First Place Honors 2001 Sussex County Delaware, Science Fair:

Factors that Affect Horseshoe Crab Nest Site Selection

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Abstract

Horseshoe crabs are remarkable creatures that have survived nearly 500 million years. They are highly valued in several diverse applications. Limulus amebocyte lysate (LAL), a product of a very specialized cell extracted from horseshoe crab blood, is vital to the detection of endotoxin contamination in our parenteral medications and vaccines. No other test for presence of endotoxin is as valid, or as dependable as the LAL assay. Horseshoe crabs are also used in the medical research of vision. In addition, they possess an essential function in the ecosystem with shorebirds. Unfortunately, the Limulus polyphemus population is declining rapidly, due to their recent increased harvest as use for eel and conch bait. There are two ways to help solve this problem. One way is to prevent further depletion of the species by decreasing the number being killed. The other way is to increase the number being born. This can be accomplished by preserving conditions favored by horseshoe crabs for spawning. Some of these conditions may or may not include sand grain size, water temperature, water salinity, and beach slope. I examined each of these factors on five beaches off the Delaware Estuary (where horseshoe crabs frequent). My conclusion supports my hypothesis that spawning horseshoe crabs prefer high water temperatures (above 20C), and low water salinity (between 20 and 30 ppt). However, I reject my hypothesis that horseshoe crabs favor smaller sand grain sizes in nest site selection and my results for beach slope are inconclusive.

Introduction

Can you imagine if a drug you depended on to live, like insulin, or a medication you needed to cure a disease, like intravenous antibiotic, actually wound up poisoning you? Well, thanks to a test called Limulus amebocyte lysate (LAL), one does not have to worry about this ever happening. It is because of this test, that millions of lives are saved every year. Without LAL, derived from horseshoe crab blood, there would be no other reliable test for the safety of our parenteral medications and vaccines.

The Limulus amebocyte lysate or LAL assay is used to test products for bacterial toxins, specifically, endotoxin. There are two different kinds of bacterial toxins, endotoxin and exotoxin.

Endotoxin is a poisonous substance that is found in the cell wall of gram-negative bacteria. Endotoxins are made up of lipopolysaccharide and lipoprotein complexes. Exotoxin is also a poisonous substance. Gram-positive bacteria, however, secrete it. Most exotoxins, which can cause diphtheria, tetanus, cholera, and whooping cough, can be destroyed by high temperature. Endotoxin, however, is immune to boiling, making it much more of a threat.

Endotoxin is released by gram-negative bacteria, such as E. coli. This generally occurs when they reproduce or die. Endotoxin is very toxic. Only a few nanograms are needed in the bloodstream to infect humans with certain diseases, particularly fever and diarrhea (A nanogram is equal to one-billionth of a gram.) Larger amounts can even cause irreversible shock, which results in death. Endotoxin is sometimes called pyrogenic because it is well known for producing fever (Pyrogen means fever.)

There is a cell found in horseshoe crab blood called amebocyte. This is the substance that is able to react in the presence of endotoxin. In nature, when a horseshoe crab is attacked or damaged, amebocyte cells swarm to the area of the wound and form a gel. This clot prevents bacteria from traveling throughout the horseshoe crab's circulatory system. This reaction is an evolutionary protective mechanism that has allowed horseshoe crabs to survive for almost 500 million years.

Not only will amebocyte form a clot in the presence of endotoxin in the bloodstream of a horseshoe crab; it will also gel in an in-vitro setting when endotoxin is introduced to it. The reason the gel forms is endotoxin activates the clotting enzyme, which then cleaves (splits) a soluble substrate. However, the activation of the clotting enzyme is not direct. There are intermediate steps prior to the activation of the clotting enzyme. These intermediate steps amplify the response to endotoxin and help give the LAL test its extraordinary sensitivity. ("Sensitivity" is the lowest endotoxin concentration to cause a clot to form under standard conditions.) Activated clotting enzyme cleaves a peptide from the inner portion of the coagulogen molecule. The two remaining peptides are linked by disulfide bridges to form insoluble coagulin (gel). Coagulin molecules associate to form a gel (Dawson, 1).

The U.S. Food and Drug Administration requires all parenteral drugs to be tested using limulus amebocyte lysate, the clotting agent derived from Limulus hemolymph. The performance of this test is necessary before a product is released for use in humans. No other test is as easy or as dependable as the LAL assay.

Before the LAL test was marketed, scientists would determine endotoxin presence by injecting the substance being analyzed into a rabbit's ear. If the rabbit developed a fever, scientists concluded that endotoxins were present. If a rabbit did not develop a fever, endotoxins were supposedly "not present." This test is much less reliable than the LAL assay due to its lack of strong sensitivity. "The LAL test is at least one hundred times more sensitive than the rabbit test" ("Pyrogens, Still a Danger," 4). In addition, the LAL test is regarded to be *specific* for the presence of endotoxins. The rabbit test is not. The rabbit test is also much more complex and takes a lot longer to perform than the LAL assay.

The LAL test has hugely changed the face of drug testing. There are many advantages to using this test in lieu of the rabbit test. Besides its extraordinary validity, the LAL test is also more humane since rabbits are not used.

(Horseshoe crabs are not harmed either; they are returned to the water after some of their blood is extracted.) It is sensational that an advancement of science is serving to also advance the humane treatment of animals as a side effect.

The Horseshoe crab is also used for vision studies because its complex eye structure is similar to the human eye. Neurophysiologists think these studies might lead to some answers about how the human eye and brain work together (Jamison, 4). In addition, horseshoe crabs play a vital role in the ecosystem. "Their eggs are the principal food source for over a million shore birds that arrive exhausted in the Delaware Bay area each year during spring migration" (Mowbray, 1). (This has little or no impact on horseshoe crab numbers because they only eat the exposed eggs on the surface that do not hatch anyway.) Horseshoe crabs are magnificent creatures that have been around for 500 million years. They deserve to be preserved even if they were not so beneficial to man and other species.

Unfortunately, their population has been declining dramatically, due to their recent increased harvest as use for eel and conch bait. In the early 1990s, European and Asian eel stocks and Caribbean conch stocks ran dry. As the price of eel and conch soared, Delaware Bay fishermen shifted their focus to meet the new demand. "Word spread quickly that horseshoe crabs, seemingly limitless and ridiculously easy to collect, make perfect bait for eel and conch" (Hawes, 5). Individual crabs sold for up to two dollars each. It is estimated that the commercial harvest, from the northeastern Atlantic coast, has increased from 10,000 pounds in 1969 to over 5,000,000 pounds in 1996, with a significant increase in 1996 (Rowan, 1). Between 1993 and 1996 alone, the harvest has increased by more than 240% ("Species Spotlight: Horseshoe Crab," 2).

There has also been a substantial decrease in spawning. It was estimated that, in 1990, there was a spawning population of 1,200,000 crabs in the Delaware Estuary; in 1995, there were fewer than 200,000 (Jamison, 1). This shows an estimated 85% drop in the population in just five years. The number is still decreasing very rapidly.

There are two ways to help solve this problem. One way is decreasing the number being killed. What makes horseshoe crabs such perfect bait for eel and conch is the scent female's release to attract males to spawn. This scent also attracts eels and conch. University of Delaware researchers have isolated the compound from horseshoe crabs that attracts American eel, and development of a suitable matrix for the compound is underway. The compound will serve as a bait extender until a cost-effective synthetic version can be produced (Gauvry, 8).

Another effort that is underway, to prevent further depletion of the species, is the manufacture and distribution of bait bags, recommended by the Atlantic States Marine Fisheries Commission. "Fewer crabs are required when bait bags are used and the bags extend the effective fishing time of the bait by preventing bait loss to non-target species" (Gauvry, 1).

"The successful use of these innovations could significantly reduce the use of horseshoe crabs as bait, an exploitation that accounts for the mortality of several million horseshoe crabs annually" (Gauvry, 8).

Another way to reduce the number of horseshoe crabs being killed is restrictions on harvesting.
For instance, in June of 2000, Governor Carper announced that Broadkill Beach should be a horseshoe crab sanctuary by the end of the summer, a declaration that will officially ban the harvesting of the species along the community's 3.8-mile coastline (Smith, 1).

Besides decreasing the number of horseshoe crabs being killed, the other way to help solve the problem of the decreasing population is to increase the number being born. This can be accomplished by preserving conditions favored by horseshoe crabs for spawning. Some of these conditions may or may not include salinity of the water, temperature of the water, grain-size of the sand, and slope of the beach. I have decided to examine each of these conditions on five different beaches: Lewes, Roosevelt Inlet, Broadkill Beach, Prime Hook Beach, and Fowler Beach. I will compare this data to the numbers of horseshoe crabs counted on each beach in the 1999 census and attempt to determine what factors affect horseshoe crab nest site selection. I will then suggest ways to preserve the horseshoe crab population by increasing the number being born. My hypothesis is that horseshoe crabs prefer a small grain size of the sand, a high temperature of the water (above 20C), a low salinity of the water (between 20 and 30 ppt), and a steep seaward slope of the beach.

Grain Size Experiment
(Performed 5/7/00 - 5/10/00)

Materials:
- Measuring tape (2 meters +)
- Tablespoon
- Centimeter ruler
- 10 petrii dishes
- Tape
- Sieve shaker
- Sieve series (3" sieves)
- Clean sheets of paper
- Top loader balance
- Weighing dish

Procedure:
1. Label each of the 10 petrii dishes as follows:
    - Lewes: marker
    - Lewes: 2 meters north of marker
    - Roosevelt Inlet: marker
    - Roosevelt Inlet: 2 m north of marker
    - Broadkill Beach: marker
    - Broadkill Beach: 2 m north of marker

- Prime Hook Beach: marker
- Prime Hook Beach: 2 m north of marker
- Fowler: marker
- Fowler: 2 m north of marker

2. Go to the first beach (Lewes) at low tide.
3. Set a marker where you will collect the first sample of sand from that beach.
4. In the intertidal zone of the beach, where you placed your marker, use the centimeter ruler to measure down 5 cm in the sand.
5. Collect a three-tablespoon sample of sand (5 cm down) and place it in the petrii dish labeled Lewes: marker and secure the lid with tape.
6. Repeat steps 4 and 5, collecting the sand sample from 2 m north of your marker and placing it in the petrii dish labeled Lewes: 2 m north of marker.
7. Repeat steps 2 through 6 for Roosevelt Inlet.
8. Repeat steps 2 through 6 for Broadkill Beach.
9. Repeat steps 2 through 6 for Prime Hook Beach.
10. Repeat steps 2 through 6 for Fowler Beach.
11. After all sand samples are collected, take the lids off all the petrii dishes and allow them to dry in the sun for two days.
12. After all of the samples have dried completely, replace and secure the lids on all of the petrii dishes.
13. Take the sand samples to the University of Delaware Lab, at Cape Henlopen (where the sieve shaker is).
14. Create a sieve series, to use in the shaker to separate samples into appropriate size fractions:
    - Top pan
    - 2.000 mm
    - 1.000 mm
    - 0.500 mm
    - 0.355 mm
    - 0.250 mm
    - 0.180 mm
    - 0.125 mm
    - 0.090 mm
    - 0.063 mm
    - Bottom pan (< 0.063 mm)
15. Weigh out 30 grams of sand from first sample (Lewes: marker). (Note: sample quantity is not critical to the results. It is the end weight that is important.)
16. Transfer sand from weighing dish to the top of the stack of sieves.
17. Cover, tighten, and secure the sieves into the shaker.
18. Put power ON and leave the sieve shaker to run for 15 minutes.
19. After the sample is finished, remove the sieve series from the shaker.
20. Invert each sieve on a clean sheet of paper, tapping the sides of the sieve to transfer the sediment.
21. Transfer sediment grains from top sieve (2.000 mm) into a tared weighing dish.
22. Record weight to 0.01 g. into data sheet.
23. Repeat steps 21 and 22 for each sieve.
24. Repeat steps 15 through 22 for the rest of the samples.

Temperature and Salinity Experiment
(Performed 6/26/00 (during spawning season))

Materials:
Temperature and salinity measuring probe

Procedure:
1. Turn power ON.
2. Dip the probe into the water at the water line on Lewes Beach.
3. Record the temperature, in degrees Celsius, to 0.01C.
4. Record the salinity, in parts per thousand, to 0.01 ppt.
5. Repeat steps 1 through 4 for Roosevelt Inlet.
6. Repeat steps 1 through 4 for Broadkill Beach.
7. Repeat steps 1 through 4 for Prime Hook Beach.
8. Repeat steps 1 through 4 for Fowler Beach.

Beach Slope Experiment
(Performed 11/5/00)

Materials used include:
Measuring tape (8 meters +)
Set of two slope measuring poles (with 0.10-meter markings)
2 boards with levels

Procedure:
(Note: This procedure requires the assistance of two people.)
1. On Lewes Beach, lay out the measuring tape from the water line, eight meters back. (8 m should be the water line and 0 m should be eight meters back.)
2. Place a leveling board at 0 meters, making sure it is level.
3. Assistant #1 (the "sighter"): Hold the sighting stick, level, on top of the board at 0 meters.
4. Assistant #1: Make your eye level with the top of the sighting pole and the horizon.
5. Assistant #2: Help keep the measuring tape straight on the sand. Assistant may have to stand on the tape, especially if it is windy the day of the experiment.
6. Principal experimenter: Hold the measuring pole, level, on top of the other leveling board, at 2 meters.
7. Assistant #1: Keeping your eye level with the horizon, observe where the top of the sighting pole meets the measuring pole. (This tells you the distance above or below mean sea level.)
8. Assistant #1: Record the change in elevation, either positive or negative, to 0.01 m.
9. Repeats steps 5 through 7, at 4 meters, 6 meters, and 8 meters.
10. Repeat steps 1 through 9 for Roosevelt Inlet, Broadkill Beach, Prime Hook Beach, and Fowler Beach.

Results: Grain Size Analysis
5/9/00 and 5/10/00

Sample I.D. Lewes: Marker, Lewes: 2 m North

Sieve Size (mm)	Dry Weight (cum)	Dry Weight (cum)
2.000	08.00 g	08.63 g
1.000	20.03 g	20.17 g
0.500	28.26 g	28.50 g
0.355	28.66 g	28.93 g
0.250	29.29 g	29.52 g
0.180	29.59 g	29.79 g
0.125	29.62 g	29.82 g
0.090	29.62 g	29.82 g
0.063	29.62 g	29.82 g
Bottom Pan (< 0.063)	29.63 g	29.82 g

Sample I.D. Roosevelt Inlet: Marker, RI: 2 m North

Sieve Size (mm)	Dry Weight (cum)	Dry Weight (cum)
2.000	00.85 g	01.58 g
1.000	05.06 g	07.17 g
0.500	19.01 g	21.73 g
0.355	24.76 g	26.64 g
0.250	28.25 g	28.94 g
0.180	28.98 g	29.30 g
0.125	29.04 g	29.33 g
0.090	29.04 g	29.33 g
0.063	29.04 g	29.33 g
Bottom Pan (< 0.063)	29.04 g	29.34 g

Sample I.D. Broadkill Beach: Marker, Broadkill Beach: 2 m North

Sieve Size (mm)	Dry Weight (cum)	Dry Weight (cum)
2.000	10.29 g	14.07 g
1.000	15.78 g	20.24 g
0.500	25.60 g	27.57 g
0.355	28.10 g	28.78 g
0.250	29.06 g	29.18 g
0.180	29.46 g	29.52 g
0.125	29.61 g	29.62 g
0.090	29.62 g	29.63 g
0.063	29.63 g	29.63 g
Bottom Pan (< 0.063)	29.63 g	29.63 g

Sample I.D. Prime Hook Beach: Marker, Prime Hook Beach: 2 m North

Sieve Size (mm)	Dry Weight (cum)	Dry Weight (cum)
2.000	02.58 g	01.29 g
1.000	19.14 g	16.49 g
0.500	29.18 g	29.48 g
0.355	29.33 g	29.56 g
0.250	29.42 g	29.61 g
0.180	29.46 g	29.64 g
0.125	29.49 g	29.65 g
0.090	29.50 g	29.66 g
0.063	29.51 g	29.67 g
Bottom Pan (< 0.063)	29.53 g	29.68 g

Sample I.D. Fowler: Marker, Fowler: 2 m North

Sieve Size (mm)	Dry Weight (cum)	Dry Weight (cum)
2.000	05.15 g	06.54 g
1.000	13.99 g	15.81 g
0.500	23.27 g	26.31 g
0.355	24.93 g	27.56 g
0.250	26.49 g	28.46 g
0.180	27.35 g	28.90 g
0.125	28.15 g	29.23 g
0.090	28.44 g	29.32 g
0.063	28.73 g	29.41 g
Bottom Pan (< 0.063)	29.41 g	29.60 g

Results: Temperature and Salinity

6/26/00

Beach	Temperature	(Time)
Lewes	16.00C	4:00 PM
Roosevelt Inlet	17.90C	4:30 PM
Broadkill	22.90C	5:00 PM
Prime Hook	23.90C	5:45 PM
Fowler	24.20C	6:15 PM

Beach	Salinity	(Time)
Lewes	28.20 ppt	4:00 PM
Roosevelt Inlet	27.00 ppt	4:30 PM
Broadkill	24.90 ppt	5:00 PM
Prime Hook	24.30 ppt	5:45 PM
Fowler	23.20 ppt	6:15 PM

Results: Beach Slope
11/5/00

Beach	(Time)	2 meters	4 meters	6 meters	8 meters
Fowler	2:20 PM	- 0.20 m	- 0.30 m	-0.50 m	- 0.80 m
Prime Hook	2:40 PM	- 0.15 m	- 0.38 m	- 0.60 m	- 0.80 m
Broadkill	3:10 PM	- 0.15 m	- 0.25 m	- 0.35 m	- 0.50 m
Roosevelt	3:40 PM	- 0.10 m	- 0.10 m	- 0.40 m	- 0.80 m
Lewes	3:50 PM	- 0.80 m	- 0.12 m	- 0.30 m	- 0.50 m

Discussion
Analysis of Data

The 1999 Horseshoe Crab Census data shows that Fowler's Beach had a total of 124,230 horseshoe crabs for the spawning season. Broadkill Beach had 20,599 and Prime Hook Beach had 37,938. Lewes and Roosevelt Inlet, however, were not included in the census. To find out why, I emailed Bill Hall at the University of Delaware. He wrote back that the beaches are chosen randomly. He also stated that there are very few horseshoe crabs on Lewes and Roosevelt Inlet. With this information, I am considering Lewes Beach and Roosevelt Inlet to have fewer horseshoe crabs than Broadkill, Prime Hook, and Fowler Beach.

This data shows that Fowler's Beach had, by far, the most spawning horseshoe crabs of the five beaches I examined. My preliminary conclusion is, now, that the conditions on Fowler's Beach are most highly favored by horseshoe crabs for spawning purposes.

Fowler had the smallest grain size of the sand out of all five of the beaches. This, however, is most likely not a condition that makes Fowler so suitable for horseshoe crab spawning. Porous, well-oxygenated sediments, and not tiny, compact sand particles, as are found at Fowler, are most suitable for egg survival and development. However, horseshoe crabs are tolerant of a wide range of oxygen levels. Physiological changes in the blood enable them to survive anaerobic conditions (Horseshoe Crab Plan Development Team, 61). The small grain size probably does not have an appreciable effect on spawning activity and is overshadowed by the other conditions that are the most suitable. I, therefore, reject my hypothesis that spawning horseshoe crabs favor small sand grain sizes.

Fowler's Beach had the highest water temperature of all five beaches. Its temperature was 24.20C. Temperatures of 20C, or greater, are the most suitable for spawning activity. This is because, at temperatures below 20C, a state of dormancy is initiated and production of ecdysone (hormone that triggers molting) is curtailed, which inhibits egg development (Horseshoe Crab Plan Development Team, 61). My data for Lewes Beach and Roosevelt Inlet further proves this. Their water temperatures were both below 20C and they had the fewest number of horseshoe crabs. I accept my hypothesis that horseshoe crabs prefer a high water temperature for spawning.

Fowler's Beach had the lowest water salinity of all five beaches; however, all of their salinities were very close. Horseshoe crabs nest on beaches where egg development is maximized. Optimal salinities from fertilization to hatching are in the range of 20 to 30 ppt (Horseshoe Crab Plan Development Team, 61). All of the beaches' water salinities I examined were greater than 20 ppt and less than 30 ppt. Due to this, it is probable that salinity is not the causative factor for the higher number of horseshoe crabs on Fowler's Beach. However, I accept my hypothesis that a low salinity, between 20 and 30 ppt, is favored by horseshoe crabs for spawning purposes.

Fowler and Prime Hook beaches both had the steepest continuous seaward slopes. Prime Hook had the second greatest number of spawning horseshoe crabs. Reproductive success is greatest on beaches with adequate slope that encourages return to the water. Horseshoe crabs generally travel downslope after spawning and appear to become disoriented on flatter beaches. They, however, show rapid seaward orientation on beaches with steep seaward slopes. Beach slope is more significant than vision in orientation behavior (Horseshoe Crab Plan Development Team, 64). This data supports my hypothesis that horseshoe crabs favor beaches with steep seaward slopes in nest site selection; however, results are questionable related to a segment of the experiment performed in such a way as to yield inconclusive results. (See below)

I measured slope from the water line at high tide. In which case, I did not obtain slope measurements from the intertidal zone, where horseshoe crabs actually lay their eggs. I, therefore, do not know what the slope is like at low tide. They lay their eggs in the intertidal zone of beaches because there is suitable oxygen-moisture ratio here. If they were to lay their eggs above the high-tide line, there would not be enough moisture in the sand and they would dry out and die. In order to accurately determine the affect beach slope has on nest-site selection, my experiment needs to be repeated, measuring the slope from the low-tide line at low tide. I plan to do this next year as I continue my research.

In addition, the slope on Lewes Beach and Roosevelt Inlet was very abrupt in some places, while the slopes on the other three beaches were relatively continuous. I would also like to investigate this further, in the future, to attempt to determine what effects, if any, this has on the suitability of the beach slope.

Future Research

Some other areas worth further research include the following: 1) repeating my beach slope experiments at low tide for each of the beaches. 2) Horseshoe crabs are known to return to the same beaches to spawn every year. However, the mechanism by which they "remember" preferred spawning locations is not completely understood. I, again, contacted Bill Hall, Marine Biologist, sharing with him my thoughts on horseshoe crabs remembering spawning locations. We discussed the possibility that it might be similar to salmon's way of finding their spawning grounds. "Salmon appear to have an innate compass or "search recognition" mechanism that operates independently of astronomical or physical signs.

Some scientists theorize that this internal compass uses the infinitely small electrical voltages generated by the ocean currents as they travel through the earth's magnetic field. Others believe that salmon's homing mechanism may take its cues from the varying salinities of the water or specific smells encountered along the journey" (Science and Technology Department of the Carnegie Library of Pittsburgh). It would be interesting to learn if and how the horseshoe crabs mechanism for locating spawning grounds relates to the salmon's.

Conservation Ideas

Some ways to preserve the horseshoe crab population by increasing the number being born include the following: 1) Keeping people off of beaches, such as Fowler, where the conditions are favorable to horseshoe crab spawning, during spawning times, so they do not interfere with the horseshoe crabs. 2) Not allowing harvesting near preferred spawning locations. 3) Horseshoe crab eggs must be buried at least five centimeters down in the sand in order to have adequate moisture for survival. One item to be considered is having census volunteers sprinkle uncovered eggs with sand to ensure all eggs are covered. This wouldn't be too hard; volunteers would just carry buckets around and scoop up sand from higher up on the beach where there is more moisture. 4) Another issue is changing the slope of beaches. If, in fact, horseshoe crabs do select beaches with steep seaward slopes, one thing to consider would be adding sand to the flatter beaches. This, however, is a tremendous task that would be very costly. What would not be such a big money investment, however, is sectioning off a piece of a flatter beach and enhancing the slope to see what happens.

Conclusion

My conclusion supports my hypothesis that spawning horseshoe crabs prefer high water temperatures (above 20C), and low water salinities (between 20 and 30 ppt). However, I reject my hypothesis that horseshoe crabs favor smaller sand grain sizes in nest site selection and my results for beach slope are inconclusive.

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