no previous foraging experience under ALAN conditions (Willmott et al., 2019).

Not all web-building spiders show this phototactic affinity to ALAN, and differences occur even within the same family. For instance, negative phototaxis has been reported for *Argiope amoena* (Araneidae) (Nakamura & Yamashita, 1997). Additionally, light does not always correlate with higher prey availability. For the giant golden orb weaver, *Nephila pilipes* (Nephilidae), lower prey capture rates were observed under lit conditions (Yuen & Bonebrake, 2017). These varying outcomes may be attributed to differences in spider and prey ecologies, such as web visibility (Craig & Freeman, 1991), prey luring strategy (Chuang et al., 2008), overall prey availability (Willmott et al., 2019), and the characteristics of the light source (e.g. intensity and spectrum).

On the population level, Sullivan et al. (2019) found a non-linear response to ALAN in the abundance of orb-weavers (Araeidae) and long-jawed orb weavers (Tetragnathidae). Specifically, a decrease in abundance was observed at moderate levels of ALAN (0.6-5 lux), while an increased abundance was found at higher levels (10-20 lux). Urban populations of yet another web-building species, the triangulate cobweb spider (*Steatoda* triangulosa, Theridiidae), have even evolved reduced light avoidance compared to rural populations (Czaczkes et al., 2018).

Our knowledge on active hunting spiders is much more limited. For the nocturnal wolf spider *Schizocosa myccooki* (Lycosidae), ALAN increased the detection distance of prey but also delayed movement initiation, resulting in no significant changes in overall predation efficiency (Amoroso, 2024). In a community study, wolf spiders and other ground-dwelling arthropod predators increased in abundance with increased ALAN (Sullivan et al., 2019), suggesting that ALAN had a positive impact on their foraging through e.g. improved visual acuity, extended activity and/or attracted prey. One of the best-known groups of active hunting spiders are the jumping spiders (Salticidae), but studies on the impact of ALAN in this group have so far been limited to anecdotal reports, with only few experimental studies.

ALAN affects jumping spider activity

Jumping spiders are diurnal visual hunters and have one of the sharpest spatial visions found among invertebrates (Steinhoff et al., 2020). This visual sensitivity may make jumping spiders particularly sensitive to light changes during the night. For two species of jumping spiders, *Menemerus confusus* and *Hasarius adansoni*, positive phototaxis towards high intensity light has been reported (Nakamura & Yamashita, 1997). Since these spiders use visual perception as their main foraging modality, ALAN also has the potential to alter their interactions with prey. Evidence for extended foraging due to ALAN is limited to a sparse number of observations made on four jumping spider species: *Menemerus bivittatus, Plexippus paykulli, Platycryptus undatus* and *Sitticus fasciger* (Edwards, 1969; Frank, 2009; Wolff, 1982). Typically, these reports describe foraging activities close to a light source (e.g. greenhouse window or porch lamp), which is thus at high light intensities. In a controlled laboratory setting under low ambient light levels down to 1.35 lux, two species of jumping spiders, *Cyrba algerina* and *C. ocellata*, visually discriminated between prey and conspecific decoys (Cerveira et al., 2019). However, the temporal activity pattern of these spiders was disturbed when transferring them to the testing arena. Therefore, it remains uncertain which light levels and spectra are sufficient to activate spontaneous nocturnal activity in jumping spiders.

In this study, we examined whether different intensity levels and spectra of ALAN can affect the activity patterns of the diurnal jumping spider *Marpissa muscosa* (Araneae, Salticidae). *M. muscosa* (♀: 7.5–14mm; ♂: 6–8.1mm) is a diurnal, active hunting spider with great eyesight (Schmitz, 2004; Steinhoff et al., 2024) and has a palearctic distribution. In its natural environment, *M. muscosa* is typically found on trees, on and underneath the bark (Elfferick, 2018; Tutelaers, 2016). The species can also be found in urbanised habitats, mainly living on man-built vertical structures (Elfferick, 2018).

To investigate the direct impact of ALAN on the activity of *M. muscosa*, we recorded their movement activity and sheltering behaviour over a 24-hour period under three different light conditions and compared it to their activity in dark night conditions. In a second stage, we examined if altered activity patterns under ALAN would lead to altered nocturnal predation rates. To examine these effects, we added fruit flies (*Drosophila sp.*) to the arena and compared the attack rate of *M. muscosa* between light and dark conditions. Overall, we hypothesised that *M. muscosa* would make use of the night light niche, leaving its shelter and increasing its nocturnal movement and foraging activity under ALAN. Furthermore, we expected that increases in nocturnal activity would lead to reduced daytime activity.

4. Material and methods

4.1. Study animals

For this study, we used adult male and female *Marpissa muscosa* spiders of a mixture of wild-caught and laboratory-reared individuals. Wild-caught individuals were collected on the Science Park Amsterdam University campus in the Netherlands, and from a garden area in Berlare, Belgium, in the summers of 2023 and 2024. Laboratory-reared spiders were raised from egg clutches laid by wild-caught females, either from matings in the laboratory or from natural matings that occurred before capture (see Table S2 for an overview of the sex ratios used per trial). Spiders were kept in solitude (when not paired for mating) in plastic boxes enriched with a mixture of bark, wood chippings, moss and egg cartons, under constant climate conditions (25°C, 16:8LD, 65% humidity), for at least one year prior to the experiment. They were fed on a diet of *Drosophila* spp.

For the predation experiment, we used flightless *Drosophila sp*. purchased from a local pet shop. Upon arrival at the university, the flies were kept in the same constant climate conditions as the spiders (25°C, 16:8LD, 65% humidity).

4.2. Light conditions

To test whether ALAN extended the activity period of diurnal *M. muscosa* jumping spiders into the night, we measured spider movement activity under three different light conditions.

To represent a variety of light levels that *M. muscosa* may encounter in the wild, two different light intensities were chosen. In a first round of experiments, we compared dark night conditions to relatively low ALAN levels of 10 ± 0.1 lux, to which we refer as low light intensity light level. With this treatment we simulated the lux levels at ground level underneath a streetlight (Boyce et al., 2009; *personal measurements*). In a second experiment, we compared dark night conditions to high intensity light levels (100 ± 1 lux), which represented the light intensity of approximately one meter underneath a streetlight (*personal measurements*). Spiders can use this higher spot, as there are often higher abundances of light attracted prey present (Frank, 2009; Wakefield et al., 2018).

Both low and high light intensity levels were first tested at a colour temperature of 5500 K, as cool white light is expected to elicit the strongest response in animals (Alaasam et al., 2018; Deichmann et al., 2021; but see Bolliger et al., 2020). To assess whether spiders responded similarly to warmer spectra of light, we also tested the effect of 100 lux at 2700 K (warmer white light).

All light conditions were obtained using Strips of LED lights (RexTech, the Netherlands; 5500 K & 2700 K), which were dimmed to the most approximate light intensity level and then further tailored by covering individual LEDs with aluminum tape. All intensity levels were measured at shelf height, 50 cm below the light source, with a LXP-10 luxmeter (Sonel, Poland). The spectral composition of each light condition was measured with a spectrophotometer (QE Pro High Performance Spectrophotometer; Ocean Insight, United States) (Fig. S1).

To control for the consecutive order in the tested light treatments, we used an independent dark control group for each light condition.

During daytime, lighting was supplied by MASTER TL-D Super 80 lamps (Philips, the Netherlands; 4000 K; suppl. material). These lights were the same for all groups (Table S1).

4.3. Study procedure

4.3.1. Movement activity and sheltering experiments

For all tested light treatments, we followed the same experimental procedure, as follows. A group of spiders of each sex were randomly sorted over two climate rooms (25°C, 16:8LD, 55% humidity). Individual spiders could acclimatise for 3 days under these climatic conditions and light–dark cycles. One group experienced dark night conditions, while the other group experienced one of the respective ALAN conditions (5500 K: 10 lux, 5500 K: 100 lux or 2700 K: 100 lux). Three days was considered sufficient for spiders to acclimatise, as spider entrainment took 1-2 days in previous studies (Jones et al., 2018; Ortega-Escobar, 2002).

During the first two days of the acclimatisation stage, individuals were kept in their familiar housing box, where they were provided with ample *Drosophila* flies so they could feed freely. After these 48 hours, spiders were moved to individual Petri dishes, where they were deprived of food until the end of the experiment. The Petri dishes contained an Eppendorf tube filled with water and a brown paper shelter.

Just before the start of the experiment, shelters were replaced. This replacement stimulated spider movement, since spiders would otherwise stay inside their day-old web built within the shelter (Okuyama, 2011; *personal observations*). To prevent jumping spiders from seeing and interacting with each other, paper walls of 1 cm high were placed between Petri dishes.

To record spider movement, Technaxx TX-117 cameras (Technaxx, Germany) were used. To adjust focal distance, +3.00 reading glasses were placed in front of the camera lenses. Cameras were hung at a 90-degree angle to avoid infrared light

reflection on the Petri dish lids during nighttime shots. Pictures were taken every 30 seconds over the course of 24 hours. To illuminate the scene at night, the cameras made use of infrared light (850nm), as spiders were not found to be sensitive to light beyond the far red (Glenszczyk et al., 2021; Tapia et al., 2020; Yamashita, 2002), suggesting this infra-red light would not cause any alterations to their behaviour.

To control for space-specific confounding factors, the light treatment was translocated to the other climate room between each trial. Spiders were kept in the same climate room but their relative position on the shelves was randomised between the trials. In this way, all spiders experienced both dark and light treatments over two consecutive trials. Most of the spiders were tested in all trials. However, spiders that died over the course of the study were replaced by another acclimatised individual at the start of a trial.

4.3.2. Predation experiment

To test if ALAN-induced activity would lead to altered predation rates, we carried out an additional predation experiment. This experiment was only conducted under the light treatment that showed a change in activity in the first experiment, which was the case in the 100 lux, 5500 K treatment.

The study procedure was similar to the one described above, but with the addition of *Drosophila* flies as prey source, which were added to the Petri dish arena. Three days prior to the experiment, flies were brought into the climate rooms at the same time as the spiders. For one day, the flies acclimatised in their regular housing box, after which five flies were transferred into each Petri dish. On the third day, a jumping spider was transferred to the Petri dish via a hole made in the bottom of the Petri dish (Fig. S2). To keep the flies separate from the jumping spider, we used a transparent plastic divider in the Petri dish (Fig. S2). To provide food for the flies, a banana slice was added to the fly compartment, while the spider compartment contained a water tube and shelter as in the previous experiments (Fig. S2). In this experiment, there was no interim replacement of the shelter as the sight of the flies through the divider kept the spiders more active.

Flies that died during the acclimatisation period were replaced by other acclimatised individuals until 6 hours prior to the start of the experiment. After this time, spiders and flies were left undisturbed.

The predation experiment started by removing the divider at midnight on the fourth day, the day after the jumping spider was introduced into the Petri dish, and when the daytime lighting turned off. Eight hours later, when the lights turned on again, the

numbers of attacked flies were counted. These counts were later checked using the camera footage of that night.

4.4. Automated picture processing

The movement and sheltering behaviour of jumping spiders in response to light was tracked using automated object detection.

We manually labelled a selection of 649 images in CVAT.ai (CVAT.ai Corporation, 2024). A distinction was made between different label categories: *spider*, *shelter* and *other*. *Other* accounted for objects that could be mistaken for spiders (such as spider droppings). These labelled images consisted of a random selection of images from a further unused test trial (291 images), gradually supplemented with images that contained examples of structural, incorrect spider identifications (358 images).

We trained an object detection model using YOLOv8n (Jocher et al., 2023) in Python 3.12.6 (Python Software Foundation., 2024). We randomly selected 80% of the labelled images as the training dataset while the remaining 20% were used as the validation dataset. The model training was completed after 100 epochs.

We then applied the best model weights via a custom tracking algorithm on all the pictures captured during the experiments so that the presence (= outside the shelter in the Petri dish) or absence (= hiding underneath the shelter) of the spider was scored. When the detection had a confidence level higher than 0.3, coordinates were extracted, and bounding boxes were automatically generated. If the bounding box surface contained less than 29000 pixels and the bounding box of the spider and the shelter would overlap, the spider was considered to be inside its shelter. Spider movement was scored when the bounding-box centre shifted by more than 70% of the previous box width along either the x- or y-axis. Spiders were also classified as moving when a spider appeared or disappeared between two frames.

The YOLOv8 model and the parameters used in the consecutive coding were verified with manual checks on a representative subset of the data. The final performance metrics of our training can be found in the supplementary material (Fig. S3-S5).

4.5. Statistical analyses

All analyses were performed with R version 4.4.0 (R Core Team, 2024). Visualisations were made using the ggplot2-package (Wickham, 2016).

4.5.1. Movement activity and sheltering experiments

To analyse the effect of light at night on jumping spiders, we compared each light condition with its own dark control, looking at the response in nocturnal and diurnal movement activity and nocturnal sheltering behaviour of each spider.

We used generalised linear mixed models with negative binomial distributions (nbinom2 family) incorporated in the glmmTMB package (Brooks et al., 2017). For each analysis, the full model contained treatment, sex, their interaction and trial as fixed factors. Trial was implemented as a fixed effect, because it only contained two levels (for each spider the respective light treatment and its dark control), which is insufficient to be added as a random effect (Harrison, 2015). Spider ID was included as a random factor in all models. The importance of including the interaction term, trial and sex was evaluated by comparing the AIC values of the full and reduced models (> Δ2 AIC). Treatment always stayed in the model, as this was our main factor of interest. When the interaction term was significant, emmeans (Lenth et al., 2025) was used to make post-hoc pairwise comparisons. A Tukey correction for multiple testing was applied. Assumptions of each model were validated with the DHARMa package (Hartig et al., 2024). Based on the DHARMa diagnostics, a zero-inflation term was added to the 10 lux daytime activity model.

4.5.2. Predation experiment

To compare the predation rate on fruit flies, we transformed the original count data into a binomial outcome: attack/ no attack. A Fisher's exact test was used. Treatment was the only explanatory variable added to this model.

5. Results

5.1. Movement activity and sheltering experiments (Table S3)

When analysing nocturnal spider movement between the light treatments and the associated dark treatments, we found no significant difference between the dark and the 10 lux, 5500 K light treatment ($\chi 2(1, N = 78) = 0.14$, $p = 0.71$) (Fig. 1). When we increased the light intensity (100 lux, 5500 K), we found significantly more nocturnal spider movement in the light treatment compared to the dark treatment ($\chi 2(1, N = 80) = 13.50$, $p < 0.001$) (Fig. 1). When switching to warmer white light with the same intensity (100 lux, 2700 K), we found a significant interaction effect between treatment and sex

($\chi 2(1, N = 78) = 6.70$, $p < 0.01$). However, in the post-hoc analysis (with Tukey correction) no single pairwise comparison came out significant (Table S4).

Further analyses showed that none of the ALAN conditions had spill-over effects on spider movements during the day (10 lux, 5500 K: $\chi 2(1, N = 78) = 0.65$, $p = 0.42$; 100 lux, 5500 K: $\chi 2(1, N = 80) = 0.04$, $p = 0.83$; 100 lux, 2700 K: $\chi 2(1, N = 78) = 0.96$, $p = 0.33$). In the experiment comparing 10 lux, 5500 K light with darkness, males were more active during the day than females, regardless of the treatment ($\chi 2(1, N = 78) = 5.56$, $p < 0.05$).

When analysing the sheltering behaviour of the spiders over the different light treatments, we found no significant effect on how much spiders sheltered at night in either of the cold white light conditions (10 lux, 5500 K: $\chi 2(1, N = 78) = 0.04$, $p = 0.84$; 100 lux, 5500 K: $\chi 2(1, N = 80) = 0.10$, $p = 0.75$). We did find that spiders exposed to the 100 lux, 2700 K treatment sheltered more than they did in the dark control ($\chi 2(1, N = 78) = 4.12$, $p < 0.05$) (Fig. 2). For the 100 lux, 2700 K experiment, where in turns each group experienced both light and darkness over the two trials, we found significantly more sheltering in the second trial compared to the first, irrespective of treatment ($\chi^2(1, N = 78) = 4.12$, $p < 0.05$).

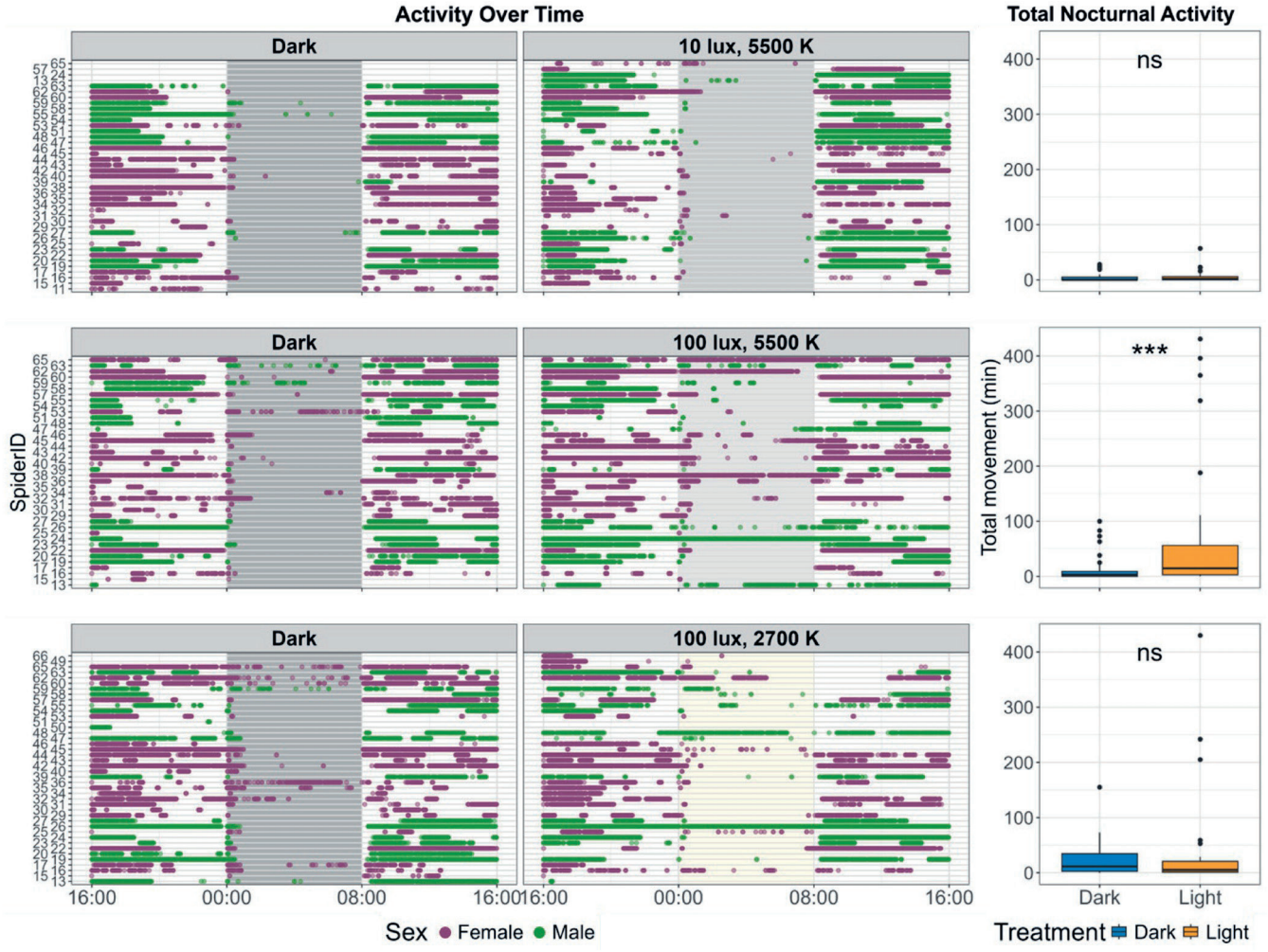


Fig. 1. Activity at different light treatments (10 lux, 5500 K (top); 100 lux, 5500 K (middle); 100 lux, 2700 K (bottom)) and their respective dark control. Left: Movement patterns over 24h. Nighttime was between 00:00 and 08:00, meaning that daylights were turned off in both treatments, but the specified light treatments were on during these hours. Right: boxplot with the total movement activity during nighttime for the respective light conditions. Significance level shown as ns: $p > 0.05$, ***: $p < 0.001$.

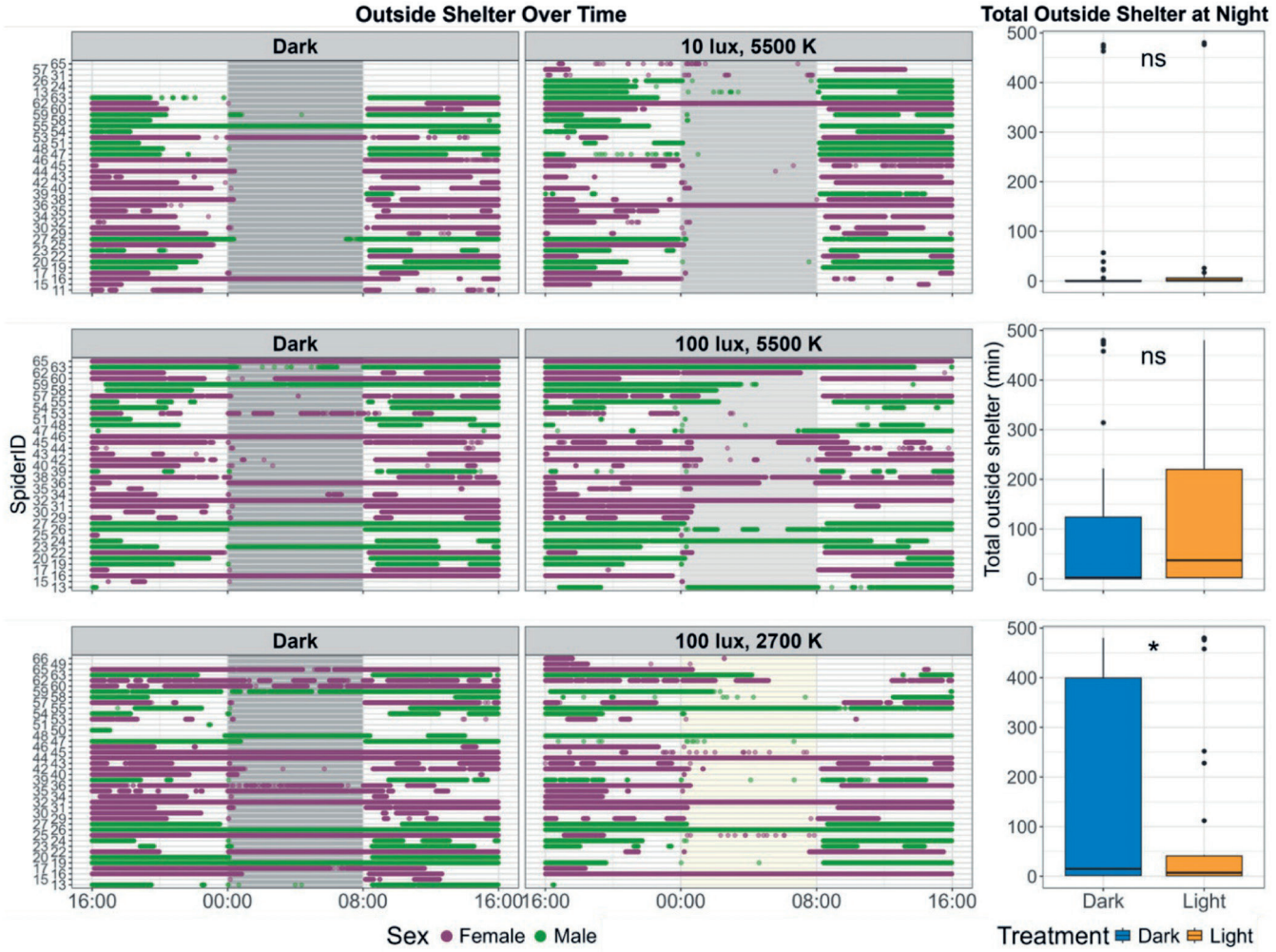


Fig. 2. Exposure at different light treatments (10 lux, 5500 K (top); 100 lux, 5500 K (middle); 100 lux, 2700 K (bottom)) and their respective dark control. Nighttime was between 00:00 and 08:00, meaning that daylights were turned off in both treatments, but the specified light treatments were on during these hours. Right: boxplot with the total observations of the spider outside the shelter during nighttime, for the respective light conditions. Significance level shown as ns: $p > 0.05$, *: $p < 0.05$.

5.2. Predation experiment

In total 9/39 spiders (23%) attacked flies between 00:00 and 08:00 in the light treatment (100 lux, 5500 K), while none of the 37 spiders attacked flies in the dark treatment (Fisher's exact test, $p < 0.01$). Since several spiders attacked multiple times, there were in total 26 dead fruit flies in the light treatment. On average, there were 0.67 ± 0.22 (SE) attacks per spider in the light treatment (Fig. 3).

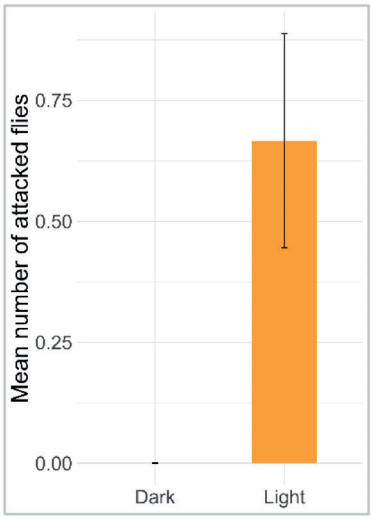


Fig. 3. The mean number of attacks per spider in the dark (n=37) and in the light (100 lux, 5500 K) treatment (n=39). Error bars show the standard error around the mean.

6. Discussion

In this study, we found that *Marpissa muscosa* increased their nocturnal movement under high intensity, cold white light, but not in lower intensities and warmer white light. ALAN had no knock-on effects on the spider's regular daytime movement patterns. Spiders sheltered significantly more under the high intensity warm white light. Unsheltered sleep was observed more in the dark compared to both high intensity light treatments. When further investigating if the increased activity under the high intensity, cold white ALAN also increased predation, we found that 23% of the tested spiders captured prey during the night in this light condition, while none of the spiders captured prey in the dark control.

As organisms use natural light-dark cycles to optimally time their activity, ALAN alters activity patterns (Gaston et al., 2013). A variety of diurnal animals have been reported to show nocturnal activity under ALAN conditions (Alaasam et al., 2018; Frank, 2009;

Gomes et al., 2021, 2024; Henke et al., 2022; Leveau, 2020; Maurer et al., 2019). However, it is generally not clear if this nocturnal activity is directly caused by ALAN or through ALAN-regulated prey availability. As we used no additional food stimulus in the initial activity experiments, we show that the increase in jumping spider activity under ALAN is a direct response to the light. However, as we observed an increase in overall spider activity when we added prey, foraging opportunities may incentivise spiders to become active even under dimmer light conditions.

In contrast to our expectation, spider sheltering behaviour was not only dependent on when spiders were active. The camera footage showed that spiders rested more outside their shelter in the dark treatments than in the light treatments, especially in the later trials of the movement and sheltering experiment. Possibly, the dark nighttime provided jumping spiders with an 'enemy free space' (Jeffries & Lawton, 1984) in which they felt safe to rest. The spiders probably also felt gradually more at ease in the arenas over the course of the experiment, at least in the dark controls. Therefore, due to the consecutive testing, we cannot determine if the pattern of resting inside or outside their shelter is due to light intensity or gained familiarity.

We found an impact on movement and sheltering only for the high-intensity white light treatments. This suggests that the behavioural effects of ALAN on jumping spiders may be confined to the immediate vicinity of a light source, although the impact zone may be larger for brighter light sources.

Even though jumping spiders are considered as strictly diurnal predators, the bright, cold white light treatment led to nocturnal foraging in 9 out of the 39 spiders tested. Some jumping spider species have been recorded to nocturnally forage in ALAN conditions (Edwards, 1969; Frank, 2009; Wolff, 1982). Even though limited lighting specifications were given in these studies, they typically describe scenarios with higher intensities (e.g. near a porch light and on greenhouse windows), which is likely closest to our 100 lux treatments.

Cerveira et al. (2019) showed that at least two species of jumping spiders (Salticidae), belonging to the genus *Cyrba*, are able to discriminate between prey and conspecific decoys at much lower light intensities (1.35 lux). However, whether they can also forage effectively on live prey under such dim conditions has not yet been tested. For a species from another genus, *Trite planiceps*, an unpublished study cited by Forster (1982) found that although individuals can detect houseflies at 1 lux, they can only hunt them effectively at 50 lux. It is unknown whether *M. muscosa* has the same visual capacities, but since we found no significant increase in nocturnal activity at 10 lux, our results suggest that such dim light conditions are not enough to activate these jumping spiders.

Under ALAN, the foraging success of the active jumping spiders may improve when insect prey are attracted to light and accumulate in lit sites (Perkin et al., 2014; Wakefield et al., 2018). Such an increase in prey captures is found for web-building spiders in lit locations (Gomes, 2020; Parkinson et al., 2020; Willmott et al., 2019; but see Yuen & Bonebrake, 2017). Increased prey catches under ALAN can have positive effects on spider body condition and mass (Gomes, 2020; Parkinson et al., 2020; but see Fuse et al., 2024), which may also benefit *M. muscosa*, especially in urbanised habitats. This increased predation success, however, comes at the expense of arthropod prey abundance, which likely contributes to insect losses associated with ALAN (van Grunsven et al., 2020; Vaz et al., 2023; Wilson et al., 2018).

ALAN may also negatively impact spiders. For example, ALAN accelerated development in the Australian garden orb-weaving spiders (*Hortophora biapicata,* Araneidae), with spiders reaching maturity at smaller body sizes, which resulted in increased juvenile mortality and lower adult fecundity (Willmott et al., 2018). Additionally, in these orb-weaving spiders short-term ALAN exposure reduced the size of brain structures involved in the primary eye visual pathway, likely due to oxidative stress or plastic alterations in neural investment (Willmott et al., 2024).

While the above-mentioned studies focused on web-building spiders, similar behavioural alterations can be expected for jumping spiders in response to ALAN. The active hunting strategy of jumping spiders may make them even more susceptible to ALAN exposure, both directly and indirectly. Our study shows that ALAN can directly impact the foraging activity of jumping spiders. Moreover, jumping spiders possess complex vision with depth perception (Nagata et al., 2012; Steinhoff et al., 2024) and exhibit strong plastic learning abilities (Liedtke & Schneider, 2014, 2017), both of which are crucial for hunting. Consequently, neural alterations like those observed in Australian garden orb-weaving spiders could potentially affect the performance of jumping spiders. Furthermore, increased nocturnal mobility may make these cryptic spiders more detectable to their own predators (Ioannou & Krause, 2009). Additionally, increased nocturnal activity may impair sleep, as evidenced by the finding that *Evarcha arcuata* (Salticidae) enters a REM sleep-like stage (Rößler et al., 2022). Since our findings indicate no knock-on effects of ALAN on the spider's daytime activity, it is likely that impaired nighttime sleep is not compensated for during the day.

To gain more insight into the ecological and physiological consequences of the altered activity patterns in jumping spiders, it would be worthwhile to determine how longer-term light exposure predation experiments affect body condition. In addition, choice experiments could inform us if jumping spiders actively seek lit habitats in their natural environment.

Our study provides an initial insight into the impact of ALAN on jumping spider behaviour, specifically focusing on the importance of light intensity and spectrum. However, conducting additional experiments at intermediate intensity and spectrum values could be informative in pinpointing the dose-dependent thresholds for spiders' sheltering, movement, and foraging behaviours.

In conclusion, our results show that high intensity white light triggers behavioural changes in *M. muscosa*, with the stimulation of movement and predation under bright, cold white light conditions and an overall increase in sheltering under bright, warm white light conditions. Increased activities under artificial illumination may enable jumping spiders to exploit the night light niche in urbanised habitats. Such behavioural advantages could significantly alter predator–prey dynamics, with cascading effects of ALAN on already vulnerable insect populations.

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10. Supplementary material

10.1. Light measurements

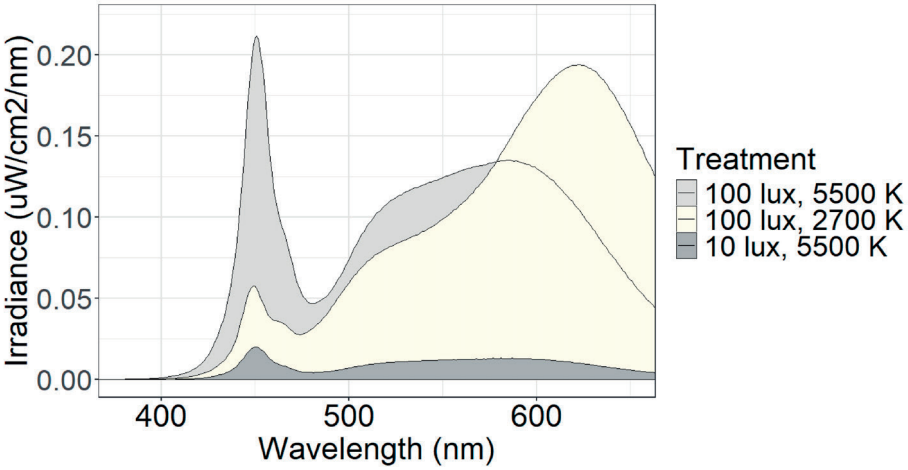


Fig. S1. Spectral composition of the three LED light treatments, measured for a representative LED strip (RexTech, the Netherlands). Measurements were taken at shelf height, 50 cm below the LED strip, with the detector oriented perpendicular to the light source. Spectral data were collected using a QE Pro High-Performance Spectrophotometer (Ocean Insight, United States). The spectrophotometer slit was connected to an optical fiber, which was coupled to a cosine corrector with a collection area of 0.119 cm^2 (OceanOptics, United States).

Table S1. Light intensity measurements of the day lights (MASTER TL-D Super 80 58W – 4000K; Philips, the Netherlands) present in the two climate rooms. Measurements are made at the centre of the shelves where spiders were placed.

	Climate room B	Climate room C
Shelf 1	2.836 kLx	2.191 kLx
Shelf 2	2.303 kLx	1.277 kLx
Shelf 3	2.553 kLx	1.174 kLx
Shelf 4	1.827 kLx	0.782 kLx

Information on the spectral composition of the daylights (MASTER TL-D Super 80 58W – 4000K; Philips, the Netherlands) can be found on *Philips product specifications*.

10.2. Study design

Table S2. Overview of the effective sample sizes for each trial, separated per treatment and per sex.

	dark		light	
	female	male	female	male
Movement activity and sheltering experiments				
10 lux, 5500 K – trial 1	12	6	12	8
10 lux, 5500 K – trial 2	12	8	12	8
100 lux, 5500 K – trial 1	12	8	12	8
100 lux, 5500 K – trial 2	12	8	12	8
100 lux, 2700 K – trial 1	12	8	10	8
100 lux, 2700 K – trial 2	11	9	13	7
Predation experiments				
100 lux, 2700 K – trial 1	12	6	13	6
100 lux, 2700 K – trial 2	12	7	13	7

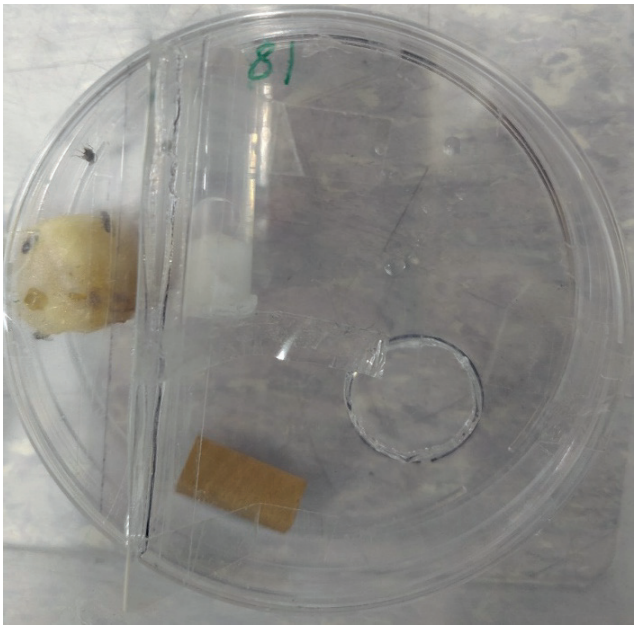


Fig. S2. The Petri dish arena used for the predation experiment. Before the start of the experiment, spider and flies are kept separate with a transparent divider. To avoid flies escaping from their compartment, a hole was made in the bottom of the Petri dish to insert the spider. The fly compartment contained a moist piece of banana, while the spider was provided with a water tube and paper shelter.

10.3. YOLO v8 model performance

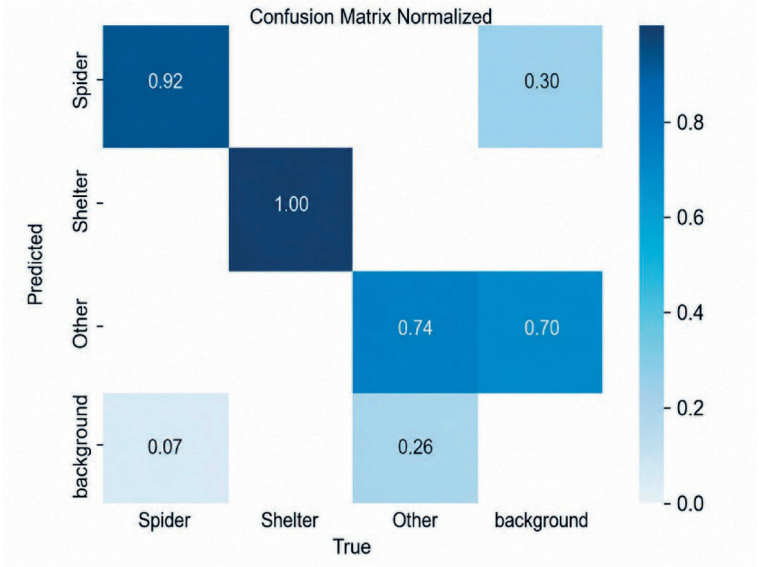


Fig. S3. The normalised confusion matrix of the implemented yolo v8 model. The matrix shows the percentages of true and false positives per labelled category (Spider, Shelter, Other and background).

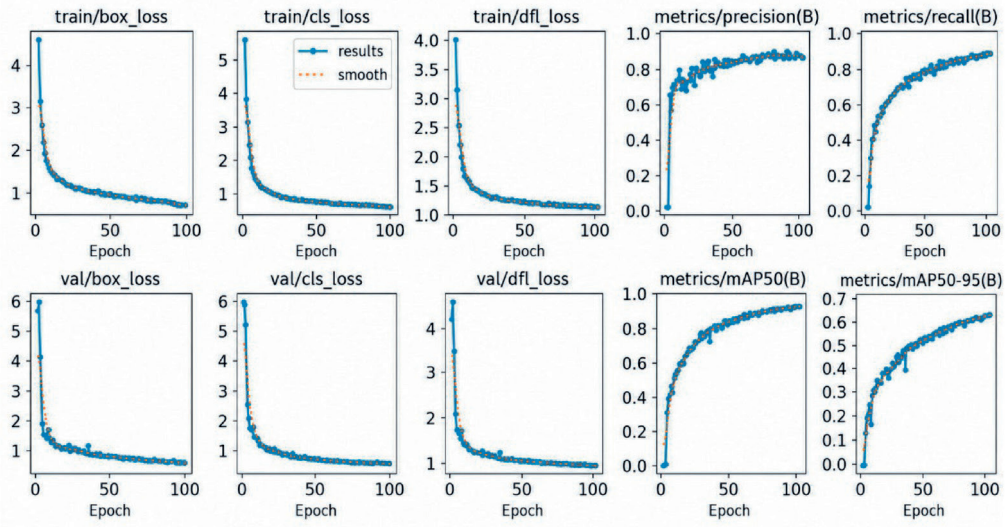


Fig. S4. Model performance metrics for the training and validation of the yolo v8 model, run over 100 epochs.

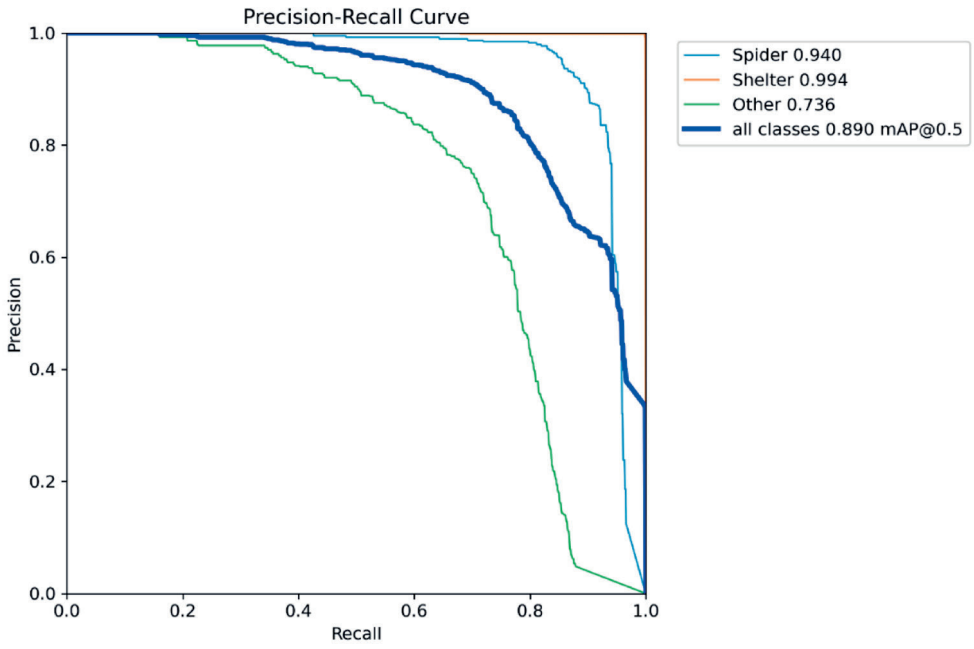


Fig. S5. The precision-recall curves of the yolo v8 model, shown per labelled category (Spider, Shelter, Other, all classes).

10.4. Model selection and statistics

Table S3. Overview of the analyses performed for the movement activity and sheltering experiments, showing all models compared during the model simplification process. For each model, the AIC (Akaike Information Criterion) values are given together with the test statistics for each of the included explanatory variables. The model that performed best is indicated in bold. For the random factor (ID), variance (S^2) and standard deviation (SD) are given. Random factors were not considered in the model simplification process.

Nighttime movement activity							
	Model	AIC	Treatment	Sex	Trial	Treatment:Sex	*ID*
10 lux, 5500 K	M1	357.2	$\chi2(1, N = 78) = 1.54, p = 0.2142$	$\chi2(1, N = 78) = 0.32, p = 0.5729$	$\chi2(1, N = 78) = 1.69, p = 0.1937$	$\chi2(1, N = 78) = 1.84, p = 0.1753$	S^2: 0.00 SD: 0.00
	M2	357.0	$\chi2(1, N = 78) = 0.25, p = 0.6149$	$\chi2(1, N = 78) = 0.22, p = 0.6388$	$\chi2(1, N = 78) = 1.50, p = 0.2214$		S^2: 0.00 SD: 0.00
	M3	355.2	$\chi2(1, N = 78) = 0.44, p = 0.5091$		$\chi2(1, N = 78) = 1.60, p = 0.2057$		S^2: 0.00 SD: 0.00
	M4	354.8	$\chi2(1, N = 78) = 0.14, p = 0.7127$				S^2: 0.00 SD: 0.00
100 lux, 5500 K	M1	633.2	$\chi2(1, N = 80) = 7.80, p = 0.0052$	$\chi2(1, N = 80) = 1.71, p = 0.1914$	$\chi2(1, N = 80) = 2.12, p = 0.1458$	$\chi2(1, N = 80) = 0.15, p = 0.6937$	S^2: 1.00 SD: 1.00
	M2	631.4	$\chi2(1, N = 80) = 14.84, p < 0.001$	$\chi2(1, N = 80) = 1.93, p = 0.1647$	$\chi2(1, N = 80) = 2.04, p = 0.1537$		S^2: 0.98 SD: 0.99
	M3	631.3	$\chi2(1, N = 80) = 14.15, p < 0.001$		$\chi2(1, N = 80) = 1.47, p = 0.2253$		S^2: 1.03 SD: 1.01
	M4	630.7	$\chi2(1, N = 80) = 13.50, p < 0.001$				S^2: 1.04 SD: 1.02
100 lux, 2700 K	M1	613.4	$\chi2(1, N = 78) = 5.43, p = 0.0198$	$\chi2(1, N = 78) = 3.72, p = 0.0537$	$\chi2(1, N = 78) = 2.93, p = 0.087$	$\chi2(1, N = 78) = 6.92, p = 0.0085$	S^2: 1.44 SD: 1.20
	M2	614.2	$\chi2(1, N = 78) = 4.61, p = 0.0318$	$\chi2(1, N = 78) = 3.45, p = 0.0633$		$\chi2(1, N = 78) = 6.70, p = 0.0096$	S^2: 1.23 SD: 1.11

Daytime movement activity							
	Model	AIC	Treatment	Sex	Trial	Treatment:Sex	*ID*
10 lux, 5500 K	M1	1062.7	$\chi2(1, N = 78) = 1.39, p = 0.2383$	$\chi2(1, N = 78) = 0.91, p = 0.3406$	$\chi2(1, N = 78) = 0.02, p = 0.8953$	$\chi2(1, N = 78) = 0.84, p = 0.3600$	S^2: 0.00 SD: 0.00
	M2	1060.7	$\chi2(1, N = 78) = 1.44, p = 0.2307$	$\chi2(1, N = 78) = 0.92, p = 0.3366$		$\chi2(1, N = 78) = 0.82, p = 0.3650$	S^2: 0.00 SD: 0.00
	M3	1059.6	$\chi2(1, N = 78) = 0.65, p = 0.4193$	$\chi2(1, N = 78) = 5.56, p = 0.0184$			S^2: 0.00 SD: 0.00
100 lux, 5500 K	M1	1103.9	$\chi2(1, N = 80) = 0.06, p = 0.8121$	$\chi2(1, N = 80) = 0.03, p = 0.8665$	$\chi2(1, N = 80) = 0.43, p = 0.5122$	$\chi2(1, N = 80) = 0.00, p = 0.9904$	S^2: 0.00 SD: 0.00
	M2	1101.9	$\chi2(1, N = 80) = 0.09, p = 0.7668$	$\chi2(1, N = 80) = 0.06, p = 0.8034$	$\chi2(1, N = 80) = 0.43, p = 0.5122$		S^2: 0.00 SD: 0.00
	M3	1099.9	$\chi2(1, N = 80) = 0.09, p = 0.7683$		$\chi2(1, N = 80) = 0.49, p = 0.4849$		S^2: 0.00 SD: 0.00
	M4	1098.4	$\chi2(1, N = 80) = 0.04, p = 0.8339$				S^2: 0.00 SD: 0.00
100 lux, 2700 K	M1	1077.7	$\chi2(1, N = 78) = 0.69, p = 0.4059$	$\chi2(1, N = 78) = 0.21, p = 0.6502$	$\chi2(1, N = 78) = 0.07, p = 0.7948$	$\chi2(1, N = 78) = 0.03, p = 0.8668$	S^2: 0.00 SD: 0.00
	M2	1075.7	$\chi2(1, N = 78) = 0.89, p = 0.3447$	$\chi2(1, N = 78) = 0.62, p = 0.4297$	$\chi2(1, N = 78) = 0.08, p = 0.7828$		S^2: 0.00 SD: 0.00
	M3	1073.8	$\chi2(1, N = 78) = 0.95, p = 0.3302$	$\chi2(1, N = 78) = 0.66, p = 0.4175$			S^2: 0.00 SD: 0.00
	M4	1072.5	$\chi2(1, N = 78) = 0.96, p = 0.3279$				S^2: 0.00 SD: 0.00
Nighttime sheltering							
	Model	AIC	Treatment	Sex	Trial	Treatment:Sex	*ID*
10 lux, 5500 K	M1	405.6	$\chi2(1, N = 78) = 0.12, p = 0.7280$	$\chi2(1, N = 78) = 1.40, p = 0.2371$	$\chi2(1, N = 78) = 3.07, p = 0.0796$	$\chi2(1, N = 78) = 4.47, p = 0.0346$	S^2: 0.00 SD: 0.00
	M2	406.7	$\chi2(1, N = 78) = 0.06, p = 0.7997$	$\chi2(1, N = 78) = 0.01, p = 0.9404$		$\chi2(1, N = 78) = 0.98, p = 0.3232$	S^2: 2.02 SD: 1.42

	M3	405.6	$\chi2(1, N = 78) = 0.10, p = 0.7525$	$\chi2(1, N = 78) = 0.81, p = 0.3681$			S^2: 2.58 SD: 1.61
	M4	404.4	$\chi2(1, N = 78) = 0.04, p = 0.8432$				S^2: 2.88 SD:1.70
100 lux, 5500 K	M1	774.0	$\chi2(1, N = 80) = 0.01, p = 0.9364$	$\chi2(1, N = 80) = 0.18, p = 0.6680$	$\chi2(1, N = 80) = 0.12, p = 0.7328$	$\chi2(1, N = 80) = 0.12, p = 0.7266$	S^2: 0.00 SD: 0.00
	M2	772.1	$\chi2(1, N = 80) = 0.15, p = 0.7025$	$\chi2(1, N = 80) = 0.06, p = 0.8026$	$\chi2(1, N = 80) = 0.11, p = 0.7411$		S^2: 0.00 SD: 0.00
	M3	770.1	$\chi2(1, N = 80) = 0.13, p = 0.7189$		$\chi2(1, N = 80) = 0.10, p = 0.7575$		S^2: 0.00 SD: 0.00
	M4	768.2	$\chi2(1, N = 80) = 0.10, p = 0.7460$				S^2: 0.00 SD: 0.00
100 lux, 2700 K	M1	775.7	$\chi2(1, N = 78) = 6.66, p = 0.0098$	$\chi2(1, N = 78) = 2.16, p = 0.1421$	$\chi2(1, N = 78) = 4.28, p = 0.0386$	$\chi2(1, N = 78) = 2.8893, p = 0.089170$	S^2: 3.29 SD: 1.81
	M2	776.6	$\chi2(1, N = 78) = 3.64, p = 0.0564$	$\chi2(1, N = 78) = 0.42, p = 0.5159$	$\chi2(1, N = 78) = 4.25, p = 0.0393$		S^2: 3.34 SD: 1.83
	M3		$\chi2(1, N = 78) = 3.88, p = 0.0487$		$\chi2(1, N = 78) = 4.12, p = 0.0424$		S^2: 3.35 SD: 1.83

Table S4. Results of the post-hoc analyses for nighttime activity in the 100 lux, 2700 K experiment, showing the pairwise contrasts between different treatment and sex (only performed when the interaction term was significant). For each analysis, the odds ratio, standard error (SE), degrees of freedom (df), *z ratio* and *p*-value (*p*) are given per pairwise contrast.

	Contrasts	*ratio*	*SE*	*df*	*z ratio*	*p*
Nighttime activity **100 lux, 2700 K**	Dark Female/ Light Female	2.62	1.08	Inf	2.33	0.0912
	Dark Male/ Light Male	0.46	0.24	Inf	-1.48	0.4497
	Dark Female/ Dark Male	3.13	1.85	Inf	1.93	0.2156
	Light Female/ Light Male	0.55	0.34	Inf	-0.97	0.7651
	Dark Female/ Light Male	1.43	0.89	Inf	0.58	0.9376
	Light Female/ Dark Male	1.19	0.72	Inf	0.30	0.9910

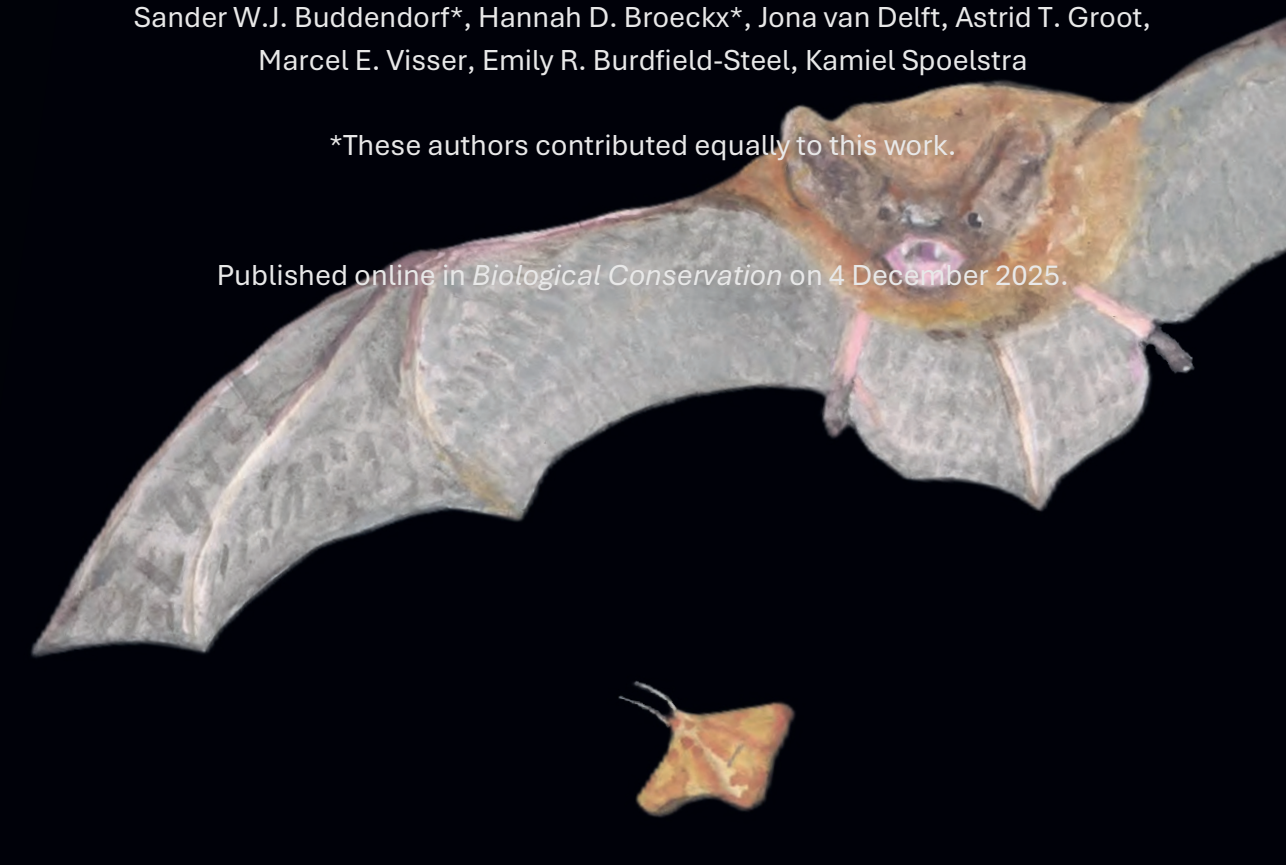
5

Dimming streetlights in urban areas reduces insect attraction but pipistrelle bats forage more in darkness

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1. Abstract

Artificial light at night (ALAN) disrupts the activity patterns of a wide range of organisms. Nocturnal animals are particularly vulnerable to this disruption, which can be especially severe in strongly illuminated urban environments. Reducing light intensity has been proposed as a mitigation measure, but the effectiveness of this strategy remains unclear. Here we studied how dimming urban streetlights affects pipistrelle bats and their prey, flying insects. We measured bat and insect activity around dimmable streetlights, with night-to-night alternating high and low light intensity, and at a dark control site nearby. We applied interval photography to assess insect arrival on sticky sheets with a high temporal resolution, and continuously recorded bat activity. Our results show that dimming streetlights did not affect pipistrelle bat activity, but reduced insect attraction. The presence of light, either dimmed or undimmed, did alter the predator-prey relationship in unexpected ways. Even though insects clustered around the lights, urban pipistrelle bats were more active and displayed higher relative foraging activity ('buzz ratio') in the relatively dark sites nearby. Nightly activity patterns of pipistrelles were not affected by dimming or light in general, whereas insect activity in the second half of the night was earlier around streetlights compared to in darkness. Limiting insect attraction by dimming urban streetlights will likely benefit insect populations, which in turn is beneficial for bats in the long term. Our findings show that darkness is not only essential for bats and insects in natural areas, but in urban habitats as well.

2. Keywords

Urban, Predator-prey interaction, Light pollution, Light intensity, Conservation

3. Introduction

Artificial light at night (ALAN) continues to increase sky brightness, with an average of 2.2–9.6 % per year (Hölker et al., 2010; Kyba et al., 2017, 2023). Artificial light is an essential part of our 24-hour society and gives many of us a feeling of safety at night (Himschoot et al., 2024). However, ALAN has also greatly transformed the natural nightscape. A wide variety of organisms are affected by light pollution, including microbes (Li et al., 2023; Maggi et al., 2020), plants (Meng et al., 2022; Segrestin et al., 2021), fungi (Li et al., 2023), invertebrates (Gomes, 2020; Levy et al., 2024; Manríquez et al., 2021) and vertebrates (Hermans et al., 2024; Jiang et al., 2020; Schligler et al., 2021). The impact for these taxa ranges from individual (Dominoni et al., 2022; Khan et al., 2018; Levy et al., 2024) up to community level, through the alteration of species interactions (Knop et al., 2017; Manfrin et al., 2017; McMunn et al., 2019; Weschke et al., 2024).

The interaction of species requires a spatial and temporal overlap in activity patterns, and ALAN can disrupt both (Seymoure et al., 2023). First, ALAN acts as a strong spatially structuring factor, as it attracts certain taxa but deters others (Kühne et al., 2021; Sanna et al., 2024; Vowles and Kemp, 2021; Willmott et al., 2019). Additionally, ALAN has a strong impact on the organisation of daily activity of organisms (Duarte et al., 2019; Georgiou et al., 2024; Henke et al., 2022; Hoffmann et al., 2018; Levy et al., 2021; Spoelstra et al., 2018), as it can blur the natural light-dark cycle that has served as strong and predictable cue throughout evolution and that has shaped the temporal niches for most organisms.

Nocturnal species are especially vulnerable to changes to the night sky, as they are behaviourally and physiologically adapted to dark conditions (Ditmer et al., 2021; van Langevelde et al., 2018). Bats and insects are known examples of nocturnal taxa that are affected by ALAN (Macgregor et al., 2017; Manfrin et al., 2025; Rydell, 1992; Stone et al., 2009), and so is their predator-prey relationship (Cravens et al., 2018; Hermans et al., 2024; Minnaar et al., 2015; Russo et al., 2019; Wakefield et al., 2015).

The attraction of insects to light during the night is a well-known phenomenon, which results in spatial accumulation of insects around light sources (Perkin et al., 2014; Wakefield et al., 2018). Flight-to-light behaviour is harmful for insects as it can increase mortality through an increased predation risk (Wakefield et al., 2015), direct burning (Gerson and Kelsey, 1997) and exhaustion (Eisenbeis and Hänel, 2009). In addition, the light can interfere with essential insect behaviours, such as feeding (van Langevelde et al., 2017), mate finding (Storms et al., 2025; van Geffen et al., 2015) and reproduction (Szaz et al., 2015). Insects that are attracted to the light source are drawn away from

the surroundings, reducing prey availability in these dark areas. This so-called "vacuum cleaner" effect (Eisenbeis, 2006) may alter foraging opportunities for predator species, depending on their response to artificial light. Several species of bats are able to exploit aggregated flying insects around light sources (Rydell, 1992). The response of bats to light depends largely on their morphological traits and foraging strategies (Voigt et al., 2021). Aerial-hawking bat species, such as bats of the *Pipistrellus* genus (Vespertilionidae), are relatively fast and agile and hence better able to evade potential predators. Pipistrelle bats have often been observed foraging on aggregated insects around light sources (Blake et al., 1994; Rydell, 1992), even though they keep a distance to the light source when foraging (Hermans et al., 2023) and generally avoid light when not foraging (Hale et al., 2015; Russo et al., 2017). In contrast, slow-flying bats, such as *Myotis sp*. (Vespertilionidae) and *Rhinolophus sp*. (Rhinolophidae), are generally light-averse, most likely because their slower flight speeds make them more vulnerable to predators (Boldogh et al., 2007; Duvergé et al., 2000; Jones and Rydell, 1994; Rydell et al., 1996, Rydell et al., 2021; Spoelstra et al., 2017).

Next to spatial disruptions, ALAN has also been found to alter the temporal activity patterns of insects (Gomes et al., 2024; Jiang et al., 2023; Taylor and Jones, 1969) and bats (Boldogh et al., 2007; Decoursey and Decoursey, 1964; Stone et al., 2009). These alterations may involve direct responses to light but can also include indirect interspecific effects. For instance, bats may extend or shorten their foraging time in response to ALAN-induced changes in insect abundance and timing (Hermans et al., 2024).

This raises the important question of whether ALAN also influences population sizes in bats and insects. For insects, Vaz et al. (2023) found no alterations in abundance, but did find species richness declines of insects related to ALAN. Specifically for moths, several studies have connected local population declines to the presence of ALAN (van Grunsven et al., 2020; Wilson et al., 2018). Van Langevelde et al. (2018) found that moth species that are nocturnally active or positively phototactic show stronger negative population trends than species without these traits. For bats, light-averse species might be outcompeted by light-opportunistic bats, as insects from surrounding dark areas are drawn to the light and consumed (Arlettaz et al., 2000; Russo et al., 2019). In the long term, even the light-opportunistic species are likely to be impeded by local insect declines caused by ALAN and their own successful foraging.

Light technology plays an important role in the increasing impact of ALAN on nocturnal wildlife. The efficiency and durability of modern Light-Emitting Diode (LED) technology have reduced the cost of public lighting and thereby facilitated the increase of light at

night (Kyba et al., 2014). In addition, LEDs typically emit broad-spectrum light, which can have a higher ecological impact due to the large proportion of short-wavelength light (Gaston et al., 2012) and increased brightness (Kyba et al., 2014). Fortunately, an advantage of LED light sources is that they are more easily (dynamically) dimmable, which can be used to reduce their impact (Gaston et al., 2012).
Traffic-regulated streetlight dimming in suburban environments was found to reduce insect attraction and consequently reduce the activity of light-opportunistic bats (Bolliger et al., 2020). Rowse et al. (2018) tested the effect of dimming on bat activity and insect counts and found that only relative feeding activity ('buzz ratio') was affected, which decreased under dimmed light. Although they could not statistically show an effect of dimming light on insect counts and pipistrelle activity, the observed pattern suggests a possible trend. Dynamically dimmable streetlights are becoming widely implemented. However, our knowledge on how dimming might mitigate the impact of artificial light on local ecosystems remains limited, especially in heavily urbanised areas. In urban areas, the background levels of light are typically higher than in rural areas (Jechow et al., 2020; Sanchez de Miguel et al., 2020), hence the impact of dimming streetlights might be diluted by other light sources. Additionally, a whole variety of other pressures act on urban populations, which might impose stronger selection and encourage different behaviours (McDonnell and Hahs, 2015). As the pressures on urban ecosystems intensify and biodiversity preservation becomes a key policy in many large cities worldwide (a.o. EU Nature Restoration Law - EU Regulation 2024/1991), with specific guidelines for bat conservation (UNEP/EUROBATS: Voigt et al., 2018; www.eurobats.org), it is important to investigate the effects of dimming streetlights in urban areas.

As ALAN is known to strongly impact local insect and bat densities, we studied how dimming urban streetlights affects the local and temporal activity patterns of highly abundant synanthropic pipistrelle bats and flying insects. We collected high temporal resolution data on bats and flying insects at dynamically dimmable streetlights and a dark control nearby.

We formulated the following hypotheses:

H1: Insects are most active (higher insect counts) under high-intensity lighting, less active under dimmed lighting and the least active in darkness.

H2: Pipistrelle bats follow insect activity and therefore show highest overall activity (bat passes) and foraging activity (feeding buzzes and buzz ratio) around high-intensity light, less at dimmed light, and least in darkness.

H3: Around light, insect activity peaks earlier in the night (earlier median arrival time in the first half of the night) and insects stay active for longer (later median arrival time in the second half of the night) compared to in darkness.

H4: Pipistrelle timing in overall and foraging activity (median timing of bat passes and feeding buzzes) follows insect activity timing and they are therefore active earlier (earlier median timing first half of the night) and stay active longer (later median timing second half of the night) around light.

4. Material and methods

4.1. Study sites and experimental setup

We selected six urban study sites located in The Hague and Rotterdam (Fig. S1), two of the largest cities in the Netherlands. All sites consisted of regular streets, illuminated with dimmable four-meter-high streetlights (average 4.15 ± 0.11 m s.e.m.). Extra selection criteria were the absence of bright light sources in the surroundings (e.g. sport fields) and the presence of some natural features (e.g. water and minimally maintained vegetation). The distance between study sites ranged between 2.4 and 24.5 km (average 13.3 ± 3 km).

At each site, we used a total of six streetlights in a row with an inter distance of 25 m. We divided these in two sets of three abreast light posts (hereafter referred to as 'set'), where we measured bat and insect activity at the middle streetlight of each set of three (Fig. S2). Within each study site, one set was configured to normal (high) light intensity and one was dimmed (low), which alternated between consecutive nights. All lights had a colour temperature of 3000 K. The high intensity averaged 17 ± 3.8 lux and the low light intensity 4.4 ± 1.4 lux, at ground level. The light intensities represent realistic lighting scenarios which were determined in consultation with both municipalities, where the high intensity resembles the original brightness and the low intensity representing the minimum level deemed necessary for public safety. For dark control measurements, we installed a separate pole (without a functional fixture) in a dark location at an average distance of 163 ± 49 m from the nearest set and at least 20 m away from the nearest light source, with an average ambient light level of 0.16 ± 0.03 lux. At two study sites we used a (mostly branchless) tree as placing a pole was not feasible.

In 2023, data were collected nightly between September 4th and 28th from sunset to sunrise. Data were only included when bats and insects were sampled uninterrupted for the full night (Fig. S3). Sampling interruptions included technical constraints or fog, when fog rendered more than 1 h of insect monitoring images unusable.

4.2. Measuring insect aggregation

Insect activity was measured at 60 cm underneath the light fixture. We used white plastic A4 sheets covered with horsefly glue (Sticky-Trap, Enter, the Netherlands) in an aluminium frame (to prevent bats and birds touching the sticky surface (Ancillotto et al., 2024)) such that the sticky side was facing the streetlight pole at 40 cm distance (Fig. S4). The sticky sheet was photographed every 5 min with a Technaxx TX-117 camera (Technaxx, Germany). Photographs taken at the dark control used infrared light to allow for adequate detectability of the insects without affecting the experimental light setup. The sheets were replaced once or twice a week, depending on the insect abundance at the different study sites.

All images taken between sunset and sunrise were manually processed, with the arrival time of each individual insect derived from photographs. Due to limited picture quality, insects were manually classified at the sheet itself, identifying them up to order level.

4.3. Measuring bat activity

Bat activity was measured with automatic bat detectors (SM4BAT-FS, Wildlife Acoustics, United States) placed in a protective case attached to the same streetlight where insect activity was measured. The case was positioned 1 m underneath the light fixture at a 45° angle relative to the front of the fixture on a horizontal plane (Fig. S5). Recordings were triggered by sounds of at least 16 kHz and a duration of at least 1.5 milliseconds. The sampling rate was set at 256 kHz, the trigger level at 12 dB and the gain at 12 dB as well. When triggered, the detector recorded for 5 s.

Bat calls were identified with the Tadarida identification software (Bas et al., 2017). After manually checking a subsample of identifications (following Barré et al., 2019), we retained identifications with a confidence of at least 0.7, as we found this minimised misidentifications. Each sound file with two or more echolocation calls identified as pipistrelle bat (*Pipistrellus spp.*) was considered a pipistrelle bat pass, which is a proxy for overall pipistrelle activity. Histogram plots of the peak frequency distribution of all pipistrelle bat passes show a dominant peak (86.2 % of all bat passes) between 42 and 50 kHz (Figs. S6–7), suggesting that the common pipistrelle (*Pipistrellus pipistrellus*) was the most frequently recorded species. Only 5.6 % of bat passes were below 42 kHz

and 4.7 % above 50 kHz, indicating that Nathusius' pipistrelles (*Pipistrellus nathusii*) or soprano pipistrelles (*Pipistrellus pygmaeus*), the other *Pipistrellus* species occurring in the Netherlands, were rare or absent.

To better link bat activity to bat-insect relationships, we isolated foraging behaviour from the recordings. To do so, each pipistrelle bat pass was checked for feeding attempts ('feeding buzzes') using a custom algorithm (Buddendorf and Spoelstra, in prep). The algorithm converts recordings into spectrogram images and analyses these using an object detection model trained specifically for this purpose, based on the YOLOv8 framework (Jocher et al., 2023). Each bat pass in which a feeding buzz was detected was classified as a feeding buzz data point for subsequent analyses. In addition, we calculated the 'buzz ratio' by dividing the number of feeding buzzes by the number of bat passes (Vaughan et al., 1997). The buzz ratio serves as an indicator of foraging effort relative to overall flight activity.

4.4. Statistical analyses

All analyses were performed with R version 4.4.2 (R Core Team, 2024). Visualisations were made using the ggplot2-package (Wickham, 2016). The fit of the linear models to their specific data was evaluated using the simulateResiduals function from the DHARMa package (Hartig, 2022). Candidate models were compared using the built-in anova function and by comparing the Akaike information criterion (>2 ΔAIC).

4.4.1. Nightly activity analysis

To test hypotheses H1 & H2, we analysed the nightly insect counts (all flying insects and separated by order), bat passes, feeding buzzes and buzz ratios using generalised linear mixed models from the glmmTMB package (Brooks et al., 2017). Forward model selection for all bat related models (bat passes, feeding buzzes and buzz ratio) was applied up to the maximal model including insect counts and treatment as fixed effects (explanatory variables). Interactions between treatment and insect counts were not tested due to strong collinearity between these predictors. Only the intercept model and the model including treatment as a fixed effect were tested for the insect related models (all flying insects and separated by order). All models included the date of the evening of each sampling night (hereafter 'date') and site as crossed random effects. A negative binomial distribution ('nbinom2' family) was used for the nightly insect counts (all flying insects and separated by order), bat passes and feeding buzzes. To most accurately analyse the buzz ratio, we used a binomial modelling approach as this is well suited for proportion data based on counts. A beta-binomial distribution, an

extension of the binomial distribution that accounts for overdispersion, provided the best fit. Post-hoc pairwise comparisons between all treatments were made using the emmeans package using the Bonferroni correction (Lenth, 2024).

4.4.2. Temporal activity analysis

To test hypotheses H3 & H4, we analysed the temporal activity patterns of bats and insects. To do so, we calculated the median activity time for the first and second half of the night, per date and pole. We chose to work with the median, as this metric is less sensitive to extreme values and it represents the spread of activity over time rather than a single event (Mariton et al., 2022, Mariton et al., 2023). Median activity times were calculated for the first and second half of the night separately, as this allowed for more detail and was better able to capture bimodal temporal patterns (Mariton et al., 2022, Mariton et al., 2023). We converted the clock time of insect arrivals and bat activity to percentages of the night to correct for seasonal variation in night length. Half-nights with fewer than three insect records were excluded because with fewer than three observations, the median would either be meaningless (with one point) or highly unstable (with two points). These median activity times per half of the night were tested using generalised linear mixed models, using the glmmTMB package (Brooks et al., 2017). We again used a forward model selection, where the maximum model for bats included treatment and insect timing, and for insect only included the treatment. Date and site were always included as crossed random effects. Based on data distribution and the model fit diagnostics, we used a Gaussian distribution for most of the models. For the analysis of feeding buzzes in the first half of the night, a Tweedie distribution was used as this model fit the data significantly better. The Tweedie distribution is part of the exponential dispersion family (Jorgensen, 1997). It is one of the more flexible distributions, as it is based on three parameters: mean, dispersion, power (Miller et al., 2013 (suppl. material)). Depending on the power term (which is automatically estimated in the glmmTMB function), Tweedie can equalise to more familiar distributions. Post-hoc pairwise comparisons were made using the emmeans package using the Bonferroni correction (Lenth, 2024).

5. Results

In total, 267 successful sampling nights were included in the analyses: 94 at dark control, 87 at high intensity and 86 at low intensity treatments. On average, this resulted in 46 ± 5 (s.e.m.) sampling nights per site. We caught a total of 13,019 insects during all sampling nights. The most abundant taxonomic groups were Diptera (9108),

Psocodea (553), Hemiptera (300), Hymenoptera (294), Coleoptera (137), with 2426 unidentified insects. In total 387,944 bat passes were recorded of which 315,513 were identified as pipistrelle bat passes. Of these, 49,190 contained feeding buzzes.

5.1. Nightly activity

The best performing model selected for flying insects included the treatment (Table S1). In this model, treatment had a significant effect on insect counts (Fig. 1, $\chi 2(2, N = 267) = 924.44$, $p < 0.001$). A post-hoc test showed that the high intensity treatment attracted significantly more insects than the low intensity treatment ($p < 0.001$). In turn, we caught significantly more insects in the light treatments compared to the dark control ($p < 0.001$).

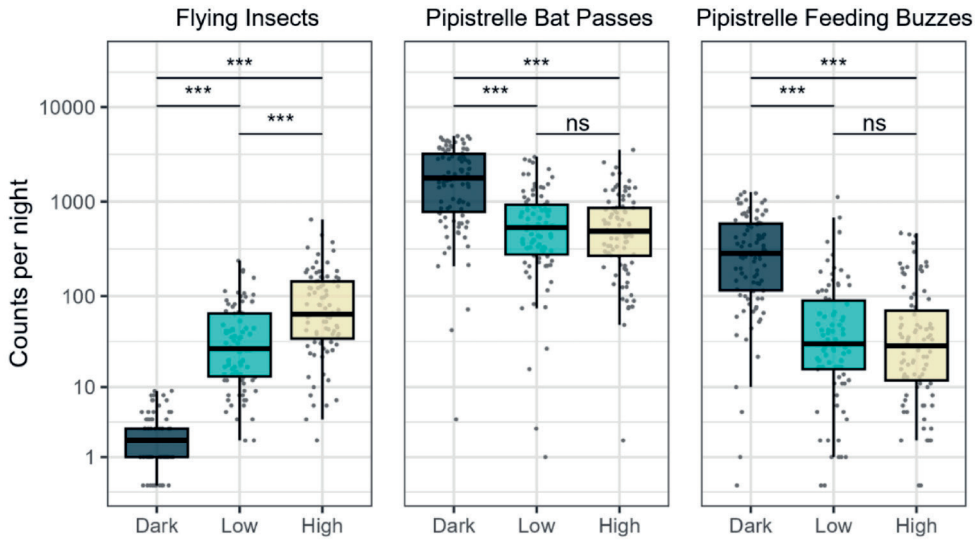


Fig. 1. Nightly counts of flying insects (left), pipistrelle bat passes (middle) and their feeding buzzes (right) per treatment (dark control: 0.16 ± 0.03 lux, low: LED 3000 K, 4.4 ± 1.4 lux, high: LED 3000 K, 17 ± 3.8 lux). Significance levels indicated as ns: $p > 0.05$, ***: $p < 0.001$.

For the separate insect order models, the one with treatment was selected (Table S1). Here we observe a similar pattern as the previous flying insect model (all orders combined) results. The overall effect of the treatment was significant for all orders (Fig. 2; Diptera: $\chi 2(2, N = 267) = 29.76$, $p < 0.001$; Psocodea: $\chi 2(2, N = 267) = 89.72$, $p < 0.001$; Hemiptera: $\chi 2(2, N = 267) = 74.65$, $p < 0.001$; Hymenoptera: $\chi 2(2, N = 267) = 53.48$, $p < 0.001$; Coleoptera: $\chi 2(2, N = 267) = 29.76$, $p < 0.001$). For Diptera, Hemiptera, Hymenoptera ($p < 0.001$) and Psocodea ($p < 0.01$) we found a strong response to dimming, with more attraction to the high intensity light compared to the low intensity

light. Only Coleoptera did not show a significant effect of dimming ($p = 0.13$). We caught significantly fewer individual insects in the dark control compared to either of the light intensity treatments for all five orders ($p < 0.001$).

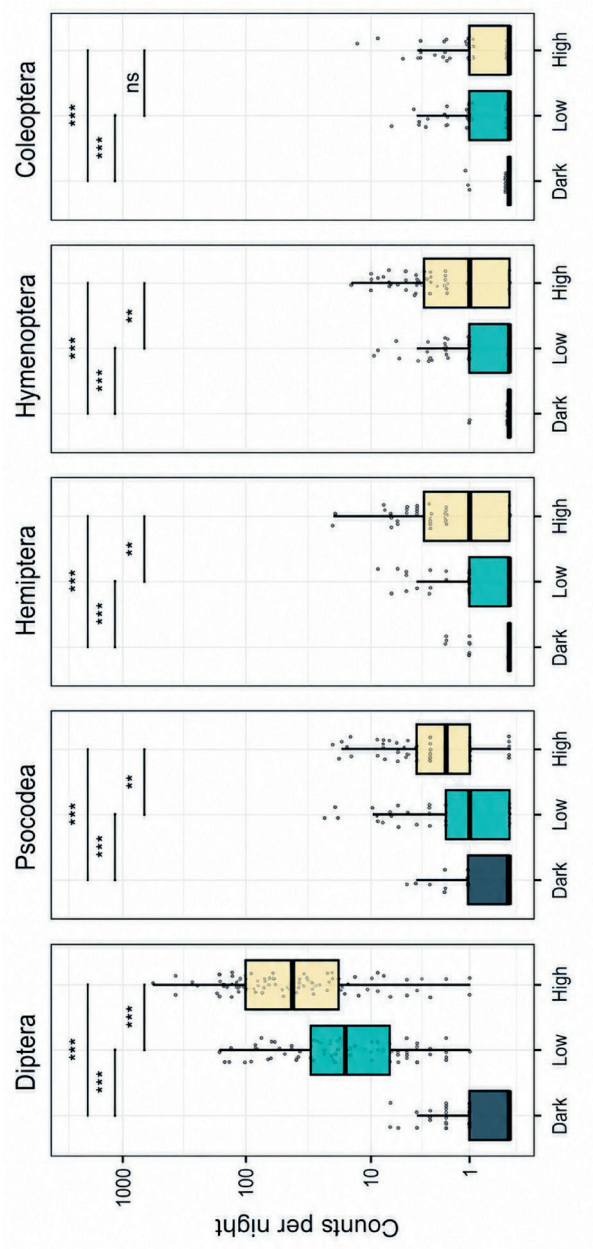


Fig. 2. Nightly flying insect counts per treatment (dark control: 0.16 ± 0.03 lux, low: LED 3000 K, 4.4 ± 1.4 lux, high: LED 3000 K, 17 ± 3.8 lux), separated per insect order. Graphs are shown for the five most abundant insect orders. Significance levels indicated as ns: $p > 0.05$, **: $p < 0.01$, ***: $p < 0.001$

The best performing model to explain pipistrelle bat passes only included treatment (Table S1). There was an overall effect of treatment (Fig. 1, $\chi 2(2, N = 267) = 110.15$, $p < 0.001$), which can be attributed to the strong, significant differences in pipistrelle bat passes in the dark control compared to both light treatments ($p < 0.001$). However, the nightly total of pipistrelle bat passes recorded at both light intensities was similar ($p = 0.76$).

For the number of pipistrelle feeding buzzes, the selected model also only included the treatment (Table S1), which had a significant overall effect on pipistrelle feeding buzzes (Fig. 1, $\chi 2(2, N = 267) = 177.56$, $p < 0.001$). Additionally, pipistrelle bats produced more feeding buzzes in the dark control compared to both the light treatments ($p < 0.001$) but did not differentiate between the two light treatments ($p = 0.80$).

The selected model for the buzz ratio included both insect counts and treatment (Table S1). Treatment had a significant effect on the buzz ratio (Fig. 3, $\chi 2(2, N = 267) = 152.5$, $p < 0.001$), with a higher buzz ratio at the dark control compared to both the light treatments ($p < 0.001$), while the two light treatments did not differ from each other ($p = 0.99$). In addition, insect counts were positively associated with the buzz ratio ($\beta = 0.0016 \pm 0.0005$, $z = 3.13$, $\chi 2(1, N = 267) = 9.77$, $p < 0.01$) within treatment.

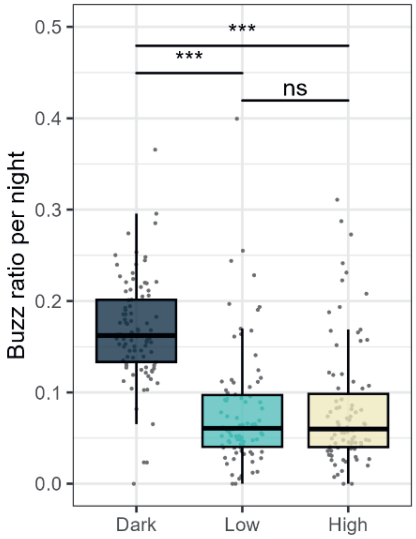


Fig. 3. Nightly buzz ratio (pipistrelle feeding buzz per bat passes) per treatment (dark control: 0.16 ± 0.03 lux, low: LED 3000 K, 4.4 ± 1.4 lux, high: LED 3000 K, 17 ± 3.8 lux). Significance level shown as ns: $p > 0.05$, ***: $p < 0.001$.

5.2. Temporal activity

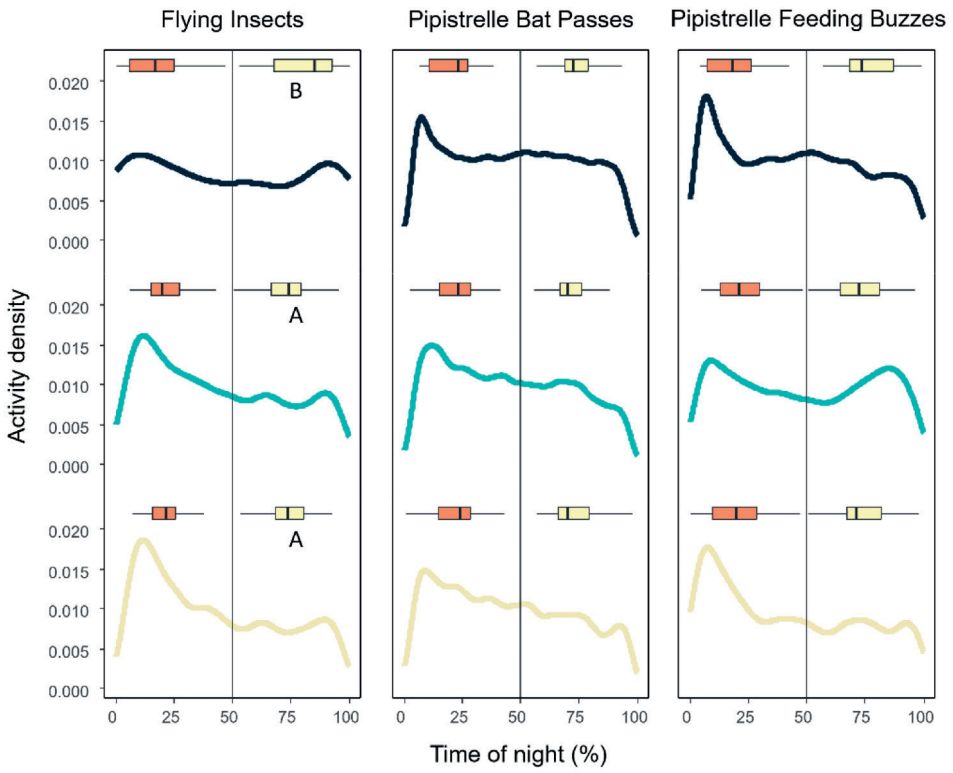


Fig. 4. Nightly temporal activity patterns of flying insects (left), pipistrelle bat passes (middle) and their feeding buzzes (right). The boxplots represent the median time of activity of the first and second half of the night, calculated per date, site and treatment (dark control: 0.16 ± 0.03 lux, low: LED 3000 K, 4.4 ± 1.4 lux, high: LED 3000 K, 17 ± 3.8 lux). The solid lines show activity density, which is the relative intensity of pipistrelle and insect activity throughout the night, with peaks indicating periods of higher activity. Capital letters indicate significant differences between treatments per group, assessed separately for the first and second half of the night. This is only tested for insect timing in the second half of the night, as only for this group the final model included treatment.

While treatment was not included in the final model to explain the median time of insect activity in the first half of the night (Table S2), it was included in the model to explain the median time on insect activity in the second half of the night (Table S2). The overall effect of treatment significantly affected timing (Fig. 4, $\chi 2(2, N = 165) = 14.04$, $p < 0.001$), where the median arrival time was later in the dark control compared to both light treatments (dark-high: $p < 0.01$, dark-low: $p < 0.001$), but did not differ between light treatments ($p = 0.73$).

For all bat related models (timing of bat passes or feeding buzzes in the first and second half of the night), the intercept model was selected, as adding insect timing, treatment or both did not improve model fit (Table S2).

6. Discussion

We found that dimming urban streetlights reduced insect attraction but did not fully eliminate it. In contrast, pipistrelle bats were not affected by dimming streetlights, and, surprisingly, favoured foraging in darkness, showing a higher relative feeding activity ('buzz ratio'). Furthermore, dimming light did not affect activity timing in bats or insects. However, the presence of light did affect insect timing in the second half of the night, showing earlier median activity around light than at the dark control. While insect activity did not help predict overall (bat passes) and foraging (feeding buzzes) activity of bats, it was positively correlated with their buzz ratio.

The finding that dimming light effectively reduces insect attraction confirms our first hypothesis (H1). This is an important finding, as research on the effects of light intensity on insect attraction is still limited. Rowse et al. (2018) did compare the insect numbers at dimmed light conditions: 0 %, 25 %, 50 % and 100 % of the original light level. Even though there was a trend, the authors could not confirm significant differences between the partly dimmed light conditions, but only between the dark (0 %) and fully lit (100 %) treatment. In another study with traffic controlled dimming (to 75 % of the original level in absence of traffic and 100 % for the duration of passing traffic), insect counts were lower in the dimmed treatment compared to the undimmed situation (Bolliger et al., 2020). However, when looking into the four most abundant orders, only the number of attracted heteropterans was reduced with dimming. At our urban study sites, where light was dimmed to 26 % of the original level (4.4 lux to the original 17 lux at ground level), we found a reduced attraction to dimmed lights by the counts of all tested orders, except for Coleoptera.

In contrast to previous studies and to our second hypothesis (H2), we found that pipistrelle bats were generally more active and foraged more in darkness compared to around the streetlights. This is remarkable as light-opportunistic pipistrelles usually forage on insects aggregated around ALAN (Blake et al., 1994; Hermans et al., 2024; Rydell, 1992). The increased feeding activity we found in darkness indicates prey availability, yet insect captures on the dark control traps were very low. This discrepancy could be explained by the insect measuring methods used in this study. Light concentrates insects, increasing the likelihood that they get caught on the insect trap placed under the light. In contrast, insect distribution in darkness is likely more

uniform, with no mechanism to attract insects to our traps. Therefore, overall insect abundance may overall still be high in the wider dark surroundings while our results do not indicate this.

While most studies were performed in rural or more suburban areas, our sampling took place in urbanised locations. As urban areas are typically much more illuminated than rural areas (Jechow et al., 2020), the perceived risk-benefit ratio associated with foraging on accumulated insects around light for pipistrelle bats may be different. When the benefits (accumulated prey) no longer outweigh the risks (increased predation pressure), pipistrelle bats may prefer to forage in darkness—consistent with their tendency to prefer darkness during other activities, such as drinking and general movement (Hale et al., 2015; Russo et al., 2017). As the density of light sources is much higher in urban areas than in rural areas, the inability to retreat into (relatively) dark spaces may keep pipistrelles from foraging on the accumulated insects. Furthermore, the ubiquitous presence of light might pull insects towards multiple sources of light, lowering insect densities near individual light sources and thereby reducing the benefit to forage there. Lastly, there is evidence that long-term light exposed urban insect populations have adapted by reducing their flight-to-light behaviour (Altermatt and Ebert, 2016), which is also associated with a decrease in mobility (Van de Schoot et al., 2024). This potentially further decreases the number of insects around streetlights in urban environments.

In addition to the clear preference of pipistrelle bats for darker foraging grounds, we found no effect of dimming on their overall activity and feeding activity, contrasting to our hypothesis (H2). This is intriguing, as dimming did significantly lower attraction of their prey. A possible explanation to this discrepancy is that the daily changing of the light intensity does not allow bats sufficient time to adjust to this change. However, Spoelstra et al. (2017) found an immediate response in pipistrelle activity to turning off lights, where foraging activity returned to levels comparable to natural dark levels as soon as the lights were left off. It is possible that this immediate response is only triggered when the light is turned off completely, whereas the dimming in our setup was insufficient to elicit a response of the pipistrelle bats. Similar to our results, Rowse et al. (2018) only found differences in pipistrelle activity between fully illuminated and darkness but not when dimming the light. At our study sites, further dimming may elicit an immediate response from pipistrelles, but this was not deemed feasible by concerns regarding public perceptions of safety.

As the adjacent sets of three light posts at a site are easily visited by the same bat, the absence of a response in pipistrelle activity to dimming may result from individuals foraging at both the sets of streetlights in quick succession. However, this scenario appears to be unlikely for two reasons. First, it does not align with typical pipistrelle

foraging behaviour, which is generally non-linear and characterised by looping or circling flight patterns, rather than continuous movement along linear features (Limpens et al., 1997). Second, our additional analysis supports the conclusion that individuals were unlikely to forage at both sets of streetlights concurrently ('Statistical independence of streetlight sets' in Supplementary material, Fig. S8).

Another unexpected result, contrasting with our hypothesis (H2), was that the pipistrelle buzz ratio was lower around light compared to dark control, independent of dimming. As insects are more clustered around light sources, we typically expect more feeding opportunities for light-opportunistic bat species underneath the light (Bolliger et al., 2020; Hermans et al., 2024). However, in line with our results, Kerbiriou et al. (2020) found a decrease in pipistrelle buzz ratio with an increase in light intensity. One possible explanation is that the lower buzz ratio represents an increased selectiveness for prey. Lights attract insects, including large bodied insects such as moths (Degen et al., 2016; van Langevelde et al., 2011), while it simultaneously interferes with the evasive manoeuvres of tympanite moths (Wakefield et al., 2015). Therefore, urban bats foraging in the light might have become more selective and possibly adapted to foraging on bigger prey (Bailey et al., 2019; Tomassini et al., 2014).

We argue that the overall bat activity (bat passes) and especially their foraging activity (feeding buzzes and buzz ratio) is a response to the insect densities rather than to light. However, the strong correlation between insect activity and the light treatments prevents a complete separation of these effects on bat activity. While we are therefore unable to formally test for our hypothesis, we do see diverging effects of light and insect activity on bat activity. The variation in the buzz ratio in best explained by insect activity together with light treatment. As buzz ratio serves as a proxy for relative foraging activity, it is indeed expected to be more closely linked to prey availability. However, for the other bat models (bat passes, feeding buzzes), treatment rather than insect counts best fit of the models. This does tell us that pipistrelles do not blindly follow insects but are also directly influenced by light.

Contrary to our hypotheses (H3, H4), dimming did not affect the timing of pipistrelle and insect activity. Only the median insect activity in the second half of the night was altered, but differently from what we initially postulated. Instead of the expected prolonged morning activity (H3), we found the median insect activity to be earlier around lights compared to their timing in the dark environment. Keeping in mind that this model only contained 10 dark control nights with sufficient insect counts (81 and 74 nights for high and low intensity, respectively), a possible explanation of this shift in activity near lights could lie in the natural bimodal activity patterns of insects. Such patterns, especially pronounced in Diptera—the most abundant insect order we caught—typically show activity peaks around dusk and dawn in natural dark conditions

(Blackwell, 1997; Gray et al., 2011; Rydell et al., 1996). Insects cluster around light sources when these sources contrast significantly with natural light levels. As sunlight diminishes, the attraction to artificial light increases, skewing the projected temporal activity patterns of insects. Additionally, the presence of ALAN can distort the timing of the perceived twilight period. By increasing ambient light intensity, ALAN could alter the cues used by insects, especially those with bimodal activity patterns. The lack of response in pipistrelle timing contrasts with previous studies, where artificial light was found to significantly alter the temporal activity patterns of rural pipistrelles (Buddendorf et al., 2025; Hermans et al., 2024; Salinas-Ramos et al., 2021). Again, our contrasting outcomes may reflect differences in light-landscapes, where brightly illuminated urban habitats cause contrast between lit and dark areas to fades, potentially blurring the differences in activity timing associated with these environments.

Our findings highlight the need to conserve the night sky and preserve dark areas throughout human-dominated landscapes. Dark locations not only serve as essential foraging sites for generally light-averse bat species, but—as our novel findings demonstrate—also for synanthropic pipistrelle bats in urban environments. Additionally, we show that dimming streetlights can mitigate the effects of ALAN on insects, although even dimmed urban streetlights remain more disruptive to bats and insects than natural darkness. While dimming cannot fully replicate natural darkness, it might provide a realistic balance between human needs and local biodiversity.

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9. Data availability

Data will be made available on request.

10. References

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11. Supplementary material



Fig. S1. An overview of the locations of our study sites, located in The Hague and Rotterdam, the Netherlands.

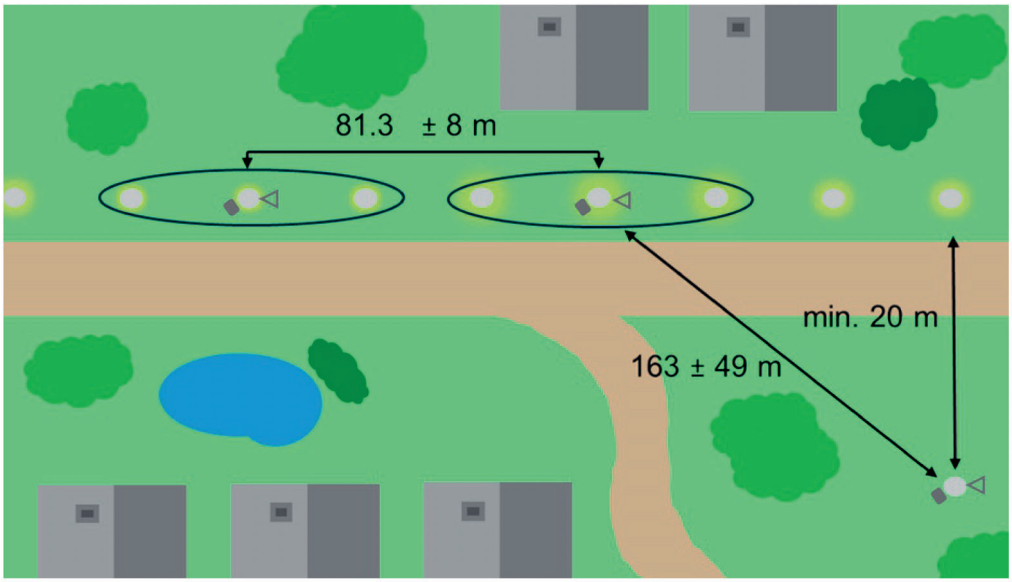


Fig. S2. A schematic overview of the study setup, showing the average distances (± s.e.m.) between the centres of the treatments. Bat and insect sampling devices were deployed at the central poles of the light treatments and at the dark control pole/tree. Selection criteria for these urban study sites were the absence of strong light sources in the surroundings (e.g. sport fields) and the presence of some natural features (e.g. water and minimally maintained vegetation).

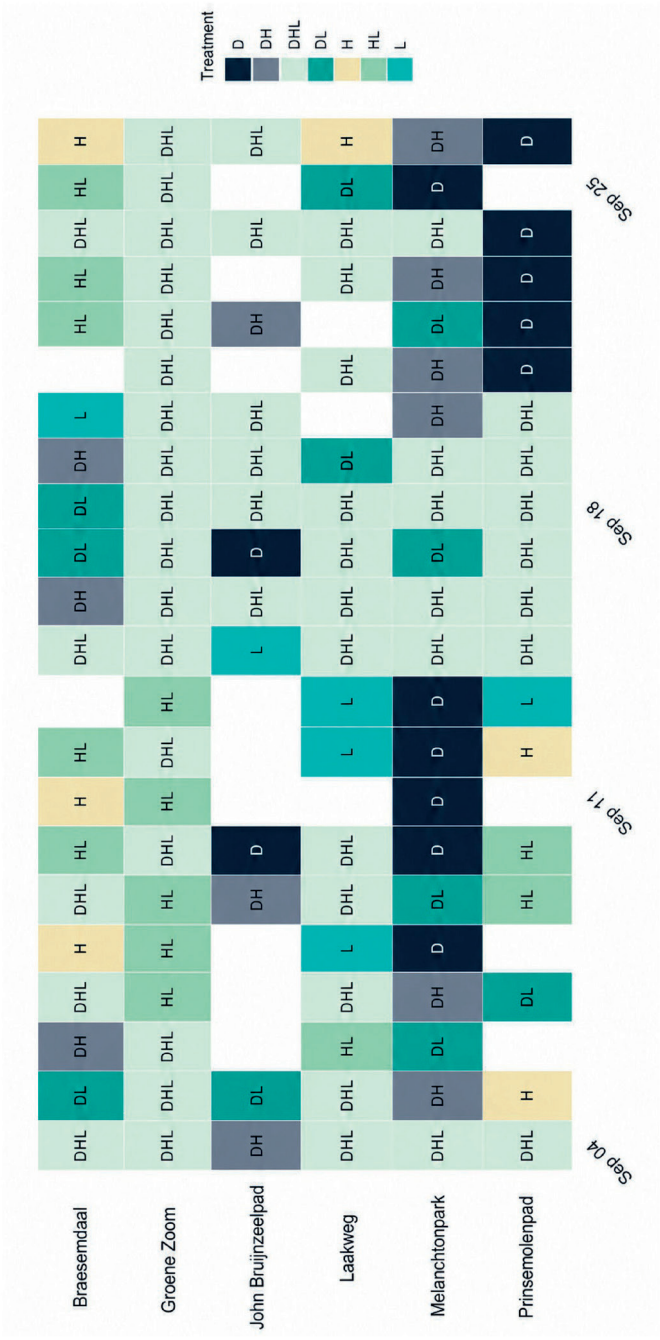


Fig. S3. Sampling nights at the treatments recorded at each study site on each night of the study period. Each cell indicates the combination of treatments (Dark = D, Low = L, High = H) recorded at a given study site on a given night. Cell colours correspond to the specific treatment combinations.



Fig. S4. The insect monitoring setup in action. It consists of a sticky sheet with a camera pointed towards it, which is programmed to photograph the sheet every 5 minutes. At the dark control, it uses infrared to visualise the insects without affecting the experimental setup.



Fig. S5. An example of the experimental setup. Here, the middle streetlight of a light treatment with insect and bat monitoring devices attached are shown.

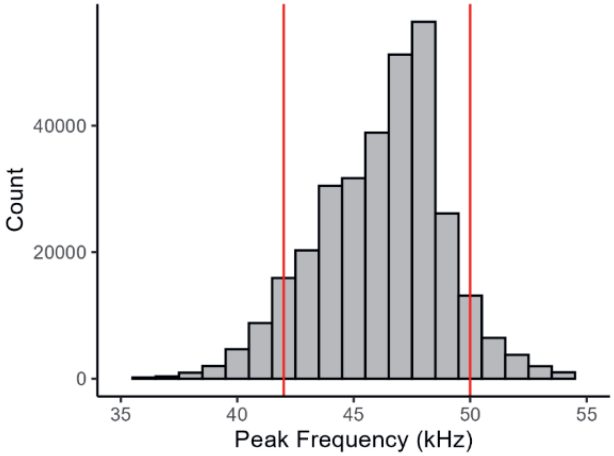


Fig. S6. The peak frequency distribution of all pipistrelle bat passes used in this study. 86.2% of bat passes had a peak frequency between 42-50 kHz, 5.6% bat passes had a peak frequency below 42 kHz and 4.7% above 50 kHz. The outcome of the statistical model did not change when excluding bat passes outside of this range.

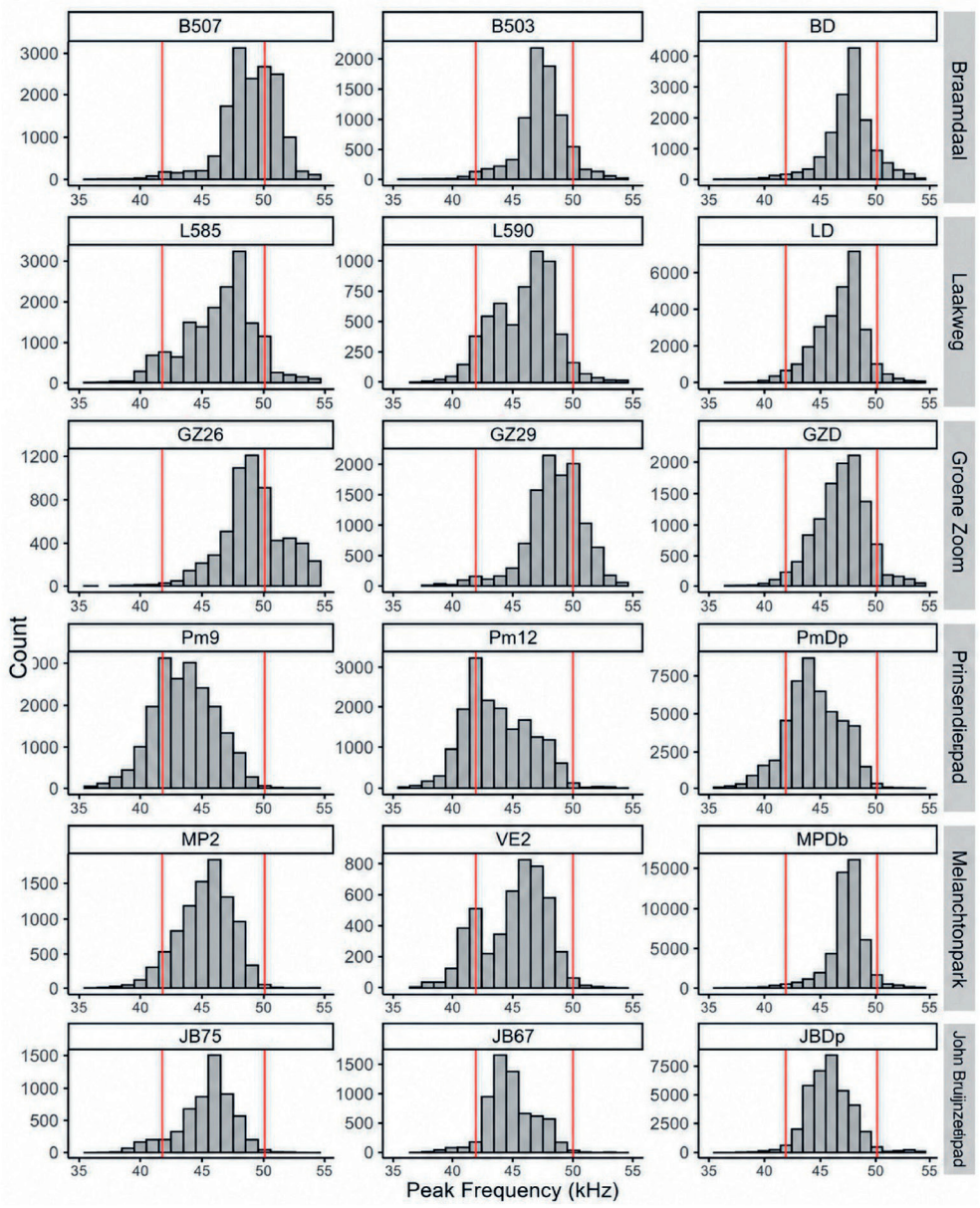


Fig. S7. The peak frequency distribution of all pipistrelle bat passes used in this study. Visualised per study site (rows) and measurement location (left and middle column: light treatments, right column: dark control).

11.1. Statistical independence of streetlight sets

As the two sets of streetlights within each study site were in close proximity, we tested whether they could be treated as statistically independent sampling locations. To assess this, we examined the correlation in pipistrelle bat passes per 15 minutes between both sets and visually evaluate the symmetry in activity levels between the two sets, as a proxy for whether individual bats were likely foraging at both in quick succession.

The correlation in pipistrelle activity between sets was low at Brasemdaal, Groene Zoom and John Bruijnzeelpad, and notably weaker at Laakweg, Melanchtonpark and Prinsemolenpad. None of the sites exhibited both a strong correlation and clear symmetry around the 1:1 reference line (grey dashed line in Fig. S7), indicating that individual bats were unlikely to be consistently moving between the two sets within these short time frames. Therefore, we treated each set as an independent sampling location in the statistical analyses.

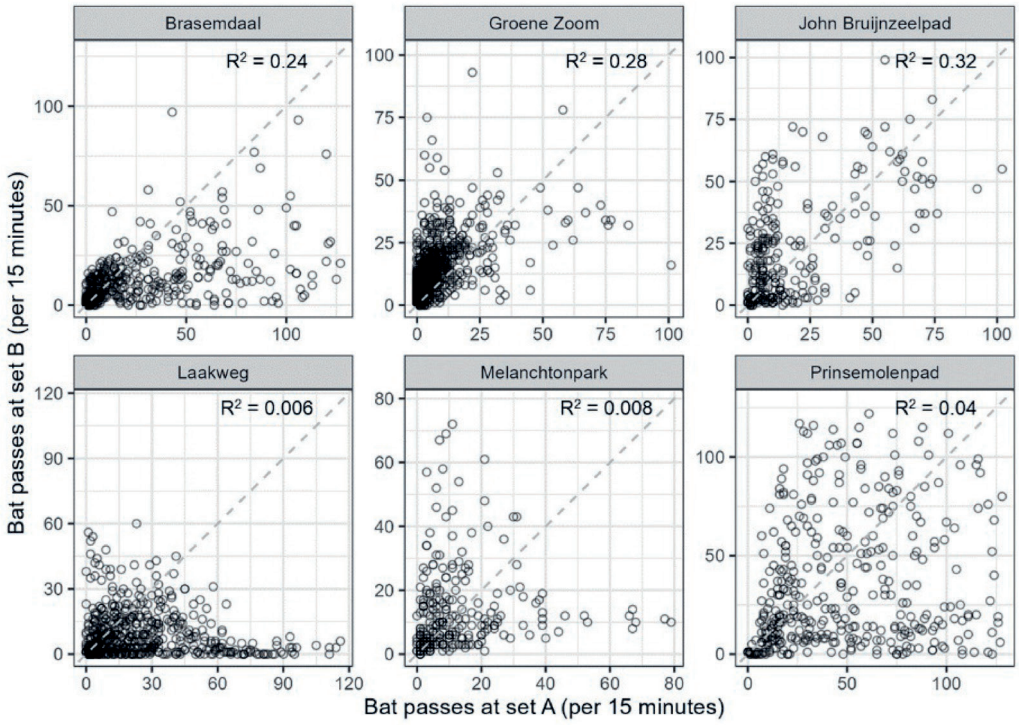


Fig. S8. Correlation of pipistrelle bat passes per 15 minutes between both streetlight sets at each study site. The grey dashed line indicates the 1:1 reference.

11.2. Model selection nightly activity models

Table S1: Forward model selection for nightly summed activity data. The maximum model for total insects and insect order was only including treatment, whereas the maximum model for pipistrelle passes, feeding buzzes and buzz ratio included insect count and treatment. Models were selected when the p-value was lower than 0.05 and the ΔAIC was higher than 2.

Diptera	**Df**	**AIC**	**BIC**	**logLik**	**deviance**	**Chisq**	**Chi Df**	**Pr(>Chisq)**
Count ~ 1 + (1 | Night_date) + (1 | Site_name)	4	2202.1	2216.4	-1097	2194.1			
Count ~ Treatment + (1 | Night_date) + (1 | Site_name)	6	1818.4	1839.9	-903.2	1806.4	387.7	2	< 0.001
Psocodea								
Count ~ 1 + (1 | Night_date) + (1 | Site_name)	4	950	964.4	-471	942			
Count ~ Treatment + (1 | Night_date) + (1 | Site_name)	6	867.2	888.8	-427.6	855.2	86.8	2	< 0.001
Hemiptera								
Count ~ 1 + (1 | Night_date) + (1 | Site_name)	4	709.6	723.9	-350.8	701.6			
Count ~ Treatment + (1 | Night_date) + (1 | Site_name)	6	635.7	657.2	-311.8	623.7	77.9	2	< 0.001
Hymenoptera								
Count ~ 1 + (1 | Night_date) + (1 | Site_name)	4	712	726.4	-352	704			
Count ~ Treatment + (1 | Night_date) + (1 | Site_name)	6	596.7	618.2	-292.4	584.7	119.3	2	< 0.001
Coleoptera								
Count ~ 1 + (1 | Night_date) + (1 | Site_name)	4	447.8	462.1	-219.9	439.8			
Count ~ Treatment + (1 | Night_date) + (1 | Site_name)	6	394.5	416	-191.3	382.5	57.3	2	< 0.001

FlyingInsect								
FlyingInsect ~ 1 + (1 | Night_date) + (1 | Site_name)	4	2467	2481.3	-1229.5	2459			
FlyingInsect ~ Treatment + (1 | Night_date) + (1 | Site_name)	6	2086.8	2108.3	-1037.4	2074.8	384.2	2	< 0.001
Bat								
Bat ~ 1 + (1 | Night_date) + (1 | Site_name)	4	4239.3	4253.6	-2115.6	4231.3			
Bat ~ FlyingInsect + (1 | Night_date) + (1 | Site_name)	5	4215.2	4233.1	-2102.6	4205.2	26.1	1	< 0.001
Bat ~ Treatment + (1 | Night_date) + (1 | Site_name)	6	4147.2	4168.7	-2067.6	4135.2	70.0	1	< 0.001
Bat ~ Treatment + FlyingInsect + (1 | Night_date) + (1 | Site_name)	7	4149.2	4174.3	-2067.6	4135.2	0.0	1	0.9747
Buzz								
Buzz ~ 1 + (1 | Night_date) + (1 | Site_name)	4	3182.1	3196.4	-1587.0	3174.1			
Buzz ~ FlyingInsect + (1 | Night_date) + (1 | Site_name)	5	3156.7	3174.7	-1573.4	3146.7	27.3	1	< 0.001
Buzz ~ Treatment + (1 | Night_date) + (1 | Site_name)	6	3041.2	3062.8	-1514.6	3029.2	117.5	1	< 0.001
Buzz ~ Treatment + FlyingInsect + (1 | Night_date) + (1 | Site_name)	7	3041.4	3066.5	-1513.7	3027.4	1.9	1	0.1714
Buzzratio								
cbind(Buzz, Bat - Buzz) ~ 1 + (1 | Night_date) + (1 | Site_name)	4	2750.5	2764.8	-1371.2	2742.5			
cbind(Buzz, Bat - Buzz) ~ FlyingInsect + (1 | Night_date) + (1 | Site_name)	Did not converge							
cbind(Buzz, Bat - Buzz) ~ Treatment + (1 | Night_date) + (1 | Site_name)	6	2635.6	2657.1	-1311.8	2623.6	118.9	2	< 0.001
cbind(Buzz, Bat - Buzz) ~ Treatment + FlyingInsect + (1 | Night_date) + (1 | Site_name)	7	2629.3	2654.4	-1307.6	2615.3	8.3	1	0.0039

11.3. Model selection temporal activity models

Table S2: Forward model selection for temporal activity, expressed in median activity of the first and second half of the night. The maximum model for timing of total insects was only including treatment, whereas the maximum model for timing of pipistrelle passes and feeding buzzes included insect count and treatment. Models were selected when the p-value was lower than 0.05 and the ΔAIC was higher than 2.

Flying Insect 1st half	**Df**	**AIC**	**BIC**	**logLik**	**deviance**	**Chisq**	**Chi Df**	**Pr(>Chisq)**
median ~ 1 + (1 | Night_date) + (1 | Site_name)	4	1247.5	1260.2	-619.7	1239.5			
median ~ Treatment + (1 | Night_date) + (1 | Site_name)	6	1246.5	1265.6	-617.3	1234.5	4.9	2	0.0847
Flying Insect 2nd half								
median ~ 1 + (1 | Night_date) + (1 | Site_name)	4	1200.2	1212.6	-596.1	1192.2			
median ~ Treatment + (1 | Night_date) + (1 | Site_name)	6	1190.7	1209.4	-589.4	1178.7	13.5	2	0.0012
Bat 1st half								
median ~ 1 + (1 | Night_date) + (1 | Site_name)	4	1250.8	1263.5	-621.4	1242.8			
median ~ Treatment + (1 | Night_date) + (1 | Site_name)	5	1252.8	1268.6	-621.4	1242.8	0.0	1	0.9299
median ~ FlyingInsect_ 1st_median + (1 | Night_date) + (1 | Site_name)	6	1253.6	1272.6	-620.8	1241.6	1.2	1	0.2648
median ~ Treatment + FlyingInsect_ 1st_median + (1 | Night_date) + (1 | Site_name)	7	1255.5	1277.6	-620.7	1241.5	0.1	1	0.7794
Bat 2nd half								
median ~ 1 + (1 | Night_date) + (1 | Site_name)	4	1123.2	1135.6	-557.6	1115.2			
median ~ Treatment + (1 | Night_date) + (1 | Site_name)	5	1123.5	1138.9	-556.7	1113.5	1.7	1	0.1889
median ~ FlyingInsect_2nd_median + (1 | Night_date) + (1 | Site_name)	6	1125.3	1143.8	-556.7	1113.3	0.2	1	0.6844
median ~ Treatment + FlyingInsect_2nd_median + (1 | Night_date) + (1 | Site_name)	7	1125.9	1147.6	-556.0	1111.9	1.4	1	0.2426

Buzz 1st half

median ~ 1 + (1 | Night_date) + (1 | Site_name)	5	1157.9	1173.2	-574.0	1147.9			
median ~ FlyingInsect_ 1st_median + (1 | Night_date) + (1 | Site_name)	6	1159.9	1178.2	-574.0	1147.9	0.0	1	0.8799
median ~ Treatment + (1 | Night_date) + (1 | Site_name)	7	1160.6	1181.9	-573.3	1146.6	1.3	1	0.2574
median ~ Treatment + FlyingInsect_ 1st_median + (1 | Night_date) + (1 | Site_name)	8	1162.5	1186.8	-573.2	1146.5	0.1	1	0.7107

Buzz 2nd half

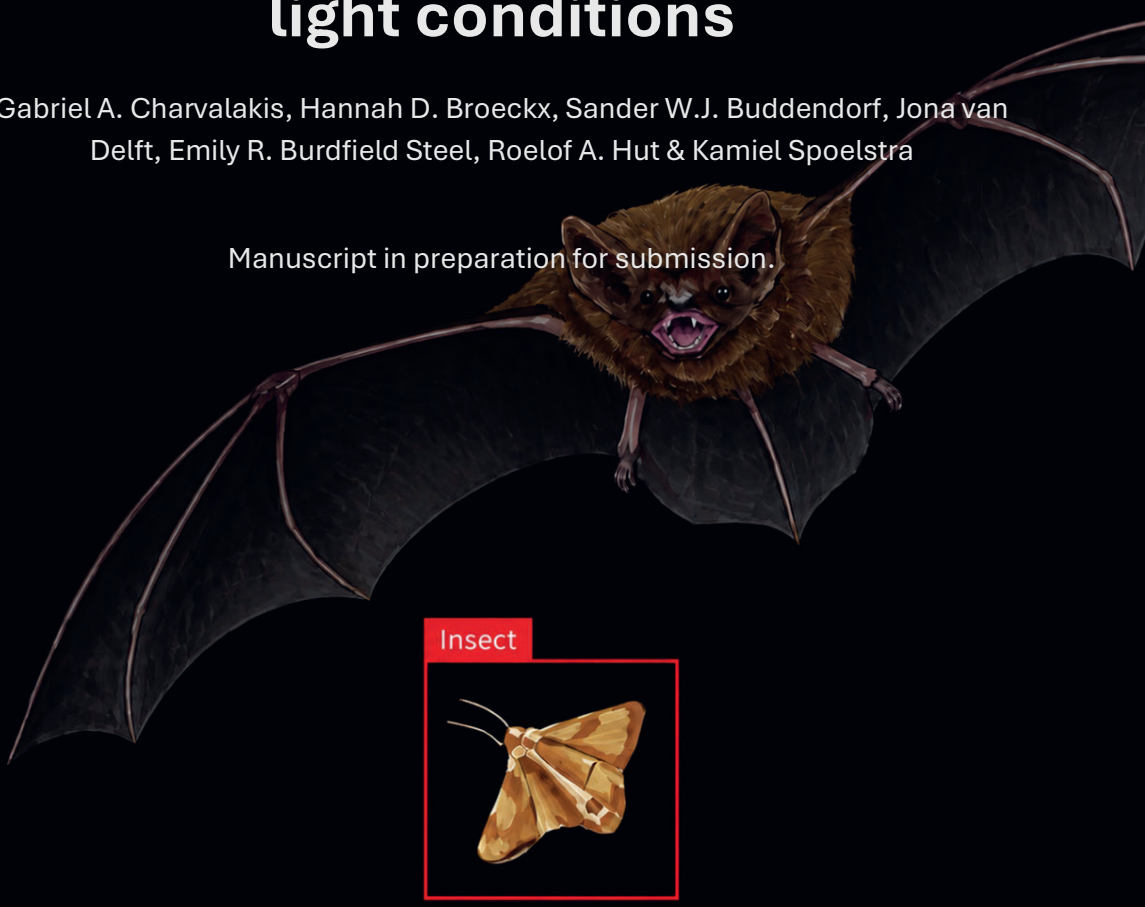
median ~ 1 + (1 | Night_date) + (1 | Site_name)	4	1031.6	1043.3	-511.8	1023.6			
median ~ FlyingInsect_2nd_median + (1 | Night_date) + (1 | Site_name)	5	1032.5	1047.1	-511.2	1022.5	1.1	1	0.2906
median ~ Treatment + (1 | Night_date) + (1 | Site_name)	6	1035.5	1053.1	-511.8	1023.5	0.0	1	1.0000
median ~ Treatment + FlyingInsect_2nd_median + (1 | Night_date) + (1 | Site_name)	7	1036.3	1056.9	-511.2	1022.3	1.2	1	0.2740

6

AI-based assessment of insect temporal activity under different light conditions

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Manuscript in preparation for submission.



1. Abstract

Insect populations are in global decline, underscoring a growing need for cost-efficient, accessible and repeatable methods to continuously assess insect densities for extended periods of time. Computer vision and neural network models allow for automated monitoring, but current approaches often emphasise taxon identification, while overlooking temporal detail and performance under variable light conditions. We present a validated workflow for quantifying nocturnal insect activity over time, using low-cost infrared camera traps and an open-source deep learning framework. An object detection model was trained on images of sticky sheet traps deployed at seven rural sites in the Netherlands. Subsequently, the model was applied to images from sticky sheet traps at six urban sites to extract individual insect arrival times. The urban insect arrival times were also manually scored to validate model performance. This validation included arrivals under darkness using an infrared light source, as well as low- and high-intensity visible light, enabling the assessment of the effect of illumination on model performance. We show that performance varies with illumination, with low and high light intensities producing more reliable results than darkness. In addition, the alignment of automated and manual arrival time scores was low at 5-minute intervals, but improved substantially when arrivals were aggregated at an hourly or coarser temporal resolution. These results show that a reliable and cost-efficient workflow for automating insect activity monitoring can be achieved under different conditions of visible light and at different temporal scales, though with limited taxonomic detail.

2. Keywords

Automated detection, Insect monitoring, Temporal activity, Light conditions

3. Introduction

Insect are not only important ecosystem service providers but also the most abundant and species rich animal class on Earth (Stork, 1993, 2018). Nonetheless, insect populations are globally declining due to multitude of anthropogenic pressures (Hallmann *et al.*, 2017; Harvey *et al.*, 2020). To accurately estimate insect losses, and associate them with possible stressors, continuous and widespread monitoring is important. However, such large-scale monitoring efforts of insect populations face practical and methodological challenges. Estimating insect densities by manually counting trapped specimens, or manually evaluating image-based records of insect activity, requires considerable time and effort. Furthermore, logistic constraints often limit the repeatability of insect monitoring efforts. Therefore, there is a growing need for accessible, cost-efficient, and reliable monitoring methodologies.

Automation of insect monitoring is therefore essential for continuous unattended assessment of insect densities over extended periods (Høye *et al.*, 2021; Teixeira *et al.*, 2023). Novel methods, such as infrared optical sensors and LiDAR (Light Detection and Ranging) based systems, have showcased the potential for the automation of insect monitoring (Kirkeby *et al.*, 2021). In parallel, advances in computer vision tools and convolutional neural networks offer comparatively cost-efficient image-based solutions for further development in automation and accessibility (Valan et al., 2019; Bjerge, Mann and Høye, 2022; Geissmann et al., 2022; Sittinger et al., 2024).

Beyond knowing how many insects are in an area, many studies could also benefit from knowing their temporally activity patterns. An increase in the temporal resolution of automated assessments of insect activity could offer deeper insights into the behaviour, ecology, and circadian biology of insects, which might be missed by conventional methods (Geissmann *et al.*, 2022; Kirse *et al.*, 2025). Despite recent advances, current approaches to image-based insect monitoring have mostly focussed on taxonomic identification, whereas less effort has been directed towards the evaluation of temporal activity patterns. One challenge in capturing such temporal patterns arises from the reassignment of new track identities (IDs) when insects leave and re-enter a picture frame, which can lead to repeated counts of the same individual (Sittinger *et al.*, 2024). Similarly, variance in the duration of insect tracks complicates temporal assessment of activity, leading either to temporal activity overestimations or underestimations (Bjerge, Mann and Høye, 2022). Instead, the use of sticky sheet traps, in combination with cameras, has been shown to produce a more efficient assessment of the temporal activity patterns of insects, even at a daily resolution

(Geissmann *et al.*, 2022), as these prevent insects from repeatedly entering and leaving the picture frame.

A major challenge in automated monitoring, for both temporal and taxonomic purposes, is the cross validation of the image-based automatic detections of insects via the use of independent datasets and methodologies (Høye *et al.*, 2021). Cross-validation is essential to know how well a method performs. Conventional validation approaches typically split a single dataset into training, validation, and test sets, thereby primarily evaluating object detection performance within that dataset. By contrast, validation using independent methods and independent experimental data makes it possible to assess the robustness of automated detection under varying environmental and operational conditions. Developing such robust automated monitoring methods is crucial, as it enables reliable assessment of insect temporal activity across a wide range of contexts.

Automating the monitoring of nocturnal insects may be especially challenging since the majority of nocturnal trapping systems depend on the attraction of insect taxa to light at night (McGeachie, 1989; Nowinszky, 2007; Ma and Ma, 2012; Wakefield *et al.*, 2018). In addition, insect monitoring devices may be deployed across a wide range of light conditions, yet the effect of lighting on model performance is to a large extent unstudied. Environmental illumination is a critical but often overlooked factor which may affect the reliability of neural network-based insect detections, similar to the demonstrated effect of sticky trap colour on model accuracy (Ong and Høye, 2025). Different light colours and intensities affect the strength of insect phototaxis (van Grunsven et al., 2014; Longcore et al., 2015; Charvalakis et al., 2025), thus such factors may also prove important for future accuracy refinements in the automated detection of nocturnal insects.

In this study, we asked the following: (i) whether AI based assessment of insect activity is reliable across a novel dataset, (ii) whether different light conditions affect the performance of automated insect detection, and (iii) how the temporal resolution of monitoring influences model reliability. To address these questions, we trained an object detection model on images from camera traps placed at seven rural sites in the Netherlands and applied the model-based algorithm to a novel dataset from six urban sites. In addition, we scored the arrivals times of insects manually and tested the model performance under different lighting conditions.

4. Material and methods

4.1. Sticky camera trap description

To monitor insect arrival times, we used infrared cameras (Technaxx TX-117, Technaxx, Germany). Each camera unit was mounted on a 90° metal hook (height: 19 cm; length: 41 cm) supported by two lateral metal arms (length: 45 cm each) connected to a plastic rectangular frame (length: 30 cm; height: 21 cm; Fig. 1C). The plastic rectangular frame (S.B. Weston Ltd, UK) was affixed to the end of a metal hook perpendicularly to the camera's optical axis. Laminated, white A4-size sheets were taped into the rectangular frames and coated with an odourless, non-toxic adhesive (Sticky-Trap, Hofman Animal Care B.V., Netherlands). The adhesive sheets were replaced at about at one- to two-week intervals, depending on the captured insect densities. Cameras were programmed for time-lapse photography at 5-minute intervals throughout the sampling period. The sticky camera traps were placed on 4 m high lampposts, approximately 0.5-0.6 m below the light source (Fig. 1B), or, for some of the dark controls, on the trunks of bare trees at the same height. The total cost of the whole setup was affordable, even for smaller research budgets (see prices in Table S1).

4.2. Experimental locations & design for the training(rural) dataset

We extracted a total of 153216 JPG image files from fourteen sticky camera traps placed at seven forested locations across the Netherlands (Fig. 1A). The aforementioned experimentally illuminated locations are described in detail in Spoelstra *et al.* (2015). At each location, one camera trap was positioned beneath a white light luminaire (Fortimo White; Philips, the Netherlands; 2700 K) and a second camera trap was mounted on the trunk of a tree located at least 50 m away from the lamppost camera trap. All lampposts were on from sunset to sunrise. The illuminance at ground level beneath the lampposts was 7.6 ± 1.2 lux. We monitored insect activity for a total of 38 days between the 21st of August and 28th of September 2023.

For each 24-h cycle, for each camera, a random two-hour window of 24 images was selected in order to create a training dataset of in total 12768 JPG files. We selected these two hours at random from the full 24-h cycle to capture the full range of variation in pixel values caused by changing lighting conditions. We trained an object detection model using the YOLOv11l architecture as described and implemented via the ultralytics python library (Jocher and Qiu, 2024). The training dataset was annotated with two classes: *insect* and *other* (*other* refers to unidentifiable or non-insect objects). We extracted the best-performing model weights derived from a training session of 250

epochs, as stored in the checkpoint file (best.pt) automatically saved by the YOLO framework. This instance was then used for inference within the analysis pipeline. During inference, each image was processed using a confidence threshold of 0.4, since this threshold provided a balance between adequate recall, accuracy and precision levels (Fig. S2).

4.3. Experimental locations & design for human scoring of the novel (urban) dataset

We extracted an additional 124416 JPG images from eighteen sticky camera traps placed at six urban study sites (three in The Hague and three in Rotterdam) from a separate experimental setup (Fig.1A; for details see Buddendorf *et al.*, 2026). Per study site, insect activity was measured under two light treatments and under one dark control. The light treatment consisted of two dimmable lampposts which alternated in intensity (high or low) on consecutive nights. Thus, two camera sticky traps were mounted under the two lampposts (of alternating intensities) and one camera sticky trap to a pole without a fixture in case of the dark control. The high intensity averaged 17 ± 3.8 lux and the low light condition 4.4 ± 1.4 lux, at ground level. The dark control pole was installed at least 20 m away from the nearest light source (0.16 ± 0.03 lux ground level). All streetlights had a colour temperature of 3000 K. Sampling took place continuously from September 4th to 28th, 2023. Based on the camera footage, the arrival of new insects on the sticky sheets was manually scored by human observers for all nights, from approximately 7:30 PM to 7:30 AM.

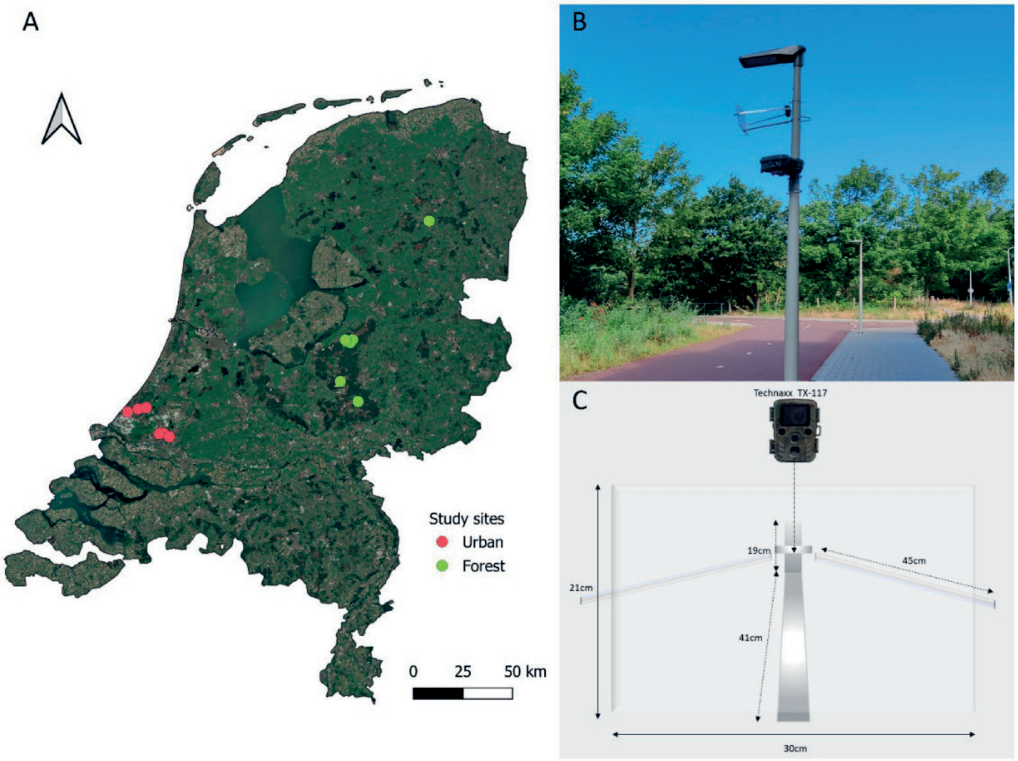


Fig.1. Experimental setup and data acquisition locations. A) Map of camera trap deployment sites. Green dots indicate rural locations used for the training dataset, and red dots indicate urban locations used for algorithm application. B) Example of a sticky sheet camera trap placed beneath a lamppost luminaire (the box beneath the camera trap is equipment from another experiment). C) Design of the sticky sheet camera trap; metal hook (height 19 cm; length: 41 cm) supported by two lateral metal arms (length: 45 cm each) connected to a plastic rectangular frame (length: 30 cm; height: 21 cm).

4.4. Insect arrival time scoring algorithm on the novel (urban) dataset

We developed a custom algorithm to automatically score the arrival time of new insects. This procedure was applied exclusively to image files of the urban dataset in order to compare the alignment of manual and automatic scores of the clock time of insect arrivals. For each new detection, the centre point of the detected bounding box (a rectangle around an object, defined by the coordinates of the corners) of an insect was compared against the previously detected centres of previous frames within the same night to identify new arrivals only. Due to potential insect movement, insect centroids were tracked and retained as previously seen within a radius of 40 pixels.

Only images taken between 7:30 PM and 7:30 AM of the next date were used since manual scoring was conducted within this period.

4.5. Statistical analyses

4.5.1. Effects of temporal resolution and light conditions on metric performance

Insect arrival events were classified by comparing algorithm detections with manual observations. Specifically, true positives (TP) were event cases where both algorithm and manual arrival time scores aligned. Conversely, false positives (FP) were defined as the event cases where algorithm detections were not confirmed via the manually scored insect arrivals. False negatives (FN) were manually observed insect arrivals missed by the algorithm. Finally, true negatives (TN) were instances where both methods agreed on the absence of insect arrivals. We evaluated performance via the following four metrics: *i*) Precision was calculated as the number of true positive insect arrivals divided by the total number of algorithm detected arrivals (Eq. S1). *ii*) We calculated recall as the number of true positive arrivals divided by the total number of manually observed insect arrivals (Eq. S2). *iii*) Accuracy was calculated as the total number of all correct algorithm classifications (true positives and true negatives), divided by the total number of algorithm classifications (true and false) (Eq. S3). *iv*) The harmonic mean of precision and recall (F1 score) was computed by taking twice the value of precision multiplied by recall, and then dividing by the sum of precision and recall, providing a single measure that balances the two metrics (Eq. S4).

To test how the temporal resolution of monitoring influences model reliability, we calculated these four metrics not only at the original observation scale (5-minute picture intervals), but also after grouping the data into larger time blocks. We therefore aggregated the insect arrival time scores for all images within one-hour (hourly) and twelve-hour (nightly) periods, and then recalculated the metrics for each of these temporal resolutions. To test whether performance differed across light condition levels, linear mixed models (LMMs) were performed, using the lme4 package (Bates et al., 2015). All analysis were executed in R, version 4.4.0 (R Core Team, 2024). Models were fitted with nightly performance metric per study site as a response variable and light condition as a fixed term. We included both study site and night as random terms in all of the metric models. Finally, we fit each performance metric model with data aggregated per 5-minute, 1-hour and 12-hour temporal resolution. Pairwise differences of performance between light levels were assessed with Tukey-adjusted post-hoc tests via the emmeans package (Lenth *et al.*, 2025).

5. Results

A total of 15753 insect arrivals were manually counted, while the algorithm detected 14850 insect arrivals. Out of those observations, 301 insect arrivals were manually observed in darkness, while the algorithm detected 535 insect arrivals in darkness. Under low light conditions we manually scored 4528 insect arrivals, while the algorithm detected 4439 arrivals. In high light level conditions 10924 insect arrivals were manually scored, and 9876 were automatically detected.

5.1. Effects of temporal resolution and light conditions on metric performance

Overall performance improved with longer aggregation windows of time resolution. At the original 5-minute resolution, overall precision, recall, F1 score and accuracy were low (0.64, 0.60, 0.62, 0.54, respectively; Table S2). However, metric performance increased with 1-hour intervals (0.76, 0.72, 0.74, 0.59, respectively; Table S2). Finally, overall precision, recall, F1 score and accuracy scores reached their highest at the 12-hour aggregations (0.81, 0.76, 0.78, 0.65, respectively; Table S2, Fig. 3). The performances per treatment group can be found in Table S2.

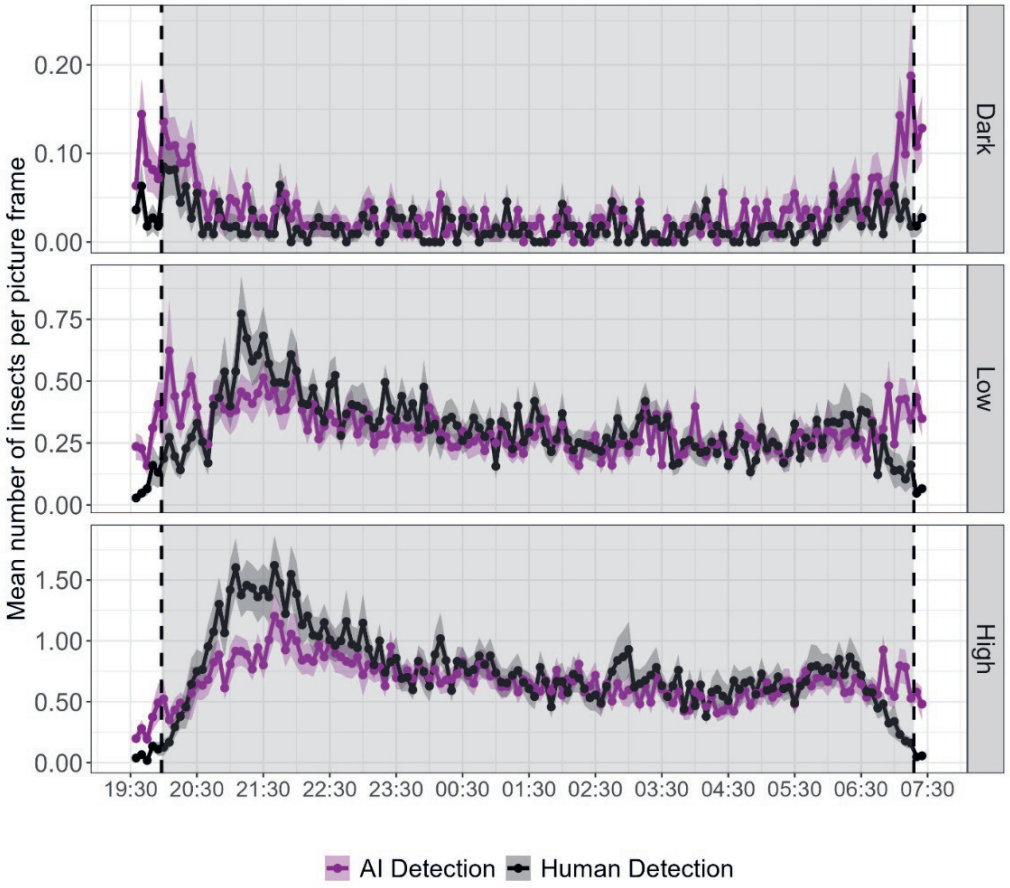


Fig. 2. Human versus AI detected mean number of insects per picture frame. Black lines represent the mean arrival of insects per 5-minute intervals (picture frame) averaged over night and location as scored via human vision, whilst purple lines represent the same as scored via AI based vision under dark, low and high light level conditions. The line-shaded areas represent the standard error (SE). The light grey area bounded by the black dotted lines represents the mean night duration from the 4th until the 27th of September 2023.

Light condition significantly affected performance across all three temporal resolutions with the exception of the mean nightly recall (Table 1). All pairwise post hoc contrasts between dark, high, and low were significant at the 5-minute resolution for all four metrics (Table 1). In the case of the hourly performance, post-hoc tests revealed significant differences between dark and light conditions (both low and high intensity) for all performance metrics (Table 1). In addition, for the mean precision and recall, there was also a significant difference between high and low intensity light, which was not found for the F1 score and accuracy (Table 1). Post-hoc tests on the nightly performance metrics revealed significant lower performances for the dark compared to both light treatments (high and low intensity), except for the mean nightly

recall (Fig. 3, Table 1**)**. No significant differences were found between the high and low intensity treatment for any of the nightly metrics (Fig. 3, Table 1**)**.

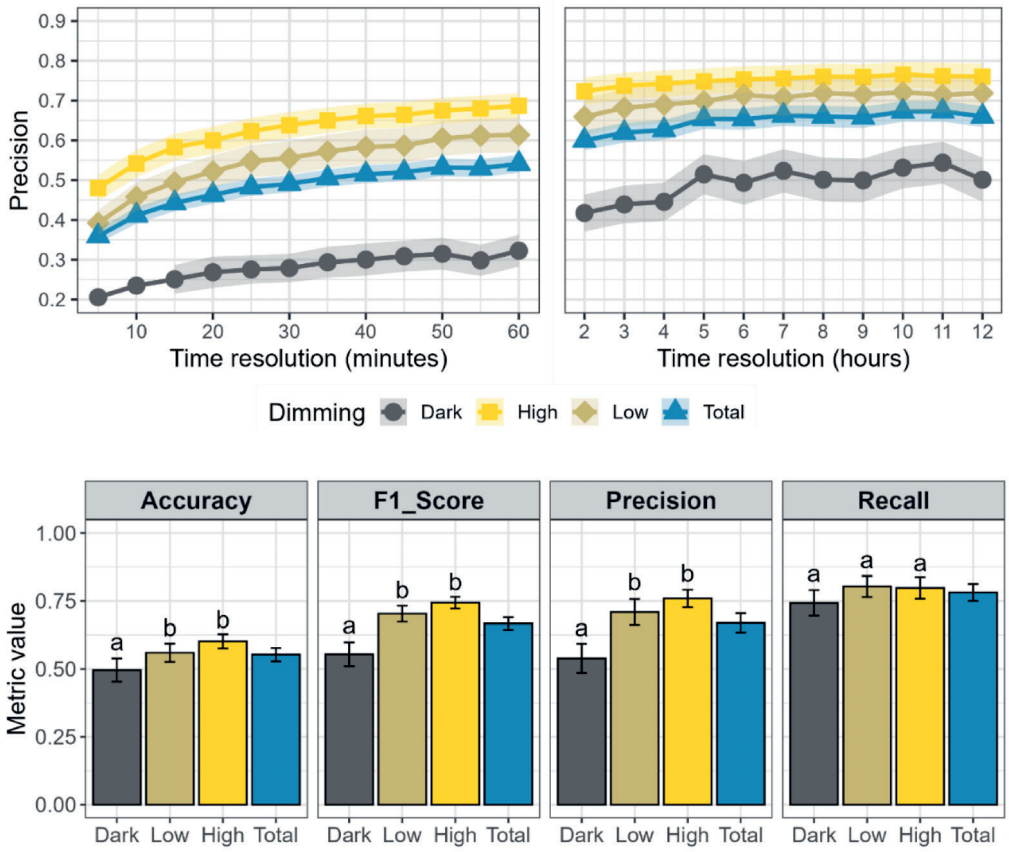


Fig. 3. Mean nightly precision averaged over location. Mean nightly precision was plotted over 5-minute bins (top left), 1-hour (top right) per dark, low, high light level conditions and averaged over sampling night and location. The blue lines (Total) refer to the same metric but also averaged over light condition. Colour-shaded areas around the lines represent the standard error. Bottom: Mean nightly metric values at the temporal resolution of the 12-hour aggregation level. Different letters indicate significance between the light condition levels as derived by the estimated mean post-hoc comparisons (Tukey, α=0.05).

Table 1. Anova and post-hoc results from linear mixed models with the mean nightly metric as a response variable. Significant p-values indicated in bold.

Models: 5-minute resolution	**Statistics**	**Contrast**	**Estimate**	***p*-value**
Precision ~ Light condition + (1/Night) + (1/Site)		Dark – High	-0.30	**< 0.001**
		Dark – Low	-0.21	**< 0.001**
Light condition	$X^2(2, N = 9675) = 258.831, p =$ **< 0.001**	High - Low	0.09	**< 0.001**
Recall ~ Light condition + (1/Night) + (1/Site)		Dark – High	-0.19	**< 0.001**
		Dark – Low	-0.12	**< 0.001**
Light condition	$X^2(2, N = 8645) = 95.14, p =$ **< 0.001**	High - Low	0.07	**< 0.001**
F1 Score ~ Light condition + (1/Night) + (1/Site)		Dark – High	0.17	**< 0.001**
		Dark – Low	0.10	**< 0.001**
Light condition	$X^2(2, N = 5072) = 171.31, p =$ **< 0.001**	High - Low	-0.07	**< 0.001**
Accuracy ~ Light condition + (1/Night)+ (1/Site)		Dark – High	0.32	**< 0.001**
		Dark – Low	0.21	**< 0.001**
Light condition	$X^2(2, N = 44674) = 5888.0, p =$ **< 0.001**	High - Low	-0.11	**< 0.001**
Models: 1-hour resolution	**Statistics**	**Contrast**	**Estimate**	***p*-value**
Precision ~ Light condition + (1/Night) + (1/Site)		Dark – High	-0.31	**< 0.001**
		Dark – Low	-0.27	**< 0.001**
Light condition	$X^2(2, N = 2601) = 165.21, p =$ **< 0.001**	High - Low	0.05	**0.0283**
Recall ~ Light condition + (1/Night) + (1/Site)		Dark – High	-0.22	**< 0.001**
		Dark – Low	-0.17	**< 0.001**
Light condition	$X^2(2, N = 2281) = 86.33, p =$ **< 0.001**	High - Low	0.05	**0.0032**
F1 Score ~ Light condition + (1/Night) + (1/Site)		Dark – High	0.13	**< 0.001**
		Dark – Low	0.11	**< 0.001**
Light condition	$X^2(2, N = 2021) = 42.00, p =$ **< 0.001**	High - Low	-0.02	0.2045
Accuracy ~ Light condition + (1/Night) + (1/Site)		Dark – High	0.26	**< 0.001**
		Dark – Low	0.27	**< 0.001**
Light condition	$X^2(2, N = 4251) = 478.29, p =$ **< 0.001**	High - Low	0.00	0.9756
Models: 12 hours resolution	**Statistics**	**Post-hoc**	**Estimate**	***p*-value**
Precision ~ Light condition + (1/Night) + (1/Site)		Dark – High	-0.22	**< 0.001**
Light condition		Dark – Low	-0.20	**< 0.001**

	$X^2(2, N = 309) = 41.34, p =$ **< 0.001**	High - Low	0.02	0.8610
Recall ~ Light condition + (1/Night) + (1/Site)		Dark – High	-0.03	0.5911
		Dark – Low	-0.06	0.1930
Light condition	$X^2(2, N = 302) = 3.05, p = 0.2178$	High - Low	-0.02	0.6949
F1 Score ~ Light condition + (1/Night) + (1/Site)		Dark – High	-0.07	**0.0345**
		Dark – Low	-0.07	**0.0492**
Light condition	$X^2(2, N = 294) = 7.66, p =$ **0.0217**	High - Low	0.00	0.9883
Accuracy ~ Light condition + (1/Night) + (1/Site)		Dark – High	-0.09	**0.0011**
		Dark – Low	-0.10	**0.0006**
Light condition	$X^2(2, N = 327) = 18.16, p =$ **< 0.001**	High - Low	-0.01	0.9866

6. Discussion

Our findings indicated that algorithms based on insect detection models are capable of reliably characterising the nocturnal activity patterns of insects at different temporal resolutions and habitats. However, performance was affected by light conditions. Algorithm-generated and manual arrival-time scores aligned under white visible light, whereas under darker conditions insect detections were overestimated by the algorithm (Fig. 2). The reduced performance of insect detection under dark conditions could be an artefact of inefficient human vision labelling under the infrared LED illumination in dark conditions. The lower mean nightly precision rates in darkness (Fig. 3) may also reflect a training bias due to a greater number of pictures taken under visible light conditions. This suggests a need for dedicated training datasets incorporating infrared imagery. Automating temporal activity monitoring under fully dark conditions holds great potential (Ruczyński *et al.*, 2020; Rydhmer *et al.*, 2022), as it enables tracking of natural insect rhythms without reliance on phototactic behaviours and light sources that can introduce density biases and increase costs.

Validation of the automated approach was based on human visual assessment. However, it is important to recognize that human assessment is not flawless. For instance, inattentional blindness (i.e. humans perceive only attended objects) can introduce additional errors in manual arrival scores (Simons and Chabris, 1999). Such human errors may have limited the validation capacity of our study. Nevertheless, manual validation remains a valuable and interpretable benchmark, as it is the predominant method that automated monitoring aims to replace. In addition to the potential for human error, it should also be noted that manual scoring used for validation in this study was highly time-consuming and labour-intensive, which may render it impractical for many studies.

Different temporal aggregations can also influence the precision of estimated insect activity patterns. At the original 5-minute resolution of our time-lapse photography, agreement between human and algorithmic arrival times was low and more sensitive to ambient light conditions. Performance improved substantially when data were aggregated into blocks longer than 1 hour (Fig. 3, Table S2). This indicates that fine-scale temporal monitoring increases misclassification errors in insect arrival times. For ecological studies, this suggests that a coarser temporal resolution may be more reliable for documenting nightly patterns (for nightly temporal patterns in September 2023, see Fig. S1). Analyses that require finer-scale temporal information may either need larger training datasets or rely more heavily on manual scoring.

In conclusion, we present an algorithm driven workflow for automating the monitoring of insect temporal patterns that balances cost efficiency with precision. While in our study lower image resolution precluded taxonomic analysis, more expensive high-performance cameras could enable the assessment of temporal activity at the level of individual taxa. Our findings highlight that both environmental lighting and temporal resolution influence model performance, but also that automated approaches can substantially reduce labour while maintaining ecological relevance. As insect declines continue globally, scalable monitoring tools will be essential for tracking population dynamics in more locations to adequately inform regional conservation strategies.

7. Funding

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9. Supplementary Information

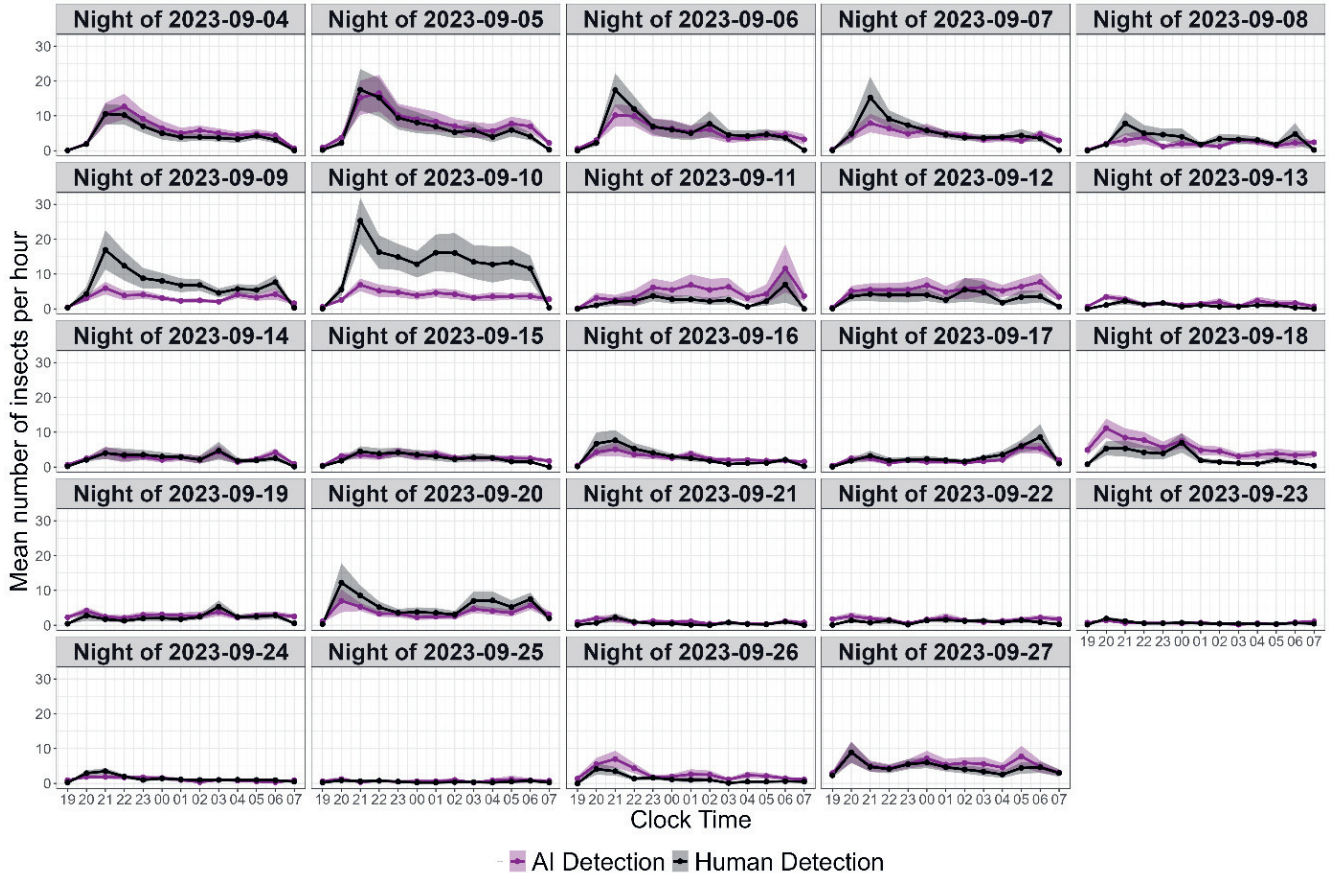


Fig. S1. Human versus AI detected mean number of insects per hour, nightly averaged over study location. Black lines represent the mean arrival of insects per 5-minute intervals (picture frame), nightly averaged over location as scored via human vision, whilst purple lines represent the same as scored via AI based vision. Shaded areas represent the standard error (SE).

$$Precision = \frac{TP}{TP + FP} \qquad [Eq. S1]$$

$$Recall = \frac{TP}{TP + FN} \qquad [Eq. S2]$$

$$F1\ score = \frac{2 * precision * recall}{precision + recall} \qquad [Eq. S3]$$

$$Accuracy = \frac{TP + TN}{TP + TN + FP + FN} \qquad [Eq. S4]$$

Equations for model metrics: precision (Eq. S1), recall (Eq. S2), F1 score (Eq. S3), and Accuracy (Eq. S4).

Table S1. Sticky camera cost per unit.

Description	Company	Price/unit	Link 2025
Infrared camera	Technaxx Deutschland	85 €	https://www.technaxx.de
Rectangular frame	S.B. Weston Ltd, UK	2.5	https://www.westonboxes.com
Metal hook base	Bouwmaat Nederland BV	2	https://www.bouwmaat.nl
Metal hook	Bouwmaat Nederland BV	2	https://www.bouwmaat.nl/
Non toxin adhesive	Hofman Animal Care B.V.	0.25	https://webshop.hofmananimalcare.nl
Metal frame holders	Intergamma B.V.	7	https://www.gamma.nl/
AA rechargeable batteries	Panasonic	15	Several retailers
Total Trap cost (2025)		**114 €**	

Table S2. Confusion matrix and metrics per temporal resolution aggregation. True positives (correct detections), False positives (incorrect detections), False negatives (missed events), and True negatives (correct non-detections), from which Precision (TP/(TP+FP)), Recall (TP/(TP+FN)), F1 Score (harmonic mean of Precision and Recall), and Accuracy ((TP+TN)/(TP+FP+FN+TN)) are computed.

Light condition	True positive	False positive	False negative	True negative	Aggregation
Dark	133	402	168	2840	5 minutes
High	6808	3068	4116	596	5 minutes
Low	2494	1945	2034	949	5 minutes
Total	9435	5415	6318	4385	5 minutes
Dark	218	317	83	91	1 hour
High	7887	1989	3037	3	1 hour
Low	3207	1232	1321	2	1 hour
Total	11312	3538	4441	96	1 hour
Dark	272	263	29	1	12 hours
High	8250	1626	2674	0	12 hours
Low	3488	951	1040	0	12 hours
Total	12010	2840	3743	1	12 hours
Light condition	**Precision**	**Recall**	**F1_Score**	**Accuracy**	**Aggregation**
Dark	0.25	0.44	0.32	0.84	5 minutes
High	0.69	0.62	0.65	0.51	5 minutes
Low	0.56	0.55	0.56	0.46	5 minutes
Total	0.64	0.60	0.62	0.54	5 minutes
Dark	0.41	0.72	0.52	0.44	1 hour
High	0.80	0.72	0.76	0.61	1 hour
Low	0.72	0.71	0.72	0.56	1 hour
Total	0.76	0.72	0.74	0.59	1 hour
Dark	0.51	0.9	0.65	0.48	12 hours
High	0.84	0.76	0.79	0.66	12 hours
Low	0.79	0.77	0.78	0.64	12 hours
Total	0.81	0.76	0.78	0.65	12 hours

7

General discussion





Insects dominate life on Earth, comprising about 80 % of all animal species (Stork, 2018). They sustain countless ecosystem processes: they regulate plant communities through pollination, herbivory and detrivory, and they form a major food source for higher trophic levels (Chowdhury et al., 2023). Yet insects are currently undergoing severe declines in mass, abundance and species richness (Hallmann et al., 2017; Seibold et al., 2019). They are suffering "a death by a thousand cuts", as multiple stressors act simultaneously upon them (Wagner et al., 2021).

Given their central role in ecosystems, there is an urgent need for effective mitigation strategies to halt further insect losses. Artificial light at night (ALAN) is recognised as one of the threats to insect biodiversity (Owens et al., 2020), and ecologically responsible lighting should therefore be part of conservation planning.

Therefore, in this thesis, I explored the impact of ALAN on insects by investigating changes in predator-prey interactions. I assessed how ALAN influences predation pressures, which directly affect insect populations. By relating predation pressure to the spatial and temporal activity patterns of both predators and prey, as well as the prey's anti-predator colouration and behaviour, I aimed to clarify how changes in predation pressure arise.

In the following paragraphs, I summarise our findings by connecting the results from different chapters and I discuss them in the broader context of how ALAN can affect food web interactions. I formulate answers to the research questions brought forward in the introduction and highlight important findings that emerged during the research. I propose directions for insect conservation efforts and end with recommendations for future ALAN research.

Does ALAN affect the activity patterns of predators and prey?

ALAN blurs the line between day and night, reshaping how animals behave after sunset. In Chapters 2–5, we explored how ALAN changes the activity patterns of both diurnal and nocturnal species. We expected clear, opposing responses: extended or increased activity in diurnal species (Frank, 2009; Gomes et al., 2021, 2024; Henke et al., 2022; Maurer et al., 2019; Spoelstra et al., 2018) and delayed or inhibited activity in nocturnal species (Jiang et al., 2023; Rotics et al., 2011; Secondi et al., 2021; Stone et al., 2009; van Geffen et al., 2015). What we found was more nuanced, with responses shaped by species' ecology.

For diurnal predators, ALAN effectively stretches the day into the night. Great tits (*Parus major*) and jumping spiders (*Marpissa muscosa*) both became more active at night when exposed to ALAN (Chapter 3 & 4). However, the conditions under which they responded strongly differed. Great tits already altered their activity patterns at very low

light levels (1.5 lux, 2700 K; Chapter 3), whereas the jumping spiders only responded under much more extreme illumination (100 lux, 5500 K; Chapter 4). This highlights that the threshold for responding to ALAN can differ across taxa.

For diurnal, active hunting spiders there is limited research on the effects of ALAN on activity patterns. Some observational studies report ALAN-aided nocturnal foraging for four jumping spider species: *Menemerus bivittatus, Plexippus paykulli, Platycryptus undatus* and *Sitticus fasciger* (Edwards, 1969; Frank, 2009; Wolff, 1982). However, the specific light levels and spectral compositions in these reports remain unknown, making it difficult to directly compare their responses to our controlled experiments with *M. muscosa*. Our results suggest that the jumping spiders only show altered activity patterns under high levels of ALAN (Chapter 4).

For birds, the literature on ALAN effects is comparatively rich and largely consistent with the patterns seen in our study. Many species start their day earlier or end it later under ALAN (Da Silva et al., 2014; de Jong et al., 2016, 2017). While we did not see an increase in nighttime foraging in our experiment (Chapter 3), nocturnal foraging has been documented for several predominantly diurnal birds, including both insectivores and wader species (Da Silva et al., 2017; Lebbin et al., 2007; Leopold et al., 2010; Leveau, 2020; Russ et al., 2015; Santos et al., 2010). ALAN-induced nocturnal foraging can become especially beneficial when it leads to an increased availability of light-attracted prey (Leopold et al., 2010). In contrast, our laboratory study described in Chapter 3 represented a closed system where the prey availability remained constant, so that we only measured a direct behavioural effect of light.

As expected for nocturnal species, ALAN reduced activity in both cryptic *Mamestra brassicae* caterpillars (Chapter 2) and *Pipistrellus* bats (Chapter 5), though each responded in different ways. The pipistrelle bats redirected their foraging efforts towards darker areas, so that the ALAN effect was spatial rather than temporal. The *M. brassicae* caterpillars became more inactive overall when exposed to ALAN. However, unlike the bats, the caterpillars were confined to the illuminated environment and could not escape to genuinely darker habitats, only to partially sheltered areas beneath leaves. It is possible that caterpillars also show spatial avoidance of lit areas under more natural conditions.

Bat emergence times are strongly influenced by predator risk and prey availability, both of which vary with ambient light conditions (Jones & Rydell, 1994; Thomas & Jacobs, 2013). ALAN has been shown to delay bat emergence time (Stone et al., 2009). However, in our study (Chapter 5), the bats had already emerged and commuted to their foraging grounds. During foraging, bats likely mainly track the temporal patterns of available insects. Under ALAN conditions, pipistrelle bats were found to shift from a

bimodal pattern to all-night foraging, correlated with increased insect presence throughout the night (Hermans et al. (2024). Intriguingly, this was not what we observed. In our study, night-flying insects shifted their temporal patterns under ALAN, but bats did not follow these shifts (Chapter 5).

In this field experiment, flying insects showed an earlier median activity in the second half of the night under lit conditions, meaning they were more active towards the middle of the night (Chapter 5). Generally, insects are most attracted to light when there is a strong contrast between the light source and the surrounding natural light. This contrast is greatest during the darkest part of the night, between the twilight periods. As a result, with the introduction of ALAN, insect activity may become even more concentrated in these darkest hours. Alternatively, this shift in timing could be explained by ALAN altering when insects perceive twilight. By increasing ambient light levels, ALAN may temporarily shift the light-intensity threshold that (de)activates insects (Gomes et al., 2024). If insects in ALAN-impacted areas perceived dawn to occur earlier, they might shift their second activity peak to earlier hours. If so, we would also expect dusk to be perceived later, delaying the first activity peak. This pattern was not evident in our data, although our ability to detect it may have been limited by the low number of insects caught under dark conditions.

While some of our results aligned with predictions for how diurnal and nocturnal species respond to ALAN, not all species were affected as anticipated. In particular, caterpillars exhibited unexpected responses. The predominantly diurnal, aposematic *Pieris brassicae* caterpillars showed no detectable change in activity in response to ALAN, whereas cryptic, nocturnal *Pieris napi* caterpillars increased their nocturnal movement and daytime feeding under ALAN (Chapter 2). Caterpillar activity patterns are not well documented, especially in relation to ALAN (Niepoth et al., 2018). While some species show clear diurnal or nocturnal rhythms, many are active both during the day and at night (Haynes et al., 2023; Heinrich, 1979; Niepoth et al., 2018). In our study, all caterpillar species were significantly more active in either the day or night, but their activity never ceased completely in either phase. This lack of strict separation in circadian activity may help explain why caterpillar responses to ALAN did not always match our expected responses. Furthermore, caterpillar behaviours are not only determined by light, but are also mediated by plant volatiles, which respond to light as well (Shiojiri et al., 2006). This thus adds an extra layer of complexity. In our experiments where we used living plants (Chapter 2), such plant responses are likely included. However, we did not measure plant volatiles, so we can only speculate as to what factors changed caterpillar behaviour under ALAN.

ALAN can have several fitness effects in nocturnal as well as in diurnal species. In nocturnal species, ALAN may reduce the time available for feeding and mating,

because ALAN can restrict their activity (van Geffen et al., 2015; van Langevelde et al., 2017). In contrast, in diurnal species, ALAN may give a larger time window to feed and perform other essential behaviours, which may thus increase their fitness. However, increased activity can have negative consequences, as such activities can also increase exposure to predators (Henke et al., 2022). Moreover, deleterious physiological effects may arise from ALAN-induced sleep disruption (Cirelli & Tononi, 2008) or from the chronic exposure to ALAN itself (Petrović et al., 2025; Schumann et al., 2025). Consistent with this idea, species that prolong their foraging activity under ALAN often show no improvement in body condition, suggesting that extended activity may come with trade-offs (Haynes et al., 2023; Russ et al., 2015; Taylor et al., 2022). Thus, ALAN-induced changes in activity patterns of predators and prey can change food web interactions, whereby winners and losers in the predatory arms race are unpredictable.

Does ALAN affect the spatial distributions of prey and predators?

In general, the way prey and predator species responded spatially to ALAN appeared to depend largely on perceived predation risk, which in turn is shaped by species traits such as escape skills and anti-predator colouration (Chapter 2,4,5). While cryptic species benefit from minimal movement and hiding, aposematic species can benefit from increased exposure (Ioannou & Krause, 2009; Nielsen & Mappes, 2020). In contrast, in many flying insects the attractive force of light seems to be an innate response that overrules the detrimental predation risk (Chapter 5).

In our laboratory experiments, caterpillars and spiders could not escape the light but could only seek shelter from it (Chapter 2 & 4). Under these conditions, ALAN increased hiding behaviour in cryptic species, including nocturnal *M. brassicae* caterpillars and diurnal *M. muscosa* jumping spiders (Chapters 2 & 4). Although we examined *M. muscosa* primarily as a predator, this spider is itself also prey for other species. As light makes cryptic species more conspicuous, hiding is likely their safest strategy. Increased sheltering under lit conditions has also been found in other palatable species (Dissegna & Chiandetti, 2025; Kenyon et al., 1995; indirect light response: Shiojiri et al., 2006). The only exception to these patterns was the cryptic, nocturnal *P. napi*. This species did not exhibit any spatial response, it only increased its movement under ALAN conditions (Chapter 2). For aposematic prey species, responses to ALAN were expected to be different, because exposure brings out their conspicuous warning signals that reduce predation risk (Nielsen & Mappes, 2020). In our laboratory experiment, diurnal *P. brassicae* caterpillars were indeed found more often on top of leaves under ALAN (Chapter 2).

In our urban field experiment, bats and flying insects were free to move and could either approach or avoid illuminated areas (Chapter 5). In this field experiment, pipistrelle bats preferred dark foraging grounds within urban environments, regardless of light dimming. These fast-flying, agile bats are known to exploit light-attracted insects around streetlights (Hermans et al., 2024; Spoelstra et al., 2017). However, pipistrelles do avoid illuminated areas during other activities, such as drinking and flying, suggesting that illumination carries costs (Hale et al., 2015; Russo et al., 2017). Although increased prey availability at lit sites can outweigh these costs during foraging, our results suggest that in extensively illuminated urban environments, this benefit–risk balance is less favourable. When lights are widespread, escaping visually hunting predators becomes more difficult because dark refuges are scarce. At the same time, prey aggregation around individual light sources may be reduced: urban insect populations show diminished flight-to-light behaviour (Altermatt & Ebert, 2016), and the insects that remain light-attracted are spread across many different light sources. Thus, even light-opportunistic species appear to reduce foraging in illuminated areas, suggesting that the benefit–risk ratio of feeding in the light is increasingly skewed towards risk in highly lit urban environments.

In our urban field experiment, we also found that insects remained attracted to light, especially at undimmed streetlights in urban environments, in contrast to the bats (Chapter 5). This persistent flight-to-light behaviour in insects is associated with negative fitness costs and negative insect population trends (Storms et al., 2025; van Langevelde et al., 2018; Wakefield et al., 2015). The detrimental impact of flight-to-light behaviour is further supported by evidence of evolutionary changes in urban moth populations, which show reduced flight-to-light responses compared to rural populations (Altermatt & Ebert, 2016; Van de Schoot et al., 2024). Thus, the attraction of insects to ALAN is a response to a misleading cue, which inadvertently traps them within an unfavourable light sphere (Fabian et al., 2024; Truxa & Fiedler, 2012).

Does the impact of ALAN depend on the visual ecology of species?

Visual acuity and colour sensitivity can vary significantly between species (Caves et al., 2024; Veilleux & Kirk, 2014), so that different light intensities or spectra can have varying impacts across taxa. Moreover, prey conspicuousness influences how effective light is for predator foraging (Medina et al., 2025). Cryptic species benefit from reduced conspicuousness to predators (Heinrich, 1979; Ioannou & Krause, 2009), whereas aposematic species gain from clearly displaying their warning signals (Nielsen & Mappes, 2020) and are generally less vulnerable to visually hunting predators (Aslam et al., 2020). However, if light levels are too dim for visual predators to distinguish warning colouration, the increased exposure of aposematic species may become

maladaptive. Similarly, the predation risk for cryptic species may increase if they fail to hide, as ALAN can make them more conspicuous.

Predator visual acuity is central to how ALAN affects predation. Great tits began their activity earlier in the day under ALAN, but did not spend their time foraging on caterpillars (Chapter 3). This suggests that these birds may be visually limited and unable to hunt effectively under low light conditions (1.5 lux, 2700 K). Visual limitations could impair the birds' ability to distinguish cryptic caterpillars from their background, thereby keeping foraging opportunities low under dim ALAN. Indeed, Kacelnik (1979) reported that light intensities below roughly 7 lux were too dim for great tits to efficiently forage on cryptic dummy caterpillars.

For the jumping spiders (*M. muscosa*), activity did not increase at 10 lux (Chapter 4), suggesting that this light level is already insufficient for effective hunting, although we cannot rule out that this response is driven by their internal clock rather than visual constraints. For two other jumping spiders of the genus *Cyrba,* 1.35 lux was found to be enough to discriminate dummy prey (Cerveira et al., 2019). Nevertheless, prey detection does not necessarily imply that the predators can successfully attack prey (Forster, 1982).

In addition to predator visual acuity, prey conspicuousness is also critical in determining the effects of ALAN. To investigate how predators respond to cryptic and aposematic prey under ALAN conditions, we conducted field experiments with clay caterpillars (Chapter 3). By using differently coloured clay models, we found no evidence of differential predation rates on aposematic or cryptic caterpillar models under ALAN conditions. We had expected that cryptic caterpillars would be more vulnerable to predation under increased illumination (Medina et al., 2025). However, a dummy predation experiment cannot account for potential behavioural changes in real caterpillars of both anti-predator colour types (Chapter 2). This is an important factor, caterpillar behaviour can directly influence the effectiveness of their colouration.

Taken together, these findings show that the impact of ALAN on predator–prey interactions cannot be predicted from the light regime alone. Instead, predation outcomes emerge from the interplay between the lighting conditions, predator visual constraints, and prey colouration and behaviour.

Does ALAN alter predation pressures?

ALAN can alter predation dynamics by enabling diurnal predators to extend or intensify their foraging into nighttime hours (Frank, 2009; Maurer et al., 2019; Russ et al., 2015), At the same time, nocturnal predators may be constrained in their ability to forage under ALAN (Secondi et al., 2021; Stone et al., 2009). In Chapters 3–5, I examined how

ALAN shapes predation by a diurnal songbird, a diurnal hunting spider, nocturnal bats, and a mixed community of diurnal and nocturnal predators. I found that predators responded differently, and that their responses were often more complex than a simple nocturnal–diurnal distinction.

When looking at the diurnal species, we indeed found that diurnal *M. muscosa* jumping spiders clearly increased their predation under ALAN (Chapter 4). Previous work on passive and active hunting spiders have shown similar foraging opportunities under ALAN (Frank, 2009; Willmott et al., 2019). These increased foraging opportunities are likely due to a combination of factors such as increased activity, enhanced visual capacity, and greater prey availability (Cerveira et al., 2019; Frank, 2009; Perkin et al., 2014). However, not all diurnal predators increased their nocturnal foraging. I found that diurnal great tits advanced the onset of their general activity pattern under ALAN conditions, while their foraging behaviour on cryptic caterpillars did not change (Chapter 3). One plausible explanation is that the birds are constrained by their visual performance, as I explain above.

When focusing on nocturnal species, we found that urban pipistrelle bats reduced their foraging under streetlights, irrespective of whether lights were dimmed or not (Chapter 5). Like many nocturnal species, bats are often light-averse, likely because light makes them more visible to their own predators (Rydell, 1992; Spoelstra et al., 2017). Still, some bat species are light-opportunistic (Rydell, 1992; Spoelstra et al., 2017). How bats respond to light is largely dependent on their traits and foraging strategies, as these determine predation risk (Voigt et al., 2021). For example, fast-flying pipistrelles are light-opportunistic and often found to exploit insects underneath streetlights (Blake et al., 1994; Hermans et al., 2024). The fact that we found reduced foraging activity of these bats may be due to the omnipresence of light in urban environments, which may have simultaneously increased risk and decreased the benefit of foraging under streetlights. Our contrasting finding thus underlines that ALAN's effects are not only species-specific, but also context dependent.

Our outdoor dummy predation experiment involving a community of both diurnal and nocturnal predators also revealed no effect of ALAN on predation (Chapter 3). Since these experiments were conducted at the Light on Nature sites, which are forest plots that have experienced more than a decade of light exposure (Light on Nature; Spoelstra et al., 2015), our results could possibly be explained by long-term effects of ALAN on predator community assemblages, as the loss of light-sensitive species may have knock-on effects on predation. Alternatively, the contrasting foraging responses of diurnal and nocturnal predators may have balanced each other out in the area.

Taken together, our variable outcomes on the effects of ALAN on predation pressures in Chapters 3–5 mirror the variable results reported in the literature (Amoroso, 2024; Eckhartt & Ruxton, 2022; Grenis et al., 2015; Jackson et al., 2025; Mcmunn et al., 2019; Miller et al., 2017; Nuñez et al., 2021). Rather than exerting a uniform directional effect, ALAN interacts with the sensory limits of predators, the traits and behavioural strategies of predators and prey, and the surrounding lightscape. The result is a mosaic of increased, decreased, or unchanged predation pressures, all of which together can highly reshape existing food webs.

ALAN affects urban and rural ecosystems differently

Urban habitats can be viewed as novel ecosystems, exposing organisms to distinct and often extreme pressures, with omnipresent artificial light likely being one of the most pervasive. Only a few common-garden experiments have compared the responses between urban and rural populations to ALAN. These studies suggest that urban populations can evolve distinct behaviours to cope with extreme urban lightscapes. For example, urban populations of the common moth *Yponomeuta cagnagella* (Yponomeutidae) show reduced flight-to-light behaviour compared to rural populations (Altermatt & Ebert, 2016), likely linked to their smaller wings and therefore reduced mobility (Van de Schoot et al., 2024). In contrast, urban populations of the spider *Steatoda triangulosa* (Theridiidae) exhibit reduced light avoidance compared to rural spider populations (Czaczkes et al., 2018).

One of our most unexpected findings was that the urban pipistrelle bats in our study behaved differently from what we know of pipistrelles studied in less urbanised areas (Chapter 5). Although pipistrelles are typically considered light-opportunistic species (Hermans et al., 2024; Spoelstra et al., 2017), we found that our urban populations preferred to forage in darkness.

As behavioural and genetic responses can differ markedly between rural and urban environments, it is important to compare rural and urban populations in the context of ALAN. Traditionally, ALAN research has focused more on natural than urban settings, partly because it is challenging to disentangle the effects of artificial light from other co-occurring stressors in cities (Halfwerk & Jerem, 2021; Liu et al., 2024). However, with the emergence of adjustable, smart lighting systems, we can now experimentally manipulate light intensity and spectrum within urban areas while keeping other factors constant, as demonstrated in Chapter 5. This opens new possibilities for understanding how urban populations respond to ALAN.

Automated monitoring holds great opportunities, but should be used with caution

Monitoring species in nature is generally labour-intensive and time-consuming, especially when data needs to be collected across large spatial and temporal scales. We experienced these human limitations first hand in Chapter 5 and started adopting machine learning methods to speed up the process. Machine learning is an advancing field within ecological research (Cipriano et al., 2025). Incorporating these methods into our research is highly valuable, especially given current ecological crises that demand faster processing and analysis of biological data. At the same time, it remains essential to be aware of their limitations, to validate automated outputs, and to continue improving these methods as the technology advances.

In Chapter 6, we trained an openly available object detection model, YOLOv8 (Jocher et al., 2023), on picture data from sticky sheets that trap insects and validated its performance against the manually collected dataset from Chapter 5. In this way, we showed that automated monitoring can greatly accelerate data collection, while also being user-friendly and cost-effective. In Chapter 4, we used the same YOLOv8 framework, but in a different context, i.e. to measure spider activity which underscores the flexibility and adaptability of this method.

Machine learning methods also have limitations. For automated object detection and tracking, it is important to carefully consider light conditions during study design; model performance was lower under dark conditions than under (un)dimmed light (Chapter 6). In addition, it is important to determine the desired temporal resolution before the start of the experiment. When higher temporal resolution is required, it is important to note that model performance may decrease, as objects are not always detected immediately and lighting effects become more pronounced.

Recommendations for insect conservation

To counteract current insect declines, it is important to mitigate the threats insects face, including ALAN. This requires a critical reassessment of our current lighting infrastructure (Owens et al., 2020).

To date, much of the research and mitigation efforts on the impact of ALAN has focused on street lighting (see also Chapter 3, 5 and 6). In this thesis, I show that dimming streetlights can help mitigate negative impacts, but also that even relatively low light levels can still alter the behaviour of insects and their predators. Since streetlights are widespread and publicly regulated, they present great opportunities to reduce the ecological impact of artificial lighting. However, our efforts must extend beyond streetlights, as many other light sources contribute to overall light pollution (Walczak

et al., 2023). In particular, private and commercial lighting is less regulated and can reach more extreme values in both intensity and colour spectrum.

Broad-spectrum lights with cool colour temperatures, such as the 5500 K used in two of our laboratory studies (Chapters 2 and 4), are more typically employed for applications such as sports field lighting, terrain lighting, garden lights, and monument illumination. Our experiments show that both caterpillars and spiders alter their behaviours under such cold white lighting. The increased herbivory and predation that we observed illustrate the direct impact of these ALAN conditions on the food web.

My recommendation is therefore to regulate lighting in public, private and commercial areas towards warmer light colours and dim lights to the lowest feasible levels. In addition, I advocate phasing out the most extreme lighting setups found in non-streetlight outdoor applications, unless their necessity can be convincingly demonstrated. However, these measures alone are not enough. Since our results show that disturbances of both predator and prey behaviours occurred even under dim conditions and warmer light colour temperatures, the primary goal should be to preserve and restore darkness, both inside and outside urban areas.

My recommendations are not new, but build further upon previous research and align with existing guidelines, such as "The Five Principles for Responsible Outdoor Lighting" (International Dark-Sky Association, 2024). These principles promote using light only when necessary, employing dim and warm-coloured lighting, limiting light spill to non-target areas, and restricting lighting to periods of actual use. Although more research is needed to evaluate novel lighting technologies in this rapidly changing field, there is already ample consensus within ALAN research to justify implementing these major guidelines now.

A key advantage of measures such as dimming lights and reducing unnecessary lighting is that they not only benefit the environment, but also save money by lowering energy use. The main barrier of turning to darkness is the strong perceived link between light and safety (Boomsma & Steg, 2014; Peña-García et al., 2015). However, evidence that light improves safety is weak (Gaston et al., 2015; Steinbach et al., 2015). Nevertheless, the feeling of safety is socially and politically important. Therefore, I advocate for efforts to disconnect safety perception from light presence. This challenging task requires translating scientific findings into compelling storytelling, implementing factual safety improvements, and fostering societal transformation.

Suggestions for future research

This thesis was primarily designed to investigate how ALAN could impact insect populations through altered predation pressures, while providing first insights into

potential patterns and pathways underlying these effects. To further disentangle the underlying mechanisms of how ALAN impacts predator-prey interactions, I recommend a more detailed examination of predators' visual capacities under different ALAN conditions. In addition, incorporating long-term studies that consider life-history traits and population dynamics within the predator–prey framework will provide a more comprehensive understanding of the ecological consequences of ALAN. Moreover, distinguishing between plastic and genetic responses to ALAN, particularly when comparing urban and rural populations, will yield important insights into the evolutionary dimensions of species' responses to ALAN.

As we move rapidly toward what has been termed the Sixth Mass Extinction (Cowie et al., 2022; Kolbert, 2014; Wake & Vredenburg, 2008), we unfortunately lack the luxury of unlimited time for exhaustive research. Identifying key traits that determine species' responses to ALAN will help us predict impacts across broader taxonomic groups rather than focusing solely on individual species (Spoelstra et al., 2017; van Langevelde et al., 2018; Chapter 2). Integration of machine-learning methods can accelerate this research by enabling the efficient processing of substantially larger datasets (Chapter 6). Evolving technologies also offer new opportunities; for example, tracking caterpillar feeding activity on live plants (Chapter 2) can now be done more efficiently using acoustic monitoring with the Insect Eavesdropper™ (Bick Lab, University of Wisconsin-Madison). These tools greatly enhance temporal resolution and allow us to address more detailed ecological questions.

Technological innovations within the lighting industry are essential to ensure that new light sources have minimal ecological impacts. Close collaboration with lighting designers and municipalities can place ecologists at the foundation of new developments, guiding innovation toward biodiversity-friendly solutions. Early-stage engagement is also essential to prevent the widespread adoption of lighting systems that could subsequently be found environmentally harmful.

Societal support is maybe even more important to develop more ecologically friendly lighting solutions in public spaces. I therefore advocate for an interdisciplinary approach that integrates ecological and social assessments. Such an approach would evaluate not only how well lighting technologies mitigate ecological impacts, but also how they are perceived in terms of safety, comfort, and suitability by residents and other stakeholders. This dual perspective is essential to ensure that ecologically responsible lighting solutions are both effective and publicly accepted.

Conclusion

The chapters in this thesis collectively show that species responses to ALAN are highly diverse. There is no single, definitive answer to whether ALAN increases or decreases

predation pressure on insects. Instead, outcomes emerge from a complex interplay of temporal, spatial, behavioural, and visual changes. These interactions generate strongly context-dependent and species-specific responses. Crucially, our variable results do not indicate a lack of impact. On the contrary, it is precisely these heterogeneous responses among species that have the potential to profoundly reshuffle food webs. Future research should distinguish more explicitly between urban and rural contexts and identify key functional traits within and between species that help predict responses to ALAN.

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Summary

Artificial light at night (ALAN) currently affects at least 23% of the Earth's land surface. ALAN strongly changes the natural night environment, obscuring light–dark cycles and celestial cues, with far-reaching consequences for animals and plants. This happens because plants and animals use these celestial cues to orient themselves and follow the light–dark cycle to time their internal processes and behaviours. Since ALAN disrupts these natural signals, ALAN can affect populations and community interactions. ALAN is now seen as one of the major drivers of the alarming insect declines observed today.

In this thesis, I investigated how ALAN may contribute to insect declines by altering predator-prey interactions. I assessed how ALAN affects predation pressures by both vertebrate and invertebrate predators. In addition to predation pressures, I examined how predator and prey activities varied in both space (where) and time (when), and how these changes in activities relate to the prey's anti-predator colouration and behaviours.

In **Chapter 2**, I first examined the effects of dim light pollution (1.5 lux, 5500K) on caterpillar behaviour in the absence of predators. Specifically, I tested larval behaviours in three lepidopteran species differing in larval anti-predator colouration and adult activity period: *Mamestra brassicae* (Noctuidae; cryptic, nocturnal), *Pieris napi* (Pieridae; cryptic, diurnal), and *Pieris brassicae (*Pieridae; aposematic, diurnal).

As larval activity patterns are poorly studied, I first tested whether larval activity patterns coincide with adult activity patterns. I found that *M. brassicae* and *P. brassicae* larval activity patterns matched their adult activity patterns, while activity patterns of the cryptic *P. napi* larvae were mostly nocturnal and thus did not match their diurnal adult activity pattern. These results suggest that larval activity is more closely tied to larval colouration than to adult activity period, as both cryptic species were primarily nocturnal, while the aposematic species was diurnal.

By observing the three species on live plants over a full 24-hour cycle in dark and ALAN conditions, I found that all species were affected by ALAN, but in species-specific ways. Under dim ALAN conditions, the aposematic, diurnal *P. brassicae* caterpillars exposed themselves more on top of the leaves, whereas the cryptic, nocturnal *M. brassicae* caterpillars hid more underneath the leaves. These opposing behaviours can be linked to their anti-predator colours. Moreover, *M. brassicae* rested more under ALAN, consistent with expectations for a nocturnal, cryptic species. In contrast, the cryptic, nocturnal *P. napi* caterpillars responded in a more puzzling way: under ALAN, larvae moved more at night and fed more during the day, a pattern that does not align with expectations for a cryptic, nocturnal species. In a complementary experiment, I measured feeding activity on differently orientated leaf disks. The caterpillars showed a general preference for feeding on the upper leaf surface, supporting the interpretation

that *M. brassicae* uses the lower side primarily to hide. This experiment also revealed an overall increase in nocturnal feeding under dim light in all three species, a pattern that I did not detect in the on-plant experiment. Together, these results demonstrate that even dim ALAN can alter caterpillar behaviour in ways that likely affect their fitness and exposure to predators.

To test the consequences for predation risk, in **Chapter 3** I focused on *M. brassicae*, which showed the strongest behavioural shifts. In this chapter, I examined the effect of ALAN on predation pressure in two complementary experiments. To assess whether changes in predator and prey behaviour translate into changes in predation rates, we conducted an indoor experiment in which *M. brassicae* caterpillars were exposed to great tits (*Parus major,* Paridae) under either dark night conditions or dim ALAN (1.5 lux, 2700 K). I found that even though great tits were more active at night and started their activity earlier under ALAN, their predation rates on caterpillars did not increase. Possibly, the light level was too low for the tits to efficiently forage. Caterpillar feeding and hiding behaviour did not differ between light treatments, although larvae hid more in response to predator presence, which likely also affected the bird's foraging efficiency. In addition, to determine the role of anti-predator colouration in prey caterpillars, we quantified community-wide predation rates on cryptic and aposematic caterpillar models in the long-term Light on Nature sites, which consist of illuminated (7.6 lux, 2700 K) and dark forest transects. I found that predation rates on both cryptic and aposematic models did not differ between illuminated and dark transects. Possibly, long-term light exposure may diminish short-term predation increases that are generally found under ALAN. Overall, our results show that changes in predator activity in response to ALAN may not result in changes in predation rates and suggest that long-term light exposure may not increase predation rates as seen in studies with short-term illumination.

In **Chapter 4**, I examined whether nocturnal light of varying intensities and spectra (10 lux, 5500 K; 100 lux, 5500 K; 100 lux, 2700 K) affect predator behaviours. I focused on a visually oriented diurnal arthropod predator, the jumping spider *Marpissa muscosa* (Salticidae). I found that *M. muscosa* extends its activity into the night only under high-intensity, cold white light (100 lux, 5500 K). I also found that *M. muscosa* effectively forages under this nocturnal light condition, while bright, warm white light (100 lux, 2700 K) increased its overall sheltering behaviour. These findings suggest that *M. muscosa* is sensitive to ALAN, but that effects may be restricted to areas close to light sources or to high-intensity lights.

In **Chapter 5**, we assessed whether dimming urban streetlights is an effective mitigation strategy to reduce ALAN impacts on flying insects and *Pipistrellus spp.* (Vespertilionidae) bats by monitoring their activity over one month under dimmed (4.4 lux, 3000 K) and undimmed (17 lux, 3000 K) streetlights and at dark locations in two Dutch cities. Dimming streetlights effectively reduced insect attraction, although

insect densities remained higher than in dark areas and insect activities throughout the night still showed slight shifts in lit conditions. Unexpectedly, pipistrelle bats did not react to light dimming and their activity patterns throughout the night were generally unaffected by light. This suggests that urban pipistrelle bats differ in their response to light than bats in less urbanised landscapes, where pipistrelles often exploit high insect densities around streetlights. Together, our results indicate that dimming urban streetlights reduces insect attraction, which thus benefits insect populations, and thereby bats, over longer timescales. Further, our findings also underscore that darkness is crucial not only in natural, but also in urban habitats.

Finally, to try to streamline insect monitoring by applying AI methods, in **Chapter 6** we customised an open-source object detection model. Using the insect data collected in **Chapter 5**, we validated the model by comparing manually scored detections with the model's automated detections, and assessed how ambient light conditions and temporal resolution influenced model performance. We found that automated detection greatly speeds up the detection of insects, although insect detection was less effective in darkness. We also found a decline in model performance and an increased sensitivity to illumination when the model was used over shorter time intervals. These findings indicate that automatisation processes can be a great help to lower the manual labour of ecological insect monitoring and increase data processing speed. However, these methods should be applied with caution, whereby selecting the study design and objectives are crucial

Samenvatting

Nachtelijk kunstlicht beïnvloedt inmiddels minstens 23% van het landoppervlak op aarde. Lichtvervuiling verandert het nachtelijke landschap ingrijpend: het vervaagt de licht-donker cyclus en maakt hemellichamen minder zichtbaar, wat grote gevolgen heeft voor dieren en planten. Zij gebruiken deze natuurlijke signalen immers om zich te oriënteren en hun gedrag en interne processen af te stemmen. Door deze verstoring kunnen hele populaties en gemeenschappen worden beïnvloed. Lichtvervuiling wordt tegenwoordig gezien als een belangrijke oorzaak van de wereldwijde achteruitgang van insecten.

In dit proefschrift onderzocht ik hoe lichtvervuiling, via verstoring van predator-prooirelaties, kan bijdragen aan de insectenafname. Ik keek naar de invloed van nachtelijk kunstlicht op predatiedruk door zowel gewervelde als ongewervelde predatoren. Daarnaast onderzocht ik hoe de activiteiten van predatoren en prooien varieerden in ruimte (waar) en tijd (wanneer), en hoe deze veranderingen samenhangen met de anti-predator kleur en het gedrag van de prooi.

In **Hoofdstuk 2** onderzocht ik eerst de effecten van zwak kunstlicht (1,5 lux, 5500K) op het gedrag van rupsen zonder aanwezigheid van predatoren. Ik testte het gedrag van rupsen van drie vlindersoorten die verschillen in anti-predator kleur en activiteit van de volwassen vlinders: *Mamestra brassicae* (cryptisch, nachtactief), *Pieris napi* (cryptisch, dagactief) en *Pieris brassicae* (aposematisch, dagactief).

Omdat er weinig bekend is over de activiteitspatronen van rupsen, onderzocht ik of deze overeenkomen met die van de volwassen vlinders. De activiteit van *M. brassicae* en *P. brassicae* rupsen kwam overeen met die van de adulten, terwijl de cryptische *P. napi* rupsen vooral 's nachts actief waren, wat niet overeenkwam met het dagactieve patroon van de adulten. Dit suggereert dat het activiteitspatroon van rupsen meer samenhangt met hun kleur dan met het activiteitspatroon van de adulten.

Door de drie soorten gedurende een volledige 24-uurs cyclus op levende planten te observeren onder donkere en verlichte nachtelijke condities, bleek dat alle soorten werden beïnvloed door kunstlicht, maar elk op een eigen manier. Onder zwak kunstlicht lieten de aposematische, dagactieve *P. brassicae* rupsen zich meer zien bovenop de bladeren, terwijl de cryptische, nachtactieve *M. brassicae* rupsen zich juist meer onder de bladeren verstopten. Deze tegengestelde gedragingen zijn te koppelen aan hun anti-predator kleuren. Bovendien rustte *M. brassicae* meer wanneer de nacht verlicht werd, wat past bij een cryptische, nachtactieve soort. De cryptische, nachtactieve *P. napi* rupsen reageerden juist onverwacht: onder kunstlicht bewogen ze meer 's nachts en aten ze meer overdag. In een aanvullend experiment heb ik de

eetactiviteit op verschillend georiënteerde bladschijfjes gemeten. De rupsen hadden een duidelijke voorkeur om te eten aan de bovenkant van het blad, wat ondersteunt dat *M. brassicae* de onderkant vooral gebruikt om zich te verstoppen. Ook nam het nachtelijke eetgedrag bij alle drie de soorten toe onder zwak kunstlicht, een patroon dat ik niet vond in het experiment met rupsen op de planten. Samen laten deze resultaten zien dat zelfs zwak nachtelijk kunstlicht het gedrag van rupsen kan veranderen op manieren die waarschijnlijk hun fitness en blootstelling aan predatoren beïnvloeden.

Om de gevolgen voor predatierisico te testen, heb ik me in **Hoofdstuk 3** gericht op *M. brassicae*, die de sterkste gedragsveranderingen liet zien. In dit hoofdstuk onderzocht ik het effect van lichtvervuiling op predatiedruk in twee complementaire experimenten. Om te beoordelen of veranderingen in het gedrag van predatoren en prooien leiden tot veranderingen in predatie, voerden we een indoor experiment uit waarin *M. brassicae* rupsen werden blootgesteld aan koolmezen (*Parus major*) onder donkere of zwak verlichte nachtcondities (1,5 lux, 2700 K). Ik vond dat koolmezen in verlichte nachten actiever waren en hun activiteit eerder begonnen, maar dat hun predatie op rupsen niet toenam. Mogelijk was het lichtniveau te laag voor de koolmezen om efficiënt te foerageren. Het eet- en verstopgedrag van de rupsen verschilde niet tussen de lichtbehandelingen, hoewel larven zich meer verstopten in aanwezigheid van predatoren, wat waarschijnlijk ook de foerageerefficiëntie van de vogel beïnvloedde. Om vervolgens ook de rol van anti-predator kleur te bepalen, hebben we predatie op cryptische en aposematische kleien rupsen gemeten in de langlopende Licht op Natuur locaties, bestaande uit verlichte (7.6 lux, 2700 K) en donkere bostransecten. Ik vond dat de predatie op zowel cryptische als aposematische modellen niet verschilde tussen verlichte en donkere transecten. Mogelijk vermindert langdurige blootstelling aan licht de kortstondige toename van predatie die doorgaans wordt gevonden onder verlichting. Onze resultaten laten zien dat veranderingen in predatoractiviteit als reactie op kunstlicht niet altijd leiden tot veranderingen in predatie, en suggereren ze dat langdurige blootstelling aan licht predatie niet verder verhoogt zoals gezien wordt in studies met kortdurende verlichting.

In **Hoofdstuk 4** onderzocht ik of nachtelijk licht van verschillende intensiteiten en spectra (10 lux, 5500 K; 100 lux, 5500 K; 100 lux, 2700 K) het gedrag van predatoren beïnvloedt. Ik richtte me op een visueel georiënteerde, dagactieve geleedpotige predator, de springspin *Marpissa muscosa*. Ik vond dat *M. muscosa* haar activiteit alleen uitbreidt naar de nacht onder fel, koud wit licht (100 lux, 5500 K). Ook bleek dat *M. muscosa* effectief foerageert onder deze nachtelijke lichtconditie, terwijl fel, warm wit licht (100 lux, 2700 K) haar schuilgedrag juist vergrootte. Deze bevindingen suggereren dat *M. muscosa* gevoelig is voor lichtvervuiling, maar dat de effecten

beperkt zouden kunnen zijn tot gebieden dicht bij lichtbronnen of bij hoge lichtintensiteit.

In **Hoofdstuk 5** onderzochten we of het dimmen van straatverlichting een effectieve mitigatiestrategie is om de impact van kunstlicht op vliegende insecten en dwergvleermuizen te verminderen. We monitorden hun activiteit gedurende een maand onder gedimde (4.4 lux, 3000 K) en niet-gedimde (17 lux, 3000 K) straatverlichting en op donkere locaties in twee Nederlandse steden. Dimmen van straatverlichting verminderde de aantrekkingskracht op insecten, hoewel de insectendichtheid toch nog steeds hoger bleef dan in donkere gebieden en de activiteit van insecten gedurende de nacht ook enigszins verstoord was in verlichte omstandigheden. Verrassend genoeg reageerden dwergvleermuizen niet op het dimmen van het licht en hun activiteitspatronen gedurende de nacht werden niet beïnvloed door de straatverlichting. Dit suggereert dat stedelijke dwergvleermuizen anders reageren op licht dan vleermuizen in minder stedelijke gebieden, waar ze vaak profiteren van hoge insectendichtheid rond lantaarnpalen. Onze resultaten tonen aan dat het dimmen van straatverlichting in steden insectenaantrekking vermindert, wat insectenpopulaties ten goede komt en op termijn ook vleermuizenpopulaties kan ondersteunen. Daarnaast onderstrepen onze bevindingen dat duisternis niet alleen in natuurlijke, maar ook in stedelijke habitats van cruciaal belang is.

Tot slot, om insectenmonitoring te stroomlijnen met AI-methoden, hebben we in **Hoofdstuk 6** een open-source objectdetectiemodel gemodificeerd voor onze doeleinden. Met de insectendata uit **Hoofdstuk 5** valideerden we het model door handmatig gescoorde detecties te vergelijken met de automatische detecties van het model, en onderzochten we hoe omgevingslicht en temporele resolutie de modelprestaties beïnvloeden. We vonden dat automatische detectie het tellen van insecten aanzienlijk versnelt, hoewel detectie minder effectief was in het donker. Daarnaast bleek dat, bij toepassing op kortere tijdsintervallen, de modelprestaties verminderden en de invloed van licht toenam. Deze bevindingen laten zien dat automatisering een grote hulp kan zijn om de handmatige arbeid bij ecologische insectenmonitoring te verminderen en de snelheid van dataverwerking te verhogen. Wel moeten deze methoden met voorzichtigheid worden toegepast, waarbij het studieontwerp en de doelstellingen van de studie cruciaal zijn.

Author contributions

Chapter 2: Artificial light at night alters caterpillar behaviour

Hannah D. Broeckx, Anne N. Haringhuizen, Alanna L. Brown, Emily R. Burdfield-Steel, Astrid T. Groot

HDB, ERB, ATG contributed to the design and conceptualisation of the study. All authors contributed to the data collection. HDB analysed the data, with the assistance of ERB. HDB wrote the first draft. ERB and ATG contributed to the revisions. All authors approved the submitted manuscript.

Chapter 3: Artificial light at night does not increase caterpillar predation

Evidence across birds and forest communities under short- and long-term exposure

Hannah D. Broeckx, Barbara M. Tomotani, Marcel E. Visser, Astrid T. Groot, Kamiel Spoelstra, Emily R. Burdfield-Steel

HDB, MEV, ATG, ERB, KS contributed to the design and conceptualisation of the study. BMT and MEV provided the experimental great tits and helped with the necessary licenses. HDB performed the experiment with the assistance of ATG and KS. HDB analysed the data, with the assistance of ERB. HDB wrote first draft. BMT, MEV, ATG, KS and ERB contributed to the revisions. All authors approved the submitted manuscript.

Chapter 4: Artificial light at night alters jumping spider behaviour: the role of light intensity and spectral composition

Hannah D. Broeckx, Gabriel A. Charvalakis, Astrid T. Groot, Emily R. Burdfield-Steel

HDB and ERB contributed to the design and conceptualisation of the study. HDB and GAC contributed to the design and conceptualisation of the automated tracking algorithm. HDB performed the experiment. HDB analysed the data, with the assistance of ERB. HDB wrote the first draft. GAC, ATG and ERB contributed to the revisions. All authors approved the submitted manuscript.

Chapter 5: Dimming streetlights in urban areas reduces insect attraction but pipistrelle bats forage more in darkness

Sander W.J. Buddendorf, Hannah D. Broeckx, Jona van Delft, Astrid T. Groot, Marcel E. Visser, Emily R. Burdfield-Steel, Kamiel Spoelstra

SWJB, HDB, ATG, MEV, ERB and KS contributed to the design and conceptualisation of the study. SWJB and HDB contributed to the data collection. SWJB curated bat data. HDB and JvD curated insect data. SWJB and HDB analysed the data. SWJB and HDB wrote the first draft. ATG, MEV, ERB, KS and ERB contributed to the revisions. All authors approved the submitted manuscript.

Chapter 6: AI-based assessment of insect temporal activity under different light conditions

Gabriel A. Charvalakis, Hannah D. Broeckx, Sander W.J. Buddendorf , Jona van Delft, Emily R. Burdfield-Steel, Roelof A. Hut & Kamiel Spoelstra

GAC, HDB, SWJB, ERB, RAH and KS contributed to the design and conceptualisation of the study. HDB and JvD curated the manual insect data. GAC trained the AI model and analysed the data. GAC wrote the first draft. HDB, SWJB, ERB, RAH and KS contributed to the revisions. All authors approved the submitted manuscript.

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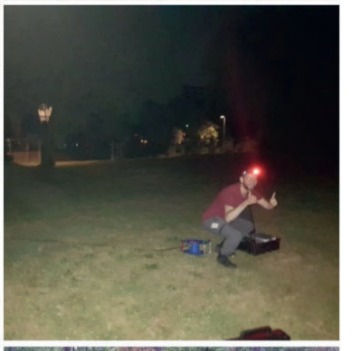
Barbara M. Tomotani

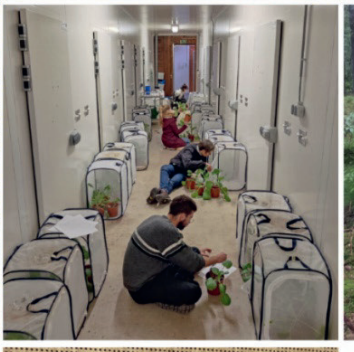

Dimming streetlights reduces insects' attraction but leaves bats in the dark















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throughout many of my experiments. **Marcel**, I'm grateful for the opportunity to conduct my first real experiment at NIOO, the first data I could actually add to my thesis.

I am also very grateful for the opportunity to supervise students. **Jip**, thanks for being my first student. As I had just started myself, we really had to work things out together, but I think you were the perfect student to do this with. In the end, your project was invaluable to learn what trapping methods did NOT work. I would also like to thank you for the later scientific outreach opportunities you gave me. **Alanna**, thank you for your optimism throughout the project. We had a good laugh over our sleep deprivation during the first 24-hour experiments, gathering the data for Chapter 2. **Britte** and **Anne**, thanks for withstanding the cold and bringing the first trials of the model predation experiment to a good end. Your efforts are immortalised in Chapter 3. Although being forced together, you formed a great team and it was fun to watch you two. Thanks also for your help in the lab, in the spider and caterpillar experiments. Although the spider data could not be used, these first trials did teach me a lot for the later experiments in Chapter 4. The caterpillar data contributed nicely to Chapter 1. **Eve**, you really won the lottery with your project, right!? Thanks for your endurance with the insect footage. Your work contributed to Chapter 5, my first published article. **Sarah**, you were a very bright bachelor student. Thank you for laying at the basis of my later model predation experiments. **Skip**, **Imre**, **Michelle** and **Sterenn**, I was less involved in your supervision, but I enjoyed our encounters and learned a little bit from each of you.

I also want to thank my dearest Amsterdam people. We started off as colleagues, but soon you became my closest friends. I am very sure I would have never finished this PhD if I did not have all your great company around me. **Renée**, you were the one showing me around after the job interview and even with the covid mask you managed to make me immediately feel at home. Your openness and enthusiasm make you a wonderful person. I'm very grateful to have had you as my desk mate, your presence always brightened my day. **Giudi**, from the very beginning, you've been the driving force behind my social life. You are always so inclusive and kind. Even from the far north, you're the one who keeps our group connected. I truly appreciate your energy and creative spirit. **Edith**, your generous heart shines through in the way you organise the biggest and most fun parties, connect people, but how you are also always up for smaller get-togethers, niche concerts, and creative events. I am very happy we shared the start and end of our PhDs. **Eileen E.**, honestly I think you are one of the coolest people I know, especially since you don't seem to realise it yourself. You are so generous and funny, it's ridiculous. Thanks, protest buddy! **Nina**, somehow our paths did not often cross in the beginning, but over time I learned what a kind, warmhearted and amazing person you are. I also look up at the amazing work you do, your care and

passion for the nature and people of the Amazon is so inspiring. **Karla**, as a whirlwind you entered my life. I love your endless energy and big smile. Your enthusiasm tilted record buying, thrift shopping and watching movies to a new level. You are so kind and engaging to everyone around you. Even my rat Liva (or Liefie, as you liked to call her) felt your love when she was sick. **Shreyas** thanks for being my cycling partner for a long time. I always enjoy your company, as you are such an open and kind person. Westies forever!

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If I forgot anyone, please don't take it personal. Let's blame it on the brain fog that arised from writing this thesis...

About the author



Hannah David Broeckx was born on September 11, 1998, in Dendermonde, Belgium. Growing up with a large garden, she developed an early fascination with nature. She spent her childhood collecting bones, monitoring garden wildlife, observing chickens as they laid eggs, and even conducting her first (though poorly replicated) plant experiments. From the start, there was no other option than to become a biologist.

In 2016, Hannah began her Bachelor's in Biology at the University of Ghent. Finally she felt a sense of belonging. She completed her bachelor's thesis on "The effect of urbanisation on colour polymorphism in weevils" under the supervision of Bram Vanthournout. She continued with a Master's in Global Change Ecology. During her master's, Hannah spent a semester abroad at NTNU in Trondheim, Norway, which broadened her horizons and revealed opportunities beyond Flanders. Back in Belgium, she completed her master's thesis on "An evaluation of agri-environment schemes to increase arthropod biodiversity and abundance" under Luc De Bruyn's guidance, and undertook a short internship on sustainable milk farming at ILVO with Veerle Van linden. In 2021, Hannah obtained her Master's degree and was finally awarded the title of biologist.

Driven by her passion for living things and her desire to reduce human impact on nature, Hannah found her first big project in 2022: tackling the issue of artificial light at night. With determination, she set out to Amsterdam, to protect the natural world she loves.

As her work progressed, she had to recognise that the reach of her scientific research alone would stay small. She therefore chose to broaden her impact by integrating public awareness, responsible personal behaviour and targeted activism, alongside her research. Having established this foundation, she is now ready to define and pursue a new project.

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 Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 30 ECTS (= 22 weeks of activities)

Review/project proposal

- The influence of artificial light at night on insect-predator interactions | 4.5 ECTS

In-depth / Topical / On-site Postgraduate Courses

- Bugs at your service | PE&RC | 2023 | 1.5 ECTS
- InsectAI | Department of Ecoscience and the Centre for Quantitative Genetics and Genomics, Aarhus University | 2025 | 2 ECTS

Methodological / Statistical Postgraduate Courses

- Generalized Linear Models | PE&RC | 2024 | 0.9 ECTS
- Mixed Linear Models | PE&RC | 2024 | 0.6 ECTS

Invited review of journal manuscripts

- Ecological Entomology | Moth mating under different light scenarios | 2024 | 1 ECTS

Competence, skills and career-oriented activities

- Mastering your PhD | UvA & Reflect Academy | 2023 | 0.75 ECTS
- Time management | UvA & Tijdswinst | 2023 | 0.3 ECTS
- Workshop Zotero | UvA | 2023 | 0.15 ECTS
- Introduction to LaTex | PE&RC | 2024 | 0.15 ECTS
- Career workshop | P(h)D councils of IBED, SILS and HIMS & FNWI career services | 2024 | 0.15 ECTS
- Workshop communication | BioClock | 2023 | 0.15 ECTS
- Module 1 You as a teacher | UvA-FNWI | 2022 | 0.15 ECTS
- Module 2 Didactical skills | UvA-FNWI | 2022 | 0.15 ECTS
- Module 3 The learning environment you create | UvA-FNWI | 2022 | 0.15 ECTS
- Module 4 You and the group | UvA-FNWI | 2022 | 0.15 ECTS
- Module 6 Tailor your supervision style | UvA-FNWI | 2023 | 0.15 ECTS

Scientific Integrity/Ethics in science activities

- Scientific integrity (as part of Mastering your PhD) | UvA & Reflect Academy | 2023 | 0.15 ECTS

- Ethics and Animal science | WUR | 2023 | 0.8 ECTS

PE&RC Retreat, PE&RC Day, and other PE&RC events

- PE&RC First year retreat | 2023 | 0.9 ECTS
- PE&RC Midterm retreat | 2024 | 0.6 ECTS
- PE&RC Last year retreat | 2025 | 0.6 ECTS

National/local scientific meetings, seminars, and discussion groups

- Future for butterflies and moths | 2025 | 0.6 ECTS
- NAEM (poster) | 2023 | 0.6 ECTS
- NAEM (PPT) | 2024 | 0.6 ECTS
- Entomologendag (poster) | 2022, 2023, 2024 | 0.9 ECTS
- Dutch Chronobiology meeting | poster in 2023, host in 2024, talk in 2025 | 0.9 ECTS
- Evolving urban communities symposium | 2024 | 0.3 ECTS
- Landelijke dag Vlinderstichting | 2023 | 0.3 ECTS
- Nationale Vlinderdag | 2023 | 0.3 ECTS
- Academische Tafel over licht en urban ecology | 2023 | 0.1 ECTS
- Academische Tafel aftrap Darkfestival | 2023 | 0.1 ECTS
- Academische Werkplaats Urban Ecology | 2024 | 0.1 ECTS

International symposia, workshops and conferences

- Behaviour (PPT) | Bielefeld, Germany | 2023 | 2.8 ECTS
- ALAN conference (PPT) | Westport, Ireland | 2025 | 1.9 ECTS

Societally relevant exposure

- Night walk for VOX-POP | 2024 | 0.1 ECTS
- Interview Folia | 2024 | 0.1 ECTS
- BioClock municipality meeting (PPT) | 2025 | 0.3 ECTS

Committee work

- BioClock Academy seminar committee | 2022-2025 | 4 ECTS

Lecturing/Supervision of practicals/tutorials

- Plant-Soil interactions | 2022 | 5 days | 1.5 ECTS
- Biodiversity and Ecology | 2022, 2023, 2024 | 16 days | 4.8 ECTS
- Evolutionary genetics | 2024 | 1 days | 0.3 ECTS
- Keystone II | 2024 | 1 days | 0.3 ECTS

BSc/MSc thesis supervision

- Research topic 1: Light Pollution affects Local Moth Community Composition | 3 ECTS

- Research topic 2: Evaluating the influence of Artificial Light at Night (ALAN) on caterpillar predator-prey dynamics and caterpillar behaviour across distinct colouration strategies and activity patterns | 2 ECTS
- Research topic 3: Shining a Light on Predator-Prey Interactions: Artificial Light at Night Affects the Behaviour of Both Caterpillars and Their Predators | 1 ECTS
- Research topic 4: The effect of artificial light at night on predator community | 0 ECTS
- Research topic 5: Illuminating Changes: The Effect of Urban Light Pollution on Flying Insects | 0 ECTS
- Research topic 6: The Effect of Light Pollution on Anti-Predator Coloration Strategies in the Lepidoptera Species *Z. filipendulae* and *M. brassicae* | 0 ECTS
- Research topic 7: Feeding the Nestlings: caterpillar biomass as a determinant of Great and Blue tits breeding success along an urban gradient | 0 ECTS

