

The effect of photoperiod and tidal cycles on the activity of juvenile Horseshoe crabs, *Limulus polyphemus*.

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Introduction

Adult American horseshoe crabs, *Limulus polyphemus*, migrate into the intertidal zone along the eastern coast of North America in the late spring-early summer in order to breed during high tides (Rudloe, 1979; Brockmann, 2003). These tidal patterns of activity have been recently shown to be endogenous and to persist at other times of year as well (Chabot *et al.*, 2004; 2007). Juveniles of this species also inhabit the intertidal zone and might be expected to exhibit circatidal patterns of activity and yet published reports suggest that they are not tidally organized but are primarily nocturnal (Casterlin and Reynolds, 1978; Borst and Barlow, 2002). Furthermore, this daily pattern of activity has been reported to persist in constant conditions and thus be controlled by a circadian clock (Borst and Barlow, 2002).

However, juveniles are known to dwell within intertidal zones for most of their lives and are thus exposed to selective pressures to synchronize with these tidal cycles. Therefore we hypothesized that juveniles would exhibit circatidal behavioral patterns much like adults. We examined the patterns of activity of individual juvenile *L. polyphemus* under both normal cyclical conditions and constant dark conditions. Our results suggest for the first time the presence of an endogenous circatidal oscillator that drives locomotor activity in juvenile horseshoe crabs.



Figure 1: Experimental Setup. Juvenile *Limulus* cannot be easily visualized in this photo because they blend in with the substrate.

Materials and Methods

Animals and Experimental Conditions:

Six juvenile *Limulus polyphemus* (5-7 cm carapace width) were collected from Pleasant Bay, MA in July from tidal flats. The animals were placed into individual 2.5 gallon tanks that were kept at a constant temperature of 22° C. The tanks were filled with approximately 1.25 gallons of estuarine water from Great Bay, NH, maintained at 24-25 psu throughout the experiment and the water was constantly aerated. The tanks had 4.0cm of small cobble (<1.5cm size) collected from Great Bay. Light:Dark cycles (LD 14:10) were created using two broad-spectrum, 20 watt, fluorescent bulbs hooked up to electric timers. Light intensity during L- 200 lux; during D - 0 lux. When they were not in experimental conditions, they were fed thawed frozen brine shrimp 2*/week.

Experimental Procedures:

LD/DD Experiment

Animals were monitored using an infrared camera and two fixed infrared lights and recorded on a time lapse video recorder. The animals were not fed throughout the duration of the experiments (20 days).

Water Pressure Experiment

This experiment took place in January, 5 months after being collected in the field. Animals were placed in a large tank that was partitioned using egg crate ceiling light grating into small activity monitoring “squares” (15 cm X 25 cm; Chabot *et al.*, 2008). There was no cobble available in this experiment. A “ceiling” (5 cm high) was used to prevent the animals from flipping over and becoming stuck. A brick was placed on the ceiling to hold it in place and create a shielded, darker, area over approximately half of each activity chamber. Button magnets (1 cm diameter) were attached to the dorsal carapace of each animal using duct tape and cyanoacrylate glue. This made their movements detectable when they moved near a reed switch that was located on the side of the square not covered by bricks.

Data Analysis:

LD/DD Experiment: Movement was scored from the videotapes using a scale from 0-3 (0- no movement; 3- several transits across tank) and stored in 5 minute bins. Water pressure locomotor activity was automatically collected and stored on computer. All data were then stored and analyzed by the ClockLab Data Analysis suite of programs. Lomb-Scargle periodograms were used to analyze significance of periodicities ($p < 0.01$).

Rhythms in LD and DD

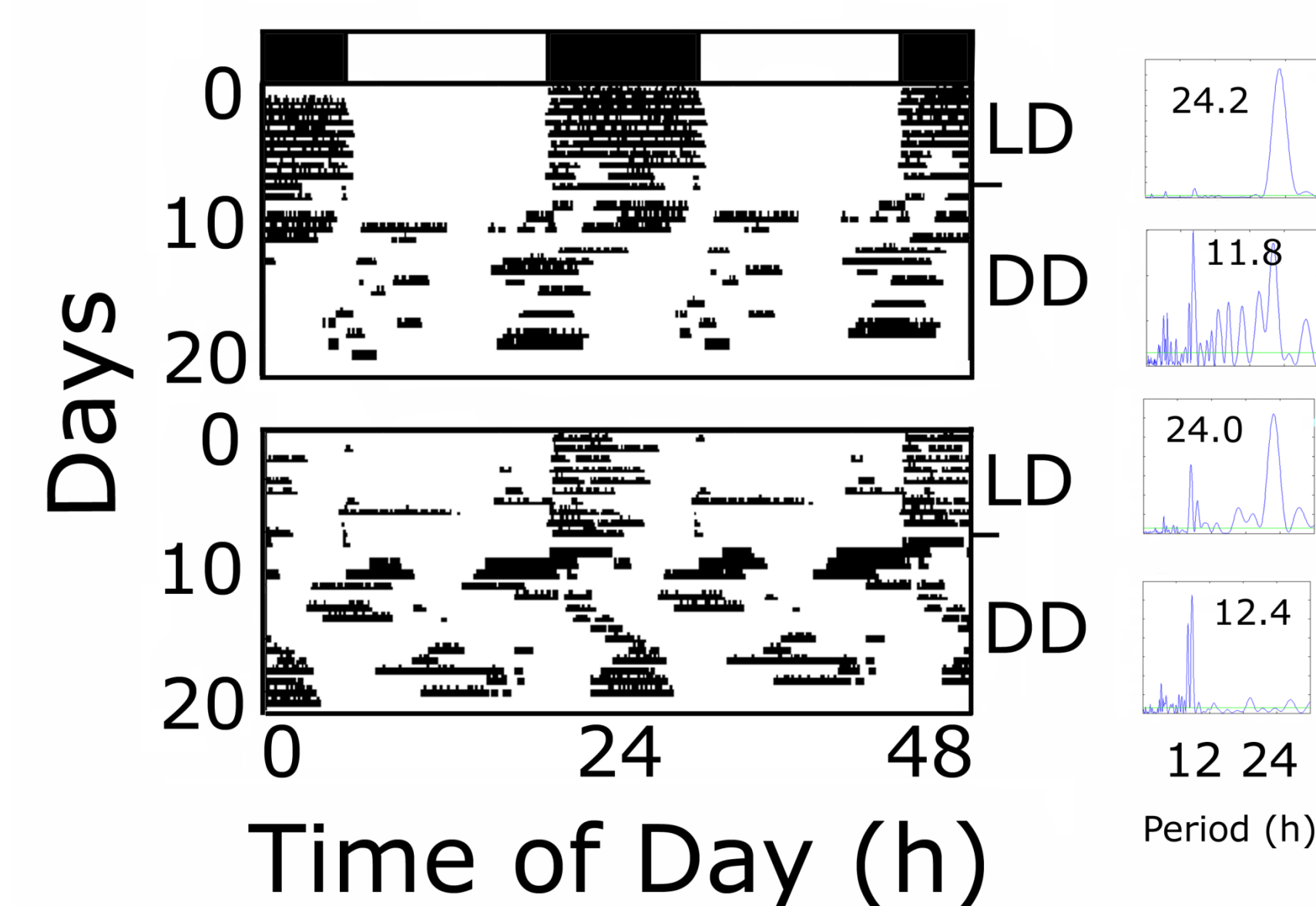


Figure 2: The effects of photoperiod on locomotor activity in two juvenile *L. polyphemus*. Left panels - actograms are double-plotted to facilitate visual inspection. The 14:10 light:dark cycle (LD) is indicated by black/white bars at the top. Right panels - Lomb-Scargle periodogram analyses of respective actogram sections; vertical scale - Relative Q(p). Largest peak value above horizontal line of significance in the circatidal or circadian range ($P < 0.01$) indicated by numerical value.

In LD conditions all animals displayed significant daily rhythms ($\tau = 24.22 \pm 0.1$ h) with most activity taking place during Dark. While these results might indicate the presence of a circadian clock, when exposed to constant-dark conditions four out of six exhibited significant circatidal rhythms ($\tau = 12.2 \pm 0.1$ h). This is the first time that circatidal rhythms have been reported in juvenile *Limulus polyphemus*.

Effects of “Tides”

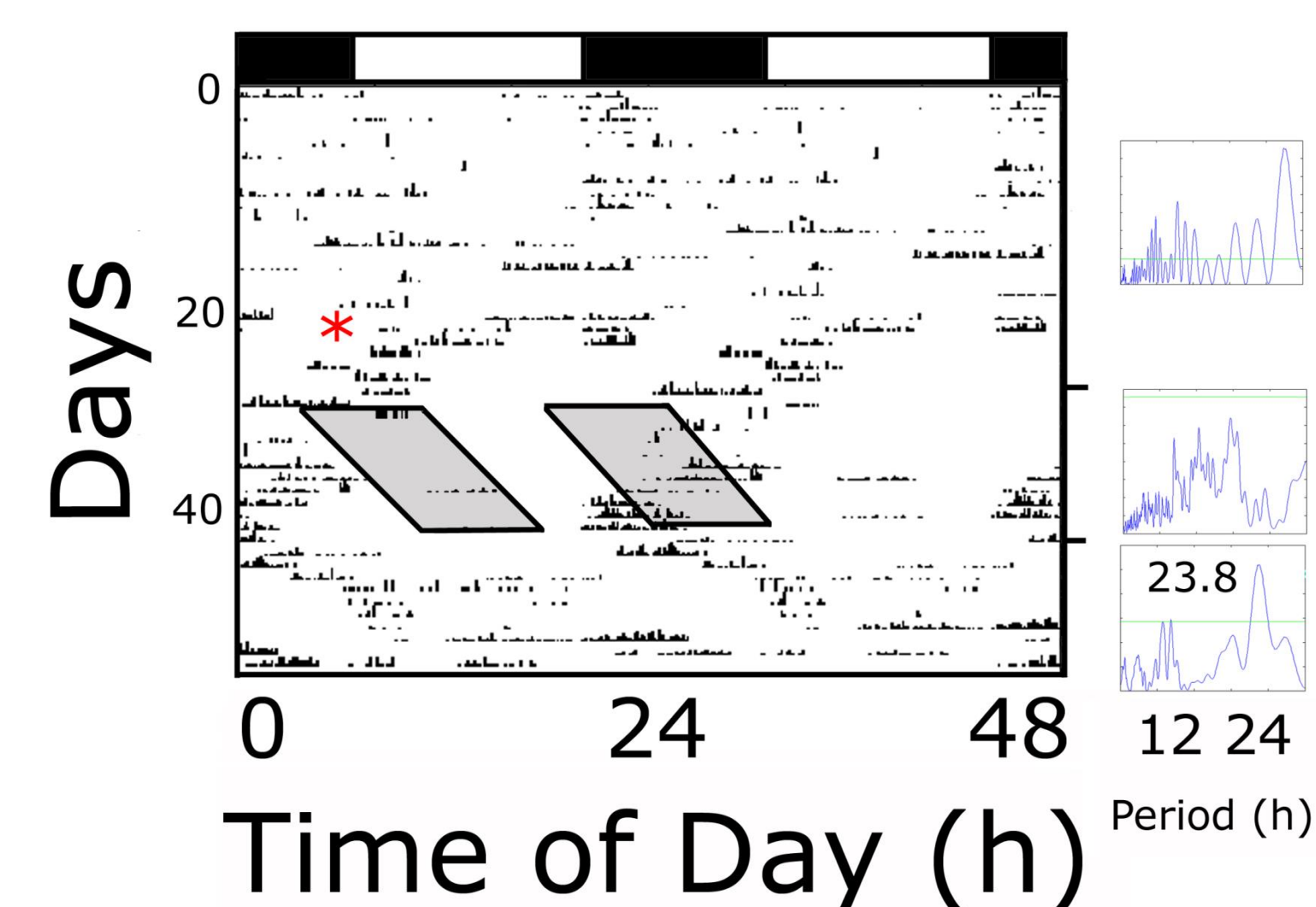


Figure 3: The effects of water pressure changes on locomotor activity in juvenile *Limulus*. Artificial tides (changes in water depth) were delivered at a period of 12.4 h as indicated by shaded boxes on the actogram (high water occurred approximately 1 hr into the time period). * - Crabs received one aberrant water pressure change beginning at this time.

Water pressure changes appeared to increase the clarity of the activity rhythms in this animal. This effect was seen in one other animal (data not shown). While there was some evidence of synchronization in this animal, it was not strong and none of the other animals exhibited similar effects. This lack of apparent entrainment, not seen in adults, may have been primarily due to the extended period of time in captivity (>150 days).

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Conclusions

- Juvenile horseshoe crabs exhibit endogenous circatidal rhythms
- The “nocturnality” previously cited by other researchers appears to be masking
- Water pressure changes may be able to synchronize these rhythms

Literature Cited

- Borst, D. and R. B. Barlow. 2002. Circadian rhythms in locomotor activity of juvenile horseshoe crabs. *Biol. Bull.* **203**: 227-228.
- Brockmann, H. J. 2003. Nesting behavior: a shoreline phenomenon. Pp. 33-49 in *The American Horseshoe Crab*. C. N. Shuster, R. B. Barlow, and H. J. Brockmann, eds. Harvard Univ. Press, Cambridge, MA.
- Casterlin, M.E. and W.W. Reynolds. 1979. Diel locomotor activity patterns of juvenile horseshoe crabs, *Limulus polyphemus*. *Rev. Can. Biol.* **38**:43-44.
- Chabot, C. C., J. Kent, and W. H. Watson. 2004. Daily, circadian and tidal rhythms of locomotor activity in the horseshoe crab *Limulus polyphemus*. *Biol. Bull.* **207**: 72-75.
- Chabot, C. C., S. H. Betournay, N. Braley, and W. H. Watson. 2007. Circadian and circatidal rhythms of locomotion in the horseshoe crab, *Limulus polyphemus*. *J. Exp. Mar. Biol. Ecol.* **345**: 79-89.
- Rudloe, A. 1979. Locomotor and light responses of larvae of the horseshoe crab, *Limulus polyphemus* (L.). *Biol. Bull.* **157**: 494-505.

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