2010

Reproductive Behavior in Horseshoe Crabs: Does Density Matter?

Jennifer Mattei
Sacred Heart University, matteim@sacredheart.edu

Mark Beekey
Sacred Heart University, beekeym@sacredheart.edu

Adam Rudman
Southern Connecticut State University

Alyssa Woronik
Stockholm University

Follow this and additional works at: https://digitalcommons.sacredheart.edu/bio_fac

Part of the Population Biology Commons, and the Terrestrial and Aquatic Ecology Commons

Reproductive behavior in horseshoe crabs: Does density matter?

Jennifer H. MATTEI [1*], Mark A. BEEKEY [1], Adam RUDMAN [2], Alyssa WORONIK [1]

[1] Department of Biology, Sacred Heart University, Fairfield, CT 06825, USA

[2] Department of Biology, Southern Connecticut State University, New Haven, CT 06515, USA

Abstract   While the four species of horseshoe crabs share many common reproductive traits with respect to their reproductive systems, they do differ with respect to their mating behavior (monogamy vs. polygynandry). Past research has attributed these differences to a number of factors including: spawning densities, operational sex ratios (OSR's), male condition (or age), environmental and/or genetic factors, or a combination thereof. Mating behaviors in the three Asian horseshoe crab species (*Tachypleus gigas, T. tridentatus*, and *Carcinoscorpius rotundicauda*) with low spawning densities and 1:1 operational sex ratios are typically monogamous. In *Limulus polyphemus*, mating behavior is more variable ranging from monogamy to polygynandry. Here we provide evidence, through a long term behavioral study, that variation in mating behavior is influenced by population density in *L. polyphemus*. Our study population on two beaches in Connecticut (Long Island Sound) have a spawning density 400 times less than that found in Delaware Bay (0.002 females/m^2 vs. 0.8 females/m^2) but similar operational sex ratios. Between 90%–95% of all spawning females in CT were paired with only one male, thus exhibiting monogamous behavior. In contrast, between 30 and 60% of spawning females in Delaware Bay have more than one mate and produce clutches of eggs with multiple paternities. Male condition played no role in mating behavior in CT populations. We also observed that on average 18% of the females on the spawning beaches are single. These results suggest that population density is an important condition that determines mating behavior. Also, low population density may lead to decreased mate finding ability and lost opportunities for spawning [*Current Zoology* 56 (5): 634–642, 2010].

Key words   *Limulus*, Mating behavior, Population density, Monogamy, Polygynandry

Among marine arthropods, horseshoe crabs (Limulacea) have a unique reproductive strategy in that all four species externally fertilize their eggs and exhibit no brooding (Sekiguchi, 1988; Brusca and Brusca, 2003). Within the Limulacea, the reproductive behavior is very similar. Spawning of all four species of horseshoe crab (*Limulus polyphemus, Tachypleus gigas*, *T. tridentatus*, and *Carcinoscorpius rotundicauda*) is synchronous. Horseshoe crabs migrate from deeper to shallow water to nest on sheltered intertidal beaches (Sekiguchi, 1988; Chiu and Morton, 1999). Nesting typically occurs on the high tides of the full and new moons. Males utilize modified prosomal appendages to attach (amplexus) to females. Females deposit eggs 7–20 cm below the sand surface where they are fertilized externally by the males. The eggs are left to develop on their own and the crabs return to the ocean (Barlow et al., 1982; Botton et al.,

1996; Brockmann and Smith, 2009). All four species exhibit sexual dimorphism with females being larger than males; F:M size ratio ranging from 1.04–1.29 with *Carcinoscorpius rotundicauda* possessing the smallest difference in size ratios (Chiu and Morton, 1999[1]; Brockmann and Smith, 2009). Females exhibit little, if any mate choice; males locate female mates using visual and chemoreceptive cues (Saunders and Brockman, 2010). Females are capable of depositing more than one clutch of eggs over the spawning season and males are capable of fertilizing more than one clutch resulting in hundreds to thousands of eggs being laid per female depending on the species (Brockmann and Penn, 1992; Brockman, 1996; Brockmann and Smith, 2009). Despite the aforementioned similarities, differences in reproductive behavior exist among the four horseshoe crab species.

---

[1] Chiu HMC, Morton B, 1999. The Biology, distribution, and status of horseshoe crabs *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda* (Arthropoda: Chelicerata) in Hong Kong: Recommendations for conservation and management. Final Report to the China Light and Power Company Limited, The Swire Institute of Marine Science, The University of Hong Kong, HK.

∗ Corresponding author. E-mail: matteij@sacredheart.edu

The mating behavior of the three Asian species of horseshoe crabs (*T. gigas*, *T. tridentatus*, and *C. rotundicauda*) has been classified in the literature as monogamous (Chatterji et al., 1992; Brockmann and Smith, 2009). Asian horseshoe crabs typically nest as pairs (one male amplexed with one female). There are some instances of seasonal monogamy in *C. rotundicauda* where the same mated pair will remain amplexed throughout the breeding season. However, serial monogamy has also been observed. If mated pairs are pulled apart and allowed to reattach in a mixed group, 66%−100% of the time males will attach to a different female in trials with both *Carcinoscorpius* and *Tachypleus tridentatus* (Chiu and Morton, 1999).

In the American horseshoe crab *L. polyphemus*, mating behavior ranges from seasonal monogamy to serial monogamy to polygynandry (Shuster and Wade, 2003). Polygynandry is typically observed in species where both sexes are variable in their mate numbers, but males are more variable than females (Shuster and Wade, 2003). During any given reproductive bout, mating may be monogamous (female and amplexed male) or polyandrous where the nesting pair is surrounded by additional satellite males. However, over the reproductive season, *L. polyphemus* females may reproduce with more than one male and during that same time period males may remain mateless, reproduce with one, or more than one female resulting in polygynandry. These polygynandrous (satellite) males engage in sperm competition with amplexed males. Polygynandrous males may be quite successful fertilizing on average 40% of the females eggs when there are one or two competitors. However, with increasing numbers of satellites their reproductive success declines (Brockmann et al., 1994). These differences in mating strategies and reproductive success among the four horseshoe crab species have been attributed to variation in spawning density, operational sex ratios, and male condition (Duffy et al., 2006; Brockman and Smith, 2009).

Spawning densities and operational sex ratios (OSR) vary between the three Asian horseshoe crab species and *L. polyphemus*. Few spawning density estimates exist for *Tachypleus gigas*, *T. tridentatus*, and *Carcinoscorpius rotundicauda* in the literature. A recent review by Brockmann and Smith (2009) report current estimates for some spawning densities in India and Japan that range from 0 to 100 females/100 meters of beach surveyed for the three Asian species but that normally fewer than 30 females were present (Sekiguchi, 1988). Botton et al. (1996) reported spawning densities of 0.035 females/m^2 from two beaches in Japan. Chiu and Morton (1999) and Shin et al. (2009) report that spawning adult horseshoe crabs are rarely seen on the beaches of Hong Kong. *L. polyphemus* populations along the Atlantic Coast range from low spawning density populations in Massachusetts (0.002 females/m^2, James-Pirri et al., 2005) to moderate spawning densities in Florida (0.55 crabs/m^2, Brockman and Smith, 2009) to high spawning densities in Delaware Bay (1.01 females/m^2 in Delaware Bay, Smith et al., 2002). OSRs for the three Asian species are generally 1:1 with little or no male bias (Sekiguchi, 1988; Botton et al., 1996; Brockman and Smith, 2009). OSRs among *L. polyphemus* populations range from a low of 1.07:1 in Maine (Schaller, 2002), to 3.5:1 in Delaware Bay (Smith et al., 2002), and a high of 9:1 in Massachusetts (James-Pirri, 2010).

It is widely known that population density and OSRs may influence male mating behavior (Calabrese and Fagan, 2004; Gascoigne et al., 2009). When population densities are high, male-male mate competition can intensify and alternative reproductive tactics may be employed by males to maximize fitness (Brockmann and Taborsky, 2008). In high density populations, male horseshoe crabs maximize fitness by choosing between two alternative reproductive tactics: males can either arrive on the beach already amplexed to females with whom they spawn or males may arrive alone and crowd around the nesting pairs as satellites (Brockmann and Taborsky, 2008). However, when population densities are low, male-male competition may decline and mate finding behaviors become more important for maintaining fitness (Gascoigne et al., 2009). In populations with high densities of spawning adult horseshoe crabs on beaches, nearly every female has at least one amplexed male and a variable number may have satellites. In addition, increased male biased operational sex ratio can lead to intense male-male mate competition which results in 30 to 60% of spawning pairs to have at least one or more satellite males (Brockmann and Penn, 1992; Brockmann, 1996; Duffy et al., 2006). The frequency of single males on a beach can be quite high (Smith et al., 2002). It has been reported in the literature that excess single males with higher frequencies of eye and clasper damage may not be as reproductively successful as amplexed males (Brockmann et al., 2000; Brockmann, 2002; Duffy et al., 2006). In fact, Brockmann and colleagues found that younger males in good condition were usually amplexed while older males in poorer condition typically exhibited satellite behavior often crowding around a mated pair and contributing sperm to

the egg mass being laid near the female's incurrent canal (Brockmann and Penn, 1992; Brockmann, 1996; Walls et al., 2002, Duffy et al., 2006; Brockmann and Taborsky, 2008). However, when population densities are low, male-male competition may decline and mate finding behaviors become more important for maintaining fitness (Gascoigne et al., 2009). Evidence for reproductive competition among all three Asian species with low densities and low operational sex ratios is limited; satellite behavior is rarely reported (Brockman and Smith, 2009).

Clearly there are differences between Asian and American horseshoe crabs with respect to reproductive strategies (i.e. monogamy vs. polygynandry). Among low density Asian populations with unbiased OSRs, serial or seasonal monogamy is the dominant mating behavior. Among high density *L. polyphemus* populations with male biased OSRs, polygynandry is more common with younger males occupying the amplexed position and older males acting as satellites. It has been reported in the literature that these differences are a function of a number of factors including: spawning densities, operational sex ratios (OSRs), male condition (or age), environmental and/or genetic differences, or a combination thereof (Loveland and Botton, 1992; Brockmann and Smith, 2009). In order to separate out some of these factors and test which may be important in determining mating behaviors, we present here behavioral data from two low density subpopulations of *L. polyphemus* that also exhibit male biased operational sex ratios (Beekey and Mattei, 2009[2]). We compare these data to previously published data from a high spawning density, male biased population in Delaware Bay.

1 Materials and Methods

1.1 Description of study sites

Milford Point, Milford, Connecticut (41°10′21.05″N, 73°6′30.27″W) is a barrier beach that includes the 8.4 acres (3.4 ha) Smith-Hubbell Wildlife Refuge and is adjacent to the 840 acre (340 ha) Wheeler Marsh Wildlife Management Area at the mouth of the Housatonic River. Milford Pt. has been a protected wildlife area for the past 18 years. It is not heavily utilized for commercial and recreational activities that could disturb *Limulus* spawning, recruitment, and shorebird foraging. Sandy Point, West Haven, Connecticut (41°16′0.59″N, 72°55′31.21″W) is an ~66 acre city-owned barrier beach

(sand spit) system with a tidal creek, an area of tidal marsh and tidal flats protruding into New Haven Harbor from the west side. Spring migrations result in a diverse array of shorebirds including terns, skimmers, plovers, sandpipers and red knots at both Milford Pt. and Sandy Pt. (National Audubon Society, 2010). Both Milford Pt. and Sandy Pt. were declared off limits to the hand harvesting of horseshoe crabs (only type of harvesting permitted in CT) in the spring of 2007 by the Connecticut Department of Environmental Protection.

1.2 Mating behavior

From 2006 through 2010, we tagged horseshoe crabs at Milford Pt. and Sandy Pt. between May 15th and June 30th on alternating days and nights during falling tides and weather permitting. All crabs were tagged as they were encountered on beaches. We assumed that the sample of tagged individuals was a random and representative sample of the spawning population at both beaches. In 2006–2007 we used yellow Floy Cinch-tags (model FT-4, 8": http://www.floytag.com) and U.S. Fish and Wildlife Service issued white disc tag from 2008−2010. Tags were attached to the crab using a #2 Yellow scratch awl (Challenge Sailcloth, Inc. Vernon-Rockville, CT) with a #8 stainless steel washer affixed to the awl with J.B. Weld. The awl was gently twisted into the lower rear of either the right or left posterior side of the prosoma. Once a hole was made, the disc tag was inserted into the hole. After tagging, the tag number was recorded and the sex of the individual was determined (based on the morphology of the pedipalps). Mating behavior was recorded for each crab as follows: single male, single female, pair (female and amplexed male), or F+N where F is equal to a female and N equals the total number of males mating with her including the amplexed male (*e.g.* F + 2 equals a female with one amplexed male and one satellite male). Thus for each tag number recorded, we were able to distinguish between solitary males and females, mated pairs, and mated pairs with satellite males. Operational sex ratios were calculated for every day/night tagging effort and averaged over the season (May 14–June 30th) for each year.

1.3 Spawning density

To estimate the density of spawning horseshoe crabs, we surveyed spawning beaches at Milford Pt. and Sandy Pt. during the summers of 2008, 2009, and 2010 using quadrat sampling. The spawning survey protocol was a modification of the spawning survey methods first

[2] Beekey MA, Mattei JH, 2009. What long term mark/recapture studies reveal about horseshoe crab population dynamics in Long Island Sound. *Proceedings of the 2008 Biennial Long Island Sound Research Conference*. Connecticut College, Groton, CT.

employed in Delaware Bay (Smith et al., 2002). The modification for the Connecticut region primarily involved increasing the quadrat size from 1 m² (1 m × 1 m) to 15 m² (5 m × 3 m) since Connecticut spawning densities are much lower than those reported from Delaware Bay. Therefore, our quadrats extended 3m into the water and were 5 m wide.

A coin flip determined the starting point (e.g., the east or west end of the beach) and the position of the first quadrat within the initial 10 m of beach was randomly determined. All subsequent quadrats were systematically placed 10 m apart. Each quadrat was located adjacent to the water's edge (swash zone) and extended 3 m into the water. Over the course of the survey the leading edge of the quadrat was moved slightly down the beach slope to keep in line with the receding tide. In 2010, we modified the survey methods to increase the area covered by the surveys. Instead of skipping 10 m between quadrats, we systematically surveyed all crabs occurring within the first 3 m of the water's edge along a stretch of 300 m at Milford Pt. and surveyed all crabs occurring along a 500 m section of Sandy Pt.

We conducted spawning surveys during a 5 day (d) period (2 d prior to, the day of, and 2 d after) the full and new moons from May through June. Surveys commenced at peak high tide and were conducted during both nighttime and daytime high tides except under extreme weather conditions. Environmental conditions (i.e., weather, wave height, water temperature) were recorded prior to the start of each survey. We counted all horseshoe crabs within each quadrat, and recorded the number of single males, single females, pairs (female and amplexed male), and females with amplexed males and satellite males.

We calculated spawning index, defined as the average number of spawning female crabs per m². Solitary females were not included in the spawning index. Yearly average spawning indices were calculated by averaging the spawning female density for each site (day and night surveys calculated separately) within each moon period from May through June (e.g., May new/full moon, June full and new moon) each year.

1.4 Condition index

During 2009, we sampled 40 males from each of 4 beaches (Rye Beach in NY, Milford Pt., Sandy Pt., and Bluff Pt. in CT) along the western coast of Long Island Sound from the southwest (NY, Westchester County) to the northeast (Groton, CT), respectively. We assessed the average condition of adult male spawning horseshoe crabs and noted whether they were part of a mated pair

(attached) or single (unattached). To assess the condition of mated males, we utilized methods previously established by Brockmann and Penn (1992) and further refined by Brockman (2002) and Duffy et al. (2006). The carapace of *Limulus* erodes over time and the dorsal prosomal surface changes in color from a light tan in newly molted males to black in older males. We scored carapace condition based on a scale of 1–3, where 1 was for a newly molted, glossy carapace, 2 for an intermediate carapace (i.e., showing scratches and other signs of wear, and with some blackened areas), and 3 for a heavily worn and mostly blackened and pitted carapace (Duffy et al., 2006) While this carapace condition cannot provide a chronological age, it has been established as a reasonable indicator of relative age: young, middle aged, and old respectively (Brockman, 2002). Males also differed with respect to the percentage of the dorsal surface covered by epibionts (barnacles, slipper shells) and we estimated the percent of the surface that was covered by epibionts on a scale of 1–5 (1 = 0–4%; 2 = 5%–9%; 3 = 10%–19%; 4 = 20%–50%; and 5 ≥ 50%). Finally, we assessed the parasitic load on the book gills. The number of parasitic cysts on five random gill lamellae were counted and each crab was categorized by total parasite load (1 ≤ 5; 2 = 5–9; 3 = 10–19; 4 = 20–29; and 5 ≥ 30 parasites). These three separate measures of condition were combined into a simple index with the lowest score (3) indicating the best overall condition and the rank of (13) indicating the poorest condition. For comparative purposes, the condition index was divided into three categories. Newly molted crabs in the best condition (score of 3–6) were given a 1, the worst condition (oldest) crabs (score of 9–13) were assigned a 3, and the middle group (score of 7–8) assigned a 2.

2   Results

In DE Bay, only 28% of the spawning females were reported with one male (Fig. 1a) as reported by Brockmann (1996) from one season of observations. Over the five years of tagging data, 95% and 90% of the observed mating behavior at Milford Pt.(MP) and Sandy Pt.(SP), respectively, involved mated pairs (one female and one amplexed male) (Fig. 1b and 1c). The difference in the frequency of observed mating patterns between Delaware Bay and Milford Pt. and Sandy Pt. was significantly different (DE vs. MP $\chi^2 = 790$, $df = 1$, $P \leqslant 0.0001$; DE vs. SP $\chi^2 = 724$, $df = 1$, $P \leqslant 0.0001$).

Operational sex ratios, defined as the number of males per female per day, for Milford Pt. and Sandy Pt.

ranged widely within each year (Table 1, minimum versus maximum). On average, over 5 years was 2.08 (range 1.8–2.4), Sandy Pt. is 2.78 (range 2.3–3.4) as compared to DE Bay ranging from 3.8 to 4.7 (Table 1).
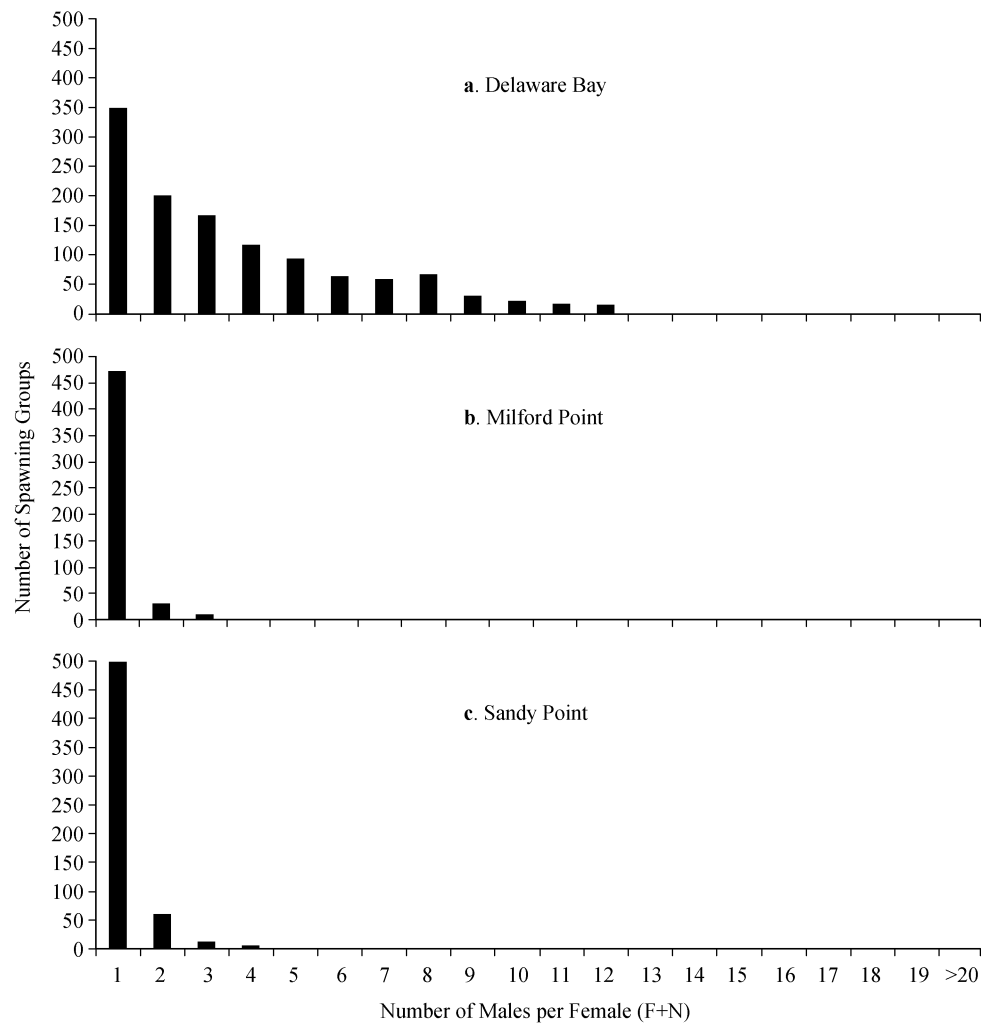
Fig. 1   The number of spawning groups observed arranged by number of males in each group

a. Spawning groups found during a beach survey in DE Bay June 7–15, 1991. Data from Brockmann (1996) (*n* = 1241). **b**. Spawning groups found during May 14–June 30, 2010 for Milford Pt. (*n* = 517). **c**. Sandy Pt. (*n* = 589) plotted by each female with the number of males encountered during tagging. DE versus MP χ^2 = 790, *df* = 1, *P*≤0.0001; DE versus SP χ^2 = 724, *df* = 1, *P*≤0.0001.

Table 1   The Average annual operational sex ratios (OSR), (# males/female) ± 1 *SE* for Milford and Sandy Points, Connecticut from 2006–2010

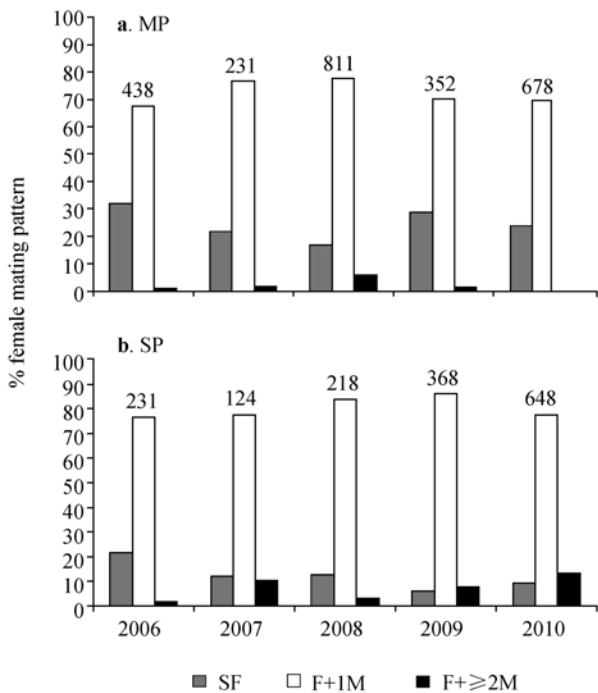| Year	Milford Point (CT)	Sandy Point (CT)	Delaware Bay (DE and NJ)[*] (M:F ± 1 *SD*)
2006	1.8 ± 0.3 0.33 – 8.0 (*n* = 33)	3.4 ± 0.8 0.83 – 13.8 (*n* = 19)	Average OSR from 1999–2008 3.8 ± 0.19 standard deviation (Michels et al., 2009[3])
2007	2.0 ±0.2 0.92 – 6.6 (*n* = 34)	2.4 ± 0.4 1.0 – 4.9 (*n* = 12)	
2008	2.4 ± 0.4 0.33 – 9.6 (*n* = 27)	2.9 ± 0.5 1.0 – 9.0 (*n* = 21)	
2009	2.1± 0.6 0.5 – 16.0 (*n* = 26)	2.3 ± 0.3 1.0 – 6.8 (*n* = 19)	4.7 (ASMFC, 2010)
2010	2.1 ± 0.3 0.74 – 6.7 (*n* = 23)	2.9 ± 0.4 1.0 – 11.3 (*n* = 25)	NA

Data are expressed as Means ± 1 SE, The range of OSRs with the minimum - maximum daily ratios (Number of days sampled)

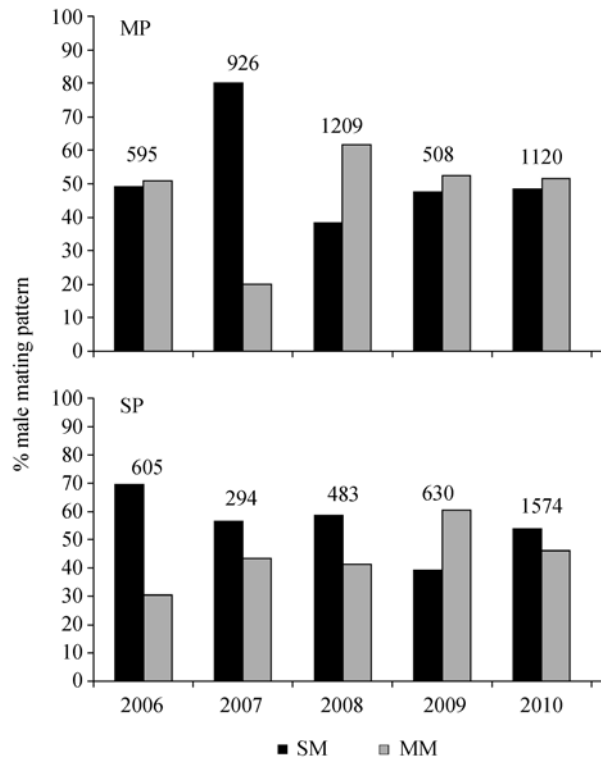[*] Comparison data is presented for Delaware Bay where available.

[3] Michels SF, Smith DR, Bennett S, 2009. Horseshoe crab spawning activity in Delaware Bay: 1999 – 2008. Report to the Atlantic States Marine Fisheries Commission's Horseshoe Crab Technical Committee (February 9, 2009).

The percentage of single females varied over time ranging from a low of 17% in 2008 to a high of 32% in 2006 at Milford Pt. and a low of 6% (2009) to a high of 22% (2006) at Sandy Pt. Paired females were the most abundant at Milford Pt. and Sandy Pt. ranging from 67%–77% and 77%–86%, respectively. Sightings of pairs with satellite males were infrequent, making up a small percentage of the females found up on the beach (Milford Pt., 0.9%–8%; Sandy Pt., 1.7%–13%, Fig. 2). The percentage of single males as compared to the percentage of amplexed and satellite males varied by year and by beach. In 2007, Milford Pt. had a high of 80% single males and a low of 38.4% the following year. Sandy Pt. had more single males than amplexed males in 4 out of the 5 years of this study. The highest percent of single males (70%) was in 2006, the lowest percent of single males was 39% in 2009 (Fig. 3).

Delaware Bay's spawning index is dramatically different compared to Milford Pt. and Sandy Pt. The average spawning index (number of spawning females per m^2) from 2008 to 2010 was 400 times greater in Delaware Bay compared to Milford Pt. and Sandy Pt. (Table 2). Low spawning densities comparable to Milford Pt. and Sandy Pt. were observed in Rhode Island and Massachusetts.

Fig. 3   Percentage of males encountered and tagged at Milford Point (MP) and Sandy Point (SP) by year as a function of mating pattern

SM = Solitary Male; MM = Mated (Attached or Satellite) Male. Total number of males tagged per year (N) is indicated above bars.

Male condition played little role in determining whether a male was more likely to be found amplexed to a female or in a satellite position. The percentage of amplexed males exhibiting poor condition was greater than the percentage of amplexed males in good condition (Fig. 4).

Interestingly, the percentage of unattached males in good condition was greater than the number of amplexed males. However, there was no significant difference between the condition and whether a male was amplexed or unattached ($\chi^2 = 1.87$, $df = 2$, $P = 0.392$).

3   Discussion

We have shown that there is a significant difference between the mating pattern of *L. polyphemus* observed at Milford Point and Sandy Point in Long Island Sound compared to the mating behavior observed in the Delaware Bay population. Significantly fewer males are found behaving as satellites ($P<0.00001$, Fig. 1) in the Sound. The frequency of monogamous behavior observed at Milford Pt. and Sandy Pt. more closely resembles the mating behavior of the Asian horseshoe crab

Fig. 2   The percent of all female horseshoe crabs encountered and tagged by year as a function of mating status

a. Milford Point (MP). **b**. Sandy Point (SP). Mating status: SF = single female; F + 1M = mated pair; and F +≥ 2M = pairs with satellite males. Total number of females (N) encountered is above bars.

Table 2   Average spawning indices (number of spawning female crabs per m² ± SE) for Milford Point and Sandy Point, Connecticut

	2008	2009	2010
Milford Point (CT)	0.002 ± 0.001 (11)	0.003 ± 0.001 (19)	0.041 ± 0.020 (17)
Sandy Point (CT)	0.058 ± 0.014 (9)	0.051 ± 0.020 (9)	0.091 ± 0.050 (9)
Monomoy (MA)	0.02 ± 0.004	0.03 ± 0.009	NA
Buzzards Bay (MA)	0.005 ± NA	0.007 ± 0.004	NA
Lone Tree Point (RI)	0.004 ± 0.004 (4)	0.004 ± 0.002 (5)	NA
Delaware Bay	0.80 ± 0.06	0.77 ± 0.06	0.82 ± 0.06

Data are combined from night surveys and averaged across all moons from May 14th – June 30th. Number of night surveys conducted is indicated in parentheses. Rhode Island (unpublished, Project *Limulus* data), Massachusetts (Commonwealth of Massachusetts, 2010) and Delaware Bay (Smith and Michels 2006.) spawning indices are included for a regional comparison.
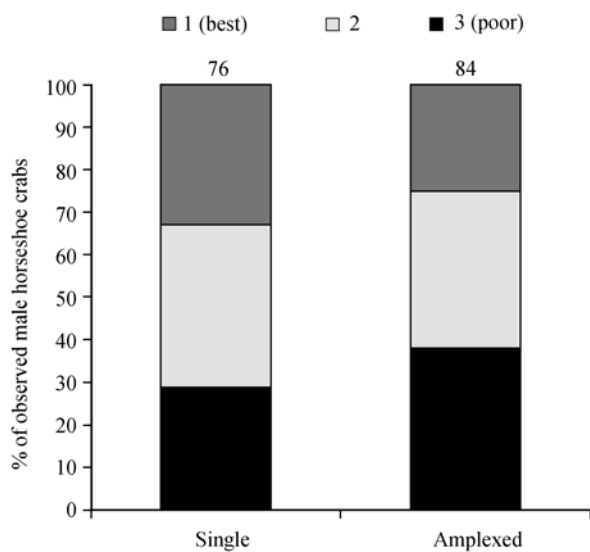
Fig. 4   Combined frequency distribution of males of different condition index categories by mating behavior from four spawning beaches (Milford Pt., Sandy Pt., and Bluff Pt. in CT and Rye Beach in NY)

Unattached males refers to single or satellite males. Attached males are considered amplexed to females. Sample sizes are given above each bar. Condition category is not significantly different between unattached and attached mating males ($\chi^2 = 1.87$, $df = 2$, $P = 0.392$).

species *Tachypleus tridentatus* (Sekiguchi, 1988; Botton et al., 1996).

One explanation for this monogamous mating pattern could be the operational sex ratio (OSR). The pattern in Asian horseshoe crab species is 1:1, i.e. the number of spawning males is equal to the number of spawning females (Botton et al., 1996; Brockmann and Smith, 2009). This was not the pattern in Long Island Sound (LIS). The operational sex ratio at Milford Pt. and Sandy Pt. was on average 2.6 over 5 years across the two beaches (see Table 1). Fig. 3 demonstrates that in any given year there were numerous single males present. In fact, in 2007, 80% of the males tagged at Milford Pt. were single over a 34 day sampling regime. Yet,

as shown in Fig. 2, in that same year (2007) only 1.7% of mated females had satellite males. We, as have others (Brockmann, personal communication), observed single males approach and swim by mated pairs. It is not clear why they behave in this way other than possibly not finding a chemical cue (Saunders and Brockmann, 2010). There are other populations both north and south of Connecticut with published OSR data: Maine (2.8; Schaller, 2002), Cape Cod Bay, MA (2.9; James-Pirri et al., 2005), Otter Island, South Carolina (1.96; Wenner and Thompson, 2000) and Florida (2.1; Brockmann and Smith, 2009). However, there are no published data from any of these sites in terms of frequency of monogamous vs. polygynandrous behavior. Therefore, based on the data from the Connecticut beaches, the low percentages of polygamous matings was not likely due to the operational sex ratio because there were single males present on the spawning beaches.

Another factor that may cause the dominance of observed monogamous behavior in Connecticut was the condition or age of the males in the population (Duffy et al., 2006). It was possible that there were only young adult males in the population and therefore they continually search for single females to mate with or that there were old blind males that cannot find mates. We tested this factor and found that mated pairs had nearly equal numbers of good, moderate, and poor conditioned males (see Fig. 4). In low density horseshoe crab populations where male-male competition was low, older "poor condition" males were as likely as younger "better condition" males to successfully attach to females (Fig. 4).   Therefore, it was not likely that the condition of the male horseshoe crabs in the Connecticut population was the cause of the mating pattern that we observed.

The one factor that could more easily influence mating behavior in all four species of horseshoe crab is the spawning adult population density. Clearly, density

plays an important role in determining alternative reproductive tactics in other species (see Courchamp et al., 1999; Stephens et al., 1999; Brockmann, 2001; Gascoigne et al., 2009 for examples). As shown in Table 2, horseshoe crab spawning densities in New England and particularly at Milford Pt, CT were significantly lower (400 times less dense; 0.002 females/m^2 vs. 0.8 females/m^2) than what has been reported in the literature for Delaware Bay (Smith et al., 2002). In fact, spawning densities at Milford Pt. and Sandy Pt. were more similar to densities reported for Asian species (ranging from 0-100 females/100 m of beach; Brockmann and Smith, 2009). Likewise, mating behavior at Milford Pt. and Sandy Pt. is also similar to mating behavior reported for Asian species in that there is little or no reproductive competition (Sekiguchi, 1988). Botton et al. (1996) suggest that the lack of male competition and satellite behavior in Asian horseshoe crab populations can be explained by their lower density. We propose this same hypothesis for subpopulations of *Limulus polyphemus*; those populations that experience locally low densities will exhibit predominantly monogamous behavior even if the OSR is male biased.

The data indicate that differences in reproductive tactics observed in male horseshoe crab mating behavior may be caused by changes in population density. Condition-dependent divergence in mating strategy depending on age does not explain what we have observed at Milford Pt. and Sandy Pt. Thus older males may maximize reproductive fitness by attaching to available single females and only employ satellite behavior if they should by chance come across a mated pair exuding a chemical cue (Saunders and Brockmann, 2010). Interestingly, when several males of the Asian species, *T. tridentatus*, were put into a tank with a mated pair, they were observed to clasp onto the paired couple (Sekiguchi, 1988). Botton et al. 1996 observed similar satellite behavior in *T. gigas* at an aquarium. While anecdotal, this artificial increase in "density" resulted in male satellite behavior in normally monogamous species. Under low population density conditions, satellite behavior is rarely, if ever, observed in Asia.

Another remarkable difference between the Delaware Bay population and the populations of *L. polyphemus* at Milford Pt. and Sandy Pt. CT is the presence of single females on the beach. It has been reported in the literature that single females do not nest (Brockmann, 1996), this may be true, but they do come up onto the beach. The only published data on the occurrence of single females is from Loveland and Botton (1992) in Cape

May, NJ who found 0.2% single females in their study population. James-Pirri (2010) noted that in the Pleasant Bay, MA population, females have been observed on spawning beaches without amplexed males; the OSR observed in this heavily harvested population is 9:1 (M:F). We have found an annual range of between 6% and 32% of the females coming up on the beach to be single across 5 years on both CT beaches. Some may still find a mate; some may have already deposited eggs and subsequently lost their mate. Usually, when female horseshoe crabs are finished spawning they do not come up on the beach again until the following year (Brockmann 2003). We propose that these lone females must be trying to spawn. The scattered but abundant number of unattached males on the same beaches as single females is evidence of reduced mate-finding efficiency in the Connecticut population of *Limulus polyphemus*.

In conclusion, low population densities are correlated with monogamous behavior in both the Asian and American horseshoe crab species. Even with male biased operational sex ratios, *L. polyphemus* exhibits monogamous behavior more than 90% of the time on Connecticut beaches. A male biased operational sex ratio does not necessarily lead to high levels of polygynandry. We need to investigate further if there are differences in the fitness of individuals in high density vs. low density populations (i.e. Allee effects, Stephens and Sutherland, 1999; Gascoigne et al., 2009). These behavioral differences, which are due to reductions in conspecific interactions, may have important consequences for the conservation of horseshoe crab species. We consider the presence of relatively high numbers of single females and the low level of polygynandrous behavior as evidence that horseshoe crabs along the CT coastline are not able to maximize their reproductive effort and are having difficulty finding mates. This could prevent the population from recovering from its already low density. Similarly, the Asian species of horseshoe crab may take a long time to recover if critical densities of adults are not present.

References

ASMFC, 2010. 2010 Review of the fishery management plan in 2009 for horseshoe crab. Washington, D.C. Atlantic States Marine Fisheries Commission, Annual Report (http://www.asmfc.org/).

Barlow RB, Ireland LC, Kass L, 1982. Vision has a role in *Limulus* mating behavior. Nature 296: 65–66.

Botton ML, Shuster CN, Sekiguchi K, Sugita H, 1996. Amplexus and mating behavior in the Japanese horseshoe crab *Tachypleus tridentatus*. Zool. Sci. (Tokyo) 13: 151–159.

Brockmann HJ, Penn D, 1992. Male mating tactics in the horseshoe crab *Limulus polyphemus*. Anim. Behav. 44: 653–665.

Brockman HJ, Colson T, Potts W, 1994. Sperm competition in horseshoe crabs *Limulus polyphemus*. Behavioral Ecology and Sociobiology 35: 153–160.

Brockmann HJ, 1996. Satellite male groups in horseshoe crabs *Limulus polyphemus*. Ethology 102: 1–21.

Brockmann HJ, Nguyen C, Potts W, 2000. Paternity in horseshoe crabs when spawning in multiple-male groups. Animal Behaviour 60: 837–849.

Brockmann HJ, 2001. The evolution of alternative strategies and tactics. Advances in the Study of Behavior 30: 1–51.

Brockmann HJ, 2002. An experimental approach to altering mating tactics in male horseshoe crabs *Limulus polyphemus*. Behavioral Ecology 13 (2): 232–238.

Brockmann HJ, 2003. Male competition and satellite behavior. In: Shuster CN, Barlow RB, Brockmann HJ ed. The American Horseshoe Crab. Cambridge, Massachusetts and London, England: Harvard University Press, 50–81

Brockmann HJ, Taborsky M, 2008. Alternative reproductive tactics and the evolution of alternative allocation phenotypes. In: Oliveira RF, Taborsky M, Brockmann HJ ed. Alternative Reproductive Tactics. New York: Cambridge University Press, 25–51.

Brockman HJ, Smith MD, 2009. Reproductive competition and sexual selection in horseshoe crabs. In: Tanacredi JT, Botton ML, Smith DR ed. Biology and Conservation of Horseshoe Crabs. New York: Springer Science, 199–221.

Brusca RC, Brusca, GJ, 2003. Invertebrates. Sunderland, Massachusetts: Sinauer Associates.

Calabrese JM, Fagan WF, 2004. Lost in time, lonely, and single: Reproductive asynchrony and the Allee effect. The American Naturalist 164 (1): 25–37.

Chatterji BP, Vijayakumar R, Parulekar AH. 1992. Spawning migration of the Indian horseshoe crab *Tachypleus gigas* (Muller) with lunar cycle. Asian Fisheries Science 5: 123–128.

Commonwealth of Massachusetts, 2009. Massachusetts compliance report for the horseshoe crab *Limulus polyphemus*, 2009 compliance report. New Bedford, MA: Massachusetts Division of Marine Fisheries.

Courchamp F, Clutton-Brock T, Grenfell B, 1999. Inverse density dependence and the Allee effect. TREE 14 (10): 405–410.

Duffy EE, Penn DJ, Botton ML, Brockman HJ, Loveland RE, 2006. Eye and clasper damage influence male mating tactics in the horseshoe crab *Limulus polyphemus*. Journal of Ethology. 24: 67–74.

Gascoigne J, Berec L, Gregory S, Courchamp F, 2009. Dangerously few liaisons: a review of a mate-finding Allee effects. Popul. Ecol. 51: 355–372.

James-Pirri MJ, 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 MJ, Tuxbury K, Marino S, Koch S, 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 (2): 296–313.

Loveland, RE, Botton, ML, 1992. Size dimorphism and the mating system in horseshoe crabs *Limulus polyphemus* L. Animal Behavior 44: 907–916.

National Audubon Society, 2010. Important Bird Areas in the U.S. Available at http://www.audubon.org/bird/iba 02/2010

Saunders KM, Brockman HJ, 2010. Male horseshoe crabs *Limulus polyphemus* use multiple sensory cues to locate mates. Current Zoology 56: 485–498.

Schaller SY, 2002. Horseshoe crab *Limulus polyphemus* spawning surveys in Maine, 2001. In: American Fisheries Society Annual Meeting. Baltimore, MD: American Fisheries Society, abstract #24261566.

Sekiguchi K, 1988. Ecology. In: Sekiguchi K ed. Biology of Horseshoe Crabs. Tokyo: Science House, 50–68.

Shin PKS, Li HY, Cheung SG, 2009. Horseshoe crabs in Hong Kong: Current population status and human exploitation. In: Tanacredi JT, Botton ML, Smith DR ed. Biology and Conservation of Horseshoe Crabs. New York: Springer Science, 347–360.

Shuster SM, Wade MJ, 2003. Mating Systems and Strategies. In: Krebs JR, Clutton-Brock T ed. Monographs in Behavior and Ecology. New Jersey: Princeton University Press, 1–533.

Smith DR, Michels SF, 2006. Seeing the elephant: Importance of spatial and temporal coverage in a large-scale volunteer-based program to monitor horseshoe crabs. Fisheries 31(10): 485–489.

Smith DR, Pooler PS, Swan BL, Michels S, Hall WR et al., 2002. Spatial and temporal distribution of horseshoe crab *Limulus polyphemus* spawning in Delaware Bay: Implications for monitoring. Estuaries 25(1): 115–125.

Stephens PA, Sutherland WJ, Freckleton RP, 1999. Consequences of the Allee effect for behavior, ecology, and conservation. TREE 14: 401–405

Walls EA, Berkson J, Smith SA, 2002. The horseshoe crab *Limulus polyphemus*: 200 million years of existence, 100 years of study. Reviews in Fisheries Science 10 (1): 39–73.

Wenner E, Thompson M, 2000. Evaluation of Harvesting Impacts and Population Trends for *Limulus polyphemus* in South Carolina. Charleston, SC: Marine Resources Research Institute, South Carolina Department of Natural Resources.