

Abstract—Estimates of the abundance of American horseshoe crabs (*Limulus polyphemus*) are important to determine egg production and to manage populations for the energetic needs of shorebirds that feed on horseshoe crab eggs. In 2003, over 17,500 horseshoe crabs were tagged and released throughout Delaware Bay, and recaptured crabs came from spawning surveys that were conducted during peak spawning. We used two release cohorts to test for a temporary effect of tagging on spawning behavior and we adjusted the number of releases according to relocation rates from a telemetry study. The abundance estimate was 20 million horseshoe crabs (90% confidence interval: 13–28 million), of which 6.25 million (90% CI: 4.0–8.8 million) were females. The combined harvest rate for Delaware, New Jersey, Virginia, and Maryland in 2003 was 4% (90% CI: 3–6%) of the abundance estimate. Over-wintering of adults in Delaware Bay could explain, in part, differences in estimates from ocean-trawl surveys. Based on fecundity of 88,000 eggs per female, egg production was 5.5×10^{11} (90% CI: 3.5×10^{11} , 7.7×10^{11}), but egg availability for shorebirds also depended on overlap between horseshoe crab and shorebird migrations, density-dependent bioturbation, and wave-mediated vertical transport.

Manuscript submitted 29 March 2005
to the Scientific Editor.

Manuscript approved for publication
21 October 2005 by the Scientific Editor.
Fish. Bull. 104:456–464 (2006).

Abundance of adult horseshoe crabs (*Limulus polyphemus*) in Delaware Bay estimated from a bay-wide mark-recapture study

David R. Smith¹

Michael J. Millard²

Sheila Eyler³

¹ United States Geological Survey Leetown Science Center
11649 Leetown Road
Kearneysville, West Virginia 25443
E-mail address (for D. R. Smith): drsmith@usgs.gov

² U.S. Fish and Wildlife Service
Northeast Fishery Center
308 Washington Avenue
Lamar, Pennsylvania 16848

³ U.S. Fish and Wildlife Service
Maryland Fisheries Resources Office
177 Admiral Cochrane Drive
Annapolis, Maryland 21401

The American horseshoe crab (*Limulus polyphemus*), having persisted largely unaltered for millions of years, is now central to a modern management debate of conflicting interests by commercial watermen, birdwatchers, biopharmaceutical companies, and eco-tourists (Odell et al., 2005). The management controversy is most acute in the Delaware Bay region where the high abundance of horseshoe crabs has resulted in the dependence of migrant shorebirds on horseshoe crab eggs to fuel their northern migration to nesting grounds (Botton and Harrington, 2003).

Because management goals have linked the horseshoe crab fisheries to the viability of other species, such as migrant shorebirds (ASMFC¹), it is not sufficient to manage on the basis of typical reference points, such as maximum sustainable yield, because sustainable harvest is not the primary issue. In Delaware Bay, the viability of shorebirds has taken precedence in decision making (Botton and Harrington, 2003; Baker et al., 2004). Effective management should reference a critical threshold of horseshoe crab abundance that provides sufficient eggs and should sustain

that threshold. Thus, abundance becomes a critical parameter because abundance estimates are useful for predicting the egg biomass that is available to migrant shorebirds and for assessing harvest rate.

Horseshoe crabs bury their eggs in beach sediment, typically 15 to 20 cm deep (Shuster and Sekiguchi, 2003). Eggs are exhumed to the beach surface and become available to foraging shorebirds through a process of entrainment in activated sediment, followed by vertical transport into surface sediments. Nest disturbance, which precedes entrainment of eggs, is predominantly due to bioturbation; whereas wave energy is only a contributing factor because typical estuarine waves do reach nest depth (Jackson et al., 2005). Given a relationship between egg exhumation and spawning density, egg availability could be predicted from current and projected horseshoe crab abundance.

¹ ASMFC (Atlantic States Marine Fisheries Commission). 1998. Interstate fishery management plan for horseshoe crab. Fishery Management Report No. 32, 58 p. ASMFC, 1444 Eye Street, NW, Sixth Floor, Washington, DC 20005.

Thus, studies to estimate abundance are an important step in the process of managing horseshoe crabs to meet the energetic needs of shorebirds.

We used results from a bay-wide mark-recapture effort during spring 2003 to estimate horseshoe crab abundance. Underlying assumptions of mark-recapture methods were accounted for in our study design and were evaluated during analysis. Recapture effort was distributed over Delaware Bay beaches by involving participants of a bay-wide spawning survey. We related our abundance estimates to reported landings to assess the recent harvest rate and used published fecundity estimates to predict egg production.

Materials and methods

We captured, tagged, and released horseshoe crabs from boats during two periods in 2003: a prespawning-season period (abbreviated to “preseason” in this article) from 26 March to 8 May and a prepeak-spawning period (abbreviated to “prepeak”) from 28 to 30 May 2003. The numbers of crabs tagged were 7221 and 10,322 during the preseason period and prepeak period, respectively. The target population was adult horseshoe crabs that were present in Delaware Bay to spawn. One boat fished throughout the bay during the preseason period, and three boats fished nearshore during the prepeak period (Fig. 1). Prepeak period captures took place within strata of equal length along the Delaware Bay shoreline (Fig. 1). During the prepeak period, two boats fished in New Jersey, but only one boat fished in Delaware because of a lack of funding. Because of the additional boat in New Jersey, fishing effort and the number of crabs tagged were higher in New Jersey. Any animal injured during capture was culled and not tagged. Adult males and females were tagged with standard button tags. Tags were 4.4 cm in diameter, bore a unique tag number and carried instructions on the tag for reporting a captured tag. Further detail on tags and tagging methods are described in Brousseau et al. (2004).

Recaptured tags came from the Delaware Bay spawning survey during the peak period of spawning (29 May, 31 May, and 2 June 2003). Although the spawning survey was conducted during spring tides in May and June, the recapture period for abundance estimation was limited to the peak spawning period to help satisfy the assumption of population-closure during the time of mark-recapture study (Borchers et al., 2002). Spawning survey volunteers were instructed to count all horse-

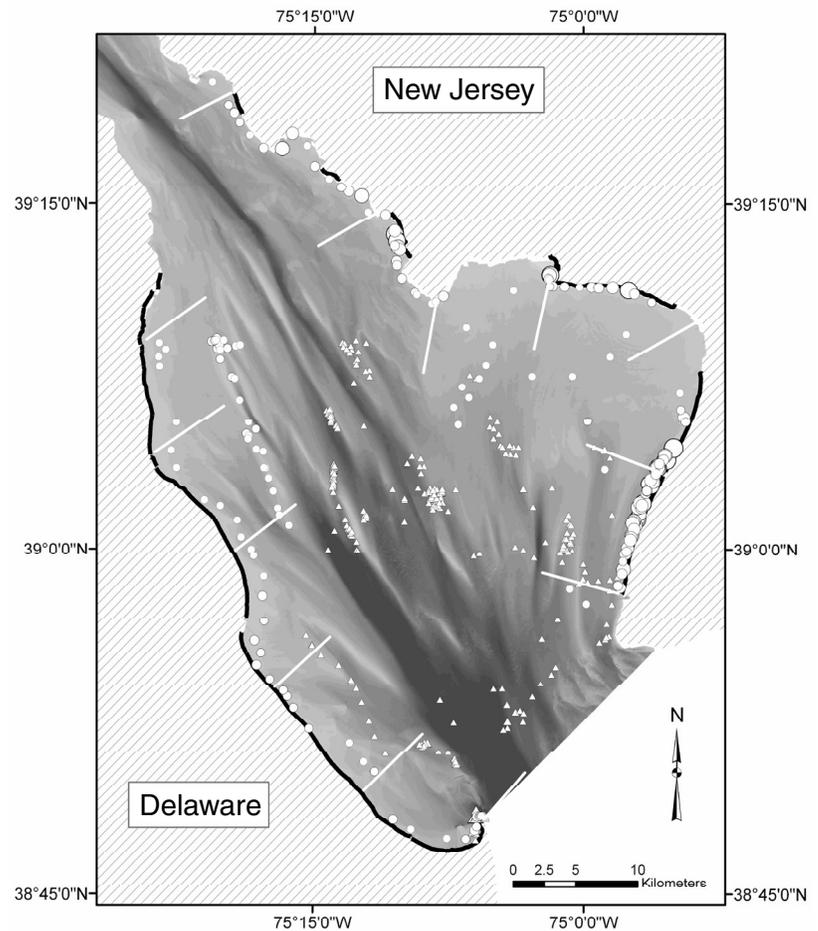


Figure 1

Locations in Delaware Bay where horseshoe crabs (*Limulus polyphemus*) were tagged and released. Size of symbols are graduated to reflect size of catches, and symbol style reflects whether releases occurred during the prespawning season period (26 March to 8 May 2003; triangle) or prepeak-spawning period (28–30 May 2003; circle). White lines perpendicular to the shoreline indicate strata within which catch effort during prepeak-spawning period was equally distributed. Black lines along the shore indicate approximate locations of spawning areas.

shoe crabs in sample quadrats and record tags that they encountered inside and outside of the sample quadrats. Flashlights were used when the survey occurred after dark. During the spring tide period around the new moon (29 May to 2 June 2003), 23 beaches were surveyed throughout Delaware Bay (Smith and Bennett²;

² Smith, D. R., and S. Bennett. 2004. Horseshoe crab spawning activity in Delaware Bay: 5 years of a standardized and statistically-robust survey, 10 p. Report to the ASMFC Horseshoe Crab Management Board, Atlantic States Marine Fisheries Commission, Washington DC. Website: <http://www.lsc.usgs.gov/aeb/2065/isa%20report%2005.pdf> [accessed on 21 October 2005].

Fig. 1). The design of the spawning survey is described in Smith et al. (2002).

Mark-recapture methods

We took two approaches to estimate abundance. The first was an application of Chapman's modification of the Petersen estimator (Borchers et al., 2002). We applied the Petersen estimator separately for each of the two release periods and the three spawning survey dates. In addition, we combined the releases and recaptures for a pooled Petersen estimate.

The second approach was based on an extension of a likelihood presented by Borchers et al. (2002: p. 118, Eq. 6.11). We extended the likelihood to allow for a temporary effect on spawning behavior due to the capture and tagging process by including separate recapture probabilities for each release cohort. The extended likelihood was

$$L_s^* = \prod_{s=1}^3 \binom{N - M_{\bullet s}}{u_s} p_s^{u_s} (1 - p_s)^{N - M_{\bullet s} - u_s} \binom{M_{1s}}{m_{1s}} p_s^{m_{1s}} (1 - p_s)^{M_{1s} - m_{1s}} \binom{M_{2s}}{m_{2s}} (p_s^*)^{m_{2s}} (1 - p_s^*)^{M_{2s} - m_{2s}}$$

where N = the abundance at the start of the recapture period at the end of May 2003;
 M_{1s} = the number of pre-season tagged animals at large at time s ;
 M_{2s} = the number of pre-peak tagged animals at large at time s ($M_{\bullet s} = M_{1s} + M_{2s}$);
 m_{1s} and m_{2s} = the recaptures of pre-season and pre-peak tagged animals at time s ;
 u_s = the survey count of untagged at time s ;
 p_s = the capture probability for untagged and pre-season tagged animals at time s ; and
 p_s^* = the capture probability of pre-peak tagged animals at time s .

We also fitted a likelihood that set all recapture probabilities to be constant through time, i.e., $p_s = p$ for all recapture surveys, which reduces to Borchers' original likelihood (Borchers et al., 2002: p. 118, Eq. 6.11).

We used maximum likelihood methods to estimate abundance \hat{N} and recapture probabilities (\hat{p}_s, \hat{p}_s^*). We used the Petersen estimate for the initial value for \hat{N} and used m_s/M_s as the initial value for recapture probabilities. Profile-likelihood intervals were calculated for the abundance estimates (Borchers et al., 2002). MathCad (vers. 12, Mathsoft Engineering and Education Inc., Cambridge, MA) and SAS (vers. 9, SAS Institute Inc., Cary, NC) were used to find numerical solutions to the likelihood and profile-likelihood equations.

The following assumptions underlie the mark-recapture methods that we applied (Borchers et al., 2002):

- 1 No emigration or mortality occurred during the period between release and recapture;
- 2 the tagged animals represented an adequate sample;
- 3 animals were captured independently of one another;
- 4 tags were not lost or overlooked; and
- 5 recapture probability depended only on recapture occasion, was equal among animals of the same sex, and was equal for tagged and untagged animals.

The study was designed through the timing and distribution of releases and recaptures to meet the first two assumptions. The pre-peak releases and recaptures were designed to be close in time to meet the assumptions of no emigration and no mortality. Immigration occurred during the time between pre-season release and recapture; therefore we estimated the number of adults in Delaware Bay at the time of recapture, which was at the end of May in 2003 (Skalski and Robson, 1992). Some mortality occurred during the time between pre-season release and recapture that we were not able to account for; however, we expect that mortality was similar for tagged and untagged animals. We ensured that both initial capture and recaptures were spatially distributed by distributing the releases throughout Delaware Bay during pre-season tagging and within strata during pre-peak tagging effort (Fig. 1) and by distributing recapture effort systematically by means of the spawning survey (Smith et al., 2002). The third assumption could be violated if horseshoe crabs moved locally in groups. However, recaptures came from widely spaced quadrats, so that even if the animals moved in groups, the whole group was unlikely to be recaptured within single quadrats. Although tag loss could be a significant factor over an extended period, we did not believe that significant tag loss occurred, especially over the five days from pre-peak release to recapture period (28 May to 2 June 2003). In a tag-loss study conducted at the United States Geological Survey Leetown Science Center with identical tags, no tag loss over ≥ 60 days was reported, indicating that tag loss between pre-season and recapture periods would not have been significant (Crawford³). Tags could have been overlooked during the spawning survey. Females bury themselves in beach sediment during spawning, and their tags could have been readily overlooked. In contrast, males do not bury themselves and the 4.4 cm white button tag is highly visible in daylight or when illuminated by flashlight. Nevertheless, tags on males could have been obscured when the horseshoe crabs piled up during peak spawning. Thus, we restricted our mark-recapture analysis to male horseshoe crabs that were counted and recaptured within 1-m² quadrats when surveyors were focusing on a small area.

³ Crawford, E. 2003. Unpubl. data. USGS-Leetown Science Center, 11649 Leetown Road, Kearneysville, WV 25430

We limited mark-recapture estimates to males because capture probability was not equal for males and females as evidenced by recapture rates. Ratios of males and females captured for the tagging study were used to estimate total abundance with the equation

$$\hat{N} = \hat{N}_m / R_m,$$

where \hat{N}_m = abundance of males; and
 R_m = the ratio of males to the total captured.

Abundance of females was estimated by subtraction, i.e., by $\hat{N}_f = \hat{N} - \hat{N}_m$.

We used patterns of recaptures among preseason releases (released from 26 March to 8 May 2003) versus prepeak releases (released from 28 to 30 May 2003) to test the assumption that tagging did not affect spawning behavior. One approach was to use a contingency table analysis that included a comparison of risks of recapture between animals tagged during preseason and prepeak periods. Another approach was based on the extended likelihood presented above (L_s^*), which included a separate set of recapture probabilities for preseason and prepeak released animals. In this way, the model allowed for a temporary effect on capture probability due to tagging, such as a temporary delay in spawning. We used model comparison techniques (i.e., Akaike's information criteria and likelihood ratio tests) to compare recapture probabilities among preseason and prepeak released animals (Burnham and Anderson, 1998).

Results from a telemetry study conducted in 2004 provided information on spawning behavior of horseshoe crabs caught and released from boats. In 2004, we attached radio transmitters to 60 adult males throughout the Delaware Bay prior to the spawning season. When the animals came on the beach to spawn the signals from the transmitters were recognized by one or more of the 14 fixed station receivers that were arrayed along the shoreline of the bay. In this article, we report the relocation rates (proportion of radio-tagged crabs that were recognized by at least one receiver) from that study because of their relevance to the assumption that tagging does not affect spawning behavior.

An important application of abundance estimates is in the calculation of harvest rates. We calculated harvest rates by dividing estimated abundance into 1) reported landings and 2) projected landings (based on recent regulations). The population that spawns in Delaware Bay disperses widely, some leaving the bay for the ocean. Calculations of harvest rate need to account for these migration patterns because landings from New Jersey and Delaware do not include landings of Delaware Bay spawning animals that were harvested in neighboring states (Virginia and Maryland). However, landings from neighboring states include animals that spawn in embayments other than Delaware Bay. Thus, use of landings from Delaware Bay states alone could underestimate harvest rate and use of landings from Delaware Bay and neighboring states could over-

estimate harvest rate. To put bounds on the problem, we calculated harvest rate 1) using landings from New Jersey and Delaware and 2) using landings from the Delaware Bay regional states (New Jersey, Delaware, Virginia, and Maryland).

Results

A total of 5398 males and 1823 females were tagged during the preseason period (26 March to 8 May 2003), and 7091 males and 3231 females were tagged during the prepeak period (28 to 30 May 2003; Table 1). Animals that were injured during capture were culled and not tagged. Culling rates were 0.19 during the preseason period, 0.05 during the prepeak period, and 0.12 overall.

During the spawning survey 22,051 males and 6675 females were counted in quadrats and examined for tags. Forty-eight tagged animals were recaptured during the spawning season surveys conducted 29 May, 31 May, and 2 June 2003. Nineteen of the recaptures were within quadrats, and 29 were between quadrats. Only three of the recaptured tags were from females. This number is not surprising; tags are difficult to detect on females because the females remain buried during spawning. The between-quadrat recaptures were not used in the estimation because we did not have a corresponding count of unmarked animals.

Using the Petersen estimator and all releases, we estimated that there were approximately 14.5 million (SE=3.2 million) adult male horseshoe crabs in Delaware Bay during peak spawning at the end of May 2003. Using just the prepeak releases, we estimated 13 million adult males (SE=3.6 million).

Model comparisons indicated that recapture probabilities were time-specific, but the temporary effect on recapture probabilities was not supported by the data (Table 2). The maximum likelihood estimate of male abundance with model 2 (Table 2) was 16.1 million (90% CI: 9.9 to 22.3 million). The difference between the Petersen estimate and the maximum likelihood estimate could have been due to model 2 that allowed for time-specific recapture probabilities, whereas the Petersen estimator did not. The maximum likelihood estimate based on constant recapture probabilities (i.e., model 1 in Table 2), matched the Petersen estimate (14.7 million for model 1 and 14.5 million for the Petersen estimate).

Sex ratios were different for the preseason and prepeak spawning periods in a pattern consistent with a sex-specific migration schedule. There was a smaller proportion of adult females in the bay during the preseason period (0.25) than when spawning activity was near its peak (0.31), which is consistent with the known observation that males migrate earlier than females. Sex ratios observed during the prepeak period were used to estimate abundance in the bay during the peak spawning period.

The assumption of equal catchability of tagged and untagged animals is an important assumption that af-

Table 1

Mark-recapture statistics for tagged horseshoe crabs (*Limulus polyphemus*) released during a prespawning season period (26 March to 8 May 2003) and a prepeak-spawning period (28 to 30 May 2003) and recaptured during spawning surveys on 29 May, 31 May, and 2 June 2003. M_{1s} is the number tagged pre-season and M_{2s} is the number tagged before the peak at the time of the survey date (i.e., occasion s); u_s is the number of untagged that were counted during the survey; and m_{1s} is the number of prespawning season crabs recaptured and m_{2s} is the number of prepeak-spawning period crabs recaptured during the survey. Number untagged along the survey portion of the beach (u_s^*) was estimated from quadrat counts, i.e., [number untagged on beach = beach length (m) \times mean number per quadrat (no./m²) - number recaptured on beach]. Survey portion of the beach was less than 1 km.

Sex	Location	s	Survey date	M_1	M_2	Along beach			Quadrats		
						u_s^*	m_{1s}	m_{2s}	u	m_{1s}	m_{2s}
Males	Baywide	1	29 May	5398	4828	55,750	3	4	6846	1	2
		2	31 May	5398	7091	48,498	10	6	5117	2	1
		3	2 June	5398	7091	96,718	10	12	10,088	4	8
	New Jersey	1	29 May	1448	3703	25,123	0	4	3890	0	2
		2	31 May	1448	5254	16,376	3	6	2113	1	1
		3	2 June	1448	5254	29,630	0	9	3524	0	6
	Delaware	1	29 May	3950	1132	30,631	2	0	2956	1	0
		2	31 May	3950	1837	32,129	7	0	3004	1	0
		3	2 June	3950	1837	67,099	10	3	6564	4	2
Females	Baywide	1	29 May	1823	2143	18,224	0	0	2149	0	0
		2	31 May	1823	3231	17,022	1	1	1712	0	0
		3	2 June	1823	3231	27,339	0	1	2814	0	1
	New Jersey	1	29 May	488	1567	8021	0	0	1169	0	0
		2	31 May	488	2268	3993	0	1	532	0	0
		3	2 June	488	2268	6859	0	0	809	0	0
	Delaware	1	29 May	1335	576	10,202	0	0	980	0	0
		2	31 May	1335	963	13,029	1	0	1180	0	0
		3	2 June	1335	963	20,480	0	1	2005	0	1

Table 2

Comparison between three likelihood-based models for mark and recapture of horseshoe crabs (*Limulus polyphemus*). Each model allowed a differed pattern of variation in recapture probabilities. The models are listed in order from least to most complex. In model 1, the recapture probabilities were set to be constant for both release cohorts (prespawning season period and prepeak-spawning period) and for all three recapture occasions. Model 2 allowed recapture probabilities to be time-specific, but equal for release cohorts. Model 3 allowed recapture to be time and cohort specific. Lower values for Akaike information criteria (AIC) and Δ AIC indicate a better model fit; Δ AIC = 0 is the best fitting model. Likelihood ratio test (LRT) compares two nested models, and a significant P -value indicates the more complex model is supported.

Model	Variation in recapture probabilities	AIC	Δ AIC	Likelihood ratio test	
				Models compared	P -value
1	Constant	-348512.08	1713	Models 1 and 2	<0.0001
2	Time-specific	-350225.72	0	Models 2 and 3	0.77
3	Time and cohort-specific	-350220.83	5		

fects the accuracy of abundance estimates. Therefore, it was important to evaluate thoroughly whether tagged animals had different catchability than untagged animals. We tested for a temporary delay in spawning by comparing relative risk of recapture among the two release periods and by fitting a likelihood model that

incorporated separate recapture probabilities for each release cohort (Table 2). Evidence did not support the hypothesis that initial capture and tagging temporarily affected spawning behavior ($\chi^2=2.03$, $df=2$, $P=0.36$). The difference between the two release periods in the risk of being recaptured during peak spawning was

Table 3

Maximum likelihood estimates of abundance for adult horseshoe crabs (*Limulus polyphemus*) in Delaware Bay during the end of May 2003. Estimates of females and for both sexes combined ("Total") are based on mark-recapture estimates of males and sex ratios among the animals caught and released for this study. Adjusted estimates take into account the possible effect of capture on spawning by reducing releases of males by 0.88, which is an observed relocation rate for radio-tagged males.

	Maximum likelihood estimates		Adjusted estimates based on relocation rates from radio-tagged males	
	Abundance	90% CI	Abundance	90% CI
Males	16,140,000	9,910,000–22,300,000	13,730,000	8,780,000–19,400,000
Females	7,350,000	4,520,000–10,160,000	6,250,000	4,000,000–8,840,000
Total	23,490,000	14,430,000–32,460,000	19,980,000	12,780,000–28,240,000

0.001 (90%CI: -0.0007 to 0.003). A model comparison test (likelihood ratio test in Table 2) did not support cohort-specific recapture probabilities ($P=0.77$). Although the difference in relative risk was in the direction of a temporary delay in spawning, the difference in recapture probabilities tended to be in the opposite direction. On two out of the three recapture occasions, the prepeak release cohort had a higher recapture probability than the preseason cohort. The maximum likelihood estimate of abundance of males with the use of model 2 (Table 2) was 20% higher for all releases (16.1 million crabs) than for prepeak releases only (13.4 million). A temporary delay in spawning would tend to cause abundance estimates based on late-releases to be higher than estimates including early releases, but in fact the opposite occurred. Thus, based on three lines of evidence, it is unlikely that there was a temporary delay in spawning due to the capture and tagging of males.

Another way in which tagged animals may behave differently from untagged animals is that tagged animals may forego spawning altogether. In 2004, we radio tagged horseshoe crabs using the same capture process that was used to tag animals in 2003. The radio-tagged horseshoe crabs were detected by radio receivers at high tide when the radio-tagged crabs emerged from the water to spawn. An array of fixed station receivers ensured nearly complete coverage of spawning habitat in Delaware Bay. Among radio-tagged males, we observed that age-specific relocation rates were 0.44, 1.00, and 0.74 for young, middle, and old-aged males, respectively. The frequencies of these age groups in the end of May 2003 tag releases were 0.07, 0.62, 0.31 for young, middle, and old-aged males. Thus, the average relocation rate predicted for the 2003 releases would be 0.88 (i.e., $0.44 \times 0.07 + 1 \times 0.62 + 0.74 \times 0.31 = 0.88$). There are several reasons for a failure to detect radio-tagged animals, namely behavioral response, movement beyond the range of radio receivers, transmitter loss or failure, and animal mortality. It is also possible that some adults migrate but do not spawn in a given year. Nevertheless, to be conservative, we adjusted the tag releases by the observed relocation rate (0.88) and computed estimates using the reduced releases. The 12% reduction in re-

leases resulted in a 15% reduction in the abundance estimates (Table 3). From currently available estimates of fecundity (88,000 eggs per female; Shuster and Botton, 1985), we estimated that egg production in 2003 was 5.5×10^{11} (90% CI: 3.5×10^{11} to 7.7×10^{11}).

The adjusted estimates of abundance were 13.7 million (90% CI: 8.8 to 19.4 million) for males and 6.25 million (90% CI: 4.0–8.8 million) for females (Table 3). Landings in New Jersey and Delaware during 2003 were 2.4% (90% CI: 2–4%) of abundance estimates (Table 4). When landings from Virginia and Maryland are included, landings during 2003 were 4% (90% CI: 3–6%) of abundance. Harvest rates were similar for males and females because the sex ratio in the landings was similar to the ratio observed in our fishery-independent catch. We believe that our fishery-independent catch was a representative sample of mature animals in the bay.

We caught horseshoe crabs prior to and during the spawning migration. Thus, a comparison of pre- and postmigration catches could indicate the proportion of the population that over-wintered in the bay. We caught, on average, 18 adults per 15-min. tow on a vessel with two 2.3-m dredges from 25 March to 3 April 2003 prior to the spawning migration, which appeared to begin in mid-April. The catch-per-tow was 39 adults per tow during the period from 13 April to 8 May 2003 and 60 adults per tow during 28 to 30 May 2003. Thus, we were catching approximately one third ($18/60=0.3$) of the animals prior to spawning migration; this fraction could represent the proportion of the population that over-wintered in the bay and that did not migrate to the ocean between 2002 and 2003.

Discussion

There have been few attempts to estimate abundance of adult horseshoe crabs in Delaware Bay during the spawning run when the population is spatially concentrated. Shuster and Botton (1985) estimated population size from surveys on Delaware Bay beaches. However, a large portion of the bay was not included in the target

Table 4

Harvest rates calculated from 2003 landings and abundance estimates of adult horseshoe crabs (*Limulus polyphemus*) in Delaware Bay at the end of May 2003. To be conservative, estimates were adjusted according to the observed relocation rate (0.88) for radio-tagged male animals (see Table 3). NJ=New Jersey; DE=Delaware; VA=Virginia; and MD=Maryland.

	Delaware Bay (NJ and DE)			Delaware Bay Region (NJ,DE,VA,MD)
	Males	Females	Total	Total
Landings ¹	318,400	151,900	470,300	745,800
Abundance	13,700,000	6,250,000	19,980,000	19,980,000
	8.8 to 19 mil	4.0 to 8.8 mil	12.8 to 28 mil	12.8 to 28 mil
Harvest rate	0.023	0.024	0.024	0.04
	0.02 to 0.04	0.02 to 0.04	0.02 to 0.04	0.03 to 0.06

¹ ASMFC (Atlantic States Marine Fisheries Commission). 2004. 2003 Review of the fishery management plan for horseshoe crab (*Limulus polyphemus*), 13 p. ASMFC, 1444 Eye Street, NW, Sixth Floor, Washington, D.C. 20005

population study and was excluded from the survey. Carmichael et al. (2003) used transects located on a grid and visual counts in a shallow clear-water estuary to estimate abundance of the population in Pleasant Bay, Cape Cod, Massachusetts. Other estimates of the Delaware Bay population have been based on offshore surveys during nonspawning periods when populations are dispersed and possibly mixed (Botton and Haskin, 1984; Botton and Ropes, 1987; Hata and Berkson, 2003). Our mark-recapture estimates apply to adult horseshoe crabs present in Delaware Bay during late May 2003 when spawning peaked.

In previous horseshoe crab population estimates that were based on offshore surveys (Botton and Haskin, 1984; Botton and Ropes, 1987; Hata and Berkson, 2003), capture efficiency was unknown, and adults that remained in estuaries (i.e., those that did not migrate to the ocean after spawning) were not sampled. Hata and Berkson (2003) concluded that the capture efficiency for their trawl survey was intermediate between that of the trawl survey reported in Botton and Haskin (1984) and that of the hydraulic dredge survey reported in Botton and Ropes (1987). The most recent estimate of offshore abundance of 7.1 million crabs was reported by Hata and Berkson (2003). Botton and Haskin (1984) reported densities that were 1.8 to 2.9 times the densities reported by Hata and Berkson (2003), which would indicate a population estimate of 12 to 20 million according to the data of Botton and Haskin (1984). Botton and Ropes (1987) reported a minimum population of 2.3 to 4.1 million. The proportion of adults that remain in the Delaware Bay and do not migrate to the continental shelf after spawning is unknown. However, if the proportion of nonmigratory adults is sizeable (e.g., on the order of 0.3, which was indicated by our fishery-independent catches) then that, along with gear inefficiencies in trawl surveys, could explain the difference between the estimates in our present study and those of Hata and Berkson (2003).

Bias due to assumption violation is another reason for differences in estimates. We designed our study to obtain a representative sample when horseshoe crabs were concentrated and to minimize the time between release and recapture periods so that only a few days separated the prepeak release and recapture occasions. We released 17,543 tagged horseshoe crabs over two periods and examined 28,738 horseshoe crabs for tags, counting them in quadrats during subsequent spawning surveys (29 May, 31 May, and 2 June 2003). Low recapture rates (<1%) were consistent with a large population. However, it was important to evaluate the potential effects of assumption violations. Tag loss and tag-induced mortality were likely to be trivial because of the short period during which crabs were at large and results from field studies and laboratory experiments have shown no tag loss or tag-induced mortality (Crawford³; Brousseau et al., 2004). Brousseau et al. (2004) attached combined acoustic and radio tags and standard button tags to 24 female horseshoe crabs along two beaches in Delaware Bay, and then tracked them for eight days. All 24 were detected at least once, and 20 spawned on the beach of release within eight days of release, indicating that handling and tagging had a minimal effect on spawning behavior. Tags could have been overlooked during the spawning survey, which is why we limited our analysis to males counted and recaptured within 1-m² quadrats.

We evaluated the effect of capture and tagging on spawning behavior, and found no evidence that tagged males delayed spawning. Using radio-tagged horseshoe crabs, we believe it is possible that tagged crabs terminated spawning, and we adjusted abundance estimates based on relocation rates of radio-tagged crabs. Thus, we based our inference on abundance estimates that were adjusted downward to account for that possibility.

Recapture rates were low over the short time period for our study. However, annual recapture rate was approximately 4% for all tag releases and recaptures.

Mark-recapture studies have been frequently applied to marine species for describing migration or estimating mortality (Hoenig et al., 1998; Bacheler et al., 2005). Mark-recapture methods are used infrequently for abundance estimates of marine species, with the exception of anadromous species, whose spawning migration concentrates the population and enhances opportunities for recapture (Schwarz and Taylor, 1998). Similarly, the unusual horseshoe crab spawning migration and behavior concentrated the population and made them assessable for recapture. The validity of our abundance estimates is founded on a large number of tag releases and animals checked for tags, a study design that ensured population closure, an adequate number of samples to represent the population, and an evaluation of the underlying assumptions.

The sex ratio in our fishery-independent catch (69% M: 31% F), which we believe is a representative sample of adults in the bay at the time of peak spawning, was similar to the sex ratio in the landings (68% M: 32% F). Hata and Berkson (2003) observed a similar sex ratio among adults in an offshore trawl survey (63% M: 37% F). Although, commercial landings in 2003 were not skewed toward the harvest of females, harvest could have selected females disproportionately in past years.

The horseshoe crab harvest has been reduced through a series of reductions mandated by ASMFC (ASMFC⁴). Although there is evidence of stock decline coincident with increased landings in the past 10–20 years (ASMFC⁴), the estimates presented in the present study indicate recent regulatory changes had achieved a low harvest level by 2003. Based on the abundance estimates reported here, harvest rate in 2003 was 0.024 (90% CI: 0.02 to 0.04) for Delaware Bay state landings and 0.04 (90% CI: 0.03 to 0.06) for Delaware Bay area landings. In 2004, additional regulations were enacted, which capped landings at 150,000 per state for Delaware and New Jersey and prohibited harvest during May and early June when migrant shorebirds stopover in Delaware Bay. As a result of the 2004 regulations, landings dropped to 173,023 (males and females) for Delaware and New Jersey combined, which is a 63% drop from 2003 landings. Sex ratio of the 2004 landings was 68% M: 32% F, consistent with the 2003 landings.

Estimating abundance is an important step in the process of determining the current capacity for horseshoe crab egg production in the bay and for managing for the energetic needs of shorebirds. We estimate that egg production in 2003 was 5.5×10^{11} (90% CI: 3.5×10^{11} to 7.7×10^{11}). The U. S. Fish and Wildlife Service Shorebird Technical Committee (USFWS⁵) estimated that a

population of 423,000 shorebirds would require 1.07×10^{11} horseshoe crab eggs as they migrated through Delaware Bay. This represents approximately 20% of all egg production, which would have to be available temporally and spatially during the shorebird migration.

Egg availability for shorebirds will depend also on overlap between horseshoe crab and shorebird migrations, density-dependent bioturbation, and wave-mediated vertical transport. Some important aspects of egg production and the process of making eggs available to foraging shorebirds are not well understood and have not been quantified. For example, if fecundity is found to be age- or size-related, then age or size would need to be incorporated in a calculation of egg abundance. Also, the process of bioturbation, which releases buried eggs to the beach surface, is known to be related to the density of spawning females, but has not been parameterized (Jackson et al., 2002). Determination of the dietary requirements of migrant shorebirds in terms of horseshoe crab eggs coupled with reliable estimates of abundance, fecundity, and bioturbation rates, will set the stage for a management of horseshoe crabs that takes into account the trophic support it provides in Delaware Bay.

Acknowledgments

We thank many individuals involved in the tagging study and the Delaware Bay spawning survey. Principal among them were Stewart Michels, Lorne Brousseau, Mike Oates, Bruce Freeman, Carl Shuster, Susan Love, staff from the DE Coastal Program and the Delaware National Estuarine Research Reserve (DNERR), Benjie Swan, Bill Hall, Glenn Gauvry, Sherry Bennett, and representatives from Audubon, The Nature Conservancy, the Slaughter Beach folks, and the USFWS. Funding came from the USGS, NJ FG&W, and Delaware Department of Natural Resources and Environmental Control (DNREC). We appreciate comments from Stewart Michels, Greg Breese, and John Young on an early draft of the paper.

Literature cited

- Bacheler, N. M., R. A. Wong, and J. A. Buckel.
2005. Movements and mortality rates of striped mullet in North Carolina. *N. Am. J. Fish. Manag.* 25: 361–373.
- Baker, A. J., P. M. González, T. Piersma, L. J. Niles, I. de Lima Serrano do Nascimento, P. W. Atkinson, N. A. Clark, C. D. T. Minton, M. K. Peck, and G. Aarts.
2004. Rapid population decline in red knots: fitness consequences of decreased refueling rates and late arrival in Delaware Bay. *Proc. Royal Soc. B*, 271:875–882.
- Borchers, D. L., S. T. Buckland, and W. Zucchini.
2002. Estimating animal abundance: closed populations, 314 p. Springer, London.
- Botton, M. L., and B. A. Harrington.
2003. Synchronies in migration: shorebirds, horseshoe crabs, and Delaware Bay. *In* The American horseshoe

⁴ ASMFC (Atlantic States Marine Fisheries Commission). 2004. Horseshoe crab 2004 stock assessment report, 87 p. ASMFC, 1444 Eye Street, NW, Sixth Floor, Washington, DC 20005.

⁵ USFWS (United States Fish and Wildlife Service). 2003. Delaware Bay shorebird-horseshoe crab assessment report and peer review. U.S. Fish and Wildlife Service Migratory Bird Publication R9-03/02, 99 p. USFWS, 4401 N. Fairfax Drive, Arlington, VA 22203.

- crab (C. N. Shuster Jr., R. B. Barlow, and H. J. Brockmann, eds.), p. 5–32. Harvard Univ. Press, Cambridge, MA.
- Botton, M. L., and H. H. Haskin.
1984. Distribution and feeding of the horseshoe crab, *Limulus polyphemus*, on the continental shelf off New Jersey. *Fish. Bull.* 82:383–389.
- Botton, M. L., and J. W. Ropes.
1987. Populations of horseshoe crabs, *Limulus polyphemus*, on the northwestern Atlantic continental shelf. *Fish. Bull.* 85:805–812.
- Brousseau, L. J., M. Sclafani, D. R. Smith, and D. B. Carter.
2004. Acoustic and radio tracking horseshoe crabs (*Limulus polyphemus*) to assess spawning behavior and subtidal habitat use in Delaware Bay. *N. Am. J. Fish. Manag.* 24:1376–1384.
- Burnham, K. P., and D. R. Anderson.
1998. Model selection and inference: a practical information-theoretic approach, 353 p. Springer, New York, NY.
- Carmichael, R. H., D. Rutecki, and I. Valiela.
2003. Abundance and population structure of the Atlantic horseshoe crab, *Limulus polyphemus*, in Pleasant Bay, Cape Cod. *Mar. Ecol. Prog. Ser.* 246:225–239.
- Hata, D., and J. Berkson.
2003. Abundance of horseshoe crabs (*Limulus polyphemus*) in the Delaware Bay area. *Fish. Bull.* 101:933–938.
- Hoenig, J. M., N. J. Barrowman, W. S. Hearn, and K. H. Pollock.
1998. Multiyear tagging studies incorporating fishing effort data. *Can. J. Fish. Aquat. Sci.* 55:1466–1476.
- Jackson, N. L., K. F. Nordstrom, and D. R. Smith.
2002. Geomorphic-biotic interactions on beach foreshores in estuaries. *Proceedings of the International Coastal Symposium (ICS 2002)*. J. Coastal Res. Spec. Issue 36:414–424.
2005. Influence of waves and horseshoe crab spawning on beach morphology and sediment characteristics on a sandy estuarine beach, Delaware Bay, New Jersey, USA. *Sedimentology* 52:1097–1108.
- Odell, J., M. E. Mather, and R. M. Muth.
2005. A biosocial approach for analyzing environmental conflicts: a case study of horseshoe crab allocation. *Bio-science* 55:735–748.
- Schwarz, C. J., and C. G. Taylor.
1998. Use of the stratified-Petersen estimator in fisheries management: estimating the number of pink salmon (*Oncorhynchus gorbuscha*) spawners in the Fraser River. *Can. J. Fish. Aquat. Sci.* 55:281–296.
- Shuster, C. N., Jr., and M. L. Botton.
1985. A contribution to the population biology of horseshoe crabs, *Limulus polyphemus*, in Delaware Bay. *Estuaries* 8:363–372.
- Shuster, C. N., Jr., and K. Sekiguchi.
2003. Growing up takes about ten years and eighteen stages. In *The American horseshoe crab* (C. N. Shuster Jr., R. B. Barlow, and H. J. Brockmann, eds.), p. 103–132. Harvard Univ. Press, Cambridge, MA.
- Skalski, J. R., and D. S. Robson.
1992. Techniques for wildlife investigations: design and analysis of capture data. Academic Press, Inc., New York, NY.
- Smith, D. R., P. S. Pooler, B. L. Swan, S. Michels, W. R. Hall, P. Himchak, and M. Millard.
2002. Spatial and temporal distribution of horseshoe crab (*Limulus polyphemus*) spawning in Delaware Bay: implications for monitoring. *Estuaries* 25(1):115–125.