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Amplexus and Mating Behavior in the Japanese Horseshoe Crab, *Tachypleus tridentatus*

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ABSTRACT—Amplexus and mating behavior in the horseshoe crab, *Tachypleus tridentatus*, were studied at Imari Bay and Kitsuki Bay, Kyushu, Japan, during Summer, 1994. The pairwise size distribution of mated pairs ($n=28$) showed a lack of size-assortative mating. Long-term amplexus is primarily maintained by the male's pair of posterior claspers, which is significantly larger than the anterior claspers. The posterior claspers always attach directly to the female's opisthosoma, just lateral to the terminal spines, but the anterior claspers attach in various anteriorward positions on the lateral edges of the female's opisthosoma. We conclude that the mating system of *T. tridentatus* is fundamentally similar to the American horseshoe crab (*Limulus polyphemus*), despite the >100 million years of isolation between the two groups. *T. tridentatus* morphologies, however, show more adaptations to long-term amplexus than those of *L. polyphemus*.

INTRODUCTION

Sexual size dimorphism is common among many species of aquatic arthropods. In a number of taxa, male reproductive success is positively correlated with size (Salmon, 1983; Christy, 1987). The greater reproductive success of larger males may occur through a number of processes, such as increased success in male-male interactions (Berrill and Arsenault, 1982; Asakura, 1987) or their ability to maintain higher quality territories (Christy and Salmon, 1984; Atema, 1986).

Horseshoe crabs (Arthropoda: Merostomata) are an ancient group of marine arthropods which has had little change in external morphology since the Cretaceous. The three Indo-Pacific species of horseshoe crabs (*Tachypleus tridentatus*, *T. gigas*, and *T. rotundicauda*) are distantly related to the American species (*Limulus polyphemus*) based on morphology (Fisher, 1984; Yamasaki *et al.*, 1988), biochemical and genetic polymorphisms (Shuster, 1962; Miyazaki *et al.* 1987, 1989; Sugita, 1988), and *in vitro* hybridization studies (Sekiguchi and Sugita, 1980). The divergence between the Asiatic and American lineages probably occurred during the Jurassic (Sekiguchi and Yamasaki, 1988).

The breeding behavior of horseshoe crabs is unique among marine arthropods; they undertake seasonal migrations to intertidal beaches, where spawning with

external fertilization occurs. Spawning activity peaks on the high tides near the full and new moon (Rudloe, 1985; Barlow *et al.*, 1986; Shuster, 1979, 1982; Shuster and Botton, 1985; Sekiguchi, 1988). Pairing (amplexus) occurs when females are in the hard-shelled condition, unlike most other aquatic arthropods. Sexual size dimorphism is characteristic of horseshoe crabs; males are typically about 78% to 80% of the size (prosoma width) of the female (Botton and Loveland, 1992).

Male horseshoe crabs have modified prosomatic appendages (claspers) which grasp tightly onto the opisthosoma of the female during amplexus. The first pair of prosomatic appendages, the chelicerae, is used in feeding; the second pair of prosomatic appendages (first walking legs) serve as claspers in all four species. In male *Tachypleus tridentatus*, *T. gigas*, and *T. rotundicauda*, the third pair of prosomatic appendages is similarly modified, but in *Limulus polyphemus* it resembles the remaining walking legs (Shuster, 1982; Yamasaki *et al.*, 1988).

There have been several recent papers describing the relationship of body size to mating success in *Limulus polyphemus* (Brockmann, 1990; Loveland and Botton, 1992; Botton and Loveland, 1992), but studies of mating behavior in the Asiatic horseshoe crabs have been less intensive. In *L. polyphemus*, mating is random with respect to size; that is, there are no significant size differences between paired and unpaired individuals and no tendency for size-assortative mating to occur (Pomerat, 1933; Cohen and Brockmann, 1983; Brockmann, 1990; Loveland and Botton, 1992; Botton and Loveland, 1992). Males that are handi-

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capped by a heavy encrustation of fouling organisms on the dorsal carapace are less likely to enter amplexus than males with clean shells (Brockmann and Penn, 1992). The width of the female's opisthosoma at the point of clasper attachment, and the dorso-ventral thickness at this site are relatively constant over the entire size range of females in the population, suggesting that even the smallest males have the capability to attach to the opisthosoma of any female (Loveland and Botton, 1992; Botton and Loveland, 1992).

This paper describes amplexus and mating behavior in *Tachypleus tridentatus* from two bays on Kyushu, Japan, and tests whether mating is random with respect to body size. We describe the components of amplexus in *T. tridentatus* based on studies of live animals and morphometric analysis of museum specimens. Comparisons are made between amplexus and mating behavior in *T. tridentatus*, a representative of the three Indo-Pacific species, and *Limulus polyphemus*; behaviors separated by at least 100 million years of isolation between the two groups of horseshoe crabs.

MATERIALS AND METHODS

All available mated pairs of horseshoe crabs were observed and measured during the breeding season in Summer, 1994. Most of the behavioral observations were conducted during daytime high tides at Tatara Beach, a protected horseshoe crab breeding area in Imari Bay (Fig. 1) from 23–25 July and 6–8 August. Comparable numbers of horseshoe crabs were seen during evening high tides, but the darkness and depth of the water precluded accurate observations. As described by Sekiguchi (1988), the beach at Tatara is limited to a 16 m by 28 m sandy area located where a concrete bank and a breakwater meet at a right angle. A second sandy area of comparable size, referred to as Tatara-South, is approximately 0.1 km away. In all, 29 spawning pairs were observed at Tatara and Tatara-South, and 24 of these were measured. After inspection of the size data, we determined that there was a high probability that 4 of these mated pairs had spawned on consecutive days. For that reason, they were only counted once, thereby reducing the sample size to 20. Additional mated pairs were collected along the shores of Koshiki Island in Imari Bay ($n=2$) and Kitsuki Bay ($n=2$), and from aquaria at the Kitsuki City Offices ($n=2$) and Kitsuki Junior High School ($n=2$).

Because of the endangered status of the horseshoe crab throughout Japan, we minimized our handling of mated pairs so as not to disrupt amplexus and egg-laying. We measured the distance between the two lateral (compound) eyes to the nearest mm using a flexible measuring tape without interrupting the egg-laying behavior; we refer to this dimension as the "curved" interocular (I/O) distance. We also recorded the presence of any epifauna on the dorsal carapace, and measured the depth of the water where the mated pair was located.

More detailed morphometric data were obtained from 99 other horseshoe crabs which could be more readily handled: 89 preserved specimens from the University of Tsukuba Institute of Biological Sciences collection at the Shimoda Marine Laboratory, 8 unpaired live animals from Kitsuki, and 2 dead animals found at Kitsuki and Imari. These animals are referred to hereafter as "single" individuals, to differentiate them from the animals found in live mated pairs. Many of the single individuals from the Shimoda group were, however, originally collected as mated pairs (K. Sekiguchi and H. Sugita, unpublished data).

Not all morphometric measurements were made on all single individuals, leading to unequal sample sizes in the results. We measured prosoma (carapace) width, telson length, height, and the straight-line interocular distance ("straight" I/O) to the nearest mm using vernier calipers, and the curved I/O using a measuring tape. For female horseshoe crabs, we measured (to the nearest 0.1 mm) the distance between the terminal opisthosoma spines, and the lateral distances between the anterior set of mating scars and the posterior set, which are depressions worn into the opisthosoma by the respective pairs of the male's claspers. The thickness of the opisthosoma at both sets of mating scars, i.e. anteriormost and posterior, were also measured. For male crabs, we measured the length and width of tibia of both sets of claspers to the nearest 0.1 mm. The volume of a clasper was then estimated by assuming that the shape of this segment approximates a cylinder.

Surface (0–5 cm) sediment samples were collected at the mid-tide region at Tatara, Tatara-South, and Kitsuki, from areas where fresh horseshoe crab eggs were seen. Grain-size analysis was performed by sieving dry sediment samples through a standard sieve series (4 μm , 2 μm , 1 μm , 500 μm , 250 μm , 125 μm , and 62 μm). Graphic mean and sorting coefficient (inclusive graphic standard deviation) were computed according to formulas in Folk (1974). Sediment organic carbon was determined as the loss of weight on ignition at 500°C for 5 hr (Byers *et al.*, 1978).

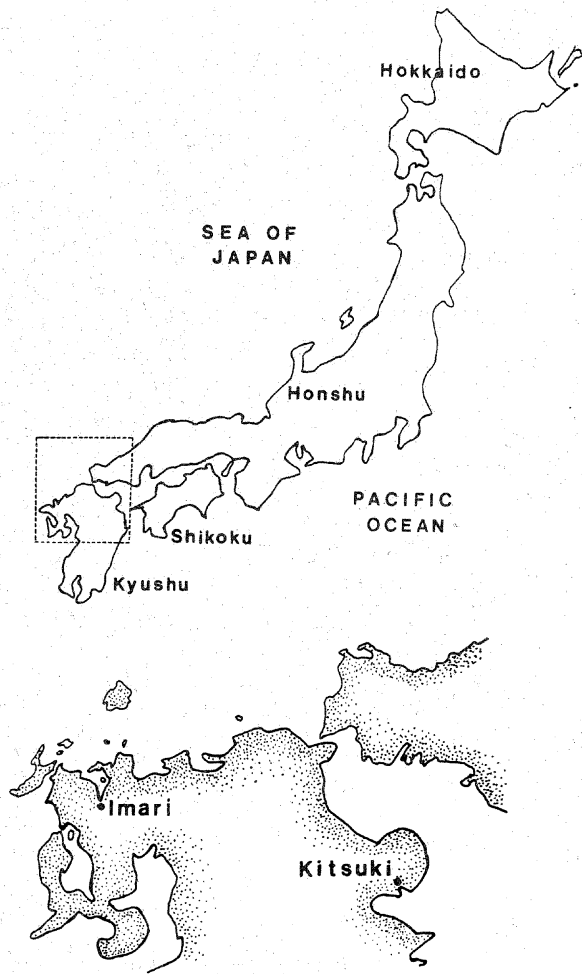
RESULTS

Spawning Habitat and Mating behavior

Tachypleus tridentatus in Imari Bay were observed spawning on sandy areas of Tatara Beach and on sandy patches along Koshiki Island. We also observed evidence of recent spawning at several locations in Kitsuki Bay, particularly along a sand island in the Yasaka River, where fresh egg masses were found buried at sediment depths of approximately 5–10 cm. Beach sediments at Tatara and Tatara-South were characterized as poorly sorted gravelly sand (Table 1); much of the coarse fraction (>2 mm) consisted of molluscan shell fragments. The spawning area in Kitsuki Bay consisted of poorly sorted medium sand. Sediment organic carbon was $<2\%$ at all three beaches.

Only mated pairs of horseshoe crabs were observed; that is, no unpaired "suitor" males or single females were seen in the vicinity of breeding beaches at the time of high tide. Egg laying took place at high tide ± 90 min on both daytime and night tides (Sekiguchi, 1988). Some mated pairs approaching Tatara Beach followed the shoreline along the concrete embankments, until they encountered a suitable area with sandy substrate. As the female began to dig, the location of the pair was revealed by a stream of fine air bubbles ("spawning foam") that was released from the porous sediments (Sekiguchi, 1988).

Egg laying typically took place about 0.5 to 2 m away from the water's edge, at an average water depth of 30 cm (range 12–62 cm, $n=10$). After depositing eggs for ca. 15–20 min, pairs often moved forward a short distance (ca. 20 cm) and the female then re-buried. This behavior was repeated over a 1–1.5 hr period of observation; therefore, each mated pair potentially deposits 3 or more egg clusters on a given high tide. We did not disturb the egg nests, not only because of their low numbers but because the size of



the eggs (3.0 mm diameter) and the numbers of eggs per nest (200–500) are well-known (Sekiguchi and Nakamura, 1979). Receding waters on the ebb tide apparently trigger the return to the sea; we did not observe any overturned or disoriented “stranded” crabs, as often occurs in *Limulus polyphemus* (Botton and Loveland, 1989).

As judged by the lack of fouling organisms and the shiny condition of the dorsal carapace, most of the horseshoe crab mated pairs from Imari Bay and Kitsuki Bay consisted of young adults. A few animals had epifaunal barnacles, oysters, or mussels, but none had the heavily fouled or deeply eroded shells that characterize older adults of *Limulus polyphemus* (Shuster, 1958; Botton and Ropes, 1988).

Morphometrics

Morphometric data based on 54 males and 43 females is presented in Table 2. Curved I/O distances were very highly correlated with straight-line I/O distances in both males ($r^2=0.972$, $P<0.001$, $n=29$) and females ($r^2=0.981$, $P<0.001$, $n=29$) in the sample of “single” *Tachypleus tridentatus* (i.e. preserved, unpaired, or dead animals). Among females, there was a high correlation between both measures of I/O and prosoma width ($r^2=0.753$, $P<0.001$ for straight I/O; $r^2=0.729$, $P<0.001$ for curved I/O) but neither correlation was as high for males (straight I/O: $r^2=0.433$; curved I/O: $r^2=0.354$). These data suggest a much greater “shape” variation in the male prosoma, which is consistent with our observations of the specimens. Some males are highly arched with a relatively narrow prosomal flare, but others are flatter and have a broad flare. Therefore, in considering the most reliable estimate of size for mated

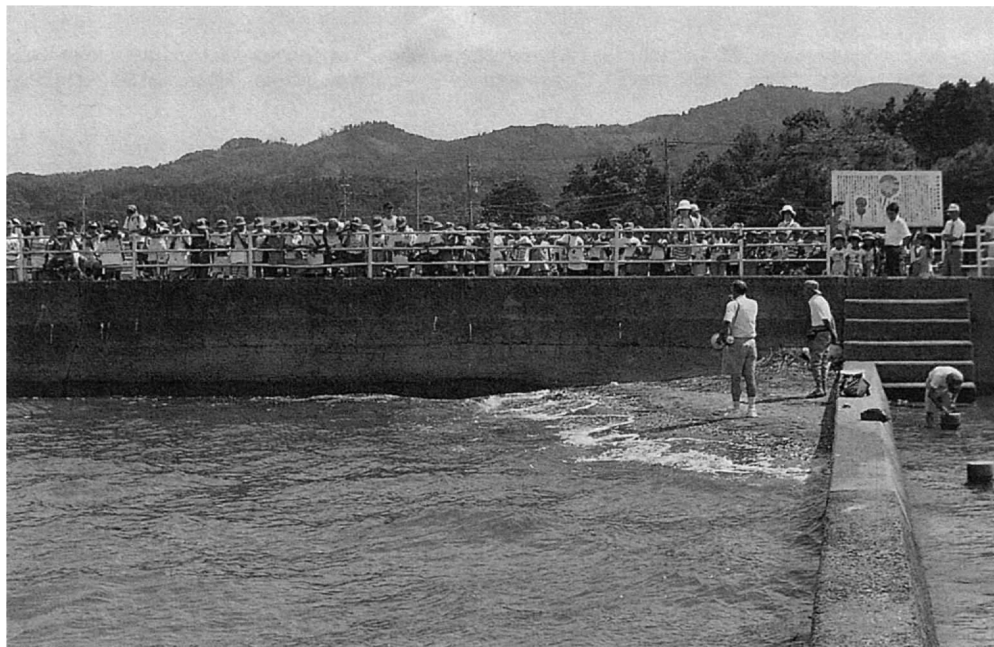


Fig. 1. Study areas. (a) Location of the study sites in Japan. (b) High tide at Tatara Beach, Imari, Japan (July 1994). K. Sekiguchi (left) and G. Yoshinaga describing the significance of the beach and the natural history of horseshoe crabs to school children.

Table 1. Physical characteristics of spawning habitat. % organic carbon is the mean of two duplicate determinations

Location	Water		Sediment Characteristics		
	Temperature (°C)	Salinity (ppt)	Mean Grain Diameter (mm)	Sorting Coeff.	% Organic Carbon
Tatara	29	33	0.54	1.059	1.52
Tatara-South	29	33	0.76	1.360	0.84
Kitsuki	—	—	0.33	1.149	1.95

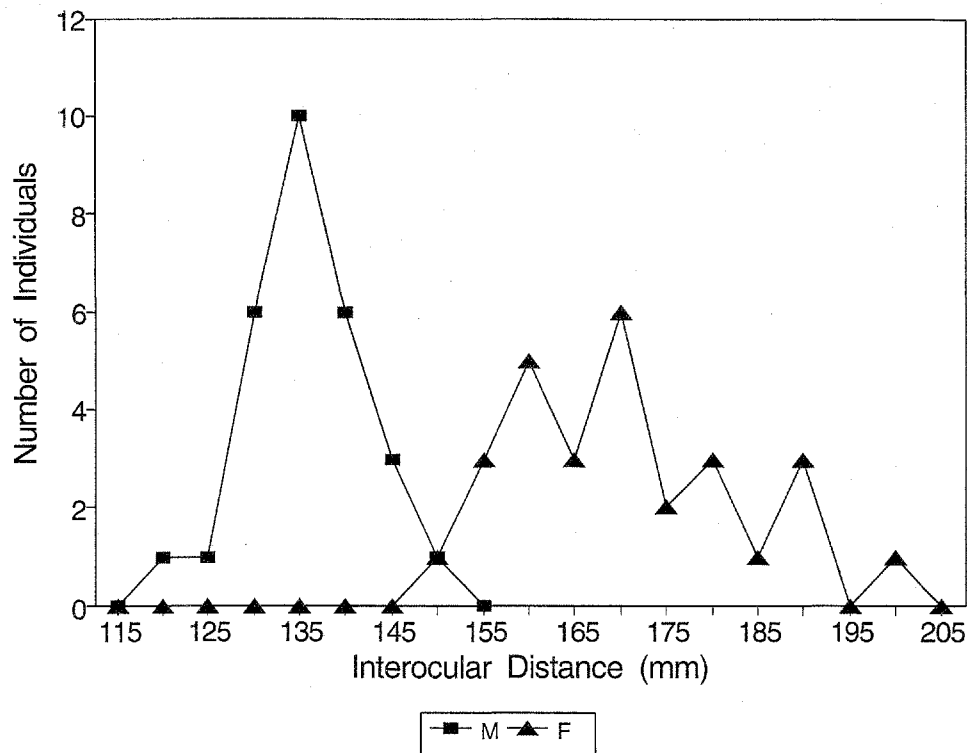


Fig. 2. Size frequency distribution of males (■) and females (▲) from 28 mated pairs of *Tachypleus tridentatus* from Kyushu, summer, 1994. Average ($\pm$ SE) male interocular distance = 138.7 mm $\pm$ 1.15; average ($\pm$ SE) female interocular distance = 168.2 mm $\pm$ 2.37.

pairs, we use I/O distance rather than back-calculated estimates of prosoma width.

The size-frequency distribution of mated pairs (Fig. 2) shows a slight size overlap between males and females. However, in each mated pair, the male was smaller than the female. The pairwise male:female size ratio was 0.829 (SD=0.067), and ranged from 0.651 to 0.953. The pairwise distribution of I/O distances within mated pairs (Fig. 3) indicated that there was no correlation between male and female size ($r^2=0.006$, $P>0.50$). In other words, there was no tendency for size-assortative mating among the 28 mated pairs.

There was no significant difference ($t=1.613$, $P>0.10$, 69 df) between the I/O distances of males from mated pairs ($\bar{x}=138.1$ mm, $n=28$) and "single" males ($\bar{x}=135.5$ mm, $n=43$). Likewise, there was no significant difference in I/O distance ($t=1.582$, $P>0.10$, 80 df) between mated ($\bar{x}=168.2$, $n=28$) and "single" ($\bar{x}=163.8$, $n=54$) females.

Components of amplexus

Adult male *Tachypleus tridentatus* have two pairs of prosomatic claspers used during amplexus. As no functional terminology exists for these appendages, we hereafter refer to them as the anterior and posterior claspers, corresponding to the second and third prosomatic appendages, respectively. The posterior clasper was significantly larger than the anterior clasper (paired t-test, $t=6.34$, $P<0.0001$, 23 df). The size of both claspers was positively correlated with male size (Fig. 4), although the rate of increase (slope of the line) was slight. The two pairs of male claspers attach to the female opisthosoma at the anterior and posterior points of attachment, where well-worn mating scars are discernable in some females which have previously mated. We carefully examined the positioning of the male claspers on several females after they had concluded their egg-laying at Tatara Beach, and we inferred the points of attachment on preserved specimens based on

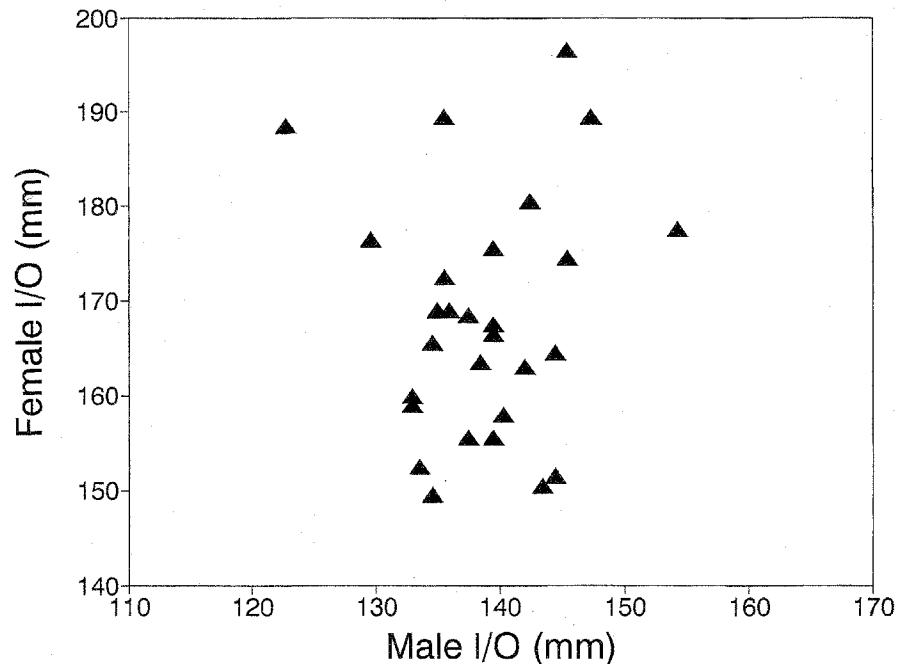


Fig. 3. Scatterplot of interocular distance of amplexed female versus amplexing male, showing the lack of a significant relationship between male and female size in mated pairs of *Tachypleus tridentatus* ($r=0.078$, $P>0.50$, $df=26$).

the mating scars (Fig. 5). In all cases, the posterior male claspers affix to the female's opisthosoma, just lateral to the terminal projections referred to as the terminal spines. In contrast, the point of attachment of the anterior claspers appeared to be more variable. In the Shimoda specimens, we noted mating scars on the marginal spines of the opisthosoma in 80% of the females; 20% had mating scars on one or more of the moveable spines. When mated pairs were disturbed, the male readily released his grip with the anterior pair of claspers but retained a firm grip with the posterior claspers. In one mated pair we examined, the left and right anterior claspers were attached asymmetrically to the female.

The anterior point of attachment showed greater statistical variability than the posterior point of attachment (Table 2). The distance between the posterior mating scars was highly correlated with female size (I/O) ($r^2=0.655$, $P<0.001$, 23 df) and with the terminal spine distance ($r^2=0.834$, $P<0.001$, 23 df). However, there was no significant relationship between the anterior mating scar distance and female I/O ($r^2=0.275$, $P>0.20$, 19 df) and a marginally significant relationship with terminal spine distance ($r^2=0.470$, $P<0.05$, 19 df).

DISCUSSION

Sexual dimorphism in horseshoe crabs is apparent from the smaller size of the males, and from secondary modifications. The size difference is demonstrated even in the juveniles of *Limulus polyphemus*, where the growth increments of the males at each moult are less than that of the females of the same instar (Shuster, 1958, 1982). When

males moult and attain sexual maturity, they develop the modified claspers and curved arch of the frontal portion of the prosoma. The latter condition permits a close fit, during amplexus, between the ventral surface of the male prosoma and the dorsum of the female opisthosoma. Sexual dimorphism in *Tachypleus tridentatus* also involves the posterior three pairs of movable spines on the opisthosoma. In adult females, these are much shortened, in comparison to the condition in juvenile females or in males (see Fig. V-8 in Yamasaki *et al.* [1988]); this modification facilitates attachment by a male.

The two pairs of male claspers function in a complementary fashion during amplexus. The larger posterior claspers always attach near the terminal spines of the opisthosoma and provide a very firm grip. On the other hand, the anterior claspers are somewhat smaller and their point of attachment is more variable. They sometimes attach to the marginal spines of the opisthosoma but they may also attach to one of the moveable spines. We believe the posterior claspers are used to maintain long-term coupling, whereas the anterior claspers probably function to maintain the side-to-side stability that helps resist displacements generated by waves, currents, or other factors. The anterior claspers may be the first to attach and thus allow the male to more accurately position the second set of claspers.

Differences in the morphometry of *Tachypleus tridentatus* and *Limulus polyphemus* are reflected in the mated positions in the two species. Specifically, the unique highly arched prosoma of *T. tridentatus*, along with the second pair of claspers, account for a male *T. tridentatus* riding further forward, hence higher, on the female's opisthosoma than occurs in *L. polyphemus*. This positioning

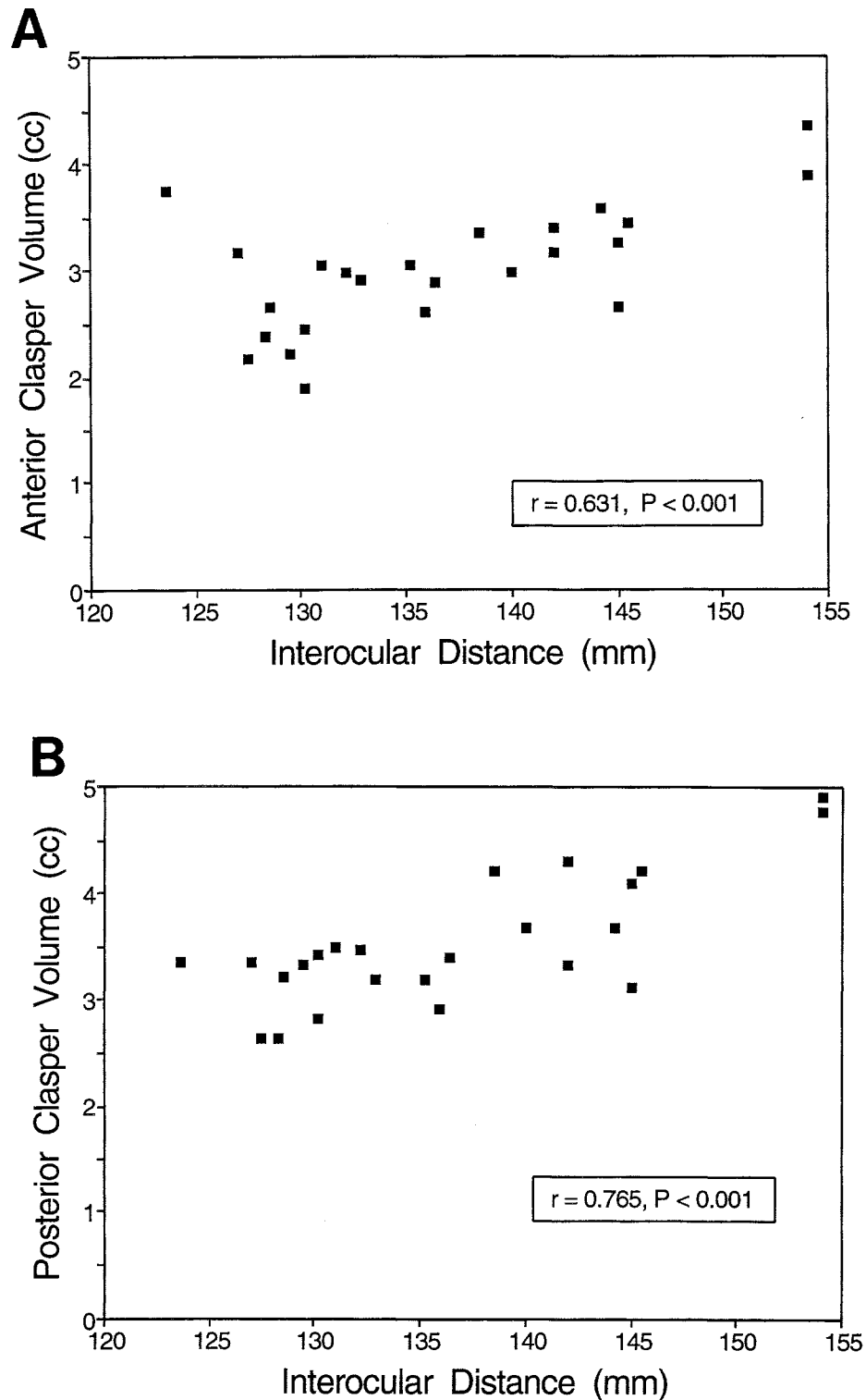


Fig. 4. Relationship of anterior (A) and posterior (B) clasper volume to interocular distance in male *Tachypleus tridentatus* (n=24).

increases the area on which the four claspers can grab. The two species differ in where they lay their eggs; *T. tridentatus* generally spawns while submerged, but *L. polyphemus* pairs are often found at the water's edge and the males are not dislodged by waves up to 30 cm in amplitude (pers. obs.).

We speculate that there may be a relationship between the relative positions of the male prosoma and the location of spawning, such that the higher profile of a *T. tridentatus* mated pair might cause it to be more easily swept off a beach by wave action than *L. polyphemus* under the same

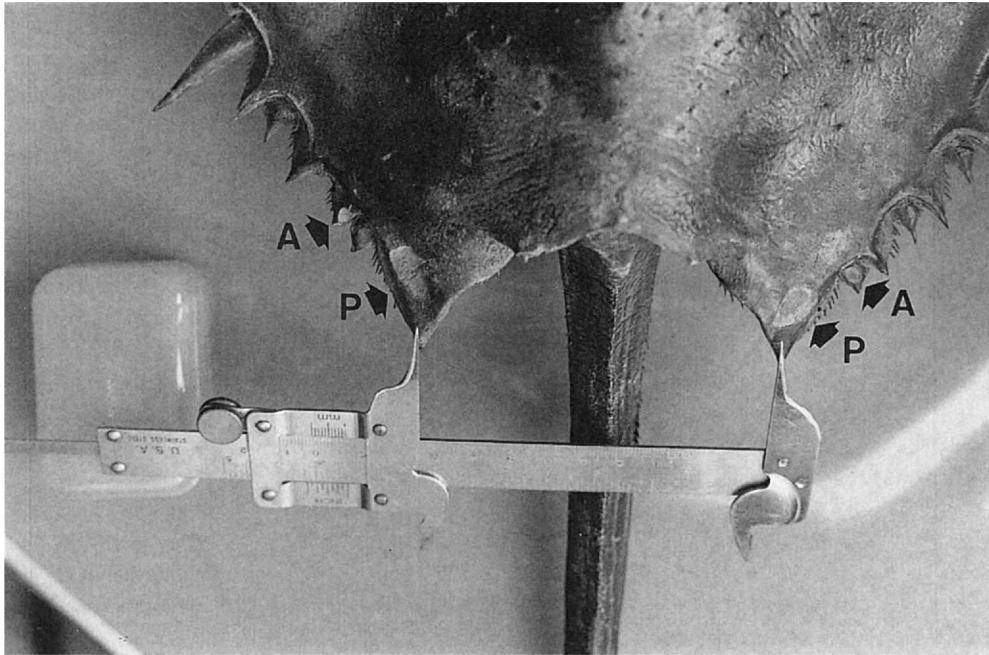


Fig. 5. Photograph of the opisthosoma of a female *Tachypleus tridentatus* showing the position of the anterior (A) and posterior (P) mating scars. Note that the posterior mating scars, caused by the larger pair of male claspers, clearly show on the terminal spines. The smaller anterior mating scars are located on the left fifth marginal spine and the right fifth moveable spine. Calipers show the terminal spines measurement.

conditions.

The relative scarcity of *Tachypleus tridentatus* in Japan may have affected our perception of the mating system. The largest population of horseshoe crabs anywhere in the world occurs in Delaware Bay, USA (Shuster and Botton, 1985), where it is common to observe >100 *Limulus polyphemus* spawning per 10 meters of beach on a single high tide during the peak of the breeding season. In contrast, *T. tridentatus* is an endangered species in the waters of Japan,

where it has suffered a dramatic decline in the past 15 years (Itow, 1993). While the hope exists that the status of the horseshoe crab in Japan can be reversed, we were fortunate to be able to study live, spawning animals. Some aspects of mating behavior can be determined from museum specimens, but that alone can not substitute for live animals in their natural habitat.

Table 3 summarizes some of the principal features of the mating systems of *Tachypleus tridentatus* and *Limulus*

Table 2. Morphometric data for *Tachypleus tridentatus*, based on preserved specimens from Shimoda, and unpaired live and dead animals from Kitsuki and Imari. I/O = interocular distance; CV = coefficient of variation; Term Spines = distance between the tips of the terminal, non-moveable projections of the female opisthosoma

Sex		Prosoma Width	I/O	Prosoma Height	Telson Length	Term Spines	Width of Mating Scar		Thickness of Mating Scar		Volume of Clasper (cc)	
							Ant	Post	Ant	Post	Ant	Post
F	Mean	271.0	163.8	106.1	292.9	62.4	90.1	67.0	2.8	4.7		
	CV	0.07	0.07	0.08	0.16	0.08	0.09	0.06	0.24	0.15		
	Min	222	124	80	209	49	74.1	60.2	1.6	3		
	Max	312	183	119	372	71.5	103.7	74.8	4.2	6.1		
	n	54	54	52	23	30	21	25	21	25		
M	Mean	242.5	135.5	87.6	253.6	61.1					3.01	3.53
	CV	0.07	0.07	0.08	0.15	0.06					0.19	0.17
	Min	201	111	69	174	53.5					1.89	2.65
	Max	273	154	102	311	71					4.35	4.91
	n	43	43	43	26	26					24	24

Table 3. Comparison of the mating systems of *Tachypleus tridentatus* and *Limulus polyphemus*, based on this study and the literature

Character	<i>T. tridentatus</i>	<i>L. polyphemus</i>	Reference
Male:female size ratio	0.83	0.78–0.80	Botton and Loveland (1992)
Mean prosoma width (mm)			
Males	242.5	200.7 ^a	Loveland and Botton (1992)
Females	271.0	258.1 ^a	
Operational sex ratio	1:1	usually male biased during peak spawning periods	Brockmann (1990) Loveland and Botton (1992)
Size assortative mating	no	no	Cohen and Brockmann (1983) Botton and Loveland (1992)
Preferred nesting sites	sandy beaches	sandy beaches	Botton <i>et al.</i> (1988)
Preferred mating times	new moon and full moon high tides	new moon and full moon high tides	Barlow <i>et al.</i> (1986) Sekiguchi (1988)
Nesting depth in water	30 cm	water's edge to 20 cm	pers. obs.
No. pairs of male claspers	2	1	Shuster (1982) Yamasaki <i>et al.</i> (1988)
Clasper morphology	didactylid	monodactylid	Shuster (1982) Yamasaki <i>et al.</i> (1988)
Attachment sites on female opisthosoma	terminal and moveable spines	terminal spines only	Shuster (1982)
Diameter of eggs	3.0–3.3 mm	1.6–1.8 mm	Shuster (1982)
No. eggs per clutch	200–500	3,650	Shuster (1982) Shuster and Botton (1985)
Eggs per female	20,000	88,000	Shuster (1982) Shuster and Botton (1985)

^a From mated pairs collected at Delaware Bay, New Jersey

polyphemus. The lack of size-assortative mating (Fig. 3) is evidence that mating in the Japanese horseshoe crab is random with respect to body size, as is true of *L. polyphemus* (Loveland and Botton, 1992; Botton and Loveland, 1992). The similar body sizes of mated and single *T. tridentatus* suggests that our sample of mated pairs was representative of the larger population. *T. tridentatus* and *L. polyphemus* both prefer to spawn on well-oxygenated sandy beaches with low organic carbon content; it is likely that these environments provide the best conditions for the development of the eggs (Botton *et al.*, 1988). Both the American and Japanese species, therefore, have the same basic features of the mating system in common - amplexus, random pairing with regard to size, a preference for sandy intertidal substrate, and lunar periodicity of spawning - despite the more than 100 million years of isolation between the lineages. Despite the larger size of *T. tridentatus*, the male:female size ratio is strikingly similar to *L. polyphemus* (Table 3). Why this size ratio has been favored by natural selection is unclear; perhaps it confers a hydrodynamic advantage relative to a more evenly size-matched mated pair, or it may optimize sperm transfer during external fertilization.

The most striking difference in mating behavior between species may be the prevalence, in *Limulus polyphemus*, of a male-biased operational sex ratio (the ratio of fertilizable females to available males) (Emlen and Oring, 1977) during the height of the spawning season. This imbalance, with

unpaired "suitor" males clustering around mated pairs during spawning, occurs because males tend to approach the beach more frequently than females, which leave the beach after having spawned (Brockmann, 1990; Brockmann and Penn, 1992; Loveland and Botton, 1992). Suitor males vigorously attempt to displace the amplexed male, and although they are usually unsuccessful (Cohen and Brockmann, 1983; Loveland and Botton, 1992), suitor sperm do fertilize a percentage of the eggs (Brockmann, 1990). In contrast, the sex ratio for *T. tridentatus* in Kyushu was 1:1, and no suitor males were seen. On days when there are few *L. polyphemus* females nesting, as in the beginning and end of the spawning season, or during neap tides, the sex ratio may be much closer to 1:1. It is possible that the 1:1 ratio in *T. tridentatus* is a consequence of the low population density and that similar suitor male behaviors would be seen if the population density were greater. We observed the interactions of a suitor male with a mated pair of *T. gigas* in the aquarium at Shimoda, and the behavior of this animal was identical to *L. polyphemus* suitors. Although anecdotal, we believe these observations suggest that suitor male behavior would probably be seen in any horseshoe crab population in which the operational sex ratio exceeds 1 male per female. On the other hand, amplexus may be a means of assuring the female with a source of sperm in the event that suitor males are not present at the time of spawning.

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