

Distribution and Development of *Limulus* Egg Clusters on Intertidal Beaches in Delaware Bay

Richard G. Weber and David B. Carter

Abstract Accurate knowledge of where spawning *Limulus* females place their egg clusters in beaches is important for sampling egg cluster density, which in turn is important in studies of habitat use, monitoring *Limulus* egg production, determining potential shorebird forage, and for investigating the effects of beach erosion or replenishment. We examined *Limulus* egg cluster placement on seven western shore Delaware Bay beaches. Depth to center of recently laid clusters ranged from 3.5–25.5 cm, with an average depth to center of 15.5 ± 3.5 cm ($n=533$). Centers of 88.0% (469) of all clusters were within 20 cm of the undisturbed beach surface, and 98.1% of all clusters (523) extended 1 cm or more into the 20 cm horizon. Clusters were found only in the upper 85% of the foreshore, beginning at the spring tide high-water mark, even though beach widths varied ($n = 6,132$ clusters in 80 transects). Intact, recently laid clusters contained from 2,524 to 16,835 eggs, with an average size of $5,786 \pm 2,834$ eggs ($n = 26$). Beach sediment temperatures in early May ranged from 13.7°C to 24.2°C (mean = $17.0^\circ \pm 3.0^\circ\text{C}$ SD); in early June from 16.4 to 29.7°C (mean = $21.6^\circ \pm 3.3^\circ\text{C}$ SD); and in early July from 22.4 to 30.4°C (mean = $26.8^\circ \pm 1.9^\circ\text{C}$ SD). Under these conditions, the first trilobite larvae (<25 larvae per beach sampled) normally began to appear in our sediment samples during the first week in June, suggesting that about 35 days are required for development of the earliest eggs in a normal spring. Because egg clusters on all beaches were confined to 85% of the upper foreshore, and most clusters were within reach of a 20 cm deep sample, future studies to assess cluster densities should be designed to sample within that portion of a beach.

R.G. Weber (✉)

Delaware National Estuarine Research Reserve, 818 Kitts Hummock Road,
Dover, DE 19901, USA
e-mail: rgweber@earthlink.net

1 Introduction

An accurate knowledge of where spawning *Limulus* females place their egg clusters in beaches is important for sampling egg cluster density, which in turn is important for studying habitat use, monitoring horseshoe crab egg production, determining potential shorebird forage, and for investigating the effects of beach erosion or replenishment. Delaware has periodically replenished selected bayfront beaches since 1961. Concerns about possible effects of beach replenishment on *Limulus* spawning resulted in a research program to find how *Limulus* eggs are distributed during spawning. During 1998–2005, we conducted a series of studies which examined *Limulus* egg cluster placement on western shore Delaware Bay beaches in May, June, and early July. We were particularly interested in the depth at which clusters were placed and in the distribution of clusters across beaches.

Literature concerning depth at which *Limulus* egg clusters are placed in Delaware Bay beaches is sparse. Hummon et al. (1976) found egg clusters “from 2–6 cm depth at neap low to 12–24 cm depth at mean high water level” in Roosevelt Inlet, Delaware. Penn and Brockmann (1994) reported a depth of 9.3 ± 3.9 cm for 112 clusters examined at Cape Henlopen State Park, Delaware. In New Jersey, Botton et al. (1994) mention 15–20 cm as the depth where “most egg clusters are initially deposited.”

Information about egg cluster distribution across Delaware Bay beaches is also sparse. Shuster (1982) discovered that “On Delaware Bay beaches, egg nests [clusters] are found in a broad band starting about 3 meters from the low-water line to the spring high-tide water mark.” Shuster and Botton (1985) reported that in New Jersey, clusters were absent from the lowest 3 m of the beach. Williams (1986), also working in New Jersey, found “the middle section of the intertidal zone contained significantly more eggs than either end . . .” Brockmann (2003b) reported that in Delaware, nesting occurred over 61% of the beach, and spawning was not concentrated near the high tide line. Bayfront beach widths are variable and relate to the extent of a beach’s tidal flat. Larger tidal flats give narrower foreshores, which seem to be preferred for spawning by *Limulus* females (Smith et al. 2002). Because beach widths vary, the area selected for egg cluster sampling must be proportional to foreshore width, rather than a fixed span (Jackson et al. 2002).

During our studies of cluster depth and distribution, we became curious about the average number of eggs per cluster in Delaware Bay beaches. Not every aggregation of *Limulus* eggs exhumed from a beach is an intact cluster, as laid by a female. Spawning females frequently damage previously laid, intact egg clusters and disturb the surrounding sediments, a process called bioturbation (Kraeuter and Fegley 1994). Even the simple act of digging up clusters with a shovel can cause parts to break off, especially if egg development has made the cluster friable. Hummon et al. (1976), examining a beach in Roosevelt Inlet, Delaware, reported finding clusters that were “comprised [of] 75–300 eggs.” In

New Jersey, Shuster and Botton (1985) examined four egg clusters and found them to average 3,650 (± 232.5) eggs. Shuster and Botton (1985) reported great variability in the amount of sediment present in each cluster, with gravel from one cluster being approximately three times the weight of the eggs.

Finally, our regular collection dates during these studies gave us an opportunity to examine beach sediment temperatures. The time required for *Limulus* eggs to develop and hatch is controlled by temperature (French 1979), although salinity also affects development rate (Jegla and Costlow 1982). Warmer temperatures give shorter times to hatching (French 1979; Jegla and Costlow 1982). French (1979) found that eggs maintained at approximately 15°C took 45 days to hatch, but eggs maintained at approximately 23°C required only 28 days.

2 Background

The American horseshoe crab, *Limulus polyphemus* L., occurs in coastal and estuarine environments from northern Maine to the Yucatán peninsula (Shuster 1982). The species is especially abundant along the mid-Atlantic coast and reaches its peak abundance in the Delaware Bay (Shuster 1982). *Limulus* spawning activity is most intense during the nocturnal full and new moon tides of May and June (Rudloe 1985; Barlow et al. 1986). However, these spawning peaks may occur later if onshore winds prevent spawning on the nights of full and new moon high tides (Shuster 1982). In Delaware Bay, considerable spawning also takes place at other times during the lunar cycle, on both day and night high tides (Shuster and Botton 1985; Penn and Brockmann 1994). As a result, within Delaware Bay, egg clusters are not concentrated along the spring high tide line as they are in other parts of their range (Brockmann 2003b). Female *Limulus* spawn near the time of high tide, on the beach foreshore, where they burrow into the beach sediments to approximately the level of their compound eyes (Cohen and Brockmann 1983). A spawning female is accompanied by at least one male, which has grasped her opisthosoma with his claspers and follows behind her. She may also be attended by one or more unattached “satellite” males (Brockmann 1990; Loveland and Botton 1992). A female burrowed into the beach, ready to spawn, moves ventral appendages (the flabellae; Sekiguchi 1988), creating a current of water beneath her body. When spawning, she pulls water under herself, from behind. It is expelled, from beneath her body, through the lateral gaps at the hinge line between her prosoma and opisthosoma (Barthel 1974; Brockmann 1994). The attached male may also assist in creating this forward flow of water (Brockmann et al. 2000; Brockmann 2003a). The moving water carries smaller sediment particles from beneath the female’s body, leaving behind particles too large to be transported by the strength of the flow she and the male generate. When a cavity has been cleared beneath her operculum, the female releases several thousand eggs into it (Penn and Brockmann 1994), which the male

fertilizes. Shortly afterward, she moves forward through the sand, away from the eggs she has just deposited. She may pause again to lay another cluster (Kingsley 1892; Brockmann 1990), or may push up and out of the beach to return to the water. As the female moves away from eggs she has just laid, the moist sand slumps down, confining the fresh eggs among whatever larger sediment particles remained in the cavity when they were laid (Shuster and Botton 1985; Shuster and Sekiguchi 2003).

As first laid, a *Limulus* egg is encased in a soft, flexible chorion and is not tightly filled or turgid. It is also covered by a sticky film (Rudloe 1979; Brown and Clapper 1981; Shuster and Sekiguchi 2003). As the female moves away, the soft, newly laid eggs are pressed together by the weight of the sand above them. They are not tightly filled spheres, so they deform slightly as they are compacted, forming numerous flattened and concave areas where they touch, thereby increasing the areas of contact between eggs (Fig. 1). During the next hour or so, the chorions become somewhat tougher and less flexible and the sticky film on the eggs cures, cementing the group of eggs and sediment particles together with a surprisingly sturdy, slightly flexible, bond. The resulting “rubbery” cluster of eggs contains variable amounts of coarse sediment particles (Fig. 2) (Shuster and Botton 1985; Shuster and Sekiguchi 2003). Clusters are not uniform in size or shape, but do tend to be ovoid, slightly flattened, and thinner

Fig. 1 Photograph of eggs inside a recently laid *Limulus* egg cluster, which has been broken apart to show the flattened and concave surfaces caused when the soft, newly laid eggs were pressed together by the moist sand around them. These broad areas of contact, coupled with the sticky material surrounding each egg when it is laid, combine to make new clusters resilient, with a “rubbery” feel when handled. As development proceeds, eggs become turgid and rounded which decreases areas of contact, the sticky bond between them breaks down, and the cluster becomes friable and is easily broken apart





Fig. 2 *Limulus* egg clusters, showing the variable sizes and amounts of beach sediment particles they contain. The *bottom* two clusters are intact; the *top* two have lost eggs along their *lower left* edges

(from top to bottom) than wide. For the next week or two, depending on temperature, the cluster will persist as a resilient, rubbery aggregation of eggs and sediment. As embryonic development progresses, the individual eggs become more rounded and turgid, which decreases the former broad contact areas, while the adhesiveness of the initial bond also begins to break down. When these changes have progressed sufficiently, a cluster becomes friable and is easily crumbled apart with a fingertip, even when still mostly supported by surrounding sand. Thus, the resilience of a cluster when probed with a fingertip can provide a useful index to how long it has been since the female deposited it. A cluster found in the beach has been laid within the previous one or two weeks, if it does not crumble when probed with a fingertip. If no storms have disturbed the beach sediments during that time, then the distance from the beach surface to the cluster is the depth at which the female originally placed it. The cluster will remain at the depth it was placed unless another spawning female disturbs the area where it rests, or the depth of sediment covering it is affected by storm waves. Depending on the angle at which storm waves strike a beach face, sediment may be removed from the beach, decreasing sediment depth above a cluster, or moved from higher to lower levels of the beach, thereby deepening sediment above a cluster (Jackson et al. 2002).

The depth at which *Limulus* egg clusters are placed is a function of the female's body size and of the depth to which she burrows into the beach (Shuster and Sekiguchi 2003). Spawning females commonly burrow into a beach until their compound eyes are near the beach surface (Cohen and Brockmann 1983), so at each subsequent spawning tide, additional females will burrow into the same section of beach, to spawn at the same depth as any previously laid clusters. Inevitably, some previously laid clusters are disturbed each time spawning takes place. This process, and the associated disturbance of beach sediments, has been called bioturbation (Kraeuter and Fegley 1994). Bioturbation from heavy spawning, combined with moderate wave action, can alter sediment depth (Jackson et al. 2005). Collisions with previously laid clusters are density dependent, and heaviest near the center of the beach foreshore; however, even at low spawning densities, as many as 20% of the existing clusters may be disturbed (Smith 2007). The effect of such a collision on the disturbed cluster is partly determined by its age. That is, whether it is new and tough, or whether it has been in place long enough for embryonic development and cement deterioration to make it friable. A collision between a spawning female and a previously laid egg cluster breaks the cluster into several smaller aggregations of eggs, or "clusterlets." Succeeding cohorts of spawning females can further break up clusterlets into individual eggs. When sediments containing clusterlets and dissociated eggs are activated by waves, eggs are moved upward toward the beach surface, where they eventually come within the reach of foraging shorebirds (Pooler et al. 2003).

In this chapter, we report studies done during 1998–2005 to refine our understanding of where female *Limulus* deposit their eggs in western shore Delaware Bay beaches and of the clusters themselves.

3 Study Beaches

Locations of our study beaches are shown in Fig. 3. All are in the mid-bay region, where western shore spawning activity is most intense (Smith and Michels 2006), from Port Mahon, south to Slaughter Beach. The straight-line distance between Port Mahon and Slaughter Beach is 28 km. Tides on these beaches are semi-diurnal, with a mean range of 1.4 m and a spring range of 1.7 m (Nordstrom et al. 2006). Through the lunar cycle, nocturnal high tides range from 0.3 to 0.5 m higher than diurnal tides, which spreads *Limulus* spawning across the upper foreshore, the span between the high tide line and the beach break at the beginning of the low tide terrace (=tidal flat). Most of these sites are low-energy, bayfront beaches, comprised of mixed sand and gravel, and having a tidal flat exposed at low tide. Exceptions are the area of Slaughter Beach we sampled, which did not have a tidal flat exposed at low tide, and Mispillion Inlet, at the mouth of the Mispillion River, which is protected from bay surf action by riprap jetties, and which also does not have an exposed tidal flat. Beaches with tidal flats exposed at low tide have shorter foreshores and, on bayfront beaches, females prefer to spawn on such narrow beaches (Smith et al. 2002).

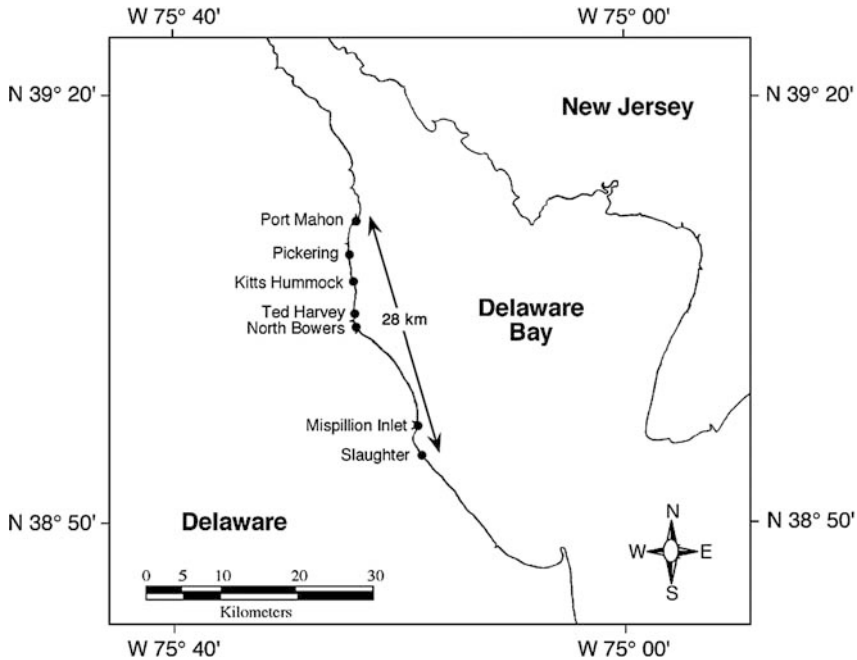


Fig. 3 Map of Delaware Bay showing locations of beaches where *Limulus* egg cluster distribution was studied. The *straight-line* distance between Port Mahon and Slaughter Beach is 28 km

4 Methods

For convenience, we have arranged this section, and the following Results section, by topic.

4.1 Egg Cluster Depth

In 1998, 1999, and 2000, we examined the depth at which female *Limulus* place their egg clusters. Port Mahon, Ted Harvey, and North Bowers beaches were sampled each year. Slaughter Beach was sampled only in 1998, and Pickering Beach was sampled only in 2000. There had not been unusually strong onshore winds for at least 10 days before any sample date, so beach surfaces had not been altered recently by wave action. Sampling was done in June, when the heaviest spawning was past and sample dates were selected to be two or more days away from new and full moon tides. This was done to minimize chances of bioturbation having altered sediment depth. When sampling, we examined 1–3 sites on each beach, measuring the depths of 15–30 clusters at each site, on a total of 25 sites. We sampled by making trenches approximately 1 m long by

0.3 m deep, at right angles to the water line, midway between the nocturnal high tide wrack line and the beach break where the tidal flat begins. A small garden trowel, oriented vertically, was used to shave away the trench face at approximately 1 cm per pass.

When a cluster was exposed, it was first probed with a fingertip to determine whether it was friable, or firm. If it seemed firm, a fingertip was used to expose its outline in the sand wall. A cluster which survived both these tests without breaking was considered to be fresh enough that its depth would be close to the depth at which the female had originally placed it. Measurements from beach surface to a cluster's top and bottom were taken at the cluster's maximum thickness and were referenced to the bottom edge of a 1 m long board laid on the beach surface along the edge of the trench. Using the board as a reference minimized the effect of any small, local surface depression or elevation on the measurements. We did not attempt to measure maximum cluster width. To do so would have required removing the cluster, which frequently caused the local sand wall to collapse, requiring removal of considerable sand to again reach unperturbed beach.

4.2 Cluster Distribution Across the Foreshore

We examined the distribution of egg clusters across beach foreshores in 2000 and 2001. We sampled Pickering, Kitts Hummock, Ted Harvey, and North Bowers beaches each year, using from 6 to 15 transects per beach. To do this, we used a single-bottom plow, pulled by a tractor, to make a series of 0.3 m deep furrows (transects) from the nocturnal high tide wrack line (approximately the spring tide high water level) down to the beginning of the tidal flat. Transects were at right angles to the water line, and spaced 3–10 m apart. We marked off each transect in 30 cm intervals, then used a garden rake and our fingers to locate all clusters present in each 30 cm span of material turned out of the furrow. Clusters were replaced into furrows as counted, and when counting in each transect was completed, it was refilled and the surface smoothed to the original level. After the next tide cycle, no evidence of the operation remained.

4.3 Cluster Distribution Along Sample Transects

We tested the foreshore cluster distribution we had observed in the 2000–2001 study, described above, by using core-sampled transects. Based on the 2000–2001 results, the transects used in this study spanned only 85% of the distance from the nocturnal high tide wrack line down toward the foot of the beach, where the tidal flat began. On beaches with no tidal flat exposed at low tide (Misphillion Inlet and Slaughter Beach), we used 85% of the distance from

the high tide wrack line to the low water level. We used a tape measure to determine the 85% point for each transect we sampled.

In this study, we sampled during 2002–2005 at Port Mahon, Pickering, Kitts Hummock, North Bowers, Mispillion Inlet, and Slaughter beaches. Half of these beaches, Port Mahon, Mispillion Inlet, and Slaughter Beach, were new beaches, which were not sampled during the 2000–2001 study of cluster distribution across the foreshore. There were five sample dates per season, each selected to fall between new and full moon tides, and more than 3 days before or after new or full moon, so that any spawning activity associated with those lunar tides would be represented in the samples. On each sample date, we used a pair of core-sampled transects per beach, separated by at least 100 m. Transects were oriented at right angles to the water line, and consisted of 20 evenly spaced core samples. Sample sediment cores were 5.7 cm in diameter \times 20 cm deep. The six beaches sampled during this study had different distances across their intertidal zones; thus, different transect lengths were required. However, it was possible to space the 20 core samples evenly, and thus proportionally, along each transect's length by using marked bungee cords. Stretching a marked bungee cord over a span to be sampled indicated the exact location where each sample core should be taken. Equal spacing of marks on the cords made the 20 cores taken across each beach fall into proportional locations, from the nocturnal high tide wrack line where the first core was taken, down to the 20th core, at the point 85% of the distance from wrack line to the tidal flat. Thus, any ranked sample core would be located at the same percentage distance down across the beach. For example, the twelfth sample core would be located at a distance 60% from the wrack line ($60\% = 12/20 \times 100$).

Upon collection, each core sample was passed through a 1.3 cm mesh screen into a bucket, to reveal any clumps of eggs. Because clusters in cores sometimes break apart as the core is taken, one, or more, firm aggregation of eggs that did not pass through the 1.3 cm mesh was recorded as a single cluster for that core. We also recorded the core position (number) where each cluster was found. Thus, by summing all clusters found in all twelfth cores (and in the other core positions as well) during this study, it was possible to obtain a composite view of cluster distribution downward across the intertidal zones of the beaches we sampled.

4.4 Egg Cluster Size

We examined cluster size by digging a 0.3 m deep trench in the mid-beach area. Each cluster that we uncovered in the trench was examined closely by two people familiar with egg clusters, to determine whether it was friable, and intact (without obvious breaks along edges). All friable clusters were discarded, and every intact cluster was collected. We counted all eggs by hand, rather than using volumetric estimates of total numbers. We collected 26 egg clusters; 14 from Port Mahon in 2003, and 12 from Pickering Beach in 2005.

4.5 Beach Temperature and Egg Development

During the spawning seasons of 2002–2005, we measured beach temperatures on Port Mahon, Pickering, Kitts Hummock, North Bowers, and Slaughter beaches. We took two measurements at each site, separated by at least 100 m. Temperatures were always measured near low tide, and beaches had been under the influence of air temperature and insolation for varying amounts of time prior to measurement. Readings were taken at a depth of 20 cm, with digital probe thermometers placed near the center of the intertidal span.

5 Results and Discussion

5.1 Egg Cluster Depth

We measured the depth and thickness of 533 clusters. Collection data and summaries of cluster depth and thickness data appear in Table 1. Cluster thickness (top to bottom) ranged 0.6–7.6 cm, with an average thickness of 3.5 ± 1.1 cm

Table 1 Summary of sampling to examine depths at which *Limulus* egg clusters were placed on Delaware Bay beaches, 1998–2000. Sampling was done approximately midway between the nocturnal high tide wrack line and beginning of the tidal flat. A total of 25 sites were examined, which yielded 533 clusters. Thickness is the distance from top to bottom of a cluster. Beaches are listed north to south

Beach	Year	Sample date	Sites examined	Total clusters	Mean depth to center (cm) \pm SD	Mean thickness (cm) \pm SD
Port Mahon	1998	8, 17, 29 June	3	48	13.2 ± 2.9	3.4 ± 1.4
	1999	8, 9, 23 June	3	75	15.0 ± 2.9	3.6 ± 1.2
	2000	13 June	2	54	16.6 ± 3.9	3.9 ± 1.1
Pickering Beach	2000	13 June	2	50	18.3 ± 3.4	4.0 ± 1.1
Ted Harvey	1998	8, 17, 29 June	3	45	15.3 ± 2.8	3.5 ± 2.8
	1999	15 June	2	50	14.1 ± 3.2	3.6 ± 0.9
	2000	14 June	1	25	14.5 ± 1.6	3.3 ± 0.7
North Bowers	1998	8, 17, 29 June	3	45	16.5 ± 2.4	3.2 ± 1.0
	1999	9 June	2	59	14.6 ± 3.1	2.9 ± 0.8
	2000	14 June	2	51	18.3 ± 3.9	3.3 ± 0.8
Slaughter Beach	1998	17, 29 June	2	31	13.2 ± 2.7	3.8 ± 0.9

SD. Depths of clusters ranged 3.5–25.5 cm, with an average depth to center of 15.5 ± 3.5 cm SD. For all clusters, centers of 4.9% (26) were within 10 cm of the beach surface (only 1 cluster was in the 0–5 cm horizon), 39.5% (210) were in the 10–15 cm horizon, 43.6% (232) were in the 15–20 cm horizon, 12.0% (64) were in the 20–25 cm horizon, and 1 cluster was below 25 cm. Figure 4 shows the depth-to-center distribution of clusters in the entire sample set.

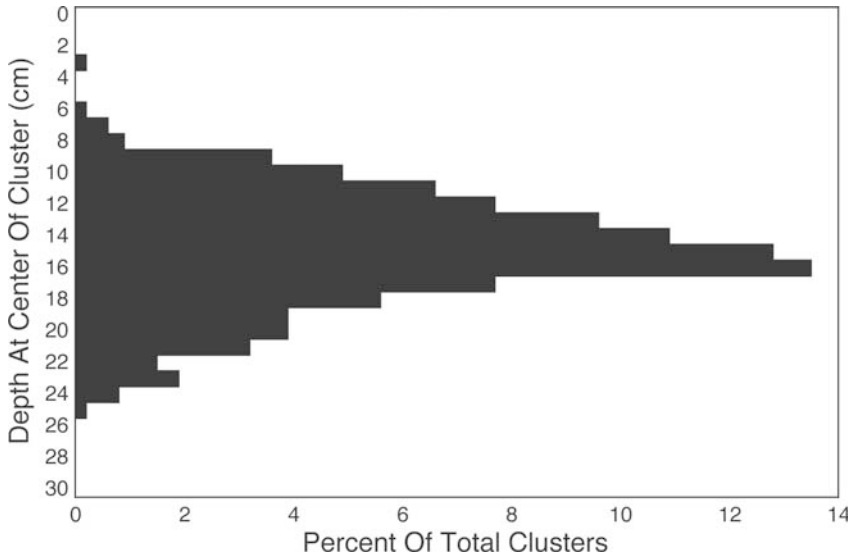


Fig. 4 Depth-to-center distribution of *Limulus* egg clusters observed at mid-beach on Port Mahon, Pickering, Ted Harvey, North Bowers, and Slaughter beaches in June, 1998–2000. A total of 533 clusters were found at the 25 locations examined. Of these, 83.1% (443) were in the 10–20 cm horizon

5.2 Cluster Distribution Across the Foreshore

We examined a total of 80 transects and found 6,132 clusters. On all beaches, all clusters were found in the upper 85% of the span from the nocturnal high tide wrack line to the beginning of the low tide terrace (tidal flat) (Fig. 5). Of these, 90.9% (5,588 clusters) were found between 25 and 75% of the distance between the nocturnal high water wrack line and the beginning of the tidal flat. These results suggest that female *Limulus* place their egg clusters only in the upper 85% of western Delaware Bay beach foreshores at the current population abundance. The average number of clusters per transect varied from beach to beach in both years, and the average number of clusters per transect was highest in 2001 (Table 2). Difference between years is most easily explained by the date of sampling. Sample dates in 2000 were in mid-May (Ted Harvey, North Bowers) and late June (Pickering, Kitts Hummock). These are slightly before,

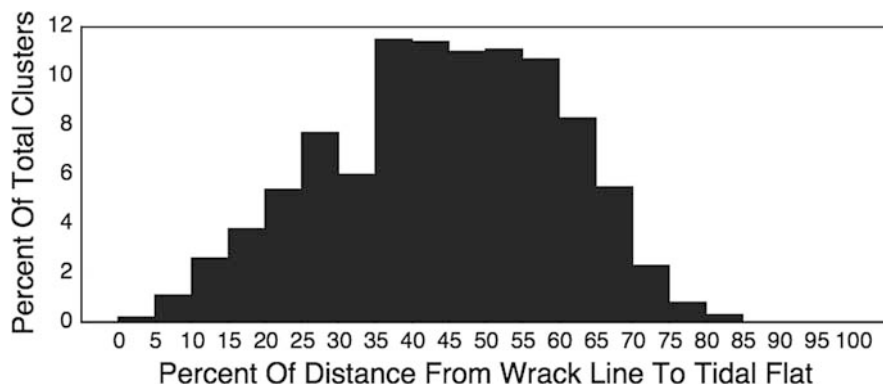


Fig. 5 Distribution of *Limulus* egg clusters observed across the foreshores of Pickering, Kitts Hummock, Ted Harvey, and North Bowers beaches in May and June, 2000 and 2001. All of the 6,132 clusters found in the 80 transects examined were in the upper 85% of beach foreshores. Of those, 90.9% (5,588 clusters) were found between 25 and 75% of the distance between the nocturnal high water wrack line (approximately the spring high tide water level) and the beginning of the tidal flat. The tidal flat would be off the chart, to the right

Table 2 Summary of sampling to examine *Limulus* egg cluster distribution across Delaware Bay beach foreshores, 2000–2001. Transects consisted of 0.3 m deep furrows, spaced 3–10 m apart, running from the nocturnal high tide wrack line (approximately the spring tide high water level), down to the beginning of the low tide terrace (tidal flat). A total of 80 transects were examined, which yielded 6,132 clusters. Beaches are listed north to south

Beach	Year	Sample date	Transects	Transect length (m)	Mean clusters per transect \pm SD
Pickering Beach	2000	30 June	6	12.5	38.7 \pm 11.7
	2001	31 May	15	14.3	213.9 \pm 74.0
Kitts Hummock	2000	27 June	10	8.5	25.8 \pm 7.6
	2001	30 May	10	11.3	60.6 \pm 20.0
Ted Harvey	2000	16 May	10	8.5	31.9 \pm 8.4
	2001	5 June	10	7.0	66.6 \pm 42.0
North Bowers	2000	15 May	9	13.7	28.1 \pm 8.6
	2001	29 May	10	14.3	64.9 \pm 24.4

and somewhat after the normal seasonal spawning peak. Spawning is just beginning in early May, and by the end of June, both the water temperature and beach sediments have warmed so that many clusters have developed to the late embryo and larval stages, which we did not count. The 2001 sample dates were in late May (Pickering, Kitts Hummock, North Bowers) and early June (Ted Harvey), very close to the normal seasonal spawning peak.

5.3 Cluster Distribution Along Sample Transects

Figure 6 shows the distribution of the 260 clusters collected along core-sampled transects during the 2002–2005 seasons. Distribution of the egg clusters found in these sample cores was similar to that observed in the 2000–2001 study of cluster distribution across the foreshore, even though half of the sampled beaches had not been part of the 2000–2001 study. Of clusters in cores, 76.9% (200) were found in the 5th through 15th cores (25–75% of the transect length). These results add additional support to evidence from the 2000–2001 test, described above, that even though beach foreshore widths may vary, sampling only the upper 85% of a west Delaware Bay beach foreshore will adequately sample the span in which female *Limulus* place their egg clusters. Thus it would seem best for studies measuring egg cluster densities to index the sampling protocol to either the spring high water level or the nocturnal high tide wrack line.

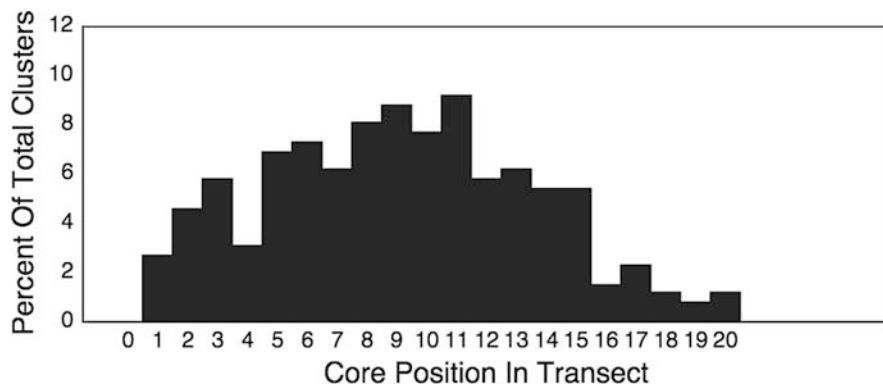


Fig. 6 Distribution of 260 *Limulus* egg clusters found in individual cores along core-sampled transects on Port Mahon, Pickering, Kitts Hummock, North Bowers, Mispillion Inlet, and Slaughter beaches in May, June and July, 2002 through 2005. Transects spanned 85% of the distance from nocturnal high tide wrack line to the tidal shelf, and were sampled with 20 cores. Cores were kept evenly, and proportionally, spaced across the varying beach widths by use of marked bungee cord lines which could be stretched to fit all beaches. Of those clusters, 76.9% (200) were found in the 5th through 15th cores (25–75% of the transect length). The zero value on the Core Position axis approximates the 0–5% position on Fig. 5. This offset is necessary because core sample dates were approximately midway between dates of the somewhat higher lunar tides when spawning is most intense. The tidal flat would be off the chart, to the *right*

5.4 Egg Cluster Size

Cluster size data appear in Table 3. After separating eggs from sediment, we found there was little uniformity in the amounts of sediment present in clusters, which agrees with the observations of Shuster and Botton (1985). For the total

Table 3 Summary of *Limulus* egg cluster size and sediment data. All eggs were counted by hand. Sediment was air dried, then weighed on a scale with 0.1 gm resolution

Beach	Year	Clusters	Eggs per cluster			Sediment per cluster (gm)		
			Minimum	Maximum	Mean \pm SD	Minimum	Maximum	Mean \pm SD
Port Mahon	2003	14	2,524	16,835	5,744 \pm 3,599	7.5	68.7	31 \pm 15.4
Pickering Beach	2005	12	2,587	9,158	5,836 \pm 1,714	7.1	95.0	40 \pm 24.8
For total sample		26	2,524	16,835	5,786 \pm 2,834	7.1	95.0	34.8 \pm 20.4

sample, air-dried sediment weights per cluster ranged from 7.1 g to 95 g (mean 34.8 ± 20.4 g SD). These sediment weights ranged from 33–352% (mean $157\% \pm 73.7\%$ SD) of damp egg weight. Sediment particle sizes present in the clusters were representative of larger particles in the surrounding sediment. Minimum numbers of eggs per cluster (2,524 eggs in 2003; 2,587 eggs in 2005) and average number of eggs per cluster (5,744 eggs in 2003; 5,836 eggs in 2005) were similar in both samples, but maximum numbers were quite different (16,835 eggs in 2003; 9,158 eggs in 2005) (Table 2). The average number of eggs we found per cluster ($5,786 \pm 2,834$ SD) for the entire sample is higher than has been previously reported for Delaware Bay beaches by Hummon et al. (1976) and Shuster and Botton (1985).

5.5 Beach Temperature and Egg Development

Mid-beach sediment temperatures in early May ranged from 13.7 to 24.2°C (mean = $17.0^\circ \pm 3.0^\circ\text{C}$ SD); in early June from 16.4 to 29.7°C (mean = $21.6^\circ \pm 3.3^\circ\text{C}$ SD); and in early July from 22.4 to 30.4°C (mean = $26.8^\circ \pm 1.9^\circ\text{C}$ SD). Temperatures did not differ appreciably between beaches on any given day. These values represent the highest temperatures to which developing eggs are exposed and exist for only a few hours during the diurnal low tide. The average temperatures at which eggs develop are somewhat lower than these values, due to the twice-daily flooding with much cooler bay water.

The first few trilobite larvae (<25 larvae per beach sampled) normally began to appear in our sediment samples during the first week in June, suggesting that about 35 days are required for development of the earliest eggs in a normal spring. These were larvae of the current season, not larvae that had overwintered (Botton et al. 1992), because larvae did not show up in our earlier spring samples.

Although we normally began to find the first larvae in samples taken during the first week of June, the date of their appearance in some years was retarded or advanced by prevailing weather. For example, May 2003 was unusually cool and overcast. As a result, average daily bay water temperatures stayed below 15°C (Smith and Michels 2006), spawning was delayed, and the first larvae did not appear in our samples until 7 July – a month later than normal. By contrast, May 2004 was unusually warm, water temperatures rose above 15°C early in May, spawning began earlier than normal (Smith and Michels 2006), and the first larvae appeared in our samples on 26 May, about 2 weeks earlier than normal. Thus, the date the first larvae of the season appear can vary widely between years.

6 Summary and Conclusions

On the western shore Delaware Bay beaches, we sampled for *Limulus* egg clusters during studies reported here, we found the average depth to center of recently laid egg clusters was 15 ± 3.5 cm SD ($n = 533$ clusters). Cluster

thickness (top to bottom) ranged from 0.6 to 7.6 cm, with an average thickness of 3.5 ± 1.1 cm SD. Most clusters (98.1%; 523 clusters) were ≥ 2 cm in thickness. Centers of 88.0% (469) of all clusters were within 20 cm of undisturbed beach surface, and centers of all egg clusters were within 25.5 cm of undisturbed beach surface. It is not necessary for a core sampler to reach to the center of a cluster for the cluster to be represented in a core. In our core sampling study, firm aggregations of eggs which did not pass through a 1.3 cm mesh were used as indication that the core included part of a cluster. Using that same criterion for the 533 clusters found in this study would mean that a 20 cm deep core could be expected to reach and sample 98% of the clusters present in an undisturbed beach, i.e., all clusters ≥ 2 cm in thickness.

All egg clusters on all beaches ($n = 6,132$ clusters in 80 transects) were found in the upper 85% of beach foreshores (the span between the high tide line and the beach break at the beginning of the tidal flat), with 90.9% (5,588) of the clusters within the 25–75% part of that span, even though foreshore widths varied. We then tested this 85% coverage of the upper foreshore using core-sampled transects, adding three beaches which had not been included in the original sample. In transects spanning only the top 85% of the foreshore, sampling with 20 evenly spaced core samples per transect produced a cluster distribution similar to that observed when using continuous trenches to sample the entire foreshore width. We found 76.9% (200) of all core-sampled clusters between 25 and 75% of the transect length ($n = 260$ clusters). These results suggest that taking evenly spaced core samples across the upper foreshore can adequately sample egg cluster density. The results also suggest that female *Limulus* place their egg clusters only in the upper 85% of western Delaware Bay beach foreshores at current population abundance, even though beach widths may vary. Thus it would seem reasonable for future studies investigating egg cluster densities to index the sampling protocol to either the spring high water level, or the nocturnal high tide wrack line, and sample downward across the foreshore, 85% of the distance to the tidal flat, or the low water level if there is no tidal flat exposed at low tide.

Intact egg clusters ranged in size from 2,524 to 16,835 eggs with an average of $5,786 \pm 2,834$ SD eggs per cluster ($n = 26$). Minimum numbers of eggs per cluster (2,524 in 2003; 2,587 in 2005) and average number of eggs per cluster (5,744 in 2003; 5,836 in 2005) were similar in both years. The average number of eggs per intact cluster ($5,786 \pm 2,834$ SD) is higher than previously reported for Delaware Bay by Shuster and Botton (1985). Extrapolations to obtain estimates of total egg numbers from counted egg clusters should take this into account.

Beach sediment temperature measured at mid-beach, and at 20 cm depth, averaged $17.0^\circ \pm 3.0^\circ$ C SD in early May, $21.6^\circ \pm 3.3^\circ$ C SD in early June, and $26.8^\circ \pm 1.9^\circ$ C SD in early July. The earliest trilobite larvae usually appeared in samples taken during the first week of June; however, prolonged cool weather caused larvae to appear a month later in 2003, while unseasonably warm weather caused them to appear 2 weeks earlier than normal in 2004. These differences appeared to be due to both to the date at which the earliest eggs were

laid and to temperatures within beach sediments where the eggs were located. Early warming of the bay causes spawning to begin earlier than normal (Smith and Michels 2006), and the warmer beach sediments accelerate larval development; delayed warming produces the opposite effects.

Acknowledgments We thank Katy O'Connell, manager of the St. Jones Center, Delaware National Estuarine Research Reserve, and previous manager Mark Del Vecchio, for making workspace available at the Center and for their attention to various research needs as they arose. Robert Scarborough, St. Jones Center Research Coordinator, worked to assure availability of special equipment. It is a pleasure to acknowledge the careful assistance of several Delaware Coastal Programs staff in this series of projects: T. Arndt, W. Conley, M. Fox, S. Love, M. Mensinger, and J. Reid. Seasonal employees H. Hudson, S. Midcap, D. Ostroff, and W. Ross provided additional support. We also thank David Smith, USGS, for providing critical insights during the course of these projects. All work on beaches, and sampling for egg clusters and eggs, was done under a series of annual permits from the Division of Fish and Wildlife, Delaware Department of Natural Resources and Environmental Control. This project was funded, in part, by a grant from the Delaware Coastal Programs with funding from the Office of Ocean and Coastal Resource Management, National Oceanic and Atmospheric Administration under award number NA05NOS4191169. The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA, or of any of its subagencies.

References

- Barlow RB Jr, Powers MK, Howard H, Kass R (1986) Migration of *Limulus* for mating: Relation to lunar phase, tide height, and sunlight. *Biol Bull* 171:310–329
- Barthel KW (1974) *Limulus*: A living fossil. *Naturwissenschaften* 61:428–433
- Botton ML, Loveland RE, Jacobsen TR (1992). Overwintering by trilobite larvae of the horseshoe crab *Limulus polyphemus* on a sandy beach of Delaware Bay (New Jersey, USA). *Mar Ecol Prog Ser* 88: 289–292
- Botton ML, Loveland RE, Jacobsen TR (1994) Site selection by migratory shorebirds in Delaware Bay and its relationship to beach characteristics and abundance of horseshoe crab (*Limulus polyphemus*) eggs. *Auk* 111:605–616
- Brockmann HJ (1990) Mating behavior of horseshoe crabs, *Limulus polyphemus*. *Behaviour* 114:206–220
- Brockmann HJ (1994) Sperm competition in horseshoe crabs (*Limulus polyphemus*). *Behav Ecol Sociobiol* 35:153–160
- Brockmann HJ (2003a) Male competition and satellite behavior. In: Shuster CN Jr, Barlow RB, Brockmann HJ (eds), *The American Horseshoe Crab*, Harvard University Press, Cambridge, pp 50–82
- Brockmann HJ (2003b) Nesting behavior: A shoreline phenomenon. In: Shuster CN Jr, Barlow RB, Brockmann HJ (eds), *The American Horseshoe Crab*, Harvard University Press, Cambridge, pp 33–49
- Brockmann HJ, Nguyen C, Potts W (2000) Paternity in horseshoe crabs when spawning in multiple-male groups. *Anim Behav* 60:837–849
- Brown GG, Clapper DL (1981) Procedures for maintaining adults, collecting gametes, and culturing embryos and juveniles of the horseshoe crab, *Limulus polyphemus* L. In: Hinegardner R, Atz J, Fay R, Fingerman M, Josephson R, Meinkoth N (eds), *Laboratory Animal Management, Marine Invertebrates*. National Academy Press, Washington, DC, pp 268–290

- Cohen JA, Brockmann HJ (1983) Breeding activity and mate selection in the horseshoe crab, *Limulus polyphemus*. *Bull Mar Sci* 33:274–281
- French KA (1979) Laboratory culture of embryonic and juvenile *Limulus*. In: Cohen E, Bang FB (eds) *Biomedical Applications of the Horseshoe Crab (Limulidae)*. Alan R. Liss, New York, pp 61–71
- Hummon, WD, Fleeger JW, Hummon MR (1976) Meiofauna-macrofauna interactions. 1. Sand beach meiofauna affected by *Limulus* eggs. *Chesapeake Sci* 17:297–299
- Jackson NL, Nordstrom KF, Smith DR (2002) Geomorphic-biotic interactions on beach foreshores in estuaries. *J Coast Res* 414–424
- Jackson NL, Nordstrom KF, Smith DR (2005). Influence of waves and horseshoe crab spawning on beach morphology and sediment characteristics on a sandy estuarine beach, Delaware Bay, New Jersey, USA. *Sedimentology* 52:1097–1108
- Jegla TC, Costlow JD (1982) Temperature and salinity effects on developmental and early posthatch *Limulus*. In: Bonaventura J, Bonaventura C, Tesh S (eds), *Physiology and Biology of Horseshoe Crabs*. Alan R. Liss, New York, pp 103–113
- Kingsley JS (1892) The embryology of *Limulus*. *J Morphol* 7:35–68
- Kraeuter JN, Fegley SR (1994) Vertical disturbance of sediments by horseshoe crabs (*Limulus polyphemus*) during their spawning season. *Estuaries* 17:288–294
- Loveland RE, Botton ML (1992) Size dimorphism and the mating system in horseshoe crab, *Limulus polyphemus*. *Anim Behav* 44:907–916
- Nordstrom KF, Jackson NL, Smith DR, Weber RG (2006) Transport of horseshoe crab eggs by waves and swash on an estuarine beach: Implications for foraging shorebirds. *Est Coast Shelf Sci* 70:438–448
- Penn D, Brockmann HJ (1994) Nest-site selection in the horseshoe crab, *Limulus polyphemus*. *Biol Bull* 187:373–384
- Pooler PS, Smith DR, Loveland RE, Botton ML, Michels SF (2003) Assessment of sampling methods to estimate horseshoe crab (*Limulus polyphemus* L.) egg density in Delaware Bay. *Fish Bull* 101:698–703
- Rudloe A (1979) Locomotor and light responses of larvae of the horseshoe crab, *Limulus polyphemus* (L.). *Biol Bull* 157:494–505
- Rudloe A (1985) Variation in the expression of lunar and tidal behavioral rhythms in the horseshoe crab, *Limulus polyphemus*. *Bull Mar Sci* 36:388–395
- Sekiguchi K (1988) *Biology of Horseshoe Crabs*. Science House, Tokyo
- Shuster CN Jr (1982) A pictorial review of the natural history and ecology of the horseshoe crab, *Limulus polyphemus*, with reference to other Limulidae. In: Bonaventura J, Bonaventura C, Tesh S (eds) *Physiology and Biology of Horseshoe Crabs*. Alan R. Liss, New York, pp 1–52
- Shuster CN Jr, Botton ML (1985) A contribution to the population biology of horseshoe crabs, *Limulus polyphemus* (L.) in Delaware Bay. *Estuaries* 8:363–372
- Shuster CN Jr, Sekiguchi K (2003) Growing up takes about ten years and eighteen stages. In: Shuster CN Jr, Barlow RB, Brockmann HJ (eds) *The American Horseshoe Crab*. Harvard University Press, Cambridge, pp 103–132
- Smith DR (2007) Effect of horseshoe crab spawning density on nest disturbance and exhumation of eggs: A simulation study. *Estuar Coasts* 30:287–295
- Smith DR, Michels SF (2006) Seeing the elephant: Importance of spatial and temporal coverage in a large-scale volunteer-based program to monitor horseshoe crabs. *Fisheries* 31:485–491
- Smith DR, Pooler PS, Loveland RE, Botton ML, Michels SF, Weber RG, Carter DB (2002) Horseshoe crab (*Limulus polyphemus*) reproductive activity on Delaware Bay beaches: Interactions with beach characteristics. *J Coastal Res* 18:730–740
- Williams KL (1986) A study of horseshoe crab egg distribution with respect to intertidal and depth gradients on two Delaware Bay beaches in New Jersey. 7 January 1986. Report to New Jersey Division of Fish, Game and Wildlife Non-Game and Endangered Species Program. 14 pp.