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Erin E. Duffy · Dustin J. Penn · Mark L. Botton
H. Jane Brockmann · Robert E. Loveland

Eye and clasper damage influence male mating tactics in the horseshoe crab, *Limulus polyphemus*

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Abstract In the horseshoe crab mating system, mated pairs are frequently accompanied by unattached satellite males as they spawn on intertidal beaches. Previous studies have shown that males locate females visually using their lateral (compound) eyes, and that attached (mated) males generally have less heavily worn or damaged carapaces than unattached males. The purpose of this study was to investigate the influences of lateral eye condition and clasper abnormalities on male mating tactics. Sexually mature males had two kinds of eye damage: deterioration caused by disease, and overgrowth by sessile invertebrates, such as bryozoans, mussels, and tube-building polychaetes. The lateral eyes of attached males had significantly less decay than unattached males. On the other hand, coverage of the lateral eyes by encrusting invertebrates was more extensive among attached than unattached males. Although overgrowth did not appear to impair a male's ability to pair with a female as severely as eye decay, it is conceivable that amplexus may have occurred before epibiont coverage was sufficient to obscure vision. Male crabs that were experimentally "blind-folded" by painting their lateral eyes with black nail polish were less likely to reattach to a female than controls. Appendage injuries were more frequent among unattached males than among attached males; in particular, 6.4% of

unattached males but 0.0% of attached males had damaged claspers (the modified first legs required for amplexus). Unattached males in the population were "older," as judged by the degree of carapace wear, than attached males. Severe visual impairment and/or clasper damage may explain the reduced pairing success of older male horseshoe crabs, and underlie their choice of the alternative satellite male mating tactics.

Key words Horseshoe crab · *Limulus polyphemus* · Mating system · Alternative mating tactics · Limb loss · Eye damage

Introduction

The American horseshoe crab, *Limulus polyphemus* (Arthropoda, Chelicerata) has proven to be a useful model system in which to study various aspects of mating behavior, including the effects of male and female size on mate selection (Brockmann 1990; Loveland and Botton 1992; Botton and Loveland 1992), male–male interactions and sperm competition (Brockmann 1996, 2003; Brockmann et al. 1994, 2000), the role of lunar and tidal rhythms in synchronizing spawning behavior (Rudloe 1980; Barlow et al. 1986), and the importance of visual cues in enabling males to locate females (Barlow et al. 1982, 1988). The horseshoe crab is an epibenthic animal, spending most of its time within bays or on the continental shelf, except for the annual spawning migration to sandy estuarine beaches. Amplexus occurs in the subtidal zone and mated pairs approach the shore during high tide. Males attach to the female's opisthosoma, anterior to the terminal spines, using an enlarged pair of subchelate pedipalps or claspers (Loveland and Botton 1992). Males attached in this position fertilize all the female's eggs if no satellite male is present (Brockmann 1990; Brockmann et al. 1994). At the time of spawning, mated pairs are typically surrounded by 1–15 satellite males (Botton and Loveland 1992; Loveland and Botton 1992). The satellites surrounding the mated pair, as well as the attached male, fertilize the female's eggs

E.E. Duffy · M.L. Botton¹ (✉)
Department of Natural Sciences, Fordham College at Lincoln Center,
113 West 60th Street, New York, NY 10023, USA
Tel. +1-212-636-6327; Fax +1-212-636-7217
e-mail: botton@fordham.edu

D.J. Penn
Konrad Lorenz Institute for Ethology, Austrian Academy of Sciences,
Savoyenstrasse 1a, 1160, Vienna, Austria

H.J. Brockmann
Department of Zoology, University of Florida, Gainesville, FL 32611,
USA

R.E. Loveland
Department of Ecology, Evolution, and Natural Resources, Rutgers
University, Cook College, New Brunswick, NJ 08901, USA

Present address:

¹Freudenthal & Elkowitz Consulting Group, Inc., 368 Veterans
Memorial Highway, Commack, NY 11725, USA

as they are being deposited in the sand (Brockmann et al. 1994). Those satellites that are able to orient themselves over the incurrent canal, partially pushing under the prosoma of the mated male, have the greatest likelihood of achieving fertilization (Brockmann et al. 2000). Fertilization by more than one male is possible because horseshoe crabs have external fertilization (Brockmann et al. 1994, 2000). Attached and unattached males do not differ significantly in overall body size (Loveland and Botton 1992; Botton and Loveland 1992). However, attached males appear to be, on average, younger (as judged by carapace condition), better at finding females, and more likely to right themselves when overturned than unattached males (Brockmann and Penn 1992; Penn and Brockmann 1995; Brockmann 1996, 2002).

Male horseshoe crabs locate females visually (Barlow et al. 1982, 1988; Herzog et al. 1996). When unattached males are searching for females, they will reorient themselves in the direction of a female at an underwater distance of up to 2 m (Barlow et al. 1982). Circadian rhythms enable the lateral eyes to increase their sensitivity by a factor of 10^6 in darkness, thereby allowing males to find females visually even at night (Barlow et al. 1980; Powers et al. 1991). It has been shown that underwater lighting also plays an important role in enabling male horseshoe crabs to detect objects that vary in contrast against a background of sand. Strobic light enhances the visibility of low-contrast objects on sandy bottoms that would otherwise be difficult for the horseshoe crab to see (Herzog et al. 1996; Passaglia et al. 1997; Krutky et al. 2000).

Despite the importance of vision in the *Limulus* mating system, many males show considerable deterioration of their lateral eyes (Brockmann and Penn 1992) which is often due to chitinoclastic bacteria and blue-green algal infections (Leibovitz and Lewbart 2003). In many horseshoe crabs, the dorsal carapace, including the eyes, is encrusted with fouling organisms such as barnacles or mussels. Brockmann and Penn (1992) showed that males with two "good" eyes were more likely to be paired than males with one or two "poor" eyes, but they did not quantify eye damage or differentiate between eye decay and epibiont coverage. Here, we extend the study by examining the separate influences of lateral eye deterioration and fouling on male mating tactics, and by conducting an experiment to evaluate the effect of eye condition on pairing success.

The condition of a male's claspers is also likely to affect pairing (Brockmann 2002). Because normal amplexus in horseshoe crabs involves the use of the claspers to attach to the female (Loveland and Botton 1992), a male that is missing a clasper would potentially be at a disadvantage in pairing with a female, or be more susceptible to displacement by competing males. We examined the frequency of clasper injuries among attached and unattached males, and in females (in females this appendage is not specially modified and remains chelate, resembling the other walking legs). We also examined the frequency of occurrence of injuries to the walking legs in both sexes to determine if male claspers were more likely to be injured than other appendages.

Materials and methods

Frequency of eye damage among attached and unattached males

Sexually mature male horseshoe crabs, which are smaller than sexually mature females, were easily identified by their monodactylus pedipalps (claspers), which closely resemble boxing gloves. Male mating status (attached vs. unattached) was determined, and eye condition was scored. Eyes were scored as "good" if they were in perfect condition versus "poor" if they were soft to the touch, as a result of infection, and/or partially or completely covered with epibionts. Males were marked to avoid evaluating the same male twice. These data were collected at Seahorse Key, Florida in April–May 1990, and at four sites on Delaware Bay (Broadkill Beach and Cape Henlopen State Park, Delaware; Reeds Beach and Cape Shore Beach, NJ, USA) in June 1990 (see Penn and Brockmann 1994; Smith et al. 2002 for environmental descriptions of each beach).

These initial observations neither quantified eye damage nor did they distinguish between damage caused by eye deterioration versus epibiont coverage. Therefore, in 2000, we examined each type of eye damage at Cape Shore Beach (Delaware Bay, NJ, USA) in relation to male mating status. Sexually mature male horseshoe crabs (260 attached and 332 unattached) were assessed for eye condition from 31 May–8 June 2000. In order to avoid any bias in the selection of males, we either evaluated every male encountered within a randomly chosen stretch of beach during high-tide spawning activity or evaluated every male within randomly selected areas on the intertidal flats at a low tide (mated males remain attached to females throughout the tidal cycle). On a few occasions, a student intern, who was not familiar with *L. polyphemus*, was shown how to identify a mated pair and unattached males and was asked to retrieve crabs for inspection. She was not informed about what was being investigated in order to diminish the possibility of a bias toward males of a particular condition. All animals were released into the bay within 15 min of capture and no obvious mortality was associated with handling or holding the crabs. The large horseshoe crab population size in this area of Delaware Bay (Loveland and Botton 1992) makes it very unlikely that the same individuals were repeatedly measured.

Visual impairment was assessed by estimating the percentage of the ommatidia of both lateral eyes of each male crab that were affected by decay or fouling by epibionts. Eye decay was recorded based on the percentage of the ommatidia that was pitted and deteriorated. The following scores were assigned: 0 for perfect eyes, 1 for $\leq 25\%$ deteriorated, 2 for 26–50% deteriorated, 3 for 51–75% deteriorated, 4 for 76–99% deteriorated, and 5 for completely deteriorated eyes. Thus, each male received a condition score of 0 through 5 for each eye; since we found that there were no significant differences between the scores for the right and left eyes, a single combined score of 0–10 for both eyes is presented. The condition of the median eyes was not

included in this study, because only the lateral eyes are used when locating mates (Barlow et al. 1982; Passaglia et al. 1997).

A similar scoring system (0–5 for each eye, combined to 0–10 for each crab) was used to assess the coverage of the lateral eyes by all species of epibionts. Encrusting organisms were identified to lowest possible taxon.

The scoring system for carapace condition was based on a scale of 1–3, where a rating of 1 was for a newly molted, glossy carapace, 2 for an intermediate carapace (i.e., showing scratches and other signs of wear, and with some blackened areas), and 3 for a highly variegated, or heavily worn, mostly blackened and pitted carapace. Although carapace condition in *Limulus* cannot be used to provide a chronological age, these categories are probably reasonable indicators of relative age (e.g., Brockmann 2002), i.e., “young,” “middle-aged,” and “old,” respectively.

Experiments to test whether eye condition affects male mating status

To test whether a male’s lateral eye condition plays a role in his ability to pair with a female, we manipulated vision and examined how this affected subsequent mating status. At high tide, we collected males and restrained them on the beach, just above the tide line. After drying the lateral eyes with a cloth and allowing them to dry for 1–2 min, we altered eye condition by painting the eyes with either black or clear nail polish. We allowed the polish to air dry for 10–15 min, and then released the crabs into the water. Control animals were handled in the same manner but no nail polish was applied. The experiment was blocked such that we manipulated approximately equal numbers of treatment and control crabs similarly and at the same time. We assigned males to a treatment group by alternating the group as we encountered each male on the beach. Males were tagged with numbers printed on plastic labeling tape (Brockmann and Penn 1992). Tagged animals were resighted during subsequent observations along the same beach at high tide, and the mating status (attached vs. unattached) of each male at the time he was first recaptured was recorded. These experiments were conducted at Seahorse Key, Florida ($n = 132$ recaptured) from 27 March–30 April 1991 and Cape Henlopen, Delaware ($n = 81$ recaptured) from 18 May–3 June 1991.

Damage to claspers and other appendages

To determine whether clasper or appendage condition has an effect on mating status, we examined 645 horseshoe crabs from Cape Shore Beach, Delaware Bay, NJ, USA (203 attached females, 193 attached males, and 249 unattached males) between 22 May and 21 June 2001. Virtually all of the females in the Delaware Bay breeding population in May and June were attached to males (Loveland and Botton 1992), so we were unable to obtain a sample of unattached females for comparison. Undamaged appendages received a score of 0, appendages missing one or more pieces of the fixed and/or moveable digit of the chela received a score of 0.5 (slight damage), and appendages that were amputated, abnormally regenerated, or incapacitated because of torn muscles, were considered to be severely damaged and received a score of 1. As in 2000, carapace condition was scored on a scale of 1 (newly molted) to 3 (highly variegated).

Results

Frequency of eye damage and male mating status

Visually impaired males were less likely to be part of a mated pair in Florida and Delaware Bay in 1990, and moreover, the number of eyes in good condition predicted their status (Table 1). Darker carapace color (which is probably associated with older age) was correlated with poor eye condition. Of the “young,” light-colored males, 89% had two good eyes and only 2% had two bad eyes, whereas the reverse was true of “old,” dark-colored males (chi-square contingency test: $\chi^2_1 = 38$, $P < 0.001$, $n = 211$).

In the 2000 Delaware Bay population, unattached males were about twice as likely to have lateral eye deterioration as attached males. Combined eye deterioration scores (pooling the scores of 0, 1–3, 4–6, and 7–10) showed significant differences between attached and unattached males ($\chi^2_3 = 20.36$, $P < 0.001$, $n = 592$); 19.2% of attached males and 34.9% of unattached males had some degree of decay of both eyes (Fig. 1). The left eyes ($\chi^2_2 = 15.13$, $P < 0.001$) and right eyes ($\chi^2_2 = 16.47$, $P < 0.001$) were both more damaged in unattached males than attached males (pooling the scores of 0, 1–3, and 4–5 for each eye).

Table 1. Male mating status (attached, unattached) in relation to the condition of the lateral eyes in *Limulus polyphemus* from three locations in 1990. 0 Both eyes poor (partially or completely eroded or covered with epibionts), 1 one eye in good condition, 2 both eyes in good condition

Location	Status	Lateral eye condition			χ^2	P
		0 (%)	1 (%)	2 (%)		
Seahorse Key, FL ($n = 101$)	Attached	3.0	12.9	38.6	10	0.007
	Unattached	8.9	17.8	18.8		
Delaware Bay, DE ($n = 427$)	Attached	1.2	4.4	31.6	200	0.001
	Unattached	40.0	12.0	10.8		
Delaware Bay, NJ ($n = 339$)	Attached	3.5	5.3	34.5	9.7	0.008
	Unattached	11.2	7.7	37.8		

Attached males were more likely to have fouled eyes than unattached males in the Delaware Bay population ($\chi^2_3 = 57.08$, $P < 0.001$, $n = 592$, Fig. 2); 29.6% of attached males had some eye fouling, compared with 6.9% of unattached males. Both left eyes ($\chi^2_2 = 48.44$, $P < 0.001$) and right eyes ($\chi^2_2 = 44.85$, $P < 0.001$) were more frequently fouled in attached males than in unattached males.

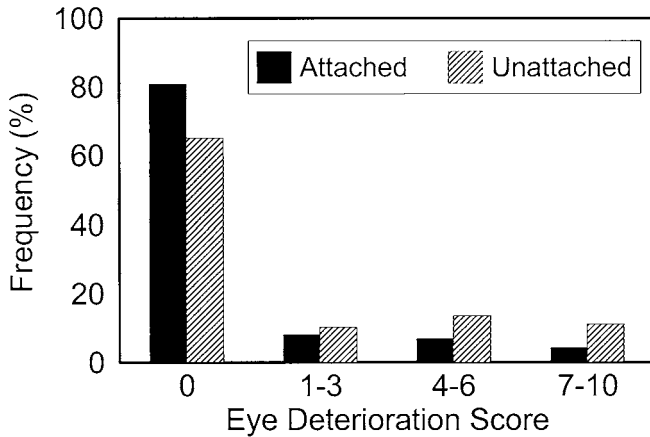


Fig. 1. Frequency distribution comparing the combined lateral eye deterioration of attached ($n = 260$) and unattached male horseshoe crabs ($n = 332$) from Delaware Bay during the 2000 mating season. Each eye was independently scored on a scale of 0 (perfect condition) to 5 (completely deteriorated) and the scores were then added to generate a combined score on a scale of 0–10

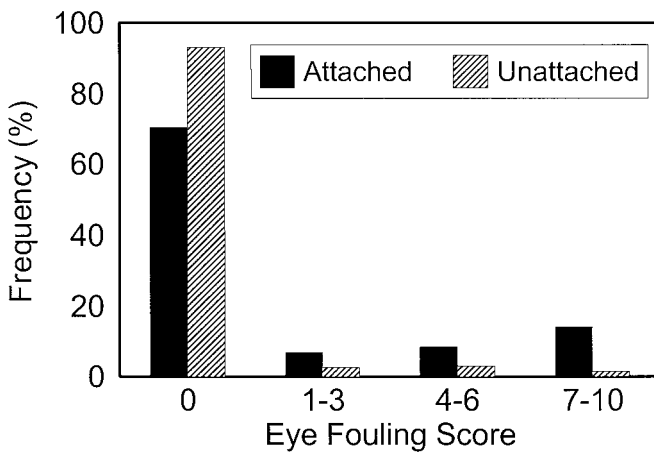


Fig. 2. Frequency distribution comparing the combined fouling coverage of the lateral eyes of attached ($n = 260$) and unattached male horseshoe crabs ($n = 332$) from Delaware Bay during the 2000 mating season. Each eye was independently scored on a scale of 0 (completely free of fouling) to 5 (100% coverage) and the scores were then added to generate the combined score on a scale of 0–10

The most frequently occurring epibionts on the lateral eyes in the Delaware Bay population were bryozoans (*Membranipora* sp.), sand tube-building polychaetes (*Sabellaria vulgaris*), and blue mussels (*Mytilus edulis*), with less frequent coverage by hydroids, barnacles, and mud-snail (*Ilyanassa obsoleta*) egg cases (Table 2). Twenty attached males (7.7%) had lateral eyes fouled by two or more species (most often *Membranipora* plus *Sabellaria*), but eye fouling by two or more species was seen only twice (0.6%) among unattached males. Seahorse Key *Limulus* were fouled with similar organisms (on the dorsal surface of the prosoma): barnacles (*Balanus improvisus*), polychaetes (*S. vulgaris*), bryozoans (*Membranipora tenuis*), oysters (*Crassostrea virginica*), hydroids, and sea squirts (*Styela plicata*).

Attached male horseshoe crabs were judged to have “younger,” i.e., less worn and variegated carapaces (mean = 2.28 on a scale of 1 = newest to 3 = most highly variegated; $n = 259$) than unattached males in the 2000 population (mean = 2.65, $n = 332$) (two-tailed t -test: $t_{590} = 7.82$, $P < 0.001$; Fig. 3).

Experiments to test whether eye condition affects male mating status

Twenty percent of the experimentally blinded males (black nail polish) did not reattach whereas all control males (clear

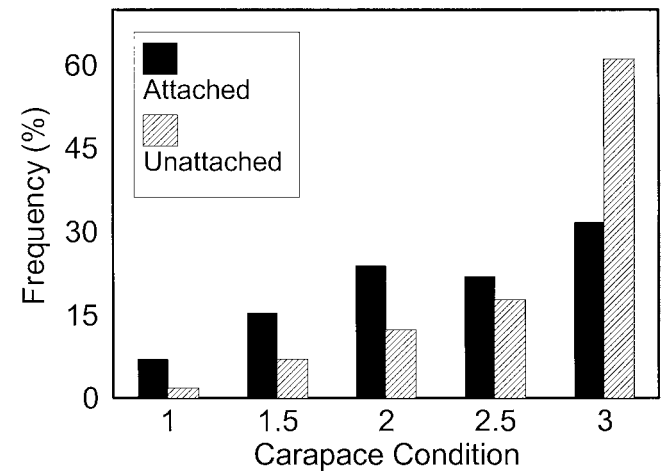


Fig. 3. Frequency distribution comparing the carapace condition scores for attached ($n = 260$) and unattached male horseshoe crabs ($n = 332$) from Delaware Bay during the 2000 mating season. A score of 1 was assigned for a newly molted, glossy carapace, 2 for an intermediate carapace, and 3 for a highly variegated, or heavily worn and pitted carapace

Table 2. Occurrence of epibionts covering the lateral eyes of horseshoe crabs in 2000

Male status	Bryozoans (<i>Membranipora</i>) (%)	Polychaete tubes (<i>Sabellaria</i>) (%)	Blue mussels (<i>Mytilus</i>) (%)	Hydroids (%)	Barnacles (%)	Mud-snail (<i>Ilyanassa</i>) egg cases (%)
Attached ($n = 260$)	22.69	7.31	6.15	2.31	1.54	0.38
Unattached ($n = 332$)	3.61	1.81	0.30	0.00	0.60	0.30

Table 3. Mating status (attached, unattached) upon recovery of *Limulus polyphemus* males whose eyes were experimentally manipulated by painting them with black or clear nail polish, or using no polish (only black and clear were used in Delaware Bay). *P* was determined using Fisher's exact test; for the Seahorse Key experiment, the categories of clear and none were combined

Location and <i>n</i> recovered	Status at time of return	Lateral eye manipulation			<i>P</i>
		Black (%)	Clear (%)	None (%)	
Seahorse Key, FL (<i>n</i> = 132)	Attached	32.6	12.1	47.0	< 0.0001
	Unattached	8.3	0	0	
Cape Henlopen, DE (<i>n</i> = 81)	Attached	0	6.2	–	0.3
	Unattached	25.9	67.9	–	

nail polish and no polish) returned to the beach shoreline at Seahorse Key within 5 days as attached males (Table 3; Fisher's exact test, $P < 0.0001$). If we exclude the nine males that lost their black paint after release from the analysis, the result is unchanged. The overall recapture rate at Seahorse Key was 69%, and black paint had no effect on return rate. Although we used many males that were initially unattached, excluding males that were originally unattached from the analysis did not change the result.

At Cape Henlopen, Delaware, we tagged 473 males (238 black polish and 235 clear polish); however, the recapture rate for all males was just 17% (81/473). Only 5/81 (6%) of the recaptured males were attached, but none of these five attached males had been experimentally blinded (Table 3).

Frequency of clasper damage and male mating status

In a sample of 249 unattached males from Delaware Bay in 2001, 16 (6.4%) had severe damage to a clasper, compared with 0 among mated males ($n = 193$) and 2 among females (0.98%, $n = 204$) (Fig. 4). Examining all five pairs of appendages, attached males had a lower frequency of damage than unattached males (all degrees of leg damage, $\chi^2_8 = 29.98$, $P < 0.001$; severe leg damage, $\chi^2_8 = 19.40$, $P < 0.025$). Among unattached males, claspers were injured more frequently than walking legs 2 and 3, and about as often as walking legs 4 and 5 (Fig. 3). Claspers were injured far more often than the first appendage in females, or the other male appendages except leg 5. In attached males and females, the frequency of occurrence of leg abnormalities was lowest for the claspers and became progressively greater in a posterior direction. Pooling all 645 animals, 123 (19.1%) had slight or severe damage to at least one appendage and 98 (15.2%) had severe damage to at least one appendage.

In the 2001 Delaware Bay population, the carapace condition score was significantly worse for those unattached males with at least one leg damaged (mean = 2.72) than unattached males with intact legs (mean = 2.27) ($t_{247} = 4.99$, $P < 0.001$). Likewise, carapace condition was worse for attached males with leg damage (mean = 2.45) than for attached males with intact legs (mean = 1.99) ($t_{191} = 3.21$, $P = 0.002$), and for females with leg damage (mean = 2.35) versus females with intact legs (mean = 2.16) ($t_{201} = 1.94$, $P = 0.054$).

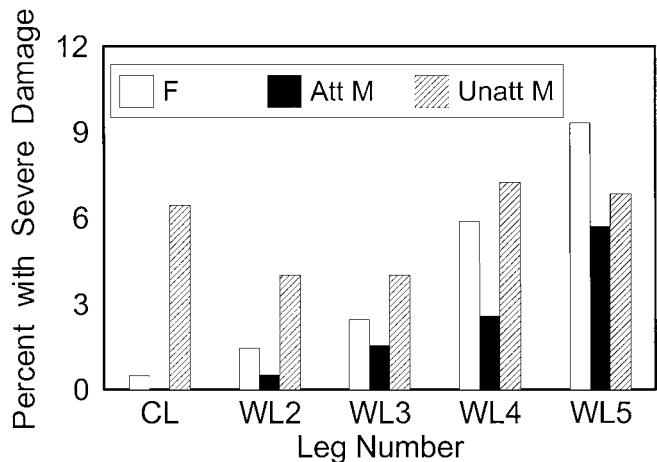


Fig. 4. Frequency distribution comparing the occurrence of severe leg handicaps in Delaware Bay horseshoe crab females (*F*) ($n = 204$), attached males (*Att M*) ($n = 193$), and unattached males (*Unatt M*) ($n = 249$) during the 2001 mating season. Legs are ordered in the anterior to posterior direction. *CL* Clasper or pedipalp (not morphologically specialized in females), *WL2* second walking leg, *WL3* third walking leg, *WL4* fourth walking leg, and *WL5* fifth walking leg

Discussion

Vision is an important factor in horseshoe crab mate location and recognition (Barlow et al. 1982). The results of this study (Fig. 1) suggest that males with deteriorated lateral eyes are less likely to pair than males with eyes in good condition. Similarly, males that are visually handicapped by experimentally covering their eyes are also less likely to pair. However, it is interesting to note that many males with eyes that are in poor condition (due to fouling, disease, or experimental manipulation) are not excluded from participation in mating, either as mated or satellite males. Within the Delaware Bay population, there were some attached males with severely or completely decayed eyes, and the majority of experimentally blindfolded males at Seahorse Key successfully located females (Table 3). Visual disabilities did not preclude unattached males from finding their way to the shoreline, and their behavior as satellites did not appear to differ from satellites with intact vision. The majority of males were recaptured as attached males at Seahorse Key, but in Delaware Bay, most were recaptured as

unattached males irrespective of eye manipulation (black vs. clear polish). These results may be related to the more heavily male-skewed operational sex ratio in Delaware Bay, which decreases the likelihood that a released male would encounter an unattached female.

The exact cause of eye deterioration in either crab population is not known, but a green algal infection has been described that leads to the degradation of the lateral eyes and other portions of the carapace, and encysted metacercariae of the trematode *Microphallus limuli* are common parasites infecting the lateral eye, especially in juvenile crabs (Leibovitz and Lewbart 2003). Younger horseshoe crabs have a cuticular secretion that prevents biofouling (Harrington and Armstrong 2000), but as crabs age, this secretion appears to become less prolific (Brockmann 1996), and overgrowth and carapace diseases become more prevalent. Unattached males from Seahorse Key had significantly darker and more heavily fouled carapaces than attached males (Brockmann and Penn 1992).

Contrary to our initial hypothesis, we found that attached males from Delaware Bay had more heavily fouled eyes than unattached males (Fig. 2), a result that also differed from the trends observed in Florida (Table 1). Behavioral differences between attached and unattached males may make the unattached males more suitable for larval settlement by epibionts (Brockmann and Penn 1992). The attached males remain paired with females during low-tide periods when they typically burrow in shallow troughs near the shoreline. Because attached males are unable to burrow as completely as unattached males, portions of their carapace may be exposed to the air at low tide, which might be less favorable for larval settlement or survival. Given the overall tendency for unattached males to be older and more heavily encrusted than attached males (Brockmann and Penn 1992), our finding that attached males had more heavily fouled lateral eyes is counterintuitive but intriguing. One possible explanation is that the overgrowth of the eyes took place after males had already located and paired with females. While the exact length of time that transpires between amplexus and mating is unknown, it is possible that it is a matter of weeks or more (Loveland and Botton 1992), which would allow time for substantial overgrowth of the eyes to take place subsequent to amplexus. Delaware Bay is also different from Florida in that the sex ratio is more strongly male biased, and there is a greater risk of beach stranding during spawning (Penn and Brockmann 1995). If males can assess their own visual capabilities, males with fouled eyes in Delaware Bay might be especially tenacious in holding onto a female, given that they would have a high risk of stranding coupled with little chance of locating another unpaired female. It has been shown by Wasserman and Cheng (1996) that fouling of the eyes by barnacles restricts visual abilities. However, it is possible that other fouling organisms allow for a greater field of view and do not significantly obstruct the crab's vision when searching for a mate. *Membranipora*, the most common fouling organism on the eyes of attached horseshoe crabs from Delaware Bay (Table 2), forms a thin film over the eye. This may be enough to blur vision, but not enough to cause blindness,

whereas a dense cover of barnacles, *Sabellaria* or *Mytilus* may cause a more debilitating loss of vision.

In agreement with Brockmann and Penn (1992), we observed that crabs with heavily eroded carapaces were less likely to be part of a mated pair (Fig. 3). Wasserman and Cheng (1996) used electroretinograms to analyze the differences between the visual response of males with fouling cover and variegated carapaces and males having non-fouled, non-variegated carapaces. Their study has shown a difference between the physiological response of perfectly clear *Limulus* eyes and fouled or darkened eyes, which correlated with carapace variegation. They did not find complete blindness for the fouled or darkened eyes, but a reduced ability to detect light with increased flash intensity and rapid flashing (Wasserman and Cheng 1996). Because light intensity and a strobic effect appear to play an important role in mate location (Herzog et al. 1996; Passaglia et al. 1997; Krutky et al. 2000), it is possible that the inability of the crabs to respond to high intensity, strobic light may reduce their ability to distinguish low-contrast females in the subtidal zone, reducing the chances for successful mate recognition. Wasserman and Cheng (1996) hypothesized that darkening of the lateral eyes may have the same effect as a cataract on a human eye, leaving the crab with functional vision, but a somewhat diminished ability to discriminate its surroundings in order to locate a mate.

Males affected by the loss of a clasper were clearly disadvantaged in pairing, and unattached males had higher frequencies of injuries to all legs than attached males (Fig. 4). These results are similar to several previous studies that have examined the effects of chela loss in species of decapod crabs that form copulatory embraces with females. Sekkelsten (1988) found that handicapped male shore crabs (*Carcinus maenas*) in certain size classes had lower mating success than males that had intact chelae. Chela loss was more common in males than females in another *C. maenas* population, and males without crusher claws were less capable of resisting displacement than normal males (Abello et al. 1994). Smith (1992) found that larger male blue crabs (*Callinectes sapidus*) tended to have higher frequencies of missing chelae, but large size compensated for this handicap such that only males with multiple injuries were disadvantaged in male-male interactions. Adult male snow crabs (*Chionoecetes opilio*) with missing walking legs were rarer in pubescent mated pairs than in the general population (Sainte-Marie et al. 1999). The deleterious effects of limb loss on mating success are not necessarily limited to marine arthropods; Brautigam and Persons (2003) showed that male wolf spiders (*Pardosa milvina*) missing four (but not one or two) legs had reduced mating success.

Limb damage and/or loss are common in decapod crustaceans, and the overall frequency of limb damage to horseshoe crabs is at the low end of the studies reviewed by Juanes and Smith (1995). Unlike decapod crustaceans, however, *Limulus* does not lose legs by autotomy. Some limb damage may occur during mating; males grip the female's opisthosoma tenaciously during amplexus, and may lose a clasper rather than release their grip during attempted displacements by satellite males (Brockmann, personal obser-

vation). In juvenile horseshoe crabs, lost limbs are not regenerated until molting has taken place (Itow et al. 1998). However, adult horseshoe crabs seldom, if ever, molt (Botton and Ropes 1988), so the loss of a male's clasper effectively limits him to the satellite mating tactic. In our study, animals with damaged legs were "older," as judged by the degree of carapace variegation, which is not surprising if leg damage accrues with age. Among crabs of both sexes, the fifth walking leg had the highest frequency of damage, which is probably a function of its more complex design and function as a "pusher" leg during burrowing (Yamasaki 1988).

Brockmann (2002) prevented males from reattaching to females by fitting their claspers with plastic bags. She found that good-condition males that were unable to attach were more likely to remain offshore, and poor-condition males with bagged claspers were more likely to come ashore as satellites. Since condition deteriorates with age, this result suggested that males base their mating tactics on an assessment of their overall condition (rather than just on their immediate ability to attach or not). The results also suggest that poor-condition males have higher reproductive success by coming ashore as satellites, whereas good-condition males maximize their reproductive success by remaining offshore and seeking females to attach to. We have shown that males with lateral eye and clasper damage are less likely to be paired with females than males that are in better condition. Nonetheless, even males in poor condition aggregate around the mated pairs at high tide, and satellite males contribute to the fertilization success of the female (Brockmann et al. 1994, 2000). Once females begin the process of nesting, they may be nearly buried in the sand, and under these conditions, it seems unlikely that satellite males could be relying on vision to locate them (Brockmann 1996). Since many satellite males are further affected by eye deterioration or fouling, at least in the Delaware Bay population, this would make it even less likely that visual cues are sufficient to explain their ability to locate mated pairs along the shoreline. Hassler and Brockmann (2001) provided evidence that chemical cues produced by female horseshoe crabs increased the number and duration of satellite male contacts with cement models of females. Chemical (and tactile) cues are sufficient for normal mating behavior to take place in visually compromised lobsters, *Homarus americanus* (Snyder et al. 1992). Chemical cues could be of particular value to horseshoe crabs in locations such as Delaware Bay, where considerable amounts of turbidity and suspended sediment typically limit nearshore water clarity to average Secchi disk depths of <0.5 m (M.L. Botton, unpublished data).

In conclusion, we suggest that males visually locate unpaired females in relatively clear subtidal water. Males that are successful at attaching are more likely to be those in good condition, i.e., with their appendages (especially claspers) intact and with little or no lateral eye deterioration. Subsequently, the high-tide searching behavior of satellite males as they seek to locate mated pairs at the water's edge may rely upon chemical and perhaps tactile cues. The success that satellites have in encountering mated pairs is

facilitated by orientation cues (wave surge, beach slope) that tend to concentrate horseshoe crabs in the vicinity of breeding beaches (Rudloe 1980; Botton and Loveland 1987).

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