VALUABLE BLUE BLOOD

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INTRODUCTION

Aquaculture is the cultivation of aquatic organisms under controlled conditions, and it is estimated that about 600 animal and plant species have been successfully introduced to marine (mariculture) or freshwater farming, mainly for food production. Based on the organism raised, aquaculture can be classified into fish, crustacean, mollusc, and seaweed farming. In 2018, the shelled mollusc (17.7 million tonnes) represented 56.3% of marine coastal aquaculture and crustacean (9.4 million tonnes) in 114.5 million tonnes of total aquaculture. Horseshoe crabs are marine arthropods that have gained a keen interest in their precious blue blood. It finds a crucial role in the pharmaceutical industry as a pharmaceutical reagent utilized to examine bacterial endotoxin. The increasing demand for testing the vaccine during this pandemic and the burgeoning pharmaceutical industry has the potential to impact the horseshoe crab populations. Therefore, the farming techniques of the horseshoe crabs are prerequisites to conserving them and for blood extraction.

HORSESHOE CRABS

Horseshoe crabs have been around for more than 300 million years, making them even older than dinosaurs. They look like prehistoric crabs but are more closely related to scorpions and spiders. The horseshoe crab has a hard exoskeleton and 10 legs, which it uses for walking along the seafloor. Horseshoe crabs belong to the family Limulidae, currently represented by four species, including *Limulus polyphemus*, which is found along the eastern coast of North and Central America, and three Indo-Pacific species, *Tachypleus gigas, Tachypleus tridentatus* and *Carcinoscorpius rotundicauda* found in the Pacific Ocean and Indian Ocean

The horseshoe crab body is segmented into three sections. The first one is the prosoma or head. The name “horseshoe crab” originates from the rounded shape of the head because, just like the shoe on a horse’s foot, the head is round and U-shaped. It's the largest part of the body and contains much of the nervous and biological organs. The head has the brain, heart, mouth, nervous system, and glands—all protected by a large plate. The head also protects the largest set of eyes. Horseshoe crabs have nine eyes scattered throughout the body and several lighter receptors near the tail. The two largest eyes are compound and useful for finding mates. The other eyes and light receptors are useful for determining movement and changes in moonlight.

The middle section of the body is the abdomen or opisthosoma. It is somewhat triangular in shape, with spines on the sides and ridges in the centre. The spines are movable and help protect the horseshoe crab. On the underside of the abdomen are muscles used for movement and gills for breathing.

The third one is the horseshoe crab's tail, known as the telson. It's long and pointed, and although it looks intimidating, it is not dangerous, poisonous, or used to sting. Horseshoe crabs use the telson to flip themselves over if they happen to be pushed on their backs.

The female horseshoe crab is about one-third larger than the males. They can grow up to 18 to 19 inches (46 to 48 centimetres) from head to tail, while the males are approximately 14 to 15 inches (36 to 38 centimetres. Horseshoe crabs utilize different habitats depending on their stage of development. The eggs are laid on coastal beaches in late spring and summer. After hatching, the juvenile horseshoe crabs can be found offshore on the sandy ocean floor of tidal flats. Adult horseshoe crabs feed deeper in the ocean until they return to the beach to spawn. Many shorebirds, migratory birds, turtles, and fish use horseshoe crab eggs as an important part of their diet.

DIET: Horseshoe crabs like dining on worms and clams at night and eating algae. A horseshoe crab picks up food with appendages located in front of its mouth. Because it has no mandible or teeth, the horseshoe crab crushes food between its legs before passing it to the mouth.

LIFE HISTORY

Adult horseshoe crabs travel from deep ocean waters to beaches along the East and Gulf coasts to breed in the late spring and early summer. The males arrive first and wait for the females. When the females come to shore, they release natural chemicals called pheromones that attract the males and send a signal that it's time to mate. Horseshoe crabs prefer to breed at night during high tides and new and full moons. The males grasp onto the females and head to the shoreline together. On the beach, the females dig small nests and deposit eggs, then the males fertilize the eggs. The process can be repeated multiple times with tens of thousands of eggs.

Horseshoe crab eggs are a food source for numerous birds, reptiles, and fish. Most horseshoe crabs will not even make it to the larval stage before being eaten. If the egg survives, the larval horseshoe crab will hatch from the egg after about two weeks or more. The larva looks like a tiny version of an adult horseshoe crab but without a tail. Larval horseshoe crabs travel into the ocean water and settle on the sandy bottom of tidal flats for a year or more. As they develop, they will move into deeper waters and begin to eat more adult food. Over the next 10 years or so, the juvenile horseshoe crabs will moult and grow. The moulting process requires shedding small exoskeletons in exchange for larger shells. Horseshoe crabs go through 16 or 17 moults during their development. At around 10 years of age, horseshoe crabs reach adulthood. They are ready to start breeding and will migrate to coastal beaches in the spring. A horseshoe crab can live for more than 20 years.

CONSERVATION

Natural threats to horseshoe crabs are their late maturity, predation of juveniles and climate change, and anthropogenic threats include habitat loss and overharvesting. Beach developments hinder horseshoe crab breeding. *Limulus polyphemus* is internationally listed as vulnerable. Therefore, their conservation is more important now than ever.

VALUABLE BLUE BLOOD!

Nowadays, vaccine saves everyone's lives, especially during this pandemic. Thus, lifesaving vaccines are required to be highly pure, which means they should not be contaminated with any other impurities, such as endotoxin. The endotoxin is mainly produced by gram-negative bacteria, which

can be detected by a clotting factor called LAL (Limulus Amoebocyte Lysate). This clotting agent, LAL is extracted from the valuable blue blood of horseshoe crabs. Since invertebrates contain copper in the blood instead of iron in vertebrates, it appears blue in colour when oxidized otherwise, it's colourless. Amoebocytes of horseshoe crab contain a clotting factor called coagulogen, induced by the entry of endotoxin and cause clump formation through a series of reactions. Earlier, the use of LAL assay was slow and less efficient as rabbit assay was practiced to test the endotoxin. Fred Bang (1956) was the first person to report horseshoe crab blood's clotting activity against gram-negative bacteria. Further, in 1977, LAL assay was commercialized and got approval from USFDA. But horseshoe crab's blue blood is so expensive as its importance; per gallon of blood costs around $60000. Currently, the LAL test is considered the most reliable endotoxin detection test. But the question is, "Will it meet the increasing future demands?". Then, culture activities have to be undertaken to increase the production of horseshoe crab for its valuable blood.

Culture method

Horseshoe crab culture may be carried out indoors or outdoors systems. In most cases, indoor culture tanks made of concrete with a depth of 120 cm are utilized for horseshoe crab farming. If ponds are built outdoors, the site should be located in upper to mid-intertidal areas in order to facilitate water exchange. Razor clam and oyster meat are favourite food organisms. However, occasionally, it consumes frozen fish. Experimental results have shown that rate is closely correlated to water temperature, with the highest feeding rate at 20-28˚C. At temperatures above 30˚ C or below 16˚C, the feeding rate declines.

The breeding techniques of horseshoe crabs have been standardized by CSIR-NIO. The live specimens were collected from the wild, the eggs were collected from the mature female using electrical stimulus, and the fertilizing fluid was collected from the male species. Then, the eggs were artificially inseminated and bathed with seawater to facilitate fertilization.

Water quality parameters of horseshoe crab in RAS culture

Parameters	Range
Water temperature	17.1˚C-21.7˚C (desired range: 15.0–25.0°C)
pH	7.5 to 8.0 (desired range: 7.5–8.8)
Dissolved oxygen	6.3–11.1 mg/L (desired range: >3.0–5.0)
Ammonia	<1.0–3.0 ppm.

Blood collection:

The animals used for blood collection are initially disinfected with 1% iodine tincture and 70% alcohol. The hypodermic needle (18G) is inserted into its artery to collect the blue blood, which flows into a clean container filled with an anticoagulant such as caffeine or theophylline. pH value of blood is buffered to 7.2 with a tris-HCI solution. About 80-100 ml of blood is collected every time from one mature crab, Blood extraction from one specimen can be done 4-8 times a year.

The isolation of haemocytes can be carried out in two methods. Blood with anticoagulant is left to stand for 48 hrs, during which the amoeba cells will sedimentate while the plasma remains on the top layer. Plasma is slowly decanted, and cells are washed 3 times with a 3 % NaCl solution. Under bacteria-free conditions, cells with 3 parts of 0.9% NaCl are

homogenized for 2 hrs and then refrigerated for 24 hrs.

Haemocytes separated from plasma by centrifugation are washed with a 3% NaCl solution. Haemocytes were diluted with distilled free pyrogen water and placed in refrigeration for 48 hrs. Two days later, the cell content is isolated.

Reagent purification:

The reagent purification is carried out by mixing one volume of haemocytes with two volumes of chloroform. It then cooled, shaken for two hours and transferred to a separator funnel to isolate the cells. CACT, MgSO4 and NaCl are added to the isolated cell portion until the concentration of calcium and magnesium ions is 0.03-0.05 ml. Finally, the desiccation is carried out by freeze-drying.

Conclusion

Aquaculture is the sunrise sector that feeds the globe by providing a required protein. Cultivable aquatic organisms have multiple potential benefits besides being a food source. Among them, pharmaceutical use has gained more importance. However, vaccine production is a meagre segment of the medical industry, it saves our lives. LAL test is more important for testing the endotoxin contamination in produced vaccines. To bridge the gap between the demand and supply of horseshoe crab's valuable blood, horseshoe crab aquaculture is the only possible and effective solution. Doing the right things at the right time is very much important, so this is a time to produce more horseshoe crabs. Try to be a smart person. Start your own horse-shoe crab farm.