

## Mating Behaviour of Sand Crab, *Portunus pelagicus* (Linnaeus)

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### Abstract

The mating behavior of *P. pelagicus* divided into five phases as Pre-Moult Guarding, Moulting, Pre-Copulatory guarding, Mating, Post-Copulatory guarding and Spawning. A hard shelled male stocked with a hard shelled female in experimental setup I. The Pre-moult guarding lasted for  $92.66 \pm 1.74$  hrs. Moulting lasted for  $4 \pm 0.5$  hrs. The Pre-copulatory guarding lasted for  $3.66 \pm 0.33$  hrs. Copulation lasted for  $7.33 \pm 0.66$  hrs. Post-copulatory guarding lasted for  $12.83 \pm 0.60$  hrs. Total mating sequence lasted for  $120.48 \pm 0.76$  hrs. For spawning the female took  $17.66 \pm 1.45$  days. A hard shelled male stocked with a newly moulted female in experimental setup II. The Pre-copulatory guarding lasted for  $6.33 \pm 1.76$  hrs. Copulation last for  $6.33 \pm 1.01$  hrs. Post-copulatory guarding lasted for  $13.33 \pm 0.54$  hrs. Total mating sequence lasted for  $25.99 \pm 1.10$  hrs. For spawning the female took  $17 \pm 1.52$  days. A hard shelled male was stocked with an eyestalk ablated female in experimental setup III. The Pre-moult guarding lasted for  $62.66 \pm 7.42$  hrs. Moulting last for  $3.83 \pm 0.44$  hrs. The Pre-copulatory guarding lasted for  $3.5 \pm 0.28$  hrs. Copulation lasted for  $6.66 \pm 0.88$  hrs. Post-copulatory guarding lasted for  $13.16 \pm 0.60$  hrs. Total mating sequence lasted for  $89.81 \pm 1.92$  hrs. For spawning the female took  $17.33 \pm 1.45$  days.

**Keywords:** Mate poaching tactics; Documentary style; Charm; Crustaceans

### Introduction

The crab fishery in India is fast developing and there is a vast scope for crab meat in both national and international markets [1]. Crabs rank third after shrimps and lobsters for their esteemed seafood delicacy and also the value of fishery they support [2]. In recent times *P. pelagicus* has been fished in large quantities from all the seas in India and finally sold as a processed food [1]. So the demands of these crabs are increasing day by day. Hatchery technology and farming activities are available for bigger sized crabs (*S. serrata*, *S. tranquebarica*) but economically viable mass seed production technology is lacking for swimming crabs in general [3] and *P. pelagicus* in particular [3]. Berried females are important for starting a commercial hatchery. The berried females are not available throughout the year from the natural populations. So the production of berried females in a controlled condition is essential. Hence in the present study mating behaviour of the blue swimming crab *P. pelagicus* was studied under controlled condition in the laboratory with three experimental setups to know which experimental setup is highly suitable for the production of berried females in controlled condition.

### Materials and Methods

The crabs for the present study were collected from the Parangipettai (Lat.  $11^{\circ} 29'N$  and Long.  $79^{\circ} 46'E$ ) landing centre. The live hard shelled male crabs of 105 - 110 mm carapace width and both hard and soft shelled females of 98 - 104 mm carapace width were selected and kept together in a 20 liters plastic container with seawater and brought to the laboratory. Crabs were immediately acclimatized in sterilized and filtered seawater (salinity 30-32 ppt; temperature 26-33°C; pH - 7.6 to 8.3 and dissolved oxygen close to saturation 5-6 mg L<sup>-1</sup>). Unilateral eyestalk ablation was performed in one set of the female crabs by cutting the right eye at its base with a fine and sterilized scissors and the wound was cauterized immediately with a hot blunt needle in order to prevent the loss of haemolymph [4]. The mating behavior of three different combinations of similar-sized pairs was closely observed.

I. Hard shelled male with hardshelled female

II. Hard shelled male with newly moulted female

III. Hard shelled male with eyestalk ablated female

Three replicates of each experimental setup were maintained. Initially the water level was maintained at 20 cm depth but the male struggled to carry the female and eventually turn her over for mating so the depth was increased to 35 cm. So that the male could easily lift the female and mate with her. Optimum environmental parameters were maintained during the experimental period (salinity 30-32 ppt; temperature 26-33°C; pH 7.5 - 8.5 and dissolved oxygen close to saturation 5-6 mg L<sup>-1</sup>). During experimental periods the crabs were fed twice a day with mixture of clam, squid and prawns at 10% of the body weight (daily morning and evening). The water quality was maintained by exchanging 50% of the water daily. The uneaten and excretory wastes from the tanks were removed during water exchange.

### Observation of Mating Behavior

For convenient, the mating behavior of *P. pelagicus* divided into five phases as follows:

#### Pre-Moult Guarding (PMG)

The hard shelled male crab introduced into the experimental tank along with female crabs at the verge of premoult stage. The female attract the hard shell male. The male crab contact the female by move towards her and extending his bigger chelate in front of her without showing any other attracting courtship display as described in other brachyuran crabs.

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The male after making contact with the female ultimately moved towards her and catch the female with the use of his chelae and embraces in a short while using his walking legs hold her beneath him to form a position called cradle-carrying position. During this position both animals are facing in the same direction. In general the male is active and aggressive but the female is defunct. The male crabs are generally in attacking mood but the female is not so when they were disturbed. Sometimes the pair is broken when disturbed but again reunited immediately in the same position. Both animals were eating during this period and movement also noticed (Figure 1a).

### Moulting (M)

At the time of female's moulting, the pair was separated from the cradle carrying position and subsequently the female start to moult. During this crucial juncture the male crab moves around the female crab and helps her to moult by removing the shell and also protect her from others still she attain normal hardness of the exoskeleton (Figure 1b and 1c).

### Pre-Copulatory Guarding (PCG)

After moulting the male and female crabs were form the cradle carrying position as earlier for sometimes until the starting of mating process (Figure 1d).

### Copulation (C)

Mating was initiated when the female's exoskeleton is soft. The male crab became very active and rotates the female by using his waking legs and by using chelate he turned over her. The assistance rendered by the male crab, she positioned herself upside down beneath him and extends her abdomen exposing gonophores allowing the male to insert his paired gonopods into her genital pores. By this time male and female crabs are facing in opposite direction. The female is in reverse position, her lower side is directed towards the males ventral side and the abdomen of both are flung backwards. During copulation the male often walked around with the female attached to its ventral surface, holding her with third and fourth walking legs (Figure 1e and 1f).

### Post-Copulatory Guarding (POCG)

After the completion of copulation the male crabs liberate the female from mating position and embraces in a short while to form the cradle carrying position continued for only few hrs. The female is inactive until she attains normal hardness of her exoskeleton (Figure 1g).

### Spawning (S)

The male crabs deposited spermatophores in the female's spermatheca during mating was stored until the female is ready for

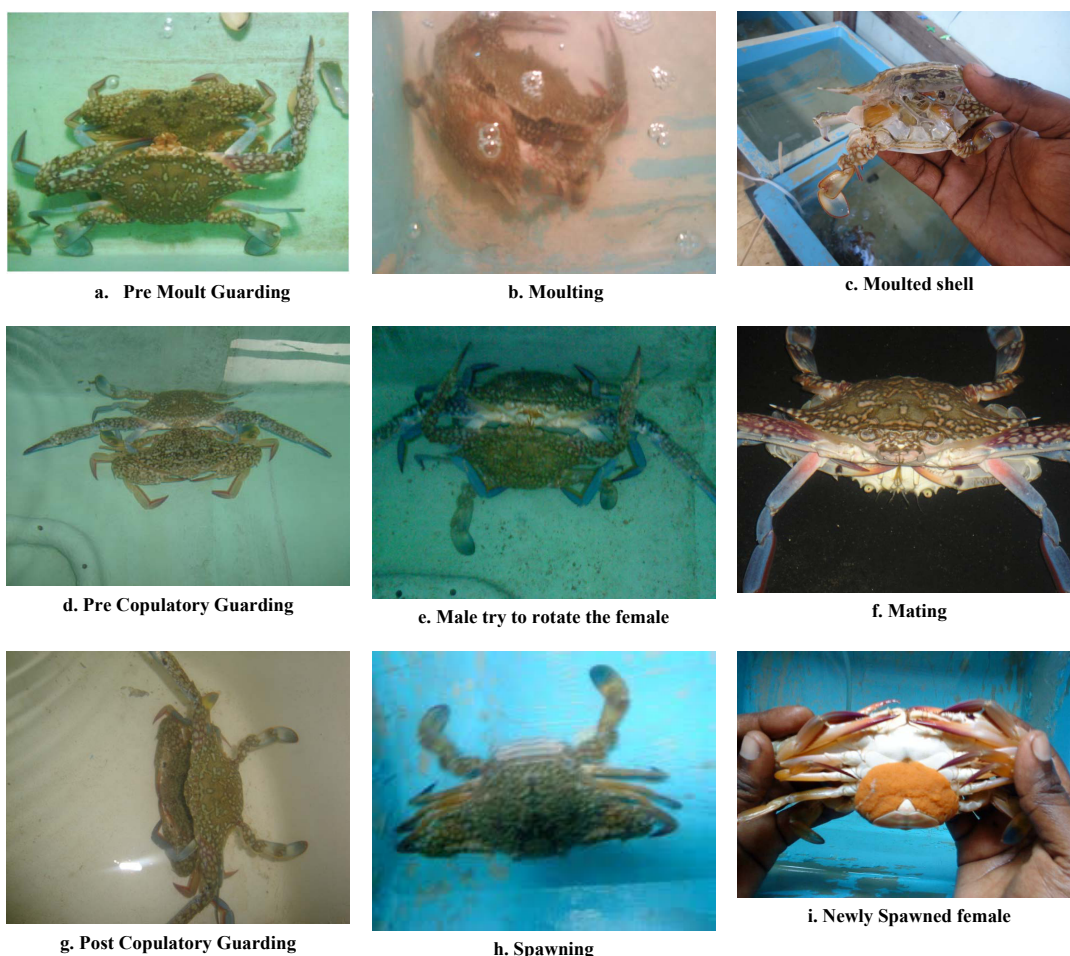


Figure 1: Various Positions of Mating Behaviour.

extrusion. During the process of spawning or extrusion, the eggs are liberated from the ovaries passing through the seminal receptacles. In seminal receptacles the stored sperms are liberated from the spermatophores to fertilize the eggs and fertilized eggs are extruded through the gonophores present in the sternites of the sixth thoracic segments of third pair of legs and these eggs become attached to the smooth setae present in the endopodites of the four pairs of pleopods in the abdominal flab. The egg mass segregated and carried on the abdominal flab is called as berry or sponge. The females carrying eggs is also called as berried crabs. The freshly extruded eggs are initially orange in colour and become black before hatched into zoeae (Figure 1h and 1i).

### Experimental setup I

A hard shelled male stocked with a hard shelled female. The Pre-moult guarding lasted for  $92.66 \pm 1.74$  hrs. Moulting lasted for  $4 \pm 0.5$  hrs. The Pre-copulatory guarding lasted for  $3.66 \pm 0.33$  hrs. The copulation (or) mating lasted for  $7.33 \pm 0.66$  hrs. Post-copulatory guarding lasted for  $12.83 \pm 0.60$  hrs. Total mating sequence (TMS) lasted for  $120.48 \pm 0.76$ . For spawning the female crab took  $17.66 \pm 1.45$  days (Figure 2).

### Experimental setup II

A hard shelled male stocked with a newly moulted female. The Pre-copulatory guarding lasted for  $6.33 \pm 1.76$  hrs. Copulation lasted for  $6.33 \pm 1.01$  hrs. Post-copulatory guarding lasted for  $13.33 \pm 0.54$  hrs. Total for mating sequence lasted for  $25.99 \pm 1.10$  hrs. For spawning the female crab took  $17 \pm 1.52$  days (Figure 2).

### Experimental setup III

A hard shelled male was stocked with an eyestalk ablated female. The Pre-moult guarding lasted for  $62.66 \pm 7.42$  hrs. Moulting lasted for  $3.83 \pm 0.44$  hrs. The Pre-copulatory guarding lasted  $3.5 \pm 0.28$  hrs. Copulation lasted for  $6.66 \pm 0.88$  hrs. Post-copulatory guarding lasted for  $13.16 \pm 0.60$  hrs. Total mating sequence lasted for  $89.81 \pm 1.92$  hrs. For the spawning the female crabs took  $17.33 \pm 1.45$  days (Figure 2).

## Discussion

In general the male partner maintains many type of mating behavior to attract the females. Teytaud [5] noted that pubertal *C. sapidus* female exhibit significant changes in particular behaviours like rocking and chelate waving when presented with a visual image of a crab and simultaneously exposed to water containing male odour. These types of movements were not observed in *P. sanguinolentus* [1] and also in the present study. Male blue crabs *C. sapidus*, has shown a courtship

display in which they elevate their body by standing high on their legs, open their chelate and paddle their swimming legs. Jaroensatasinee [6] studied the courtship in *U. paradussumieri*. They have observed male display in the form of claw waving to attract the females to the burrows of males for mating. Lucas [7] suggested that the conspicuous colouring of some male Hymenosomatids might be evidence for visual displays. The portunid crabs, *Ovalipes punctatus* from South Africa, after pairing, the male would bury himself and the female in the sand with only their eyes protruding [8]. This type of behaviours had never been observed in the present study.

Cradle carrying is a pre-copulatory mate guarding behaviour which is required to guarantee a successful mating and to ensure paternity and is therefore of great ecological importance. Male mate-guarding is energetically expensive and considered a measure of male fitness [9,10]. In *C. sapidus* pupertal females are frequently initiate the cradle carrying position themselves by approaching and repeatedly bumping against non displaying males [11]. This type of approach by pupertal female was not observed in *P. sanguinolentus* in the laboratory conditions [1]. In the present study the males only initiate the cradle-carry position by approaching the pupertal females and the pupertal females has not shown any sort of approach towards the male. The female is protected by a male before and after her pubertal moult by being grasped by the male's first pair of walking legs and hold right side up in a cradle carry position under the male. The same behaviour has been reported previously in various crabs by [12-14].

During the pubertal moult of female portunids, the abdominal flab changes from a triangular to oval shape and from being tightly to loosely fix to the cephalothorax [15,16]. Similar changes are also reported in the present study. In crustaceans, the female moulting and mating are intimately linked, postcopulatory mate guarding may also shield the post-moult females (and the males reproductive investment) from predators until her shell has hardened enough to offer protection [17,18]. Crustaceans are unique in mating is often coupled with female moulting [16,19], when female are especially vulnerable to predation and physically incapable of exerting control over mating. Guarding ensures that females are protected during their vulnerable moulting period, while males gain exclusive paternity of the offspring during the ensuring intermoult period of the females [16,20]. In the present study the male and female crab are separated from cradle carrying position before moulting and the male crab moves around the female crab during this crucial time and helps her to moult by removing her shell and protect until her exoskeleton is hardened. In many brachyuran crabs, the moulting cycle is an important activity during copulation hence the mating males are invariably hard, so that male gonopods must be able to successfully penetrate the female vulvae. Therefore, all males must have a hard exoskeleton in order to mate successfully. Typically, females are physically able to mate only when their exoskeleton is soft, immediately after moulting. It is very common in the crabs belonging to the families of Cancridae and Portunidae [21-23].

The mating of *P. pelagicus* is basically corresponding with the pattern of brachyuran crabs [24], generally occurred between hard shelled males and soft shelled females [24]. Previously soft-female mating had been reported in crabs belongs to Portunidae [25-28]. Alternatively some species where multiparous females can mate in a hard-shell condition, there is often little pre-copulatory guarding, but the male may guard the female for sometime following copulation. As females of these species are not especially vulnerable to predation, post-copulatory mate guarding is likely to result from male-male competition. This type of post copulatory guarding is not seen in *P.*

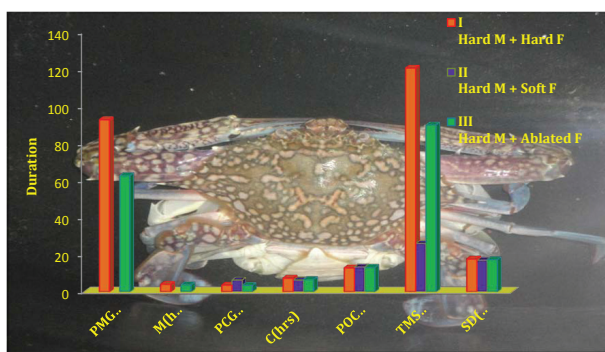


Figure 2: Mating behavioural durations of three different experimental pairs.

*sanguinolentus* [1] but it's observed in the present study. Mating was reported when the females were in hard shelled condition in *Pugettia product* [29], in goldan crab, *Geryon fenneri* [30], in *Cyclograpsus lavauxi*, *Helice crassa*, *Hemigrapsus crenulatus*, *H. sexdentatus* [31] and in portunid species *T. prymna*, *T. sima* [32]. In other crabs, such as *C. pagurus* and *S. serrata*, pair formation takes place just before the female undergo moulting [33].

During copulation the male positions the ventral parts of its body facing upwards and the female places herself on top of the male with the ventral side facing that of the male [17,34]. Similar type of position has not been observed in the present study. The first pair of pleopods of the male is the functional intromittent organs, each receiving spermatophores and semen from the respective penis. The second pair of pleopods are inserted into the posterior foramen of the first pleopods, forcing the spermatophores and semen through the tube like, first pleopods. During copulation semen and spermatophores are passed from the first pleopods of the male into the paired oviducts and eventually the seminal receptacles of the female [35]. At the time of mating the brachyuran males transfer their spermatophores to the female seminal receptacles. The female store the spermatophores until fertilization [36]. The eggs are subsequently fertilized at the time of extrusion found that sperm in female *C. sapidus* seminal receptacles can remain viable for at least 12 months [37,38]. This is also applicable to *P. pelagicus*. Eggs are fertilized when passing from the ovaries to the seminal receptacles before being extruded onto the female's pleopods. Egg extrusion onto endopodites of the female's pleopods may be complete within 2 hrs [38].

The female spider crab of *Inachus phalangium* commonly extrude a brood shortly after mating [39], but may store sperm for over six months and fertilize as many as six broods before mating again [40]. Females usually extrude a clutch of eggs within 1-5 days of moulting, whether mated or not [41,42]. Both fertilized and unfertilized eggs attached to the pleopods; those fertilized are incubated for upto 2 years; those not fertilized are lost within 5-6 months of attachment [43-45]. The number of eggs extruded in each brood was defined as the reproductive success of females because egg loss through the breeding period is quite small [46] and almost all eggs extruded is fertile under natural conditions [47,48]. Laboratory rearing of sand crabs has shown that female sand crabs have to maturity moults and each of these moults can extrude upto four batches of eggs [49].

The male and female will form a pre-copula for eight to ten days before ecdysis of the female. After female ecdysis, when the female is soft shelled, copulation takes place over a six to eight hrs period [50]. The mating behavior of *P. pelagicus* follows the typical portunid crabs patterns as described by [17], consists of three typical phases. They are precopulatory behaviour, ecdysis and mating. The precopulatory behavior lasts 63hrs, ecdysis lasts 96 minutes, mating took place more than 2hrs and 30minutes and spawning took place after 2 weeks [51]. In the present study the experimental setups I and III had Premoult guarding  $92.66 \pm 1.74$  hrs and  $62.66 \pm 7.42$  hrs respectively. When compared to experimental setup I, the experimental setup III perform quick premoult guarding due to eyestalk ablation in the females (The eyestalk ablation will reduce the intermoult period. X – Organ sinus gland complex with eyestalk secretes moult inhibiting hormone. Y – Organ secretes moulting hormone. When the removal of eyestalk will reduce the (MIH) moult inhibiting hormone. So increasing the moulting hormone. The presence of moulting hormone shortens the intermoult duration). The moulting lasted  $4 \pm 0.5$  hrs and  $3.83 \pm 0.44$  hrs in experimental setups I and III respectively. The precopulatory

guarding was lasted  $3.66 \pm 0.33$ hrs,  $6.33 \pm 1.76$ hrs and  $3.5 \pm 0.28$ hrs in experimental setups I, II and III respectively. In experimental setup II had more precopulatory guarding than the experimental setup I and III may be due to introduction of newly moulted female. Cannibalism was observed among soft shelled females in the present study. Cannibalism was occurred more often when soft shelled female was introduced with hard shelled male. It may be due to the lack of premoult guarding between the pairs. The mating lasted  $7.33 \pm 0.66$  hrs,  $6.33 \pm 1.01$  hrs and  $6.66 \pm 0.88$  hrs respectively in experimental setups I, II and III. The postcopulatory guarding was lasted for  $12.83 \pm 0.60$  hrs,  $13.33 \pm 0.54$  hrs and  $13.16 \pm 0.60$  hrs respectively in experimental setups I, II and III. The total mating sequence lasted  $120.48 \pm 0.76$  hrs,  $25.99 \pm 1.10$  hrs and  $89.81 \pm 1.92$  hrs respectively in experimental setups I, II and III. For spawning the female crab took  $17.66 \pm 1.45$ ,  $17 \pm 1.52$  and  $17.33 \pm 1.45$  days for experimental setups I, II and III respectively.

In experimental pair II the females were already moulted, so it takes more pre copulatory guarding time when compared to experimental setup I (Hard) and III (Ablated). However the total durations of mating sequence is less in experimental pair II when compared to other experimental pairs I and III. The days taken to spawning were more or less equal in three experimental pairs. From this study it is suggested that the soft shelled females with hard shelled males are suitable for quick production of berried females under controlled condition.

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