

ATLANTIC HORSESHOE CRAB CONSERVATION



T H E A N C I E N T M A R I N E R



Limulus polyphemus, known as the Atlantic horseshoe crab, is an ancient mariner with a lineage that dates back 440 million years. Though they look similar to crustaceans, they actually belong to the subphylum *Chelicerata*, relating them more closely to spiders and scorpions. *Limulus polyphemus* is found along the Atlantic and Gulf coasts from northern Maine to the Yucatán Peninsula, with the Delaware Bay as the center of the population. This interesting animal is important, not only for the pivotal role it plays in its ecosystems, but for its valuable contribution to the field of biomedical research and its applications. Decades of scientific study of this living fossil have resulted in Nobel prize-winning advances in physiology and medicine, with perhaps the greatest discovery being the animal's contribution to the detection of bacterial contamination in drugs.

A T L A N T I C H O R S E S H O E C R A B C O N S E R V A T I O N



The Atlantic Horseshoe Crab

As keepers of the environment, we have a responsibility to preserve, protect and live harmoniously with the animals which share our planet. With its sturdy helmet-like shell and intimidating pointed “tail,” the Atlantic horseshoe crab looks perfectly capable of protecting itself. This is true to an extent, but this amazing creature’s important contribution to our everyday health and safety grants it our special attention.

As a source of critical proteins that detect the presence of endotoxins in products and instruments used by humans, the Atlantic horseshoe crab has long been treasured by the biopharmaceutical industry. For this reason, it is critical that we serve as advocates for the humane treatment of these animals, and strive to achieve balance between our need for this valuable material and the livelihood of the animal that provides it. Charles River is proud to play a role in alleviating pressures on horseshoe crab populations through tireless conservation efforts, active animal welfare campaigns, and decades of research and development.

Whether you’re a drug manufacturer, a health care professional or an animal lover, we invite you to help raise awareness about these amazing creatures, the role they play in our everyday lives, and what we can do to protect them.

Horseshoe Crab Reproduction

During spring and early summer, nesting females will make several trips up the beach to spawn. With each trip, she will dig a small nest and lay a series of four to five discrete egg clusters. These clusters are located in dense masses near the front of her shell, and typically contain 4,000 eggs per cluster. By the end of the season, she will have deposited as many as 20 clusters. As an important source of food for shore birds, many of these thousands of eggs will not live past the larval stage. Survivors hatch at two weeks and move into shallow waters where they will live for a year or more, moving deeper into the ocean as they mature.

Anatomy

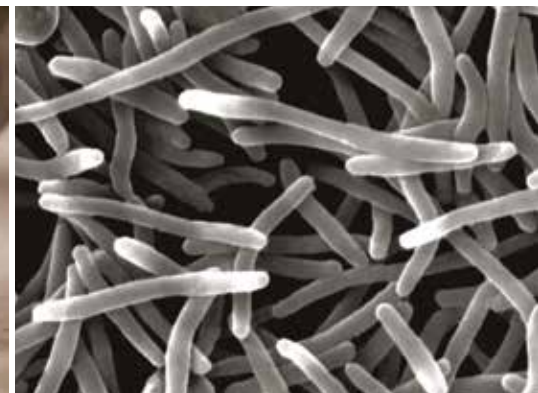
The hardened exoskeleton of the crab consists of three sections: the prosoma (the largest section), the opisthosoma (the middle section) and the telson (tail). Many mistake its spike-like tail for a poisonous stinger or mechanism of defense. In fact, the horseshoe crab primarily uses its telson to maneuver or to flip itself upright if it is overturned.

The simplicity of this ancient creature’s exterior is a dramatic contrast to the complexity of its mysterious blood. Unlike the blood of vertebrates that uses hemoglobin to carry oxygen, the blood of the horseshoe crab instead uses copper-containing hemocyanin, which turns the animal’s blood a striking shade of blue.

The true beauty of this remarkable creature, however, is seen in its primitive immune system. Instead of developing antibodies in response to infection, the immune system releases proteins that can bind and kill bacteria. In addition, other immune proteins clot when exposed to waterborne bacteria, a mechanism which forms the basis of the LAL test.

The earliest horseshoe crab fossils are found in geologic layers from the late Ordovician period, roughly 440 million years ago.

LIMULUS AMEBOCYTE LYSATE AND ENDOTOXINS



Limulus amebocyte lysate (LAL) is an aqueous extract of blood cells (amebocytes) from the horseshoe crab. Comprised of proteins, LAL is used to detect the presence of endotoxins. In the 50 years since this discovery, the blood of the horseshoe crab has served as a natural indicator of bacterial contamination in critical medical treatments all over the world. So why is endotoxin detection so important? Endotoxins are a cell wall component of gram-negative bacteria that cause a pyrogenic response (fever). They are extremely potent, heat stable, and present everywhere bacteria are or have been. With its specificity, simplicity and remarkable sensitivity, LAL ensures the safety of products such as intravenous drugs, vaccines and medical devices.

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Bacterial Endotoxin Testing

Prior to FDA approval of LAL in the 1980s, the rabbit pyrogen test was the standard FDA-approved test for endotoxins. This test, however, was labor intensive, lengthy and expensive. Years of research and application have proven LAL to be unequivocally recognized as the most sensitive method available for the detection of endotoxins. To illustrate, LAL can detect as little as 1 pg/mL of harmful bacterial endotoxin (equivalent to one grain of sand in an Olympic-sized pool).

Approximately 70 million LAL tests are performed each year, and there have been no FDA-confirmed pyrogenic outbreaks due to the failure of LAL detecting the presence of endotoxin. As a highly regulated product, manufactured LAL undergoes biannual inspections by the FDA and must conform to cGMP requirements. Of course, to manufacture LAL, the blood of the horseshoe crab must be carefully collected through a controlled bleeding program. Policies such as hand-harvesting and same-day return to the sea ensure the animal's safety. With a focus on innovation, humane care and environmental protection, Charles River has responsibly manufactured LAL since 1987.

While there are synthetic alternatives to LAL currently on the market, they do not provide the same level of performance in sensitivity as the natural LAL proteins. Additionally, these alternatives are not FDA-licensed and would require a significant amount of time and risk assessment to be validated for use on products. Due to the limited experience in the testing of a wide variety of pharmaceutical products, there is a great amount of uncertainty in the ability of synthetic LAL to detect all waterborne bacteria that can be seen in pharmaceutical manufacturing.

Innovations in Testing:

Reducing LAL with Cartridge Technology

Charles River has been a pioneer in the realm of endotoxin detection, developing an FDA-licensed LAL cartridge that further safeguards the 440-million-year-old species by using 20 times less raw material than traditional LAL tests. If all tests were performed using this optimized technology, today's entire worldwide demand could be met with the blood collected from our current annual quota of animals.

Our cartridge technology is the most environmentally conscious strategy because it significantly reduces the amount of LAL per test and minimizes the need for retesting that is often necessary with traditional methods.

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of bacterial contamination in critical medical treatments.


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C H A R L E S R I V E R A D V O C A C Y



We are committed to employing only fishermen licensed by the Department of Natural Resources to hand-harvest horseshoe crabs from the coastal waters of South Carolina. Our highly controlled and monitored procedures enable us to collect enough raw material for 24 months of LAL production from a minimum number of donor animals. Once the crabs are brought into our lab, they are carefully inspected by trained employees who determine the animal's health and maturity. After careful collection of a measured amount of blood, the crabs are returned unharmed to their natural habitat within the same day, and their blood volume rebounds quickly. These practices have allowed Charles River to achieve an industry-leading survival rate of donor animals.

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Involvement with Government Regulations

Much of the continued survival of the horseshoe crab can be attributed to its inherent adaptability, as well as modern-day government protection. Prior to 1991, there were no laws or regulations in South Carolina dealing with horseshoe crabs. Increased fishing industry demands for *Limulus polyphemus* made it necessary to develop a state-wide management plan to conserve this resource. In 1992, Dr. James Cooper (a pioneer in the research and development of the LAL assay) wrote draft legislation that called for the management and regulation of horseshoe crab fisheries.¹ As a result, the South Carolina state legislature enacted laws to protect the indigenous horseshoe crab population. In South Carolina, horseshoe crabs must be harvested by hand and can only be used for biomedical applications (LAL production) and marine biological research, not as bait for the eel and whelk industries. The addition of six island sanctuaries makes the horseshoe crabs in South Carolina one of the most protected species on the East Coast of the United States.

The State of the Population

In 2013, the Atlantic States Marine Fisheries Commission (ASMFC) Stock Assessments emphasized that the horseshoe crab population in the Southeastern Atlantic Coast has increased.² Additionally, the Southeast Area Monitoring & Assessment Program (SEAMAP) Coastal Trawl Survey, conducted by the South Carolina Department of Natural Resources, shows a population increase over the last several years.³ Results from a South Carolina tagging study also demonstrated that bled horseshoe crabs are able to return to spawning beaches in subsequent years.

With a passion for innovation and a commitment to humane care, Charles River is proud to be an industry leader in both the manufacture of life-saving LAL and the protection of nature's most important blood donors.



For more information about our commitment to the conservation of the Atlantic horseshoe crab, related resources and the references cited in this brochure, please visit us at www.criver.com/hsc. To request our Atlantic horseshoe crab conservation poster, email us at hsc@crl.com.


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