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## ABSTRACT:

*Limulus polyphemus*, the Atlantic horseshoe crab, is important for its environmental role and human uses. Yet little is known about horseshoe crabs beyond their spawning behavior. The goal of this project was to follow the movements of horseshoe crabs throughout their life cycle in the Great Bay estuary. Working out of the Rutgers University Marine Field Station (Fig. 1), a stratified survey of four beaches was designed to locate where and when horseshoe crabs spawned in the Great Bay Estuary. Sediment grain size in each strata was also analyzed to identify relationships between spawning site selection and sediment characteristics. Eggs obtained for beach samples were reared in the laboratory to document larval development and larval behavior. Some of these larvae were used in flume experiments to observe larval behavior in realistic currents. Concurrently plankton sampling was conducted in Little Sheephead Creek to determine when larval *Limulus* appear in the water column and if there were any diel differences. Adult subtidal movement was tracked with ultrasonic acoustic telemetry. Eggs were found at higher densities in the more elevated beach strata. *Limulus* appeared to strongly prefer spawning on beaches with coarse to medium grained sand. Of the four adult horseshoe crabs tagged with ultrasonic transmitters, two disappeared after release and the second pair were successfully tracked for several hours immediately after release and showed limited movement.



Fig. 3. Tagged adult male horseshoe crab (The Librarian)

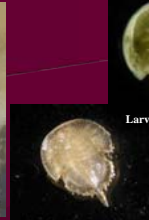
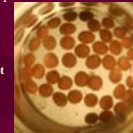


Fig. 4. Larval horseshoe crabs (Not to scale)

**Fig. 5. Time Series photos.** Eggs from the 6/29 beach samples were photographed every other day to record the developmental process. The first embryos can be seen at day 13 and the first hatching was at day 20. Eggs increase in diameter as they develop so some were removed periodically to save space.



July 1 Day 3



July 6 Day 8



July 8 Day 10



July 11 Day 13



July 15 Day 17



July 19 Day 21



July 22 Day 24

Table 2. Effect of time, beach, and strata on egg density and variability.

Analysis of Variance					
Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TIME	96.414	5	19.283	2.890	0.020
BEACH	532.731	3	177.577	26.619	0.000
STRATA	81.006	2	40.503	6.071	0.004
TIME*BEACH	117.562	15	7.837	1.175	0.311
TIME*STRATA	156.491	10	15.649	2.346	0.019
BEACH*STRATA	99.741	6	16.624	2.492	0.030
TIME*BEACH*STRATA	618.668	30	20.622	3.091	0.000
Error	480.323	72	6.671		

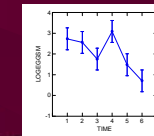


Fig. 7A. Egg density over time across all beaches and strata.

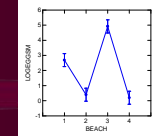


Fig. 7B. Egg density at each beach across all time points and strata.



Fig. 6. Horseshoe crab at Graveling Point beach.

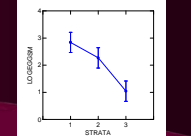


Fig. 7C. Egg density at each strata across all beaches and time points.

## MATERIALS AND METHODS:

**BRIDGENET SAMPLING:** Bridge sampling occurs off the Little Sheephead creek. At the sampling point Little Sheephead Creek is nominally ~4m deep. Monday night at either 9:30pm or 2:00am on the flood tide a 1m circular plankton net with 1mm mesh is lowered to a depth of 1.5m and fished for half an hour. Temperature, salinity, and volume filtered are measured. Three tows are performed and the samples sorted for larval fish and horseshoe crabs. After crabs appeared in the night samples on July 11 a day sample was taken July 19 at 2:00pm. **BEACH SAMPLING:** Four local beaches (Fig. 2) where horseshoe crab adults had been reported were selected for stratified surveys. Every week from June 7 to July 13 on Tuesday or Wednesday at low tide, six core samples were taken at each beach. Samples were taken in two transects with one from each strata of the beach: top, middle, and bottom. Each sample was 7cm in diameter and 10cm deep for a volume of 1540cm<sup>3</sup>. Samples were transported to the wet lab and eggs were removed from the sediments by upwelling water through a 500 micron sieve. Eggs were then counted and egg density per sample calculated. Samples were also saved for grain size analysis.

**TAGGING AND TRACKING:** I captured adult crabs (one male and one female) off the Graveling Point beach and kept them in tanks outside during the tagging process. A section of their carapace above and behind the left compound eye was scraped with sand paper and cleaned with alcohol before applying the cement. The tag was put in a short piece of 1/2cm inner diameter PVC pipe and cemented to the crab's carapace with marine cement. A spaghetti tag was also fixed to the acoustic tag. I held the crabs over night to assure that the cement had fully hardened and released them the morning of June 11 at the Graveling Point Beach. On subsequent Fridays I went mobile tracking for these crabs. July 14 another male and female pair were tagged and released the next day off Graveling Point. I tracked the second pair immediately after releasing them. Mobile tracking is supplemented by a permanent array of 12 moored LOTEK WHS\_1000 wireless units that retransmit acoustic contacts via VHF for remote monitoring. Fixed location tracking also occurred on a weekly basis; fixed GPS locations covering Great Bay and the Mullica River were surveyed for the presence/absence of tagged animals.

Table 1. Tagged horseshoe crabs

June 7 05	Name	Tag number	Sex	Weight (kg)	Length of Prosona (cm)	Length of Opisthosoma (cm)	Length of Telson (cm)	Width (cm)	Interocular Width (cm)
8	Mrs. Cake	169	F	2.27	16	13.5	22.3	23.5	15.5
9	The Librarian	152	M	0.7	11.3	8.5	15.7	18	10.5
July 15 05									
10	Rylee Hira	126	F	3.17	18	14.5	24.5	24.5	16.3
11	M. Bienvenue	99	M	1.13	12.5	10.2	18	19.5	11.7

**REARING:** Eggs saved from the beach samples were kept in glass dishes, the water changed every other day. When the eggs hatched I removed the larvae to other glass dishes with sand in the bottom. At the first post hatch molt (Fig. 4) I began feeding them small amounts of frozen brine shrimp. I kept a few eggs separate in order to take time series photos (Fig. 5). I also did some preliminary behavioral observations to examine their responses to water flow and turbulence, as well as their motile abilities, for larval crabs both before and after their first post hatch molt.

## RESULTS AND CONCLUSIONS:

### BRIDGENET SAMPLING

Larval crabs usually appear in the bridgenet samples the last week of June. The appearance of crabs this year was two weeks late, probably due to unseasonably cool weather and slower development. The highest abundance of crabs was on July 11 (when they initially appeared) at an average of 34.05 crabs/1000m<sup>3</sup>. No crabs were collected during the day sampling, indicating that larval horseshoe crabs are active at night, although further sampling would be needed to verify this observation. Diel behavior was not examined in the lab, however, larval crabs appeared active during the day. Preliminary behavioral observations suggest that turbulence or water flow will induce activity in larval crabs during day time. Larvae are also easily advected into the water column in even modest currents.

### BEACH SAMPLING

Egg density decreased over time (Fig. 7A), with the exception of the spike in the fourth sampling week (June 29). Horseshoe crabs are known to begin spawning in early May with the first high spring tide of that month and continue into June, so it is unsurprising that the egg densities decreased over the sampling period as eggs hatched (larvae were not found in the sediment samples). The highest two sampling days, June 7 and June 29 occurred after the new and full moons, respectively. The June 29 increase, since the eggs from these samples appeared newly spawned, indicates that adults were spawning up to the end of June.

Egg density at each beach (Fig. 7B) was highly variable, indicating that spawning horseshoe crabs have beach preferences. Beach 1 (Graveling Point) and beach 3 (Little Sheephead) consistently had the most eggs. Beach 2 (First Bridge) occasionally had low numbers of eggs and beach 4 (Field Station) had few to none. Egg density was also variable with strata (Fig. 7C), decreasing towards the low tide water line. This decrease is unsurprising since horseshoe crabs spawn at high tide and thus leave their eggs higher up the beach. The character of both the strata and the beach appeared to have a lot to do with the egg numbers. If the sediment was too muddy or too pebbly there would be no eggs in the sample. To quantify these observations the grain size for each beach at each strata was analyzed. Horseshoe crabs only spawned on beaches with grain size ranging from 0.5 to 2.5ϕ (Fig. 8A), which correspond to coarse to medium sands. Eggs were absent from beaches with sizes above and below this range, even if most of the grain sizes overlapped the ideal range (Fig. 8B). Eggs were also absent from beaches with the ideal range, indicating that grain size is not the only factor when horseshoe crabs select their spawning beach.

### TRACKING

The first pair of tagged horseshoe crabs were tracked a week after their release to avoid post release responses, but were never located, either during mobile tracking or on the permanent array. There are several possible explanations for the disappearances; the crabs may have died and either been buried or left on land, or they may have moved into a small marsh creek beyond the range of the hydrophones, among other possibilities.

The second pair were tracked immediately upon release for five hours. Since I was unable to track them subsequently these data may indicate post release behavior and not the normal movements of adult crabs (Fig. 9). The male (99) moved more than the female (126) and over the limited amount of observation time appears to be leaving the area. Fixed location tracking the next week (July 20, data not shown) revealed that the female was still present but the male was not.

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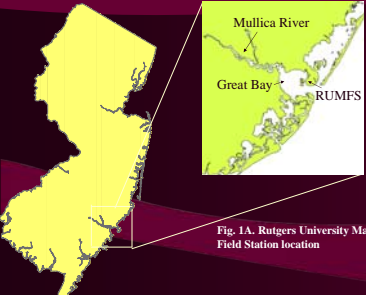


Fig. 1A. Rutgers University Marine Field Station location



Fig. 1B. Rutgers University Marine Field Station (RUMFS)

## INTRODUCTION:

*L. polyphemus* has existed for 350 million years but now faces an unknown future due to human impacts. Over the past century their population has seen a dramatic decline. Historically crabs were harvested in the millions to make fertilizer. Today they are harvested as bait for conch and eels (Shuster, 2001). Recently it was discovered that they have a medical significance as well; their blood is the source of Limulus Amebocyte Lysate, which is used to detect or diagnose bacterial contamination in medical products. Horseshoe crabs are caught by hand on beaches, bled, and released within 72 hours, ideally with little impact on the crab. Mortality studies have shown that only 10% of bled crabs die upon release (Swan, 2001). However, the impact of collection on spawning success is unknown. At the same time horseshoe crabs play a vital role ecological role. Every year when the crabs are spawning migrating shore birds (including the endangered red knot) stop off on their trek north and gorge on horseshoe crab eggs. They can increase their weight by 40%, providing the extra energy they need to finish their journey (Tanasredi, 2001). Thus *L. polyphemus* is not only important in and of itself as an ancient and declining species, but also because of the key role they play in nature and in human use.

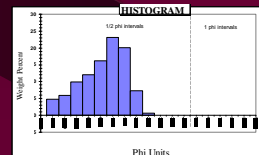
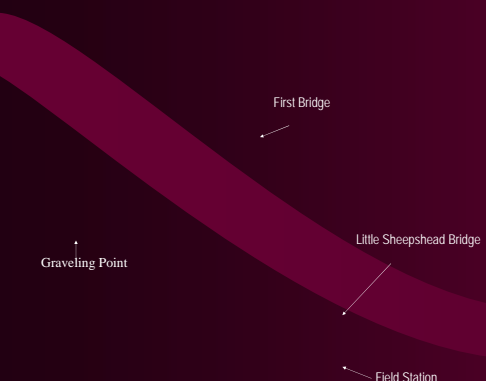


Fig. 8A. Grain size distribution on a spawning beach (Graveling Point, middle strata).

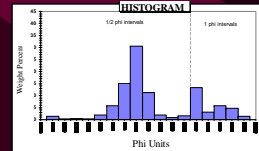


Fig. 8B. Grain size distribution on a non-spawning beach (First Bridge, middle strata).

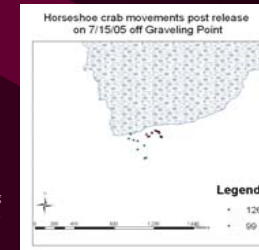


Fig. 9. Adult Horseshoe crab movements after release from tagging.

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