

## **Research Design**

Minerva University

NS51: Empirical Analyses

Prof. Richard

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## Research Design

### Background summary:

The world is becoming brighter every day. Literally. The use of artificial light at night (ALAN) is significant. The effects of increased ALAN on animal physiology and behavior have been widely observed (Gaston et al., 2013). Insects like moths, beetles, and stink bugs demonstrate positive phototaxis (e.g., the attraction of organisms toward natural light sources such as the moon or reflection of light on the water surface) to navigate or find an optimal habitat for foraging (Heiling, 1999). Likewise, species that prey on these insects (e.g., spiders) are also attracted to the same sites. Therefore, ALAN also influences the physiology and behavior of spiders that live in well-lit areas. For instance, a study has found that female orb-web spiders evolved to prefer lit areas for building their web (Heiling, 1999). Willmott et al. (2018) found that ALAN exposure on nocturnal orb-web spiders resulted in early maturation, a higher mortality rate, and a smaller body size. Furthermore, Willmott et al. (2018) suggest that the fewer egg production can be mainly related to the smaller body size. Therefore, I want to look more closely at their egg production and how the exposure to ALAN is correlated with the number of egg sacs produced by each female spider. This is important because it can show whether there is the risk of extinction that ALAN poses on orb-web spiders if the ALAN keeps decreasing the egg production of nocturnal orb-web spiders.<sup>1</sup> **[Hypothesis:]** Therefore, I hypothesize that high exposure to artificial lights at night (ALAN) on nocturnal orb-web spiders is associated with fewer egg sacs production because spiders that are highly exposed to ALAN have smaller body sizes and early maturation, which can have a direct impact on their reproduction process. The prediction is that female nocturnal orb-web spiders of highly artificial light exposed environments will produce fewer egg sacs on average than those living in areas with low or no

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<sup>1</sup> #sourcequality: identifies and uses high quality sources that is most relevant for the context.

ALAN.<sup>2</sup>

**Outline of Research Design:**

- Selection of the study type
- A careful description of how the samples would be chosen.
- Description of the variables that will be measured: The number of egg sacs produced by each female spider (dependent variable), areas with: high exposure to ALAN, low exposure to ALAN (dependent variable).
- How the sampling methods would mitigate potential biases
- Methods on how statistical analysis on the collected data would be made
- Confounding variables
- Possible results from the data analysis, and alternative explanations for refuting results
- Importance of the findings
- Further topics to explore
- Limitations

**Type of research design:** Cross-sectional observational study

**Sampling strategy:** Stratified sampling

**Treatment:** N/A

**Variables:**

The number of egg sacs made by each female spider. The team will count the number of egg sacs found on the web made by each spider from the sample.

Dependent variable: Exposure to light. The dependent variable will be qualitative: high exposure to ALAN, low exposure to ALAN.

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<sup>2</sup> #hypothesisdevelopment: provides background by describing the researches and finding that has been found in the past; links the topic of interest from one of the studies; explains the importance of the hypothesis; generates a hypothesis that includes the premises that it builds on.

Controls: Since the number of egg sacs produced by the spider could depend on what time of the year the study is conducted, the study should collect the data in late summer to autumn, when most of the orb-web spiders lay their eggs ("Orb Weaver Spiders," 2022). Plus, the measurements should be taken simultaneously and year for both groups to eliminate the differences in time.

Since different areas have different light densities (e.g., homes have 150 lux, while street lamps have 10 lux of light density (Read, 2017)), the study will take samples from areas with around 10 lux of ALAN for the case group. This is due to the assumption that the higher the ALAN, the more the effect it has on spiders' physiology. Thus, this criteria will control differences in ALAN effects on spider body size. Similarly, stratified sampling also controls the size differences (how it controls is elaborated in the Narrative description). In addition, the samples should be taken from the same city to control the differences between the physiology of the spiders from region to region.

Potential confounding variables: The availability of the insects that feed on spider eggs

### **Narrative Description:**

An observational study is chosen because it requires less time, money, and effort than interventional studies. It also gives insights that are closer to real-life than interventional studies.

A cross-sectional study is used for its simplicity and the time duration because we want to see the correlation between ALAN and the evolution of the egg production of orb-web spiders.

Especially because we are looking at the evolution, the cross-sectional study is effective because we can sample from already ALAN exposed and unexposed areas, which eliminates the need to wait for generations of spiders to see the evolution in their egg production due to ALAN. A cross-sectional study sufficiently tests the hypothesis by looking at the correlation between the

two variables. However, interventional study would be better if we want to see a causal relationship between the variables.

Since we will measure the number of egg sacs, we will only sample pregnant female orb-web spiders from different families. The nocturnal orb-web spiders would be sampled from 2 different types of places:

- 1) Case group: Urban areas with many streetlights (areas with a light density between 10 lux and 12 lux) [the measure is chosen by looking at typical street lighting levels].
- 2) Control group: Rural areas without any streetlights at night (areas with less than 1 lux).

The assumption is that the spiders that live in these specific areas have been here for generations and that the natural selection process of the living condition has influenced the evolution of the species. The control group is used to compare with the case groups as a baseline number of egg sacs produced by the orb-web spiders in an environment with little or no ALAN. This is important because it allows to come up with more accurate and comprehensive conclusions. By choosing spiders from rural areas as a control group can help test the hypothesis by showing the differences between their number of egg sacs and those by the spiders from high ALAN environments.

Since it is impossible to get information on all nocturnal orb-web spiders worldwide, simple random or systematic sampling would be impractical. Therefore, stratified sampling would be used in each location to eliminate size confirmation bias in choosing a sample. It is a confirmation bias because if the team is expecting fewer egg sacs for each spider as a result of ALAN, they could consciously or unconsciously choose a small-sized spiders' sample that will give a result that they are expecting. If the sampled spiders are already small, the number of eggs produced would also be small, largely due to their size (Willmott et al., 2018). Stratified

sampling is beneficial because it makes sure that size variability is consistent in the groups since the size is an important factor that could impact the result. This is important because it helps divide the population into subgroups that share important characteristics that could have potentially skewed the results otherwise. Thus, the population would be divided into two strata: smaller than average-sized female orb-web spiders, bigger than average-sized female orb-web spiders. The average length for females is 3 cm in length ("Orb-Weaver Spider Facts," 2021). Then, 30 female spiders would be randomly selected from each stratified group. As a result, the whole sample would consist of 60 pregnant nocturnal orb-web spiders. To further mitigate the size confirmation bias, the research team could hire a group of qualified people to sample the spiders. The sampling group of people should be blinded, meaning that they will not know the study's hypothesis or have any prior expectations. Both control and the case groups have to consist of an equal number of smaller than average and bigger than average-sized orb-web spiders. The sample size is chosen as 60 because it has to be big enough to give insightful information but also not too many as it will make the data collection process time, energy, and money costly. Each female spider's number of egg sacs will be measured as an independent variable. When the samples and the measurements are completed, the research team can proceed with the data to analyze and interpret whether the data supports the hypothesis.<sup>3 4</sup>

After data are collected, the research team should compute the mean number of egg sacs produced by both control and case groups. It is also useful to plot-relevant histograms and computes statistical significance using p-values. Looking at the outliers in the datasets and coming up with possible explanations can spark further research questions.

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<sup>3</sup> #biasidentification: identifies the potential bias and which type it is; explains why it is considered as an confirmation bias; explains how it could affect the sample choice and the result; explains how the sampling strategy could help mitigate the bias.

<sup>4</sup> #sampling: identifies an appropriate sampling; explains why the chosen sampling method is effective while other are not; explains how the chosen method eliminates bias; gives details on how sampling should be conducted; sets the sample size and justifies it.

Since the study is an observational study that uses samples from nature, there could have been many different confounding variables that have affected the species throughout generations. Thus, we cannot decrease all the impacts or sometimes identify the confounding variables that would affect the number of egg sacs produced. One example is the availability of insects that prey on spiders' eggs. It could be the case that the egg sacs in the well-lit areas are less because predators can see them well and feed on them easily. When the number of predators is high, it can significantly impact the result. However, it also means that a natural selection process could have led to an evolution in fewer egg sacs production.<sup>5</sup>

### **Conclusion:**

The hypothesis will be proven if the average number of egg sacs is smaller in the case groups than in the control group and if the result is statistically significant. The finding will be important to identify the risk ALAN poses on the orb-web spider population since the constant decrease in offspring can lead to their extinction. However, if results refute the hypothesis, the alternative explanation would be that there is no correlation between ALAN and nocturnal orb-web spiders' egg production. Another explanation could be that the study was poorly designed and that many confounding variables affected the result, and new hypothesis should be explored in the future. Further studies should look more closely at the causal relationship between ALAN and spiders' physiology in a controlled environment. More specifically, studies should look at the possibility of how ALAN could lead to orb-web spider extinction. However, these kinds of experiments could take many years, and there could be ethical controversies about whether the study should be conducted or not.

Since it is a cross-sectional study, the limitation of the study is that it can only show a difference

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<sup>5</sup> #comparisongroups: identifies and describes a control group; explains why it is useful and how it can lead to a better result; explains how the why the certain control group is selected; provides how the control group will be used in the study and gives details on what aspects should be consistent in both groups.

between two different environments at one point in time. Therefore, it will be useful to replicate a study in the following years while the study structure remains the same. The exact (close) study replication will help best to determine whether the result is generalizable and valid. It is expected that the sample would be renewed since orb-web spiders live around 12 months. It is also acceptable to change the location of the experiment since it can add to the generalizability of the finding. This is important because the prior study's result could have been affected by the factors specific to the time of the period or/and the location.

While the study described in the paper has its advantages and disadvantages, I believe that it can add insight to the past findings of the relationship between ALAN and spiders and encourage others in the future.<sup>6 7 8 9</sup>

**Words:1477**

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<sup>6</sup> #observationalstudy: carefully describes how the observational study would be conducted; explains why it was selected over interventional study; explains how it better tests the hypothesis; explains the limitations and the advantages;

<sup>7</sup> #studyreplication: explains why the limitation of this study type can be improved by study replication; identifies the type of study replication and why it is useful; explains what are aspects that could vary between the initial study and the replication;

<sup>8</sup> #evidencebased: provides appropriate evidence to back up arguments; explains how the information adds to the discussion; explains why some aspects of the study have been selected using evidence from other sources.

<sup>9</sup> #testability: provides a testable prediction from the hypothesis; explains how the connection between the variables would help test the prediction and the hypothesis; provides an alternative explanation in case the results refute the hypothesis; gave suggestions on future directions; explains the limitations of the study.



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