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Circadian Rhythms in Locomotor Activity of Juvenile Horseshoe Crabs

Douglas Borst[1] and Robert Barlow[2] (Marine Biological Laboratory, Woods Hole, Massachusetts 02543)

The American horseshoe crab, *Limulus polyphemus,* is a classic model for vision research. Not only are the lateral eyes of *Limulus* relatively simple, but they share many processes with the eyes of more advanced animals, including humans (1). The eyes of *Limulus* also exhibit a remarkable circadian rhythm: they become nearly 1,000,000-fold more sensitive at night, accommodating for the roughly 1,000,000-fold decrease in ambient light (2). Is visual sensitivity the only process controlled by a circadian oscillator in *Limulus*? Other marine animals have been shown to possess circadian as well as circatidal rhythms in locomotor activity (3). Does *Limulus* exhibit a rhythm in locomotor activity? Past attempts in this laboratory to determine the locomotor activity cycle of adult horseshoe crabs were largely unsuccessful, with only one of more than 100 crabs tested exhibiting circadian activity in darkness. Here we report a study to determine the locomotor activity rhythm of juvenile crabs. We found that most juveniles were nocturnally active under natural cyclic lighting, and that some maintain rhythmic activity in constant darkness.

We assessed the locomotor activity of 10 juveniles maintained in isolation. The animals were collected in the environs of Woods Hole, Massachusetts, and were placed in a 39 × 49-cm tank (water depth: 8 cm) filled with about 2 cm of sand. The tank was placed in a lightproof box with a porthole pressed directly against a laboratory window to provide natural light. The tank was continuously illuminated with infrared light sources (Sony: Model HVL-IRC) so that we could observe the activities of the animals day and night with a video camera (SeaView Video Technology, Inc.) sensitive to infrared light. Using Snappy Video Snapshot (Play, Inc.) hardware and SnapRecorder (SB Software) software, we took one picture per minute and measured activity with the following algorithm: if an animal moved onto an imaginary line drawn across the tank, it scored a point; if the animal turned approximately 120° or moved along the line at least one-half body length, it scored a point; if an animal remained in the same place on the line, crossed it without being caught on camera, or moved less than one-half body length along the line, no points were awarded. When collected in 30-min bins, activity scores ranged from 0 to 36. We considered scores of 9 or more to represent significant locomotor activity. When kept in constant darkness, the group was cyclically active with 77% of their daily activity occurring during their subjective night (data not shown). The period of the endogenous rhythm was about 23.5 h, which is less than 24 h, indicative of a circadian rather than a circatidal clock.

Did the circadian oscillators of all 10 crabs have the same period? Frame-by-frame inspection indicated that after a prolonged period of inactivity, one crab would become active first and was soon followed by the rest. We could not differentiate between crabs, so we do not know if an individual crab possessed the short period of 23.5 h and thus was an "early riser" who "woke up" all the rest. To answer this question we monitored the locomotor activity of five crabs separated into individual small plastic pens 15 cm in diameter. All five were predominantly active at night under cyclic lighting, but only two exhibited clear rhythmic activity in constant darkness.

Figure 1 plots the locomotor activity of an individual crab during 4 days in diurnal lighting and 9 days in constant darkness. The crab's activity was observed for 1-h periods and analyzed with a different algorithm: active 0–6 min, score = 0; active 6–49 min, score = 1; active 50–69 min, score = 2. Figure 1 shows activity with a score of 2. Under diurnal lighting, the crab was nocturnally active; 91% of its activity occurred during the night. When placed in constant darkness, the crab remained cyclically active; 72% of its activity occurred during its subjective night. The endogenous rhythm appears to be drifting with a period of less than 24 h, but no firm conclusions can be drawn about the length of the period because we analyzed activity in 1-h bins. We conclude that circadian oscillators in *Limulus* modulate both visual sensitivity and locomotor activity. It is not known

[1] Reed College, Portland, Oregon.
[2] SUNY Upstate Medical University, Syracuse, New York.

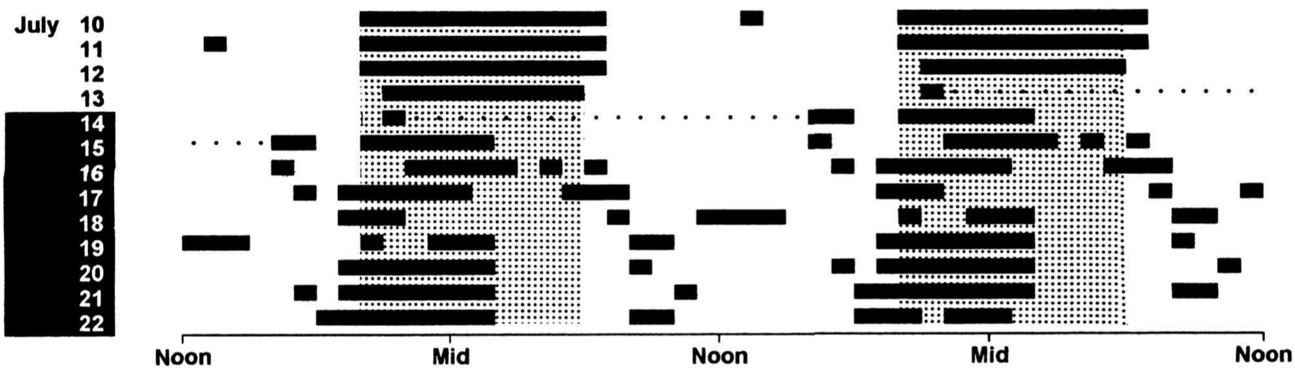

Figure 1. *Locomotor activity of a juvenile horseshoe crab maintained first in natural cyclic lighting and then in constant darkness. The data are double-plotted to emphasize periodic events. Black bars indicate periods of activity; large dots indicate no data collection. Stippled regions indicate the solar night (sunset to sunrise). The juvenile was exposed to cyclic lighting from July 10 to July 14, when the porthole was closed to produce constant darkness until July 22.*

whether these processes are modulated by one or more circadian oscillators.

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