Chapter 19
Mating Tactics of the American Horseshoe Crab (*Limulus polyphemus*)

H. Jane Brockmann, Sheri L. Johnson, Matthew D. Smith, and Daniel Sasson

Abstract The reproductive behavior of horseshoe crabs (*Limulus polyphemus*) is easily observed, yet until recently, little was known about their unusual mating habits. Fertilization is external and occurs beneath the female as her eggs are being laid in the sand. Spawning is often synchronized to the highest spring tides of the year, so the time available for breeding is limited. Males have alternative mating tactics, some search and pair with a female offshore and migrate inshore to spawn, while others search onshore for nesting pairs and engage in sperm competition in group spawning. Females also have alternative tactics such that some spawn with a single attached male and others spawn with multiple males, which results in multiple male paternity of their offspring. Both male and female tactics are condition and context-dependent and are affected by breeding density, operational sex ratio, and the interplay between male and female tactics. To better understand horseshoe crab reproductive behavior, we review studies conducted during the past 25 years in one Gulf of Mexico population in northern Florida at Seahorse Key. We discuss the costs, benefits, and tradeoffs of alternative tactics for males and females. We synthesize the recent literature on mating tactics, resolve some conflicting results, and point to the future by identifying the questions that remain.

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19.1   Introduction

Reproduction is the most conspicuous feature of horseshoe crab biology, since it is the only time when horseshoe crabs are seen in large numbers. During their spring-time breeding, horseshoe crabs travel to low energy, coastal beaches and estuaries, sometimes in enormous numbers (Teale 1957; Myers 1986; Kerlinger 1998). They arrive with the incoming tide, usually in amplexus, the male clasping the posterior margin of the female's shell with his modified claws. The pair nests at the top of the high tide line, sometimes entirely out of the water, and quite apparent to any beach visitor. Yet, it has only been in the last 25 years that this highly visible mating and nesting behavior has been studied in detail.

The female lays her eggs in beach sediments where the eggs are fertilized externally as they are being laid by aquatic, free-swimming sperm (Levitan 1995; Brockmann 2003b). The fertilized eggs incubate in the sand for several weeks before emerging as larvae. No other arthropod, indeed no other animal, reproduces in this way. In addition to their unique mode of reproduction, *L. polyphemus* spawning is synchronized and temporally constrained. Spawning begins in the spring as the water warms (Smith et al. 2010) and continues for about 2 months (the dates depend on latitude); most females lay all their eggs for the year during just a few, consecutive high tides (i.e. determinate spawners, Leschen et al. 2006). In contrast, males return repeatedly to the spawning area (Brockmann and Penn 1992). For this reason, there are usually many more males on the nesting beach than females, i.e. the operational sex ratio (males: females or OSR) is usually male biased (Rudloe 1980; Sekiguchi 1988; Leschen et al. 2006; Smith et al. 2010; Brockmann and Johnson 2011). In most areas breeding is synchronized with the high tides around the new and full moon, i.e. the highest tides in a month (Rudloe 1985; Barlow et al. 1986; Penn and Brockmann 1994; Brockmann and Johnson 2011). Where mating and nesting occurs at the extreme high tide line, it results in eggs being left in an environment that is conducive to embryonic and larval development (Penn and Brockmann 1994; Brockmann 2003b; Vasquez et al. 2015). Synchronization with spring high tides means that spawning is usually confined to a limited number of hours each month during a 2–3 month breeding season. Such constrained and synchronized spawning has been referred to as "explosive breeding" with males engaging in scramble competition for females (Emlen and Oring 1977; Alcock 2009).

Horseshoe crabs share a number of mating patterns with other explosively breeding species such as frogs, toads (Brockmann 1990; Wells 2007; Roberts and Byrne 2011), and fish (Martin et al. 2004; Byrne and Avise 2009). For example, like male *L. polyphemus*, males of explosively breeding frogs and toads search out females, whereas prolonged breeding anurans sit and wait for females to arrive (Höglund 1989). Among explosively breeding anurans where scramble competition for mates is the rule, there is little female choice or direct male combat (Wells 2007), no size-assortative mating, and no differences in size between amplexed and non-amplexed males, as there is among prolonged breeders (Höglund and Saterberg 1989). Similarly, studies have found only weak or no size-assortative mating in *L. polyphemus* (Pomerat 1933; Cavenaugh 1975; Botton and Loveland 1992; Suggs

et al. 2002), no differences in body size (Brockmann and Penn 1992) or clasper size (Botton et al. 1996) between paired and unpaired males, and no evidence of female choice during pairing (Loveland and Botton 1992). Many features of horseshoe crab mating behavior can be understood in light of their external fertilization and the constraints imposed by "explosive" breeding and scramble competition for mates.

Understanding the reproductive patterns and mating behavior of horseshoe crabs is crucial to their management. Horseshoe crabs play an ecologically important role as predators of mollusks and other invertebrates and as food for sea turtles, intertidal fishes, and migratory shorebirds (Botton et al. 2003; Botton 2009). Commercially, horseshoe crabs are used by the biomedical industry and in the whelk (conch) and eel fisheries (Kreamer and Michels 2009). Dramatic declines in horseshoe crab numbers during the 1990s led to their management (ASMFC 1998; Berkson and Shuster 1999; Shuster et al. 2003). Much has been learned in the last 15 years about horseshoe crab reproduction, which is used to inform management decisions. For example, information on the effect of changes in OSR informed the decision to institute a male-only harvest in Delaware Bay in 2008 (ASMFC 2008). Long-term studies at a single location are particularly valuable for understanding annual and seasonal variation in reproduction as well as the behavior and reproductive success of individuals that experience different conditions.

A long-term study on the reproductive behavior of *L. polyphemus* has been conducted at Seahorse Key, FL, an island breeding site along the west coast of northern peninsular Florida (Brockmann and Johnson 2011). At Seahorse Key (SK), horseshoe crabs nest along a 1 km beach where their eggs develop in the sand. Emerging larvae quickly settle just offshore in an extensive mud, grass and sand nursery where the juveniles feed and grow. Since 1990, the Seahorse Key studies have focused on many aspects of horseshoe crab biology, behavior, life-history traits, and population status. Here we pull together what is known about the mating behavior of *L. polyphemus* at SK, resolve some discrepancies and conflicting results, make comparisons with other populations, and point to the future by identifying some of the questions and puzzles that remain.

19.2   Male Mating Tactics

The evolution and maintenance of two reproductive tactics in a population is puzzling. In horseshoe crabs some males clasp on to a female out at sea and arrive on a beach to spawn in amplexus (i.e., the attached tactic), whereas other males come to the breeding area alone (unattached tactic), roam the shoreline, and join spawning pairs or groups (Rudloe 1980; Cohen and Brockmann 1983; Shuster and Botton 1985; Barlow et al. 1986; Loveland and Botton 1992). These unattached males vie for position around the female and engage in sperm competition with other males, (i.e., satellite behavior, defined as an unattached male that is in physical contact with the female, her attached male or other satellites; Fig. 19.1, Brockmann 1990). Populations differ in the sizes of satellite groups (group sizes are correlated with nesting density), but at most *L. polyphemus* breeding sites, males show both attached
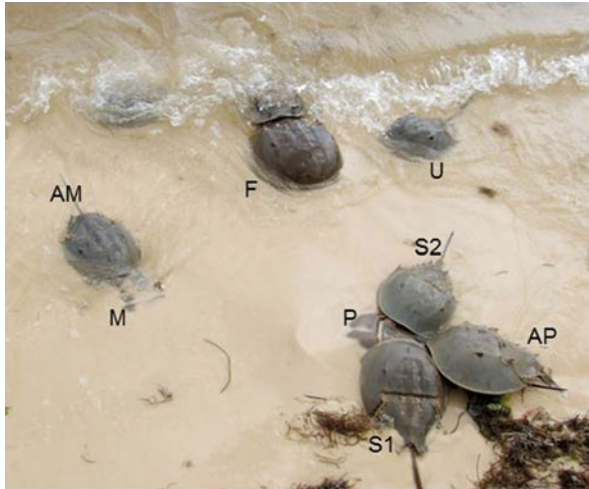
Fig. 19.1 Photograph of horseshoe crabs spawning at Seahorse Key, FL. A pair arrives on the beach, the larger female in front (*F*) with her attached male behind. An unattached male (*U*) approaches the beach and the arriving pair. A monandrous female (*M*) is nesting on the beach, well buried in the sand with her attached male (*AM*) visible behind her. A polyandrous female (*P*) is nesting nearby well buried in the sand and her attached male (*AP*) is visible behind her. There are two satellite males on either side of the polyandrous female (S1 and S2) both touching the AP male's carapace. These two males are in the 1F position with the S1 satellite under the front margin of the AP male's prosoma. This is the position that yields the highest paternity (Photograph by S.L. Johnson)

and unattached tactics (Brockmann and Smith 2009). Normally we would expect that if one tactic were even slightly less successful than the other, selection would eliminate that tactic over time (Brockmann 2001). Hence, in order for two tactics to be maintained within a population, each must be more successful than the other under some situations. In some species, alternative tactics are maintained as a genetic polymorphism, but in most species the tactics depend on the individual's phenotype (condition-dependent tactics), such as body size or age, and the circumstances in which they are living (context-dependent tactics), such as population density or sex ratio (Gross 1996; Zamudio and Chan 2008). These studies suggest the possibility that there may be differences among male horseshoe crabs that are using different tactics or there may be circumstances in which males are consistently attached or unattached.

19.2.1 Are Individual Males Consistently Attached or Unattached?

Mark-recapture studies are a useful tool for studying horseshoe crab mating tactics since males repeatedly return to the same nesting beach. These studies show that males are consistent in their attachment status: if a male is unattached on one high

tide, he is very likely to be unattached on the next high tide (Brockmann 2002). This consistency was also demonstrated through a series of experimental field manipulations in which we compared the behavior of males using different tactics. Males that came to the breeding beach unattached were compared with males that arrived attached to females but were then detached (Brockmann and Penn 1992). Unattached males were much more likely to return still unattached on the next high tide (49 %, $N=81$) than the attached males that had been detached (11 %; $N=129$, $\chi^2=37$, $df=1$, $P<0.0001$). When detached (i.e. originally attached) and unattached males were put together in a wading pool along with detached females (not with their original mates), the detached males paired much more quickly than the unattached males (36 %, $N=52$, originally attached males reattached and 0 %, $N=55$ unattached males attached within the first 5 min, $\chi^2=7$, $df=1$, $P<0.02$). They were also more likely to stay attached when released at sea. These studies demonstrate that within a breeding season, individual males are fairly consistent in the mating tactics they use.

19.2.2   Is There Anything Different About Attached and Unattached Males?

Attached and unattached males differ in appearance but not size. There are no differences in the body sizes of males using different mating tactics (Botton and Loveland 1992; Loveland and Botton 1992), and even when one attached male replaces another, he is not larger than the original male that was paired with the female (Brockmann and Penn 1992). This finding is surprising given the competitiveness of horseshoe crab mating behavior. However, on average, attached males are lighter in color compared to unattached males, and their eyes and claspers are more likely to be in good condition (Brockmann and Penn 1992; Duffy et al. 2006). Brockmann (2002) investigated the condition and mating status of 252 males that were marked and returned to the spawning beach at least three times, finding that 26 % were attached (75 % or more of the time), 39 % were unattached (75 % or more of the time), and 35 % showed a mixture of behavioral tactics. These animals differed in original attachment status ($\chi^2=43$, $df=2$, $P<0.0001$) and condition: 40 % of the animals in the best condition category were attached and 24 % were unattached, whereas in the lowest condition category 7 % were attached and 61 % were unattached ($\chi^2=26.9$, $df=6$, $P=0.002$). When original attachment status and condition were considered together, only condition affected whether males spent most of their time unattached (ANOVA; original status: $F=0.016$, $df=1$, $P=0.9$, condition: $F=12.8$, $df=1$, $P=0.0004$, original status by condition $F=2.9$, $df=1$, $P=0.09$; Fig. 19.2). These results suggest that male mating tactics are based on male condition (condition-dependent tactics).

   The physical condition of the carapace deteriorates over time (Brockmann and Penn 1992; Brockmann 1996) because horseshoe crabs do not molt as adults (Koon 1883; Shuster 1954; Sokoloff 1978; Shuster and Sekiguchi 2003; Smith et al. 2010).
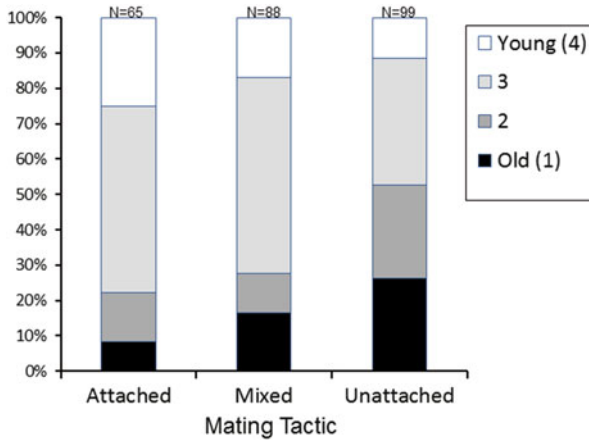
Fig. 19.2 The percent of males using each male mating tactic (attached, mixed, unattached) separated by age (condition) category. The age categories range from young (light color, no pitting or epibionts, mucus present and eyes and spines in perfect condition) to old (deteriorated, dark colored prosoma with pitting, no mucus, and with soft or covered eyes and worn spines). These data are from males (N=252) that were marked, their condition evaluated and released back to sea. They were then re-sighted at least three times on subsequent high tides at the breeding beach to be included in this dataset. Attached refers to males that returned attached on more than 75 % of their visits; mixed refers to those that showed a mixture of tactics on their multiple visits; and unattached were those that returned unattached on more than 75 % of visits (Redrawn from Brockmann 2002)

The prosoma becomes progressively darker; pits and scratches develop; and many fouling organisms invade their shells (Grant 2001). While there is no method to measure absolute adult age in horseshoe crabs, relative age can be determined based on shell condition. For example, the darker color is associated with more pitting caused by chitinoclastic bacteria, algae, or other infections (Leibovitz and Lewbart 2003). Darker animals also tend to have reduced quantities of mucus, which is exuded through pores in the carapace as an anti-fouling and surface immunity agent (Harrington and Armstrong 2000; Harrington et al. 2008). Horseshoe crabs with more deteriorated carapaces also have, on average, larger slipper shells, *Crepidula fornicata* living on them (Botton and Ropes 1988). Since slipper shells attach as larvae, this correlation means that when a crab carapace is in worse condition, the animal has been an adult longer than when the carapace is in better condition (Brockmann 1996, 2002). This age-dependent deterioration and biofouling means that carapace condition can be used as a proxy for relative adult age (Sasson et al. 2012). Moreover, the condition of the carapace correlates with physical performance such as righting behavior (Penn and Brockmann 1995) and mating tactics (Duffy et al. 2006).

The correlation between physical condition and attachment status means that male horseshoe crabs exhibit condition-dependent mating tactics (Brockmann 2001; Brockmann and Taborsky 2008). Our hypothesis (Brockmann 2003a) is that these mating tactics are alternative reproductive strategies in which males that are

Fig. 19.3 Threshold model for the evolution of alternative mating tactics in male horseshoe crabs. The *arrow* shows the threshold age below which males that show the attached tactic (pairing with females at sea) have higher fitness and above which males that show the unattached tactic (joining spawning pairs on shore) have higher fitness (Redrawn from Brockmann 2002)
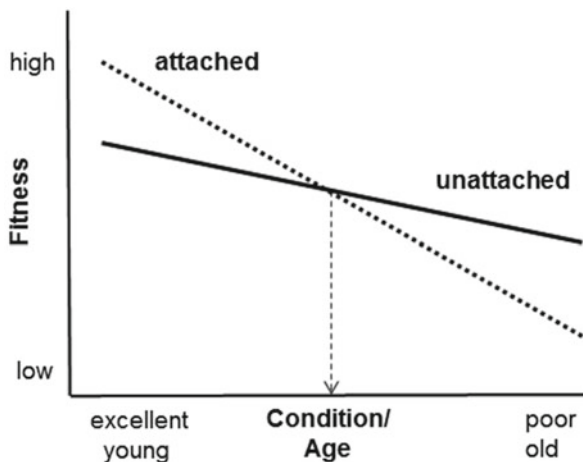
younger and in better condition have higher success when adopting the attached tactic, and those that are older and in poorer condition have greater success when adopting the unattached tactic (Fig. 19.3). At a certain threshold age or condition, males maximize fitness by switching from attaching to females out at sea (attached tactic) to coming ashore and looking for mating groups to join (unattached tactic).

While this model is difficult to fully evaluate, there is some supporting evidence from a field experiment in which males (both unattached males and attached males that we detached) were prevented from attaching by placing plastic bags over the claws they use for clasping females (Brockmann 2002). If males are coming ashore as satellites just because they cannot find or hold onto a female (using a making-the-best-of-a-bad-job tactic), then all males with bags should search onshore for pairs to join because none can attach. But, this is not what happened. The experiment showed that males that were in poor condition were much more likely to come ashore as satellites than males that were in good condition (these males apparently stayed offshore searching for females at sea). Both condition and original attachment status (but not size) significantly affected whether males with bags became satellites (logistic regression, $N=225$, $\chi^2=5.55$, $P=0.01$; for condition $\chi^2=10.59$, $P=0.001$; for original status $\chi^2=5.07$, $P=0.02$). These results suggest that the two mating tactics are the product of males making condition-dependent decisions, rather than males making the best-of-a-bad-job.

19.2.3   What Are the Trade-Offs (Costs and Benefits) for Each Male Tactic?

Several tradeoffs involving differences in costs and benefits have been identified for each male mating tactic. During each 1 week spawning cycle (the period of extra high tides around the new and full moon), attached males normally mate with only

one female, whereas satellite males may join several mating pairs (Brockmann 2003a). With each clutch of eggs laid, satellite males have similar paternity success compared to attached males (Brockmann et al. 2000). Attached males do not always compete for paternity with satellites because groups do not always form when nesting densities are low (Brockmann 1996), and some nesting pairs do not attract satellite males at all (Johnson and Brockmann 2012). In contrast, satellite males must always engage in sperm competition (Brockmann et al. 1994, 2000). While unattached males appear to mate more often than attached males (Brockmann and Penn 1992), they do not always find a spawning pair and when they do, some older males are unable to get into the best positions around the female (Smith and Brockmann 2014). Lastly, horseshoe crabs are often overturned on the beach, leaving them vulnerable to desiccation and predation (Botton and Loveland 1989). Attached males are less likely to be overturned than satellites and when this happens, younger males are better able to right themselves than older males (Penn and Brockmann 1995). Taken together, attached males appear to have higher success overall than unattached males (Brockmann et al. 2000). If an age or condition threshold for switching tactics has evolved that maximizes fitness, however, then we expect that there should be compensatory costs associated with the attached tactic. One previously unexplored, potential cost to the attached male tactic is nutritional stress caused by a reduced ability to feed while attached to a female (Smith et al. 2013b). During amplexus, the attached male's mouth is dorsal to and covered by the female's telson (Loveland and Botton 1992) and the male cannot bury himself in the substrate to feed in the normal manner by manipulating food into the mouth using the gnathobases that surround the mouth (Botton 1984). If the attached tactic inhibits feeding, then attached males should produce less fecal material than satellite males. In a waste production experiment, satellite males produced 57 % more waste than attached males, and females produced more waste than either type of male (likely due to their larger size) suggesting that attached males were indeed eating less. When gut contents were compared, the gut fullness of satellites was 150 % greater compared to attached males. While lower fecal production and an emptier gut could be due to attached males being less motivated to feed rather than the physical constraint of being attached, a subsequent feeding experiment showed that attached and satellite males ate the same amount of food, supporting the reduced feeding hypothesis. In Florida, males typically remain attached for a 1 week spawning cycle (mean ± SD length of attachment is 3.7±6.1 days, Brockmann and Penn 1992), but occasionally may stay attached up to 51 days (Brockmann 2003a). In other populations, pairs can remain attached much longer (Shuster 1954); for example, in New England, attached pairs have been observed overwintering together (Barlow et al. 1987; Moore 2004). Overall, these studies suggest that attached males may be fasting for several weeks during the breeding season.

It might seem obvious that fasting is costly but this cost may be offset by the energetic benefits of being attached. Attached males do not spend energy locating spawning pairs as unattached males do, and attached males are 'carried' along by females as they travel to the beach. . Nutritional stress due to a period of fasting (when an animal forgoes feeding in favor of other activities) or starvation (when

feeding is prevented due to some extrinsic limitation; McCue 2010) can be inferred from stable isotope values in animal tissues (Hobson et al. 1993; Castillo and Hatch 2007; McCue and Pollock 2008). If an animal is in a negative energetic balance, ^{15}N is preferentially retained, while ^{14}N is excreted, and as a result, $\delta^{15}N$ values increase in tissues over time as the animal feeds on itself (McCue and Pollock 2008). A comparison of $\delta^{15}N$ values in the feces of wild-caught individuals demonstrated that mean $\delta^{15}N$ values for attached males were higher than those for satellite males and females (there was no difference between satellite males and females). A subsequent 4 week starvation experiment demonstrated increased $\delta^{15}N$ values in feces of individuals that were not given food, whereas the values did not change for individuals that were fed. These results support the idea that reduced consumption of food and a period of nutritional stress are costs of the attached tactic. Horseshoe crabs that remain attached longer than those sampled in the Seahorse Key population, may suffer even greater costs of adopting the attached tactic.

Low-energy, alternative phenotypes often evolve as a release from the energetic demands of 'preferred' phenotypes (Taborsky 1998; Widemo 1998; Brockmann and Taborsky 2008; Cummings and Gelineau-Kattner 2009), and in some systems, a male's success for a given tactic may partially depend on his energy reserves (McCauley et al. 2000). We suggest that older males in poorer condition may not be able to afford the cost of reproduction (i.e. periodic fasting) that accompanies being attached to a female during breeding. Consequently, the satellite tactic may allow males to maintain (or regain) a positive energy balance while still obtaining reproductive success. Investigating this hypothesis is the next step to fully understand the evolution and maintenance of alternative tactics in horseshoe crabs.

19.2.4   Sperm Competition and Multiple Paternity

Horseshoe crab sperm remain quiescent until they are in close proximity to the eggs; then chemical cues from the eggs activate the sperm (Brown 1976). Millions of sperm may attach to each egg and undergo acrosome reactions, but only one sperm ultimately succeeds in fertilizing the egg. Sperm competition occurs when the sperm of two or more males compete for the same ova (Parker 1970). As the number of satellite males around a female increases, so does the intensity of sperm competition (Parker 1998). Attached males generally fertilize a majority of the eggs when competing against just one satellite (Brockmann et al. 1994), but attached males are less successful than satellite males when two satellites are present (although there is a lot of variation among males, Brockmann et al. 2000). In larger groups, the paternity share of additional satellite males comes at the expense of the other satellites, and the attached male normally fertilizes at least some of the eggs.

The most important factor affecting fertilization success during sperm competition is the position of the male around the female. Attached males are directly behind the female and thus are close to where eggs are released. Satellite males can take any position around the female, but when satellites first arrive at a pair, they

nearly always move into positions over the female's incurrent canals (the channel between the prosoma and opisthosoma, Eldredge 1970), called the 1F positions (Fig. 19.1, Brockmann et al. 2000). When in this position, satellite males have close access to the site of egg release and consequently the highest percentage of satellite paternity comes from males in this position (Brockmann et al. 1994, 2000). Additionally, satellites in this position may work their way under the anterior margin of the attached male's prosoma. This "under" position may result in higher paternity for that satellite male, especially when additional satellite males are present. Once the 1F positions have been occupied, later arriving satellite males must take up other positions around the female, which have reduced paternity (Fig. 19.4).

When sperm from multiple males are in close proximity to the eggs, the outcome of sperm competition likely hinges on the quantity and quality of sperm released (Snook 2005). Sperm quantity is a function of two traits: ejaculate size and sperm concentration (the number of spermatozoa in an ejaculate of a particular size). Augmenting either trait increases the number of spermatozoa that are competing during mating. All else being equal, the male that releases the most sperm should have an advantage over competing males (Parker 1982). We found that attached males have, on average, higher sperm concentrations (N=102, mean = 8.5×10^9 sperm/mL, S.E. = 7.2×10^8) than satellite males (N=44, mean = 6.5×10^9 sperm/mL, S.E. = 6.0×10^8, $P=0.04$) (Sasson et al. in press).

Age is another factor that affects sperm quantity in horseshoe crabs: younger males have larger ejaculates and more concentrated sperm compared to older males (Sasson et al. 2012). Thus, given the relationship between age and mating tactics, we expect younger attached males to have higher sperm quantity compared to older satellite males. While reproductive tactic is correlated with sperm concentration, we found no difference in ejaculate size between attached (N=84) and satellite males
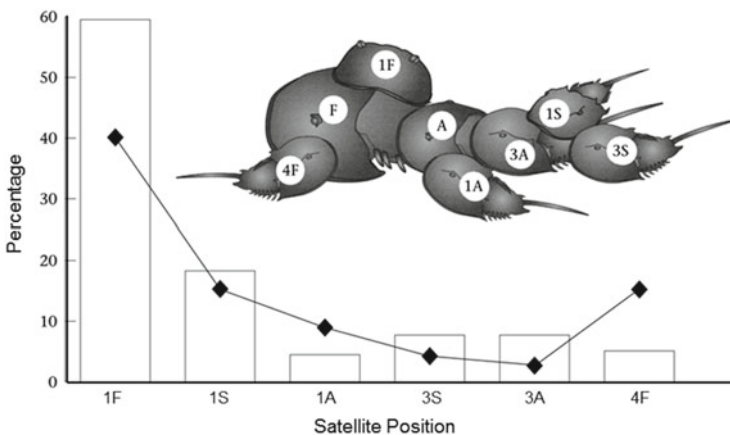

Fig. 19.4 Frequency distribution of satellite male positions and the associated paternity. The *bars* show the frequency with which satellite males were found in different positions around a nesting pair (positions are shown in the picture) and the *line* shows the mean paternity for satellite males when they were in each position (Modified from Brockmann et al. 2000)

(N=37, GLM: $F_{1,114.7}=0.43$, P=0.51) (Sasson et al. in press). One explanation for this apparent contradiction is that attached males may adjust their sperm output when faced with sperm competition, as has been found in some externally fertilizing fish (Candolin and Reynolds 2002; Pilastro et al. 2002). However, we have previously found that ejaculate size remains relatively constant within males across a number of days (Spearman rank correlation, $r_s=0.85$, N=19, P<0.001), suggesting that ejaculate size is not affected by a male's immediate competitive environment (Sasson et al. 2012). While fertilization success in competitive situations can also be affected by sperm quality (e.g., velocity or viability, Snook 2005), neither age nor mating tactic correlates with any measure of sperm quality among horseshoe crabs in the Seahorse Key population (Sasson et al. 2012, in press).

Higher sperm competition risk should select for sperm traits that make males more successful at fertilizing eggs under competitive conditions (Parker 1998; Snook 2005; Alvarez et al. 2014). Populations differ in the risk of sperm competition because of differences in OSR. For example, the Seahorse Key (SK) population of horseshoe crabs averages about 2.1 males per female, while the population in Delaware Bay (DB) has 3.5 males per female (Brockmann and Smith 2009). The higher OSR means that attached males in DB are more likely to face sperm competition than attached males at SK. Accordingly, we found differences in sperm traits between DB and SK populations. The horseshoe crabs in DB had larger ejaculates and more concentrated sperm than those in SK (Sasson and Brockmann in prep). While a number of factors may be involved, sperm competition risk seems to be an important driving force for changes in sperm concentration and a strong contributor to a change in ejaculate size across populations that differ in sperm competition risk (Sasson and Brockmann in prep). Similar patterns have been found across closely related species that differ in sperm competition risk such as frogs and butterflies (Gage 1994; Byrne et al. 2003) and in other externally fertilizing species with alternative reproductive tactics (Fu et al. 2001; Vladic and Jarvi 2001).

19.3   Female Mating Tactics

Group spawning by satellite males can also be viewed as multiple mating by females. Even when the OSR is strongly male biased, some females nest only with their attached males (monandrous females), so their offspring are fathered by one male. At the same time, nearby females may spawn with satellites (polyandrous females), which results in multiple paternity of their offspring (Fig. 19.1, Brockmann 1990; Brockmann et al. 1994, 2000). The frequency of monandry declines as nesting density and OSR increase (Brockmann and Smith 2009), but there are always some monandrous pairs present on the nesting beach. For example, when nesting densities are high at SK, 20–80 % of pairs are monandrous (Fig. 19.5a, Johnson and Brockmann 2012) and in DB, where the OSR is much higher, 5–20 % of pairs are monandrous (Fig. 19.5b, Brockmann 1996). As with males, do females have two alternative mating tactics, or are monandry and polyandry a by-product of male competition for females?

19.3.1   Are Individual Females Consistently Monandrous or Polyandrous?

Two research methods have been used to assess whether females consistently maintain monandrous and polyandrous behavior. (1) Data from mark-recapture studies show that individual monandrous females are more likely to remain monandrous from one nesting to the next than are polyandrous females (Hassler 1999), even when their attached males were removed and they paired again with a new attached male ($\chi^2=21.3$, $df=1$, $P<0.001$; Johnson and Brockmann 2012). (2) Results of field experimental manipulations show that monandrous pairs are more likely to stay monandrous than nearby polyandrous pairs that had all their satellites removed ($\chi^2=96$, $df=1$, $P<0.001$; from data given in Brockmann 1996). These results support the view that individual females are consistently monandrous or polyandrous. Our results (Fig. 19.5) also show that when there are large numbers of unattached males present, all polyandrous females have satellites, whereas when few unattached males are present, some polyandrous females nest without satellites (and thus are recorded as monandrous). Hence, the frequency of monandry is affected by the environment, in this case by nesting density and OSR.

19.3.2   There Are Two Types of Monandrous Females

Horseshoe crabs are an ideal model species in which to study mating tactics because you can experimentally remove and "add" satellite males to females to address questions about the mating system. Satellites are not attracted to monandrous pairs,
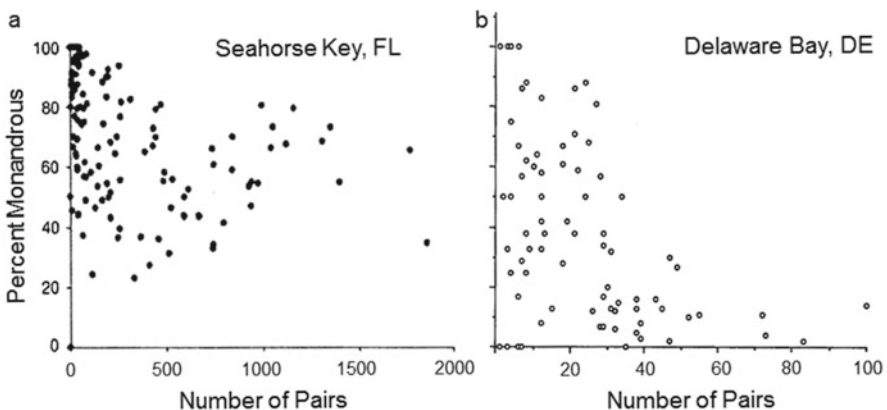

Fig. 19.5 The relationship between the proportion of female horseshoe crabs that were monandrous and the nesting density (number of pairs present on the breeding beach). (**a**) Proportion of monandrous females at SK on 157 tides on which we counted one or more pairs along the 1 km beach (Redrawn from Johnson and Brockmann 2012), (**b**) Proportion of monandrous females at Cape Henlopen, Delaware Bay on 12 high tides from eight 100 m beach sections (Redrawn from Brockmann 1996)

but we could encourage a male to join a monandrous pair by gently guiding him toward the nesting couple (and the control pair was allowed to remain monandrous, Johnson and Brockmann 2010). More than half of the monandrous females (62 %) left the nesting beach within 2 min rather than nest with the added satellite male. We call these exclusively monandrous females "intolerant" (Johnson and Brockmann 2012). Other monandrous females were "tolerant," since they would continue to nest with experimentally added satellite males, although they did not attract (or were not attractive to) satellites naturally on their own.

19.3.3   Are Monandrous and Polyandrous Females Different?

Monandrous females were, on average, slightly smaller (mean prosoma width 13.75 cm $\pm$ 0.03 S.E., $N=1,059$) and in better condition (younger) than polyandrous females (13.85 cm $\pm$ 0.05 SE, $N=507$), and intolerant monandrous females were slightly smaller than tolerant monandrous females (Brockmann 1996; Hassler 1999; Schwab and Brockmann 2007; Johnson and Brockmann 2012). Monandrous females lay fewer eggs on average during each nesting bout than polyandrous females (Table 19.1, Schwab and Brockmann 2007); but there were no differences between monandrous and polyandrous females in the rate of laying eggs, egg size (Johnson and Brockmann 2012), energy content of the eggs (Hassler 1999), fertilization success of the eggs (monandrous: mean$=99.6$ %; polyandrous: mean$=99.2$ %, $N=29$; Wilcoxon test, $P=0.39$) or any measure of developmental success of the offspring (Johnson and Brockmann 2010, 2012). Although smaller females tend to lay fewer eggs (Leschen et al. 2006), the smaller size of the monandrous females does not fully explain the smaller numbers of eggs that she lays (Johnson and Brockmann 2012), i.e. female mating status explains a significant amount of the variation in egg number (Johnson and Brockmann 2010). While we found no differences between monandrous and polyandrous females in the size or

Table 19.1 Median number of eggs laid in three clusters and the proportion of the clusters that contained eggs for monandrous and polyandrous females (Data from Johnson and Brockmann 2012)

Year	Median number of eggs laid			Proportion of clusters with eggs (N)	
	Mon	Poly	P^a	Mon	Poly
1993	3,300	5,280	0.36	0.88 ($N=36$)	0.88 (N=8)
1994	4,004	4,840	0.38	0.82 (N=22)	1.0 (N=6)
1995	3,696	6,820	0.03	0.86 (N=69)	0.93 (N=28)
2004	3,476	5,192	0.03	1.0 (N=33)	1.0 (N=35)
2005	2,728	3,080	0.76	0.84 (N=50)	0.88 (N=60)
2008	5,500	5,720	0.25	0.95 (N=40)	0.96 (N=55)

[a]Mann-Whitney U Test

condition of their attached males, the attached males of intolerant females were in better condition than the attached males of tolerant females (Johnson and Brockmann 2012).

Differences have been found between the sperm concentration of males attached to monandrous and polyandrous females. The attached males of monandrous females have on average higher sperm concentrations (N=59, mean = 9.6×10^9 sperm/mL, S.E. = 1.2×10^9) than the attached males of polyandrous females (N=43, mean = 6.9×10^9, S.E. = 5.3×10^8, Tukey HSD: P=0.04) or satellite males (N=44, mean = 6.5×10^9 sperm/mL, S.E. = 6.0×10^8, Tukey HSD P=0.01) (Sasson et al. in press). Overall, the evidence suggests that monandry and polyandry are stable tactics (alternative reproductive tactics) that are associated with condition-dependent (e.g. size, age, physical condition) and context-dependent (the particular male that is attached) differences among females, with differences in reproductive investment in egg number (per nesting bout).

19.3.4   What Do Females Gain from Multiple Mating?

Multiple mating is widespread among animals, even when females can acquire all the sperm they need by mating with a single male and even when mating is costly (Jennions and Petrie 2000). So why do females mate with multiple males and, perhaps even more puzzling, why are both patterns maintained in horseshoe crabs? Many explanations for multiple mating have been proposed including increasing the opportunity for a female to mate with a high quality (good genes) or genetically compatible male and increasing the genetic diversity of offspring by mating with multiple males (Zeh and Zeh 2003; Simmons 2005; Slatyer et al. 2012). Alternatively, multiple mating might just be a by-product of male competition (i.e. convenience polyandry, Alcock et al. 1977; Thornhill and Alcock 1983). We explored potential costs and benefits of multiple mating in the field using both observational and manipulative experiments where we monitored nesting time, clutch numbers, and numbers of eggs laid. When females were allowed to choose freely between nesting with or without satellites (i.e., naturally occurring monandrous and polyandrous females), they had equal nesting success and similar duration of nesting, number of eggs, and developmental success.

In an experiment in which satellite males were removed from polyandrous females, we found that spawning with multiple males was costly for these females. Polyandrous females without satellites laid more eggs, largely because they stayed on the beach longer, than polyandrous females nesting with satellites (Johnson and Brockmann 2010). However, to our surprise the developmental success of the eggs from polyandrous females whose satellites had been removed did not differ from the success of polyandrous females with satellites. When we conducted paternity analyses on larvae reared from the satellites-removed nests, we found that 78 % of the clutches had been fertilized by satellites-even when all satellites had been removed 20 min earlier! This finding means that somehow satellite sperm are

retained or pulled along by the female as she lays successive clutches of eggs in the sand. When satellites were experimentally added to monandrous females, they laid fewer eggs than monandrous females nesting alone, mainly because "intolerant" females left the beach immediately and hence laid fewer eggs. Overall, these manipulative experiments suggest that mating with multiple males is costly for all females by reducing nesting success, and monandrous females can avoid this costly nesting with satellites by not attracting them in the first place.

19.3.5   Are There Trade-Offs or Benefits for Polyandrous But Not for Monandrous Females?

Artificial fertilization experiments can be conducted easily in horseshoe crabs by obtaining unfertilized eggs and sperm through gentle electro-ovulation and – ejaculation (Brown and Clapper 1981) and *in vitro* fertilization (IVF). These techniques eliminate the male or female pre-mating behavioral component of mate choice and mating success. In one IVF study, we fertilized eggs from polyandrous females with sperm from either their own attached male or a randomly chosen satellite male. The result was lower hatching success with their attached male than with the satellite male. In contrast, eggs from monandrous females that were fertilized by sperm from their attached males had equal success compared to eggs fertilized by satellite males (Fig. 19.6, Johnson and Brockmann 2013). This finding suggests that there was something different about the quality or compatibility of the attached males of
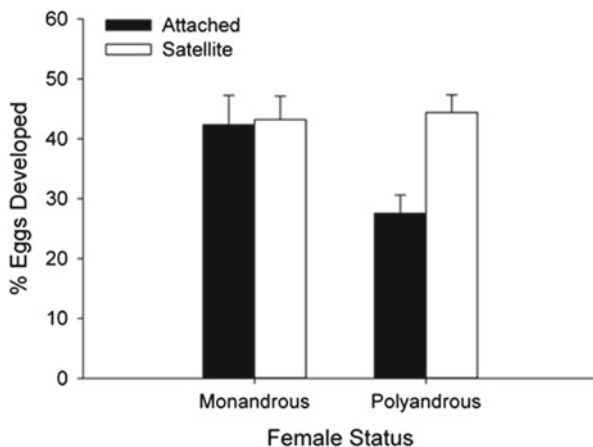

Fig. 19.6 The developmental success (mean ± SE) of eggs from females mated with attached (*black bars*) and satellite (*white bars*) males for monandrous and polyandrous females. Using *in vitro* fertilization methods, a total of 21 females of each type were crossed with their respective attached males and with 2 randomly chosen satellite males (Redrawn from Johnson and Brockmann 2013)

monandrous and polyandrous females, and that there may be indirect (post-mating) benefits to polyandrous females who mate with satellite males.

Both genetic compatibility and good genes (mate quality) may be important factors that polyandrous females derive from mating with multiple males. Further IVF experiments demonstrated that no single male was best for *all* females when it comes to hatching success of their embryos; instead, there was some evidence that compatible male-female crosses result in higher hatching success. However, the rate of development (number of larvae that reach metamorphosis in just 45 days), was affected by both the intrinsic quality of males and male-female compatibility. We also found evidence of female effects, both intrinsic female effects such as female or egg quality and female status effects: polyandrous females tended to have a lower proportion of offspring achieving early metamorphosis compared to monandrous females (4.7 % vs. 8.0 %; when females were crossed with random satellite males). It is reasonable to assume that the heavily armored juveniles are more likely to survive than the non-feeding, unarmed trilobite larvae. Hence, early metamorphosis should result in fitness benefits for horseshoe crabs. Taken together, these results suggest that monandrous and polyandrous females differ in the costs and benefits of multiple mating and that genetic incompatibility between males and females and/or male quality may offset the costs of multiple mating in this system.

19.4   Finding and Attracting Mates

19.4.1   How Males Find Females

The two mating tactics of male horseshoe crabs require different mate-searching behavior (Brockmann 2003a), and the different female mating tactics require different responses to males. Pairing typically occurs offshore exclusively in an aquatic environment, whereas satellite behavior requires locating and identifying spawning pairs that are above or below the high tide line where there may be constant interference from waves. Using cement casts of female horseshoe crabs placed offshore in shallow water, Barlow and his colleagues observed males orienting to females or horseshoe crab shapes when they came within visual range (Barlow et al. 1982, 1986, 1988; Powers et al. 1991; Barlow and Powers 2003). Similarly, unattached males approached cement models of females (Hassler and Brockmann 2001) or pairs (Schwab and Brockmann 2007) placed along the shoreline. In a field choice test, unattached males preferred the larger of two model females that differed by only 2.5 cm (both within the natural range of female sizes; Fig. 19.7). Clearly, then, male horseshoe crabs can locate mates visually. Although horseshoe crab eyes are remarkably sensitive (males can detect horseshoe crab-like objects equally well at night and in the day and when contrast is low, Powers et al. 1991; Passaglia et al. 1997; Herzog et al. 1996), visual cues are not always available because of diseased eyes (Brockmann and Penn 1992; Wasserman and Cheng 1996; Duffy et al. 2006),

Fig. 19.7 The number of satellite males that were attracted to the large (*dark symbol*) or small (*light symbol*) cement model casts of a pair of horseshoe crabs nesting on the beach. Mean (± SE) number of satellites coming within 1 m of a model (= enter arena), coming within 10 cm of a model (= approach) or touching the model (= join). The inset shows the two cement models used during the tests with a satellite male joining the model in the foreground. The models differed in size by 2.5 cm (Modified from Schwab and Brockmann 2007)
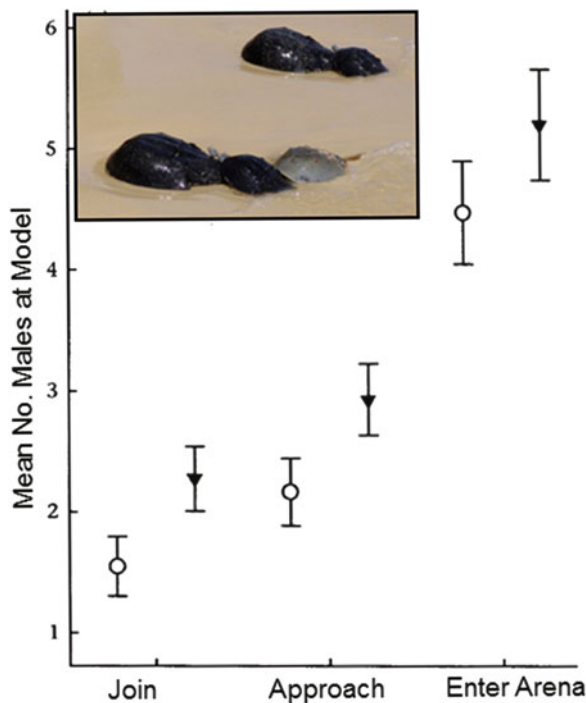
turbid water, or because nesting females are buried in the sand (Schwab and Brockmann 2007). Like many other species (Candolin 2003; Partan and Marler 2005), horseshoe crabs may use multiple cues when searching for mates (Saunders et al. 2010).

Horseshoe crabs are well endowed with chemoreceptors and the neurons for processing chemical information (Barber 1956; Hayes and Barber 1982; Loesel and Heuer 2010), and there is evidence that males use chemical cues to locate mates (Brockmann 2003a). In a field experiment, identical cement horseshoe crab casts (models) were placed over a spot on the beach where a monandrous female had been nesting moments before (the pair was removed), where a polyandrous female had been nesting (and was removed) and a third (control) model was placed over a nearby spot on the sand where no female had been nesting (Hassler and Brockmann 2001). The effect was strong. For up to 10 min after the experiment started, more satellites arrived at the models placed over the polyandrous nesting spots than at the monandrous nesting spots or the controls (Friedman ANOVA: $N=36$, $df=2$; $P=0.01$ at 0 min; $P=0.002$ at 1 min, $P=0.04$ at 4 min, $P=0.04$ at 6 min, and $P=0.004$ at 10 min). In another field experiment, sponges were placed under the two cement models, one sponge was filled with water from underneath a polyandrous female and the other (control) was filled with seawater. Approaching satellites preferred the model with water from the polyandrous female (Hassler and Brockmann 2001; Saunders et al. 2010). We also know that the cues that attract males from a distance

are not coming from eggs (which are known to have a sperm attractant, Shoger and Bishop 1967), since satellites approach females as they arrive on the beach well before any eggs are laid. Further, when newly laid eggs are placed under one of the two cement models, satellites are no more attracted to the model with eggs than to the model without, but once in contact with the model, they remain longer with the one that has eggs (Schwab and Brockmann 2007). Nothing is known about the nature of the chemicals that attract males from a distance except that they can remain in the sand for many minutes despite repeated washing by small waves (Hassler and Brockmann 2001).

19.4.2 Do Females Attract Males with Chemical Cues?

Given that males are using chemical cues or semiochemicals to find mates, the question then becomes, who is producing those cues? There are several possibilities. (a) Females might be producing attractants (polyandrous) or deterrents (monandrous) or (b) the attached males might be attracting or deterring satellite males. It is unlikely that selection would favor attached males that produced cues that attracted competitors, but he might produce inadvertent cues that would reveal that he was of low quality. For example, if an attached male had lower sperm volumes, which would render him unable to compete with satellites, then he might be attractive to unattached males if they could detect the sperm. (c) It is also possible that males copy the behavior of other males, i.e. that satellites are attracted to pairs that have already attracted satellites. This behavior would be particularly likely if the presence of a satellite improved the success of subsequent satellites or indicated the presence of a non-competitive attached male or tolerant female. It is difficult to resolve which of these explanations is most likely. In a field experiment, satellites were attracted to a pair nesting in shallow water but hidden from view by an enclosure (the enclosure had many small holes drilled in each side so chemical cues were available) and they were more attracted to a pair than to a lone female or a lone male covered by the enclosures (Saunders et al. 2010). These results suggest that the semiochemicals come from a nesting pair alone, without satellites. But is the female or the attached male producing the mate-attracting semiochemical?

Monandrous females are consistent in not attracting satellites from one nesting bout to the next, even when their attached males are removed and replaced with a new male (Johnson and Brockmann 2012). Polyandrous females also consistently attract satellites, even when their attached male has been replaced. Moreover, some monandrous females do not attract and are intolerant of satellite males and will leave the beach rather than nest as a polyandrous female. These results suggest that the presence of satellites around a female is a property of the female, or an interaction between female and male (e.g. male quality and female mating status) and not due exclusively to the attached male. The female, therefore, may control the amount of sperm competition her attached male faces. Whether the female is monandrous or polyandrous determines the male's reproductive success, as males attached to

monandrous females fertilize 100 % of the eggs, while males attached to polyandrous females may have their paternity share drastically reduced. Unless males can detect the reproductive tactic of the female prior to amplexus and before arriving at the beach, it may be impossible for males to predict whether they will face sperm competition. If females become polyandrous when attached to a poor quality or incompatible male, however, then it would be possible for a male to predict his chances of facing sperm competition. Clearly, we need to know much more about pre-copulatory mate choice in this species.

19.5 Conclusions and Future Directions

Research on the reproductive tactics of horseshoe crabs has revealed the importance of studying male and female behavior concurrently. Previous studies failed to consider how female behavior affects males, and as a result, we missed important elements of horseshoe crab reproductive biology.

19.5.1 Mate Choice in Horseshoe Crabs

It is not clear what if any mate choice occurs at the time of amplexus because little is known about the behavior of pairs prior to arriving on the beach. There is ample evidence that attachment is non-random. For example, when males are detached from their mates and then allowed to re-attach to a new female in a wading pool, they pair much more quickly than satellite (unattached) males that have been placed in the same pool (Brockmann and Penn 1992; Duffy et al. 2006). These results demonstrate non-random pairing, which suggests mate choice, but it is not clear whether this non-random pairing is due to motivational differences among males or to choice by the male, the female, or both.

Females are limited in the time available for nesting, and they must have an attached male to spawn (Brockmann 1990). Like other explosive breeders, horseshoe crabs may have few opportunities for active choice prior to mating (amplexus). A lack of pre-copulatory mate choice means that the best way for females to control mate choice may be through their control of polyandry. We have shown that monandrous females that retain their attached males were more likely to remain monandrous than females whose attached males were removed and replaced with new males. This result, together with the finding that polyandrous females have lower developmental success with their attached males than with satellites, suggests that a female's mating tactic is affected by her attached male and is context-dependent. Since chemical cues are used by satellites to identify polyandrous females, it is possible that females are attracting satellite males by producing attractants when an attached male is of low quality or genetically incompatible.

19.5.2 Can Females Discriminate Attached Male Quality or Compatibility?

Mechanisms for detecting underlying male quality (good genes) might include male size or condition, sperm quantity or quality, or parasite load. We have not detected size or condition differences between the attached males of monandrous and polyandrous females, although the attached males of intolerant monandrous females tend to be in better condition (Johnson and Brockmann 2012, 2013). We have detected differences in sperm concentration (Sasson et al. in press), indicating it is possible that females assess male quality based on a male's sperm traits. Parasite load has not been properly investigated, but would be worth studying (Wedekind 1994) since older males have more parasitic flatworms (unpubl. data). It would be advantageous for females to detect genetically compatible males to prevent inbreeding, to increase heterozygosity or compatibility, or to avoid selfish genetic elements or heritable microorganisms (Jennions and Petrie 2000; Tregenza and Wedell 2000; Zeh and Zeh 2003; Chandler and Zamudio 2008). Perhaps the high heterozygosity observed in *L. polyphemus* (King et al. 2005) populations is the result of mate choice for compatible males to avoid inbreeding (Johnson and Brockmann 2013). Studies investigating the relatedness of attached pairs are needed to test this hypothesis. In general, if a female has information about the quality or compatibility of her attached male, we expect that she should attract additional mates when her attached male's sperm are less compatible with her eggs or when the male is of low quality, such as when he has a low sperm concentration.

19.5.3 Multiple Mating: Convenience Polyandry or Female Tactics?

When multiple mating is costly, yet present in a system, as in horseshoe crabs, the usual explanation is sexual conflict, sexual harassment, or convenience polyandry (Thornhill and Alcock 1983; Maklakov and Lubin 2006; Sztatecsny et al. 2006). Our in-depth studies of female mating behavior and reproductive success allow us to reject these hypotheses as the primary explanation for multiple mating in horseshoe crabs. Some females benefit from multiple mating while other females have mechanisms for reducing the effect of coercing males such as leaving the beach or laying fewer eggs, mechanisms that are similar to those observed in other species (Sirot et al. 2003; Hettyey et al. 2009). We find that multiple mating is more likely explained by the interplay between male and female mating tactics. A male may adopt the unattached tactic when he stands to benefit from being a satellite male. A female may attract (or fail to prevent) satellite males when she stands to gain either through improved mate quality or compatibility. However, it seems unlikely that

females would need to attract more than a few additional satellite males to ensure that she mates with a high quality or compatible male. Hence, very large group sizes may result from convenience polyandry, where it is more costly for females to resist or stop mating than to allow additional satellites, particularly for polyandrous females.

19.5.4   The Dynamic Interplay Between Male and Female Tactics

Overall, the evidence demonstrates that male tactics are affected by male age (condition) and behavior (what other males are doing and female behavior). Female tactics are the result of male traits and behavior as well as female mating decisions. The different types of females choose different mating tactics, depending on their condition (condition-dependent) and circumstances (context-dependent), when they are free to do so. Female decisions change the opportunities and payoffs for male tactics, just as male tactics affect the reproductive success of females. This interplay between male and female mating tactics and mate choice decisions suggests that we should be studying the interactions within and between the sexes concurrently (Alonzo 2008). Further, the decisions that individuals make are dynamic, affected by OSR, density and other social (e.g. how aggregated is the nesting) and environmental (e.g. wave height that increases the risk of being overturned) factors. Such complexity cannot be evaluated piecemeal, which suggests that modeling may be needed to understand reproductive decisions in horseshoe crabs.

Condition-dependent tactics can be maintained in populations as a result of trade-offs as has been suggested here: males switch tactics at a threshold age or condition that maximizes fitness. However, condition-dependent tactics may also be maintained by frequency-dependent selection (Brockmann and Taborsky 2008) or through an interaction between frequency and density dependence (Lucas and Howard 2008). When condition-dependence and frequency dependence are involved, the success of alternative tactics is not equal (Calsbeek et al. 2002; Shuster and Wade 2003; Hazel et al. 2004), but there is a unique and stable switch point to which the population returns when perturbed (Repka and Gross 1995; Gross and Repka 1998). The mating tactics of male horseshoe crabs are density dependent, and they are likely to be frequency dependent, since the success of the unattached tactic depends on its frequency in the population; for example, if there were no satellites, then a rare mutant satellite would be highly successful, but as the frequency of satellites increased, their success would decline (Brockmann 2001). Understanding the interacting and dynamic effects involved in the mating tactics of horseshoe crabs may require a dynamic game theoretic modeling approach (Clark and Mangel 2000).

19.5.5 Application to Other Populations: Effects of Density and OSR

Our studies have focused mainly on the *Limulus polyphemus* population at SK, so how might these findings apply elsewhere? Other populations differ from SK in a number of respects, including the timing and seasonality of nesting, and the OSR and density of nesting horseshoe crabs (Brockmann 1996). Genetically distinct populations may also show adaptation to local conditions (King et al. 2005). These factors may affect mating tactics and the intensity of sexual selection (Brockmann and Smith 2009). For example, Mattei et al. (2010) compared the mating behavior of horseshoe crabs from two different areas in Connecticut (CT) that differ from the DB population in spawning density, but still maintain a similar male-biased OSR. They found that the extremely low density of animals in CT resulted in females being almost exclusively monandrous when paired, and many females arrived on the beach without an attached male, which is rare at SK (Brockmann and Johnson 2011). Mattei et al. (2010) suggested that at such low densities horseshoe crabs may have difficulty finding and attracting mates, both when attaching in the water and once they have arrived at the beach. In such low-density environments, the selection pressures acting on reproductive traits may shift. For instance, females may be forced to be monandrous even if they would benefit from mating multiply, potentially lowering their reproductive success. Thus, there may be stronger selection in these populations to choose high quality or compatible mates when pairing. Since sperm competition would be rare for males in these populations, rather than acting on sperm traits, selection should favor a male's ability to find and hold onto females. Furthermore, because at these densities unattached males may have little reproductive success, we predict that the threshold age at which males switch to the unattached tactic would shift to an older age compared to higher density populations. While Mattei et al. (2010) still found many unattached males, they also found no difference in condition between attached and unattached males, suggesting that older males may forgo a switch to the unattached tactic and continue to attach at an older age than males in the SK population. Such a decision may mean that these males are less able to hold onto females and could result in females losing their attached males as they come ashore. This scenario would explain why unattached females are more common in the CT population compared to SK. Overall, these findings suggest that horseshoe crab density, at least at certain levels, may be more important than the OSR in affecting mating behavior and reproductive success. We know little, however, about how pairing occurs at sea other than the cues used, and we do not know whether males change tactics when density or OSR changes.

19.5.6 Implications for Management

Where populations are being depleted to the point that densities are very low, such as parts of New England, Florida's east coast and the northern Gulf of Mexico (Carmichael et al. 2003; James-Pirri et al. 2005; Gerhart 2007; Fulford and Haehn

2012), per capita reproductive success is likely to be affected. Since horseshoe crabs have external fertilization, a female can lay viable eggs only when a male is attached and at very low densities it may be difficult for males and females to find optimal, or any, mates (Mattei et al. 2010). In addition, horseshoe crab spawning may be social in the sense that females may be attracted to and gain from the presence of other females, for example, if many females nesting in close proximity swamp predators (Brockmann and Smith 2009). Low population density would result in negative density-dependent effects on reproduction, which may affect the ability of populations to recover from overharvest, habitat destruction or disease (Mattei et al. 2010).

In 2008 a male-only harvest was instituted in DB as a management strategy to prevent over-harvest of female horseshoe crabs (ASMFC 2008) and more recently an adaptive management approach has been used (McGowan et al. 2011; ASMFC 2012). Adaptive management of horseshoe crabs in DB is designed to allow sustainable harvest while maintaining an adequate supply of horseshoe crab eggs as a food resource for migrating shorebirds (Smith et al. 2013a). Our studies of horseshoe crab mating suggest some additional management considerations. First, since spawning is strongly aggregated, large numbers of females may arrive all at once, each requiring an attached male before laying eggs. Most males do not easily switch from satellite to attached tactics, so the OSR is not a good measure of the males available for pairing. Since younger males are more likely to attach than older males, the male-only harvest should exclude young or already attached males. Second, some females may attract satellite males when the male to which they are paired is of low quality or incompatible. This behavior means that unattached males are not "excess males" but an important part of the mating system of this species. Third, when nesting densities are low, even when the OSR is male biased, some females do not pair and hence do not lay eggs (and at extremely high densities females fail to lay eggs due to interference from other females). Hence, the relationship between nesting density and surface egg density will not be linear. Fourth, satellite males are costly to certain females, and some females will not nest with satellites. Therefore at very high OSRs, spawning would decline due to interference from satellite males. Exceptionally male-biased OSRs may result from selective harvest of females in some populations (Widener and Barlow 1999; James-Pirri et al. 2005), and our findings suggest that nesting at these sites may be particularly costly to females. Finally, there are likely to be population differences in male and female mating behavior, depending on the natural and sexual selection history of the population. For example, if a low OSR is the rule, then we expect different levels of sperm competition than when OSR is often high. Hence, populations are likely to differ in their reproductive response and vulnerability to harvest, habitat loss and disease.

19.5.7 Explosive Breeding in Horseshoe Crabs

Horseshoe crabs are often thought of as explosive breeders with scramble competition among males and, indeed, they seem to share many traits with other explosive breeders (Brockmann 1990). Explosive breeding is thought to allow few opportunities for mate choice, male combat or other sexually selected traits (Wells 1977), yet the OSR is often strongly male biased which usually means strong selection for sexually selected traits (Kvarnemo and Ahnesjö 1996). If some males do not mate under these conditions, then this further intensifies sexual selection (Shuster and Wade 2003). In fact, sexual selection for traits that enable a male to reproduce may be especially intense in explosive breeding scramble competition systems because males only have a brief opportunity to mate each year, which means that many males may be unable to find and secure a mate during that short window of opportunity (Greene and Funk 2009). Under these conditions males would be expected to have particularly well-developed sensory organs, high mobility for locating females, good spatial memory (Andersson and Iwasa 1996) and other traits that make them faster and more effective at pairing (Sztatecsny et al. 2012). Studies on a species of explosively breeding frog, for example, show that although overall size is not associated with mating success, small differences in the structures for holding onto females affect fitness (Greene and Funk 2009). These traits are not associated with male combat, female choice, or the ability of males to take over females once amplexus occurs, but rather with the ability of males to hold onto females until oviposition. We do not know the proportion of male horseshoe crabs that are not breeding, and hence, we do not know the intensity of sexual selection in our system. However, based on studies of anurans, we suspect that mating is not random with respect to characters that affect the ability of males to find and hold onto females.

Explosive breeding also increases opportunities for alternative tactics such as multi-male spawning, clutch piracy and sneaking behavior (Zamudio and Chan 2008). Such behavior may increase female coercion and even injury to females as males vie for position around a female (Sztatecsny et al. 2006). Explosive breeding is a particularly dynamic mating system and many species show differences between years and populations in OSR and the time available for breeding, and hence in the intensity of scramble competition (Olson et al. 1986; Reichard et al. 2007; Alvarez et al. 2014). When population size, the degree of female asynchrony, and the time available for mating competition vary (as they might on a daily to yearly basis), selection will favor an associated change in mating behavior and different mating tactics under different conditions (Mendoza-Cuenca and Macias-Ordonez 2010). Horseshoe crabs have similarly large differences in the amount of time available for breeding and in the intensity of scramble competition from year to year or at different breeding sites. This variation likely results in differences in the frequency of different female and male mating tactics between years and breeding locations.

References

Alcock J (2009) Animal behavior, 9th edn. Sinauer Associate, Inc, Sunderland

Alcock J, Eickwort GC, Eickwort KR (1977) The reproductive behavior of *Anthidium maculosum* (Hymenoptera: Megachilidae) and the evolutionary significance of multiple copulations by females. Behav Ecol Sociobiol 2:385–396

Alonzo SH (2008) Conflict between the sexes and alternative reproductive tactics within a sex. In: Oliveira RF, Taborsky M, Brockmann HJ (eds) Alternative reproductive tactics: an integrative approach. Cambridge University Press, Cambridge, pp 435–450

Alvarez D, Viesca L, Nicienza AG (2014) Sperm competitiveness differs between two frog populations with different breeding systems. J Zool 292:202–205

Andersson M, Iwasa Y (1996) Sexual selection. Trends Ecol Evol 11:53–58

ASMFC (1998) Interstate fishery management plan for horseshoe crabs. Atlantic States Marine Fisheries Commission, Washington, DC

ASMFC (2008) Addendum V to the interstate fishery management plan for horseshoe crabs. Atlantic States Marine Fisheries Commission, Washington, DC

ASMFC (2012) Addendum VII to the interstate fishery management plan for horseshoe crabs for public comment. Atlantic States Marine Fisheries Commission, Washington, DC

Barber SB (1956) Chemoreception and proprioception in *Limulus*. J Exp Zool 131(1):51–73

Barlow RB, Powers MK (2003) Seeing at night and finding mates: the role of vision. In: Shuster CN Jr, Barlow RB, Brockmann HJ (eds) The American horseshoe crab. Harvard University Press, Cambridge, MA, pp 83–102

Barlow RB Jr, Ireland LC, Kass L (1982) Vision has a role in *Limulus* mating behaviour. Nature 296(5852):65–66

Barlow RB Jr, Powers MK, Howard H et al (1986) Migration of *Limulus* for mating: relation to lunar phase, tide height, and sunlight. Biol Bull 171:310–329

Barlow RB Jr, Powers MK, Howard H et al (1987) Vision in *Limulus* mating and migration. In: Herrnkind WF, Thistle AB (eds) Signposts in the sea. Florida State University Press, Tallahassee, pp 69–84

Barlow RB Jr, Powers MK, Kass L (1988) Vision and mating behavior in *Limulus*. In: Atema J, Fay RR, Popper AN, Tovolga WN (eds) Sensory biology of aquatic animals. Springer, New York, pp 419–434

Berkson J, Shuster CN (1999) The horseshoe crab: the battle for a true multiple-use resource. Fisheries 24(11):6–10

Botton ML (1984) Diet and food preferences of the adult horseshoe crab *Limulus polyphemus* in Delaware Bay, New Jersey, USA. Mar Biol 81:199–207

Botton ML (2009) The ecological importance of horseshoe crabs in estuarine and coastal communities: a review and speculative summary. In: Tanacredi JT, Botton M, Smith DR (eds) Biology and conservation of horseshoe crabs. Springer, New York, pp 45–63

Botton ML, Loveland RE (1989) Reproductive risk: high mortality associated with spawning by horseshoe crabs (*Limulus polyphemus*) in Delaware Bay, USA. Mar Biol 101(2):143–151

Botton ML, Loveland RE (1992) Body size, morphological constraints, and mated pair formation in four populations of horseshoe crabs (*Limulus polyphemus*) along a geographic cline. Mar Biol 112:409–415

Botton ML, Ropes JW (1988) An indirect method for estimating longevity of the horseshoe crab (*Limulus polyphemus*) based on epifaunal slipper shells (*Crepidula fornicata*). J Shellfish Res 7(3):407–412

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

Botton ML, Harrington BA, Tsipoura N et al (2003) Synchronies in migration: shorebirds, horseshoe crabs, and Delaware Bay. In: Shuster CN Jr, Barlow RB, Brockmann HJ (eds) The American horseshoe crab. Harvard University Press, Cambridge, MA, pp 5–32

Brockmann HJ (1990) Mating behavior of horseshoe crabs, *Limulus polyphemus*. Behaviour 114:206–220

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

Brockmann HJ (2001) The evolution of alternative strategies and tactics. Adv Study Behav 30:1–51

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

Brockmann HJ (2003a) Male competition and satellite behavior. In: Shuster CN Jr, Barlow RB, Brockmann HJ (eds) The American horseshoe crab. Harvard University Press, Cambridge, MA, pp 50–82

Brockmann HJ (2003b) Nesting behavior: a shoreline phenomenon. In: Shuster CN Jr, Barlow RB, Brockmann HJ (eds) The American horseshoe crab. Harvard University Press, Cambridge, MA, pp 33–49

Brockmann HJ, Johnson SL (2011) A long-term study of spawning activity in a Florida Gulf coast population of horseshoe crabs (*Limulus polyphemus*). Estuar Coast 34:1049–1067

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

Brockmann HJ, Smith MD (2009) Reproductive competition and sexual selection in horseshoe crabs. In: Tanacredi JT, Botton M, Smith DR (eds) Biology and conservation of horseshoe crabs. Springer, New York, pp 199–221

Brockmann HJ, Taborsky M (2008) Alternative reproductive tactics and the evolution of alternative allocation phenotypes. In: Oliveira RF, Taborsky M, Brockmann HJ (eds) Alternative reproductive tactics: an integrative approach. Cambridge University Press, Cambridge, pp 25–51

Brockmann HJ, Colson T, Potts W (1994) Sperm competition in horseshoe crabs (*Limulus polyphemus*). Behav Ecol Sociobiol 35:153–160

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

Brown GG (1976) Scanning electron-microscopical and other observations of sperm fertilization reactions in *Limulus polyphemus* L. (Merostomata: Xiphosura). J Cell Sci 22:547–562

Brown GG, Clapper DL (1981) Procedures for maintaining adults, collecting gametes, and culturing embryos and juveniles of the horseshoe crab, *Limulus polyphemus* L. In: Hinegardner R, Atz J, Fay R, Fingerman M, Josephson R, Meinkoth N (eds) Laboratory animal management, marine invertebrates. National Academy Press, Washington, DC, pp 268–290

Byrne RJ, Avise JC (2009) Multiple paternity and extra-group fertilizations in a natural population of California grunion (*Leuresthes tenuis*), a beach-spawning marine fish. Mar Biol 156:1681–1690

Byrne PG, Simmons LW, Roberts JD (2003) Sperm competition and the evolution of gamete morphology in frogs. Proc R Soc Lond B Biol 270:2079–2086

Calsbeek R, Alonzo SH, Zamudio K et al (2002) Sexual selection and alternative mating behaviours generate demographic stochasticity in small populations. Proc R Soc Lond B Biol 269:157–164

Candolin U (2003) The use of multiple cues in mate choice. Biol Rev 78:575–595

Candolin U, Reynolds JD (2002) Adjustments of ejaculation rates in response to risk of sperm competition in a fish, the bitterling (*Rhodeus sericeus*). Proc R Soc Lond B Biol 269:1549–1553

Carmichael RH, Rutecki D, Valiela I (2003) Abundance and population structure of the Atlantic horseshoe crab *Limulus polyphemus* in Pleasant Bay, Cape Cod. Mar Ecol Prog Ser 246:225–239

Castillo LP, Hatch KA (2007) Fasting increases delta N-15 values in the uric acid of *Anolis carolinensis* and *Uta stansburiana* as measured by nondestructive sampling. Rapid Commun Mass Spectrom 21:4125–4128

Cavenaugh CM (1975) Observations on mating behavior in *Limulus polyphemus*. Biol Bull 149:422

Chandler CH, Zamudio KR (2008) Reproductive success by large, closely related males facilitated by sperm storage in an aggregate breeding amphibian. Mol Ecol 17:1564–1576

Clark CW, Mangel M (2000) Dynamic state variable models in ecology: methods and applications. Oxford University Press, Oxford

Cohen JA, Brockmann HJ (1983) Breeding activity and mate selection in the horseshoe crab, *Limulus polyphemus*. Bull Mar Sci 33(2):274–281

Cummings ME, Gelineau-Kattner R (2009) The energetic costs of alternative male reproductive strategies in *Xiphophorus nigrensis*. J Comp Physiol 195:935–946

Duffy EE, Penn DJ, Botton ML et al (2006) Eye and clasper damage influence male mating tactics in the horseshoe crab, *Limulus polyphemus*. J Ethol 24(1):67–74

Eldredge N (1970) Observations on burrowing behavior in *Limulus polyphemus* (Chelicerata, Merostomata), with implications on the functional anatomy of trilobites. Am Mus Novit 2436:1–17

Emlen ST, Oring LW (1977) Ecology, sexual selection, and the evolution of mating systems. Science 197:215–223

Fu P, Neff BD, Gross MR (2001) Tactic-specific success in sperm competition. Proc R Soc B Biol Sci 268:1105–1112

Fulford RS, Haehn RA (2012) An evaluation of Mississippi barrier islands as spawning and nesting habitat for the American horseshoe crab, *Limulus polyphemus*, with implications for island restoration. Gulf Caribb Res 24:51–62

Gage MJG (1994) Associations between body-size, mating pattern, testis size and sperm lengths across butterflies. Proc R Soc B Biol Sci 258:247–254

Gerhart SD (2007) A review of the biology and management of horseshoe crabs with emphasis on Florida populations. Fish and Wildlife Research Institute, St. Petersburg. http://research.myfwc.com. Accessed 1 June 2014

Grant D (2001) Living on *Limulus*. In: Tanacredi JT (ed) *Limulus* in the limelight. Kluwer Academic, New York, pp 135–146

Greene AE, Funk WC (2009) Sexual selection on morphology in an explosive breeding amphibian, the Columbia spotted frog (*Rana luteiventris*). J Herpetol 43:244–251

Gross MR (1996) Alternative reproductive strategies and tactics: diversity within sexes. Trends Ecol Evol 11:92–97

Gross MR, Repka J (1998) Game theory and inheritance in the conditional strategy. In: Dugatkin L, Reeve HK (eds) Game theory and animal behavior. Oxford University Press, Oxford, pp 168–187

Harrington JM, Armstrong PB (2000) Initial characterization of a potential anti-fouling system in the American horseshoe crab, *Limulus polyphemus*. Biol Bull 199:189–190

Harrington JM, Leippe M, Armstrong PB (2008) Epithelial immunity in a marine invertebrate: a cytolytic activity from a cuticular secretion of the American horseshoe crab, *Limulus polyphemus*. Mar Biol 153(6):1165–1171

Hassler CL (1999) Satellite male groups in horseshoe crabs (*Limulus polyphemus*): how and why are males choosing females. M.S. thesis, University of Florida

Hassler C, Brockmann HJ (2001) Evidence for use of chemical cues by male horseshoe crabs when locating nesting females (*Limulus polyphemus*). J Chem Ecol 27:2319–2335

Hayes WF, Barber SB (1982) Peripheral synapses in *Limulus* chemoreceptors. Comp Biochem Physiol 72A(2):287–293

Hazel W, Smock R, Lively CM (2004) The ecological genetics of conditional strategies. Am Nat 163(6):888–900

Herzog ED, Powers MK, Barlow RB (1996) *Limulus* vision in the ocean day and night: effects of image size and contrast. Vis Neurosci 13:31–41

Hettyey A, Baksay S, Vagi B et al (2009) Counterstrategies by female frogs to sexual coercion by heterospecifics. Anim Behav 78:1365–1372

Hobson KA, Alisauskas RT, Clark RG (1993) Stable nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analyses of diet. Condor 95:388–394

Höglund J (1989) Pairing and spawning patterns in the common toad, *Bufo bufo*: the effects of sex ratios and the time available for male-male competition. Anim Behav 38:423–429

Höglund J, Saterberg S (1989) Sexual selection in common toads: correlates with age and body size. J Evol Biol 2:367–372

James-Pirri MJ, Tuxbury K, Marino S et al (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

Jennions MD, Petrie M (2000) Why do females mate multiply? A review of the genetic benefits. Biol Rev 75:21–64

Johnson SL, Brockmann HJ (2010) Costs of multiple mates: an experimental study in horseshoe crabs. Anim Behav 80:773–782

Johnson SL, Brockmann HJ (2012) Alternative reproductive tactics in female horseshoe crabs. Behav Ecol 23:999–1008

Johnson SL, Brockmann HJ (2013) Parental effects on early development: testing for indirect benefits of polyandry. Behav Ecol 24:1218–1228

Kerlinger P (1998) Showdown at Delaware Bay. Nat Hist 107:56–58

King TL, Eackles MS, Spidle AP et al (2005) Regional differentiation and sex-biased dispersal among populations of the horseshoe crab *Limulus polyphemus*. Trans Am Fish Soc 134(2):441–465

Koons BF (1883) Sexual characters of *Limulus*. Am Nat 17:1297–1299

Kreamer G, Michels SE (2009) History of horseshoe crab harvest in Delaware Bay. In: Tanacredi JT, Botton M, Smith DR (eds) Biology and conservation of horseshoe crabs. Springer, New York, pp 299–313

Kvarnemo C, Ahnesjö I (1996) The dynamics of operational sex ratios and competition for mates. Trends Ecol Evol 11:404–408

Leibovitz L, Lewbart GA (2003) Diseases and symbionts: vulnerability despite tough shells. In: Shuster CN Jr, Barlow RB, Brockmann HJ (eds) The American horseshoe crab. Harvard University Press, Cambridge, MA, pp 245–275

Leschen AS, Grady SP, Valiela I (2006) Fecundity and spawning of the Atlantic horseshoe crab, *Limulus polyphemus*, in Pleasant Bay, Cape Cod, Massachusetts, USA. Mar Ecol Evol Persp 27(1):54–65

Levitan DR (1995) The ecology of fertilization in free-spawning invertebrates. In: McEdward L (ed) Marine invertebrate larvae. CRC Press, New York, pp 123–156

Loesel R, Heuer CM (2010) The mushroom bodies – prominent brain centres of arthropods and annelids with enigmatic evolutionary origin. Acta Zool 91:29–34

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

Lucas JR, Howard RD (2008) Modeling alternative mating tactics as dynamic games. In: Oliveira RF, Taborsky M, Brockmann HJ (eds) Alternative reproductive tactics: an integrative approach. Cambridge University Press, Cambridge, pp 52–82

Maklakov AA, Lubin Y (2006) Indirect genetic benefits of polyandry in a spider with direct costs of mating. Behav Ecol Sociobiol 61:31–38

Martin KLM, van Winkle RC, Drais JE et al (2004) Beach-spawning fishes. Terrestrial eggs and air breathing. Physiol Biochem Zool 77:750–759

Mattei JH, Beekey MA, Rudman A et al (2010) Reproductive behavior in horseshoe crabs: does density matter? Curr Zool 56(5):634–642

McCauley SJ, Bouchard SS, Farina BJ et al (2000) Energetic dynamics and anuran breeding phenology: insights from a dynamic game. Behav Ecol 11:429–436

McCue MD (2010) Starvation physiology: reviewing the different strategies animals use to survive a common challenge. Comp Biochem Physiol A 156:1–18

McCue MD, Pollock ED (2008) Stable isotopes may provide evidence for starvation in reptiles. Rapid Commun Mass Spectrom 22:2307–2314

McGowan CP, Smith DR, Sweka JA et al (2011) Multispecies modeling for adaptive management of horseshoe crabs and red knots in the Delaware Bay. Nat Resour Model 24(1):117–156

Mendoza-Cuenca L, Macias-Ordonez R (2010) Female asynchrony may drive disruptive sexual selection on male mating phenotype in a Heliconius butterfly. Behav Ecol 21:144–152

Moore S (2004) The Taunton Bay assessment. Unpublished manuscript

Myers JP (1986) Sex and gluttony on Delaware Bay. Nat Hist 95:68–76

Olson DH, Blaustein AR, O'Hara RK (1986) Mating pattern variability among western toad (*Bufo boreas*) populations. Oecologia 70:351–356

Parker GA (1970) Sperm competition and its evolutionary consequences in the insects. Biol Rev 45:525–568

Parker GA (1982) Why are there so many tiny sperm – sperm competition and the maintenance of 2 sexes. J Theor Biol 96:281–294

Parker GA (1998) Sperm competition and the evolution of ejaculates: towards a theory base. In: Birkhead TR, Møller AP (eds) Sperm competition and sexual selection. Academic, New York, pp 3–54

Partan SR, Marler P (2005) Issues in the classification of multimodal communication signals. Am Nat 166:231–245

Passaglia CL, McSweeney ME, Stewart KM et al (1997) Visual performance of horseshoe crabs: role of underwater lighting. Biol Bull 193:205–207

Penn D, Brockmann HJ (1994) Nest-site selection in the horseshoe crab, *Limulus polyphemus*. Biol Bull 187:373–384

Penn D, Brockmann HJ (1995) Age-biased stranding and righting in horseshoe crabs, *Limulus polyphemus*. Anim Behav 49:1531–1539

Pilastro A, Scaggiante M, Rasotto MB (2002) Individual adjustment of sperm expenditure accords with sperm competition theory. Proc Natl Acad Sci USA 99:9913–9915

Pomerat CM (1933) Mating in *Limulus polyphemus*. Biol Bull 64(2):243–252

Powers MK, Barlow RB Jr, Kass L (1991) Visual performance of horseshoe crabs day and night. Vis Neurosci 7(3):179–190

Reichard M, Le Comber SC, Smith C (2007) Sneaking from a female perspective. Anim Behav 74:679–688

Repka J, Gross MR (1995) The evolutionarily stable strategy under individual condition and tactic frequency. J Theor Biol 176:27–31

Roberts JD, Byrne PG (2011) Polyandry, sperm competition and the evolution of anuran amphibians. Adv Study Behav 43:1–53

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(3):177–183

Rudloe A (1985) Variation in the expression of lunar and tidal behavioral rhythms in the horseshoe crab, *Limulus polyphemus*. Bull Mar Sci 36(2):388–395

Sasson DA, Brockmann HJ (in prep) Factors influencing geographical variation in sperm traits of the American horseshoe crab, *Limulus polyphemus*

Sasson DA, Johnson SL, Brockmann HJ (in press) Sperm traits in a system with condition-dependent alternative reproductive tactics. Behav Ecol Sociobiol

Sasson DA, Johnson SL, Brockmann HJ (2012) The role of age on sperm traits in the American horseshoe crab, *Limulus polyphemus*. Anim Behav 84:975–981

Saunders KM, Brockmann HJ, Watson WH et al (2010) Male horseshoe crabs *Limulus polyphemus* use multiple sensory cues to locate mates. Curr Zool 56(5):485–498

Schwab RL, Brockmann HJ (2007) The role of visual and chemical cues in the mating decisions of satellite male horseshoe crabs, *Limulus polyphemus*. Anim Behav 74:837–846

Sekiguchi K (1988) Ecology [of horseshoe crabs]. In: Sekiguchi K (ed) Biology of horseshoe crabs. Science House Co., Ltd., Tokyo, pp 50–68

Shoger RL, Bishop DW (1967) Sperm activation and fertilization in *Limulus polyphemus*. Biol Bull 133:485

Shuster CN Jr (1954) A horseshoe "crab" grows up. Ward's Nat Sci Bull 28(1):1–6

Shuster CN Jr, Botton ML (1985) A contribution to the population biology of horseshoe crabs, *Limulus polyphemus* (L.) in Delaware Bay. Estuaries 8(4):363–572

Shuster CN Jr, Sekiguchi K (2003) Growing up takes about ten years and eighteen stages. In: Shuster CN, Barlow RB, Brockmann HJ (eds) The American horseshoe crab. Harvard University Press, Cambridge, MA, pp 103–132

Shuster SM, Wade MJ (2003) Mating systems and strategies. Princeton University Press, Princeton

Shuster CN Jr, Botton ML, Loveland RE (2003) Horseshoe crab conservation: a coast-wide management plan. In: Shuster CN, Barlow RB, Brockmann HJ (eds) The American horseshoe crab. Harvard University Press, Cambridge, MA, pp 358–377

Simmons LW (2005) The evolution of polyandry: sperm competition, sperm selection, and offspring viability. Ann Rev Ecol Evol Syst 36:125–146

Sirot LK, Brockmann HJ, Marinis C et al (2003) Maintenance of a female-limited polymorphism in *Ischnura ramburi* (Zygoptera: Coenagrionidae): evaluation of hypotheses and new insights. Anim Behav 66:763–775

Slatyer RA, Mautz BS, Backwell PRY et al (2012) Estimating genetic benefits of polyandry from experimental studies: a meta-analysis. Biol Rev 87:1–33

Smith MD, Brockmann HJ (2014) The evolution and maintenance of sexual size dimorphism in horseshoe crabs: an evaluation of six functional hypotheses. Anim Behav 96:127–139

Smith DR, Brousseau DJ, Mandt MT et al (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. Curr Zool 56(5):563–574

Smith DR, McGowan CP, Daily JP et al (2013a) Evaluating a multispecies adaptive management framework: must uncertainty impede effective decision-making. J Appl Ecol 50:1431–1440

Smith MD, Schrank HE, Brockmann HJ (2013b) Measuring the costs of alternative reproductive tactics in horseshoe crabs, *Limulus polyphemus*. Anim Behav 85:165–173

Snook RR (2005) Sperm in competition: not playing by the numbers. Trends Ecol Evol 20:46–51

Sokoloff A (1978) Observations on populations of the horseshoe crab *Limulus* (=Xiphosura) *polyphemus*. Res Popul Ecol 19:222–236

Suggs DN, Carmichael RH, Grady SP et al (2002) Effects of individual size on pairing in horseshoe crabs. Biol Bull 203:225–227

Sztatecsny M, Jehle R, Burke T et al (2006) Female polyandry under male harassment: the case of the common toad (*Bufo bufo*). J Zool 270:517–522

Sztatecsny M, Preininger D, Freudmann A et al (2012) Don't get the blues: conspicuous nuptial colouration of male moor frogs (*Rana arvalis*) supports visual mate recognition during scramble competition in large breeding aggregations. Behav Ecol Sociobiol 66:1587–1593

Taborsky M (1998) Sperm competition in fish: 'bourgeois' males and parasitic spawning. Trends Ecol Evol 13:222–227

Teale EW (1957) The oldest migration. Nat Hist 66:364–369

Thornhill R, Alcock J (1983) The evolution of insect mating systems. Harvard University Press, Cambridge, MA

Tregenza T, Wedell N (2000) Genetic compatibility, mate choice and patterns of parentage: invited review. Mol Ecol 9:1013–1027

Vasquez MC, Johnson SL, Brockmann HJ et al (2015) Nest site selection minimizes environmental stressor exposure in the American horseshoe crab, *Limulus polyphemus*. J Exp Mar Biol Ecol 463:105–114

Vladic TV, Jarvi T (2001) Sperm quality in the alternative reproductive tactics of Atlantic salmon: the importance of the loaded raffle mechanism. Proc R Soc B Biol Sci 268:2375–2381

Wasserman GS, Cheng Z (1996) Electroretinographic measures of vision in horseshoe crabs with uniform versus variegated carapaces. Biol Signals 5:247–262

Wedekind C (1994) Mate choice and maternal selection for specific parasite resistances before, during and after fertilization. Phil Trans R Soc B 346:303–311

Wells KD (1977) The social behaviour of anuran amphibians. Anim Behav 25:666–693

Wells KD (2007) The ecology and behavior of amphibians. University of Chicago Press, Chicago

Widemo F (1998) Alternative reproductive strategies in the ruff, *Philomachus pugnax*: a mixed ESS? Anim Behav 56:329–336

Widener JW, Barlow R (1999) Decline of a horseshoe crab population on Cape Cod. Biol Bull 197:300–302

Zamudio KR, Chan LM (2008) Alternative reproductive tactics in amphibians. In: Oliveira RF, Taborsky M, Brockmann HJ (eds) Alternative reproductive tactics: an integrative approach. Cambridge University Press, Cambridge, pp 300–331

Zeh JA, Zeh DW (2003) Toward a new sexual selection paradigm: polyandry, conflict and incompatibility. Ethology 109:929–950