COAST

Written by Willie Heard

On the cutting edge…
There are only four species of horseshoe crabs in existence in the world today. These are *Limulus polyphemus, Tachypleus gigas, Tachypleus tridentatus, and Carcinoscorpius rotundi-cauda.* These creatures are sometimes called "living fossils" because they have changed little from their fossilized relatives; the earliest species identified is approximately 450 million years old. Horseshoe crabs are a valuable resource, commercially as a fertilizer and as a source of calcium for enriching fowl grains and medicinally in identifying endotoxins. The most persistent study on these animals has focused on the properties of their blue blood. In 1977, The Food and Drug Administration of the United States approved a new test for identifying endotoxins using *Limulus* Amoebocyte Lysate (LAL) purified from horseshoe crab blood. In spite of their commercial and medical importance, horseshoe crabs are threatened by the loss of living and breeding habitats. This habitat degradation has resulted in a rapid population decline over the last few decades.

Horseshoe Crabs

Lesson Objectives: Students will be able to do the following:
- Describe the difference between a true crab and a horseshoe crab
- Briefly discuss the life history of the horseshoe crab
- Give some medical and commercial uses of the horseshoe crab

Key Concepts: chitin, *Limulus* Amoebocyte Lysate (LAL), chitosan, exoskeleton, pedipalps, molt, arthropod, decapod, chemoreceptors, pheromone

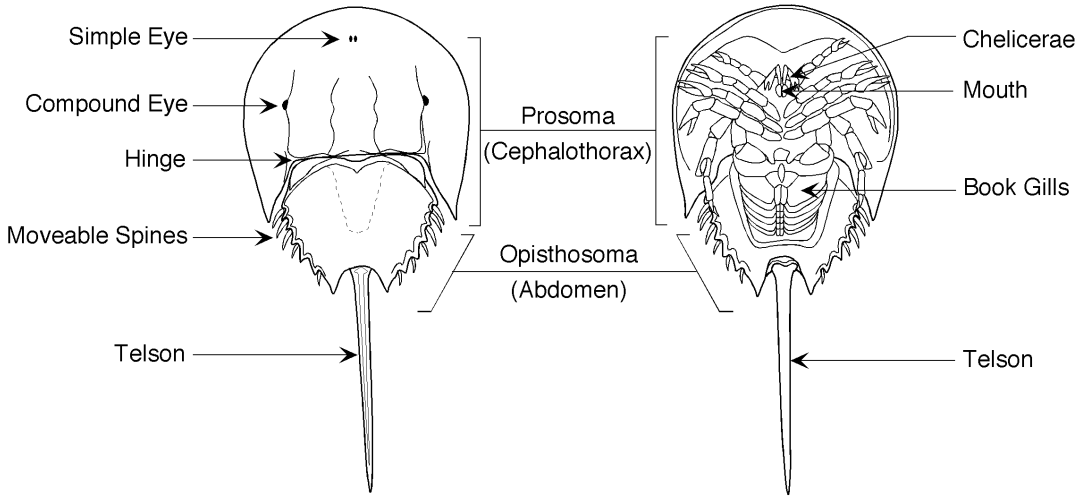
What is a Horseshoe Crab?

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.

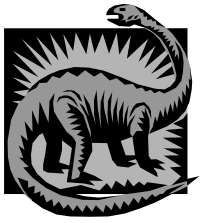
True crabs have two pairs of antennae and a pair of mandibles, or jaws; horseshoe crabs lack these structures. Further, comparing the legs of a true crab with the legs of a horseshoe crab reveals another significant difference. True crabs classified as **decapod** crustaceans, have five pairs of legs, 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.

Horseshoe Crab Anatomy



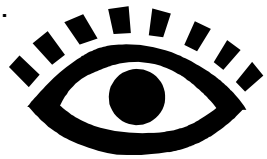
Biology/Life History of the Horseshoe Crab



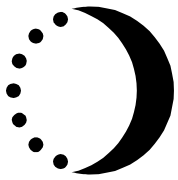
Horseshoe crabs are among the world's oldest and most fascinating creatures. The earliest horseshoe crab species had already inhabited Earth at least 200 million years before the dinosaurs arrived or about 400 million years ago.

Today, there are four species of horseshoe crabs in the world, but only one is found on North American shores. Our native horseshoe crabs live along the Atlantic coast from Maine south to Florida and the Yucatan Peninsula. They are also found in several places along the eastern shores of the Gulf of Mexico. The largest concentration of horseshoe crabs is found in Delaware Bay, located between Delaware and New Jersey. 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.



Males patrol the nearshore waters and use their pedipalps to attach to the abdomen of a female as she moves toward the beach. She drags him to the water's edge and scoops out a series of five to seven crude nests, where she deposits tens of thousands of eggs. The attached males and other males that gather around the female fertilize the eggs as she lays them. The new eggs are about 1.5 mm, or 1/16 inch, in diameter, and are an opaque, pastel green color. In a few days, they double in size and the outer layer peels away, leaving the eggs transparent. Moisture from the tides, and the warmth of the sun, allows the eggs to hatch in the two-week period between spring tides



(the higher-than-normal tides that occur at the new and full moons.) After hatching, the juvenile horseshoe crabs dig their way "out of" the sand. When born, the tiny horseshoe crabs look very much like their adult counterparts. They begin life as miniature adults, about three mm (1/8 inch) across, but lack a fully functional digestive system and a movable tail. For about a week, they "swim about," eating their yolk sac as their digestive systems mature. They swim upside down, moving their legs and gills in a progressive wave-like 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 then 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 by 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, which "backs 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. No one really knows how long horseshoe crabs live, but some scientists believe that 30 years is possible. Generally, an animal which does not begin reproduction until age nine or older would have a life span enabling reproduction for a number of years.

Commercial Importance of Horseshoe Crabs

Though the eggs and flesh of *Limulus polyphemus* are not toxic, they are not eaten by people today. Years ago, indigenous American people did eat the lump of meat in the abdomen which moves the tail. They also used the shell to bail water from their canoes and the tails as spear tips. More recently, horseshoe crabs were used as fertilizer and as feed for chickens and hogs.

Chicken and hogs fed on horseshoe crab developed a bad taste, so using horseshoe crabs as a food source was discontinued.

Today horseshoe crabs are important to people for their use in medicine. For over fifty years scientists have used horseshoe crabs in eye research. Scientists can easily study the large eyes and optic nerve (the nerve that sends signals from the eye to the brain) of the horseshoe crabs. Scientists have learned a great deal about how human eyes function from research on horseshoe crab eyes.

Chitin is a substance found in the shells, or exoskeleton, of horseshoe crabs, as well as other arthropods, such as lobsters, crabs, shrimp, spiders, beetles, and mosquitoes. Chitin has received a lot of attention from scientists because it is non-toxic and biodegradable. When chitin is processed, another substance, **chitosan**, is produced and can be used as a raw material to manufacture a variety of important products.

Contact lenses, skin creams, and hair sprays can also be manufactured from chitin. Chitin can be used to remove lead and other harmful chemicals from wastewater. Chitin joins the fight against fat when added to foods. It has the ability to bind with fats and then pass them through the body without being absorbed. Besides absorbing fat to promote weight loss, chitosan also inhibits "bad" cholesterol uptake and boosts "good"

cholesterol. Other chitosan uses include: promoting the healing of ulcers and lesions; serving in anti-bacterial action; acting as an antacid; helping to control high blood pressure; and treating and preventing irritable bowel syndrome. Lastly, chitosan can be made into string used to suture surgical wounds and in wound dressings. People are less likely to experience an allergic reaction to the chitosan-based stitches, which dissolve slowly, and the dressings actually promote healing.

In 1950, Frederick Bang discovered the blue-colored blood of the horseshoe crab contained special cells that react to certain kinds of disease causing bacteria. ***Limulus***
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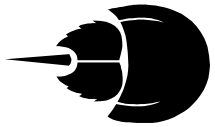
Amoebocyte Lysate (LAL) was found in Factor C, the first enzyme to be activated by the endotoxin from gram negative bacteria. LAL is now used as a fast and effective way of testing drugs to make sure they are free of these harmful bacteria before they are administered to people. Pharmaceutical companies used the LAL to test sterility of antibiotics and kidney dialyzers, to detect some cancers, and to detect spinal meningitis.

Sometimes during the peak summer months, more than 1,000 crabs per week are harvested for their blood. Up to one-third of the animal's blood is painlessly removed by medical researchers. The cost of horseshoe crab blood has reached $15,000 dollars per quart. A horseshoe crab collector can make up to $1,000 dollars a night.

Activity: Horseshoe Crab "Hands On"

The horseshoe crab is compared to the giant cyclops *(polyphemus*) in Greek mythology. Yet, despite this frightening comparison, the horseshoe crab with its large spiny body and spiked tail is not poisonous, but completely harmless.

Objectives: Students—in groups of two or four—will be able to do the following:
- Touch and hold the horseshoe crab.
- Identify the external anatomy of the horseshoe crab.
- Identify the gender of the horseshoe crab.



Materials*:*
- Live horseshoe crabs, male and female
  (There are a number of vendors for living horseshoe crabs, however, one vendor is: Dynasty Marine Associates, 10602 7th Avenue, Gulf Marathon, FL 33050. Phone: 305-745-7666. Horseshoe crabs are approximately $12 each depending on size and seasonal price variations.)
- Molts from various sizes of horseshoe crabs
- Paper towels
- Hand soap

Procedures:
1. The instructor should review the history of the horseshoe crab and research uses, as well as discuss the safe handling techniques for these animals.
2. Removing the live animal from its tank, the instructor should present the animals to the class, identifying the major external, anatomical structures.
3. Students should be given time to carefully feel the tail, legs and pinchers of both the live animals and the molts.
4. The instructor should review, using male and female animals, the distinguishing characteristics of the claws between the two sexes.
5. Students should wash hands thoroughly at the conclusion of the class.

Extensions:
1. Compare the horseshoe crab with a true crab such as the blue crab (*Callinectes sapidus*).
2. Locate recent and historical research reports utilizing horseshoe crabs by accessing via internet the web sites of Delaware Sea Grant College Program.
3. Construct a paper model of the horseshoe crab using Dr. Bill Hall's published model available through Delaware Sea Grant College Program.

Activity: Biodiversity Quiz

Objectives: Students will be able to do the following:
- Identify which niche horseshoe crabs fill.
- Define "niche".
- State the importance of horseshoe crabs in research.

Materials*:*
- Live horseshoe crabs
- Paper
- Pencil

Procedure:
Two camps of animal rights activists argue concerning the use of marine invertebrates for biomedical purposes. Some individuals believe passionately that research involving living subjects of any kind is cruel and unnecessary. Other individuals believe invertebrates can be substituted for warm-blooded research animals, i.e., mammals. A list of uses for horseshoe crabs has been provided below. Explore your value system by ranking the uses from most to least acceptable. Tell which uses should be continued and discontinued, and why. Be prepared to explain your answers.

1. Horseshoe crab nerve cells are large, accessible, and good for modeling human nervous system mechanisms. Horseshoe crabs' relatively large and simply constructed compound eyes have an easily accessed optic nerve; and because they are easy to keep in the laboratory, horseshoe crabs have contributed greatly to human eye research.
2. American Indians began using horseshoe crabs thousands of years ago. They ate the abdominal meat, the tail muscles, and possibly other parts. Shells were not discarded, but used to "bail out" dugout canoes. Spear tips were constructed from horseshoe crab tails, and Indians buried the crabs as a time released, high-nitrogen fertilizer.
3. American settlers also used horseshoe crabs for fertilizer, leading to an industry that lasted until the 1950s. A few farmers continue the practice today. Horseshoe crabs have also been used as stock feed, but imparted a fishy flavor to hogs and chickens. Horseshoe crabs' eggs are still used as eel bait in a small fishery supporting U.S. and European markets.
4. The chitin of horseshoe crab shells is extremely pure, and chitin-coated sutures reduce healing time in humans by 35-50%. A Japanese company spins pure chitin dressings for burns, surface wounds, and skin-graft donor sites.
5. *Limulus* Amoebocyte Lycate (LAL) is a clotting agent extracted from horseshoe crab blood. LAL is the standard test for injectable and intravenous drugs, during which it clots in the presence of toxins and impurities. LAL is also used to diagnose diseases such as spinal meningitis. Large horseshoe

crabs are caught and bled for LAL production through a stainless steel tube inserted into the circulatory system. After 24 hours, the crabs are returned to the bays where they were caught with little serious harm. (Source: <www.txdirect.net/sitc/sea-hrs.htm>. Used by permission.)

Possible Extension:

1. Devise a food web with the horseshoe crab at the center of the web.

Student Information: Horseshoe Crabs

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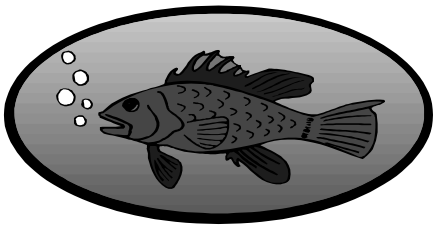
Chitin is a substance found in the shells, or exoskeletons, of horseshoe crabs, as well as other arthropods, such as lobsters, crabs, shrimp, spiders, beetles, and mosquitoes. Chitin is non-toxic, biodegradable, and is processed to produce another substance called **chitosan (aminopolysaccharide)** that can be used to produce a variety of important products. Horseshoe crabs grow larger by **molting,** or shedding their outer shell, which is actually their skeleton (**exoskeleton**).

Too often, we learn the value of something only after it is gone. In the case of the horseshoe crab, we now know its value and as a result, many people are concerned about the horseshoe crab's future. We should learn from the experience of the Japanese, whose horseshoe crab species is considered endangered**.** The medical value of the horseshoe crab is easily measured economically, yet environmentally, the picture is much more complex when we consider the millions of shorebirds and other animals—fish, turtles, and the entire estuarine food chain—that depend on horseshoe crab eggs as a food source.



Horseshoe Crab Vocabulary

Carapace-the front top portion of the shell of the horseshoe crab

Chelicerae-the first pair of pincers located in front of the horseshoe crab's mouth

Chemoreceptors-tiny hairs located on the spiny projections that surround the horseshoe crabs' mouths that allow them to "smell" prey

Chilaria-appendages located in the rear of the horseshoe crab's mouths

Chitin-a cellulose-like substance found in the shells, or exoskeleton of horseshoe crabs, as well as other arthropods

Chitosan-processed chitin; used in a variety of manufactured products

Decapod-creatures that have five pairs of jointed legs

Dorsal-top portion of the carapace where the shell splits during molting

Exoskeleton-the outer structure (skeleton) of the horseshoe crab's body

Gnathobases-the heavy, spiny projections that surround the horseshoe crab's mouth

***Limulus* Amoebocyte Lysate**-a clotting agent extracted from horseshoe crab blood

Molting-the process by which crabs grow by shedding their exoskeleton

Pedipalps-the second pair of claws of the male which has a "boxing glove" shape and which are used during spawning to grasp the female's abdomen

Pheromone-a natural chemical produced by female horseshoe crabs that acts as a sexual stimulant to attract males

Prey-an animal that is hunted for food

Telson-the long, spike-like tail of the horseshoe crab

Ventral-the bottom portion of the carapace where the horseshoe crab's shell splits during molting

Horseshoe Crab References

Brockmann, H. Jane and James A. Cohen (1983). *Bulletin of Marine Science*. 33(2) pp. 274-381.

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