Horseshoe Crab (*Limulus polyphemus*) Spawning at Cove Point, Maryland: Final Report and Evaluation of Marsh Restoration

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Abstract

This report summarizes a four-year survey of horseshoe crab spawning at Cove Point, Maryland. A total of 219 spawning groups were observed. Crabs emerged during nighttime full or near moon tides in June and early July. Crabs were only observed at night, and never moved above the wave line. Spawning groups were largely restricted to the southern portion of the beach. This unequal distribution was examined by measuring beach slope, sand grain size, and pore water sulfide levels. Beach slope did not significantly vary between the northern and southern beach and was not significantly associated with crab distribution. However, both sand grain size and sulfide levels were significantly associated with crab distributions, with sulfide showing the greatest degree of association. The percentages of fine sand and silt and sulfide levels were significantly greater in the northern beach. These physical characteristics are indicative of anaerobic conditions in the sand, which would lead to lower survivorship of horseshoe crab eggs and larvae. It is likely that sand erosion and deposition differs between the northern and southern beaches, leading to the physical differences that are inversely associated with horseshoe crab spawning. A proposed marsh restoration project would stabilize the northern marsh, but likely eliminate the northern beach. The proposed work would not extend into the primary spawning area, so there are likely to be few direct effects on horseshoe crab spawning. A proposed series of breakwaters, extending southward into the spawning area, could stabilize the southern beach and lessen storm erosion. This could enhance the beach as a spawning area. However, this project could also indirectly impact spawning if it blocks southward sand movement, leading to erosion, or leads to the deposition of more fine grained sand on the southern beach. The southern beach should be monitored after completion of the marsh restoration project for stabilization and its continued utilization as a horseshoe crab spawning habitat.

Introduction

Horseshoe crabs (*Limulus polyphemus*) are ecologically and economically important along the Atlantic coast of the United States. They spawn and deposit eggs on suitable beaches, usually during nighttime high tides in May or June (Rudloe, 1980; Shuster and Botton, 1985). These eggs are important food sources for many migrating shorebirds (Castro and Myers, 1993; Botton et al., 1994; Tsipoura and Burger, 1999). Adult horseshoe crabs are economically important as the source of *Limulus* amebocyte lysate, used for detecting contamination in human blood products (Berkson and Shuster, 1999).

There is evidence that horseshoe crab populations in many areas may be in decline, due to harvest for bait or degradation of spawning habitat (Berkson and

Shuster, 1999; Widener and Barlow, 1999). There is thus interest in determining population numbers, spawning success and recruitment. Horseshoe crabs spawn on the Atlantic coast from Maine to Mexico, with their greatest abundance centered on Delaware Bay (Shuster, 1979). Chesapeake Bay is a smaller but important spawning area for this species and contains a horseshoe crab population genetically distinct from the Delaware Bay population (Pierce et al., 2000).

Population numbers and spawning areas have received less study in the Chesapeake than in Delaware Bay. Based upon conversations with workers at the Maryland Department of Natural Resources and US Fish and Wildlife Service, there are few published reports quantifying horseshoe crab spawning in the Chesapeake and none for some years.

Previous reports have identified Cove Point, Maryland as a spawning area for horseshoe crabs. Based on the 2007 report, it appears that horseshoe crabs spawn primarily on the southern beach. Spawning groups were observed only during nighttime high tides. Spawning females never moved completely out of the water during spawning. These observations differ from those from Delaware Bay, where spawning may occur during daytime high tides and spawning groups can completely emerge from the water. The 2007 report showed that beach pore water sulfide levels tended to be higher along the northern beach, which might account for the spawning distribution.

The current final report will provide a further year of spawning observations and measurements of beach sulfide. It will also report measurements of beach slope and sand grain size. These physical parameters may affect spawning success by affecting oxygen levels in the beach. Horseshoe crab eggs require oxygenated sand for development. Beach sand saturated with water is generally anoxic, and this anoxic conditions can persist if beach slope is slight, keeping the sand saturated through capillary action (Gordon, 1960). Beach sand with small grain size can also trap water for longer periods and thus produce anoxic conditions. Sulfide, in addition to being toxic, is indicative of anaerobic bacterial activity and thus anoxia. For these reasons, all these physical characteristics have been suggested to affect horseshoe crab spawning success (Penn and Brockman, 1994; Botton et al., 1988). The data presented here may help to describe the necessary conditions for spawning success, and may shed light on why only one section of the beach is apparently suitable.

Lastly, this report will discuss the proposed restoration of the northern marsh. The restoration proposal will be evaluated for possible impacts on the southern beach as a spawning habitat for horseshoe crabs.

Materials and Methods

Horseshoe crabs were surveyed over a four-year period beginning in May 2005. Prior to the surveys, benchmarks were established approximately every 50 meters from the boardwalk to the lighthouse using GPS. This generated 18 benchmarks which divided the beach into 17 sections (Fig. 1). Benchmarks and sections were numbered in ascending order from the boardwalk south to the lighthouse.

Spawning Activity - The surveys evaluated spawning activity on three or four nights of full or new moons during May, June and July in 2005, 2006, 2007 and 2008. Each survey night, the beach was walked as a single line transect within one hour of high tide. The positions of spawning groups, defined here as a female and one or more males, were recorded using GPS. Each survey represented a single pass down the beach. The data thus provide a "snapshot" of animal density during the height of spawning activity. The number of males per female was recorded for each spawning group. GPS positions of spawning animals were overlaid in the laboratory onto maps containing benchmarks and sections.

Beach Sulfide Levels – Measurement of beach pore water sulfide levels followed a protocol modified from van Handel (1987). This assay was conducted twice in June and July of 2007 and twice in June and July of 2008. Water samples were taken during the daytime on a different date than the surveys. Within two hours of low tide, a water sample was taken at each benchmark, 1 meter inland from the waterline. To sample, 4.5 ml water was collected by suction 15 cm below the beach surface. To this sample, 0.5 ml of sulfide reagent was added immediately. A methylene blue reaction product was proportional to the sulfide present in the water sample. Methylene blue concentrations were measured in the laboratory with a spectrophotometer and sulfide concentrations determined (O.D. = 0.17 per microgram sulfide at 650 nm). Calculations were pooled and averaged to produce a single sulfide reading for each benchmark.

Beach slope – At low tide, GPS positions were recorded of low and high tide marks at each benchmark. Tidal elevation for that tide and day was recorded from publicly available information (Maryland DNR). These data formed a right triangle at each benchmark, with GPS positions forming the hypotenuse and tidal elevation forming the adjacent side. The angle formed by the hypotenuse and adjacent side then represented the slope of the beach at that benchmark, in degrees, and was calculated for all benchmarks.

Beach sand grain size – Sand cores were taken at each benchmark. Each core was 2 cm in diameter, and extended 20 cm into the sand. Cores were taken 1 meter seaward from the highest tide mark visible. Each sand core was dried, weighed, and sifted through a stack of soil sieves with standard size numbers 5, 35, 60, 120 and 230. These represent mesh sizes of 4, 0.595, 0.250, 0.125 and 0.063 mm, respectively. Grains retained by the #120 and #230 sieves, as well as those small enough to pass through all sieves, were collected. Grains retained by these mesh sizes are considered fine sand and silt. They were weighed and used to calculate the percent fine sand and silt in each core sample.

Figure 1. Locations of sections and benchmarks established for this study. Numbers represent sections. Vertical lines represent location of benchmarks.

Results

Figure 2 shows spawning locations recorded over the four-year period of the survey. A total of 219 spawning groups were recorded. The first survey on June 7 in Year 1 found 45 spawning groups. The second survey, on June 22, found 42 groups. Sixty-six of the 84 spawning groups in Year 1 contained single males, while 17 had double males and 4 had triple males. Year 2 surveys failed to find any spawning groups on the first survey night. The second survey on June 12 found only 4 spawning groups. In the third year, the first survey on June 1 found 31 spawning groups. The second survey on June 15 found 19 spawning groups. Four surveys were conducted in Year 4. The first, on May 20, found only 2 spawning groups. The second, on June 6, found 55 groups. The third, on June 18, found 3 groups, while the last, on July 3, found 18 spawning groups.

The number of spawning groups observed in each section was averaged over all surveys. The mean number of spawning groups per section is examined in Figure 3A. When all years are combined, a pattern showing differential use of Cove Point beach emerges. Sections 1-9 contained only 20 spawning groups over the three year period, or 9% of the total. Sections 10-17 contained 199 spawning groups, or 91% of the total. The sections were therefore divided into two groups for further analysis: sections 1-9 representing the upper, northern beach, and sections 10-17 representing the lower, southern beach. These two areas were statistically different. When mean spawning groups per section for northern and

Figure 2. Numbers of spawning groups observed over the four year study. Numbers represent the total number of spawning groups observed. Arrow A marks the approximate southward extent of the proposed revetment. Arrow B marks the southward extent of the proposed breakwaters.

southern beach areas were compared, the southern beach area contained significantly more spawning groups per section than the northern beach area. A regression of mean spawning groups per section and the section number (Fig. 3B) was statistically significant based on a regression ANOVA. This suggests that increasing section number (i.e., moving to the southern beach) is associated with increasing numbers of spawning groups.

Three physical beach characteristics were examined: beach slope, sand grain size, and pore water sulfide levels. Figure 4 shows the measurements for these three characteristics at each benchmark. Benchmarks were divided similarly to sections, in that benchmarks 1 through 9, representing the northern beach, were compared with benchmarks 10 through 18, representing the southern beach. When means from northern and southern beach areas were compared there was no statistical difference in beach slope between areas (Fig. 4A). The smallest slopes were found in the northern beach area, near the boardwalk. This is consistent with the large storm overwash zones in that part of the northern area. By benchmark 5, however, beach slopes are high, and there is no consistent trend after that. When sand grain size was examined, the percent of fine sand and silt present in sand cores was significantly higher in the northern beach area (Fig. 4B). Similarly, sulfide levels were significantly higher in northern beach samples compared with those from the southern beach (Fig. 4C).

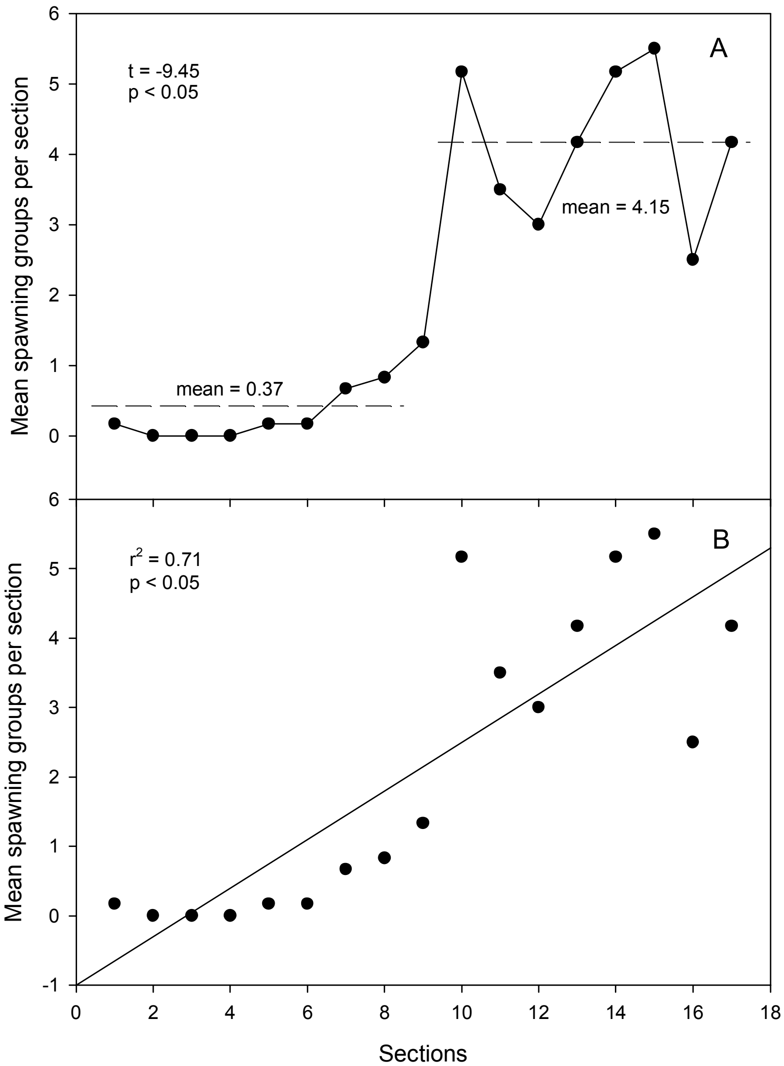

Figure 3. Mean spawning group numbers compared with beach section. 3A: Mean spawning group numbers for the northern beach compared with mean group numbers for the southern beach. 3B: Regression of mean spawning group numbers per section against section number.

Measurements of beach characteristics were then compared with spawning group numbers (Fig. 5). When a regression was performed comparing beach slope at each benchmark with mean spawning group numbers at the adjacent section, there was little association and no statistical significance (Fig. 5A). However, the percent of fine sand and silt in benchmark samples was significantly and inversely associated with mean spawning group numbers (Fig. 5B). There was a similar significant inverse association between pore water sulfide levels and mean spawning group numbers (Fig. 5C).

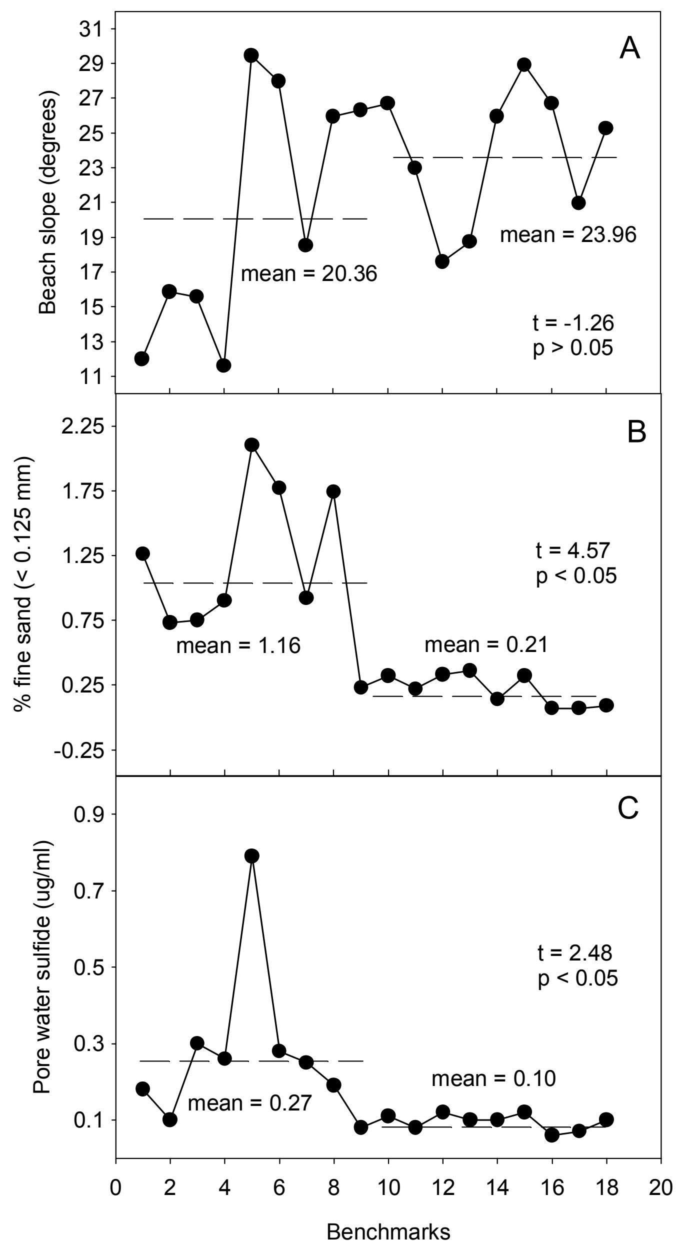

Figure 4. Comparisons of beach physical characteristics between northern and southern areas. 4A: beach slope. 4B: percent fine sand and silt. 4C: pore water sulfide levels.

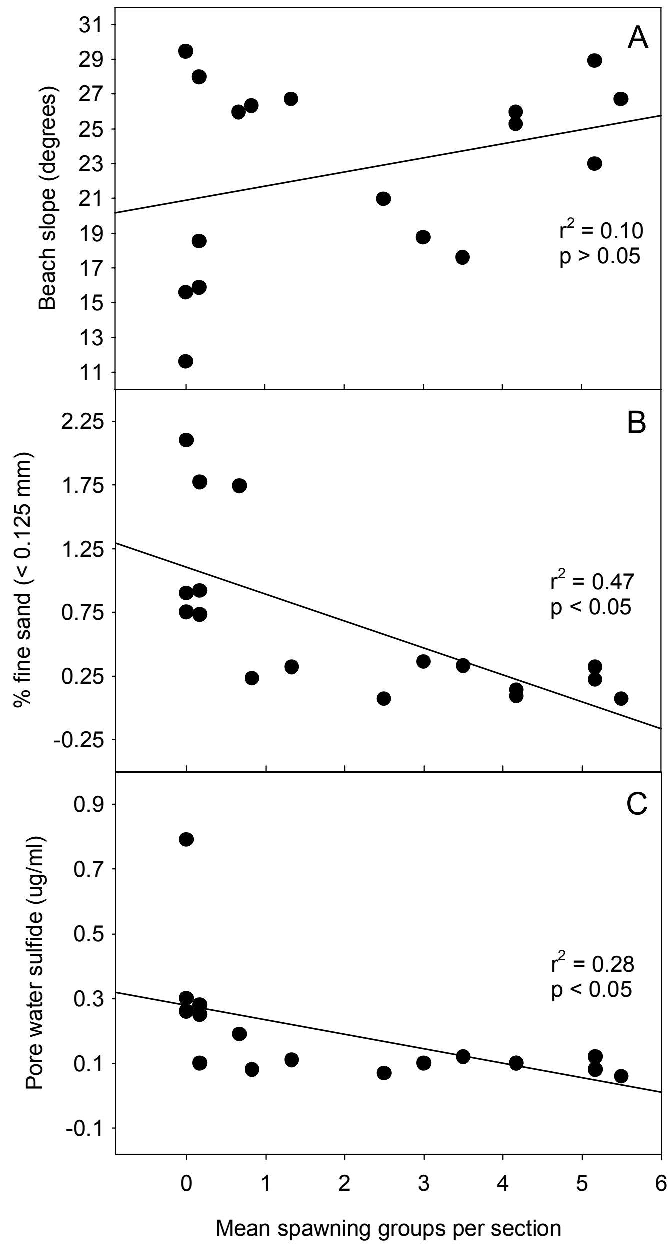

Figure 5. Comparisons of beach physical characteristics at each benchmark with mean spawning group numbers in the adjacent section. 4A: beach slope. 4B: percent fine sand and silt. 4C: pore water sulfide levels.

Discussion

Horseshoe crabs clearly utilize Cove Point as a spawning beach. Spawning begins during high tides in late May, and continues through June. Surveys were not done in late July, but a decline in spawning would be expected as the summer advances. Spawning appears to occur only during nighttime high tides. While on the beach, females were never observed leaving the wave zone. This is different from what is commonly observed in the Delaware Bay population, where spawning can occur during daytime and females often emerge from the wave zone.

The southern portion of the beach is utilized for spawning to a much greater degree than the northern portion. This differential use of Cove Point beach by spawning groups is interesting and perhaps important for horseshoe crab conservation. Although some areas of the northern beach are clearly unsuitable, with little open sand before *Phragmites australis* stands begin, the beach in other areas appears usable, with an adequate depth of sand for spawning. It has been suggested that a very shallow slope can retain water between the sand grains for a longer period, resulting in greater periods of anoxia (Gordon, 1960). Although there were a few places in the northern beach with very shallow slope, in general beach slope did not differ significantly between northern and southern beaches. Beach slope was likewise not significantly associated with the observed pattern of horseshoe crab spawning.

The percentages of fine sand and sulfide levels were significantly higher in the northern beach, and these physical characteristics were significantly and inversely associated with observed patterns of crab spawning. The first six sections, in particular, had high levels of both fine sand and sulfide, and very few spawning groups. A higher percentage of smaller grains in beach sand can retain water for longer periods, thus extending anoxic conditions and lowering crab egg and larvae survivorship. Anoxic conditions in the sand can result in anaerobic bacterial activity and the production of hydrogen sulfide. Both of these physical characteristics, therefore, are associated with low oxygen levels in the sand. It is thus reasonable for horseshoe crab females to avoid these areas when spawning.

Sulfide levels are likely to be particularly important cues for spawning. Crabs may avoid areas with higher sulfide levels both because it is indicative of anoxic conditions and because of sulfide toxicity (Botton et al., 1988). In either case it represents a poor spawning beach with likely low egg survival. Further, of the physical characteristics examined in this report sulfide levels could be best evaluated by crabs before choosing an area for spawning. However, it has not been demonstrated that spawning adults can directly detect either sulfide or hypoxic water.

High sulfide levels in beach pore water can also indicate decaying vegetation in the beach sand. It is likely that sulfide levels are related to both erosion and the marsh behind the beach. The freshwater marsh is primarily behind the northern beach. In addition, the northern beach is eroding and receding toward the marsh. Beach erosion may bring underlying vegetation closer to the surface, even where none is exposed. This, combined with the marsh behind the northern beach, could lead to reduced sediments and hydrogen sulfide production in beach pore water. These conditions present both toxicity and hypoxia problems for developing horseshoe crab eggs. Sand appears to be accreting on the southern beaches, burying vegetation. The land behind the southern beaches is also higher, with less marsh water working into the beach sediments. Thus differential beach conditions could lead to differential utilization by spawning groups.

If true, this suggests that Cove Point beach, and other Chesapeake beaches, may become less suitable for spawning in the future. Sea levels are rising, primarily due to thermal expansion, and this rise is accelerated in many areas along the Chesapeake due to land subsidence. Behind many beaches is marsh, or hard structure, preventing beach migration as water levels rise. Many beaches will experience erosion and greater exposure of underlying vegetation. It is possible that this will produce more sulfide-rich and hypoxic conditions and may thus lead to decreased horseshoe crab reproduction on those beaches.

Proposed Marsh Restoration

Recently a marsh restoration project has been proposed. Due to the erosion on the northern beach, a breach formed several years ago, and has been widening. The breach is permanent, with flowing water at about 0.5 m depth at low tide. The freshwater marsh behind the beach has thus been converted to a tidal marsh. Much of the freshwater vegetation has disappeared. The tidal nature of the current marsh has likely contributed to the higher sulfide levels measured along the northern beach. The proposed restoration project would lay a porous rock revetment along the northern beach, and fill material behind the barrier. This will stabilize the shoreline, and presumably allow the marsh behind to convert back to freshwater. The revetment would extend along the beach in a southward direction to an approximate position marked as “A” on Figure 2. A series of breakwaters would then be constructed approximately 100 ft offshore, extending southward to an approximate position marked as “B” on Figure 2. While the marsh may be restored behind the revetment, there will likely be no beach southward until the breakwaters begin.

The installation of the revetment would likely not directly affect horseshoe crab spawning. Although there will likely be no usable beach until the breakwaters, almost all (95%) of the observed spawning groups used the beach southward of this position. The breakwaters could enhance the southern beach as a spawning habitat. There has been some beach loss over the last decade due to hurricanes and other large storms. By reducing wave energy, the proposed breakwaters may lesson storm erosion and help to better stabilize the southern beach.

There may also be indirect negative effects. Although storms have had impacts, the southern beach currently appears to be stable, with a coarser grain size and low sulfide level that indicates suitable spawning habitat. It is possible that alteration to the northern beach, while not directly affecting the crab spawning area, may change the pattern of sand deposition at the southern beach. If this results in erosion, leading to exposure of underlying vegetation, the southern beach may eventually exhibit the fine sand and sulfide levels typical of the current northern beach. In that case, Cove Point beach would be diminished as a spawning habitat for horseshoe crabs.

Following completion of the revetment and breakwaters, the southern beach should be monitored for changes that could impact horseshoe crab spawning success. With knowledge of beach conditions and spawning activity before the restoration, measurements of these parameters following the project should allow an evaluation of any changes. It should thus be possible to determine if restoration of Cove Point marsh will have any effect on the southern beach and its utilization as a spawning area for horseshoe crabs.

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