Horseshoe Crab – A Valuable Marine Creature

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The ocean is a treasure trove of many living and non living resources. About 26 phyla of marine organisms are found in the ocean, whereas arthropods (jointed limbs and an outer shell, which the animals moult as they grow), with over 35,000 varieties, contribute four-fifth of all marine animal species. Surprisingly, a number of marine organisms, suspected to be extinct, still flourish as living animals. The horseshoe crab, a chelicerate arthropod, is one such amazing creature and is considered to be the oldest 'living fossil'.

The horseshoe crab has descended from mud dwelling primitive arthropods, named trilobites, which lived in the Precambrian seas, nearly 600 million years ago. After 150 million years or so, the horseshoe crab evolved into its present shape and, surprisingly,the archeal animal body has not shown any phenotypic change, even after a span of 350 million years. While so many animals, including dinosaurs and many other latecomers to the Mesozoic era, have become completely extinct, it is a wonder how the humble horseshoe crab has survived for such a long period. It seems that this animal can overcome all kinds of adverse situations in its estuarine and coastal shallow habitat. It can also tolerate wide ranges of salinity, temperature, desiccation and submergence.

The ancestors of the horseshoe crab are believed to have inhabited brackish or freshwater environments. Fossil records show that the oldest horseshoe crabs were similar to the aglaspids, with less abdominal segments but without well defined appendages. The five pairs of walking legs, discontinuing at the abdomen, were present in the primitive forms. Gradually, the first four pairs started developing pinching claws, whereas, the last pair terminated in primitive spines. In modern horseshoe crabs, these five pairs are highly specialized appendages with broad, flat and overlapping plates. The external gills of the horseshoe crab were partly developed from these appendages.

Horseshoe crab habitat

The horseshoe crab belongs to the benthic community. They prefer calm seas or estuaries with muddy sandy bottoms for their biogenic activities. They migrate to the shore from the deeper waters specifically for breeding purposes. During this shoreward migration the animal is subjected to a wide range of environmental conditions including salinity and temperature. Although detailed knowledge of the complete life cycle of the animal is not yet known, it is generally believed that the animal inhabits the deeper zone of the sea for most parts of its life. The fluctuating habitats encountered by the horseshoe crab during their different stages of life reflect their amazing ability to tolerate and adapt to different environmental conditions.

The horseshoe crab has a patchy distribution pattern. They have highly specialized methods for detecting their selected habitat for reproduction and dispersal. These methods ensure the animals their safe arrival to their respective destinations and reduce the chance of mortality during their migration. Such characteristics could possibly contribute to the evolutionary

T. gigas migrating on sandy beaches for spawning

advantage of the horseshoe crab for their very long survival.

In contrast, uniformly distributed animals are subject to more competition for their biological needs and have fewer opportunities to survive. The horseshoe crab has a difficult distribution pattern because the animal is totally dependent on two important physical stimuli – the tide and the lunar periodicities. There have been a number of attempts to study the distribution of the horseshoe crab in the recent past decades. For example, the occurrence of a species of the horseshoe crab, Tachypleus hoeveni, was reported for the first time off the coast of Moluccas. Later, the occurrence of two more species of the horseshoe crab, Limulus and Cancer mollucensis were reported from the same region.

The diversified Paleozoic group is represented by only four species with confirmed discernible morphology and three genera of the horseshoe crab worldwide. It is interesting to note that among these four species, Limulus polyphemus and Tachypleus tridentatus occur in a north-south-north direction, whereas, Tachypleus gigas and Carcinoscorpius rotundicauda occur in an east-west-east direction (Plate 4). This includes Malaysia. It is an interesting phenomenon to have an abundance of three species of the horseshoe crab, namely Carcinoscorpius rotundicauda, Tachypleus gigas and Tachypleus tridentatus along the coast of Malaysia. A record breaking large specimen of a T. tridentatus (7.5 kg) was collected for the first time along the coast of Paper Sabah. Although there are high degrees of similarity among all three Asian species of the horseshoe crab, T. gigas is morphologically more closely related to T. tridentatus than C. rotundicauda.

rotundicauda in a mangrove area

Optic nerves

Modern researchers find the horseshoe crabs' unique physiology of the heart and the functioning of its optic nerves interesting. The crab has altogether ten eyes which are located on the upper surface of the body. Out of these, two pairs are known as true eyes though the rest of the organs are also sensitive to light. The compound eyes of the horseshoe crab can polarize light and the crystal lining cones can concentrate it tenfold. This is an adaptation for their continuous living in translucent and dark muddy waters. The vision is also sensitive to infrared and ultraviolet lights. These structures help the horseshoe crabs to find their way on cloudy days when the sun is not visible - a small patch of clear sky is enough for the animal to locate the sun.

This principle has been useful in developing a compass for navigation in the Polar Regions where magnetic compasses are not reliable and celestial navigation is also difficult as the sun and stars are not visible in the long polar twilight. The design of eye structure of the horseshoe crab has also been successfully adopted in designing solar energy collectors. The optic nerve cells of the horseshoe crab are cross connected in such a way that when one nerve is stimulated, its partner nerve gets inhibited, resulting in an increase in contrast of the images.

A student of UMT working on a horseshoe crab

In 1967, Prof H K Hartline of USA shared a Nobel Prize for his notable work on the functioning of the optic nerves of the horseshoe crab. A video system based on this principle was developed by General Electrical Company USA to provide sharper television images and is presently in common use. The principle has also helped in developing improved radar systems.

Remedies and ornaments

For the past several decades, the horseshoe crab has had many uses for humans. It is believed that the early Indians used the tail spines of the crab as spear tips. After grinding the body, it was used as fertilizer for their fields and ponds. In India, some of the tribes inhabiting the north-east coast of Orissa still use the tail piece for relief from different types of pain. They tie the tail on their arms or prick their foreheads with it. It has been reported that the tail tips are used for healing arthritis or other joint pains and they are sold by faith healers in West Bengal, India. It is believed that Indians in the early days used to eat the appendages of the horseshoe crab. Additionally, the dead carapace of the crab is boiled with mustard oil and used for treating rheumatic pain in India. The Indians also used the hard carapace as a utensil for eating food and drinking water.

In China, people use the big carapace of the T. tridentatus species as a hat or ladle. They also use the tail of the horseshoe crab to make small ornaments. In Korea, fishermen sell hundreds of dried horseshoe crab shells as decorative pieces to tourists. In Singapore, Malaysia, and Borneo, the eggs of horseshoe crab are regarded a delicacy while people in the southern part of China blend the meat of the appendages with sauces and eat it as part of everyday food. Pregnant women in Singapore are said to eat the egg mass of the horseshoe crab for immunity for their foetus.

Diagnosing endotoxins

The blood of the horseshoe crab is generally known as haemolymph which is white in colour but it turns blue when exposed to air. Hence, horseshoe crabs are also known as blue blooded animals. The blue colour of the blood is attributed to haemocyanin, a copper based molecule that carries oxygen via the circulatory system of the crab. The white blood cells of horseshoe crabs are known as amoebocytes which are elliptical in shape and with a prominent nucleus. The cytoplasm in the blood cells is densely packed with large refractile granules consisting of elements required for blood clotting. The blood cells aggregate quickly when exposed to minute amounts of endotoxins or harmful bacteria. In the presence of harmful bacteria, the amoebocyte shape changes and,

The largest specimen of a horseshoe crab ever reported

TACHYPLEUS TRIDENTATUS (POCOCK)

Western & Southern Japan, Taiwan, Philiphines & North Borneo, Malaysia

LIMULUS POLYPHEMUS (MULLER)

Atlantic Coast of North America from Maine to Yucatan

TACHYPLEUS GIGAS (MULLER)

Bay of Bengal (North - East Coast), Thailand, Malaysia, Philiphines, Borneo & Torres Straits

CARCINOSCORPIUS ROTUNDICAUDA (LATREILLE)

Bay of Bengal, Thailand, Malaysia, Philiphines, Borneo & Torres Straits.

simultaneously, long processes extend from one amoebocyte to the other and appear in a pseudopod-like fashion.

This process is followed by the degranulation of the amoebocyte and release of all the clottable proteins or coagulogen that immobilizes and engulfs the endotoxins to form a firm gel or clot. Endotoxin is present in the triple layered cell wall of gram-negative bacteria which is composed of polysaccharide lipids and smaller peptides (lipopolysaccharide or LPS). The amount of endotoxins in a cell is reported to be 10-4 g. The release of endotoxins in the surrounding medium is due to the rupture or lysing of the bacterial cell wall. The toxicity of endotoxin is basically due to the presence of lipid-A fraction with LPS. The endotoxin has an extraordinary resistance to a wide range of temperature and to all types of detergents.

The LAL test

In human beings, the presence of these endotoxins is generally assessed with the help of a rabbit vaccine test. This method is not very sensitive and does not produce accurate results and it results in the death a large number of rabbits. In India, approximately 50,000 rabbits are sacrificed every day for the purposes of the detection of endotoxins. In addition, maintaining a rabbit colony requires large amounts of money. The other limitation in the rabbit vaccine test is that it takes about 8-10 days to get a clinical report and this delay to confirm results may result in the death of the patient.

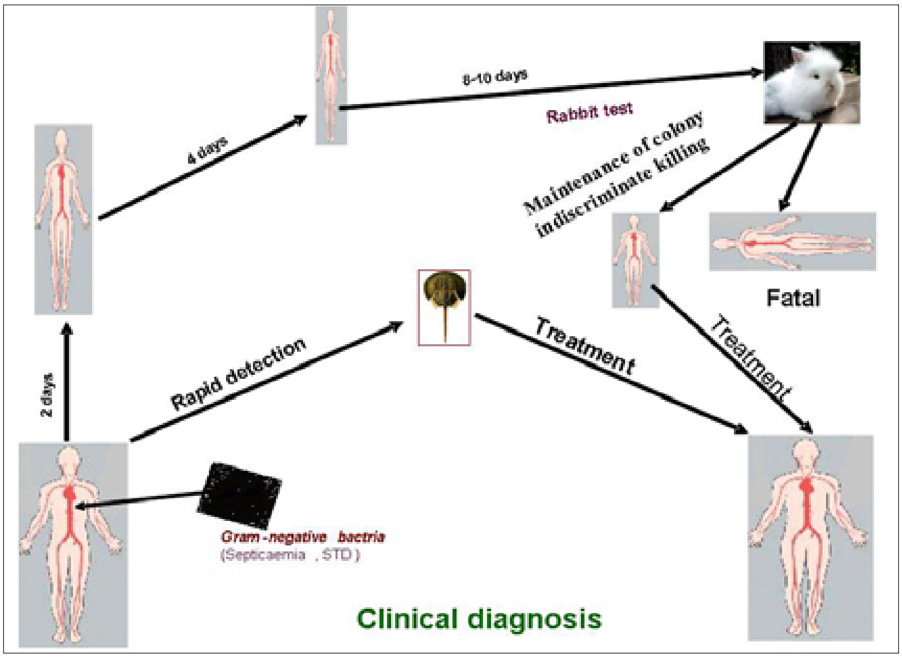
However, the pioneer research on endotoxin diagnosis with the help of a reagent prepared from the blood of the horseshoe crab has opened a new chapter in the history of the diagnosis of endotoxins. This process is popularly known as Limulus Amoebocyte Lysate or LAL test. Dr R Nandan, an Indian scientist based in USA, was the first to patent the LAL test in 1956.

Blue blood of horseshoe crab

In recent times, the biomedical properties of the horseshoe crab have assumed greater importance due to the unanticipated marketing of Limulus Amoebocyte Lysate as an endotoxin tester in food, drug and pharmaceutical industries. The lysate test has been proved to be a better substitute for the rabbit vaccine test because of its efficiency in terms of quick results and costs.

With the help of lysate, the pyrogen test is simple, sensitive, accurate and easy to perform. The test does not require any special type of equipment. The test sample (0.1 ml) is mixed with lysate (0.1 ml) in a sterile test tube and incubated in a water bath at a constant temperature of 370 C for a period of two hours. If the solution is clear, liquid, and runs down the wall of the test tube, the sample is considered to be free from gram-negative bacteria. But, if the solution turns into a firm opaque gel and sticks to the bottom of the test tube when inverted, the sample is considered to be contaminated with gram-negative bacteria.

The amoebocytes of the horseshoe crab have a clotting enzyme - lysate which clots and forms a firm opaque gel in no time when it is exposed to minute amounts of endotoxin or bacterial pyrogens. The rate of gelation of lysate is directly related to the concentration of endotoxins, which in turn, is dependent on the protein content of lysate. In lysate, the enzymatic activity basically controls the rate of gelation at a particular



concentration of endotoxin. Lysate is so sensitive that it can detect one millionth of a billionth gram of endotoxin in just less than an hour.

The endotoxin testing by lysate is currently a promising and sensitive method for the detection of endotoxins (gram-negative bacteria), even up to 100 pg/ml. It has become a basis for the in vitro test for endotoxins associated with parenteral pharmaceuticals and drugs which are intended for human use. The lysate test is recognized by drug regulatory authorities and industries in most countries and is commonly used as an end-product testing procedure for endotoxin in humans, animal injectables and drug products. Endotoxaemia, urinary infection, infection of the eye and bacterial meningitis can also be detected efficiently with the help of lysate. Very recently, it has also been discovered that the bacterium responsible for gonorrhea (Neiserria gonorthea) is a gram-negative bacterium, possessing an endotoxin which is extremely reactive with lysate (Plate 8).

Homeopathic healing

In 1970, the Federal Food and Drug Administration of USA permitted lysate tests for testing radiopharmaceuticals, viral vaccines, and various pharmaceutical products. Very recently, an important protein, which can detect vitamin B12 deficiency in human beings, has also been discovered in the lymph part of the haemolymph of the horseshoe crab.

The haemolymph of the horseshoe crab is also important in homoeopathic practices. Limulus medicine, introduced by Dr C Herring in homoeopathic therapies, is prepared from the haemolymph of the horseshoe crab. There are a number of applications of this medicine for the treatment of mental exhaustion, gastro-enteric symptoms, cholera, and drowsiness after sea bathing.

The gastro-intestinal tract of the human being is a house for gram-negative bacteria. The most common gram-negative bacteria belong to the families of Psudomonadaceae, Entererobacteriaceae (mainly Eschrichia coli), Bacteriodaceae and Neisseriaceae. For mutual benefit, an important symbiosis exists between the gram-negative bacteria and human being. A little LPS of gram-negative bacteria is beneficial in producing stimulation in our immunological surveillance and increases non-specific body resistance to different pathogens. Endotoxins have also been reported to induce necrosis and regression of malignant tumours and helpful in providing protection against the vascular and haemodynamic effects of radiation.

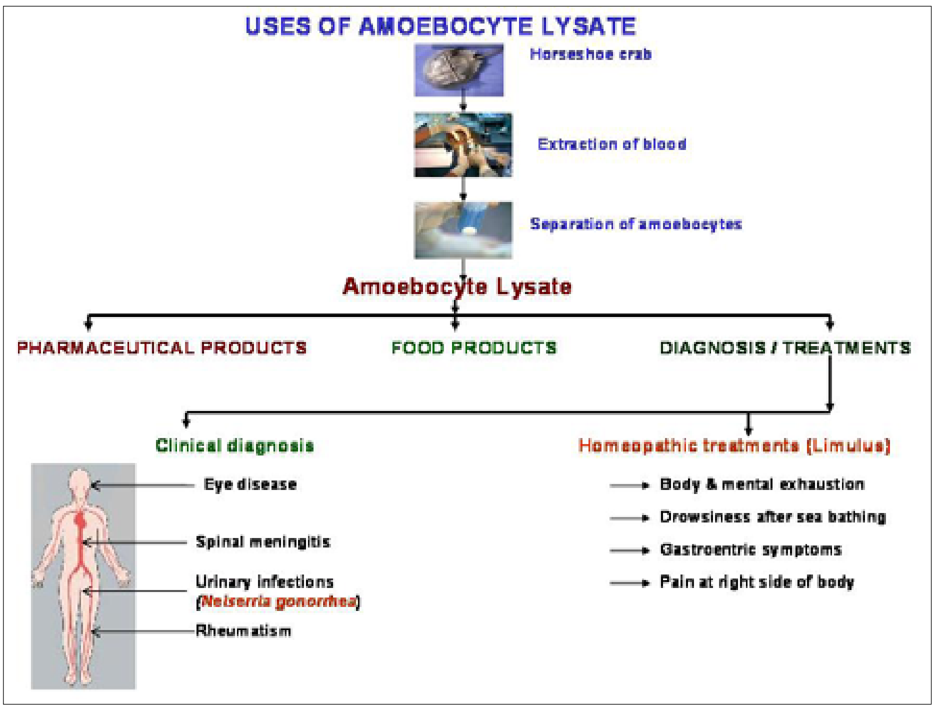
The prevention of absorbing endotoxins from the gastro-intestinal tract to the blood circulatory system is mainly controlled by the liver-regulated mechanisms which involve the detoxification of endotoxins. But, if by any chance even a small fraction of endotoxin of these gram-negative bacteria enters into the blood stream, it becomes extremely toxic and affects the body, especially the hypothalamus of the brain, the blood cells, the hormonal system, the metabolic system and the immunological surveillance system.

These endotoxins can result in the dissemination of intravascular coagulation, haemorrhage, hypertension, circulatory collapse or even death. The most common example of the effect of gram-negative bacteria is septicaemia where a 30% mortality rate has been reported, in spite of taking all the necessary precautions.

Diagnostic device

Recently, the National Aeronautics and Space Administration (NASA) of USA has realized the importance of the enzymes from horseshoe crab's blood cells and have included them in hand-held diagnostic devices for astronauts. The astronauts require a sensitive and rapid device as there are no clinical laboratories in space and they need to know quickly what is wrong when they are sick. Previously, the station astronauts used Petri dishes to culture and identify microorganisms found onboard. This process takes days.

A new device, called Lab-on-a-Chip Application Development Portable Test System - the LOCAD-PTS – which, although may not identify the bacteria, will deliver results quickly. The LOCAD-PTS is a handheld device that helps the crew to perform complex laboratory tests on a thumb-sized cartridge with a press of a button. Every thumb-sized plastic cartridge has four channels



and each channel contains a dried extract of horseshoe crab blood cells and a colourless dye. In the presence of bacteria and fungi, the dried extract reacts strongly to turn the dye to a green colour in a few minutes, confirming the presence of toxic materials - the more the green dye, the more the presence of microorganisms in the samples.

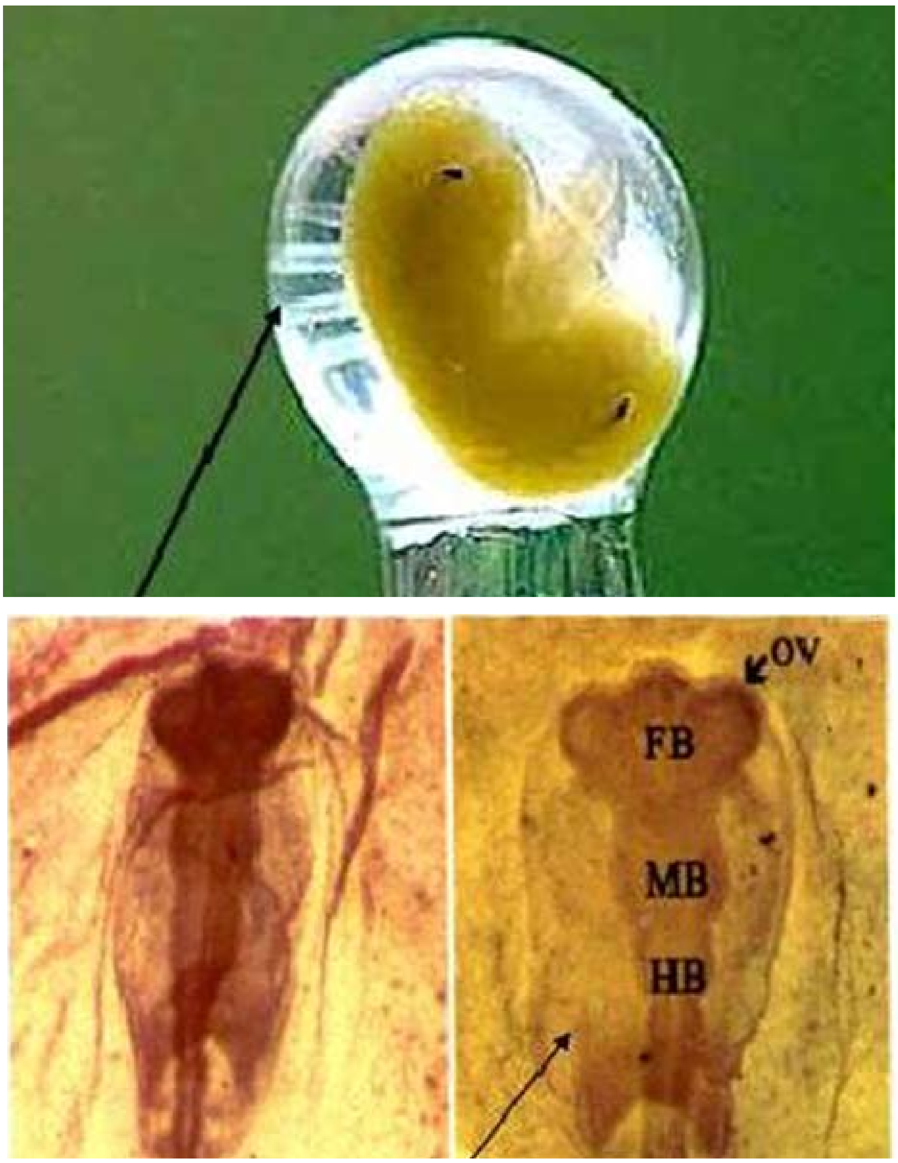
The LOCAD-PTS is meant to provide quicker warnings about any potential health threats. It takes only 15 minutes to identify germs swabbed from station instrument panels and other surfaces. The LOCAD tests have worked so well that NASA plans to permanently leave the device onboard the station.

Peri-vitelline fluid

The egg of the horseshoe crab is also surprising and valuable. It is larger than the sperm, greenish in colour, and surrounded by an extra cellular egg. The fluid between the outer envelops and embryo is known as peri-vitelline fluid. The peri-vittelline fluid contains important primitive types of proteins. An attempt was made to investigate the uses of peri-vitelline fluid. It has been observed that the peri-vitelline fluid helps in the proliferation of β-cells that may be used in insulin production in human beings. This may help in developing effective anti-diabetic drugs.

The peri-vitelline fluid contains proteins such as hemagglutinins and hemocyanin, which may have an important role during embryogenesis. Studies on chick developing embryo showed that the peri-vitelline fluid contained important biological active compounds that influenced early vertebrate embryonic development. Chick embryo explants cultured in vitro are used as a model system as several features of chick embryo development are remarkably similar to mammals, including human development and these embryos are more amenable to in vitro culture and experimentation.

It has been observed that peri-vitelline fluid tends to stimulate several aspects of chick embryonic development. For example, peri-vitelline fluid stimulates the development and differentiation of specific organs such as the brain and heart. This clearly indicates that peri-vitelline fluids contain molecules that stimulate growth and the differentiation of specific organs.



An embryo of a horseshoe crab with fluid and the effect of fluid on chick developing embryo

It has also been observed that peri-vitelline fluid of the developing embryo of horseshoe crab showed the presence of both anti- and pro-angiogenic activities useful in cardiac disorders and cancer therapies. This is an important observation, since inhibition of angiogenesis is a way to "suffocate" developing tumours by depriving them of vascular irrigation and oxygen supply. This is of biomedical and economic importance, especially for cancer research.

Organogenesis and cell replacement therapy will soon be a popular method to handle several dreaded medical complications including diabetes and degenerative diseases related to the heart. In vitro organ development and stem cell biology are two of the approaches that will underline the success of such a strategy. However, in order to achieve ideal organ growth, differentiation and/or regeneration, it would be essential to identify, purify and employ specific molecules, either individually or in various combinations.

The same is true for successful applications of stem cell biology wherein one needs to provide a defined milieu to naive stem cells so as to coax them to take one or the other developmental pathway. The possible source for such novel molecules is, again, the amazing horseshoe crab. The PVF of the Indian horseshoe crab contains peptide(s) capable of positively influencing differentiations of specific organs. Such peptides are likely to be present in minute quantities and may be as proteins.

Threat of extinction

The eggs of the horseshoe crab are reported to be eaten by striped bass, striped killi fish, silver perch, northern king fish, Atlantic silverside and flounders. The appendages and gill hooks of the young limuli are sought after by the puffer and cat fish. Plovers, sand pipers and gulls are also considered to be regular predators of the horseshoe crab eggs and larvae in the beach nest. The other predators causing considerable damage to the horseshoe crab population are tiger sharks, leopard sharks, loggerhead turtles, rays and sword fish. The horseshoe crab also has a great fear of mosquitoes and will die if bitten by a mosquito.

In most Asian countries, the local fishermen are not fully aware of the economic importance of the horseshoe crab and discard them. This is because the horseshoe crabs damage their fishing nets when they are trapped. Fishermen usually catch these crabs and throw them on the shore, leading to a high mortality rate of the animals. In addition, the indiscriminate exploitation of the horseshoe crab for food also threatens its extinction all over the world.

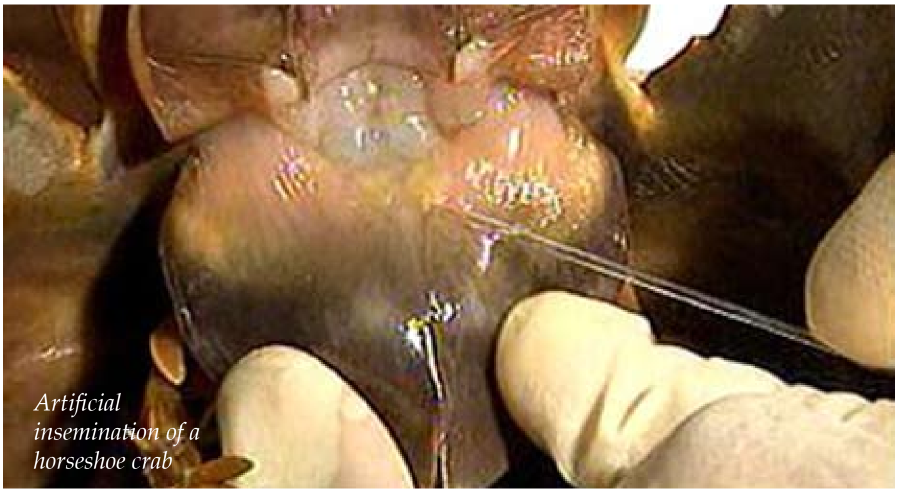
In the USA itself, about 50,000 brooder crabs are sacrificed every year for the preparation of Limulus Amoebocyte. This activity has resulted in the considerable depletion of the horseshoe crab population along the coast of the USA. Japan was the first country to realize the declining population of the horseshoe crab (T. tridentatus) and subsequently implemented conservative measures by dedicating a coast as a national monument for horseshoe crabs. The decrease in the population of the horseshoe crab in several places is also the result of the degradation and destruction of their habitat, especially their breeding ground,.

In Malaysia, research on the biotechnological potential of the horseshoe crabs has been minimal. Some researchers in Malaysia, particularly from University Putra Malaysia have studied the characterization of its blue blood, its reproductive biology and mass production. Studies on T. gigas were limited to its morphology and the developmental stages of the embryo. There is an urgent need to carry out comprehensive biotechnological studies on the three species of horseshoe crabs found along the coasts of Malaysia.

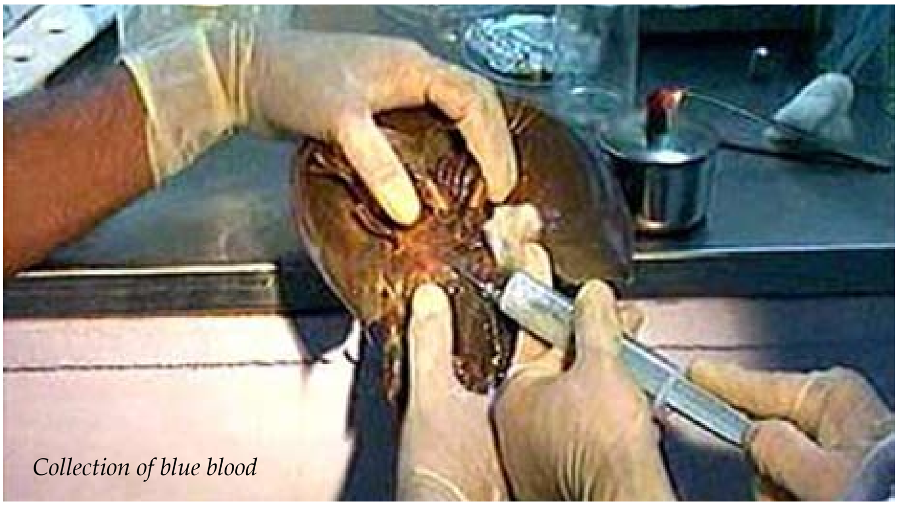
Conservation of horseshoe crabs

As the horseshoe crab has become significant in biomedical research, it is essential to protect this valuable creature from extinction. It is possible to rear and breed the horseshoe crab under confined environments as the animal is hardy and can tolerate differences in temperature, salinity and other environmental conditions. The Institute of Tropical Aquaculture, University Malaysia Terengganu has developed the technique for artificial breeding of the horseshoe crab with the help of electrical stimuli. The non-destructive extraction of the blood without sacrificing the horseshoe crab is also one of the important techniques standardized at the Institute of Marine Biotechnology of University Malaysia Terengganu.

As the horseshoe crab regularly migrates towards the shore for breeding purposes, efforts should be made to protect their breeding beaches from destruction and environmental degradation. Environmentalists should ensure regular planting of trees along the coasts to control beach erosion, protecting sand dune vegetation on the beaches, ban the removal of sand gravel for construction purposes from the breeding beaches, ban the deforestation of mangroves, and ensure the technique of removal of blood without sacrificing and disturbing the normal life cycle of the horseshoe crab. These measures will protect this valuable creature from becoming extinct.



Artificial insemination of a horseshoe crab



Collection of blue blood

This marine creature can play a vital role in improving the economy of the people of Malaysia. Besides meeting the local demand of the horseshoe crab for research and other purposes, there is financial gain in exporting the final or semi final products to companies that are actively involved in producing pharmaceutical products from horseshoe crabs on a commercial scale.

Such a project would require a dedicated and competent team, as well as the polic makers to ensure implementation. The team should comprise economists, urban specialists, architects, marketing survey specialists, medical specialists, as well as research administrators. The active and sincere support from the local authorities will play a vital role in implementing such an important programme for the benefit of society.