Spiders in the city

Timing and clocks in orbweaving nocturnal spider species

Monique Suelmann
Msc student Science communication
S2833778

29/10/2020

Supervisors:
prof. dr. M.E. (Martine) Maan
prof. dr. B. (Barbara) Helm

university of groningen

faculty of science and engineering

Abstract

Humans can cause rapid changes in the environments of animals and other organisms. One of the Human Induced Environmental changes (HIREC) known is urbanization. Artificial light at night (ALAN) is one of the features of urbanization and can interfere with circadian clocks and timings of organisms. This can cause problems in various organisms. However, some species seem to thrive well in urban areas and it is not well known why or how these species thrive so well in urban areas. Two of these species are the nocturnal orbweavers *Zygiella x-notata* and *Larinioides sclopetarius* and are taken in this research as a model species. To determine timing, circadian rhythmicity, and the possible correlation between the two, locomotor activity was monitored. To determine the timing, different phase markers were calculated based on locomotor activity in light: dark cycles. To determine circadian rhythmicity, the free-running period was measured and expressed in terms of Tau. A regression analysis for *Zygiella x-notata* was performed for onset with tau. *Zygiella x-notata* had a relatively early onset of activity compared to *Larinioides sclopetarius*. Tau was notably longer for *Larinioides sclopetarius* (mean 26.17 h, N=2) than for *Zygiella x-notata* (mean 23.06 h, N=9). Furthermore, the results from this research point to a relationship between timing and the free-running period. This could mean that there is a genetic component in circadian timing. However, more research should be done before it can be concluded that there has been genetic adaptations in the populations where the tested spiders were offspring off. More research on this subject is necessary to explain how and why these two spider species can thrive so well in urban areas.

Table of contents

1 Introduction

Humans can cause rapid changes in the environments of animals and other organisms. One of the Human Induced Environmental changes (HIREC) known is urbanization (Sih, Stamps, Yang, McElreath, & Ramenofsky, 2010) (Dahirel, Dierick, De Cock, & Bonte, 2017). According to the United Nations, in 1950, 56.1 percent of the Dutch population lived in urban areas and this percentage has risen to 91.5 percent of the Dutch population living in urban areas in 2018 (United Nations, 2018). The remaining population is living in so-called rural (countryside) areas (United Nations, 2018). Different definitions of urbanization can be used and in this report the definition from Johnson et al., will be adopted: *'Urbanization is the process by which humans form dense settlements constructed of buildings, roads, and supporting infrastructure*' (Johnson MTJ & Munshi-South J, 2017).

The ongoing urbanization still creates different novel environments and therefore challenges for many different organisms. Temperatures in urban areas are often higher than in rural areas, (air) pollution can be higher and artificial light at night (ALAN) is generally present in urban areas (Johnson MTJ & Munshi-South J, 2017) (Hopkins, Gaston, Visser, Elgar, & Jones, 2018). Organisms have to cope with these changes.

Although the changes to the environment of organisms can cause ecological problems, some species seem to flourish in cities (Kralj-Fišer & Schneider, 2012). For example, *Larinioides sclopetarius*, the bridge spider, does well in urban areas and, as their name already suggests, are often found on (artificially illuminated) bridge handrails (Heiling, 1999) (Kralj-Fišer & Schneider, 2012). The spider *Zygiella x-notata,* the missing sector orb-weaver, also seems to do well in cities and can often be found near windows, bus stops, and traffic signs (own observation). Furthermore, you see many species in and around the city like pigeons and seagulls (own observation).

How urban-dwelling species can do so well in urban areas is not completely understood (Kralj-Fišer & Schneider, 2012). This knowledge, that some species flourish in urban areas and some do not, raises bigger questions. Some of the urban-dwelling species perhaps already have the characteristics to survive in cities. But what about the species that didn't seem to possess the right characteristics to survive in cities? The species that had and have to cope with a rapidly changing environment? How do these species cope with a rapidly changing environment such as urbanization?

One of the factors in urbanization is the presence of artificial light at night (ALAN)(Hopkins et al., 2018), and ALAN can be due to (public) street lightning, lightning from advertising, public and private buildings, and vehicles(Gaston & Bennie, 2014) (Gaston, Davies, Bennie, & Hopkins, 2012).

It is known that ALAN can disrupt natural daily, seasonal and lunar cues for the timings of different biological activities and has a great impact on ecological systems (Gaston, Davies, Nedelec, & Holt, 2017). Since five of the twenty-five most light-polluted places from Europe are in the Netherlands (Falchi et al., 2019), and the biological clock can be influenced by ALAN, it makes sense to research this phenomenon in The Netherlands.

In this project, we will investigate daily timing and circadian rhythmicity in two spider species which thrive well in ALAN and non-ALAN areas. Although this research will not focus directly on the role of ALAN on biological clocks and urban evolution, the insights gained in this research can be valuable for research on the subject in the future.

Two spider species are chosen as model organisms to investigate the biological clock: the silver-sided sector spider (*Zygiella x-notata*) and the bridge spider (*Larionioides sclopetarius*).

Both species belong to the Araneidae family and are orb-web weavers (Roberts, 1996).
Zygiella x-notata is mainly found around houses, on window frames (Roberts, 1996). Their webs are easily recognizable: they weave orb-webs and leave a small sector open in the upper part of the web (Roberts, 1996).

Larinioides sclopetarius make orb webs and are mostly found near water and in particular on bridges (Roberts, 1996).

Figure 1. At the left a Zygiella x-notata spider and at the right Larinioides. Both orb-web weaving spiders. (Koomen, 2015) (Wikimedia, sd)

1.1.1 Biological rhythms

The biological clock regulates and influences a variety of processes in an organism(Yerushalmi S & Green RM, 2009) (Krittika S & Yadav P, 2019). It regulates metabolic processes, activity, and reproduction (Yerushalmi S & Green RM, 2009) and it can influence an organism's biological fitness, life-history traits, physiology, and behavior of an organism (Krittika S & Yadav P, 2019). Organisms fitness will be maximized if activities are correctly timed with respect to their environment (Kronfeld-Schor, Visser, Salis, & van Gils, 2017)

The circadian clock can be entrained by different Zeitgebers (Time givers) and the daily light-dark (LD) cycle is the most important Zeitgeber for clock synchronization (Kronfeld-Schor et al., 2017) (Helfrich-Förster, Winter, Hofbauer, Hall, & Stanewsky, 2001). A wide range of organisms have evolved biological rhythms which are entrained by the circadian (circa = about, dies = day) clock (Yerushalmi S & Green RM, 2009)(Krittika S & Yadav P, 2019). Patterns of light and darkness can be used by organisms to regulate circadian cycles, to determine day length and therefore even trigger some seasonal events(Gaston et al., 2012).

Following Ferraz et al., chronotypes are a '*chronobiology classification based on the preferential times for beginning and ending activities throughout the day'*(Ferraz, Borges, & Vianna, 2008). Even though the time giver in a certain environment is the same for a species, individuals can have different timings and therefore chronotypes. Humans for example have a variation in their timing of daily behavior(Brown et al., 2008). Different chronotypes such as 'larks' and 'owls' in humans are known(Roenneberg, Wirz-Justice, & Merrow, 2003). Larks tend to wake up when extreme owls fall asleep(Roenneberg et al., 2003).

Since Zeitgeber information in the city is compromised by light and noise, organisms in urban environments might benefit from a relatively labile clock (Dominoni D M, Helm B, Partecke J, Lehmann M, & Dowse H B, 2013). One hypothesis for explaining why some species or populations of species thrive well in cities is that these species need and have more labile clocks than organisms in a more natural environment(Dominoni D M et al., 2013). In a study where chronotypes and rhythmicity of birds were investigated, city birds were less rhythmic than forest birds which supports the hypothesis that organisms in urban areas thrive better with a relatively labile clock (Dominoni D M et al., 2013).

However, if light is completely absent or changes in LD cycles are absent, most organisms still have a rhythm close to 24 hours(Yerushalmi S & Green RM, 2009)(see Figure 2). This rhythm, which is measured in the absence of environmental cues, is also called the free-running period.

Light is not only important for entraining daily timing, but for seasonal timing as well. Photoperiod, the interval of illumination received by an organism per day, remains constant between years at any given geographic location in a natural environment. Therefore it is in a natural environment a reliable cue for entraining suitable seasonal responses to the environment (Kronfeld-Schor et al., 2017) (Science Direct, 2013).

1.1.1.1 Artificial light at night

ALAN possibly influences the circadian clock in an organism since it causes continuous (dim) light to occur. Nocturnal vertebrates and invertebrates are most vulnerable to ALAN(Owens ACS & Lewis SM, 2018). If ALAN is present, activity and seasonal timing can then be disturbed as well. For example, a study from 2017 shows that chronic exposure to dim night lighting resulted in a reduction in adult longevity in *Drosophila melanogaster* (McLay, Green, & Jones, 2017).

The circadian rhythmicity and timing may differ between organisms living in ALAN-exposed areas and organisms living in non-ALAN areas. In non-ALAN areas, the circadian rhythm is entrained by the natural LD cycle. In ALAN areas, where it is continuous light, an organism may have different daily and seasonal rhythms. It is not known what the long term consequences of this phenomenon are.

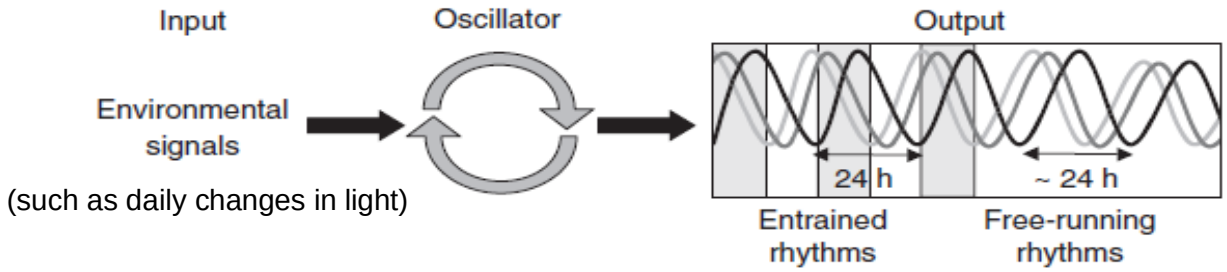

Figure 2 The circadian system. This picture is adapted from Yerushalmi et al., 2009. Environmental signals such as daily changes in light are used to entrain the oscillator. In turn, this oscillator controls many different processes in an organism's body. In a natural environment, with changes in light: dark cycles, rhythms are 24 hours and entrained by these light: dark cycles. Under constant conditions, such as constant dark, the rhythm of an organisms is most likely close to 24 hours but most often not exactly 24 hours. In the output, the grey and white boxes represent dark and light. Even if different organisms experience the same environmental cues, their output rhythms can be different.

1.2 Adapting to a new environment

There are different mechanisms involved in coping with a new and or changing environment. At the individual level, phenotypic plasticity can occur. This is the ability of an organism to produce different, relatively fit phenotypes in different environments during an organism's lifetime (DeWitt, Sih, & Wilson, 1998). It is seen as a key mechanism for enabling organisms to survive environmental changes during their lifetime (Murren CJ et al., 2015).

If phenotypic plasticity would be a perfectly working mechanism, the organism would possess the perfect and correct information on the current events, and at the same time, have the mechanisms to produce the appropriate phenotypic response at all points in development (Murren CJ et al., 2015). However, research suggests that this type of phenotypic plasticity is rare or even sometimes absent in natural populations (Murren CJ et al., 2015).

Although some responses and behaviors of organisms can be explained by phenotypic plasticity, this does not account for all traits. Organisms can also be genetically adapted to a certain environment. Mutations can occur and can make organisms more fit for the current environment. If a mutation is given to the next generation and establishes a change of allele frequencies in a population then we speak about evolution (Johnson MTJ & Munshi-South J, 2017).

Mutations are not the only thing which can affect allele frequences changes in populations. Natural selection, gene flow, and genetic drift can affect allele frequencies in populations (Johnson MTJ & Munshi-South J, 2017).

Following Johnson et al. and Hopkins et al., many recent studies suggest that urbanization alters the evolution of life around us (Johnson MTJ & Munshi-South J, 2017) (Hopkins et al., 2018). It is now recognized that observable evolutionary change can occur in as little as two generations (Johnson MTJ & Munshi-South J, 2017).

1.2.1.1 ALAN as a driver of evolutionary change

Hopkins et al. (2018) present a conceptual framework of ALAN acting as a driver of evolutionary change(Figure 3)(Hopkins et al., 2018). They hypothesize that ALAN can act as a driver of genetic mutations, natural selection, gene flow, and genetic drift and therefore of evolutionary change(Hopkins et al., 2018). Although Hopkins hypothesizes that ALAN can act as a driver of genetic mutations, this is still unknown and more research on this subject should be done(Hopkins et al., 2018). However, the other mentioned mechanisms seem plausible to contribute to (urban) evolution with ALAN as a driver(Figure 3)(Hopkins et al., 2018). As reviewed in Johnson & Munshi-South (2017), it has been documented in cities that evolution can be caused by some of these mechanisms(Johnson MTJ & Munshi-South J, 2017).

If an urban population of organisms is living in an ALAN area this can alter the behaviors and physiology of the animals and therefore affect their fitness(Hopkins et al., 2018). In that case, ALAN possibly acts as a selection pressure for masked Zeitgebers(Hopkins et al., 2018). Gene flow can be restricted between ALAN and non-ALAN areas because the two populations have different daily or seasonal timing(Hopkins et al., 2018). Genetic drift is the most prominent in small, isolated populations, possibly populations in ALAN areas, and thus evolutionary influence through genetic drift within ALAN areas is expected(Figure 3)(Hopkins et al., 2018) (Johnson MTJ & Munshi-South J, 2017). For a visual overview of these concepts, see Figure 3.

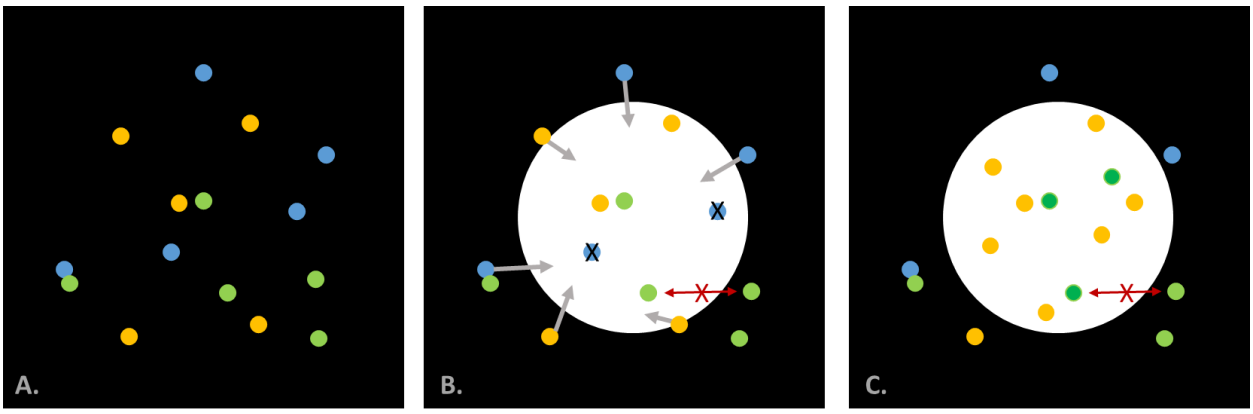

Figure 3: Adapted from ***(Hopkins et al., 2018)***. Conceptual illustration of ALAN acting as a driver of evolutionary change. Every dot represents one individual. **A.** This is a hypothetical population (N=15) composed of fifteen spider individuals from one species with each one of the three alleles for a particular trait living in a naturally dark at night environment. Alleles are marked by color. **B.** An area of the environment is artificially illuminated (white circle). The black 'X' symbols represent the selection against the blue allele. This selection eliminates the blue allele from the population. The green alleles are repelled by light, and will therefore not cross the boundary, reducing gene flow both into and out of the population (red arrow with 'x' symbol). Individuals with the blue and orange allele are attracted to light and join the lit population. This may be adaptive (orange) or maladaptive (blue). These maladapted individuals are then quickly selected against. **C.** Genetic drift plays a strong role in influencing evolution in the small resultant population in the lit habitat. Finally, the resultant lit population is further isolated from the outside population by being phenologically mismatched (red arrow with 'x' symbol), as light at night causes a change in seasonal reproductive timing (the different shade of green). The final artificially lit population now has a higher frequency of orange alleles, and a lower relative frequency of green and blue alleles than the source population. Thus, evolution has occurred.

1.3 Spiders, light, and rhythmicity

1.3.1 Reduced light avoidance and light attraction in spiders

Several studies provide evidence that ALAN may influence spider behavior and survival.

Kralj-Fiser et al., observed through experiments that the behaviors related to activity in a novel environment and the voracity towards prey were not heritable in *L. sclopetarius* (Kralj-Fišer & Schneider, 2012). This suggests that the expression of these behaviors is largely shaped by the environment (Kralj-Fišer & Schneider, 2012). This could be one of the reasons why some species seem to flourish in novel urban environments.

Czaczkes et al. (2018) found that *Steatoda triangulosa* spiders originating from urban areas have reduced light avoidance. In a light choice experiment, they found that spiderlings originating from rural areas built their webs 37 percent of the time in light, while for urban spiders this was 49 percent. The authors, therefore, hypothesized that the reduced light avoidance of *Steatoda triangulosa* spiderlings may be the beginning of evolution towards light attraction. As a light source predicts high prey abundance, lower light repulsion may lead to higher food intake. This can result in higher fecundity and therefore even select for light attraction (Czaczkes, Bastidas-Urrutia, Ghislandi, & Tuni, 2018). On the other hand, Willmott et al., found that prey availability was a stronger cue than LED light for remaining in a foraging site within the Australian garden orb-web spider *Eriophora biapicata* (Willmott, Henneken, Elgar, & Jones, 2019). Perhaps some urban spiders learned that prey availability is higher in the light and/or underwent genetic adaptations which makes them less light avoidant.

Heiling (1999) observed that adult females of the bridge spider actively choose artificially lit sites for web construction (Heiling, 1999). It didn't matter if these spiders were reared in laboratory conditions and never experienced ALAN or if the individuals had already experienced ALAN(Heiling, 1999). Heiling suggested that therefore, this behavior is genetically predetermined(Heiling, 1999). She also observed that insect density and prey capture rates were significantly higher in lit areas than in unlit areas. She describes that the behavior for web site choice building is uniquely linked to prey availability and not a response to light per se (Heiling, 1999). The results from this investigation indicate that bridge spiders have evolved a specialized foraging behavior that is tied to the behavior of nocturnal insects that are attracted to light(Heiling, 1999). This research suggests that light preference in spiders has a genetic background and could be contributing to ALAN-driven evolutionary change in city spiders.

1.3.2 Circadian rhythmicity in spiders

If some spider populations are attracted to light and others are not, this could affect the (urban) evolution of spider species. While the rural spiders would live according to the natural light and dark cycle, the city spider may have to cope with the time givers in different ways. Especially since the organism is exposed to continuous (dim) light. Could this cause different circadian rhythms in spider populations? And if it does, does it for example cause a difference in reproductive timing between spiders from the city and spiders from rural areas?

Circadian rhythmicity can be determined by measuring the free-running period of an organism. Free-running period is '*the lenght of time it takes for an organism's endogenous rhythm to repeat int he absence of environmental time cues'* (UC San Diego, unknown).

FRP can be determined by exposing an organism to a constant, non-changing environment. For example, in a constant dark environment. For some spiders, it is known what their free-running period is. The spider species *Cyclosa turbinata* has an exceptionally short clock from 19 hours (Moore, Watts, Herrig, & Jones, 2016). This is not the case in all spider species. In the orb-web weaver *Metazygia wittfeldae* (Araneae: Araneidae) the free-running period is 22,7 h(Jones, Wilson, & Moore, 2018), which is notable longer than in *Cyclosa turbinata*.

1.4 Research questions

To learn more about the possible influence of ALAN on (urban) evolution, it is important to first get more understanding of the timing in relation to light: dark circumstances and the circadian rhythmicity under constant conditions.

Specifically, I will address the following questions:

1) What is the timing of the daily activity of *Zygiella x-notata* and *Larinioides sclopetarius*?

It is important to determine timing in the chosen model species in a light: dark environment. Timing will be determined by monitoring the activity of the spider species. Determining the timing of the spiders will be done by calculating so-called 'phase markers'. These timepoints tell us more about when the onset and offset of activity occurs. Onset is the time at which an organism's passive phase ends, and the active phase begins (UC San Diego, unknown). Offset of activity is the timepoint where the active phase ends and the passive phase starts. Also, based on the onset relatively to sunset, the chronotype can be assigned.

2) What is the circadian rhythmicity of *Zygiella x-notata* and *Larinioides sclopetarius*?

The circadian rhythmicity of the spiders can be determined by measuring the free-running period and calculate Tau (period length) under constant conditions. Free-running period will be determined by monitoring the locomotor activity of the spiders in constant darkness.

3) Is there a relationship in timing between the onset of activity and Tau(period length)?

Changes in timing concerning light: dark cycles, can implicate also changes in the underlying clock (Dominoni D M et al., 2013). Therefore the relation between the onset of activity (timing) and Tau (circadian rhythmicity) will be investigated. If the time of onset is related to the period lenght, this would strenghten the case that the tested spiders are genetically adapted to the environment.

To sum up, in this research it will not be possible to make a clear statement about ALAN nor if the timing or rhythmicity characteristics are genetic or non-genetic. However, this research will make a start at unraveling timing and circadian rhythmicity in the two spiders species.

2 Material and methods

2.1 Collection of spiders

Spiders were collected in and around Groningen, The Netherlands, during daylight hours from the 5th of November 2019 until the 12th of November 2019 (Table 1). Spiders were mainly found in and on traffic signs, bus stops, windows, and garbage bins. I wore a headlight so I could easily look in the, often dark, hiding spots of spiders. Once I caught the spider I put it in a lunch box with tiny air holes in the lid Spiders were assigned a 'tag', an individual name. With 'Vs' (abbreviation for *venstersectorspin,* Dutch name for *Zygiella x-notata*) denoting *Zygiella x-notata* and Bs (abbreviation for brugspin, name for *Larinioides sclopetarius)* for *Larinioides sclopetarius*. The last letter of their tag was a random letter assigned to the boxes in which spiders were kept individually, in this way, spiders could be kept apart. For all tags and the origins of the spiders see Table 1.

Table 1 Spiders of Zygiella x-notata and Larinioides sclopetarius that were collected in and around Groningen, The Netherlands. All of the spiders shown in this table are females. Not all exact dates were registered, but the weeks were. Offspring of VsB, VsF, and BsA were used for experiments. In the tag, Vs was code for Zygiella x-notata (vs is an abbreviation for the Dutch name: Venstersectorspin) and BS was code for Larinioides sclopetarius (bs is an abbreviation from the Dutch name: Brugspin). The last letter was a random letter that was used to code the boxes with individual spiders.

Tag	Species	Origin	Coordinates	Collection Date	Collection Week
VsA	*Zygiella x-notata*	Kleine Butjesstraat	53°13'15.1"N 6°33'54.2"E	12-11-2019	46
VsB	*Zygiella x-notata*	Kleine Butjesstraat	53°13'15.1"N 6°33'54.2"E	12-11-2019	46
VsC	*Zygiella x-notata*	van Houtenlaan, backyard	53°11'49.2"N 6°35'02.9"E	9-11-2019	45
VsD	*Zygiella x-notata*	Hoornse Plas, playground	53°10'40.5"N 6°32'58.7"E	7-11-2019?	45
VsE	*Zygiella x-notata*	Hoornse Plas, busstop	53°10'50.1"N 6°32'47.7"E	7-11-2019	45
VsF	*Zygiella x-notata*	Hoornse Plas	53°10'45.0"N 6°32'56.8"E	5-11-2019	45
VsG	*Zygiella x-notata*	Traffic Sign, Groningen, Hoornse Plas	53°10'48.7"N 6°32'46.3"E	7-11-2019	45
BsA	*Larinioides sclopetarius*	Busstop Groningen, Eisenhowerstraat	53°11'22.4"N 6°33'36.3"E	5-11-2019	45
BsB	*Larinioides sclopetarius*	Hoornse Plas	53°10'45.0"N 6°32'56.8"E	5-11-2019	45
BsC	*Larinioides sclopetarius*	Beginning of Hoornse Plas	53°10'47.2"N 6°33'08.4"E	?	45
BsE	*Larinioides sclopetarius*	Hoornspe Plas, Playground	53°10'40.5"N 6°32'58.7"E	7-11-2019	45
BsD	*Larinioides sclopetarius*	Hoornse Plas, Parking place, other side	53°10'28.2"N 6°32'55.9"E	?	45
BsF	*Larinioides sclopetarius*	Hoornse Plas?	53°10'45.0"N 6°32'56.8"E	?	45

2.2 Identifying spiders

After collection, spiders were brought into the lab. When identifying spiders, spiders were placed individually in a magnifying container [insectenpot, HEMA] (Figure 4). Spiders were identified and sexed with the aid of 'Basisgids spinnen' (Ellferich, 2018), a 'zoekkaart' (a card to identify the species of the spider) from ARK (ARK, 2019) and a 'zoekkaart' (EIS, 2019). By using multiple sources for determining spider species, it was easier to determine the right species due to differences in color and illustrations in the different sources. Spiders that did not belong to the species *Zygiella x-notata* or *Larinioides sclopetarius* were not registered.

Figure 4: Magnifying container which is used to identify the species and sex of the spider [HEMA].

1.1 Maintaining spiders and origin

Each spider was provided with a moist cotton plug to keep humidity high and was kept individually in boxes. Once a week spiders were fed with +- five fruit flies (three to six fruitflies) per spider. The time point of changing boxes was based on how boxes smelled or looked. When fungus was suspected, spiders got new, clean boxes. Once per couple of weeks (see Appendix G for lab journal) spiders were placed into new, clean, boxes and boxes were cleaned with water, soap, and ethanol to prevent fungus to grow. Three of the collected spiders produced offspring, spider VsB, VsF, and BsA (Table 2) (fathers unknown), and this offspring was used for experiments.

Spider VsB was caught on a balcony in the city of Groningen, The Netherlands. This is presumably an ALAN area since the balcony was in the city center of Groningen (exact light measurements not available). The egg sack of spider **VsB** was first observed on the 29th of November 2019. Spiderlings of VsB emerged between the 20th of December and the 6th of January (exact date unknown).

Spider VsF was caught at the Hoornse Plas nearby/in the city of Groningen, The Netherlands. This area is presumably a non-ALAN area (own observation). The egg sack of **VsF** was first observed on the 29th of November 2019. The exact date that spiderlings emerged is unknown, but this was before the 20th of December 2019.

Spider BsA was caught at a bus stop in Groningen, The Netherlands. This is presumably an ALAN area since bus stop lights are often also on at nights (light measurements were not taken). The egg sack of BsA was first observed on the 6th of December 2019. Spiderlings emerged at the Christmas break somewhere between the 20th of December and the 6th of January (exact date unknown).

Table 2:Origin spiders used for experiments

Tag	Species	Origin	Coordinates	Collection date	Trial 1	Trial 2
VsB	Zygiella x-notata	Kleine Butjesstraat	53°13'15.1"N 6°33'54.2"E	12-11-2019	1	8
VsF	Zygiella x-notata	Hoornse Plas	53°10'42.9"N 6°32'59.6"E	5-11-2019	1*	1
BsA	Larinioides sclopetarius	Busstop Groningen, Eisenhoswerstraat	53°11'22.4"N 6°33'36.3"E	5-11-2019	1	11

1.2 Specifics per trial

Two experiments, from now on called trials, were performed. In trial 1, four spiders were tested and in trial 2 twenty spiders were tested. All spiders were offspring of the wild-caught spiders VsB, VsF, and BsA described above (Table 2). Spiderlings hatched in a lab room where temperatures were around 20°C, humidity unknown, and variance in light differed since different people could enter and leave the room (and switch the lights on or off). Presumably, lights were off at night in the lab. Spiderlings hatched in boxes or in vials. Specific details about the difference between the trials and the difference in the age of the spiders between the trials are given below.

To answer the research questions, the locomotor activity of the two spider species was monitored. The activity of spiders was monitored for up to 14 days. In the first couple of days, spiderlings were exposed to a light: dark environment. This was to gain more insights into the timing of daily activity in

the spider species. After this period, spiders were exposed to only dark cycles. By measuring the locomotor activity of spiders in only dark cycles, Tau could be determined and the circadian rhythmicity of the spiderlings could be determined. After tau and phase markers were established, the relationship in timing between the onset of activity and tau (period length) could be determined.
A Trikinetics system, DAM2 Drosophila Activity Monitor, 5 mm tubes, 32 tubes per monitor, 1 beam per tube, was used to monitor the activity of spiderlings and the sum of activity per minute was calculated (TriKinetics Inc USA , 2018). Spiders were continuously monitored for activity during the trial.

When the trial began, spiders were individually placed in transparent tubes (Figure 5) which were placed in the Trikinetics system(Figure 5). When a spider walks back and forth within the tube, the infrared beam of the Trikinetics system, which is in the middle of the tube, is interrupted and one activity event of the spider is recorded (Trikinetics). At one side of the tube, agar was added so spiderlings could eat during their time in the Trikinetics monitor(Figure 5).

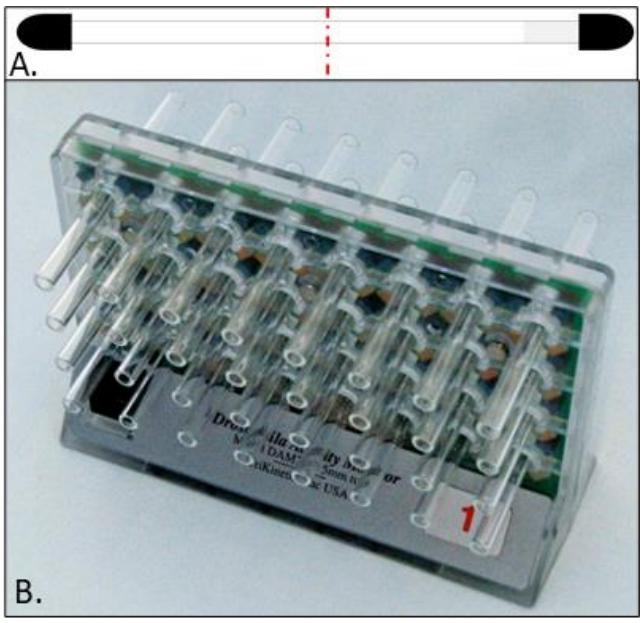

Figure 5: Trikinetics system. ***A.*** *A schematic drawing of a tube placed in the Trikinetics system. The black objects are caps that are placed on the tubes once the tubes are in the Trikinetics monitor. The red line is the place of the infrared beam. The grey area is the agar agar that spiders could eat from.* ***B.*** *A Trikinetics monitor. Spiders could be placed individually in tubes. (TriKinetics Inc USA , 2018)*

1.2.1 Trial 1

Four spiderlings were tested in the first trial. From one of the spiders, it is unknown which spider species this is due to missing lab notes. This spider is not used for further analysis.

One of the tested spiders in trial 1 was the offspring of VsB and one was the offspring of BsA (Table 2). These spiderlings were between three and twenty days old when the trial started.
The third of the tested spiderlings in trial 1 was the offspring of VsF. The egg sack was first observed on the 29th of November and the exact age of the spiderling during testing is unknown. The spiderling emerged before the 20th of December, the exact age is unknown. The age of this spiderling was between twenty and forty days old.

The Trikinetics system monitored the locomotor activity of these three spiderlings from 9th of January 2020 20:03 until the 26th of January 09:52. Conditions during the Trikinetics monitoring were as follows: 20°C, Humidity: unknown. The light: dark cycle was approximately 12: 12 hours respectively, from the 9th of January until the 13th of January, for exact times, see Table 3). Lights switched on and off without gradually intensifying light brightness. When the data of the machine was retrieved, it appeared in the data that the light-dark cycle was not 12: 12 but slightly differed the first days (Table 3). It is unknown how this is possible.

Lights were off when spiderlings entered the monitor on the 9th of January. Lights went permanently off on the 13th of January at 19:52 until the 26th of January 9.52 (Table 3). The free-running period was measured during this constant darkness.

Table 3: Light regime in trial 1. The activity of spiderlings was monitored from 9th of January 2020 20:03 until 26th of January 09:52. Spiderlings entered the setup on 9/1/19 at 20:03.

Real time date		Lights On	Lights Off
9	January	x	20:03
10	January	09:00	20:02
11	January	08:59	20:02
12	January	08:59	20:03
13	January	08:59	19:52
14	January	Lights stayed off	
15	January		
16	January		
17	January		
18	January		
19	January		
20	January		
21	January		
22	January		
23	January		
24	January		
25	January		
26	January		

1.2.2 Trial 2

Twenty spiderlings were tested in trial 2. Spiderlings were moved to a climate room on the 10th of January 2020. The climate room had a constant temperature of 15°C and relative humidity of 80 percent. Choices for relative humidity were based on the relative humidity in The Netherlands in autumn and winter (Koninklijk Nederlands Metereologisch Instituut, Ministerie van Infrastructuur en Waterstaat, 2019). Lights in the climate room went on at 8:30 and reached at 9:30 their maximum of light. At 16:00 it started to get darker and at 17:00 it was completely dark. Times of L: D cycles are comparable to late autumn (December) in the Netherlands.

Nine of the tested spiderlings were from the species *Zygiella x-notata.* Eight of these were the offspring of VsB2 (Table 1). On the 24th of January, these spiderlings were divided over two boxes to avoid crowding in the boxes. Spiders 23, 26, 28, and 30 were transferred to box I and spiders 1, 3, 5, 7 to box II. One of the tested *Zygiella x-notata* (spider 4) was offspring from VsF2(Table 1).

Eleven of the tested spiderlings were *Larinioides sclopetarius*, offspring of BsA2 (Table 1). The egg sack laid by the mother was first observed on the 6th of December 2019.

On the 31st of January 2020 spiderlings were divided over two boxes to avoid crowdedness in the boxes(instead of staying together in one box). Spiders 10, 12, 14, 16, 17, 19, and 21 were in box I, and spiders 8, 25, 31, and 32 were in box II.

Spiderlings were transferred to the Trikinetics monitoring system on the 4th of February, 11:11. Lights were on when spiderlings entered the Trikinetics system. At ~8:00 lights went on and at ~20:00 lights went off, with slight deviations between days (Table 4). The temperature was 20°C. Humidity unknown. Lights were on when spiders entered the monitor on the 4th of February 11:11. Measurements started on Tuesday the 4th of February at 11:11. Lights went permanently off on 7th February at 20:00, so a free-running period could be measured. Measurements continued until the 17th of February, 11:53.

Table 4: Light regime in trial 2. Activity of spiderlings was monitored from the 4th of February 11:11, when lights were on until 17th of February, 11:53 when lights were off.

Date		Lights on	Lights off
4	February		19:59
5	February	07:57	19:59
6	February	07:58	19:43
7	February	Lights stayed off	
8	February		
9	February		
10	February		
11	February		
12	February		
13	February		
14	February		
15	February		
16	February		
17	February		

2.3 Data quality control and moving errors

After trials were performed, data was retrieved from the Trikinetics monitors as a text file and converted to a .csv file. When data was retrieved, it was seen that activity looked high on the time points when lights were switched on. However, this was also the case in tubes where no spiders were placed. To avoid wrong or inaccurate results, these time points were replaced with values which in the further analysis was seen as 'missing' points instead of 'high activity' points.

Further analysis was done with software that is designed for circadian analysis in behavioral data, Chronoshop 1.1.

2.3.1 Data analysis: timing of activity

Three metrics were used to analyze the timing of locomotor activity with respect to lights off: onset, CoG, and offset. These metrics are also called 'phase markers' and, as the name already suggests, are in this research used to determine when which activity phase an organism is in. The value of onset

shows '*the first moment the activity exceeds the average activity in the current cycle*' and is used to indicate the chronotype of the spiders. So the onset of activity is the point where the activity first exceeds a higher activity than the average activity, the offset is the other way around. The onset value and other phase markers are given in minutes after lights are off. So, for example, if lights were off at 20.00 and activity started at 20.02 the onset will be '2'. The phase marker Center of Gravity (CoG) can be defined as the point where activity is, at average, the highest.

These three phase markers were calculated for each spider. The calculation was only based on activity levels during the light: dark period (so the first days of the trials, see Table 3 and Table 4)and not on the completely dark period.

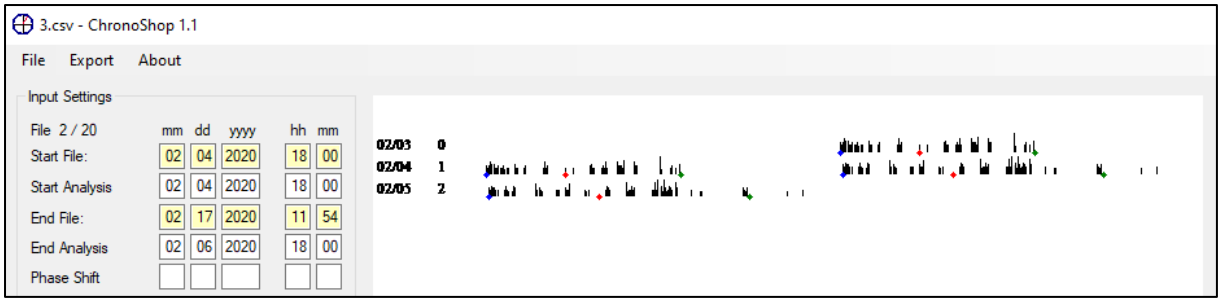

Figure 6 Screenshot of a double-plotted quantitative actogram for cycle 1 and 2 of trial 2 for spider 3. Lights switched on at 8.00 and switched off at 20.00. The higher the black bars in the actogram, the more activity at that specific time point. Blue, red and green dots are, respectively, onset, CoG and Offset of activity.

For **trial 1**, three cycles were analyzed to establish phase markers. Each cycle was 24 hours long and data from the 10th of January 18.00 until the 13th of January 18.00 was analyzed. This period was chosen because there was still a light: dark regime in the Trikinetics system during this period. No spider data or cycles were deleted.

For **trial 2**, two cycles were analyzed for determining phase markers. Each cycle was again 24 hours long. Analyzing was done for data from the 4th of February 18:00 until the 6th of February 18:00.

First, quantitative actograms were produced for the cycles that were used for calculating phase markers. Phase markers were added to the actograms in Chronoshop(**Figure 6**). So for trial 1, actograms of three cycles were made and for trial 2, actograms of two cycles were made. For the actograms see Appendix A and B.

Some data was excluded after actograms were observed. Different exclusion rules were established based on looking at the actograms.

Cycles were excluded if:

- No activity at all was measured. This was visible if no activity was visible on the actogram and/or if there were only grey bars were showing in the actogram. It is unknown what the grey bars exactly are, but it looks like the grey bars corresponded to artifact data. Therefore, the decision was made to exclude these data.
- Phase markers onset, CoG, and offset seem all to occur at the same timepoint. This is highly unlikely to be a trustworthy result. Therefore it was deleted.

Excel export files with the different phase markers were retrieved from Chronoshop and further analyzed. First, further exclusion of data was done. Data were excluded if multiple cycles had the same values for the phase markers. If this was the case, it was likely that something went wrong in data analysis by Chronoshop or that that specific spider died during the trial, therefore, these data were excluded from further analysis.

For trial 1, no data was deleted. For trial 2, different cycles were excluded from further analysis (for details see Appendix C).
For trial 2, data of eight *Zygiella x-notata* spiderlings were used for further timing analysis. For *Larinioides sclopetarius*, the full data set of one spiderling was used for further analysis. Furthermore, imcomplete datasets from three spiderlings were used. From two of those spiderlings only cycle 2 was used for further analysis and from one spiderling only cycle 1.

Once phase marker data was retrieved from Chronoshop, the retrieved excel files were analyzed. All values were first calculated relative to the time point 'lights off'. So, afterward, if the onset was '-5' this would mean that the onset of activity occurred five minutes before lights were switched off. If the onset value was '5', this would mean the onset occurred five minutes after lights off.

2.3.2 Data analysis: circadian rhythm

Spiders were exposed to an environment of constant darkness. In this way, the free-running period, and therefore Tau could be calculated. Tau was calculated with the aid of a Lomb-Scargle periodogram. Lomb-Scargle periodograms were retrieved in Chronoshop (Appendix D and E). Following Ruf, The Lomb-Scargle algorithm may serve as a suitable method for studying biological rhythms(Ruf, 1999). Lomb-Scargle periodograms were made in Chronoshop within the window of a circadian period of 16 and 32 hours, a resolution of 2 bins, and a significance threshold of alpha 0.05. After periodograms were made, values for Tau were further analyzed in Excel.

For trial 1, Tau was calculated by Chronoshop based on the data from the 14th of January 00:00 until the 26th of January 00:00. No data were excluded. For trial 2, Tau was calculated based on data from the 7th of February 00:00 until the 17th of February 00:00. For some spiderlings, tau was not calculated based on all cycles due to missing data(see Appendix F). After exclusion of data, seven Tau values for *Zygiella x-notata* were left, and one *Larinioides sclopetarius* tau value was left.

2.4 Statistical analysis

Two-sided, paired T-tests were performed in Excel for the different cycles of *Zygiella x-notata in the* light: dark phase. This was to test if the measured phase markers differed significantly from each other per cycle. If they would not significantly differ from each other, this could mean that no habituation period for the spiderlings was required. Also, if no significant result would be found, the mean of the cycles could be used for further calculation. This should give more clear data.

2.4.1 Correlation between Tau and phase markers

To test whether there is a correlation between timing (onset) and circadian rhythm, a regression analysis for onset and Tau was done in Excel for *Zygiella x-notata*. Period length (Tau) was plotted as a dependent factor on the x-axis and the onset as an independent factor on the Y-axis.

Since only two samples for Tau and phase markers in *Larinioides sclopetarius* were available, no regression analysis was done for this species.

3 Results

3.1 Daily timing in relation to the light: dark regime in *Zygiella x-notata*

Zygiella x-notata spiders did not seem to have a habituation period in the Trikinetics system. For both trials, no significant differences were found for the phase markers between the different cycles (Table 5 and Table 6). This does not mean that there is no difference at all. However, for clarity sake, in the further analysis of the phase markers of *Zygiella x-notata*, cycles were pooled.

1.2.3 Results in trial 1

A two-sided, paired, T-test (N=2)(P0.05) was performed in order to establish if the phase markers were influenced by habituation(Table 5). Based on this data, habituation period was not necessary present. Data of two *Zygiella x-notata* spiderlings were analyzed. The averages of the cycles for onset, CoG and offset were 70.33, 524.44, and 1048.7 minutes after lights were switched off, respectively, and for the other spiderling 4.7, 423.7, and 844 minutes after lights off. Since there are two individuals tested in this trial with each three cycles, it is not possible to make a hard statement about these results or do further statistics.

Table 5 Trial 1, Outcome of a paired, two-sided T-test (N=2)(P<0.05). Testing if phase markers are subjected to a habituation period. P-values are given in the table. Two individuals with each three cycles were tested.

		Onset	CoG	Offset
Cycle	1 & 2	0,50	0,99	0,27
	2& 3	0,48	0,46	0,19
	1 & 3	0,49	0,29	0,54

1.2.4 Trial 2

The average onset of the activity of the eight *Zygiella x-notata* spiders was relatively close to lights off (at average 9.9 minutes before lights went off). CoG was 5.36 hours after lights were switched off. With the offset of activity being 869.4 minutes after lights were switched off, the time of offset took place in the light phase (Figure 7).

Table 6 Zygiella x-notata *Onset, CoG and offset. Minutes are respectively from lights off.*

		Onset	CoG	Offset
Trial 2	Average	-9,9375	335,9375	869,4375
(N=8)	Median	-0,5	322,25	888,25

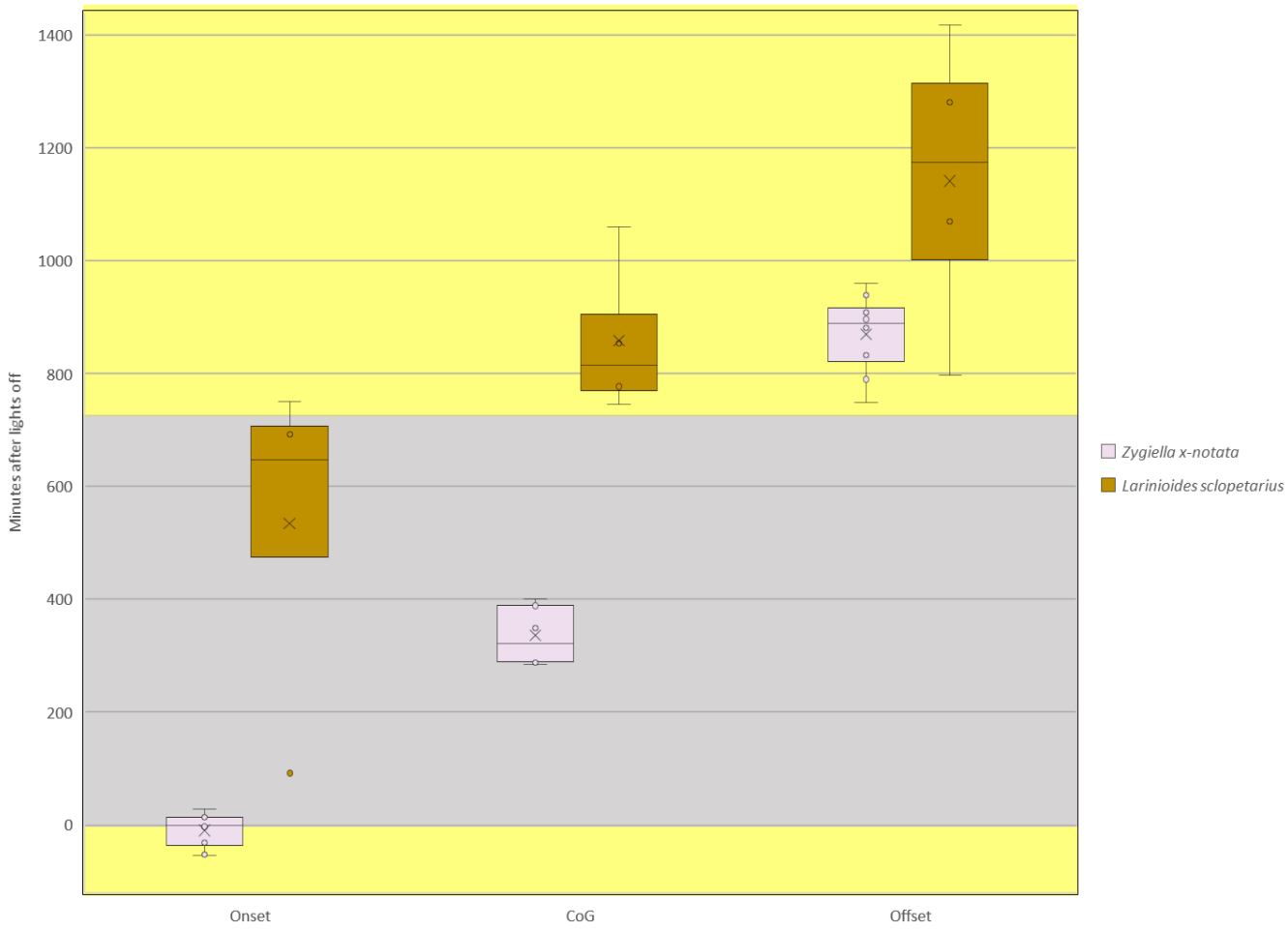

Figure 7 Onset, Center of Gravity (CoG) and offset for locomotor activity in trial 2 for Zygiella x-notata *(N=8) and Larinioides sclopetarius* (cycle 1 N=2, cycle 2 N=3). *Where available, measurements were taken for two cycles and the average of those two cycles is plotted in this figure. Onset, CoG, and offset were standardized against lights off.*
In the yellow areas, lights were on, (-100 minutes until 0 minutes and 720 minutes until 1000 minutes). Dark grey areas are the minutes where lights were off (0 minutes until 720 minutes).

Boxplots were calculated inclusive the median. The 'X' in the boxplot is the mean. Symbols in the boxplot represent individual values. The boxplot represents, from top to bottom: Highest value, upper quartile, above the median, median, below the mean, lower quartile, lowest value.

1.3 Timing in relation to the light: dark regime in *Larinioides sclopetarius*

Due to the limited sample size, no T-tests were performed for *Larinioides sclopetarius*.

1.3.1 Trial 1

Three cycles were measured for *Larinioides sclopetarius* (N=1). The average onset of the *Larinioides sclopetarius* was 169 minutes after lights off (2.8 hours after lights off), CoG was 475.7 minutes after lights off, and the activity offset was 734.7 minutes after lights off. Three cycles were measured and the average of these three cycles was taken.

1.3.2 Trial 2

On average, onset was 445.8 minutes after lights switched off, CoG at 842.2 minutes, and Offset 1196.4 minutes after lights were switched off (cycle 1 N=2, cycle 2 N=3).

1.4 Free-running circadian rhythm in *Zygiella x-notata* and *Larinioides sclopetarius*

To determine the free-running circadian rhythm, tau was calculated for *Zygiella x-notata* (N=9) and *Larinioides sclopetarius* (N=2). Results of trial 1 and trial 2 were combined. On average, Tau for *Zygiella x-notata* (N=9) was 23.06 hours (mean = 23.06, se = 0.22) and for *Larinioides sclopetarius* 26.17 (N=2) hours.

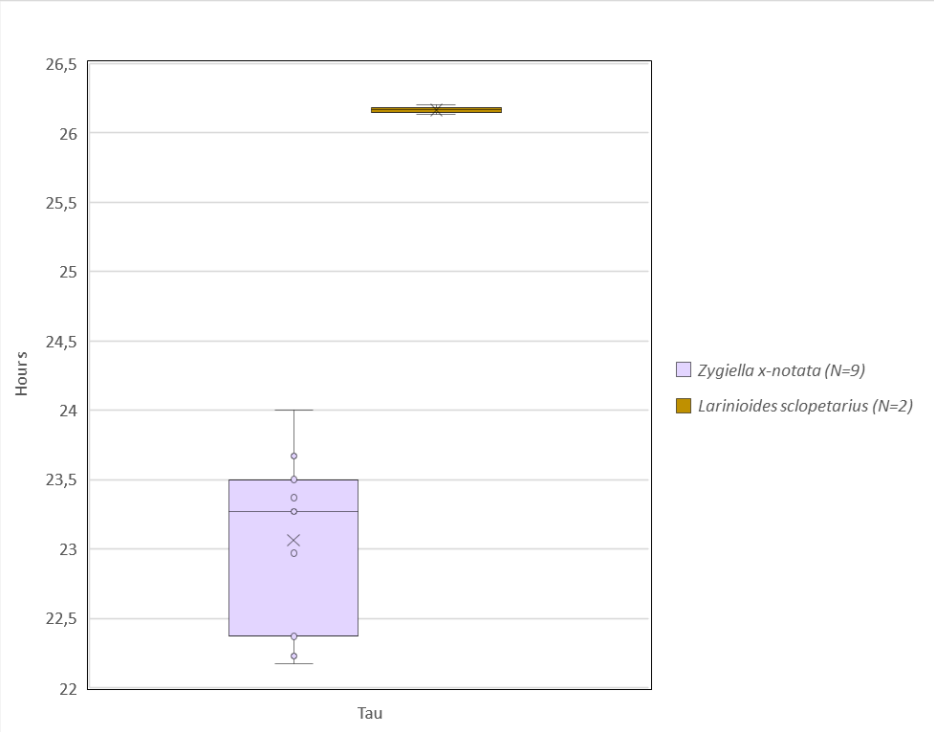

Figure 8 Tau (period length) for Zygiella x-notata (N=9)(se 0.22) and Larinioides sclopetarius (N=2). Boxplots were calculated inclusive the median. The 'X' in the boxplot is the mean. Dots in the boxplot represent values. The boxplot represents, from top to bottom: Highest value, upper quartile, above the median, median, below the mean, lower quartile, lowest value.

1.5 Correlation between Tau and Onset for *Zygiella x-notata*

To test whether the data would fit a linear model, a regression analysis for *Zygiella x-notata* is performed. Onset values from trial 2 was calculated relatively to sunset (lights off) and was taken as the indicator of chronotype. Period length (Tau) was plotted as a dependent factor on the x-axis and the onset as an independent factor on the Y-axis (Figure 9). A very weak correlation could be seen (R=0.47).

Since only two samples for Tau and phase markers in *Larinioides sclopetarius* were available, no regression analysis was done for this species.

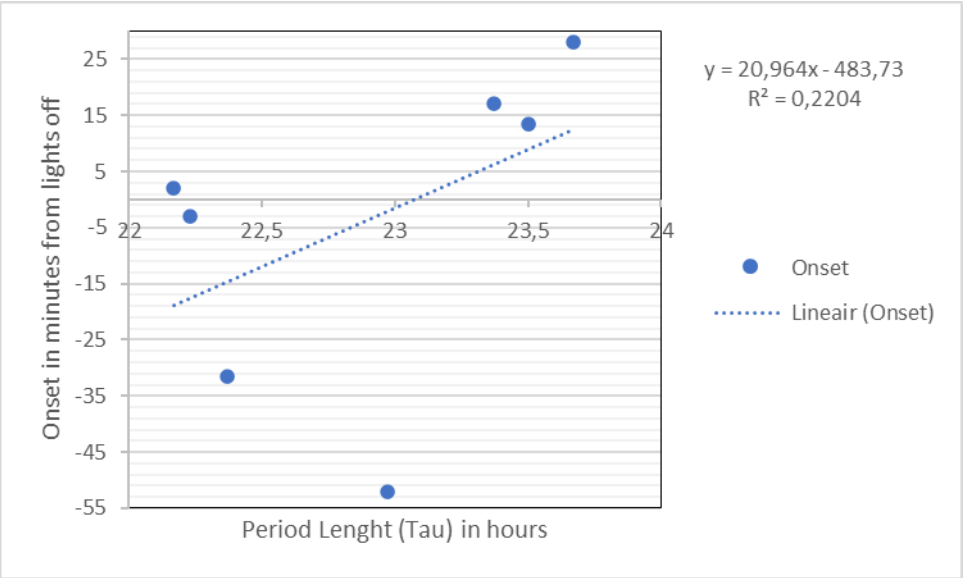

Figure 9 Regression analysis for Tau and Onset in Zygiella x-notata (N=7)(trial 2)R=0.47.

4 Discussion & conclusion

To find out more about timing under light-dark circumstances and clocks in two different spider species, phase markers were determined under light-dark circumstances and the period length was determined during completely dark periods. Afterwards, the correlation between timing and rhythm was investigated.

4.1 Zygiella x-notata

4.1.1 Zygiella x-notata

Zygiella x-notata is a spider species that is wide-spread in northern Europe and it builds its web mostly in or nearby human buildings(Anotaux, Toscani, Leborgne, Châline, & Pasquet, 2014). Following Smith (Smith, 2019), *Zygiella x-notata* is classified as nocturnal. This corresponds to our results. Under variable light: dark conditions, *Zygiella x-notata* was mainly active during dark hours. However, Smith points out that while *Zygiella x-notata* can be classified as nocturnal, it will still respond to prey caught in their webs during daylight hours (Smith, 2019). In the used experimental setup it was not possible to catch prey, therefore it was impossible to measure activity while prey was in their webs.

Although *Zygiella x-notata* seems to be mostly active in the scotophase and is therefore nocturnal, the offset of activity was in the light phase. This means that *Zygiella x-notata* shows also activity in the light phase without the opportunity to catch prey. Although *Zygiella x-notata* is still active in the light phase, it still looks like its activity stops under the influence of light. Important to mention here is that the lights in these experiments were 'on' or 'off' and did not gradually increase or decrease, which is most likely the case in natural habitats or cities and can give different results. Although most cities are artificially illuminated at night, this does not mean the intensity of the light is high enough to stop the activity of the *Zygiella x-notata*.

Based on the onset of locomotor activity relatively to lights off, *Zygiella x-notata* will be classified as an early chronotype. In the literature, some examples are known where chronotype seems to be intertwined with period length. Brown et al. concluded, based on their research on human fibroblasts, that period length may be influencing chronotypes (Brown et al., 2008). In this research, no clear correlation between the onset of activity and period length in *Zygiella x-notata* is found.

The average period length of *Zygiella x-notata* was 23.06 hours and can be described as a typical free-running period (Jones et al., 2018). Tau between individuals did not have a large variation.

Based on the regression analysis where tau was plotted as a dependent factor against onset as an independent factor, no correlation between onset and tau can be confirmed. Of an orb-weaving spider species, *Metazygia wittfeldae*, it is known that the onset of activity becomes more precise after two days of entraining (Jones et al., 2018). Therefore, based on these results, which are the onset values of the first two cycles of the trials, it cannot be concluded that there is no correlation between onset and Tau.

4.2 Larinioides sclopetarius

Larinioids sclopetarius is mainly found on bridges, fences, tunnels, and other manmade objects nearby water and it is also common in the Netherlands to find them in residential areas (Ellferich, 2018). Webs are mostly made on or nearby outdoor lamps (Ellferich, 2018). They are classified as nocturnal (Heiling, 1999).

Little results were obtained from *Larinioides sclopetarius*, therefore it is hard to conclude something based on these results. Results of timing in *Larinioides sclopetarius* seem to differ between trial 1 and 2, which makes sense since light conditions were different. Where in trial 1 the onset of *Larinioides sclopetarius* was 169 minutes after lights off, in trial 2 this was, at average, 445.8 minutes after lights off.

Although Larinioides sclopetarius is classified as a nocturnal species, results of trial 2 showed that activity started late in the dark phase, and their CoG, peak of activity, even took place during the light. In a study on the spider *Metazygia wittfeldae* Jones et al (2018) observed that the onset of activity after lights out became more precise after two days of entraining. Since we only analyzed two cycles of entrainment, it is not known if the spiders would have different outcomes for phase markers if they would have entrained for longer than three cycles. This should be investigated in the future.

If we have to classify Larinioides into a chronotype base on the results of these trials, one could say that *Larinioides sclopetarius* can be classified as a late chronotype. It is suggested that late chronotypes have a longer period length (Brown et al., 2008) (Dominoni D M et al., 2013). This is what we see in the results of this spider species. Although measurements for tau in *Larinioides sclopetarius* were limited (N=2), the measured tau likely represents a bigger group of the species. Since the results of the two individuals were so similar. If these results hold up in further researches, this could mean that chronotype and timing are genetically intertwined and this would strengthen the case for urban evolution.

The mother of these two tested spiderlings was caught at a bus stop, where it is highly likely that there is a situation of continuous light. The population they come from may be adapted to continuous light.

4.3 Comparing *Zygiella x-notata* and *Larinioides sclopetarius*

Both species were most active during the night and showed clear rhythms. The observed timing in a 12: 12 L: D cycle suggests that *Zygiella x-notata* has an earlier chronotype than *Larinioides sclopetarius*. However, it is known that next to light intensity, the temperature can also be an important Zeitgeber (Sharma & Chandrashekaran, 2005). Since *Zygiella x-notata* tend to live more often nearby buildings where temperature fluctuations in 24 hours can be less than near water, where *Larinioides sclopetarius* mostly lives, temperature differences between the habitats can occur. The fluctuating temperature near water can be an important Zeitgeber for *Larinioides sclopetarius*, and this can explain the differences in timing in this experiment.

Zygiella x-notata had a shorter free-running period than *Larinioides sclopetarius*. This is in line with the hypothesis that if the onset of activity is early, free-running period will be shorter (Dominoni D M et al., 2013). Therefore, it is likely that timing is genetically determined.

A circadian period within two hours of a 24-our daily cycle is called typical(Jones et al., 2018). This makes the free-running period of *Zygiella x-notata* typical and the free-running period of *Larinioides sclopetarius* non-typical. However, spiders sometimes have exceptionally clocks. For example, the orbweaver *Cyclosa turbinata* has a free-running period of 19 hours (Moore et al., 2016). On the other hand, the nocturnal orbweaver *Metazygia wittfeldae* has an observed free-running period of 22.7 hours, which makes it 'typical' (Jones et al., 2018). More research on the free-running period of *Larinioides sclopetarius* should be done.

Aschoff's rule states that FRP of nocturnal animals is longer in LL than in DD (Center for circadian biology, unknown). If this rule holds up and the free running-period results for the two tested spider species are representative, this would mean that the two tested spiderlings would have a longer circadian rhythm in an urban environment since there is often continuous light. Since in a natural habitat it would be most beneficial to have an internal clock of +- 24 hours(Mah A, Ayoub N, Toporikova N, Jones TC, & Moore D, 2020), this could indicate that these spiderlings come from a genetically adapted population.

4.4 Limitations and future recommendations

Spiderlings were monitored in small tubes, which made it impossible to mimic their natural behavior. They could not make full-sized webs and therefore spiderlings could probably not behave as they would do in their natural habitat. Jones et al. asks the question of what laboratory locomotor activity represents in orb-web weaving spiders(Jones et al., 2018). In natural conditions, orb-web weavers remain motionless unless capturing prey or working on their webs(Jones et al., 2018). Jones

et al., speculate that what they measured as locomotor activity in orbweavers corresponds to exploratory behavior that is observed almost exclusively at night(Jones et al., 2018). This can also be true for the two spider species measured in this research. One way to solve this problem in the future is to measure the activity of the spiders in a more natural environment where spiders can also build naturally sized webs. It is not known if the spiderlings that were tested in these experiments, already exposed the web building behavior and if the outcome of these experiments were influenced by the age of the spiderlings. This should be tested. However, it is likely that the spiderlings couldn't present their natural behavior in the small tubes.

Next to the problem that spiderlings probably did not have enough space to mimic their natural behavior, the light conditions given in these experiments did not mimic the real world. In these experiments lights were 'on' of 'off' and not gradually increasing or decreasing in light intensity. In a next research, it is recommended to perform two experiments: one with light conditions that mimic the light conditions in cities and one that mimics natural light conditions. If possible, experiments would be performed with two different populations of the two tested species: one derived from a non-ALAN natural habitat and one derived from an ALAN urban habitat. Based on the outcome of the study one could say more about the perhaps adapted species.

For measuring the free-running period also two light different conditions are advised: one group in a constant dark environment, just as what was done in this research, and one group in a constant low light intensity instead of completely dark. In this way, free-running period can be measured in constant light which may influence the locomotor activity behavior of the spiders and mimics living in an ALAN area.

Furthermore, it is observed that the behavior of the orbweaver *Metazygia wittfeldea* became more precise after two days of entraining (Jones et al., 2018) . Therefore it is important that in the next experiments spiders get at least four days of entraining. In this way, the phase markers of the days after day 1 and 2 of entraining will be more representable for natural conditions.

4.5 Conclusion

In this research timing and the period length of two orb-web weaving spiders were measured to start finding an answer to how these spiders can thrive so well in urban environments. Although both *Zygiella x-notata* and *Larinioides sclopetarius* live in urban and non-urban environments, their chronotypes (onset) and period length (tau) differed from each other. Where *Zygiella x-notata* showed an onset of activity around the lights off, the activity of *Larinioides sclopetarius* started later and they, therefore, had a later chronotype. It is likely that temperature is also an important Zeitgeber in these species, especially in *Larinioides sclopetarius*.

Furthermore, the period length from *Larinioides sclopetarius* was rather long and that of *Zygiella x-notata* was relatively close to a 24-hour rhythm.

The results in this research points to a relation between timing and the free-running period and this should be further investigated.

Acknowledgements
I would like to thank many people who supported me during this thesis. First of all, I would like to thank my supervisors Barbara Helm and Martine Maan. Thank you for discussing with me. Having the patience for it. I know, I hear you both think and say on a modest tone '*but of course, it is my work*'. Nevertheless, I was really happy that this job was done by the both of you. Every time we had discussions, I had the confidence to pick it up again. Thank you for giving me the amount of freedom in my research that I wanted. Thank you for thinking with me. Also when the Corona crisis started, I never had the feeling that you didn't take enough time for me or that I '*wasn't supposed to ask you something*'. I am really happy with that! Thank you!
I would like to thank Pauline Romeyer for letting me use precious space in the Trikinetics system. Thank you for all the help!
Hylke, when I was new I was really glad someone offered to help me and think with me! Thank you!
I also would like to thank Aude, who I see nowadays as my 'study buddy'. Thank you for your feedback and for the motivating words online and in real life!
Furthermore, I would like to thank al the people who were prepared to have a certain kind of ladder for hopefully catching spiders in their backyards or balconies. I would like to think the 'kinderdagverblijven' and organization that helped me hunting for spiders!

So thank you all!

5 References

ARK. (2019, September). *Zoekkaart Spinnen*. Opgehaald van Ark: https://webwinkel.ark.eu/producten/zoekkaarten-tekeningen/zoekkaart-spinnen

Center for circadian biology. (unknown). *3. Addressing Oversimplifications*. Opgehaald van CCB UCSD Edu: https://ccb.ucsd.edu/the-bioclock-studio/education-resources/basics/part3.html

EIS, N. B. (2019, September). *Spinnen in huis en tuin.* Opgehaald van Tuintelling: https://www.tuintelling.nl/downloads/Spinnenzoekkaart_2014.pdf

Ellferich, C. (2018). *Basisgids Spinnen.* Zeist: KNNV Uitgeverij.

Koninklijk Nederlands Metereologisch Instituut, Ministerie van Infrastructuur en Waterstaat. (2019, October). *Klimaatatlas*. Opgehaald van Klimaatatlas: http://www.klimaatatlas.nl/klimaatatlas.php

Koomen, P. (2015). *Venstersectorspin*. Opgehaald van Waarneming: https://waarneming.nl/species/8723/

Roberts, M. J. (1996). *Collins Field Guide.* London: HarperCollins Publishers Ltd.

Science Direct, .. (2013). *Science Direct*. Opgehaald van Photoperiod: https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/photoperiod

Smith, T. (2019). *Discovering the daily activity pattern of Zygiella x-notata and its relationship to light.* Groningen: Rijksuniversiteit Groningen.

TriKinetics Inc USA . (2018). *DAM2 Drosophila Activity Monitor.* Opgehaald van Trikinetics: https://trikinetics.com/Downloads/DAM2%20Data%20Sheet.pdf

UC San Diego. (unknown). *Center for circadian biology*. Opgehaald van ccb ucsd: https://ccb.ucsd.edu/the-bioclock-studio/education-resources/basics/glossary.html#:~:text=Free%2DRunning%20Period%20(FRP)%3A,absence%20of%20environmental%20time%20cues.

United Nations, .. (2018). *Country Profiles*. Opgeroepen op Februari 7, 2020, van Population United Nations: https://population.un.org/wup/Country-Profiles/

Wikimedia. (sd). Opgehaald van Wikimedia.org: https://nl.wikipedia.org/wiki/Brugspin#/media/Bestand:Larinioides_sclopetarius_WP_070910.jpg

Zondsondergang tijden. (Unknown). *Zonsondergang tabel 2019 vergroting*. Opgeroepen op March 26, 2020, van Zonsondergang tijden: http://www.zonsondergangtijden.nl/tabel-zonsondergang-2019.html

References

Brown, S. A., Kunz, D., Dumas, A., Westermark, P. O., Vanselow, K., Tilmann-Wahnschaffe, A., . . . Kramer, A. (2008). Molecular insights into human daily behavior. *Proceedings of the National Academy of Sciences of the United States of America, 105*(5), 1602-1607.

Dominoni D M, Helm B, Partecke J, Lehmann M, & Dowse H B. (2013). Clocks for the city: Circadian differences between forest and city songbirds. *Proceedings of the Royal Society B: Biological Sciences, 280*(1763) doi:10.1098/rspb.2013.0593

Jones, T. C., Wilson, R. J., & Moore, D. (2018). Circadian rhythms of locomotor activity in metazygia wittfeldae (araneae: Araneidae). *The Journal of Arachnology, 46*(1), 26-30.

Mah A, Ayoub N, Toporikova N, Jones TC, & Moore D. (2020). Locomotor activity patterns in three spider species suggest relaxed selection on endogenous circadian period and novel features of chronotype. *Journal of Comparative Physiology.A, Neuroethology, Sensory, Neural, and Behavioral Physiology,* doi:10.1007/s00359-020-01412-y

Suter, R. B., & Benson, K. (2014). Nocturnal, diurnal, crepuscular: Activity assessments of pisauridae and lycosidae. *The Journal of Arachnology, 42*(2), 178-191.

References

Anotaux, M., Toscani, C., Leborgne, R., Châline, N., & Pasquet, A. (2014). Aging and foraging efficiency in an orb-web spider. *Journal of Ethology, 32*(3), 155-163.

Brown, S. A., Kunz, D., Dumas, A., Westermark, P. O., Vanselow, K., Tilmann-Wahnschaffe, A., et al. (2008). Molecular insights into human daily behavior. *Proceedings of the National Academy of Sciences of the United States of America, 105*(5), 1602-1607.

Czaczkes, T. J., Bastidas-Urrutia, A. M., Ghislandi, P., & Tuni, C. (2018). Reduced light avoidance in spiders from populations in light-polluted urban environments. *The Science of Nature, 105*(11-12), 64.

Dahirel, M., Dierick, J., De Cock, M., & Bonte, D. (2017). Intraspecific variation shapes community-level behavioral responses to urbanization in spiders. *Ecology, 98*(9), 2379-2390.

DeWitt, T. J., Sih, A., & Wilson, D. S. (1998). Costs and limits of phenotypic plasticity. *Trends in Ecology & Evolution, 13*(2), 77-81.

Dominoni D M, Helm B, Partecke J, Lehmann M, & Dowse H B. (2013). Clocks for the city: Circadian differences between forest and city songbirds. *Proceedings of the Royal Society B: Biological Sciences, 280*(1763)

Falchi, F., Furgoni, R., Gallaway, T. A., Rybnikova, N. A., Portnov, B. A., Baugh, K., et al. (2019). Light pollution in USA and europe: The good, the bad and the ugly. *Journal of Environmental Management, 248*

Ferraz, E., Borges, M. C., & Vianna, E. O. (2008). Influence of nocturnal asthma on chronotype. *Journal of Asthma, 45*(10), 911-915.

Gaston, K. J., & Bennie, J. (2014). Demographic effects of artificial nighttime lighting on animal populations. *Environmental Reviews, 22*(4), 323-330.

Gaston, K. J., Davies, T. W., Bennie, J., & Hopkins, J. (2012). Reducing the ecological consequences of night-time light pollution: Options and developments. *Journal of Applied Ecology, 49*(6), 1256-1266.

Gaston, K. J., Davies, T. W., Nedelec, S. L., & Holt, L. A. (2017). Impacts of artificial light at night on biological timings. *Annual Review of Ecology, Evolution, and Systematics, 48*(1), 49-68.

Heiling, A. M. (1999). Why do nocturnal orb-web spiders (araneidae) search for light? *Behavioral Ecology and Sociobiology, 46*(1), 43-49.

Helfrich-Förster, C., Winter, C., Hofbauer, A., Hall, J. C., & Stanewsky, R. (2001). The circadian clock of fruit flies is blind after elimination of all known photoreceptors. *Neuron, 30*(1), 249-261.

Hopkins, G. R., Gaston, K. J., Visser, M. E., Elgar, M. A., & Jones, T. M. (2018). Artificial light at night as a driver of evolution across urban-rural landscapes. *Frontiers in Ecology and the Environment, 16*(8), 472-479.

Johnson MTJ, & Munshi-South J. (2017). Evolution of life in urban environments. *Science (New York, N.Y.), 358*(6363)

Jones, T. C., Wilson, R. J., & Moore, D. (2018). Circadian rhythms of locomotor activity in metazygia wittfeldae (araneae: Araneidae). *The Journal of Arachnology, 46*(1), 26-30.

Kralj-Fišer, S., & Schneider, J. M. (2012). Individual behavioural consistency and plasticity in an urban spider. *Animal Behaviour, 84*(1), 197-204.

Krittika S, & Yadav P. (2019). Circadian clocks: An overview on its adaptive significance. *Biological Rhythm Research,*

Kronfeld-Schor, N., Visser, M. E., Salis, L., & van Gils, J. A. (2017). Chronobiology of interspecific interactions in a changing world. *Philosophical Transactions of the Royal Society B: Biological Sciences, 372*(1734), 20160248.

McLay, L. K., Green, M. P., & Jones, T. M. (2017). Chronic exposure to dim artificial light at night decreases fecundity and adult survival in drosophila melanogaster. *Journal of Insect Physiology., 100*, 15-20.

Moore, D., Watts, J. C., Herrig, A., & Jones, T. C. (2016). Exceptionally short-period circadian clock in cyclosa turbinata: Regulation of locomotor and web-building behavior in an orb-weaving spider. *Journal of Arachnology,* , 388-396.

Murren CJ, Auld JR, Callahan H, Ghalambor CK, Handelsman CA, Heskel MA, et al. (2015). Constraints on the evolution of phenotypic plasticity: Limits and costs of phenotype and plasticity. *Heredity, 115*(4), 293-301.

Owens ACS, & Lewis SM. (2018). The impact of artificial light at night on nocturnal insects: A review and synthesis. *Ecology and Evolution, 8*(22), 11337-11358.

Roenneberg, T., Wirz-Justice, A., & Merrow, M. (2003). Life between clocks: Daily temporal patterns of human chronotypes. *Journal of Biological Rhythms, 18*(1), 80-90.

Ruf, T. (1999). The lomb-scargle periodogram in biological rhythm research: Analysis of incomplete and unequally spaced time-series. *Biological Rhythm Research, 30*(2), 178.

Sharma, V. K., & Chandrashekaran, M. K. (2005). Zeitgebers (time cues) for biological clocks. *Current Science, 89*(7), 1136-1146.

Sih, A., Stamps, J., Yang, L. H., McElreath, R., & Ramenofsky, M. (2010). Behavior as a key component of integrative biology in a human-altered world. *Integrative and Comparative Biology, 50*(6), 934-944.

Willmott, N. J., Henneken, J., Elgar, M. A., & Jones, T. M. (2019). Guiding lights: Foraging responses of juvenile nocturnal orb-web spiders to the presence of artificial light at night. *Ethology, 125*(5), 289-297.

Yerushalmi S, & Green RM. (2009). Evidence for the adaptive significance of circadian rhythms. *Ecology Letters, 12*(9), 970-81.