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Mating behaviour in the penaeid shrimp *Penaeus vannamei*

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Abstract

Mating behaviour of *Penaeus vannamei* was observed during January, 1986. Mating behaviour was divided into four phases: (1) approach, (2) crawling, (3) chasing, and (4) mating. Male mating and spermatophore transfer to the mature female take place at intermolt stage C_4. The glutinous spermatophore emitted from the male can be transferred onto the female open thelycum during the ventral-to-ventral position in mating in *P. vannamei*. Though male *P. vannamei* often chase males or immature females with undeveloped ovaries, no males were ever mated and mating only occurred in females with ripe ovaries.

Introduction

Spermatophore transfer is the essential final step of a series of events comprising mating behaviour in decapod crustaceans. Many workers have described the events occurring during mating and the mechanism of spermatophore transfer in a variety of shrimps and prawns (Kamiguchi 1972, Bauer 1976, Berg and Sandifer 1984). However, little information is available on mating behaviour in the penaeid shrimp. Mating behaviour has been described for the closed thelycum species: *Penaeus japonicus*, *P. monodon* and *P. paulensis* (Hudinaga 1942, Primavera 1979, De Saint-Brisson 1985), where mating takes place before ovarian maturation. Little is known about the mating behaviour of open thelycum species like *P. vannamei*, in which mating takes place after ovarian maturation (Aquacop 1977). Descriptions of mating behaviour in *P. vannamei* have not been possible because a mature female is essential to observe mating behaviour and *P. vannamei* is difficult to mature in captivity. Fortunately, *P. vannamei* can be fully matured in captivity at the Oceanic Institute, USA, and a study of mating behaviour in *P. vannamei*, an open thelycum penaeid shrimp, was carried out.

Materials and methods

Thirty-six female *Penaeus vannamei* (40 to 60 g in body weight) with undeveloped (20 individuals) and ripe ovaries (16 individuals) were used for observation. Females were housed with 36 mature males (40 to 55 g in body weight) in a 15 m^3 tank (water level 70 cm) with flowing (600 liters h^{-1}) filtered sea water. Identification of sex of experimental shrimps during the present observation was achieved by painting them with colored enamel. Water temperature was 26° to 27 °C and the photoperiod was 14 h light:10 h dark (2.0 to 8.5 $\mu E\ m^{-2}\ s^{-1}$). The fluorescent light was switched on at 00.00 hrs and off at 14.00 hrs. Salinity ranged from 33.4 to 35.2‰ S. The shrimps were fed pelleted diets, frozen squid and bloodworm twice daily at 08.00 and 16.00 hrs. Mating behaviour was observed twice between 09.00 to 14.00 hrs during January, 1986. Numbers of chasing, distances chased, and mating were counted during the observation of mating behaviour. After observation of mating behaviour, achievement of spermatophore transfer (presence of spermatophore in the thelycum) was checked in all females at 15.00 hrs. To investigate the relationship between mating and molting stages of females, small integument pieces were removed from the uropods of two females that had received spermatophore transfer and fixed in Bouin's solution. Samples were dehydrated in ethanol, embedded in a paraffin-celloidin mixture, sectioned (6 μm), and stained in Delafield hematoxylin plus eosin. Molt stage was determined by the degree of development of sampled integument (Drach 1939, 1944, Stevenson 1985).

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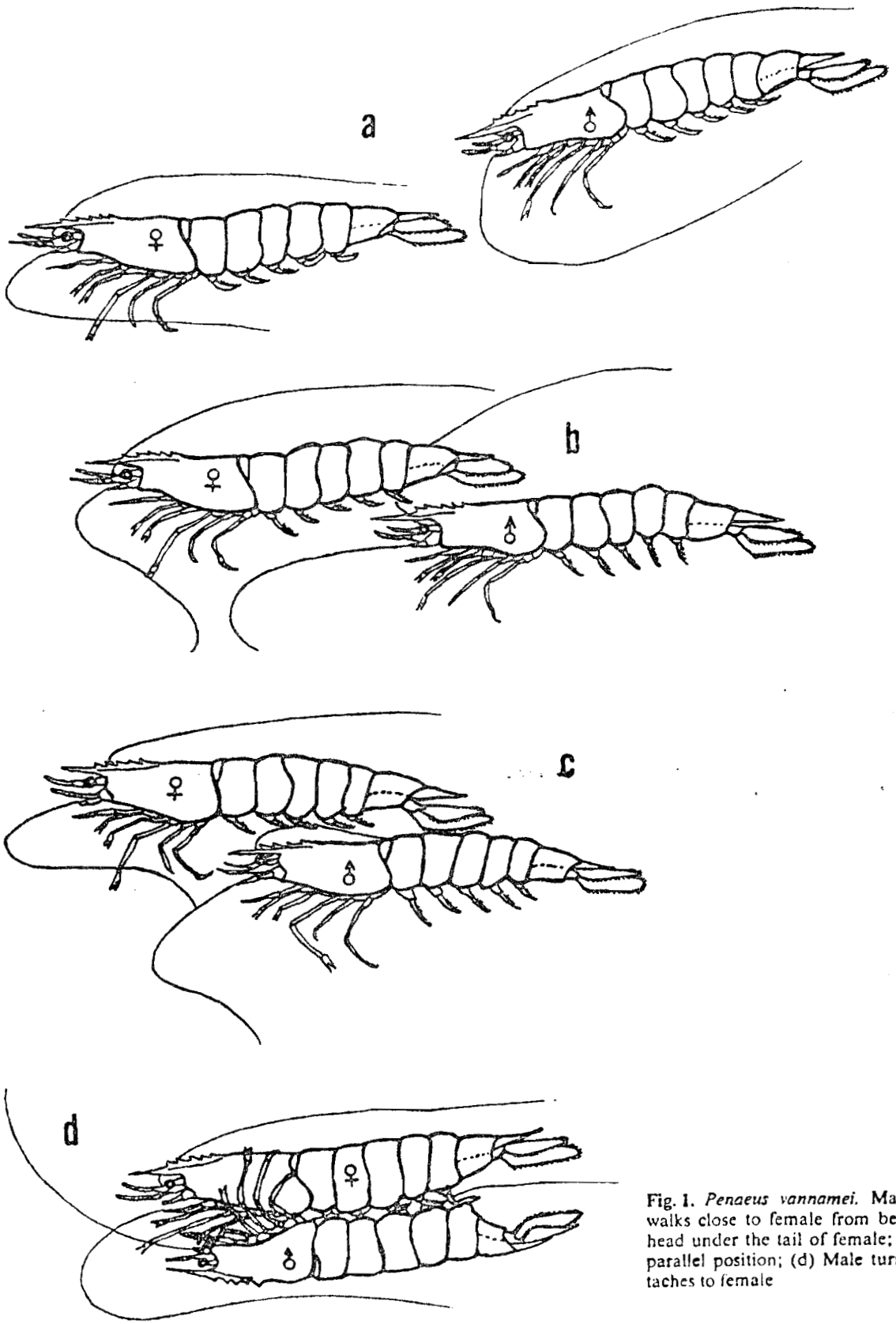

Fig. 1. *Penaeus vannamei.* Mating behaviour. (a) Male walks close to female from behind; (b) Male crawls his head under the tail of female; (c) Male chases female in parallel position; (d) Male turns ventral side up and attaches to female

Table 1. *Penaeus vannamei.* Frequency of chasing, mating and spermatophore transfer in mature males ($n = 36$), and immature ($n = 20$) and mature ($n = 16$) females during a 10-h period of observation

	No. of chasings	No. of matings	Mating/chasing (%)	No. of females with spermatophore	Spermatophore transfer/mating (%)
Male/immature female	12	0	0.0	0	0
Male/mature female	33	10	30.3	2	20
Male/male	76	0	0.0	0	0

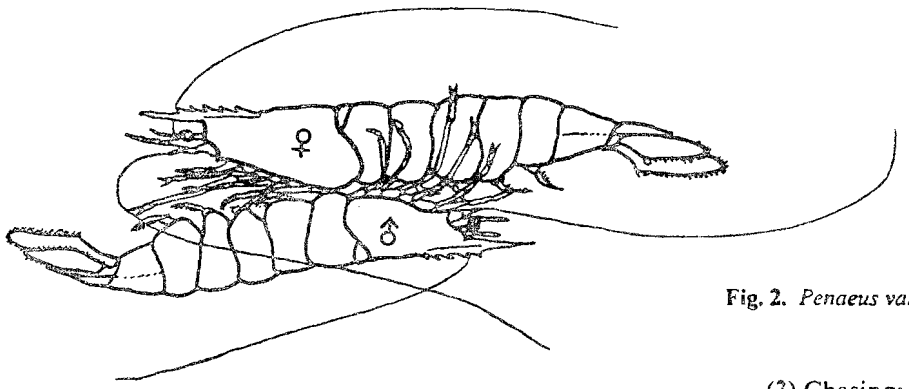

Fig. 2. *Penaeus vannamei.* An inverted position in mating

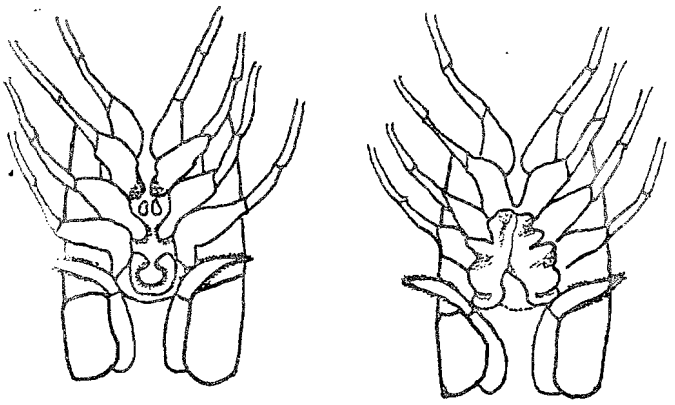

Fig. 3. *Penaeus vannamei.* Female thelycum (left) and spermatophore attached to the female thelycum (right)

Results

Mating behaviour can be divided into four phases, taking place within 3 to 16 s:

(1) Approach: The male repeatedly walks close to females from behind (Fig. 1a). At this time, the male walks actively on the bottom of the tank. There is no apparent sign of recognition or display behaviour of any kind by the male.

(2) Crawling: After approaching the female from behind, the male crawls his head under the tail of the female (Fig. 1b). This advance often results in the female moving away from the male. Apparently females are not always passive and some resist the male's approach.

(3) Chasing: Simultaneous with the male's crawling under the female's tail, the female begins to swim quickly upwards. The female swims in a slightly curved line along the wall of the tank or straight across the center of the tank up to 2 to 3 m in distance. At this point, the male chases the female from below and in a parallel swimming position (Fig. 1c), following her every change in direction. The swimming male remains between 10 and 30 cm above the bottom, but the female may move near the water surface during the chasing. A single female may be chased by 2 to 3 males at once. Females with both undeveloped or ripe ovaries are receptive to chasing, but ripe females are more often chased. Table 1 lists the frequency of male chasings and mating with mature and immature females.

(4) Mating: After chasing a female, the male turns ventral side up and grasps her (Fig. 1d). The ventral-to-ventral position is maintained for 1 to 2 s. Usually a face-to-face position is assumed but occasionally an inverted position is observed (Fig. 2). If spermatophore transfer is not achieved, the male immediately returns to the former upright position, still trying to swim parallel to the female. Then the male again turns ventral side up and attaches himself to the female. This male mating behaviour can be repeated 2 to 3 times with the same female. Usually the male repeats chasing and mating other females immediately after mating. Though males chase females with undeveloped ovaries, only females with ripe ovaries are receptive to male mating (Table 1). The integument of mating males is hard. Spermatophore transfer was only found in ripe females. Judging from the numbers of females mated and receiving spermatophore, frequency of successful spermatophore transfer is low (20%) as shown in Table 1.

Usually, after mating, spermatophore was found attached to the female thelycum (Fig. 3). Spawning was observed in ripe females with spermatophore within 2 h after mating in *Penaeus vannamei.* Fresh spermatophores at-

tached to ripe females are very sticky in seawater. In fact, glutinous spermatophore removed from the terminal ampullae of males can be easily attached to the female thelycum. The attached spermatophore comes off the thelycum shortly after spawning.

Male chasing males

Males often approach other males and crawl under their tails, and then chase. The frequency of males chasing males is high compared to that of females, but no male mating with males was observed (Table 1).

Molt stage of mated female

The integument of females that had received spermatophore transfer consisted of epicuticle, exocuticle and endocuticle. Each cuticle was complete and the epidermis was shrunken. Newly formed cuticle was not found to occur under the endocuticle in the integument sampled ($n = 2$). These results indicate that spermatophore transfer occurs when ripe females are at intermolt stage C_4.

We observed mating in *Penaeus vannamei* under fluorescent illumination (2.0 to 8.5 μE m^{-2} s^{-1}). These results indicate that mating is found to occur even under illumination in penaeid shrimp. On the other hand, mating was most active just before the lights were turned off (13.00 to 14.00 hrs). After the lights had been switched off, active mating was also observed.

Discussion

Penaeus vannamei mating behaviour can be divided into four phases: (1) approach, (2) crawling, (3) chasing and (4) mating. In *P. japonicus, P. monodon* and *P. paulensis,* the male swims parallel and below the female and turns ventral side up and attaches himself to the female (Hudinaga 1942, Primavera 1979, De Saint-Brisson 1985). These processes are the same as observed in *P. vannamei,* and therefore, appear to be a common pattern for mating behaviour for penaeid shrimp. Several workers have described that the male flicks his head and tail and rotates 90 degrees in relation to the female, assuming a "hooked" position during mating in *P. monodon* and *P. paulensis.* In *P. vannamei,* however, this complex position was never observed during mating. In comparison with *P. monodon* and *P. paulensis* which have closed thelycum in which the spermatophore is inserted, *P. vannamei* has an open thelycum to which the spermatophore is attached. In *P. vannamei,* the glutinous spermatophore, which can be removed from the terminal ampullae of males, can be easily attached to the female thelycum. This indicates that the glutinous spermatophore emitted from the male can be transferred onto the female's open thelycum during the ventral-to-ventral position in mating.

It is well known that mating in captivity in penaeid shrimp is affected by environmental factors such as water temperature, depth, light intensity and photoperiod, and by biological factors such as aerial density and sex ratio. In addition, many authors have deduced the presence of sex pheromones which attract a mate and initiate the appropriate courtship and mating behaviour in decapod crustaceans (for review, Dunham 1978). Though male *Penaeus vannamei* often chase males or immature females with undeveloped ovaries, no males were ever mated and mating only occurred with females with ripe ovaries. These results show that male *P. vannamei* only mate with mature females. At this time, males apparently recognize mature females by sex attractants from the mature female shrimp in *P. vannamei.* Water from a tank containing mature females is effective in inducing searching and chasing behaviour in males even in the absence of female *P. vannamei* (Yano et al., unpublished data). Antennal contact by a male with a receptive female appears important in recognizing sexual receptivity in shrimp and lobster (Burkenroad 1947, Atema et al. 1979, Berg and Sandifer 1984). However, before chasing and mating, there was no significant sign of recognition and display behaviour of any kind by male or female *P. vannamei.* Males often approach males and crawl, and then chase. Frequency of chasing males is higher than chasing females (Table 1). These observation indicate that chasing takes place without recognition of sexes in *P. vannamei.* Based on our observations, we deduce the presence of two different sex attractants which may be produced and released by mature females for signalling sexual receptivity, and chasing and mating behaviour in the penaeid shrimp, namely (1) chasing-stimulating pheromone (CSP), and (2) mating-stimulating pheromone (MSP).

Mating generally takes place during the night-time in decapod crustaceans (Berry 1970, McKoy 1979). In *Penaeus vannamei,* mating was most active just before and after darkness (sunset).

Usually after mating, the transferred spermatophore is found attached to the female thelycum in *Penaeus vannamei.* The spermatophore is sometimes, however, transferred onto places other than the thelycum and when this occurs, fertilization was found not to occur in the eggs spawned after mating (Yano et al., unpublished data). This suggests that sperm may not be released from the spermatophore unless attached to the thelycum in *P. vannamei.* These results imply that there is a close relationship between the thelycum and sperm-release from an attached spermatophore in female *P. vannamei.*

In a closed thelycum penaeid shrimp, males mate with newly molted females. The present results show that male *Penaeus vannamei* mate with ripe females that are intermolt stage C_4. This indicates that mating and spermatophore transfer take place in ripe females which have fully matured shortly after molting in *P. vannamei.*

In penaeid shrimp with a closed thelycum, mating and spermatophore transfer take place before ovarian maturation and mating does not accelerate ovarian maturation directly (Yano 1987). In open thelycum shrimp, such as

Penaeus vannamei, mating and spermatophore transfer closely follow ovarian maturation and take place immediately before spawning. This suggests that mating and spermatophore transfer may play a role in final maturation of oocytes and spawning in open thelycum penaeid shrimp.

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