PRECONSTRUCTION HORSESHOE CRAB EGG DENSITY MONITORING AND HABITAT AVAILABILITY AT KELLY ISLAND, PORT MAHON, AND BROADKILL BEACH STUDY AREAS, DELAWARE

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Contract Number: DACW61-01-P0212

15 September, 2001

Submitted to:

Philadelphia District Corps of Engineers Wannamaker Building 100 Penn Square East Philadelphia PA 19107-3390

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Background

Several species of migratory shorebirds and resident laughing gulls feed extensively on eggs of the horseshoe crab, *Limulus polyphemus* L., during its spring spawning season (Botton 1984, Burger and Gochfeld 1991, Castro and Myers 1993). For some shorebird species migrating to their arctic nesting grounds, the stopover on Delaware Bay beaches to feed on *Limulus* eggs may represent the most critical part of their annual reproductive cycle (Castro and Myers 1993). Migrating shorebirds have been shown to make body weight gains of 40%, or more, during their two to three-week stopover on Delaware Bay beaches in May (Castro, et al. 1989).

In Delaware Bay, most *Limulus* spawning occurs from April through July, with May and June being the peak months of activity (Shuster and Botton 1985). Female Limulus spawn near the high tide line beneath the beach surface in "nests", where they produce one or more clusters of adhering eggs. Clusters are deposited below the feeding zone of shorebirds. However, many of these clusters become dissociated before the eggs hatch, and their constituent eggs are dispersed through beach sediments, toward the surface. A simple census, for egg clusters only, can underestimate actual egg numbers present on a beach (Weber 1998, 1999a, 2000). Several studies have sampled beaches to determine the populations of horseshoe crab eggs present in beach sediments. Researchers examining Limulus spawning behavior have taken a variety of approaches, however no standardized sampling method for determining densities of *Limulus* eggs dispersed in beach sediments has emerged from the literature. Such a method would facilitate a variety of comparisons that would be especially useful in making coastal and estuarine management decisions. Examples include: quantification of dispersed-egg population densities on beaches most heavily used by migrating shorebirds, comparisons of dispersed-egg populations in heavily used beaches with egg populations of less-used beaches, comparison of annual variations in spawning activity on a particular beach, and investigation of the effects of beach erosion or beach replenishment on Limulus spawning.

The Army Corps of Engineers is proposing to use dredged material from deepening the Delaware River Federal Navigation Channel for shoreline restoration projects at Kelly Island, Port Mahon, and Broadkill Beach, areas on the Delaware Bay known to attract shorebirds and spawning horseshoe crabs. These projects are expected to increase the amount and quality of horseshoe crab spawning habitat, significantly improving the habitat quality for both horseshoe crabs and shorebirds. In order to determine whether the completed shoreline restoration has benefited these species at the site, it is necessary to collect and analyze quantitative and qualitative baseline data on horseshoe crab egg density prior to construction.

Currently an environmental window exists that prevents construction (ie. sand placement) to take place from 15 April to 31 August to prevent impacts to spawning horseshoe crabs. This

window follows the recommendations of the Atlantic States Marine Fisheries Commission's Interstate Fishery Management Plan for Horseshoe Crab (1998). These projects will be extremely difficult to build if no construction is done during this period. It may not be possible to complete the Kelly Island wetland restoration. The Delaware Department of Natural Resources and Environmental Control has stipulated that unless the Corps of Engineers can provide site specific information to indicate that 1) the site is not being used as a horseshoe crab nursery area or 2) that horseshoe crab spawning and egg incubation has ceased for the year, then the above window would be applied. Site specific information will be needed for confirmation of these conditions if sand placement is requested within the general 15 April to 31 August closure window. During 2001, this study will estimate the amount of potential horseshoe crab spawning habitat that exists at each site, will sample horseshoe crab egg densities at these sites, and compare those egg densities to egg densities on other horseshoe crab spawning areas examined on the Delaware Bay coast in Delaware during the same period.

Objectives Of This Study

This study was conducted on Kelly Island, Port Mahon (both in Kent County), and Broadkill (Sussex County) beaches, in Delaware during the summer of 2001. The study collected information about the seasonal distribution and relative abundance of horseshoe crab (*Limulus polyphemus* L.) eggs in these beaches. It further provided estimates of spawning habitat currently available on each beach.

This report presents information about horseshoe crab egg densities gained during studies conducted on Kelly Island, Port Mahon, and Broadkill beaches during the summer of 2001. In it, I summarize my findings, discuss them in relation to the literature of horseshoe crab spawning, compare them to data collected in a parallel 2001 study on three other Delaware beaches (North Bowers, Kitts Hummock, and Pickering), and further compare them to data collected during studies conducted on several other Delaware beaches during recent summers.

Materials And Methods

Descriptions of the study beaches Kelly Island is not actually an island, but rather a marshy peninsula lying between the Mahon River and Delaware Bay. The southern tip of Kelly Island, near the mouth of the Mahon River, is the area where a restoration project is being considered. Figure 1, Appendix A is an aerial photograph of the study area, taken in 1997. The shoreline runs more-or-less true north. Seen looking shoreward from a boat, at low tide, most of the shoreline consists of irregular, vertical peat "cliffs", ranging in height from ca. 0.5–1.3 meters above low water. There are few locations where the sandy upper beach grades smoothly down to the low water line. The upper edge of the beach is separated from the background marsh by a variable wrack line, consisting mostly of coarse vegetable detritus, deposited during periods of storm flooding. Seaward from this storm wrack line, and running irregularly along beside it, is a discontinuous band of sand of varying depth, deposited on the peat substrate. Depth of this band ranges from approximately 40 cm at the upper edge to 2 cm at the lower edge. The band ranges in width from 2.1 m (7') to 8.5 m (28'), and in all but a few narrow places, is discontinuous with the tide flats, being separated from the low water line by variable expanses of peat substrate which is well above the low water line All egg clusters and eggs that I found on this beach were in this band of sand.

The two study transects sampled on Kelly Island during this study were "North", and "South", whose upper (high beach) ends were located at N39°12.679', W075°23.913' and N39°12.431', W075°23.849', respectively. Locations of these points are shown on **Figure 1**, **Appendix A**. Approximate distance between the two transects was 418 m (1,373').

Port Mahon beach has a northeasterly-oriented Delaware Bay shoreline. **Figure 2, Appendix A** is an aerial photograph of the study area, taken in 1997. A sand road closely parallels the shoreline. The southern midsection of the beach has several sections of vertical metal breakwater, which persist from early attempts to protect the roadway. Breakwater sections parallel the shoreline 1-2 m out past the low tide line. The road is separated from the water by a variable band of riprap which consists principally of boulders in the 30-120 cm (1'-4') size range. The lower edge of the riprap runs variously up and down through the intertidal area. In some places the lower edge of the riprap reaches out nearly to the low tide line. In other cases the lower edge rises somewhat above the middle part of the intertidal area. At lunar tides, water rises completely over some sections of riprap, and wave action erodes the roadway. As a result, the road is subject to continual grading and repair, with additional sand being added several times each year. Sand from this erosion and subsequent replenishment works downslope through the riprap, to create the sections of sandy beach upon which the horseshoe crabs spawn.

On the bay side of the riprap, the beach contains varying amounts of smaller (≤ brick size) miscellaneous chunks of macadam, masonry rubble, etc., applied long ago in attempts to stabilize and maintain the road. This trash material, together with random layers of shell, is variably covered with sand. The color and size uniformity of the sand particles along the riprapped beach areas suggest that most sand present is the result of erosion from the material used to repair the road. Much of what appears to be sandy beach is actually shallow sand underlaid by clay hardpan, dense layers of shell, or miscellaneous trash material, and is generally unsuitable for spawning. Female horseshoe crabs seldom spawn in situations where the sand is not at least deep enough to nearly cover their bodies, approximately 10 cm (4").

The two study transects sampled on Port Mahon during this study were "North", and "South", whose upper (high beach) ends were located at N39°11.114', W075°24.071' and N39°10.794', W075°24.297', respectively. Locations of these points are shown on **Figure 2**, **Appendix A**. Approximate distance between the two transects was 671 m (2,203').

Broadkill Beach differs from the other beaches studied, being a wide, continuous band consisting almost entirely of clean sand and small (<2 cm) gravel. Sediment depths are greater than 30 cm in most sections. The beach is currently protected by a series of regularly-spaced breakwater structures extending from high on the beach, out into the water at right angles to the shoreline. Shoreward, the beach is backed by varying widths of sparsely vegetated dunes, and a dense residential area. Figure 3, Appendix A is an aerial photograph of the study area, taken in 1997. This beach is the southernmost of the beaches studied approximately 26 miles from Port Mahon.

The two study transects sampled on Broadkill beach during this study were "North", and "South", whose upper (high beach) ends were located at N38°49.961', W075°12.958' and

N38°49.713', W075°12.692', respectively. Locations of these points are shown on **Figure 3**, **Appendix A.** Approximate distance between the two transects was 577 m (1,894').

Sampling procedures In Delaware Bay, *Limulus* spawning activity seems to be more intense during the full and new moon tides (Rudloe 1985). During the 2001 spawning season, full moon tides were on May 7; June 5; July 5, and new moon tides were on April 23, May 22; June 21. I sampled the beaches several days after each of these tides. It was not possible to sample all three beaches on a single day. Typically, the Kelly Island and Port Mahon samples were taken on one day, and Broadkill was sampled another day. For simplicity in this report, sample dates are listed as a single date (the day Kelly Island and Port Mahon were sampled), rather than two. Sample dates were April 26; May 10, 25; June 11, 25; July 9. On these dates, I sampled each beach along two transects which were at right angles to the waterline. Upper (high beach) transect endpoints were located by reference to permanent visual markers, and recorded as GPS readings, and the same section of beach was sampled on each date. (The exception to this sampling schedule is that I could not sample the Kelly Island N transect on 25 May because the boat sank at anchor while I was collecting the sample on S transect.) All transects were within the intertidal zone, where spawning activity is more concentrated (Botton, et al. 1994, Shuster and Botton 1985, Weber and Ostroff 1997, Williams 1986, Williams 1987).

I took 25 evenly-spaced core samples along each transect, spanning 83% of the distance from the nocturnal high tide wrack line to the foot of the beach, where the flat began. I used 83% of the total distance because a pilot study I did in 2000 (unpublished) showed that 100% of all eggs in each of four Delaware beaches were located in the upper 83% of the nocturnal-tide-wrack-line-to-flat span. Although intertidal beach widths varied at the points where transects were located, the 25 sample cores along each transect were kept evenly, thus proportionally, spaced across the sample distance by use of transect lines made from bungee cord. These lines were marked off into 25 equal units of distance. Bungee cord lines can be stretched to fit beaches of varying widths, and since the marks spread apart at the same ratio as the line is stretched, cores are always equally spaced across the span to be sampled.

Sample cores consisted of beach sediment cores, 5.7 cm (2.25") in diameter x 20 cm (8") deep. The 20 cm depth of the sample cores spans the reported range at which most egg clusters are placed during spawning (Hummon et al. 1976, Rudloe 1979, Weber 1998, Weber 1999a, Weber 2000). Surface area (cross section) of each core was 25.7 cm², giving a total cross-section of the 25 cores taken per transect of 641 cm². After each core was lifted, it was separated into two fractions: 0–5 cm and 5–20 cm depth.

Core sample fractions from each transect were combined into the appropriate bucket as they were collected, and all material collected was processed to extract the eggs. Upon collection, each fraction of the core sample was passed through a 13 mm (0.5") mesh screen into a collection bucket, to remove any large gravel or shells, and to reveal clumps of eggs. (When *Limulus* eggs are laid, they adhere together in tight clusters [Rudloe 1979], and they continue to adhere tightly to each other during the first weeks of development.) One, or more, tight aggregations of eggs that did not pass through the 13 mm mesh was recorded as a single cluster. Thus, a single 20 cm core could have up to two clusters: one each from the 0–5 cm and 5–20 cm fractions. After being recorded, clumps were broken apart to pass through the 13 mm screen, into the appropriate

sample container, and their component eggs included in the final egg volume values. The 25 sample cores from a single transect (0–5 cm and 5–20 cm fractions, considered together) had a total volume of approximately 13.3 liters (3-1/2 gallons).

Extracting and quantifying eggs Samples were processed at the Delaware National Estuarine Research Reserve Center. The contents of each bucket were flushed through a series of screens with running water to separate eggs from most of the beach substrate material. Mesh size of the first screen was 6.4 mm (1/4"); of the second, 3.2 mm (1/8"). All eggs were captured on the third screen, of copper window screening (mesh size, $1 \times 1.5 \text{ mm} = 0.04$ " x 0.06"), which retained all eggs encountered, plus beach sediment particles in the same size range. Eggs were separated from the remaining sediment and most other materials retained on the third screen, by elutriation with running tap water as described previously (Weber 1998).

Residual peat particles and meiofauna were separated from *Limulus* eggs, embryos and trilobite larvae by hand picking. I then used a 10% (v/v) solution of MgSO4 and tap water to separate smaller, greenish undeveloped eggs ("eggs") from the larger, visibly embryonated eggs ("embryos") by differential flotation. "Embryos" float, "eggs" sink, in this solution, giving a good separation. The separation is not absolute however, and some items that appear to be "eggs" float, while some apparent "embryos" sink. "Eggs" that float are not viable. Most hatchlings (trilobite larvae) swim, or float passively, in the MgSO4 solution. All material that floated in the MgSO4 solution was discarded, and only the eggs were quantified. It is not necessary to also quantify embryos and trilobite larvae, because the eggs take sufficient time to develop that they are present in the beach for at least two sample periods before they hatch. (See Beach temperature, below.)

As each sample is being separated from remaining sediments by the elutriation process, a few viable eggs are also rinsed out. All material coming out of the elutriation system was checked, and any viable eggs present were hand counted. When sample egg numbers were small, I made direct counts. When numbers were too great for direct counting to be efficient, I measured the extracted eggs volumetrically, using standard graduated cylinders. Volumes were measured by pouring the sample, with tap water, through a funnel into a graduated cylinder (25, 50, 100, 250 and 500 ml, as appropriate to sample size). The cylinder was then stoppered, inverted several times to distribute the sample evenly in the water column, set upright and allowed to settle. After settling, the cylinder was bumped against the benchtop several times to further consolidate the sample, then volume was read and recorded.

By counting measured volumes of eggs, some taken during each sampling period, I found there was an average of 178 eggs (n= 20 samples) per ml. Eggs used for these counts were taken from among those extracted from the core samples on each sample date. They were not selected from a single cluster, core, or transect. This correlates well with Shuster and Botton's (1985) report of 176 eggs/ml (n=9 samples from a single cluster). I used the average value 178 to calculate egg numbers from their respective volumes.

Results And Discussion

Beach temperature The time required for *Limulus* eggs to develop and hatch is controlled by ambient temperature. I measured beach temperatures within the transects on each date when core

samples were taken. This was always near low tide, usually between 7 and 11 AM, so transects had been under the influence of air temperature and insolation for several hours prior to measurement. Readings were taken with digital probe thermometers at a depth of 20 cm, at the upper, middle, and lower end of each transect. On several transects, subsurface rock, shell, etc., required that readings be taken at less than 20 cm, however, no readings were taken at less than 10 cm.

There was little variation in beach temperature within or between transects. On 26 April, during the first sampling, average temperature of the 3 beaches was 12.9°C (55.6°F). Average beach temperatures increased steadily through the sampling period to 23.0°C (73.8°F) on the last sample date (9 July). This is an average increase of $\approx 1.8^{\circ}\text{C}$ ($\approx 3.2^{\circ}\text{F}$) per week of the study period. In the laboratory, French (1979) found that *Limulus* eggs took more than 6 weeks to hatch at $15-17^{\circ}\text{C}$ (59–63°F), and 3–4 weeks to hatch at 25°C (77°F). This suggests that eggs laid within the study transects both before sampling began, and during the course of this study, were present in the sand for sufficient time to be sampled at least twice.

Egg clusters and total egg population The summer's sampling yielded considerable information about egg populations on the sampled beaches. I found a total of 43 egg clusters on the Kelly Island and Port Mahon transects during the 2001 sampling period. No clusters were ever found on Broadkill Beach, although a few dispersed eggs were regularly recovered. The number of clusters found in any single transect on one sampling date ranged from 0 to 7 (for Port Mahon, south transect, on 11 June). For purpose of illustration, 7 clusters per transect would equate to 109.2 clusters per m². Figure 4 shows the distribution of total egg clusters by sampling date. There were no clusters from any transect on the first sampling date, and only four clusters on the last sampling date, indicating that the sampling season spanned the period of heaviest spawning. Thus, data collected during this study should be representative of *Limulus* spawning on these transects during the 2001 spawning season.

Table 1 shows beaches and transects ranked by total numbers of egg clusters, and compares the 2001 season's cluster totals observed on the Port Mahon N and S transects to totals from previous years. No earlier data exists for Kelly Island because it has not been sampled previously. Cluster totals from previous years are not directly comparable to the 2001 values, since the 2001 season sampling was done at right angles to the water line, and in previous years was done parallel with the water line.

All clusters were in the 5–20 cm fractions of cores, except for one cluster found in the 0–5 cm fraction on Port Mahon N on 11 June. Of interest is the fact that in 2001 Port Mahon S had approximately twice as many clusters as Port Mahon N (**Table 1, Appendix B**). The previous year, both Port Mahon transects had nearly equal numbers of clusters, and in 1999, total clusters were highest on Port Mahon N. It is tempting to attribute the changes in egg cluster numbers observed on these transects, in these three seasons, to qualitative changes in the beach associated with erosion. However, that is not possible, in part because correlated sand depth and beach sediment studies have not been done on this beach.

The total number of eggs found in any single transect on one sampling date (0-5 cm and 5-20

cm fractions combined) ranged from 0 to 122,000 (Port Mahon N, 11 June). For purpose of illustration,122,000 eggs per transect would equate to 1,900,000 eggs per m². **Table 2** (Appendix B) ranks the transects by total number of eggs collected during the 2001 season. For these beaches and transects, the ranking by total egg numbers is the same as the ranking by cluster totals, which is not always the case. Most eggs were in the 5–20 cm fractions of cores, but substantial numbers were also present in the 0–5 cm fractions. On Kelly Island and Port Mahon, eggs present in the 0–5 cm fractions ranged from 3% to 19% of total eggs collected (**Table 2**).

Broadkill beach, where no clusters were found, represents a curious case, since considerably more eggs were found in the 0–5 cm fractions than in the 5–20 cm fractions. The very high percentages of eggs found in the top 5 cm (N transect, 69%; S transect, 58%), and the very low total numbers of eggs found (**Table 2**), might suggest that many of the eggs found in the samples had washed down to this beach from more heavily used spawning beaches to the north. However, on the last sample date, I found an estimated several hundred trilobite larvae in the 5–20 cm fractions from both transects. These, and the eggs found in the 5–20 cm fractions verify that some spawning did actually take place on these areas of Broadkill beach, since eggs will not become reburied into beach sediments after they have come up out of the sand. This fact was noted by Williams (1986), and is the basis of most methodologies used to separate *Limulus* eggs from beach sediment samples.

There are two components to the *Limulus* egg population in a beach: clusters as laid by spawning individuals, and the subsequently-dissociated eggs dispersed throughout beach sediments. Both these components must be sampled, and the resultant total egg volume quantified, to obtain the most accurate estimate of transect (and thus beach) egg load. Because dissociated eggs are present throughout the spawning season, a simple census for egg clusters only will seriously underestimate actual egg numbers present. Conversely, excluding egg clusters from total egg volume calculations would also underestimate egg numbers. In this study I enumerated clusters as they were found in the sample cores, using the 13 mm (0.5") screen. Then I replaced their component eggs into the samples so they would be included in the total egg population. Finally, I extracted all eggs from the entire quantity of material collected in the sample cores.

If it is assumed that clusters in this study contained the same number of eggs per cluster, 3,650, reported by Shuster and Botton (1985) for a study of Delaware Bay beaches, it is possible to estimate the fractions of eggs that were represented in clusters in this study. If the total number of clusters found on Kelly Island and Port Mahon during the 2001 sampling is multiplied by 3,650, and the resulting value is divided by the total eggs found on each beach, then only 23.1% (Port Mahon) and 40.6% (Kelly Island) of the eggs collected on these transects would have been contained in the clusters. Thus, dispersed eggs were substantially more abundant on these transects than the number of clusters would indicate. Moreover, these estimated percentages are likely to be high because complete clusters are seldom recovered with core sampling, and therefore the true percentages of eggs found in clusters during this study would be lower.

Kelly Island, Port Mahon and Broadkill beaches varied widely in the egg loads they held. **Table 3, Appendix B** compares their egg totals to totals from Kitts Hummock, Pickering, and

North Bowers beaches, which were also studied during 2001 in a parallel study. Port Mahon had approximately twice as many eggs as the next most populous beach, Kitts Hummock (248,000). In turn, Kitts Hummock and Pickering (201,000) beaches yielded more eggs than did Kelly Island. Pickering was approximately twice as productive as Kelly Island (104,000). North Bowers had approximately half as many eggs as Kelly Island (55,000). Broadkill beach had a season total from both transects of 431 eggs.

Evaluation of spawning habitat and 2001 beach egg loads Unless transect endpoints are permanently marked with stakes, it is impossible to sample the same transect exactly. In fact, it would not be good practice to repeatedly sample the same transect line precisely, taking the same number of equally-spaced cores each time. This practice could be expected to deplete egg stocks in the areas where sample cores were repeatedly removed, yielding an inaccurate estimate of the real egg load.

In actual sampling, using visual markers to position the upper end of a vertical transect as in this study, the true seasonal "transect" sampled is not a single line. Instead, it is a narrow band encompassing all transects of the season, and reaching from the nocturnal tide line downward 83% of the distance to the tide flat. It is reasonable to expect that the sample transects taken at one site over a summer will fall within a band 1.5 m (4.9') wide. By extension, the season total egg number from one such vertical transect can be used as an estimate of egg load for other 1.5 meter widths of spawning habitat in the local area where the transect was located.

Estimating egg loads based on linear frontage of spawnable area should be more accurate than more traditional estimates based on beach area. This is because *Limulus* eggs are not distributed evenly across the intertidal area, but instead are more frequent at about midspan. Vertical sample transects pass through all intertidal areas where eggs may be found, thus making them ideal for estimates based on length of spawning habitat shoreline. As can be seen from data presented above, the full length of a beach does not usually have the same egg load. In fact, considerable differences between total N and S transect egg loads are commonplace. To damp this variability, and provide a more accurate estimate of egg load for parts of a beach not sampled, the pertransect egg loads for a beach need to be averaged. The average value is then used to multiply the spawning frontage of the beach to obtain an estimate of beach egg load for that season. **Table 4, Appendix B** provides egg load estimates for each of the study beaches, which are discussed individually, below.

Kelly Island I walked 2,203 m (7,234') of frontage on this beach, to determine the amount of spawning habitat present. I began at the southern tip of Kelly Island (N39°11.577', W075°23.781') and continued northward along the storm wrack line to N39°12.872', W075°23.855. I used a GPS unit to record the lengths of sand stretches having sufficient depth for spawning. Center widths of these stretches were measured with a tape, so estimates of their surface areas could be calculated. There were 901 m (2,957') of spawning habitat along this 2,203 m (7,234') of bay frontage. This represents 40.8% of the length I examined. The combined area of these sections of spawning habitat was 0.39 hectare (0.96 acre). The 2001 estimated egg load for the 901 m spawning frontage of this beach, based on the calculations described above, is 15.6 million eggs (Table 4, Appendix B).

This is the first time Kelly Island has been evaluated as a *Limulus* spawning site. Judging from the evidence of a rapidly eroding shoreline—both on-site, and from aerial photographs and the relevant USGS Quadrangle—the spawning habitat I evaluated will be drastically altered next season. Indeed, the impression gained from repeated sampling on the beach, and walking the sandy upper edge, is that this is not at all a constant or consistent spawning area.

Port Mahon I examined the entire 1,672 m (5,491') frontage of the beach at low tide, to determine the amount of spawning habitat present. I began at the southern end of the beach (N39°10.654' W075°24.491') where a culvert passes under the road, and continued northerly to N39°11.358', W075°23.909' at the bait store. I used a GPS unit to record the waterline lengths of sand stretches with sufficient depth for spawning. At the same time, center widths of these stretches were measured with a tape, so their approximate surface areas could be calculated. There were 450 m (1,478') of spawning habitat along the beach. This represents 26.9% of the total length of Port Mahon beach. The combined area of these lengths of habitat was 0.44 hectare (1.08 acre). The amount of spawning habitat on this beach has remained essentially the same since I examined it in 1999. At that time, total area of spawning habitat was 0.39 hectare (0.96 acre), and 28.5% of total beach length (Weber, 1999b). The 2001 estimated egg load for the 450 m spawning frontage of this beach, based on the calculations described above, is 150.4 million eggs (Table 4, Appendix B).

Typically, Port Mahon transects have been among the top transects for numbers of *Limulus* eggs. **Table 5, Appendix B** compares total egg numbers from the Port Mahon N and S transects over three years, during which period, total egg numbers for the beach have ranged between 400,000 and 500,000, while per-transect values have been 174,000 or higher. The 2001 total egg values from Port Mahon transects S and N, 268,000 and 233,000 respectively, were considerably higher than from any other transect sampled in a parallel study done that same season. The next highest 2001 egg total observed was from Kitts Hummock S (135,000 eggs). In 2000, total egg values from Port Mahon transects N and S were 174,000 and 229,000, respectively. These were less than the value observed on Ted Harvey S (312,000) that year. The 1999 Port Mahon transect totals were both higher than any others, with the next highest being Ted Harvey S (140,000).

Comparing the *Limulus* egg data from Port Mahon beach with similar data collected on other beaches sampled in this, and earlier, studies is problematic. For example, the approximately milelong frontage of Port Mahon contains a rather small percentage of shoreline where there is sufficient sand to allow spawning, and where coupled *Limulus* pairs come up to the water's edge. While other beaches generally provide a meter of spawning beach for each meter of shoreline, this is definitely not the case at Port Mahon. It seems probable that female *Limulus* in the waters along Port Mahon beach are forced to concentrate into the few areas where they can spawn. This seems unlikely to be the case on the most other beaches where shoreline and suitable spawning habitat are essentially equal. While the N and S transects typically have high cluster and total egg counts, these may be high simply because individuals spread along the Port Mahon shoreline are forced to come to the same few locations suitable for spawning. This could account for the high cluster counts and total egg numbers observed there. However, this concentration effect should be partly offset by the fact that *Limulus* are legally harvested from Port Mahon beach two days a week during the spawning season. Personal observations, and discussions with those harvesting, suggest that females coming onto the beach to spawn are the primary catch. These potential

spawners are taken before they have a chance to spawn, since females full of eggs are more desirable as bait, their intended use. No data are available on numbers harvested each season, but the favored places to harvest are the few spawning areas, which include areas surrounding both the N and Stransects.

Broadkill The area I evaluated began at N38°50.347', W075°13.493' and continued southward to N38°48.408', W075°11.397', at the boundary with Beach Plum Island Nature Reserve. Total frontage length, 4,723 m (15,506'), was determined by measurements taken from beach restoration project plans provided by USACE personnel. At 13 locations distributed along the frontage, I measured beach width from nocturnal tide wrack line down to the foot of the beach slope. Widths for Broadkill beach ranged from 11.9 m (39') to 16.1 m (53'), with an average width of 14.4 m (54'). Frontage length of the beach was multiplied by the average width value to estimate the amount of spawning habitat present. The full length of shoreline consisted of sandy sediments, which appeared suitable for *Limulus* spawning. The potential spawning habitat on the beach was 6.4 hectares (15.8 acres). The 2001 estimated egg load for the 4723 m spawning frontage of this beach, based on the calculations described above, is 680,000 eggs (Table 4, Appendix B).

Although the entire area of Broadkill beach appears to be suitable for spawning, the very low numbers of eggs found there during this study indicate that it definitely is not. It is unclear why this is so, although I usually found the wave height, and corresponding surf, to be greater than found on more northerly Delaware beaches on the same day, and within an hour or two. On more northerly beaches, *Limulus* spawning does not take place when onshore winds create waves over ca. 30 cm (12") (personal observation). Whatever the cause of the low egg numbers on this beach, the extremely low numbers suggest that this beach currently has very low value as a *Limulus* spawning area.

Acknowledgements

I thank David Carter, Environmental Program Manager, Delaware Coastal Management Program, for his assistance in in various aspects of this study. In this regard, he assigned two of his staff (Susan Love, and Sharon Midcap) to assist in this, and a parallel egg density study. I also thank Mark Del Vecchio, Manager of the Delaware National Estuarine Research Reserve Center, for making work space available at the Center, and for his constant attention to other needs as they arose. In addition, he assigned Heather Hudson, Summer Intern, to work with me on the project. It is a pleasure to acknowledge the careful assistance of Susan, Sharon, and Heather in collecting samples and other data, and especially in helping to separate and quantify the large quantities of extracted eggs. Their attention to details, coupled with their pleasant humor during the rigors of this study, made workdays pass more quickly. Special recognition is also due Daniel Ostroff for his ingenious proposal to use marked bungee cord transect lines to obtain equally- and proportionally-spaced cores on beaches of varying widths. Funding for this study was provided by the U. S. Army Corps of Engineers, Philadelphia District.

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APPENDIX A

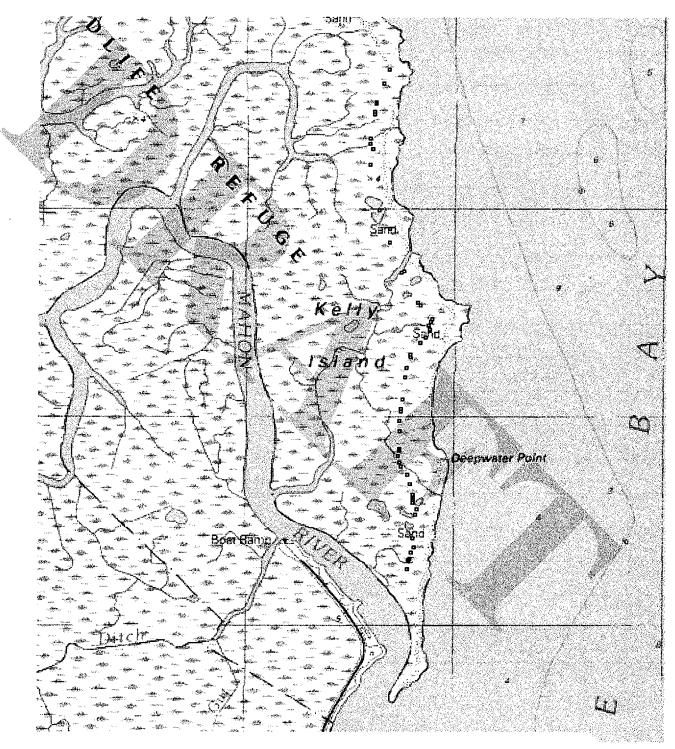


Figure 1 Aerial photograph of Kelly Island, taken in 1997, showing locations of 2001 study transects N and S, as well as linear frontage of spawning habitat.

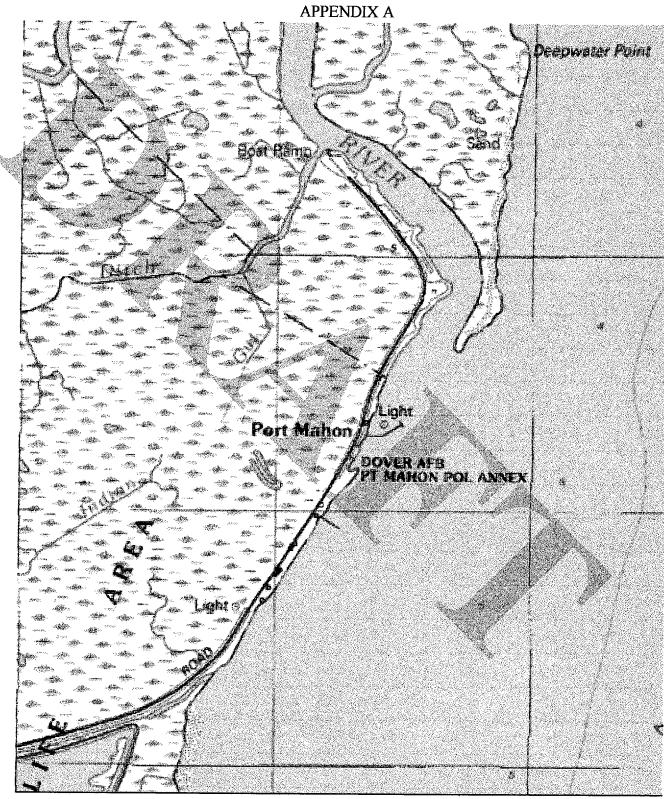


Figure 2 Aerial photograph of Port Mahon, taken in 1997, showing locations of 2001 study transects N and S, as well as linear frontage of spawning habitat.

APPENDIX A



Figure 3 Aerial photograph of Broadkill beach, taken in 1997, showing locations of 2001 study transects N and S. The entire linear frontage of this beach is spawning habitat of low quality.

APPENDIX A

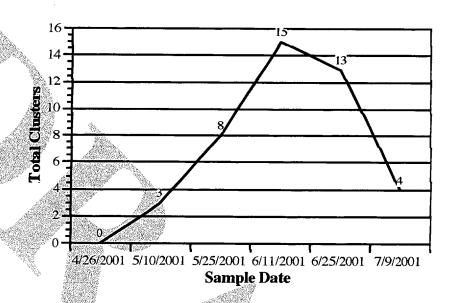


Figure 4 Distribution of the 43 egg clusters collected on Kelly Island and Port Mahon transects over the 2001 sampling period. No clusters were found on Broadkill beach. Values above dates are total clusters collected from all transects on that date

APPENDIX B

**		1	otal Cluster	rs	
	Beach & Transect	2001	2000	1999	
	Port Mahon, S	21	29	10	
	Port Mahon, N	11	25	27	
	Kelly island, N	8			
	Kelly island, S	4			
	Totals	44	54	37	

Table 1 Kelly Island and Port Mahon transects, ranked by total number of egg clusters found during the 2001 sampling period, and compared with cluster totals observed on Port Mahon during 2000 and 1999. The 2001 Port Mahon N and S transects are the same locations that were sampled in 2000 and 1999. Transect orientation was vertical in 2001, and horizontal in 2000 and 1999, so totals are not directly comparable. No clusters were found on Broadkill beach. The Kelly Island N total does not include a sample from 25 May, when only the S transect could be sampled, so the actual total might have been slightly higher.

7%
19%
4%
3%
69%
58%
COLUMN TO THE PARTY OF THE PART

Table 2 Kelly Island, Port Mahon, and Broadkill beach transects, ranked by total numbers of eggs found in 2001. Values in the Total Eggs column are the sums of egg numbers extracted from all core samples taken in that transect during the season. Values in the 0–5 cm and 5–20 cm columns were obtained by various combinations of direct counts and volumetric extrapolations, so they have been truncated at the thousands level, except for Broadkill beach, where every egg was counted. The Kelly Island N total does not include a sample from 25 May, when only the S transect could be sampled, so the actual total might have slightly been higher.

APPENDIX B

ş	Beach	Eggs, 0–5 cm	Eggs; 5–20 cm	Total Eggs	% in 0-5 cm
	Port Mahon	62,000	439,000	501,000	12%
	Kitts Hummock	16,000	232,000	248,000	6%
	Pickering	23,000	178,000	201,000	11%
	Kelly Island	4,000	100,000	104,000	4%
	North Bowers	2,000	53,000	55,000	4%
	Broadkill S	284	147	431	66%

Table 3 Comparison of 2001 Kelly Island, Port Mahon, and Broadkill beach egg totals, to totals observed on Kitts Hummock, Pickering and North Bowers beaches during the same period. Values in the Total Eggs column are the sums of egg numbers extracted from all core samples taken in that transect during the season. Values in the 0–5 cm and 5–20 cm columns were obtained by various combinations of direct counts and volumetric extrapolations, so they have been truncated at the thousands level, except for Broadkill beach, where every egg was counted.

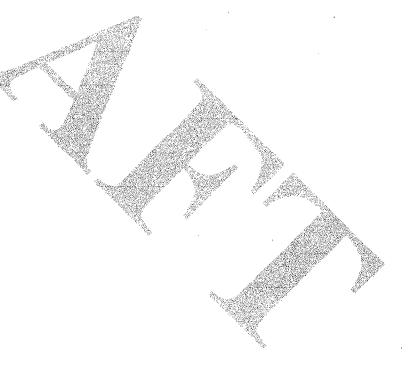
Beach & Transect	Total Eggs per Transect	Total Eggs per Beach	Average Eggs per Transect		Egg Load Estimate
Port Mahon, S Port Mahon, N	268,000 233,000	501,000	250,500	901	150,467,000
Kelly Island N Kelly Island S	73,000 31,000	104,000	52,000	450	15,600,000
Broadkill S Broadkill N	325 106	431	216	4,723	680,112

Table 4 Egg load estimates of Port Mahon, Kelly Island and Broadkill beaches, based on averages of beach N and S transect egg totals observed in 2001. Average Eggs per Transect is used as an estimate of egg load in 1.5 meters of spawning habitat frontage of that beach. Spawnable Frontage is the combined length of all sections of spawnable frontage found on that beach in 2001. Egg Load Estimates were derived by dividing Spawnable Frontage by 1.5, then multiplying the result by Average Eggs per Transect. The Kelly Island N total does not include a sample from 25 May, when only the S transect could be sampled, so the actual egg total might have been slightly higher.

APPENDIX B

Total Egg Numbers					
Beach & Transect	2001	2000	1999		
Port Mahon, S	268,000	229,000	234,000		
Port Mahon, N	233,000	174,000	239,000		
Totals	501,000	403,000	473,000		

Table 5 Comparison of total numbers of eggs found on Port Mahon transects in 2001 with numbers found the preceding two seasons (0–5 cm and 5–20 cm values combined). Note that totals listed here for 2000 and 1999 represent only the eggs found, and do not include embryo numbers, as was done in reports for those years. Values have been truncated at the thousands level.



2001 Study

Date	samples	taken:	26	4	1.1	01	I
F			77	A	ar.	101	_

Date counted: 26 April 01 30 April 01
Initials: PSW/SC/SM

Date samples extracted: ___

Kelly Isla	nd N clusters;	Kelly Island S clusters:
Transect	Counted Eggs	Egg volume (ml)
Kelly Islan Kn 5 cm	nd .	,45
Kn 20 cπ	d	Ø
Ks 5 cm		CS
Ks 20 cm	(á	4

Port Mahon N clusters:

Port Mahon S clusters:_

Ø	

	265,65			64,	
Transect	Counted			Egg olume (ml)	
	Eggs	100000000000000000000000000000000000000	- V	olume (ml)	1
Port Mahon				4	
Mn 5 cm	59			φ	
Mn 20 cm	37			න් ්	
WIII ZU CHI		- 6	Application of the second of t	A TASE S	65/28
Ms 5 cm	<u> </u>) D	
Ms 20 cm	1			Ø	

Broadkill N clusters: Broadkill S clusters:

Transect	Counted Eggs	Egg volume (ml)
Broadkill BRn 5 cm	4	O C
BRn 20 cm	1	ø
BRs 5 cm	Ø	Ø
BRs 20 cm	Ø	Ø

2001 Study

Date samples taken: 10 May 01

Date samples extracted: 10 woy 6/

Date counted: 14 M2 y 01

Initials: 126w | 140/4 | 5 m

Kelly Islan	nd N clusters;	Kelly Islan	d S clusters:	
Transect	Counted Eggs		Egg volume (ml)	
Kelly Islan Kn 5 cm			Ø	
Kn 20 cm	80		Ø	
Ks 5 cm	25		- 3	
Ks 20 cm	33		Ø	

Port Mahon N clusters:

Port Mahon S clusters:

	5355	170,50x 197149-90x-00090909	1,39%	
Transect	Counted Eggs		Eg: volume	
Port Mahon Mn 5 cm	13		35 m	2
Mn 20 cm	27		33 n	26
Ms 5 cm	9		6.2	me
Ms 20 cm	5		76.	n L

Broadkill N clusters:		Broadkill S clusters:		
Transect	Counted Eggs	Egg volume (ml)		
Broadkill BRn 5 cm	26	Ø		
BRn 20 cm	12	Ø		
BRs 5 cm	3			
BRs 20 cm	9	Ø		

2001	Study

		2001 Study		
Date samp	les taken: 24 M2y	②/ Date counted	: 4 June	01
Date samp	les extracted: 24 m			m/ HCH
	75 m2	y 01		
Kelly Islan	id N clusters:	Kelly Island	S clusters:	1
Transect	Counted Eggs		Egg volume (ml)	
Kelly Islan Kn 5 cm	■85088802873F	pled on this d	1	to bost
Kn 20 cm	51.1K. 5	while we were	taking t	Le 5 5200 /0
Ks 5 cm	3		5 ml	
Ks 20 cm	97		37ml	·
Port Maho	n N clusters: 2	Port Mahon S	clusters:	5
Transect	Counted Eggs		Egg volume (ml)	
Port Maho Mn 5 cm			12 mC	
Mn 20 cm	<i>251</i>		185 m.L	
Ms 5 cm	15		36 m L	
Ms 20 cm	21		270mL	
		**		
Broadkill N	Clusters:	Broadkill S cl	usters: 👤 🙎	<u> </u>
Transect	Counted Eggs		Egg volume (ml)	*
Broadkill BRn 5 cm	3		Ø	
BRn 20 cm	20		Ø	
PDa 5 am	18		B	

BRs 20 cm

		2001 Study	
Date samples	taken: 11 June 0	. 🗲	
Date samples	extracted: // June		n/
	12 Jime	601	
Kelly Island l	N clusters: 3	Kelly Island S clusters:	
Transect	Counted Eggs	Egg volume (ml)	
Kelly Island Kn 5 cm	4	16 ml	
Kn 20 cm	61	185 an l	
Ks 5 cm	643	.0	
Ks 20 cm	7.0	68ml	
		10 these was in the 0-5 cm sect	hom
Port Mahon !	N clusters: $5^{\prime\prime}$	Port Mahon S clusters: 7	
Transect	Counted Eggs	Egg volume (ml)	
Port Mahon Mn 5 cm	96	199me	
Mn 20 cm	6	637.5	
Ms 5 cm	27	60 ml	
Ms 20 cm	25	690mL	
Broadkill N c	lusters: Ø	Broadkill S clusters:	á

Transect	Counted Eggs	Egg volume (ml)	
Broadkill BRn 5 cm	26	Ø	
BRn 20 cm	5	Ø	
BRs 5 cm	146	Ø	
BRs 20 cm	37	<u> </u>	

2001 Study

	2001 Study			
Date samples taken: 25 June 0/ 27 June '0/ Date samples extracted: 25 June 0/		Initi	ted: 25 June 'C ials: RGCU/SM	
Keny Island	N clusters: 4	Kelly Islan	nd S clusters:	ø
Transect	Counted Eggs		Egg volume (ml)	
Kelly Island Kn 5 cm	20		1.6 ml	
Kn 20 cm	64		154 ml	
Ks 5 cm	166		Ø	
Ks 20 cm	12		7ml	
Port Mahan	N objectors: 3	Port Mahr	on S christore	6

Port Mahon N	clusters: <u> </u>	Port	Mahon S clusters:	<u> </u>
Transect	Counted Eggs		Egg volume (mi)
Port Mahon Mn 5 cm	Z		5.2 m	4
Mn 20 cm	6		142 m	4
Ms 5 cm	ø		1.8 m	(
Ms 20 cm	22		1950	٠. ا

Broadkill N clusters:		Broadkill S clusters:	
Transect	Counted Eggs	Egg volume (ml)	
Broadkill BRn 5 cm	2		
BRn 20 cm	Z	Ø	
BRs 5 cm	50	Ø	
BRs 20 cm	31	Ø	

2001	Study
------	-------

Date samples taken:	9 Jul 01	Date counted:	16	Jul	01	
	12 /01/01					

Date samples extracted	: ラノいしひノ	Initials: RGW/SM/HCH/S/
	12 14101	
400000		

Kelly Island N clusters: 1		Kelly Island S clusters:	
Transect	Counted Eggs	Egg volume (ml)	
Kelly Islan Kn 5 cm	d	4.4 ml	
Kn 20 cm	412	52 ml	
Ks 5 cm	54	Ø	
Ks 20 cm	47	58m(

Transect	Counted Eggs			gg ie.(ml)
Port Mahon Mn 5 cm	104		9	8
Mn 20 cm	1		100	mL
Ms 5 cm	119	***	9	
Ms 20 cm	Ø		177.	ml

Broadkill N clusters:		Broadkill S clusters:	
Transect	Counted Eggs	Egg volume (ml)	
Broadkill BRn 5 cm	4	G C	
BRn 20 cm	5	Ø	
BRs 5 cm	6		
BRs 20 cm	6	Ø	