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Discovering the daily activity pattern of *Zygiella x-notata* and its relationship to light

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Abstract

As global urbanization is on the rise, unintended consequences of this modern advancement continue to plague our ecosystems. One of these consequences is artificial light at night (ALAN). Up to 18.7% of landmass experiences ALAN currently, and that figure is only expected to grow. ALAN can threaten biodiversity in urban locations by altering life histories and disrupting the functioning of ecosystems. Many organisms living under ALAN exposure alter their daily rhythm patterns in response to ALAN. This study aimed to investigate the baseline daily activity levels and rhythmic patterns of a common nocturnal urban species, the Silver Sided Sector Spider (*Zygiella x-notata*). Using the Zantiks AD unit, individuals were tested in-lab for 48-hours by monitoring their activity responses to a 12-hours light/12-hours dark (with ramping transitions) light treatment. *Zygiella x-notata* was confirmed to be a nocturnal species that displayed clear daily activity patterns under a baseline 12-hours light/12-hours dark treatment. Future studies can now look at the effect of ALAN on *Zygiella x-notata* and determine how strongly ALAN causes deviation from the activity pattern baseline.

1. Introduction

Global urbanization is steadily rising as the global population continues to grow dramatically. It is estimated that the human population on the planet increases at a rate of over 1% per year, with the total human population count projected to be close to 10 billion by the year 2050 (Bongaarts, 2009). Approximately 55% of the human population live in urban locations currently, and that number is expected to grow to 68% by 2050 (*World Urbanization Prospects: The 2018 Revision*, n.d.). As such, urban areas will expand along with our population, covering more landmass and impacting more ecosystems.

Urbanization leads to high levels of ecosystem disturbance, such as increased air pollution, soil pollution, water pollution, noise pollution, and light pollution (Martínez-Bravo & Martínez-del-Río, 2019). These different types of disturbances can wreak havoc on local biodiversity. Species have evolved to flourish within specific niche environments, and even minor disruptions can lead to failure to thrive via multiple different mechanisms (Johnstone et al., 2016). With global biodiversity decreasing rapidly, the need to counteract these negative effects from urbanization becomes apparent, and as such the field of urban ecology was formed during the 1970s (Niemela, 2011). Simply put, urban ecology aims to study the impact of humans, particularly high human aggregation, on the planet.

Light pollution is a phenomenon that has affected our planet for approximately only the past 100 years (Stone, 2017). Modern technology gave rise to lighting instruments that are now globally ubiquitous and able to be used under any circumstances and during any time of day. Historically, humanity tended to orient themselves around the rising and setting of the sun, but modern lighting now allows for social activities, work shifts, and more to extend beyond daylight hours. In addition to indoor lighting, urban areas especially tend to

experience external light pollution at night from sources such as streetlights, buildings, vehicles, and billboards. All this unnatural lighting is referred to collectively as artificial light at night, or ALAN.

Nightly measurements show that lux levels within urban areas are between three and six times higher than rural areas, showcasing the intensity of light in urban locations. However, the impact of ALAN from urban areas is so strong that rural areas are still not excluded from it. While urbanization might only be estimated to be at around 3% of the global landmass (Global Rural Urban Mapping Project (GRUMP), 2005), studies based off satellite imagery have estimated that, due to the phenomenon known as 'skyglow,' up to 18.7% of global landmass may experience ALAN (Cinzano, Falchi, & Elvidge, 2001). Skyglow is the nighttime brightness in the sky that builds up from a multitude of artificial sources that are clustered together (i.e. in urban areas). Another study estimated that 83% of the global population are living in an area that experiences light pollution (Drake, 2019). The prevalence of skyglow and the intensity of ALAN is predicted to further increase. One study predicts that ALAN will grow by 6% yearly (Hölker et al., 2010). Regardless, this growth trend will expose more ecosystems to ALAN, altering systems on both an ecological scale and on an organismal scale. Figure 1 shows the ratio of artificial sky brightness to natural sky brightness and gives a visual overview of the global prevalence of ALAN.

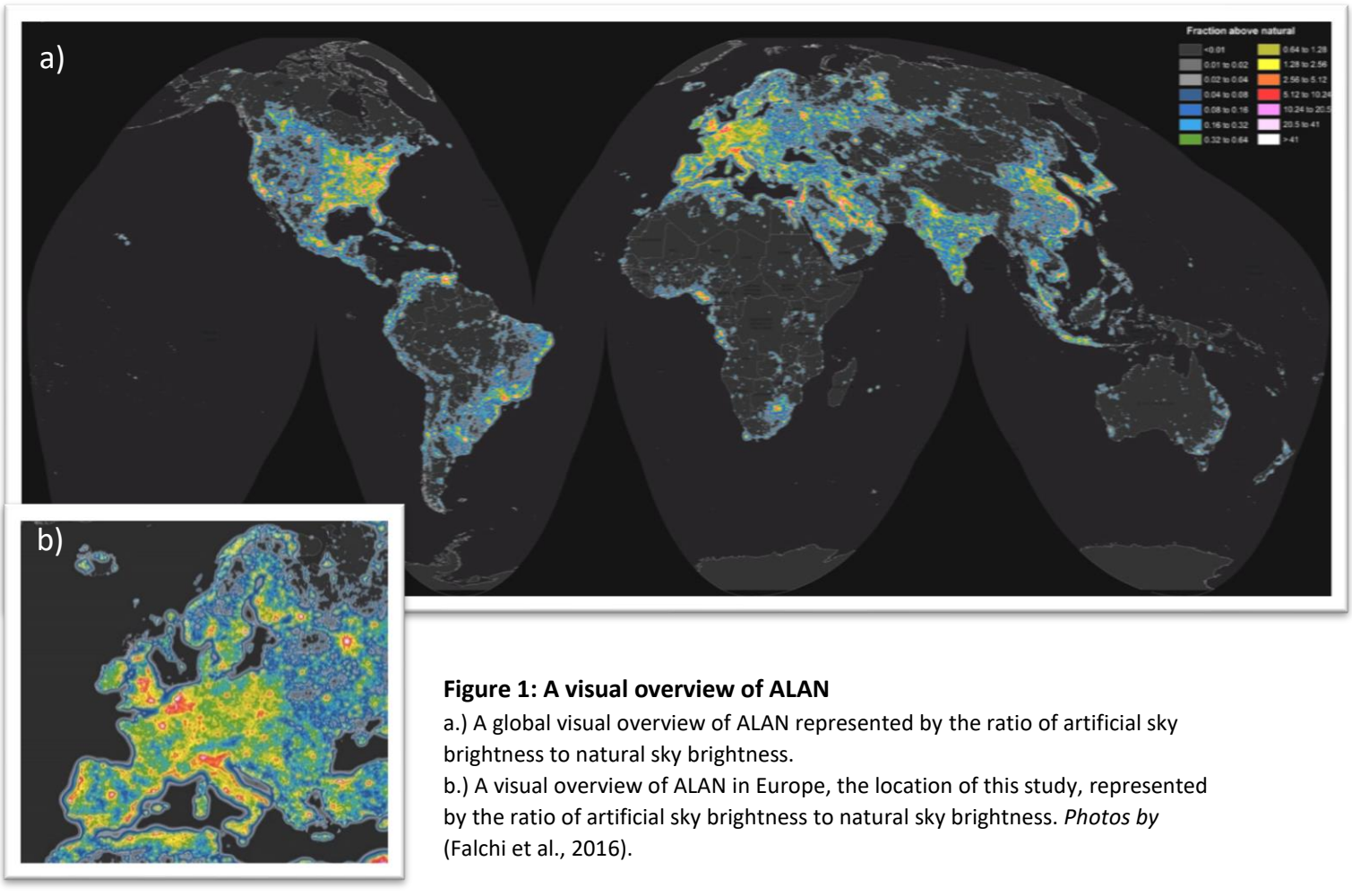

Figure 1: A visual overview of ALAN
a.) A global visual overview of ALAN represented by the ratio of artificial sky brightness to natural sky brightness.
b.) A visual overview of ALAN in Europe, the location of this study, represented by the ratio of artificial sky brightness to natural sky brightness. *Photos by* (Falchi et al., 2016).

Artificial light at night has documented physiological, epidemiological, reproductive, and behavioral effects on living organisms (Bedrosian, Fonken, Walton, & Nelson, 2011; Gaston, Bennie, Davies, & Hopkins, 2013; Navara & Nelson, 2007). Studies have found ALAN to disrupt reproductive strategies, reproductive success, metabolism, and foraging, as well as increase the chances of predation and cause oxidative stress (Fobert, Burke Da Silva, & Swearer, 2019; Gaston et al., 2013; Navara & Nelson, 2007). Such effects on life histories makes ALAN a driver of ecosystem changes and species adaptation.

Species have evolved within a specific environment, which includes aspects such as ambient lighting, daily lighting rhythms, and seasonal lighting rhythms. Biological clocks have evolved primarily in response to these light cycles, and other predictable rhythmic changes in the environment (e.g. temperature) (Kreitzman & Foster, 2011). A biological clock is the innate mechanism that governs the physiological activities of an organism on a temporal scale (Lexico, 2020). Biological clocks in turn produce circadian rhythms, which regulate an organism based on an approximate 24-hour period (NIGMS, 2017). This clock can exist without external cues, therefore allowing organisms to anticipate rhythmic changes rather than simply reacting to them (Helm et al., 2017). One of the adaptations that species have evolved is the tendency to sync-up their biological clocks with certain exogenous cues – one of the most common ones being light. This has allowed individuals to best profit from their environment; for example, if a given species' prey is most active at night, then it can be beneficial for said species to also be active at night. Conversely, if a predator of said species is also active at night, then it might be more beneficial for the species to be active during the day. However, this statement is simplified, as there are naturally more factors to consider here outside of predator-prey relationships, regardless of their importance for fitness. Largely converging to a basic cost-benefit analysis, the evolution of species and their biological clocks have led to the development of temporal niches. A temporal niche can be defined, simply, as the period of the day in which an individual displays (prominent) locomotor activity (Hut et al., 2012). The most common temporal niches are diurnality and nocturnality, which respectively mean that an individual is more active during the daytime or during the nighttime.

Temporal niche phenotypes can be subjected to selective pressure, and therefore may change within a species overtime. Additionally, on both the species level and the individual level a phenomenon known as "temporal switching" can be seen, which is a form of plasticity that allows for the switching between temporal niches based on external environmental cues (Hut, Kronfeld-Schor, van der Vinne, & De la Iglesia, 2012). As such, the presence of ALAN and the resulting disruption of normal light-based cues can significantly alter an organism's behavior, both directly and indirectly. Predator-prey relationships are one of the main determinants of whether a species is nocturnal or diurnal. Many species use the cover of darkness to hide from predators while they forage for their own food, and therefore the addition of ALAN to their ecosystem may result in much higher predation of this species. If a significant portion of their population is preyed upon, this could have indirect effects that cascade up or down the food web, therefore further impacting an ecosystem. Since urbanization and the resulting ALAN will not be reduced, but rather continue to spread, the cataloging of the species found in urban environments, their life

histories, and their reaction to the relatively recent introduction to artificial light at night is necessary for the management of urban ecosystems.

It is believed that the circadian clock is involved with temporal memory. An individual is able to adjust its behavior based on successful previous experiences, i.e. based on amount of prey caught. *Zygiella x-notata*, a small, urban, orb-weaving spider, has been found to be plastic in its (web-building) behavior, even from one web-build to the next. *Zygiella x-notata* tends to renew its web daily, and may make adjustments to its web or foraging behavior based on the prey catch-rate of the previous night (Venner et al., 1999). This is an important adaptation, as foraging decisions can consequently impact an individual's energetic gains, growth, and reproductive success. Therefore, prey availability can be a likely cause for inducing temporal switching and/or plasticity in foraging behavior. Multiple studies across varying climates and regions have reported prey availability for the orb-weaving spider guild to be higher during the daytime than the nighttime (Crouch, 2000; Moore, Watts, Herrig, & Jones, 2016). However, presence of the predators of orb-weaving spiders (e.g. birds) tends to be higher during the daytime as well. In this manner, the orb-weaving spider has found a quite advantageous environment with ALAN, as it can avoid predators by being nocturnal while also increasing its prey interception by selecting habitats close to sources of ALAN, as insects exhibit positive phototaxis.

Orb-weavers are spiders that come from the family Araneidae and are best identified by their recognizable spiral spoked orb web. Certain orb-weaver spiders tend to only be found in rural environments, while other ones tend to only be found in urban environments. Some species can be found within both habitat types (Elfferich, 2018). Orb-weaver spiders tend to be nocturnal species, and as they feed on insects, which display positive phototaxis, they have become the focus of many urban studies on the impacts of ALAN. A well-known study within the field by Astrid Heiling looked at *Larinioides sclopetarius* web distribution on a bridge that had alternating dark and lit sections spread over identical structures. She found that *L. sclopetarius* exhibited positive phototaxis, as there was a tendency to build their webs significantly more within the lit sections than within the dark sections. Upon measuring nighttime prey density, Heiling found that the lit sections had significantly more prey availability, which is the most likely explanation behind this phototaxis (Heiling, 1999).

However, while orb-weaving spiders tend to benefit from ALAN due to increased prey availability, they also experience negative side effects. A study on the orb-weaving spider *Cyclosa turbinate* created a model to evaluate whether the daily activity cycles of orb-weaving spiders could be explained as risk-averse behavior against daytime predation. Indeed, their findings indicated that *C. turbinate*'s nocturnality is a predator-avoidance adaptation (Watts, Jones, Herrig, Miller, & Tenhumberg, 2018). Nocturnal predators that could previously not view orb-weavers during nighttime may now have the opportunity to prey on the spiders if they become visibly-lit by ALAN. The presence of ALAN could therefore increase the chances of predation-based mortality for the orb-weaver spider, as they are no longer able to forage under the cover of darkness. Another study found that ALAN exposure accelerated juvenile development of the Australian garden orb-weaver spider (*Eriophora biapicata*). This nocturnal species was found to undergo fewer molts, leading to an earlier

maturation time and therefore a smaller body size at maturation (Willmott, Henneken, Selleck, & Jones, 2018). Additionally, females were found to produce fewer eggs under ALAN conditions, likely due to their smaller body size.

The versatility of the orb-weaver spider under ALAN conditions makes it a prime study subject for light pollution studies, as it allows for both light-based adaptation studies as well as for studies on the negative interactions of ALAN with local ecosystems and organisms. They also seem to exhibit clear responses to light-based signals, as one study that observed colonial orb-weaving spiders (*Metepeira incrassate*) during a solar eclipse found that spider behavior immediately switched to typical nocturnal behavior upon totality but resumed to typical diurnal behavior once the solar eclipse had finished (Uetz et al., 1994).

The orb-weaver spider known as the silver-sided sector spider, or the missing sector orb weaver (*Zygiella x-notata*) was selected for this study, as it is found within a variety of habitat types in urban environments and there is currently very little information known on its life history, and consequently its relationship with ALAN and biological clocks. While it is assumed to be nocturnal, similar to other orb-weaver spiders, there remain few studies on this species, so confirmation could be used. Additionally, no known studies have evaluated its baseline daily activity patterns (here, the patterns that emerge under a stable lab environment without ALAN). Before we can determine if ALAN has any effect on the silver-sided sector spider, we first must characterize its baseline daily activity patterns under a 12-hour light/12-hour dark lighting conditions. Experimental design and setup were loosely inspired by a study by Moore et al., (2016), which found the orb-weaving spider *C. turbinate* to perform web-building behavior under total darkness, and discovered likely endogenous circadian control on the locomotion behavior of this species. However, their study was inconclusive concerning whether web-building behavior was regulated by endogenous or exogenous cues (Moore et al., 2016).

Now that *Zygiella x-notata*'s environment is changing, it is important to know if it will adhere to its internal biological clock, or if it will respond to light cues in artificial environments. One way in which to test this is to know whether spiders in urban environments even have clear temporal niches, and if so if these niches are strict or flexible. Simply put, we need to know if this species is rhythmic in the first place. Therefore, the aim of this study is to determine the daily activity levels and rhythmic patterns of *Zygiella x-notata* and characterize it as nocturnal or diurnal, as well as evaluate any phenotypic traits that might affect this. It is expected that *Zygiella x-notata* will exhibit nocturnality and daily activity patterns similar to other orb-weaver spiders. This translates to little-to-no activity during lit hours, with activity beginning during twilight and continuing throughout the period of darkness until dawn. Providing a clear baseline point for other studies on *Zygiella x-notata* to measure from will allow for more accurate interpretations of *Zygiella x-notata*'s behavior, as well as pave the way for critical future studies on the effects of artificial light at night on this species.

2. Methodology

Methods for collecting, maintaining, studying, and housing orb-weaving spiders were largely adapted from papers by Zscholle and Herberstein (2005) and Witt (1971) (Witt, 1971; Zschokke, Samuel Herberstein, 2019). The design and construction of the experimental frames and other experimental material was also adapted from these papers.

2.1 Specimen collection

40 female spiders were collected from eight different and ecologically-distinctive sites within Groningen, the Netherlands (53.2194° N, 6.5665° E). The spiders were visually sexed and identified by species in the field and then confirmed again in the lab based on morphology. The collection sites were chosen based on visual confirmation of high *Zygiella x-notata* populations, distance from other locations, ALAN intensity, and site type (Fig. 2). Site type included bridges (above water), fences, brick walls, and bus stops (immediately in the area). The bridges and fences all had varying structures, heights, exposure, and ALAN intensities. The collection dates ranged from 16/5/19 to 27/6/19, and the collection time ranged from 23:30 to 3:12. *Zygiella x-notata* were primarily found after sunset, with peak activity seeming to be approximately 1-2 hours after complete darkness. During the collection period, the latest sunset was at 22:05, with complete darkness occurring approximately 1-2 hours afterwards. Spider collection would typically begin at complete darkness. Spiders were spotted using multiple methods: (i) visualization of their web, which tends to reflect under ALAN, (ii) visualization of the spider itself as it sits in the hub of its web, (iii) visualization of the spider itself as it actively builds its webs, (iv) the use of a flashlight to locate spiders in darker locations (*Note*: some spiders can shy away from this sudden onset of light, making capture difficult), (v) actively seeking out locations that are known *Zygiella x-notata* habitats, such as bridges, fences, and bus stops underneath ALAN. Many of these sites were discovered while performing field work on a concurrent orb-weaving spider study (Florez Blesgraeft, 2019). Spiders were most easily found in locations with ALAN. Specimens were collected in containers along with parts of their web and transported to the lab.

Lab conditions were set to 20°C and 60% relative humidity. The lab was also equipped with a 12-hour ramping light/dark cycle, with “sunrise” starting at 4:00 and ramping up to full light at 5:00, and “sunset” starting at 16:00 and ramping down to complete darkness at 17:00 as a standardized way to represent natural outdoor lighting conditions. The in-lab light source was one overhead light. The lab room had no windows and was completely sealed from other external light sources.

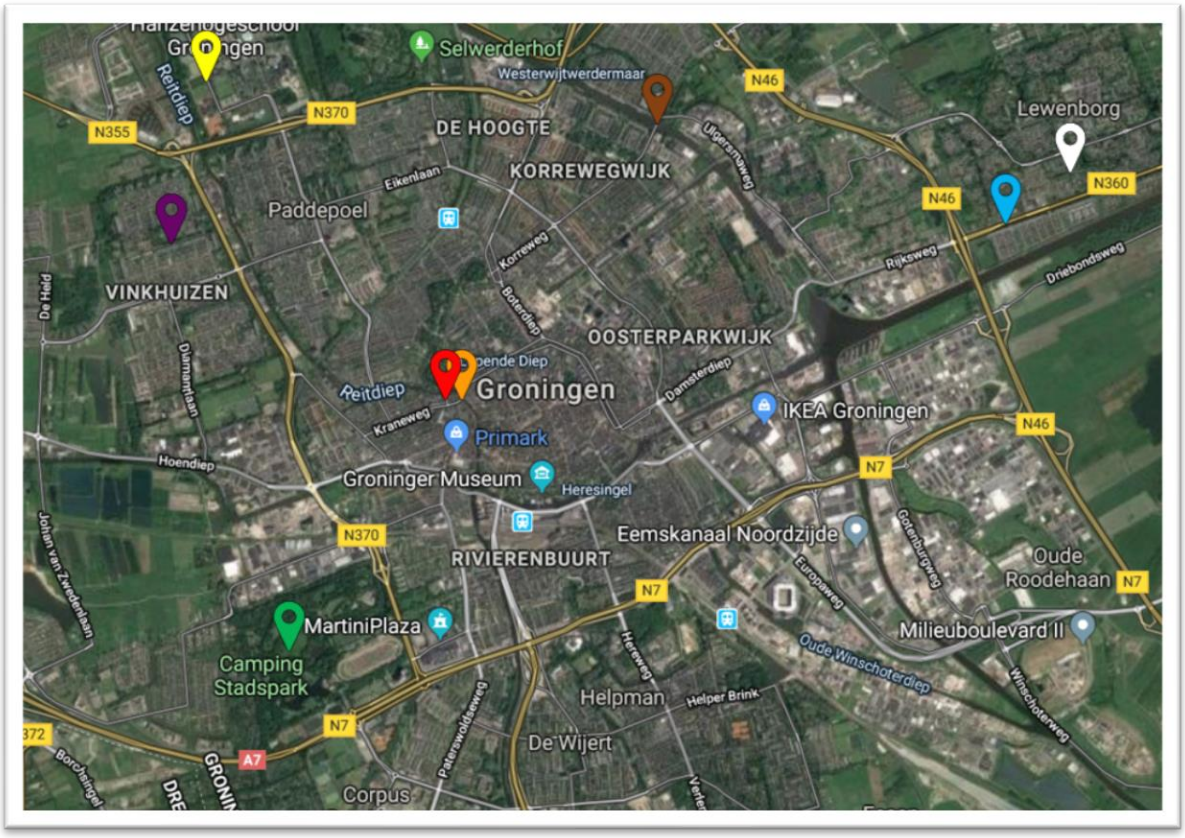

Figure 2. Map of Collection Areas in Groningen
A map of Groningen, the Netherlands, with all of the *Zygiella x-notata* collection areas marked. The following locations correspond with the colors above.
Yellow: A fence at the University of Groningen, Purple: A bridge (unnamed) found in Vinkhuizen, Green: A bridge (unnamed) found in a wooded area in Stadspark, Red: A bridge (Plantsoenbrug) found right next to the Noorderplantsoen park, Orange: A bridge (Visserbrug) found on Visserstraat, Blue: A bride (unnamed) found in Lewenborg, White: The fences and walls of an apartment complex (Meerpaal) found in Lewenborg, Brown: A very large bridge (Gerrit Krol-brug) found on the Korreweg.
Image created from Google Maps, 1/12/2019.

2.2 Species Information

Species identification was performed manually based on morphology. A spider species identification book was primarily used (Elfferich, 2018), with supplementary information gathered from the internet. Three primary orb-weaving species were identified: *Zygiella x-notata* (the Silver-Sided Sector Spider), *Larinioides sclopetarius* (the Bridge Spider), and *Nuctenea umbratica* (the Walnut Orb-Weaver Spider). Originally, all three species were collected and tested, but due to time constraints, *Zygiella x-notata* was chosen as the primary species of this study due to its ubiquitous presence in local urban environments, and its apparent quick web-building tendencies. Its smaller body also seemed to make it superior for in-lab and in-frame web-building. *Zygiella x-notata* is most recognizable from its grey banded legs, wavy dark grey dorsal pattern, and silver sheen on the side of its abdomen. They are small for orb weavers, with females ranging from 5 – 11 mm in length. *Zygiella x-notata* also has a very specific web morphology, as most webs tend to have a missing sector built in near the top of the web (Fig. 3). No male specimens were

used – spiders were discarded based on male-like morphological traits, such as enlarged pedipalps, a smaller, less-swollen abdomen, and with the front legs seeming to dominate the body and stick out at a larger angle (Fig. 4). Males typically do not display web-building behavior.

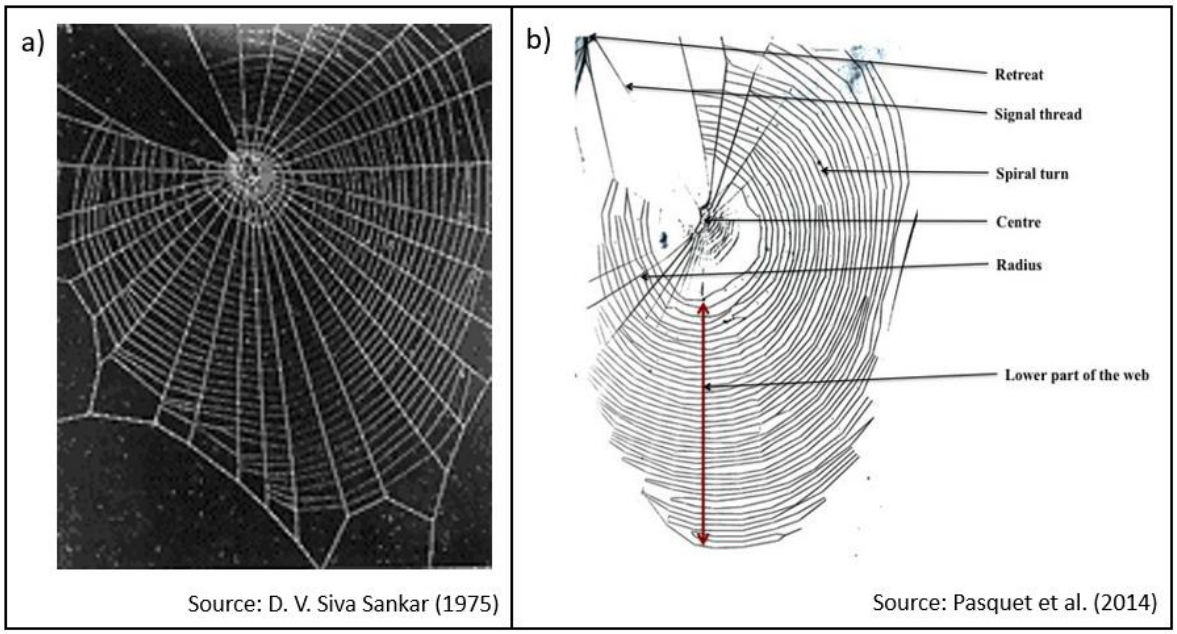

Figure 3. The web morphology of *Zygiella x-notata*

a.) Actual image taken of a well-constructed *Zygiella x-notata web*. Note the missing sector and signal thread. b.) An illustration of the typical structure of a *Zygiella x-notata web*, with labels.

For this study, 20 of the *Zygiella x-notata* individuals that were collected underwent experimental testing. *Zygiella x-notata* individuals were named via a simple code. All codes started off with Zx, in lieu of the full name "*Zygiella x-notata*." They were then assigned a number based on the order in which they entered the lab and were processed. The tested individuals ranged from Zx7 to Zx30. Data from Zx1 – Zx6 was discarded as the methodology was updated after testing these initial six, and Zx25 – Zx28 were never tested before the end of the experiment.

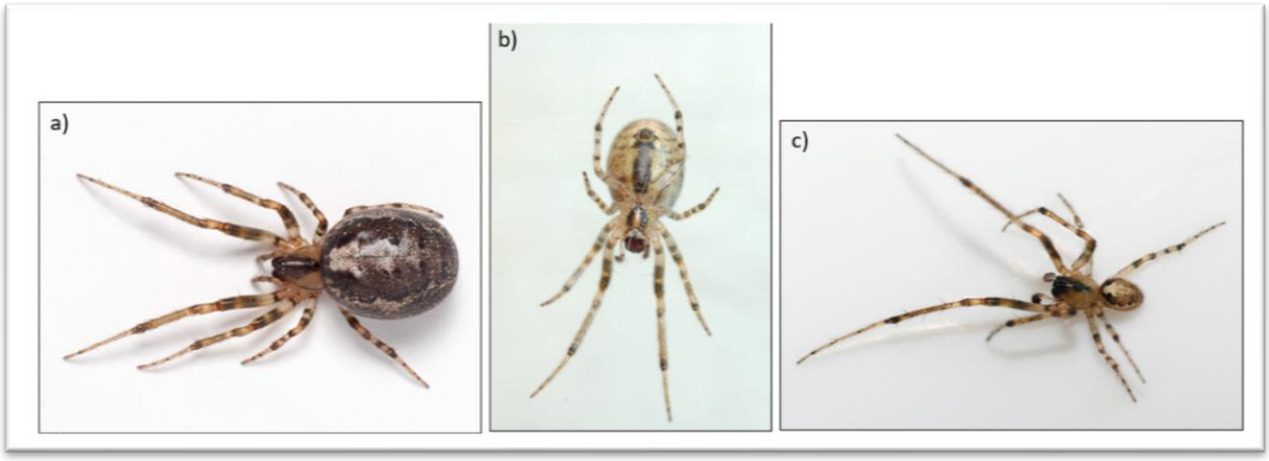

Figure 4. Zygiella x-notata

a.) A dorsal view of a female *Zygiella x-notata*. (Source: Peter Kooman)

b.) A ventral view of a female *Zygiella x-notata* (Source: John Brooks)

c.) A dorsal view of a male Zygiella x-notata. Notice the large pedipalps, the overall shape of the body, and the positioning of the legs. (Source: D.E. Chemnitz)

2.3 Specimen Preservation

At the end of the experimental testing period, spiders were preserved for possible future testing. Specimens were placed within tubes and put in the freezer at -20°C for one hour. At the end of the freezing period, specimens were then put into labelled Eppendorf tubes and filled with 96% analytical ethanol. Webs from post-experimental spiders were also collected from the respective frames and preserved in Eppendorf tubes with 96% ethanol for potential future DNA extraction for species identification confirmation.

2.4 Experimental Setup

In-lab, spiders' body length, abdominal width (W_A), and cephalothorax width (W_C) were measured and recorded. Specimens were then paired with the one closest to their size (based on body length) and each were inserted into one half of a custom-built housing frame (Fig. 3). The frames were crafted from 3 x 0.75 cm wooden slats, which served as the structure, and with two pieces of 18.5 x 14 cm acrylic plexiglass serving as the sides of the frames. The wooden slats were cut with a hand saw and then attached together with a cyanoacrylate adhesive, and the acrylic plexiglass was attached to the wooden frame with a glue epoxy specially made for plexiglass. A 14 cm tall wooden slat split the frame into two chambers, so that two spiders could be tested concurrently. The top of the frame consisted of a loose wooden slat (18.5 cm in length), that could slide in and out between the plexiglass walls to allow for spider introduction and removal. Two holes were drilled in the top of this wooden slat (one per each chamber side) to allow for both air flow and feeding. To prevent the spiders from escaping, this hole was covered with a piece of mesh, which was simply taped down to secure it. More detail can be seen in Figure 5.

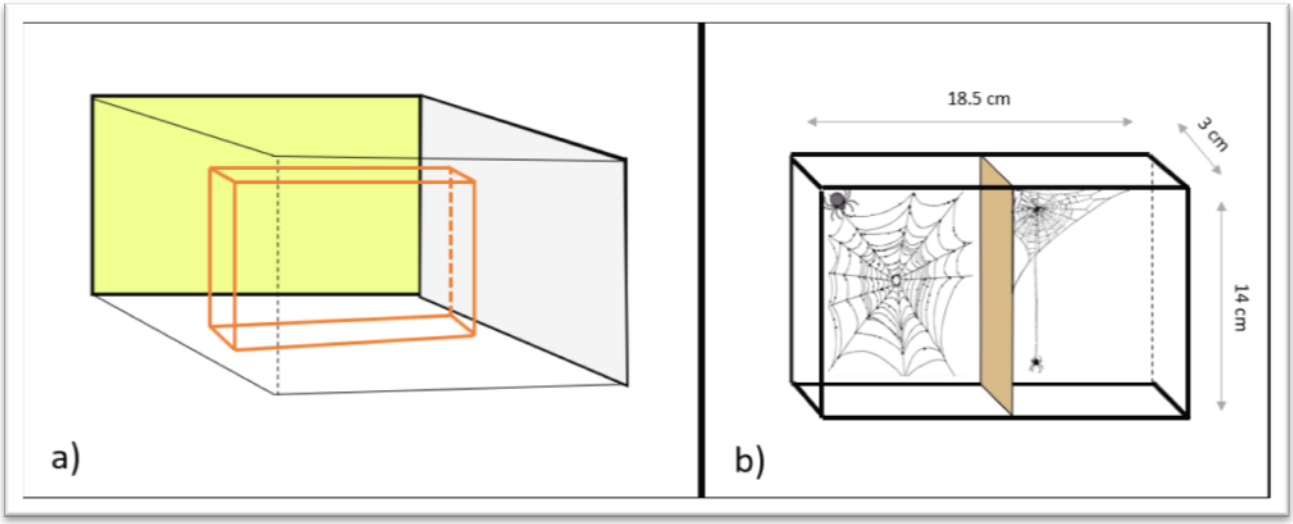

Figure 5. Experimental Setup of Zantiks Unit and Frames

a.) The experimental setup as seen inside the Zantiks unit. The far yellow vertical wall represents the screen that served as the light source. The grey vertical wall on the right side represents the back of the Zantiks unit. The open vertical wall on the left represents the opening of the Zantiks unit (which is closed off with a black piece of foam during experimental runs). The front open vertical wall represents where the camera is positioned. The red box inside represents the positioning of the frames during experimental runs.
b.) An overview of the frames used in this experiment. These frames were handcrafted to have two separate chambers so that two spiders might be tested simultaneously. A wooden slat (here highlighted in brown) served as a divider between the two chambers. All of the frame's sides, as well as the top and bottom, were also made from wood slats. The front and back facing vertical walls were made from solid acrylic plexiglass sheets.

2.5 Habituation

10 *Drosophila melanogaster* were fed to each spider on Day 1 to encourage web-building and to sustain energy during their time in the lab. They were fed with an apparatus that was designed for similar experiments with *Drosophila*. It consisted of a plastic, flexible tube of less than one cm in diameter. Gauze was stuffed in one end to serve as a filter, and the other end had a large pipette tip attached to it. This allowed for the user to remove *Drosophila melanogaster* from their stock tubes by sucking them into the pipette tip, and then blowing them into the chamber (via the holes drilled into the top of the frame) of each frame. Every frame was left in-lab for approximately three days to acclimate the spiders to the lab conditions and the standardized light cycle. Spiders left in-lab for longer than five days received 10 more flies, as well as water, which was applied to the web via a mister spray bottle. Every day spiders were observed and scored based on presence of web-building. If both spiders in a frame had built a proper orb-web, they could be used that day for daily rhythm testing. If one or neither of the spiders had built a (preferably) full orb web, they would remain within the acclimation period until both webs were present. Frames were cleaned-out in-between uses with a duster, and then disinfected with a clothed soaked in 70% ethanol. Frames were left to dry and air-out before inserting the next specimen to allow for the dissipation of the ethanol fumes.

2.6 Data Collection

Orb weaver time profile rhythm data was collected via the Zantiks AP unit from Zantiks Inc. The unit was inverted on its side to best film the relevant specimens. Frames were inserted within the unit, with the plexiglass walls parallel to the light screen and the camera, so that the webs were vertical. Spiders were inserted with their dorsal side facing the lit screen of the Zantiks unit (Fig. 5; also see Fig. 27A in the Appendix). Zanscripts (code to control the Zantiks unit) were written that allowed for the mimicking of lab light conditions (a ramping 12-hour light/dark cycle). The unit used a red, green, and blue LED-based white light source. Frames were only selected if the individuals on both sides had built a full orb web, so that activity would be normalized (i.e., a web-less spider constructing a web during the experimental treatment would likely present with higher activity patterns than one who had already built a web). Data collection began at 14:00 every other day. This time was selected to allow for the synchronicity of the Zantiks unit's lighting cycle with the laboratory condition's lighting cycle used for habituation. In this manner, bias would be controlled for in that the spiders would not receive their light treatment on a different schedule than what they had become used to during their lab habituation. The Zanscripts were written to allow for a 2-hour full light acclimation period, and then began an hour of ramping down. By setting experimental time for 14:00, the spiders inside the Zantiks unit would not enter the ramping down phase until 16:00, the same schedule that they had been exposed to previously. A dark foam door was used to seal the unit off and block all light. The Zantiks unit was run by a Zanscript designed by members on Zantiks staff, and everything could be operated externally by a computer. The unit was programmed to run through a 24-hour cycle twice. The phases are represented by LTON (lights on), LTOFF (lights off), RAMPON (lights ramping on), and RAMPOFF (lights ramping off). First, a two-hour acclimation period was set, which was in the LTON setting. This was to account for any disturbances in behavior that might result from the physical transfer from the lab setting to inside the Zantik's unit. Afterwards, the following cycle was run twice: RAMPOFF (1 hour), LTOFF (11 hours), RAMPON (1 hour), LTON (11 hours). Two days later, the Zantiks unit was stopped at 14:00 again, and the frame was swapped out for a different one with two new individuals (Fig. 6). The Excel file with all the locomotor activity was downloaded from the Zantiks server.

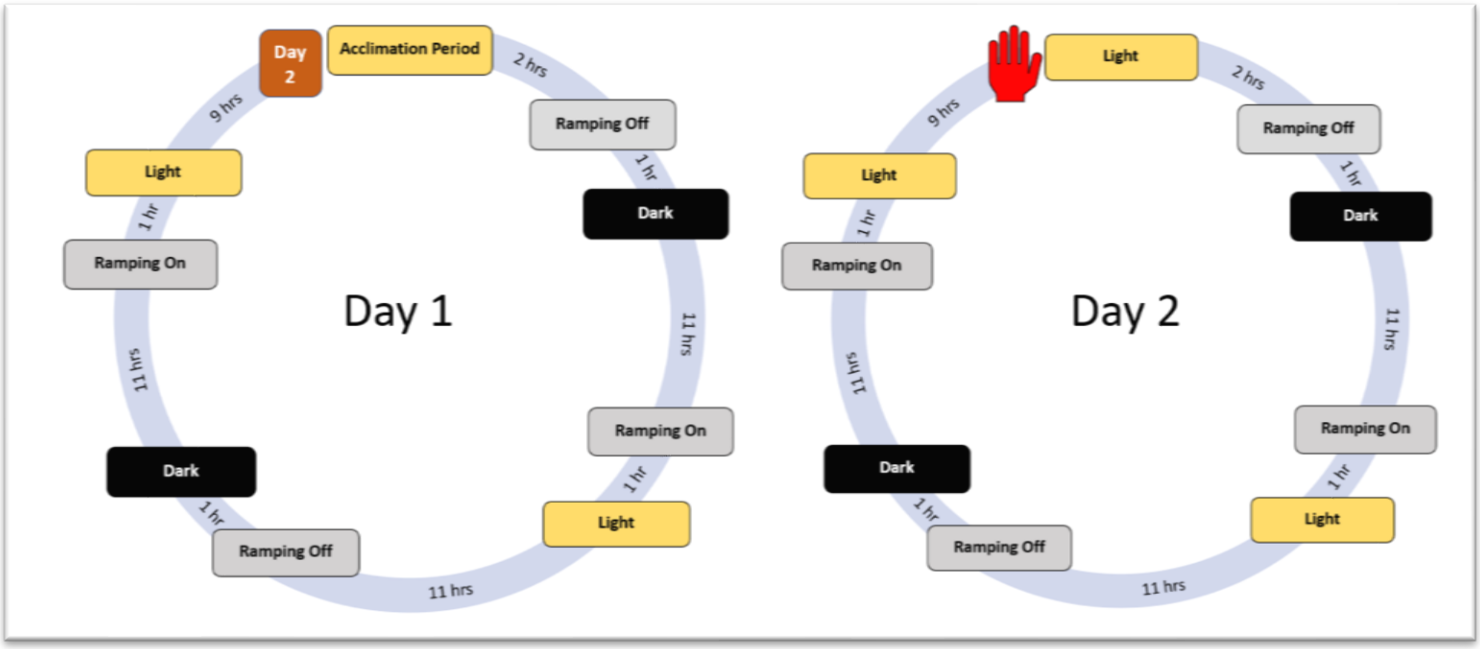

Figure 6. Process of the Light Treatment Phases in the Zantiks Unit

Day 1 begins with an acclimation period of two hours, and then continues with the typical 12-hour light/12-hour dark cycle, which includes a 1-hour transferring time of light ramping either on or off. As such, the second light treatment phase carries over into Day 2 by two hours. At the end of Day 2 the experiment is terminated (represented by the red hand), and a new frame was inserted into the machine for testing.

The primary Zanscript (the 12-hr light/dark cycle, with ramping transitions) was designed to test the baseline activity pattern of *Zygiella x-notata*. Two more scripts were to be designed and run to further analyze daily activity patterns of this species. One would have had a 48-hour period of full light, which would test for the species' free-running pattern and circadian period. The second was to have a 12-hr full light and 12-hr dim light cycle, to mimic urban conditions with artificial light at night ALAN. The effect of ALAN would be determined based on comparisons to the data gathered from the baseline run. However, due to problems formatting the Zantiks unit, leading to time restrictions, these last two scripts were unable to be performed, leaving opportunity for future research.

2.7 Statistical Analyses

Data was collected within one Excel 'master sheet' and imported into RStudio. Separate plots were made of each individual to visualize the data, with time of day as the independent variable and activity as the dependent variable. Plots were used to identify possible errors and to visualize the effect of the acclimation period to determine if the first two hours of data could be trustworthy. Zx14, Zx15, Zx17, Zx18, Zx19, Zx21, and Zx24 all appeared to react somewhat to the transfer into the Zantiks unit, and so the first hour of data (half of the acclimation period, as this is the period that spiders appeared to show restlessness) of all individuals was removed from the analysis. Spider Zx7 was removed, as it had no recorded movement during the entire 48-hours. Spiders Zx9 and Zx11 were also removed, as there was a malfunction within the unit, and the lighting cycle did not begin until 3.67 hours after starting the program. The (lighting) environment inside the unit cannot be known, and therefore their data must be discarded.

The log of the variable *Activity* was taken to transform the data due to two individuals (Zx14 and Zx24) having some exceptionally high data points. A linear mixed model (LMM) was used to evaluate the effects of the variables. Using R (version 3.6.1, package *lmerTest*), a maximum explanatory model (MEM) was created (Table 1b, see Appendix) with *Spider ID* modeled as a random effect to account for behavioral and physiological variation between individuals. Arena, light intensity at collection site, experimental light treatment stage, the first 24-hours versus the second 24-hours, time as a sine and cosine variant, length of the spider, and days habituated were modeled as explanatory variables. The MEM was subjected to stepwise elimination (using Likelihood Ratio Tests) to find the minimum adequate model (MAM); the best fit model with only the effects of significant (p = 0.05) variables remaining. QQ plots were made from the MAM for model diagnostics. Expected versus actual values were plotted. A Loess curve plot was also created for the collective data of all individuals.

Variables Glossary

Activity (logAct2): an interval variable that represents how far an individual moved (in millimeters) that has been log transformed.

Arena (Arena): a binary factor that distinguishes between the two categories of arenas, or chambers (Arena 1 and Arena 2), that a spider could have been placed in during experimental testing.

Light intensity at collection site (Lux): a six-level factor that distinguishes between the 6 assigned levels of light intensity that were visualized at the collection site. 0 = complete darkness, 1 = low light, 2 = low-medium light, 3 = medium light, 4 = medium-high light, and 5 = high light.

Light treatment stage (LT123): a three-level factor that distinguishes between the three characterizations of light seen during the experimental treatment stage. Darkness = 1, Ramping Off/On = 2, and Light = 3.

First 24-hours vs. second 24-hours (Day1v2): a binary factor that distinguishes between the two sets of 24-hours that were measured during this experiment. Day 1 = the first 24-hours, and Day 2 = the second 24-hours in the 48-hour experimental period.

Time as a sine and cosine variant (sin(time.circ), cos(time.circ)): variables used to test time as a circular function. Time was converted to radians, and then its sine and cosine were included for testing rhythmicity.

Spider length (Length): an interval variable that represents the body length of the spider at the start of the habituation process. Range: 0.29 – 0.65 cm.

Days habituated (HabDays): an interval variable that represents the amount of days the spider spent in habituation before entering the experimental process. Range: 1 – 8 days.

3. Results

Initial visual examination of the individual plots indicated synchronicity between individuals and a nocturnal classification for the species. The minimal adequate model (MAM) derived from the general linear mixed model with log activity as the dependent variable showed that logAct2 is predicted by SpiderID (random factor) + Arena + Lux + Day1v2 + HabDays*LT123 + sin(time.circ)*Length + cos(time.circ)*Length. The MAM showed highly significant results (Table 1a).

The MAM found a highly significant effect of *Day1v2*. This variable had a negative coefficient. This implies that spiders were overall more active during the first 24-hours than during the second 24-hours. Habituation days (*HabDays*) had a significant effect, with the spiders that had longer habituation days displaying higher levels of activity. The light treatment stage (*LT123*) was highly significant and had a negative coefficient, indicating that activity was highest during the dark phase. Light intensity at the origin collection location (*Lux*) was highly significant, with a negative coefficient. This implies that spiders collected from darker locations presented with higher activity than those collected from better-lit locations. The effect of spider length (*Length*) was significant. This variable had a positive coefficient, indicating that larger spiders moved further. The interactions between habituation days and origin light intensity and light treatment and origin light intensity were both highly significant. The interaction between spider length and the cosine of *time.circ* was highly significant, with a positive coefficient; however, the interaction between spider length and the sine of *time.circ* was not significant. However, due to the significance of the interaction of the cosine of *time.circ* and spider length, it will remain within the model. The cosine of *time.circ* was highly significant, with a negative coefficient, but the sine of *time.circ* was not significant, and it also had a negative coefficient. Table 1 contains the summary output for the minimum adequate model and the maximum explanatory model.

The QQ plot for the MAM showed good fit for a large part of the model (Fig. 1A, see Appendix). A Loess curve plot was created to increase the interpretability and reduce the noise shown within Figure 7, and to show the approximate sine wave (Fig. 8).

Table 1a – Linear Mix Model (LMM) Results

The summary of the minimum adequate model.

Fixed Effects – Minimum Adequate Model (MAM)				
	Estimate	**Std. Error**	**t-value**	**Pr(>|z|)**
(Intercept)	-0.172	0.783	-0.220	0.826
Day1v2	-0.811	0.019	-41.650	< 2e-16 ***
HabDays	0.252	0.111	2.265	0.024 *
LT123	-0.972	0.042	-23.156	< 2e-16 ***
Lux	-0.564	0.161	-3.508	0.0005 ***
sin(time.circ)	-0.066	0.067	-0.985	0.325
Length	2.527	0.981	2.576	0.010 **
cos(time.circ)	-0.153	0.063	-2.418	0.016 *
HabDays*LT123	-0.086	0.005	-18.064	< 2e-16 ***
LT123*Lux	0.175	0.007	23.719	< 2e-16 ***
sin(time.circ)*Length	0.063	0.130	0.484	0.628
Length*cos(time.circ)	0.630	0.124	5.084	3.69e-07 ***

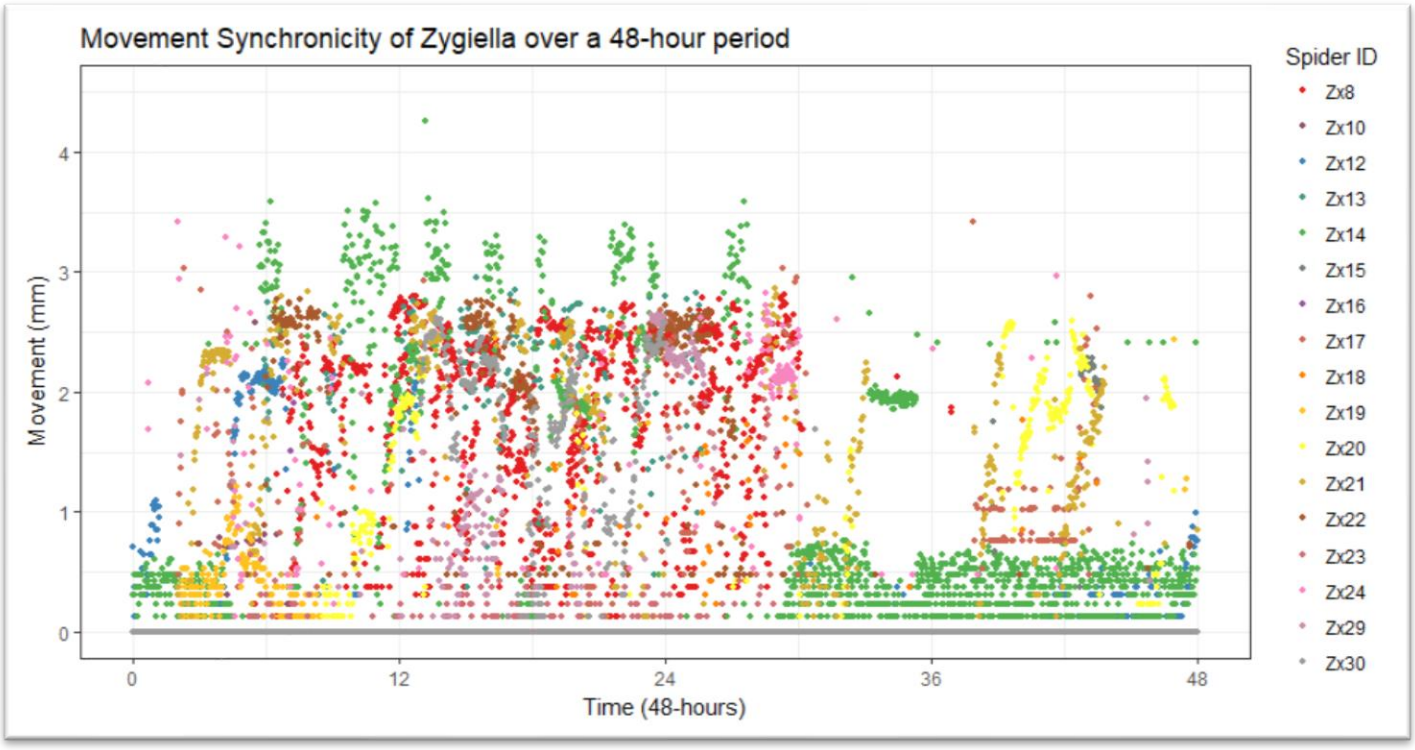

Figure 7. Scatterplot of activity of all tested individuals

All movement displayed by all tested individuals during the 48-hour experimental phase.

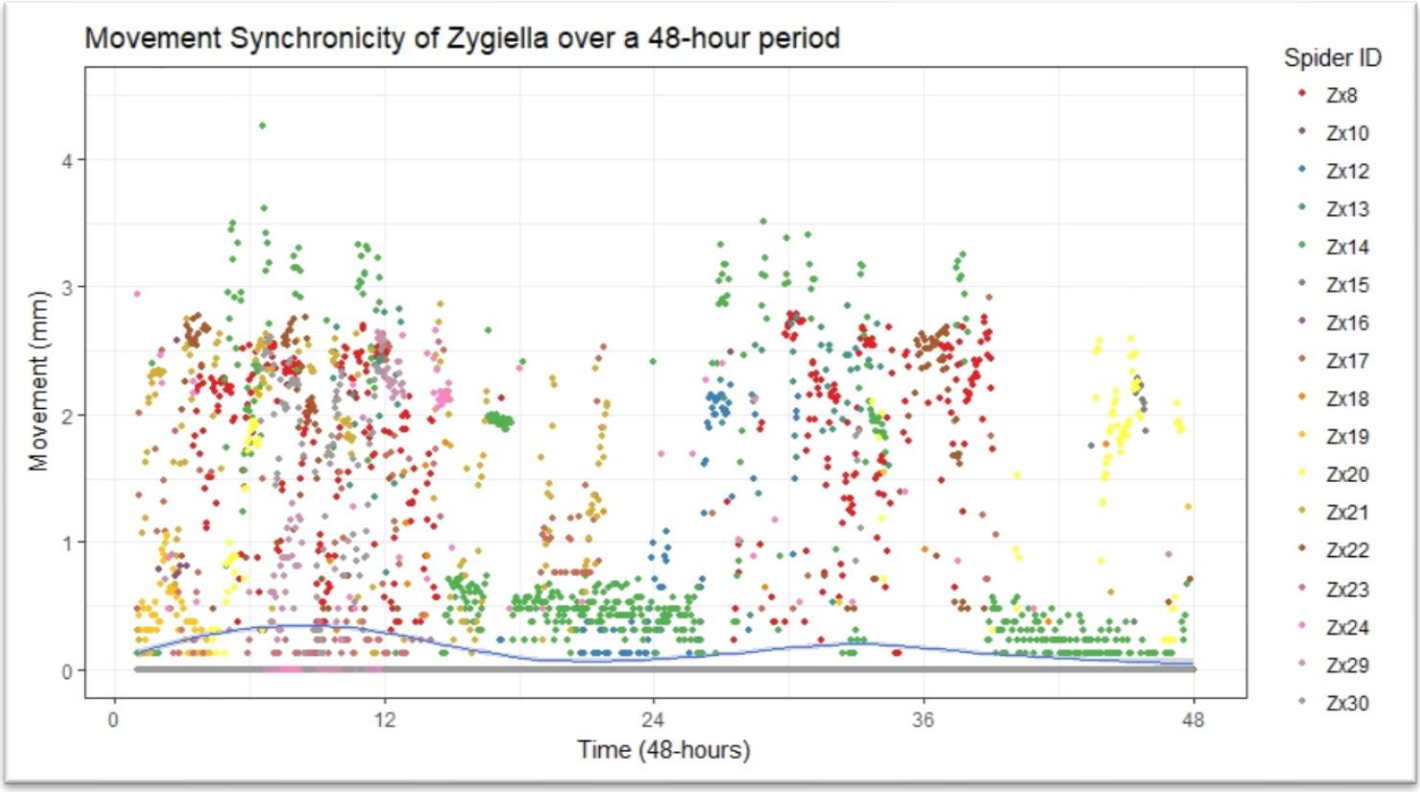

Figure 8. Loess curve of all tested individuals

Movement (33%) displayed by all tested individuals during the 48-hour experimental phase. A Loess curve shows the synchronicity of these individuals. Spider ID can be seen in the legend on the right.

4. Discussion

Zygiella x-notata is an organism that is largely under-represented in research, making many of the results found here to be original but also sometimes preliminary. *Zygiella x-notata* was determined to be a nocturnal species whose temporal niche is likely to be impacted by ALAN, as seen by the significant role that origin light intensity plays in *Zygiella x-notata*'s reaction to exogenous light cues. Additionally, this species appears to rely heavily on its previous experiences, going as far as to alter its web-building and foraging behavior based on temporal memory (Venner, Pasquet, & Leborgne, 2000). Larger spiders also appear to be more active, while also more finely selecting when they exert their energy, which is likely due to their increased experience. In reference to their daily activity, all individuals appeared to display a sort of collective synchronicity, as seen in the Loess curve (Fig. 8). As these individuals were all exposed to the same light treatment, this would indicate the presence of an endogenous biological clock in this species that leads individuals to react to the changing of their environment in regard to light very similarly.

The MAM presented with many highly-significant variables, allowing for a variety of analysis and conclusions. While the QQ plot had an initial good fit, the actual values deviated from the expected values at some point, which is likely due to the two individuals with a few exceptionally high activity points (>3000mm/minute). This individual-specific

deviation is likely due to a sampling error. With this consideration, a lot of the variance in the samples can be explained by the MAM.

Many individuals displayed what appears to be predictive behavior for the ramping-off lighting phase, as activity peaks right before RAMPOFF were seen in 33.3% of the cases, showing probable anticipation of day/light cycles. Further studies would have to be performed to classify and prove the existence of the behavioral phenotype.

4.1 Classifying *Zygiella x-notata* as nocturnal

While already assumed to be nocturnal, the results of this study have shown that the orb-weaving spider *Zygiella x-notata* can indeed be classified as a nocturnal species. The MAM showed high significance for the light treatment variable, indicating more movement in the dark treatment phase. 58% of activity points were measured during the dark treatment phase, 9% of activity points were measured during the ramping light treatment phases, and 33% of activity points were measured during the light treatment phase. However, this was simply just the count of times moved, and did not take into consideration the magnitude of that movement. The average of the movement for each light treatment phase showed that spiders travelled over 11 times further on average during the dark treatment phase than during the light (Fig. 20A, see Appendix). While this all indicates a high preference for nocturnal activity, it does also suggest that *Zygiella x-notata* has some crepuscular tendencies as well. Most other orb-weaving spiders are nocturnal, so this falls in-line with expectations.

However, field observations did show the *Zygiella x-notata* to respond to prey caught in its web during daylight hours. Upon sensing the vibrations from a struggling prey, the spider would leave its retreat, catch the prey, and then quickly return to the retreat with the prey. This phenomenon was observed countless times; here, the benefit the spider receives from quickly grabbing the prey likely outweighs the risk of getting seen by a predator, as the spider is only exposed for mere seconds. An activity such as web-building, however, which takes the spider much longer, is safer to be performed under the cover of darkness. Displaying some activity outside of a species' temporal niche is not uncommon for spiders that are sit-and-wait foragers, as they must capitalize on prey as soon as it becomes available to them.

4.2 *Zygiella x-notata* presents with a daily rhythm pattern

The sine and cosine of *time.circ* were evaluated together, with the results being significant, therefore implying synchronicity of the individuals used in this study. This indicates that *Zygiella x-notata* has a clear daily activity pattern, as opposed to random, unpredictable periods of activity and inactivity. Species evolved within a specific temporal niche environment, of which lighting is a factor (Hut et al., 2012). By having a clear (in this case, nocturnal) pattern, *Zygiella x-notata* is avoiding its natural predators while still having significant enough prey availability for survival. As ALAN attracts flying insects, and some orb-weaving spiders seem to have a preference for building their webs in areas lit by ALAN

(Florez Blesgraeft, 2019; Heiling, 1999), establishing a web near a source of ALAN is rather ideal for the nocturnal *Zygiella x-notata*, as it is able to stick to its temporal niche, thereby avoiding predation while capitalizing on prey availability.

The Loess curve (Fig. 8) showed that all individuals displayed a similar synchronicity throughout a given day. The curve fits with the model and with expectations: activity peaked during the dark hours and dropped during the light hours. Due to the magnitude of the data, a Loess curve using the original 1-minute time bin measurements was unable to be plotted on a standard computer, and would need to instead be computed on a cloud-based system. Therefore, time bin sizes were increased until computation was possible; the final result was a plot that had time bins that were 3x larger and included 33% of the original data (across the entire original period). A Loess curve with delineated light treatment phases can be found in the Appendix for easier interpretation (Fig. 19A, see Appendix).

4.3 The correlation between body size and activity

Length, which was used as a proxy for body size of the spider, was found to be a significant variable within the model. It indicated that the larger the spider, the more it travelled. This could possibly stem from larger spiders having more energy, or simply because their longer legs allow them to traverse distances throughout their frame more easily and quickly. Smaller spiders would have to exert more energy to travel across the same distance. Additionally, their larger body size would require more energy, and therefore they may stop being satiated more quickly after a feeding than the smaller spiders would. The increased movement of larger spiders could be related to higher foraging activity.

Smaller spiders were active earlier than larger ones, and appeared to display a longer period of activity than their larger counterparts as well (Fig. 23A, see Appendix). These findings were in-line with expectations, as well as the field observations made during collection times, where smaller spiders appeared to be active (i.e. sitting in the hub of their web or engaging in web-building activity) earlier during the twilight/dusk stage than larger spiders. This biological explanation for this could simply be that larger spiders are more exposed to predators due to their superior body size, and therefore waiting until the complete cover of darkness is the better choice to increase their survival chances. Additionally, larger spiders are more experienced, and therefore may be able to better predict when twilight and/or full-darkness will begin. As *Zygiella x-notata* renew their web most nights, it could be that larger spiders' superior experience has taught them that web-building is best-performed after foraging, when they have more energy. Spiders' learned behavior is also linked to prey availability (Venner et al., 2000), and therefore larger and older spiders are more experienced concerning the best time for prey interception and web renewal. Prey size and density may also vary based on the time of night; perhaps larger prey is more active later in the night, allowing larger spiders to exert less energy to catch more prey mass because they entered their foraging stage later. Further research would need to be done to link this possible explanation to spider size and activity periods.

4.4 Higher activity during the first 24-hours

The Day1v2 variable showed that spiders were significantly more active during the first 24-hours than during the second 24-hours. 64% of the activity points were measured during the first 24-hours, and 36% were measured during the second 24-hours. However, this was simply just the count of times moved, and did not take into consideration the magnitude of that movement. The average of the movement for the two 24-hour periods showed that spiders traveled over 1.5 times further on average during the first 24-hours than during the second 24-hours.

The differences between the two days could be due to the moving of the frame from the habituation section into the Zantiks unit, as the physical movement might have disturbed them. Additionally, in the habituation section of the lab the spiders sat approximately one meter away from the lights, while in the Zantiks unit they sat approximately eight centimeters away from the light source. Additionally, the light source during the habituation phase and the experimental phase might differ in intensity and light type (e.g. LED, fluorescent). These changes in environment have the potential to induce behavioral abnormalities, which may lead to the agitation of the spider and therefore increased movement following its transfer. Recommendations for future experiments would be to run the experimental phase for 72+ hours instead of 48 to see if activity levels stabilize or further decrease, or at the very least habituate the spiders in near-exact conditions to those inside the Zantiks unit.

4.5 Habitation days correlated with activity

The MAM model found the variable 'HabDays' to be significant, indicating that spiders that had a longer habituation phase were more active (i.e. travelled further) than those with shorter habituation days. This is assumedly due to the spiders being more accustomed to the light cycles (12-hour light/12-hour dark with a 1-hour ramping transition), as well as the lab temperature, humidity, and prey availability. These spiders might be better at predicting the light cycles, and therefore not only act in-sync with the light transitions, but also act more boldly. Additionally, as the spiders were provided with prey on their first day of habituation, the ones tested earlier on into their habituation period might feel more satiated, and therefore forage and travel less during their experimental testing.

The model also found a significant interaction between days habituated and the light treatment phase. Plotting out this interaction (Fig. 22A) showed that those who had been habituated for longer were more active during the dark treatment phase; however, this effect was not linear. Habituation times of two and four days led to the lowest average overall activity levels, with habituation times of one day and six days being very similar in overall activity levels. However, a habituation time of eight days was correlated with significantly more activity. This is likely due to the same reasons as listed above: familiarity, predictive behavior, and a lower foraging drive. Additionally, as shown by the study by Venner et al. (1999), *Zygiella x-notata* will adjust their web-building and foraging behavior if their currently strategies have either proven to be a success or a failure. By the eighth day,

the individuals may be foraging for prey more strongly than the newly-habituated ones, and as a result they could be renewing their webs more frequently to test different web phenotypes.

4.6 Origin light intensity and activity

Spiders found in locations with lower light intensity were found to have travelled further than those found in locations with higher light intensity. This could be due to these individuals having a more inherent 12-hour light/12-hour dark rhythm due to the low intensities of ALAN around where they had built their webs. Bringing these individuals into the lab would likely not prompt them to change their behavior, as the lab conditions were also set to 12-hour light/12-hour dark cycles. As such, these individuals would not have to undergo a 'learning curve,' where they would adjust to an alternative light cycle. Spiders originating from high-light intensity locations would be expecting ALAN during the night hours but would not receive it in the lab. The habituation period may not have been long enough for them to adjust their behavior to this new light regime.

The GLMM model found an interaction between origin light intensity and the light treatment stage. Using a basic bar chart, it is seen that individuals originating from low-lux environments displayed the highest activity levels during the dark light treatment phase relative to the other origin groups, as well as during the light treatment phase as well (though at a much-reduced rate). Individuals originating from low-medium light intensities displayed higher activity during the ramping phases relative to the other origin groups. Individuals coming from high-light intensity environments tended to move the least out of all groups during all light treatment phases. This can likely be explained by the same assumptions as listed above, as the spiders coming from high-light origins may have needed a longer habituation time. An interesting future comparison would be to repeat this laboratory experiment with similarly-sourced individuals, except having the light treatment consist of ALAN (during the dark phases), and observing whether this trend switches in favor of the high-light origin spiders.

5. Future Directions & Limitations

Research on the orb weaver spider guild has been extremely limited in more recent times, as the majority of research was published during the 1960s – 1980s. The rising trend of urbanization and increasing levels of ALAN make urban ecology a very vital field for the preservation of our natural environment and biodiversity levels, and the orb weaving spider provides an excellent model for such research. Its size makes it ideal for laboratory experiments and its nocturnality makes it a good test subject for studying ecological and biological responses to ALAN. Additionally, many orb weavers display strict circadian rhythms, therefore making them ideal test subjects for chronobiology studies.

Modern technology and trends of rising urbanization and urban pollution call for more modern testing to be performed to understand and quantify the effects of

urbanization on ecosystems and biodiversity. This study had originally aimed to evaluate (i) the baseline activity pattern of *Zygiella x-notata* under 12-hour light/dark lighting conditions, (ii) the activity pattern of *Zygiella x-notata* under 12-hour light/12-hour dim light lighting conditions, and (iii) the circadian period based off a free run investigation (48-hours of constant dim light). Only the first part was completed due to time constraints. The experimental setup in part II would represent an environment with ALAN, and therefore the information gleaned from this project could be crucial to our understanding of how species respond to light pollution. Part III would allow for the testing of the species inherent circadian rhythm, which is useful for any studies that aim to characterize the effects of ALAN on the circadian rhythm of these species.

Originally this study had aimed to test multiple orb-weaving spider species, but this was not possible within the established time period. Repeating this protocol (along with parts II and III mentioned above) on multiple other urban orb-weaving spider species would generate a helpful database of baseline activity patterns and circadian rhythms that would add to the limited knowledge of this species, as well as to our knowledge of the effects of urban pollution on species. For future experiments done within the Netherlands, I recommend the Bridge Spider (*Larinioides sclopetarius*), the Walnut-Orb Weaver (*Nuctenea umbratical*), and the Cross Spider – also known as the European Garden Spider (*Araneus diadematus*). Both *L. sclopetarius* and *N. umbratical* are found predominantly in urban environments and tend to adhere to strict nocturnal schedules, allowing for comparison to this *Zygiella x-notata* study. *A. diadematus* tends towards nocturnality but is not as strict as many other orb-weavers. Additionally, it can be found in both urban and rural environments.

The varied habitat type of *Araneus diadematus* makes it the ideal candidate for a study that compares the behavioral differences of urban versus rural species. Future studies should determine if activity level patterns and circadian rhythms vary between these two populations, as not only does this provide potential insight on ALAN and urban-based pollution types, but also has possible implications for the future evolution of this species.

I would also recommend repeating this same experiment, but with a larger sample size to draw more accurate and confident conclusions.

6. Conclusion

The orb-weaving spider *Zygiella x-notata* was found to display nocturnal tendencies, aligning itself with most of the others in the orb-weaver guild. It also displayed a clear daily rhythm, courtesy of its biological clock. *Zygiella x-notata*, along with many other orb-weaving spiders, use nocturnality as an adaptation to avoid common diurnal predators, but suffer from reduced prey availability. The presence of ALAN offers a great benefit for these species: the possibility of increased prey availability without having the switch temporal niches, where they would be more exposed to predation. As such, the urban *species Zygiella x-notata* might be one of the few species to adapt well to the fast-changing urban world.

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Appendix

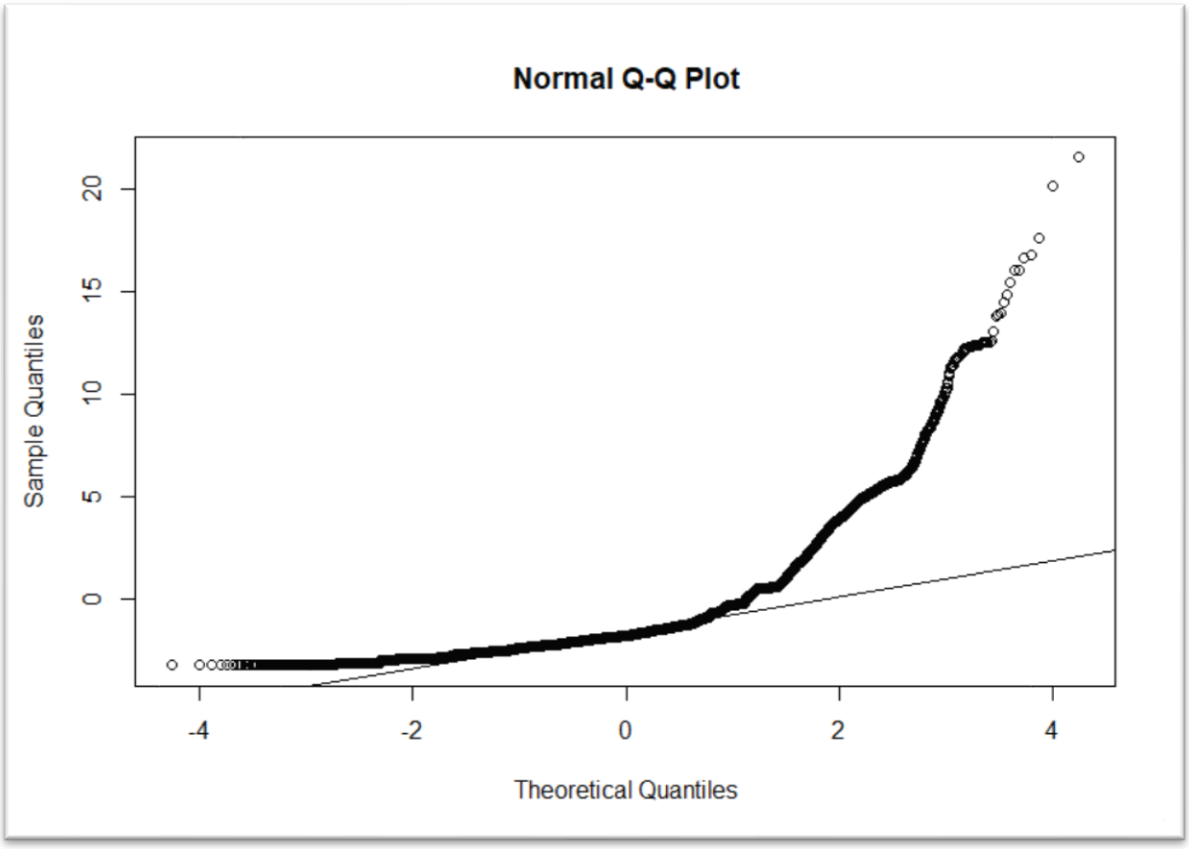

Figure 1A. QQ Plot of Expected vs. Actual Quantiles of the MAM

A QQ plot displaying a good fit line until approximately 1.5 on the x axis. This deviation is theorized to be due to abnormally high activity values present within two individuals.

Table 1b. The Maximum Explanatory Model (MEM)

The summary of the maximum explanatory model.

Fixed Effects – Maximum Explanatory Model (MEM)				
	Estimate	**Std. Error**	**t-value**	**Pr(>|z|)**
(Intercept)	-1.123	1.037	-1.084	0.278
Arena	0.111	0.509	0.219	0.827
Lux	-0.204	0.169	-1.204	0.228
Day1v2	-0.743	0.020	-37.789	< 2e-16 ***
HabDays	0.064	0.119	0.535	0.592
LT123	-0.526	0.105	-5.026	5.01e-07 ***
Length	2.885	1.683	1.715	0.086
sin(time.circ)	0.005	0.117	0.045	0.964
cos(time.circ)	-0.148	0.099	-1.495	0.135
LT123*Length	-0.346	0.215	-1.605	0.109
Length*sin(time.circ)	-0.124	0.238	-0.521	0.602
Length*cos(time.circ)	0.795	0.205	3.884	0.0001 ***

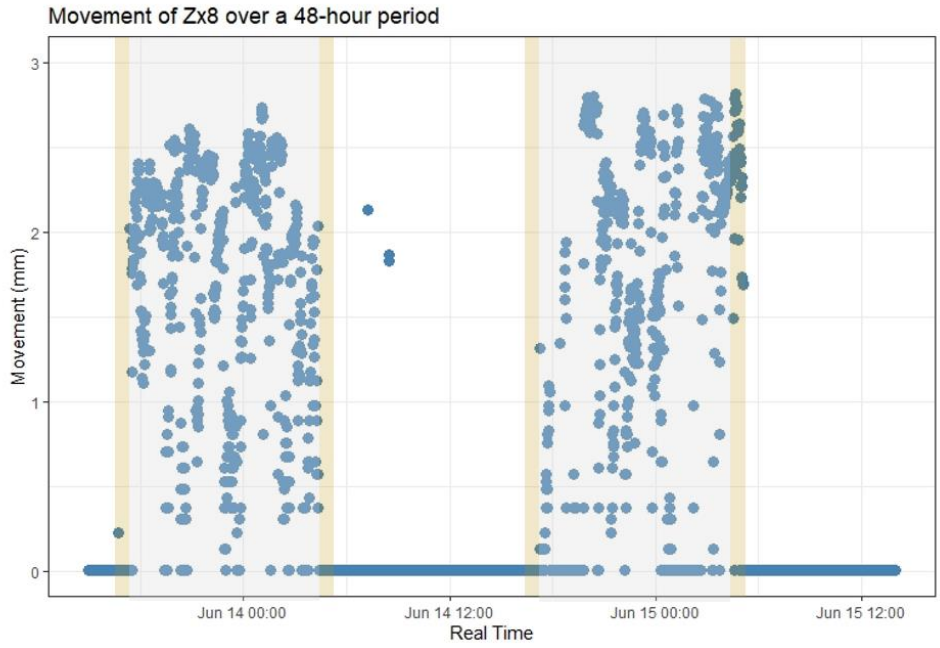

Figure 2A. The movement of individual Zx8 over a 48-hour period.

The x-axis indicates the date and time at which the measurements were taken, and the y-axis indicates the distance in which the spider travelled within a 1-minute time bin. The grey background represents the hours during which the spider received no light (darkness) in the experimental setup, while the brown background represents the ramping off or ramping on period. A plain white background indicates where the spider received a positive light treatment.

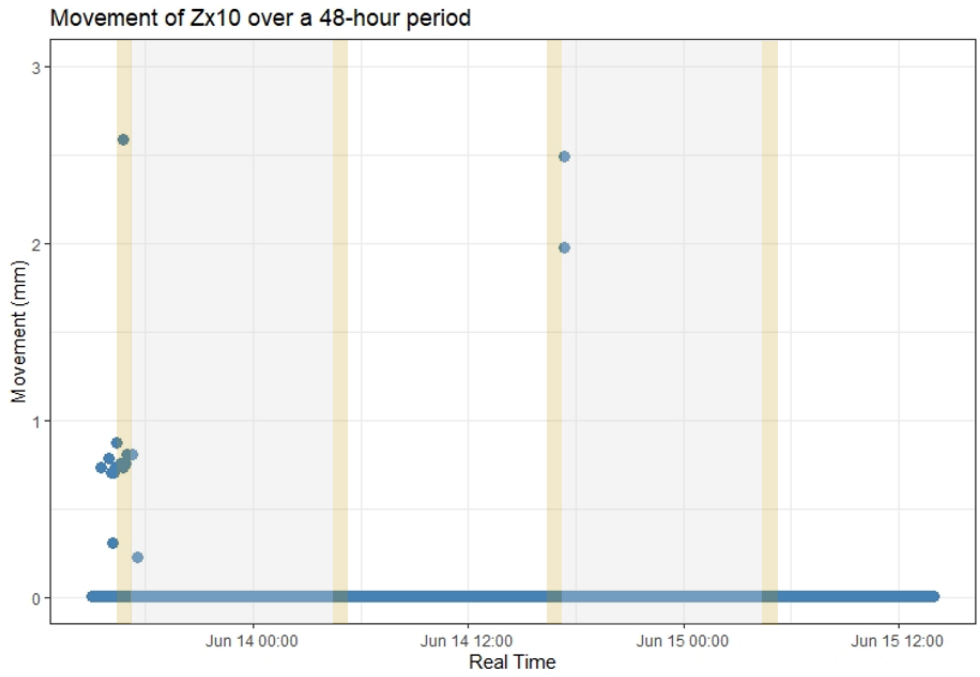

Figure 3A. The movement of individual Zx10 over a 48-hour period.
The x-axis indicates the date and time at which the measurements were taken, and the y-axis indicates the distance in which the spider travelled within a 1-minute time bin. The grey background represents the hours during which the spider received no light (darkness) in the experimental setup, while the brown background represents the ramping off or ramping on period. A plain white background indicates where the spider received a positive light treatment.

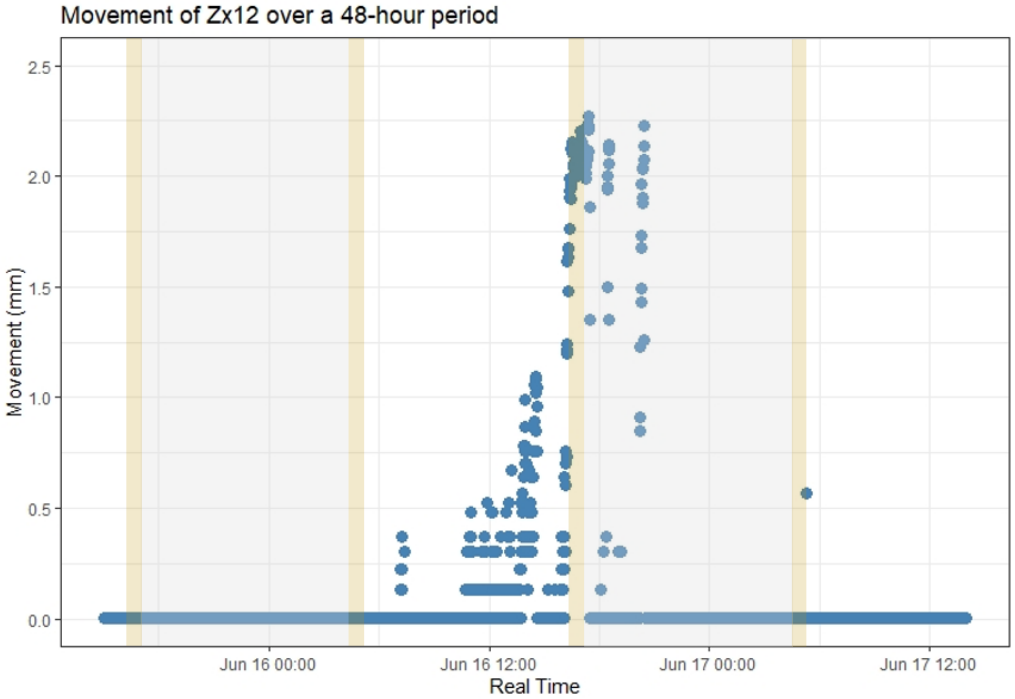

Figure 4A. The movement of individual Zx12 over a 48-hour period.
The x-axis indicates the date and time at which the measurements were taken, and the y-axis indicates the distance in which the spider travelled within a 1-minute time bin. The grey background represents the hours during which the spider received no light (darkness) in the experimental setup, while the brown background represents the ramping off or ramping on period. A plain white background indicates where the spider received a positive light treatment.

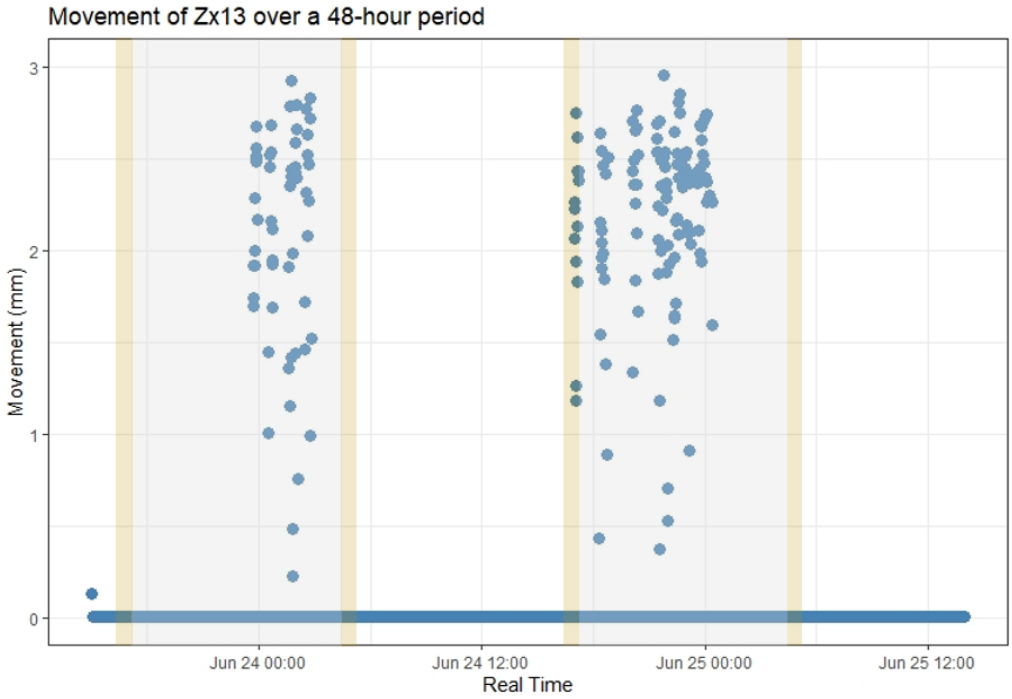

Figure 5A. The movement of individual Zx13 over a 48-hour period.
The x-axis indicates the date and time at which the measurements were taken, and the y-axis indicates the distance in which the spider travelled within a 1-minute time bin. The grey background represents the hours during which the spider received no light (darkness) in the experimental setup, while the brown background represents the ramping off or ramping on period. A plain white background indicates where the spider received a positive light treatment.

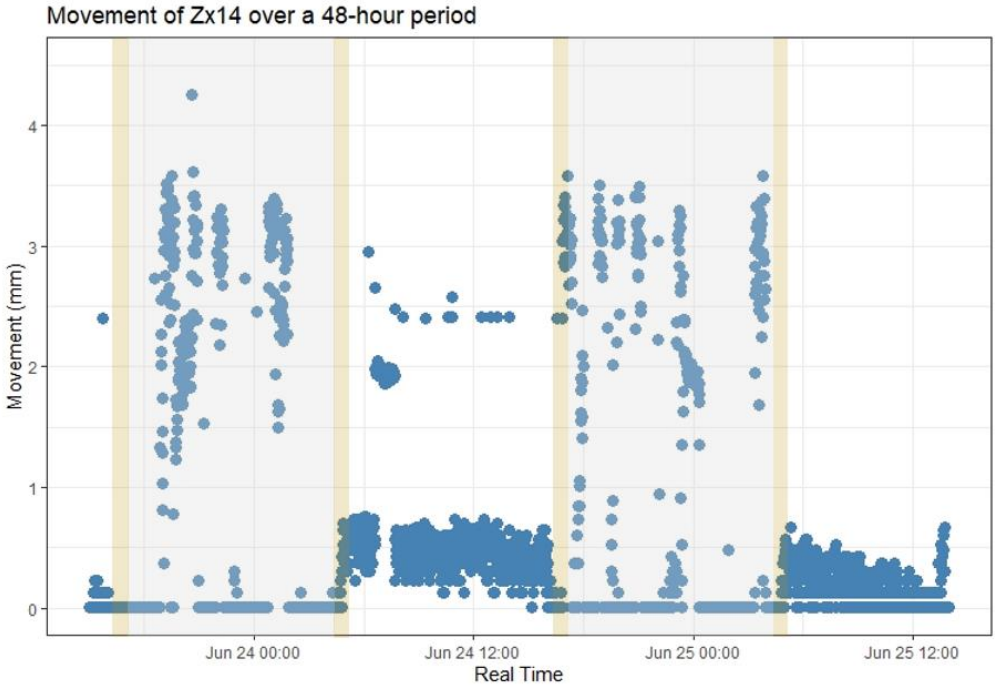

Figure 6A. The movement of individual Zx14 over a 48-hour period.
The x-axis indicates the date and time at which the measurements were taken, and the y-axis indicates the distance in which the spider travelled within a 1-minute time bin. The grey background represents the hours during which the spider received no light (darkness) in the experimental setup, while the brown background represents the ramping off or ramping on period. A plain white background indicates where the spider received a positive light treatment.

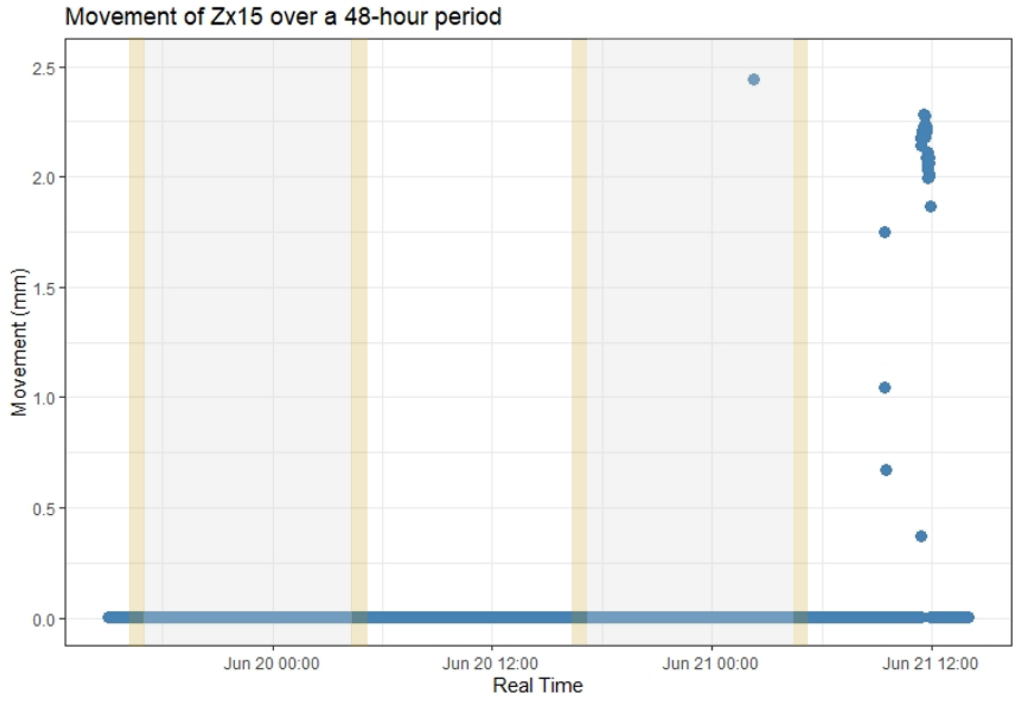

Figure 7A. The movement of individual Zx15 over a 48-hour period.
The x-axis indicates the date and time at which the measurements were taken, and the y-axis indicates the distance in which the spider travelled within a 1-minute time bin. The grey background represents the hours during which the spider received no light (darkness) in the experimental setup, while the brown background represents the ramping off or ramping on period. A plain white background indicates where the spider received a positive light treatment.

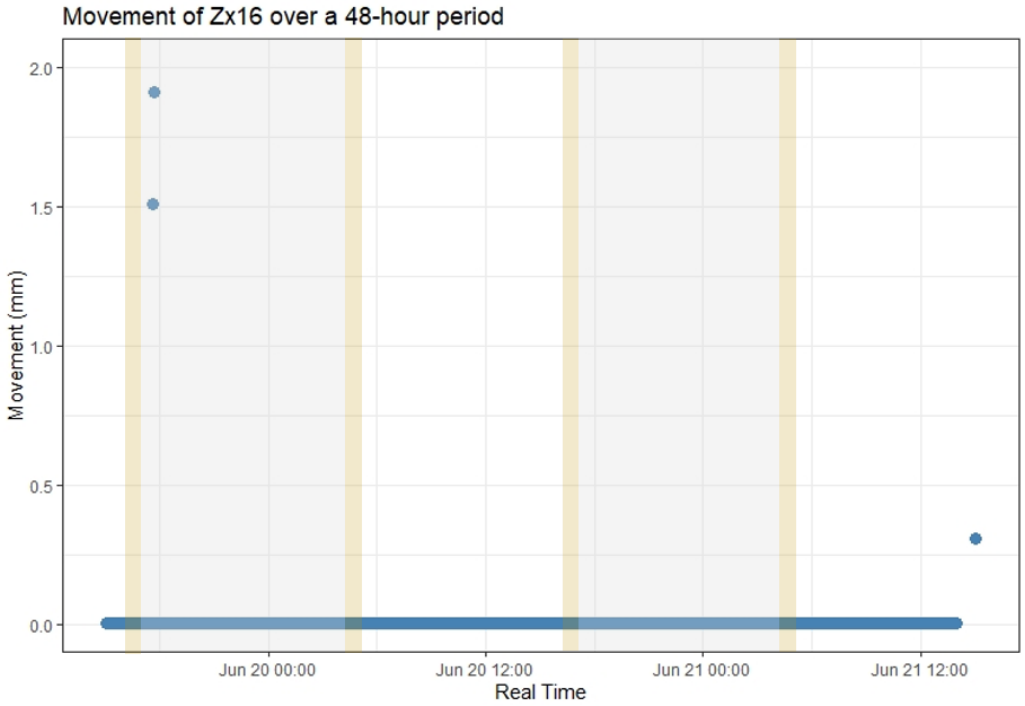

Figure 8A. The movement of individual Zx16 over a 48-hour period.
The x-axis indicates the date and time at which the measurements were taken, and the y-axis indicates the distance in which the spider travelled within a 1-minute time bin. The grey background represents the hours during which the spider received no light (darkness) in the experimental setup, while the brown background represents the ramping off or ramping on period. A plain white background indicates where the spider received a positive light treatment.

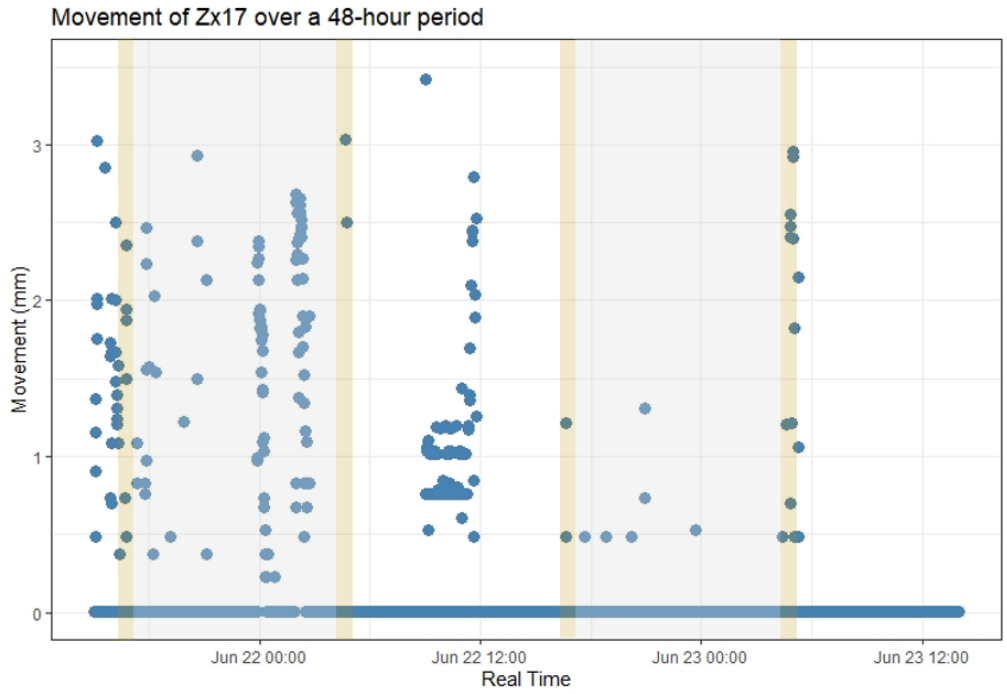

Figure 9A. The movement of individual Zx17 over a 48-hour period.
The x-axis indicates the date and time at which the measurements were taken, and the y-axis indicates the distance in which the spider travelled within a 1-minute time bin. The grey background represents the hours during which the spider received no light (darkness) in the experimental setup, while the brown background represents the ramping off or ramping on period. A plain white background indicates where the spider received a positive light treatment.

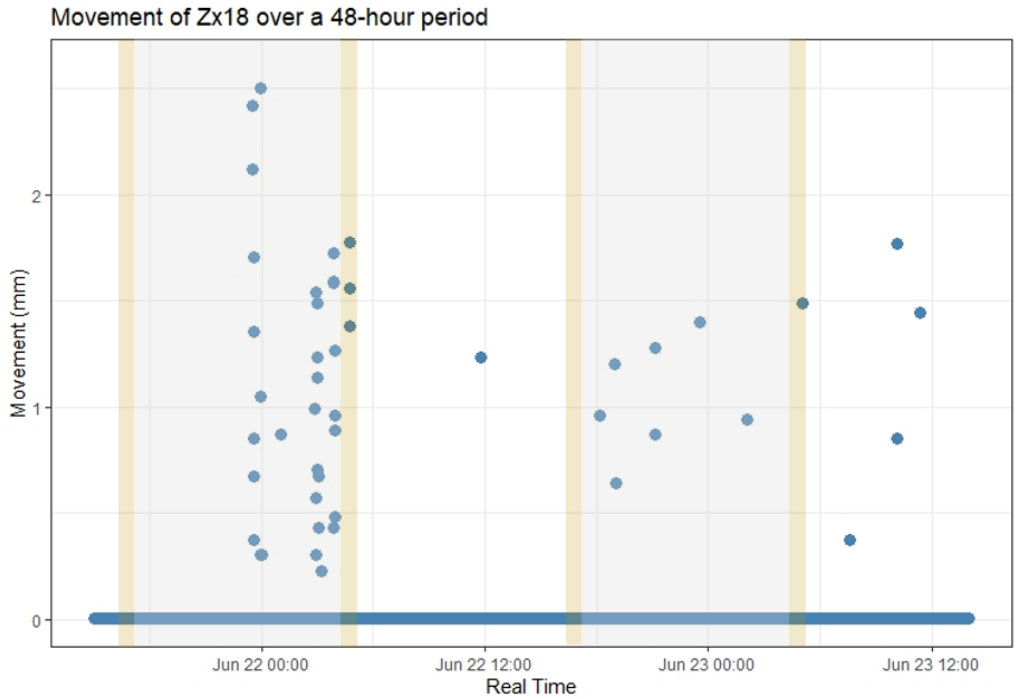

Figure 10A. The movement of individual Zx18 over a 48-hour period.
The x-axis indicates the date and time at which the measurements were taken, and the y-axis indicates the distance in which the spider travelled within a 1-minute time bin. The grey background represents the hours during which the spider received no light (darkness) in the experimental setup, while the brown background represents the ramping off or ramping on period. A plain white background indicates where the spider received a positive light treatment.

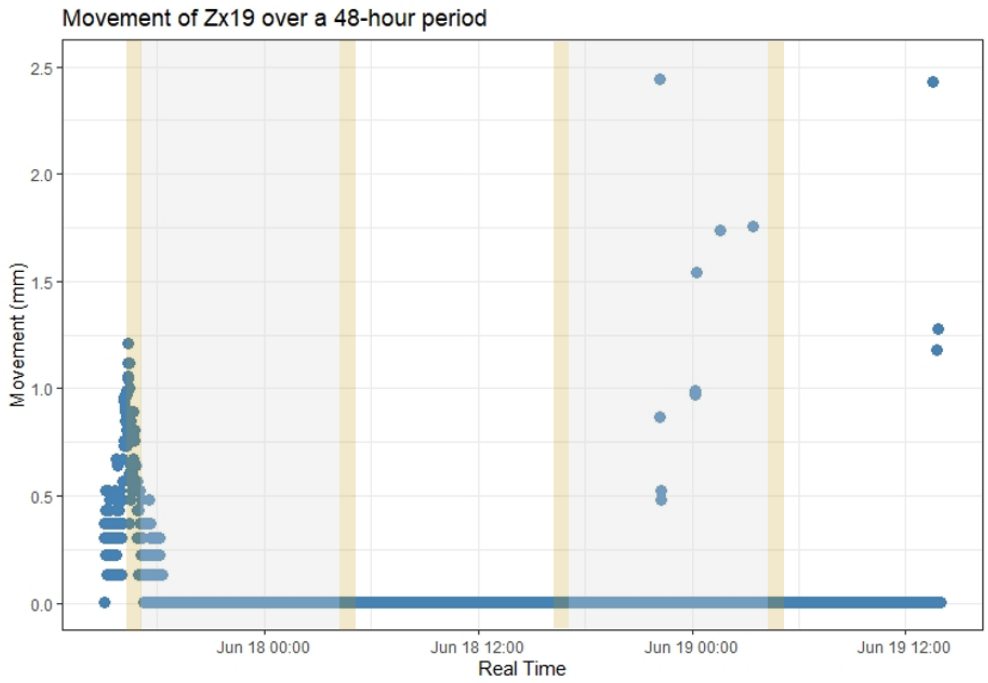

Figure 11A. The movement of individual Zx19 over a 48-hour period.
The x-axis indicates the date and time at which the measurements were taken, and the y-axis indicates the distance in which the spider travelled within a 1-minute time bin. The grey background represents the hours during which the spider received no light (darkness) in the experimental setup, while the brown background represents the ramping off or ramping on period. A plain white background indicates where the spider received a positive light treatment.

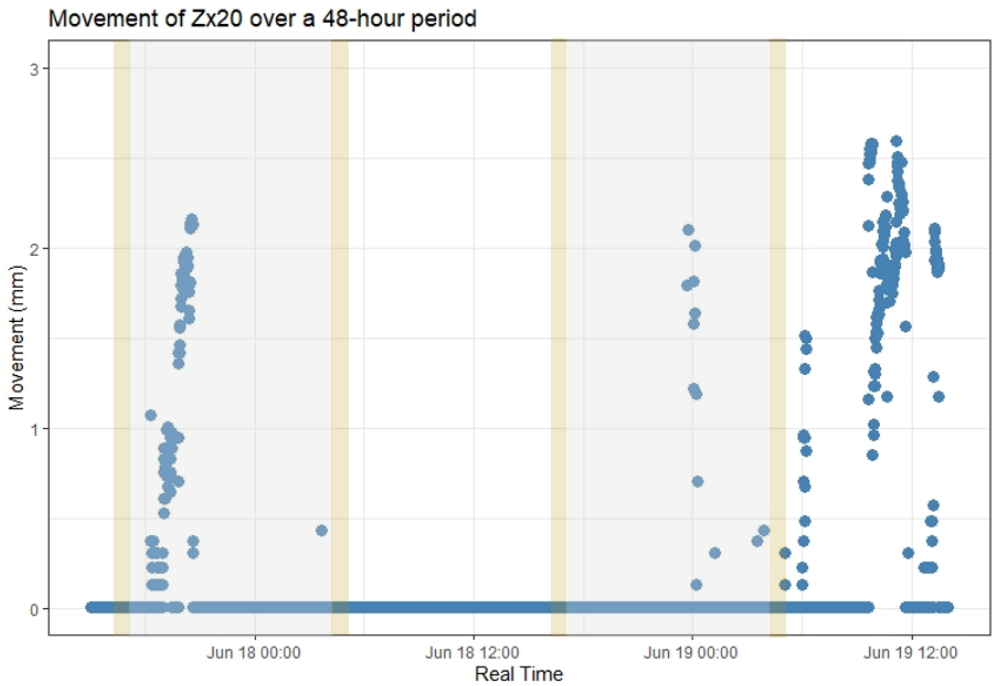

Figure 12A. The movement of individual Zx20 over a 48-hour period.
The x-axis indicates the date and time at which the measurements were taken, and the y-axis indicates the distance in which the spider travelled within a 1-minute time bin. The grey background represents the hours during which the spider received no light (darkness) in the experimental setup, while the brown background represents the ramping off or ramping on period. A plain white background indicates where the spider received a positive light treatment.

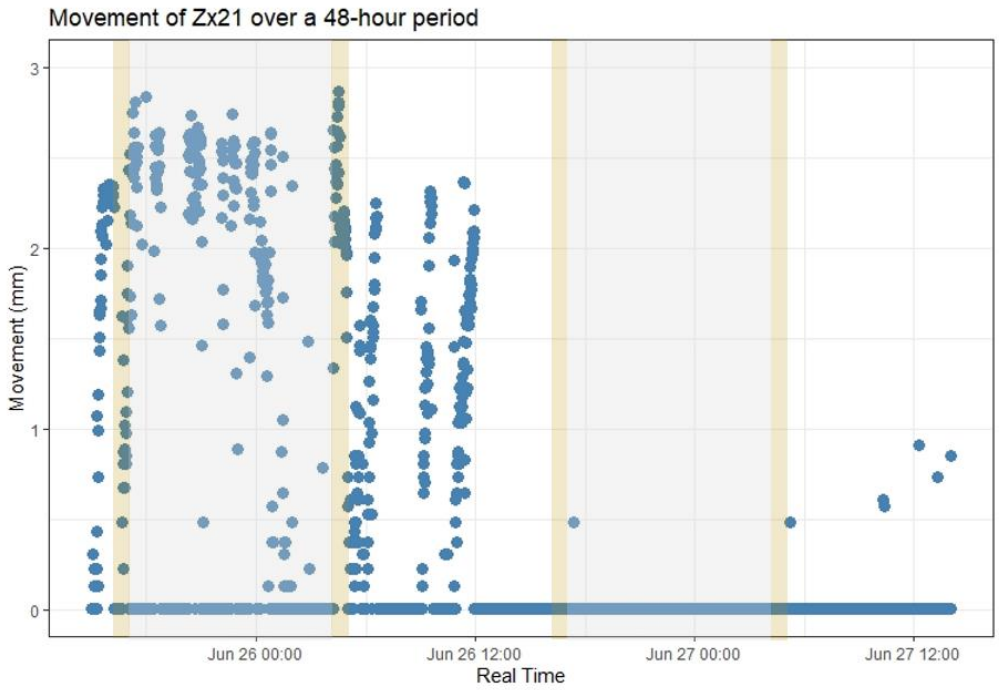

Figure 13A. The movement of individual Zx21 over a 48-hour period.
The x-axis indicates the date and time at which the measurements were taken, and the y-axis indicates the distance in which the spider travelled within a 1-minute time bin. The grey background represents the hours during which the spider received no light (darkness) in the experimental setup, while the brown background represents the ramping off or ramping on period. A plain white background indicates where the spider received a positive light treatment.

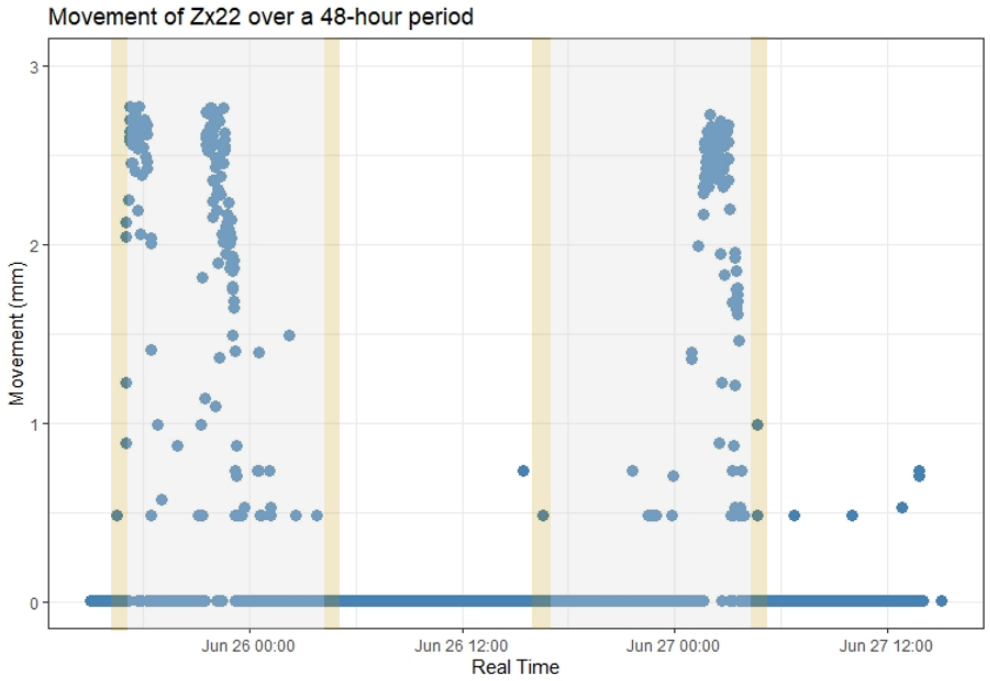

Figure 14A. The movement of individual Zx22 over a 48-hour period.
The x-axis indicates the date and time at which the measurements were taken, and the y-axis indicates the distance in which the spider travelled within a 1-minute time bin. The grey background represents the hours during which the spider received no light (darkness) in the experimental setup, while the brown background represents the ramping off or ramping on period. A plain white background indicates where the spider received a positive light treatment.

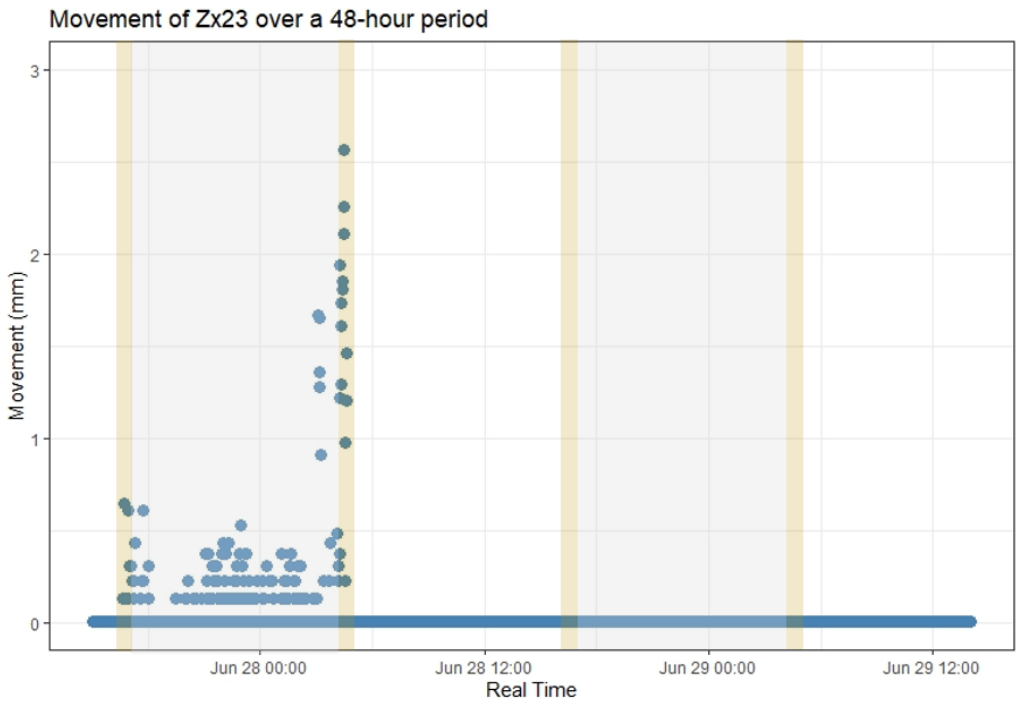

Figure 15A. The movement of individual Zx23 over a 48-hour period.
The x-axis indicates the date and time at which the measurements were taken, and the y-axis indicates the distance in which the spider travelled within a 1-minute time bin. The grey background represents the hours during which the spider received no light (darkness) in the experimental setup, while the brown background represents the ramping off or ramping on period. A plain white background indicates where the spider received a positive light treatment.

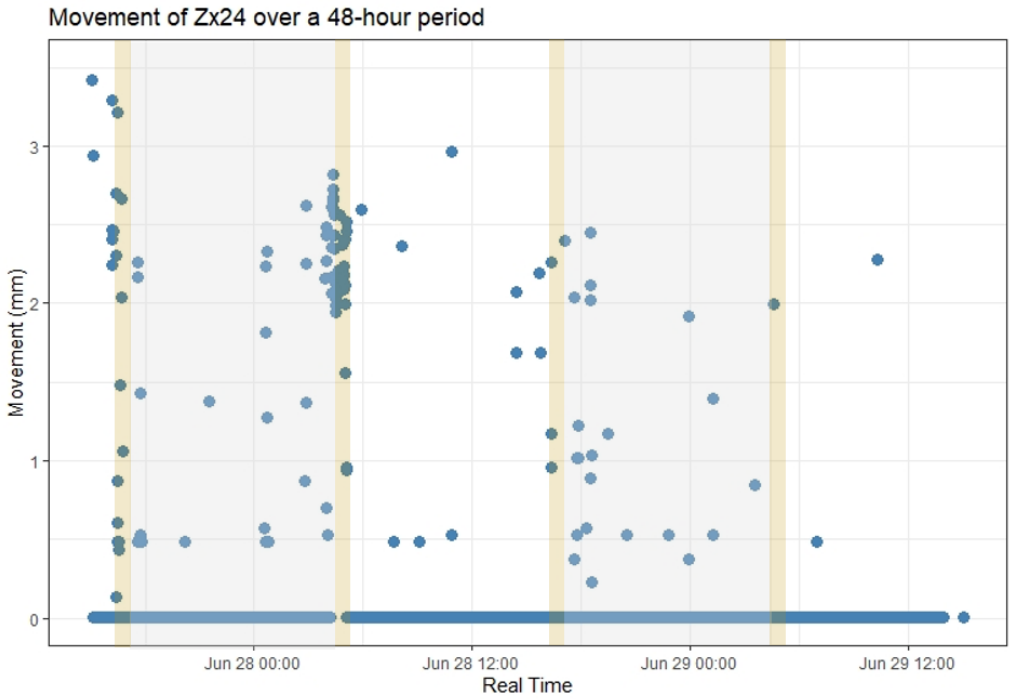

Figure 16A. The movement of individual Zx24 over a 48-hour period.
The x-axis indicates the date and time at which the measurements were taken, and the y-axis indicates the distance in which the spider travelled within a 1-minute time bin. The grey background represents the hours during which the spider received no light (darkness) in the experimental setup, while the brown background represents the ramping off or ramping on period. A plain white background indicates where the spider received a positive light treatment.

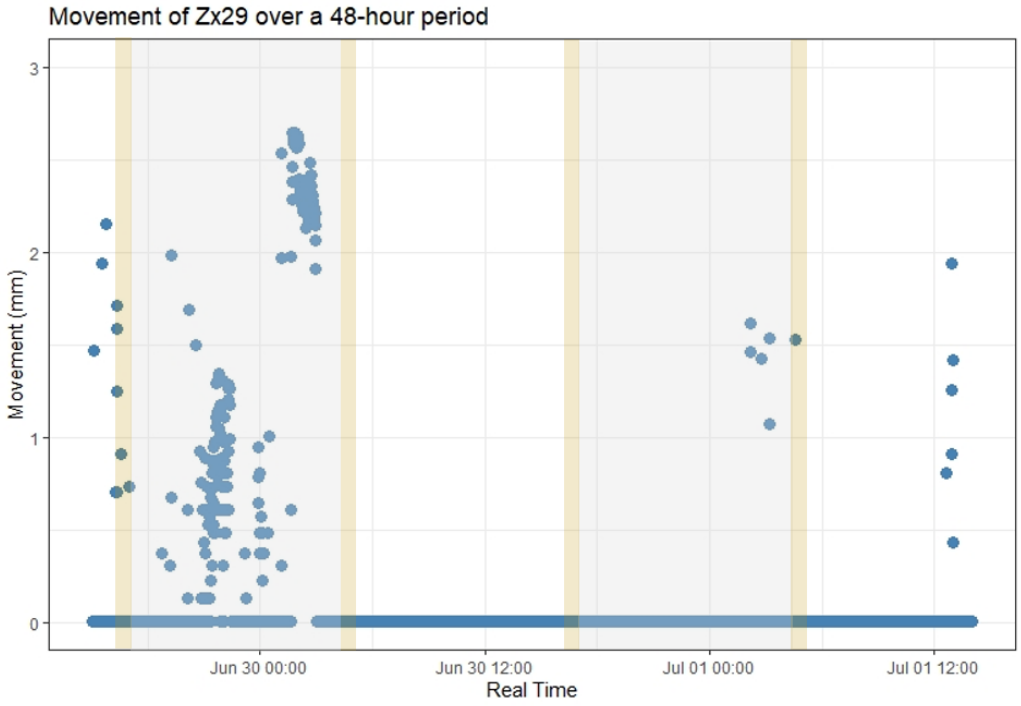

Figure 17A. The movement of individual Zx29 over a 48-hour period.
The x-axis indicates the date and time at which the measurements were taken, and the y-axis indicates the distance in which the spider travelled within a 1-minute time bin. The grey background represents the hours during which the spider received no light (darkness) in the experimental setup, while the brown background represents the ramping off or ramping on period. A plain white background indicates where the spider received a positive light treatment.

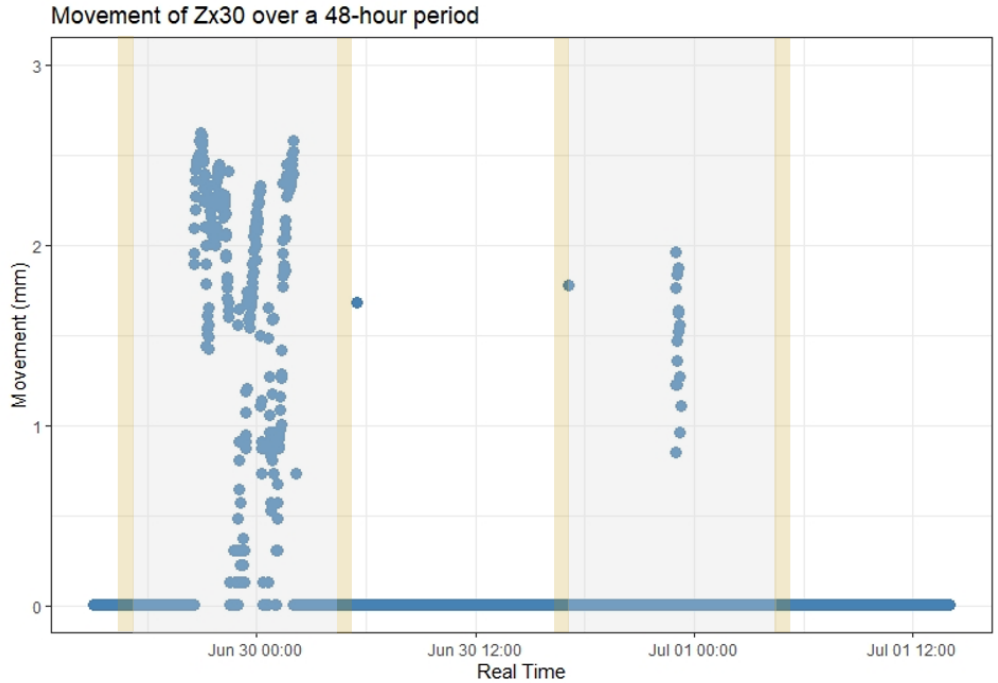

Figure 18A. The movement of individual Zx30 over a 48-hour period.
The x-axis indicates the date and time at which the measurements were taken, and the y-axis indicates the distance in which the spider travelled within a 1-minute time bin. The grey background represents the hours during which the spider received no light (darkness) in the experimental setup, while the brown background represents the ramping off or ramping on period. A plain white background indicates where the spider received a positive light treatment.

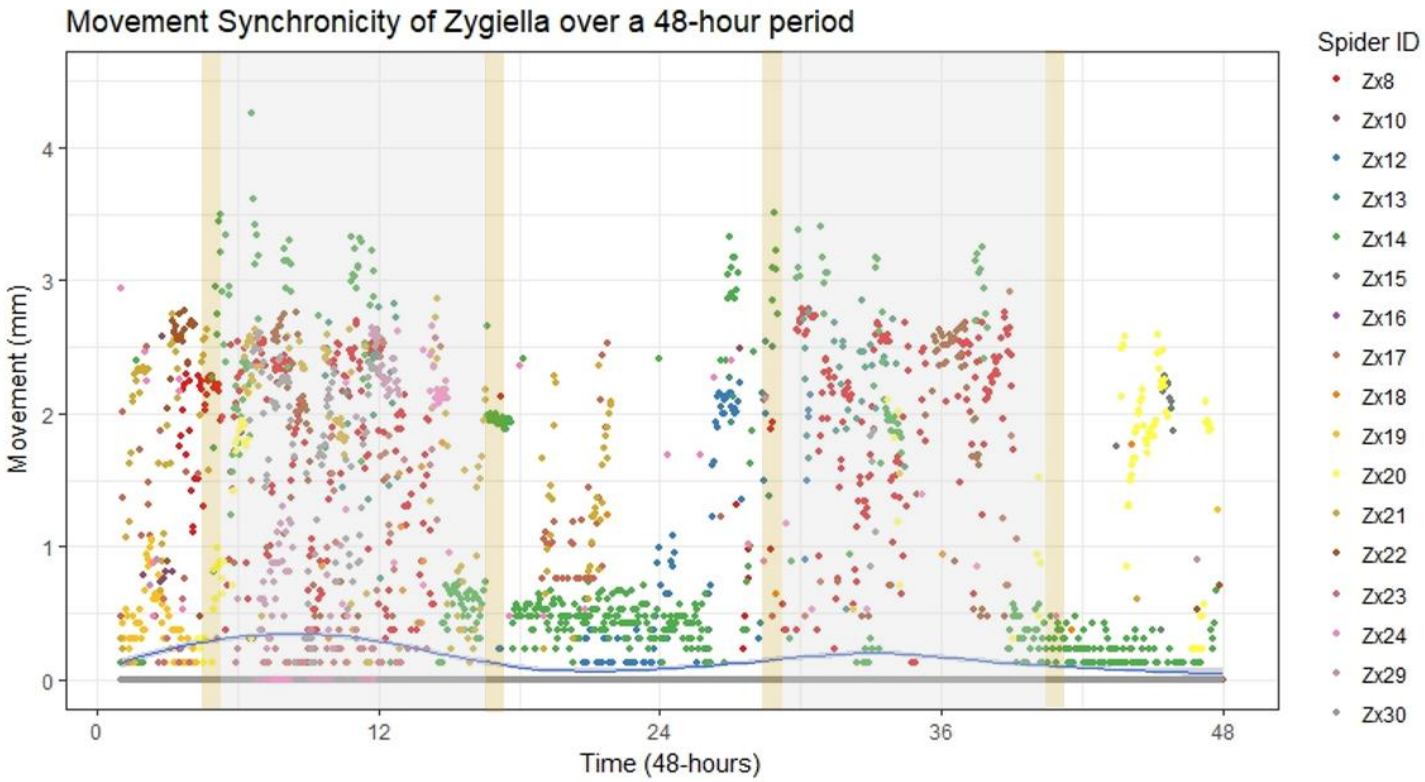

Figure 19A. Movement Synchronicity of *Zygiella x-notata* over a 48-hour period

A repeat of Figure 8, with the backgrounds altered to represent the different light treatments for easier interpretation. The x-axis indicates the experimental time at which the measurements were taken, and the y-axis indicates the distance in which the spider travelled within a 3-minute time bin. The grey background represents the hours during which the spider received no light (darkness) in the experimental setup, while the brown background represents the ramping off or ramping on period. A plain white background indicates where the spider received a positive light treatment.

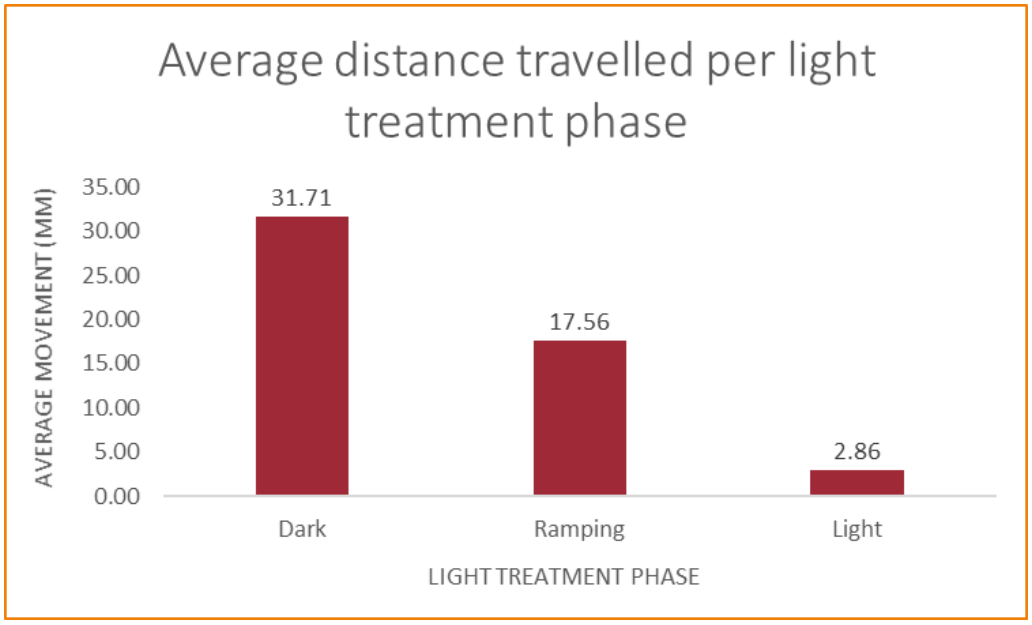

Figure 20A. Average distance travelled per light treatment phase

The average movement (in mm) of all individuals relative to the different light treatment phases. 58% of activity points were measured during the dark treatment phase, 9% of activity points were measured during the ramping light treatment phases, and 33% of activity points were measured during the light treatment phase. Spiders travelled 11 times further during the dark treatment phase than they did during the light treatment phase.

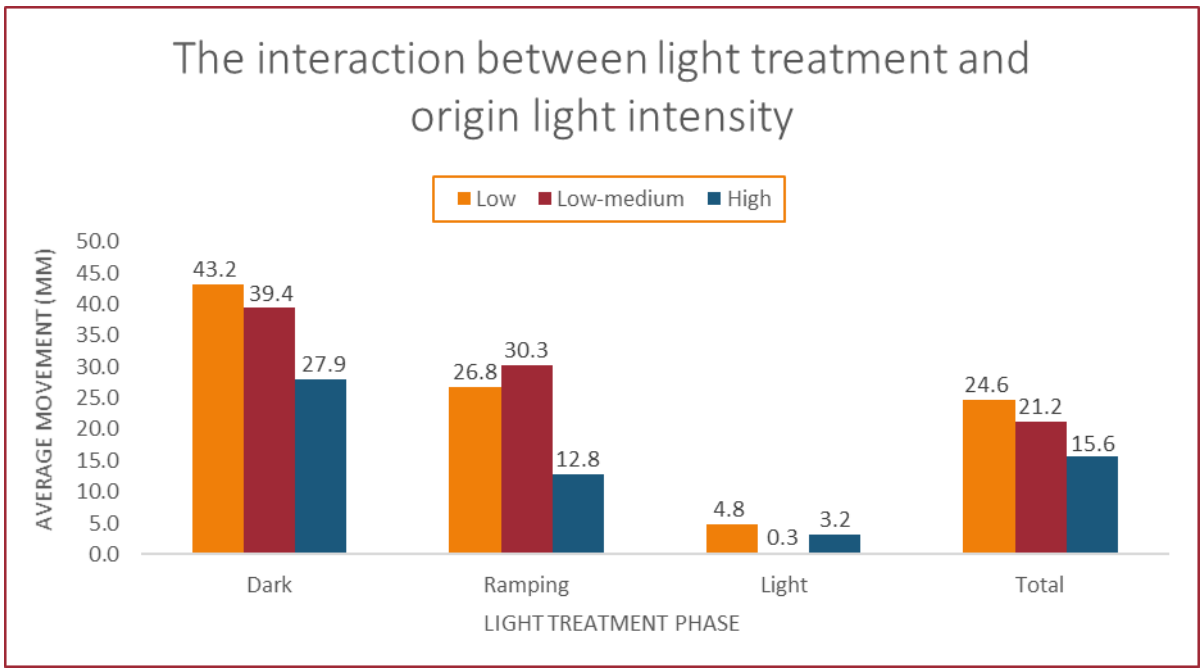

Figure 21A. The interaction between light treatment and origin light intensity

The MAM showed a highly significant interaction between the light treatment phase and the origin light intensity. This plot is for the visualization of the interaction. Individuals originating from low-light intensity environments displayed the highest average movement, while individuals originating from high-light intensity environments displayed the lowest average movement.

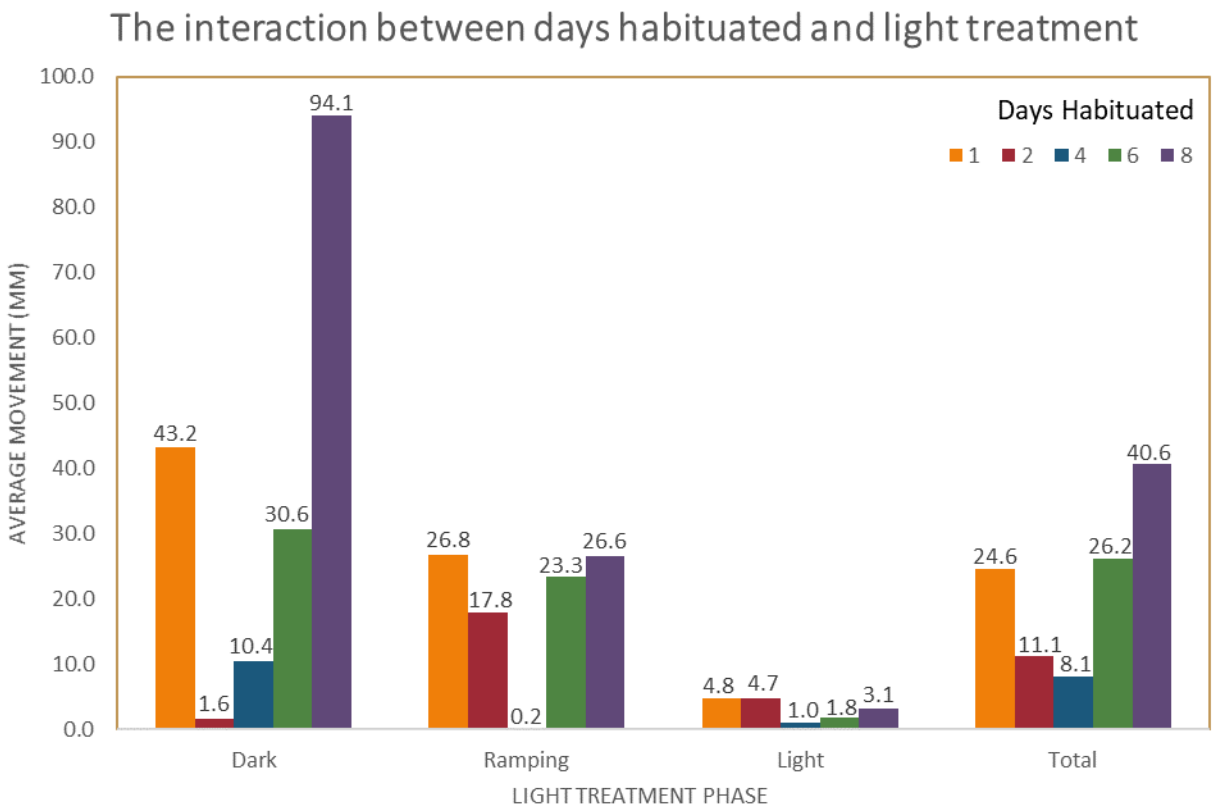

Figure 22A. The interaction between days habituated and light treatment

The MAM showed a significant interaction between days habituated and light treatment phase. This plot allows for the visualization of the data, and shows that individuals that underwent eight days of habituation moved significantly more during their experimental phase than those who underwent less habituation. Two and three days of habituation seemed to result in spiders with the least amount of movement.

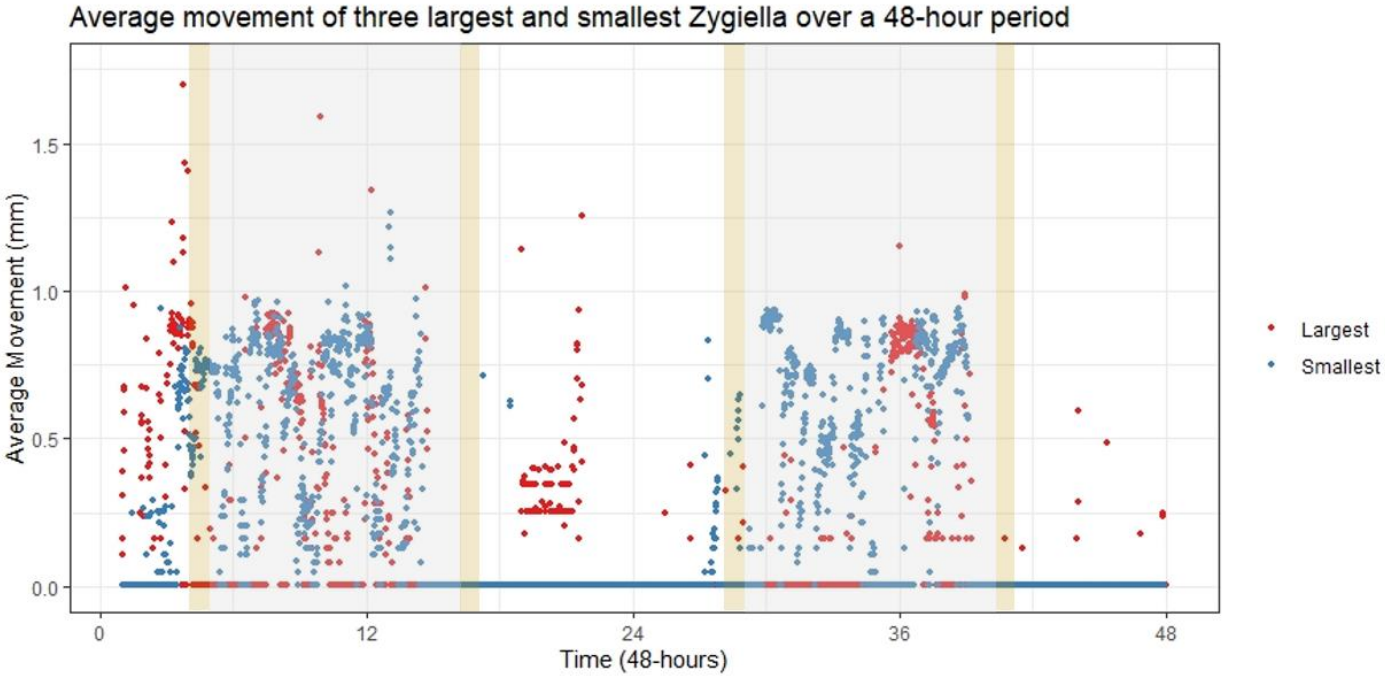

Figure 23A. The average movement of largest and smallest *Zygiella x-notata* over 48-hours
This graph shows the average movement of the three largest and the three smallest individuals that underwent experimental testing. The MAM showed a significant interaction between spider length (a proxy for spider size) and the *time.circ* variable. In order to determine which phenotype tended to be active earlier, these averages were plotted as representation of larger and smaller spiders. Smaller spiders (blue) were found to be active slightly earlier than larger spiders (red). The x-axis indicates the experimental time at which the measurements were taken, and the y-axis indicates the distance in which the spider travelled within a 1-minute time bin. The grey background represents the hours during which the spider received no light (darkness) in the experimental setup, while the brown background represents the ramping off or ramping on period. A plain white background indicates where the spider received a positive light treatment.

Figure 24A. A visual overview of the Zantiks AD unit

The Zantiks AD unit, complete with all its basic equipment. This photo shows the normal, upright position. For this study the unit was flipped 90° to the right so that it could like on its side. The display lighting screen is located at the inside bottom of the unit (here the inner white base on which a tank is sitting on). The camera is located on the upper inside of the unit (directly across from the display screen).

Figure 25A. A screenshot of the Zantiks live video feed

A screenshot of the Zantiks live video of inside the experimental chamber. Here you can see the outline of the wooden frame that housed the spiders during both the habituation and experimental phases of this project. These two individuals are displaying typical inactive behavior and are sitting in their retreats at the top of their orb webs.

Figure 26A. A screenshot of the Zantiks live video feed

A screenshot of the Zantiks live video of inside the experimental chamber. Here you can see the outline of the original wooden frame that housed the spiders during both the habituation and experimental phases of this project. The individual in the left chamber is a *Zygiella x-notata* displaying typical inactive behavior. The individual in the right chamber *is Araneus diadematus*, and it is currently sitting inactive the in the hub of its web. Active vs inactive behavior will be somewhat species-specific.

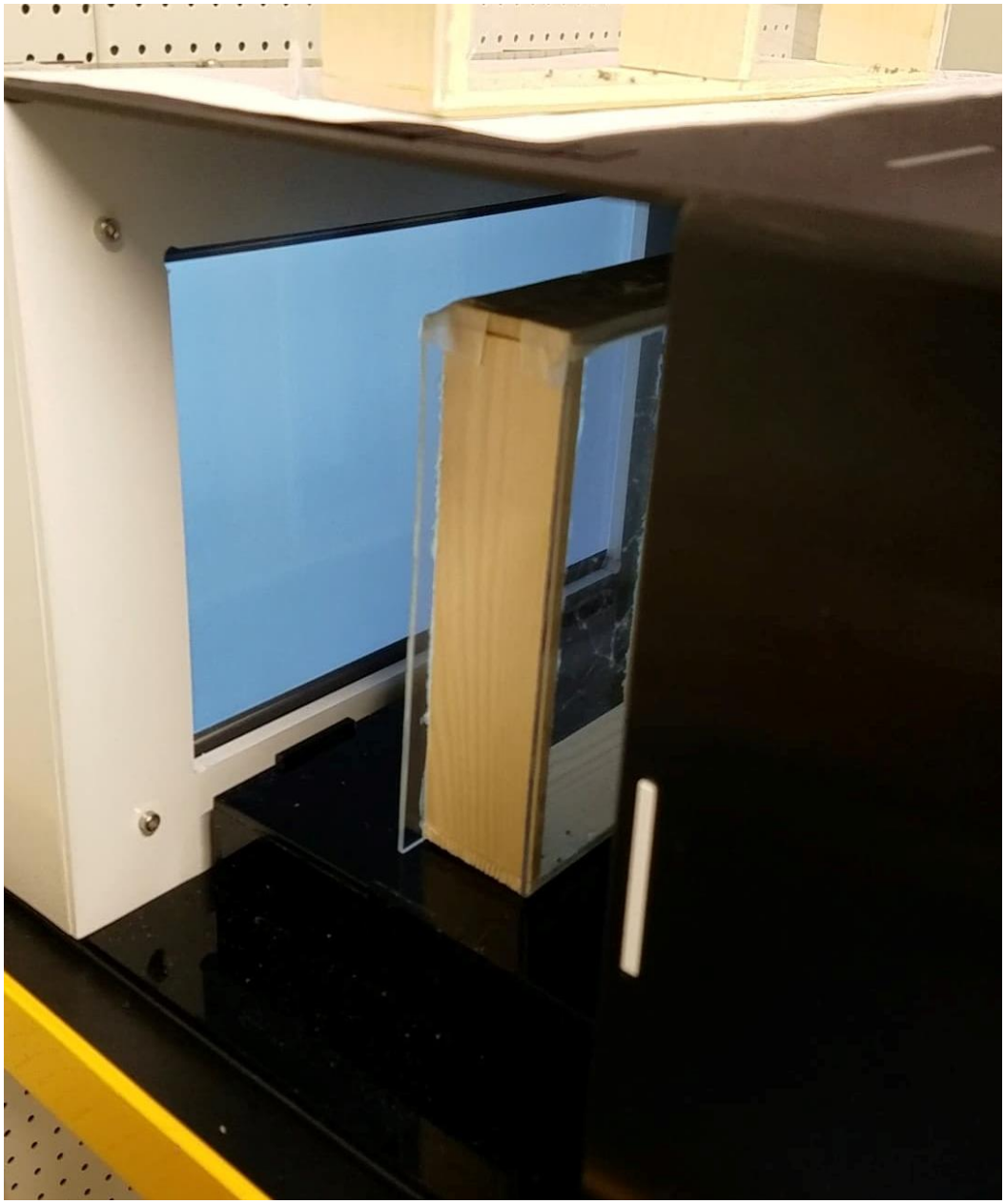

Figure 27A. The experimental chamber of the Zantiks unit.
A view of inside the experimental chamber of the Zantiks unit, with a wooden frame inserted to show orientation. The display screen is currently lit as it would be during the LIGHTSON light treatment phase. The camera is located opposite of the display screen. During experimental testing, the opening to this chamber would be sealed with a black foam insert.