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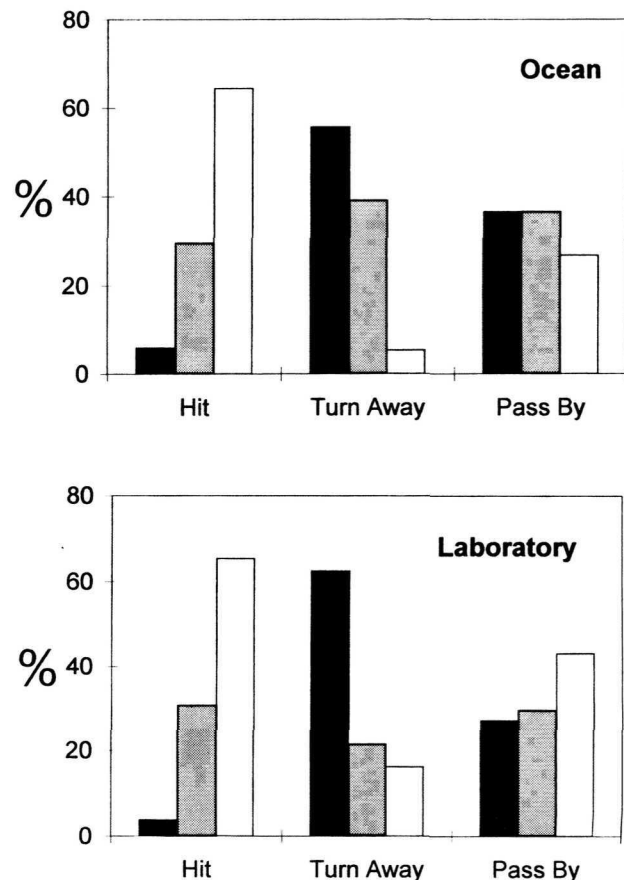
**Visual Behavior of Juvenile *Limulus* in Their Natural Habitat and in Captivity**  
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*Limulus polyphemus*, the common horseshoe crab, inhabits coastal estuaries of North America and the Yucatan peninsula. It has been an admirable model for vision research because its relatively simple visual system has marked functional similarities to those of many species, including humans (1). Adult male horseshoe crabs use their lateral eyes to find mates (2), and their ability to see underwater has been studied in the estuaries where mating occurs (3, 4, 5). However, studies of the visually guided behavior of adults in captivity have not been successful (R.B., pers. obs.). On the other hand, juvenile horseshoe crabs do exhibit visually guided behavior under laboratory conditions (6). Here we report a comprehensive investigation into the visually guided behavior of juveniles, both in their natural habitat and in captivity.

Between May 15 and June 30, 2002, we studied the behavior of juvenile specimens of *Limulus* in Nantucket Sound, along the Western shore of the North Monomoy Island Refuge in Chatham, Massachusetts. The sand bars on the tidal flats of the island are a protected natural habitat for both juveniles and adults; the waters are relatively clear and calm. Juveniles were most active near shore at depths ranging from 30–120 cm, usually during the late afternoon and evening. In testing the visually guided behavior of a juvenile horseshoe crab, we followed the procedure of a previous study of such behavior in adults (4). First we determined its direction of travel along the bottom and then placed a small object directly in front of it at a distance of 20–40 cm. We used three cylindrical acrylic plastic objects (diameter 7.6 cm, height 5.2 cm): one was painted matte black, one matte gray, and one remained unpainted. Because these objects were designed for experiments with adults, their size was reduced proportionally for small juveniles (prosomal width range = 2–14 cm). We filmed 713 trials, defined as an approach of an animal to an object, on a Sony camcorder (model DCR-PC110 NTSC; underwater housing by Gates Underwater Products) and then analyzed the behavior with a computer.

All videotaped trials were analyzed with an eMac computer using iMovie software (copyright 2001–2002 Apple Corporation). We categorized each trial as representing one of three behaviors: “hit,” an animal moves directly toward an object and makes direct contact with it; “pass-by,” an animal passes by an object without changing direction; and “turn-away,” an animal turns away from an object. For turn-away behavior, we measured the distance from the center of the object to the middle of the animal at the point where it turned. For the pass-by behavior, we measured the distance of closest approach to the object. Figure 1 (top) shows percentages for all three behaviors.

We performed a similar set of experiments in the laboratory, where we observed the behavior of 10 juveniles in a large trough (130 × 62 cm; the water 6.5 cm deep). The bottom of the trough was covered with a thin fiberglass mesh screen, giving the crabs traction for walking and righting themselves if overturned. The crabs were maintained in ambient diurnal lighting in a separate trough before being placed in the experimental trough, where we used diffuse overhead illumination to simulate, as closely as possible, an overcast day. The same three objects used in the ocean observations were now placed in the center of the trough and rotated every 15 min. The crab’s behavior was filmed and analyzed, as in the ocean trials. We defined a behavioral event as a crab entering the camera’s field of view (51 × 74 cm). We filmed 797 trials, mostly in the evening hours when the crabs were most active. Figure 1 (bottom) gives percentages for the laboratory trials.



**Figure 1.** Percentages of behavioral responses to black, gray, and clear objects in the ocean and in the laboratory.

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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  $16.2 \pm 6.4$  (SD) cm and  $14.9 \pm 6.5$  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  $15.0 \pm 5.7$  cm and  $11.8 \pm 4.1$  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 ( $\sim 4.0$  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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### Literature Cited

1. Ratliff, F. 1974. *Studies on Excitation and Inhibition in the Retina*. The Rockefeller University Press, New York.
2. Barlow, R. B., L. C. Ireland, and L. Kass. 1982. *Nature* 296: 65–66.
3. Powers, M. K., R. B. Barlow, and L. Kass. 1991. *Visual Neurosci.* 7: 179–189.
4. Herzog, E. D., M. K. Powers, and R. B. Barlow. 1996. *Visual Neurosci.* 13: 31–41.
5. Krutky, M. A., J. Atherton, S. Smith, F. Dodge, and R. Barlow. 2000. *Biol. Bull.* 199: 178–180.
6. Errigo, M., C. McGuiness, S. Meadors, B. Mittman, F. Dodge, and R. Barlow. 2001. *Biol. Bull.* 201: 271–272.
7. Smith, K., C. Ridings, F. Dodge, and R. Barlow. 2002. *Biol. Bull.* 203: 222–223.