

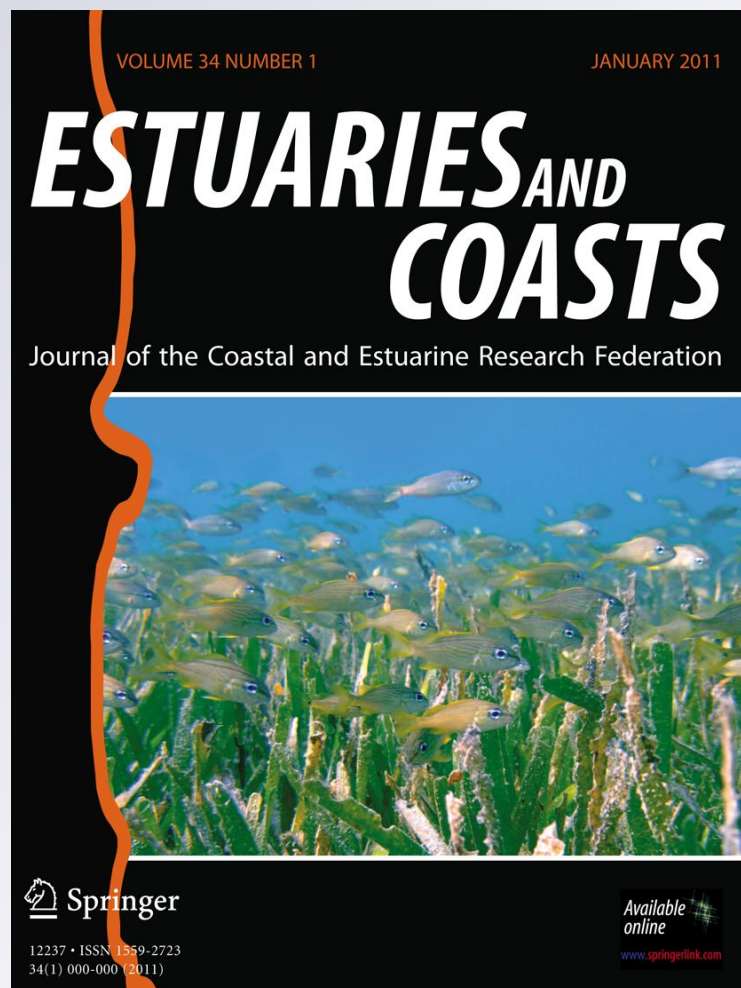
*A Long-Term Study of Spawning Activity in a Florida Gulf Coast Population of Horseshoe Crabs (**Limulus polyphemus**)*

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A Long-Term Study of Spawning Activity in a Florida Gulf Coast Population of Horseshoe Crabs (*Limulus polyphemus*)

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Abstract Populations of the American horseshoe crab (*Limulus polyphemus*) differ in broad areas of their biology. We observed a non-harvested, marked Florida Gulf coast population during their spring spawning (March–May) in 11 years across a 17-year period (1992–2009). Long-term changes occurred in the number of spawning pairs: the population was stable from 1992 to 2000 but increased markedly after 2000. Short-term variation in numbers of spawning pairs, unpaired females, unpaired males, and operational sex ratios was explained by changes over the season and during each week of spring tides and by differences in actual (not predicted) maximum high tide height. Wind direction strongly affected tidal inundation and the number of spawning horseshoe crabs. Tagging individuals revealed that females returned to the nesting beach less often than males and most females were resighted only within 1 week of spring tides. No animals were seen across more than 6 years. Implications for management are discussed.

Keywords *Limulus polyphemus* · Horseshoe crab · Florida population · Spawning numbers · Operational sex ratio

Introduction

The American horseshoe crab, *Limulus polyphemus*, is harvested in some parts of its range as bait by the whelk and eel fishery and for its blood by biomedical companies (Berkson and Shuster 1999; Kreamer and Michels 2009; Walls et al. 2002). As a result of the greatly expanded use of horseshoe crabs during the 1990s and a profound decline in the numbers of spawning animals in the Delaware Bay (DE Bay) and elsewhere (Shuster 2001; Shuster et al. 2003; Widener and Barlow 1999), the Atlantic States Marine Fisheries Commission introduced a management plan (ASMFC 1998). Because of the lack of information about horseshoe crab biology, this plan required states to develop monitoring programs. Subsequently, a number of studies have increased our understanding of the population biology and spawning habits of horseshoe crabs in DE Bay (Smith et al. 2002a, b; Swan 2005), New York (Hanna 2001), Massachusetts (Carmichael et al. 2003; James-Pirri et al. 2005; Leschen et al. 2006), and Maine (Moore and Perrin 2007). Recently, a male-only harvest was instituted in DE Bay (ASMFC 2008), but little information is available on natural breeding sex ratios (operational sex ratio or OSR) or the likely effect of sex-biased harvesting on horseshoe crab populations.

As horseshoe crab use was controlled in some areas, new areas were exploited where much less was known about their biology. One of those areas was Florida where horseshoe crabs occur on both the east and west coasts and in the Florida Keys (Brockmann 1990; Ehlinger and Tankersley 2007; Rudloe 1985). Most of the Florida harvest of horseshoe crabs (20,000–40,000 annually) is for the marine-life fishery including adult specimens for education and research and juveniles for the aquarium trade (Gerhart 2007). When an estimated 99,000 adult horseshoe crabs

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were harvested during one 44-day period from the Port St. Joe area (Gulf County, Florida) in March 1999 after fisheries had closed elsewhere (Wallace 1999), the Florida Division of Marine Fisheries established harvest regulations (Florida Statutes, Chapter 68B-46). However, management has been hampered by a lack of information on population sizes, spawning habitats, or life histories of Florida horseshoe crabs (Wallace 1999). The one exception is a genetically distinct population (King et al. 2005) in the Indian River Lagoon on the east coast of Florida, which appears to be in decline (Ehlinger and Tankersley 2007).

The study reported here examines a Florida population of horseshoe crabs that spawn on an island along the northern Gulf coast, Seahorse Key (Fig. 1). The 11-year study spans a 17-year period, during which time little or no harvesting of horseshoe crabs occurred in this area. We examine (a) long-term, seasonal, and short-term trends in the abundance and OSR of horseshoe crabs spawning at this site and (b) the physical and environmental factors that influence the number of spawning crabs including tides, wind, and temperature. Further, (c) by marking and recapturing horseshoe crabs, we assess the pattern of individuals returning to the same nesting site within and between years. The results provide a baseline for evaluating the status of a non-harvested population of horseshoe crabs in Florida.

Methods

Location and Dates of the Study

We conducted this study at the University of Florida Marine Laboratory at Seahorse Key (SHK), a 2-km-long by 0.5-km-wide (at its widest point) island 5.6 km from Cedar Key (Fig. 1) in the Big Bend region of Florida's west coast. The island is part of the Cedar Keys National Wildlife Refuge, so there has been no development at this site. Substantial erosion of the island has occurred over the past 25 years due to sea level rise (1.8 mm/year on average since 1910; NOAA Tides and Currents 2010). North of the island is a large estuary formed by the Suwannee River. The south side of the island faces the Gulf of Mexico and has a narrow sandy beach where horseshoe crabs spawn and lay their eggs (Penn and Brockmann 1994). The island is surrounded by shallow banks for at least 2 km in all directions (Fig. 1), which creates a low-wave-energy environment (Davis 1997). The SHK beach is also protected by Seahorse Reef (7 km south), which is occasionally above water during extreme low tides.

Each year, the study covered 5–7 weeks of spring tides, when maximum high tides (high water level) were unusually high (i.e., predicted tide heights >91 cm above

mean lower low water—mllw). Previous studies had revealed that horseshoe crabs were not normally present during neap tides (Barlow et al. 1986; Cohen and Brockmann 1983), which is why we focused our study on spring tides (the week of the new or full moon). We began each year with the first spring tides in late February or early March (initial dates varied because of storms or cold weather). We continued into June in the first 2 years of the study, but discontinued the June surveys thereafter because so few crabs were present. During these survey periods, we observed horseshoe crabs on all high tides that occurred from 2 days before the new or full moon to 5 days after (the two high tides per day fell between 11:15 and 17:30 hours and 00:15 and 06:30 hours) for a total of 57–88 high tides per year except in 2009 when we observed horseshoe crabs on only 35 high tides (Table 1). Over the 11 years of study, a few (one or two) high tides were missed each year due to unsafe conditions (e.g., electrical storms).

Procedures

Spawning Survey

The spawning survey covered the 1-km, south-facing beach at Seahorse Key (very few crabs nested outside this portion of the beach). The survey began at either end of the beach (haphazardly determined), 10–15 min before the predicted maximum high tide at SHK (which is 15 min prior to the predicted high tide at Cedar Key) and normally ended 20–30 min later at the other end, but on six occasions, the survey took an hour to complete because there were exceptionally large numbers of horseshoe crabs. One or two observers walked the beach and counted all horseshoe crabs present on or within 2 m of the shoreline. Females were classified as unpaired or paired; a female was paired if a male was attached by his claspers (modified first pair of appendages) to her posterior, opisthosomal spines. Nearly all females arrived on the beach with an attached male that they retained throughout the nesting process (Brockmann 2003a). Males were defined as paired (attached to a female) or unpaired (Brockmann 2003b). In 1992, unpaired males were not counted reliably so they were not included in our analyses. Over the 17 years spanned by this project, we made every effort to keep the spawning survey procedures identical from year to year as other parts of the study changed. Since this spawning survey always covered the same area (1 km) and since the vast majority of animals nested along a narrow strip of beach at SHK at the time of the maximum high tide, the spawning survey provides a measure of spawning density. The mean number of pairs present per tide is comparable to the spawning index used in other studies (Smith et al. 2002b).

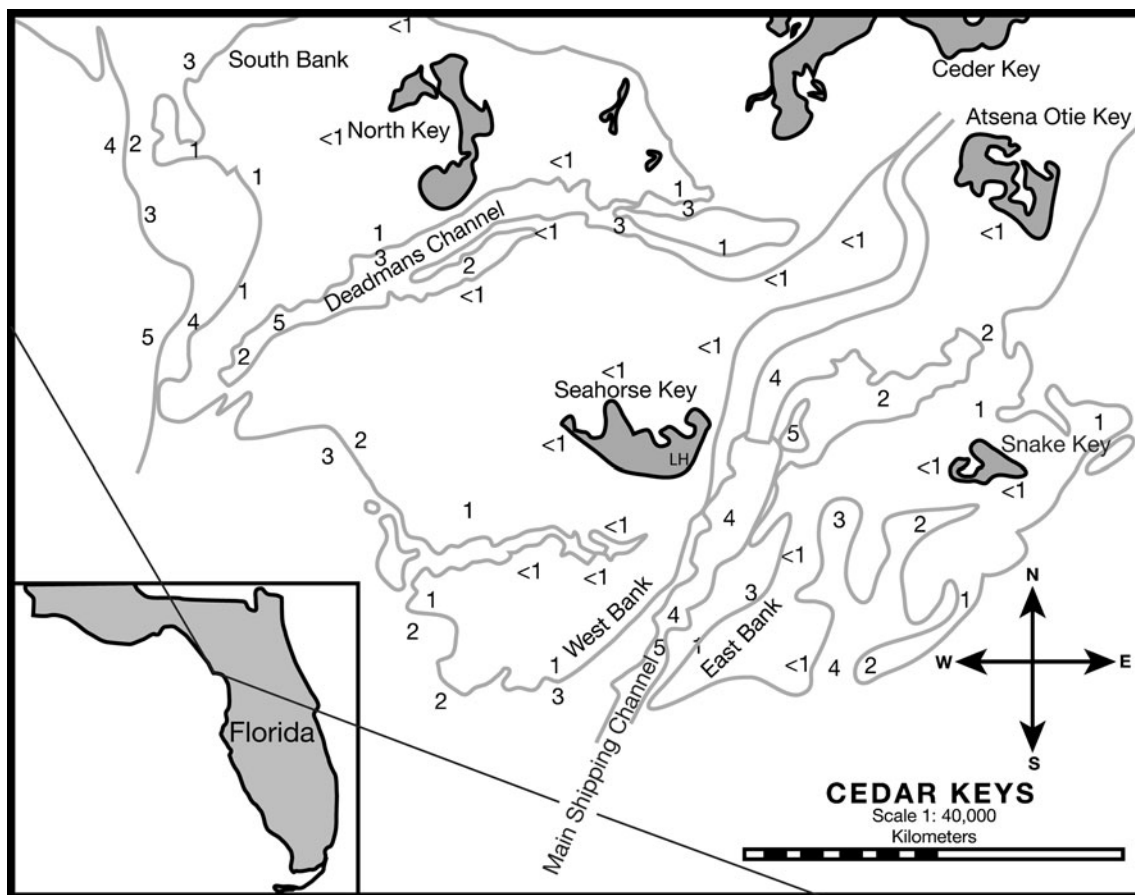


Fig. 1 Map showing the west coast of Florida and the location of Seahorse Key ($29^{\circ}5'47''$ N, $83^{\circ}3'55''$ W). The detailed map shows Seahorse Key (SHK) and the surrounding area with water depth indicated (*small numbers*) in meters above mean low water (modified from NOAA nautical chart). Unlike the other islands in the area,

which are 0.5–2.5 m above sea level at their highest points, SHK is an ancient sand dune and the center of the island rises 18 m above sea level (the location of the lighthouse). The study was conducted along the south-facing beach of SHK, from 400 m west of the lighthouse to 600 m east

Physical and Environmental Measurements

Measurements were recorded at the time of the maximum high tide, i.e., when the person taking the spawning survey passed the midpoint on the beach (directly below the SHK lighthouse). In the early years of the study (1992–2000), we recorded the following data: water temperature 10 cm below the surface 5 m from shore; air temperature at 2 m (using a shaded mercury thermometer); wave height (estimated visually as the waves began to curl onto the shoreline); and actual high tide height measured with a set of reference stakes located perpendicular to the shore line. Wind speed and direction were obtained from NOAA weather radio reports for Cedar Key at the start of each spawning survey, which closely matched our personal perception of the wind at SHK (not collected in 1992). We also recorded the moon phase and the predicted time and height for the maximum high tide from published NOAA

tide tables for Cedar Key. From 2004 to 2009, we used data from on-line databases collected from NOAA sensors at Cedar Key (NOAA Tides and Currents 2010): the time and maximum water level (above mllw) of the predicted and actual high tides; air temperature, wind direction, and velocity from meteorological data; and moon phases from astronomical data. Water temperature was available from NOAA sensors at Cedar Key in 2004–2005 but not thereafter. In 2008–2009, we installed a temperature sensor on a post 50 m from shore (but we were unable to retrieve these data after 11 March 2009).

In 1997 ($N=37$ tides) and 2000 ($N=47$), we measured the direction and flow rate of currents (General Oceanics mechanical flow meter, model 2030 with low-speed rotor) during the high tide. The flow meter was placed below the SHK lighthouse, 2–8 m from the shoreline and suspended on a line 15–16 cm below the surface of the water. The flow meter turned so that the head of the instrument faced directly into the current; we recorded the direction of flow

Table 1 The numbers of male and female horseshoe crabs spawning along a 1-km beach at Seahorse Key, FL during spring 1992–2009

Measure	1992 ^a	1993	1994	1995	1996	1997	2000	2004	2005	2008	2009
A. Total number of horseshoe crabs observed in each year of the study (paired and unpaired females and males)											
Sum crabs each year		5,796	5,730	4,580	11,864	4,913	11,404	29,359	49,806	25,852	15,760
Total number of tides	77	88	78	65	64	61	63	57	87	66	35
Crabs/tide		65.9	73.5	70.5	185.4	80.5	181	515.1	572.5	376.4	450.3
SE		12	16	15	39	26	39	135	108	105	134
Range		0–752	0–785	0–449	0–1,163	0–956	0–1,149	0–4,544	0–5,634	0–3,048	0–3,346
No. (%) of high tides without any crabs	7 (9%)	17 (19%)	26 (33%)	16 (25%)	19 (30%)	21 (34%)	10 (16%)	10 (18%)	16 (18%)	21 (32%)	1 (3%)
B. Total number of pairs observed (data set includes high tides on which at least one pair was present; number of excluded tides given in the last row)											
Sum pairs each year	2,699	2,262	1,846	1,438	3,682	1,929	3,374	10,159	14,845	8,085	6,507
No. of high tides w/ pairs	69	67	50	46	43	40	48	46	69	43	32
Pairs/tide	39.1	33.8	37.0	31.3	85.6	48.2	70.3	220.8	215.1	188.0	203.3
SE	8.0	7.3	7.6	6.6	18.5	15.4	14.1	59.3	40.8	51.6	60.1
Range	1–264	1–313	1–240	1–172	1–453	1–403	1–325	1–1,774	1–1,863	1–1,126	1–1,359
No. (%) of high tides without pairs ^b	8 (10%)	21 (24%)	28 (36%)	19 (29%)	21 (33%)	21 (34%)	15 (24%)	11 (19%)	18 (21%)	23 (35%)	3 (9%)
C. Total number of unpaired females (data set includes high tides on which at least one pair was present; number of excluded tides given in the last row of B above)											
Sum unpaired females		102	20	40	42	27	24	109	226	95	161
% total females that are unpaired		4.3	1.1	2.7	1.1	1.4	0.7	1.0	1.5	1.2	2.4
Unpaired females/tide		1.5	0.4	0.83	0.98	0.68	0.46	2.4	3.3	6.3	5.6
SE		0.23	0.14	0.18	0.25	0.24	0.12	0.95	0.69	1.32	1.72
Range		0–7	0–6	0–6	0–7	0–8	0–3	0–36	0–23	0–20	0–30
D. Total number of unpaired males (data set includes high tides on which at least one unpaired male was present; number of excluded tides given in the last row)											
Sum unpaired males		1,170	2,018	1,664	4,458	1,028	4,632	8,932	19,890	9,587	2,585
% total males that are unpaired		41	49	53	57	37	54	48	54	47	32
No. of tides with unpaired males		54	40	39	33	27	42	41	63	36	28
Unpaired males/tide		21.7	50.5	42.7	135.1	38.1	110.3	217.9	326.1	282.5	92.4
SE		3.6	11.8	8.4	21.6	11.6	23.6	49.7	57.3	75.9	26.8
Range		1–124	1–306	1–185	1–397	1–187	1–599	1–1,228	1–1,892	1–1,467	1–600
No. (%) of high tides without unpaired males ^b		34 (38%)	38 (49%)	26 (40%)	31 (48%)	34 (56%)	21 (33%)	16 (28%)	24 (27%)	30 (45%)	7 (20%)
E. Operational sex ratios (data set includes high tides on which at least one pair was present; number of tides observed and excluded is given in B above)											
% total crabs/tide that are female		42	39	35	36	43	36	37	34	39	44
SE		1	1.3	1.8	2	2	2	1.3	1.3	1.8	1.4
Range (per tide)		17–67	16–55	9–50	11–67	11–67	11–80	14–55	16–60	10–50	16–59
OSR males/females		1.45	2.07	2.10	2.18	1.51	2.36	1.86	2.30	2.16	1.36

Dates of the study are 19 March to 19 June 1992; 7 March to 8 June 1993; 10 March to 27 May 1994; 15 March to 18 May 1995; 16 March to 19 May 1996; 7 March to 9 May 1997; 4 March to 7 May 2000; 5 March to 3 May 2004; 23 February to 26 May 2005; 19 February to 17 May 2008; 9 March to 27 April 2009

^a Unpaired males and females were not recorded in this year

^b Percent is of the total number of tides observed given in A above

by this angle using a compass. To measure flow rate, we collected two readings 5 min apart, 1 h before the predicted maximum high tide (flood tide) and 1 h after the time of the predicted maximum high tide (ebb tide;

measurements were in revolutions per minute converted to centimeter per second using a calibration curve). Occasionally, measurements could not be taken due to waves or turbid water conditions.

Mark and Recapture

During each high tide between 1992 and 2000, male and female horseshoe crabs were picked up, given unique tags and placed temporarily in 7–10 plastic wading pools (1.2 m diameter, 20 cm high) that we located at 100-m intervals along the shoreline and filled with seawater (new water was added prior to each high tide). We recorded the status (paired or unpaired) of each crab as it was collected. These initial tags were 3 cm strips of embossing tape pierced with a thumb tack, which was pressed into the crab's prosoma 1–2 cm above the ventral margin directly below the eye (Cohen and Brockmann 1983; Sokoloff 1978). After the tide receded, the crabs were removed from the pools and given a second and more permanent, unique tag (Floy Tag Co. FD-94 T-bar Anchor Tag), a 30×2-mm polyolefin tube (printed with a unique number) attached to a 15-mm monofilament with a T-bar at one end. This T-bar was inserted into the inside of the carapace by puncturing a 1-mm diameter hole in the prosoma (near the location of the first tag) with the needle of the application gun and inserting the T-bar anchor tag. On days when there were few crabs on the beach, all individuals were marked. On days when many horseshoe crabs were present, we picked up spawning pairs and unattached males that were close to the pools until we reached a pre-determined number (which depended on the number of field assistants available to mark the crabs after the tide).

On each subsequent high tide, we searched for tagged crabs along the shoreline to 2 m offshore (conducted before and after the spawning survey). Considerable emphasis was placed on finding marked crabs so nearly all animals that came to the beach were checked. Tags were color coded for each year of the study so we could immediately identify crabs returning from previous years. Old tags in poor condition were replaced with new tags when necessary.

Data Analysis

We were interested in understanding two broad categories of questions: (a) the factors that influenced the presence or absence of crabs during spring high tides in which case the entire data set was used; and (b) the factors that influenced the numbers (i.e., the density per tide) and OSR (proportion of animals present that were female) of horseshoe crabs, in which case the analysis included only those high tides on which pairs were present. We treated these two problems separately because factors that influenced the presence or absence may be different from the factors that influenced density or OSR. Data from all years of the study were combined, but since there were differences among the early (1992–2000) and later (2004–2009) years of the study in the way that we collected physical and environmental measurements, we analyzed these two data sets separately for these variables (SigmaStat 2006).

Presence and absence data were analyzed using the chi-square test. We tested for normality and used non-parametric statistics when the dependent variables (number of pairs, unpaired males, unpaired females, and OSR per tide) were not normally distributed. The median is reported when the data were not normally distributed. When using the Kruskal–Wallis analysis of variance test on ranks, we have reported only those results shown to be significant by the pair-wise multiple comparison procedure (Dunn's method). Many variables co-varied, such as wind direction, wind velocity, and tidal inundation (actual high tide height). For this reason, we use multiple and logistic regression analyses to evaluate the factors that explain patterns of nesting behavior.

In both the 1992–2000 and 2004–2009 data sets, some measurements had to be converted into categorical variables to facilitate data analysis. The categories were chosen prior to analysis. The high tides within each week of spring tides were numbered consecutively and categorized into early (first seven high tides of the week) and late (tides 8–15). Actual high tide height was categorized into low (<100 cm above mllw), medium (>100 and ≤125 cm), and high (>125 cm). Wind direction was categorized into four cardinal directions, “north” was centered at 0°±45°, “east” at 90°±44°, “south” at 180°±45°, and west at 270°±44°. Water temperature was categorized into below 20°C, 20–25°C, and above 25°C. Wave height was categorized into low (<10 cm), medium (10–19 cm), and high (≥20 cm). To make the seasons comparable in various years, we set the first day of each year at 1 March.

Results

We first describe long- and short-term patterns of spawning and then the effect of a number of variables that were thought to influence horseshoe crab spawning. These results are summarized in Table 2. Because many of these variables co-vary, we use multivariate models to determine the factors that had the greatest impact on spawning patterns.

*Patterns of Spawning Activity**Patterns over Years*

In all years of the study, horseshoe crabs were present on only some high tides (3–34% of high tides had no crabs; Table 1 A). Although there were significant differences between years ($\chi^2=25.7$, $df=9$, $p=0.002$), there was no trend across the years ($R^2=0.14$, $p=0.16$) and the earlier (1992–2000) and later (2004–2009) years of the study did not differ in whether crabs were present (early: 77% of high tides crabs were present on the beach; later: 80%; $\chi^2=3.2$, $df=1$, $p=0.07$). Years differed in whether pairs ($\chi^2=18.4$, $df=$

Table 2 Variables that affect horseshoe crab spawning with significance level (*p* values given in parentheses)

Variable	Presence/absence		Numbers of horseshoe crabs (HSC) present on each high tide				Operational sex ratio		Conclusion
	Probability of any HSC present during high tide		Number of pairs ^a		Number of unpaired females ^a		Male bias ^a		
	1992–2000	2004–2009	1992–2000	2004–2009	1992–2000	2004–2009	1992–2000	2004–2009	
Year	S* (0.002) ^b NS (0.16) ^d		S* (0.001) ^c + (0.001) ^d		S* (0.001) ^c + (0.001) ^d		S* (0.001) ^c NS (0.87) ^d		More in later years
Week of season	+ (0.04) ^b		+ (0.001) ^c		+ (0.001) ^c		+ (0.001) ^c		More later in season
Day in week	NS (0.94) ^b		NS (0.18) ^c		NS (0.09) ^c		+ (0.002) ^c		More males later in week of high tides
Day vs. night	NS (0.11) ^b		NS (0.31) ^c		NS (0.72) ^c		+ (0.003) ^c		More males at night
New vs. full moon	NS (0.084) ^b		+ (0.02) ^c		NS (0.18) ^c		+ (0.02) ^c		More on new moon high tides
Actual high tide height	+ (0.001) ^b	+ (0.001) ^b	+ (0.001) ^c	+ (0.001) ^c	+ (0.001) ^c	+ (0.001) ^c	+ (0.008) ^c	NS (0.10) ^c	More when high tides are higher
Tidal current flow: flood ^f	NS (0.98) ^b	—	NS (0.60) ^b	—	NS (0.61) ^b	—	+ (0.007) ^b	—	More male-biased when flood tides are faster
Tidal current flow: ebb tides ^f	NS (0.24) ^b	—	+ (0.006) ^c	—	NS (0.19) ^c	—	+ (0.006) ^c	—	More when ebb tide currents are faster
Wind direction	+ (0.001) ^b	+ (0.001) ^b	+ (0.006) ^c	NS (0.29) ^c	NS (0.66) ^c	NS (0.11) ^c	+ (0.004) ^c	NS (0.43) ^c	More when wind is from the south
Wave height	+ (0.001) ^b	—	+ (0.04) ^c	—	+ (0.003) ^c	—	NS (0.17) ^c	—	More when waves are higher
Temperature	+ (0.001) ^b	+ (0.001) ^b	— (0.01) ^c	+ (0.001) ^c	NS (0.12) ^c	+ (0.001) ^c	NS (0.13) ^c	+ (0.001) ^c	More when water temperature is higher

“+” means that the measure of horseshoe crab activity (top row) is significantly positively correlated with the variable (left column). “—” means that the measure of horseshoe crab activity (top row) is significantly negatively correlated with the variable (left column). NS means that the relationship between the measure of spawning activity and the variable is not significant. S* means that there are significant differences between years. “—” means that no data are available for these dates

^aThe data set includes only those tides on which at least one pair was present

^bAnalyzed with chi-square test

^cAnalyzed with Kruskal–Wallis test

^dAnalyzed with linear regression across years

^eAnalyzed with Mann–Whitney test

^fData for this variable were collected only in 1997 and 2000

9, $p=0.03$; Table 1 B), unpaired females ($\chi^2=28.6$, $p<0.001$; Table 1 C), or unpaired males ($\chi^2=27.0$, $p=0.001$; Table 1 D) were present on the nesting beach, but there were no significant trends over the years (pairs: $R^2=0.05$, $p=0.53$, $N=10$; unpaired males: $R^2=0.37$, $p=0.06$, $N=9$; unpaired females: $R^2=0.1$, $p=0.38$, $N=9$). There was a significant difference in whether pairs (early: 70% of high tides pairs were present; later: 77%; $\chi^2=3.9$, $df=1$, $p=0.05$, Table 1 B) or unpaired males were present (early: 56% of high tides unpaired males were present; later: 67%; $\chi^2=7.2$, $df=1$, $p=0.01$, Table 1 D) in the early and later years of the study, but not unpaired females (early: 40.5%; later: 49%; $\chi^2=3.0$, $df=1$, $p=0.08$; Table 1 C).

The total number of horseshoe crabs observed in a year ranged from 4,580 crabs in 1995 (65 observed tides for 70.5 crabs/tide) to 49,809 (87 tides for 573 crabs/tide) in 2005 (Table 1 A). There was a significant trend toward more horseshoe crabs ($R^2=0.47$; $p=0.03$, $N=9$) and a higher number per tide ($R^2=0.77$; $p<0.001$) in more recent years. Therefore, the earlier (1993–2000) and later (2004–2009) years of the study differed significantly in total crab density (Mann–Whitney U test, $p<0.001$; early: median=7.0 crabs/tide, $N=419$; later: median=29 crabs/tide, $N=245$).

We found considerable variation in spawning density when considering only those high tides on which horseshoe crabs were present. (a) The number of pairs nesting per high tide observed varied from 31 to 221 (Table 1 B) with significantly more pairs nesting in recent years ($R^2=0.85$, $p<0.001$, $N=10$). Therefore, the later years of the study had a significantly higher density of nesting pairs than the earlier years (Mann–Whitney U test, $p<0.001$; early: median=9 pairs/tide, $N=363$; later: median=39, $N=190$). (b) The number of unpaired females per high tide varied from 0.4 to 6.3 (Table 1 C) with more unpaired females being present in the later years of the study ($R^2=0.77$, $p<0.001$, $N=9$). However, the proportion of all females that were unpaired did not change over the years ($R^2=0.09$, $p=0.40$). At least one unpaired female was present on the nesting beach on 44% of high tides when pairs were present. There was a strong correlation between the number of unpaired females present and the number of pairs present ($R^2=0.7$, $p<0.001$, $N=484$). (c) The number of unpaired males per tide varied from 22 to 326 (Table 1 D). Although unpaired males were more common in recent years ($R^2=0.51$, $p=0.02$), the proportion of all males that were unpaired did not change over the years ($R^2=0.06$, $p=0.5$; Table 1 D). In only 2.8% of tides (11 of 399) were unpaired males on the beach when no pairs were present. Unpaired male density was strongly correlated with pair density throughout the study (Fig. 2).

The OSR was male-biased on most tides (Table 1 E): on only 2.1% of high tides (10 of 484) were more females

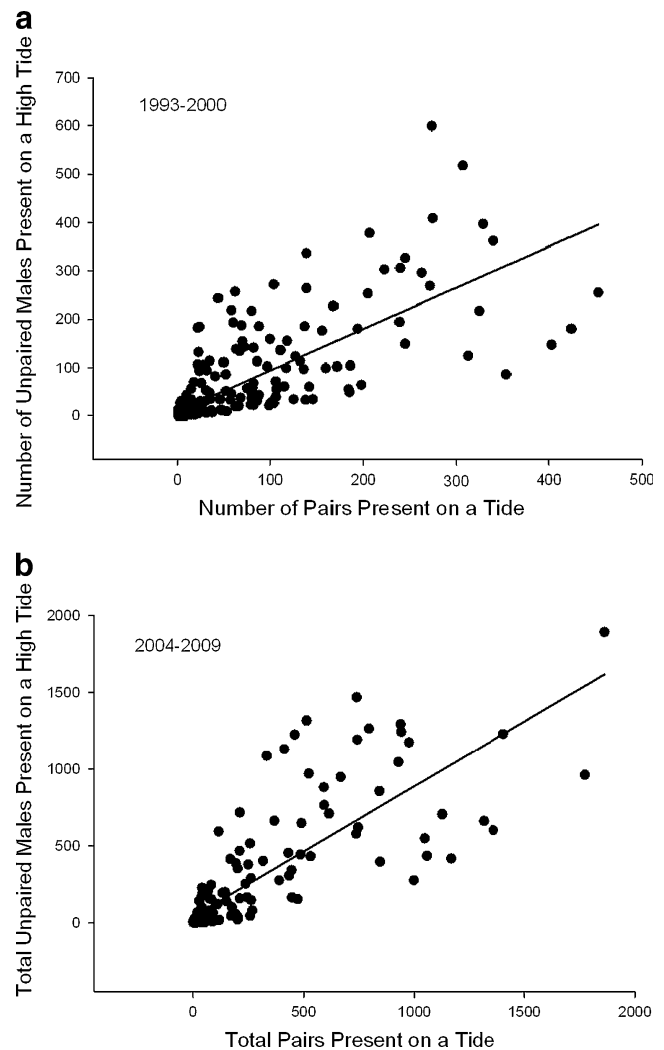


Fig. 2 The correlation between the number of pairs present on the beach during a high tide at SHK and the number of unpaired males present (only high tides with both males and females present were included). The *line* shows the linear regression. **a** 1993–2000, $R^2=0.578$, $p<0.001$, $N=294$ tides ($y=8.6+0.855x$). **b** 2004–2009, $R^2=0.656$, $p<0.001$, $N=190$ ($y=39.2+0.847x$)

present than males (5 in early years, 5 in later). The sex ratio on the beach differed significantly between years (Kruskal–Wallis test $H=44.5$, $df=9$, $p<0.001$) but did not show a trend across years ($R^2=0.01$, $p=0.87$). This means that the OSR did not differ between the early and later years of the study (Mann–Whitney U test, $p=0.28$; early: median=41% females, $N=294$ tides; later: median=39% females, $N=190$).

Changes over the Season

Across all years, there were significant differences between the weeks of a season in whether crabs were present or not ($\chi^2=15$, $df=7$, $p=0.04$) with the third week of spring tides being the most likely to have spawning horseshoe crabs.

There were also marked seasonal changes in the density of nesting pairs. The number of pairs increased each week during the first three spring tide weeks of the season (late February and March or March and early April depending on the year) and declined later in the season (significant differences between weeks, Kruskal–Wallis test, $H=112$, $df=7$, $p<0.001$; Fig. 3). The number of unpaired males also showed significant increases over the first 3 weeks and decreases over the last 3 weeks of the season ($H=58$, $df=7$, $p<0.001$). The most male-biased sex ratios occurred during the third and fourth weeks of the season (35% and 34% female) and the least biased were the first two (48% and 45%, February and March) and the last two (39% and 44%, late April and early May) weeks of the season ($H=52$, $df=7$, $p<0.001$).

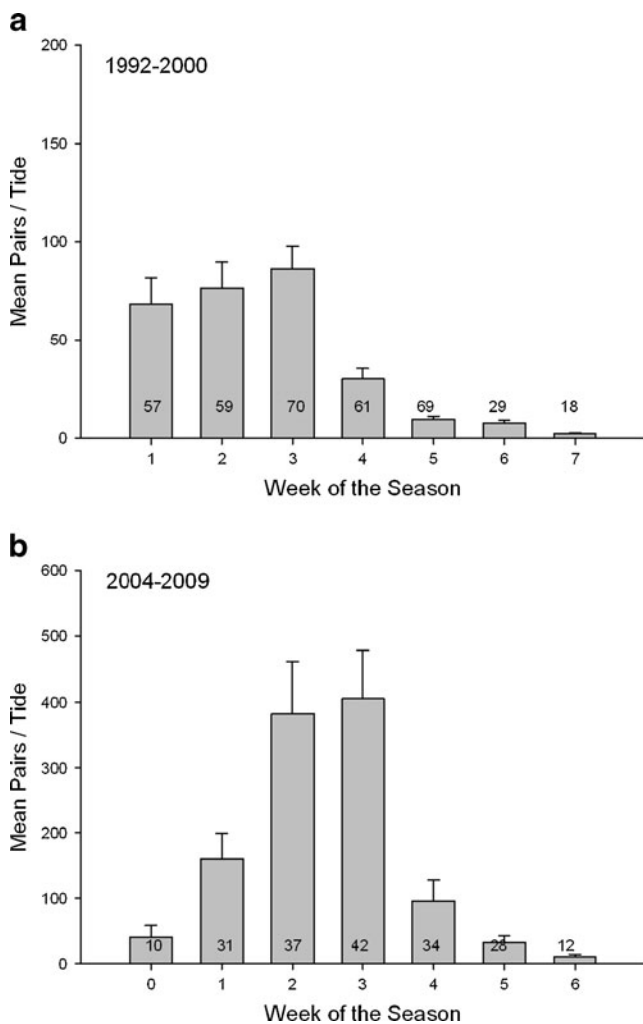


Fig. 3 Seasonal changes in the abundance of nesting horseshoe crab pairs at SHK (for high tides on which at least one pair was present; week 1 begins with the first new or full moon in March; week 2 is the next new or full moon week of high tides). **a** In 1992–2000, there are significant differences between the first three and the last 3 weeks of the season; **b** in 2004–2009, there are significant differences between weeks 2–3 and 5–6 (sample sizes are given on each bar)

Changes Within a Week of Spring Tides

Crabs were equally likely to be present at the beginning (first seven tides) and end of a week of spring tides ($\chi^2=0.01$, $df=1$, $p=0.94$). There were also no differences in nesting density early as compared with later in a week (Mann–Whitney rank sum test, $p=0.18$; first seven tides of the week: median=12, $N=319$; last 8 tides: median=20, $N=234$; Fig. 4). Throughout the study, however, unpaired male density was significantly higher later in a week (for tides on which unpaired males were found, $p=0.002$; first seven tides of the week: median=13, $N=214$; last 8 tides: median=39, $N=185$). Likewise the OSR showed a significant increase over the spring tide week (for tides with males and females present, $p<0.001$; first seven tides of the week: median=43% female, $N=274$; last 8 tides: median=35% female, $N=210$).

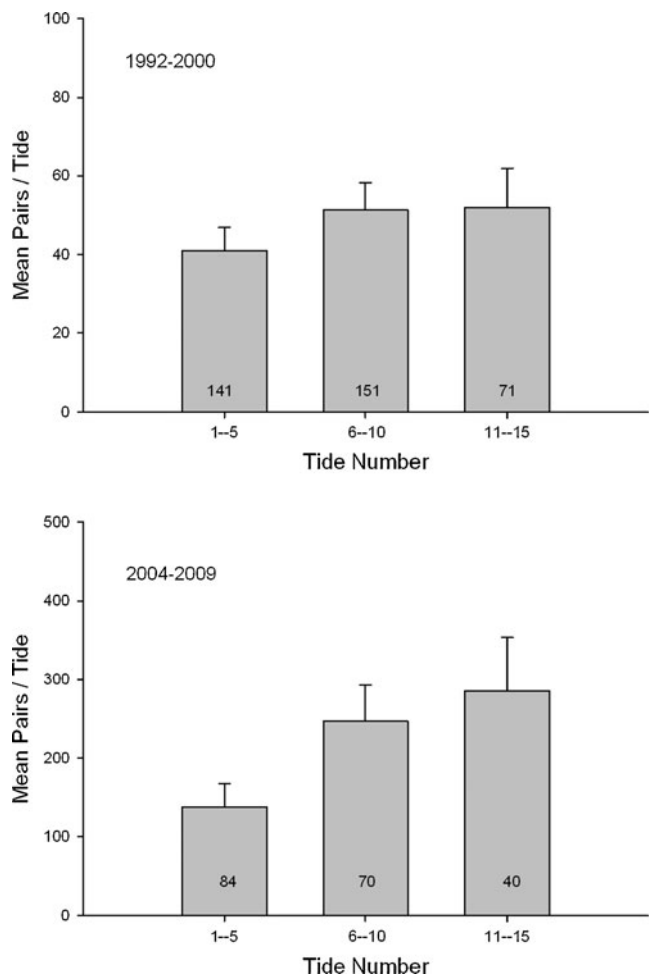


Fig. 4 Changes in the density of nesting pairs of horseshoe crabs at SHK during a week of spring tides. The first five high tides of the week are before the new or full moon, the second five high tides are at the time of the new or full moon or just after, and the third five high tides are after the new or full moon (for tides on which at least one pair was present; sample sizes are given on each bar)

Daily Patterns

Day and night tides did not differ in whether horseshoe crabs were present or not (crabs present on 79% of daytime high tides, $N=352$, and 73% of nighttime high tides, $N=312$; $\chi^2=2.6$, $df=1$, $p=0.11$). The total number of nesting pairs during the day and night high tides did not differ (Mann-Whitney U test, $p=0.31$; day: median=12; night: median=18.5) but there were more unpaired males present at night ($p=0.003$; day: median=4.5, $N=268$; night: median=17, $N=216$). This means that the OSR was significantly higher at night than in the day ($p<0.001$; day, 43% female; night, 36% female).

Factors Affecting Spawning Patterns

New and Full Moon High Tides

New and full moon high tides did not differ in whether horseshoe crabs were present or not (crabs present on 77% of full moon high tides, $N=341$, and 76% of new moon high tides, $N=323$; $\chi^2=0.04$, $df=1$, $p=0.84$). However, more pairs were present on the new than on the full moon high tides (Mann-Whitney U test, $p=0.02$; new: median=21, $N=267$; full: median=11.5, $N=286$). There were also more unpaired males present on the new than on the full moon high tides ($p=0.02$; new: median=14, $N=235$; full: median=6, $N=249$), but the OSR did not differ between the new and full moon high tides ($p=0.08$; new, 39% female; full, 42% female).

High Tide Height (Tidal Inundation)

The actual (as opposed to predicted) height of the maximum high tide affected the presence of horseshoe crabs in all years of the study. In 1992–2000, we observed nesting crabs on 59% ($N=163$) of the high tides that were under 100 cm, on 79% of tides of intermediate height ($N=276$), and on 96% ($N=52$) of tides that were over 125 cm ($\chi^2=35.9$, $df=2$, $p<0.001$). Similarly in 2004–2009, we observed nesting pairs on 35% ($N=43$) of the high tides that were under 100 cm, on 86% ($N=129$) of the high tides in the intermediate range, and on 88% ($N=73$) of the tides that were over 125 cm ($\chi^2=54.6$, $df=2$, $p<0.001$) in actual maximum high tide height.

Spawning density was also strongly correlated with actual maximum high tide height (Fig. 5). In 1992–2000, the median number of nesting pairs present was three on tides <100 cm, 10 on intermediate tides, and 37.5 on tides that were over 125 cm (Kruskal–Wallis test, $H=24.0$, $df=2$, $p<0.001$), and there was a similar pattern for unpaired males ($H=19.2$, $p<0.001$). In the 2004–2009 data set, the median number of nesting pairs present was

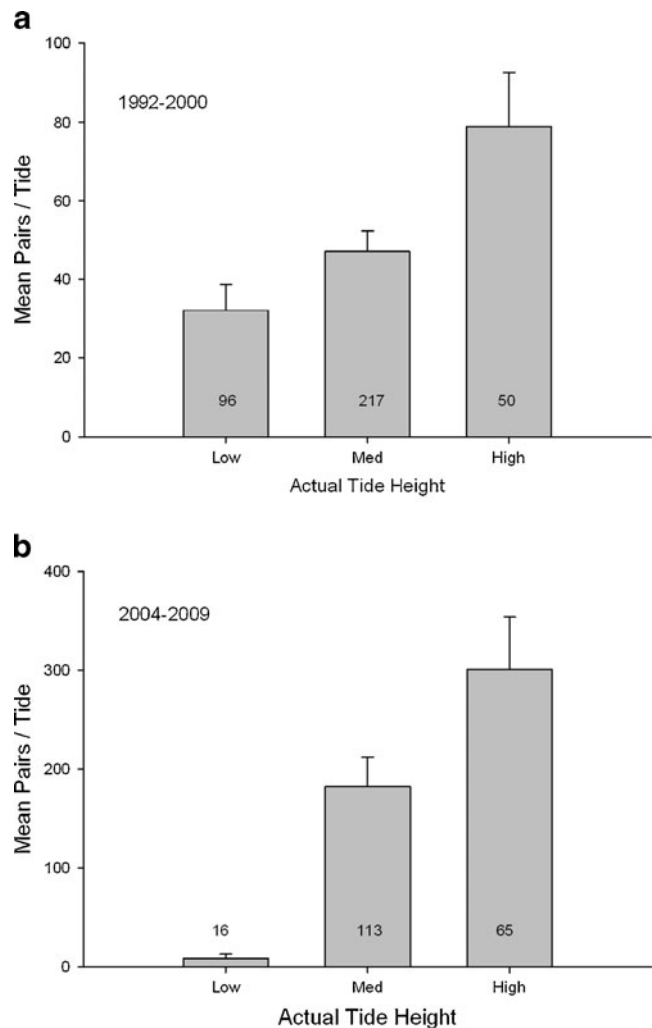


Fig. 5 a, b Changes in the density of nesting horseshoe crabs at SHK with differences in actual high tide height (above mllw). Low: tidal inundation <100 cm; medium: ≥ 100 and ≤ 125 cm; high: >125 cm (sample sizes are given on each bar)

three on tides <100 cm, 31 on medium tides, and 83 on tides that were over 125 cm (Kruskal–Wallis test, $H=20.3$, $df=2$, $p<0.001$), and there was a similar pattern for unpaired males ($H=15.8$, $p<0.001$). The OSR in 1993–2000 was more male biased on high tides of medium height (<100 cm: 50% female; medium tide height: 39% female; >125 cm: 44% female; $H=9.6$, $p=0.008$), but in 2004–2009, the OSR was not significantly more male biased on the higher tides (<100 cm: 43% female; medium tide height: 40% female; >125 cm: 36%; $H=4.6$, $p=0.1$).

Of course, the actual and the predicted maximum high tide height were correlated (1992–2000: $R^2=0.15$, $p<0.001$, $N=363$; 2004–2009: $R^2=0.30$, $p<0.001$, $N=194$), but the number of nesting pairs on a high tide was significantly predicted by the actual (1992–2000: $p=0.002$; 2004–2009: $p<0.001$) and not by the predicted (1992–2000: $p=0.5$;

2004–2009: $p=0.94$) maximum high tide height (multiple linear regression: 1992–2000: $F=5.9$, $p=0.008$; 2004–2009: $F=8.7$, $p<0.001$).

Tidal Flow Rates

Tidal currents measured 1 h before the maximum high tide flowed from west to east (flood tide) along the shoreline at a rate that varied from 2 to 30.6 cm/s (mean 11.5 ± 0.79 cm/s, $N=83$). These flow rates were not correlated with the actual maximum high tide height ($R^2=0.004$; $p=0.26$, $N=76$). Tidal flow rates measured 1 h after the maximum high tide ran from east to west (ebb tide) and varied from 2 to 28.2 cm/s (mean 9.6 ± 0.86 cm/s, $N=83$) and were correlated with actual maximum high tide height ($R^2=0.04$; $p=0.05$, $N=76$). Current flow rates did not explain variation in whether pairs were present or not (Mann–Whitney U , flood: $p=0.98$; ebb: $p=0.24$; $N=56$). However, more pairs nested when the ebb tide flow rates were faster, but there was no significant association between numbers nesting and flood tide flow rates (Kruskal–Wallis test; flood tides: $H=1.0$, $df=2$, $p=0.6$; ebb tides: $H=10.2$, $p=0.01$; Fig. 6). Similarly, more unpaired males were present when ebb tide flow rates were faster (flood: $H=3.0$, $p=0.23$; ebb: $H=16.0$, $p=0.001$) and the OSR was more male-biased (flood: $H=10.0$, $p=0.01$; ebb: $H=10$, $p=0.01$).

Wind and Waves

High tides with a north wind were significantly less likely and high tides with a south wind were more likely than predicted (i.e., predicted if nesting were independent of wind) to have nesting pairs present (χ^2 test, $df=3$; 1992–2000: $\chi^2=26$, $p<0.001$; 2004–2009: $\chi^2=22$, $p<0.001$). In 1993–2000,

crabs were present on 82% ($N=124$) of high tides with a south wind and 47% ($N=66$) of north wind high tides, and in 2004–2009, on 91% ($N=74$) of high tides with a south wind and 51% ($N=37$) of high tides with a north wind.

In 1993–2000, the number of pairs nesting on the beach (Kruskal–Wallis test, $df=3$; $H=12$, $p<0.006$) and the number of unpaired males present ($H=13$, $p<0.004$) were strongly associated with wind direction, but in 2004–2009, no association was found between wind direction and number of nesting pairs ($H=3.8$, $p<0.29$) or number of unpaired males present ($H=2.8$, $p=0.43$). Operational sex ratio was not correlated with wind direction (1993–2000: $H=5.6$, $p=0.13$; 2004–2009: $H=3.9$, $p=0.28$).

Actual high tide height was significantly higher than predicted when the wind was from the south and significantly lower than predicted when the wind was from the north (Kruskal–Wallis test, $df=3$; 1993–2000: $H=123$, $p<0.001$; 2004–2009: $H=25$, $p=0.001$). Wind direction also affected tidal current speed ($H=10$, $p=0.02$): when the wind was from the west, i.e., moving in the same direction as the current, current speed increased (mean 16.5 ± 2.2 cm/s, $N=11$), but when the wind was from the east, the incoming tide was slowed (mean 6.4 ± 1.3 cm/s, $N=9$) when compared with the north (10.4 ± 1.5 cm/s, $N=25$) or south (11.9 ± 1.2 cm/s, $N=36$) winds. The outgoing tide was not affected by wind direction ($H=1.4$, $p=0.64$).

Waves were never very high at SHK (range 0–35 cm; only 11 of 295 tides had waves over 20 cm), but the waves were significantly higher when the wind was from the south (1993–2000: $H=31$, $p<0.001$), and when wind velocities were significantly higher (1993–2000: $H=8.0$, $p=0.045$). Somewhat more pairs were observed nesting when waves were higher (Kruskal–Wallis test, $df=2$, $H=6.7$, $p=0.04$) and the presence of unpaired females was strongly associated with higher waves ($p=0.003$).

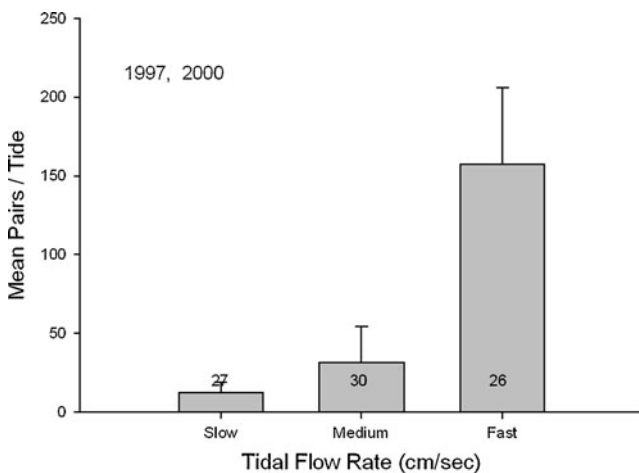


Fig. 6 Horseshoe crab nesting activity as a function of the rate of tidal current flow during ebb tides. Slow: 0–6.9 cm/s; medium: 7–15 cm/s; fast: >15 cm/s (sample sizes are given on each bar)

Temperature

Horseshoe crabs were present on the beach when water temperatures were between 11°C and 33°C, but their presence was more likely when the water temperature was above 20°C (1992–2000, 53% of tides had crabs when water temperature was below 20°C, 76% when above, $\chi^2=16.0$, $df=2$, $p<0.001$; 2004–2009, 45% when water temperatures were below 20°C, 84% when above, $\chi^2=29.6$, $df=2$, $p<0.001$). The number of pairs found nesting on a tide was correlated with temperature in 1992–2000, with fewer crabs nesting at higher temperatures (Kruskal–Wallis test, 1992–2000: $H=8.6$, $p=0.01$), but in 2004–2009, more pairs nested ($H=22$, $df=2$, $p<0.001$), more unpaired males were present ($H=27$, $p<0.001$), and sex ratios were more male-biased ($H=17$, $p<0.001$) when temperatures were higher. The coldest water temperatures occurred when the wind

was from the north, air temperature was low, and actual maximum tidal inundation was low (<100 cm).

Effect of Multiple Physical and Environmental Variables

The variables associated with spawning behavior (Table 2) co-vary so multivariate analyses are required. A logistic regression model for the presence or absence of nesting crabs during a high tide as the dependent variable included actual high tide height as the most important coefficient in both 1992–2000 ($p < 0.001$) and 2004–2009 ($p < 0.001$) data sets, with tide number (whether a high tide was early or late in a week of spring tides) as a second significant variable ($p < 0.005$). The presence or absence of unpaired males during a tide was predicted by actual high tide height and tide number as the most important coefficients in both 1993–2000 and 2004–2009. A multiple linear regression shows that nesting density can be predicted from a linear combination of the independent variables: actual high tide height, week of the season, and tide number. Much of the variation in unpaired male density was explained by the total number of pairs on the beach (logistic regression $R^2 = 0.49$), but additional variation was explained by the actual high tide height and tide number.

Patterns of Repeated Spawning by Individuals Returning to the Same Nesting Site

Within a given year, 31–60% of females and 45–67% of males returned to the beach at least once after being marked (Table 3). Of these, 40–76% of females and 26–61% of males were seen only once. Males returned to the nesting beach more frequently than females in all 7 years of the marking study (Table 3). Many females (41–87%) and males (15–77%) were seen only during 1 week of spring tides (Table 4).

Of the 8,633 animals that were tagged between 1992 and 1997 (Table 5), 1,132 or 13% were re-sighted in another year (from 1993 to 2000). In 1997, we observed two animals marked in 1992; in 2000, we observed 11 previously marked animals: 2 had been marked in 1995, 5 in 1996, and 4 in 1997. This means that no animals were recaptured across more than 6 years. Most (88%) of these individuals were seen only twice (the year marked and one additional season spanning 1 to 4 years), but some were observed three times (9.8%) across three to five seasons and a few (1.4%) were observed four times across 4 to 6 years and three (0.2%) were seen five times across 5 to 6 years. A number of animals (209 or 18.4%) were not seen in consecutive years, but females and males did not differ in this respect ($\chi^2 = 2.68$, $df = 1$, $p = 0.11$). Males and females did not differ in the span of years over which they were re-sighted (Mann–Whitney U test, $p = 0.23$) or in the number of years in which they were seen ($p = 0.61$).

Discussion

Patterns of Spawning Activity

Patterns Across Years

The first 7 years of surveys at SHK reveals that the spawning population size was stable, but after 2000, the numbers increased markedly (Table 1). Since we used the same survey procedures throughout the study, methodological explanations for this increase are unlikely. In some years, we may have missed some spawning animals. For example, in 1997, large numbers were present during the first week of the study suggesting that the horseshoe crabs may have spawned earlier in that year (and fewer total animals were observed that year). Horseshoe crabs may also spawn during neap tides (when we do not conduct surveys) if there are exceptionally high tides due to offshore storms, which may account for some of the variation in total numbers (this was likely in 2009). But our results show that the number of tides without crabs did not differ between the earlier and later years of this study (Table 1 A); rather, it was the spawning densities of horseshoe crabs at SHK that increased over the years. We can speculate that beach erosion and shoreline development in the nearby Cedar Key area may have resulted in more animals nesting at SHK (which is part of a National Wildlife Refuge and therefore not developed) although casual observation suggests that few animals move between the two nesting beaches. Another possibility is that after a ban on fishing with entangling nets was implemented in July 1995 (Adams et al. 2000), the number of horseshoe crabs may have increased. Large numbers of horseshoe crabs were caught in these nets and had to be forcibly removed, which resulted in considerable damage and mortality to the animals. Furthermore, following the net ban, a thriving commercial clam aquaculture industry has developed in the Cedar Keys area (Colson and Sturmer 2000). The clams are reared in net bags to an adult size and they usually spawn before they are harvested, which means there are now probably large numbers of free-living clams in the area. Horseshoe crabs are known to eat adult and juvenile hard clams, including *Mercenaria mercenaria* (Botton 2009; Botton and Shuster 2003), so clam farming of this species might have increased the amount of food available to adult and juvenile horseshoe crabs in the area and this might have resulted in an increased population size since 2000 at SHK. Our results raise the important point that even unharvested populations of horseshoe crabs may fluctuate from 1 year to the next.

A few unpaired females were often found on the beach among the spawning pairs during high tide. These females are often in poor condition and do not lay eggs (Brockmann

Table 3 The number of individually marked horseshoe crabs that returned to the nesting beach at SHK within a year

	1992	1993	1994	1995	1996	1997	2000
Females							
Total no. marked	659	876	428	383	576	325	606
No. (%) returning after marking	375 (57%)	442 (50%)	216 (50%)	229 (60%)	333 (58%)	146 (45%)	186 (31%)
Mean number of times females returned (mean ± SE) ^a	2.0±0.06	2.1±0.07	1.9±0.08	1.1±0.05	1.8±0.06	1.8±0.06	1.4±0.06
% observed once ^a	43	40	42	45	50	56	76
% observed twice ^a	33	30	34	30	30	27	17
% observed three or more times ^a	24	30	24	25	20	17	7
Males							
Total no. marked	877	1,038	739	1,105	1,041	586	1,176
No. (%) returning after marking	559 (64%)	462 (45%)	389 (53%)	643 (58%)	702 (67%)	292 (50%)	549 (47%)
Mean number of times males returned (mean ± SE) ^a	2.5±0.07	2.4±0.08	2.9±0.09	1.6±0.06	2.9±0.08	2.0±0.06	1.6±0.04
% observed once ^a	35	36	31	33	26	43	61
% observed twice ^a	24	26	25	25	24	28	25
% observed three or more times ^a	41	38	44	42	50	29	14
Males and females differ in their probability of returning ^b	0.008 ^{c, d}	0.01 ^{d, e}	0.51	0.63	<0.001 ^{c, d}	0.18	<0.001 ^{c, d}
Males return more often than females ^f	<0.0001 ^d	0.02 ^d	<0.0001 ^d	<0.0001 ^d	<0.0001 ^d	0.02 ^d	<0.001 ^d

^a Based on those that returned at all (% returning after marking in second row)

^b Chi-square test probability, *df*=1

^c Males more likely to return than females

^d Indicates significant differences

^e Females more likely to return than males

^f Mann–Whitney *U* test probability

1990). The number of unpaired females increases as the number of pairs on the beach increases, but they were particularly common when waves were higher. Our data support the view that the unpaired females are those that have lost their attached males. This is particularly likely to occur in rough weather when pairs are overturned in the surf as they travel to the beach.

The OSR at SHK is usually male-biased (Fig. 2) due to unpaired males that join the attached spawning pairs. These males engage in sperm competition with the attached male and with other unpaired males that surround the spawning pair and they can fertilize a high proportion of the eggs laid by the female (Brockmann et al. 1994, 2000). Our results suggest that the male bias in spawning numbers is due to

Table 4 The proportion of individually marked horseshoe crabs that were observed at SHK only during 1 week of spring tides on which they were marked

Year marked	Females		Males		χ^2 test
	Number returning after marking	% (no.) re-sighted only during 1 week of spring tides	Number returning after marking	% (no.) re-sighted only during 1 week of spring tides	
1992	375	59% (222)	559	52% (289)	4.8, <i>p</i> =0.03 ^a
1993	442	50% (222)	462	69% (319)	32, <i>p</i> <0.0001 ^b
1994	216	70% (151)	389	61% (236)	4.8, <i>p</i> =0.03 ^a
1995	229	75% (172)	643	54% (350)	29, <i>p</i> <0.0001 ^a
1996	333	41% (136)	702	15% (103)	86, <i>p</i> <0.0001 ^a
1997	146	73% (106)	292	73% (212)	0, <i>p</i> =0.91
2000	186	87% (162)	549	77% (424)	7.8, <i>p</i> =0.005 ^a

^a Females significantly more likely than males to return during only 1^o week of spring tides

^b Males significantly more likely than females to return to the beach during only week of spring tides

Table 5 The number of horseshoe crabs marked in a year and the number of years over which those animals were re-sighted, for example, 1,536 individuals were marked in 1992 and two of those animals were re-sighted in 1997 (some individuals were recaptured multiple times but this table shows the years spanned by those observations)

Year marked	No. marked	Years spanned by observations						Total recaptured
		1993	1994	1995	1996	1997	2000	
1992	1,536	169	39	14	2	2	0	226
1993	1,914	–	258	71	33	13	0	375
1994	1,167	–	–	79	57	22	0	158
1995	1,488	–	–	–	180	53	2	235
1996	1,617	–	–	–	–	129	5	134
1997	911	–	–	–	–	–	4	4

males returning to the nesting beach more frequently than females (Table 3) rather than being due to a population-wide male bias. As the number of nesting pairs increased over the years of this study, the number of unattached males also increased proportionally (i.e., the OSR remained the same). Similar correlations between the number of unpaired males and nesting pair density have been found in most (Brockmann 1996; Brockmann and Penn 1992; Brockmann and Smith 2009; Rudloe 1980; Smith et al. 2002b) but not all horseshoe crab populations (Hanna 2001; Schaller 2002). The mean OSR at SHK (1.5–2.4 males/female; Table 1) is similar to other unharvested populations in Maine (Schaller 2002), Massachusetts (James-Pirri et al. 2005), Connecticut (Long Island Sound; Mattei et al. 2010), South Carolina (Wenner and Thompson 2000), and the Florida panhandle (Rudloe 1980) and much less male-biased than harvested populations in Massachusetts (Carmichael et al. 2003; James-Pirri et al. 2005) or DE Bay (Shuster and Botton 1985; Smith et al. 2002b) where the mean OSR is normally 3–6 males/female, presumably due to female-biased harvesting (Kreamer and Michels 2009). Our results suggest that a male-biased OSR that averages 1.5–3 males per female is the norm for unexploited populations of horseshoe crabs.

Changes over the Season

Horseshoe crab spawning activity at SHK is clearly seasonal. The density of nesting pairs and the number of unattached males increase from late February through March into April and decline in May and June (Fig. 3). A few horseshoe crabs continue to spawn at SHK during July and August and there is a smaller peak of spawning in the autumn (Cohen and Brockmann 1983). Prior to 1998, horseshoe crabs were regularly caught in nets by fishermen during February and early March before they appeared on the shoreline (Henry Coulter, pers. comm.) but not from November to early February, suggesting that they are not active during this period. This means that horseshoe crab breeding is seasonal in north Florida (Rudloe 1980), which is consistent with breeding patterns farther north (Baptist et al. 1957; Barlow et al. 1986; Botton and Ropes 1987; James-Pirri et al. 2005;

James-Pirri 2010; Leschen et al. 2006; Moore and Perrin 2007; Schaller et al. 2010; Shuster 1979; Sokoloff 1978; Thompson 1998; Watson et al. 2009) but at odds with data from south Florida (Ehlinger et al. 2003) and the Yucatan (Britton and Morton 1989; Zaldivar-Rae et al. 2009) where horseshoe crabs breed throughout the year. The trigger for the seasonal spawning migration may be photoperiod (Cohen and Brockmann 1983; Ehlinger et al. 2003; Penn and Brockmann 1994; Watson et al. 2009) or a combination of photoperiod and temperature, since the animals are not active when water temperatures were below 10°C (Schaller et al. 2010; Watson et al. 2009). At SHK, most spawning occurs at water temperatures above 20°C. It is known that horseshoe crab eggs and larvae do not develop at temperatures below 15°C (Brown and Clapper 1981; French 1979), which may provide an explanation for the seasonality. However, embryos and larvae are very tolerant of environmental extremes, and once they are returned to normal temperatures, they will continue to develop normally (Botton et al. 1992; Jegla and Costlow 1982).

Spawning Within a Week of Spring Tides

Although there is no change in the density of nesting pairs over a week of spring high tides, there is a pattern to the number of unpaired males visiting the spawning beach: fewer unpaired males appear early in the week and more appear later. This means that the operational sex ratio becomes more male-biased on the later high tides of a spring tide week. This result is consistent with the view that males that did not attach to females switched from searching for females offshore to searching for pairs onshore (Brockmann 1996, 2002) toward the end of a spring tide week when most available females were either already paired or had already completed their egg laying.

Diurnal Patterns

Horseshoe crabs breed during both day and night high tides, a pattern that has been described for other sites (Barlow et al. 1986; Chabot et al. 2007; Rudloe 1980).

However, we have found that unpaired males at SHK are somewhat more likely to come to the beach at night than during the day even though no more pairs were present. Unpaired males locate spawning pairs onshore using both visual and chemical cues even under new moon conditions (Hassler and Brockmann 2001; Schwab and Brockmann 2007; Saunders et al. 2010). Unpaired males are on average in worse condition than attached males (Brockmann and Penn 1992; Duffy et al. 2006; Wasserman and Cheng 1996), and they are more likely to be overturned by waves or other crabs and less likely to right themselves once overturned (Penn and Brockmann 1995). Nocturnal spawning may be less risky than daylight activity for these males due to lower waves at night and reduced desiccation and lower predation by gulls if they are overturned.

Factors Affecting Spawning Patterns

Horseshoe crab spawning is well known for its variability and for its strong association with the new and full moon: in Massachusetts (Barlow et al. 1986), DE Bay (Smith et al. 2002b) and Apalachee Bay, Florida (Rudloe 1979; Rudloe 1980), horseshoe crabs nested on the new and full moon high tides. For this reason, it is widely assumed that nesting is triggered by tidal or lunar rhythms. At SHK, where nesting clearly occurred on the new and full moon high tides, we found that spawning was more closely associated with actual high tide height than with moon phase, predicted high tide height or tidal flow rates. Wind direction and wind speed correlate with spawning, but wind direction and speed strongly influence high tide water levels at this site (a south wind causes higher than predicted and a north wind causes lower than predicted tidal inundation), so we conclude that it is actual high tide height and not wind that influences spawning density. Multivariate analyses support the conclusion that actual maximum high tide height is the most important factor in determining whether horseshoe crabs will spawn or not and in affecting the number of spawning pairs and unpaired males and females present on the beach.

In other areas, the evidence for the role of water depth in affecting spawning density is mixed. In Massachusetts where tidal inequalities occur, horseshoe crabs prefer to nest on the higher of the two high tides in a 24-h period (Barlow et al. 1986) and nesting activity was roughly correlated with the difference in height between the two tides. In New Hampshire, water depth was found to have the greatest impact on horseshoe crab activity (Watson and Chabot 2010; Watson et al. 2009). Horseshoe crabs were found to entrain their daily activity to artificial tides in which water depth was increased by only 20 cm (Chabot et al. 2008). In St. Joe Bay in northwest Florida (Gulf County), which is microtidal (regular tidal inundation does not occur),

horseshoe crab nesting was correlated with water depth and not phases of the moon (Rudloe 1985). However, in a population of horseshoe crabs from the Indian River Lagoon, a microtidal environment in southeast Florida (Brevard County), spawning was episodic and not associated with environmental changes (Ehlinger and Tankersley 2007; Ehlinger et al. 2003). A study from Cape Cod reports no differences in spawning activity from mid-May to mid-June “regardless of lunar phase” (Leschen et al. 2006), but actual water levels were not measured. In the DE Bay, Smith et al. (2002b) concluded that “tide height alone was a poor predictor of spawning activity”, but this conclusion was based on the predicted and not on the actual maximum water level attained during each spawning survey. At Cedar Key, at least, NOAA predicted and actual maximum water levels were not tightly correlated (2004–2009: $R^2=0.31$, $N=249$, $p<0.001$). Our results suggest that even in areas where tidal inundation is much greater than in Florida, spawning may be influenced by actual water depth.

In DE Bay, spawning activity is affected by wave height with fewer crabs nesting when waves exceed 30 cm (Smith et al. 2002b; Thompson 1998). This is presumably due to the risk associated with nesting in the high intertidal (Botton 1993; Botton and Loveland 1989; Duffy et al. 2006; Penn and Brockmann 1995). At SHK, wave height does not correlate with spawning density, except insofar as water depth tends to be higher when waves are higher (this occurs when the wind is from the south). However, the beach at SHK is lower energy than the beaches of DE Bay, and during our study, very few high tides had waves over 30 cm.

When high tide water levels are higher, horseshoe crabs nest higher on the beach. Evidence suggests that nesting height affects reproductive success, particularly for crabs that live in areas with low tidal amplitude (Penn and Brockmann 1994). In Florida, higher water levels (i.e., greater tidal or wind-driven inundation) allow the crabs to nest higher (Penn 1992) and thus to avoid anoxic sediments where their eggs do not develop. On wider, higher energy beaches with greater tidal fluctuations, which occur in DE Bay, oxygen levels across the beach do not differ, but temperature varies and the lower temperatures at the bottom of the beach retard egg development (Jackson et al. 2008). Lower parts of the beach also have an increased risk of egg loss due to erosion. In areas with greater tidal inundation, horseshoe crabs may spawn on neap high tides (Barlow et al. 1986), but in Florida, they rarely do (Cohen and Brockmann 1983; Rudloe 1980). This can be explained by differences in tidal inundation: in Florida, neap tides rarely rise above the anaerobic zone at the bottom of the beach slope, but farther north, neap tides may be high enough to ensure egg development. By waiting to migrate toward shore until the water reaches a minimum depth (Shuster and Botton 1985), crabs avoid areas with reduced egg development.

If we are to understand the factors that affect variation in spawning numbers in different populations, then actual high tide height (water depth) needs to be measured. These measurements need to be made at the sites where the spawning surveys are taking place since small differences in geography can alter tidal inundation in unpredictable ways (NOAA 1992–2000). Further, the factors that influence tidal inundation, including wind direction and velocity, wave height and barometric pressure also need to be measured. Given the variability, reliable measures of spawning densities require multiple spawning surveys (Smith et al. 2002b) and should focus on the number of females present, since male densities are closely correlated with numbers of pairs.

Patterns of Repeated Spawning by Individuals Returning to the Same Nesting Site

No SHK horseshoe crabs were found more than 6 years after tagging (Table 5). This estimate of adult lifespan could have been affected by tag loss, but we used two tags and many animals were seen repeatedly and re-tagged each year, so 6 years may be a reasonable measure of adult survivorship in this population. Since all individuals were mature when they were marked, these data provide an estimate of adult life span in a Florida population, an estimate that is similar to that found for horseshoe crabs in Plum Island Sound, Massachusetts (5–7 years, Ropes 1961) and DE Bay (8 years, Botton and Ropes 1988; 5–10 years, Swan 2005). The lower re-sighting rate in 2000 (0.4% of animals re-sighted after 3 years) compared with previous years (3.6% of animals re-sighted after 3 years of those marked in 1994 and 1993, 0.9% of those marked in 1992) is probably due to the lack of re-tagging between 1997 and 2000 (Table 5). Although males return to the beach more often than females during a season (Table 3), they do not return across more years nor live longer as adults than females. Since juvenile male horseshoe crabs molt into adults at a younger age than females (Smith et al. 2009), our results suggest that the total lifespan of males may be shorter than that of females.

Many horseshoe crabs at SHK return to nest on the same beach year after year. This study did not include an analysis of other beaches in the area, although occasional and haphazardly collected observations suggest that SHK was the largest and most active spawning beach in the area. A few individuals that were marked at SHK were found on these beaches (12 at Snake Key, 2 km southeast of SHK; 6 at North Key, 2 km north; 21 at Atsena Otie Key, 2 km east; and 10 on Cedar Key, 3 km northeast) so we know that individuals marked at SHK do not nest exclusively at SHK. No horseshoe crabs marked on other beaches were ever found at SHK, but the numbers were small (<20/year). A

systematic survey of the region is needed to determine the extent of beach fidelity and whether there are differences among sites in the pattern of nesting, as others have observed in DE Bay and Cape Cod (James-Pirri et al. 2005; James-Pirri 2010; Smith et al. 2002b, 2010).

Individual males and females differ in their spawning patterns. Most females are re-sighted on average once or twice (of those that return at all; Table 3) whereas males return three or more times during one spring tide week either unpaired or attached to the same or different females (Brockmann 1992, 2002). Further, in 5 of 7 years, males were significantly more likely than females to return across multiple weeks (Table 4), a pattern that has been noted in other populations (Leschen et al. 2006). These observations are consistent with calculations that suggest females can lay the 60,000 mature eggs they carry in about 4 h of nesting (Leschen et al. 2006; Shuster and Botton 1985; Sokoloff 1978). Females from different populations differ in size (Brockmann and Smith 2009) and larger females lay more eggs (Leschen et al. 2006), but the SHK horseshoe crabs are only slightly smaller than those that Leschen et al. (2006) studied so these are reasonable estimates for SHK females. While it is quite possible that some females nested on other beaches in the area, there is no reason to believe that females are more likely to do so than males. On the contrary, males are known to range over greater distances than females (King et al. 2005). We think it likely then that many (50–87%) females complete their nesting for the year within 1 week of spring tides (Brousseau et al. 2004; James-Pirri 2010; Schaller et al. 2010).

Conclusions

Although SHK horseshoe crabs are genetically distinct from Florida east coast and more northern populations (King et al. 2005), the patterns of nesting that we have observed are similar to those that have been observed in other unharvested populations. We have shown that spawning density can undergo substantial changes from 1 year to the next and from one high tide to the next. Horseshoe crab spawning is strongly seasonal with maximum numbers during the third or fourth week of the season (late March and early April). The presence and density of spawning horseshoe crabs is strongly associated with actual maximum high tide height. Secondary factors include season and whether it is early or late in a week of spring high tides. Wind direction and speed influence numbers through their effect on high tide height. Females usually arrive on the beach paired with a male, but unpaired females sometimes occur. Their numbers are in proportion to the number of pairs present, but there are particularly large numbers of unpaired females when waves are higher and pairs are

overturned in the surf. The number of unpaired males is also strongly influenced by the number of nesting pairs and secondarily by whether it is early or late in a spring tide week (more unpaired males at the end of a week). There are normally many more males present during spawning than females (1.5 to 2.4 males/female) due to the fact that individual males visit the beach more frequently than females. Males and females returned to the beach at SHK for up to 6 years suggesting that this is their adult lifespan.

Our results have implications for the way in which spawning surveys are conducted and the conclusions that can be drawn from those surveys. Spawning surveys have been used in DE Bay and elsewhere to estimate breeding population size. Our tagging study suggests that individual females and males return to the same beach repeatedly within a breeding season (Table 3), so a spawning survey that does not mark animals will overestimate the actual numbers. But spawning surveys do not capture all animals: some have left before the survey begins and some arrive after it has ended. We also show that horseshoe crab spawning is strongly seasonal, and there are changes in spawning density over a week of spring tides. This means that the timing of a survey is critical for obtaining reliable measures of spawning density and operational sex ratio. Our results also suggest that even in areas where tidal inundation is much greater than in Florida, spawning may be influenced by actual water depth. Since spawning was more closely associated with actual high tide height than with moon phase or predicted tide height, the timing of the surveys should take these factors into account. Furthermore, horseshoe crabs nest on the SHK beach in a consistent manner: the last 100 m on the west end and the last 200 m on the east end of the beach had consistently fewer nesting crabs than the center (Hassler 1999). In fact throughout this study, 82% of pairs nested in the center 600 m of the beach. Similar preferences for certain portions of the beach have been observed elsewhere (Barlow et al. 1986; Botton et al. 1988). This means that spawning densities may vary depending on the scale at which measurements are taken as Smith and Michels (2006; Smith et al. 2002a, b) have discussed.

The results reported here also have implications for horseshoe crab management. First, if the large increase we observed in the number of spawning animals at SHK reflects an increase in population size, as we suspect, then even unharvested populations of horseshoe crabs may fluctuate considerably from 1 year to the next. Further, if the change in numbers at SHK was due to decreased horseshoe crab bycatch after the net ban (since 1995) and/or the Cedar Key clam farming (since 1997), as we suggest, then it appears that this population has responded quickly to changes in mortality or food supply. Second, our results suggest that a male-biased OSR of 1.5–3 males/female is

the norm for unexploited populations of horseshoe crabs. More extreme biases, such as what occur in DE Bay and Cape Cod, likely result from males remaining inshore longer than females or from differential mortality such as preferential harvesting of females. Third, if many females complete their nesting in 1 week, as we suggest, then restrictions that close beaches during even some parts of the breeding season will likely protect a portion of the breeding population in that year. Finally, the observed patterns of returning to the beach by male and female horseshoe crabs mean that one cannot use standard mark–recapture techniques on spawning horseshoe crabs to estimate population size because the assumptions about recapture rates will be violated. Rather, one has to mark animals prior to the breeding season (Smith et al. 2006) or use non-breeding animals (Hata and Berkson 2003) or genetic techniques (Faurby et al. 2010) to estimate population size.

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References

- Adams, C., S. Jacob, and S. Smith. 2000. *What happened after the net ban?* Gainesville: Food and Resource Economics Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.
- ASMFC. 1998. *Interstate fishery management plan for horseshoe crabs*. Washington: Atlantic States Marine Fisheries Commission.
- ASMFC. 2008. *Addendum V to the Interstate fishery management plan for horseshoe crab*. Washington: Atlantic States Marine Fisheries Commission.
- Baptist, J. P., O. R. Smith, and J. W. Ropes. 1957. Migrations of the horseshoe crab, *Limulus polyphemus* in Plum Island Sound, Massachusetts. United States Fish and Wildlife Service Special Scientific Report—Fisheries No. 220:1–15
- Barlow Jr., R.B., M.K. Powers, H. Howard, and L. Kass. 1986. Migration of *Limulus* for mating: Relation to lunar phase, tide height, and sunlight. *Biological Bulletin* 171: 310–329.
- Berkson, J., and C.N. Shuster. 1999. The horseshoe crab: The battle for a true multiple-use resource. *Fisheries* 24: 6–10.

- Botton, M.L. 1993. Predation by herring gulls and great black-backed gulls on horseshoe crabs. *Wilson Bulletin* 105: 518–521.
- Botton, M. 2009. The ecological importance of horseshoe crabs in estuarine and coastal communities: A review and speculative summary. In *Biology and conservation of horseshoe crabs*, ed. J. T. Tanacredi, M. Botton, and D.R. Smith, 45–63. New York: Springer.
- Botton, M.L., and R.E. Loveland. 1989. Reproductive risk: High mortality associated with spawning by horseshoe crabs (*Limulus polyphemus*) in Delaware Bay, USA. *Marine Biology* 101: 143–151.
- Botton, M.L., and J.W. Ropes. 1987. Populations of horseshoe crabs, *Limulus polyphemus*, on the Northwestern Atlantic Continental Shelf. *Fisheries Bulletin* 85: 805–812.
- Botton, M.L., and J.W. Ropes. 1988. An indirect method for estimating longevity of the horseshoe crab (*Limulus polyphemus*) based on epifaunal slipper shells (*Crepidula fornicata*). *Journal of Shellfish Research* 7: 407–412.
- Botton, M.L., and C.N. Shuster Jr. 2003. Horseshoe crabs in a food web: Who eats whom? In *The American horseshoe crab*, ed. C. N. Shuster, R.B. Barlow, and H.J. Brockmann, 133–153. Cambridge: Harvard University Press.
- Botton, M.L., R.E. Loveland, and T.R. Jacobsen. 1988. Beach erosion and geochemical factors: Influence on spawning success of horseshoe crabs (*Limulus polyphemus*) in Delaware Bay. *Marine Biology* 99: 325–332.
- Botton, M.L., R.E. Loveland, and T.R. Jacobsen. 1992. Overwintering by trilobite larvae of the horseshoe crab *Limulus polyphemus* on a sandy beach of Delaware Bay (New Jersey, USA). *Marine Ecology Progress Series* 88: 289–292.
- Britton, J.C., and B. Morton. 1989. *Shore ecology of the Gulf of Mexico*. Austin: University of Texas Press.
- Brockmann, H.J. 1990. Mating behavior of horseshoe crabs, *Limulus polyphemus*. *Behaviour* 114: 206–220.
- Brockmann, H.J. 1992. The effects of age on male behavior in *Limulus polyphemus*. *Galaxea* 11: 61–62.
- Brockmann, H.J. 1996. Satellite male groups in horseshoe crabs, *Limulus polyphemus*. *Ethology* 102: 1–21.
- Brockmann, H.J. 2002. An experimental approach to altering mating tactics in male horseshoe crabs (*Limulus polyphemus*). *Behavioral Ecology* 13: 232–238.
- Brockmann, H.J. 2003a. Nesting behavior: A shoreline phenomenon. In *The American horseshoe crab*, ed. C.N. Shuster, R.B. Barlow, and H.J. Brockmann, 33–49. Cambridge: Harvard University Press.
- Brockmann, H.J. 2003b. Male competition and satellite behavior. In *The American horseshoe crab*, ed. C.N. Shuster, R.B. Barlow, and H.J. Brockmann, 50–82. Cambridge: Harvard University Press.
- Brockmann, H.J., and D. Penn. 1992. Male mating tactics in the horseshoe crab, *Limulus polyphemus*. *Animal Behaviour* 44: 653–665.
- Brockmann, H.J., and M.D. Smith. 2009. Reproductive competition and sexual selection in horseshoe crabs. In *Biology and conservation of horseshoe crabs*, ed. J.T. Tanacredi, M. Botton, and D.R. Smith, 199–221. New York: Springer.
- Brockmann, H.J., T. Colson, and W. Potts. 1994. Sperm competition in horseshoe crabs (*Limulus polyphemus*). *Behavioral Ecology and Sociobiology* 35: 153–160.
- Brockmann, H.J., C. Nguyen, and W. Potts. 2000. Paternity in horseshoe crabs when spawning in multiple-male groups. *Animal Behaviour* 60: 837–849.
- Brousseau, L.J., M. Sclafani, D.R. Smith, and D.B. Carter. 2004. Acoustic-tracking and radio-tracking of horseshoe crabs to assess spawning behavior and subtidal habitat use in Delaware Bay. *North American Journal of Fisheries Management* 24: 1376–1384.
- Brown, G.G., and D.L. Clapper. 1981. Procedures for maintaining adults, collecting gametes, and culturing embryos and juveniles of the horseshoe crab, *Limulus polyphemus* L. In *Laboratory animal management, marine invertebrates*, ed. R. Hinegardner, J. Atz, R. Fay, M. Fingerman, R. Josephson, and N. Meinke, 268–290. Washington: National Academy Press.
- Carmichael, R.H., D. Rutecki, and I. Väliela. 2003. Abundance and population structure of the Atlantic horseshoe crab *Limulus polyphemus* in Pleasant Bay, Cape Cod. *Marine Ecology Progress Series* 246: 225–239.
- Chabot, C.C., S.H. Betourmay, N.R. Braley, and W.H. Watson. 2007. Endogenous rhythms of locomotion in the American horseshoe crab, *Limulus polyphemus*. *Journal of Experimental Marine Biology and Ecology* 345: 79–89.
- Chabot, C.C., S.J. Skinner, and W.H. Watson. 2008. Rhythms of locomotion expressed by *Limulus polyphemus*, the American horseshoe crab: I. Synchronization by artificial tides. *Biological Bulletin* 215: 34–45.
- Cohen, J.A., and H.J. Brockmann. 1983. Breeding activity and mate selection in the horseshoe crab, *Limulus polyphemus*. *Bulletin of Marine Science* 33: 274–281.
- Colson, S., and L.N. Sturmer. 2000. One shining moment known as clamelot: The Cedar Key story. *Journal of Shellfish Research* 19: 477–480.
- Davis, R.A. 1997. Geology of the Florida coast. In *The geology of Florida*, ed. A.F. Randazzo and D.S. Jones, 155–168. Gainesville: University of Florida Press.
- Duffy, E.E., D.J. Penn, M.L. Botton, H.J. Brockmann, and R.E. Loveland. 2006. Eye and clasper damage influence male mating tactics in the horseshoe crab, *Limulus polyphemus*. *Journal of Ethology* 24: 67–74.
- Ehlinger, G.S., and R.A. Tankersley. 2007. Reproductive ecology of the American horseshoe crab *Limulus polyphemus* in the Indian River Lagoon: An overview. *Florida Scientist* 70: 449–463.
- Ehlinger, G.S., R.A. Tankersley, and M.B. Bush. 2003. Spatial and temporal patterns of spawning and larval hatching by the horseshoe crab, *Limulus polyphemus*, in a microtidal coastal lagoon. *Estuaries* 26: 631–640.
- Faurby, S.T., L. King, M. Obst, E.M. Hallerman, C. Pertoldi, and P. Funch. 2010. Population dynamics of American horseshoe crabs—Historic climatic events and recent anthropogenic pressures. *Molecular Ecology* 19: 3088–3100.
- French, K.A. 1979. Laboratory culture of embryonic and juvenile *Limulus*. In *Biomedical applications of the horseshoe crab (Limulidae)*, ed. E. Cohen, 61–71. New York: Alan R. Liss.
- Gerhart, S.D. 2007. A review of the biology and management of horseshoe crabs, with emphasis on Florida populations. *Fish and Wildlife Research Institute Technical Reports* 12(i–ii): 1–24.
- Hanna, D.H. 2001. An estimate of population sizes of two horseshoe crab (*Limulus polyphemus*) sites in Jamaica Bay. In *Limulus in the limelight*, ed. J.T. Tanacredi, 147–156. New York: Kluwer.
- Hassler, C. L. 1999. Satellite male groups in horseshoe crabs (*Limulus polyphemus*): How and why are males choosing females. M.S. thesis, University of Florida, Gainesville, FL
- Hassler, C., and H.J. Brockmann. 2001. Evidence for use of chemical cues by male horseshoe crabs when locating nesting females (*Limulus polyphemus*). *Journal of Chemical Ecology* 27: 2319–2335.
- Hata, D., and J. Berkson. 2003. Abundance of horseshoe crabs (*Limulus polyphemus*) in the Delaware Bay area. *Fishery Bulletin* 101: 933–938.
- Jackson, N.L., D.R. Smith, and K.F. Nordstrom. 2008. Physical and chemical changes in the foreshore of an estuarine beach: Implications for viability and development of horseshoe crab *Limulus polyphemus* eggs. *Marine Ecology Progress Series* 355: 209–218.
- James-Pirri, M.J. 2010. Seasonal movement of the American horseshoe crab *Limulus polyphemus* in a semi-enclosed bay on

- Cape Cod, Massachusetts (USA) as determined by acoustic telemetry. *Current Zoology* 56: 575–586.
- James-Pirri, M.J., K. Tuxbury, S. Marino, and S. Koch. 2005. Spawning densities, egg densities, size structure, and movement patterns of spawning horseshoe crabs, *Limulus polyphemus*, within four coastal embayments on Cape Cod, Massachusetts. *Estuaries* 28: 296–313.
- Jegla, T.C., and J.D. Costlow. 1982. Temperature and salinity effects on developmental and early posthatch stages of *Limulus*. In *Physiology and biology of horseshoe crabs: Studies on normal and environmentally stressed animals*, ed. J. Bonaventura, C. Bonaventura, and S. Tesh, 103–113. New York: Alan R. Liss.
- King, T.L., M.S. Eackles, A.P. Spidle, and H.J. Brockmann. 2005. Regional differentiation and sex-biased dispersal among populations of the horseshoe crab *Limulus polyphemus*. *Transactions of the American Fisheries Society* 134: 441–465.
- Kreamer, G., and S.E. Michels. 2009. History of horseshoe crab harvest in Delaware Bay. In *Biology and conservation of horseshoe crabs*, ed. J.T. Tanacredi, M. Botton, and D.R. Smith, 299–313. New York: Springer.
- Leschen, A.S., S.P. Grady, and I. Valiela. 2006. Fecundity and spawning of the Atlantic horseshoe crab, *Limulus polyphemus*, in Pleasant Bay, Cape Cod, Massachusetts, USA. *Marine Ecology* 27: 54–65.
- Mattei, J.H., M.A. Beekey, A. Rudman, and A. Woronik. 2010. Reproductive behavior in horseshoe crabs: Does density matter? *Current Zoology* 56: 634–642.
- Moore, S., and S. Perrin. 2007. Seasonal movement and resource-use patterns of resident horseshoe crab (*Limulus polyphemus*) populations in a Maine, USA estuary. *Estuaries and Coasts* 30: 1016–1026.
- NOAA 1992–2000. Tide tables. East coast of North and South America. International Marine, McGraw Hill.
- NOAA Tides and Currents. 2010. <http://tidesandcurrents.noaa.gov/geo.shtml?location=8727520> Accessed 15 Mar 2011.
- Penn, D. 1992. The adaptive significance of nest-site selection and spawning synchronization in horseshoe crabs (*Limulus polyphemus*). M.S. thesis, University of Florida, Gainesville, FL.
- Penn, D., and H.J. Brockmann. 1994. Nest-site selection in the horseshoe crab, *Limulus polyphemus*. *Biological Bulletin* 187: 373–384.
- Penn, D., and H.J. Brockmann. 1995. Age-biased stranding and righting in horseshoe crabs, *Limulus polyphemus*. *Animal Behaviour* 49: 1531–1539.
- Ropes, J.W. 1961. Longevity of the horseshoe crab, *Limulus polyphemus* (L.). *Transactions of the American Fisheries Society* 90: 79–80.
- Rudloe, A. 1979. Locomotor and light responses of larvae of the horseshoe crab, *Limulus polyphemus* (L.). *Biological Bulletin* 157: 494–505.
- Rudloe, A. 1980. The breeding behavior and patterns of movement of horseshoe crabs, *Limulus polyphemus*, in the vicinity of breeding beaches in Apalachee Bay, Florida. *Estuaries* 3: 177–183.
- Rudloe, A. 1985. Variation in the expression of lunar and tidal behavioral rhythms in the horseshoe crab, *Limulus polyphemus*. *Bulletin of Marine Science* 36: 388–395.
- Saunders, K., H.J. Brockmann, W. Watson, and S.J. Jury. 2010. Male horseshoe crabs, *Limulus polyphemus*, use multiple sensory cues to locate mates. *Current Zoology* 56: 485–498.
- Schaller, S.Y. 2002. *Survey of Maine horseshoe crabs (Limulus polyphemus) spawning populations, 2001*. Boothbay Harbor: Maine Department of Marine Resources.
- Schaller, S.Y., C.C. Chabot, and W.H. Watson. 2010. Seasonal movements of American horseshoe crabs *Limulus polyphemus* in the Great Bay Estuary, New Hampshire (USA). *Current Zoology* 56: 587–598.
- Schwab, R.L., and H.J. Brockmann. 2007. The role of visual and chemical cues in the mating decisions of satellite male horseshoe crabs, *Limulus polyphemus*. *Animal Behaviour* 74: 837–846.
- Shuster Jr., C.N. 1979. Distribution of the American horseshoe “crab”, *Limulus polyphemus* (L.). In *Biomedical applications of the horseshoe crab (Limulidae)*, ed. E. Cohen, 3–26. New York: Alan R. Liss.
- Shuster Jr., C.N. 2001. Two perspectives: Horseshoe crabs during 420 million years worldwide, and the past 150 years in Delaware Bay. In *Limulus in the limelight*, ed. J.T. Tanacredi, 17–40. New York: Kluwer.
- Shuster Jr., C.N., and M.L. Botton. 1985. A contribution to the population biology of horseshoe crabs, *Limulus polyphemus* (L.) in Delaware Bay. *Estuaries* 8: 363–572.
- Shuster Jr., C.N., M.L. Botton, and R.E. Loveland. 2003. Horseshoe crab conservation: A coast-wide management plan. In *The American horseshoe crab*, ed. C.N. Shuster, R.B. Barlow, and H.J. Brockmann, 358–377. Cambridge: Harvard University Press.
- SigmaStat. 2006. SigmaStat 3.5 for Windows. Point Richmond, CA, Systat Software, Inc.
- Smith, D.R., and S.F. Michels. 2006. Seeing the elephant: Importance of spatial and temporal coverage in a large-scale volunteer-based program to monitor horseshoe crabs. *Fisheries* 31: 485–491.
- Smith, D.R., P.S. Pooler, R.E. Loveland, M.L. Botton, S.E. Michels, R.G. Weber, and D.B. Carter. 2002a. Horseshoe crab (*Limulus polyphemus*) reproductive activity on Delaware Bay beaches: Interactions with beach characteristics. *Journal of Coastal Research* 18: 730–740.
- Smith, D.R., P.S. Pooler, B.L. Swan, S. Michels, W.R. Hall, P. Himchak, and M.J. Millard. 2002b. Spatial and temporal distribution of horseshoe crab (*Limulus polyphemus*) spawning in Delaware Bay: Implications for monitoring. *Estuaries* 25: 115–125.
- Smith, D.R., M.J. Millard, and S. Eyler. 2006. Abundance of adult horseshoe crabs (*Limulus polyphemus*) in Delaware Bay estimated from a bay-wide mark-recapture study. *Fishery Bulletin* 104: 456–464.
- Smith, D.R., M.T. Mandt, and P.D.M. MacDonald. 2009. Proximate causes of sexual size dimorphism in horseshoe crabs (*Limulus polyphemus*) of the Delaware Bay. *Journal of Shellfish Research* 28: 405–417.
- Smith, D.R., L.J. Brousseau, M.T. Mandt, and M.J. Millard. 2010. Age and sex specific timing, frequency, and spatial distribution of horseshoe crab spawning in Delaware Bay: Insights from a large-scale radio telemetry array. *Current Zoology* 56: 563–574.
- Sokoloff, A. 1978. Observations on populations of the horseshoe crab *Limulus (=Xiphosura) polyphemus*. *Researches on Population Ecology* 19: 222–236.
- Swan, B.L. 2005. Migrations of adult horseshoe crabs, *Limulus polyphemus*, in the Middle Atlantic Bight: A 17-year tagging study. *Estuaries* 28: 28–40.
- Thompson, M. 1998. Assessments of the population biology and critical habitat for the horseshoe crab, *Limulus polyphemus*, in the South Atlantic Bight. M.S. thesis, Medical University of South Carolina, Charleston, South Carolina.
- Wallace, G. E. 1999. Potential effects of a horseshoe crab fishery on migratory shorebirds in the state of Florida. Bureau of Wildlife Diversity Conservation, Florida Fish and Wildlife Conservation Commission. Panama City.
- Walls, E.A., J. Berkson, and S. Smith. 2002. The horseshoe crab, *Limulus polyphemus*, 200 million years of existence, 100 years of study. *Reviews in Fisheries Science* 10: 39–73.
- Wasserman, G.S., and Z. Cheng. 1996. Electroretinographic measures of vision in horseshoe crabs with uniform versus variegated carapaces. *Biological Signals* 5: 247–262.

- Watson, W.H., and C.C. Chabot. 2010. High resolution tracking of adult horseshoe crabs *Limulus polyphemus* in a New Hampshire estuary using fixed array ultrasonic telemetry. *Current Zoology* 56: 599–610.
- Watson, W.H., S.Y. Schaller, and C.C. Chabot. 2009. The relationship between small- and large-scale movements of horseshoe crabs in the Great Bay Estuary and *Limulus* behavior in the laboratory. In *Biology and conservation of horseshoe crabs*, ed. J.T. Tanacredi, M. Botton, and D.R. Smith, 131–147. New York: Springer.
- Wenner, E., and M. Thompson. 2000. *Evaluation of harvesting impacts and population trends for Limulus polyphemus in South Carolina*. Charleston: Marine Resources Research Institute, South Carolina Department of Natural Resources.
- Widener, J.W., and R. Barlow. 1999. Decline of a horseshoe crab population on Cape Cod. *Biological Bulletin* 197: 300–302.
- Zaldivar-Rae, J., R.E. Sapien-Silva, M. Rosales-Raya, and H.J. Brockmann. 2009. American horseshoe crabs, *Limulus polyphemus*. In *Biology and conservation of horseshoe crabs*, ed. J.T. Tanacredi, M. Botton, and D.R. Smith, 97–113. New York: Springer.