The Incredible Horseshoe Crab: Modern Medicine's Unlikely Dependence on a Living Fossil

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Introduction

Horseshoe crabs, those unassuming, overturned bowls of the sea, have an amazing hidden superpower. What makes them so special? There are a few factoids you might see in a biology textbook—they have blue blood, 10 eyes, are more closely related to spiders and scorpions than crabs, and, during their approximate 450-million-year existence, have survived more than one mass extinction and global catastrophes[1]—but those facts aren't what really makes them special.

Horseshoe crabs play an important part in marine ecosystems stirring up sediment layers, act as a source of food for fish, crustaceans, and shorebirds, and act as predators to mollusks and polychaetes. But that's not what makes them special either.

It's all about the incredible defensive properties in their blue blood. Those properties have helped save the lives of anyone who has had a vaccine, needed a medical injection, or used a medical device in the past 30 years. The discovery and application of those remarkable properties is what has driven efforts to protect and conserve this surprisingly precious marine animal. But are current conservation efforts adequate?

Blue-Blooded Buckets

The Atlantic horseshoe crab, *Limulus polyphemus*, uses copper-based protein called hemocyanin in its blood to transport oxygen, which affords it that baby-blue color.

The horseshoe crab's circulatory system is open, like that of a spider. If you think of the circulatory system of higher vertebrate animals (like us) as plumbing in a house—a series of pipes delivering fluid to crucial areas, while the rest of the house stays dry—a horseshoe crab's circulatory system would be a bucket of water. All of its internal surfaces are bathed in a combined mix of blood and interstitial fluids called hemolymph. This means that any dangerous foreign body or toxic element introduced into the blood could have widespread access to vital organs. To combat this, the horseshoe crab's blood contains amoebocytes that detect incredibly small traces of bacterial presence and trap them in clotted, gel-like masses via the release of coagulogen. The blood itself will rapidly isolate any infectious or dangerous agents before they can spread, and can also plug any wounds with gel clots.

Limulus Amoebocyte Lysate (LAL) to the Rescue: Horseshoes, Rabbits, and People Rejoice

Before the pharmaceutical and biomedical industries started utilizing the unique properties of the horseshoe crab's blood, the agricultural and fishing industries were already aware of the usefulness of the horseshoe crab—as ground-up fertilizer on land and chopped-up bait for catching eel and conch. In some areas along the East Coast, the horseshoe population was nearly wiped out as a result.[2]

While the horseshoe crab was being used as bait and fertilizer, the pharmaceutical industry was testing various implants and injectables for contaminants using rabbits. Then researchers discovered a way to take advantage of the clotting enzymes in the horseshoe crab's blood. They could extract coagulogen and use it to detect contamination in liquid down to 1 part per trillion. This led to the development of the LAL, which became the industry standard for detecting bacterial endotoxin contamination in vaccines, medical devices, and pharmaceutical products. To date, LAL is the most sensitive, reliable, and accurate screening tool of its kind,[3] and the raw material for LAL comes from the horseshoe crab's blood, which can be harvested without killing the crab. Scientists will remove 30% or less[4] of the horseshoe crab's hemolymph during a harvest, and the horseshoe crab will be released to recuperate back in the ocean.

In the 1970's, Associates of Cape Cod (one of the four major companies in the LAL industry) was granted the first license from the FDA to manufacture and sell LAL for use in the medical field for endotoxin detection and quality control testing.[5] Today, the FDA requires LAL testing on every drug for certification, along with medical implants and prosthetics.[5] This means that each person who has received a medical injection has been impacted by LAL and, by extension, the horseshoe crab. This makes the horseshoe crab an essential component of modern medicine.

The Business of Protecting Horseshoes and People

Because the FDA required LAL testing, the pharmaceutical industry needs a large amount of horseshoe crab blood. The worldwide market for LAL test products is estimated at more than $200 million.[6]

Why don't we see large horseshoe crab farms? It turns out that raising horseshoe crabs in captivity results in a steadily declining quality of hemolymph.[6] This means wild horseshoe crabs are caught, bled, and returned to the ocean each year. Reports vary as to the exact number, but the hemolymph from approximately 600,000 horseshoe crabs was collected in 2012.[1,6]

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Given the precious nature of the horseshoe crab's blood, conservation efforts have been put in place in some regions to mitigate population decline and to ensure the global demand for LAL is met. In South Carolina, laws have been established to govern the management and regulation of horseshoe crab fisheries, restricting horseshoe crab use to biomedical purposes and marine research instead of bait.[2,3] This is leading to an increase in crab populations, but some states are still seeing a population decline.[2,6,7] Connecticut has established zones where harvesting is prohibited.[7]

For LAL production, horseshoe crabs are typically collected during the spawning season and transported to a laboratory where they are cleaned of sand and debris.[4,8] The animals without notable injuries are bled with a large-gauge needle until up to 30% of their hemolymph is removed.[3] Once bled, the animals are returned to the ocean, typically within 72 hours.[9]

Charles River, another major LAL manufacturer, sees that blood is collected under tightly controlled procedures from horseshoe crabs harvested by hand by personnel licensed by the Department of Natural Resources.[3] They report that crabs are released back into the water within 24 hours of collection.[3] They have also developed extremely sensitive cartridge technology that uses 20-times less hemolymph than conventional methods, and claim that over the years, the amount of raw material needed to perform LAL testing has declined.[3]

Lonza, another major firm invested in LAL production, has a synthetic version of the LAL test that does not use any wild horseshoe crab blood.[9] Other companies have also worked on synthetic versions of the LAL test.[9] Currently, synthetic LAL tests are not in the United States Pharmacopeia, leading pharmaceutical companies to shy away from it in favor of the FDA-approved LAL tests from natural hemolymph.[9]

Horseshoe Date Night: Romance, Moonlit Beaches, and Bleeding?

While the process of extracting hemolymph has a low mortality rate, questions have been raised as to how harmful the bleeding is to the horseshoe crabs. Female horseshoe crabs are preferentially bled due to

> The unassuming horseshoe crab is indispensable to human health.

their larger size compared to males.[4] According to researchers, fewer females are showing up to spawning beaches, and egg counts have decreased as a result.[6,9] This prompted researchers and environmental managers to question the potential non-lethal effects of the LAL harvest process, effects which may be exacerbated by the harvesting that takes place during the spawning season. To some researchers, it seemed reasonable to assume that when a non-insignificant amount of blood is taken from an animal plucked out of peak of its mating time for a few days, the animal would experience some depression in mating success.[6]

Researchers at the University of New Hampshire and Plymouth State University conducted a study to examine the sublethal effects of the LAL harvest process. They outfitted 28 female horseshoe crabs in the Durham, NH area with accelerometers and divided the crabs into several groups. They then recreated a traditional LAL harvest process. Afterwards, the crab's motility and hemocyanin levels were recorded.[4]

The researchers discovered that crabs that were bled moved less frequently and moved in altered patterns and frequency compared to their non-bled counterparts for approximately 2 weeks after the bleeding procedure. The altered movement in the bled crabs meant these crabs were less likely to follow the tides and were therefore less likely to show up to the spawning beaches. The bled crabs also exhibited fewer hemocyanin levels. Researchers theorize that with reduced movement and reduced immune responses, crabs that have been bled are less likely to contend with typical stresses and can be more susceptible to infection. As the Anderson, et al, report, "The changes we observed in activity levels, movement velocity, and expression of tidal rhythms may interfere with daily *L. polyphemus* activities, which would be particularly pronounced during the spawning season. Spawning necessitates several energetically costly trips to the intertidal zone ... An activity deficit, such as that caused by biomedical bleeding, may influence either the number of those trips or their timing. In the case of the latter, females may delay spawning activity while they are recuperating, and this could reduce their spawning output."[4]

Essentially, Anderson found that female crabs that have been bled may be less apt to mate, which would adversely affect horseshoe crab populations. It should be noted, however, that the LAL industry tends to use a more careful collection and shorter handling procedure than that employed in the study.[3,5]

Conservation Considerations

Perhaps additional considerations are needed to help conserve horseshoe crab populations. Researchers suggested that delaying the harvest to avoid the spawning season may help any detrimental impact on populations due to the behavioral changes caused by the bleeding process.[6] By bleeding the crabs after the spawning season, they may have time to recover to a more robust health by the time the next breeding (and bleeding) occurs the following year.[6]

Whether the biomedical field will make further adjustments to their considerable conservation efforts based on the findings of Anderson, et al, remains to be seen. One thing remains clear: The unassuming horseshoe crab is indispensable to human health. Keeping their population healthy has a direct, if unexpected, impact on the health of our own population.

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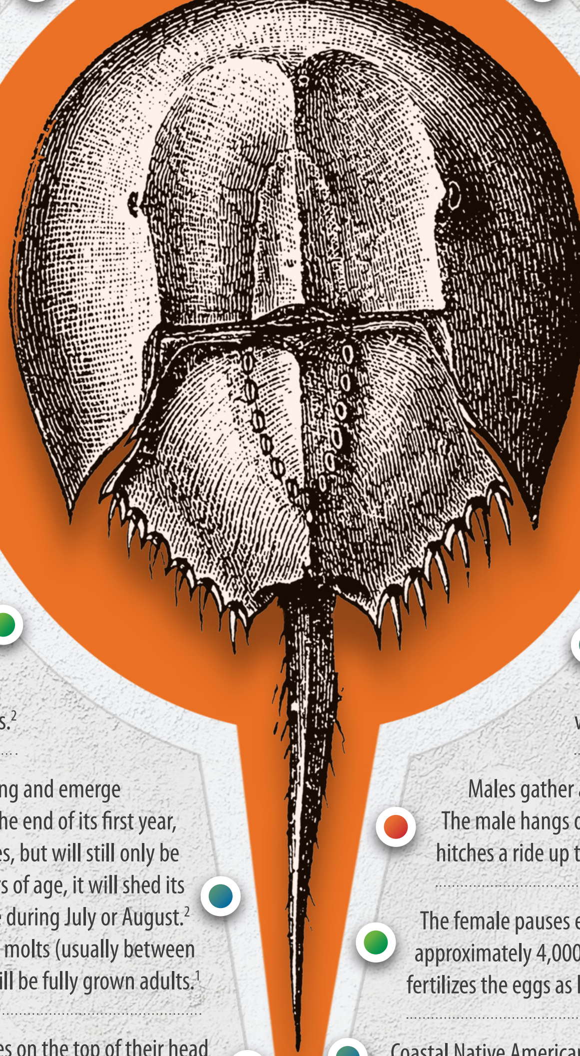
Meet the Horseshoe Crab

Limulus Polyphemus



They have a hard exoskeleton like a crab, but are more closely related to scorpions and spiders than crabs.

A horseshoe crab's tail is not a weapon, and is in fact very delicate. The tail is used to help propel the crab through sediment, to act as a rudder, and to right the crab when it accidentally tips over.[1]

The species is estimated to be at least 300 million years old.[1]

Horseshoe crabs can swim upside down in the open ocean using their 12 legs and 5 book gills to propel themselves.[1]

Juvenile horseshoe crabs generally spend their first and second summers on the intertidal flats, feeding before the low tides of daytime and burrow into the sand the rest of the day. As the crabs grow, they move into deeper waters.[2]

Horseshoe crabs grow by molting and emerge 25 percent larger with each molt.[1] By the end of its first year, the crab will have molted several times, but will still only be about a half-inch wide. By 3 or 4 years of age, it will shed its shell only once a year, sometime during July or August.[2] After approximately 16 molts (usually between 9 and 12 years) they will be fully grown adults.[1]

They have 2 compound eyes on the top of their head and 10 optic sensors that let them see UV light.[1]

Horseshoe crab eggs and larvae are an important food source for at least 11 species of migratory shore birds, including the red knot, which relies strictly on horseshoe crab eggs for food during migration. Sea turtles also eat the eggs and larvae. Many shoreline ecosystems depend on horseshoe crab eggs for survival.[5]

They have been known to survive up to one full year without food.[3]

Horseshoe crab eggs are important food for migratory shore birds that pass through during the spring mating season.[1]

In the 1900s, horseshoe crabs were dried for use as fertilizer and poultry food supplements before the advent of artificial fertilizers.[1]

They inhabit coastal bays and near shore waters from spring to fall, and move offshore to depths of 60 to 600 feet in the winter.[1]

Horseshoe crabs spawn each spring during the high tides of the new and full moons, and begin spawning once the water temperature reaches approximately 60°F. [1]

Males gather along the water's edge as the females arrive. The male hangs on to the female's shell with its pincers and hitches a ride up the beach to the high tide line.[1]

The female pauses every few feet to dig a hole and deposit approximately 4,000 greenish, bb-sized eggs. The male fertilizes the eggs as he is pulled over the nest.[1]

Coastal Native Americans used to affix horseshoe crab tails to the tips of their fish spears.[4]

The 1967 Nobel Prize was awarded to Dr. H. Keffer Hartline based on studying the electrical impulses from the horseshoe crab's optic nerve in its later eye, leading to a greater understanding of the principles underlying all visual systems.[4]

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