

Age and sex specific timing, frequency, and spatial distribution of horseshoe crab spawning in Delaware Bay: Insights from a large-scale radio telemetry array

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Abstract To study horseshoe crab *Limulus polyphemus* spawning behavior and migration over a large-spatial extent (>100 km), we arrayed fixed station radio receivers throughout Delaware Bay and deployed radio transmitters and archival tags on adult horseshoe crabs prior to their spawning season. We tagged and released 160 females and 60 males in 2004 and 217 females in 2005. The array covered approximately 140 km of shoreline. Recapture rates were >70% with multi-year recaptures. We categorized adult age by carapace wear. Older females tended to spawn earlier in the season and more frequently than young females, but those tendencies were more apparent in 2004 when spawning overall occurred earlier than in 2005 when spawning was delayed possibly due to decreased water temperatures. Timing of initial spawning within a year was correlated with water temperature. After adjusting for day of first spring tide, the day of first spawning was 4 days earlier for every 1 degree (°C) rise in mean daily water temperature in May. Seventy nine % of spawning occurred during nighttime high tides. Fifty five % of spawning occurred within 3 d of a spring tide, which was slightly higher than the 47% expected if spawning was uniformly distributed regardless of tidal cycle. Within the same spawning season, males and females were observed spawning or intertidally resting at more than one beach separated by >5 km. Between years, most (77%) did not return to spawn at the same beach. Probability of stranding was strongly age dependent for males and females with older adults experiencing higher stranding rates. Horseshoe crabs staging in the shallow waters east of the channel spawned exclusively along the eastern (NJ) shoreline, but those staging west of the channel spawned throughout the bay. Overall, several insights emerged from the use of radio telemetry, which advances our understanding of horseshoe crab ecology and will be useful in conserving the Delaware Bay horseshoe crab population and habitats [Current Zoology 56 (5): 563–574, 2010].

Key words Spawning behavior, Marine arthropod, Xiphosurid, *Limulus polyphemus*, Delaware Bay, Radio telemetry

It is difficult to study spawning migration of horseshoe crabs *Limulus polyphemus* over a large embayment with shoreline >100 km. Spawning behavior has been investigated extensively within an individual beach (Shuster and Botton, 1985; Penn and Brockmann, 1995; Widener and Barlow, 1999; Brockmann, 2003). Studies have recorded spawning at multiple beaches during the same time periods (Smith et al., 2002; James-Pirri et al., 2005). However, because of the demands for observing continuously over large spatial extent, some compromise has been necessary in temporal or spatial coverage – either the same time can not be observed at multiple sites or the same site can not be observed at multiple times. Telemetry in combination with field deployed data loggers is a possible approach for simultaneous and

continuous monitoring of animal movements and behaviors over a variety of spatial and temporal scales (Cooke et al., 2004).

Telemetry has been applied previously to study horseshoe crab movements and behavior at different spatial and temporal scales (Brousseau et al., 2004; Kurz and James-Pirri, 2002; Moore and Perrin, 2007; Watson et al., 2009). Brousseau et al. (2004) and Kurz and James-Pirri (2002) tracked animals for less than a month over several kilometers of shoreline. Watson et al. (2009), Moore and Perrin (2007), and James-Pirri (2010) tracked animals over multiple seasons within whole estuary or adjacent estuaries (≤10 km). Acoustic telemetry was employed in most of these studies (Kurz and James-Pirri, 2002; Moore and Perrin, 2007; Watson

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et al., 2009; James-Pirri, 2010). A combination of acoustic and radio telemetry was employed by Brousseau et al. (2004), which demonstrated the feasibility for remotely observing spawning behavior with radio telemetry. Signals from radio transmitters in marine environments can be detected as the animal emerges from the water to spawn. Radio telemetry transmitters when combined with data logging receivers have the potential to remotely observe spawning behavior over a larger extent (>100 km shoreline) than previously attempted.

Delaware Bay is the location of an ecologically and economically significant horseshoe crab population (Berkson and Shuster, 1999; Smith et al., 2009). The largest population of American horseshoe crabs spawns along the shores of Delaware Bay (Fig. 1). Information on timing, frequency, and spatial distribution of spawning within Delaware Bay has important implications to conservation and management of horseshoe crabs and the species that depend on them. For example, migratory shorebirds depend on consuming horseshoe crab eggs during their stopover in Delaware Bay (Botton, 2009). The ecological relationship between shorebirds and horseshoe crabs requires that their respective migrations match in time and space (Mizrahi and Peters, 2009). Factors causing a delay in spawning increase the risk that sufficient eggs will not be available during the

shorebirds' stopover in Delaware Bay.

Our purpose was to use telemetry to address basic questions about the timing, frequency, and spatial distribution of horseshoe crabs throughout Delaware Bay over multiple spawning seasons. Prior to the spawning seasons in 2004 and 2005, we tagged adult horseshoe crabs with radio transmitters. During the spawning seasons in 2004 through 2006, we tracked the tagged crabs using fixed station receivers that were arrayed along the shoreline of Delaware Bay. The tags transmitted uniquely coded radio signals for multiple seasons. Here we provide a descriptive summary of the tagging data.

1 Materials and Methods

The Delaware Bay is an approximately 1600 km² funnel shaped estuary connecting the Delaware River to the Atlantic Ocean (Fig. 1). The bay is within the political boundaries of the states of Delaware on the western shore and New Jersey on the eastern shore. The Delaware Bay shoreline is comprised of sandy beach, peat bank, salt marsh, or developed lands, and less than a third of the shoreline is considered suitable or optimal for horseshoe crab spawning (Lathrop and Allen, 2005). Undisturbed sandy beach is considered optimal spawning habitat (Botton et al., 1988). Mean tidal range in Delaware Bay is approximately 1.5 m.

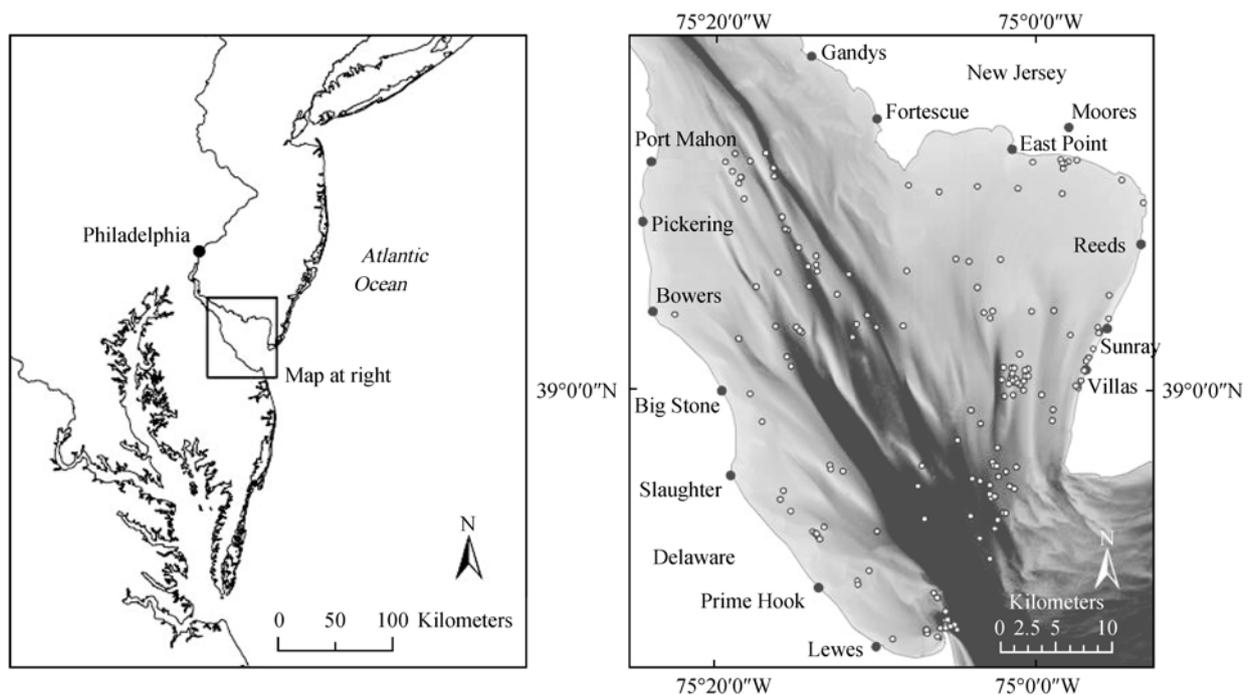


Fig. 1 Map of Delaware Bay showing locations of 14 radio receiver/data loggers along the shoreline and release locations for telemetry tagged horseshoe crabs

During April and through the first week of May of 2004 and 2005, we captured and radio tagged adult horseshoe crabs. Captures and releases were distributed throughout Delaware Bay with roughly equal numbers in the east and west sides of the bay (Fig. 1). Animals were captured from fishing boats, which were equipped with dredges used typically in Delaware Bay for horseshoe crab harvest. We tagged 220 in 2004 and 217 in 2005 (total of 60 males and 377 females). Males were tagged in 2004 only.

Carapace wear, prosomal width, and weight were recorded for each radio-tagged horseshoe crab. Carapace wear has been shown to indicate age class in horseshoe crabs (Brockmann and Penn, 1992; Smith et al., 2009; Shuster, 2009). We classified each horseshoe crab as young, middle-aged, or old based on carapace wear. Sexual maturity was determined by the presence of modified pedipalps in males and the presence of mature eggs in females (Smith et al., 2009). Detailed descriptions of carapace wear within those age classes and determination of sexual maturity were reported by Smith et al. (2009).

Transmitters and receivers were manufactured by Advanced Telemetry Systems (ATS), Isanti, MN, USA. Two sizes of transmitter (i.e., radio tag) were deployed. The radio tags differed in weight and life span, but had identical range. The small radio tag weighed 20 g in air and had a battery capacity of 510 d. The large radio tag weighed 32 g in air and had a battery capacity of 1140 d. The range of the signal determined by field trial was 3.2 to 8.9 km. Tags were <2% of body weight on average. The small radio tags were attached to all males and half of the females tagged in 2005. Tag size did not affect recapture rate ($\chi^2=0.18$, $df=1$, $P=0.67$). Each tag transmitted a unique frequency and code combination so that individual animals could be identified. In addition to attaching a radio transmitter, button tags (US Fish and Wildlife Service, Annapolis, MD, USA) were inserted into a 4 mm diameter hole drilled at the medial point along the posterior angle of the prosoma. Tag attachment was described and illustrated in Brousseau et al. (2004).

Fourteen fixed-station receivers were arrayed along the Delaware Bay shoreline (Fig. 1). Locations were selected to provide nearly complete bay-wide coverage. Sunray and Villas was 4.1 km apart, and the rest were > 5 km apart (Fig. 1). Each station included an ATS R4500C receiver/datalogger, 2 6-element yagi antennas, and an antenna switchbox. The receiver was powered by a 12 v deep-duty marine battery, which was periodically

replaced and recharged.

Archival tags were attached to 33 radio-tagged females in 2005. The archival tags (Lotek, LTD1100, Ontario, Canada) weighed 2g in water / 4g in air, were rectangular (9 mm × 16 mm × 27 mm), and recorded time, pressure (± 0.5 m), and temperature ($\pm 0.2^\circ\text{C}$) every 8 seconds. A telescoping log was used for memory management, so that the interval between records depended on the length of time the archival tag was deployed. For the animals recaptured within the same year, the interval between records was between 4 to 7 min. Downloading data from an archival tag required that the animal be physically recaptured. Thus, we used the radio signal at the time of high tide to locate the animal as it was spawning and available for physical recapture. Eight of the archive-tagged crabs were physically recaptured, but only four had useable data because of tag malfunction. Pressure was converted to depth and adjusted for tidal amplitude during post processing. CARIS HIPS Single-beam software (CARIS, New Brunswick, Canada) was used to apply a weighted average of tide values to the archival depth data to bring the archival data to the chart datum of Mean Lowest Low Water (MLLW). The tide values from the NOAA Brandywine and Lewes tide stations were used to create the tide zone that was subtracted from the archival depth data to create a depth at MLLW dataset. Tag attachment was the same as for the radio-tag (Brousseau et al., 2004).

Radio signals were detected and logged by the fixed station receivers when a transmitter was within range. Multiple detections of the same transmitter with relative signal strength (a signal to noise ratio) exceeding 100 were recorded as an observation. The signals were only detected by the receiver when the transmitter was at the water's edge or out of the water because the radio signal does not transmit through saltwater. The time of the observation was compared to the time of low and high tides adjusted for the station location. Based on that comparison, the observation was categorized into the behavioral states of spawning, intertidal resting, or stranding. When tagged animals are spawning, their signal will be received at high tide, but will be absent before and after high tide. When tagged animals are resting in the intertidal zone, then their signal will be received at low tide, but will be absent before and after low tide. If tagged animals are stranded, then their signal will be received continuously regardless of tidal cycle. An observation was categorized as spawning if the record was within 2 hours of a high tide and there was not a sequence of records that spans low to high

tides. An observation was categorized as intertidal resting if the record was within 3 hours of a low tide and there was not a sequence of records that spans low to high tides. An observation was categorized as a stranding if there was a sequence of records that spans one high tide to another. We attempted to relocate individuals stranded for more than 48 hours to assess possible mortality. Behavioral categorization assumes that animals did not move throughout the tidal cycle to remain at the water's edge. Watson and Chabot (2010) observed that horseshoe crabs were not uniformly active through the tidal cycle and were most active during the two hours on either side of the peak high tide. We use the term 'recapture' to denote generally when an individual is detected by a receiver.

To compare the timing of spawning in relation to temperature, we adjusted the day that a female was first observed spawning by subtracting the day of first spring tide for each year. In Delaware Bay, the initiation of spawning tends to coincide with tidal cycle, and the heaviest spawning occurs during the second spring tide in May (Michels et al., 2009). However, the dates of the spring tides differ among years. So, the adjustment was necessary so that year to year comparisons were not confounded by timing of spring tide.

Statistical analysis was conducted in R (2.7.2), SAS (9.1), and S-Plus (6.2). Regression methods included logistic regression for binary responses (e.g., recapture rate) and poisson regression for counts. Quantile regression was used for highly heteroscedastic data patterns and to test whether higher quartile responses were greater than median or mean responses (Cade and

Noon, 2003). Distributions were compared using a Kolmogorov-Smirnov test for 2 groups and Kruskal-Wallis test for more than 2 groups. Spearman correlations were computed to assess bivariate associations. To assess spatial distribution and spawning migration, we conducted a cluster analysis of release and recapture locations in SAS (9.1). We used Euclidian distances and Ward's method for hierarchical clustering, which minimized variance between clusters (McGarigal et al., 2000). Pseudo t^2 statistics suggested four clusters.

2 Results

Size distributions were similar for all carapace-wear based age classes, which is consistent with terminal molt at maturity (Kruskal-Wallis $\chi^2=3.4$, $df=2$, $P=0.18$ for females; $\chi^2=1.5$, $df=2$, $P=0.48$ for males). Mean prosomal width was 271 mm (21.3 SD) for females and 210 mm (13.4 SD) for males. Mean weight was 2.7 kg (0.54 SD) for females and 1.1 kg (0.31 SD) for males.

2.1 Recapture rate

First year recapture rates varied little among age classes for females (Table 1). Females were recaptured during their first year after release at rates of 71% in 2004 and 75% in 2005. For females released in 2004, 82% were recaptured in at least one year from 2004 to 2006. In contrast, young males were recaptured at a much lower rate than older males in the first year after release (Table 1). Young males were recaptured at a rate of 44% and middle or old males were recaptured at a rate of 85% in the first year after release.

Table 1 Recapture rates of adult horseshoe crabs released in 2004 – 2005 and recaptured in 2004 – 2006

Released	Sex	Age class	<i>n</i>	Recaptured					
				2004		2005		2006	
				Rate	<i>SE</i>	Rate	<i>SE</i>	Rate	<i>SE</i>
2004	F	Young	47	0.70	0.067	0.57	0.073	0.23	0.062
		Middle	65	0.72	0.060	0.46	0.067	0.15	0.049
		Old	47	0.72	0.066	0.38	0.072	0.09	0.041
	M	Young	27	0.44	0.098	0.30	0.090	NA	
		Middle	14	1.00		0.36	0.114	NA	
		Old	19	0.74	0.104	0.26	0.104	NA	
2005	F	Young	56	NA		0.71	0.061	0.25	0.058
		Middle	91	NA		0.80	0.042	0.22	0.044
		Old	69	NA		0.74	0.053	0.17	0.046

Animals were captured, tagged with a radio transmitter, and released during April or the first week of May within Delaware Bay. Recaptures were made by fixed station receivers arrayed along the shoreline of Delaware Bay (F=female, M=male, *n*= sample size, *SE* = standard error, NA = not available)

For both females and males recapture rate declined with each year after release due to mortality, emigration from the bay, or radio loss (Table 1). The decline increased with age, and the pattern was generally the same for both sexes. We did not observe any radio-tag loss, but neither can we rule it out. Each radio-tagged horseshoe crab was also tagged with a button tag. However, there were no recaptures where the radio-tag was lost, and the button tag remained.

Logistic regression indicated that animal size as measured by weight or prosomal width was not related to the likelihood of future recapture for either sex. There were 377 females released in 2004 or 2005 and 122 were recaptured one year after release. There were 60 males released in 2004 and 18 were recaptured one year after release. Recapture of an adult female one year after release was not related to weight ($t=0.29$, $df=346$, $P=0.77$) or prosomal width ($t=-0.25$, $df=346$, $P=0.80$). Recapture of an adult male one year after release was not related to weight ($t=-0.80$, $df=49$, $P=0.43$) or prosomal width ($t=0.67$, $df=49$, $P=0.51$).

2.2 Timing of spawning

Males tended to spend more time at the water's edge either spawning or intertidal resting than females (Fig. 2). (Here we combine spawning and intertidal resting to compare the amount of time that males and females remain near spawning beaches. Elsewhere we analyze spawning as a separate activity). A typical male was observed spawning or intertidal resting 11 times; whereas a typical female was observed spawning or intertidal resting 7 times. The distribution of observations at waters edge was significantly more right-skewed for males than

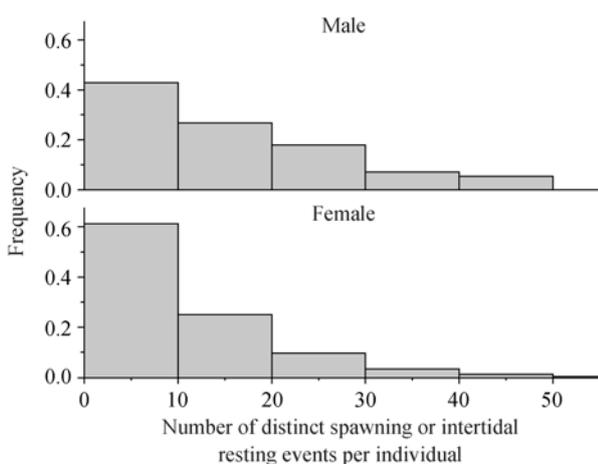


Fig. 2 Frequency distribution of the number of distinct spawning or intertidal resting events per individual for male and female adult horseshoe crabs tagged with radio transmitters and released prior to the spawning season in Delaware Bay

females (Kolmogorov-Smirnov $KS = 0.49$, $P < 0.001$).

Older females tended to spawn earlier than young females (Fig. 3). In 2004, the median day of first spawning was 13 May for old, 14 May for middle, and 18 May for young aged females. In 2005, the median day of first spawning was 26 May for old, 24 May for middle, and 28 May for young aged females. Poisson linear regression revealed that the relationship between age class and first day of spawning among females was negative and significant in both years of release, but was strongest in 2004 (slope=-0.11, $SE=0.0312$, $P < 0.001$).

In 2005, females that had been classified as young in 2004 initiated spawning at similar dates compared to females classified as young in 2005. Median day of first spawning in 2005 was 27 May for females released as young in 2004 and 28 May for females released as young in 2005. Frequency distribution did not differ among years of release (Kolmogorov-Smirnov $KS = 0.17$, $P = 0.74$). Thus, the additional year of maturation did not cause the young released in 2004 to behave as older age classes in 2005.

The day of first spawning by females was inversely related to water temperatures (Table 2). After adjusting for day of first spring tide, the day of first spawning was 4 days earlier for every 1°C rise in mean daily water temperature in May (slope = -4.05, $df=1$, $P = 0.09$; Spearman $\rho = -1$, $P = 0.08$). Data from 4 archival tagged females indicated that approaches to the waters edge, as indicated by depth $\cong 0$, coincided with rising water temperatures (Fig. 4).

Spawning activity tended to be at night and near the spring tides. Seventy nine % of spawning (82% in 2004 and 76% in 2005) occurred after 20:00 h and before 06:00 h, which corresponds approximately to nighttime during May and June in Delaware Bay. The relationship between the proportion of spawning activity and the number of days on either side of a spring tide closely followed a power curve (least squares regression $t = 92$, $df=2$, $P < 0.0001$). The relationship was $y = 0.26x^{0.68}$, where y is the proportion of spawning activity and x is the number of days on either side of a spring tide within May and June ($1 \leq x \leq 7$). For example, 26% of the spawning occurred within 1 d of a spring tide, 55% occurred within 3 d, and 78% occurred within 5 d. If spawning was distributed uniformly through time regardless of tidal cycle then 20%, 47%, and 73% would occur within 1 d, 3 d, and 5 d of spring tide, respectively.

2.3 Frequency of behavioral events

There was a tendency for older females to spawn more frequently than young females, but the magnitude

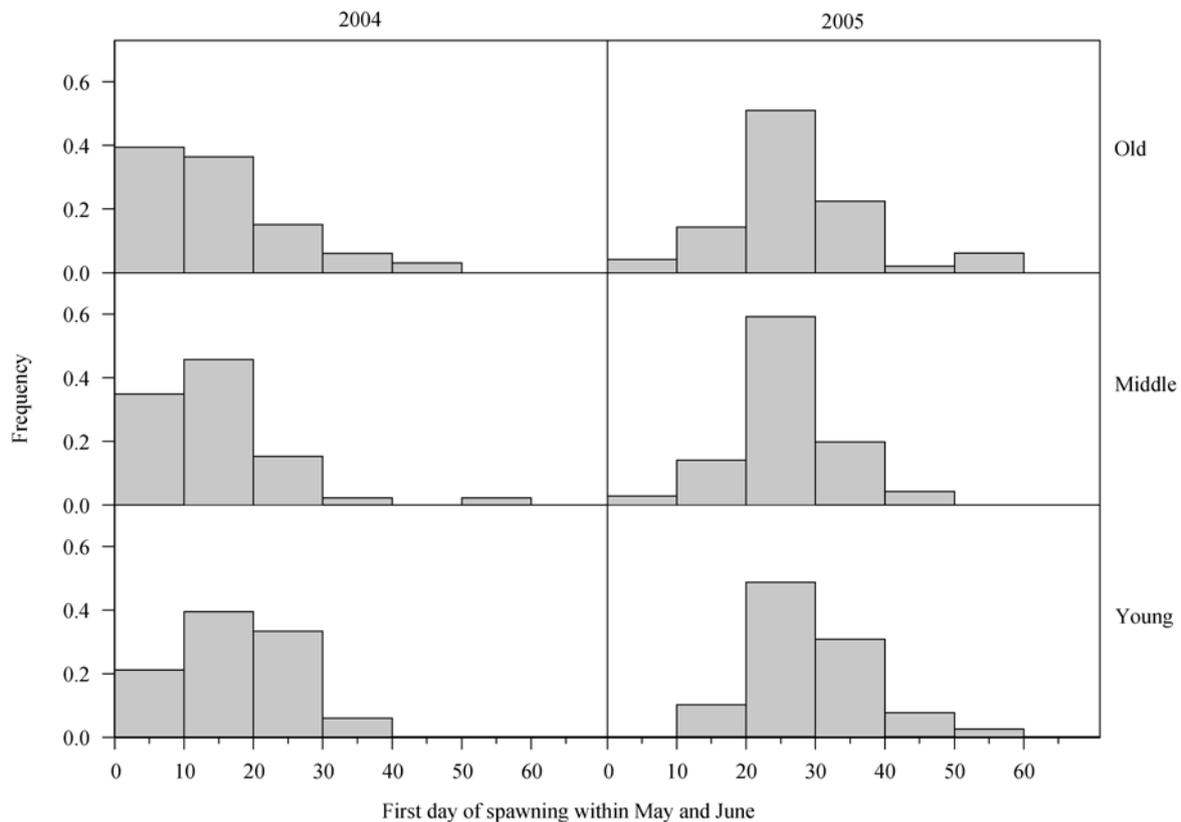


Fig. 3 Frequency distribution of the first day of spawning within May and June (i.e., within the spawning season) for adult female horseshoe crabs tagged with radio transmitters and released prior to spawning season in Delaware Bay

Observations for animals released in 2004 are shown on the left and those released in 2005 are shown on the right. Age classes were based on carapace wear.

Table 2 Day of the first spring tide and mean first day of spawning for adult females tagged with radio transmitters and released in Delaware Bay for 2004 – 2006

Year	Day of first spring tide	Mean day of first spawning	Adjusted mean day of first spawning	Mean daily water temperatures (C) in May
2004	4 May	16 May	12 May	15.7
2005	8 May	27 May	19 May	13.7
2006	13 May	21 May	8 May	16.3

The mean first day of spawning was adjusted by subtracting the day of the first spring tide for comparison to mean daily water temperature in May. Water temperatures were recorded at the National Ocean Service station at Lewes, DE and reported in (Michels et al., 2009).

of the difference was small and it was significant only in 2005 (slope = 0.13, $df=157$, $P = 0.006$). In 2005, the median number of spawning bouts was 5 for old, 5 for middle, and 4 for young aged females. In 2004 and 2005, the maximum numbers of spawning bouts tended to be higher for the older age classes than for the young age class females (Fig. 5).

There was an indication that lighter weight females spawned more frequently than heavier females (Spearman $\rho = -0.15$, $P = 0.11$). The pattern was strongest among middle age class females (Fig. 6). The pattern

was distinctly wedge shaped so quantile regression was applied. The slope of the relationship between spawning events and weight became increasingly steep as quantile increased (Fig. 6). The pattern was similar when weight was adjusted for prosomal width.

The probability of stranding increased with age for females ($\chi^2 = 8.01$, $df=2$, $P = 0.02$) and males ($\chi^2 = 15.8$, $df=2$, $P < 0.001$). The increase in probability of stranding with age was most pronounced among males (Fig. 7). None of the young or middle age class males were stranded, but 50% (7 out of 14) of the old age class

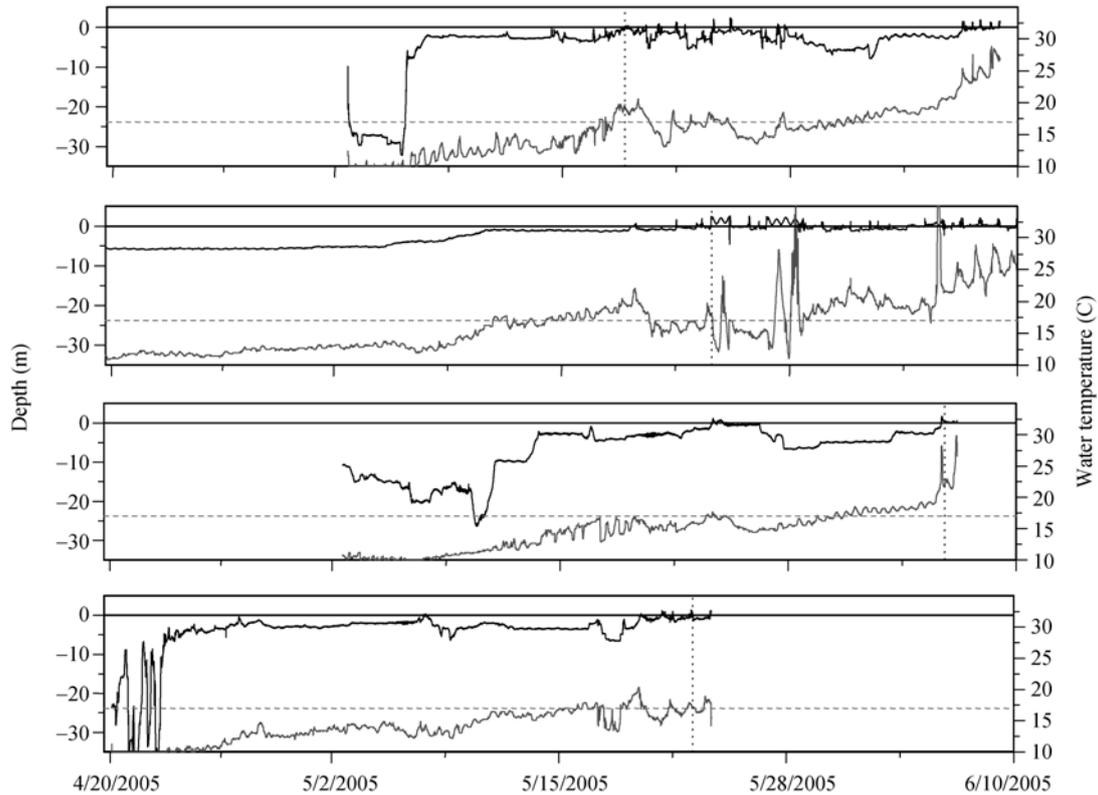


Fig. 4 Profiles of depth (m) and temperatures (°C) recorded on archival tags deployed on four adult female horseshoe crabs released in Delaware Bay prior to spawning season in 2005

Solid horizontal line indicates zero depth, and dashed horizontal line indicates 17°C. The 17°C reference is suggested by the literature (cf Chabot and Watson, 2010). Dotted vertical line indicates the first spawning observed by radio telemetry. Animals were recaptured by hand opportunistically when they were detected spawning and found by radio tracking. Depth was adjusted for tidal amplitudes.

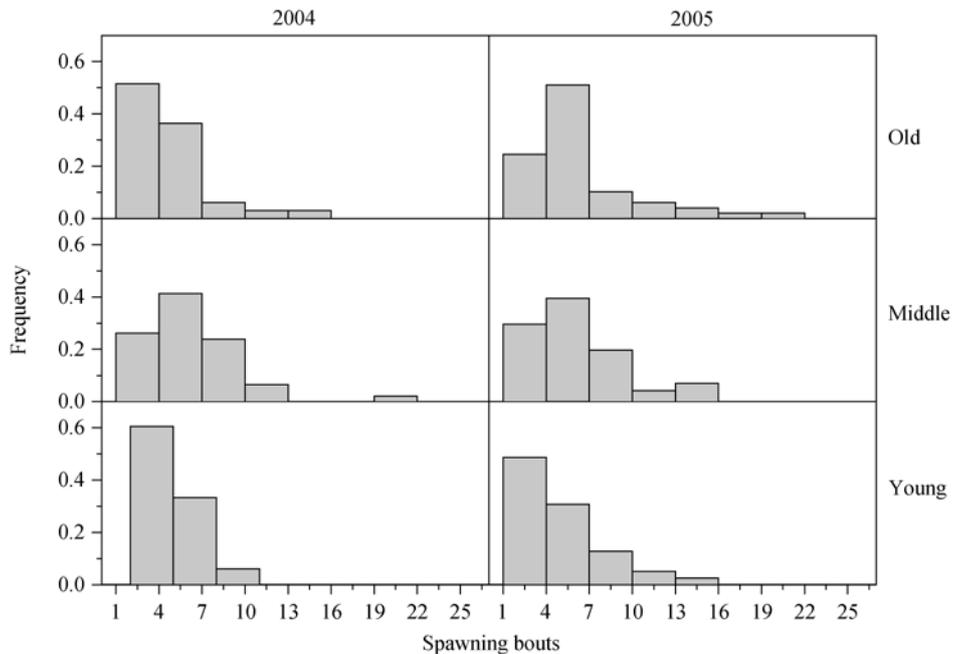


Fig. 5 Frequency distribution of the number of observed spawning bouts for adult female horseshoe crabs tagged with radio transmitters and released prior to spawning season in Delaware Bay

Intertidal resting is not included. Observations for animals released in 2004 are shown on the left and those released in 2005 are shown on the right. Age classes were based on carapace wear.

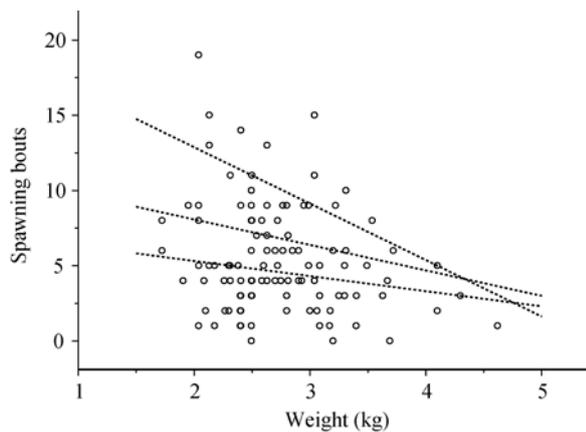


Fig. 6 Spawning bouts and weight for middle age class females tagged with radio transmitters and released prior to spawning season in Delaware Bay in 2004 and 2005

Intertidal resting is not included. Quantile regression lines are shown for quantiles of 0.5 (lowest), 0.7 (middle), and 0.9 (highest).

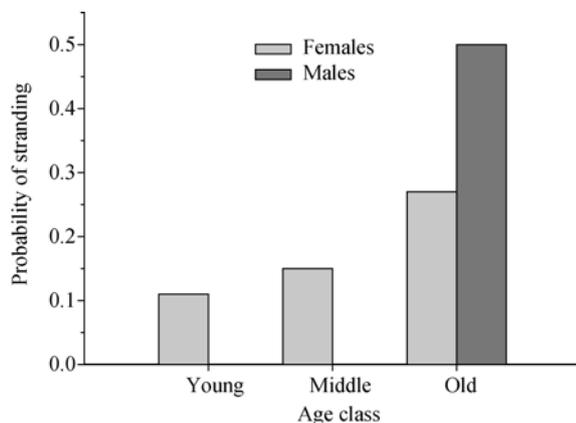


Fig. 7 Probability of stranding for male and female horseshoe crabs tagged with radio transmitters and released prior to spawning season in Delaware Bay in 2004 and 2005

Stranding occurred when an animal remains out of the water for at least one tidal cycle. We observed no stranding among males in the young or middle-age class.

males were stranded. Among females 11% of the young, 15% of the middle, and 27% of the old age class animals were stranded. Logistic regression indicated that stranding was not related to weight for females ($P = 0.79$) or males ($P = 0.72$). Out of the 86 strandings, 70 (81%) were followed by resumption of normal behavioral activities (i.e., spawning or intertidal resting), six (7%) resulted in confirmed mortality, and ten (12%) ended with unknown dispositions. This indicates mortality due to stranding was between 7% (6 out of 86) and 19% (16 out of 86; assuming all unknown dispositions were mortalities).

2.4 Spatial distribution

Within the same spawning season, males and females were observed spawning or intertidally resting at more than one beach. Thirty of the 280 times (11%) females were observed spawning or resting at two receiver stations. Eight of the 40 times (20%) males were observed spawning or resting at two receiver stations.

Between years, most (77% overall) did not return to spawn at the same beach. Among those observed spawning in 2004 and 2005, 71% (54 out of 76) did not return to spawn within the range of the same receiver station. Among those observed spawning in 2005 and 2006, 87% (35 out of 40) did not return to spawn within the range of the same receiver station.

Hierarchical clustering of release and recapture coordinates resulted in four clusters characterized by the combination of spawning and release locations (Fig. 8). Northeastern shoreline (Fig. 8A) included Gandys, Fortescue, East Point and Moores. Individuals that spawned along the northeastern shoreline tended to be released east of the channel (see Fig. 1), but included some released in the lower bay west of the channel. The southeastern shoreline (Fig. 8B) included Moores, Reeds, Sunray, and Villas. Individuals that spawned along the southeastern shoreline tended to be released east of the channel relatively near shore, but also included some released in the lower bay west of the channel. The western shoreline included all DE receivers (Fig. 8C). Individuals that spawned along the western shoreline were released in the lower bay, either near the channel or near the western shore. The upper bay shoreline (Fig. 8D) included Slaughter, Big Stone, Bowers, Pickering, and Port Mahon on the west side and Gandys and Fortescue on the east side. Individuals that spawned along the upper bay shoreline were released in upper bay waters either near or on the west side of the channel.

3 Discussion

Radio telemetry was an effective approach to study spawning migration of horseshoe crabs over a large estuary. Recapture rates were high for all sexes and sizes, >70%, with the exception of younger males where recaptures were only 44% (which we discuss below). Spatial and temporal coverage exceeded previous approaches such as a spawning survey requiring simultaneous deployment of large numbers of volunteers (Smith and Michels, 2006). Radio telemetry does have limitations. For example, migratory pathways could not be observed directly, which can be done with acoustic

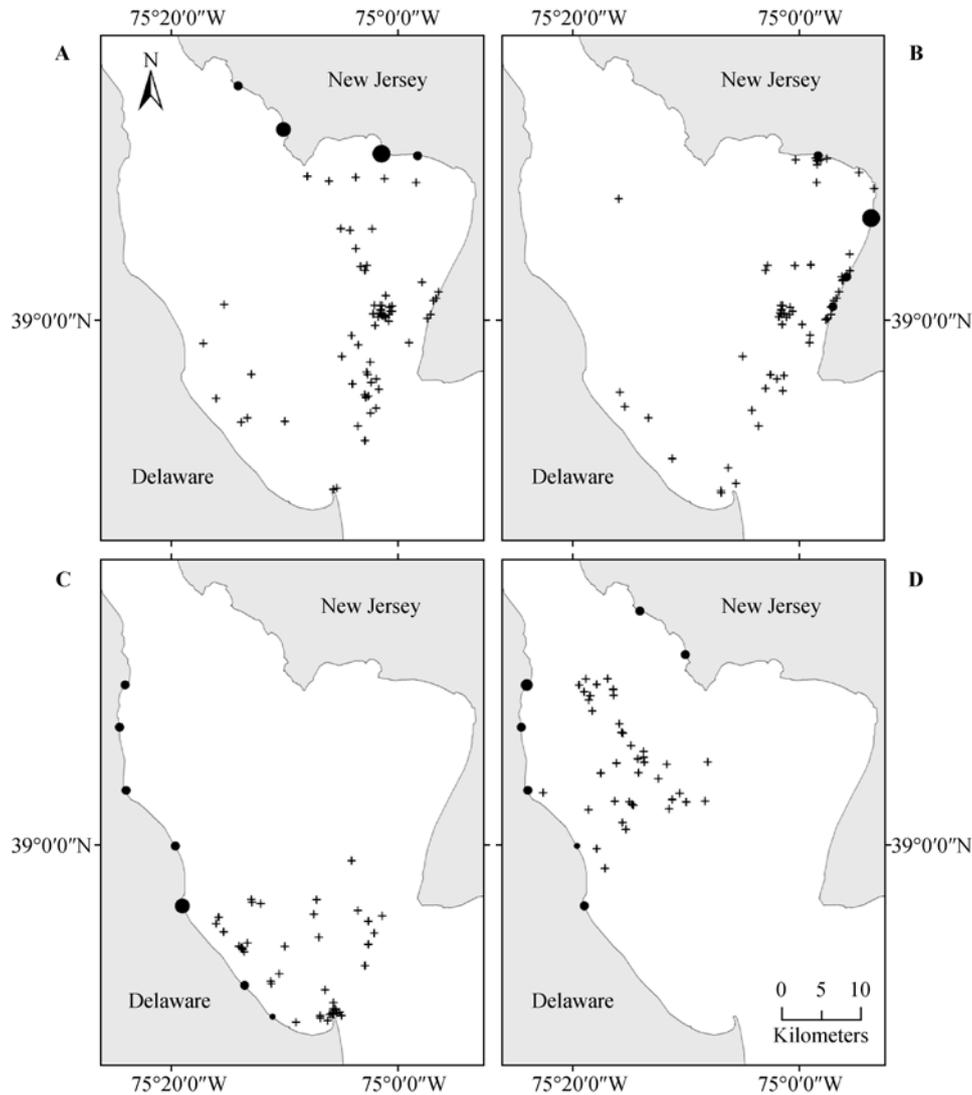


Fig. 8 Map of releases (+) and spawning (●) locations of horseshoe crabs grouped by hierarchical cluster analysis

Release of radio-tagged horseshoe crabs occurred prior to spawning season in 2004 and 2005. Relocations occurred during spawning. Spawning locations were for the first observed spawning in the year of release. Symbol for spawning location is at the receiver station, and its size is proportional to the number spawning at that station. Clusters, as characterized by spawning location, were northeastern shoreline (A), southeastern shoreline (B), western shoreline (C), and upper bay shoreline (D).

telemetry (Moore and Perrin, 2007; Watson et al., 2009). Overall, several insights emerged from the use of radio telemetry, which advances our understanding of horseshoe crab ecology and will be useful in conserving horseshoe crab populations and their habitats within Delaware Bay.

Older females tended to spawn earlier and more frequently than young females. The direction, but not the magnitude, of that tendency was consistent among years. It was more apparent in 2004 when spawning overall occurred earlier than in 2005. An implication of this pattern is that older age class females disproportionately will contribute to spawning during May when migratory

shorebirds stopover in Delaware Bay to forage on excess horseshoe crab eggs (Mizrahi and Peters, 2009). The early spawning was observed in the middle and old age class females. Thus, the delayed spawning is likely to be restricted to the first years after recruitment. Overharvest could reduce the proportion of older females in the population and increase the chance of a temporal mismatch between horseshoe crab egg availability and shorebird stopover. In years when abiotic factors, such as cool water temperature and wave-generating storms, cause delayed spawning, the importance of demographically controlled timing of spawning could be diminished, as we saw in 2005.

Initiation of spawning was correlated to water temperature. We found that the day of first spawning was 4 days earlier for every 1 degree (°C) rise in mean daily water temperature in May. Other factors, such as wind-generated wave energy, could have contributed to the initiation of spawning. However, the correlation between spawning and temperature has been seen in spawning survey data (Smith and Michels, 2006). Watson et al., (2009) reported temperature mediated behavior and noted that pre-spawning migratory movements followed temperatures reaching 10–11°C. Chabot and Watson (2010) experimentally showed that water temperature was a key factor in determining activity associated with tidal rhythm. Temperature is an important factor in egg development, which could be the underlying evolutionary mechanism linking the temperature spawning relationship to fitness (French, 1979).

Males spent more time at the beach than females, which has been reported widely elsewhere (Rudloe, 1980; Brockmann and Penn, 1992; Brockmann, 2003; Smith et al., 2002; James-Pirri et al., 2005). This behavioral pattern predominately causes male-skewed operational sex ratio, but sex-specific natural or fishing mortality also can affect operational sex ratio (Brockmann, 2003).

Recapture rate was lower for young males (44%) compared to middle or older aged males (85%). The low recapture rate for young males could be explained by male mating tactics and competition for females in Delaware Bay (Brockmann and Penn, 1992; Brockmann, 2002). Brockmann and Penn (1992) reported that attached males tend to have less carapace wear than unattached males. Through a novel experiment, Brockmann (2002) demonstrated that males with less carapace wear were more likely to come ashore to spawn if attached to a female; whereas, males in poor condition came ashore regardless of amplexus and spawned as satellites if unattached. Over 2002–2008 in Delaware Bay, the population sex ratio averaged 2.1 and the operational sex ratio was 3.8 males to female (Atlantic States Marine Fisheries Commission, 2009; Michels et al., 2009). These highly male-biased sex ratios create competition for females. Young males that cannot find a female for amplexus would therefore be less likely to spawn at all, which is consistent with our observation of low recapture rate for young males. In contrast, Mattei et al. (2010) found no difference in condition between attached and unattached males in a low density population in Long Island Sound and attributed this to density dependent male competition. Thus, age-specific recapture rate in

males might not be seen in low density populations.

We observed that 79% of spawning activity occurred during nighttime tides, which is the higher of the diurnal high tides in Delaware Bay. Rudloe (1980) found that spawning activity was higher on nighttime high tides in Florida. Barlow et al. (1986) reported that spawning activity was highest on the higher of the diurnal high tides in Massachusetts. Smith (2007) assumed in a simulation study of Delaware Bay spawning that 80% of spawning activity occurred on the higher diurnal high tide, which closely matches our observations. However, James-Pirri et al. (2005) reported a high amount of spawning during daytime high tides on Cape Cod. Watson et al. (2009) found only a slight tendency to spawn at night in the Great Bay estuary, NH, but there was only a minor difference in diurnal tide height (Chabot and Watson, 2010).

Consistent with the conclusions of Smith et al. (2002), we found that spawning activity in Delaware Bay was not tightly correlated to spring tide. Only 55% of spawning activity was within 3 d of the springtide, which is slightly higher than the 47% that would be expected if spawning was uniformly distributed through time.

Unexpectedly, the frequency of spawning bouts was higher for lighter weight females than for heavier females, regardless of adjustment for prosomal width. The increased frequency was most prominent for the upper quantiles, so that the females that spawned the most often were most likely to be lighter females. Our *a priori* expectation was that heavier females meant more eggs, which in turn, would result in a higher frequency of spawning. The pattern was marginally significant, but was apparent across age class. We believe that it is worth presenting here as a hypothesis and offering a possible mechanism to explain the pattern. Assuming that low relative weight implies low relative fecundity, then an adaptive strategy to mitigate the effect of low fecundity might be to spread the risk of nest failure across time and space. This hypothesis relates to fractional spawning in fishes (Winemiller and Rose, 1992). Egg development depends on the location of the nest along and across the shoreline (Botton et al., 1988; Penn and Brockmann, 1994). Nest location across shore is largely determined by tidal height (Weber and Carter, 2009). Thus, spawning frequently increases the opportunities that the nest will be located in an environment conducive for egg development determined by substrate, oxygen, moisture, and temperature.

The probability of stranding was strongly related to

age class. Stranding represents a significant source of natural mortality among adult horseshoe crabs (Botton and Loveland, 1989). Our estimate of stranding related mortality was at least 6% for confirmed mortalities and as high as 18%, if all unknown dispositions were treated as mortalities. Penn and Brockmann (1995) noted that relationship for males and attributed it to senescence and cumulative parasite (epibiont) load of old aged males. We observed a sharp increase in stranding for the old age class. Among females, we observed a gradual increase in probability of stranding as age increased. Stranding was not related to weight.

Movement between years was extensive and confirmed prior observations that horseshoe crabs in Delaware Bay lack inter-annual fidelity to spawning beaches (Swan, 2005). Between years the majority (77%) did not return to the same beach to spawn. This is consistent with the lack of genetic differentiation within the Delaware Bay region (King et al., 2005). In contrast, most, but not all, horseshoe crabs exhibited within season fidelity to an area of shoreline. We did observe males and females spawning at multiple locations separated by >5 km within a spawning season. Within season movement at this scale was more frequent in males (20%) than females (11%).

Migration pathways and spatial distribution within Delaware Bay have not been studied directly although migration patterns have been suggested (Shuster and Botton, 1985; Swan, 2005). Shuster and Botton (1985) suggested that horseshoe crab migration is determined by dominant currents, which run strongly towards the eastern shoreline. Swan (2005) concluded from tag returns that horseshoe crabs rarely move across the bay within the same spawning season. We concur with Swan (2005) that an individual will rarely spawn on both the eastern and western shoreline within the same spawning season. However, horseshoe crabs staging west of the channel, even those near the western (DE) shoreline spawned throughout the bay including on the eastern (NJ) shoreline. The cross-bay movement was not symmetric. Horseshoe crabs staging in the shallow waters east of the channel spawned exclusively along the eastern (NJ) shoreline and did not spawn along the western (DE) shoreline. This asymmetry in movement is consistent with Shuster and Botton's (1985) suggestion that movements are influenced by relative currents. Also, consistent with current-driven migration was the observation that horseshoe crabs staging in the upper bay where flood tide currents were less directional tended to spawn along the upper shoreline on both sides of the bay.

sides of the bay.

We present a descriptive summary of the radio telemetry data with emphasis on behavioral observations. Tagging data is also useful for estimation of demographic parameters and population level inference (Pollock et al., 2002; Williams et al., 2002). Brousseau et al. (2004) fit multistate mark-recapture models to combined radio and acoustic telemetry observations to estimate daily probabilities of spawning and departure from the vicinity of spawning beaches. When applied to data from multi-year studies, mark-recapture models can incorporate population parameters for survival and regional movement as well as behavioral transitions. We see application of these mark-recapture models as the next step for drawing insights from radio telemetry observations of horseshoe crabs in Delaware Bay.

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