Reproductive Competition and Sexual Selection in Horseshoe Crabs

H. Jane Brockmann and Matthew Denman Smith

Abstract The four species of horseshoe crabs share many similarities but differ in the intensity of reproductive competition. Although all horseshoe crabs nest synchronously, only *Limulus polyphemus*, the American horseshoe crab, has intense male–male competition (high operational sex ratios and multi-male groups around nesting pairs) and very high female nesting densities. These differences in reproductive competition are reflected in differences between American and Asian species (*Tachypleus gigas*, *T. tridentatus*, or *Carcinoscorpius rotundicauda*) in their reproductive structures and in egg and sperm size. However, the American and Asian species do not differ in the degree of sexual dimorphism in body size. The intensity of reproductive competition is correlated with nesting density in *L. polyphemus*, but this is not the case in the Asian species, which rarely have multi-male groups or highly male-biased operational sex ratios.

1 Introduction

Horseshoe crabs have an unusual reproductive system unlike that of any other arthropod (Sekiguchi 1988b, Giese and Kanatani 1987): all four species have external fertilization and broadcast spawning with close pairing but no brooding. The unusual reproductive behavior of the four extant species is in many ways very similar (Table 1). In all four species males attach to females (in amplexus) with modified prosomal appendages; the male–female pairs migrate into shallow water where they spawn; females oviposit in clusters by injecting their eggs into the substrate; and the eggs are fertilized as they are laid with aquatic free-swimming sperm (Brockmann 2003a; although females occasionally arrive in the nesting area without males, they do not lay eggs). These clusters of eggs are left buried 7–20 cm below the surface where they develop and the pair returns to the sea. Despite similarities, differences exist in the

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H.J. Brockmann (✉)
Department of Zoology, University of Florida, Gainesville, FL 32611-8525, USA
e-mail: hjb@zoo.ufl.edu

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Table 1 Comparison of reproductive patterns in horseshoe crabs

Characteristic	*Limulus polyphemus*	*Tachypleus tridentatus*	*Tachypleus gigas*	*Carcinoscorpius rotundicauda*
Nesting habitat	Bays and estuaries on undisturbed sandy beaches with low organic content in the upper intertidal; nest in 20 cm water or less[2,3,17, 20,22, 27,29]	Sheltered, low-profile sandy beaches with low organic content between the upper and lower tide lines in 30 cm water[3,10,12,16,22,23]	Brackish estuaries and rivers and sandy estuarine beaches near the high-tide mark[6,8,22,23,24]	Brackish estuaries and rivers in muddy sand and mangrove swamps[6,10,14,23,24]
Length of breeding season	MA: May–June[13,15,28] NY: late May–July[4] DE Bay: May–June[26] FL: March–Nov[20]	Japan: mid-July to mid-August[6,12,16,22,23] S. China: May–August[11]	Gulf of Thailand (lat. 10°N): April–August[22,23] Bay of Bengal, India (lat. 21°N): throughout the year[6,8]	Gulf of Thailand: throughout the year[22,23] Bay of Bengal, India: early pre-monsoon[14]
Timing of nesting	Nest on high tides of new and full moon[1,20,30]	Nest on high tides of new and full moon[5,12,22]	Nest on high tides of new and full moon[6,8,24]	Nest on high tides of new and full moon[6,14]
Density: pairs/100 m	Variable, 1–1863 pairs, median = 40 (FL; see Table 2)	1–48, normally 30 pairs[22]	1–20 pairs[6,8]	50–100 pairs[14]
OSR (M:F)	1.5–6.9[1,20,30]	Normally 1.0[3,22]	1.09[6,8,31]	1.0–1.4[14]
Egg numbers/ female/ year	14,500–63,500[15] 88,000[28]	20,000[16,23,28]	1242–6565[6,7] 8000[23,28]	3540–13,490[7,14,23,28] 4217–10,982[6,5]
Eggs/cluster	3650[26,28]; 640–1280[15]	561–888[22]; 200–300[5,23,28]; 100–631[11]; up to 1000[7,12]	40–213[24]; 200–300[6]; 400[16,23,28]	20–72[14]; 43–139[6]; 80–150[23,28]

Table 1 (continued)

Characteristic	*Limulus polyphemus*	*Tachypleus tridentatus*	*Tachypleus gigas*	*Carcinoscorpius rotundicauda*
Egg size	1.6–1.8 mm[15,23,25,28]	3 mm[5,12,23,25]	3.25–4 mm[19,24,25,28]	2–2.2 mm[7,23,24,28]
		3–3.3 mm[16,28]	1.54–2.09 mm[7,16]	1.5–2.3 mm[5,25]
			4.0 mm[23]	1.25–1.5 mm[14]
Female:male size ratio;	1.28 (see Table 2)	1.14–1.20[3,32]	1.29[32]	1.04[32]
Prosomal width*	Male: 200.2 mm Female: 256.9 mm[32]	Male: 244.2 mm Female: 278.4 mm[32]	Male: 163 mm Female: 210 mm[32]	Male: 128.7 mm Female: 133.8 mm[32]
Claspers on male	First pair thickened; hemichelate[32]	First and second thickened; hemichelate[32]	First and second thickened; hemichelate[32]	First and second slightly swollen; chelate[32]

* Size varies considerably between sites in all four species (see Table 2 for variation in *L. polyphemus*).

[1]Barlow et al. (1986); [2]Botton et al. (1988); [3]Botton et al. (1996); [4]Botton et al. (2006); [5]Chatterji and Parulekar (1992); [6]Chatterji (1994); [7]Chatterji (1995); [8]Chatterji et al. (1992); [9]Chen et al. (2004); [10]Chiu and Morton (2003); [11]Chou and Cheng (1950); [12]Goto and Hattori (1929); [13]James-Pirri et al. (2005); [14]Khan (2003); [15]Leschen et al. (2006); [16]Mikkelsen (1988); [17]Penn and Brockmann (1994); [18]Rama and Rao (1972); [19]Roonwal (1944); [20]Rudloe (1980); [21]Sekiguchi (1988a); [22]Sekiguchi (1988b); [23]Sekiguchi and Nakamura (1979); [24]Sekiguchi et al. (1977); [25] Sekiguchi et al. (1988); [26]Shuster and Botton (1985); [27]Shuster (1950); [28]Shuster (1982); [29]Smith et al. (2002); [30]Smith et al. (2002b); [31] van der Meer Mohr (1941); [32] Yamasaki et al. (1988).

reproductive biology of the four species, which may be associated with ecological and environmental differences.

All four species of horseshoe crabs migrate for spawning into shallow water. *Limulus polyphemus*, *Tachypleus gigas*, and *T. tridentatus* nest on sheltered, intertidal beaches (Chiu and Morton 2003, Sekiguchi 1988b) where the sand is well aerated, low in organic matter, and low in mud and silt (Botton et al. 1988, 2006, Penn and Brockmann 1994, Smith et al. 2002a). This substrate provides a suitable environment for development until the larvae emerge and metamorphose into juveniles (the timing of which depends on temperature and population; Laughlin 1983). These nesting areas are usually in estuaries and bays that are protected from high waves and that have shallow, nearby flats that provide feeding grounds for the young juveniles (Brady and Schrading 1997, Chiu and Morton 1999, 2003, Sekiguchi 1988b). As they grow larger, juveniles move into progressively deeper water (Shuster 1982). Unlike the other three species, *Carcinoscorpius rotundicauda* nests well up into the mouths of rivers, on mudflats, and in mangroves (Khan 2003, Mikkelsen 1988). Horseshoe crabs are capable of picking out favorable habitat within much larger, unsuitable areas (Botton et al. 2006), so their nesting is often concentrated in a few high-quality patches.

The concentrated nesting of horseshoe crabs may also be due to two additional factors. First, it is possible that horseshoe crab nesting may be social, i.e., that horseshoe crabs are attracted by the presence of conspecifics. Social nesting is often thought to evolve in response to predation, which may be important for *L. polyphemus*, at least at some points in their long life cycle (Botton 2009). Second, there is good evidence that horseshoe crabs are philopatric. Larvae metamorphose shortly after emerging from the nest and often settle near their natal beaches (Botton et al. 2003a, Rudloe 1981). Both tagging (Baptist et al. 1957, Leschen et al. 2006, Rudloe 1980, Sokoloff 1978, Swan 2005) and tracking studies of animals equipped with sonar (Moore 2004, Brousseau et al. 2004) demonstrate that most individuals remain within a limited area throughout the year. Phenotypic studies (Botton and Loveland 1992, Burton 1983, Itow 2004, Kato et al. 2005, Miyazaki et al. 1987, Palumbi and Johnson 1982, Riska 1981, Rudloe 1985, Sekiguchi et al. 1976, Sekiguchi et al. 1978, Shuster 1955, 1982, Sokoloff 1978) and genetic analyses (Itow 2004, King et al. 2005, Pierce et al. 2000, Saunders et al. 1986) demonstrate substantial differences among populations that could only be maintained with little gene flow. Such high levels of philopatry mean that horseshoe crabs will concentrate in areas where eggs and juveniles develop successfully.

In addition to being concentrated in certain areas, nesting is also highly synchronized. In all four species, migration to the nesting area for spawning is associated with high water levels (Sekiguchi 1988b). Pairs of horseshoe crabs leave the tidal flats with the incoming tide, apparently in response to increased water flow rates (Brockmann in prep) that might be detected by water flushing through the book gills and pressure-sensitive areas on the carapace (Chen et al. 2004, Kaplan et al. 1976, Sekiguchi 1988b, Shuster and Botton 1985, Waterman and Travis 1953). Specifically, migration to nesting sites is timed to the spring tides, i.e., to the several days of extra high tides just before, during, and after the new or full moons (each such period is called a tidal cycle) (Barlow et al. 1986, Cohen and Brockmann 1983, Sekiguchi 1988b, Smith et al. 2002b). In general, the number of horseshoe crabs migrating to shore is higher when tides are higher (Barlow et al. 1986, 1987, Barlow et al. 1986, Brockmann in prep, Chatterji et al. 1992, Sekiguchi 1988b, Schaller 2002; although this is apparently not true of all populations, Smith et al. 2002a). Also, they prefer the higher of two unequal tides in a day (Barlow et al. 1986, 1987, Cohen and Brockmann 1983, Rudloe 1980). In areas with little or no tide, migration occurs at times of high water, such as when wind-blown surge increases water levels (Brockmann unpublished ms, Ehlinger et al. 2003, Rudloe 1985). The strong synchronization with high tides or high water levels means that large numbers of horseshoe crabs may nest at the same time.

Further, horseshoe crab spawning is seasonal. In areas where the temperature falls below 16–20°C, horseshoe crabs are inactive (Chiu and Morton 2003, Sekiguchi 1988b), apparently moving to deeper water and burying in the substrate (Sekiguchi 1988b, Wenner and Thompson 2000; but the most northern population of *L. polyphemus* in Maine remains active down to 12–14°C; Schaller 2002). This limited temperature range for activity means that horseshoe crabs in many parts of their range have a limited breeding season. But temperature is not the only factor limiting reproduction because all four species of horseshoe crabs seem to have a well-defined breeding season of 1–3 months (Barlow et al. 1986, Chatterji 1994, Rudloe 1980, Sekiguchi and Nakamura 1979), which further contributes to their synchrony of spawning (Sekiguchi 1988b).

In all four species, the synchronous and seasonal spawning of horseshoe crabs that is associated with high tides (or high water), and that is focused on a few suitable sites, can result in high nesting densities. The extreme occurs in Delaware Bay, where beaches may be literally paved with nesting *L. polyphemus*. In fact, there are so many nesting horseshoe crabs that females dig into nests laid earlier in the season (Smith 2007). Also in this species, large numbers of unpaired males converge on the nesting beaches and crowd around the nesting pairs as satellites to form spawning groups. Paternity analyses demonstrate that in these mating groups, satellite males (in addition to attached males) fertilize many of the eggs that females lay (Brockmann et al. 1994). High nesting densities and high operational sex ratios (OSR, males:females) with strong sperm competition mean that both males and females can face high levels of reproductive competition. But in other areas or at other times, when population sizes are smaller or spawning is less synchronous or less focused on a few favored beaches, females rarely exhume the nests of others (female competition) and few satellites gather around the nesting pairs (male competition) (Brockmann 1990, Carmichael et al. 2003, Leschen et al. 2006, Penn and Brockmann 1994, Shuster and Botton 1985). This means that in *Limulus* the level of reproductive competition is highly variable. *Tachypleus* and *Carcinoscorpius* are consistently described as showing little or no reproductive competition (Sekiguchi 1988b). Botton et al. (1996) suggest that the lack of satellite males and group spawning in the Asian horseshoe crabs may be explained by their much smaller population sizes.

In this chapter we first detail the pattern of reproductive competition in *L. polyphemus* and evaluate whether differences in the presence of satellites and satellite groups within and among populations can be explained as flexible responses to different female nesting densities. We then return to the Asian species of horseshoe crabs to evaluate whether their lower levels of reproductive competition can be explained by their lower densities.

2 Reproductive Competition in Male *L. polyphemus*

2.1 Reproductive Competition in Limulus

Reproduction in *L. polyphemus* is often highly competitive. Female nesting densities may be remarkably high (e.g., 10–80 females m^{-2}; Smith et al. 2002b), in fact, so high that females frequently dig over previous nesting sites. This causes eggs to be exhumed by the later arriving individuals and results in considerable egg mortality (Botton et al. 2003b, Kraeuter and Fegley 1994, Smith 2007). Even at high female densities, male-biased OSRs (operational sex ratio, males:females) are the rule. Male biases result from the fact that only a small proportion of females in the population nest at any one time (Brockmann 2003b, Cohen and Brockmann 1983) and females normally complete their nesting with only a few visits to the beach, whereas males return repeatedly throughout the breeding season (Leschen et al. 2006, Rudloe 1980, Sekiguchi 1988b). Females are determinate layers, i.e., all eggs to be laid in a given breeding season are mature at the onset of spawning, so once all eggs are laid the female leaves the breeding area (Leschen et al. 2006). Attached males normally remain with the same female throughout her nesting but then detach when the female's breeding is complete and she returns to offshore feeding areas (Brockmann and Penn 1992). There may be differences among populations in the length of attachment: attached pairs have been observed overwintering on Cape Cod (Barlow et al. 1987, Moore 2004) whereas in Florida (Seahorse Key; SHK) animals usually remain together for only one tidal cycle (mean length of attachment is 3.7 ± 6.1 s.d. days; Brockmann and Penn 1992) but occasionally may stay together longer (up to 51 days; Brockmann 2003b).

Spawning groups form when unattached (i.e., unpaired) males crowd around a nesting pair as satellites (Barlow et al. 1987, Shuster 1953, Shuster and Botton 1985, Sydlik and Turner 1990). They are attracted to a pair either before nesting has begun or after nesting is underway (Schwab and Brockmann 2007); they use visual cues and are equally sensitive during the day and at night (Barlow et al. 1987, Barlow and Powers 2003). Males also approach cement models of horseshoe crabs that have been placed in shallow water or along the shoreline. When encountering two models, males prefer to approach the one that contrasts more strongly with the background, the one that is shaped like a horseshoe crab (Barlow et al. 1982, 1987, Barlow and Powers 2003) and the larger of the two models (Schwab and Brockmann 2007). Males also prefer to approach a model placed over a site where females have been nesting previously; they are particularly attracted to a site where a female with many satellites has nested before (Hassler and Brockmann 2001); and when satellite males are removed from a female, that female is more likely to regain satellites than a female that had not previously attracted satellites (Brockmann 1996), which suggests that chemical cues are involved.

Whether approaching a model or a nesting female, the satellite males take up characteristic positions around the pair (Brockmann 1990). The favored position over the female's incurrent canal (Fig. 1) results in particularly high levels of paternity, but satellites in all positions may achieve at least some paternity (Brockmann 2003b, Brockmann et al. 1994, 2000). Males jockey for position around the female, sometimes working their way under the front margin of the attached male's prosoma. This position results in somewhat higher paternity for the satellite (Brockmann et al. 1994, 2000). Other factors also affect paternity including tidal current speed (faster current favors satellites) and female size (attached males have higher paternity with larger females) (Brockmann et al. 2000). In many externally fertilizing invertebrates, both sperm limitation and

Fig. 1 Photograph of a spawning group of *L. polyphemus* at Seahorse Key, FL (Levy County). The attached male (*center*) is lighter and has fewer encrusting epibionts than the five satellites that surround him. Two of the satellites have taken up positions over the female's incurrent canal (indicated with *arrows*). Paternity analyses reveal that these two satellites will fertilize most of the eggs that the female lays (photograph by HJB)

sperm competition are involved (Levitan 1998, Yund and McCartney 1994), but in *Limulus* nearly all eggs (96–100%) are fertilized whether satellites are present or not (Brockmann 1990).

2.2 Assortative Mating and Alternative Tactics

High levels of reproductive competition are associated in many species with size-assortative mating (Andersson 1994, Harari et al. 1999, McLain 1987). In *L. polyphemus* unattached and paired males do not differ in overall size (Botton and Loveland 1992, Brockmann 1990, 1996, Brockmann and Penn 1992, Cohen and Brockmann 1983, Penn and Brockmann 1995) or in clasper size (Botton et al. 1996, Brockmann 1990, Cohen and Brockmann 1983, Duffy et al. 2006). Replacement males (i.e., when a female returns to the beach with a different attached male than on the previous tide) are not different in size from the original mates (Brockmann 1990, Brockmann and Penn 1992). This means that there is no assortative mating based on size (Brockmann 1990, Cohen and Brockmann 1983, Pomerat 1933) and no size advantage for large males. Botton and Loveland (1992), Loveland and Botton (1992) and Suggs et al. (2002) argue that this is because there are no size-related constraints on amplexus.

Nonetheless, differences in physical condition exist between males that are found paired and unpaired males in both Florida and Delaware Bay populations (Fig. 1; Brockmann 2003b). Attached males are less likely to be covered with epibionts; their eyes are less likely to show deterioration or fouling; their prosoma is lighter and less pitted; and they are less likely to show damage or to be missing the claws used to clasp females (Brockmann 2002, Brockmann and Penn 1992, Duffy et al. 2006, Penn and Brockmann 1995, 1996). These lighter males have less damage from the flatworms (*Bdelloura candida*) that lay eggs on their gills (Groff and Leibovitz 1982, Watson 1980a,b) and they have a heavier mucous secretion on their prosoma (Harrington and Armstrong 2003), which is thought to protect them from fouling, infections, and possibly UV light (Brockmann 2003b). Taken together, these data suggest that attached males are younger (their terminal molt occurred more recently) than unattached males (Brockmann 1996, 2003b).

Younger males are also behaviorally different from older individuals. They are more active and more likely to pair; they pair more quickly; they are more likely to remain paired; and they are more likely to find a female if they are detached (Brockmann and Penn1992). Younger males are also less likely to return to the nesting area and return less frequently than males in poorer condition (Brockmann 2002, Brockmann and Penn 1992). In addition, males in good condition are more likely to right themselves if overturned and less likely to become stranded after the tide (Penn and Brockmann 1995), a major cause of mortality among spawning males (Botton and Loveland 1989).

The physical and behavioral differences between attached and unattached males mean that there are two condition-dependent mating tactics in *L. polyphemus* (Brockmann and Penn 1992). Although some males switch between tactics, most of the time they are confined to one tactic or the other (Brockmann 2002). Brockmann (2002) has also shown that being unattached is not just a result of males not finding females: males in good condition that are prevented from attaching do not just come ashore and take up satellite positions around the nesting pairs. Rather, males in good condition are less likely to become satellites even when they cannot attach, which further supports the view that younger and older males use different mating tactics. There are no effects of satellite male condition on paternity but when the attached male is in worse condition, the satellite male's paternity improves (Brockmann et al. 2000).

2.3 Sexual Size Dimorphism and Reproductive Competition

In many species of animals, the degree of sexual size dimorphism is positively correlated with reproductive competition (Clutton-Brock et al. 1977, Jehl and Murray 1986, Mitani et al. 1996). In turn, reproductive competition is positively correlated with density (Conner 1989) and with a male-biased OSR (Kvarnemo and Ahnesjö 1996). *L. polyphemus* is sexually dimorphic with females larger than males. Populations differ in the degree of sexual dimorphism (F:M ratio; $\bar{x} = 1.29 \pm 0.04$ SD; range = 1.18–1.35; $n = 15$; Table 2) and in the level of reproductive competition: nesting density averages 0.74 ± 1.27 s.d. crabs/m^2 (range = 3.54–0.01; $n = 10$; Table 2) and OSR averages 2.9 ± 1.28 s.d. males per female (range = 1.1–5.8; $n = 15$; Table 2). Despite large differences in reproductive competition, the degree of sexual dimorphism does not correlate with the level of reproductive competition in females (nesting density) or males (OSR) (least-squares multiple regression, $F_{3,8} = 0.31$, $p = 0.813$). Instead, variation in size dimorphism may be due to other ecological factors such as resource availability or to environmental factors such as temperature.

2.4 Group Size, OSR, and Spawning Density

The intensity of sperm competition within a population varies markedly because mating group sizes and OSR are highly variable. Even when many pairs are nesting, at least some are without satellites; and even when few pairs are nesting some usually have satellites (Fig. 2; Brockmann 1996, Cohen and Brockmann 1983, Hassler and Brockmann 2001). In Delaware Bay (DE) the mean number of satellites per pair ranges from 0.2 to 5.2 and increases with the number of pairs on the beach (Fig. 2B), and 5–87% of the nesting pairs have no satellites (this is more likely at lower nesting densities; Fig. 2A; Brockmann 1996). Similarly, in a population from the west coast of Florida, Seahorse Key

Table 2 Comparisons of factors associated with reproductive competition among populations of *Limulus polyphemus*

State	Location	Study year	Sex	Prosomal width (cm) ± SD (*n*)	Dimorphism (F:M)	OSR (M:F) (n = # of tides)	Density	Source
ME	Hog Bay	2001	Male	14.7 (951)	1.183	2.8 (17)	0.16 crabs/m^2	Schaller (2002)
			Female	17.4 (337)				
	Thomas Point Beach[a]	2001	Male	14.8 (26)	1.297	1.7 (17)	0.30 crabs/m^2	Schaller (2002)
			Female	19.2 (14)				
	Bagaduce River[a]	2001	Male	13.9 (9)	1.259	1.07 (8)	0.06 crabs/m^2	Schaller (2002)
			Female	17.5 (12)				
MA[b,c]	Monomoy NWR	2000–2002	Male	18.8 ± 1.5 (909)	1.298	1.9 (14)	0.06 crabs/m^2	James-Pirri et al. (2005)
			Female	24.2 ± 2.2 (447)				
	Pleasant Bay[d]	2000–2002	Male	17.9 ± 1.3 (1775)	1.279	5.8 (12)	0.04 crabs/m^2	James-Pirri et al. (2005)
			Female	22.9 ± 2.4 (298)				
	Nauset Estuary	2000–2002	Male	17.5 ± 1.7 (433)	1.337	1.6 (6)	0.01 crabs/m^2	James-Pirri et al. (2005)
			Female	23.4 ± 2.1 (256)				
	Cape Cod Bay	2000–2002	Male	17.4 ± 1.6 (2942)	1.304	2.9 (27)	0.007 crabs/m^2	James-Pirri et al. (2005)
			Female	22.7 ± 1.9 (759)				
NJ[e]	Cape May	1977	Male	20 ± 1.4 (1058)	1.283	5		Shuster and Botton (1985)
			Female	25.7 ± 2.0 (653)				
	Cape May	1979	Male	20 ± 1.4 (1058)	1.283	3		Shuster and Botton (1985)
			Female	25.7± 2.0 (653)				
	Cape May	1999	Male	20 ± 1.4 (1058)	1.283	3.5[f] (86)	0.75 females/m^2 ≈2.63 crabs/m^2	Smith et al. (2002b)
			Female	25.7 ± 2.0 (653)				
DE[f]	DE Bay	1977 1979	Male	20 ± 1.4 (75)	1.275	3		Shuster and Botton (1985)
			Female	25.5 ± 1.9 (50)				

Table 2 (continued)

State	Location	Study year	Sex	Prosomal width (cm) ± SD (*n*)	Dimorphism (F:M)	OSR (M:F) (n = # of tides)	Density	Source
	DE Bay[c]	1999	Male	20 ± 1.4 (75)	1.275	3.5 [f] (86)	1.01 females/ m^2 ≈3.54 crabs/ m^2	Smith et al. (2002b)
			Female	25.5 ± 1.9 (50)				
SC[d]	Otter Island	1999	Male	23.7	1.299	1.96		Wenner and Thompson (2000)
			Female	30.8				
FL	Apalachee Bay	1976–1978	Male	16.9 ± 1.3 (1552)	1.324	3.6 (66)		Rudloe (1980)
			Female	22.3 ± 2.2 (742)				
	SHK	2004–2005	Male	16.6 ± 1.2 (586)	1.35	2.1 (115)	0.55 crabs/m^2	Brockmann, unpublished data
			Female	22.5 ± 1.5 (322)				

[a] Several survey dates were clearly past the breeding season and were not included in calculating average density.
[b] Density was averaged from all years and day and night surveys.
[c] Numbers were estimated from graph.
[d] Populations at these sites have been heavily harvested, thus reported OSR may be an overestimate due to a harvesting preference for large females.
[e] Data on body size from Loveland and Botton (1992).
[f] Unpublished data on body size from H.J. Brockmann.
[g] At beaches with a spawning density >0.35 females/m^2.

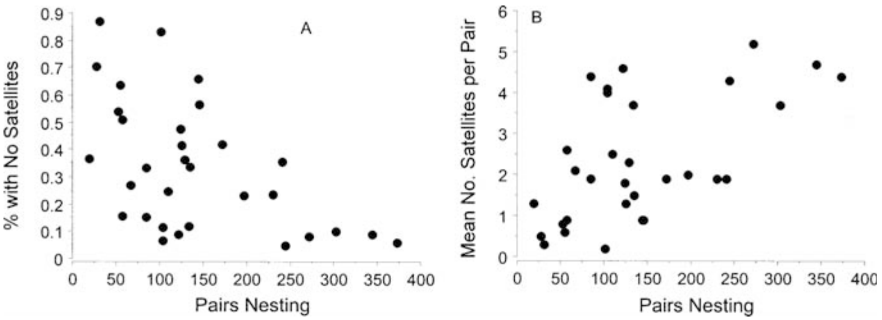

Fig. 2 Correlation between nesting density and spawning groups at Cape Henlopen, Delaware Bay (May and June 1991–1993; data from Brockmann 1996). (**A**) The percent of the population without satellites is negatively correlated with nesting density ($r^2 = 0.28$, $p = 0.002$, df = 30); (**B**) The mean number of satellites per pair is positively correlated with density ($r^2 = 0.33$, $p = 0.0007$, df = 30)

(SHK, 2004–2005), mean group size varies from 0 to 2.5 and is correlated with nesting density ($r^2 = 0.13$, $p < 0.0001$, df = 114); 23–100% of the pairs are without satellites, and this is negatively correlated with nesting density ($r^2 = 0.3$, $p < 0.0001$, df = 114). In these studies the number of lone males (unattached males that are not in groups) varies greatly (38–91% of all males present in DE; 0–100 % at SHK) and correlates with density (DE: $r^2 = 0.28$, $p = 0.002$, df = 30; SHK: $r^2 = 0.1$, $p = 0.004$, df = 114). However, OSR as a measure of reproductive competition does not always correlate with nesting density (SHK: $r^2 = 0.1$, $p = 0.004$, df = 114) because there are both high and low OSRs at low densities.

The OSR varies widely within and among populations (Table 2; Carmichael et al. 2003). For example, in DE the median OSR is 3.5 (males:females) at times when many crabs are nesting (>0.35 females/m^2) and 1.5 when fewer crabs are present (Smith et al. 2002b). On Cape Cod, MA, some populations show low mean OSR (Nauset Estuary: 1.6), whereas other nearby locales show much higher OSR (Pleasant Bay: 5.8), but overall there is no correlation between OSR and spawning density ($r^2 = 0.03$, $p = 0.47$, $n = 10$; James-Pirri et al. 2005). A number of factors may account for variation in OSR. Unattached males are more vulnerable to being overturned in the waves than attached males (Penn and Brockmann 1995) and when overturned many of these unattached, poor-condition males are unable to right themselves. Perhaps for this reason, OSR is lower with higher waves (Smith et al. 2002b). At SHK the OSR is higher toward the end of a tidal cycle and toward the end of the season (Brockmann, unpublished ms) but in Maine the numbers of unattached males decline later in the season (Schaller 2002). Perhaps because of these many interacting factors, in general, some populations show a higher OSR when more nesting pairs are present (Rudloe 1980, Smith et al. 2002b) and several show no such relationship including populations from Maine (Schaller 2002) and New York (Hanna 2001). Taken together, these within-population and among-population comparisons mean that differences in male competition (i.e., differences in OSR and frequency of group spawning) are not simply a reflection of changes in the number of nesting females between tides.

3 Differences in Reproductive Competition Among Horseshoe Crab Species

3.1 Reproductive Competition in Asian Horseshoe Crabs

The evidence for reproductive competition in *Tachypleus* and *Carcinoscorpius* is quite limited. Population sizes and nesting densities of *T. tridentatus* and *C. rotundicauda* are thought to be low throughout most of their range and most studies describe population OSR as 1:1 (Chen et al. 2004, Chiu and Morton 1999, Khan 2003). *T. tridentatus* normally nest as pairs where the maximum number of pairs present is 48 and normally fewer than 30 (Sekiguchi 1988b). Satellite male behavior appears to be almost non-existent. Sekiguchi (1988b) observed several males in a tank clasping onto paired couples, but states that males do not normally associate in this way under natural conditions where a male–female pair is the normal condition. Botton et al. (1996) observed no unpaired males during their study of *T. tridentatus*. Although he does not comment on seeing satellites or groups, a picture in Mikkelsen (1988, p. 82) clearly shows two male *T. tridentatus* traveling in tandem with the accompanying legend “a cluster of males surrounding a female of the same species” from a site near Beihai, China, on the Beibu Gulf (where the numbers of nesting pairs are higher than elsewhere in the range). Botton et al. (1996) observed a satellite male with a mated pair of *T. gigas* in an aquarium and the behavior of this animal was identical to that of satellite *L. polyphemus*. Chatterji (1994) describes *T. gigas* populations in the Bay of Bengal as low with 1–17 females nesting on a beach each day with a total of 235 females and 255 males over the entire nesting season and an OSR of 1.09, but he has no descriptions of groups. The picture of reproductive competition in the Asian horseshoe crabs, then, is very different from that observed in *L. polyphemus*.

3.2 Is Low Reproductive Competition the Result of Low Density?

The available data suggest that *T. gigas* may have the highest levels of reproductive competition of the Asian species. Chatterji et al. (1992) provide sufficient data for *T. gigas* to test the hypothesis that unattached males are more common when more nesting pairs are present. They observed 1–16 pairs on 36 high tides nesting along the Balramgari coast of Orissa. Although the pairs often nested close together (based on figures in Chatterji 1994), there was never more than one unattached male present on any tide and no mention is made of satellite groups. Unattached males were no more likely to be present on a high tide when there were many nesting pairs than when there were few pairs (Mann–Whitney U Test, $N_1 = 17$, $N_2 = 19$, $p = 0.36$). For comparison, we examined a similar data set from SHK using Florida *L. polyphemus*, i.e., presence or absence of unattached males on all tides where there were 1–16 pairs nesting (0–49 unattached males were present on those tides; 2004–2005 data). Unattached males were significantly more likely to be present when there were more nesting pairs even within this limited range of nesting densities (Mann–Whitney U Test, $N_1 = 16$, $N_2 = 28$, $p = 0.01$). We conclude then (assuming overall equality of population sex ratios) that the pattern of male mating tactics in *T. gigas* is different from that found in *L. polyphemus* and that differences in the presence of satellites is not just a reflection of low nesting densities.

3.3 Sexual Size Dimorphism

The Asian horseshoe crabs are sexually dimorphic with males smaller than females (Table 1; Shuster 1982, Yamasaki et al. 1988). As in *L. polyphemus*, considerable variation in body size can be found throughout the range of the Asian species, but the relationship between male and female sizes remains about the same (Chiu and Morton 2001, Itow et al. 2004, Kato et al. 2005, Sekiguchi et al. 1978). The mechanism underlying size dimorphism is a difference in the growth pattern between males and females (Botton and Loveland 1992). Sekiguchi et al. (1988) showed that male *L. polyphemus* and *T. tridentatus* take 16 molts to reach maturity while females mature in 17. Thus, the consistency of dimorphism between populations is a result of a difference in the number of molts, regardless of adult body size. This is further demonstrated by Wenner and Thompson (2000) who found that the adult female to male size ratio (1.29) was the same as the mean juvenile pre-molt to post-molt ratio (size increase per molt) calculated over the first 11 instars. Despite the fact that the American horseshoe crabs face far more intense reproductive competition than any of the Asian species, the level of sexual dimorphism in *L. polyphemus* does not differ from the Asian species. This is consistent with the view that sexual selection in externally fertilizing animals is similar for males and females (Levitan 1998). For horseshoe crabs it might also be that factors other than sexual selection are affecting sexual dimorphism. For example, because larger females lay more eggs (Chatterji 1995, Chatterji et al. 1992, Khan, 2003, Leschen et al. 2006, Schwab and Brockmann 2007), fecundity selection may favor delayed sexual maturation of females, whereas selection may favor earlier maturation for males since there appears to be no size advantage in male–male competition. Another hypothesis suggests that physical constraints related to amplexus may favor males that are smaller than females (Botton and Loveland 1992).

3.4 Correlates of Reproductive Competition

Differences in reproductive competition between Asian and American horseshoe crabs are mirrored by differences in their internal and external anatomy. In all female horseshoe crabs, eggs are produced by the lining of the oviduct; mature eggs evaginate through the ovarian walls, break off, and accumulate in the lumen of the oviduct (Dumont and Anderson 1967) leading to the paired gonopores, located on the operculum. The posterior oviducts of female *L. polyphemus* are more developed than in the other three species and divided into several tubes (Yamasaki et al. 1988). During the breeding season these posterior branches are filled with the ovulated eggs that will be laid during that season. The more highly developed and numerous oviducts allow female *L. polyphemus* to store larger numbers of eggs prior to spawning and to lay far more eggs compared to the Asian species (Mikkelsen 1988, Sekiguchi and Nakamura 1979, Shuster 1982). In all four species larger females lay more eggs (Chatterji 1994, 1995, Chatterji and Parulekar 1992, Khan 2003, Leschen et al. 2006, Schwab and Brockmann 2007), presumably because of their greater storage abilities.

L. polyphemus eggs are substantially smaller and females carry many more eggs and deposit many more at one time than in any of the three Asian species (Table 1). Smaller eggs mean smaller newly hatched larvae, and *Limulus* has the smallest larvae of the four species (Shuster 1982). This variation represents different strategies in the life history trade-off between egg size and egg number. The smaller, more numerous eggs laid by *L. polyphemus* may be an adaptation to their higher OSR, higher densities, and much greater female competition or to higher predation on their eggs and larvae compared with the Asian species. We are currently investigating variation in egg size and number and the potential causes of differences among populations of *L. polyphemus*.

The reproductive system of male *L. polyphemus* also differs from that of the other horseshoe crabs in several important ways (Yamasaki et al. 1988). Sperm are produced in the walls of a reticulated network of fine tubules that make up the testes with a complex network of sperm ducts leading to the paired gonopores, which are located on the operculum. Unlike the Asian species, *L. polyphemus* has more complexly branching and proliferated distal sperm ducts and "sperm sacs" or organs that store mature sperm in the testes (Yamasaki et al. 1988). This means that male *L. polyphemus* are capable of storing mature sperm, perhaps as an adaptation for sperm competition or as an adaptation for fertilizing the eggs of the highly fecund females. It may even be possible for males to adjust their ejaculate to the intensity of sperm competition as has been demonstrated in some insects (Schaus and Sakaluk 2001, Wedell 1999) and other species. Selection may have favored the loss of these structures in the Asian horseshoe crabs because of the lower risk of sperm competition and the fact that fewer eggs are laid at one time.

Limulus sperm are quite different from the sperm of the Asian species. The internal microstructure of the flagellum is different and because of this *Limulus* sperm swim much faster than the sperm of the Asian species (Ishijima et al. 1988). After reaching the egg, fertilization begins when the sperm's acrosomal filament is explosively released through the sperm head and literally screws through the jelly of the egg (Fahrenbach 1973, Sekiguchi et al. 1988, Tilney 1975). Presumably because of the reduced size of the egg and surrounding jelly, the acrosomal filament of American horseshoe crab sperm is shorter than that found in the sperm of the Asian species although the overall sperm size is the same (Hong et al. 1995). *Limulus* eggs are fertilized after multiple sperm attachment (Brown and Knouse 1973) and multiple acrosomal reactions (Fahrenbach 1973, Yamasaki et al. 1988). All horseshoe crabs must have mechanisms to prevent polyspermy, but *Limulus* must have particularly strong blocks since there are often many spawning males (and hence more sperm) with one female. The differences in sperm structure and behavior among horseshoe crab species may be adaptations to differences in the level of sperm competition.

The external anatomy of the four species also differs in ways that may parallel differences in reproductive competition (Yamasaki et al. 1988). *L. polyphemus* has one pair of claspers that hold the terminal spines (posterior processes) of the female. In contrast *T. tridentatus*, *T. gigas*, and *C. rotundicauda* have two pairs of claspers that are used in amplexus (Botton et al. 1996, Shuster 1982, Yamasaki et al. 1988). The anterior pair attaches along the opisthosomal margin or to the moveable spines and the posterior pair attaches to the terminal spines. The result is that attached male *Tachypleus* are more firmly attached and ride farther forward on the female's opisthosoma than male *Limulus*. Additionally, the anterior portion of the male's prosoma is more arched than in the female, and this is particularly noticeable in *T. tridentatus*. The presumed function of this prosomal arch is to allow the male to fit over the female's opisthosoma (Shuster 1982). While differences in reproductive competition may be responsible, there are many alternative hypotheses to explain variation in patterns of amplexus and body shape between American and Asian species. Unfortunately, there have been no studies on the costs and benefits associated with differences in either (1) the number of claspers (or the resulting attachment strength and ride height) or (2) prosomal arch size. Despite the lack of data, some potential trade-offs have been discussed. First, Botton et al. (1996) suggest that the different patterns of amplexus in the Asian species have been selected to assure fertilization by the attached male since, unlike *Limulus*, satellites are rarely if ever present during spawning. Alternatively, past competition could have selected for morphology that reduced vulnerability to sperm competition (i.e., dual claspers that increase attachment strength), thus making satellite behavior less successful than in *Limulus*. Second, the increased attachment strength of the Asian species may make them less likely to become detached from females in rough waves (especially beneficial because unattached males are more likely to become stranded). In contrast, Botton et al. (1996) suggest that the higher ride height (because of the two pairs of claspers) of the Asian species may actually make them more vulnerable to wave action. Lastly, variation in prosomal arch size may affect how males are attached to females. The resulting fit between a male and a female may influence susceptibility to both wave action and sperm competition. Variation in arch size between species has not been quantified. However, in *Limulus*, arch height varies significantly within populations and potentially among populations (M. D. Smith unpublished data). We are currently investigating the fitness costs and benefits of arch variation and how factors such as density, OSR, and individual condition influence fitness trade-offs. These studies will help us to understand if and how reproductive competition influences external anatomy and patterns of amplexus in the four species.

4 Conclusions

In addition to their unusual external fertilization, horseshoe crabs differ from many other species in having a limited amount of time for breeding. In most populations pairs are limited to only a few hours a day over 5 days every 2 weeks for 1 or 2 months if the seas are relatively calm and if they can find a suitable nesting site. Males engage in scramble competition, clasping females and remaining with them through much of their breeding period. This means that horseshoe crabs, and particularly *L. polyphemus*, have a mating system that is closer to the explosive breeding system found in some anurans and fishes than it is to the mating patterns of most other arthropods (Brockmann 1990). Like other explosive breeders with scramble competition, males seek out females rather than females approaching males; there is no assortative mating based on size; there are no size differences between paired and unpaired males; males often clasp inappropriate objects; and they mate guard by remaining in amplexus for days. As in other explosive breeders (Myers and Zamudio 2004, Tennessen and Zamudio 2003, Zamudio and Chan 2008), *L. polyphemus* males show condition-dependent alternative mating tactics and relatively high levels of multiple mating and multiple paternity. In several groups of anurans and *L. polyphemus*, high sperm production is correlated with a high probability of group spawning (Byrne et al. 2002), which suggests that satellite behavior is not merely the result of unusually high population densities, but rather is an evolved trait that increases individual reproductive success under particular ecological conditions. Short-term changes in densities or operational sex ratios (OSR) select for the ability to change tactics quickly (Arak 1983, Wells 1977). Populations of *Limulus* in different regions experience much greater variation in local densities, OSR, and lengths to their breeding seasons than do the Asian species, and this selects for individuals that can fine-tune their behavioral responses and maximize fitness by varying their mating tactics depending on the immediate social context (Semlitsch 1985, Sullivan 1989). Under these conditions selection favors males that can switch to satellite behavior when the payoff to searching is low, thus favoring the evolution of satellite behavior and spawning groups as we see in the American horseshoe crab. Alternatively, when there is little variation in population density, OSR, or breeding season length, and especially if densities are so low that females are hard to find, selection favors males that search out females and stay with them, as in the Asian horseshoe crabs. Although low population densities may be a recent phenomenon due to habitat destruction and exploitation, the morphological differences between the four species in reproductive structures, eggs, and sperm suggest long-standing patterns of far greater reproductive competition in American than in Asian horseshoe crabs.

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