5-2019

Circadian Rhythms of the Spider Pholcus phalangeoides in Activity Monitors and Web Boxes

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Circadian Rhythms of the Spider *Pholcus phalangeoides* in Activity Monitors and Web Boxes

Thesis submitted in partial fulfillment of Honors

By

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April (26), 2019

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Abstract: Circadian rhythms are endogenous molecular clocks that correspond to the 24-hour day and are regulated by light stimulus, allowing organisms to entrain to the dawn-dusk cycle. These clocks may allow organisms to anticipate daily events, influencing their behavior. In arthropods, including spiders, circadian rhythmicity is tested using activity monitors, which house individuals in tubes. However, this does not reflect the natural habitat of many spiders. We compared the locomotor activity of the cellar spider *Pholcus phalangiodes* in activity monitors with the locomotor activity in web boxes. After being entrained to a 12:12 light:dark cycle, the spiders were recorded in constant darkness. The resulting free-running periods demonstrated similar clock data for spiders in tubes as in boxes. This validates the activity-monitor research method.

Introduction

Circadian rhythms are endogenous molecular clocks corresponding with the 24-hour day. Although internal, these clocks are regulated by light stimulus. This allows organisms to synchronize to the dawn-dusk cycle. The circadian mechanism is important for organisms because it allows them to “anticipate” recurring environmental changes. Circadian rhythms are found in many different types of organisms, including spiders. Some of the behaviors that spiders present are circadian, and some are non-circadian. For example, the timing of web-building and a spider’s position in its web may allow the spider to reduce conspicuousness to visual predators (Moore, et al. 2016). Spider metabolism is also regulated by the circadian clock, as evidenced by respirometry (Cloudsley-Thompson 1987).

Ecologically, spiders occupy a position as both predator and prey (Wise 1993). Therefore, circadian behaviors may allow spiders to minimize risk by timing behaviors like foraging and web maintenance. Furthermore, circadian patterns can influence responses to stimulus. For example, antipredator response-behavior in orb-weaving spiders has also been shown to have circadian oscillation in intensity of response, and the anterior median eyes of a nocturnal orb-weaver have been shown to have circadian patterns in sensitivity to light. (Jones et al. 2011, Yamashita and Nakamura 1999). Interestingly, the anterior median eyes of the spider *Lycosa tarentula* are not able to entrain the spider to a light/dark cycle, while all other eyes can (Ortega-Escobar 2002).

Spider locomotor activity has been shown to have circadian patterns in several families, including lycosids (Seyfarth 1980, Schmitt et al. 1990, Ortega et al. 1992, Ortega-Escobar 2002), linyphiids (Suter 1993), theridiids (Suter 1993, Wolf 2011), ctenids (Soriano-Morales et al. 2013), diplurids (Soriano-Morales et al. 2013), and araneids (Moore et al. 2016). However, data showing circadian rhythmicity in pholcids have not been published.

Pholcus phalangiodes is commonly referred to as "daddy-longlegs spiders." *Pholcus phalangiodes* is an Araneomorph spider species that builds non-sticky space webs, often on the ceilings of places like cellars, basements, and garages. *P. phalangiodes* preys upon arthropods which become stuck in or trip the lines of its web. Furthermore, *P. phalangiodes* invades the webs of other spiders, vibratory-mimics a prey item, and feeds upon that web's spider (Jackson and Brassington 1987).

Typically, circadian rhythms in animals are measured using locomotor activity, which is widely believed to represent neurological arousal. For example, in experiments involving rodents, wheel-running behavior is used as a proxy for when the animal would normally be moving about and foraging. In experiments involving arthropods the most common research method is to use activity monitors which contain banks of tubes with infrared light beam emitters and receptors. When the arthropod interrupts the beam, an instance of locomotion is recorded. However, web-building spiders like *Pholcus phalangiodes* do not live in tubes *in natura*. They live in webs. Furthermore, when in their webs, spiders are largely stationary, moving only to capture prey or perform web maintenance. This leads to the question of what is actually being measured by the locomotor activity in tubes of web-building spiders? If the locomotor activity in tubes does reflect underlying patterns of neurological arousal, then there should be agreement in temporal patterns of activity when measure in tubes or in webs. Therefore, to test the reliability of data collected from activity monitors, we compared locomotor activity from *P. phalangiodes* in activity monitors and locomotor activity from *P. phalangiodes* in web-boxes. This follows similar research which found that *Latrodectus mactans* show similar patterns of activity whether in tubes or webs in both a 12:12 light:dark cycle and constant darkness (Gauck, unpublished work 2018).

Methods

Study Species

Adult female *Pholcus phlangeoides* (Family: Pholcidae) cellar spiders were collected in East Tennessee (Washington Co.). All spiders were fed crickets and housed individually in screen boxes.

Behavioral Tests

Rhythmicity in boxes

Rhythmicity was tested by allowing spiders to form webs in screen boxes. The spiders were given prey items in advance of filming and given no prey items during filming. The spiders were entrained in 12 hours light / 12 hours dark (LD) cycle. After entrainment for at least 72 hours, the spiders were video recorded in LD to observe the times and duration of locomotor activity. The spiders were then switched to a constant dark (DD) cycle. The spiders were again recorded to observe locomotor activity. The footage was reviewed, and time-of-activity data were inputted into the free, open-source software BORIS (Friard and Gamba). The data were then exported from and inputted into Clocklab (Actimetrics, Wilmett IL) for analysis.

Rhythmicity in activity monitors

Rhythmicity was also tested in tubes, with an infrared light beam recording instances of locomotion across the length of the tube. Like in boxes, the spiders were entrained in a 12 hours light / 12 hours dark (LD) cycle. After entrainment for at least 72 hours, the spiders were recorded in LD to observe the instances of locomotor activity. The spiders were then switched to constant dark (DD). The data were then inputted into Clocklab for analysis. These data were produced by Andrew P. Shields and Nathaniel R. Wyatt.

Results

The results of most of the spiders did not show a significant circadian pattern:

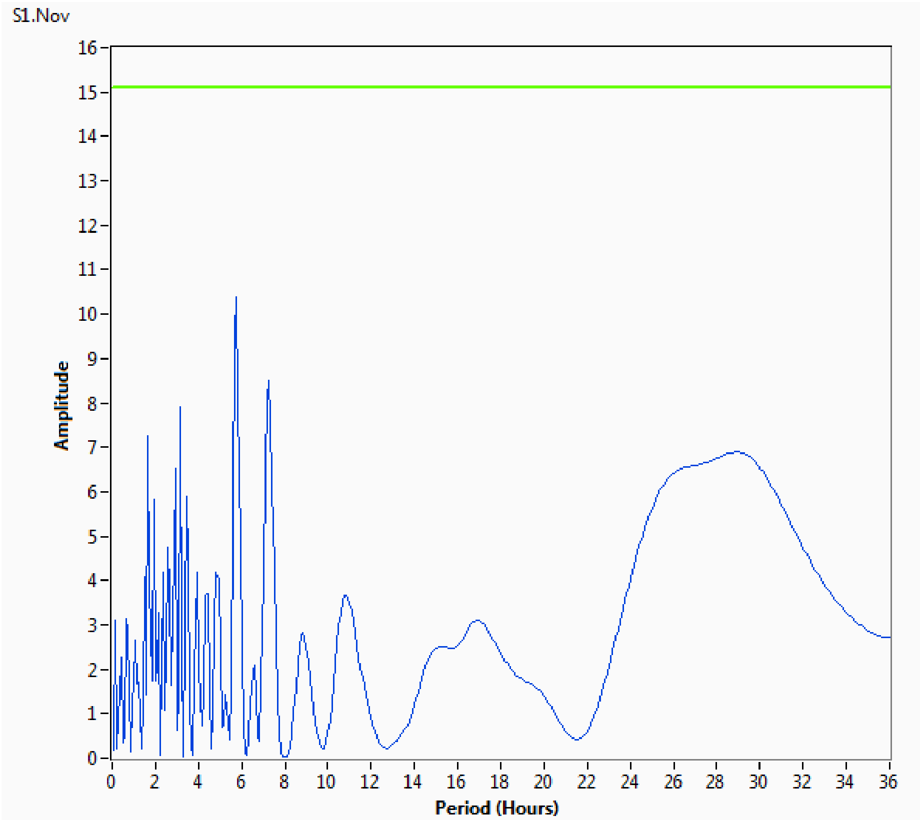

Figure 1: *Periodigram of spider 1.* Note the horizontal line indicating statistical significance.

The data for spider 4 indicate a significant circadian pattern, with a period of 25.75 hours:

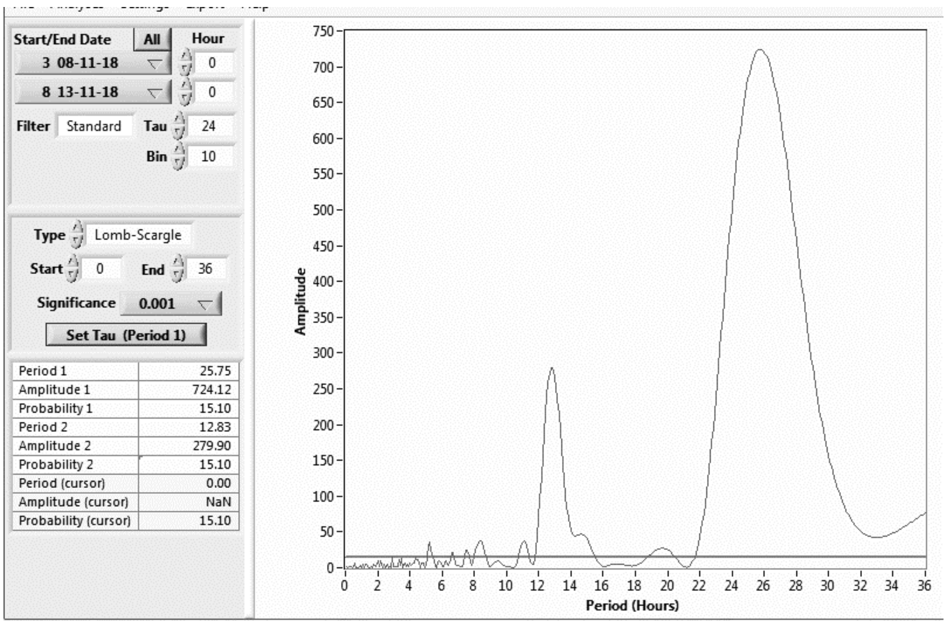

Figure 2. *Periodigram of spider 4.* Note the horizontal line indicating statistical significance.

Interestingly, the data also present a secondary, less intense peak in behavior at 12.83 hours. This corresponds with half of the 26 hour day that the spider anticipates.

These data correspond to the data gained from pholcids in tubes. This reconciles the two methods.

During the LD light cycle, the spiders had an entrainment profile displaying activity starting approximately one-half to one hour after the lights turned out at 19:00. This activity proceeded in short bursts throughout the night, stopping approximately one-half to one hour before the lights turned on at 7:00. Most individuals were inactive during the day:

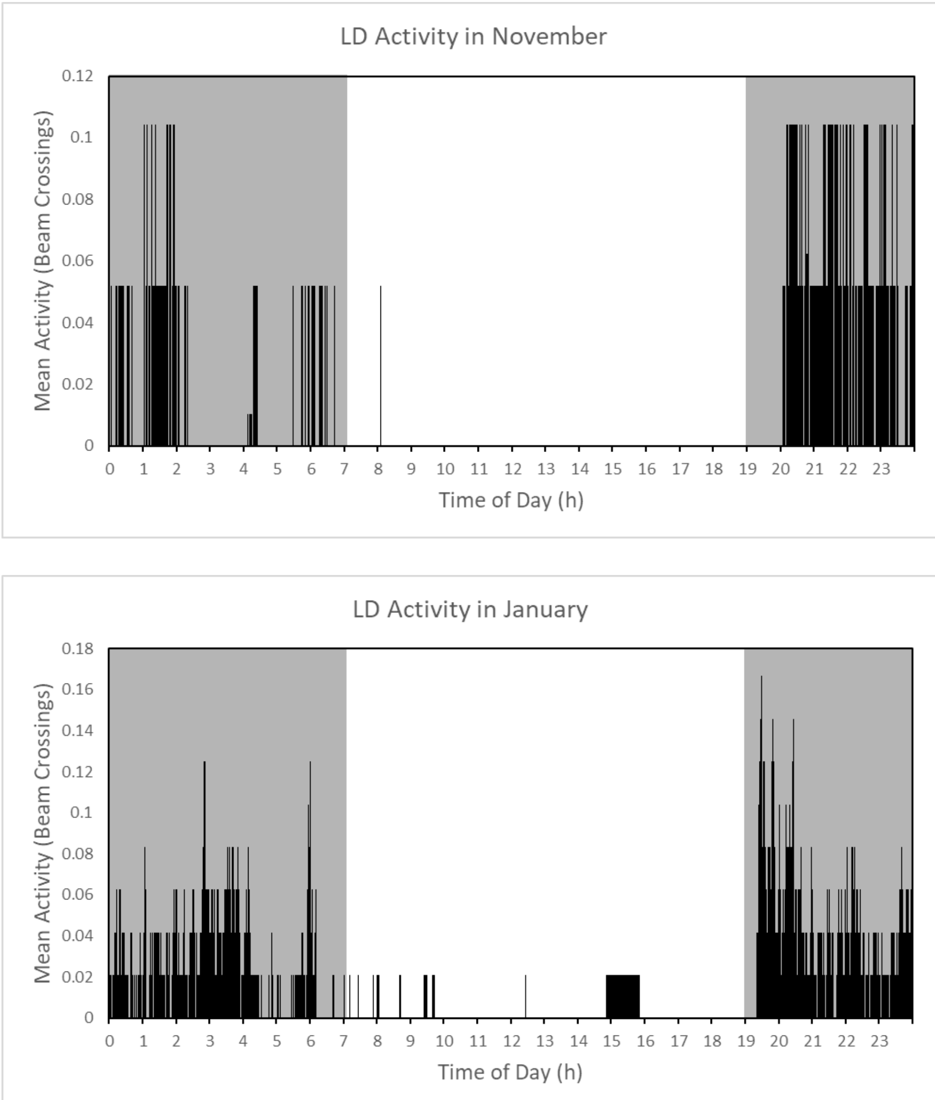

Figure 3. *Entrainment profiles of spiders.*

Of the data from spiders in boxes, one spider produced significant circadian rhythmicity. In DD, this spider exhibited a free-running pattern, that is, a periodicity that persists in the constant conditions where re-entrainment is prevented:

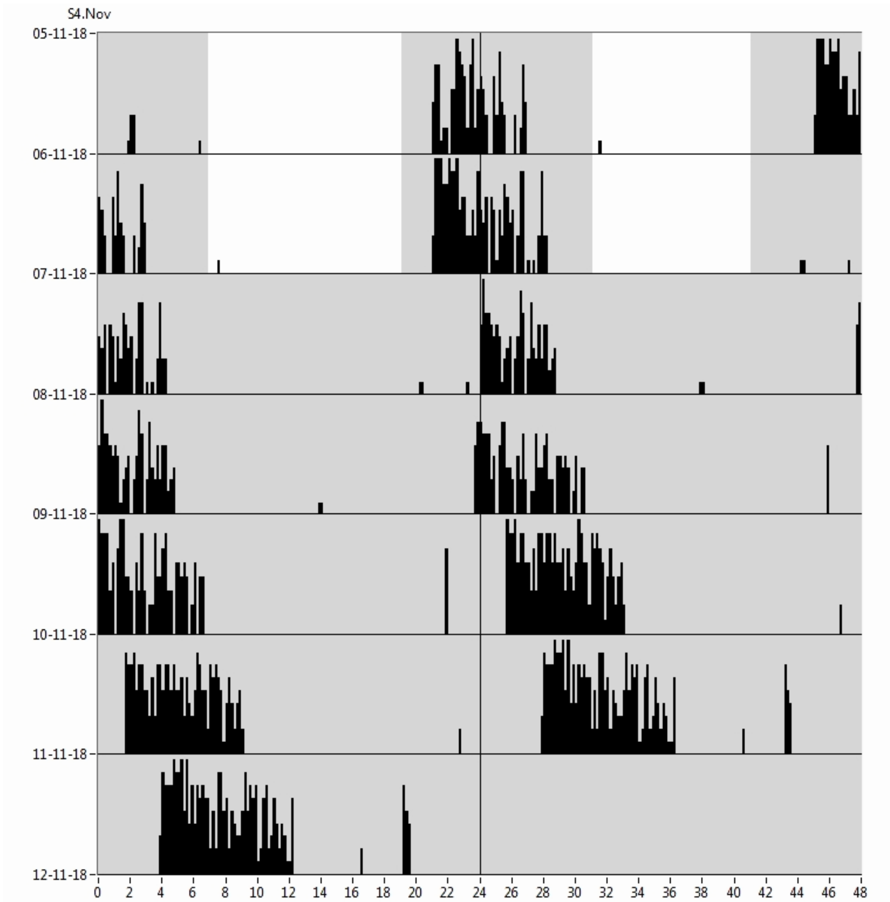

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Figure 4. *Actogram of spider 4.* Note that this spider is in a web box.

In the actogram of spider 4, the spider can be seen to be entrained to the light:dark cycle. When the light pattern switches to D:D, the spider's locomotor activity begins to shift rightward. The spider's >24 hour clock causes it to begin and end locomotor activity later and later each day. Were it to not be freerunning, it would resemble the actogram for spider 1:

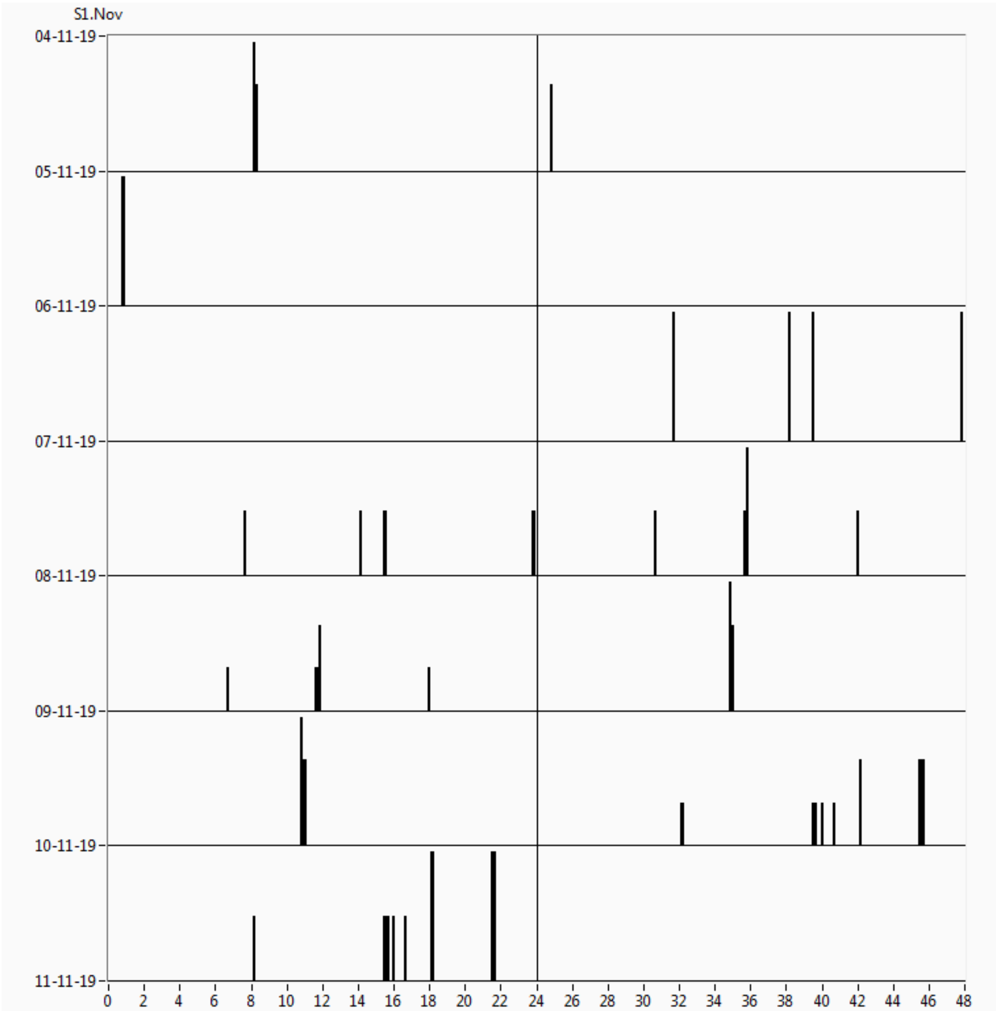

Figure 5. *Actogram of spider 1.* Note that this spider is in a web box.

Again, these data for spider 4 correspond with the data of the spiders in tubes. For example, the actogram of spider 15 is provided:

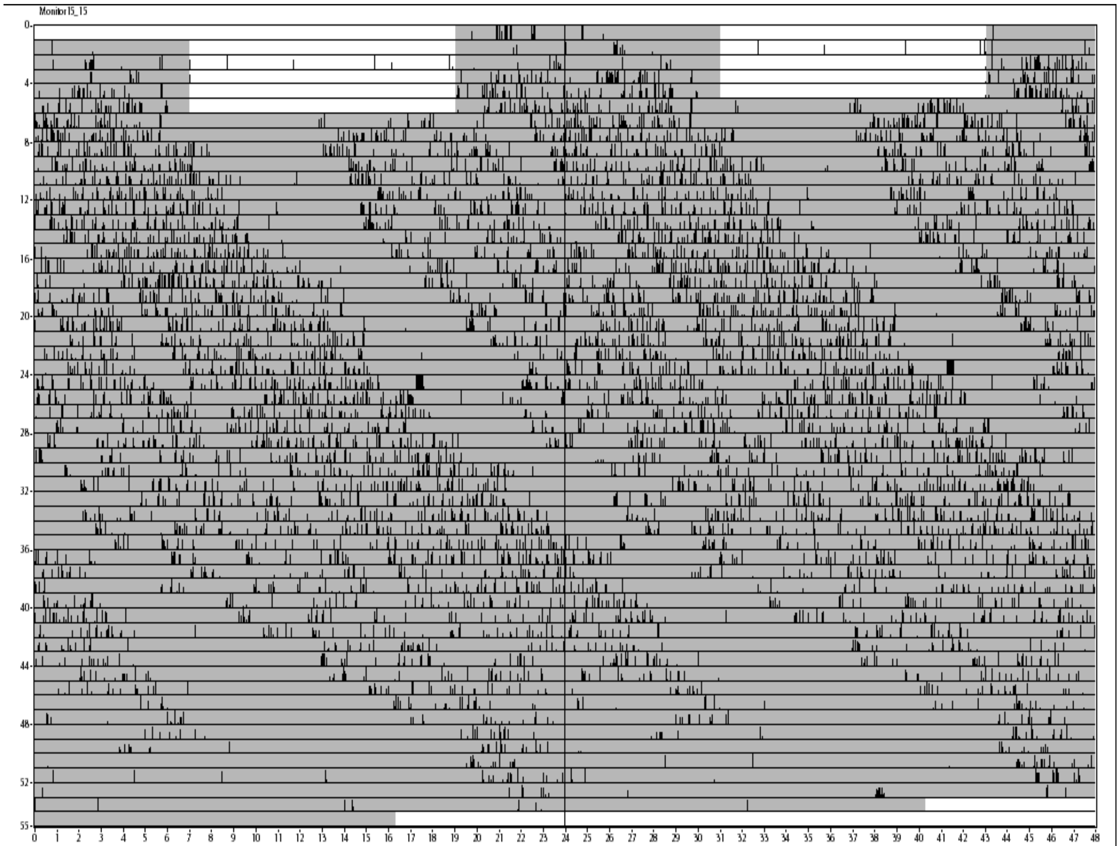

Figure 6. *Actogram of spider 15*. Note that this spider is in an activity monitor

Recall that spider 4 exhibited a primary rhythm at 25.75 hours and a secondary rhythym at 12.8 hours. When we examine the periodogram of spider 15, we find similar rhythms:

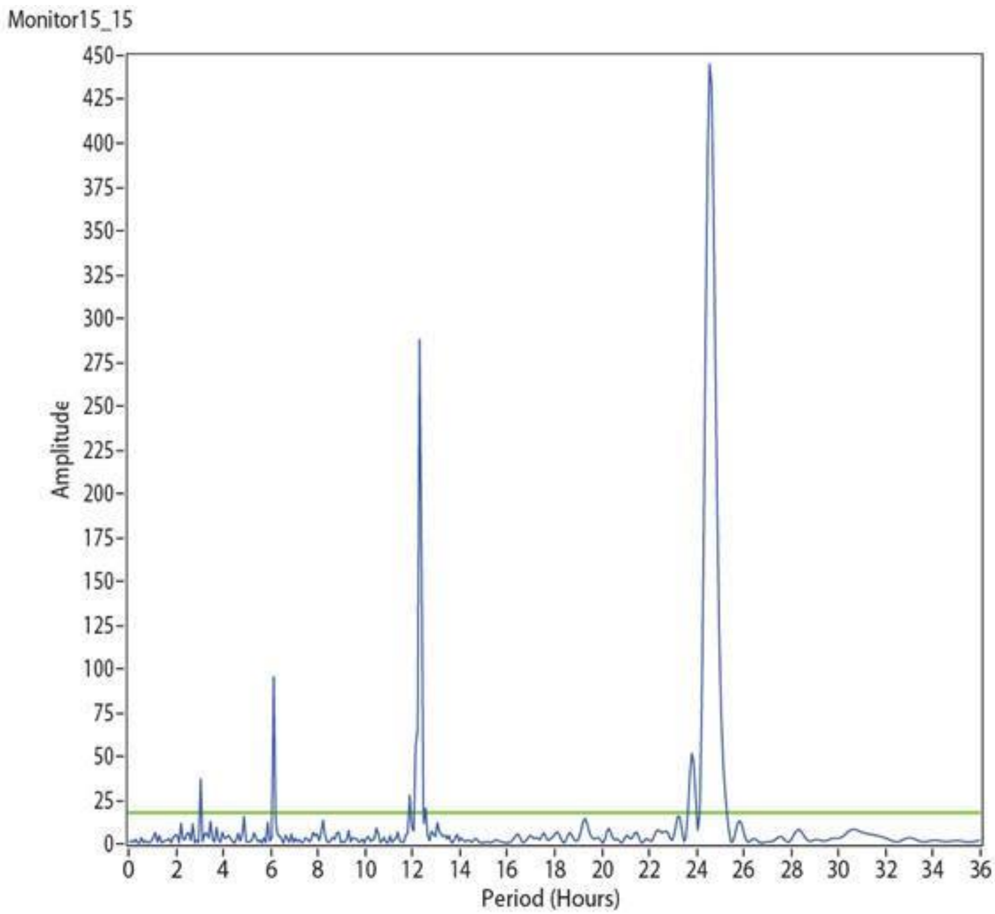

Figure 7. *Periodigram of spider 15*. Note that this spider is in an activity monitor.

Discussion

Because of the natural history of *P. Phalangiodes*, we expected this spider to exhibit circadian rhythmicity in its locomotion, particularly locomotion related to araneophagic foraging. Our results demonstrate that *P. Phalangiodes* is rhythmic in its locomotor activity. This rhythmicity includes diel periodicity, which confirms the presence of an endogenous, circadian clock. Most of the locomotor activity recorded in the web boxes consisted of the spider moving around the perimeter of the box. This may be related to searching for prey items or probing the box for an escape. However, the web boxes were large enough for the spider to build a full-sized web of approximately equal size to the webs from which the spiders were harvested.

The data from the spiders in web boxes recorded fewer instances of locomotion, which is most likely due to the research method. Only locomotion events in which spider's body moved greater than approximately 1 cm were recorded. Additionally, locomotion was divided into 1-minute bins, in which locomotion was considered only as a binary condition—true and false. That is, the intensity of the locomotion was not considered, nor the duration within the 1-minute bins.

Interestingly, spider 15 also exhibits further rhythmicity at approximately 6 hours and 3 hours. These rhythms follow a pattern, where each period is at approximately half the time of the next. Further research into the molecular biology of these spider's circadian rhythms could prove useful in explaining this pattern.

The extreme variance in circadian rhythms between spider families presents great potential for increased understanding of the evolutionary significance of the circadian mechanism and circadian behavior. Validation of the results of activity monitors will allow chronobiologists to continue to use activity monitors where possible, allowing for the collection of more data with less effort.

Bibliography

Cloudsley-Thompson, John L. 1987. "Ecophysiology of Spiders." In *Ecophysiology of Spiders*, by John L. Cloudsley-Thompson, 371-379. Berlin: Springer.

Gauck, Megan. 2018. Unpublished work.

Huber, B A. 2003. "High species diversity in one of the dominant groups of spiders in East African montane forests (Araneae : Pholcidae : Buitinga n. gen., Spermophora Hentz)." *Zoological Journal of the Linnean Society* 137 (4): 555-619. https://onlinelibrary.wiley.com/doi/pdf/10.1046/j.1096-3642.2003.00053.x.

Jackson, R R, and Roxanne J Brassington. 1987. "The biology of Pholcus phalangioides (Araneae, Pholcidae): predatory versatility, araneophagy and aggressive mimicry." *The Journal of Zoology* 211 (2): 227-238. doi:10.1111/j.1469-7998.1987.tb01531.x.

Jones, Thomas C, Tamer S Akoury, Christopher K Hauser, and Darrell Moore. 2011. "Evidence of circadian rhythm in antipredator behaviour in the orb-weaving spider Larinioides cornutus." *Animal Behavior* 82 (3): 549-555. https://www.sciencedirect.com/science/article/pii/S000334721100251X#bib31.

Moore, Darrell, J Colton Watts, Ashley Herrig, and Thomas C Jones. 2016. "Exceptionally short-period circadian clock in Cyclosa turbinata: regulation of locomotor and web-building behavior in an orb-weaving spider." *The Journal of Arachnology* 44 (3): 338-396. doi:10.1636/JoA-S-16-014.1.

Ortega, J, M Ruiz, and C Fernandez-Montraveta. 1992. "Daily patterns of locomotor activity in a Lycosid spider." *Journal of Interdisciplinary Cycle Research* 23 (4): 295-301. doi:10.1080/09291019209360188.

Ortega-Escobar, Joaquin. 2002. "Circadian Rhythms of Locomotor Activity in Lycosa tarentula (Araneae, Lycosidae) and the Pathways of Ocular Entrainment." *Biological Rhythm Research* 33 (6): 561-576. doi:10.1076/brhm.33.5.561.13934.

Schmitt, Alain, Martin Schuster, and Friedrch G. Barth. 1990. "Daily Locomotor Activity Patterns in Three Species of Cupiennius (Araneae, Ctenidae): The Males Are the Wandering Spiders." *The Journal of Arachnology* 18 (3): 249-255. https://www.jstor.org/stable/3705430.

Seyfarth, Ernst-August. 1980. "Daily patterns of locomotor activity in a wandering spider." *Physiological Entomology* 5 (2): 199-206. doi:10.1111/j.1365-3032.1980.tb00227.x.

Soriano-Morales, Sara, Omar Caballero-Hernandez, Mayra Davila-Montres, and Manuel Miranda-Anaya. 2013. "Circadian locomotor activity and entrainment by light cycles in cave spiders (Dipluridae and Ctenidae) at the cave Los Riscos, Qro. México." *Biological Rhythm Research* 44 (6): 949-955. doi:10.1080/09291016.2013.781330.

Suter, Robert B. 1993. "Circadian Rhythmicity and Other Patterns of Spontaneous Motor Activity in Frontinella pyramitela (Linyphiidae) and Argyrodes trigonum (Theridiidae)." *The Journal of Arachnology* 6-22. https://www.jstor.org/stable/3705374.

Wise, David H. 1993. *Spiders in Ecological Webs.* New York: Cambridge University Press. Accessed 2019. https://books.google.com/books?hl=en&lr=&id=qP0fYU3FTDwC&oi=fnd&pg=PR11&ots=qd7Gi5DjDE&sig=zWNgH3dxNFswDlohNK8NrgSfug8#v=onepage&q&f=false.

Wolf, Elise. 2011. ""Diel Periodicity in Activity and Location in the Web of the Common House Spider (Achaearanea tepidariorum)." *Undergraduate Honors Theses.* http://dc.etsu.edu/honors/13.

Yamashita, Shigeki, and Takiko Nakamura. 1999. "Circadian oscillation of sensitivity of spider eyes: diurnal and nocturnal spiders." *Journal of Experimental Biology* 202: 2539-2542. http://jeb.biologists.org/content/jexbio/202/18/2539.full.pdf.

Acknowledgements

I would like to thank Andrew P. Shields and Nathaniel R. Wyatt for producing the activity monitor data for *P. phalangiodes* in activity monitors, and I would like to thank Dr. Darrell J. Moore for sharing these data. I would like to thank Dr. Thomas C. Jones (TJ), who has been a wonderful mentor. I could not have done it without him.