

## Assessment of sampling methods to estimate horseshoe crab (*Limulus polyphemus* L.) egg density in Delaware Bay

**Penelope S. Pooler**

**David R. Smith**

U.S. Geological Survey  
Leetown Science Center  
11700 Leetown Road  
Kearneysville, West Virginia 25430

E-mail address (for D. R. Smith, contact author): david\_r\_smith@usgs.gov

**Robert E. Loveland**

Department of Ecology and Evolution  
Cook College  
Rutgers University  
New Brunswick, New Jersey 08901

**Mark L. Botton**

Fordham University  
113 West 60<sup>th</sup> Street  
New York, New York 10023

**Stewart F. Michels**

Delaware Division of Fish and Wildlife  
P.O. Box 330  
Little Creek, Delaware 19961

Each spring horseshoe crabs (*Limulus polyphemus* L.) emerge from Delaware Bay to spawn and deposit their eggs on the foreshore of sandy beaches (Shuster and Botton, 1985; Smith et al., 2002a). From mid-May to early June, migratory shorebirds stopover in Delaware Bay and forage heavily on horseshoe crab eggs that have been transported up onto the beach (Botton et al., 1994; Burger et al., 1997; Tsipoura and Burger, 1999). Thus, estimating the quantity of horseshoe crab eggs in Delaware Bay beaches can be useful for monitoring spawning activity and assessing the amount of forage available to migratory shorebirds.

We evaluated procedures to estimate horseshoe crab egg density by asking three questions that address sampling at a different spatial scale. 1) How many samples of sediment are needed

for precise estimation of egg density within a segment of beach? 2) Does egg density within a segment of beach adequately represent egg density across a larger stretch of beach? 3) How many beach segments should be sampled to monitor bay-wide egg density? We chose these three questions because the objective of egg studies might focus on any of these scales. We ask the first question to determine the sampling effort necessary to detect changes in egg density over time within a specific beach segment. The second question allows us to examine the reliability of using egg density in a beach segment to infer egg density over a larger stretch of beach. The third question deals with the level of precision in estimates of bay-wide egg density and how many beaches must be sampled to detect bay-wide declines in density over time.

Understanding the reliability of egg density estimates at multiple scales will help develop effective monitoring programs.

We addressed all three questions with respect to eggs found in both shallow (0–5 cm) and deep (0–20 cm) sediments. Horseshoe crabs are generally thought to lay most of their eggs at a depth of 15–20 cm (Brockmann, 1990; Botton et al., 1994). Through processes of bioturbation and wave-generated sediment activation, horseshoe crab eggs are brought onto the beach and made available to foraging shorebirds (Botton et al., 1994; Kraeuter and Fegley, 1994; Jackson et al., 2002).

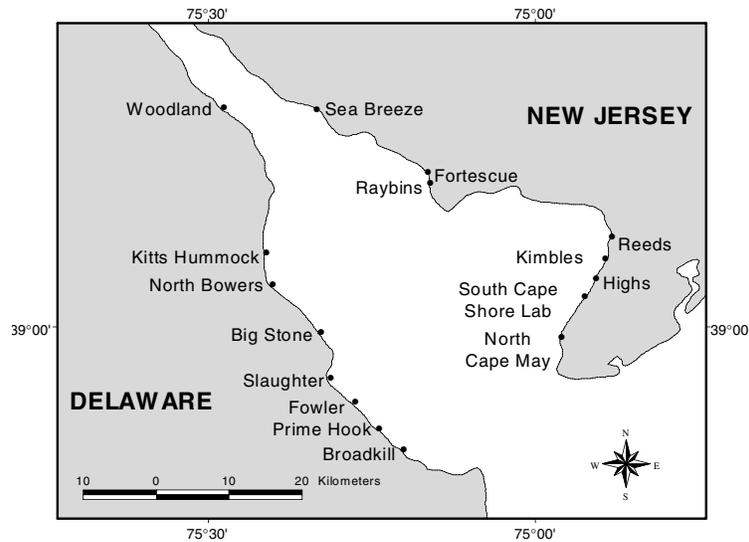
### Materials and methods

During May and June 1999, we collected sediment on 16 beaches in Delaware Bay (Fig. 1), eight along the eastern shore (New Jersey) and eight along the western shore (Delaware), to estimate egg density. Methods used to collect sediment and extract horseshoe crab eggs are summarized in the present study, but are presented in detail in Smith et al. (2002b). Beach sediment was collected in cores (5 cm diameter) within a 3-m wide strip along a 100-m segment of beach. Each 3-m wide strip was centered on the mid-beach elevation where a majority of horseshoe crab nests occur (Botton et al., 1988). The mid-beach elevation is halfway between the spring high water level and the beach break at the low tide terrace. Within each egg-sampling strip, 40 locations were selected randomly for sediment collection. At each location, a pair of core samples was taken: one to a depth of 5 cm and the other to a depth of 20 cm. We sampled eggs on 25–26 May and 14–15 June 1999, which followed the heaviest spawning activity in Delaware Bay that year (Smith et al., 2002a). We mixed the entire core contents thoroughly and then removed 80-mL aliquots. We ran the aliquots

Manuscript approved for publication  
12 February 2003 by Scientific Editor.

Manuscript received 4 April 2003 at NMFS  
Scientific Publications Office.

Fish Bull. 101:698–703 (2003).



**Figure 1**

Delaware Bay beaches (•) where horseshoe crab eggs were sampled in May and June 1999.

through a 1-mm sieve to separate eggs and larvae from ambient sediments and then counted eggs (dead or live) and larvae in each aliquot. Depth of aerobic sand varied; thus we measured core volume prior to extrapolating egg counts to totals per core and then estimated the total density of eggs and larvae. The larvae comprised a small fraction of total eggs and larvae, and for the purposes of this paper we evaluated the sampling of eggs only.

### Question 1: How many sediment cores should be sampled per beach segment?

We addressed this question in two steps. First, we determined the precision of egg-density estimates as a function of egg density and sample size. Second, we translated the precision of the estimates into statistical power to detect change in egg density over time. For simplicity, variance of the egg-density estimate was calculated from a random sample from an infinite population. Coefficient of variation (CV) was calculated as

$$CV = \sqrt{\text{var}(y) / n} / \bar{y},$$

where  $\text{var}(y)$  = variance of eggs among cores; and  $\bar{y}$  = egg density.

We modeled the relationship between egg density and variance among cores (i.e.  $\text{var}[y]=f[\bar{y}]$ ) to predict coefficient of variation (CV) for different sample sizes and across the observed range of egg densities (i.e.  $CV = \sqrt{f[\bar{y}] / n} / \bar{y}$ ). Using predicted CVs, we estimated the probability of detecting a change in egg density over time. The probability of detecting decline (i.e. statistical power) was calculated by using a one-tailed *t*-test with a type-I error rate of 0.2 and a constant rate of annual change for CVs = {0.1, 0.2, 0.3, 0.4} with the software program TRENDS (Gerrodette, 1993).

### Question 2: Is egg density within a beach segment representative of egg densities along a larger stretch of beach?

Smith et al. (2002b) modeled the relationship between counts of spawning females and egg densities within beach segments. Spawning females are counted annually as part of a bay-wide survey of spawning activity (Smith et al., 2002a), and in 1999, egg sampling was conducted on some of the same beaches as the spawning survey (Smith et al., 2002b). For eggs that were sampled in May 1999 on six New Jersey beaches, the relationship was fairly strong, linear, and predictive ( $r^2=0.62$ ; Smith et al., 2002b). Although we sampled for eggs on only one 100-m segment of beach, we used the above relationship to predict egg densities for all 100-m segments along the beach where spawning females were counted. We limited the predictions to the six New Jersey beaches where we felt the relationship between spawning females and egg densities was sufficiently strong (Smith et al., 2002b). We compared egg density in the observed 100-m segment to the distribution of densities predicted in all 100-m segments on the beach. If the observed density was within the interquartile range of the distribution of predicted densities, we concluded that the 100-m segment was representative of the larger stretch of beach.

### Question 3: How many beaches should be sampled?

Using the observed variation in egg density among the 16 beaches sampled in 1999, we predicted the CV for bay-wide egg density estimates as a function of the number of beaches sampled and under a stratified sampling design where the two strata were New Jersey and Delaware. We could not evaluate CV across a range of bay-wide densities because the 1999 results provided only one datum point, and we expected variation among beaches to be a function of egg

**Table 1**

Mean eggs per core and standard errors (SE) for horseshoe crabs (*Limulus polyphemus*) at 16 beaches sampled in Delaware Bay that were sampled in May and June 1999. Cores were 5 cm in diameter. At 40 random locations on each beach, a pair of sediment cores were sampled: one core at 5 cm depth (shallow sediment) and another at 20 cm depth (deep sediment).

State	Beach	No. of eggs per core on 25–26 May 1999				No. of eggs per core on 14–15 June 1999			
		Shallow sediment	SE	Deep sediment	SE	Shallow sediment	SE	Deep sediment	SE
Delaware	Broadkill	0.0	0.00	1.5	1.47	1.2	0.36	101.7	60.42
	Prime Hook	0.2	0.08	81.9	76.17	7.4	2.06	223.9	112.14
	Fowler	0.1	0.05	1.8	0.65	2.7	1.22	211.3	116.56
	Slaughter	11.7	2.69	814.8	186.04	41.7	5.35	664.5	97.81
	Big Stone	0.1	0.05	11.3	5.09	0.7	0.53	24.2	14.27
	North Bowers	23.0	6.49	950.3	234.18	105.1	23.54	400.4	70.81
	Kitts Hummock	26.4	8.23	325.1	78.63	15.2	5.49	124.8	43.94
	Woodland	0.5	0.17	0.1	0.06	7.0	3.68	60.9	29.75
New Jersey	North Cape May	0.3	0.25	0.0	0.00	0.5	0.33	0.7	0.37
	South Cape Shore Lab.	25.5	0.86	1085.4	140.29	4.5	0.81	1399.2	144.03
	Highs	2.1	0.71	1128.6	96.99	4.4	0.94	1456.8	173.80
	Kimbles	9.7	4.80	1561.3	286.32	1.7	0.55	1008.0	105.63
	Reeds	2.0	0.52	540.4	79.90	18.2	2.52	468.0	62.67
	Raybins	3.5	1.91	65.8	43.88	0.1	0.06	6.7	4.57
	Fortescue	2.0	0.43	645.9	108.71	20.6	3.85	465.7	193.64
	Sea Breeze	27.5	7.95	347.3	94.70	0.2	0.09	3.1	2.01

density. However, we examined the probability of detecting a percentage change in bay-wide egg density over time as a function of the number of beaches sampled by using the 1999 bay-wide egg density as the initial value in the time series.

## Results

When the objective is to monitor egg density within a segment of beach, a sample size of 40 sediment cores is sufficient for detecting substantial changes in egg density in the top 20 cm of sediment, but >40 cores would be needed to monitor egg density in the top 5 cm of sediment. Distributions of egg densities were skewed right with median densities of 3 and 275 eggs per core for shallow and deep cores, respectively (Table 1). A sample size of 40 cores resulted in a CV of 0.26 for a median density of eggs 0–20 cm deep (Fig. 2B). In contrast, about 100 cores would need to be sampled to bring the CV down to 0.3 when sampling shallow sediment and when egg density was at the median (Fig. 2A). A CV of 0.3 corresponds to a 75% chance of detecting a 50% decline in egg density over 5 years (Fig. 3A) and an 80% chance of detecting a 40% decline over 10 years (Fig. 3B). A sample size of 60 shallow cores would result in CV of 0.4 for median egg density (Fig. 2A), which would be sufficient for monitoring over 10 years, but not over 5 years. A CV of 0.4 would lead to a better than 85% chance of detecting a 50% decline in density over 10 years (Fig. 3B). Precision and power would improve when sampling higher densities of eggs (Fig. 2).

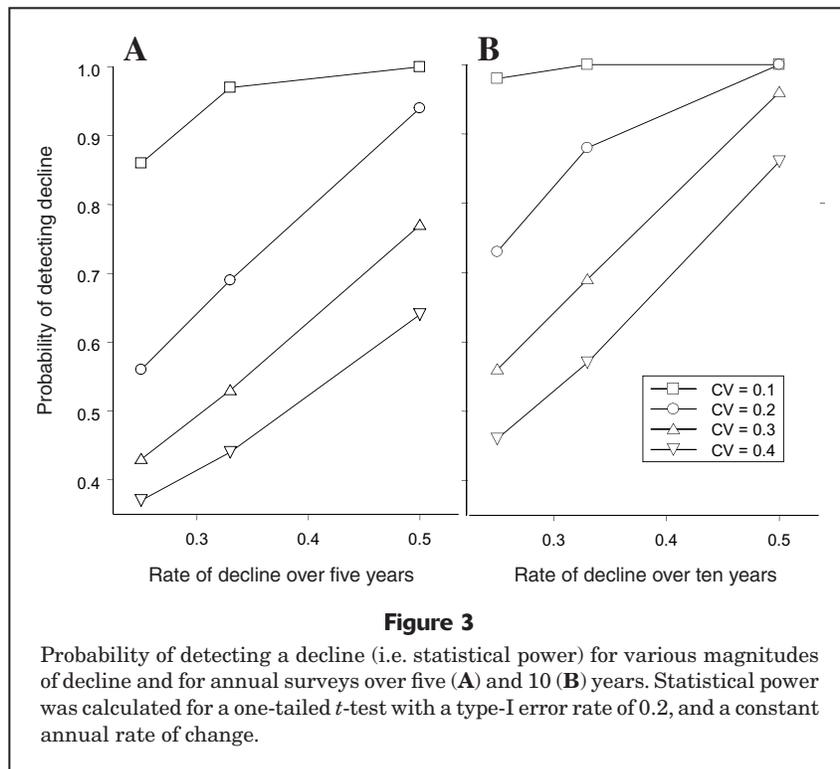
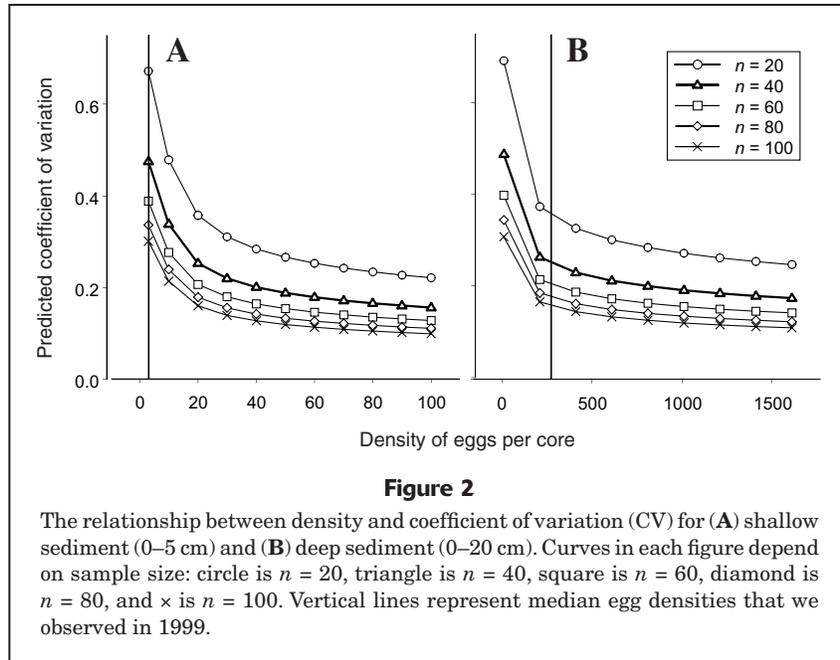
At most beaches, observed egg densities within a 100-m segment of beach were not representative of egg densities throughout a larger beach. On only two of the six New Jersey beaches examined (South Cape Shore Lab and Reeds) did the observed egg density fall within the interquartile range of beach-wide densities (Fig. 4). On three beaches the observed egg density was greater than all predicted densities, and on one beach observed egg density was less than all predicted densities.

With egg density at the 1999 level and sampling at 16 beaches (i.e. eight beaches per state) distributed throughout the bay, the CV for densities of eggs in 0–20 cm of sediment was 0.26 in May and 0.29 in June (Fig. 5). For densities of shallow eggs, the CV was 0.33 for egg densities in May and 0.43 in June. Variability in egg densities among beaches was greater for sampling in June 1999 than in May 1999.

## Discussion

Eggs in shallow sediment (0–5 cm) consistently yielded lower densities and higher variability than eggs in deep sediment (0–20 cm). A sample size of 40 sediment cores was sufficient for estimating and monitoring density of eggs 0–20 cm deep within a 100-m beach segment. However, a larger sample size ( $\geq 60$  sediment cores) would be needed for estimating and monitoring density of eggs 0–5 cm deep within a segment of beach.

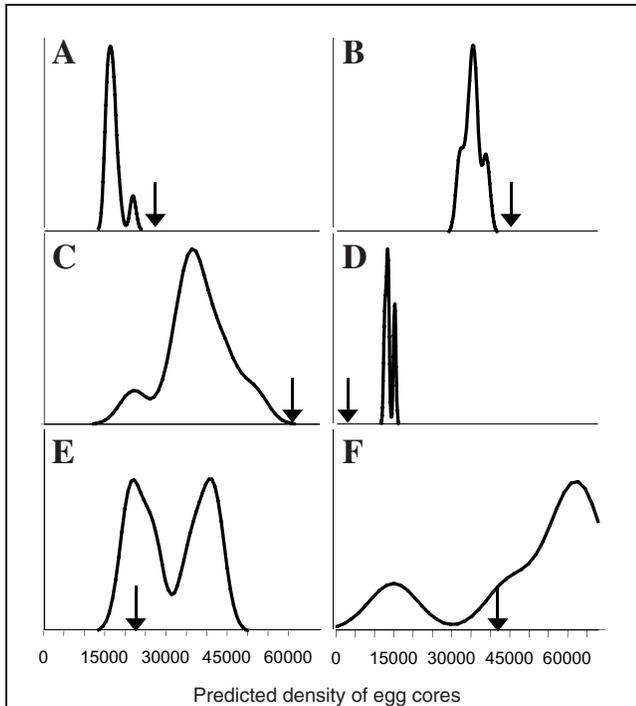
Because egg density in a 100-m segment of beach is not necessarily representative of the larger surrounding beach,



it is important to realize that if sampling is restricted to a short segment of beach, then the scope of inference is strictly limited to that segment. If a reliable estimate of egg density along a beach is required, then it will be necessary to take samples along the entire beach. Because of the logistics of sampling sediment it would be difficult to sample throughout a long stretch of beach in one stage of

sampling. However, a two-stage sampling design could be considered in which beach segments are selected at the 1<sup>st</sup> stage and sediment cores within segments are selected at the 2<sup>nd</sup> stage.

Consistent with our findings on sampling within a beach, bay-wide egg density can be more precisely estimated for eggs 0–20 cm deep than for eggs 0–5 cm deep. A stratified

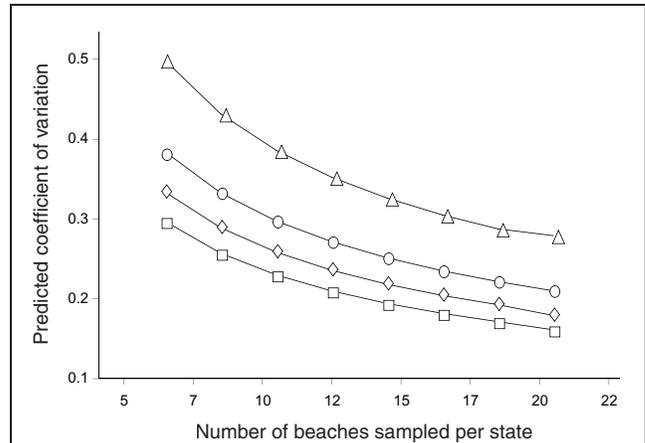


**Figure 4**

Density curves of predicted egg densities on 100-m beach segments at six New Jersey beaches. An arrow represents the egg density in the observed 100-m segment. These density curves were generated by dividing the area surveyed for spawning females into 100-m segments and using the observed relationship between egg densities and spawning females to predict egg density for each segment. The beaches shown are (A) Fortescue, (B) Highs, (C) Kimbles, (D) North Cape May, (E) Reeds, and (F) South Cape Shore Laboratory.

random sample of eight beach segments per state would result in  $CV \leq 0.3$  for estimates of egg densities 0–20 cm deep. If this level of effort were maintained, it would be sufficient to detect biologically significant declines in egg density over a 5- or 10-year period. However, greater effort would be required to monitor change in egg densities 0–5 cm deep. According to results from the May samples, to estimate egg densities in shallow sediment with  $CV \leq 0.3$ , a stratified random sample of 10 segments per state would be required.

Sampling eggs is a costly process; therefore sampling efficiency and reducing sample size are important considerations. Although sediment can be collected quickly, the process of extracting and enumerating eggs from the sediment can be time consuming. Quantifying the eggs in surface sediments to assess shorebird forage biomass is likely to be the main objective of many egg sampling studies because horseshoe crab spawning activity can be assessed by other methods, such as through counts of spawning horseshoe crabs (Smith et al., 2002a). However, a primary finding in the present study is that estimating eggs in 0–5 cm



**Figure 5**

Predicted coefficient of variation (CV) shown for the possible range of number of beaches sampled per state. This figure is based on the observed levels of bay-wide density during the two sampling periods in 1999. Curves are based on egg densities found at different depths and time periods: triangle is shallow sediment in June, circle is shallow sediment in May, diamond is deep sediment in June, and square is deep sediment in May. Shallow sediment is 0 to 5 cm deep, and deep sediment is 0 to 20 cm deep.

of sediment will be more costly than estimating eggs in 0–20 cm of sediment. In the future, alternatives in survey design, such as stratification of the beach foreshore, should be considered to reduce the amount of sediment that needs to be collected for precise estimates of horseshoe crab egg density.

## Acknowledgments

This work was funded through the USGS/State Partnership Project (no. 99HQAG0050). Additional funding was provided through the New Jersey Endangered & Nongame Species Program.

## Literature cited

- Botton, M. L., R. E. Loveland, and T. R. Jacobsen.  
1988. Beach erosion and geochemical factors: influence on spawning success of horseshoe crabs (*Limulus polyphemus*) in Delaware Bay. *Mar. Biol.* 99:325–332.  
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, H. J.  
1990. Mating behavior of horseshoe crabs, *Limulus polyphemus*. *Behaviour* 114:206–220.
- Burger, J., L. Niles, and K. E. Clark.  
1997. Importance of beach, mudflats and marsh habitats to migrant shorebirds on Delaware Bay. *Biol. Conserv.* 79: 283–292.

- Gerrodette, T.  
1993. TRENDS: software for a power analysis of linear regression. *Wildl. Soc. Bull.* 21:515–516.
- Jackson, N. L., K. F. Nordstrom, and D. R. Smith.  
2002. Geomorphic-biotic interactions on beach foreshores in estuaries. *J. Coast. Res.* 36:414–424.
- Kraeuter, J. N., and S. R. Fegley.  
1994. Vertical disturbance of sediment by horseshoe crabs (*Limulus polyphemus*) during their spawning season. *Estuaries* 17:288–294.
- Shuster, C. N., Jr., and M. L. Botton.  
1985. A contribution to the population biology of horseshoe crabs, *Limulus polyphemus* (L.), in Delaware Bay. *Estuaries* 4:363–372.
- Smith, D. R., P. S. Pooler, B. L. Swan, S. F. Michels, W. R. Hall, P. J. Himchak, and M. J. Millard.  
2002a. Spatial and temporal distribution of horseshoe crab (*Limulus polyphemus*) spawning in Delaware Bay: implications for monitoring. *Estuaries* 25:115–125.
- Smith, D. R., P. S. Pooler, R. E. Loveland, M. L. Botton, S. F. Michels, R. G. Weber, and D. B. Carter.  
2002b. Horseshoe crab (*Limulus polyphemus*) reproductive activity on Delaware Bay beaches: interactions with beach characteristics. *J. Coast. Res.* 18:730–740.
- Tsipoura, N., and J. Burger.  
1999. Shorebird diet during spring migration stopover on Delaware Bay. *Condor* 101:635–644.