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Mating behaviour and broodstock development of commercially important blue swimming crab, *Portunus sanguinolentus* (Herbst)

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Abstract: The study investigates the mating behavior and broodstock development of commercially important crab, *Portunus sanguinolentus*. In first experimental setup two different sized hard shell males (85g and 110g) and a soft shell female (85g) was stocked in a tank. The female crab avoided the small crab and accepted the bigger crab for its aggressive interaction and eventually successful mating was happened. In second experimental setup two same sized males (100g) in which one male had one chelate and another one with two chelae stocked with a soft shell female (95g). The female always readily accepted to mate with two chelate male than single chelate male. The breeding behaviour encompassing 3 phases *viz*., contact, cradle carry position and mating were described in detail. Finally the fertilized eggs were deposited in the abdomen of the female. The berried females were maintained in the laboratory until the eggs hatched into I zoeae. The crab fishery in India is fast developing and there is a vast scope for the crab meat both national and international markets. To start year round seed production the availability of berried females throughout the year is essential.

Keywords: *Portunus sanguinolentus*, mating behaviour, crab culture

Introduction

The crab fishery in India is fast developing and there is a vast scope for the crab meat both national and international markets. Crabs rank 3rd after shrimps and lobsters for their esteemed seafood delicacy and also value of fishery they support (Mohammed Saved & Rajeev Raghavan, 2001). The economically important portuid crabs found along east coast of India are *Scylla serrata,* S. *tranquebarica, P. sanguinolentus, P. pelagicus, Charybdis feriata, C. lucifera and C. truncata* (John Samuel *et al.,* 2004). In recent times the blue swimming crabs are fished in large quantities from all the seas in India and finally sold as a processed food. So demand for these crabs is increasing day by day. Hatchery technology and farming activities are available here and there for bigger sized crabs (S. *serrata*, S. *tranquebaric)*. But economically viable mass seed production technology is lacking for swimming crabs in general (Soundarapandian *et al.,* 2007) and *P. sanguinolentus* in particular (John Samuel, 2008). To start year round seed production the availability of berried females throughout the year is essential. It is not possible from the natural resources. So the production of berried females in the controlled conditions is need of the hour. To produce broodstock in the laboratory one should know the mating behaviour. Such studies are very much lacking in commercial crabs especially *P. sanguinolentus*. So in the present investigation an attempt has been made to know the mating behavior and broodstock development of commercially important crab, *P. sanguinolentus.*

Materials and methods

The experimental male and female crabs of *P. sanguinolentus* were collected from the Parangipettai (Lat. 11˚ 29´ N; Long. 79˚49˚ E) landing center in plastic bucket containing sea water. The live female water crabs or soft-shell crabs were selected and checked for any loss of appendages, healthy and disease free. The size of the females was ranging from 80-90g. Simultaneously the hard shell male crabs were also collected and the sizes were ranging from 85-110g. They were transported to the laboratory for further experiment. Once they reached in the laboratory they were acclimatized in the tanks containing sterilized and filtered sea water (salinity of 30-32 ppt; temperature 26-31˚C; pH 7.5-8.2 and dissolved oxygen 5 ppm).

After acclimatization, the male and female crabs were stocked in two different combinations. In first experimental setup 2 different sized hard shell males (85g and 110g) and a soft shell female (85g) was stocked in a tank. In second experimental setup 2 same sized males (100g) in which one male had one chelate and another one with two chelae were stocked with a soft shell female (95g). Triplicate was maintained for each experimental setup. At the beginning the water level was maintained at 18 cm depth and it was observed that the males struggled to carry the females and eventually turn over for matting. In order to avoid the above problem the water depth was increased up to 25 cm. So the males were carried the females very easily for mating. Optimum environmental parameters were maintained during the experimental period (salinity 30-32 ppt, temperature 26-31°C, pH 7.5-8.2 and dissolved oxygen 5 ppm) (Plate 1).

Results

Observations of mating behavior

Experimental setup 1: Two different sized hard males (85g and 110g) were stocked along with one soft female (85g). The bigger male crab had robust and bigger chelate where as the smaller ones had smaller chelate. When these males introduced in a tank containing pubertal female were triggered and ultimately moved towards the female. The small male invaded the breeding territories of larger male. Since the larger male had bigger chelate which displayed aggressive interaction with small male and chased away and finally placed himself in the cradle carry position. The female crab avoided the small crab and accepted the bigger crab for its aggressive interaction and eventually successful mating was happened.

Experimental setup 2: Two same sized hard shell males (100g) were stocked along with soft shell female (95g).

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Among these 2 males one had one chelate and another with two chelate. The two chelate male dominated over single chelate male. The female always readily accepted to mate with two chelate male than single chelate male. In general the mating with two chelate male was much easier to turn her over and also positioned her upside down for mating.

For convenient, the mating behavior of *P. sanguinolentus* is divided into three phase as follows:

I. Contact

When soft shell female introduced into the experimental tank had some attraction by releasing sex pheromones in the water and ultimately the male triggered towards the female (Glesson, 1980). In turn the females were able to utilize chemical cues (possibly a male pheromone) to locate males which are an important mechanism for optimizing reproduction success. This chemicals released by the female was to attract the male for the selective capture and contact.

II. Cradle – carry position

When the aggressive dominant male encounter newly moulted female tried to chase the other male and started approaching the female by moving in front extending his bigger chelate legs and force the female to the corner of the tank so that it can not move away from male. Then the male climbed over the female and the male started to hold the female by the third and fourth walking legs. Subsequently the male became exited and started releasing air bubbles vigorously from the mouth. In short while the male crab with the help of swimming legs carried the female to the suitable place for copulation. The copulation started at night 7.30-8.00 pm (Plate 2). This pair formation was usually starts 3-4 days before female moulting. But in the present experiment the moulted female introduced directly in the tank where already hard shell male was present.

III. Mating

In the presence of female the male crabs became very active, moved towards the female and grab her with one of his chelate vigorously and turned her over. The assistance rendered by the male crab, she positioned herself upside down beneath him and extends her abdomen, allowing the male to insert his paired copulatory appendages into her genital pores (Plate 3). Then the male crab deposited spermatophor into the spermathecae, which last for more than 8-12 hrs. During copulation the male often walked around with the female attached to its ventral surface, holding her with third and fourth walking legs. After copulation the cradle- carry position were continued for only few hrs (10-14hr).The female found inactive till she attain normal hardness of her exoskeleton.

Extrusion of eggs

After male crab deposited spermatophores in the female's seminal receptacles and it was stored until the female was ready to extrusion. During the process of extrusion the optimum environmental parameter were maintained as earlier. During the process of extrusion the stored sperms were liberated from the spermatophores to fertilize the eggs and the fertilized eggs were extruded through the genital openings present in the sternites of sixth thoracic segment. And these eggs became attached to the smooth setae present in the endopodits of four pairs of abdominal pleopods. The egg mass segregated and carried on the abdominal flap is called as berry. Those females are also called as berried or ovigerous crabs. The freshly extruded eggs were orange in colour. These berried crabs were maintained in the laboratory until the eggs were hatched into I zoeae.

Discussion

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 gonopores. 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 Cancridae and Portunidae (Edwards, 1966; Hartnoll, 1969; Edwards, 1979; Berrill & Arsenault, 1982; Elner *et al*., 1985). Female about to moult is release a pheromone which attracts the male (Ryan, 1967) and this male then protects the female during the critical time surrounding the female and mates with her shortly. In the present observation mating was took place between hard shelled male and newly moulted female as in many crabs. In golden crab, *Geryon fenneri* (Hines, 1988) and in *Cyclograpsus lavauxi*, *Helice crassa*, *Hemigrapsus crenulatus* and *H. sexdentatus* (Brockrehoff & Mclay, 2005) mating was reported when the females were in hard shell condition.

Male blue crab, *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. This courtship display is not reported in other swimming (Portunid) crabs (Michiya Kamio, 2008). Jaroensatasinee and Jaroensatasinee (2003) 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 (1980) suggested that the conspicuous colouring of some male Hymenosomatids might be evidence for visual displays. Such sort of attractive displays were not observed in *P. sanguinolentus* of the present study.

In the families of Cancridae and Portunidae, a male might guard the female until she moults, then copulate with her and resume guarding until the females integument has hardened (Hartnoll, 1969). In the present study, *P. sanguinolentus* male guarding the female by preventing rival males to mate and also protecting until her shell became hard. 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 some time following copulation.

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Plate 1. Experimental set up for easy mating

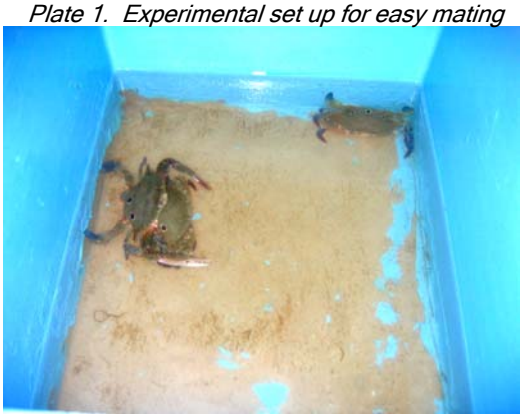

Plate 2. Cradle carry position

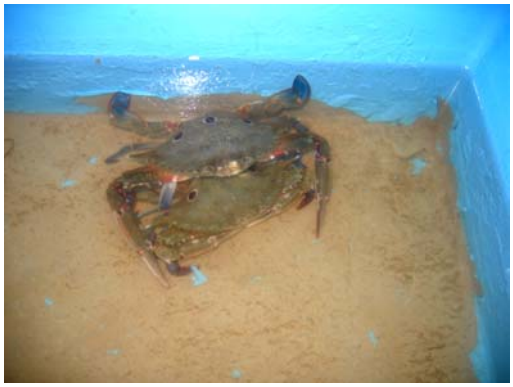

Plate 3. Mating of P. sanguinolentus

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. sanguinolentus*. In C*. sapidus* pubertal females will frequently initiate the cradle-carry themselves by approaching and repeatedly bumping against non displaying males (Glesson, 1982). These sorts of approach by pubertal female were not observed in *P. sanguinolentus* at laboratory condition.

According to Glesson (1980) the pheromone signal is of primary important for initiating the male's courtship behavior and that visual cues are secondarily used to orient this behaviour towards any crab in the immediate vicinity. In the present investigation it is not sure whether the male *P. sanguinolentus* was attracted by pheromone or visual cues. Teytaud (1971) 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 odor. These types of movements were not observed in *P. sanguinolentus.*

In many species, agonistic behaviour mediates competition for mates or for resources necessary for mating, often, but not always, between males (Huntingford & Turner, 1987; Harvey & Bradbury, 1991). Strong competition between individuals is therefore expected when a resource such as mates used by one individual is consequently less available to other (Emlen & Oring, 1977; Anderson, 1994). In these cases, reproductive success of males may be largely dependent on contest success, giving rise to intrasexual selection for morphological or behavioural characteristics associated with agonistic capability. In several species of crab, reproductively successful males tend to be larger than unpaired males (Edwards, 1966; Hazlett *et al*. 1977; Hazlett, 1979; Berrill & Arsenault, 1982; Wilber, 1986; Asakura, 1987; Christy, 1987; Diesel, 1988; Sekkelsten, 1988; Smith, 1992; Norman & Jones, 1993) and the chelipeds, which are used in agonistic displays, are often sexually dimorphic (Dingle, 1983). In several species there is pre- and post-copulatory pairing of males and females (Hartnoll, 1969) and observations in captivity indicate that males fight when a single male attempts to displace a paired male and take over the female (Edwards, 1966; Jachowski, 1974; Berrill & Arsenault, 1982; Smith, 1992). In the present study also competition was existed between males, finally the bigger chelate possessing crab dominated over the smaller one and successfully mates the soft shell female crab.

Display patterns of chelipeds have an important role in agonistic and aggressive interactions. Of the five pairs of pereiopods, the chelae are versatile organs of chelipedis. In *U. pugilator* there is a marked difference in display patterns between mature and immature males (Salmon *et al*., 1978). Since the degree of dominance is

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expressed by the type of chelae morphometry. An animal with robust and bigger chelae has easy access to mate during inter- male competition and also through sexual selection by females. In present study two chelate male dominated the single chelate male even though they are of same size. So it is very much evident that chelate plays major role in mating.

In many species, small males invade the breeding territories of larger males by avoiding direct competition altogether. Once enters breeding territories, these sneaker" or "satellite" males surreptitiously mate with receptive females, as occurs in some isopods (Shuster, 1992), amphipods (Borowsky, 1980; Clark, 1997; Kurdziel & Knowles, 2002) and in many decapods (*Macrobrachium* spp. Ra'anan & Sagi,1985; sand bubbler crabs, *Scopimera globosa*, Koga *et al.,* 1993; spider crabs, *Libinia emarginata*, Sagi *et al*. 1994; Ahl & Laufer 1996; and rock shrimp, *Rhynchocinetes typus*, Correa *et al.,* 2003). In each of these species stolen mating were reported. Such mating is not observed in *P. sanguinolentus* of the present study.

The mating and reproductive patterns are observed among polymorphic males of *M. rosenbergii*. The dominant blue-clawed males (bulls, BC males) effectively court and protect mates (Ra'anan & Sagi, 1985), while intermediate males (OC males) show reduced reproductive activity in the presence of BC males (Ra'anan & Cohen, 1985). Submissive small males are also sexually less competent, but mate successfully in the absence of BC and OC males (Sagi, 1984). It is evidenced in the present observation that the bigger and aggressive crab dominated the smaller crab as in *M. rosenbergii.*

Multiple mating and egg fertilization with stored sperm were also known to occur in a variety of spider crabs (Hartnoll, 1969; Paul, 1984). In mud crabs the moulted female allows two different males to copulate one after another with an interval of 1 to 2 days. Finally the spermatophores deposited by two male co-exist in the seminal receptacle of one female. In the present investigation it is not very sure whether the female allowed two male to mate and spermatophore deposited by second male was only used.

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