

USGS-BRD State Partnership Program

Project Report I: Surveys of Spawning Activity and Egg Density to Monitor Status and Trend of Horseshoe Crabs in Delaware Bay

State Partnership Project Title: Status, Trends, and Genetic Population Structure of Horseshoe Crabs (*Limulus polyphemus*) in Delaware Bay

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REPORT SUMMARY

The state partnership project entitled “Status, Trends, and Genetic Population Structure of Horseshoe Crabs (Limulus polyphemus) in Delaware Bay” proposed to meet the following objectives (condensed and reordered from the proposal):

- 1) Assess temporal variation of spawning horseshoe crabs during May and June. Use data from 1999 surveys to test the hypothesis that horseshoe crab spawning activity does occur predominantly during full and new-moon high tides and to provide assistance to the state partners in developing a statistically rigorous long-term monitoring program to assess status and trend in horseshoe crab spawning activity in the Delaware Bay.
- 2) Examine the form and strength of the relationship between indices of spawning horseshoe crabs, dispersed eggs, and egg clusters; and determine if spawning activity can be monitored effectively by tracking 1 or 2 rather than all 3 of these indices.
- 3) Develop species-specific microsatellite DNA loci in L. polyphemus, and utilize microsatellite DNA markers to identify and quantify genetic diversity present in a range-wide survey among geographic populations, with emphasis placed on the Delaware Bay region.

This report is the final project report for the first 2 objectives. The genetics work, identified in objective 3 is ongoing and a report will be issued at a later date. The organization of the report corresponds to project objectives.

Chapter 1, entitled “Spatial and Temporal Distribution of Horseshoe Crab (Limulus polyphemus) Spawning in Delaware Bay: Implications for Monitoring”, addresses the 1st objective. In early 1999 a workshop was held at the request of the Atlantic States Marine Fisheries Commission to design a statistically valid survey of horseshoe crab spawning in Delaware Bay. The survey that resulted was a redesign of a volunteer-based spawning survey that began in 1990, and its network of volunteers was relied on to implement the 3-stage sampling design in 1999. During May and June of 1999, 163 participants surveyed during the highest of the daily high tides on 16 beaches (8 on each side of Delaware Bay). During the first half of the spawning season, spawning was associated with lunar phases; however, it was moderated by wave height. Disproportionately more spawning occurred within 3 d of the first new and full moons ($t = 4.27$, 79 df, $p < 0.001$), and spawning activity was correlated inversely to the % of beaches with waves $\geq 0.3\text{m}$ ($r = -0.558$, $p = 0.011$). Spawning was heaviest on the Delaware shore around the full moon in May in spite of low waves in New Jersey during the new and full moons in May. Number of beaches sampled was the most important factor in determining the precision of the spawning index and power to detect a decline. Explicit consideration of statistical power has been absent from the current debate on horseshoe crab status and harvest. Those who argue against harvest restrictions because of a lack of statistically significant declines take on a burden to show that the surveys they cite have high statistical power. We show the Delaware Bay spawning survey will achieve high statistical power with sufficient sampling intensity and duration. We recommend that future Delaware Bay spawning surveys 1) sample on 3 d during the 7-d period centered on the new and full moons in May and June and 2) increase the number of beaches to ensure high statistical power to detect trends in baywide spawning activity. For example, a 25% decline in spawning activity over 10 yr would be detectable (power ≥ 0.80 , $\alpha = 0.20$) if 13 beaches were sampled per state on 3 d around each new and full moon.

Chapter 2 is entitled “Comparison Between Indices of Horseshoe Crab (Limulus polyphemus) Spawning and Eggs on Delaware Bay Beaches” and addresses, in part, the 2nd objective. In this chapter we raise and attempt to answer the following question – can monitoring eggs and predicting spawning females provide sufficient data for both assessing horseshoe crab

stock and quantifying shorebird food base? If so, then horseshoe crab monitoring needs could be met at much less cost. However, the answer depends on the strength of the relationship between indices of spawning females and their eggs. We compared indices of spawning female horseshoe crabs and their deposited eggs using observations from 16 beaches in Delaware Bay. We explored that relationship and examined how it is affected by geography, time within a spawning season, and beach characteristics. Finally, we discussed implications of our findings to horseshoe crab monitoring programs. We found that the relationship between indices of spawning horseshoe crabs and their eggs depended on geography and time of sampling. Only on New Jersey beaches early in the spawning season did we find a strong relationship between indices of spawning and eggs buried 0 – 20 cm deep. Eggs that had been brought to the beach surface, and were thus available to shorebirds, were not related to the amount of spawning that had occurred on the beach. We suggest different explanations for the failure to observe strong relationships on Delaware beaches, during late-season sampling, and for eggs 0 – 5 cm deep. We believe a strong relationship was not observed on Delaware beaches because the sampling protocol failed to account for variation in the distribution of eggs across the foreshore. Also, mid-season sampling (i.e., June sampling) introduced a temporal mismatch between numbers of spawning females and live, unhatched eggs. Finally, we hypothesize that density of eggs 0 – 5 cm deep was determined by a complex relationship involving an interaction between physical factors (i.e., beach morphology and wave energy) and density of spawning females. We concluded that spawning biomass cannot be reliably predicted from an index of egg density (and vice versa). Thus, if egg density is to be monitored, then a separate bay-wide survey of horseshoe eggs must be designed and implemented. We also suggest that some aspects of the current egg sampling protocol need to be reconsidered. In particular, a robust protocol to identify the center of the egg distribution in the beach profiles needs to be developed that will apply across a range of beach types. The current practice of sampling over a 3 m strip is unlikely to be robust to errors in locating the strip relative to egg distribution. Further, research is needed to determine whether certain beach characteristics make it more likely that eggs become available to shorebirds. Such information might also be helpful in identifying potential shorebird habitat.

Chapter 3, entitled “Assessment of Horseshoe Crab (*Limulus polyphemus*) Egg Sampling Methods and Sample Size Determination”, is a brief report, which combines with Chapter 2 to address the 2nd objective. In this chapter we examine horseshoe crab egg data (presented in Chapter 2) to assess adequacy of egg sampling procedures. We structure the assessment by 3 specific questions. Each addresses sampling at a different spatial scale: 1) How many sediment cores should be sampled to monitor density within a 100 m segment of beach? 2) Does egg density within a 100 m section 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 found that a sample size of 40 sediment cores is sufficient for estimating density of eggs 0 – 20 cm deep within a 100 m beach segment, but a larger sample size (≥ 60 sediment cores) would be needed to estimate density of eggs 0 – 5 cm deep. However, our results suggest that a 100 m segment of beach is unlikely to be representative of a larger stretch of beach. If accurate information about egg density is required for the larger stretches of beach, then it would be necessary to include the entire stretch of beach in the sample design, perhaps through a 2-stage sampling design. With regard to sampling to estimate bay-wide egg density, a stratified random sample of 8 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 is maintained, it should be sufficient to detect biologically significant declines in egg density. However, we conclude that greater effort would be required to monitor change in egg densities 0 – 5 cm deep. Based on shallow egg densities in May, a stratified random sample of 10 segments per state would result in $CV \leq 0.3$. Based on the lower densities found in June, a stratified random sample of 17 segments per state would be needed to result in comparable CVs. Before increasing effort at this scale, managers must assess the

importance of monitoring baywide egg density. Throughout these analyses, data from the shallow cores consistently yielded lower densities and higher variability than the data from the deep cores. A primary recommendation is that current levels of sampling effort are sufficient for eggs 0 – 20 cm deep, but sampling effort needs to be increased to estimate density of eggs 0 – 5 cm deep.

At the time of this report, the redesigned spawning survey has been implemented 2 consecutive years, 1999 – 2000. To this project report, we appended a memo, which was submitted to the ASMFC Horseshoe Crab Technical Committee, on the index of spawning activity for 1999 – 2000. Results from the 2000 survey were similar to those from 1999. Spawning activity was slightly higher in New Jersey on the first lunar period in May, but during the other lunar periods in May and June, spawning activity was higher in Delaware. In both years, the spawning index for all lunar periods combined was higher in Delaware than in New Jersey. There was no discernable change in baywide spawning index between 1999 and 2000. The lack of change, or apparent stability, in the spawning index between 1999 and 2000 does not support any broad conclusions about horseshoe crab population trends. Such conclusions will require a longer time series of data. We stress that two years of results do not provide an informative time series for trends in spawner numbers of iteroparous species such as horseshoe crabs. Also, a pattern of stability does not address the concern that current levels of spawning may not be sufficient to support shorebird trophic demands. As such, we also stress the importance of continuing this survey as currently designed, which demands that annual funding be pursued and secured.

This State Partnership project proposed to deliver the following products:

- 1) A report to the ASMFC Technical Committee and a presentation at annual meeting of the American Fisheries Society on a statistically valid survey design for monitoring horseshoe crab spawning activity in Delaware Bay that takes into account both spatial and temporal variation. The design will be evaluated for estimator variance, cost, and power to detect change over time.
- 2) A report to the ASMFC Technical Committee and a published, co-authored manuscript on an assessment of the strength and form of the relationship between counts of spawning horseshoe crabs and of dispersed eggs and egg clusters.
- 3) A report to the ASMFC Technical Committee on within-season temporal variation of spawning.
- 4) At least two publications in peer-reviewed journals on genetics work are anticipated; one describing marker development and one describing the genetic population structure of horseshoe crabs in Delaware Bay.
- 5) Associated metadata to comply with FGDC and NBII standards. A web site will be designed and maintained to describe the USGS-BRD State Partnership horseshoe crab project. We will consult with partners and ASMFC Technical Committee for specific content of the web site. Possible content includes information on how horseshoe crabs are being monitored and who to contact to volunteer help with monitoring efforts. Web documents will be uploaded to the web server maintained by the AEL (<http://ael.er.usgs.gov>).

This project report delivers products for numbers 1, 2, and 3. Chapters 1 of this report has been submitted for possible publication in a peer-reviewed journal, and Chapters 2 and 3 are undergoing further editing prior to submission. The web site mentioned in product number 5 has been available at <http://ael.er.usgs.gov/groups/stats/Limulus/>. Metadata is being developed. Products listed under number 4 will be part of a forthcoming report on the genetic aspects of the project.

Chapter 1 – Spatial and Temporal Distribution of Horseshoe Crab (Limulus polyphemus) Spawning in Delaware Bay: Implications for Monitoring

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Introduction

Increases in harvest of Atlantic horseshoe crabs (*Limulus polyphemus*) for bait and loss of their spawning habitat have raised questions about their current status (Botton and Ropes 1987, Berkson and Shuster 1999, Widener and Barlow 1999). Much concern has focused on shorebird, horseshoe crab interactions in Delaware Bay. Horseshoe crab spawning on Delaware Bay beaches coincides with shorebird migration through the region. Shorebirds, such as redknot (*Calidris canutus*), ruddy turnstone (*Arenaria interpres*), sanderling (*Calidris alba*), and semipalmated sandpiper (*Calidris pusilla*), stopover in the Delaware Bay and feed heavily on horseshoe crab eggs, as well as infaunal benthic invertebrates, to fuel nesting and reproduction in the Arctic (Castro and Myers 1993; Botton et al. 1994; Burger et al. 1997; Tsipoura and Burger 1999). In addition to being harvested for bait, horseshoe crabs are caught and bled to produce *Limulus* amoebocyte lysate, which is used to detect pathogenic endotoxins in medical products (Berkson and Shuster 1999). Bled horseshoe crabs are returned to their point of capture, and Rudloe (1983) reported that survival of bled horseshoe crabs was 90% of the survival of unbled horseshoe crabs.

In spite of the emergence of multiple threats on the long-term stability of horseshoe crabs and the potential that their decline will have a ripple effect through the ecosystem, few resources had been directed at collecting data for stock assessment. In response, the Atlantic States Marine Fisheries Commission developed a Fisheries Management Plan (FMP) with a mandate that certain states “formulate standardized and statistically robust methodologies ... for spawning surveys” (Atlantic States Marine Fisheries Commission 1998a). As part of the mandate, a workshop was held January 1999 to discuss the design of a logistically feasible and scientifically valid spawning survey. Horseshoe crab researchers, natural resource managers, and biological statisticians attended the workshop. Primary goals of the workshop were to develop an index of spawning activity and design a statistically valid survey to track that index over time.

Since 1990, there has been a volunteer-based survey with the expressed goal of indicating status and trends of Delaware Bay horseshoe crabs (Finn et al. 1991). However, the volunteer-based survey has been criticized because frequent methodological changes have put into question its reliability and accuracy (Atlantic States Marine Fisheries Commission 1998c). Workshop participants recognized that, given available funds, the existing volunteer workforce would continue to be needed to implement a baywide spawning survey. At the same time, the volunteer-based survey would be strengthened by a statistical redesign. However, outstanding issues regarding survey design remained, such as when and how often to survey beaches especially in relation to new and full moons, which are the times that horseshoe crabs spawning is believed to be heaviest (Rudloe 1980; Shuster and Botton 1985; Maio 1998). Other survey design issues, such as sample size calculations that rely on large-scale spatial and temporal variation, needed to be more fully addressed than was possible with available data.

In this paper we have 3 broad objectives. First, we describe a survey design and present results from the 1999 spawning survey in Delaware Bay. In the presentation of results, we describe the patterns of spawning spatially and temporally within the Delaware Bay. Second, we use the results from the 1999 survey to evaluate the survey design and make recommendations for future horseshoe crab spawning surveys in Delaware Bay. Finally, we discuss statistical power of the spawning survey in the context of risks that management decisions present to other species and fisheries that depend on the horseshoe crab.

Methods

We implemented a 3-stage survey design in which beaches were selected at the first stage. Dates within a beach were selected at the second stage. At the third stage quadrats within a beach and date were selected. The target population was identified as that portion of the horseshoe crab population that spawns in Delaware Bay in a given y. The survey objective was to estimate an index of spawning activity useful for measuring trends in spawning activity over time. We derived formulae for estimating the index based on this survey design and presented them in the Appendix.

The first stage of sampling involved selection of spawning habitat within the bay and thus required defining a baywide sampling frame, which was a list of all spawning habitat that could be sampled in Delaware Bay. However, because of physical constraints not all spawning habitat in Delaware Bay was accessible to a volunteer-based survey. As an alternative sampling frame, we included only beaches that could be accessed by public road. The restricted sampling frame, which cannot be used to estimate abundance or population size, is appropriate for trend detection under the assumption that trends in spawning activity on accessible beaches are parallel to trends for the target population. We stratified the beach-level sampling frame by state (Delaware and New Jersey) because in past surveys spawning activity was typically higher on 1 side of Delaware Bay in a given y (B. L. Swan, unpublished data) and managers were interested in state-specific assessments. In the 1999 survey, 16 beaches (8 on each side of Delaware Bay) were selected by stratified random sampling (Table 1). We limited sampling to a beach section ≤ 1 km in length because 1 km could be surveyed within 2 h; most spawning activity is likely to be completed within 2 h of the time of high tide (Maio 1998).

Table 1-1. Beaches where spawning horseshoe crabs were sampled during 1999 in Delaware Bay.

State	Beach	Length of beach section (km)	Sampling schedule
DE	Woodland	0.4	3 d at full/new moon plus 1 neap d
	Kitts Hummock	1.0	every 2 nd or 3 rd d
	North Bowers	1.0	3 d at full/new moon plus 1 neap d
	Big Stone	1.0	every 2 nd or 3 rd d
	Slaughter	1.0	3 d at full/new moon plus 1 neap d
	Fowlers	1.0	every 2 nd or 3 rd d
	Prime Hook	1.0	3 d at full/new moon plus 1 neap d
	Broadkill	1.0	3 d at full/new moon plus 1 neap d
NJ	Sea Breeze	0.2	3 d at full/new moon plus 1 neap d
	Gandys	0.4	3 d at full/new moon plus 1 neap d
	Fortescue	1.0	every 2 nd or 3 rd d
	Reeds	1.0	every 2 nd or 3 rd d
	Kimbles	0.8	3 d at full/new moon plus 1 neap d
	Highs	0.5	every 2 nd or 3 rd d
	South Cape Shore Lab	0.8	3 d at full/new moon plus 1 neap d
	North Cape May	1.0	3 d at full/new moon plus 1 neap d

The second stage of sampling involved the time dimension and thus required defining a temporal sampling frame. Peak spawning activity occurs during May and June in Delaware Bay (B. L. Swan, unpublished data). So, the temporal sampling frame could comprise all dates in May and June or it could be restricted to a subset of those dates (similar to the beach-level sampling frame). The issue of which dates to include in the temporal sampling frame was

unresolved at the time of this investigation. Thus, sampling in the 1999 survey was temporally intensive to examine the temporal distribution of spawning and evaluate alternative temporal sampling frames. In the 1999 survey, 16 beaches were scheduled to be sampled 3 d around the new or full moon (2 d prior, day of, and 2 d after) and 1 d at the neap tide. To augment the temporal sampling even further, 6 of the 16 beaches (3 beaches on each side of the Delaware Bay) were sampled every 2nd or 3rd d (Table 1 and Fig. 1). We made a heuristic judgment to sample 1 of the 2 daily high tides so that volunteers' limited time could be distributed over more beaches and dates. In past surveys, more spawning activity tended to occur during the higher of the daily high tides (B. L. Swan, unpublished data in Delaware Bay; Rudloe 1980 in Gulf of Mexico; Maio 1998 in mid-Atlantic coastal bays). Thus, we sampled on the higher of the daily high tides, which was invariably the evening or nighttime high tide.

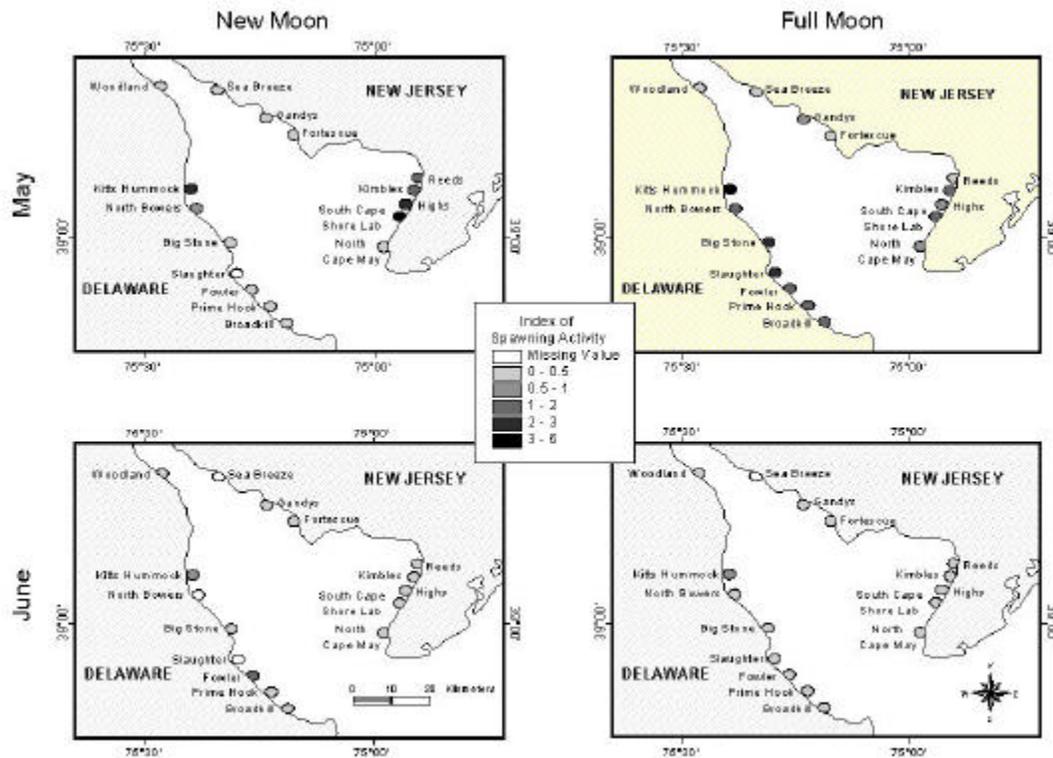


Figure 1-1. Spatial distribution of spawning activity in Delaware Bay during the new and full moons of May and June, 1999 at 16 beaches selected for the 1999 horseshoe crab spawning survey.

The third (and ultimate) stage of sampling involved subsampling a beach to count horseshoe crabs. A 1 m² quadrat was chosen as the sampling unit to increase survey efficiency based on evidence that horseshoe crabs tend to cluster spatially along a beach. Horseshoe crabs were clustered on 82% of the sampling occasions during the 1996 and 1997 surveys (B. L. Swan, unpublished data); this based on the equality of variance to mean in a Poisson series (Elliott 1977:40). In the case of spatial clustering, Elliott (1977:128) recommends, and cites other studies that support, the use of a relatively small sized quadrat.

We placed quadrats along a beach by systematic sampling with 2 random starts (Thompson 1992). Systematic sampling is an attractive alternative to simple random sampling

because it is convenient to apply under field conditions. Also, systematic sampling performs well for spatially clustered populations (Christman 2000). Multiple random starts allow estimation of variance and decrease the chance that the interval between quadrats coincides with a spatial pattern in the distribution of spawning horseshoe crabs. Hedayat and Singh (1991) recommend a systematic sample with 2 random starts because more random starts would compromise the advantage of easy application. We expected to sample 100 quadrats per beach; thus, the interval between quadrats depended on the length of the beach.

Counting horseshoe crabs began when the tide began to recede from the high tide mark. Quadrats were placed systematically along the line formed by the spawning horseshoe crabs that were highest on the beach. Horseshoe crabs that were at least halfway in the quadrat were counted, and sex was recorded. Weather conditions, such as wave height, were also recorded. Protocol and data sheets were distributed to volunteers at training workshops and made available on internet (<http://ael.er.usgs.gov/groups/stats/Limulus/protocol.html>).

We believe that most of the spawning females can be encountered ≤ 1 m of the high tide line. Brockman (1990) observed 94% of nesting females ≤ 1 m of the maximum high tide line. Maio (1998) found highest density at shallow depths (≤ 33 cm). However, we attempted to count horseshoe crabs that were ≤ 2 m (rather than ≤ 1 m) from the high tide line by locating 2 adjacent quadrats extending into the bay at each systematically located point along the beach. However, in our trials of this approach we encountered 2 problems. First, the quadrat furthest in the bay was frequently in water too deep to be observed effectively. Second, the additional quadrats were time consuming so that surveying a beach exceeded the allotted time (2 h). Thus, we discontinued this practice.

The index of spawning activity was the average number of spawning females per quadrat (or per 1 m^2) at the high tide line on the highest of the daily high tides at a given beach. We formulated the index of spawning activity in terms of spawning females because the female segment of the population best represents the fluctuations in reproductive potential and egg availability to shorebirds. A female-based spawning survey was also recommended by a panel assembled by ASMFC to review the horseshoe crab stock assessment (ASMFC 1998b). We averaged across nights to estimate the index for a beach-specific index at a given period and averaged across beaches to estimate the index for the bay. Averages were weighted appropriately to account for survey design attributes, such as stratification and unequal beach length (Appendix).

There were 163 participants in the 1999 Delaware Bay horseshoe crab spawning survey. The participants, who were mostly volunteers, surveyed beaches for a total of 190 hours, not counting the travel time or the time waiting for the tide to be at the right height. Typically, a crew of 3 to 4 took 53 minutes to sample a beach.

We used data from the 6 beaches that were most frequently sampled to compare the mean and variance of spawning activity that resulted from several alternative temporal sampling frames. For each alternative, we computed mean and among-beach variance for dates that were sampled within the frame. Our objective was to find the temporal sampling frame that minimized variance in relation to the mean. The alternative temporal sampling frames were defined as

- 1) all d in May and June,
- 2) 7 d centered on new or full moon plus 1 d at neap tide,
- 3) 7 d centered on new or full moon,
- 4) 7 d beginning on new or full moon,
- 5) 5 d beginning on new or full moon, and

- 6) 1 d on new or full moon.

Sample size and power to detect trends are important for effective implementation of the survey. We used the 1999 survey results to assess variation in the index of spawning activity and to calculate sample size and statistical power. The three sources of variation in the estimate of the index are

1. between beach,
2. between dates within each beach, and
3. between systematic sample (i.e., quadrats) within each beach and date (Appendix).

We simulated how changing the sample size for beaches, dates, or quadrats affects coefficient of variation (CV). Based on our comparisons of the alternative temporal sampling frames, we used only 1 temporal sampling frame in our simulations; the 7-d periods centered on the full and new moons in May or June. For simplicity, the beach-level sampling frame was set to 25 accessible beaches per state; this was slightly larger than the sampling frame in current use (21 accessible beaches in New Jersey and 19 in Delaware). We calculated CV for estimates of baywide and state-specific spawning. Coefficient of variation is important because it measures reliability of the index and is related inversely to the probability of detecting a change in the index. We used program TRENDS (Gerrodette 1993) to calculate power to detect trends in the index and assess the effectiveness of the survey to detect declines in spawning activity. Type I error rate (α) was set to 0.10 and 0.20. We calculated power for exponential declines in spawning activity of 25, 33, and 50% over 5 and 10 yr. Power was based on a 2-sided t-test, which is a conservative choice because power would be higher if based on a 1-sided t-test. An examination of the 1996 and 1997 survey results (B. L. Swan, unpublished data) indicated that beach-level CV was proportional to $1/\sqrt{\text{spawning activity}}$, so we assumed that relationship in the power calculations. We calculated cost of a spawning survey in terms of person-nights or the number of participants required if each person surveys only 1 night assuming a crew of 3.

Results

Spatial and Temporal Distribution of Horseshoe Crab Spawning

During the first half of the spawning season, spawning was associated with the lunar phases (Fig. 2). Disproportionately more spawning occurred around the first new and full moons ($t = 4.27$, 79 df, $p < 0.001$); 50% of spawning activity occurred within 3 days of the first new and full moons, but only 23% would be expected if spawning was uniformly distributed through May and June. However, spawning was also affected by wave height (Fig. 3). When spawning began on the New Jersey shore (early to mid-May), a high percentage of beaches on the Delaware shore were experiencing wave heights in excess of 0.3 m (Fig. 3). Then as wave action subsided on the Delaware shore, spawning increased until it peaked again in early June shortly after the May full moon (Fig. 3).

Tide height alone was a poor predictor of spawning activity. During the first 2 lunar cycles (prior to 7 June) when most of the spawning occurred, tide height accounted only for 12% of the variation of females and 6% of males. Continuing to look at results from the first 2 lunar cycles, correlation of spawning activity with tide height was higher for females ($r = 0.325$, $p = 0.106$) than for males ($r = 0.196$, $p = 0.338$). In contrast, spawning activity correlated more strongly with high waves (% of beaches with waves > 0.3 m), and the correlation was similar for females ($r = -0.531$, $p = 0.005$) and males ($r = -0.576$, $p = 0.002$). Effective modeling of spawning activity required a combination of temporal and geographic strata, weather, and tide height (Table 2).

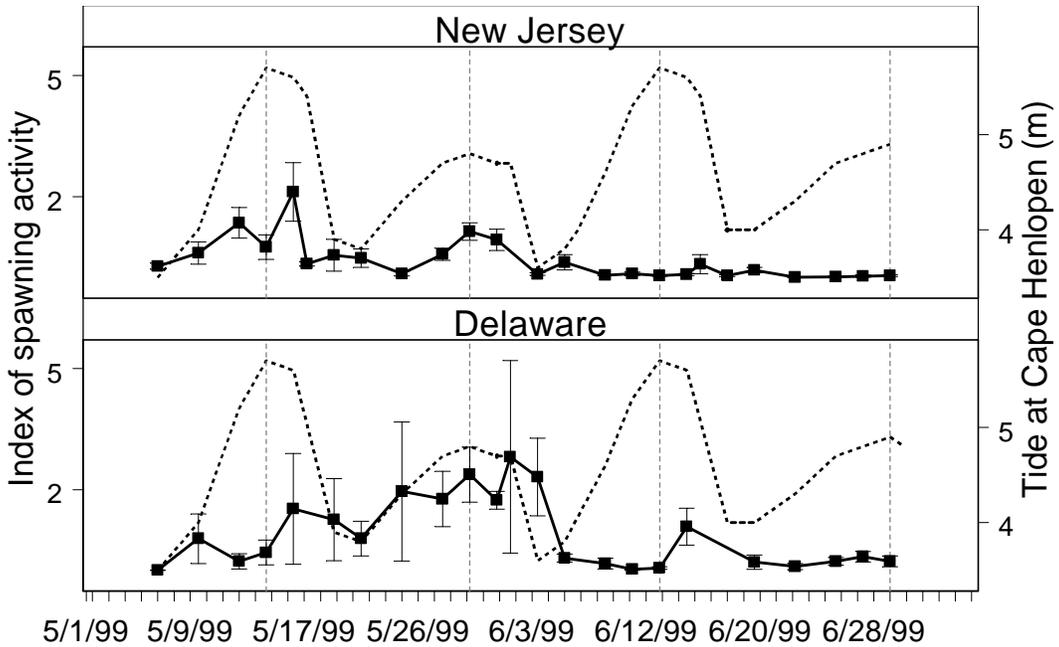


Figure 1-2. Index of spawning activity during May and June for beaches on the New Jersey and Delaware sides of the Delaware Bay. The index of spawning activity is based on the density of spawning female horseshoe crabs. Tide height at high tide (m) at Cape Henlopen is the dashed line. New and full moon dates are indicated by vertical reference lines.

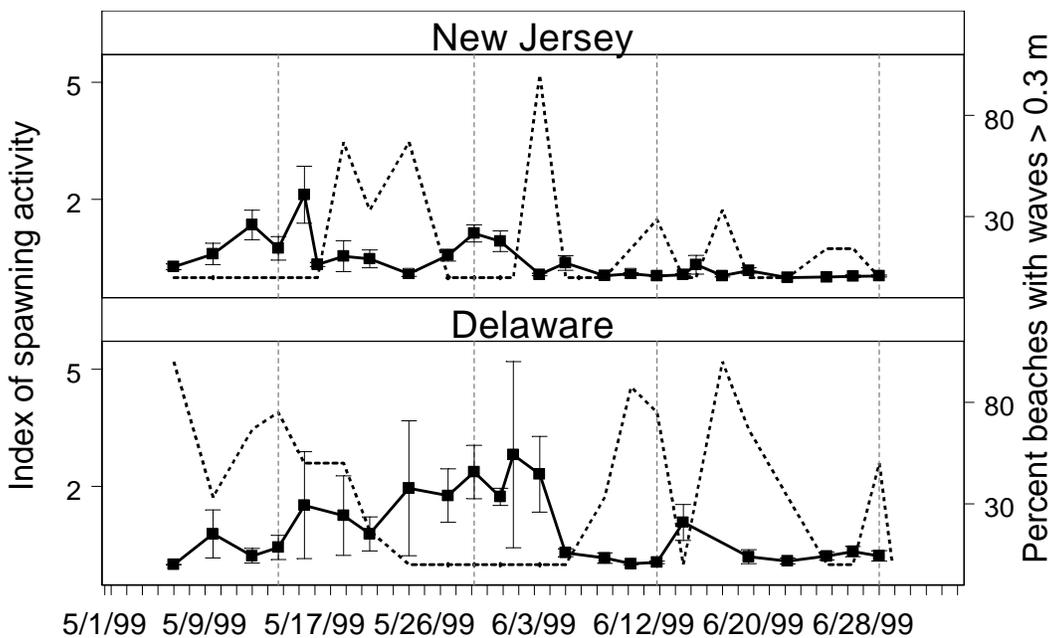


Figure 1-3. Index of spawning activity during May and June for beaches on the New Jersey and Delaware sides of the Delaware Bay. The index of spawning activity is based on the density of spawning female horseshoe crabs. Percent of the beaches with waves exceeding 0.3 m is the dashed line. New and full moon dates are indicated by vertical reference lines.

Table 1-2. Summary statistics for models relating spawning to state, time, tide height, and wave height. The r^2 were 0.63 for females and 0.67 for males. Spawning activity was transformed to the log scale for the regression. Time was categorized into dates before and after 7 June, which was halfway between the full moon in May and the new moon in June. Wave height was the percent of beaches with waves ≥ 0.3 m.

Variable	Females			Males		
	Parameter estimate	SE	p	Parameter estimate	SE	p
Intercept	4.619	1.195	0.0004	-2.913	1.262	0.0261
State	1.183	0.329	0.0009	1.031	0.348	0.0050
Time	2.105	0.328	0.0001	2.183	0.346	0.0001
Tide height	0.432	0.239	0.0787	0.377	0.253	0.1438
Wave height	-2.128	0.505	0.0001	-3.279	0.533	0.0001

At the new moon in May, spawning was concentrated in the vicinity of Reeds, Kimbles, Highs, and South Cape Shore Lab beaches (Fig. 1). From the end of May to early June spawning in Delaware was widespread. The exception was Kitts Hummock where spawning was relatively intense from early May and into early June, perhaps because it was locally sheltered from prevailing winds by a breakwater.

Sex ratio was affected by the magnitude of spawning activity, and that effect differed between the states ($F = 9.24$, 1 and 190 df, $p = 0.003$). At beaches with spawning activity >0.35 females m^{-2} , median sex ratio was 3.5 males to females (90% of those ratios ranged from 1.9 to 5.9) regardless of state ($F = 0.21$, 1 and 85 df, $p = 0.648$). However, at lower spawning activity (≤ 0.35 females m^{-2}), sex ratios were higher and more variable in New Jersey, where the median was 2.8 and 90% ranged from 1.0 to 9.4. In contrast, sex ratios were lower and less variable in Delaware at low spawning activity, where the median was 1.5 and 90% ranged from 0.8 to 3.3.

Not all beaches were sampled as scheduled. Two of the 8 Delaware beaches (Slaughter and North Bowers) were not sampled on any of the 3 d around the new moon in June. Also, 1 New Jersey beach (Sea Breeze) was not sampled in June because of problems with access. This missing information is unlikely to weaken our conclusions because spawning appeared to taper off considerably throughout the Delaware Bay during June. However, missing sampling dates as scheduled is a concern, especially in a volunteer survey, and we pick up on this issue in the discussion section.

Evaluation and Recommendation of Survey Design for Monitoring an Index of Spawning Activity

Among-beach variance to mean ratio was lowest and magnitude of the spawning activity was intermediate when the temporal (second-stage) sampling frame was restricted to the 7 d centered on new or full moons (Fig. 4; Case 3). The magnitude of spawning activity was greatest for a temporal sampling frame of 5 or 7 d beginning on and after the new or full moon (Cases 4 and 5). The magnitude was least when dates around neap tide were included (Cases 1 and 2). Sampling only on the new or full moon (Case 6) decreased the magnitude of the spawning index compared to Cases 3, 4, and 5.

The variance of the spawning activity index, $\hat{v}ar(\bar{y})$, can be partitioned into 3 components for sampling beaches, dates within beaches, and quadrats within dates and beaches (Appendix). Using data from the 6 most frequently sampled beaches and the Case 3 temporal sampling frame, the beach-level variance component was 116 times the date-level variance component and over 10,000 times the quadrat-level variance component. Removing stratification

of dates by lunar period caused the date-level variance component to increase by a factor of 3.7. These results highlight the value of temporal stratification and suggest that precision of the index will be determined largely by number of beaches.

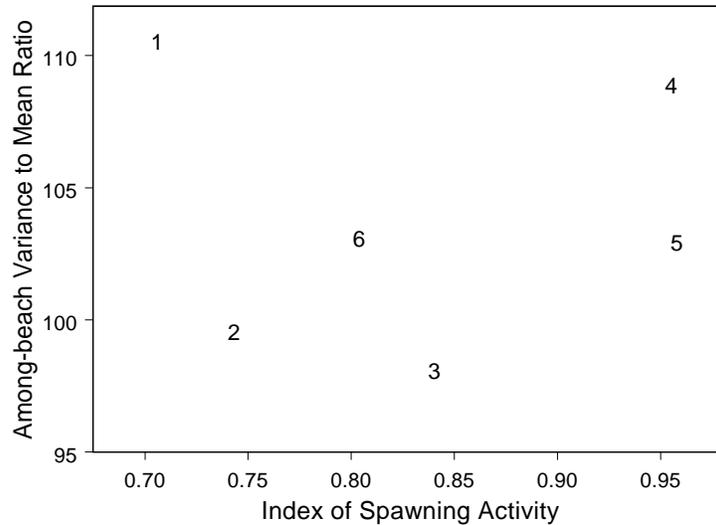


Figure 1-4. Ratio of among-beach variance to mean spawning activity for 6 alternative temporal sampling frames. Numbers indicate alternatives: 1) all d in May and June, 2) 7 d centered on new or full moon plus 1 d at neap tide, 3) 7 d centered on new or full moon, 4) 7 d beginning on new or full moon, 5) 5 d beginning on new or full moon, and 6) d of new or full moon.

The effect of the number of beaches, nights, and quadrats on CV was the same whether spawning activity was estimated by state or baywide. Thus, we present only comparisons from the baywide index. Number of beaches was most important in determining CV ($F = 32034.54$, 1 and 1256 df, $p < 0.0001$; Fig. 5); 96% of the reduction in CV (as measured by sum of squares) was due to number of beaches. Number of nights was statistically significant in determining CV ($F = 102.79$, 1 and 1256 df, $p < 0.0001$). However, <1% of the reduction in CV was due to number of nights. Number of quadrats, in contrast, did not significantly affect CV of the baywide index ($F = 0.04$, 1 and 1256 df, $p = 0.85$).

Precision of the state-specific index was lower than for a baywide index. For sample sizes of 5 to 15 beaches per state, on average CV for a state-specific index was 41% higher than for the baywide index. The degree of clustering and the CV for spawning males and females indicated that the distribution of males was more variable than of females (Fig. 6). Thus, a female-based index of spawning activity tended to be more precise.

Probability of detecting a decline in spawning activity (statistical power) is determined by 4 factors: sample size, sampling variance, decline in spawning activity (magnitude and form), and Type I error rate. Because we found that sampling variance was chiefly determined by number of beaches, we focused on the effect of the number of beaches on statistical power and set the number of sampling occasions to 3 nights per lunar period and the number of quadrats to 100 per beach (Table 3).

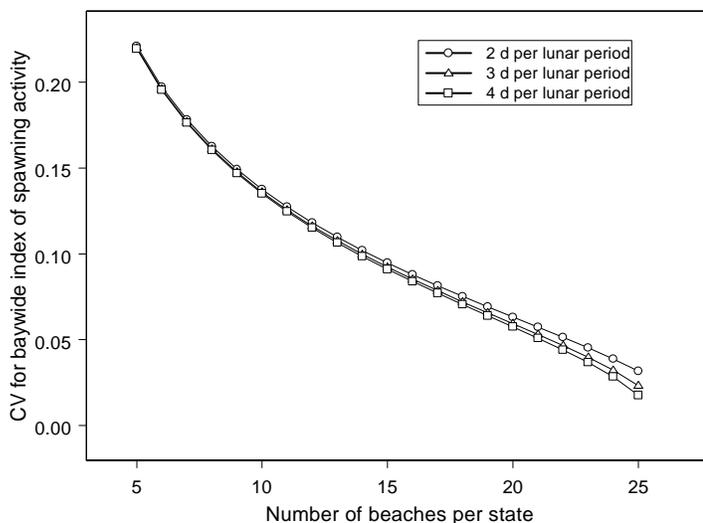


Figure 1-5. Coefficient of variation as a function of the number of beaches sampled per state and number of dates sampled per lunar period.

Power to detect declines increased with number of sampled beaches, duration of monitoring, Type I error rate (α), and magnitude of the decline (Table 3). For 5 yr of monitoring, power did not exceed 0.80 unless the decline was at least 50% when $\alpha = 0.10$. Increasing α to 0.20 resulted in adequate power for smaller declines. For example, power exceeded 0.80 ($\alpha = 0.20$) for a decline $\geq 33\%$ if 15 beaches or more were sampled per state annually for 5 yr. A 25% decline over 5 yr was not detectable (power < 0.80) for ≤ 15 beaches sampled per state. However, a 25% decline over 10 yr was detectable (power ≥ 0.80) if 15 beaches were sampled per state and $\alpha = 0.10$, or if 13 beaches were sampled per state and $\alpha = 0.20$.

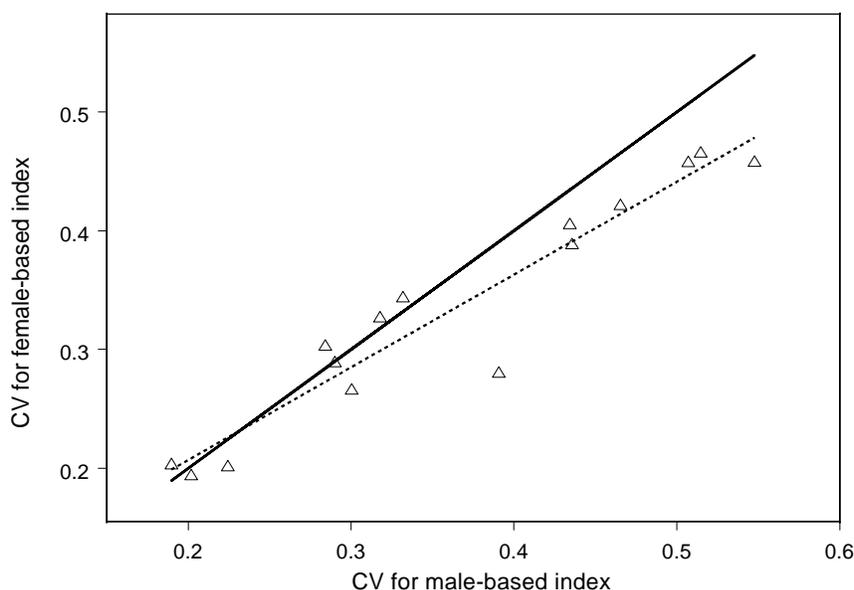


Figure 1-6. Coefficient of variation for an index of spawning activity based on females and males for beaches surveyed during May and June, 1999, in Delaware Bay. Solid line shows the 1-to-1 isopleth where CV for males and females based indices would be equal.

Table 1-3. Sample size (beaches per state) for a survey of spawning horseshoe crabs and the resulting cost, CV, and power to detect exponential decline in spawning activity. In all cases, 3 nights were sampled in each of the 4 lunar periods in May and June, 100 quadrats were sampled per beach per night. Calculations were based on 25 accessible beaches per state, 7 day lunar periods, and 1 km long beaches. Unit of measurement for cost was person-nights or the number of participants required if each participant surveys only 1 night. Power was based on a 2-tailed t-test and the assumption that CV was a nonlinear function of spawning activity. Power ³ 0.80 are underlined.

Years of monitoring	No. of beaches sampled per state	Cost (person-nights)	CV for baywide index	5 yr of monitoring						
				$\alpha = 0.10$			$\alpha = 0.20$			
				25% decline	33% decline	50% decline	25% decline	33% decline	50% decline	
5	5	360	0.22	0.17	0.22	0.39	0.30	0.38	0.59	
	6	432	0.20	0.18	0.24	0.44	0.32	0.41	0.64	
	7	504	0.18	0.20	0.27	0.50	0.34	0.45	0.70	
	8	576	0.16	0.22	0.32	0.57	0.38	0.50	0.77	
	9	648	0.15	0.23	0.34	0.62	0.40	0.53	<u>0.81</u>	
	10	720	0.14	0.25	0.37	0.67	0.42	0.57	<u>0.85</u>	
	11	792	0.13	0.28	0.41	0.72	0.45	0.61	<u>0.88</u>	
	12	864	0.12	0.30	0.45	0.77	0.49	0.66	<u>0.92</u>	
	13	936	0.11	0.34	0.51	<u>0.83</u>	0.53	0.71	<u>0.95</u>	
	14	1008	0.10	0.38	0.57	<u>0.88</u>	0.58	0.77	<u>0.97</u>	
	15	1080	0.09	0.43	0.64	<u>0.93</u>	0.64	<u>0.83</u>	<u>0.99</u>	
	10	5	360	0.22	0.27	0.41	0.73	0.43	0.57	<u>0.85</u>
		6	432	0.20	0.31	0.46	0.79	0.46	0.63	<u>0.90</u>
		7	504	0.18	0.35	0.53	<u>0.86</u>	0.51	0.69	<u>0.94</u>
		8	576	0.16	0.41	0.61	<u>0.92</u>	0.57	0.76	<u>0.97</u>
9		648	0.15	0.44	0.66	<u>0.95</u>	0.61	<u>0.80</u>	<u>0.98</u>	
10		720	0.14	0.49	0.71	<u>0.97</u>	0.65	<u>0.84</u>	<u>0.99</u>	
11		792	0.13	0.53	0.76	<u>0.98</u>	0.70	<u>0.88</u>	<u>1.00</u>	
12		864	0.12	0.59	<u>0.82</u>	<u>0.99</u>	0.75	<u>0.91</u>	<u>1.00</u>	
13		936	0.11	0.65	<u>0.87</u>	<u>1.00</u>	<u>0.80</u>	<u>0.95</u>	<u>1.00</u>	
14		1008	0.10	0.72	<u>0.92</u>	<u>1.00</u>	<u>0.85</u>	<u>0.99</u>	<u>1.00</u>	
15		1080	0.09	<u>0.80</u>	<u>0.96</u>	<u>1.00</u>	<u>0.90</u>	<u>0.99</u>	<u>1.00</u>	

Effort for the 1999 survey as scheduled was 894 person-nights. That cost is similar to the cost of sampling 12 or 13 beaches per state under the proposed design, which calls for sampling fewer dates than in 1999 (Table 3). Thus, effort allocated to sampling dates in 1999 should be shifted to sampling more beaches in future surveys to increase power to detect trends.

Discussion

In 1999, spawning was varied geographically and associated with lunar phase. However, spatial and temporal distribution of spawning was moderated by wave height. During the new moon in May, spawning was greater on New Jersey beaches at a time when wave height might have prevented spawning on Delaware beaches. As waves subsided and the full moon in May approached, spawning increased on the Delaware side of the bay. As predicted by Shuster and Botton (1985) spawning in Delaware Bay did not follow lunar phase as sharply as Rudloe (1980) observed for beaches in Apalachee Bay, Florida. However, our results contradict their (Shuster and Botton 1985) assertion that spawning activity is greatest along the Cape May shore of New Jersey unless prevailing northwesterly winds drive spawning to wind protected Delaware Bay shore. In 1999, spawning was heaviest on the Delaware shore around the full moon in May in spite of low waves in New Jersey during the new and full moons in May. Spawning tapered off in June throughout the bay.

We recommend that the spawning index be based on females for 3 reasons. First, female abundance determines reproductive potential, which is an important parameter in population assessment. Egg production available for shorebirds is also a function of female abundance (as well as abiotic factors that cause scouring and deposition of beach sediment). Second, the distribution of males was more variable than distribution of females. Third, counting only spawning females would require less time to survey a beach because the majority of the total count was males (ASMFC 1998b; Maio 1998). Thus, a female-based index would be biologically informative, more sensitive to trend detection, and less costly than an index based on males or total counts.

Concentration of spawning around the new or full moons suggests that future surveys need only sample during those times. We recommend sampling during the 7-d periods centered on the new and full moons of May and June because that temporal sampling frame minimized among-beach variance in spawning activity relative to the mean. The validity of this restricted temporal sampling frame depends on the assumption that trends in spawning activity during lunar periods are parallel to trends in spawning activity during periods of neap tides. The pattern of decline in spawning activity reported by Widener and Barlow (1999) supports this assumption. Widener and Barlow (1999) surveyed spawning horseshoe crabs at a Cape Cod beach 3 times over 15 yr and found that spawning activity during the peaks (lunar period) and valleys (neap tidal periods) dropped uniformly.

Volunteer training, coordination, and dedication can greatly influence the success and credibility of the survey. For example, Slaughter Beach was only sampled around the full moons and several beaches were sampled only 1 out of the scheduled 3 d around a new or full moon. The effect of missing data on the index of spawning activity depends on the reason for not sampling as scheduled. If the beaches were omitted at random, then the index will not be biased. However, if the decision not to sample was influenced by a perception of the magnitude of spawning then the index will be biased. This issue is critical in a volunteer-based survey because volunteers, unless instructed otherwise, may decide subjectively whether to sample or not.

It is important to note that the value of the spawning survey to conservation of horseshoe crab and associated species is in trend detection. The index of spawning activity cannot be used to estimate population abundance or population size of horseshoe crabs in Delaware Bay.

Consider that we counted horseshoe crabs only 1) within 1 m of the high tide line, 2) on the beaches near land-based access points, and 3) on the highest of the daily high tides. For practical reasons we restricted the sampling frames. For example, certain habitat, such as inaccessible beaches and tidal creeks, were left out of the beach-level sampling frame. Furthermore, it is not known what proportion of the horseshoe crab population spawns in a given yr. As a result we did not attempt to estimate population size or absolute magnitude of spawning activity. Rather, the underlying assumption was that the trends in spawning activity for the Delaware Bay could be represented by the trends in spawning activity on accessible beaches during the weeks centered on new or full moons. (This assumption can be tested by a separate study to compare trends in spawning activity among spawning habitats.) Use of the proposed index to compute a population size by simply expanding the number by the length of beaches in Delaware Bay would be highly biased and misleading.

The most effective way to increase the probability of detecting a trend in spawning activity was to increase the number of beaches sampled (Fig. 6). Sampling more quadrats on a beach did not significantly increase the precision of a baywide index. Although sampling more dates per lunar period increased precision, the increase was minor in comparison to the effect of sampling more beaches. Nevertheless, we recommend sampling multiple dates (3 d per lunar period) for statistical and pragmatic reasons. First, stratification on lunar period (Appendix) is important for reducing sampling variance; thus, at least 2 d must be sampled per lunar period to estimate sampling variance. Second, we believe that 3 d are preferable to 2 because 3 d allows dates to be selected systematically in a ‘balanced’ pattern (before, during, and after the new or full moon). Third, rather than sampling ≥ 4 d per lunar period, effort should be allocated to sampling more beaches. Power analysis, which highlights the importance of sampling more beaches, is an approximation useful for planning. The survey design that we present calls for an independent random selection of accessible beaches in each yr. Power to detect change could be improved somewhat by a design that allows a combination of fixed and random selection of beaches (Fuller 1999).

Trend detection involves balancing risks. The risk of concluding a decrease in spawning activity (or increase) when in fact it has not changed is the Type I error rate (α). We call this risk the fishers’ risk because falsely concluding a decrease in spawning activity could lead unnecessarily to harvest reduction. In contrast, there is the species’ risk, which is the risk of failing to detect a true and biologically meaningful decrease in spawning activity. In statistical notation, species’ risk is Type II error rate (β), and statistical power is its complement ($1-\beta$). We call this the species’ risk because failing to detect a biologically important change in spawning activity could lead to a stock collapse or declines in species that depend on horseshoe crabs. The fishers’ risk, which is measured by α , is set prior to data analysis and is thus known. The species’ risk, which is only known if statistical power is calculated, depends on sample size, sampling variance (which is a function of survey design and underlying population variance), true trend in spawning activity, and the fishers’ risk (α). All else being equal, the lower the fishers’ risk the higher the species’ risk. To balance the 2 risks, sample size needs to be increased or survey design must be improved to reduce sampling variance.

Under the precautionary principle (Buhl-Mortensen and Welin 1998), it would be risk prone to assume that species’ risk is low unless it has been shown to be low by calculation of statistical power, even when survey data shows no statistically significant trend. Peterman and M’Gonigle (1992) outline 3 outcomes when statistical power is incorporated into monitoring programs and regulatory decision-making. First is the case where survey data show a statistically significant decline of a magnitude deemed to be biologically important. As a result, harvest restriction would be recommended. Second is the case where survey data fail to show a statistically significant decline, and statistical power has been evaluated and found to be high for

a decline deemed to be biologically important. In this case, harvest restriction would not be recommended. Third is the case where survey data fails to show a statistically significant decline, and statistical power has been evaluated and found to be low for a biologically important decline. In such a case, species' risk is high because the possibility of an important decline cannot be ruled out. The precautionary principle stipulates that, in the face of this uncertainty, harvest reduction should be recommended as a risk adverse strategy.

Many sampling programs that have encountered horseshoe crabs have failed to show significant coastwide declines (ASMFC 1998c). Although statistical power has not been considered explicitly in assessments of these sampling programs, past efforts to monitor horseshoe crabs have suffered from methodological inconsistencies and gear inefficiencies (ASMFC 1998c). In the face of this uncertainty and consistent with the precautionary principle, Atlantic States Marine Fisheries Commission (1998a) has adopted a risk adverse management strategy and has called for harvest reduction. Others may point to the lack of statistically significant declines and argue against harvest reduction. However, in doing so they take on a burden to show that the surveys they cite have high statistical power.

We proposed a survey and assessed its statistical power to detect declines in baywide spawning activity. The amount of acceptable risk and magnitude of decline deemed biologically important are judgments that must be debated by stakeholders in the management of horseshoe crabs. We argue for a balance of risks (i.e., fishers' risk = species' risk), suggest biologically important declines should be determined through population modeling, and present results in Table 3 that can be used to guide sample size decisions.

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Appendix. Formulae for estimating the index of spawning activity.

The survey design that we recommend can be described as a 3-stage sampling design. At the first stage, beaches are selected; at the second stage, dates within beaches are selected; and at the third stage, quadrats within dates and beaches are selected. (By beach we mean a section of beach the length of which can be sampled at the third stage of sampling.) Selection at the first and second stages is stratified. Beaches are stratified by state (i.e., NJ or DE) with equal allocation and random sampling within strata. Dates are stratified by lunar period (e.g., 7-d period centered on the new or full moons in May and June) with equal allocation and systematic sampling of nights within lunar period (e.g., selecting 2 d prior to, the day of, and 2 d after the new or full moon). Quadrats on a beach are selected by systematic sampling with 2 random starts. Given the design of the survey we derive formulae for an estimate of the spawning index and for its variance.

Notation:

H = number of beach-level strata

N_h = number of accessible beaches in the h^{th} strata

P = number of lunar periods in the spawning season

T = number of dates in a lunar period

L_{hi} = length of i^{th} beach in h^{th} strata

S_{hi} = number of possible systematic samples on i^{th} beach in h^{th} strata

n_h = number of beaches sampled in h^{th} strata

t = number of dates sampled during the a lunar period

s = number of random starts for a systematic sample

m_{hijkl} = number of quadrats in the l^{th} systematic sample on the k^{th} date in the j^{th} lunar period on i^{th} beach in h^{th} strata

y_{hijklp} = count of spawning female horseshoe crab in the p^{th} quadrat in the l^{th} systematic sample on k^{th} date in j^{th} period on i^{th} beach in h^{th} strata

The index of spawning activity for the k^{th} date of the j^{th} period on the i^{th} beach in the h^{th} strata is

$$\bar{y}_{hijk} = \frac{S_{hi}}{L_{hi}} \frac{\sum_{l=1}^s y_{hijkl}}{s},$$

where $y_{hijkl} = \sum_{u=1}^{m_{hijkl}} y_{hijklp}$ is the count of spawning female horseshoe crabs in each systematic sample. To compute the index for the i^{th} beach in the h^{th} strata, we have

$$\bar{y}_{hi} = \frac{\sum_{j=1}^P \bar{y}_{hij}}{P},$$

where $\bar{y}_{hij} = (t)^{-1} \sum_{k=1}^t \bar{y}_{hijk}$. Because beaches vary in length, we use the ratio estimator (Thompson 1992:60) to estimate the index for the h^{th} strata

$$\bar{y}_h = r_h \bar{L}_h,$$

where $r_h = \frac{\sum_{i=1}^{n_h} \bar{y}_{hi}}{\sum_{i=1}^{n_h} L_{hi}}$ and $\bar{L}_h = \frac{\sum_{i=1}^{N_h} L_{hi}}{N_h}$. Finally, because of stratification of beaches, the baywide index is

$$\bar{y} = \frac{\sum_{h=1}^H L_h \bar{y}_h}{\sum_{h=1}^H L_h},$$

where $L_h = \sum_{i=1}^{N_h} L_{hi}$. Variance of the baywide index can be estimated by

$$\hat{\text{vâr}}(\bar{y}) = \left(\sum_{h=1}^H L_h \right)^{-2} \sum_{h=1}^H L_h^2 \hat{\text{vâr}}(\bar{y}_h),$$

where the estimate of strata-specific variance is based on 3 stage sampling (Thompson 1992:137)

$$\hat{\text{vâr}}(\bar{y}_h) = \left(\frac{N_h - n_h}{N_h} \right) \frac{\hat{\mathbf{S}}_h^2}{n_h} + \frac{1}{N_h n_h P^2} \sum_{i=1}^{n_h} \sum_{j=1}^P \left[\left(\frac{T-t}{T} \right) \frac{\hat{\mathbf{S}}_{hij}^2}{t} + \frac{1}{Tt} \sum_{k=1}^t \left(\frac{S_{hi} - s}{S_{hi}} \right) \frac{\hat{\mathbf{S}}_{hijk}^2}{s} \right].$$

There are 3 population variance estimates in $\hat{\text{vâr}}(\bar{y}_h)$: variance among beaches ($\hat{\mathbf{S}}_h^2$), variance among dates within lunar period and beach ($\hat{\mathbf{S}}_{hij}^2$), and variance among systematic samples within date, lunar period, and beach ($\hat{\mathbf{S}}_{hijk}^2$). To estimate these population variances we have

$$\hat{\mathbf{S}}_h^2 = \frac{\sum_{i=1}^{n_h} (\bar{y}_{hi} - r_h L_h)^2}{n_h - 1},$$

$$\hat{\mathbf{S}}_{hij}^2 = \frac{\sum_{k=1}^t (\bar{y}_{hijk} - \bar{y}_{hij})^2}{t - 1}, \quad \text{and}$$

$$\hat{\mathbf{S}}_{hijk}^2 = \frac{\sum_{l=1}^s (\bar{y}_{hijkl} - \bar{y}_{hijk})^2}{s - 1}.$$

Chapter 2 – Comparison Between Indices of Horseshoe Crab (Limulus polyphemus) Spawning and Eggs on Delaware Bay Beaches

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Introduction

There is substantial interest in monitoring distribution and abundance of horseshoe crab (Limulus polyphemus) because of their role in estuarine ecology along the mid-Atlantic coast, use for production of Limulus amebocyte lysate (LAL) for detection of bacterial contamination of injectable drugs and implantable medical devices, and use as bait for commercial harvest of whelk and eel (Berkson and Shuster 1999). Programs have been proposed to monitor horseshoe crab spawning activity in Delaware Bay by counting intertidal (spawning) horseshoe crabs or their eggs (ASMFC 1998). Spawning horseshoe crabs and eggs are both a function of reproductive activity; however, which to select for monitoring depends on program objectives. If population dynamics of horseshoe crabs is the primary objective, then monitoring spawning stock (i.e., number of spawning females) and recruitment to reproductive maturity are useful for stock assessment (Shepherd 1977). Alternatively, if food base for migrating shorebirds is the primary objective, then monitoring horseshoe crab eggs in habitats used by shorebirds is important (Botton et al. 1994, Margraf and Maio 1998).

Long-term monitoring of horseshoe crabs is expensive. A survey to count spawning females requires a large labor force distributed simultaneously throughout the Bay. For example, over 160 individuals participated in the 1999 spawning survey in Delaware Bay (Chap 1). Moreover, horseshoe crabs spawn in greatest numbers at the higher daily high tide, which often occurs at night or early morning. It may be difficult to organize volunteers to survey at odd hours. In contrast, a survey of horseshoe crab eggs is not as time sensitive and requires fewer personnel. Eggs can be collected at whichever low tide is more convenient; and a crew of 3 to 5 can survey multiple beaches per day. So there is the potential for egg surveys to be considerably cheaper than surveys of spawning horseshoe crabs.

Because the number of eggs is a function of the number of spawning female horseshoe crabs, it is possible that eggs could be predicted from an index of spawning females (or vice versa). This raises the question – can monitoring eggs and predicting spawning females provide sufficient data for both assessing horseshoe crab stock and quantifying shorebird food base? If so, then horseshoe crab monitoring needs could be met at much less cost. However, the answer depends on the strength of the relationship between indices of spawning females and their eggs.

In this paper, we compare indices of spawning female horseshoe crabs and their deposited eggs using observations from 16 beaches in Delaware Bay. We explore that relationship and determine how it is affected by geography, time within a spawning season, and beach characteristics. Finally, we discuss implications of our findings to horseshoe crab monitoring programs.

Methods

During May and June 1999, spawning horseshoe crabs and their eggs were counted at 16 beaches in Delaware Bay. The beaches were selected randomly as part of a survey of spawning activity, which we described in Chapter 1. In this chapter, we describe methods to sample horseshoe crab eggs and relate spawning females to their eggs.

To sample horseshoe crab eggs, beach sediment was collected in cores (5 cm diameter) within a 3 m by 100 m strip of beach. Each strip was centered on the mid-point of the beach foreshore, which is the point halfway between the spring wrack line and the beach break. Within a strip, 40 locations were selected randomly for sediment collection. At each location a pair of core samples were taken: one to a depth of 5 cm and the other to a depth of 20 cm. Botton et al. (1992) found that horseshoe crabs in Delaware Bay deposit most of their eggs 10 – 20 cm deep.

Beach sediment was processed under the direction of Drs. Loveland and Botton using standard procedures that they have applied over the last decade. Horseshoe crab eggs in 3 separate 80 ml subsamples from a core were counted, and then the counts were extrapolated to estimate the total count per core using estimates of core volume. Eggs were classified by stage (egg, embryo, or trilobite) and viability (live or dead). We expressed the egg counts in terms of density or number per 19.6 cm², which is the surface area of a core. Essentially, we summed counts across the depth dimension to reduce counts to an area basis rather than a volume basis. This was done because not all cores collected the same volume of sediment due to variation in depth of soft sediment. From estimates of egg density, we estimated total numbers of eggs for the 3 m by 100 m strip.

We determined the number of spawning females in the vicinity of the strip where eggs were sampled. Spawning was surveyed over a longer stretch (≤ 1 km) at each beach (Chap 1); however, we focused on spawning that occurred over a 200 m stretch of beach centered on the 100 m strip where eggs were sampled. For comparison to total numbers of eggs, we estimated cumulative (or total) number of spawning females across 100 m of beach during the 15 days prior to egg sampling. The period was limited to 15 d because eggs deposited earlier could have developed to a larval stage (Brockman 1990). We sampled eggs at 2 times: May 24th – 25th and June 14th – 15th.

In addition to sampling individual eggs, egg clusters were sampled across the entire span of the beach where spawning were surveyed. Sampling egg clusters was also conducted at 2 time periods: May 17th – 20th and June 1st – 3rd. Sediment from a core (5 cm diameter, 20 cm deep) was dropped through a 1.3 cm mesh, and occurrence of at least 1 egg cluster was recorded. Cores were taken at 300 – 400 locations at each beach. Locations for these cores were determined as follows: first 100 positions were selected systematically along the spring wrack line, then at each of the 100 random positions core locations were selected systematically at 2 m intervals (with a

random start) along a line perpendicular to the beach break. At the first sampling period (May 17th – 20th) cores were taken from 0 – 6 m from the spring wrack line. At the second sampling period (June 1st – 3rd) cores were taken from 1 – 9 m from the spring wrack line. We sampled a greater distance of the foreshore at the second sampling period because we suspected that the upper foreshore (0 – 6 m from the wrack line) was not capturing the full spatial distribution of eggs.

Relationships between indices of spawning females, eggs, and egg clusters were correlated and explored graphically and through regression-tree modeling. Because of the presence of outliers, Spearman rank correlations were used to gauge the strength of linear relationships. Regression-tree modeling was used to explore the relationship between egg density and a variety of potential predictor variables including spawning females, side of the bay (DE vs NJ), time of sampling (first vs second sampling period), % of spawning-survey days with waves > 0.33 m, beach azimuth, beach slope, and beach width. We applied regression-tree modeling because it is a useful exploratory technique, is robust to nonlinear relationships between response and predictor variables, and incorporates interactions between predictor variables in an intuitive and transparent fashion (Anderson et al. 2000). Results from regression-tree modeling can be interpreted similar to those of standard multiple regression. However, the tree-based method splits observations (beaches in this case) into groups with similar levels of the response variable (egg density). The splits are determined by variation in the explanatory variables. For example, the regression tree might show that in certain habitat types high egg density in 0 – 5 cm is associated strongly with high numbers of spawning females, but that association is weak in other habitat types.

Results

Egg density, spawning activity, and beach characteristics

We found only 1 significant relationship between indices of spawning females and deposited eggs (Table 1). Deeply buried eggs (0 to 20 cm deep) at the first period of sampling were related to numbers of spawning females on New Jersey beaches ($p = 0.002$). However, deeply buried eggs at the second period and shallow eggs (0 to 5 cm deep) at both periods were unrelated to numbers of spawning females (Figs. 2-1 and 2-2).

Table 2-1. Spearman rank correlations and p-values in parentheses for numbers of spawning females and their eggs deposited over 2 depths.

Time of egg sampling	Eggs 0 to 5 cm deep		Eggs 0 to 20 cm deep	
	Delaware beaches	New Jersey beaches	Delaware beaches	New Jersey beaches
May 25 -- 26	0.3571 (0.3851)	0.3333 (0.4198)	0.0476 (0.9108)	0.9048 (0.0020)
June 14 -- 15	-0.4762 (0.2329)	0.3214 (0.4821)	-0.2619 (0.5309)	0.3571 (0.4316)

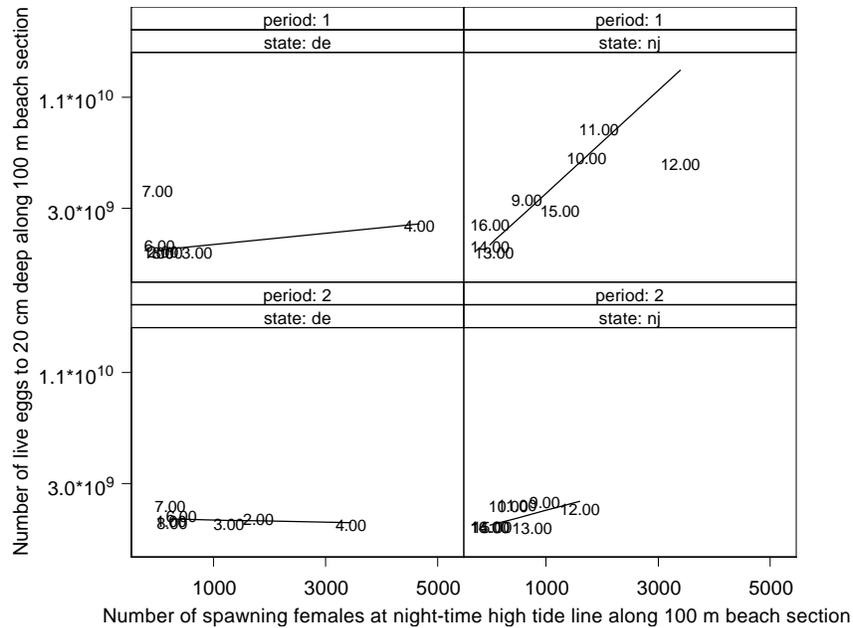


Figure 2 - 1. Relationship between eggs 0 to 20 cm deep and number of spawning females at nighttime high tide line during the 15 d prior to egg sampling on Delaware Bay beaches at 2 time periods in 1999. For the first time period eggs were sampled May 24th – 26th, and spawning was surveyed during the May 10th – 23rd. For the second time period, eggs were sampled June 14th – 15th, and spawning was surveyed during May 31st – June 13th. Delaware beaches are labeled 1) Broadkill, 2) Big Stone, 3) Fowlers, 4) Kitts Hummock, 5) North Bowers, 6) Prime Hook, 7) Slaughter, and 8) Woodland. New Jersey beaches are labeled 9) Fortescue, 10) Highs, 11) Kimbles, 12) South Cape Shore Lab, 13) North Cape May, 14) Raybins, 15) Reeds, and 16) Sea Breeze.

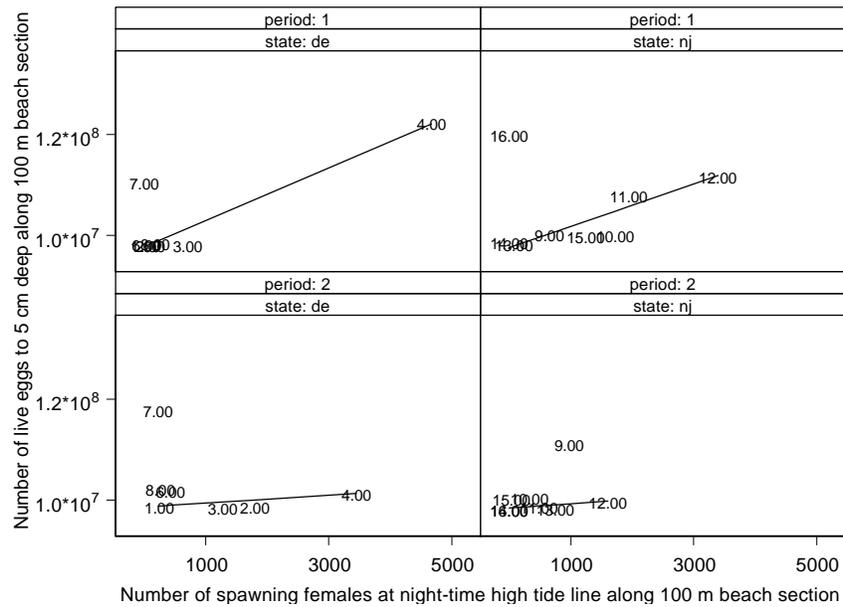


Figure 2 - 2. Relationship between eggs 0 to 5 cm deep and number of spawning females at nighttime high tide line during the 15 d prior to egg sampling on Delaware Bay beaches at 2 time periods in 1999. For the first time period eggs were sampled May 24th – 26th, and spawning was surveyed during the May 10th – 23rd. For the second time period, eggs were sampled June 14th – 15th, and spawning was surveyed during May 31st – June 13th.

Delaware beaches are labeled 1) Broadkill, 2) Big Stone, 3) Fowlers, 4) Kitts Hummock, 5) North Bowers, 6) Prime Hook, 7) Slaughter, and 8) Woodland. New Jersey beaches are labeled 9) Fortescue, 10) Highs, 11) Kimbles, 12) South Cape Shore Lab, 13) North Cape May, 14) Raybins, 15) Reeds, and 16) Sea Breeze.

Forty three % of variation in buried eggs (0 – 20 cm deep) was explained by the interaction of spawning females and beach width according to results from regression-tree modeling (percent reduction in error [PRE] = 43%; PRE is equivalent to the R^2 statistic in linear regression). Other variables did not explain variation in eggs beyond what was explained by spawning females and beach width. The regression-tree model indicated that high spawning activity (number of spawning females along 100 m > 1440 at nighttime high tide line during 15 d before egg sampling) was associated with high numbers of eggs (mean number of eggs along 100 m = 4.4×10^9 , SD = 3.2×10^9). Among those beaches with fewer spawning females (number of spawning females along 100 m < 1440), egg density was lower (mean number of eggs along 100 m = 6.9×10^8 , SD = 1.0×10^9) on wide beaches (width > 16.5 m) and higher (mean number of eggs along 100 m = 2.8×10^9 , SD = 1.6×10^9) on narrow beaches (width < 16.5 m).

Forty five % of variation in surface eggs (0 – 5 cm deep) was explained by the interaction of beach width and time of sampling according to results from regression-tree modeling (PRE = 45%). Narrow beaches (width < 16.5 m) were associated with higher density of surface eggs (mean number of eggs along 100 m = 6.5×10^7 , SD = 5.8×10^7). Density of surface eggs was lower (mean number of eggs along 100 m = 9.0×10^6 , SD = 1.1×10^7) on wider beaches (width > 16.5 m).

There was a tendency for New Jersey beaches to be more narrow and steep than Delaware beaches (Fig. 2-3). Thus, effects due to beach width and slope are confounded, at least in the extremes, by state. An effect of beach width must be interpreted as an effect of both width and slope because narrow beaches tended also to be steep ($r = -0.62$).

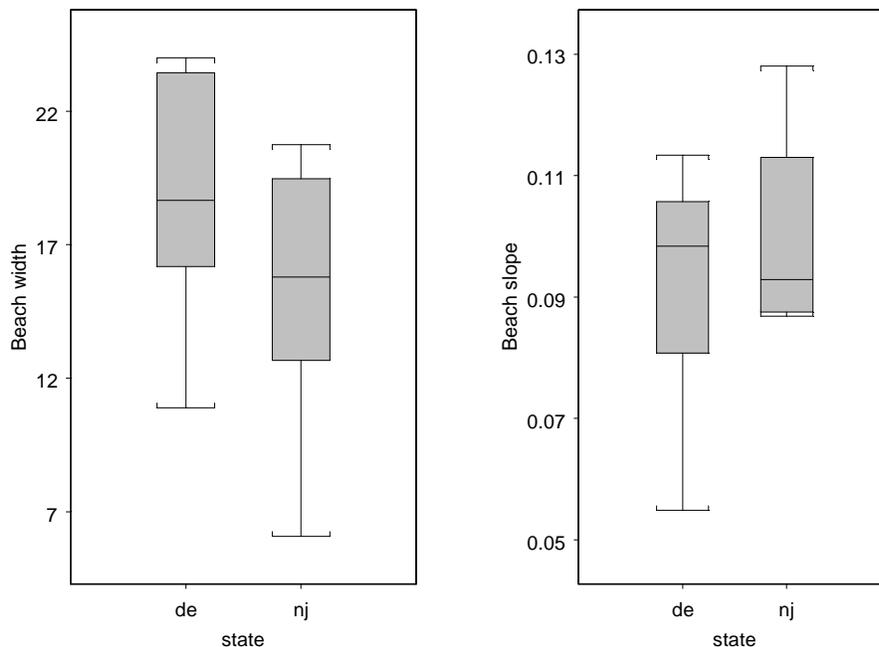


Figure 2 - 3. Box plots (showing quartiles, min and max) of beach widths and slopes for Delaware Bay beaches that were sampled for eggs in 1999.

Occurrence of egg clusters

Occurrence of egg clusters was associated with buried eggs (0 to 20 cm deep) on New Jersey beaches, but not on Delaware beaches (Table 2, Fig. 2-4).

Table 2-2. Spearman rank correlations and p-values for relationships between egg clusters and eggs 0 to 20 cm deep.

Time of egg sampling	Delaware	New Jersey
May 25 -- 26	0.3416 (0.4076)	0.7381 (0.0366)
June 14 -- 15	0.2156 (0.6081)	0.8469 (0.0162)

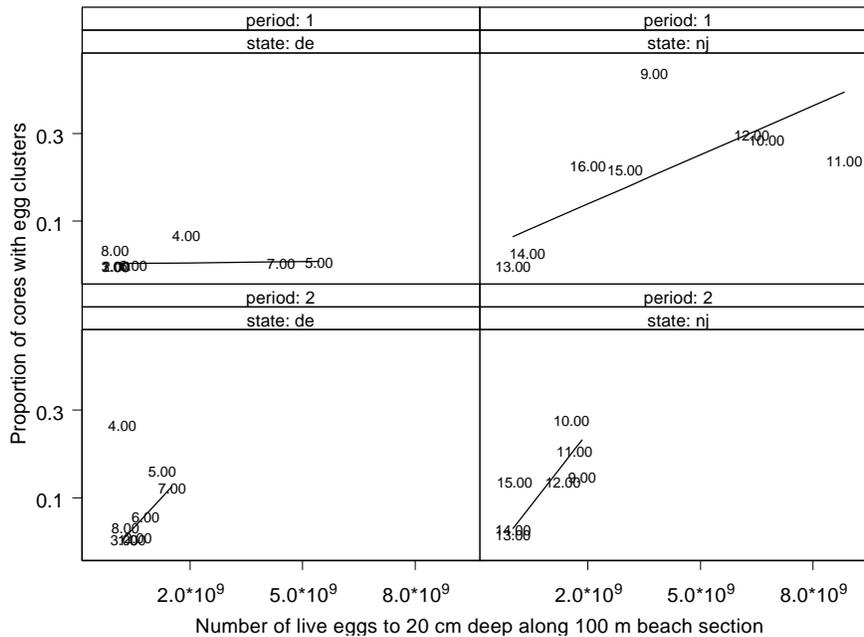


Figure 2 - 4. Relationship between occurrence of egg clusters and egg density on Delaware Bay beaches at 2 time periods in 1999. Eggs were sampled May 24th – 26th (period 1) and June 14th – 15th (period 2). Delaware beaches are labeled 1) Broadkill, 2) Big Stone, 3) Fowlers, 4) Kitts Hummock, 5) North Bowers, 6) Prime Hook, 7) Slaughter, and 8) Woodland. New Jersey beaches are labeled 9) Fortescue, 10) Highs, 11) Kimbles, 12) South Cape Shore Lab, 13) North Cape May, 14) Raybins, 15) Reeds, 16) Sea Breeze, and 17) Gandys.

Similar to egg density, we found no strong relationships between occurrence of egg clusters and spawning activity (Table 3 and Fig. 2-5).

Table 2-3. Spearman rank correlations and p-values for relationships between egg clusters and spawning activity.

Time of egg sampling	Delaware	New Jersey
May 17 -- 20	0.3366 (0.4604)	0.5714 (0.1390)
June 1 -- 3	0.4671 (0.2433)	0.4144 (0.3553)

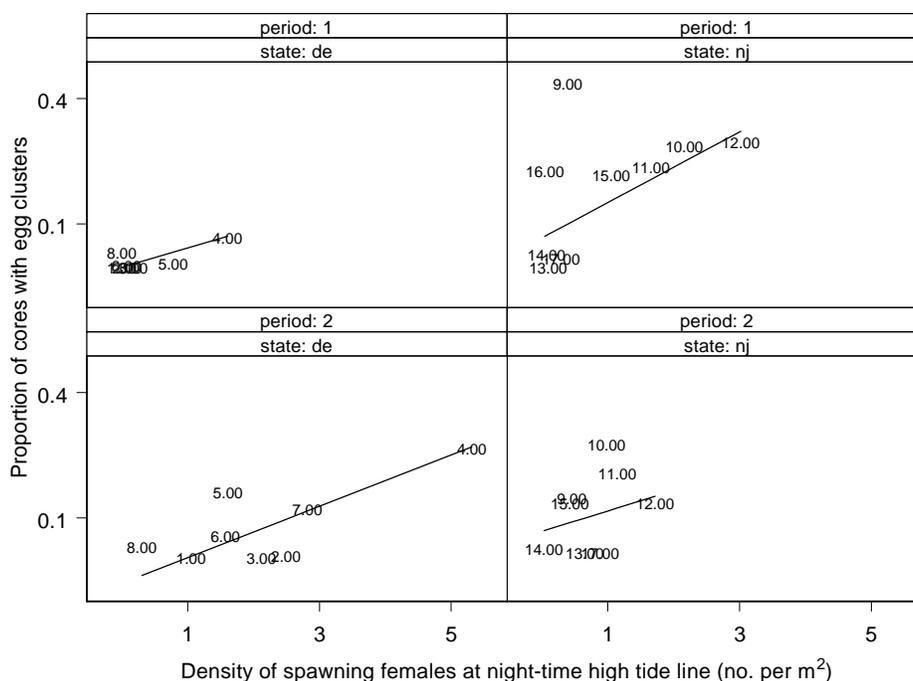


Figure 2 - 5. Relationships between egg clusters and spawning activity on Delaware Bay beaches in Delaware and New Jersey at 2 time periods in 1999. For the first time period egg clusters were sampled May 17th – 20th, and spawning was surveyed during the May 13th – 17th. For the second time period, egg clusters were sampled June 1st – 3rd, and spawning was surveyed during May 28th – June 1st. Delaware beaches are labeled 1) Broadkill, 2) Big Stone, 3) Fowlers, 4) Kitts Hummock, 5) North Bowers, 6) Prime Hook, 7) Slaughter, and 8) Woodland. New Jersey beaches are labeled 9) Fortescue, 10) Highs, 11) Kimbles, 12) South Cape Shore Lab, 13) North Cape May, 14) Raybins, 15) Reeds, and 16) Sea Breeze.

Table 2-4. Proportion of cores with egg clusters on 16 Delaware Bay beaches. Number of cores is in parentheses. The 1st period of sampling occurred May 17th – 20th and the 2nd period occurred June 1st – 3rd. Spatial extent of the sampling differed between the periods of sampling. Sampling was 0 – 6 m from the spring wrack line during 1st period and 1 – 9 m during the 2nd period.

State	Beach	1 st period	2 nd period	Length of beach (m)
NJ	No. Cape May	.00 (310)	.02 (310)	1000
	So. Cape Shore Lab	.30 (300)	.14 (400)	1000
	Highs	.29 (300)	.28 (400)	500
	Kimbles	.24 (300)	.21 (400)	800
	Reeds	.22 (300)	.14 (400)	1000
	Raybins	.03 (350)	.03 (288)	200
	Fortesque	.44 (300)	.15 (388)	1000
	Gandys	.02 (282)	.09 (396)	400
	Sea Breeze	.23 (173)	-	100
DE	Broadkill	.00 (295)	.008 (399)	1000
	Primehook	.003 (300)	.06 (400)	1000
	Fowler	.00 (300)	.008 (398)	1000
	Slaughter	.007 (300)	.126 (398)	1000
	Bowers	.01 (299)	.165 (400)	1000
	Big Stone	.00 (289)	.013 (391)	1000
	Kitts Hummock	.07 (300)	.27 (397)	1000
	Woodland	.036 (300)	.034 (383)	400

Spatial distribution of eggs

Occurrence of egg clusters across the beach profile showed geographic (DE vs NJ) and temporal (first vs second sampling period) differences in spatial distribution of eggs (Fig. 2-6). During mid-May just after the peak spawning on New Jersey beaches, distinct peaks in egg cluster occurrence were observed within 6 m from the spring wrack line on 5 of 8 beaches. During the second sampling period (early-June) on New Jersey beaches, egg cluster occurrence was more evenly distributed across 1 to 9 m of the beach profile. On Delaware beaches during the first sampling period, egg clusters occurred infrequently, which was consistent with low spawning activity in mid-May. During the second sampling period on Delaware beaches, maximum occurrence was between 7 to 9 m from the spring wrack line on 6 of 8 beaches. Average location of egg clusters was closer to the spring wrack line on New Jersey beaches than on Delaware beaches ($p < 0.001$; Fig. 2-7).

Results from a pilot study conducted by Dr. Richard Weber and David Carter (Delaware Department of Natural Resources) to examine distribution of eggs across the beach profile at 2 Delaware beaches (Ted Harvey and North Bowers) offer further evidence of large-scale variation in egg distribution. At each beach, trenches (9 at North Bowers and 10 at Ted Harvey) were dug perpendicular to the beach break, and location and number of egg clusters were recorded. There was no difference in number of clusters per transect between the 2 beaches ($p = 0.74$). However, the position of the eggs relative to the nocturnal wrack line and the spread of the eggs did differ ($p < 0.0001$ and $p = 0.0006$, respectively). On Ted Harvey eggs were closer to the nocturnal wrack line and less spread across the foreshore. The differences were meaningful. At Ted Harvey approximately 2/3 of the eggs were between 0.3 to 2.75 m from the nocturnal wrack line. However, at North Bowers 2/3 of the eggs were between 1.5 to 4.5 m from the nocturnal wrack line. These data also show that results (egg densities) depend on where sampling occurs (Table 2-5). For example, if on Ted Harvey beach a 3 m strip was centered 1.5 m above the center of the egg distribution (offset = -1.5), then the strip would cover 49% of the eggs. In contrast, if on

North Bowers beach a 3 m strip was centered 1.5 m below the center of the egg distribution (offset = 1.5), then the strip would cover 77% of the eggs. In each case the strip was misplaced by only 1.5 m however the net result is that 36% less eggs would have been available for sampling at Ted Harvey than at North Bowers. Use of a wider strip to sample eggs would be more robust. For example, a 6 m strip offset in either direction by 1.5 m would still cover >90% of the eggs at both beaches.

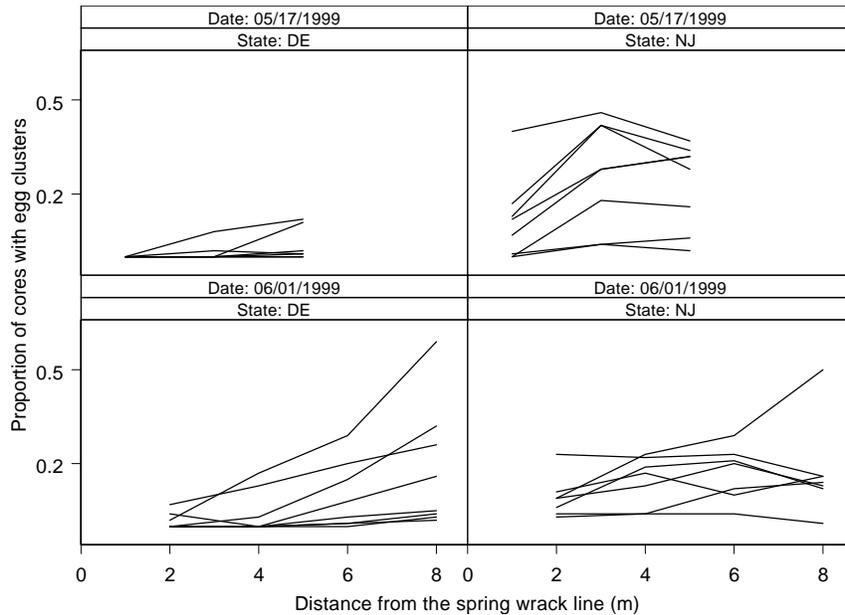


Figure 2 - 6. Occurrence of egg clusters across the beach profile on Delaware Bay beaches sampled for eggs in 1999. Plot shows proportion of cores with egg clusters at mid-point of 2 m intervals. Each line is from a different beach. During the first period of sampling (beginning 5/17/99), sediment cores were sampled across a distance of 0 to 6 m from the spring wrack line. During the second period (beginning 6/1/99), cores were sampled 1 to 9 m from the spring wrack line.

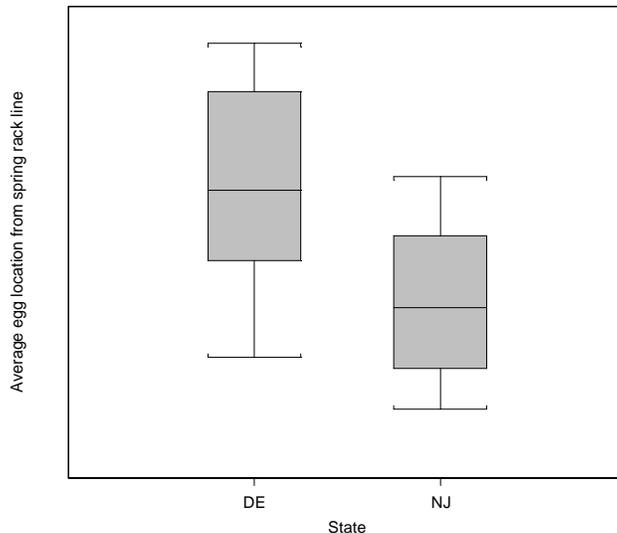


Figure 2 - 7. Box plots of the average location where egg clusters occurred among Delaware Bay beaches sampled in 1999. During the first period of sampling (beginning 5/17/99), sediment cores were sampled across a distance of 0 to 6 m from the spring wrack line.

During the second period (beginning 6/1/99), cores were sampled 1 to 9 m from the spring wrack line.

Table 2-5. Percent of eggs that would be sampled in 3 m and 6 m wide strips at 2 Delaware beaches based on egg distribution data collected in May 2000. The % depends on the offset of the strip from central location of the eggs and strip width.

Offset from central location (m)	% of eggs in a 3 m strip		% of eggs in a 6 m strip	
	Ted Harvey	North Bowers	Ted Harvey	North Bowers
-1.8	39	23	90	86
-1.5	49	32	94	91
-1.2	58	46	98	95
-0.9	68	58	100	97
-0.6	77	66	100	97
-0.3	86	75	100	99
0.0	88	84	100	100
0.3	89	88	100	100
0.6	92	87	100	100
0.9	82	85	100	100
1.2	70	84	99	99
1.5	61	77	98	98
1.8	51	68	95	96

Discussion

A strong, predictive relationship between indices of horseshoe crab spawning and eggs was observed, but only for New Jersey beaches in May after the first significant spawn of the year and for eggs 0 – 20 cm deep. We suggest different explanations for the failure to observe strong relationships in Delaware, during late-season sampling, and for eggs 0 – 5 cm deep. We believe a strong relationship was not observed on Delaware beaches because the sampling protocol failed to account for variation in the distribution of eggs across the foreshore. Also, late-season sampling (i.e., June sampling) introduced a temporal mismatch between numbers of spawning females and live, unhatched eggs. Finally, we hypothesize that density of eggs 0 – 5 cm deep was determined by a complex relationship involving an interaction between physical factors (i.e., beach morphology and wave energy) and density of spawning females.

Our results indicated that the proportion of eggs within a 3 m strip varied among beaches, and the proportion might have been lower on Delaware beaches, which could explain, in part, why weak relationships were observed on those beaches. Beach width was associated with egg density – the narrow beaches tended to have a higher density of eggs on beaches with similar (low to moderate) levels of spawning. (Beach width and slope tended to be inversely related [$r = -0.62$], thus beaches tended to be narrow and steep, wide and low-sloped, or have intermediate width and slope.) We suspect that it was not the overall density of eggs that was affected by beach width and slope. Rather, beach width and slope affected the proportion of eggs on a beach that were available to be sampled (i.e., the proportion that was covered by the 3 m wide sampling strip). Beach slopes ranged from 3° to 7°. The difference between semidiurnal tides is maximal at new and full moons when the majority of horseshoe crabs spawn (Barlow et al. 1986), and that difference covers more of the foreshore on low-sloped beaches. For example, a tidal fluctuation of 0.3 m translates to a coverage of 5.7 m on a beach with a 3° slope and 2.5 m on a beach with a 7° slope (distance covered = tidal fluctuation/sin[slope]). Thus, eggs are likely to be distributed in a tighter pattern across the foreshore of narrow, steep beaches, and a 3 m strip would cover a higher proportion of those eggs. New Jersey beaches tended to be more narrow and steep than

beaches in Delaware. Also, egg clusters sampled across 8 – 9 m of the upper foreshore exhibited greater spread and indicated that the center of the distribution of eggs was located further from the wrack line on Delaware beaches.

The proportion of eggs subject to sampling on a beach is important because it will affect the apparent relationship between spawning females and deposited eggs. Suppose there is a true regression parameter (\mathbf{b}) describing the slope between spawning females and eggs. Let the proportion of eggs covered by the sampling strip be denoted by I_1 for narrow/steep beaches and I_2 for wide/low-sloped beaches. Then, the expected regression slope would be $\hat{\mathbf{b}}_1 = I_1 \mathbf{b}$ for narrow/steep beaches and $\hat{\mathbf{b}}_2 = I_2 \mathbf{b}$ for wide/low-sloped beaches. The difference in the 2 slopes would be $(I_2 - I_1) \mathbf{b}$, and the % difference would be $(I_2 - I_1)/I_1$. For example, the regression slope for wide/low-sloped beaches would be 33% lower than on narrow/steep beaches if a 3 m strip covered 75% of the eggs on narrow/steep beaches, but only 50% on wide/low-sloped beaches. All else being equal, there would be an equal % difference in the strength of the apparent relationship as measured by a correlation coefficient. We suggest this illustrates one possible reason why the relationships appeared stronger in New Jersey, where beaches tended to be more narrow and steep.

The relationship between spawning and eggs is likely to change as the spawning season progresses. Counts of spawning females were summarized and totals estimated for the 15 d period prior to egg sampling because of development to larval stage (Brockman 1990). The majority of spawning on New Jersey beaches occurred around the new moon in May (i.e., May 15th), and the first period of egg sampling began 9 d after. However, on Delaware beaches the majority of spawning occurred around the full moon in May (i.e., May 30th), and the second period of egg sampling began 16 d after. So, it is possible that development of eggs or loss due to sediment transport contributed to weak relationships for the second period of sampling.

We hypothesize that the quantity of eggs available to shorebirds (i.e., eggs in the top 5 cm of sediment) is determined by a complex relationship between physical factors and density of spawning females. Horseshoe crabs deposit their eggs 10 – 20 cm below the beach surface and out of reach of shorebirds (Botton et al. 1992, Loveland et al. 1996). Sediment disturbance, activation, and mixing are required to bring the eggs to the surface and make them available to foraging shorebirds. Kraueter and Fegley (1994) demonstrated that horseshoe crabs disturb sediment to typical depths of 11.1 cm and, in certain beach habitat, to 17.7 cm. As a result, we would expect eggs to be brought to the surface by the burrowing and nesting behavior of spawning females if their density is sufficiently high (i.e., density is above a threshold). However, below that threshold, at low or moderate densities of spawning females, we expect wave action to be primarily responsible for mixing sediment and bringing eggs to the surface. Jackson and Nordstrom (1993) observed sediment activation to 15 cm for wave heights of 0.5 m, and they confirmed that depths of sediment activation are greater for steeper beaches. Thus, high-energy events are sufficient to bring eggs to the surface, and beach characteristics (e.g., slope) are likely to interact with both wave energy and density of spawning females to determine forage biomass for migrating shorebirds.

Implications for monitoring and recommendations for future research

Because the relationship between indices of spawning horseshoe crabs and deposited eggs depends on temporal and geographic variation, indices of egg density cannot be used to predict spawning biomass. The reverse also holds, that egg density cannot be predicted reliably from the results of a spawning survey. As an example, consider the one strong relationship that we observed on New Jersey beaches from the first period of sampling. Shuster and Botton (1985)

reported that in Delaware Bay a typical nest contains 2 or 3 clutches with 3,650 eggs per clutch. Thus, 11,000 eggs per female could be used to predict eggs from an estimate of spawning females. However, observed eggs averaged 3,000 times greater than predicted eggs for New Jersey beaches from the first period of sampling. The spawning survey was designed to estimate an index or relative abundance of spawning females useful for monitoring change over time (Chapter 1), but it seems implausible that the relative abundance would be 1/3,000 of the true abundance. Presence of a spawning female does not guarantee a fixed amount of eggs deposited in beach sediment, and there is uncertainty about deposited eggs per female. Brockman (1990) found considerable variation in eggs per female reporting nests at 80% of the locations where females paused 5 min or more, nests with 1 – 15 clutches of eggs, and clutches with 50 – 7750 eggs. Some of the discrepancy between observed and predicted eggs (using the 11,000 eggs per spawning female) could be due to an underestimate of eggs per female, and further research is needed to determine variation in fecundity. Nevertheless, it is clear that predicting deposited eggs from an index of mature females, as was done by Castro and Myers (1993), leads to a gross underestimate given existing data and knowledge.

If managers decide that horseshoe crab eggs should be monitored - in particular if shorebird forage biomass is to be monitored - then a survey must be designed specifically for sampling eggs. Protocol for sampling horseshoe crab eggs needs to be considered. Protocol to identify the center of egg distribution needs to be studied across a range of beach types. Sampling over a 3 m strip is unlikely to be robust to errors in locating the strip relative to distribution eggs across the foreshore. The sampling strip needs to be wider, perhaps as wide as 6 m. Sample size and power calculations for monitoring eggs are needed (and reported in Chapter 3). Also, further research is needed to determine the extent to which certain beach characteristics (e.g., slope and sediment type) make it more likely that eggs become available to shorebirds. Such information could be helpful in identifying and preserving critical shorebird foraging habitat.

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Chapter 3 – Assessment of Horseshoe Crab (Limulus polyphemus) Egg Sampling Methods and Sample Size Determination

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Introduction

In this chapter we examine Horseshoe crab (Limulus polyphemus) egg data (presented in Chapter 2) to assess adequacy of egg sampling procedures. We structure the assessment by 3 specific questions, each addresses sampling at a different spatial scale.

- 1) How many sediment cores should be sampled if the objective is to monitor density within a 100 m stretch of beach?
- 2) Does egg density within a 100 m section of beach adequately represent egg density across the larger stretch of beach surveyed for spawning activity?
- 3) How many beaches should be sampled if the objective is to monitor bay-wide egg density?

We chose these 3 questions because conclusions about egg density might be made at any of these scales. We ask the first question to assess if current sampling effort (40 cores per 3 m by 100 m beach strip) is sufficient to detect beach-strip level changes in density over time. The second question allows us to examine the reliability of using egg density in a 100 m beach-strip 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 reliability of egg density estimates at multiple scales will help determine future monitoring efforts.

We addressed all three questions with respect to both shallow (0 – 5 cm) and deep (0 – 20 cm) cores. Horseshoe crabs are generally thought to lay most of their eggs at a depth of 15 – 20 cm (Brockman 1990, Botton et al. 1994). So, if egg production were of primary interest then

sediment to 20 cm would be sampled. However, if estimating food availability for migrating shorebirds is the goal, then we would look specifically at egg density in shallow (0 – 5 cm deep) sediments (Botton et al. 1994).

Methods

Details of how core samples were collected are described in Chapter 2. We describe the analyses in sections separately for each of the 3 questions.

Question 1: How many cores should be sampled per 100 m beach section?

We addressed this question in 2 steps. First, we determined precision of egg density estimates as a function of density and sample size. Second, we translated precision into power to detect change in egg density over time. For simplicity, density and variance at this level were calculated as if cores were a random sample from an infinite population. Coefficient of variation (CV) was calculated as: $CV = \sqrt{\text{var}(y)/n} / \bar{y}$, where $\text{var}(y)$ is variance of egg density among cores and \bar{y} is average egg density. The observed relationship between egg density and variance was used to predict coefficient of variation (CV) for different sample sizes and across the range of observed densities. Using predicted CV's, we estimated the probability of detecting a percentage change in density over time. Probability of detecting decline (i.e., statistical power) was calculated using a 1-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} using program TRENDS (Gerrodette 1993).

Question 2: Is a 100 m section representative of a larger stretch of beach?

We partitioned the spawning survey area on each beach into 100 m segments and used the observed relationship between spawning and egg densities to predict egg densities for each of the 100 m sections within each beach. The predictive relationship was built using data from New Jersey beaches during the first period of sampling (May 10 – May 26), which was the only state and sampling period where we found the relationship between spawning and egg density to be discernable and reliable (see Chap. 2). We compared egg density in the observed 100 m segment to the distribution of densities predicted in all 100 m segments. If the observed density was within the inter-quartile range of the distribution of predicted densities, then we concluded that the 100 m segment was representative of the larger stretch of beach.

Question 3: How many beaches should be sampled?

Using similar procedures as those used to answer Question 1, we predicted the probability of detecting a percentage change in bay-wide egg density over time. We could not predict CV across a range of bay-wide densities because the 1999 results provided only 1 data point to work with. Thus, we examined statistical power as a function of number of beaches sampled, but used the 1999 bay-wide egg density as the initial value in the time series.

Results

Question 1: How many sample cores should be selected per beach?

The sample size of 40 sediment cores per 3 m by 100 m strip is sufficient for detecting substantial changes in egg density over time when looking at the 0 – 20 cm depth but > 40 cores

would be needed for monitoring egg density in the top 5 cm of beach sediment. Median egg density in shallow sediment was 3 per core (core area is $\sim 20 \text{ cm}^2$), and CV at that density was 0.48 when 40 cores were sampled (Fig. 3-1A). In contrast, 40 cores resulted in a CV of 0.26 for median density of eggs 0 – 20 cm deep (275 per core; Fig. 3-1B). Distributions of egg densities were skewed right (Fig. 3-2). A CV of 0.3 corresponds to a 75% chance of detecting a 50% decline in egg density over 5 years and an 80% chance of detecting a 40% decline over 10 years (Fig. 3-3).

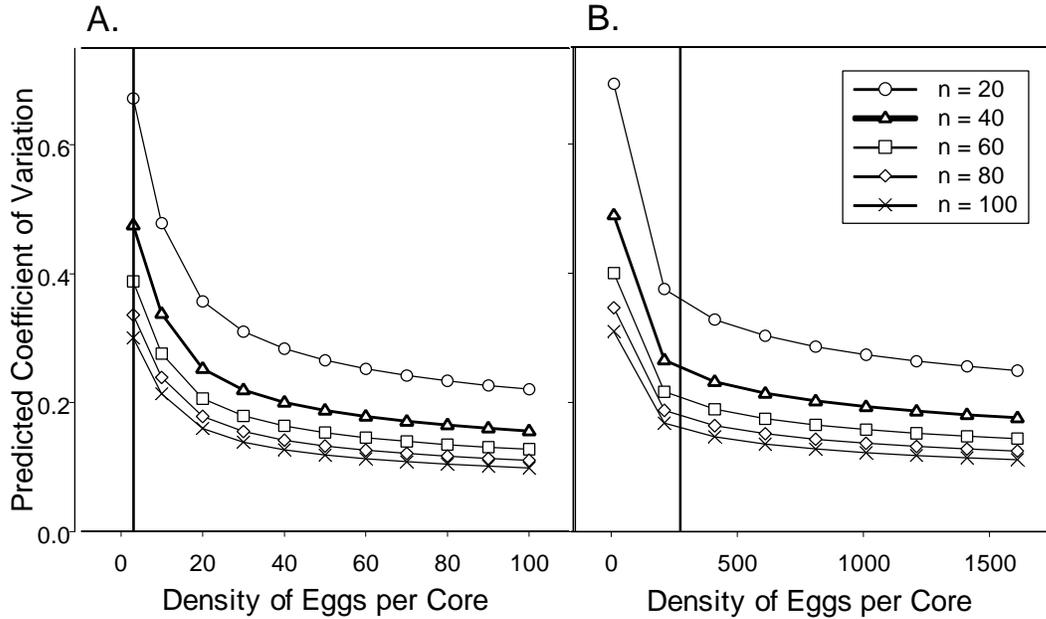


Figure 3-1. The relationship between density and CV for A. shallow sediment (0 – 5 cm deep) and B. deep sediment (0 – 20 cm deep). Lines in each figure refer to different sample sizes. Median egg density is represented by a vertical line.

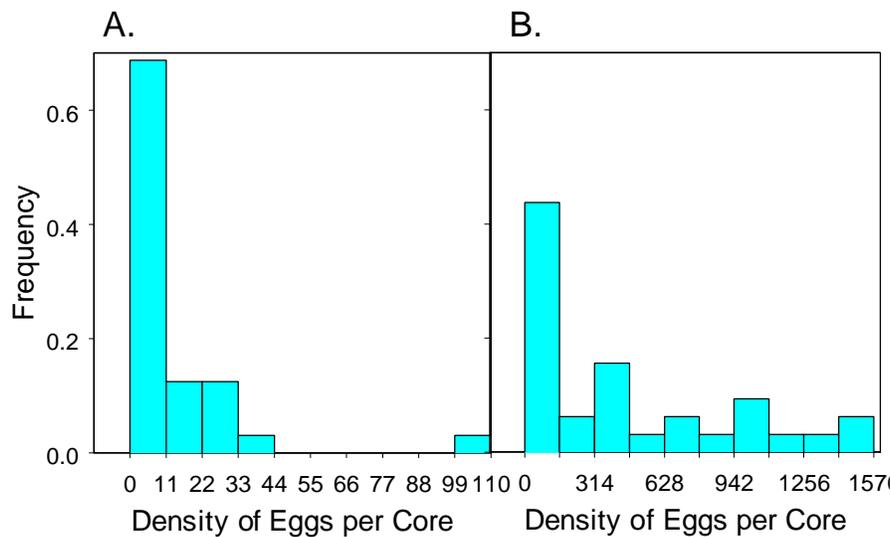


Figure 3-2. The distribution of egg density for A. shallow sediment (0 – 5 cm deep) and B. deep sediment (0 – 20 cm deep) in 100 m beach segments sampled in 1999.

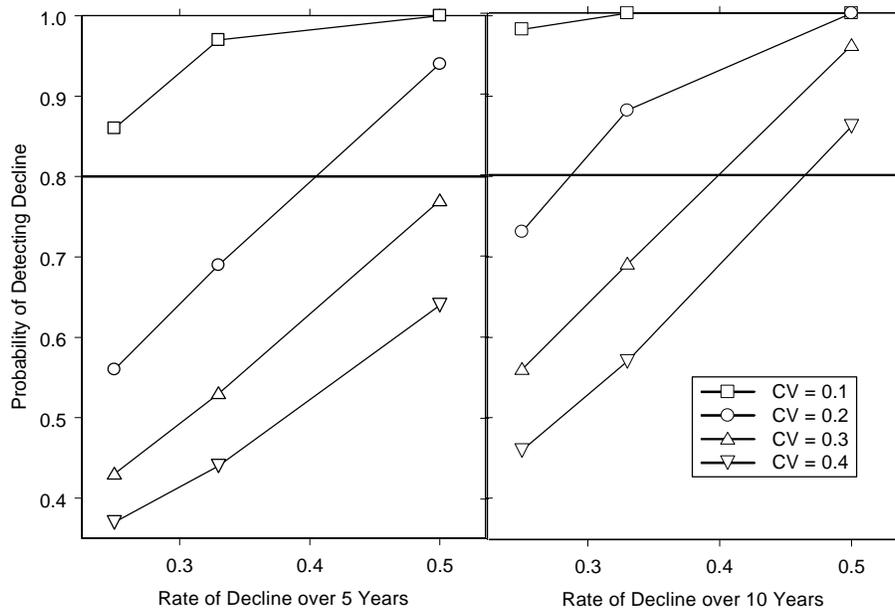


Figure 3-3. Probability of detecting a decline (i.e., statistical power) for various magnitudes of decline and for annual surveys over 5 and 10 years. Statistical power was calculated for a 1 tailed t-test with a Type 1 error rate of 0.2, and a constant annual rate of change.

Question 2: How should core samples be located on the beach?

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 2 of the 6 New Jersey beaches examined (South Cape Shore Lab and Reeds) did the observed egg density fall within the inter-quartile range of beach-wide densities (Figure 3-4). On 3 beaches the observed egg density was greater than all predicted densities, and on 1 beach observed egg density was less than all predicted densities. These results indicate that egg density estimates based on sampling a 100 m beach section are not necessarily representative of the surrounding beach. Note that we limited this comparison to New Jersey in the first period of sampling, when the relationship between spawning and deep egg core densities was most reliable (Figure 2-1).

Question 3: How many beaches should be sampled?

With initial egg density at the 1999 level, 16 beach segments (100 m) distributed throughout the bay would be sufficient to detect substantial decreases in egg density (0 – 20 cm deep). However, greater effort would be required for monitoring egg density 0 – 5 cm deep. Also, we found that variability in egg density among beaches was greater when sampling in May than in June. The CV for densities of deep eggs was 0.26 in May and 0.29 in June. For densities of shallow eggs, the CV was 0.33 for egg densities in May and 0.43 in June. A CV of 0.3 indicates that there is about a 75% chance of detecting a 50% decline in egg density over 5 years and an 80% chance of detecting a 40% decline over 10 years (Figure 3-3).

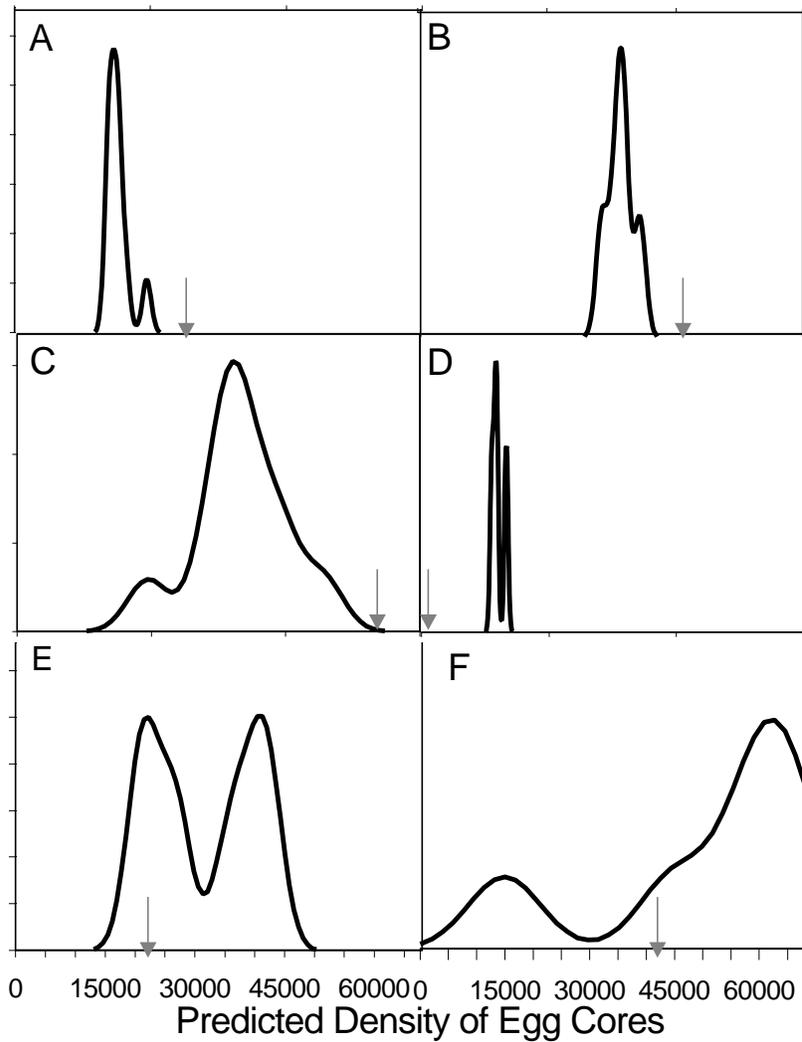


Figure 3-4. Density curves of predicted egg densities on 100 m beach segments at 6 New Jersey beaches. An arrow represents the observed egg density at each beach. These density curves were generated by dividing the area sampled for spawning into 100 m segments and using the observed relationship between egg densities and spawning to predict egg density for each segment based on the level of spawning. The beaches shown are (A) Fortescue, (B) High's, (C) Kimbles, (D) North Cape May, (E) Reeds, and (F) South Cape Shore Lab.

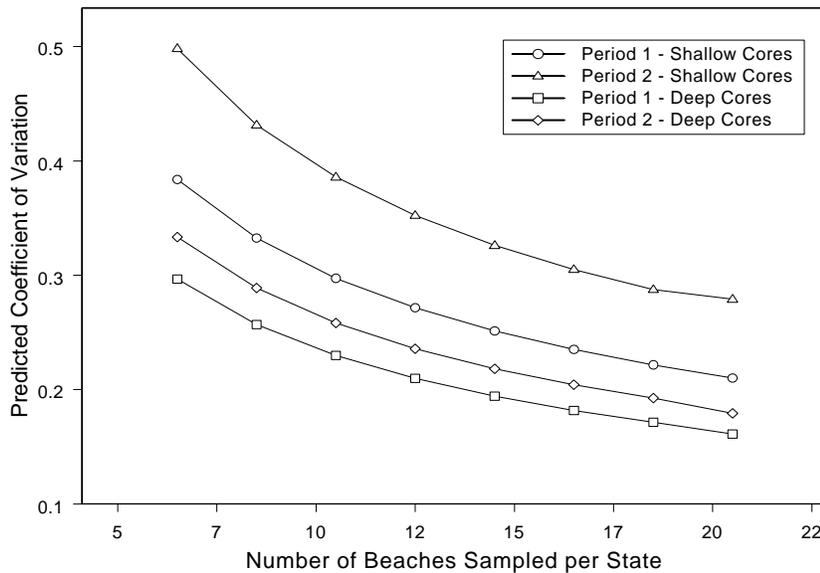


Figure 3-5. Predicted CV shown for the possible range of number of beaches sampled per state. This figure is based on the observed levels of bay wide density during the two sampling periods in 1999.

Discussion

Question 1: How many sample cores should be selected per beach?

If we are only interested in estimating density within a 100 m strip, then a sample size of 40 sediment cores is sufficient for estimating egg density to 20 cm deep, but a larger sample size would be needed to estimate egg density in shallow sediment (i.e., to 5 cm deep). Increasing the sample size of shallow cores to 60 per beach section would seem to be sufficient; CV would be below 0.4 for 3.1 eggs per core, which was the median observed in 1999. A CV of 0.4 would lead to a better than 85% chance of detecting a 50% decline in density over 10 years. If the survey objective is to provide information about shorebird food availability (density of eggs 0 – 5 cm deep), we recommend a sample size of ≥ 60 sediment cores. However, if the objective is to estimate egg density to 20 cm depth then we recommend 40 sediment cores.

Question 2: How should core samples be located on the beach?

We conclude that egg density in a 100 m segment of beach is not necessarily representative of the larger surrounding beach. If reliable information about egg density at beach level is required, then it will be necessary to include the beach in the sampling scheme. Because of the logistics of sampling sediment it would be difficult to sample throughout a beach in one stage of sampling. However, a 2-stage sampling design could be considered, which would select segments of beach in the 1st stage and cores with segments in the 2nd stage. If the current protocol of sampling eggs within one 100 m segment per beach is continued, then it is important to realize that the scope of inference is strictly limited to that 1 segment.

Question 3: How many beaches should be sampled?

Consistent with our conclusions under question 1, bay-wide egg densities 0 – 20 cm deep were more precisely estimated than eggs densities 0 – 5 cm deep. A stratified random sample of 8 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 is maintained, it should be sufficient to detect substantial declines in egg density. However, we conclude that greater effort would be required to monitor change in egg densities 0 – 5 cm deep. Based on shallow egg densities in May, a stratified random sample of 10 segments per state would result in $CV \leq 0.3$. Based on the lower densities found in June, a stratified random sample of 17 segments per state would be needed to result in comparable CVs. Before increasing effort at this scale, managers must assess the importance of monitoring baywide egg density.

Throughout these analyses, data from the shallow cores consistently yielded lower densities and higher variability than the data from the deep cores. Our primary recommendation is to increase sampling effort particularly if shallow egg density is the focus.

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Appendix – Horseshoe Crab Spawning Activity in Delaware Bay (1999 – 2000): A Report to the ASMFC Horseshoe Crab Technical Committee

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The Atlantic State Marine Fisheries Commission (ASMFC) sponsored a workshop on horseshoe crab surveys in January 1999. The workshop resulted in recommendations for the design and implementation of a statistically valid survey of spawning horseshoe crabs in the mid-Atlantic region. In Delaware Bay, the recommendations were used to redesign the volunteer-based spawning survey that began in 1990. Funds were awarded from the USGS State Partnership Program in 1999 and the U.S. Fish and Wildlife Service in 2000 to implement the Delaware Bay Horseshoe Crab Spawning Survey. In 1999 and 2000, the survey, which was coordinated through a contract with Ms. Benjie Swan (Limuli Labs), involved a large volunteer workforce (> 160 participants per year) and received both local and national media coverage.

The Delaware Bay Horseshoe Crab Spawning Survey was designed to accomplish several important objectives: (1) provide a reliable index of spawning activity to monitor the temporal and spatial distribution of horseshoe crab spawning activity for comparing baywide spawning among years, beach-level spawning within Delaware Bay, and distributions of spawning horseshoe crabs and shorebirds; (2) increase our understanding of the relationship between environmental factors (tidal height and wave height) and spawning activity; and (3) promote public awareness of the central role of horseshoe crabs in shorebird population dynamics, Atlantic coast fisheries, and human health through production of Limulus ameocyte lysate (LAL).

Here we summarize the statistical analysis of results from the 1999 and 2000 Delaware Bay Horseshoe Crab Spawning Surveys. Survey protocol and methods to estimate an index of spawning activity have been described in a previous report (Smith et al. 2000). With this report, we hope to initiate an annual series of statistical reports on the survey.

In 2000, 22 beaches were surveyed on as many as 12 nights in May and June (3 nights per lunar period, where lunar period is defined as the 7-day period centered on a new or full moon). Half of the beaches were in Delaware and the other half in New Jersey. This represents an increase in the number of beaches sampled and a reduction in

number of nights sampled compared to the 1999 survey. This change was based on results from the 1999, which showed that the number of beaches sampled was the most important factor in determining the precision of the spawning index and power to detect a decline (Smith et al. 2000).

Results from the 2000 survey were similar to those from 1999 (Fig. 1, Tables 1 and 2). Spawning was slightly higher in New Jersey on the first lunar period, but then switched during the other lunar periods with Delaware showing a higher spawning index (Fig. 1). In both years, the spawning index computed for all lunar periods combined was higher in Delaware (Table 1). There was no discernable change in the spawning index between 1999 and 2000 (Table 2).

The lack of change, or apparent stability, in the spawning index between 1999 and 2000 does not support any broad conclusions about horseshoe crab population trends. Such conclusions will require a longer time series of data. We stress that two years of results do not provide an informative time series for trends in spawner numbers of iteroparous species such as horseshoe crabs. Also, a pattern of stability does not address the concern that current levels of spawning may not be sufficient to sustain horseshoe crab populations or support shorebird trophic demands. As such, we also stress the importance of continuing this survey as currently designed, which demands that annual funding be pursued and secured.

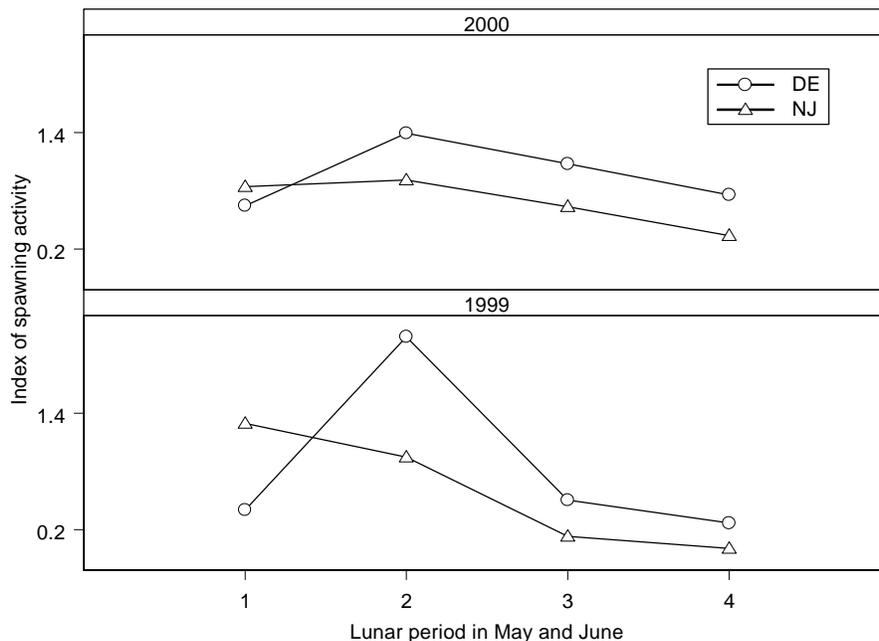


Figure 6. Index of spawning activity for Delaware Bay in each of the 4 lunar periods in May and June. The index is the number of spawning females within 1 m of high tide line on beach index sites. Surveys were conducted within 3 days of the new or full moons, and these periods were termed ‘lunar periods’. The index is shown separately for beaches in Delaware (DE) and New Jersey (NJ).

Table 1. Index of spawning activity (ISA) computed for May and June 1999 and 2000. Index is shown separately for Delaware and New Jersey.

Year	State	ISA	90% Confidence Interval
1999	Delaware	1.0528	0.7933, 1.3971
1999	New Jersey	0.6076	0.4643, 0.7951
2000	Delaware	1.0137	0.7091, 1.4492
2000	New Jersey	0.6862	0.5521, 0.8530

Table 2. Index of spawning activity (ISA) for the Delaware Bay in 1999 and 2000. Standard error (SE) and 90% confidence intervals are also presented.

Year	ISA	SE	90% Confidence Interval
1999	0.8320	0.1039	0.6777, 1.0214
2000	0.8513	0.1196	0.6754, 1.0730

REFERENCE:

Smith, D. R., P. S. Pooler, B. L. Swan, S. Michels, W. R. Hall, P. Himchak, and M. J. Millard. Spatial and temporal distribution of horseshoe crab (Limulus polyphemus) spawning in Delaware Bay: implications for monitoring. <http://aer.usgs.gov/groups/stats/Limulus/1999/Chap1.pdf>