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Impact of different light sources on the responses of moth

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Abstract

In recent decades, the negative effects of artificial light at night on natural ecosystems have attracted the attention of ecologists. Studies have shown that artificial light at night leads to a considerable reduction in insects and has a worrying impact on terrestrial ecosystems, including nocturnal insects (e.g. Lepidoptera) such as moths. Warm white light is generally expected to have a lower ecological impact compared with cold white light which has a higher proportion of blue light (< 500 nm). The aim of this study is to investigate the impact of three light emitting diode (LED) light sources with different spectral power distributions on the responses of the greater wax moth (*Galleria mellonella*) under controlled experimental settings. In this experiment, three light sources with different spectral power distribution and dark condition were used and the start response time, time active, time flap wings, time flap wings / time active, main activity area and stop area of the greater wax moth were investigated. The light treatment used were: (1) darkness (n = 13); (2) warm-white light (correlated color temperature of 2675 K, n = 12); (3) white light (4070 K, n = 4); (4) cold white light (6200 K, n = 8). The experiment was performed in a rectangular light-tight box under controlled conditions. Main activity area showed significant difference between warm-white light and cold white light. With cold white light, a larger proportion of the moths were active in the area with the highest light levels, while the main activity area in the warm white light was in the zone with the lowest light levels. Other variables, however, did not show significant differences. The conclusion is that warm white light had a lower ecological impact than cold white light due to a larger proportion of moth is attracted to areas with stronger light. The results support the notion that in outdoor

environments, warm white LEDs (maximum 3000 K) should be preferred compared to cold white light LEDs (over 3000 K), to reduce the impact on insects such as moths. The low correlated color temperature light sources used outdoors deserves more in-depth development and research.

Keywords:

Artificial light at night (ALAN), Greater wax moth, Light Attraction, light emitting diodes, LED, *Galleria mellonella*

1.Introduction

1.1 Background

Illumination of nocturnal landscapes by anthropogenic light sources, for example, street lamps, façade lighting, and vehicle head-lights, hereafter referred to collectively as artificial light at night (ALAN), seems to decrease populations of nocturnal animal species present in affected habitats (Davies & Smyth, 2018; Sanders et al., 2021). In the last decade, considerable attention has been showed in the continuing global increase of ALAN and its effects on ecosystems (Hölker et al., 2010). The widespread use of electric lamps has extensively changed the night-time environment, creating "artificial light ecology" and "ecological light pollution" (Rich & Longcore, 2006). Recently, since the lighting technology has advanced and the decline of the cost of per luminaire, both the intensity and number of light installations have increased worldwide (Kyba, 2018). ALAN is used to support and enhance human activities, but its profligacy use is a potential threat to local biodiversity (Hölker et al., 2010). Currently, ALAN is no longer confined to urban centres but radiates outwards through the atmosphere and along with road networks that run into or around otherwise pristine areas (Gaston et al., 2015). ALAN is gradually invading biodiversity hot spots (Owens & Lewis, 2018). Global surveys of night sky brightness have revealed that 88% of Europe and 47% of the United States, covering 23% of land surfaces between 75° N and 60° S, experience "light pollution" at night and the brightness increase at least 8% above the natural level (Falchi et al., 2016).

The light output of the most used luminaires is more than enough to radically disturb the entire habitat of insects which may exhibit positive phototaxis (Donners et al., 2018). Positive phototaxis causes insects to move in the direction of increasing light intensity (Donners et al., 2018). One-third of insects attracted to stationary artificial light sources die before morning because of exhaustion or predation (Eisenbeis, 2006).

A common but still not fully convincing and complete explanation for their flight-to-light behavior is that moths mistake a strong light source for the moon and fly towards it (van Langevelde, 2011). Many effects of artificial lights are already known or suspected and insects attracted to artificial lights may be injured or killed by contact with the hot surface of a lamp (Justice, 2016). Insects that escape immediate death may become trapped in a "light sink", then they will circulate around the light, unable to perform activities essential to fitness (van Langevelde et al., 2017). Flight-to-light behaviors and time spent around lights can also disrupt normal behaviors and rhythms of behavior, including perturbations of hatching, feeding, dispersal, pupation, emergence, mate searching, mating, and oviposition (Frank, 2006; Perkin et al., 2013; van Geffen et al., 2014). ALAN may have several effects on foraging and reproduction activities of moths and their interspecific interactions (Frank, 2006). For example, moths flying around streetlights at night may experience increased predation by bats and other nocturnal predators which have learnt to take advantage of these artificial feeding stations (Rydell, 2006; Kuijper et al., 2008). Insects that manage to escape the cone of light beneath a luminaire can still be affected by skyglow, which emanates far beyond urban centres at levels sufficient to obscure or alter vital environmental cues including polarized moonlight, starlight, moon phase, and day-length (Owens et al., 2020).

During the recent decades, ALAN caused the abundance of insects to decline significantly and researchers have reported declines in insect diversity and biomass across geographically distinct areas with worrying implications for terrestrial ecosystems (Owens & Lewis, 2018; Owens et al., 2020). Habitat degradation and climate change may be the drivers of these declines (Fox et al., 2014), where ALAN is a potentially important contributing factor (Fox, 2013). It is suspected that ALAN has unprecedented destructive effects on nocturnal insects (such as Lepidoptera) (Eisenbeis, 2006; van Langevelde et al., 2011). The increasing amount of nocturnal illumination may be a driver of the current widespread decline of many moths observed in industrialized countries (Roy, 2014).

The impact of ALAN is determined by two factors: the quantity of the light and the wavelengths of the light (spectral power distribution, SPD). Different light sources will have different quantities and SPDs, and will therefore result in various impacts on insects. The spectral dependence of attraction is relatively well documented, with strong, direct attraction of insects to ultraviolet light and blue light whereas longer wavelengths (red light, yellow light) attract less insects (Spoelstra, 2020). Light with wavelengths below 500 nm shows more attractive results for insects (van Langevelde et al., 2011). Warm color temperatures (correlated color temperature (CCT) of ca 3000

K) have more yellow and red light in the SPDs. Light sources with more blue “cool” color temperatures (CCT of ca 5000 K) are believed to cause more atmospheric scattering and thus lead to increases in light pollution (Falchi et al., 2011). The warm-white light source is therefore argued to have a lower ecological impact and general recommendations often suggest to use light sources of as low correlated color temperature (CCT) as possible to avoid ecological impact (Bara et al. 2020), because the relative amount of emitted blue light can be reduced by using warm-white light (preferably lower than 2700 K) (Spoelstra, 2020).

In the past few decades, the number of people using artificial lighting outdoors at night has increased dramatically (Cinzano et al., 2001) and forms one of the major anthropogenic changes to our environment (Hölker et al., 2010). To date, knowledge on the impact of ALAN on our ecosystems is limited. With progressive urbanization and concurrent increase in the use of ALAN, a better understanding of its impact is urgently needed (Gaston et al., 2012). As different types of artificial lights are being used, knowledge about the effects of different SPDs on moths is important for their conservation. The light sources can largely differ in both intensity and spectral composition, which determine their attraction to insects (van Langevelde, 2011). Hitherto, no previous study has examined the impact of different SPDs on insects in a fully controlled experiment. The project will therefore result in new knowledge on the effect of ALAN on insects.

1.2 Aim

The aim of this project is to investigate the impact of three light emitting diodes (LEDs) light sources with different SPDs (warm-white light 2675 K, white light, 4070 K, cold white light, 6200 K; and dark) on the responses of the greater wax moth (*Galleria mellonella*) under controlled experimental settings. The results will be valuable for improved recommendations on how to mitigate the adverse impact of ALAN on insects.

1.3 Hypothesis

It is assumed that the dark conditions and the light sources will have different impacts on the responses of the wax moth. In particular, it is likely that both darkness and the warm-white light source has a lower impact on insects. Warm-white light is expected to have a lower impact due to the lower amounts of blue light in the wavelengths below 500 nm. Such results support the use of warm-white light sources over more white or "colder" light sources in the outdoor environment since it will probably have

a general lower ecological impact.

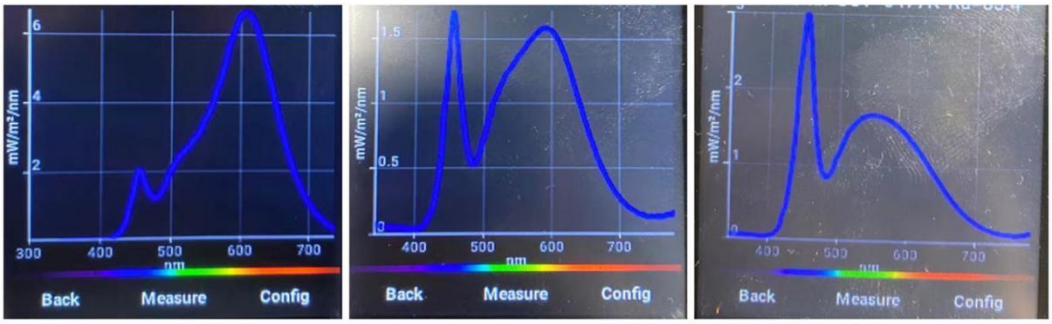
2.Materials and methods

2.1 Study species

The greater wax moth (*G. mellonella*) was used to test the response of different light sources in a controlled experiment. Commercially available greater wax moth (*G. mellonella*) was bred from purchased larvae. The larvae were cultivated in an opaque box with a heating blanket at a constant temperature of 26°C, mature adults were taken out of the box and used in the experiment.

2.2 Light source

Light emitting diodes (LED) was used as the light source. The responses to three types of SPD of LEDs were compared: (1) a warm-white LED with low color temperature (2675 K) and (2) a white LED with middle color temperature (4070 K) and (3) a cold white LED (6200 K). To ensure the light level was the same for all light sources in the experiment, we measured the illuminance and luminance in the box and adjusted the output until the same levels as used for previous light source was obtained. At the centre of the experiment box the illuminance in the horizontal direction was 0.07 lux and the vertical illuminance was 0.17 lux. The illuminance in the vertical direction was 0.07 lux in the centre of the wall facing the light source. The SPDs of different light sources are shown in Figure 1. The warm-white light had less blue light compared with white light and cold white light.



warm-white white cold white

Figure 1. The SPDs of three different light sources.

2.3 Experiment design

The effect of four different types of light treatments (dark, warm-white light, white

light and cold white light) on moth response variables (time start response, time active, time flap wings, ratio of time flap wings to time active, main active area and main stop area) were tested. The experiment was performed in a rectangular light-tight box 60 cm × 45 cm × 90 cm (W × H × L), see Figure 2 and 3. The light source was a sphere-based luminance source with a mechanical shutter and is placed at one of the short sides. At the beginning of the experiment, the mature moth was taken out of the incubator. During this period, it was ensured that there is no other light source in the surrounding environment, but a pure red light. The illuminance of the red light was less than 100 lux at all times at a distance of ca 10 cm from the lamp. The red wavelengths are believed to be beyond the visible light range of the moth. To ensure accuracy regarding temperature conditions, the experiment was carried out at room temperature (23 °C). Infrared LEDs are used to ensure clear visibility of the insects (this is out of the visible range for the insects), see Figure 2. Infrared LEDs were installed on the top of the light-tight box. Two monochrome cameras with wide angles objectives monitor the movements of the insects in the observation time.

The newly hatched moth was given priority for each experiment. However, when the number of hatchlings was too low to continue the experiement, the moth used in the previous experiment was used. It was ensured that they spent a long enough time in the dark and rest to eliminate the influence of the light source (at least 12 hours) or previous handling in the experiment.

Figure 2. The outside appearance of the light-tight box. Infrared LEDs are installed on the top of the box.

Figure 3. The inside look of the light-tight box. The monochrome cameras with wide angles are installed in the top corners.

Photos of the luminance distribution were taken and developed by an LMK Mobile Advanced imaging luminance photometer and the associated computer software LMK laboratory Software, see Figure 4. The photos were taken by Canon EOS 550D which has a digital single-lens reflex camera with a CMOS sensor with 18.0 effective megapixels resolution and has a working temperature range of 0–40 and working humidity of 85% or less.

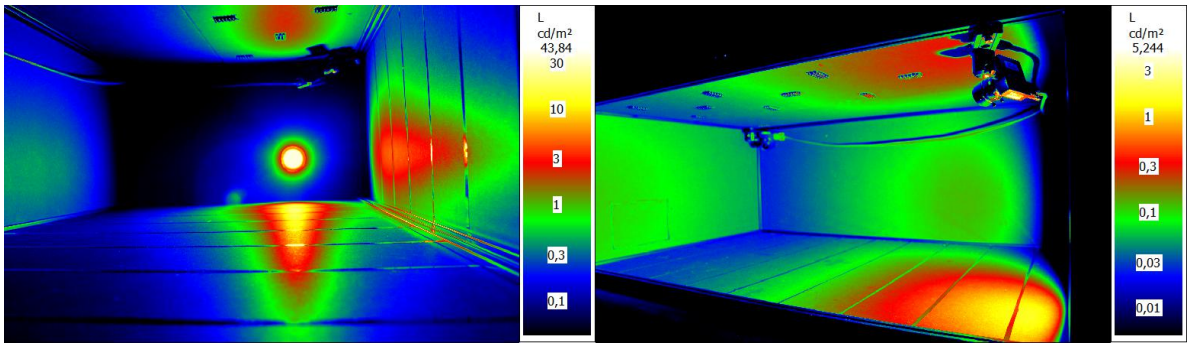


Figure 4. The photos show how the luminance is distributed. Luminance photos and evaluations have false colors. The photos are used by courtesy of Annika Jägerbrand.

2.4 Data collection

Each single experiment used one moth, a single experiment was ten minutes, and after each experiment with a light source, an experiment with a dark control group (without any light source) was executed with a new moth. In the experiment, two wide-angle cameras were used to record the response of moth to different light sources. All recorded videos were used as initial data for studying the moth response of different

light sources. Hagner Universal Photometer S4 was used to measure the light intensity and luminance at different positions in the light-tight box. The response variables tested were: Time start response, the time when the moth moved for the first time, Time active,the total time of movement in the observation time, Time flap wings, the total time of flapping in the observation time, Main active area,the area moth spent most time in the observation time and Stop area,the area where the moth stop move completely.

2.5 Data analysis

Data analysis variables were extracted by measuring the time of various actions through the recorded moth response videos. The variables extracted were the start response time, total active time, flapping time and the ratio of flapping time to the total active time. Start response time (in seconds) was measured as time from when the moth was placed in the box until it moved. Total active time (in seconds) was the amount of time the moth kept on moving and did not sit still, the time was counted from the moth was placed in the box until the experiment ended.

The light-tight box was divided into three areas, which were based on the light intensity in the box (see Figure 5) and were used to analyze the movement trajectory of the moth and the main activity area by counting the active time of the moth in different areas (in seconds) and by registering the area where the movement completely stopped. The moths usually stopped moving after adapting to the environment.

Forty five moths were used in this experiment, thirty seven moth had response. The moth with no response were not used in analysis. The final sample size were:(1) darkness (n = 13); (2) warm-white light (n = 12); (3) white light (n = 4); (4) cold white light (n = 8).

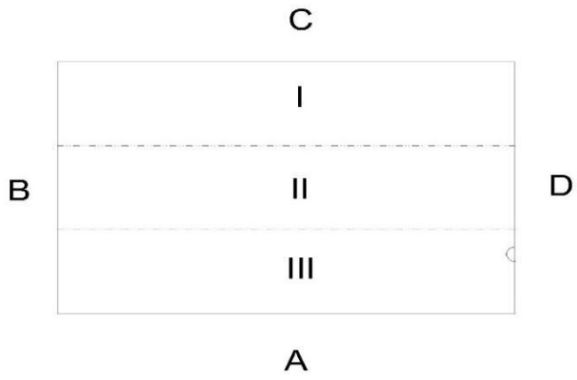


Figure 5. The three different areas used to analyse the movement trajectory of the moth and the hot spot movement area. A, B, C, D are the names of walls. The light source was in area III on wall D, as indicated by the semi-circle.

2.6 Statistical Analyses

All the statistical analyses were done using SPSS version 26. One-way ANOVAs (if the data meet the assumptions for normal distribution) and Kruskal-Wallis tests (if the data did not meet the assumptions for normal distribution) were performed to test if there were significant differences between response variables to darkness and the different light sources. Mann-Whitney U test was used when Kruskal-Wallis test had a significant difference. The response variables were, as described in section 2.4, time start response, time active, time flap wings, time flap wings/time response, main activity area and the stop area.

3.Results

3.1 Response to different light sources and darkness

There is no significant difference between the different light treatments on start response time (Kruskal-Wallis test, $p = 0.134$; see Figure 6) or time response (Kruskal-Wallis test, $p = 0.221$; see Figure 7). Furthermore, there was no significant difference between the different light treatments on time flap wings (one-way ANOVA, $p = 0.133$; see Figure 8) or time flap wings/time response (one-way ANOVA, $p=0.061$; see Figure 9). However, there is a trend that the mean start response time in darkness showed higher values compared to the mean start response times for the other light treatments (see Figure 6). Similarly, responses in darkness (not significant) were lower for mean active time and mean time flap wings compared with the other light treatments (see Figure 7 and 8).

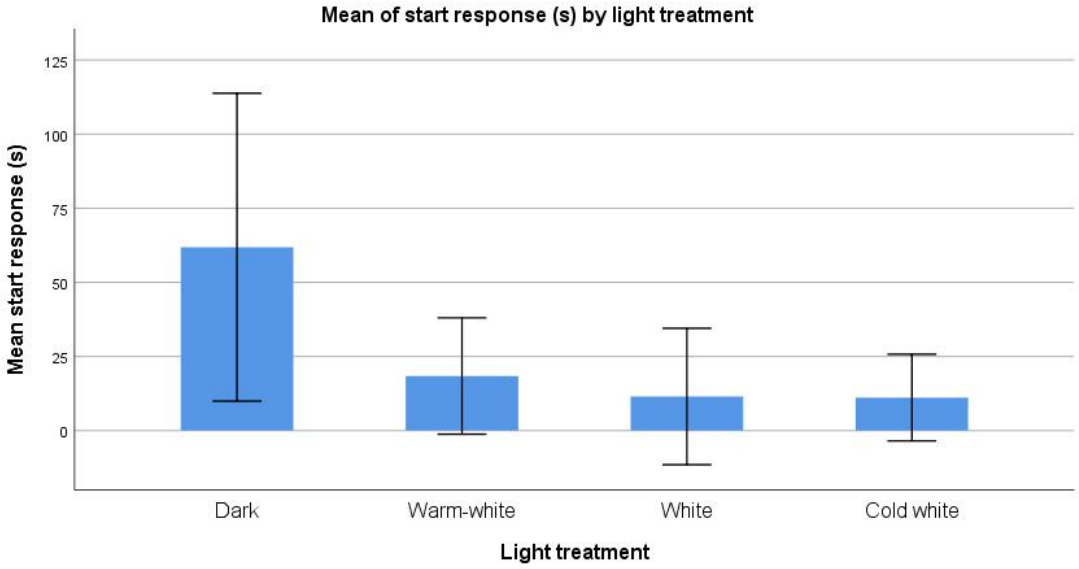


Figure 6. Mean values of time start response (in seconds) between darkness and the three light sources (dark, n = 13; warm-white, n=12; white, n = 4; cold white, n = 8). Error bars show ± 2 standard error.

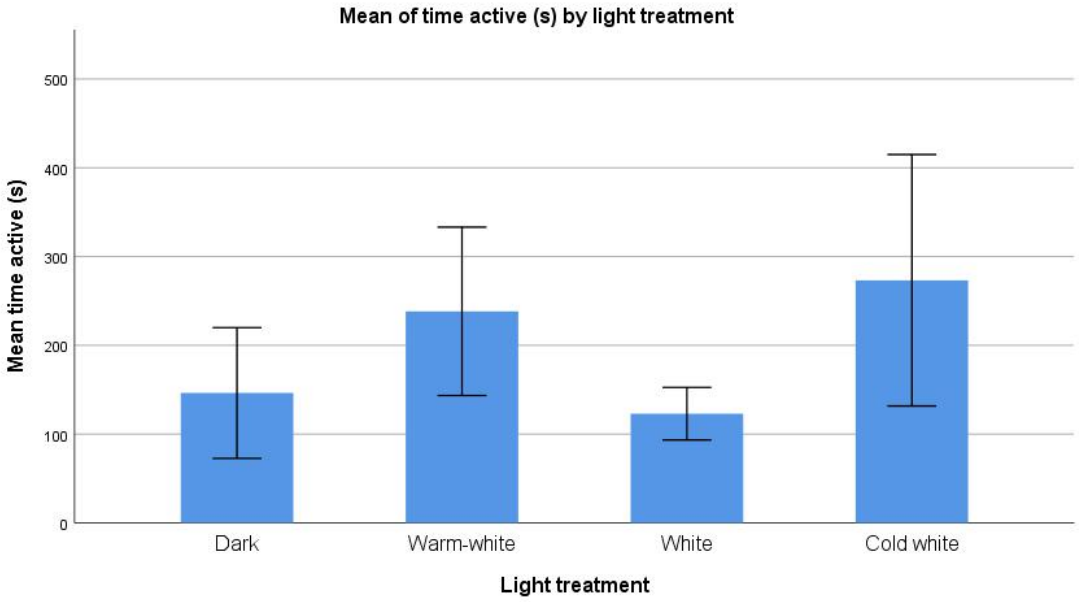


Figure 7. Mean values of time active (in seconds) between darkness and the three light sources (dark, n = 13; warm-white, n=12; white, n = 4; cold white, n = 8). Error bars show ± 2 standard error.

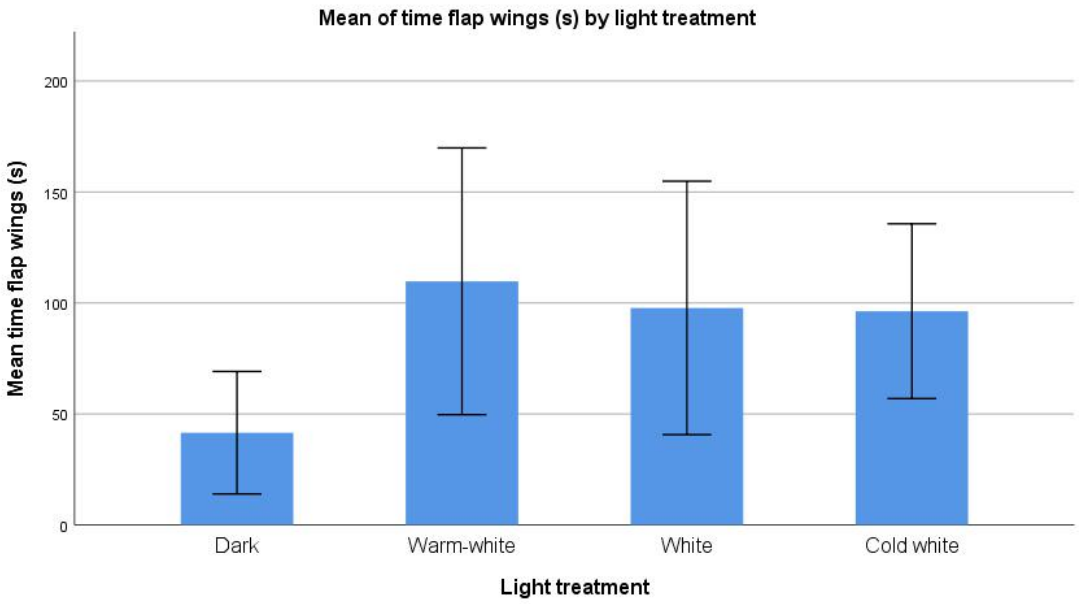


Figure 8. Mean values of time flap wings (in seconds) between darkness and the three light sources (dark, n = 13; warm-white, n=12; white, n = 4; cold white, n = 8). Error bars show ± 2 standard error.

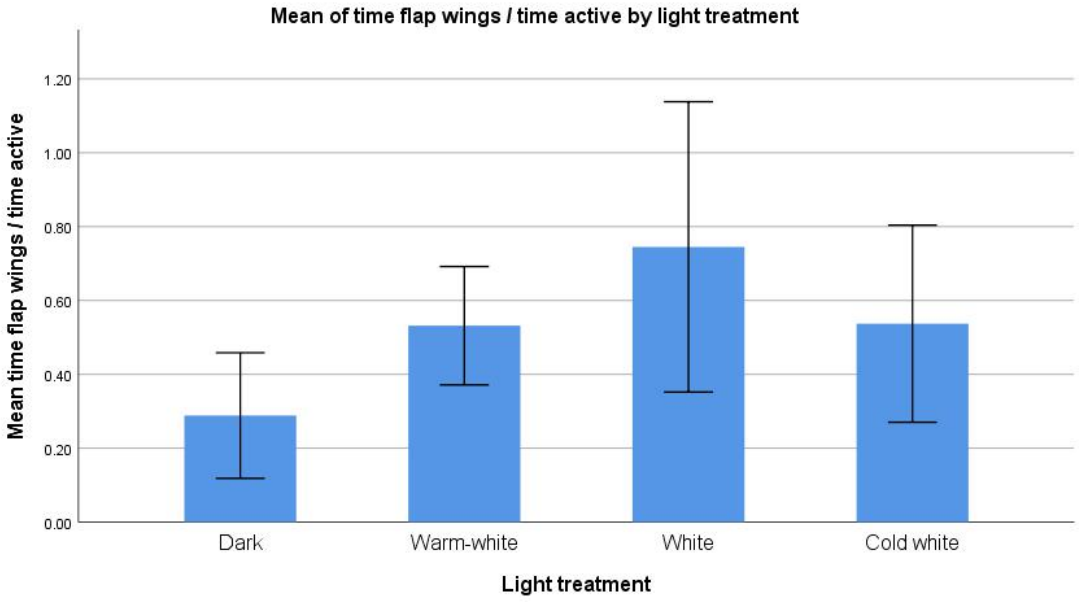


Figure 9. Mean values of time flap wings / time active between darkness and the three light sources (dark, n = 13; warm-white, n=12; white, n = 4; cold white, n = 8). Error bars show ± 2 standard error.

3.2 Moth main activity area and stop area

There is a significant difference between the main activity area (Kruskal-Wallis test, H (2) = 6.119, p = 0.047), where cold white light and warm-white show a significant difference (Mann-Whitney U test, u = 15, p = 0.014), see Figure 10. The moth spent more time in area III when exposed to cold white light (i.e. the area with the highest luminance) while more time in the warm-white light was spent in area I that also had the lowest luminance. However, for the stop area there is no significant difference between the three light sources and darkness (Kruskal-Wallis test, p = 0.952), see Figure 10.

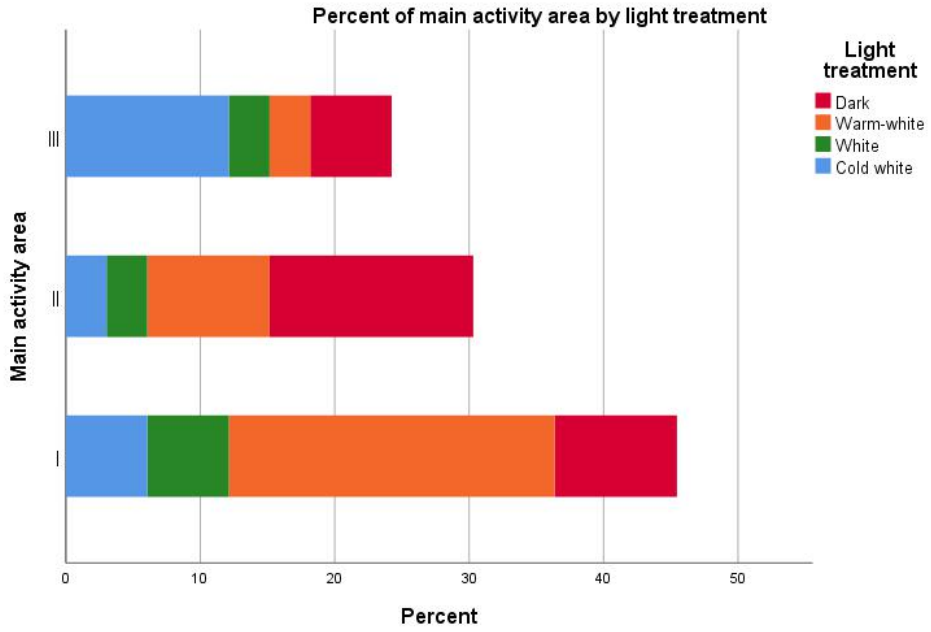


Figure 10. Main activity area (%) between darkness and the three light sources (dark, n = 13; warm-white, n=12; white, n = 4; cold white, n = 8).

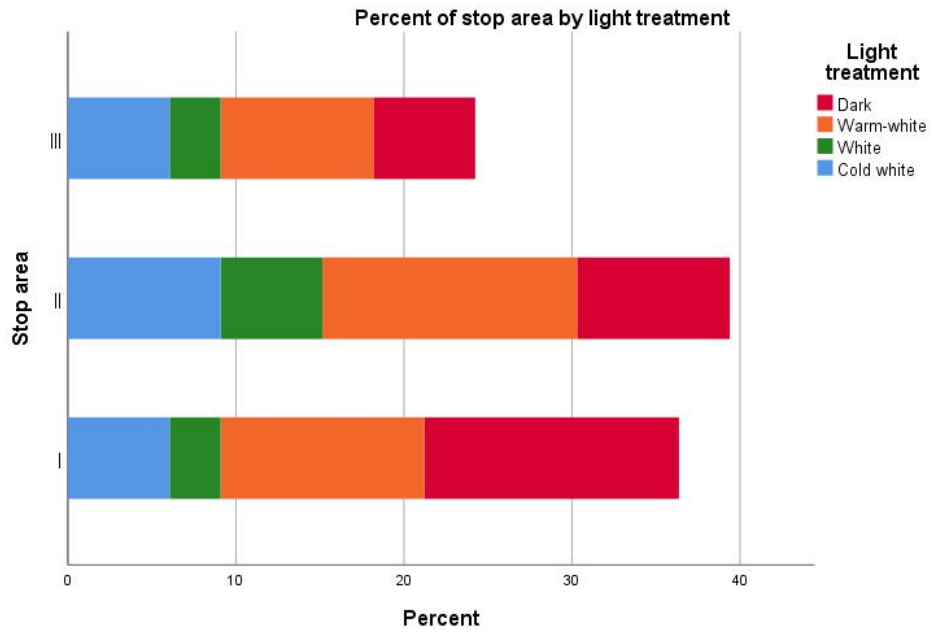


Figure 11. Stop area between darkness and the three light sources (dark, n = 13; warm-white, n=12; white, n = 4; cold white, n = 8).

4. Discussion

This study shows that dark conditions and the light sources have different impacts on the responses of the wax moth. Moths in darkness had a trend (non-significant) of a delayed mean start response time and shorter mean active time and mean flap wings time. When cold white light was used as a light source, a significant larger proportion of moth was active in area III where the luminance distribution was the highest. Therefore, this study indicates that darkness and the warm-white light source have a lower impact on insects. Even though the results are not exactly the same as the expected experimental result, they show, based on the differences in main activity area between the light sources, that cold white light sources (with higher color temperature and more blue light) are more attractive for moths than warm-white light. The results therefore indicate that the warm-white light might have a lower ecological impact on moth.

In the experimental results, there was no statistically significant difference in the reaction start time between darkness and warm-white light, white light, and cold white light. The reason for these unclear results may be that in the process of transferring the moth from the incubator to the experiment box, the moth might have been impacted by the handling from incubator to a separate container and then into the experimental box. During this period, it seemed that the moth was somewhat affected by the handling and was not always completely calmed down when it entered the

experimental box. The moths did not show any significant difference in the total activity time, the time of flapping wings, or in the proportion of the time of flapping wings to the total activity time. This means that the light sources with different color temperatures and darkness did not have a significant effect on moth's behavior in this experiment. Due to the low number of replicates used in this study, the responses might be statistically different if the experiment would include more samples.

The main active area was the only variable with statistical differences, which showed that the influence of different light sources on the response behavior of moth has a limit. The results in the main active area showed that cold white light was more attractive to moth, because a larger proportion of moths were active in area III than other light sources. The experiment showed that for the area where the moth finally stops moving and the main moving area, it did not matter what light source was used, a larger proportion of the moth eventually stopped moving in area I and II where the luminance distribution is relatively weak instead of area III where it was the strongest. Furthermore, in the selection of the stop area, more than 50% of the moths did not choose a position facing the light source or the strong luminance distribution. Eight individuals finally stayed behind the camera in the corner of the experiment box. This demonstrates that the moth will not stop opposite the light source after clam.

The greater wax moth is morphologically similar to wild moth species and shows phototaxy, but comparisons to other species or groups may be inappropriate since they have different eye morphology and ecology, for example mayflies (Durmus et al. 2021). Therefore, this species can only valid as a model organism for moth species with phototaxis.

This experiment showed differences in the response of moths to cold white light with high color temperature compared to warm-white light with low color temperature, although the effect was only significant for one of the response variables. This is consistent with the previous field experimental results but not as significant as the previous results. For example, in one previous experiment, the experiments were conducted in open forest environment and the intensity of the light sources were much greater (over 8 lux) than used in this experiment. The moths attracted and captured were also of several different species (Spoelstra et al., 2015; van Langevelde et al., 2011). Light source attraction is correlated with morphological characteristics of moths (van Langevelde et al., 2011). The difference in attraction result may be caused by the selection of a single moth species in this experiment and the low intensity of the light source. Experiments in open areas will also attract birds and bats that may influence the outcome when studying insects due to increased predation.

ALAN will affect moths' behaviors. It is reported ALAN will reduce moths' feeding frequency (van Langevelde et al., 2017) and diapause inhibition and sex-specific life-history changes (van Geffen et al., 2014). Flapping wings is courtship and mating behavior of a moth. This study shows no significant differences between the three light sources. It might also be because rather low luminance and illuminances was used in this experiment. However, it shows that the light sources have a limited impact on the behavior of wax moth. But when comparing the darkness and the light sources, the moths showed a trend of longer mean start response time and shorter active time and wings flapping time in the dark environment. For further ecological effect study, population consequences and cascading effect are mentioned in previous experiment (Spoelstra & Visser, 2013). Experimental light affects individual moths, but the effect of light at the population level might be limited and possibly only emerges after several years (Spoelstra et al., 2015). The greater wax moth lives in beehives so it might be that the species is not completely comparable with "wild" species. Therefore, short-term experiments like the ones used in this study will not perhaps reflect the impact on the moth population.

Some studies have carried out modeling analysis on the impact of ALAN on moths and other insects (Gaydecki, 2018; van Grunsven et al., 2014) in order to predict the ecological impact. However, under field condition, it is often not possible to isolate the influence of a single light source but there will be mutual influence of multiple light sources. Currently available models predicting the attractiveness of a light source are not completely adequate for modern light sources because they only analyze a single light source (van Grunsven et al., 2014). Although the development of less attractive light sources can greatly benefit from this model (van Grunsven et al., 2014), and the type of lighting will not only be a tool to reduce the impact of artificial light but also reduce the attractiveness of insects. Therefore, a better predictive model suitable for modern light sources is needed.

Activity near lamps is likely to have crepuscular peaks among Diptera, while Lepidopterans likely have a nocturnal peak (Nowinszky, 2004). Therefore, the timing of the lamp illumination may influence the ecological impacts on insects. This should be considered if the lamp is turned off at some point to balance light pollution and user needs. Compact fluorescent lights (CFLs) use mercury to create ultraviolet light which in turn causes a powdered coating to fluorescent (Justice et al., 2016). CFLs have also been used in lighting historically, which have created a stronger attraction for moth and a greater impact. In the method of avoiding the influence of ALAN, it can be a feasible way to change to use a low-impact single light LED as the light source. This study confirms that cold white light should not be used outdoors but it

still used. However, the results did not show much difference between warm-white and white. This should be analysed in future studies.

Future study should use larger sample sizes and larger experiment areas to enable studies of the effects in the moth populations. During the experiment, the interference to moths should be minimized, and the influence of the sex and reproduction of moth on the experimental results can also be considered. If the warm-white LEDs consistently attracts fewer moths compared with cold white LEDs, warm-white LEDs should be favored. This will also benefit a decrease in light pollution since warm-white LEDs have lower emissions in the blue spectrum (Falchi et al., 2011; Somers-Yeates et al., 2013).

5. Conclusions

This study concludes that the warm-white light attracts moth less than cold white in main activity area. However, other response behaviors and the location of the stop area did not show any significant responses. However, there was a trend of difference between darkness treatments compared to light sources for start response time, time active, time flap wings, time flap wings / time active. The results support the notion that warm-white light has a lower impact on moth due to the lower amounts of blue light in the wavelengths below 500 nm. Using a light source of lower color temperature outdoor (for example 2675 K) can therefore reduce the potential detrimental impacts of ALAN on insects. The result can be valuable for improved recommendations on how to mitigate the adverse impact of ALAN on insects. The low color temperature light sources used outdoors deserves more in-depth development and research.

6. Acknowledgements

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