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Effects of temporal variation on ambient light in Northwest Arkansas

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By

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Abstract:

An animal's life success is determined solely by its fitness, which makes choosing a mate one of its most important life decisions. Natural selection plays a big part in an animal's phenotype, but so does sexual selection. Even though females are usually thought to be the choosier sex, in many species or seasons males are also choosy. Male mate preference is an understudied topic compared to female mate preference and therefore, even less is known about the outcomes of a male's prior mating experience's influence on future mating experiences. Therefore, I dove deeper into this topic with the highly studied species of butterfly, *Bicyclus anynana. Bicyclus anynana* males have shown a predisposed preference for females with no UV-reflective spots as opposed to two UV-reflective spots on their hindwings. Given this preference, I designed a full factorial experiment to test how males allocate spermatophore to the preferred (0 spot) and unpreferred (2 spot) female phenotypes in a second mating. Male *B. anynana* have a cryptic preference, meaning they give a more attractive female a higher quality spermatophore, which she uses to lay more eggs. Therefore, male preference was to be assessed by the number of eggs laid by the second mated female. However, COVID-19 interrupted these plans. Due to the restrictions and shutdowns, I discontinued this project and switched to analyzing the variation in ambient light observed when butterflies make mate choice decisions in field conditions. Light measurements were collected at three sites in Northwest Arkansas over three years (2018-2020), from May to November in the morning, noon, and evening. These data were tested to assess whether time of day, site, season, and year had an effect on the total amount of light available,

wavelength of peak intensity, total amount of UV light available, UV peak intensity, and wavelength of UV peak intensity. I found an effect of time of day on amount of light, with noon light environments receiving the greatest amount light and morning light environments receiving greater amounts of light than evening light environments. I also found an effect of season, as there was a decrease in amount and variation of light as the year went on. Lastly, I found an interactive effect of time of day and month, as noon light environments decreased in brightness and morning light environments increased in brightness as the year progressed. These findings suggest daily and seasonal changes in light could serve as drivers of animal behavior change.

Introduction

An animal's life success is determined solely by its fitness, which makes choosing a mate one of the most important decisions of its life. Natural selection plays a big part in an animal's phenotype, but so does sexual selection. Reproduction includes surviving long enough to reach maturity and attracting the opposite sex.

Mate preference differs on a number of different parameters from appearance, age, pheromones, auditory cues, behaviors, and season (Liu et al., 2010)(Ryan, 1992)(Nieberding et al., 2012). Which sex has the stronger preference and is more choosy is another factor. Males or females can be the choosy sex in different species and in some cases even in different seasons (Prudic, Jeon, Cao, & Monteiro, 2011). Knowing the preferences of both males and females of a population can help predict trends and changes in phenotype.

Not much is known about male preference and resource allocation after a first mating in most animal species, including most insects. One study on male mate preference in the cricket, *Gryllus bimaculatus,* showed males did not show a preference in the first mating, but became more choosy as they continued to mate (Bateman & Fleming, 2006). These results suggest that males may not have enough resources to allocate as they continue mating, and therefore start to choose higher quality females over time. However, this experiment did not assess the effect of mating on males that have an innate preference, and only tested the patterns of a SLSL (small, large, small, large) and LSLS (large, small, large, small) body types, instead of testing the same phenotype twice.

The male butterflies of *Pieris rapae* and *Pieris brassicae* give more insight on the effect of experience on preference by examining successive ejaculates from polyandrous and monandrous males. It was found that monandrous males were limited in nutritious ejaculates and polyandrous males successively had high protein content and quality in nuptial gifts (Bissoondath & Wiklund, 1996). Another study took these findings a step further to relate mating systems to the protein content inside the spermatophore. These results found that increased degree of polyandry in pierid and satyrid male butterflies produced higher quality spermatophore, including higher protein content and higher ejaculate mass (Bissoondath & Wiklund, 1995). These studies further show the value of nuptial gifts to females and therefore making it a costly gift for monandrous males who have been shown to have a limit on the quality of their spermatophore. This would provide support to the hypothesis that males will become choosier as they continue to mate.

Bicyclus anynana (Figure 1A) make a perfect test subject to assess whether male mate preference is influenced by a prior mating experience because they are a highly studied species with a known predisposed male preference. *Bicyclus anynana* alternate by season on which sex is the choosy sex (Prudic et al., 2011). In the wet season, resources are plentiful so females become the choosier sex. In the dry season, resources are limited so females use males' spermatophore as a meal due to its high nutrition (Karlsson, 1998) (Prudic et al., 2011). This makes nuptial gifts costly for the males and desired by the females, which results in male preference in the dry season.

Just like in many other species, extensive studies have been done on female preference in B. *anynana*, but male preference is largely unexplored (Robertson & Monteiro, 2005)(Prudic et al., 2011)(Westerman & Monteiro, 2013). A previous study showed that male *Bicyclus anynana* have a cryptic sexual preference, meaning they will mate with the females regardless of phenotype, but will give more spermatophore to the female that they find more attractive (no UV-reflective spots), which subsequently lay more eggs (Siebemorgen, in prep). However, as males in this species mate multiply, it is unclear if this cryptic preference is maintained over time and across multiple matings.

There are three most likely possible outcomes of this experiment. One, the first mating does affect the second mating. In this case, results would show the preferred phenotype introduced second (2 spot, 0 spot) laying more eggs than the preferred phenotype introduced twice (0 spot, 0 spot). Another result would be the unpreferred phenotype introduced second (0 spot, 2 spot) laying fewer eggs than

the unpreferred phenotype introduced twice (2 spot, 2 spot). A second outcome is that the first mating has no effect on the second mating. In this case the preferred phenotype females would lay more eggs than the unpreferred, regardless if the preferred phenotype was presented first, second, or twice. A third outcome is that all of the treatments will result in the same average number of eggs laid, suggesting males do not show preferences for a certain phenotype in a second mating. Any of these outcomes contribute new insight to preference of polyandrous male *B. anynana* and give insight to how males partition resources after a prior mating experience.

Materials and Methods

Study animal and husbandry

Bicyclus anynana is a sub-tropical African Nymphalid butterfly species that has been reared in laboratory conditions since 1988 (Westerman & Monteiro, 2013). The population at the University of Arkansas was started from thousands of eggs from a population in Singapore in 2017. The adult butterflies' diet consists of mashed bananas, while the caterpillars feed on young *Zea mays* plants. Butterflies are kept in a climate controlled greenhouse at 27˚C, 80% humidity, and 13:11 light:dark cycle. *B. anynana* is seasonally polyphonic in morphology and behavior, and these conditions ensure a wet season butterfly. All butterflies were kept in large (0.6096 x 0.6096 x 1.2192 meters) pop-up, net cages where rearing, mating, and feeding takes place.

Experimental design

This experiment tested male mate preference of female hindwing UV-reflective spots after a first mating. Four treatment groups were set up. In the first treatment a male was mated with the preferred phenotype twice (0 spot, 0 spot). The second treatment, a male was mated with the preferred phenotype first and the unpreferred phenotype second (0 spot, 2 spot). The third treatment, a male was mated with the unpreferred phenotype first and the preferred phenotype second (2 spots, 0 spots). The fourth treatment, a male was mated with the unpreferred phenotype twice (2 spot, 2 spot). All females used in this experiment were 2 spot females, and all females received testers enamel glossy black 1147 paint on their hindwings to eliminate any preference for paint or effect on female behavior. The female's wings were either painted to cover the UV-reflective spots (0 spot females) or painted next to the UV-reflective spots (2 spot females) on their hindwings. Females were painted as 2-day old females and given a day to rest before mating. Since all females were the same and only painted to look different, the only difference among the females were the manipulated hindwings.

Immediately following emergence, males were put in their own isolated cage with plastic barriers around the net cage to prevent males from learning mating preferences from other males. A three-day old painted female was put in a three-day old male's cage and given 8-10 hours for copulation. After the first mating, the first mated females were taken out of the cage and frozen for future analysis. Males were then given two days until the second copulation with another three-day old female. After the second mating, the male was taken out of the cage and frozen for future analysis, while the female was given a corn plant for egg laying (Figure 1B) and a

mashed banana for nutrition. Females were given a week to lay eggs, and then eggs were counted in order to observe any effect on the number of eggs laid.

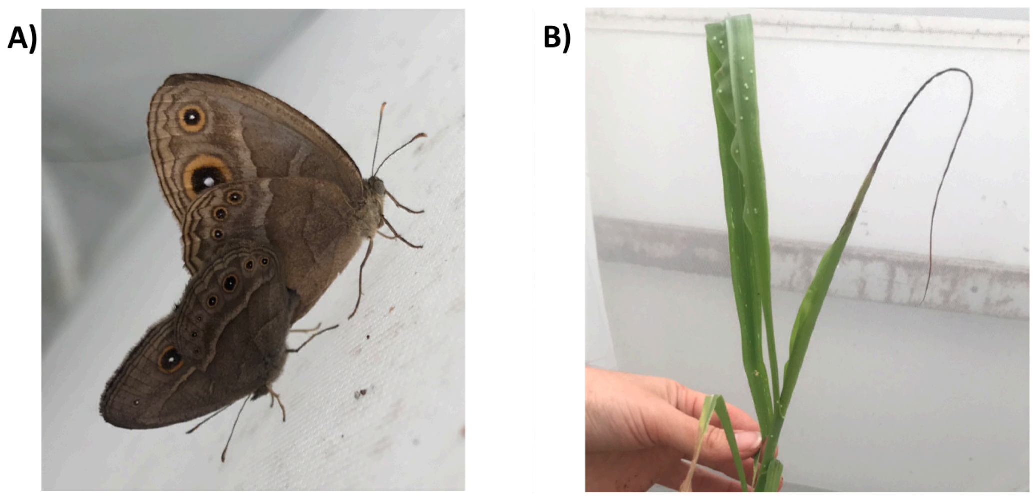


Figure 1: A) *B. anynana* male and female during copulation. **B)** *B. anynana* eggs laid on a corn plant (photos by Anderson).

Egg laying data from 20 females per treatment was used in this study. First mated females were frozen and those eggs were not counted.

Ethical Note

B. anynana population in the lab was maintained by the guidance of USDA-APHIS Permit #P526P-17-00343 and #P526P-20-00417. All caterpillars were given corn plants *ad libitum* to feed on and all butterflies were given a mashed banana and dampened cotton to receive water *ad libitum*. All butterflies used in this experiment were disposed of humanely either by natural death or by freezing. Frozen butterflies were placed in individual glassine envelopes and placed in a -30 degree C freezer for future analysis.

Statistical Analysis

Analysis was done to compare the results of the number of eggs counted from the second mating, using either an ANOVA (parametric) followed by a Tukey Test or a Kruskal-Wallis test (nonparametric) followed by a Steel-Dwass Test.

--------And then, COVID-19 happened-------

The first data point was about to be collected when the University of Arkansas shutdown due to COVID-19. Therefore, I discontinued this project and switched to analyzing the variation in ambient light used when butterflies make mating choice decisions in nature.

Light Measurement Collection

This project analyzed irradiance data taken from Chesney Prairie and Stump Prairie in Siloam Springs, and Woolsey Prairie in Fayetteville, measured for three years and still ongoing (2018, 2019, 2020) from May to November. Irradiance measurements were taken every two weeks 1 hour after sunrise, 1 hour before sunset, and at solar noon from May to November in 2018 and August to November in 2019 and 2020. In May through July in 2019 and 2020, data points were only taken at solar noon. Data collection was altered after 2018 due to the observation of wing color change in *Junonia coenia* (the common buckeye butterfly), which was the focal species of the larger study. At each site there were five sampling points. At each sampling point, three irradiance measurements were taken using an Ocean Optics Jaz Spectrometer, which were then averaged using a pipeline in R. This

resulted in a sample size of 3,057 measurements over three years. Data were analyzed to statistically test for an effect of time of day, site, season, and year on amount of light available, wavelength of peak intensity, amount of UV light available, UV peak intensity and wavelength of UV peak intensity.

Data Processing

I began analysis of the data by sorting and renaming excel data sheets in order for all of the files to follow a standard naming format as well as compiling all data into an accessible folder to make it easier to input and analyze in R Studio. R version 3.6.3 and R Studio version 1.2.5033 were used for analyzing data.

I began analyzing data in R Studio by running a pipeline to read and compile the data into a large, analyzable data frame. For this code, I used multiple R packages. I downloaded *readr* to help read data. I used the library *data.table* to organize data sets, and libraries *hyperSpec* and *colorSpec* to help read and analyze the data with spectral properties from the spectrometer. Next, I used *photobiology*, *photobiologyWavebands*, and *photobiologyInOut* to read data from spectrometers and calculate irradiances, fluence rates, transmittance, reflectance, absorptance, and absorbance of spectral data. I used the library *pavo* to analyze and organize color from spectral data. I used *knitr* as a general-purpose tool for dynamic report generation. The libraries *dplyr*, *tidyr*, *stringr*, and *lubridate* were added to help manipulate data. I used *dplyr* for shortcuts for subsetting, summarizing, rearranging, and joining data sets. *Tidyr* includes tools that help change the layout of data sets. *Stringr* includes tools for regular expressions and character strings. *Lubridate*

includes tools that make working with dates and times easier. I added *ggplot2* to help visualize data by making graphs, and used *ggspectra* for additional annotations, stats and scales for plotting light spectra with *ggplot2*.

Using these libraries, I averaged the data sample points and created a loop to extract five variables from 3,057 different files to make one large data set. The five variables included mean area under the curve, mean wavelength of peak intensity, mean area under the curve for UV light (wavelengths 300nm-400nm), mean UV peak intensity and mean wavelength of UV peak intensity (Figure 2). The area under the curve represented the total amount of light available. The wavelength of peak intensity corresponded to the most prominent wavelength (color) of light present when the light measurement was taken. The area under the curve for UV light represented the amount of UV light presented at the time and place of the data point. The UV peak intensity showed the maximum amount of UV light energy at a given point. Lastly, the wavelength of UV peak intensity represented the most prominent wavelength of UV light at the given time. I used this data for the following statistical analyses.

Statistical Analyses

I assessed the effect of each individual independent variable (time, season, year, and site) on the amount of light, wavelength of peak intensity, amount of UV light, UV peak intensity and wavelength of peak intensity of UV light. Due to a difference in the collection of data, I separated the data into two data frames, one

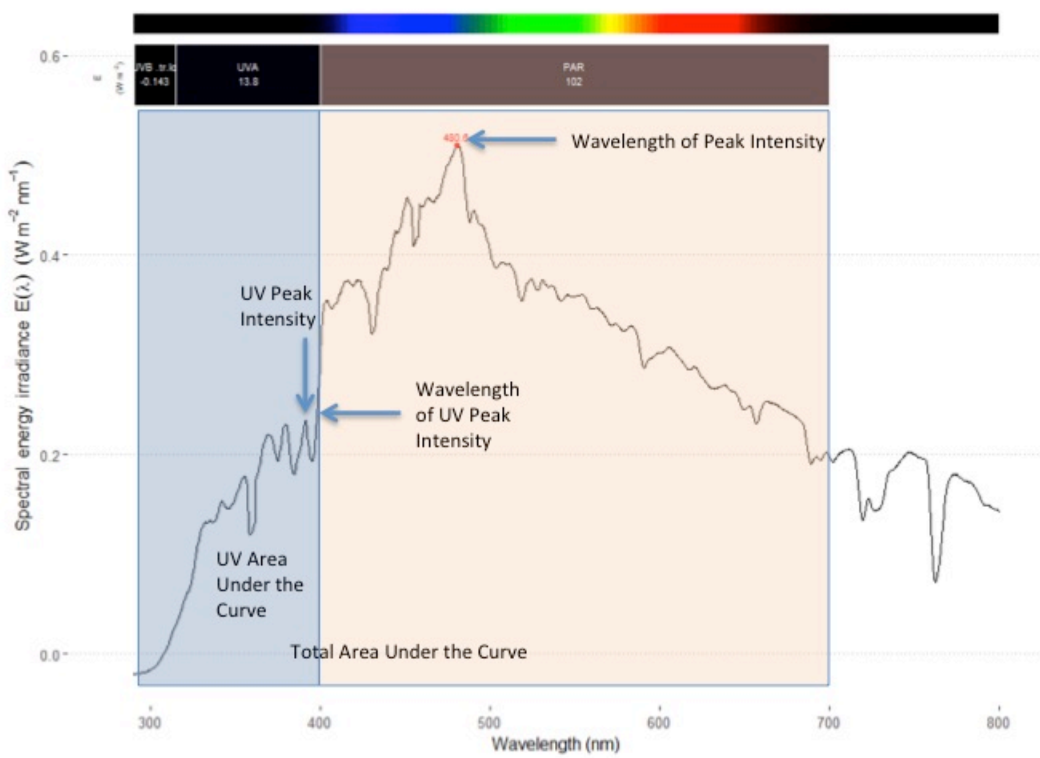


Figure 2. Spectrum with area under the curve, wavelength of peak intensity, UV area under the curve, UV peak intensity, and wavelength of UV peak intensity labeled. Light spectra courtesy of G.E. Hirzel.

that extracted data taken from 2018 only and one that extracted data points only taken at noon. I used the 2018 data frame to test the effects of time of day on amount of light, and used the noon data frame to test the effects of site, month, and year on amount of light. I first used a histogram and a Shapiro-Wilk test to check the distribution under the curve in order to identify whether the data were normally distributed. Since the data were not normally distributed, I performed Kruskal-Wallis tests to test whether there was a difference between time of day, season, year, or location on amount of light, wavelength of peak intensity, amount of UV light, UV peak intensity and wavelength of peak intensity of UV light. If a difference was found of the independent variable on the dependent, I performed a post-hoc Steel-Dwass test to determine which components of the independent variables were different.

In addition, I ran generalized linear models to look for a combinatorial effect or interactions of time of day, location, season, and year on the amount of light, wavelength of peak intensity, amount of UV light, UV peak intensity and wavelength of UV peak intensity. Two generalized linear models were run for each dependent variable, one using the 2018 data frame to test interactions between time of day, site, and month and the other using the noon data frame to test interactions between site, month, and year.

Results

Data Were Not Normally Distributed

Total amount of light, wavelength of peak intensity, total amount of UV light, UV peak intensity, and wavelength of UV peak intensity all were not normally distributed (Table 1, Supplemental Figure 1).

Effect of Time of Day on Light Environment

There was an effect of time of day on total amount of light (Kruskal-Wallis, N= 443, df=2, χ^2= 376.9, p-value= 1.438e-82). Morning, noon, and evening all had a distinct amount of light with noon receiving the most and morning receiving more light than evening (Table 2, Figure 3, Figure 4.a). There was a statistical effect of time of day on wavelength of peak intensity (Kruskal-Wallis, N= 443, df=2, χ^2= 24.94, p-value= 3.849e-06). There was a difference in wavelength of peak intensity between evening (average= 511.7nm) and noon (average= 482.4nm), indicating evening contained more green/yellow light than noon which contained more blue light, and between morning (average= 483.2nm) and noon, but not between morning and evening (Table 3, Figure 4.b). There was an effect of time of day on total amount of UV light (Kruskal-Wallis, N= 443, df= 2, χ^2= 298.3, p-value=3.222e-87). Morning, noon, and evening all received a distinct amount of UV light with noon receiving the most and morning receiving more UV light than evening (Table 4, Figure 4.c). There was also an effect of time of day on UV peak intensity (Kruskal-Wallis, N= 443, df= 2, χ^2= 387.5, p-value= 7.134e-85). Morning, noon, and evening all had a distinct UV peak intensity with noon receiving the highest and morning receiving higher UV peak intensities than evening (Table 5, Figure 4.d). There was

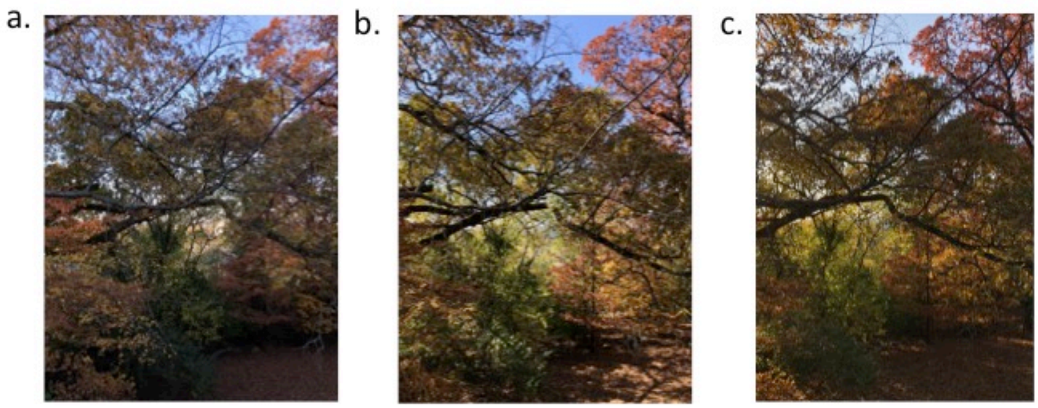


Figure 3. Pictures taken of light environment (a.) one hour after sunrise, (b.) at solar noon, and (c.) one hour before sunset at 36.0644˚N, 94.1461˚W on November 5, 2020.

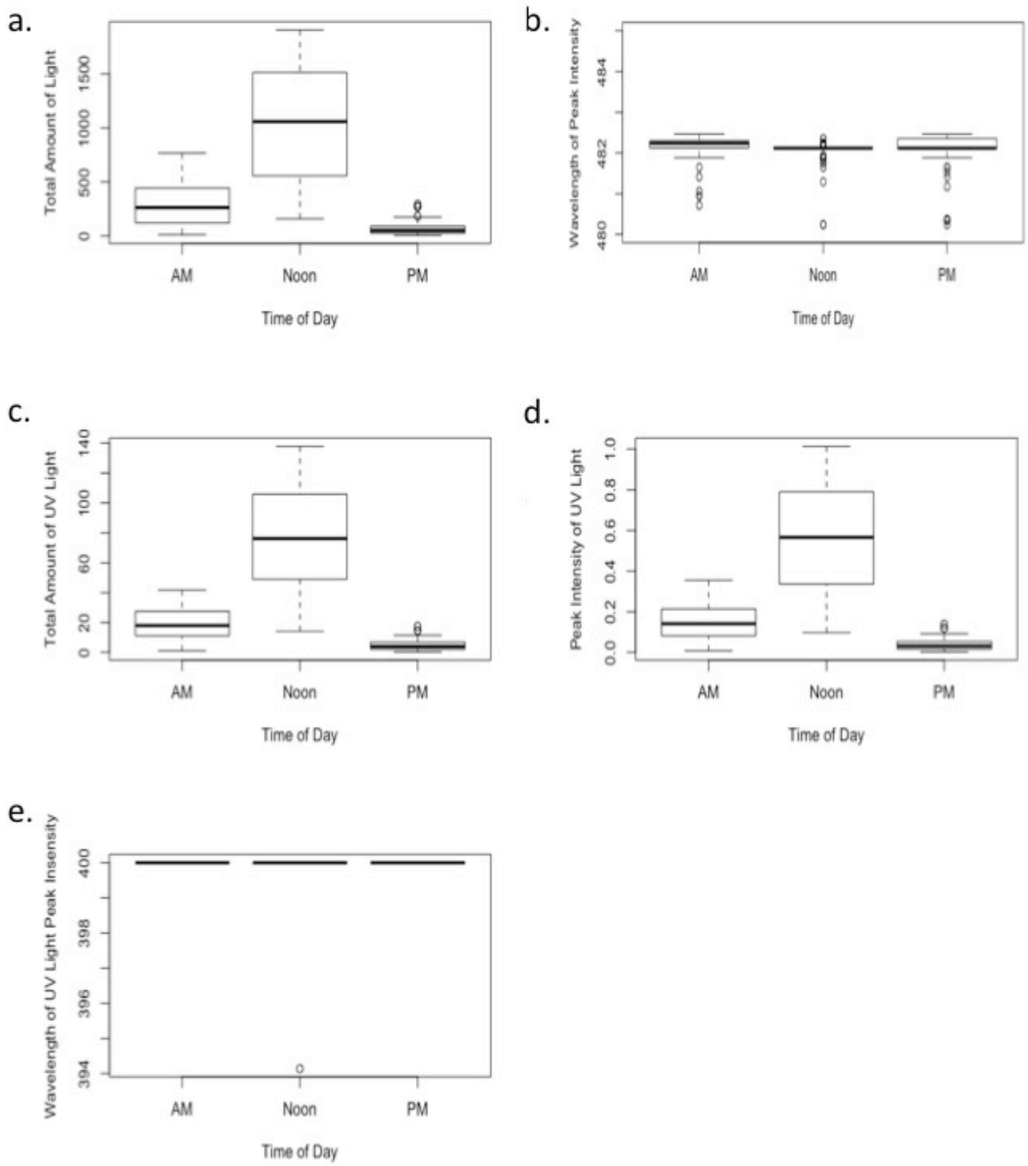


Figure 4. There is an effect of time of day on (a.) total amount of light, (b.) wavelength of peak intensity, (c.) total amount of UV light, and (d.) peak intensity of UV light, but there is not an effect of time of day on (e.) wavelength of UV peak intensity.

not an effect of time of day on wavelength of UV peak intensity (Kruskal-Wallis, N= 443, df= 2, χ^2= 1.973, p-value= 0.3729, Figure 4.e).

No Effect of Site on Light Environment

There was no effect of site on the total amount of light (Kruskal-Wallis, N= 354, df= 2, χ^2= 6.32, p-value= 0.04243, Figure 5.a); wavelength of peak intensity (Kruskal-Wallis, N= 354, df= 2, χ^2= 5.483, p-value= 0.06450, Figure 5.b); total amount of UV light (Kruskal-Wallis, N= 354, df= 2, χ^2= 7.043, p-value= 0.02956, Figure 5.c); UV peak intensity (Kruskal-Wallis, N= 354, df= 2, χ^2= 8.234, p-value= 0.01630, Figure 5.d); or wavelength of UV peak intensity (Kruskal-Wallis, N= 354, df= 2, χ^2= 3.139, p-value= 0.2081, Figure 5.e).

Effect of Month on Light Environment

There was an effect of month on the total amount of light (Kruskal-Wallis, N= 354, df= 6, χ^2= 20.15, p-value= 0.002601). A difference in amount of light was found between October and November with October receiving more light than November and between September and July with July receiving more light than September (Table 2, Figure 6, Figure 7.a). There was a statistical effect of month on the wavelength of peak intensity (Kruskal-Wallis, N= 354, df= 6, χ^2= 30.88, p-value= 2.67e-05). Statistically distinct wavelengths of peak intensities were found between October and August, between October and July, between October and June, between October and November, and between September and October (Table 3, Figure 7.b). However, when the wavelengths of peak intensity for each month were averaged,

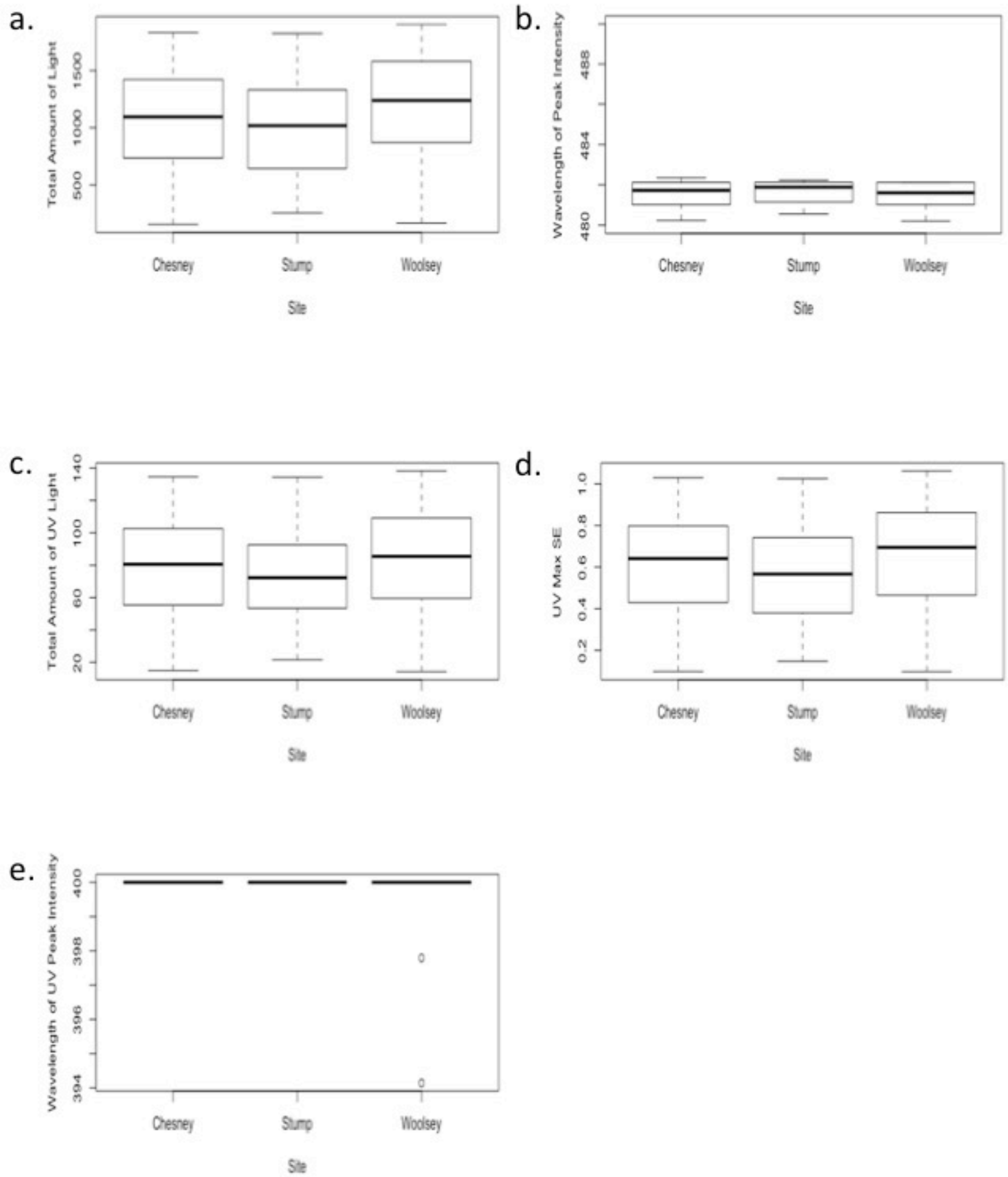


Figure 5. There was not an effect of site on (a.) total amount of light, (b.) wavelength of peak intensity, (c.) total amount of UV light, (d.) UV peak intensity, nor (e.) wavelength of UV peak intensity.

there did not seem to be a biological significance, with the shortest average wavelength of 481.4nm in July and the longest average wavelength of 482.0nm in October. There was also an effect of month on the total amount of UV light (Kruskal-Wallis, N= 354, df= 6, χ^2= 40.38, p-value= 3.83e-07). Distinct amounts of UV light were found between September and May with more UV light received in May, between October and November with more UV light received in October, and between September and July with July receiving more UV light (Table 4, Figure 7.c). There was an effect of month on the UV peak intensity (Kruskal-Wallis, N= 354, df= 6, χ^2= 27.62, p-value= 1.108e-04). Distinct UV peak intensities were found between September and July with July receiving a higher UV peak intensity (Table 5, Figure 7.d). Lastly, there was not an effect of month on the wavelength of UV peak intensity (Kruskal-Wallis, N= 354, df= 6, χ^2= 5.468, p-value= 0.4853, Figure 7.e).

Effect of Year on Light Environment

There was an effect of year on wavelength of peak intensity (Kruskal-Wallis, N= 354, df= 2, χ^2= 240.6, p-value= 5.751e-53). The years 2018, 2019, and 2020 all had statistically distinct wavelengths of peak intensity, but are probably not biologically significant (Table 3, Figure 8.b). The year of 2018 received an average wavelength of peak intensity of 482.4nm, 2019 received an average wavelength of peak intensity of 481.3nm, and 2020 received an average wavelength of peak intensity of 481.1nm. There was an effect of year on UV peak intensity (Kruskal-Wallis, N= 354, df= 2, χ^2= 11.63, p-value= 0.002985). Distinct UV peak intensities

a.

b.

c.

Figure 6. Pictures taken in (a.) June, (b) July, and (c.) October at Stump location 36.205007˚ N, 94.495596˚ W. Images taken by GE Hirzel.

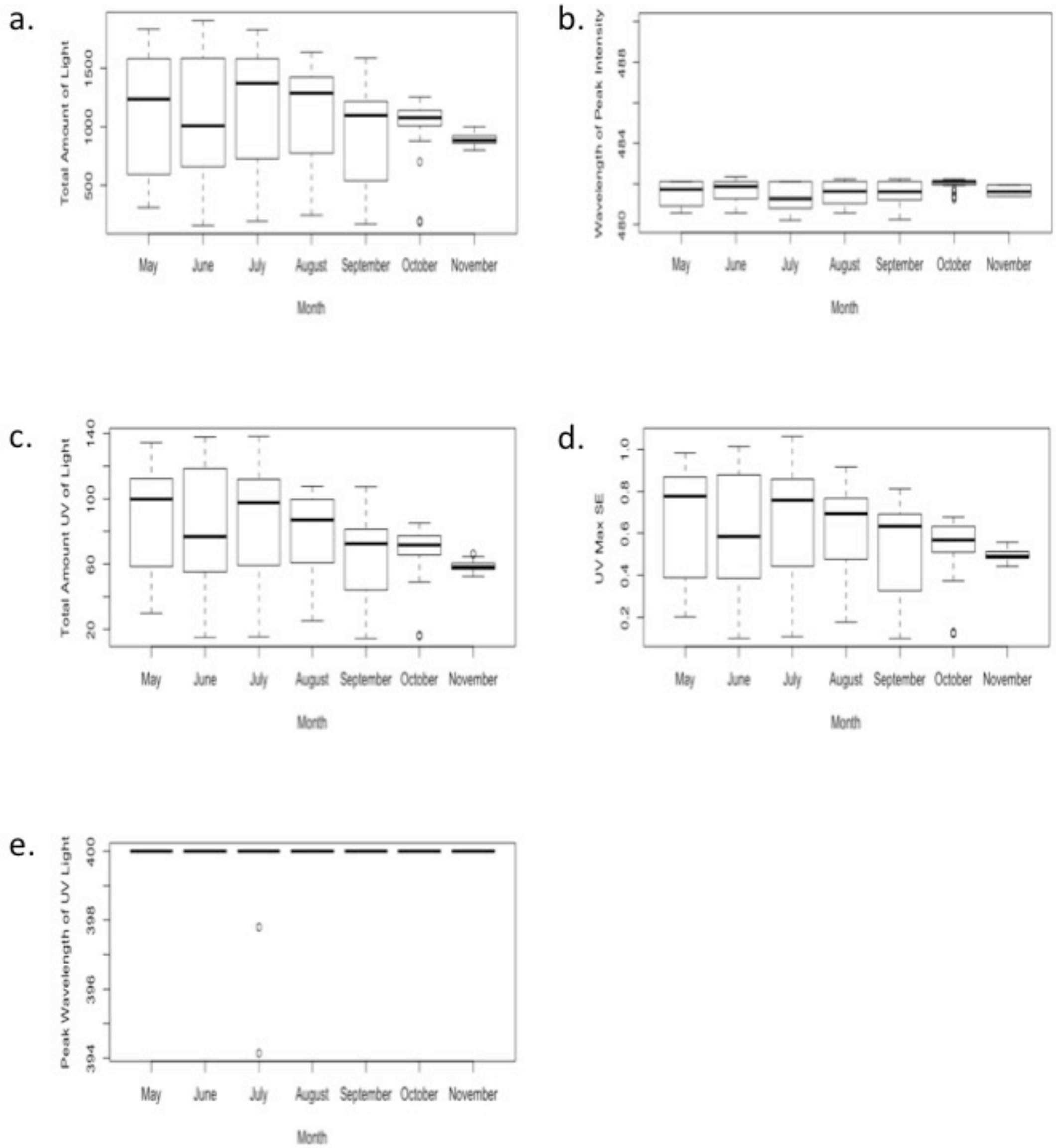


Figure 7. There is an effect of season on (a.) total amount of light, (b.) wavelength of peak intensity, (c.) total amount of UV light, and (d.) peak intensity of UV light, but there is not an effect of time of day on (e.) wavelength of UV peak intensity.

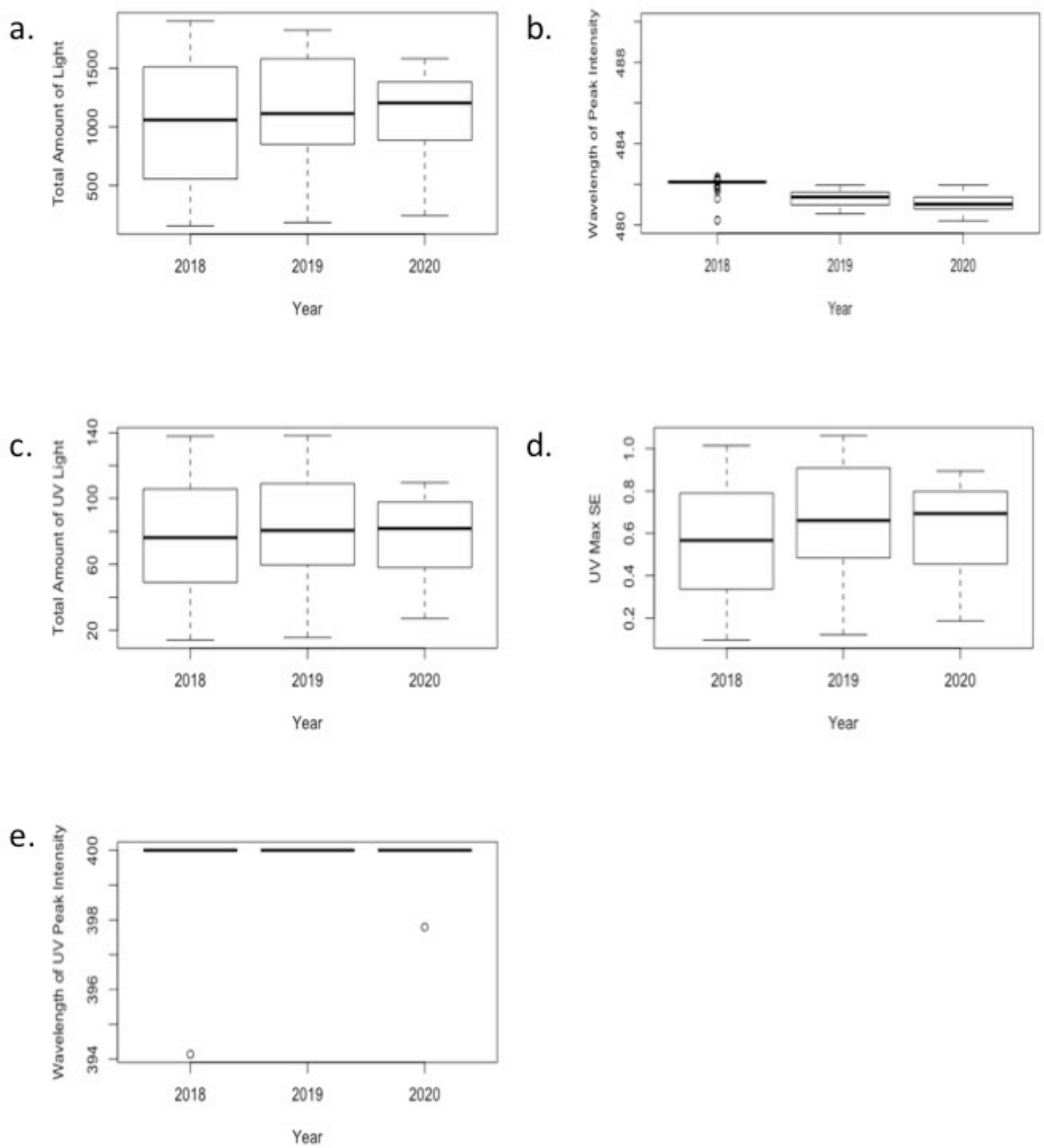


Figure 8. There is not an effect of year on (a.) total amount of light, (c.) total amount of UV light, and (e.) wavelength of UV peak intensity, but there is an effect of year on (b.) wavelength of peak intensity and (d.) UV peak intensity.

were found between 2018-2019 only with greater UV peak intensities in 2019 (Table 5, Figure 8.d). There was no effect of year on the total amount of light (Kruskal-Wallis, N= 35, df= 2, χ^2= 2.609, p-value= 0.271, Figure 8.a); total amount of UV light (Kruskal-Wallis, N= 354, df= 2, χ^2= 4.674, p-value= 0.09662, Figure 8.c); or wavelength of UV peak intensity (Kruskal-Wallis, N= 354, df= 2, χ^2= 1.198, p-value= 0.5493, Figure 8.e).

Combinatorial Effects on Light Environment

In 2018, interactions between site and month, between time of day and month, and between site, time of day, and month affected the total amount of light (Table 6, Figure 9.a, Figure 10.a), total amount of UV light (Table 8, Figure 9.c, Figure 10.c), and UV peak intensity (Table 9, Figure 9.d, Figure 10.d). The interactive effect of time of day and month influenced noon light environments to follow a downward trend and morning light environments to follow an upward trend from May to November. Interactions of site and month in 2018 affected September, with the Chesney location receiving more light than the other two locations. Interactions between site and time of day and between site and month also statistically affected the wavelength of peak intensity in 2018, but are likely not be biologically significant, given the small change in peak wavelength (Table 7, Figure 9.b, Figure 10.b). Lastly, in 2018 interactions between variables did not show an effect on wavelength of UV peak intensity (Table 10, Figure 9.e, Figure 10.e).

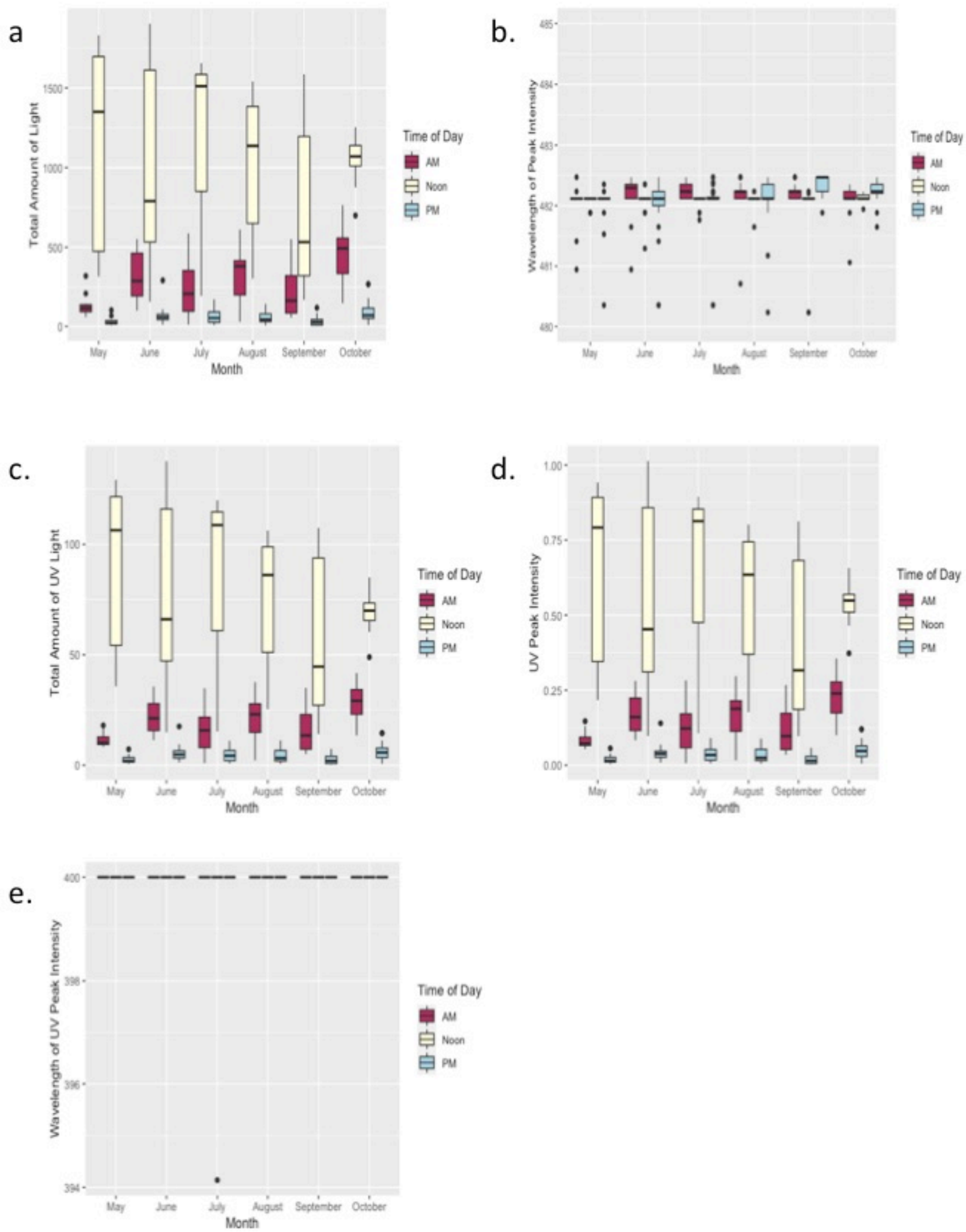


Figure 9. Effect of time of day and month in 2018 on (a.) total amount of light, (b.) wavelength of peak intensity, (c.) total amount of UV light, (d.) UV peak intensity, and (e.) wavelength of UV peak intensity.

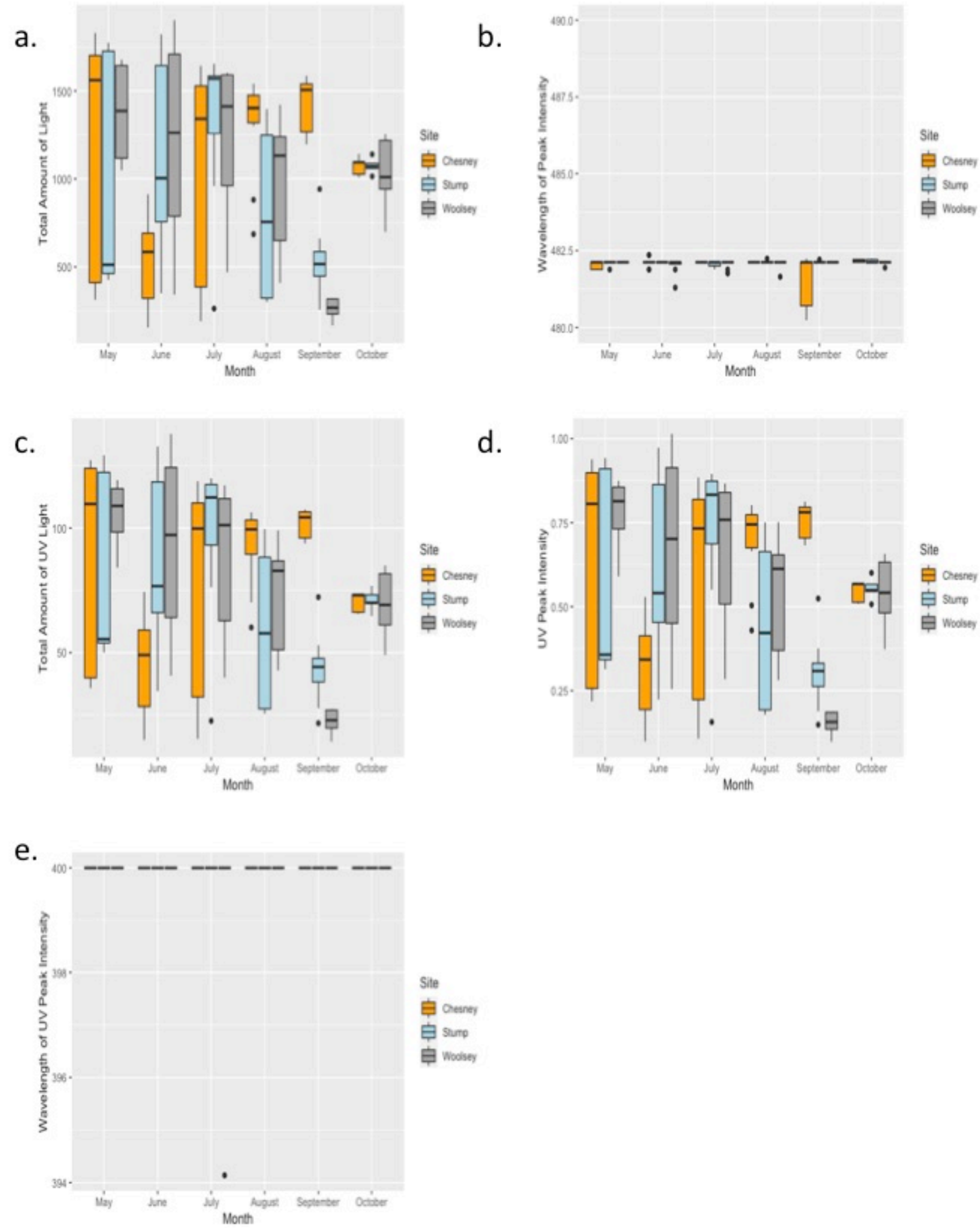


Figure 10. Interactions of site and month in 2018 on (a.) total amount of light, (b.) wavelength of peak intensity, (c.) total amount of UV light, (d.) UV peak intensity, and (e.) wavelength of UV peak intensity.

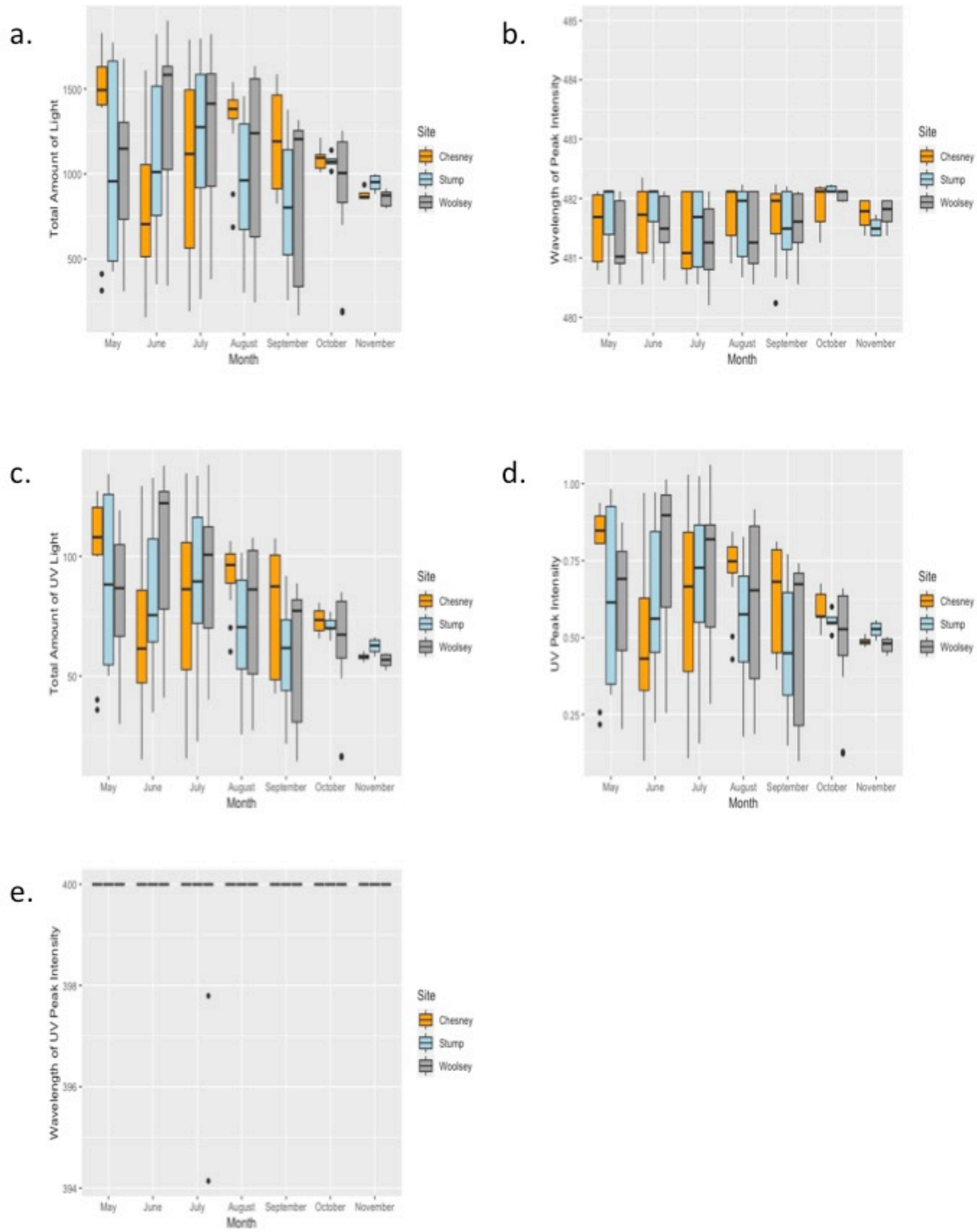


Figure 11. Interactions of month and site at noon on (a.) total amount of light, (b.) wavelength of peak intensity, (c.) total amount of UV light, (d.) UV peak intensity, and (e.) wavelength of UV peak intensity.

Interactions between site and month and between site, year, and month affected the total amount of light (Table 11, Figure 11.a), total amount of UV light (Table 13, Figure 11.c), and UV peak intensity at noon (Table 14, Figure 11.d). Interactions between year and month and between site, year, and month statistically affected the wavelength of peak intensity at noon, but are likely not biologically significant (Table 12, Figure 12.b). Lastly, the wavelength of UV peak intensity at noon was not affected by interactions between independent variables (Table 15).

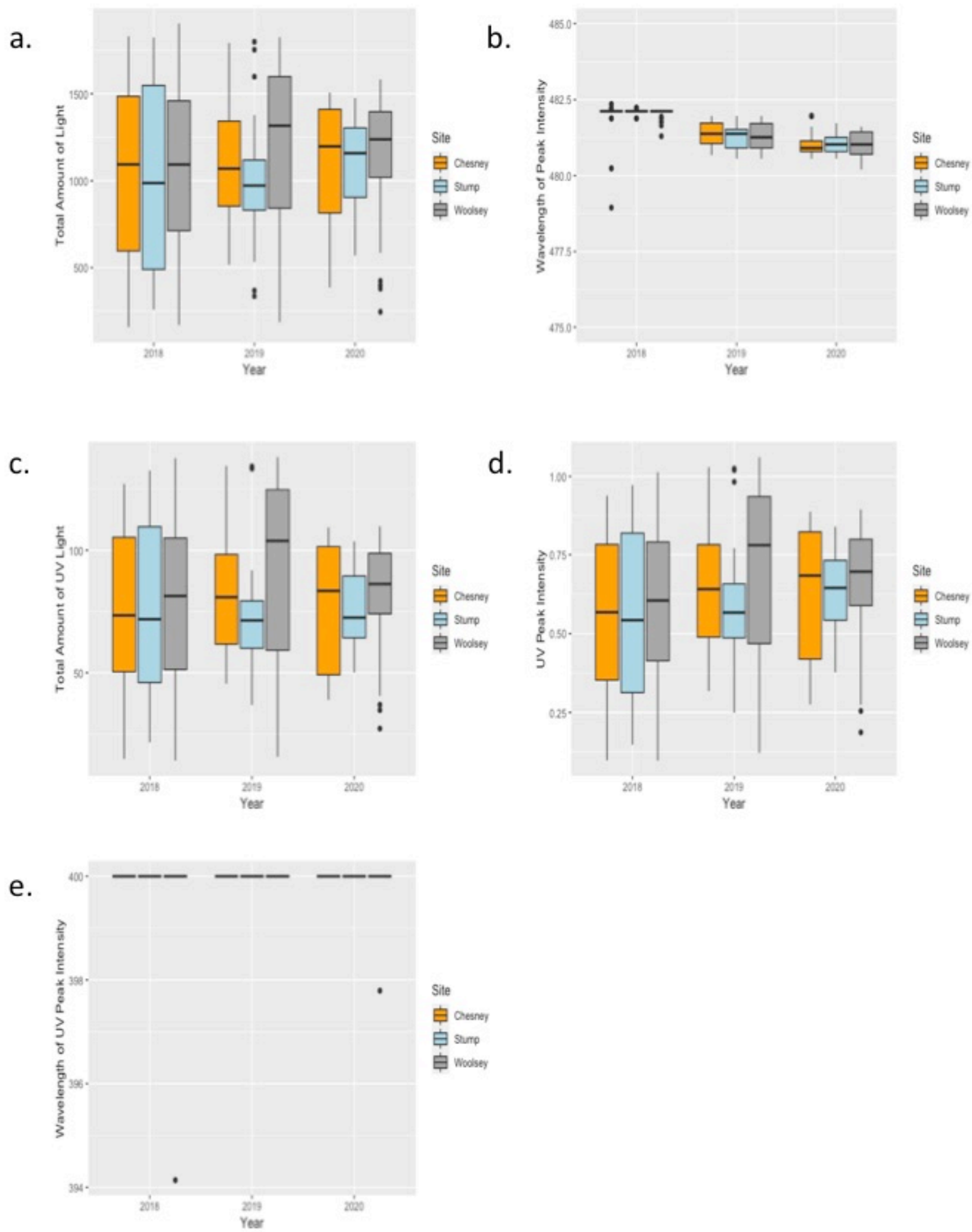


Figure 12. Interactions of year and site at noon on (a.) total amount of light, (b.) wavelength of peak intensity, (c.) total amount of UV light, (d.) UV peak intensity, and (e.) wavelength of UV peak intensity.

Discussion

I found an effect of time of day and season on the light environment in Northwest Arkansas prairies. Noon light environments consistently received the greatest amount of light, amount of UV light, and UV peak intensities while morning received greater amounts than evening light environments. Time of day also differed with evening light containing more green/yellow light than noon, which contained more blue light. I also found season contributed to a decrease in amount and variation of light as the year went on. Lastly, I found an interactive effect of time of day and month influenced noon light environments to follow a downward trend and morning light environments to follow an upward trend from May to November.

Different light environments can contain distinct light profiles, and change the perceived appearance of plants and animals due to interactions of ambient light color and the reflectance color of plants and animals (Endler, 1993). Time of day may influence the differing angles of incoming light and blocking of light by other plants or animals providing plants and animals with more or less light. Season may influence light due to the changing weather and foliage. In turn, these differences can influence the daily and seasonal behaviors of animals.

My finding of changing light environments throughout the day in Northwest Arkansas prairies suggests that ambient light could be used for the development and maintenance of circadian rhythms in local animals. Circadian rhythm in animals is an advantageous trait, which allows them to expend maximum energy at optimal times (Chittka, Stelzer, & Stanewsky, 2013). Optimal times may vary in different climates and different species due to different utilization and perception of light or

temperature (Chittka et al., 2013). Animals use different components of light in development of circadian rhythms. The fly, *Dacas tryoni*, uses light intensity as a guide for activity during the day, mating at the optimal light intensity of ca 0.8 lux (Lazzari & Insausti, 2008). While on the other hand, bumblebee (*Bombus Terrestris*) circadian rhythms are synchronized by daily UV light levels in the presence of continuous light (Chittka et al., 2013). Additionally, many types of birds such as the blackheaded bunting (*Emberiza melanocephala*) maintain their circadian clock due to the daily variation in light wavelengths and light intensities (Kumar, Gupta, Naseem, & Malik, 2017). Additional changes in behavior due to time of day are influenced by light intensity in the Buckeye butterfly, *Junonia coenia*. The readiness of male Buckeye butterflies to court females is most likely under high light intensities in the late morning or early afternoon (McDonald & Nijhout, 2000). This lines up with results of my experiment, as noon was found to receive the most light.

Changes in light throughout the year can also serve as drivers of behavioral change. As the wavelength and intensity of light changes from different seasons, birds like a robin (*Erithacus rubecula*) depend on short light wavelengths to orient them during migration, while longer wavelengths disorient them (Kumar et al., 2017). Therefore bird migration takes into account not only temperature, but other aspects of light as well. With bird migrations typically occurring in the fall and consistent findings of differences in total amount of light, wavelength of peak intensity, total amount of UV light, and UV peak intensity between October and November, the effect of ambient light on bird behavior in Northwest Arkansas could be an aspect of avian biology to look into further.

Seasonal effects of temperature and light intensity have shown to elicit changes in mating behavior and dispersal patterning in the satyrine butterfly, *Lethe diana*, suggesting that butterflies adapt to the light intensity and temperature of their environment (Ide, 2002). Seasonal change in the dispersal pattern of *L. diana* was caused by the seasonal change in mating behavior and in preference for specific light conditions. Butterflies were most active in July-August, utilizing the widespread forest shade for thermoregulation due to abundance of high intensity light. While in October-September and May-June, butterflies sought out brighter light environments and remained there, being less active (Ide, 2002). Due to similar results in my experiment with light environments being the most intense in July and easing up on surrounding months, butterflies in Northwest Arkansas could exhibit this same pattern.

With differing light conditions throughout the day and throughout the year, morphological changes as well as behavioral changes in animals continue to emerge, however it is not always understood how animals interpret the changes in light environments. Future research should investigate the influence of light on behavioral and morphological changes animals. For example, *Junonia coenia* (the common buckeye butterfly) experience a color change in their wings around August and elicit a subtle change in behavior (Hirzel et al, preliminary data), but it is unknown why. Future research could look into a possible change in vision adapting to the wings as the composition of the light changes with the seasons.

B. anynana is a seasonal butterfly that displays sexual dimorphism and plasticity in eye morphology (Macias-Muñoz, Smith, Monteiro, & Briscoe, 2016). The

different seasoned butterflies have adapted their eyes to meet the different environmental requirements by upregulating expression of vision genes in the wet season and down regulating expression of vision genes in the dry season to conserve energy (Macias-Muñoz et al., 2016). With colder seasons corresponding to less light as found in my results, decrease in vision could also be associated with changes in light environment. While *B. anynana* is not native to the United States, it is possible its native seasonal light environment changes similarly to that of Northwest Arkansas. Therefore, looking for an effect of season on vision in an Arkansas-native species like *J. coenia* could result in similar findings.

In conclusion, changing light environments fuel a large variety of animal behaviors and morphologies, especially in butterflies. Daily changes in light environment can influence readiness to court a mate at different times of the day, possibly due to visual cues. Seasonal changes in light could contribute to plasticity in vision, changes in phenotype, and changes in mating preference directly or indirectly in butterflies through fluctuation in the amount of light, color of light, and amount of UV light present at different times of the year. An interaction of time of day and season could show a trend in butterflies mating earlier or later in the day during different seasons in order to receive the optimal amount of light.

References:

Bateman, P., & Fleming, P. (2006). Males are selective too: mating, but not courtship, with sequential females influences choosiness in male field crickets. *Behavioral Ecology and Sociobiology*, *59*(4), 577–581. https://doi.org/https://doi.org/10.1007/s00265-005-0083-y

Chittka, L., Stelzer, R. J., & Stanewsky, R. (2013). Daily changes in ultraviolet light levels can synchronize the circadian clock of bumblebees (Bombus terrestris). *The Journal of Biological and Medical Rhythm Research*, *30*(4), 434–442. https://doi.org/https://doi.org/10.3109/07420528.2012.741168

Endler, J. A. (1993). The color of light in forests and its implications. *Ecological Society of America*, *63*(1), 1–27.

Ide, J.-Y. (2002). Mating behaviour and light conditions cause seasonal changes in the dispersal pattern of the satyrine butterfly Lethe diana. *Ecological Entomology*, *27*(1), 33–40. https://doi.org/https://doi.org/10.1046/j.1365-2311.2002.0372a.x

Karlsson, B. (1998). Nuptial gifts, resource budgets, and reproductive output in polyandrous butterfly. *Ecology*, *79*(8), 2931–2940. https://doi.org/https://doi.org/10.1890/0012-9658(1998)079[2931:NGRBAR]2.0.CO;2

Kumar, J., Gupta, P., Naseem, A., & Malik, S. (2017). Light spectrum and intensity, and the timekeeping in birds. *Biological Rhythm Research*, *48*(5), 739–746. https://doi.org/https://doi.org/10.1080/09291016.2017.1345449

Lazzari, C., & Insausti, T. (2008). *Circadian rhythms in insects*.

Macias-Muñoz, A., Smith, G., Monteiro, A., & Briscoe, A. D. (2016). Transcriptome - wide differential gene expression in Bicyclus anynana butterflies: female vision - related genes are more plastic. *Molecular Biology and Evolution*, *33*(1), 79–92. https://doi.org/https://doi.org/10.1093/molbev/msv197

McDonald, A. K., & Nijhout, F. H. (2000). The effect of environmental conditions on mating activity of the Buckeye butterfly, Precis coenia. *Journal of Research on the Lepidoptera*, *35*, 22–28.

Prudic, K. L., Jeon, C., Cao, H., & Monteiro, A. (2011). Developmental plasticity in sexual roles of butterfly species drives mutual sexual ornamentation. *REPORTS*, *331*(6013), 73–75. https://doi.org/10.1126/science.1197114

Robertson, K. A., & Monteiro, A. (2005). *Female Bicyclus anynana butterflies choose males on the basis of their dorsal UV-reflective eyespot pupils*. *272*(1572), 1541–1546. https://doi.org/10.1098/rspb.2005.3142

Westerman, E., & Monteiro, A. (2013). Odour influences whether females learn to prefer or to avoid wing patterns of male butterflies. *Animal Behaviour*, *86*(6), 1139–1145. https://doi.org/https://doi.org/10.1016/j.anbehav.2013.09.002

Table 1: Shapiro-Wilk Test

Variable	P-value	Test Statistic
Total Amount of Light***	< 2.2e-16	0.8554
Wavelength of Peak Intensity***	< 2.2e-16	0.1608
Total Amount of UV Light***	< 2.2e-16	0.8586
UV Peak Intensity***	< 2.2e-16	0.8576
Wavelength of UV Peak Intensity***	< 2.2e-16	0.02082

Shapiro-Wilk test testing the normality of the data for each dependent variable. P-value < 0.0125 indicates significance.

Table 2: Steel-Dwass Test for Total Amount of Light

Independent Variables	Variables Compared	P-Value	Test Statistic
Time of Day	PM - Noon***	< 2.22e-16	-21.08
	AM - Noon***	< 2.22e-16	-17.14
	AM - PM***	< 2.22e-16	-16.07
Site	Woolsey - Stump	0.05	-3.315
	Woolsey - Chesney	0.2095	-2.388
	Stump - Chesney	0.5826	-1.401
Month	Oct-Nov**	0.002167	-5.461
	Sept-July**	0.00332	-5.306

Steel-Dwass test indicating distinct differences between components of independent variables in total amount of light. Data from 2018 only were used for analyses of time of day and data taken at noon only were used for analyses of site, month, and year. *** denotes p-value < 0.001. ** denotes p-value < 0.01.

Table 3: Steel-Dwass Test for Wavelength of Peak Intensity

Independent Variables	Variables Compared	P-Value	Test Statistic
Time of Day	PM - Noon***	3.79E-10	-9.095
	AM - Noon***	3.30E-14	-11.73
	AM - PM	0.9841	-0.241
Year	2018 - 2019***	< 2.22e-16	-19.17
	2018 - 2020***	< 2.22e-16	-18.40
	2020 - 2019***	8.04E-05	-5.94
Month	Oct-Aug**	6.66E-03	-5.044
	Oct-July***	6.34E-06	-7.241
	Oct-June**	6.69E-03	-5.042
	Oct-Nov**	5.11E-03	-5.146
	Sept-Oct**	0.003006	-5.343

Steel-Dwass test indicating distinct differences between components of independent variables in wavelength of peak intensity. Data from 2018 only were used for analyses of time of day and data taken at noon only were used for analyses of site, month, and year. *** denotes p-value < 0.001. ** denotes p-value < 0.01.

Table 4: Steel-Dwass Test for Total Amount of UV Light

Independent Variables	Variables Compared	P-Value	Test Statistic
Time of Day	PM - Noon***	< 2.22e-16	-21.13
	AM - Noon***	< 2.22e-16	-18.87
	AM - PM***	< 2.22e-16	-17.00
Site	Woolsey - Stump	0.02758	-3.632
	Woolsey - Chesney	0.2442	-2.267
	Stump - Chesney	0.5005	-1.587
Month	Sept-May**	0.0023	-5.440
	Oct-Nov**	0.001951	-5.499
	Sept-July***	3.15E-05	-6.799

Steel-Dwass test indicating distinct differences between components of independent variables in total amount of UV light. Data from 2018 only were used for analyses of time of day and data taken at noon only were used for analyses of site, month, and year. *** denotes p-value < 0.001. ** denotes p-value < 0.01.

Table 5: Steel-Dwass Test for UV Peak Intensity

Independent Variables	Variables Compared	P-Value	Test Statistic
Time of Day	PM - Noon***	< 2.22e-16	-21.11
	AM - Noon***	< 2.22e-16	-18.23
	AM – PM***	< 2.22e-16	-16.57
Site	Woolsey - Stump	0.01863	-3.828
	Woolsey - Chesney	0.1215	-2.776
	Stump - Chesney	0.6104	-1.340
Year	2018 - 2019**	0.003088	-4.624
	2018 - 2020	0.07417	-3.087
	2020 - 2019	0.689	-1.164
Month	Sept-July***	0.0003383	-6.085

Steel-Dwass test indicating distinct differences between components of independent variables in peak intensity of UV light. Data from 2018 only were used for analyses of time of day and data taken at noon only were used for analyses of site, month, and year. *** denotes p-value < 0.001. ** denotes p-value < 0.01.

Table 6: Generalized Linear Model for Total Amount of Light in 2018

Independent Variables	df	P-value
Site	2	0.2276
Time of Day***	2	< 2.2e-16
Month***	5	0.0005728
Site: Time of Day	4	0.2456
Site: Month***	10	1.852e-11
Time of Day: Month***	10	8.646e-07
Site: Time of Day: Month***	20	3.074e-08

Generalized linear model indicating interactions between independent variables affecting the total amount of light in 2018.

Table 7: Generalized Linear Model for Wavelength of Peak Intensity in 2018

Independent Variables	df	P-value
Site**	2	0.003548
Time of Day***	2	9.151e-09
Month	5	0.4921
Site: Time of Day***	4	0.000445
Site: Month**	10	0.004724
Time of Day: Month	10	0.7945
Site: Time of Day: Month	20	0.02442

Generalized linear model indicating interactions between independent variables affecting the wavelength of peak intensity in 2018.

Table 8: Generalized Linear Model for Total Amount of UV Light in 2018

Independent Variables	df	P-value
Site	2	0.4211
Time of Day***	2	< 2.2e-16
Month**	5	0.001275
Site: Time of Day	4	0.3667
Site: Month***	10	4.359e-13
Time of Day: Month***	10	4.153e-09
Site: Time of Day: Month***	20	4.543e-10

Generalized linear model indicating interactions between independent variables affecting the total amount of UV light in 2018. *** denotes p-value < 0.001. ** denotes p-value < 0.01.

Table 9: Generalized Linear Model for UV Peak Intensity in 2018

Independent Variables	df	P-value
Site	2	0.2856
Time of Day***	2	< 2.2e-16
Month***	5	0.0005795
Site: Time of Day	4	0.2691
Site: Month***	10	1.087e-12
Time of Day: Month***	10	3.034e-08
Site: Time of Day: Month***	20	1.880e-09

Generalized linear model indicating interactions between independent variables affecting the peak intensity of UV light in 2018. *** denotes p-value < 0.001. ** denotes p-value < 0.01.

Table 10: Generalized Linear Model for Wavelength of UV Peak Intensity in 2018

Independent Variables	**df**	**P-value**
Site	2	0.3687
Time of Day	2	0.3899
Month	5	0.763
Site: Time of Day	4	0.4167
Site: Month	10	0.8991
Time of Day: Month	10	0.8372
Site: Time of Day: Month	20	0.959

Generalized linear model indicating interactions between independent variables affecting the wavelength of UV peak intensity in 2018. *** denotes p-value < 0.001. ** denotes p-value < 0.01.

Table 11: Generalized Linear Model for Total Amount of Light at Noon

Independent Variable	**df**	**P-value**
Site	2	0.01154
Year	2	0.05729
Month	4	0.01147
Site: Year	4	0.63430
Site: Month***	8	4.465e-09
Year: Month	8	0.01049
Site: Year: Month***	14	7.476e-06

Generalized linear model indicating interactions between independent variables affecting the total amount of light at noon. The months of October and November were removed from analysis due to unfinished data collection. *** denotes p-value < 0.001. ** denotes p-value < 0.01.

Table 12: Generalized Linear Model for Wavelength of Peak Intensity at Noon

Independent Variable	**df**	**P-value**
Site	2	0.6021762
Year***	2	0.0001814
Month	4	0.0505433
Site: Year	4	0.2824817
Site: Month	8	0.1018062
Year: Month***	8	0.0003748
Site: Year: Month**	14	0.0016001

Generalized linear model indicating interactions between independent variables affecting the wavelength of peak intensity at noon. The months of October and

November were removed from analysis due to unfinished data collection. *** denotes p-value < 0.001. ** denotes p-value < 0.01.

Table 13: Generalized Linear Model for Total Amount of UV Light at Noon

Independent Variable	**df**	**P-value**
Site**	2	0.0030187
Year***	2	0.0006994
Month***	4	1.912e-07
Site: Year	4	0.7164385
Site: Month***	8	1.256e-09
Year: Month	8	0.0766474
Site: Year: Month***	14	1.496e-06

Generalized linear model indicating interactions between independent variables affecting the total amount of UV light at noon. The months of October and November were removed from analysis due to unfinished data collection. *** denotes p-value < 0.001. ** denotes p-value < 0.01.

Table 14: Generalized Linear Model for UV Peak Intensity at Noon

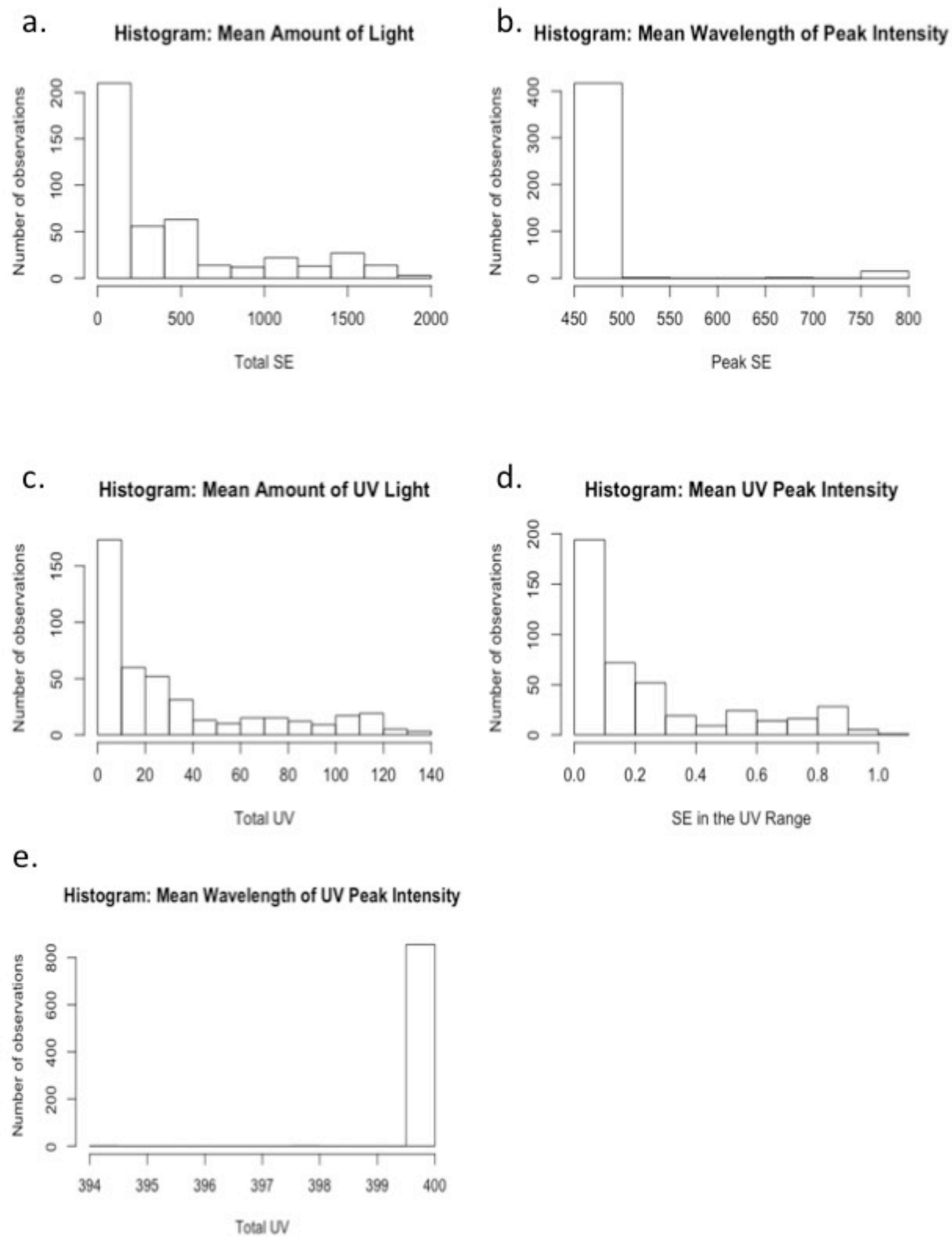
Independent Variable	**df**	**P-value**
Site**	2	0.0013240
Year***	2	2.865e-05
Month***	4	0.0003774
Site: Year	4	0.6264193
Site: Month***	8	6.844e-09
Year: Month	8	0.0141443
Site: Year: Month***	14	5.957e-07

Generalized linear model indicating interactions between independent variables affecting the peak intensity of UV light at noon. The months of October and November were removed from analysis due to unfinished data collection. *** denotes p-value < 0.001. ** denotes p-value < 0.01.

Table 15: Generalized Linear Model for Wavelength of UV Peak Intensity at Noon

Independent Variable	**df**	**P-value**
Site	2	0.2962
Year	2	0.4899
Month	4	0.5329
Site: Year	4	0.6933
Site: Month	8	0.8286
Year: Month	8	0.9124
Site: Year: Month	14	0.9960

Generalized linear model indicating interactions between independent variables affecting the wavelength of UV peak intensity at noon. The months of October and November were removed from analysis due to unfinished data collection. *** denotes p-value < 0.001. ** denotes p-value < 0.01.



Supplemental Figure 1. Data showing non-normal distribution of (a.) total amount of light, (b.) wavelength of peak intensity, (c.) total amount of UV light, (d.) UV peak intensity, and (e.) wavelength of UV peak intensity.