Larval Sampling, Rearing, and Acoustic Tracking of Limulus polyphemus

Rachel Sargent¹, Mark Sullivan†, John Quinlan‡

1Rutgers University Marine Field Station, Institute of Marine and Coastal Sciences, 132 c/o 800 Great Bay Blvd., Tuckerton, NJ 08087
2Rutgers University, Institute of Marine and Coastal Sciences, 71 Dudley Road, New Brunswick, NJ 08901

INTRODUCTION:
Limulus polyphemus has existed for 350 million years but now faces an unknown future due to human impacts. Over the past century their population has shown a dramatic decline. Historically crabs were harvested in the millions to make fertilizer. Today they are harvested as bait for fishing and crabbing (Swan, 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. Limulus 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.

MATERIALS AND METHODS:
BRIDGENET SAMPLING: Bridge sampling stations off the Little Sheepshead Bridge in Little Sheepshead Creek were sampled on a weekly basis; fixed GPS locations covering Great Bay and the Mullica River were surveyed. Pre-existing GPS tracks allowed for the identification of spawning areas in Great Bay (Fig. 1A). Egg density varied across the spawning area at different time points and strata. Each sample was 7 cm in diameter and 10 cm deep for a volume of 425 cm³. Samples were 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 post as a sheet pass of 0.15 cm inner diameter PVC pipe and cemented to the crab’s carapace with marine cement. A scaphocerous tag was also fixed to the acoustic tag. I fed the crabs every other day to ensure that the tag had fully hardened and released them the morning of June 12 at the Graveling Point Beach. On subsequent Fridays I was unable to locate either of the crabs. July 14 marked eggs and female pair were tagged and released the next day at Graveling Point. I tracked the second pair immediately after release. The tracking of a male horseshoe crab is a permanent art of 2 mm round LOTEX WHO 1000 wireless tags that transmit 6 m per day through contacts via VHF for remote monitoring. Fixed GPS location tracking also occurred on a weekly basis covering Great Bay and the Mullica River, which were surveyed for the presence/absence of tagged animals.

RESULTS AND CONCLUSIONS:
BRIDGENET SAMPLING:
Larval crabs usually appear in the bridge net samples the last week of June. The appearance of small larval crabs was two weeks late, probably due to a relatively cool and wetter summer weather. The highest abundance of larvae was on July 11 when they initially appeared at an average of 34.05 crabs/1000 m³. No eggs 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. Did behavior was witnessed in the lab, however, live crabs appeared active during the day. Preliminary behavioral observations suggest that turbulence or water flow will induce activity in larval horseshoe crabs during the day. Larvae are also easily/adapted into water column in even moderate currents.

REFERENCES:

Table 1: Effect of time, beach, and strata on egg density and variability.

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum-of-Squares</th>
<th>df</th>
<th>Mean-Square</th>
<th>F-ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEACH</td>
<td>90,439</td>
<td>2</td>
<td>45,219</td>
<td>7.7</td>
<td>0.001</td>
</tr>
<tr>
<td>STRATA</td>
<td>9,782</td>
<td>2</td>
<td>4,891</td>
<td>0.79</td>
<td>0.001</td>
</tr>
<tr>
<td>TIME*BEACH</td>
<td>9,782</td>
<td>2</td>
<td>4,891</td>
<td>0.79</td>
<td>0.001</td>
</tr>
<tr>
<td>TIME*STRATA</td>
<td>9,782</td>
<td>2</td>
<td>4,891</td>
<td>0.79</td>
<td>0.001</td>
</tr>
<tr>
<td>TIME<em>BEACH</em>STRATA</td>
<td>9,782</td>
<td>2</td>
<td>4,891</td>
<td>0.79</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 2: Summary of larval horseshoe crab length at each location.

<table>
<thead>
<tr>
<th>Location</th>
<th>Length (cm)</th>
<th>Telson (cm)</th>
<th>Interocular (cm)</th>
<th>Carapace Width (cm)</th>
<th>Carapace Height (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graveling Point</td>
<td>8</td>
<td>1.5</td>
<td>1.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Great Bay</td>
<td>7</td>
<td>1.5</td>
<td>1.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Mullica River</td>
<td>7</td>
<td>1.5</td>
<td>1.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Fig. 1A. Rutgers University Marine Field Station Location
Fig. 1B. Rutgers University Marine Field Station (RUMFS)
Fig. 2. Beach and Bridge sampling locations
Fig. 3. Tagged adult male horseshoe crabs (The Librarian and M. Bienvenue) from Great Bay and the Mullica River.
Fig. 4. Larval horseshoe crab (Great Bay) with ultrasonic transmitter attached.
Fig. 5. Time Series photos. Eggs from 20 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 size as they develop so some were removed periodically to avoid space constraints.
Fig. 6. Larval horseshoe crabs in the flume.
Fig. 7A. Egg density across all beaches and time points.
Fig. 7B. Egg density at each beach across all time points.
Fig. 7C. Egg density at each strata across all beaches and time points.
Fig. 8A. Rutgers University Marine Field Station Location
Fig. 8B. Grain size distribution on a non-spawning beach
Fig. 8C. Grain size distribution on a spawning beach
Fig. 8D. Grain size distribution on a spawning beach (First Bridge, middle strata)
Fig. 8E. Grain size distribution on a non-spawning beach (First Bridge, middle strata)
Fig. 9. Adult horseshoe crab movements after release tagging.