The Possible Role of Extra-ocular Photoreceptors in the Entrainment of Lobster Circadian Rhythms to Light:Dark Cycles

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Abstract
The American lobster, *Homarus americanus*, is a decapod crustacean whose daily nocturnal rhythms of activity are driven, in part, by an internal biological clock. While this circadian clock is capable of producing a rhythm of approximately 24 hours on its own, it can also be entrained to light:dark cycles. Recent findings in our laboratory suggest that lobsters have photosensitive neurons throughout their nervous system (extra-ocular photoreceptors). The objective of this research was to test the hypothesis that these extra-ocular photoreceptors aid in entraining their daily rhythm of activity. First, the locomotion of juvenile American lobsters was measured in trials under a normal 24 hour light:dark cycle. Then, after this “control” period, the lobsters’ eyes were painted with black nail polish and their activity was monitored under the same conditions. The lobsters were also exposed to DD conditions with their eyes covered in one of the trials. During the control LD cycle, lobsters expressed increased locomotion at night, demonstrating their daily rhythm. The DD cycle had a consistent rhythm with an elongated period of activity. Interestingly, half of the lobsters continued to follow the same pattern of activity despite the visual impairment and half expressed a diurnal rhythm. Although there was variation in the locomotor activity, a periodicity of ~24 hours was maintained throughout the LD cycles. This indicates that *H. americanus* receives photoperiod input from both their eyes and extra-ocular photoreceptors that help synchronize their locomotor activity to LD cycles.

Keywords
COLSA, Biological Sciences, Animal Science

Subject Categories
Behavioral Neurobiology

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The Possible Role of Extra-ocular Photoreceptors in the Entrainment of Lobster Circadian Rhythms to Light:Dark Cycles.

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Abstract

The American lobster, *Homarus americanus*, is a decapod crustacean whose daily nocturnal rhythms of activity are driven, in part, by an internal biological clock. While this circadian clock is capable of producing a rhythm of approximately 24 hours on its own, it can also be entrained to light:dark cycles. Recent findings in our laboratory suggest that lobsters have photosensitive neurons throughout their nervous system (extra-ocular photoreceptors). The objective of this research was to test the hypothesis that these extra-ocular photoreceptors aid in entraining their daily rhythm of activity. First, the locomotion of juvenile American lobsters was measured in trials under a normal 24 hour light:dark cycle. Then, after this “control” period, the lobsters’ eyes were painted with black nail polish and their activity was monitored under the same conditions. The lobsters were also exposed to DD conditions with their eyes covered in one of the trials. During the control LD cycle, lobsters expressed increased locomotion at night, demonstrating their daily rhythm. The DD cycle had a consistent rhythm with an elongated period of activity. Interestingly, half of the lobsters continued to follow the same pattern of activity despite the visual impairment and half expressed a diurnal rhythm. Although there was variation in the locomotor activity, a periodicity of ~24 hours was maintained throughout the LD cycles. This indicates that *H. americanus* receives photoperiod input from both their eyes and extra-ocular photoreceptors that help synchronize their locomotor activity to LD cycles.
Introduction

Many organisms possess an internal biological clock. An endogenous clock acts to control, in part, the biological rhythms of activity. These activities include feeding, sleeping, mating, and locomotion. While it is capable of producing a rhythm of approximately 24 hours on its own, the circadian clock can also be entrained by a variety of exogenous environmental cues, including the natural photoperiod, or cycles of light and dark. Almost all organisms are exposed to daily changes in photoperiod, which are important in saving energy for daily activities such as reproductive and locomotive behaviors (Strauss & Dircksen, 2010), including the American lobster, Homarus americanus.

Research has shown that H. americanus, like many other decapod crustaceans, expresses a nocturnal daily rhythm (Jury et al., 2005). Field studies examining the biological rhythmicity of H. americanus in its natural habitat show a large variation in locomotor activity and daily rhythm, most likely due to seasonal foraging and molting behaviors, or environmental cues such as the presence of predators or competition for shelter (Golet et al., 2006). In laboratory studies devoid of exogenous influences, the endogenous clock of H. americanus has been shown to influence locomotor activity, which primarily occurs at night (Jury et al. 2005).

Mollusks, arthropods, and insects have organized photoreceptive structures and many possess extra-retinal or extra-ocular photoreceptors. In invertebrates they are primarily used for the entrainment of circadian rhythm and measuring day length (Page, 1982). Recent findings in our laboratory have suggested that American lobsters also have photosensitive neurons throughout their nervous system that potentially play a part in controlling circadian rhythms of locomotor activity.
Previous research in our laboratory indicates the presence of putative cryptochrome (CRY) neurons in the abdominal ganglia, located in the ventral nerve cord. CRY is a photosensitive protein that is activated by UVA/blue light and is the only photosensitive clock protein that has been found in crustaceans. It has been shown to help reset the circadian clock through a negative feedback loop in the fruit fly, *Drosophila*. Once the protein is exposed to light, it binds to the heterodimer of two other clock proteins period (PER) and timeless (TIM), and causes the proteasome-mediated degradation of TIM, ending the inhibition of its gene transcription (Strauss & Dircksen, 2010). Research has shown that CRY levels cycle in a crepuscular pattern, with the highest levels at dawn and dusk, suggesting a role in controlling circadian rhythm (Strauss & Dircksen, 2010). It has also been suggested that CRY has a role in synchronizing the locomotor activity to LD cycles in the crayfish, *Cherax destructor* (Sullivan *et al.*, 2009). This information leads us to infer that *H. americanus* may have extra-ocular photoreceptors on their abdomen that help coordinate locomotor activity.

Caudal photoreceptors located on the sixth abdominal ganglion (caudal ganglion) have been found in at least nine species of crayfish, and are thought to be present in the spiny lobster *Panulirus*, and the pistol shrimp *Alpheus*, but there is an apparent absence of such photosensitive neurons in *Homarus* (Wilkens & Larimer, 1976). In other experiments involving removal of the retina and sixth abdominal ganglion in crayfish, a level of entrainment was still exhibited, indicating the possibility of extra-ocular and extra-caudal photoreceptors that aid in entrainment (Page & Larimer, 1972). Extra-ocular photoreception has also been studied in a variety of other marine organisms, such as echinoderms like the green sea urchin, *Strongylocentrotus droebachiensis*, (Lesser *et al.*, 2011) and mollusks like the gastropods *Aplysia* and *Bulla* (Block & Davenport, 1982).
Despite the research present on extra-ocular photoreception and daily rhythms, these topics have not been thoroughly investigated in regards to *H. americanus*. The purpose of my research is to test the hypothesis that American lobsters possess extra-ocular photoreceptors that aid in entraining their daily rhythm of activity and will continue to be entrained to the LD cycle after their eyes are covered. The hypothesis was tested using analyses of locomotion and levels of activity in different photoperiodic conditions. We found that although the patterns of activity varied, a consistent rhythm of approximately 24 hours was maintained in lobsters without ocular input, demonstrating the presence of extra-ocular photoreceptors that are able to synchronize biological rhythms to light:dark cycles.

**Materials and Methods**

Juvenile American lobsters were obtained from the New England Aquarium and raised in the laboratory. A total of 5 were selected at random to be used in this experiment. They were placed in separate arenas consisting of circular plastic buckets 19 cm. in diameter and a rectangular shelter in the center that was slightly shorter than the lobsters’ body length. The arenas contained seawater that had its salinity continuously checked and were housed in a cold room set to 15 degrees Celsius. Figure 1 shows the experimental setup depicting both light and dark conditions.

The trials took place from November 21-December 5, 2012 (fall), containing two lobsters, and January 4-22, 2013 (winter), containing three lobsters. The lobsters were first subjected to a “control” light:dark (LD) cycle of 12 hours of light beginning at dawn and 12 hours of dark beginning at dusk (12:12 LD) to demonstrate the juveniles, like adults, exhibit a daily rhythm entrained to the natural photoperiod. Dawn and dusk were defined as 5am and
5pm, respectively, but were changed to 7am and 7pm in the winter trial due to ease of starting and stopping the videos at the appropriate times. They subsequently had their eyes and eyestalks covered with a mixture of glue and black nail polish and were subjected to the same LD cycle to see if they continued to express a daily rhythm, showing that the lobsters have extra-ocular photoreceptors that can detect light and may be involved in entraining the circadian clock to natural LD cycles. In the January trials, the “blinded” lobsters were also exposed to 24 hour darkness, demonstrating the daily rhythm of activity is under the control of an endogenous circadian clock that will continue to operate with no exogenous light cues.

A video camera and digital time lapse system were used to continually monitor lobster activity. An infrared (IR) sensitive camera and IR lights made it possible to obtain video recordings at night. The videos were analyzed Ethovision XT software, which tracked the animals’ movements, and the resulting data were plotted and analyzed using Clocklab software.

![Figure 1. Experimental design for locomotion assay. The January trial contained subjects 1-3, arranged from bottom to top. The light (left) and dark (right) cycles are depicted. The red dot and line are used to visually track the lobsters’ movements.](image)

**Results**
The lobsters spent most of the day hidden inside the shelters and came out during the night to walk around and explore their enclosure, similar to the natural behaviors documented in field studies (Golet et al., 2006). Both of the juvenile lobsters expressed a clear daily rhythm during the control 12:12 LD cycle with an average $\tau$, the measurement of periodicity, of 23.98h (Figure 2). The peaks of activity occurred in a crepuscular, rather than a nocturnal, rhythm, observed at the onset of dawn and dusk, consistent with patterns of CRY cycling (Strauss & Dircksen, 2010). After the lobsters’ eyes were covered, the daily rhythm continued to persist with a similar periodicity of 25.2h, but there also appeared to be an increase in activity in light conditions, compared to the control cycle (Figure 2). The second lobster only had eyes covered LD data from 11/26-11/30, with a $\tau$ of 24.8h. The LD cycle with eyes covered lasted for ten days, but after approximately seven days the rhythm started to shift, with a greater time of increased movement at night and a periodicity of 28.8 hours. Only one actogram was included in the study (Figure 2), because there was only partial data available from the other juvenile that would not accurately depict the locomotor activity.
Figure 2. Actogram and periodograms of juvenile lobster activity from the November-December trial data. The Actogram (left) depicts the control LD cycle from 11/21-11/26, showing a crepuscular rhythm with peaks of activity at dawn and dusk, each followed by a gradual decline in movement. This pattern continues after the juvenile lobsters are blinded on 11/26, but with a higher activity level. After approximately seven days, there is a shift in periodicity and a further increase in activity. The periodograms (right) show the $\tau$ values for the control LD (23.95h) and eyes covered LD cycles before (25.2h) and after (28.8h) the rhythm shifts. The periodogram data for eyes covered LD (bottom right) is partial and only covers from 11/26-12/04.

Winter Trial

The animals in the second trial also had the same 24 hour daily rhythm in the control 12:12 LD cycle (average $\tau$=23.45h), with peaks of activity at dawn and dusk (Figures 3&4). Interestingly, once the animals’ eyes were covered there was a switch to a diurnal rhythm, also with an increased level of activity (Figures 3&4). Despite the major switch in activity pattern, the average periodicity continued to measure ~24 hours (average $\tau$=23.85h), showing that a daily rhythm was still present and synchronized to the LD cycle. The lobsters expressed some individuality in their behaviors after their eyes were covered. As a result, only two out of the
three actograms were used in the experimental results, because subject 1 had continuous activity once the eyes were covered that did not cease or follow any pattern, and would not contribute to the experiment. When the lobsters were subjected to 24 hour darkness while their eyes were still covered there was still an apparent rhythm which slightly shifted and had an average periodicity of 26.88 hours. In order to explain the diurnal pattern, the temperature and light intensity data from the experimental cold room was examined using HOBOware software and found that the average temperature from 01/07-01/22 was 16.84 degrees Celsius. This could help explain the switch to diurnal activity.

![Figure 3. Actogram and Lomb-scargle periodograms of the first juvenile lobster’s activity from the January trial data.](image)

The actogram (left) depicts the control LD cycle from 01/04-01/09, showing a crepuscular rhythm with peaks of activity at dawn and dusk, each followed by a gradual decline in movement. After the lobsters’ eyes were covered on 01/09, there was a complete switch to a diurnal daily rhythm with increased activity, but a similar periodicity was maintained. The DD cycle starting on 01/18 shows a slight shift in the periodicity. The periodograms (right) show the $\tau$ values in control LD (24h), eyes covered LD (23.8h), and eyes covered DD (28.85h) cycles.
Figure 4. Actogram and Lomb-scargle periodograms of the second juvenile lobster’s activity from the January trial data. The actogram (left) depicts the control LD cycle from 01/04-01/09, showing a crepuscular rhythm with peaks of activity at dawn and dusk. After the lobsters’ eyes were covered on 01/09, there was a complete switch to a diurnal daily rhythm with increased activity, but a similar periodicity was maintained. The DD cycle starting on 01/18 shows a slight shift in the periodicity and decrease in activity. The periodograms (right) show the $\tau$ values in control LD (22.9h), eyes covered LD (23.9h), and eyes covered DD (24.9h) cycles.

Discussion and Conclusions

Many organisms synchronize their biological clocks to the natural photoperiod to anticipate daily activities such as exploration and locomotion. The American lobster, *H. americanus*, proves to be no exception. The juvenile lobsters in the experiment all demonstrated a daily rhythm that was entrained to the LD cycle, exhibiting the majority of their activity during the night. Upon further analysis, the periodicity was shown to be around 24 hours, proving that the lobsters had indeed synchronized their locomotor activity to the LD cycle. This result is consistent with data from previous research on lobster daily rhythms (Jury *et al.*, 2005; Williams & Dean, 1989).
Eyestalk ablation experiments in crayfish have shown a persistence of locomotor rhythmicity in LD cycles (Page & Larimer, 1972), consistent with the results from the fall trial. Extra-ocular photoreceptors that have the ability to sense the presence of light should continue to entrain the animal to the active day length. Interestingly, the January subjects switched to a diurnal rhythm (more active in the day) once their eyes were covered. A number of variables could have triggered a shift in activity period, including the high temperature in the cold room.

A similar transition to diurnal behavior was demonstrated in eyeless *Bulla gouldiana* and *Aplysia*, marine gastropods which also exhibit a circadian rhythm in locomotor behavior (Block & Davenport, 1982). The researchers hypothesized that the diurnal rhythm was likely due to extra-ocular photoreceptors functioning without the influence of an ocular circadian pacemaker that acts to inhibit locomotor activity directly induced by illumination (Block & Davenport, 1982). This may not relate to the juvenile lobsters, as their eyes were only covered and not completely removed, but it could suggest that visual input from the eyes is required for the ocular pacemaker to work, and essential for mediating light onset activity. Also, if CRY is not super-sensitive to light, as demonstrated in eyeless *Drosophila* which were not entrainable in dark conditions (Hall, 2000), this might explain the switch to a diurnal pattern.

In the fall trial, the “blinded” lobsters’ daily rhythm began to drift after a period of seven days during the LD cycle, demonstrating an activity pattern and periodicity similar to the eye capped DD conditions from the winter trial. This could suggest that the abdominal photoreceptors are not sufficient for solid continual entrainment. A lack of CRY activation from the eyes could also affect the synchronization of the daily rhythm to the normal LD cycle. Each trial yielded different results in eyes covered LD behavior, and due to the low number of lobsters
and trials in this experiment, it is impossible to come to a conclusion as to which is the probable natural reaction to these conditions.

Some lobsters also showed an apparent circadian rhythm in DD, suggesting the presence of an endogenous circadian clock. In previous research, the 24 hour circadian rhythm continued to persist in DD cycles (Jury et al., 2005), but when the eyes are covered the periodicity seems to elongate slightly and become more arrhythmic. This could demonstrate the importance of the eyes in maintaining the circadian rhythm, even without any light input.

Research has also been done that shows crustaceans can exhibit rhythm damping and time structure changes under constant conditions, but a rhythm should still persist (Aréchiga et al., 1993). In previous laboratory tests in DD conditions on the crayfish, Procambarus clarki, the period shortened to approximately 22 hours, while it lengthened in LL to ~26 hours (Aréchiga et al., 1993), which seems to be the expected results of such trials. The experiment did not achieve these results in DD with eyes covered, possibly because the putative CRY neurons throughout the abdominal ganglia are triggered to reset the clock by the onset of light, as demonstrated in Drosophila (Strauss & Dircksen, 2010). Without any light cues, the nocturnal activity rhythm would continue to persist for a longer period of time, but the biological clock would still allow the organism to maintain a circadian rhythm.

In this experiment, the juvenile lobsters were shown to produce both daily and circadian rhythms, as adult American lobsters do. Animals in both trials expressed a clear daily rhythm in LD activity after their eyes were covered, which supports the hypothesis that they have extra-ocular photoreceptors which aid in entraining daily locomotor activity rhythms. In the future to continue the experiment, the trials should be repeated in order to have more accurate
and reliable data and we will work to find the precise location of the putative CRY photoreceptors throughout the abdominal ganglia.
References


**Acknowledgements**

I would like to thank Win Watson for the use of his laboratory and equipment, Scott Finnance for help with data collection and troubleshooting, Kyle Harris for instruction in the use of the computer programs and graphical analysis, as well as Cody White and Colin Lee for help with experimental setup and continual support. I would also like to thank NH INBRE for helping to fund this project.