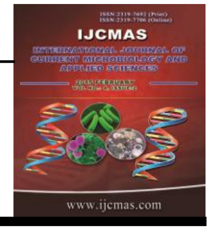

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Review Article

Horseshoe crab and its medicinal values

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A B S T R A C T

The medicinal value of horseshoe crab blood comes from its ability to clot in the presence of bacteria, rendering the bacteria harmless. Even though bacteria are usually destroyed by modern sterilization techniques, some bacteria, called endotoxins, may survive the sterilization process. This blood-clotting ability of the horseshoe crab makes it very valuable in testing for injectable medicines, vaccines and sterile medical equipment. The horseshoe crab has also proved valuable to the medical field in another way. Studies involving the nerve pathways in the eyes of horseshoe crabs have led to many discoveries in human eye research. The outer shell of a horseshoe crab is made primarily of chitin. Scientists discovered that when used as a coating for suture material and burn dressings, chitin rapidly increased wound healing, cutting the time by half.

Introduction

Horseshoe crabs are marine arthropods of the family *Limulidae* and order Xiphosura or Xiphosurida, which live primarily in and around shallow ocean waters on soft sandy or muddy bottoms. They occasionally come onto shore to mate. They are commonly used as bait and fertilizer. In recent years, a decline in the population has occurred as a consequence of coastal habitat destruction in Japan and overharvesting along the east coast of North America. Tetrodotoxin may be present in the row of species inhabiting the waters of Thailand [1]. Because of their origin 450 million years ago, horseshoe crabs are considered living fossils [2].

Even though the horseshoe crab has a hard shell and numerous appendages with claws, it is not really a crab. Horseshoe crabs belong to the phylum, Arthropoda, along with crabs, insects, and other invertebrates with jointed legs, but their closest living relatives are spiders and scorpions. True crabs have two pairs of antennae and a pair of mandibles, or jaws; horseshoe crabs lack these structures. Further, comparing the legs of a true crab with the legs of a horseshoe crab reveals the other significant difference. True crabs classified as decapod crustaceans, have five pairs of legs, which include a pair of claws. Horseshoe crabs have seven pairs of legs under their helmet-like shells; five of these seven pairs of legs are equipped with claws. In adult males, the second pair of claws (pedipalps) has a “boxing-glove” appearance and is used to grasp females during spawning. Horseshoe crabs also have four simple eyes on the top of their carapace instead of two as with the true crab. Our North American species has been named *Limulus Polyphemus* – *Limulus* meaning "a little askew or odd" and *Polyphemus* after the giant Cyclops in Greek mythology.

Classification

Horseshoe crabs resemble crustaceans, but belong to a separate Subphylum, Chelicerata, and are closely related to arachnids. The earliest horseshoe crab fossils are found in strata from the late Ordovician period, roughly 450. The *Limulidae* are the only recent family of the order Xiphosura, and contain all four living species of horseshoe crabs [3]:

- *Carcinoscorpius rotundicauda*, the mangrove horseshoe crab, found in Southeast Asia
- *Limulus polyphemus*, the Atlantic horseshoe crab, found along the American Atlantic coast and in the Gulf of Mexico
- *Tachypleus gigas*, found in Southeast and East Asia
- *Tachypleus tridentatus*, found in Southeast and East Asia

Taxonomy of the Horseshoe Crab

Kingdom: Animalia
Phylum: Arthropoda
Subphylum: Chelicerata
Class: Merostomata (Dana, 1852)
Subclass: Xiphosura (Latreille, 1802)
Order: Xiphosura (Latreille, 1802)
Suborder: Limulina (Richter and Richter, 1929)
Superfamily: Limulacea (Zittle, 1885)
Family: Limulidae (Zittle, 1885)
Genus: Carcinoscorpius
Limulus (O. F. Müller, 1785)
Mesolimulus (extinct)
Tachypleus
Species: *Carcinoscorpius rotundicauda*
Limulus polyphemus (Linnaeus, 1758)
Tachypleus tridentatus
Tachypleus gigas
Mesolimulus walchi (extinct)

Biology/life history of the horseshoe crab

Horseshoe crabs are among the world's oldest and most fascinating creatures. The earliest horseshoe crab species had already inhabited Earth at least 200 million years before the dinosaurs arrived or about 400 million years ago. Horseshoe crabs are animals of the temperate seas. During the cold months, they lie half-buried in the ocean sediments. Horseshoe crabs have been observed mating from April through December, although the peak reproduction period occurs during the highest tides in late May and early June, at the time of the full or new moon. Most spawning is at night because of the protection afforded by darkness. In spring, males arrive at the shorelines first, followed by the females a week or two later. Females average being 30% larger than males and attract the males by releasing a pheromone, or natural chemical stimulant, into the water. Horseshoe crabs also use their relatively good vision to help locate potential mates. The males patrol the near shore waters and use their pedipalps to attach to the abdomen of a female as she moves toward the beach. They swim upside down, moving their legs and gills in a progressive wavelike oscillation from front to back.

Horseshoe crabs push their way along the bottom, digging small furrows in search of food. They use their first pair of legs as feelers to determine the presence of prey. When the crab feels or smells a worm, clam, or dead fish, one of the claws picks it up and pushes it toward the heavy, spiny projections that surround the mouth; the horseshoe crab has no nose; but the tiny hairs on the spiny projections around its mouth are chemoreceptors, allowing the crab to smell prey. Since the horseshoe crab has no jaws to chew its food, it must bring all of its legs together and use the spiny projections around its month than the first set of legs to crush the worm or clam. Horseshoe crabs also have gizzards containing sand and small bits of gravel to help grind their food. Horseshoe crabs continue to grow for nine to ten years until they reach maturity. The young horseshoe crabs molt, or shed, their outer skeleton (exoskeleton) often, until they reach sexual maturity, then molting slows, occurring only about once annually. The animals increase in size from 25-30% with each molt by pumping in water to expand their new shells, which will harden in approximately 24 hours. Males are sexually mature at their sixteenth molt or ninth year. Females need at least 17 molts and mature in their tenth year. Unlike the blue crab, this breaks out of its old shell, the horseshoe crab crawls forward out of its shell through a split that develops along the junction of the dorsal (top) and ventral (bottom) surfaces.

Horseshoe crabs face dangers from a variety of predators throughout their lifecycle. These include mollusks, crustaceans, fish, leopard sharks, eels, birds, sea turtles, and, maybe most importantly, the man. A partial list of organisms known to prey on horseshoe crabs during various stages of their lives is given in Table 1 [4, 5, 6].

Anatomy and behavior

The entire body of the horseshoe crab is protected by a hard carapace. It has two compound lateral eyes, each composed of about 1000 ommatidia, plus a pair of median eyes that are able to detect both visible light and ultraviolet light, a single endoparietal eye, and a pair of rudimentary lateral eyes on the top. The latter becomes functional just before the embryo hatches. Also, a pair of ventral eyes is located near the mouth, as well as a cluster of photoreceptors on the telson. Despite having relatively poor eyesight, the animals have the largest rods and cones of any known animal, about 100 times the size of humans' [7, 8]. The mouth is located in the center of the legs, where their bases have the same function as jaws and help grinding up food. It has five pairs of legs for walking, swimming, and moving food into the mouth, each with a claw at the tip except the last pair. The long, straight, rigid tail can be used to flip it over if turned upside down, so a horseshoe crab with a broken tail is more susceptible to desiccation or predation.

Behind their legs, they have book gills, which exchange respiratory gases and are also occasionally used for swimming. As in other arthropods, a true endoskeleton is absent, but the body does have an endoskeletal structure made up of cartilaginous plates that support the book gills [9].

Horseshoe crab normally swims upside down, inclined at about 30° to the horizontal and moving at about 10–15 cm/s (0.22–0.34 mph) [10, 11, 12]. Despite this, they usually are found on the ocean floor searching for worms and molluscs, which are their main food. They may also feed on crustaceans and even small fish. Females are larger than males; *C. rotundicauda* is the size of a human hand, while *L. polyphemus* can be up to 60 cm (24 in) long (including tail). The juveniles grow about 33% larger with every molt until reaching adult size [13].

Breeding

During the breeding season, horseshoe crabs migrate to shallow coastal waters. A male selects a female and clings to her back. The female digs a hole in the sand and lays her eggs while the male fertilizes them. The female can lay between 60,000 and 120,000 eggs in batches of a few thousand at a time. Shore birds eat many of the eggs before they hatch. The eggs take about two weeks to hatch. The larvae molt six times during the first year. Raising horseshoe crabs in captivity have proven to be difficult. Some evidence indicates mating only takes place in the presence of the sand or mud in which the horseshoe crab's eggs were hatched. Neither what is in the sand that the crab can sense, nor how they sense it is known with certainty [14].

The nervous system

In the horseshoe crab, there are several large nerves that supply the crab with information. From the anterior portion of the brain, a pair of optic nerves runs directly to the two lateral eyes. There are also 8 pairs of haemal nerves that extend into the body of the crab. These contain motor and sensory fibers and are distributed mainly to the membrane and other tissues. The sixth pair sends branches to the heart and intestine. All of the haemal nerves are essentially the same, except the first one, the lateral line nerve. It runs close to the surface, just outside the bases of the appendages, begins to branch at the base of the 6th leg. It extends the whole length of the branchial chamber, sending one small branch to the base of each of the five gills. It is a purely sensory nerve and supplies the skin lining the channel along which water is carried to the gills.

The Circulatory System

The horseshoe crab's heart is a long tube that lies along the opposite side of the body of the nerve cord and extends almost the entire length of its body. On average, the heart rate of the horseshoe crab is 32 beats per minute. It has eight pairs of slit-like openings, or ostia, each opening having two valves through which the blood enters the heart from the pericardial chamber. The blood is pumped forward and escapes through three pairs of aortae, one pair of cerebral arteries, and a frontal artery. Two types of hemocytes exist in the blood of the horseshoe: an abundant number of granulocytes and a few cyanocytes. The granulocytes are responsible for the rapid clotting of blood, especially in the presence of bacterial endotoxins.

Horseshoe crab importance in human medicine

The Horseshoe crab is really important to the medical community. The horseshoe crab has a simple, but the amazing immune system. When a foreign object (bacteria) enters through a wound in their body, it almost immediately clots into a clear, gel like material, thus effectively trapping the bacteria. If the bacterium is harmful, the blood will form a clot. Horseshoe crabs are proving to be very helpful in finding remedies for diseases that have built immunities against penicillin and other drugs.

The horseshoe crab has the best-characterized immune system of any long-lived invertebrate. The study of immunity in horseshoe crabs has been facilitated by the ease of collecting large volumes of blood and from the simplicity of the blood. Horseshoe crabs show only a single cell type in the general circulation, the granular amebocyte. The plasma has the salt content of sea water and only three abundant proteins, hemocyanin, the respiratory protein, the C-reactive proteins, which function in the cytolytic destruction of foreign cells, including bacterial cells, and α2-macroglobulin, which inhibits the proteases of invading pathogens. Blood is collected by direct cardiac puncture under conditions that minimize contamination by lipopolysaccharide (endotoxin, LPS), a product of the Gram-negative bacteria. A large animal can yield 200 - 400 ml of blood. Unlike vertebrates, horseshoe crabs do not have hemoglobin in their blood, but instead use hemocyanin to carry oxygen. Because of the copper present in hemocyanin, their blood is blue. Their blood contains amebocytes, which play a role similar to white blood cells of vertebrates in defending the organism against pathogens. Amebocytes from the blood of *L. polyphemus* is used to make *Limulus* amebocyte lysate (LAL), which is used for the detection of bacterial endotoxins in medical applications. The blood of horseshoe crabs is harvested for this purpose [15]. Harvesting horseshoe crab blood involves collecting and bleeding the animals, and then releasing them back into the sea. Most of the animals survive the process; mortality is correlated with both the amount of blood extracted from an individual animal, and the stress experienced during handling and transportation [16]. Estimates of mortality rates following blood harvesting vary from 3-15% [17] to 10-30% [18].

The LAL test represents one of a number of pharmacological significant, chemical constituents found in marine flora and fauna [19]. A wealth of significant compounds has been isolated from marine animals. These include compounds derived from the sea cucumber used in anti-cancer chemotherapy, hormones from gorgonians used for birth control, against peptic ulcers and asthma and lowering blood pressure, as well as compounds derived from red algae that can prevent atherosclerosis [19]. The discovery, commercialization, and use of LAL have been an important improvement in the pharmaceutical industry. Prior to the use of LAL, compounds were tested for the presence of endotoxins in a variety of ways that involved living animals or living parts of animals [19]. Thus, LAL provides a means to detect endotoxins without having to kill or disable animals [19].

Limulus Amebocyte Lysate is extremely useful in detecting those toxins that cause fever – the bacterial "pyrogens" or endotoxins. Endotoxins occur as a structural component of the cell wall of a large group of bacteria known as Gram negative [20]. Most aquatic bacteria are of the Gram-negative variety, as studies at the Woods Hole Oceanographic Institution have shown that seawater contains over 1 million Gram-negative bacteria per milliliter and that almost 1 billion bacteria can be found per gram of sand near the shore [21]. Thus, the horseshoe crab habitat contains vast amounts of endotoxin, making it no coincidence that the horseshoe crab evolved a vital system to protect itself against endotoxins. The horseshoe crab blood includes amebocytes that contain the clotting enzymes and other factors with the ability to immobilize and engulf an endotoxin [19]. When exposed to endotoxin, the amebocytes change shape, adhere to the sides of the vascular channels, and from the resultant gel clot [4]. This phenomenon is at the heart of the LAL assay, as the formation of a clot shows the presence of endotoxin.

The major use of LAL today is in the detection of endotoxins in pharmaceutical products [20]. Since its original description, however, it has also been used in the diagnosis of endotoxemia in conjunction with cirrhosis, cancer, meningitis, eye disease, dental problems, gonorrhea, boutonneuse fever, and water-quality analysis [21], as well as urinary tract infections [20]. In addition, new applications for LAL continue to be found, including the detection of bacterially contaminated meat, fish, and dairy products, including frozen items [20, 22].

Study of blood chemistry

For a study of the plasma, blood cells are immediately removed from the plasma by centrifugation and the plasma can then be fractionated into its constituent proteins. The blood cells are conveniently studied microscopically by collecting small volumes of blood into LPS-free isotonic saline (0.5 M NaCl) under conditions that permit direct microscopic examination by placing one of more LPS-free cover glasses on the culture dish surface, then mounting those cover glasses in simple observation chambers following cell attachment. A second preparation for direct observation is to collect 3 -5 ml of blood in an LPS-free embryo dish and then explanting fragments of aggregated amebocytes to a chamber that sandwiches the tissue between a slide and a cover glass. In this preparation, the motile amebocytes migrate onto the cover glass surface, where they can readily be observed. The blood clotting system involves aggregation of amebocytes and the formation of an extracellular clot of a protein, coagulin, which is released from the secretory granules of the blood cells. Biochemical analysis of washed blood cells requires that aggregation and degranulation does not occur, which can be accomplished by collecting blood into 0.1 volumes of 2% Tween-20, 0.5 M LPS-free NaCl, followed by centrifugation of the cells and washing with 0.5 M NaCl [23].

Horseshoe crabs have a total of 10 eyes (Fig 1) used for finding mates and sensing light. The most obvious eyes are the 2 lateral compound eyes. These are used for finding mates during the spawning season. Each compound eye has about 1,000 receptors or ommatidia [24]. The cones and rods of the lateral eyes have a similar structure to those found in human eyes, but are around 100 times larger in size. The ommatidia are adapted to change the way they function by day or night. At night, the lateral eyes are chemically stimulated to greatly increase the sensitivity of each receptor to light. This allows the horseshoe crab to identify other horseshoe crabs in the darkness. The horseshoe crab has an additional five eyes on the top side of its prosoma. Directly behind each lateral eye is a rudimentary lateral eye. Towards the front of the prosoma is a small ridge with three dark spots. Two are the median eyes and there is one endoparietal eye.

Each of these eyes detects ultraviolet (UV) light from the sun and reflected light from the moon. They help the crab follow the lunar cycle. This is important to their spawning period that peaks on the new and full moon. Two ventral eyes are located near the mouth but their function is unknown. Multiple photoreceptors located on the telson constitute the last eye. These are believed to help the brain synchronize to the cycle of light and darkness. The research into their eyes has helped the study and understanding of how the human eye works.

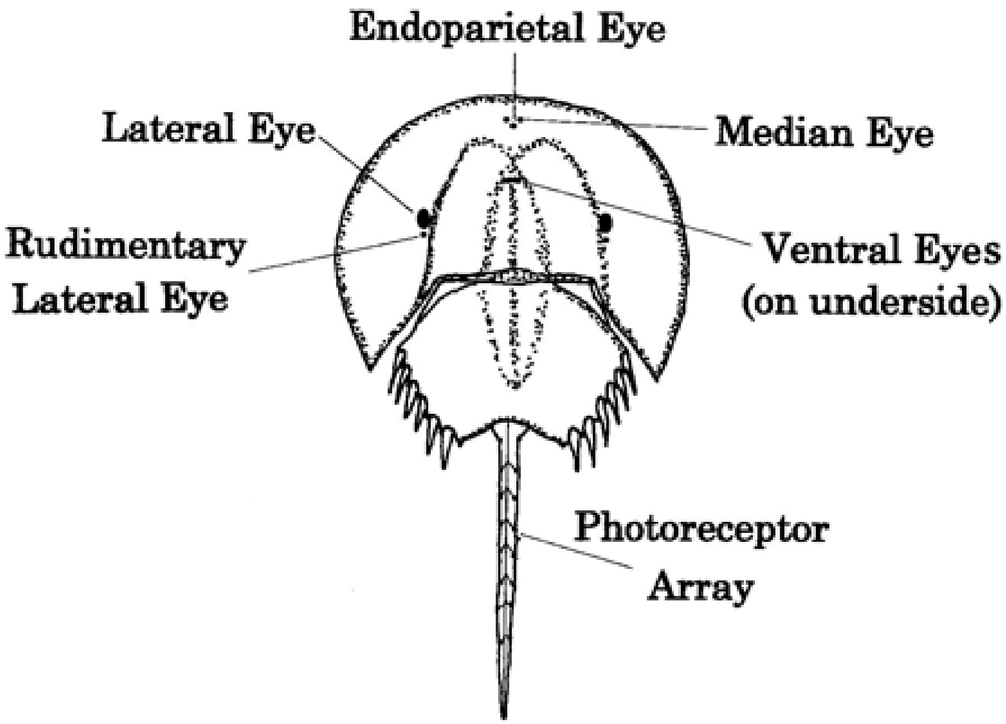
The horseshoe crab (also known as the king crab), is a hard-shelled invertebrate found on the sea floor in warmer climates. Despite its name, the horseshoe crab is not actually a crab (or crustacean) and is in fact more closely related to arachnids such as spiders and scorpions. The horseshoe crab is a primitive-looking arthropod with a hard, brownish-green exoskeleton and a spike-like tail. Horseshoe crabs have bright blue blood and it gets clotted when it comes into contact with foreign bacteria.

Table.1 Organisms Known to Prey on the Horseshoe Crab, Limulus polyphemus

Group	Species	Group	Species
Bird	Semipalmated plover (*Charadrius semipalmatus*) (eggs)	Fish	Silver perch (*Bairdiella chrysura*) (eggs and larvae)
	Black bellied plover (*Pluvialis squatarola*) (eggs)		Weak fish (*Cynoscion regalis*) (eggs and larvae)
	Pectoral sandpiper (*Calidris melanotos*) (eggs)		Northern kingfish (*Menticirrhus saxatilis*) (eggs and larvae)
	Least Sandpiper (*Calidris minutilla*) (eggs)		Atlantic silverside (*Menidia menidia*) (eggs and larvae)
	Semipalmated sandpiper (*Calidris pusilla*) (eggs)		Summer flounder (*Paralichthys dentatus*) (eggs and larvae)
	Sanderling (*Calidris alba*) (eggs)		Winter flounder (*Pseudopleuronectes americanus*) (eggs and larvae)
	Laughing gull (*Larus articilla*) (eggs)		Leopard shark (*Triakis semifasciatus*) (adults)
	Boat tailed grackle (*Cassidix major*) (eggs)		Catfish (eggs)
	Red knots (*Calidris canutus*) (eggs)		Puffers (*Tetraodontidae*) (juveniles)
	Ruddy turnstones (*Arenaria interpres*) (eggs)		Devil ray (*Mobula hypostoma*)
	Herring gull (*Larus argentatus*) (adults)		Sword fish (*Xiphiidae*)
	Greater black backed gull (*Larus marinus*) (adults)		Mullet (*Mugilidae*) (eggs and larvae)
Arthropods	Sand shrimp (*Crangon septemspinosa*) (eggs)		Striped bass (*Morone Saxatilis*) (eggs)
	Fiddler crab (*Uca pugnax*) (first and second tailed stages)		White perch (Morone americana) (eggs)
	Blue crab (*Callinectes sapidus*) (juveniles)		American eel (*Anguilla rostrata*) (eggs and larvae)
	Green crab (*Carcinides maenus*) (juveniles)	Mollusks	(*Melongena* spp.) (adults)
	Spider crab (*Libinia* spp.) (juveniles)	Reptiles	Loggerhead sea turtle (*Caretta caretta*) (adults)
	Amphipods (larvae)		

[4, 5, 6]

Figure.1 Horseshoe crab eyes location



In other words, this stuff's ideal for detecting impurities and in human medicine, it is used to develop products like pharmaceutical drugs. Horseshoe crabs have 2 large compound eyes located on the top of the shell. These eyes are made up of a thousand light sensors that see in shades of gray. The crab combines all these separate sensors together as an image that they see. These eyes are probably used for finding a mate. The compound eyes are larger and have an optical nerve that is easy to identify making the crabs ideal for studying how an eye works. The eyes have the ability to detect UV light and are sensitive enough that the horseshoe crab sees as well at night as it does during the day. Scientist and researchers are putting effort in horseshoe crab eyes to know the mechanism how their eyes work so that it can give some light in human eyes working mechanism.

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