THE USE OF THE INLAND BAYS BEACHES AS HORSESHOE CRAB (LIMULUS POLYPHEMUS) NESTING SITES

By:

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A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Bachelor of Science of Wildlife Conservation with Distinction.

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ABSTRACT

Horseshoe crabs are important creatures. They support the whelk and eel fisheries, provide an important food source for migrating shorebirds, and are important to the biomedical industry. All three of these industries contribute approximately $35.7 to $48 million annually to the region. Therefore, it is important to monitor and conserve their population. For eight years, spawning counts have occurred on 24 Delaware Bay beaches (DE and NJ). However, the objective of this project was to determine if beaches in the Inland Bays, Delaware, are important nesting sites for the American horseshoe crab (*Limulus polyphemus*). Using established methods, I performed spawning counts on James Farm Beach in Indian River Bay at Full and New Moon periods in the summers of 2007 and 2008. In addition, I counted spawning adults on beaches at Camp Arrowhead, Holts Landing/Ellis Point, and Bay Colony in 2008. I compared numbers of spawning adults per meter of shoreline for these four Indian River Bay beaches to spawning adult counts performed on Delaware Bay beaches in 2008. Data from James Farm, with many samples recording 7 or more horseshoe crabs per meter, were within range, and even surpassed some from the Delaware Bay's beaches. In addition, I performed egg counts on Holts Landing and James Farm in the summer of 2007 and the summer and fall of 2008. I then compared these data to one another, as well as to previous egg counts from beaches in the Delaware Bay in 1999 and in 2006. Although it was difficult to compare the numbers of eggs per square meter from different years, the numbers of eggs per square meter at James Farm and Holts Landing, roughly 200,000 per square meter were

comparable to values from Delaware Bay beaches. In addition, I performed egg counts on James Farm and Holts Landing/Ellis Point in November of 2008, and I found eggs and larvae thriving in the sand, and I believe that they may “over-winter”. I recommend that sampling methodologies for spawning adult and egg be modified by sampling on more Inland Bays beaches, extending the counts into July, and increasing sample sizesThese data indicate that beaches in Indian River Bay do support spawning activity and larval productivity comparable to better known and studied beaches in Delaware Bay. These results provide a stepping stone for research determining whether the Inland Bays are important breeding grounds for Mid Atlantic horseshoe crab populations, as well as to better understand the ecological and habitat value of beaches within the Inland Bays.

Chapter 1

INTRODUCTION

1.1 Overview of Limulus polyphemus

There are four extant species of horseshoe crabs (Shuster 1982). *L. polyphemus* is found from Maine to the Yucatan Peninsula, They spawn in large numbers during spring tides in the Mid-Atlantic (with highest densities in the Delaware Bay) (Shuster 1982, Walls and Berkson 2003).

Although given the common name 'horseshoe crab,' *Limulus polyphemus* is not a crab at all. In fact, it is more related to spiders (subphylum Chelicerata) than to actual crustaceans. Scientists believe that horseshoe crabs evolved at least 300 million years ago, and have not changed much over this time, hence their nickname, 'Living Fossils'. In fact, their larvae look much like prehistoric trilobites of the Paleozoic era.

The biology of the horseshoe crab is unique. It has a pair of book lungs, a pair of compound eyes on its carapace, a pair of chelicera, and 5 pairs of leg-like appendages. Males also posess a pair of modified appendages (claspers) that they use during spawning. To spawn, horseshoe crabs gather in large numbers at the spring tides at new and full moons in early summer. The female digs a large nest approximately 20 cm deep, and deposits up to 20,000 eggs. As she digs her nest, males approach and attempt to use their claspers to attach to her carapace, and deposit their sperm toward her eggs (Shuster 2003).

1.2 The Importance of *Limulus polyphemus* to Organisms

The American Horseshoe Crab (*Limulus polyphemus*) is a very important creature for its role in the food chain. Horseshoe crabs, which nest in abundance in greatest numbers in the Delaware Bay area (Shuster 2003), serve as an important food resource to many organisms. The eggs produced by the adult horseshoe crabs that come to spawn every summer are consumed by many migratory shorebirds, such as the charismatic red knot (Weber 2006). Without these eggs, the birds would not have enough energy to complete their migration to the arctic (Eilperin 2005). Also, the adult horseshoe crabs serve as a food source for the endangered Loggerhead Sea Turtle, which comes into the area to consume the horseshoe crabs (Spotila 1997).

1.3 The Importance of Limulus polyphemus to Humans

Humans also use horseshoe crabs; the adults are used to supply bait for the whelk and American eel industry. As of the year 2006, these fisheries had a combined estimated regional annual economic value of $2.2 to $2.8 million dollars (Davis et al. 2006). In addition, their blood contains Limulus Amebocyte Lysate (LAL), a compound used to detect endotoxins within intervenes drugs and other objects that are implanted into humans. Regionally, the medical use of horseshoe crabs generates $26.7 to $34.9 million dollars annually. Finally, recreational bird watchers who come to the Delaware bay area to observe shorebirds contribute an estimated $6.8 to $10.3 million dollars to the regional economy each year (Davis et al. 2006). Therefore the survival of horseshoe crabs is of great economic importance (Walls and Berkson 2003).

However, despite the dependency of these industries on horseshoe crabs, they still contribute to the decline in the horseshoe crab population. Over fishing has caused the population to decline significantly (Davis et al. 2006); in 1998, before restrictions, an estimated 2 million horseshoe crabs were harvested. Also, studies show that the bleeding of horseshoe crabs results in a mortality rate of about 7.5%, with an estimated 260,000 horseshoe crabs bled in 1997 (Walls and Berkson 2003). Attempts have been made to lessen the impact these industries have on the horseshoe crab population.

For instance, over the years, the debate over the state of the horseshoe crab population has heightened. In 2005, a moratorium was enacted by John Hughes (Secretary of the Department of Natural Resources and Environmental Control) that made it illegal to harvest any horseshoe crabs. This moratorium was created after a decline in the migratory shorebird population was observed (Murray 2007). However, in May, 2007, the moratorium was lifted, and commercial fishermen were allowed to harvest a total of 100,000 males. This new ruling has caused a great uproar from environmentalists who fear that if the horseshoe crab population were to decline, shorebirds would once again be in danger (Murray 2007).

Although the debate continues, a decline in the horseshoe crab population could harm not only shorebirds, but the whelk industry, as well as human health. In light of the debate between fisherman and environmentalists, the horseshoe crab population must be assessed and monitored (including determining human mortalities (Walls and Berkson 2003) and its habitats are conserved so that it may once again become a sustainable natural resource.

1.4 Goals of Current Work

This project seeks to determine the importance of the inland bays beaches, more specifically, Indian River Bay, as horseshoe crab (*Limulus polyphemus*) nesting sites. Beaches in Indian River Bay will be compared to beaches in Delaware Bay through determining the approximate number of spawning horseshoe crabs per meter and number of eggs per meter on various Inland Bay Beaches. In addition, the spatial, along-beach pattern of horseshoe crab nests will also be explored, as well as the proportion of eggs and larvae that "over-winter" on Indian River Bay beaches. This project is a continuation of an NSF REU Summer Internship and Science and Engineering Scholar project at the College of Marine and Earth Studies begun during the summer of 2007.

Chapter 2

EXPERIMENTAL METHODS

2.1. Study Area Description

The Inland Bays are brackish to high salinity bodies of water with an average depth of about 3 to 8 feet (1 to 2.4 meters). They are home to a variety of other organisms such as shellfish (oysters, clams, etc.), fish (summer flounder, bluefish, tautog, etc.), diamondback terrapins, and a variety of species of birds. People also use the Inland Bays for fishing, crabbing, hunting, and other recreational activities. The Inland bays have a variety of habitats, such as eel grass beds, salt marshes, mudflats, and sandy beaches (http://www.inlandbays.org). The sandy beaches are the areas most utilized by spawning horseshoe crabs when they enter the Inland Bays.

These beaches were selected based on local knowledge, as well on the presence of white sand observed on satellite images from Google Earth identifying sandy beaches using Google Earth (Figure 1 and 2).

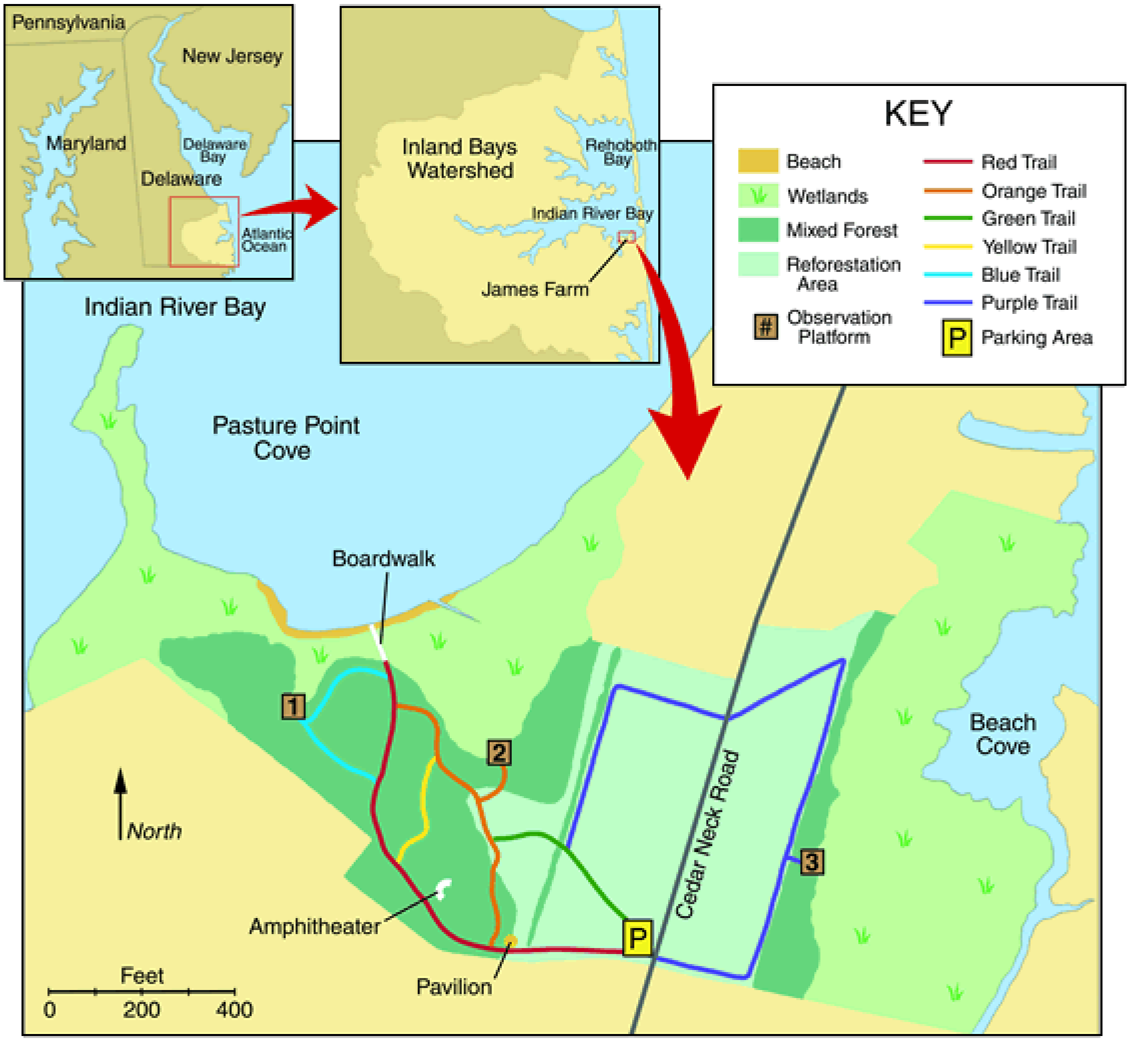

Figure 1. A map of the James Farm Ecological Preserve. James Farm beach is located at the end of the indicated boardwalk.

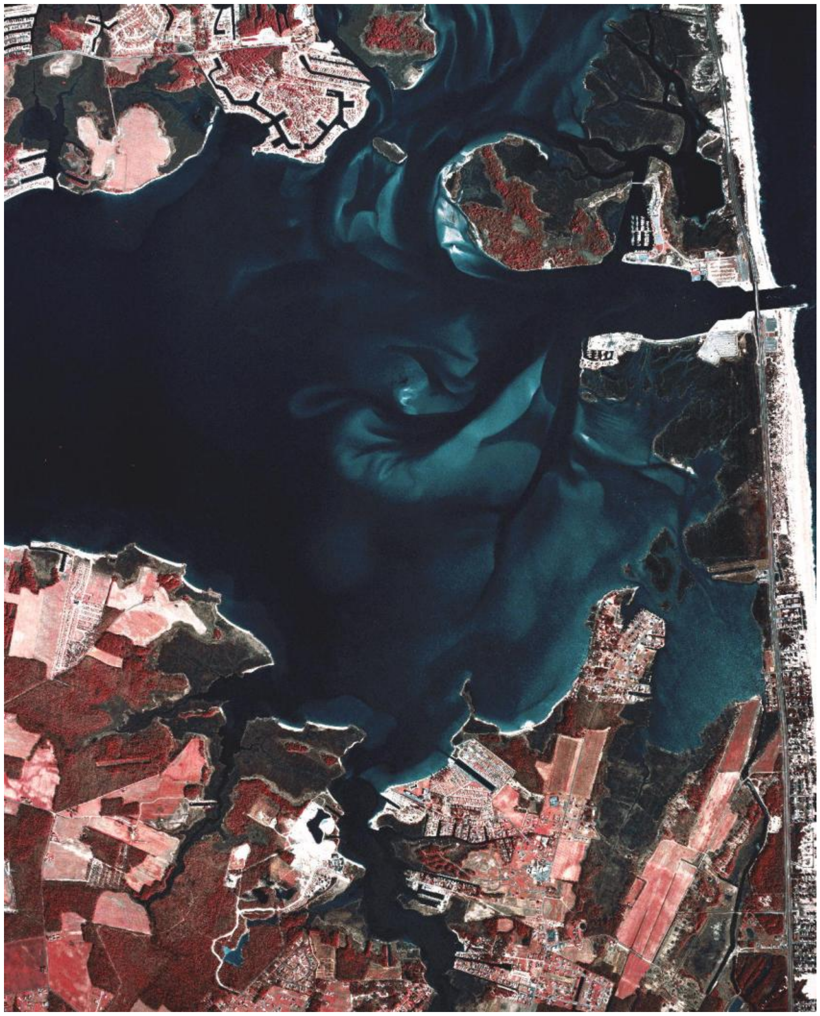

Figure 2. A satellite image of the Inland Bays. Sandy beaches were identified by looking at photos such as this one.

2.2. Spawning Adult Counts

Members of the laboratory of Doug Miller at the University of Delaware and volunteers from the Center for the Inland Bays (Miller and McCole 2007) counted horseshoe crabs at spring high tide at James Farm, Holts Landing, Bay Colony, and Camp Arrowhead (See dates in Table 1).

Table 1. Dates of spawning counts on Inland Bays beaches, 2007 and 2008

Dates of Spawning Adult Counts Summer 2007 and 2008

Date	James Farm	Holts Landing	Camp Arrowhead	Bay Colony
5/31/2007	**X**			
6/14/2007	**X**			
6/28/2007	**X**			
5/5/2008	**X**			
5/17/2008	**X**			
5/19/2008	**X**	**X**	**X**	**X**
6/1/2008	**X**			
6/3/2008	**X**	**X**	**X**	**X**
6/16/2008	**X**			
6/20/2008	**X**			
7/2/2008	**X**			
7/18/2008	**X**			

2.2.1. Sampling Methods

Within an hour of high water on the chosen night, an 8-m transect tape was used to define the beginning of a segment along the beach where counts would take place. The transect was laid at the water line so spawning crabs were directly below the tape. Each count started at the east end of the beach (for James Farm, the count began at the eastern most start of sandy beach), and within the 8-m transect, 2 counts were taken at randomly chosen areas. All male and female horseshoe crabs present within a 1 by 1 meter square quadrat were counted. Males can be identified by their smaller size and the presence of hook like appendages that are used to grasp the female during mating. This 8-m segment was leaf-frogged along the beach and the counting repeated so that the entire length of the beach (approximately 250 meters) was counted.

Figure 3. A photo showing the spawning adult count taking place. We placed the quadrat at one of the two random sites along the 8m transect, and then counted and recorded the number of males and females within the quadrat.

2.2.2 Analysis Methods

The number of horseshoe crabs per meter for the four Inland Bays Beaches on May 19, 2008 and June 3, 2008 were compared to the number of horseshoe crabs per meter from the Delaware Bay beaches on those same dates. In addition, the number of horseshoe crabs for each quadrat was plotted against their individual locations along the beach (meters) for all counts on James Farm (2007 and 2008) to see if a spatial distribution pattern existed.

2.3. Egg Census

2.3.1 Sampling Methodology

James Farm and Holts Landing were cored for eggs during the month of June once in 2007 and twice in 2008, as seen in the table below.

Table 2. Dates of egg counts on James Farm and Holts Landing, 2007 and 2008.

Season	James Farm	Holts Landing
Summer 2007	6/27/2007	7/6/2007
Summer 2008	6/18/2008	7/2/2008
Fall 2008	11/7/2008	11/8/2008

I modified the methodology published by Smith et al. (2002): specifically: at low tide, 15-m transect was laid along the beach and within each transect (right above the tape), and 10 cores were taken at points selected by the use of a random numbers table. Cores (20 cm deep, 6.7 cm core diameter) for eggs were taken at the tide line halfway between the spring high tide line and the low tide line. The contents from each core were mixed together in the tray, a 50-ml subsample is taken from the mixed contents, run over a 1-mm sieve, and the eggs were counted. This procedure is repeated twice more yielding a total of 30 cores from a total length of 45 meters along the beach. Subsample results were expressed on a per meter squared of beach width basis. Counts done in November of

2008 to assess the percent of "over-wintering" eggs and larvae occurred at the exact same areas as determined by handheld GPS.

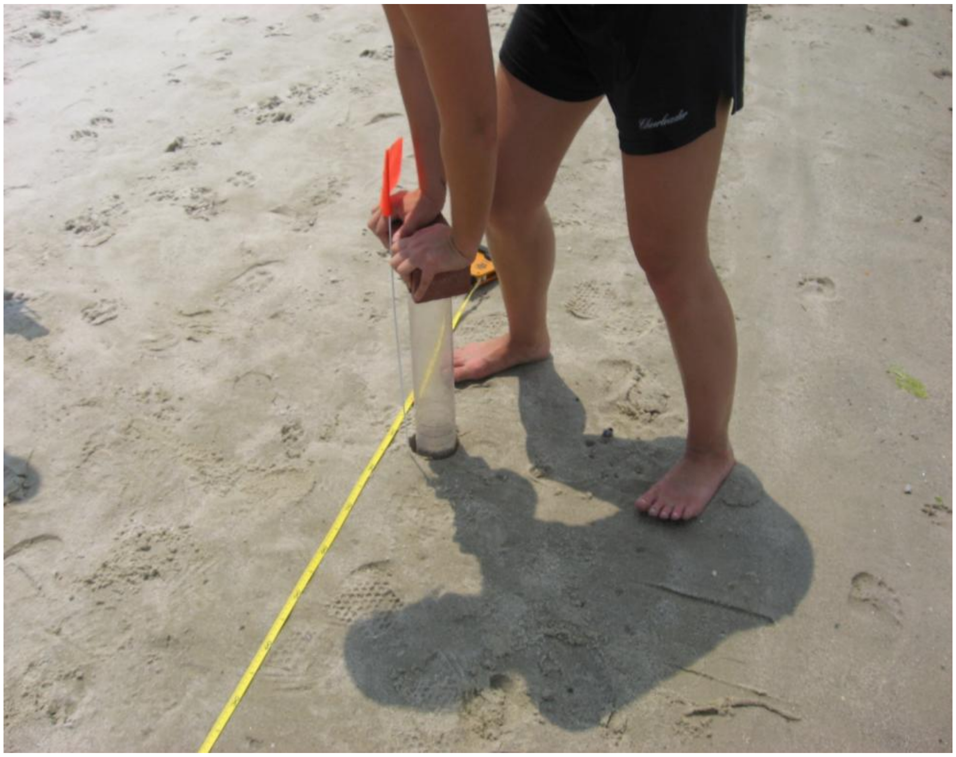

Figure 4. A photo showing a core taken along one of the three 15m transects. We took 10 total cores at random sites along each transect, for a total of 30 cores.

Figure 5. A photo showing the contents of the core which we dumped into the white tub and mixed together. A 50ml sub-sample was taken and run through a 1 mm sieve, and then the eggs counted.

2.3.2 Analysis Methods

Once egg sampling had been conducted, egg counts were copied into an Excel spreadsheet. I calculated the number of eggs per meter squared along the beach as n / (π/4·(6.7^2)) ·10,000, where n= average number of eggs per sub sample In addition, I extrapolated the number of eggs along the entire beach, and compared this to previous studies which explored egg data in the Delaware Bays.

Chapter 3

RESULTS

3.1. 2007 Adult Counts at the James Farm

Three counts occurred on May 31, June 14, and June 30 of 2007 (Figure 6 and 7). The average number of horseshoe crabs per meter for these three counts was 4.03. The average male to female ratio for the three counts was 4.4:1, the peak male to female ratio was 6.2:1 on May 31. We explored the spatial distribution of spawning adults on James Farm in 2007 and 2008 (Figures 8 and 9).

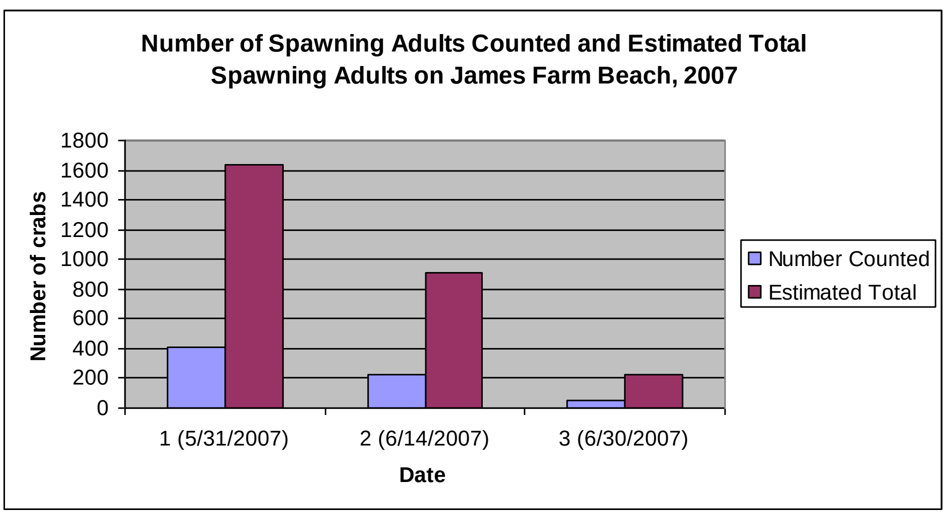

Figure 6. The number of spawning adults counted as well as the estimated total number of horseshoe crabs present on the beach for each particular census date.

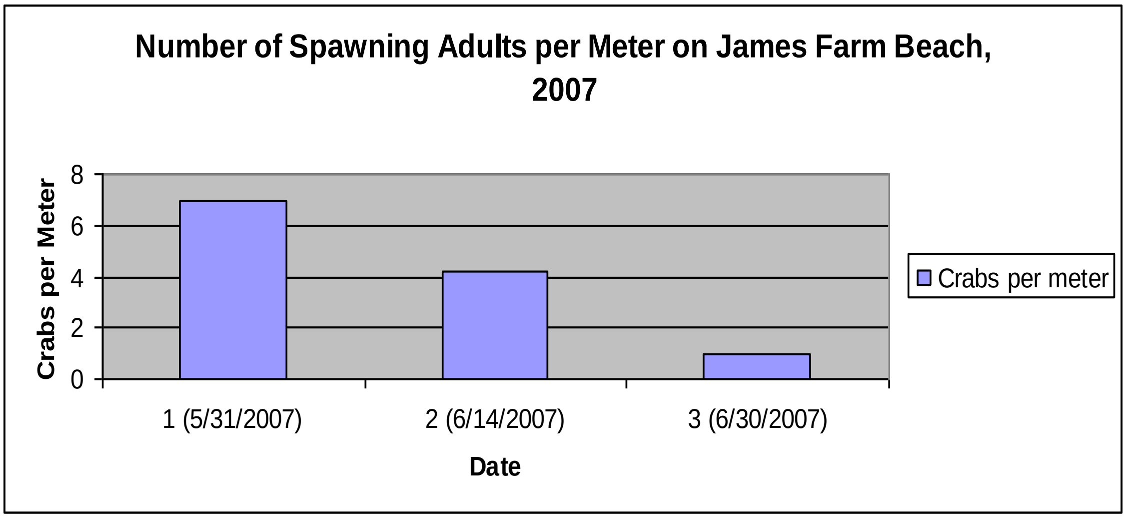

Figure 7. The number of average spawning adult horseshoe crabs per meter on James Farm beach on the three count dates during 2007.

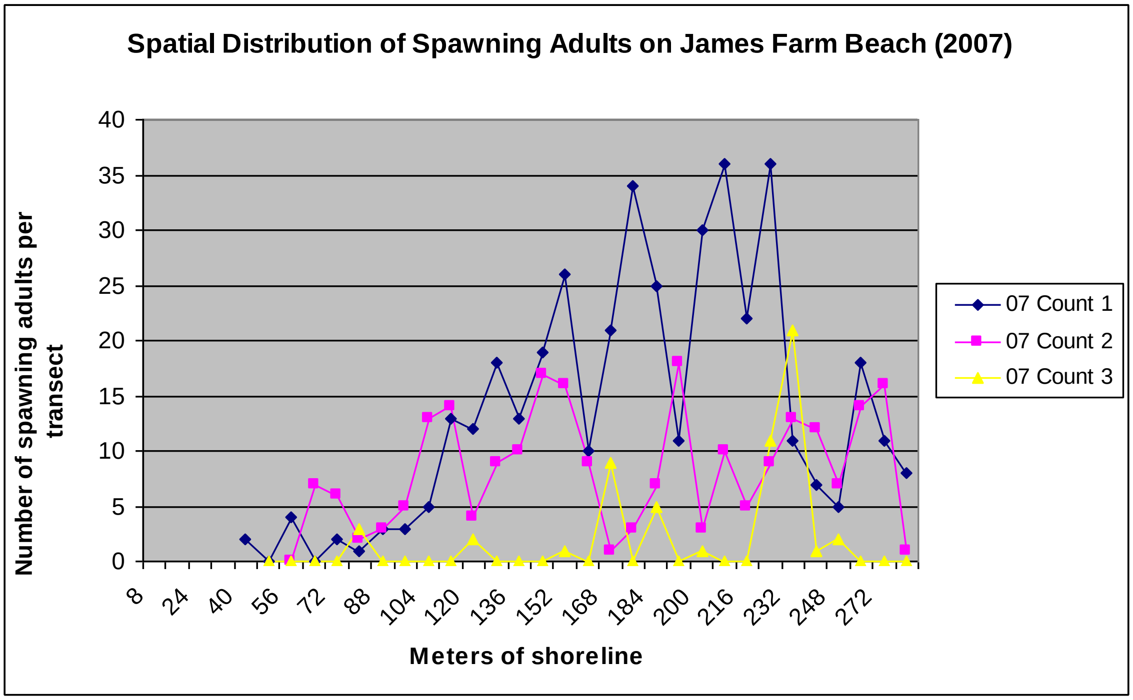

Figure 8. Spatial distribution of spawning adults on James Farm during the three separate count dates in 2007.

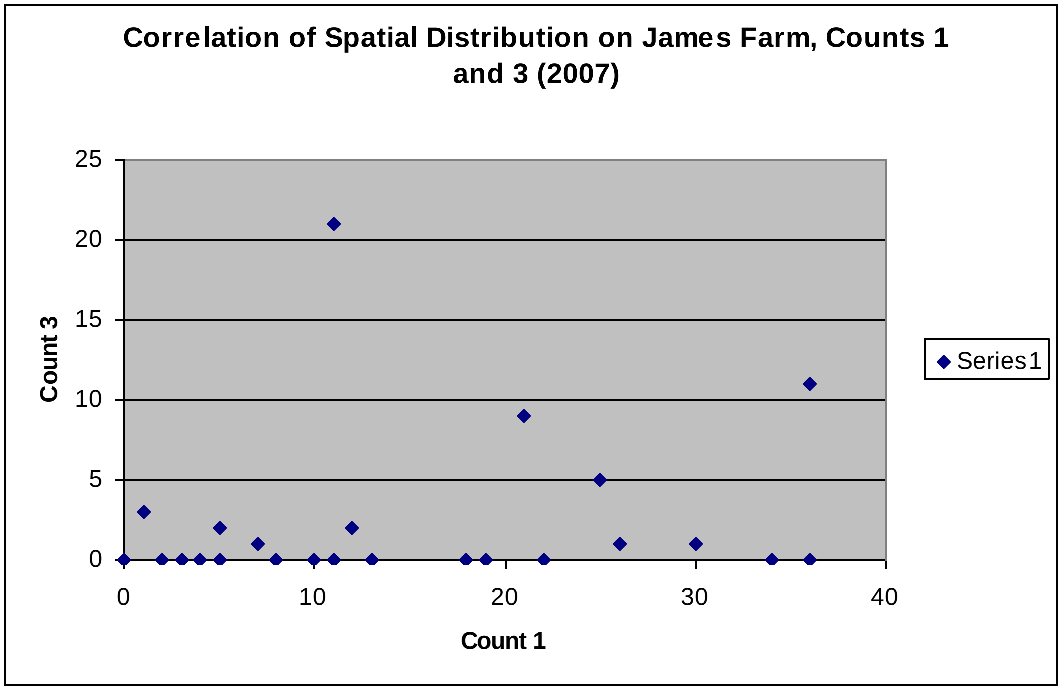

Figure 9. A correlation test for counts 1 and 3 (2007). R=0.19

3.2. 2008 Adult Counts at the James Farm

Spawning adult counts occurred on May 5, 17, 19, June 1, 3, 16, 20, and July 2 and 18 of 2008. The average number of horseshoe crabs per meter for all 9 counts was 1.67. The date with the highest number of horseshoe crabs per meter was June 3, followed by July 2. The average male to female ratio was 3.3:1. The highest male to female ratio for all 9 counts was 4.8:1.

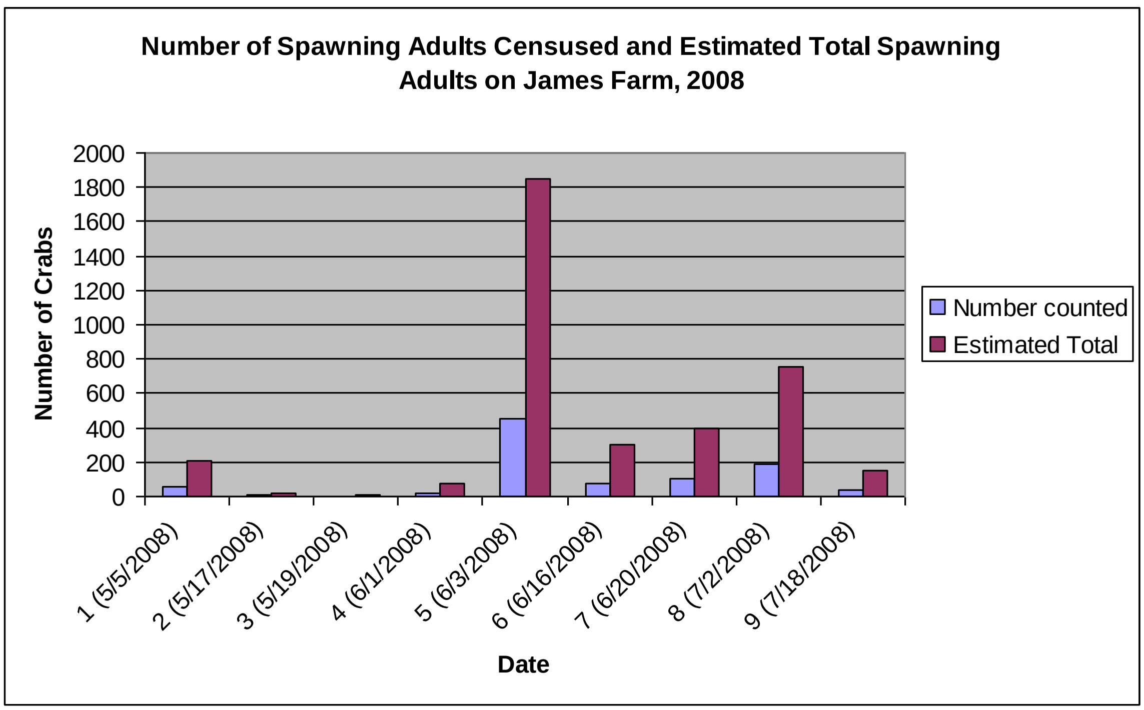

Figure 10. Number of spawning adults counted and the estimated total number of adults on the beach for each census that occurred in 2008 on James Farm

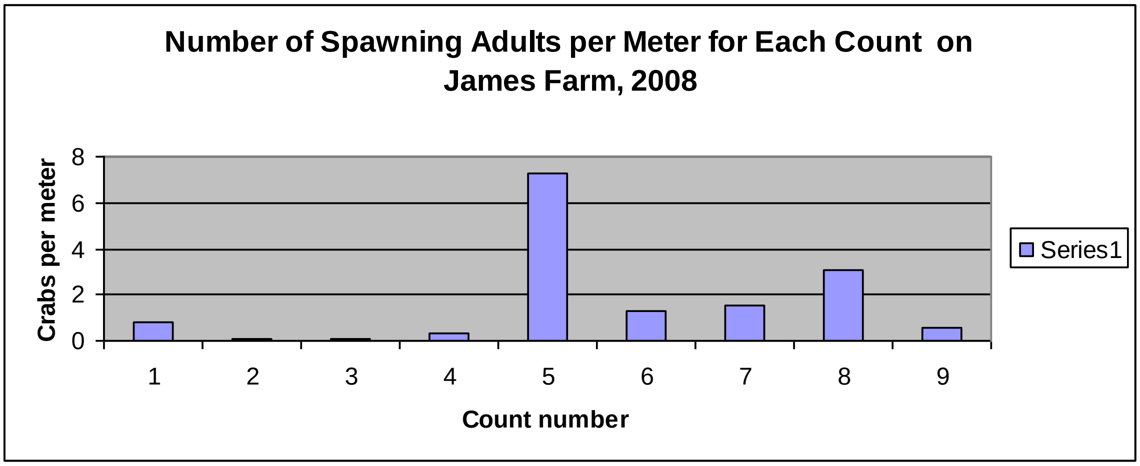

Figure 11. Number of spawning adults per meter on James Farm for all 9 counts, 2008. The peak count occurs on June 3, followed by July 2.

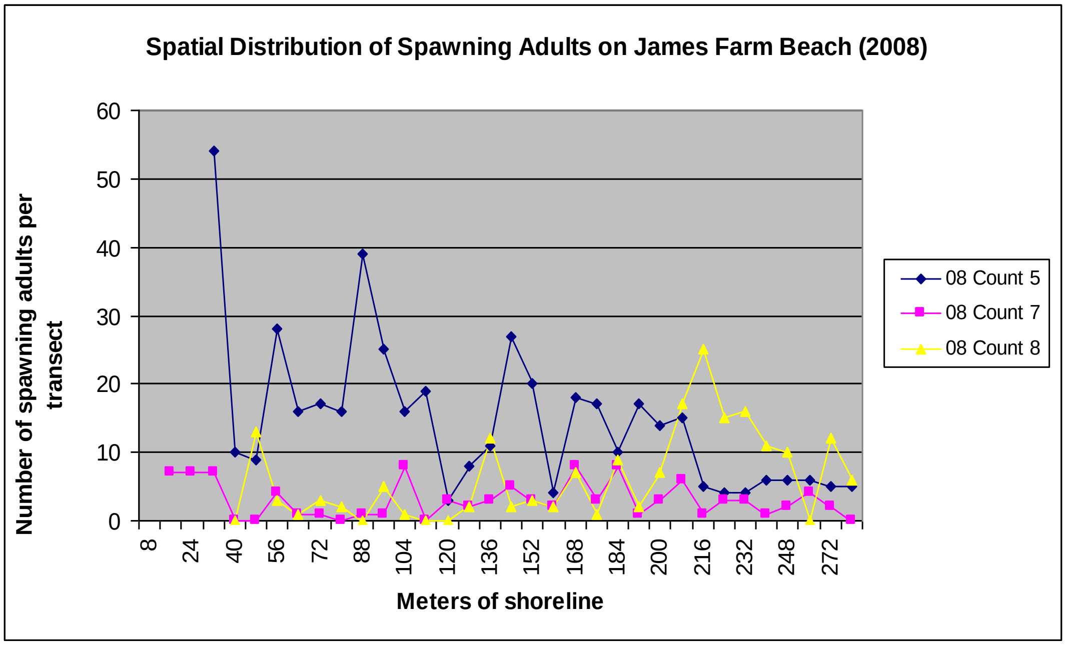

Figure 12. Samples of Spawning adults taken at their exact meter locations, for counts 5, 7, and 8 that took place in 2008 on James Farm.

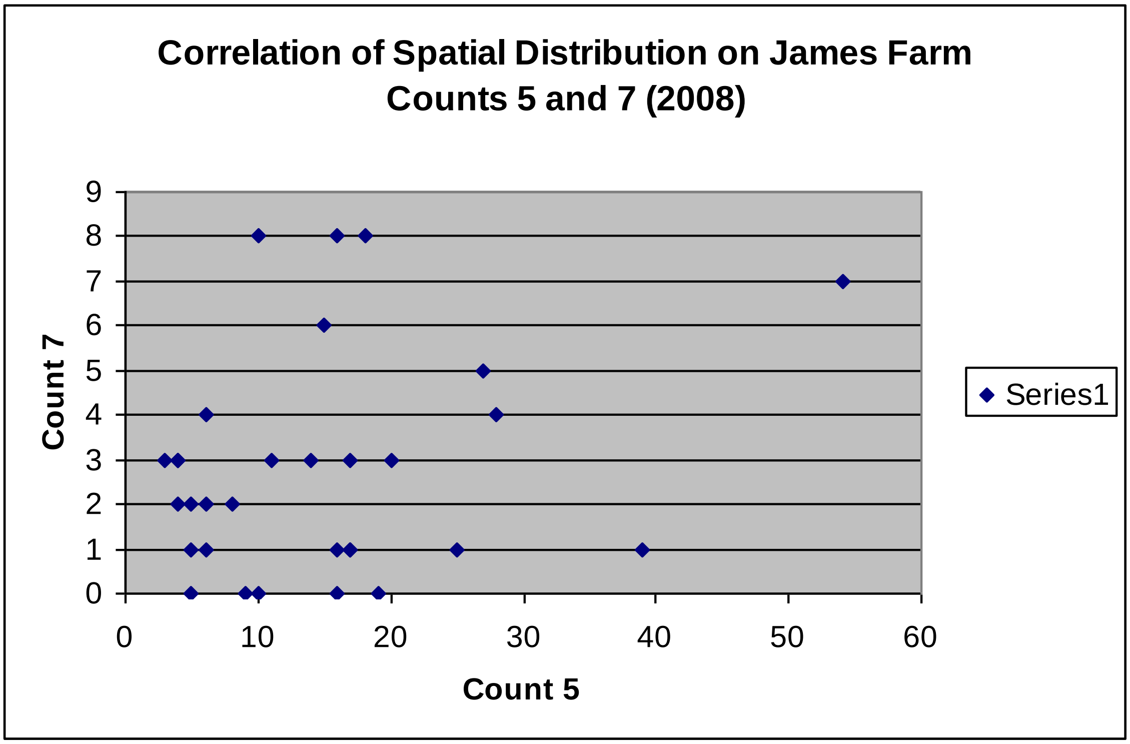

Figure 13. A Pearson's Correlation for Counts 5 and 7. r= 0.27

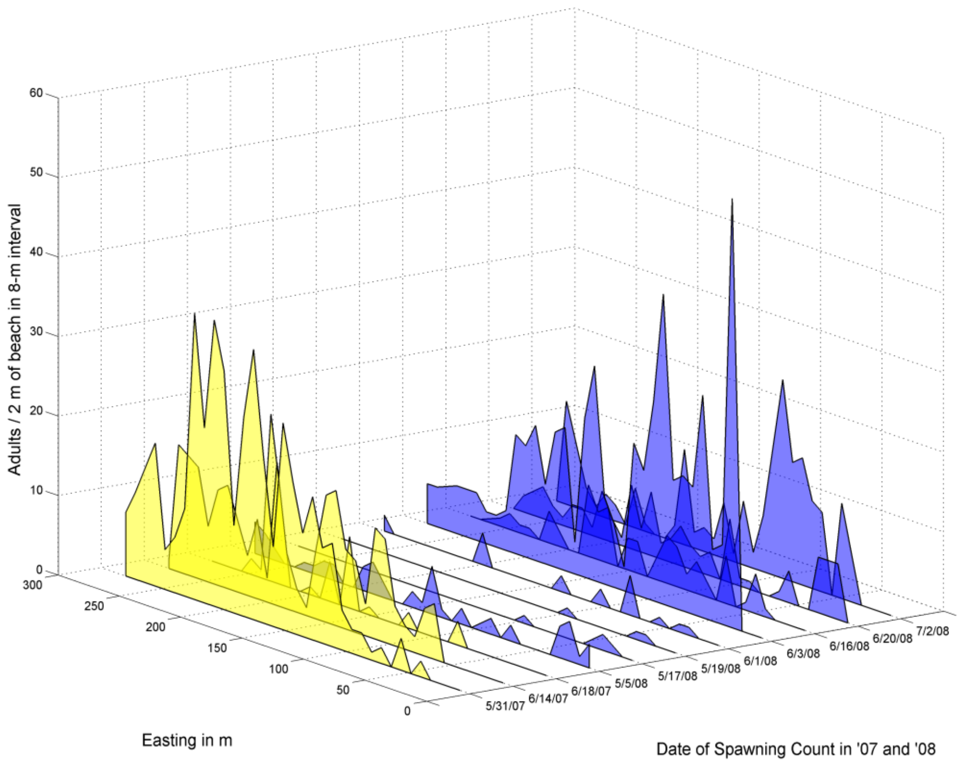

Figure 14. Spatial distribution of spawning adults on James Farm, all counts, 2007 and 2008.

3.3 Comparison of Spawning Adult Counts on Four Indian River Bay Beaches

In 2008, I counted spawning adults on four Indian River Bay beaches; Camp James Farm, Holts Landing, Camp Arrowhead, and Bay Colony. Savages Ditch (Rehoboth Bay) was identified as a sandy beach, however when cored for eggs, none was found (therefore it is presumably not a spawning beach).

James Farm and Holts Landing have a higher number of adults counted, as well as a higher density of adults than Bay Colony and Camp Arrowhead (Figure 15). The average male to female ratios for Holts Landing, Bay Colony, and Camp Arrowhead were 3.5:1, 3.6:1 and 3.3:1 respectively.

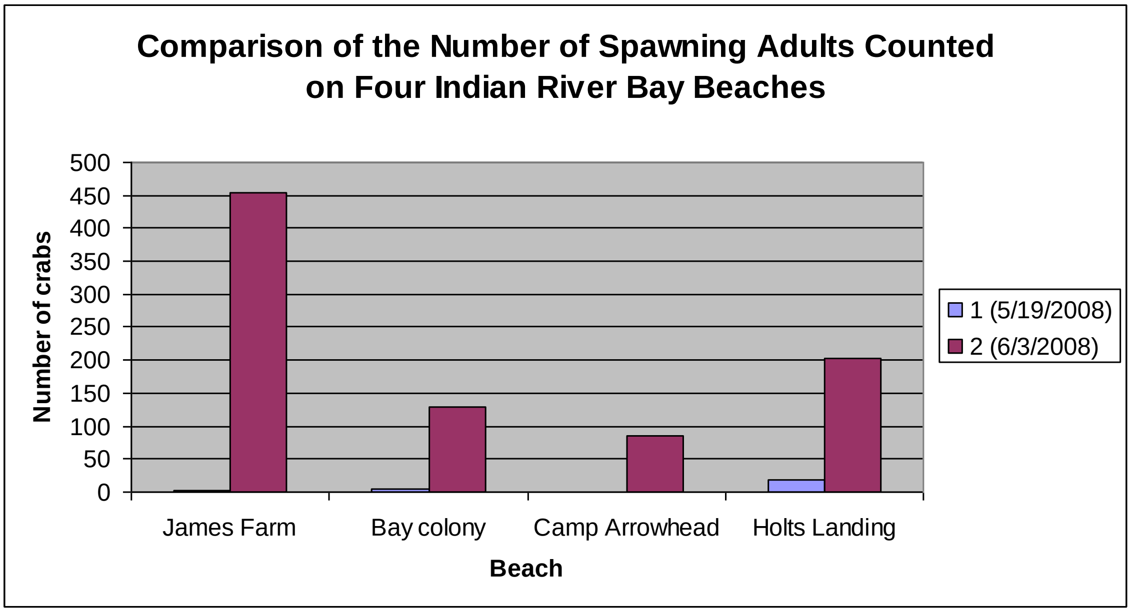

Figure 15. Spawning adults counted on the four Indian River Bay beaches, for May 19, 2008 and June 3, 2008.

We compared the spawning adult activity on the Inland Bays Beaches to that of the Delaware Bay's beaches. The comparison of the results obtained from Indian River Bay (bars in pink) to those from the Delaware Bay (in yellow) (Figure 16). These values represent the average number of spawning adults (both males and females) per meter from May 19, 2008 and June 3, 2008. The average number of spawning adults per meter at James Farm (Indian River Bay) surpasses that of Cape Henlopen and Woodland (Delaware Bay).

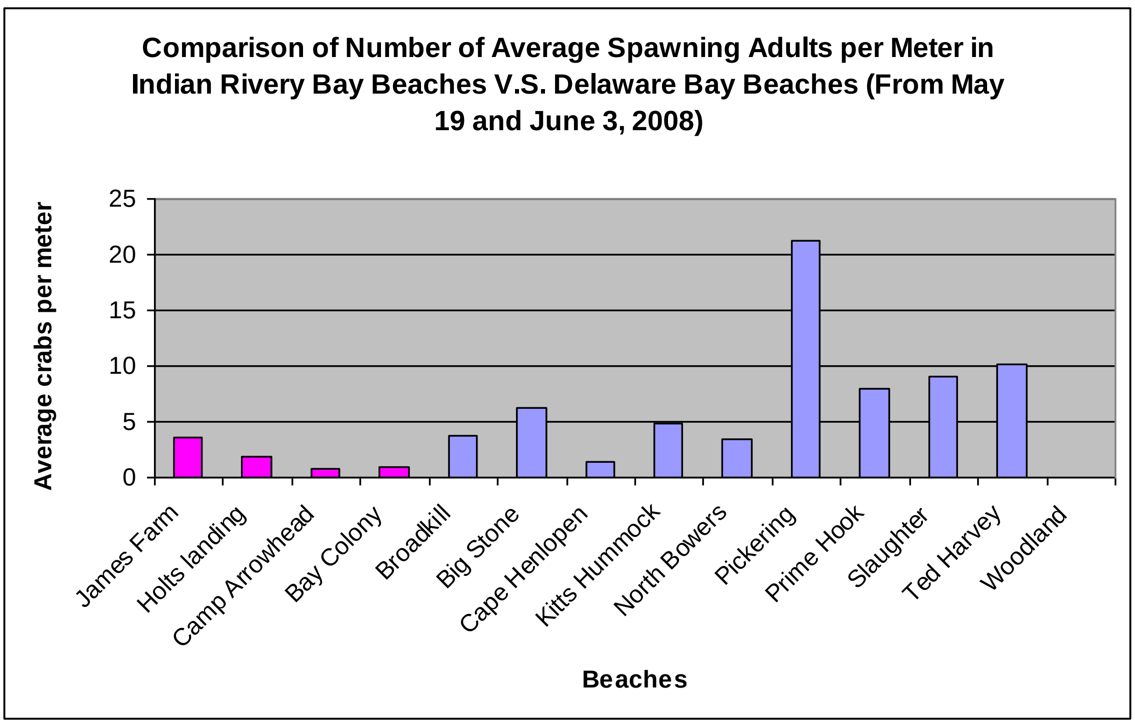

Figure 16. A comparison of Inland Bays beaches and the Delaware Bay beaches in the average number of spawning adults per quadrat for May 19 and June 3, 2008.

3.4 Horseshoe Crab Egg Densities at the James Farm and Holts Landing Ellis Point

Egg count methodology was tested in 2007 by comparing number of eggs per meter squared to the estimated number of eggs per meter squared as described by Smith et al. (2002) in 1999. The number of eggs per meter squared in 2007 was within range of those in 1999, indicated that we replicated the methodology correctly. We also compared the number of eggs per square meter on James Farm and Holts Landing (2007 and 2008) to those on Part Mahon, North Bowers, and Mispillion Inlet (2007) from Weber (2006). The egg densities are much higher on Delaware Bay beaches in 2006 than those on the Inland Bays beaches in 2007 and 2008, however they are, once again, within range.

On James Farm, the number of eggs per meter squared from 2007 to 2008 decreased by half from 266,000 to 148,000 eggs per square meter, whereas the number of eggs per square meter on Holts Landing from 2007 to 2008 increased from 182,000 to 369,000. In addition, the density of eggs on James Farm was much higher than that of Holts Landing in 2007, whereas the opposite is true in 2008. Finally, the percent of estimated "over-wintering" eggs and larvae is much higher on Holts Landing (149,000 or 40%) than on James Farm (3990 or 2.7%) (Figure 19).

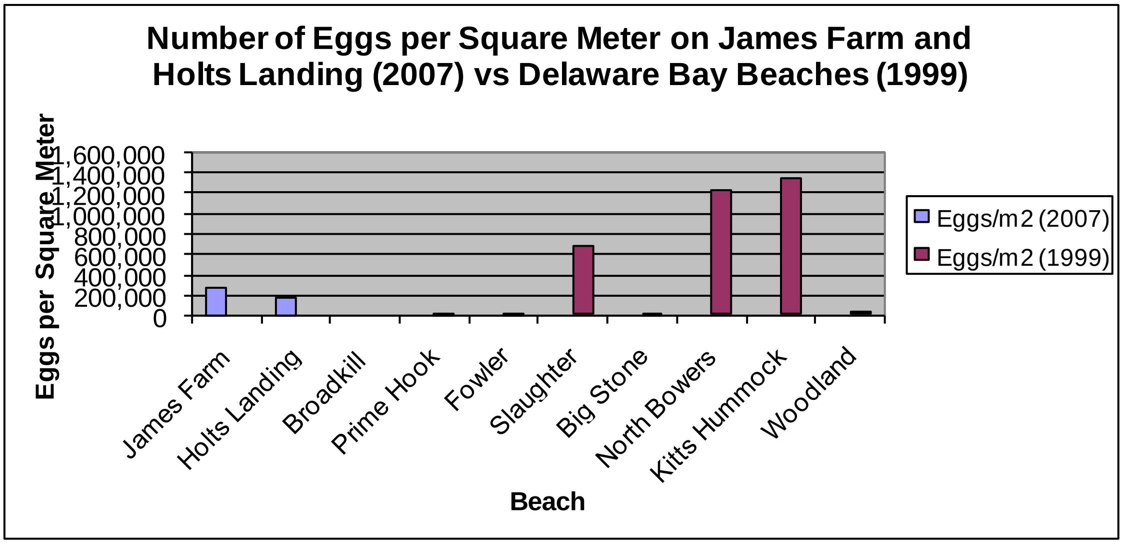

Figure 17. Comparison of the number of eggs per meter squared on Indian River Bay beaches (2007) to those in the Delaware Bay (1999).

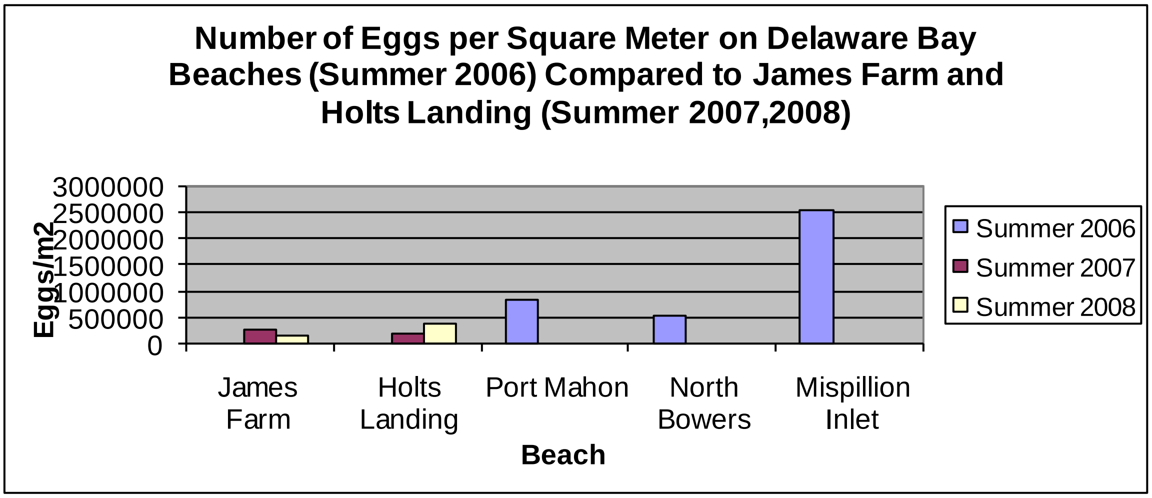

Figure 18. Eggs per square meter from Delaware Bay Beaches (2006) compared to those of James Farm (2007, 2008)

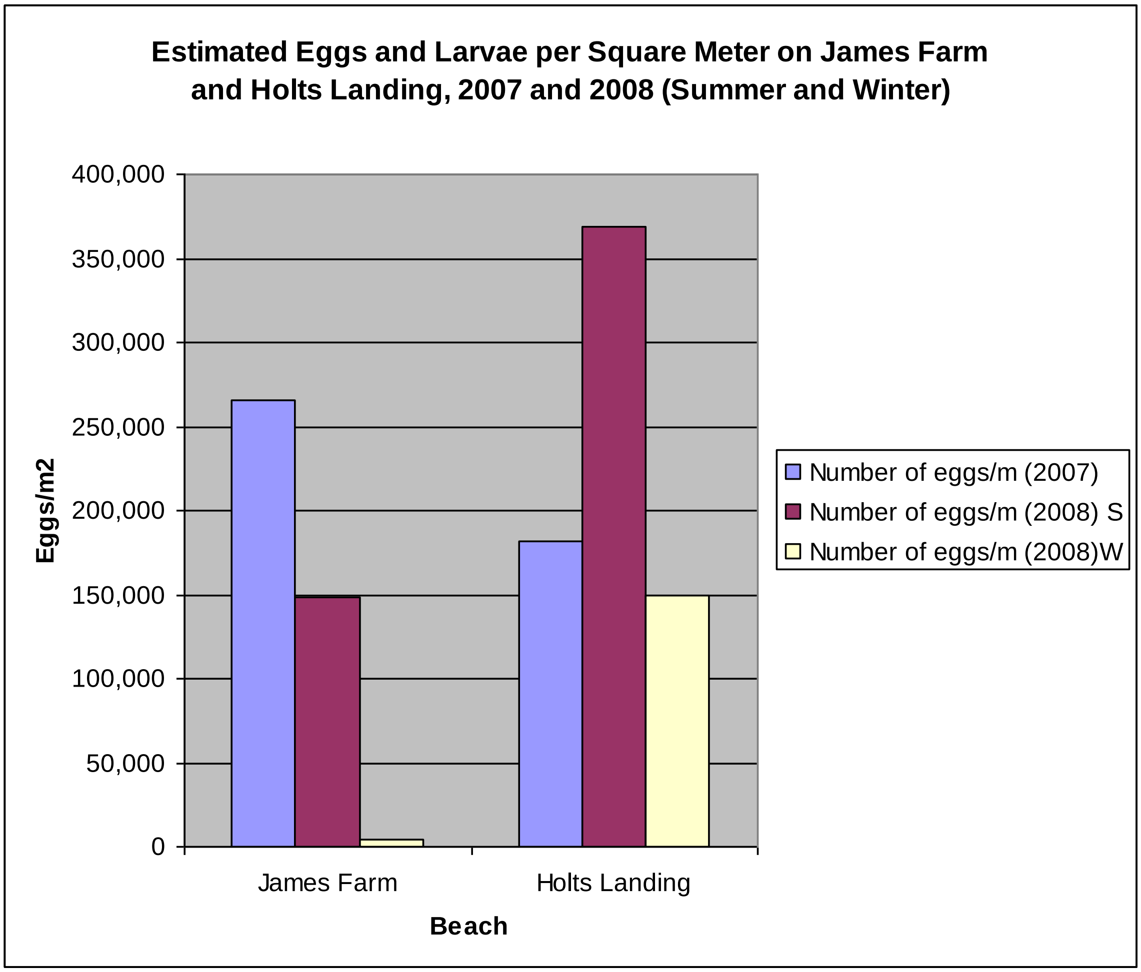

Figure 19. Comparison of the number of eggs per meter squared for both Holts Landing and James Farm, 2007 and 2008. Also includes a comparison of the number of over-wintering eggs and larvae per meter squared.

Chapter 4

DISCUSSION

4.1 Adult count in the Inland Bays compared to the Delaware Bay

The average number of spawning adults per square meter from Indian River Bay were within range of those from the Delaware Bay (Figure 16). More Specifically, James Farm not only exceeded the number of horseshoe crabs per meter of the other two Indian River Bay beaches, but also that of Cape Henlopen and was very close to Broadkill. Holts Landing also yielded a high density of horseshoe crabs, close to that of Cape Henlopen, and all four beaches surpassed that of Woodland beach.

The reason for why James Farm yielded such a higher density of spawning horseshoe crabs than the other three Inland Bays beaches is unknown, however it could have been be due to the closer proximity of James Farm to the Indian River Inlet where the horseshoe crabs come in from the ocean (Figure 1). However, causes of crab movement from one beach to another could be due to many unknown variables that must be further explored, such as the impact of currents on spawning adult dispersal and beach selection. In addition, the high density of horseshoe crabs at James Farm could have been attributed to the inverse relationship of foreshore width to the number of horseshoe crabs per meter as discussed in previous research (Smith et al. 2002). This research states that the wider the foreshore width the more scattered the spawning horseshoe crabs appear to be. Since James Farm has a foreshore width of only about 4 meters compared to Holts landing which is about 8 meters, as well as the beaches of Delaware Bay which are much wider, this relationship could explain the high density of horseshoe crabs on James Farm.

More importantly, although the number of spawning adults per meter at these four beaches were not within range of the 'Big Beaches' like Pickering, these results show that the Inland Bays do provide important spawning habitat for horseshoe crabs, and this answers the question that this project was designed to answer. In addition, because the inland bays do provide significant spawning habitats for horseshoe crabs, we must take these data into consideration when discussing management of the Inland Bays, as well as discussing horseshoe crab and shorebird survival.

My data also suggests that spawning adults may nest on particular areas along the beach. A relationship appeared to exist between where spawning adults nested during counts one, two and three in 2007 (Figure 8), and again for counts 5,7 and 8 in 2008 (Figure 12). It seems that horseshoe crabs tended to spawn in large numbers between meters 150 and 250 on James Farm in 2007, but were more widespread in 2008. Peaks appear to match up in these figures, however the exact relationship could not be determined. Correlation tests were performed for the counts that yielded the most horseshoe crabs for both seasons. For 2007, counts 1 and 3 resulted in an r value of 0.19, and for 2008, counts 5 and 7 resulted in an r value of 0.27. Although these values would be considered low (far from 1) for a laboratory experiment, they may be of some significance in this case, because many unknown variables exist when observing wild organisms and their behavior. Despite these values however, the idea that horseshoe crabs do prefer the same areas of the beach to spawn from one event to another must be explored further.

In addition, when comparing all counts that occurred on James Farm during 2007 and 2008, it does not appear that horseshoe crabs nested in the same general area for both summers (Figure 12). It seems that horseshoe crabs in 2007 nested in the center of the beach, whereas those in 2008 were more widespread. These potential relationships within seasons however, must be further explored in the upcoming years, in order to determine to what extent it exists. Other beaches such as Holts Landing, and even Delaware Bay beaches should be explored to see if horseshoe crabs are nesting in the same area during spawning events. Understanding these relationships will help to improve the methodology of adult counts.

Reasons for why certain areas on the beach are more preferable to spawning adults should also be investigated. For instance, one potential reason for this phenomenon could be the variation of oxygen in the sediment from one area of the beach to another (Button et al. 1988). For instance, when cores of eggs were taken and 0 eggs were consistently found, there seemed to be reduced conditions at around 10 cm. When cores were taken and eggs were found, reduced conditions were observed at areas deeper than 20 cm or not at all. It has been stated in previous research that horseshoe crabs may be able to detect anoxic layers of sediment, and as a result, do not reproduce in that area (ERDG). This could be an interesting topic to study at James Farm in the future.

4.2 Egg densities in the Inland Bays compared to the Delaware Bay

The decrease in the number of eggs per square meter on James Farm from 2007 to 2008 versus the increase in the number of eggs per square meter on Holts Landing (Figure 16, Table 3) could be due to some sampling error at James Farm, in that samples were taken too close to the water line. In addition, a storm had occurred on James Farm the previous week, and eggs may have been washed away.

Studies performed by Weber (2005), and Weber and Kalasz (2005), estimated densities of eggs per square meter for Delaware Bay beaches. Weber's results portray the number of eggs per square meter for the top 5 cm of sediment, whereas ours are from 20 cm, therefore it is inaccurate to compare these numbers. In addition, Weber and Kalasz found average densities, however, are the average of eggs per square meter from 5 counts that occurred throughout the summer, and were also only for the top 5 cm of sediment. However, Weber also estimated the number of eggs per square meter down to 20 cm in 2006 for Port Mahon, North Bowers, and Mispillion Inlet. He took a total of 20 cores for each transect. We compared the number of eggs per square meter on James Farm from 2007 and 2008 to these numbers (Figure 17). As expected, Delaware Bay beaches have much higher densities of eggs than those in the Inland Bays, however it may be inaccurate to compare numbers from different years, as well as numbers from different authors who used different methodologies. We must obtain data from all beaches in the Delaware Bay and the Inland Bays from the same year for future studies, and use the same methodology each time. Having an accurate dataset in the next few years will allow us to better understand old data, and understand where comparisons can be made.

In addition to exploring densities of eggs on Holts Landing and James Farm from one year to another, "over-wintering" of eggs and larvae was explored. Previous literature does not mention much about the phenomenon of over-wintering horseshoe crab larvae, however Weber (2003-2006) does indicate that over-wintering can occur in the Delaware Bay beaches into October. Holts Landing yielded a very high number of "over-wintering" eggs and larvae, whereas James Farm did not (Figure 19). An estimated number of 40% over-wintered, whereas James Farm only had 2.7% over-winter (Table 3). While there may be a variety of reasons for the differences in percentages, however the main point is that the eggs and larvae are still viable in the sediment into the fall, and it is a significant proportion of the original eggs laid. Weber (2002) sampled for larvae on October 31. He found that although larvae were still present, the density "dropped down to zero" in October, especially in bay-front beaches like Pickering and North Bowers. This could mean that the Inland Bays, because they are more sheltered from wave action and storms than the Delaware Bay, provide protection and allow more eggs to survive into November. However, more research must be done on this topic. Up-to-date data must be collected in the Delaware Bay regarding eggs and the proportion of "over-wintering" eggs, in order to compare them to the Inland Bays. Also, it would be wise to sample for over-wintering eggs in early spring, to see if "over-wintering" larvae are still present and alive.

4.3 Significance of Inland Bays to Mid Atlantic horseshoe crab populations

For each spawning survey, the Delaware-New Jersey counting program extrapolates the number of total spawning adults on each beach by multiplying the density of spawning adults by the number of meters of beach. With these numbers, it then estimates the total number of spawning horseshoe crabs for the season. Since we used the exact same methodology, we are able to estimate these numbers as well in order to estimate the total number of horseshoe crabs on James Farm for each count in 2008 (Figure 16).

The length of the beach plays an important role in comparisons of the total number of spawning adults on each entire beach, All four of the Inland Bays beaches are fairly short compared to Delaware Bay beaches, with Bay Colony being the longest at 0.4 km, whereas Delaware Bay beaches are many kilometers long. In fact, for large beaches, only 1km of the beach is counted, such as for Big Stone Beach, which is 5 km. Therefore, even if the Inland Bays beaches had the same average densities of spawning adults as those in the Delaware Bay, total estimates of horseshoe crabs would be much higher for the Delaware Bay. For instance, James Farm is only approximately 0.240 kilometers long. Therefore, for the peak spawning date on June 3, 2008, the estimated total spawning adults for that date would be 1845, whereas Cape Henlopen which had similar spawning adult densities in 2008, would have a 6.5 times higher abundances than James Farm due to its length of 1.5 km. Therefore, it can be concluded that the Delaware Bay Beaches have much higher total estimates of horseshoe crabs per season than do the Inland Bays. However, as described in section 4.4 below, estimating total numbers of spawning adults throughout whole beaches may be inaccurate.

4.4 Evaluation of published methods to horseshoe crab studies in the Inland Bays

First, nature provides a variety of obstacles that result in sampling error for both spawning adult and egg counts. For instance, while counting adults, murky water, as well as water level, could cause sampling error. Murky water could cause an observer to see fewer horseshoe crabs than are actually present. Also, sand bars are located past the shoreline at James Farm, and spawning adults in this area could be difficult to see. In addition, weather disturbances play a large role in sampling. Storms can affect the number of horseshoe crabs counted, as many horseshoe crabs may not come to spawn on days following a storm. Also, storms may wash away eggs that would otherwise be present in the sand, creating errors in analysis. Although not much can be done to correct these errors, it is important to note these as observations.

Second, for eight consecutive years, the Delaware and New Jersey counting program (http://www.ocean.udel.edu/SeaGrant/mas/bhall/hsccensus/index.html) has performed spawning adult counts during the summer months on 13 of the Delaware Bay beaches, using the methodology described above. In addition, other scientists such as Smith (2002) and Kalasz and Weber (2005) have conducted egg surveys using the methodology previously described. Although these methodologies provides a basis for estimating the abundances of spawning adults and eggs, the methodology for both spawning adult and egg counts could be further modified in order to improve future counts. In addition, this project has raised questions that merit additional sampling with methodologies that include counting adults and eggs on more Inland Bays beaches, increasing the number of counts on each beach, and increasing the sample size on each beach.

Smith and Michels (2006) state that temporal variation in the number of spawning adults does exist, and therefore, location and time of spawning surveys can have great effects on the general outcome of the 'big picture.' Because surveys only occur in the Delaware Bay during peak spawning times, results could be skewed (Smith and Michels 2006). This project supports Smith and Michels conclusions, in that peak spawning times were on different dates for 2007 and 2008. In particular, July 2, 2008 had the second highest number of spawning adults per meter out of all counts for 2008. Previous Delaware Bay surveys state that horseshoe crab spawning decreases in late June, and therefore, no surveys are performed into July. However, this statement is inaccurate, at least for the Inland Bays (Figures 10 and 11). Therefore a large number of spawning adults may not have been included in population estimates. These data may provide justification for conducting counts on more days (and into July) out of the summer on the Delaware Bay.

We also found that there may be patterns of spatial distribution along the beach (Figure 14). Correlation tests merit further study of the spatial distribution of spawning adults. Although these correlation tests yielded r values too low to make the conclusion that spawning adults nest in the same areas along the beach within each season (figures 9 and 12), horseshoe crabs appear to nest in clumps along the beach (figures 8, 12, 14). Since our methodology used random sampling with only two count sites per transect, detection probability was probably low, and clumps were skewing the data. In addition, For instance, although we sampled using the same methodology as that used in the Delaware Bay, we decreased our transect length from 20 (used in the DE Bay) to 8 because the Inland Bays beaches are much shorter than Delaware Bay beaches. Differences in sample sizes could cause an issue, and the potential impact of this difference must be further explored. Greater sample sizes would help to correct these issues, and should be considered for future counts.

In addition to spawning adult counts, the methodology for egg counts must requires modification. For instance, Pooler et al. concluded that 40 cores at approximately 20 cm deep would accurately detect changes in egg densities for beaches up to 100 meters long. He also states that samples must be taken along the entire beach, not just in one segment, in order to make inferences for that entire beach (Pooler et al. 2003). Our data support Pooler's conclusions. With our raw numbers taken from 30 cores, we extrapolated the number of eggs and larvae per meter squared for the whole beach. However, eggs and larvae were not evenly distributed along Indian River Bay beaches, rather, they existed in clumps or sometimes existed as individuals. Weber and Carter (2002) found that egg clusters consist of an average of 5,786 eggs plus or minus 2834 eggs. Therefore, using our numbers to estimate abundances as well as total numbers of eggs and larvae for whole beaches is inaccurate (Weber 2006). Also, previous studies by Weber (2005) and both Weber and Kalasz (2005) have shown that the density of eggs on beaches changes throughout the summer. For instance, on May 25, 2005, Kitts Hummock had an average density of 455 eggs/m2 for the top 5cm of sand, however, on June 9, 2005, it had an average density of 103,000 eggs/m2 for the top 5 cm of sand (Weber 2005). Weber and Kalasz (2005) also support these findings. They provide means for the density of eggs for 5 dates (May 15, 22, 29, and June 5, and 12). Their results showed different average densities of eggs for each date, with the highest occurring on June 5 and 12. For this project, we only sampled James Farm and Holts Landing once per summer, on different days for each beach (see table of dates in section 2.2.1 of methodology) taking a total of 30 cores each time. To make future counts more accurate, we must sample for eggs many times throughout the summer, on the same dates for each beach. Also, we must first increase the number of cores taken to cover the entire beach, as well as to find the probability of detecting a clump, so more accurate inferences can be made.

Therefore, I recommend that for both future egg and spawning adult counts, we count on more Inland Bays beaches, increase the number of counts that occur within the summer, and increase the number of egg samples taken on each of these beaches. This will allow for a more accurate estimate of the horseshoe crab population in the Inland Bays.

4.5 Future surveys and data needs

Spawning counts at James Farm, Holts Landing, Camp Arrowhead, and Bay Colony will continue for the summer of 2009. However, in order to accurately compare the Inland Bays spawning adult horseshoe crab populations with those of the Delaware Bays by estimating the total number of spawning horseshoe crabs for each night and for the entire season, we must have enough volunteers to count horseshoe crabs on different Inland Bay beaches for each date that the Delaware and New Jersey counting program performs its counts on Delaware Bay beaches. In 2008, spawning adult counts only occurred on May 19, 2008 and June 3, 2008, on Bay Colony, Camp Arrowhead, and Holts Landing, therefore it may be difficult to estimate accurately the total number of spawning adults for the entire season of 2008 and compare to the Delaware Bays.

In addition, more Inland Bays beaches (identified through tips from locals and map observations) that provide spawning sites for horseshoe crabs must be identified, and the spawning adults must be counted for the same dates as described above. Counting on more beaches will create a more accurate picture of the spawning horseshoe crab population in the Inland Bays. Finally, it is important to continue to count horseshoe crabs into July, because as we have seen in 2008, peak spawning dates are unpredictable, and it should not be assumed that spawning ends at the end of June.

For egg counts, as described in section 4.1, we must increase our sample size, sample eggs many times within the summer, and take into account clumping when estimating the density of eggs per square meter.

4.6 Summary recommendations and conclusions

The original objective of this project was to determine if the Inland Bays provide important spawning sites for *L. polyphemus*. Based on the spawning adult counts we performed on James Farm Beach, Camp Arrowhead, Bay Colony, and Holts Landing, I conclude that a significant number of horseshoe crabs, comparable to that of Delaware Bay on a per meter of beach basis, spawn in the Inland Bays, and that the Inland Bays beaches provide crucial habitat that allow this population of horseshoe crabs to exist. In addition, egg sampling in throughout the summer of 2007 and 2008, reveal that this spawning results in viable eggs and larvae. Finally, egg sampling in November 2008 showed that live embryos and larvae are present on these beaches at least four months after the observed peak spawning period and these eggs and larvae may “over-winter”.

The previous data collected throughout the past two years, is preliminary. The Delaware and New Jersey counting program has performed annual spawning surveys for 8 consecutive years in the Delaware Bay, with a total number of approximately 24 sites (11 in New Jersey and 13 in Delaware) on 12 separate dates, analyzed by Limuli Laboratories and compiled by Benjie Lynn Swann, William R. Hall, and Carl N. Shuster. However, the database for the Inland Bays consist of only these data present in this report, from four beaches. It is important that these adult and egg counts continue in the Inland Bays, in order to monitor the population of horseshoe crabs in the Inland Bays over time, as well as compare it to the Delaware Bays.

This project has provided an immense amount of information about the population of horseshoe crabs that spawn in the Inland Bays. Further research must be conducted in order to better understand this population of horseshoe crabs, and this project provides an incentive to do so. For instance, this project also brought up many more questions to be answered and potential experiments to be conducted regarding horseshoe crabs in the Inland Bays. Some examples include perhaps quantifying shorebirds that utilize these eggs in the Inland Bays, determining if grain size and levels of oxygen in the sand have impacts on where horseshoe crabs spawn, determining if the location of the Inland Bays provide more protection for horseshoe crabs than the Delaware Bays, exploring the population genetics of the Inland Bay horseshoe crabs to determine if they are a sub-species of those found in the Delaware Bay, and many more. This project is a very important start to answering all of these questions, and it is important that spawning adults and eggs are counted on the Inland Bays beaches every year, in order to obtain a larger dataset, and better understand the number of horseshoe crabs present in the Delaware area. These results can have large conservation and management impacts, especially when assessing the environmental impacts of certain projects that could occur on Inland Bays beaches, such as the shoreline hardening (e.g., bulkheading and rip rap), or beach nourishment by sand pumping onto the beach. In addition, it is these numbers that conservationists and policy makers could use to have an accurate picture of all Atlantic horseshoe crap populations and therefore make accurate decisions to conserve horseshoe crabs through the placement of a future moratorium, or the decision to lift a moratorium. Having an accurate understanding of the horseshoe crab population will therefore help to conserve the red knot and other shorebirds, as well as improve the whelk and eel fishing industries.

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