

LIMULUS POLYPHEMUS: A REVIEW OF THE ECOLOGICALLY SIGNIFICANT LITERATURE

ANNE RUDLOE

Department of Biological Science
Florida State University
Tallahassee, Florida 32306

Despite its abundance, phylogenetic importance and significance as a major neuro-physiological preparation, remarkably little has been known of the ecology and behavior of the horseshoe crab, Limulus polyphemus.

The life cycle in essence is as follows: sexually mature adults deposit eggs in the sand of a low energy beach through the spring and summer at the high water mark on a spring high tide. The eggs hatch in approximately 5 weeks in the northern Gulf of Mexico, producing trilobite larvae. The larvae remain in the sand for several weeks and are released into the water column on an appropriate high tide. They then molt through a series of juvenile instars, morphologically similar to adults, and are found on the intertidal sand flat adjacent to the breeding beach. As they grow, they move lower in the intertidal, becoming subtidal by the time they mature. Adults are found to depths of approximately 30 meters, but major concentrations are found in depths of about 5.6 meters on sand bottom. They remain offshore for the duration of the life cycle, returning to the beach only to breed. The species ranges from Maine to Yucatan on the Atlantic and Gulf Coasts of North America.

The most comprehensive work to date on the general biology of Limulus polyphemus is that of Shuster (1950, 1952, 1953, 1954 a,b) who provides extensive information on the external anatomy and morphology, comparative serology and natural history of Limulus. His morphological and serological work (1962) sheds light on the relationships

of the genera of horseshoe crabs.

The horseshoe crab, is an especially good model for studying ecologically dictated modifications of behavior. It moves during the course of the life cycle from the high supralittoral through the intertidal and finally into the subtidal environment. The demands imposed by each environment change, requiring adoptive changes in biological responses, most particularly in behavior. All phases are accessible to field as well as laboratory observations.

This species is also valuable as a subject for ecological study for a variety of additional factors.

Due to Limulus' position as a "living fossil", eventual comparisons with ecologically similar crustacea could conceivably reveal something of the course of behavioral evolution, both in terms of more "primitive" and more "advanced" means of accomplishing common behavioral objectives, and in terms of alternate sensory capabilities. Limulus polyphemus is one of the few surviving species of the Xiphosura, the sword tails, the horseshoe crabs. A widespread and diverse group in the Paleozoic, today only 4 species in 3 genera survive. In addition to Limulus, which ranges from Maine to Yucatan on the North American coast, Tachypleus tridentatus and T. gigas range from Japan through Southeast Asia. The 4th species, Carcinoscorpius rotundicaudata is a freshwater form from Malaysia. The majority of the species in the fossil record occurred in brackish to freshwater environments. Forms nearly identical to Limulus date from the Triassic some 230 million years ago, and very similar horseshoe crabs appear in the Devonian, some 400 million years ago.

The presently accepted taxonomic organization of the Xiphosuridae was established by Pocock (1902) who recognized five species in three genera. The phylogenetic history and fossil taxonomy of the Xiphosuran line is presented by Stormer (1952) who traces a continuous line of development from the Silurian, some 425 million years ago. More recent paleontological work on this group has included publications by Fisher (1975, 1977) on the biomechanics and locomotion of Limulus and Mesolimulus as well as work by Eldredge (1970) on burrowing behavior.

The lateral compound eye of the horseshoe crab is "one of the best understood examples of sensory receptor function" (Adolph, 1971) at the electrophysiological level, as a result of almost 40 years of research using Limulus preparations.

There is a vast literature concerning the neurophysiology of the Limulus compound eye. Hartline (1927) first introduced the preparation, which has since received an enormous amount of attention (Wolbarsht & Yeandle, 1967) from physiologists. The advantages it offers include the size and constancy of the optic nerve fibers and electrical responses that parallel data from more complex eyes. Recently Barlow et al (1977) have reported a circadian rhythm in optic fiber responses that parallels rhythms observed in intact animals. And yet the visual system is not at all well known in terms of its relevance to the intact animal.

Nor have the reams of work on the visual neurophysiology and biochemistry of Limulus been paralleled by an equivalent quantity of research concerning the physiology, behavior, or ecology of the horseshoe crab.

Scattered observations on various aspects of the natural history of Limulus have appeared occasionally. In recent years these have included reports of hermaphroditism, estimates of the life span, descriptions of burrowing and swimming movements, and tagging studies employing Petersen tags (Baptist, 1953; Ropes, 1961; Eldredge, 1970; Vosatka, 1970; Baptist, et. al., 1957).

In addition to these older publications, several physiological studies have appeared in the last few years. Crabtree and Page (1974) and Thompson and Page (1975) have described external oxygen receptors on the book gills. Studies of osmoregulation have included McMannus (1969), Robertson (1970) and Hannan and Evans (1973), while respiratory physiology has been examined by Hammen and Lum (1964), Johansen and Peterson (1975), and Magnum (1976). Eagles (1973) has identified the lateral spines as mechanoreceptors. Frankel (1960) has described upper temperature limits, while Bursey (1977) has described the histological response of Limulus to injury. Brown and Kanouse (1973) provide a study of fertilization of Limulus eggs, and Hummun (1976) describes the interaction of beach neurofauna

with developing Limulus eggs. Larval molting physiology has been studied by Jelga (1972) and Jelga, Costlow and Alspaugh (1972). Selander et. al. (1970) have examined Limulus for genetic variation.

The limited work on learning in invertebrates has included some research on Limulus, (Lahue, 1973), as studies of conditioned and unconditioned reflexes have appeared (Smith and Baker, 1960; Wasserman and Patton, 1970; Wasserman, 1973; Wasserman, in press).

Research on the sensory capabilities of Limulus has been substantial using neurophysiological approaches. Work by Waterman (1950; 1954a, b) has demonstrated the sensitivity of the lateral eye to polarized light. Wald and Krainan (1963) extended this work with electroretinograms of the median eye of young Limulus and found they are sensitive to ultraviolet light. Spectral sensitivities of the lateral, ventral and median eyes were measured (Millecchia and Mauro, 1969a, b; Nolte and Brown, 1969, 1970, 1972), while attempts to measure the animals' ability to discriminate visual patterns have been inconclusive (Campenhausen, 1967). Adolph (1971) recorded optic nerve spikes underwater and found continuous optic nerve activity under dim light in turbid water. Lall and Chapman (1973), working with intact animals in nature, found positive responses to full spectrum sunlight mediated through the lateral eyes and dorsal ocelli. Positive responses to near ultraviolet were shown to be mediated by the dorsal ocellus.

Work on the non-visual sensory systems of Limulus has included descriptions of chemoreceptors associated with respiration, responses to chemical, thermal, tactile and proprioceptive stimuli and descriptions of articular proprioceptors of the walking legs (Barber, 1951, 1956, 1958, 1960; Waterman and Travis, 1953; Pringle, 1956; Barber and Segel, 1960; Hayes and Barber, 1967; Ward, 1969). Eagles (1973) described the anatomy and innervation of the tailspine system relative to the behavioral function of the telson, and Wyse (1971) describes the receptors of Limulus chelae. Most of the literature, however, treats receptor physiology rather than exploring the functional significance of the receptor to intact horseshoe crabs. Nevertheless, these studies establish a broad spectrum of possible modes of reception for guidance stimuli.

The emerging importance of Limulus to biomedical research, as the source of Limulus lysate, requires more complete knowledge of the biology of the species, so that it can be wisely managed as a natural resource. This biochemical preparation from horseshoe crab blood provides a rapid and accurate assay for endotoxemia or gram negative sepsis (Levin et. al., 1970). There has developed in recent years a substantial harvest of horseshoe crabs for this purpose. Such harvesting has most often occurred on breeding beaches. If the resource is not to be depleted, we must have a better knowledge of Limulus populations and growth rates.

Limulus polyphemus, a Paleozoic relic, is behaviorally a relatively simple creature.

Yet it has succeeded evolutionarily. The eggs are protected from most potential predators, buried in the highest intertidal, and nearly all survive to hatching. The trilobite larva is a stage specialized for dispersal with an initial "swimming frenzy" and positive phototaxis that facilitates release from the nest. The juveniles are found in an environment that, due to its physiological rigor at low tide, permits few potential predators or competitors access to them, and their burial after low tide protects them from the pelagic invaders of the rising tide (Rudloe, in press). The adults orient precisely to wave surge and other orientational stimuli as they move to and from the breeding beaches (Rudloe and Herrnkind, 1976). Even as the continents have drifted, the Tethys has given way to the Atlantic and sea levels have fluctuated over hundreds of meters, there has evidently always been - somewhere - a sandy beach, a sandy somewhat estuarine tide flat next to it, and sand bottoms offshore. A horseshoe crab has needed little else. Our current interest in Limulus as a biomedical resource must not endanger the elegantly adapted species and bring its long story to an end.

LITERATURE CITED

- Adolph AR (1971). Recording of optic nerve spikes underwater from freely moving horseshoe crabs. Vision Research 11: 979.
- Baptist J (1953). Record of a hermaphroditic horseshoe crab, Limulus polyphemus. Biol Bull 64:243.

- Baptist J et al (1957). Migrations of the horseshoe crab, Limulus polyphemus, in Plum Island Sound, Mass. US Fish and Wildlife Ser Sp Sci Rep Fish 220.
- Barber S (1951). Contact chemoreception in Limulus. Anat Rec 124:561.
- Barber S (1956). Chemoreception and proprioception in Limulus. J Exp Biol 131:51.
- Barber S (1958). Properties of Limulus articular proprioceptors. Anat Rec 132.
- Barber S (1960). Structure and properties of Limulus articular proprioceptors. J Exp Zool 143:283.
- Barber S, Segel M (1960). Structure of Limulus articular proprioceptors. Anat Rec 137:336.
- Barlow RB, Balanowski SJ, Brachman ML (1977). Efferent optic nerve fibers mediate circadian rhythms in the Limulus eye. Science 197:86.
- Brown GG, Knouse JR (1973). Effects of sperm concentrations, sperm aging, and other variables on fertilization in the horseshoe crab, Limulus polyphemus. Biol Bull 144(3): 462.
- Bursey CR (1977). Histological response to injury in the horseshoe crab, Limulus polyphemus. Am J Zool 55:458.
- Campanhausen, C (1967). The ability of Limulus to see visual patterns. J Exp Biol 46:557.
- Crabtree RL, Page CH (1974). Oxygen-sensitive elements in the book gills of Limulus polyphemus. J Exp Biol 60(3):631.
- Eagles DA (1973). Lateral spine mechanoreceptors in Limulus polyphemus. Comp Biochem Physiol A Comp Physiol 44(2):557.
- Eldredge N (1970). Observations on burrowing behavior in Limulus polyphemus (Chelicerata, Merostomata) with implications on the functional anatomy of trilobites. Am Mus Novitates No. 2436.
- Fisher DC (1975). Swimming & burrowing in Limulus and mesolimulus. Fossils & Strata 4:281.
- Fisher DC (1977). Functional significance of spines in the Pennsylvanian horseshoe crab, Euproops danae. Paleontology 3:175.
- Frankel G (1960). Lethal high temperatures for 3 marine invertebrates, Limulus polyphemus, Littorina littorea & Pasurus longicarpus. Oikos 11:171.
- Hammon CS, Lum SC (1964). Carbon dioxide fixation in marine invertebrates. Quantitative relations (Bdelloura candida, Limulus polyphemus, Heterakis gallinae). Nature Zol (4917)416.

- Hannan, JV, Evans DH (1973). Water permeability in some euryhaline decapods and Limulus polyphemus. Comp Biochem Physiol A Physiol 44:1199.
- Hummun WD et al (1976). Meio-Fauna-macrofauna interactions. I. Sand beach meiofauna affected by maturing Limulus eggs. Chesapeake Sci 17:292.
- Jelga TC (1972). Development and molting physiology of horseshoe crab larvae. Am Zool 12:724.
- Jelga TC, Costlow JD, Alspaugh J (1972). Effects of ecdysones and some synthetic analogs on horseshoe crab larvae. Gen & Comp Endocrin 19:159.
- Johansen K, Peterson JA (1975). Respiratory adaptations in Limulus polyphemus. In Vernberg J (ed): "Physiol Ecol Estuarine Organisms", University of South Carolina Press, Columbia, SC.
- Lahue R (1973). Learning in chelicerata. In Corning WC, Dyal JA, Williams (eds): "Invertebrate Learning", Plenum Press, New York.
- Lall A, Chapman R (1973). Phototaxis in Limulus under natural conditions: evidence for reception of near-ultraviolet light in the median dorsal ocellus. J Exp Biol 58:213.
- Levin J, Poore TE, Zauber NP, Oser RS (1970). Detection of endotoxin in the blood of patients with sepsis due to gram-negative bacteria. New Engl J Med 283:1313.
- Magnum CP (1976). The ionic environment of hemocyanin in Limulus polyphemus. Biol Bull 150:453.
- McMannus AMP (1969). Osmotic relations in the horseshoe crab Limulus polyphemus. Am Mid Nat 81:569.
- Millecchia R, Mauro A (1969). The ventral photoreceptor cells of Limulus. II. The basic photoresponse. J Gen Physiol 54:310.
- Millecchia R, Mauro A (1969). The ventral photoreceptor cells of Limulus. III. A voltage clamp study. J Gen Physiol 54:331.
- Nolte J, Brown J (1969). The spectral sensitivities of single cells in the median ocellus of Limulus. J Gen Physiol 54:636.
- Nolte J, Brown J (1970). The spectral sensitivities of single receptor cells in the lateral, median, and ventral eyes of normal and white eyed Limulus. J Gen Physiol 55:787.
- Nolte J, Brown J (1972). Ultraviolet induced sensitivity to visible light in ultraviolet receptors of Limulus. J Gen Physiol 59:186.

- Pocock R (1902). The taxonomy of recent species of Limulus. Ann Mag Nat Hist 9:256.
- Pringle J (1956). Proprioception in Limulus. J Exp Biol 33:658.
- Robertson JD (1970). Osmotic and ionic regulation in the horseshoe crab Limulus polyphemus (Linnaeus). Biol Bull 141(3):157.
- Ropes J (1961). Longevity of the horseshoe crab Limulus polyphemus. Trans Am Fish Soc 90:79.
- Rudloe AD, Herrnkind WF (1976). Orientation of Limulus polyphemus in the vicinity of breeding beaches. Mar Beh Physiol 4:75.
- Selander RK, Yang SY, Lewontin RC (1970). Genetic variation in the horseshoe crab (Limulus polyphemus), a phylogenetic relic. Evol 24(2): 402.
- Shuster C (1950). Observations on the natural history of the American horseshoe crab, Limulus polyphemus. Woods Hole Ocean Inst Contr 564:18.
- Shuster C (1953). Odyssey of the horseshoe crab. Audubon Mag 55:162.
- Shuster C (1954). A method for recording measurements of certain mollusks, arthropods, and fishes. Prog Fish Cult 16:39.
- Shuster C (1954). On morphometric and serological relationships within the Limulidae, with particular reference to Limulus polyphemus. unpubl PhD dissertation, New York University.
- Shuster C (1962). Serological correspondence among horseshoe crabs. Zoologica 47:1.
- Smith J, Baker H (1960). Conditioning in the horseshoe crab. J of Comp and Physiol 53:279.
- Stormer L (1952). Phylogeny and taxonomy of fossil horseshoe crabs. J Paleont 26:630.
- Thompson C, Page CH (1975). Nervous control of respiration: oxygen-sensitive elements in the prosoma of Limulus polyphemus. J Exp Biol 62:545.
- Vosatka E (1970). Observations on the swimming, righting, and burrowing movements of young horseshoe crabs, Limulus polyphemus. Ohio J of Sci 70:276.
- Wald G, Krainin J (1963). The median eye of Limulus: an ultraviolet receptor. Proc Natl Acad Sci 50:1011.
- Ward D (1969). Leg extension in Limulus. Biol Bull 136:288.
- Wasserman G (1973). Unconditioned response to light in Limulus: mediation by lateral, median, and ventral eye. Loci Vision Research 13:95.

- Wasserman G (in press). Unconditioned response to light in Limulus: role of time of day, day of testing, and intertrial interval. Vision Res.
- Wasserman G, Patton D (1970). Limulus visual threshold obtained from light-elicited unconditioned tail movements. J Comp and Physiol Psych 73:111.
- Waterman T (1950). A light polarization analyzer in the compound eye of Limulus. Science 111:252.
- Waterman T (1954). Relative growth and the compound eye in Xiphosura. J Morphology 95:125.
- Waterman T (1954). Polarized light and angle of stimulus incidence in the compound eye of Limulus. Proc Nat Aca Sci 40:258.
- Waterman T, Travis D (1953). Respiratory reflexes and the flabellum of Limulus. J Cell and Comp Anatomy 41:261.
- Wolbarsht M, Yeandle S (1967). Visual processes in the Limulus eye. A Rev Physiol 29:513.
- Wyse GA (1971). Receptor organization and function in Limulus chelae. Z Vergl Physiol 73:249.