

The animals behaved about the same toward each of the three objects in the ocean and in the laboratory (Fig. 1). In both settings, most animals hit the clear object and turned away from the black and gray objects. The juveniles appear to be avoiding objects with high visual contrast; the higher the contrast, the greater the avoidance. Because juveniles avoided black objects more than the lower contrast gray and clear ones, we conclude that these are visually guided behaviors. In short, juveniles exhibit functional vision.

Averaging the distances of turn-away events and pass-by events reveals the differential effects of contrast on the crab's behavior. For example, crabs in the ocean turned away from the black and gray objects at average distances of 16.2 ± 6.4 (SD) cm and 14.9 ± 6.5 cm, respectively, indicating that the black object can be seen at a greater distance; that is, it is more visible. Likewise, the average distance of closest approach in pass-by events was 15.0 ± 5.7 cm and 11.8 ± 4.1 cm for the black and gray objects respectively, indicating that juveniles will pass closer to the lower contrast gray object without responding to it.

The width of the eyes (~ 4.0 mm) of the juveniles we tested (prosomal width 4.6 cm) was about 25% of that of an adult eye (15–20 mm), and juvenile eyes have fewer and smaller ommatidia (7). Even though the visual system of juvenile crabs is not fully developed, we show here that they have functional vision. In particular, juvenile crabs have contrast-sensitive vision, as do adult crabs. Adult females avoid objects of high contrast, whereas adult males are attracted to them; thus juveniles behave like adult females. As the animal grows, the eyes mature in parallel with

growth of the brain and organization of the brain's map of the visual world. Because *Limulus* juveniles can see and distinguish contrast, they may serve as a useful alternative model for understanding not only how the brain processes visual information, but also how behavior changes during the developmental process.

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Effects of Individual Size on Pairing in Horseshoe Crabs

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During late spring and early summer, spawning horseshoe crabs (*Limulus polyphemus*) can be readily observed along the beaches of Cape Cod, Massachusetts. Males use specially modified claws to clasp onto the carapace of females (1). This pairing is called amplexus. Amplexed pairs attract unattached males that cluster around them during spawning. These satellite males are frequently successful in fertilizing a portion of the female's eggs (2). What determines which males amplex with females, while others do not, has not been clearly defined. Numerous studies have investigated factors thought to influence pairing in *L. polyphemus*, such as visual (3, 4) and chemical cues (5, 6) and body size. Studies regarding how body size may affect pairing have generated conflicting results—some research suggests size-based pairing is random (7, 8), while other work has found a weak relationship between body size and mate selection (2, 9).

In this study we examined the relationship between male and female body size and its influence on mate selection for amplexus in *L. polyphemus* in field and laboratory studies. To describe the

reproductive categories within a horseshoe crab population during the spawning season, we carried out field surveys within a known spawning area along the eastern shore of Pleasant Bay, Cape Cod. Field surveys were conducted 15 May–15 June in 2001 and 25 June–25 July in 2002. The 2001 survey spanned the peak of the spawning season; the 2002 survey spanned only the latter part of the spawning season. We collected data regarding sex, spawning state, and size (prosomal width) from 56 pairs along 125 m of beach in 2001, 84 pairs throughout the bay in 2002, and an additional 761 crabs, including single males and females and satellite males.

To determine whether pairing was mediated by horseshoe crab size, we first compared sizes of paired crabs in the field; and second, we collected animals from the field for laboratory studies on mate selection. Experimental studies were conducted in 140 cm \times 90 cm \times 40 cm recirculating aquaria, containing 4 cm of native beach sand. Trials were performed by pairing a single male (155–215 mm prosomal width) with 2, 3, 4, or 7 females (190–282 mm). Each male was used in three trials, and trials were allowed to run for 12 h or until amplexus was achieved. We examined the effect of number of individuals in these trials and found that density did not affect success of pairing. Similarly, there was no

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difference between the trials in which males were used more than once and those in which males were used once. Hence, all data were pooled in Fig. 1C.

The majority of animals surveyed in intertidal areas during the spawning season were single males (79%), followed by amplexed pairs (18%), single females (2%), and satellite males (1%) (Fig. 1A). Most males were unpaired and most females were paired. These results are consistent with observations that unpaired males tend to gather on the beach, while females tend to remain offshore unless approaching the beach to spawn (4, 9, 10). Adult females are larger than adult males, which is characteristic of the species (1). In addition, the mean prosomal width of paired females was larger than that of paired males, suggesting that males may tend to pair with females having a larger prosomal width than their own (Fig. 1A).

To ascertain whether there was size-based mate selection in amplexed pairs in the field, we compared the prosomal widths of paired males and females (Fig. 1B) measured in the field surveys done in the two years. There was no significant difference in size of paired males and females sampled in 2001 and 2002. This finding implies that there were also no differences in pairs observed during the peak of spawning compared to those sampled post-peak (Males: $t = 1.17$, $P = 0.24$; Females: $t = -0.67$, $P = 0.50$). Hence, data were combined in Fig. 1B. The aggregated data indicated no significant size-based selective mating across the full range of sizes observed ($R^2 = 0.01$, $df = 83$, $P > 0.05$). Pairings did not, however, occur between the smallest [Fig. 1A, B (area to the lower left of —)] or largest [Fig. 1A, B (area to the lower right of ·····)] males and females in the general field population. These results suggest that some of the smallest animals may not have reached sexual maturity and that some size selectivity may occur among larger males. In addition, size reversals among pairs (females having a smaller prosomal width than their male partner) were rarely observed (2%).

To further test the effect of size on mate selection, we compared the size of males to the size of the females chosen for amplexus during laboratory trials (Fig. 1C). In 35 trials, small males (154–170 mm) tended to select the smallest females offered (regardless of the females' absolute size). Intermediate-sized males (171–190 mm) tended to prefer the smallest or intermediate-sized females, and the only large male (215 mm) to accomplish amplexus chose the largest female offered. Males did not amplex with the first female they encountered and took at a minimum 35 min to pair. Overall, small and medium-sized males were more successful in achieving amplexus than large males (Fig. 1C). These results suggest that some size-based mate selection may occur at the largest and smallest sizes only, as observed in the field, and that male size may also affect success of amplexus.

To further examine whether male size affects the likelihood of amplexus in the field, we compared the success of males of different sizes tested in the laboratory to those observed in the field. The percent of amplexus as a function of size was similar for both field and laboratory animals (Fig. 1D), where smaller male crabs (up to 180 mm) were generally more successful than larger crabs.

In sum, for the majority of male and female prosomal widths, size did not appear to affect mate selection. At the largest sizes,

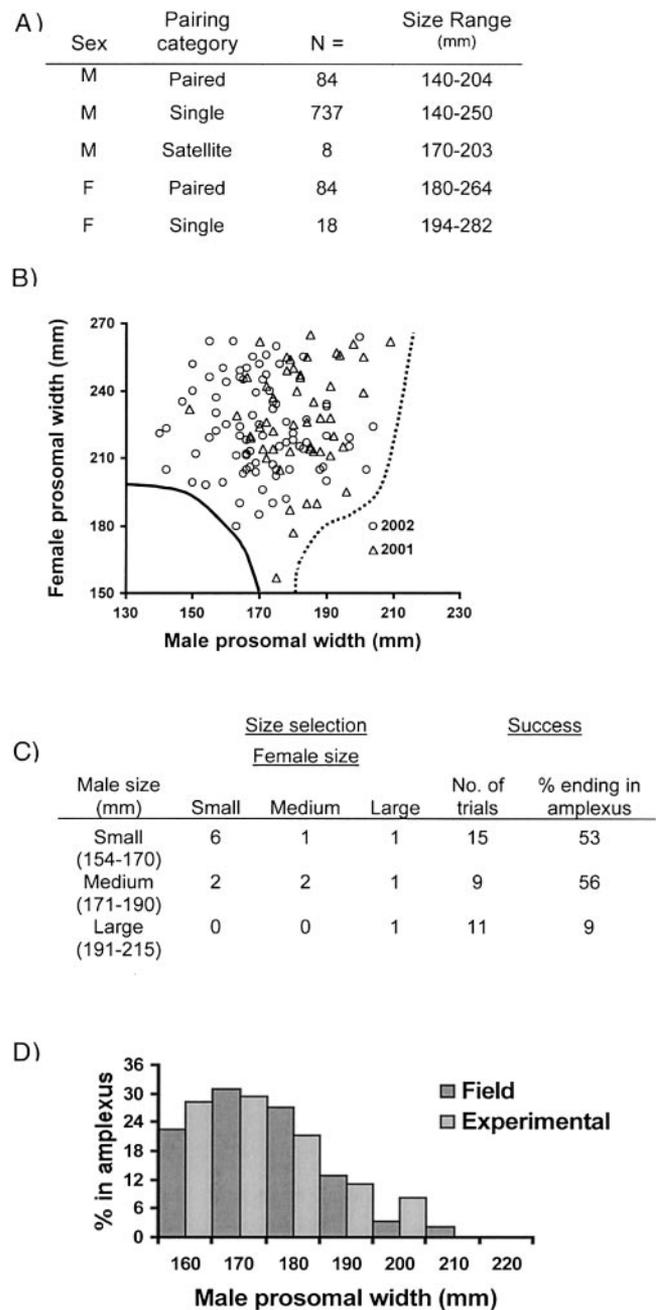


Figure 1. (A) Sex ($M =$ male, $F =$ female), pairing category, number, and size range of sexually-mature horseshoe crabs observed during the spawning season in Pleasant Bay. (B) Prosomal widths of male and female crabs in amplexus measured in two years. Solid line indicates approximate size boundary below which most crabs are not sexually mature. Dashed line indicates upper size boundary of paired males. (C) Number of females of three sizes selected by males of three sizes for amplexus in laboratory trials, number of trials, and success rate of amplexus for the three sizes of males. (D) Size distribution of males in amplexus among experimental and field observations.

however, males tended to prefer larger-sized females and were generally less successful in achieving amplexus. Hence, our results are consistent with previous reports that selection is not size-based

(7, 8) and also with reports that some size-based selection may occur (2, 9). Size selectivity and apparent random pairing can both occur, but do so within different size ranges.

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Circadian Rhythms in Locomotor Activity of Juvenile Horseshoe Crabs

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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

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