



# Article The Effect of Electric Bridge Lighting at Night on Mayfly Activity

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Abstract: Phototactic and polarotactic aquatic insects, such as mayflies, can be drawn to electric lighting on bridges at night. Past research investigating the effect of light intensity, polarization, and spectrum on mayflies suggests that a combination of different techniques can reduce the number of mayflies attracted to bridges. Here, various lighting strategies are systematically tested on Veterans Memorial Bridge in Pennsylvania to investigate the effect of lighting on mayflies and address safety concerns caused by their mass crowding. Isolated trials on different parts of the bridge tested the effectiveness of correlated color temperature, chromaticity, ultraviolet radiation, shielding, and polarization. Results indicate that mayflies were more attracted to ultraviolet radiation, blue and green light, and polarized light than other lighting conditions. Shielding was minimally effective in reducing the number of mayflies on the bridge when supported by the change in light source spectrum. While the correlated color temperature did not result in a statistically significant impact, the spectral power distribution of the light sources was a major influencer for mayfly activity. Future research should investigate the effect of radiant intensity and timing on mayfly activity. Smart solid-state lighting systems and controls can also be used to adjust the light levels when needed to reduce adverse effects on aquatic insects and aid traffic safety.

**Keywords:** outdoor nighttime lighting; disruptive nocturnal light exposure; correlated color temperature; light pollution; ecosystem; diel periodicity; phototactic response; aquatic insects; sustainability; polarization

# 1. Introduction

Outdoor lighting design requires careful consideration of the conflicting needs of humans, animals, and plants. Unwarranted electric light at night can cause disruption for animals and plants, and the attraction of insects to light sources can reduce visibility and safety for humans, especially in urban areas. Recent research on the ecological impacts of electric light has focused on contemporary lighting technologies, such as solid-state lighting (SSL) devices. For example, a field study comparing light traps of a phosphor-converted white LED (4000 K) and high-pressure sodium (HPS) lamp found that flying invertebrates were more attracted to LEDs than HPS lamps [1]. When LEDs at different correlated color temperatures (CCTs) were normalized between 400 nm and 500 nm (alleged peak sensitivity for most insects), the CCT did not significantly affect the number in the trap catch. The results underscore the poor performance of metrics that are based on human visual sensitivity, such as CCT and illuminance, rather than shortcomings of a specific lighting technology. Since the spectral output of SSL devices can be adjusted, it is possible to replace older technologies like HPS with LEDs, which enable connectivity and adaptive lighting solutions (e.g., automatic adjustment of light levels depending on environmental data collected from sensors).



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**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). The effect of constant spectral power at certain wavelengths [1] suggests that the spectral optimization methods can help balance the needs of local fauna and human observers, especially if the spectral sensitivity of the animals is known and the number of light-sensitive species is limited in a given location at a specific time. Spectral optimization studies have been widely used to solve indoor lighting problems to achieve optimal color quality, energy efficiency, reduce damage to artwork, and synchronize human circadian rhythms [2–5]. The SSL devices can also provide efficient, high-quality lighting solutions for plants by fine tuning the spectral power distribution to plant action spectra (e.g., *chlorophyll a, chlorophyll b, beta-carotene,* and *phytochrome*) [6]. Spectral flexibility of SSL output, intelligent sensors, and controls can enable developing adaptive lighting systems that detect specific plant responses and adjust light output in real-time to improve yield quality and quantity [7].

While SSL devices have been widely used to solve complex problems in indoor environments, outdoor lighting poses different challenges. The use of electric light outdoors at night often includes consideration of aspects beyond energy efficiency and human visual response. One of these considerations is the potential disruption to the ecological environment (wildlife), which typically harbors many species of plants and free-living animals [8]. Balancing the needs of organisms as well as safety and security for humans can be challenging due to several unknown and uncontrolled factors, such as animal behaviors and human activities, visual and circadian responses, and other ever-changing environmental factors. Despite the complexity of the issues, there have been proposals to balance the trade-offs between ecological, social, and economic impacts of electric light at night [8,9]. The recommended practice for sustainable outdoor electric light at night includes reducing lighting intensity, duration, short-wavelength energy (including ultraviolet (UV) radiation), uplight, and shielding luminaires [9].

Bridge lighting may affect various insects, including mayflies, especially during their upstream compensatory flights (female mayflies' flights to compensate for river flow to lay eggs). A similar occurrence at the Veterans Memorial Bridge, in south-central Pennsylvania (PA), USA, initiated this research project to study the effects of electric lighting on mayfly activity. Here, we examine the effects of some of the recommended design measures (shielding, ultraviolet radiation, CCT, light polarization, and careful luminaire placement and aiming) on mayfly activity by conducting a field study. The field study aims to determine sustainable strategies to retrofit or augment the existing bridge lighting system to improve traffic safety and reduce ecological impacts of light at night.

#### 2. Mayfly Response to Light

# 2.1. Physiology

Mayflies are aquatic insects that belong to the *Ephemeroptera* order, with over 3000 species worldwide [10]. Adult mayflies do not feed but rely on reserves collected during their nymphal life [11]. The adults live between a couple of hours to a few days (some species can live up to 14 days) and spend most of their lives in aquatic environments. While adult mayflies have short life spans, nymphal life spans range from 3–4 weeks to about 2.5 years [11]. An important activity for mayflies is the emergence from the water and transitioning to the adult form. Environmental factors, such as radiant and thermal energy, are major factors in determining the timing of swarming [11].

Nymphs show more diversity in habit and appearance compared to adult mayflies. Their respiration rates are affected by temperature, light intensity, and growth stage. Mayfly movements can be random, directional, daily, or seasonal. During the final stages of nymphal life, they concentrate in the shallower areas of lakes and rivers and emerge to the water surface (usually at dusk or dawn) during the transition from nymph stage to terrestrial *subimago*, the final molt of the nymph stage before the full adult form, and they can elevate up to 5 m [11].

Mayflies' diel activity patterns vary according to the species and location, but the main driver of emergence is known to be temperature [12]. However, emergence can

also be affected by photoperiod [13], wind, humidity, precipitation, turbidity, and irradiance [11]. Mayflies' en-masse emergence from water may cause a nuisance to humans. For example, tree limbs drop under their weight, they can create odors, they fly into people's faces, and in extreme cases, they may cause highway bridges to close due to safety concerns [14]. The mayfly *cuticle* can also cause allergic reactions in certain human populations [14]. Conversely, a benefit of mayflies is that they are pollution-sensitive (they cannot fly long distances from toxicity); therefore, the presence of mayflies indicates high water quality [14,15].

# 2.2. Light Sensitivity

# 2.2.1. Spectrum

The effect of light source spectral power distribution (SPD) on mayflies has been investigated at different stages of their maturity, especially in the adult stage [16]. Research suggests that male mayflies have *dorsal* and *lateral* eyes while females have only lateral eyes [17]. Male mayfly dorsal eye sensitivity peaks at around 340 nm (gaussian single peak sensitivity curve), and lateral eye sensitivity peaks at around 535 nm (dual spectral sensitivity with a smaller peak at 350 nm) [17]. Although only one photoreceptor has been encountered in mayflies, the dual peak in lateral eye sensitivity indicates that they might have more than one photoreceptor. It has also been suggested that dorsal eyes help males to discriminate females as females fly through a swarm of dancing males, where males can capitalize on a dark night as the background to detect females.

Nymphs have mostly dorsal light response, and light reflected from white painted substrate might disequilibrate them [18]. Light sources also provide orientation for nymphs in water, and it is an essential cue for their emergence. A research study suggests that mayfly nymphs react strongly to green (550 nm) and red (650 nm) light (0.95 mW tungsten filtered ~= 11  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>), but not to infrared (IR) radiation at 950 nm [19]. However, researchers conducted this study in a controlled lab environment, which did not include shelter or flow of the stream, which may affect mayfly behavior. In a different study, lotic mayflies (*nousia* sp.) did not respond to green (568 nm), red (635), or IR (950 nm) at a flux density of 18–19  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> [20], and only half of the mayflies responded to light in still flow. In a moving flow, most mayflies did not respond to any of the lighting conditions. Results of this study indicate that mayflies may require moving water to orient themselves. However, it should be noted that the lighting conditions were not randomized, which might cause residual effects.

The ultraviolet (UV) radiation was also shown to affect mayfly grazers and algae interaction. In a study conducted in New Zealand where ground-level UV levels are high due to ozone depletion, *Deleatidium* larvae detected and avoided natural levels of UV and grazing on exposed surfaces more than other conditions when the UV radiation was attenuated [21]. In short, research suggests that mayfly spectral sensitivity peaks around 535 nm and with possible UV detection abilities, with no concrete proof of infrared response.

## 2.2.2. Intensity

Nymphs are known to be positively *thigmotactic* (seeking contact) and negatively *phototactic* (avoiding light) [22]. A research study found that red light did not affect nymphs in dim lighting conditions (3 lx), and the high nocturnal activity started at illuminance levels between 5 lx and 20 lx and finished at light intensities between 2 lx and 60 lx [22]. High nocturnal activity started at a higher light intensity in July (around 20 lx) than in January and March (around 5 lx) and finished at different light intensities in July (around 60 lx), January (around 2 lx), and March (around 40 lx). During the high nocturnal activity, nymphs moved onto the upper surface of a stone and did not react to low light levels (1 lx or less) or to a red light. Only a small number of nymphs reacted at 3 lx, while most nymphs moved away from the light during night. Although other researchers have specified a definite threshold of 40 lx for nymphs of *Baetis rhodani* [23], the negative phototactic

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threshold might not apply to all mayfly species due to the differences between species and the presence of fish, which affect the invertebrate drift periodicity [24].

Another study found that nymphs were more affected by light and shade than light reflected from a *substrate* in the stream [25]. When the illuminance was around 80,000 lx (~ 8000 fc) under bright sunlight, nymphs preferred to stay under the shade of 1500 lx (~ 150 fc) [25]. However, when there was a black object between nymphs and a light source, they moved toward the light source and even made physical contact. On the other hand, continuous high light levels (500 lx) or low light levels (below 0.5 lx) did not impact a change in their activity pattern compared to the activities seen under natural illumination, but very few nymphs moved onto the upper surface of the stone in high light levels, and some nymphs were always found on the upper surface of the stone under low light levels [22].

It is evident that the variation in lighting conditions provides a cue to mayflies for diel vertical migration. In a study, a halogen lamp was used to simulate natural lighting changes (from  $7.9 \times 10^2$  to  $6.9 \times 10^{-2} \,\mu\text{W} \,\text{cm}^{-2}$  at a constant rate of relative light change;  $-1.9 \times 10^{-3} \,\text{s}^{-1}$ ) in controlled lab conditions, and locomotor activity and vertical movements among individual mayflies were found to be highly variable under controlled conditions [26]. Similarly, the timing of both increased locomotor activity and changes in the vertical location on mayfly nymphs was strongly correlated with the relative reduction in light levels [27]. Locomotor activities started earlier for nymphs that adapted for 60 min at a constant but reduced optical radiation level at the water's surface of 8  $\mu$ W cm<sup>-2</sup> [27]. Individual nymph responses to light periodicity were dependent on their initial activity during the light-adaptation periods. The results suggest that physiological differences influence mayfly responses to environmental conditions, especially the timing and intensity of the light stimuli.

# 2.2.3. Time of the day

The time of the day can also influence mayfly activity. Research suggests that the nocturnal activity is more significant than the diurnal activity, and the number of mayfly nymphs follows a random distribution close to a low mean during the day, and a high mean at night [22]. However, another study found no adverse reaction to lighting conditions (mayfly nymphs did not react to light), but light levels were low and consisted of green (568 nm), red (635 nm), and infrared (950 nm) [20]. As previously mentioned, mayflies are not sensitive to long-wavelength radiation (red and infrared), and lighting conditions in this experiment were not randomized.

# 2.2.4. Polarization

Earlier research suggested that the male mayfly dorsal eye is not sensitive to polarized light [10], but there is growing evidence for an opposite effect. For example, several studies indicate that mayflies, similar to other aquatic insects, are sensitive to polarized light (positive polarotaxis), which peaks at around 450 nm–480 nm, allowing them to detect water and orient themselves [28,29]. Research also shows that mayflies can be attracted to cars with polarized surfaces. The attractiveness of black car surfaces to polarotactic insects may depend on the surface roughness (e.g., shiny, matte), mayfly species (e.g., *dolichopodids, tabanids*), and intensity [30]. Surprisingly, mayflies were more attracted to the matte dark grey car finish than cars with matte black finish. The study also suggested that the polarized light from black cars with specular surfaces cannot be mitigated using a matte paint finish [30].

Horizontally polarized light may elicit positive photo- and polarotaxis for mayflies. Research suggests that horizontally polarized light attracts mayflies more than unpolarized light, and vertically polarized light is the least attractive given that the optical radiation levels and spectral compositions are the same [31]. For example, mayfly *C. robusta* was attracted to vertically polarized light if light intensity was about two and five times higher

than unpolarized and horizontally polarized light sources, respectively. The polarotaxis may be explained by an evolutionary advantage to mayflies that enables them to differentiate water from vegetation. The open water surface reflects horizontally polarized light, while the shadow and mirror image of vegetation at the riparian zone (the edge of the water and land) reflect weak non-horizontal (mainly vertical) polarized light [31].

# 2.3. Bridge Lighting and Mayflies

Illuminated bridges may act as an optical barrier and attract mayflies to continue their upstream compensatory flights by heading towards the lights on a bridge. In a field study, the upstream-flying mayflies typically turned back when faced with a bridge, and 86% of the mayflies never crossed the bridge [32]. Mayflies' lack of physical contact with the bridge suggests that the bridge was an optical barrier rather than a mechanical one for the polarotactic mayflies. The authors used imaging polarimetry to examine how the bridge disrupted the horizontally polarized light guiding the flight of mayflies above the river. Energy loss and time constraints forced female mayflies to lay eggs only downstream [32].

In another study, 99% of adult females performed upstream compensatory flights and were attracted to the unpolarized light source at the bridge and away from the river's horizontally polarized light [33]. Imaging polarimetry analysis confirmed that the bridge's asphalt surface was strongly and horizontally polarized, providing a supernormal ovipositional cue to mayflies (*Ephoron virgo*). The researchers concluded that *Ephoron virgo* is attracted to both unpolarized and polarized light sources [33]. In a similar study, both male and female adults (*Rhithrogena semicolorat, Epeorus silvicola*) were attracted to bridges, but mostly to shiny black plastic sheets (high polarization, low brightness); only somewhat to matte black cloth (low polarization, low brightness); and not at all to white matte/shiny cloth or shiny aluminum (low polarization, high brightness) [34].

The direction of compensatory flight is known to be mostly upstream, facing the river's flow direction [35]. Therefore, illuminating a river from under the bridge facing the compensatory flight (in the direction of river flow) can guide females to lay eggs to the water and prevent them from dying in locations beyond riverside [36]. Attracting mayflies with electric lighting may alter the feeding habits of insectivorous animals, but this effect could be minimized by restricting the use of the lamp to mayfly non-swarming periods [36]. The research on mayfly activity suggests that the intensity of the light sources should be significantly higher than the average levels of the surrounding urban lighting, independent of the moonlight. Similarly, *E. virgo* females gathered in front of a bridge, were attracted to the urban lights due to the increased light intensity compared to the moonlit environments [33].

The body of knowledge indicates that light source characteristics (spectrum, intensity, polarization) have a significant impact on mayfly physiology and behavior. Coupled with knowledge from interventional field studies, it is likely that lighting design strategies can be applied to mitigate the unwanted effects of light at night on mayflies.

## 3. Methods

A series of major mayfly emergences at a bridge in Pennsylvania (PA) initiated this project to study the effects of light on mayfly activity. Building on previous research studies, we conducted a field study to test various lighting conditions and observe the effects on mayfly behavior. The study was conducted on the Veterans Memorial Bridge, which is a 2 km (1.25 mile) long concrete structure spanning the Susquehanna River in south-central PA (40°01′50″ N, 76°30′41″ W), connecting the municipalities of Columbia, Lancaster County on the eastern shore, and Wrightsville, York County on the western shore. Initially, the bridge featured lantern-style light fixtures that were appropriately scaled for pedestrians, carriages, and cars and had a design that integrated with the bridge's architecture. By the 1970s, the bridge's original fixtures had been replaced with modern streetlights consisting of "cobra head" style luminaires with refractive lenses and HPS lamps. These fixtures were arm-mounted at the top of tall aluminum poles and occupied a fewer number of piers

than the original fixtures. In 2014, a rehabilitation project was completed to replace the HPS light sources with LEDs, and the modern streetlights with (back to) historic-style lanterns.

Flowing underneath the bridge from north to south, the Susquehanna River provides a fertile breeding ground for a large population of mayflies every year, and they have been attracted to light sources at night, as shown in Figure 1 (left image). Historically, the mayflies in the vicinity of the Veterans Memorial Bridge have been a nuisance but never posed a major issue. However, in late spring and early summer of 2015, the year following the most recent light fixture replacement, a larger number of mayflies than usual was attracted to the new lights, as shown in Figure 1. With the life span of the adult mayfly typically being only 24 h, many spent carcasses began to pile up on the roadway and sidewalk. These piles of slick carcasses caused traction problems for vehicles while the swarms of live mayflies caused visibility issues, resulting in several vehicle crashes. This study was set up to isolate some of the variables associated with the existing lighting system on the Veterans Memorial Bridge, investigate different lighting elements that could potentially be used as part of a supplemental lighting system, and attempt to draw a correlation between the different characteristics of the electric lights and the behavior of the mayflies.



**Figure 1.** (**left**) Mayflies swarm at a light fixture along the riverbank near Veterans Memorial Bridge. (**right**) Accumulation of mayfly carcasses at bridge light fixture and concrete pier.

In order to isolate some of the variables related to the existing lighting on the bridge, different trial scenarios were temporarily installed on-site; data was collected, and observations were made during each trial. The data collection in the field took place in June, July, and August 2020, which is the primary time for mayflies to emerge from the river. Three locations (Spans 12, 20, and 28) separated by darkness were used to test the effect of different lighting conditions on the bridge in each trial, as shown in Figure 2.

The light source spectral power distributions were measured using a spectrometer (Asensetek Lighting Passport model no. ALP-01 Pro, serial no. 78620L20002AG, calibration no. AHZA04007). The illuminance values were measured using a NIST-calibrated Konica-Minolta T-10 illuminance meter (calibration no. 366281), and air temperature and relative humidity were recorded using a data logger (Onset HOBO MX temperature and relative humidity logger device). Water temperature was recorded by data loggers in the river (data provided by the Pennsylvania Department of Environmental Protection (PA DEP)); sky conditions, precipitation, and wind were noted as general observations.



**Figure 2.** Schematic of the Veterans Memorial Bridge shows the three spans (span 12, 20, and 28) used to test experimental lighting conditions. Sections between the test spans and at each end of the bridge were left unlit to differentiate the test locations.

To obtain the number of mayflies in the different spans at each trial, several processing steps were followed. The images were recorded using digital cameras by positioning them in three different test locations along each bridge. Images were extracted every ten minutes from the recorded video footage, as shown in the second step of Figure 3. ImageJ, an open-source image processing program for multi-dimensional image data, is built for a variety of needs of scientific imaging [37]. Due to its key function of image processing and analysis based on user-defined thresholds, ImageJ was used to develop an automated counting method analysis for processing and quantifying the number of mayflies in the images documented on-site.



**Figure 3.** A step-by-step data extraction method was utilized to count the number of mayflies (white dots in steps 2 and 3) in each trial.

The trials and the nominal characteristics of the lighting conditions in each trial are given in Table 1. The data from seven trials (CW1, CW2, CW3, CW4, CW5, CW6, and CW7) were collected across six non-consecutive days. Lights at the top of the bridge were the existing, permanently installed roadway luminaires in all cases, except where tape light was used in CW7. The conditions at Span 12 were held constant, while lighting conditions at the other spans were iteratively changed to systematically test the effect of different lighting characteristics, such as CCT, shielding, spectrum, and location. The spectral characteristics of the light sources used throughout the trials at the Veterans Memorial Bridge are shown in Figure 4.

		Baseline Condition	CW1	CW2	CW3	CW4	CW5	CW6	CW7
	Date	1 June 2020	25 June 2020	25 June 2020	26 June 2020	20 July 2020	23 July 2020	24 July 2020	5 August 2020
Span 12	Top of bridge	4800 K	4800 K	4800 K	4800 K	4800 K	4800 K	4800 K	4800 K <sup>1</sup>
	Shielding	-	-	-	-	-	-	-	-
	Underneath	-	-	-	-	-	-	-	-
Span 20	Top of bridge	4800 K	2200 K	2200 K	2200 K	2200 K	2200 K	2200 K	2200 K <sup>1</sup>
	Shielding	-	-	-	Yes <sup>6</sup>	-	-	-	-
	Underneath	-	-	-	-	-	-	-	-
Span 28	Top of bridge	4800 K	2200 K	2200 K	2200 K	2200 K	2200 K	2200 K	3000 K <sup>4</sup>
	Shielding	-	-	-	Yes <sup>6</sup>	-	-	-	Yes <sup>2</sup>
	Underneath	-	-	UV	-	Yes <sup>5</sup>	Yes <sup>3</sup>	-	-

**Table 1.** Lighting characteristics on top and under the bridge at each span for seven trials (from CW1 to CW7) and the reference baseline condition.

<sup>1</sup> Light intensity was reduced by turning off the light sources at the north side of the bridge; <sup>2</sup> Provided by parapet walls; <sup>3</sup> Green and blue polarized light; <sup>4</sup> Tape light along roadside of parapet wall; <sup>5</sup> Green light; <sup>6</sup> House sides of roadway luminaires were blocked with cardboard.



**Figure 4.** The normalized spectral power distribution of light sources used in the study (**a**) on the bridge: 4800 K (blue line, left graph, left axis), 2200 K (red line, left graph, left axis), and 3000 K (yellow line, left graph, left axis) white light sources; (**b**) under the bridge: blue light (blue line, right graph left axis) and green light (green line, right graph, left axis) lights, and ultraviolet radiation (purple line, right graph, left axis) are shown against the spectral sensitivity of the mayfly lateral eyes (black dotted line, right axes) (after Horridge et al. 1982 [17]).

# 4. Results

Since daily changes in environmental factors may contribute to measurement uncertainty (e.g., mayfly hatching), the absolute data were normalized to the mayfly appearance rate per day (total number of mayflies in one day divided by the number of time slots). The data from the site visits were analyzed step by step. At each step, different hypotheses were tested using appropriate statistical significance testing tools. The temporal mayfly activity as a function of time of day for each lighting solution is given in Figure 5.



**Figure 5.** The normalized number of mayflies attracted to the bridge under each test scenario: baseline reference condition of 4800 K light at Span 12 (black continuous line), 2200 K test condition at Spans 20 and 28 in trials CW 1, 2, 4, 5, and 6 (orange continuous line), 2200 K combined with shielding at Spans 20 and 28 in trial CW3 (grey continuous line), 2200 K on the bridge and UV radiation under the bridge at Span 28 in trial CW2 (grey dashed line), 2200 K on the bridge and green light under the bridge at Span 28 in trial CW4 (green continuous line), and 2200 K on the bridge and green-blue polarized light combination under the bridge at Span 28 in trial CW5 (blue dotted line).

A comparison of the average normalized number of mayflies as a function of temporal change indicates three trends. First, the peak mayfly activity is around 21:30–22:00 h regardless of the tested lighting condition, which seems consistent with the findings of another study [36]. However, other studies found a slightly earlier emergence time [29,33]. Interestingly, the data indicate a second, lesser peak activity time at about 23:00 in the baseline condition with 4800 K lighting and in some of the 2200 K conditions. Second, most of the tested lighting scenarios perform slightly better than the baseline lighting conditions at reducing mayflies, especially within the peak mayfly activity hours. Among all design trials, the greatest reduction of mayfly numbers is associated to the lighting condition with 2200 K correlated color temperature on the bridge and the green-blue polarized light under the bridge. Last, the variations in the number of mayflies across the total time range are relatively smaller for certain lighting conditions, such as 2200 K + green light and 2200 K + green light.

# 4.1. Normality

The data were tested using two widely used and recommended normality tests: Shapiro–Wilk [38] and d'Agostino–Pearson [39]. All the data were normally distributed, except Spans 12 and 20 in CW6 (Shapiro–Wilk p = 0.002 and p = 0.001, d'Agostino–Pearson p = 0.002 and p = 0.0001, respectively). Therefore, a series of parametric and non-parametric tests was used when the data were normal and non-normal in distribution, respectively.

#### 4.2. Baseline Condition and Different Days

In the second step, Span 12 trials from CW1 to CW6 were compared using a oneway analysis of variance (ANOVA) test [40]. In this case, there were no statistically significant differences between group means as determined by the one-way ANOVA test (F(5,51) = 1.67, p = 0.15).

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The parametric and non-parametric tests comparing the same lighting conditions at different nights (CW1 vs. CW6) for all spans, showed that there were no significant differences (Span 12: Mann–Whitney U = 35, z = -1.20, p = 0.11; Span 20: Mann–Whitney U = 44.5, z = -0.53, p = 0.30; Span 28: t(17) = 1.73, p = 0.09). The rest of the calculations were based on these findings of assumed similarity between conditions over different days.

# 4.3. Location on the Bridge

The potential bias that can be introduced by the position of the light sources on the bridge was investigated by comparing Span 20–Span 28 pairs in trials CW1, CW3, and CW6. A combination of parametric and non-parametric tests showed that there were no significant differences between the location of light sources on the bridge (between Span 20 and Span 28). The statistical significance testing resulted in CW1: t(10) = 1.82, p = 0.21 (effect size r = 0.25, Cohen's d = 0.48); CW3: t(16) = 1.75, p = 0.37 (effect size r = 0.08, Cohen's d = 0.16); and CW6: Mann–Whitney U = 83, z = -1.20, p = 0.23 (common language effect size f = 0.37) [41]. Since the comparisons were made within the same day, the absolute values were used in all three tests.

# 4.4. Correlated Color Temperature

The effect of CCT on mayfly behavior was analyzed using student's *t*-test in trials CW1, CW2, CW4, CW5, and CW6 between Span 12 and Span 20. Results showed that although the number of mayflies at Span 12 (2200 K) was lower compared to Span 20 (4800 K), there were no statistically significant differences between group means (t(51) = 1.46, p = 0.07).

# 4.5. Under-Bridge UV Radiation

Since there was no statistically significant difference between positions along the bridge (as established in step 3), the effect of the under-bridge lighting was analyzed by comparing Span 20 vs. Span 28 in CW2. The first under-bridge lighting condition was the UV radiation. Student's *t*-test results on the absolute data showed that there was a significant difference between conditions t(7) = 1.88, p = 0.03 with a large effect size r = 0.61. Results indicate that using UV radiation under the bridge reduced the number of mayflies on top of the bridge.

# 4.6. Cardboard Shielding

The effect of shielding using cardboard on the roadway luminaires was tested by comparing Span 20 in trial CW3 with Span 20 in CW6; and Span 28 in CW3 with Span 28 in CW6. Since data collected on different days were compared, normalized values were used in the student's *t*-test. Results indicate that there were no statistically significant difference in either condition t(21) = 0.56, p = 0.29 for CW3-CW6 Span 20, and t(21) = 1.72, p = 0.25 for CW3-CW6 Span 28.

# 4.7. Under-Bridge Green Light

Similar to step 5, the effect of under-bridge green light was analyzed by comparing the absolute number of mayflies at Span 20 with Span 28 in trial CW4. Student's *t*-test results indicate a statistically significant difference t(24) = 1.71, p = 0.04 with a small effect size r = 0.35. The results indicate that the number of mayflies on top of the bridge decreased when the green light was used under that specific bridge section (Span 28).

# 4.8. Under-Bridge Green/Blue Polarized Light

An analysis similar to step 5 was conducted to investigate the effect of polarized under-bridge light. The absolute number of mayflies was compared at Span 20 vs. Span 28 in trial CW5 using Student's *t*-test. There was a statistically significant difference between conditions t(14) = 1.76, p = 0.01 with a large effect size r = 0.57.

The difference between green (non-polarized) and green/blue polarized light was also analyzed by comparing Span 28 in CW4 vs. Span 28 in CW5. There was a statistically

significant difference as found by Student's *t*-test t(2) = 2.58, p = 0.01 with a large effect size r = 0.96, where CW4 (green light only) had a larger amount of mayflies on top of the bridge

compared to CW5 (green/blue polarized light). This indicates that green/blue polarized light was more effective in reducing the number of mayflies on top of the bridge. In addition, UV radiation and green/blue polarized light under the bridge were

compared (Span 28 in CW2 and CW5). A significant difference (t(17) = 1.74, p = 0.02 with a medium effect size r = 0.46) between these conditions highlight that green/blue polarized light was more effective at reducing the mayflies on top of the bridge than UV radiation.

# 4.9. Wall Shielding and 3000 K Tape Light

The effect of shielding and the use of 3000 K tape light at the parapet wall were analyzed by comparing Span 28 in trial CW6 and CW7 using the student's *t*-test. There was a statistically significant difference between conditions t(23) = 1.71, p = 0.02 with a medium effect size r = 0.43 when normalized values were used. This result suggests that shielding provided by parapet walls and a change in light source spectrum can reduce the number of mayflies on the bridge. In addition, results from this trial and previous shielding analysis suggest that shielding can be an effective solution only when it is combined with other measures.

# 4.10. Light Intensity

In the final trial (CW7), a tape light was installed at Span 28 on the roadway side of the parapet wall, while the roadway lights were turned off at that span. At Spans 12 and 20, the roadway light sources on the north side of the bridge were turned off to test the effect of light intensity. Span 12 in trials CW6 and CW7 were compared using the Mann–Whitney U test (Span 12 at CW6 is not normal distribution) and normalized values. No statistical significance was found (Mann–Whitney U = 93, z = 0.77, p = 0.22). The results indicate that light intensity did not have a significant impact on the number of mayflies.

#### 5. Discussion

The results suggest that placing light underneath the bridge can significantly reduce the number of mayflies on top of the bridge. Blue and green light and UV radiation all resulted in reductions, with blue and green polarized light leading to a greater reduction than non-polarized green light or UV radiation. While the results suggest that adding light to the underside of the bridge could be beneficial in reducing the number of mayflies on top of the bridge, doing so could have negative consequences, such as affecting other wildlife or causing an accumulation of mayfly carcasses on the light fixtures. If light fixtures are added underneath the bridge, negative effects should be taken into account by limiting the under-bridge lighting to certain times of the year (or hours of the night), locating and orienting the fixtures with the lens facing down to minimize build-up of dirt and insects on the lens, and performing regular maintenance on the under-bridge fixtures.

Results regarding the effect of light source CCT on mayfly activity were inconclusive. Although the 3000 K tape light in conjunction with the parapet wall shielding suggested a reduction in mayfly quantity, no significant difference was found between the conditions with the 2200 K roadway retrofit lights and original 4800 K LEDs. Based on visual observations during data collection, the intensity of mayfly swarms seemed to vary throughout a given night, at the different roadway lights at different times, without necessarily correlating to the CCT of the light source. However, the aftermath of the mayfly swarms seemed to indicate that overall, a greater number of mayflies were attracted to the light sources with a higher CCT. We observed that a higher number of mayfly carcasses accumulated at the Span 12 light fixtures (4800 K) over the study period as compared to the Span 20 and 28 fixtures (2200 K). During trial CW7, photos were taken of the roadway and sidewalk areas adjacent to the Span 12 and 20 light fixtures, as shown in Figure 6.



(a)

(b)

**Figure 6.** Photos of roadway and sidewalk near the base of the light fixtures on the piers of the Veterans Memorial Bridge: (a) at Span 12 (4800 K); (b) at Span 20 (2200 K) taken within minutes of each other during trial CW7. Visual observations suggested that more mayflies were attracted to the higher CCT light source (left image), despite no significant difference in statistical analysis between the trials. It should be noted that due to the car headlamps, the image on the right appears to be higher CCT, but the roadway luminaire in that image does indeed have the 2200 K light source.

The images shown in Figure 6 reflect the research team's observations—there appeared to be more mayflies on the piers, roadway, and sidewalk near the 4800 K fixtures than at the 2200 K fixtures. During trial CW7, the video camera that was typically located at Span 20 had been located at Span 28 to provide a second vantage point of data capture for the tape light installed at that location. With no video footage at Span 20, a comparison could not be made between Spans 12 and 20 for trial CW7. Therefore, the analysis for CCT was completed using data from other trials. As stated in the previous section, there was no statistically significant difference in mayfly attraction due to different CCTs.

It should be noted that a lack of significant difference due to CCT does not conclusively demonstrate that the spectrum of the light source has no effect. Firstly, CCT is a one-dimensional metric that is limited in quantifying the properties of light source spectrum. Also, the results of this field study may be limited due to the experimental design (e.g., selection of light sources) and environmental conditions, and it is possible that a significant result can be discovered with different experimental set up or different environmental conditions. For example, future studies can investigate a more narrowband (spikier spectrum) light source, as opposed to the warm white broader spectrum LED used in the retrofit kits in the current study. Narrow band, long-wavelength light source spectrum can help prevent sea turtles from disorienting, to ensure that they safely reach shore to lay eggs and the hatchlings return to the sea [42]. Future studies can utilize amber or orange light sources to verify if they have a similar non-attractive effect on mayflies. Use of the lower CCT (3000 K) tape light in conjunction with shielding by the parapet wall showed a statistically significant difference compared to the same bridge span with the 2200 K roadway lights on and no tape light. While the data analysis for the CCT change and cardboard shielding conditions did not show a significant difference, combining the two approaches indicate there might be a beneficial effect in reducing the number of mayflies.

The light source spectrum can also be tuned for mayfly spectral sensitivity. Since female mayflies, who have only lateral eyes, lead the compensatory flight, we hypothesize that it may be adequate to use LEDs with little emission in wavelengths where mayfly lateral eye sensitivity peaks (shown in Figure 4) to prevent mayflies from being attracted to the bridge. The data collected in this study provide partial evidence for this hypothesis. However, field or lab studies should be conducted to directly test the "tuning for lateral eye spectral sensitivity" hypothesis.

Since the illuminance on the roadway was held constant by request from project sponsor Pennsylvania Department of Transportation (PennDOT), the light intensity was not an independent variable in this study. However, during trial CW7, when the roadway lights were turned off at Span 28 to isolate the tape light mock-up, our research team discovered that the roadway fixtures on the north side of the bridge at Spans 12 and 20 were connected to the same circuit as the roadway lights at Span 28, and thus turned off at the same time. This provided an opportunity to briefly look at the effect of changing the total amount of light emitted by the roadway lights at a given span. While the analysis showed no significant difference, it is worthwhile to study radiation levels (e.g., radiance, irradiance) as a variable in the future. Following studies can also utilize lower light levels for the first few hours after sunset when mayfly activity is at its highest or using motion sensors to maintain low light levels when the road is unoccupied and increase the light levels when vehicles or pedestrians approach the bridge.

Another important finding was the effect of polarized light, which echoed studies conducted in Europe [28–35]. The results obtained here also suggest that mayflies are attracted to linearly polarized electric light sources, which mimic the horizontally polarized surface of the river. At the Veterans Memorial Bridge during trial CW5, the polarized light sources were lowered close to the river surface, on the south (downstream) side of the bridge, aimed downstream, and tilted up slightly. The hypothesis was, as the mayflies are traveling upstream (as is their natural tendency when emerging as adults) and encounter the polarized lights, they would be attracted to those lights as if they were the surface of the river; and as a result, would remain near the surface and not travel up to the top of the bridge.

The analysis of the data recorded at the top of the bridge showed a significant reduction in mayfly quantities on top of the bridge when the polarized light was used, compared to non-polarized light, UV radiation, and no under-bridge light at all. Although this suggests that having light fixtures with polarized lenses located as they were in the trial could reduce the number of mayflies on the bridge, care must be taken when introducing light fixtures near the river's surface. The fixtures and conduit should be very durable and watertight to resist high water levels, which would likely increase the cost of the system. Additionally, a lighting system close to the water surface could negatively affect other wildlife such as fish, birds, or bats. If such a system is implemented, the operation times should be limited to the hours of peak mayfly activity and only during the time of the year of mayfly emergence.

# 6. Conclusions

Electric outdoor lighting at night may have unwanted consequences for the ecological system. Here, the effect of bridge lighting on mayflies has been investigated in the field by testing several different lighting conditions. The use of under-bridge lighting (blue and green light, or UV radiation) significantly reduced the number of mayflies attracted to the lights on top of the bridge. When installed under the bridge, blue/green polarized light performed better at reducing the number of mayflies on top of the bridge compared to only green (non-polarized) light or UV radiation.

Although light source CCT did not have a significant impact, the effect of light source spectral power distribution on mayflies was apparent in other sections of the experiment. Since CCT is an incomplete metric with little relevance to mayfly vision, radiometric quantities (e.g., irradiance) should be used in the experimental design of future mayfly studies.

Similarly, shielding the light sources provided mixed results. When only sheets of cardboard were used for shielding, there were no significant effects. However, shielding the lights completely by locating the light source below the level of the parapet wall and changing the CCT resulted in a lower number of mayflies on the bridge. Very limited light intensity testing was conducted by turning off half of the roadway lights at two of the spans, which did not result in a significant difference from the all-lights-on fixture condition. It should also be noted that the effect of varying the radiance was not taken into account in this study since illuminance had to be held constant to meet roadway lighting requirements (maintained illuminance of minimum 2 lx at any point on roadway [43]). It is possible that absolute optical radiation levels will make a difference in mayfly activity.

While electric lighting may negatively impact certain species, light is needed for human activities and crucial for driving. Recommended lighting design practices can help control the negative effects of lighting on animals by reducing electric lighting where possible, limiting the extent, intensity, and duration of illumination, reducing blue-enriched light and UV, reducing light pollution and sky glow, and eliminating inefficient lighting technologies [8,44]. In addition to these recommendations, adaptive lighting systems based on SSL devices, sensors, and smart controls can be used to reduce the negative impacts to local fauna and energy consumption while maintaining minimum lighting for vision and safety.

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# References

- 1. Pawson, S.M.; Bader, M.-F. Led lighting increases the ecological impact of light pollution irrespective of color temperature. *Ecol. Appl.* **2014**, *24*, 1561–1568. [CrossRef]
- Durmus, D.; Davis, W. Appearance of achromatic colors under optimized light source spectrum. *IEEE Photonics J.* 2018, 10, 1–11. [CrossRef]
- 3. Durmus, D.; Davis, W. Object color naturalness and attractiveness with spectrally optimized illumination. *Optics Exp.* **2017**, *25*, 12839–12850. [CrossRef] [PubMed]
- 4. Vázquez, D.; Fernández-Balbuena, A.A.; Canabal, H.; Muro, C.; Durmus, D.; Davis, W.; Benitez, Z.; Mayorga, S. Energy optimization of a light projection system for buildings that virtually restores artworks. *Digital Appl. Archaeol. Cult. Heritage* **2020**, *16*, e00128. [CrossRef]
- Durmus, D. Impact of Surface Reflectance on Spectral Optimization for Melanopic Illuminance and Energy Efficiency. In Optical Devices and Materials for Solar Energy and Solid-State Lighting; Optical Society of America: Burlingame, CA, USA, 2019; p. PT2C-5. [CrossRef]
- Pattison, P.M.; Tsao, J.Y.; Krames, M.R. Light-emitting diode technology status and directions: Opportunities for horticultural lighting. In Proceedings of the VIII International Symposium on Light in Horticulture, East Lansing, MI, USA, 22–26 May 2016; pp. 413–426.
- Durmus, D. Real-Time Sensing and Control of Integrative Horticultural Lighting Systems. J. Multidiscip. Sci. J. 2020, 3, 266–274. [CrossRef]
- 8. Jägerbrand, A.K. Synergies and Trade-Offs Between Sustainable Development and Energy Performance of Exterior Lighting. *Energies* **2020**, *13*, 2245. [CrossRef]
- 9. Jägerbrand, A.K. New framework of sustainable indicators for outdoor LED (light emitting diodes) lighting and SSL (solid state lighting). *Sustainability* **2015**, *7*, 1028–1063. [CrossRef]
- 10. Balian, E.; Lévêque, C.; Segers, H.; Martens, K. Freshwater animal diversity assessment. In *Developments in Hydrobiology*; Springer: Dordrecht, The Netherlands, 2008; Volume 198, pp. 339–350. [CrossRef]
- 11. Brittain, J.E. Biology of mayflies. Annu. Rev. Entomol. 1982, 27, 119–147. [CrossRef]
- 12. Brittain, J.E. Experimental studies on nymphal growth in leptophlebia vespertina (l.)(ephemeroptera). *Freshw. Biol.* **1976**, *6*, 445–449. [CrossRef]
- 13. Langford, T.; Daffern, J. The emergence of insects from a british river warmed by power station cooling-water. *Hydrobiologia* **1975**, 46, 71–114. [CrossRef]
- 14. Fremling, C.R. Mayfly Distribution as a Water Quality Index; US Government Printing Office: Washington, DC, USA, 1970; pp. 5–28.
- 15. Fremling, C.R. Mayfly distribution indicates water quality on the upper Mississippi river. *Science* **1964**, 146, 1164–1166. [CrossRef] [PubMed]
- 16. Horridge, G.A.; McLean, M. The dorsal eye of the mayfly Atalophlebia (Ephemeroptera). *Proc. R. Soc. Lond. B Biol. Sci.* **1978**, 200, 137–150.
- 17. Horridge, G.A.; Marčelja, L.; Jahnke, R. Light guides in the dorsal eye of the male mayfly. *Proc. R. Soc. Lond. B Biol. Sci.* **1982**, *216*, 25–51.
- 18. Hughes, D. On the dorsal light response in a mayfly nymph. Anim. Behav. 1966, 14, 13–16. [CrossRef]
- 19. Heise, B.A. Sensitivity of mayfly nymphs to red light: Implications for behavioural ecology. *Freshw. Biol.* **1992**, *28*, 331–336. [CrossRef]
- 20. Barmuta, L.A.; Mckenny, C.E.; Swain, R. The responses of a lotic mayfly nousia sp.(ephemeroptera: Leptophlebiidae) to moving water and light of different wavelengths. *Freshw. Biol.* **2001**, *46*, 567–573. [CrossRef]
- 21. Johansson, J.; Nyström, P. Effects of ambient UV-radiation on the behaviour of mayfly larvae of the genus deleatidium from trout bearing and fishless streams in new zealand. *Arch. Hydrobiol.* **2004**, *161*, 403–415. [CrossRef]
- 22. Elliott, J. The daily activity patterns of mayfly nymphs (ephemeroptera). J. Zool. 1968, 155, 201–221. [CrossRef]
- 23. Scherer, E. Phototaxtisches Verhalten von Fliesswasser-Insektenlarven. Nuturwissenschaften 1962, 49, 477–478. [CrossRef]
- 24. Flecker, A.S. Fish predation and the evolution of invertebrate drift periodicity: Evidence from neotropical streams. *Ecology* **1992**, 73, 438–448. [CrossRef]
- 25. Hughes, D. The role of responses to light in the selection and maintenance of microhabitat by the nymphs of two species of mayfly. *Anim. Behav.* **1966**, *14*, 17–33. [CrossRef]
- 26. Schloss, A.L. A laboratory system for examining the influence of light on diel activity of stream macro-invertebrates. *Hydrobiologia* **2002**, *479*, 181–190. [CrossRef]

- 27. Schloss, A.L.; Haney, J.F. Direct observations of the activity responses of mayfly nymphs to relative light change and light intensity. *Arch. Hydrobiol.* **2002**, *154*, 353–374. [CrossRef]
- 28. Schwind, R. Spectral regions in which aquatic insects see reflected polarized light. J. Comp. Physiol. A 1995, 177, 439–448. [CrossRef]
- 29. Kriska, G.; Bernáth, B.; Horváth, G. Positive polarotaxis in a mayfly that never leaves the water surface: Polarotactic water detection in palingenia longicauda (ephemeroptera). *Naturwissenschaften* **2007**, *94*, 148–154. [CrossRef]
- 30. Blaho, M.; Herczeg, T.; Kriska, G.; Egri, A.; Szaz, D.; Farkas, A.; Tarjanyi, N.; Czinke, L.; Barta, A.; Horvath, G. Unexpected attraction of polarotactic water-leaving insects to matt black car surfaces: Mattness of paintwork cannot eliminate the polarized light pollution of black cars. *PLoS ONE* **2014**, *9*, e103339. [CrossRef]
- 31. Farkas, A.; Száz, D.; Egri, Á.; Barta, A.; Mészáros, Á.; Hegedüs, R.; Horváth, G.; Kriska, G. Mayflies are least attracted to vertical polarization: A polarotactic reaction helping to avoid unsuitable habitats. *Physiol. Behav.* **2016**, *163*, 219–227. [CrossRef]
- Málnás, K.; Polyák, L.; Prill, E.; Hegedüs, R.; Kriska, G.; Dévai, G.; Horváth, G.; Lengyel, S. Bridges as optical barriers and population disruptors for the mayfly palingenia longicauda: An overlooked threat to freshwater biodiversity? *J. Insect Conserv.* 2011, 15, 823–832. [CrossRef]
- Szaz, D.; Horvath, G.; Barta, A.; Robertson, B.A.; Farkas, A.; Egri, A.; Tarjanyi, N.; Racz, G.; Kriska, G. Lamp-lit bridges as dual light-traps for the night-swarming mayfly, ephoron virgo: Interaction of polarized and unpolarized light pollution. *PLoS ONE* 2015, 10, e0121194. [CrossRef]
- 34. Kriska, G.; Horváth, G.; Andrikovics, S. Why do mayflies lay their eggs en masse on dry asphalt roads? Water-imitating polarized light reflected from asphalt attracts ephemeroptera. *J. Exp. Biol.* **1998**, 201, 2273–2286. [CrossRef]
- 35. Flecker, A.S.; Allan, J.D. Flight direction in some rocky mountain mayflies (ephemeroptera), with observations of parasitism. *Aquat. Insects* **1988**, *10*, 33–42. [CrossRef]
- 36. Egri, Á.; Száz, D.; Farkas, A.; Pereszlényi, Á.; Horváth, G.; Kriska, G. Method to improve the survival of night-swarming mayflies near bridges in areas of distracting light pollution. *R. Soc. Open Sci.* **2017**, *4*, 171166. [CrossRef]
- 37. Schindelin, J.; Arganda-Carreras, I.; Frise, E.; Kaynig, V.; Longair, M.; Pietzsch, T.; Rueden, C.; Saalfeld, S.; Schmid, B.; Tinevez, J.Y.; et al. Fiji: An open-source platform for biological-image analysis. *Nat. Methods* **2012**, *9*, 676–682. [CrossRef]
- 38. Shapiro, S.S.; Wilk, M.B. An analysis of variance test for normality (complete samples). Biometrika 1965, 52, 591-611. [CrossRef]
- 39. D'Agostino, R.B.; Belanger, A.; D'Agostino, R.B., Jr. A suggestion for using powerful and informative tests of normality. *Am. Stat.* **1990**, *44*, 316–321.
- 40. Howell, D.C. Statistical Methods for Psychology, 8th ed.; Cengage Learning: Belmont, CA, USA, 2009; pp. 325–368.
- 41. Nachar, N. The Mann-Whitney U: A test for assessing whether two independent samples come from the same distribution. *Tutor. Quant. Methods Psychol.* **2008**, *4*, 13–20. [CrossRef]
- 42. Witherington, B.E.; Bjorndal, K.A. Influences of wavelength and intensity on hatchling sea turtle phototaxis: Implications for sea-finding behavior. *Copeia* **1991**, *4*, 1060–1069. [CrossRef]
- Pennsylvania Department of Transportation. Publication 13M (DM-2) Design Manual; Chapter 5: Lighting; Commonwealth of Pennsylvania: USA. Available online: https://www.dot.state.pa.us/public/Bureaus/design/PUB13M/Chapters/Chap05.pdf (accessed on 18 May 2021).
- Hermoso-Orzáez, M.J.; Gago-Calderón, A.; Rojas-Sola, J.I. Power quality and energy efficiency in the pre-evaluation of an outdoor lighting renewal with light-emitting diode technology: Experimental study and amortization analysis. *Energies* 2017, 10, 836. [CrossRef]