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Mating behaviour of the orange mud crab, *Scylla olivacea*: The effect of sex ratio and stocking density on mating success

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ABSTRACT

Mud crabs from the genus *Scylla* have high commercial value and are considered as one of the highly sought luxury seafood items. Thorough understanding about their biology and mating behaviour is vital in providing important information for a sustainable exploitation and future incorporation into the aquaculture industry. The mating process of *S. olivacea* lasted 82.0 ± 10.8 h was divided into four phases: precopulation, molting, copulation, and postcopulation. Courtship displays and fighting were shown by mature males while they were courting females. Precopulatory position lasted for 55.2 ± 10.8 h before the pairs disengaged for the female to molt. The molting process was 4.6 ± 0.3 h. Copulation (mean duration was 6.6 ± 0.5 h) occurred while the female's exoskeleton was still soft. Postcopulatory guarding lasted for 13.6 ± 0.6 h. Separation of the mating pairs indicates the end of postcopulation phase. Mating success percentage was unaffected by sex ratio, but inversely affected by stocking density. Cumulative mortality increased with increasing stocking density and unequal sex ratios. Postcopulatory guarding duration was significantly shorter in treatment with 1 male:2 females ratio and treatment with the lowest stocking density (2 crabs m^{-2}). We proposed rearing of mud crab broodstocks for mating purpose using sex ratio of 1 male:2 females and stocking density of 6 crabs m^{-2} to maximize output (successful mating pairs) while maintaining low mortality percentage and shorter postcopulatory guarding duration.

1. Introduction

Mud crabs of the genus *Scylla* (Brachyura: Portunidae) are distributed throughout the Indo-Pacific region and are commonly found in intertidal zones of mangrove forests and estuaries (Keenan et al., 1998). These crabs are of high demand both in the local and international markets because of the sweet and delicate taste of its meat. Out of the four species of *Scylla* (*S. serrata*, *S. olivacea*, *S. tranquebarica* and *S. paramamosain*) *S. olivacea* is popularly used for aquaculture because of its hardy nature and ease of capture. Recently, mud crab farming has gained much attention in Malaysia. However, most of these farming activities involve only crab fattening and the production of soft-shelled crabs, with the use of captured juveniles from the wild (Ikhwanuddin et al., 2013). This dependency on wild caught mud crab juveniles is due to the unavailability of adequate knowledge on the biology and larval rearing of mud crabs (Williams and Primavera, 2001).

Understanding of the mating behaviour is a paramount component of the biology of any organism. Mating in mud crabs is known to take place in intertidal zones and estuaries before the females migrate as far as 50 km offshore to spawn their eggs (Koolkalya et al., 2006; Robertson and Kruger, 1994). The mating strategies in eubrachyuran crabs can be divided into six groups based on the difference in mating-molting link (soft-shell mating or hard-shell mating), growth pattern (indeterminate growth or determinate growth) and seminal receptacle structure (dorsal or ventral seminal receptacle) (McLay and López Greco, 2011). These 6 groups are classed into two main groups based on their growth patterns, separating those with determinate growth (i.e., Portunoids and Majoids) and indeterminate growth (i.e., Eriphioids, Xanthoids, Cancroids, and Grapsoid-Ocypodoids). Portunoids, having dorsal seminal receptacle and mate during soft-shell condition are grouped separately from Majoids (possess ventral seminal receptacle and mate during hard-shell condition). Among the mating strategies employed by members in Portunidae family are the display of courtship behaviors, prolonged precopulatory and postcopulatory guarding, the presence of sperm plug, and the absence of hinged opercula in vulvae (McLay and López Greco, 2011). Previous

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studies (Davis, 2004; Koolkalya et al., 2006; Robertson and Kruger, 1994) have looked into the spawning biology and characteristics of wild-caught berried females from of the genus *Scylla*, but little has been published on their mating behaviour prior to spawning.

Sex ratio and stocking density are important elements affecting the mating success of mud crabs when reared in a confined area where space is a limiting factor (Naranjo-Paramo et al., 2004). This is because mud crabs are well known for their territorial, aggressive and cannibalistic behaviors (Mirera and Moksnes, 2013). A thorough understanding on the mating behaviour of mud crabs and the effect of different sex ratio and stocking densities on the mating process is essential to ensure selective breeding and for mating of mud crabs to be successful in hatchery conditions.

This study describes the mating behaviour of orange mud crab, *S. olivacea* under controlled laboratory conditions and the effect of different sex ratios and stocking densities on mating success.

2. Materials and methods

2.1. Sampling

S. olivacea were collected from Matang Mangrove Forest Reserve, Perak, Malaysia (4°45′58″N; 100°37′31″E). Standard crab pots with trash fish as bait were set up during low tides, left overnight, and the crabs were harvested the next day during high tide. The identification of the *S. olivacea* species was based on the morphological description provided by Keenan et al. (1998). The carapace width (CW) of selected mature males ranged from 110.23 mm to 124.49 while the CW of immature juvenile females ranged from 65.33 mm to 78.25 mm (Ikhwanuddin et al., 2010, 2011). Immature juvenile females that were about to undergo pubertal moult were used to ensure that they were unmated. The specimens were transported back in dry condition on the same day to marine hatchery of Institute Tropical Aquaculture, Universiti Malaysia Terengganu, Malaysia.

2.2. Mating behaviour

Crabs were first disinfected with 150 ppm formaldehyde for 30 min and acclimatized for 3 days in tank with a salinity of 30 ppt (Baylon, 2011). The crabs were marked using water-resistant markers on their carapace and divided into 2 tanks (2 m length × 1 m width × 1 m height). Each tank consisted of 10 males and 5 females, hence a total of 30 crabs were used. They were reared until spawning occurred, with a maximum rearing period of 60 days. All males were used only once and promptly removed from tank after mating with a female had ended. The water level had a depth of 50 cm and 5 PVC pipes with open ends (diameter = 10 cm, length = 20 cm) per tank were provided as shelter. Optimum environmental conditions were maintained throughout the experiment (salinity 25 ppt; temperature 26–30 °C; natural photoperiod). Feeding with a mixture of squid *Loligo* sp. and trash fish *Decapterus* sp. at approximately 10% of crab's body weight was done twice a day while water exchange was conducted once every 3 days. Uneaten feed and excretory wastes were siphoned out 1 h after each feeding period. Observation for any mating behaviour was carried out twice daily (12 h interval). Once mating behaviour was observed, continuous observation of the mating pairs was conducted. During observations, PVC pipes were gently pulled away for a brief period to reveal the hiding pairs. Mating behaviour and duration of mating phases (hours ± standard error) for each mating pair were described and recorded via direct observation with the aid of another 4 researchers in shifts. Total mating duration was inclusive of precopulation, molting, copulation and postcopulation process. The observation for precopulation process started when any crabs showed mating behaviors towards the opposite sex. Molting phase involves crabs showing no movement, followed by shedding of their old carapaces. Copulation process is identified when the male started to flip over the newly-molted female and subsequently positioned his sternum within the female's opened abdomen, and the male's gonopods were inserted into the female's gonopores. The postcopulatory guarding was recorded when the male released the female and positioned her underneath his abdomen, caging her with his legs.

2.3. Sex ratio and stocking density

To test the effect of sex ratio on the mating success of *S. olivacea*, 27 pairs of mud crabs ($n = 54$) were randomly divided into three different male:female sex ratios, i.e., 1:1 (9 males:9 females), 2:1 (12 males:6 females) and 1:2 (6 males:12 females).

The effect of stocking density on the mating success of *S. olivacea* was separately investigated as well by stocking 2, 6 or 10 pairs of crabs in each tank ($n = 36$). This is equivalent to approximately 2, 6, or 10 crabs m^{-2}. A sex ratio of 1 male:1 female was applied.

Both the sex ratio and stocking density experiments were conducted in triplicate, using a total of 270 crabs. Disinfection and quarantine period were the same as described in the previous experiment. Environmental conditions, water level, feeding regime, tank dimensions, marking of individual crabs and water exchange were similar to that used in mating behaviour experiment. Crabs were monitored daily throughout the experiment. Dead or weak crabs were recorded, removed from the tanks and replaced with new crabs to compensate for mortality and maintain stocking density. The experiment was carried out with a maximum rearing period of 60 days.

Copulated females were transferred to individual tanks after their male counterparts released them from post-copulatory guarding. They were maintained for at least a week before being brought into laboratory to check for the presence of sperm in the females' seminal receptacle (i.e., spermatheca). Successful sperm deposition by males into females after copulation was considered as mating success.

2.4. Sperm examination

Copulated females were anesthetized by dipping into ice water for 20 min. They were then dissected to examine the presence of sperm packets in their seminal receptacle which are located inside the gonopores. The deposited spermatophores appeared to be small, round, solid and whitish in colour when viewed with the naked eye. For confirmation, they were diluted with few drops of saline solution and examined under stereozoom microscope (Nikon AZ100 M).

2.5. Data analysis

The effect of sex ratio and stocking density on mating success, cumulative mortality and duration of copulation and post-copulation guarding stages in *S. olivacea* were compared. All data were checked for normality of distribution and homogeneity of variance using Wilks-Shapiro and Bartlett's test, respectively. Mating success percentage data were arcsine transformed and analysed using One-Way Analysis of Variance (ANOVA) to find out if there was significant differences among the different treatments. This was followed by Tukey's test to find out which treatments were significantly different from each other (Sokal and Röhlf, 1981; Zar, 1984). Cumulative mortality of crabs in relation to time for each treatment (sex ratios and stocking densities) were fitted via polynomial regression and compared graphically. Statistical analyses were performed using Microsoft Excel 2010.