Effect of Water Salinity on Mating Success of Orange Mud Crab, *Scylla olivacea* (Herbst, 1796) in Captivity

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**ABSTRACT**

This study was conducted to determine the effects of salinity on the mating success of orange mud crab, *Scylla olivacea*. Male crabs and immature female crabs were reared at the salinity of 15, 20, 25 and 30 ppt in 30 days study period. Brood stocks were fed with squids and maintained until the pre-copulation observed. The pre-copulated crab pairs were isolated from the other brood stock and observation was done for duration of pre-copulation, copulation and post-copulation. The mating duration was recorded. Sperm deposition in the female’s spermatheca was also examined. Mating was successful in all tested salinities and mean pre-copulation and copulation period for *S. olivacea* was 22.4 and 2.9 h, correspondingly. The mating success was 50, 30, 20 and 10% for salinity of 25, 15, 20 and 30 ppt, respectively. However, there were no significant relationship between the salinity level and mating success of *S. olivacea* where 25 ppt salinity was the optimum salinity preferable by mud crab pairs to achieve successful mating with sperm deposition.

**Key words:** Mating success, salinity, orange mud crab, *Scylla olivacea*

**INTRODUCTION**

Mud crab, *Scylla* spp. are distributed naturally in the estuary zone and mangrove areas (Ikhwanuddin *et al.*, 2011; 2010a, b) where the salinity varies over time (Davenport and Wong, 1987). Mud crab are increasingly known to be part of the nowadays aquaculture cultivated species. However, mud crab production in aquaculture for commercialization is still not well developed in Malaysia due to lack of knowledge in captive hatchery breeding.

Mating of mud crab occurs when the female mud crabs are ready to molt. As the mating occurs, spermatophore from the male will be inserted into the females and stored until the eggs are matured (Phelan and Grubert, 2007). In order to achieve the eggs maturation, the female’s mud crab will migrates offshore into deeper water as the environment is more suitable for the eggs development (Le Vay, 2001) and larval survival (Talpur and Ikhwanuddin, 2012). The natural mating of the mud crab occurs in suitable environment condition with surrounding factors that contribute to the mating success.

Water salinity seems to be affecting the habitat preferences and many functional responses of the organisms including animal survival and reproduction (Talpur and Ikhwanuddin, 2012). Salinity is an important parameter in regulating physiology of marine organisms (Lesser, 2003).
S. serrata is the most distributed one among the four mud crab species, genus Scylla throughout the Indo-pacific and is most common at high salinities area (Keenan et al., 1998). Meanwhile, S. paramamosain and S. olivacea are more common to be found in lower salinities area (Keenan et al., 1998). Due to the facts that they inhabit mostly in mangroves and coastal area, the salinities fluctuation of the water would surely influences the behaviours of the mud crab towards the mating attempts. Hence, optimum water salinity preferences for mud crab to induce mating can be established.

Successful mating can be achieved for production of berried females in captivity and contributed to the reduction usage of exploited seed supply from the wild. This study was done to determine the effect of different water salinity regimes on the mating success of orange mud crab, S. olivacea in captivity via (1) Mating attempts and duration at different mating stages of pre-copulation, copulation and post-copulation and, (2) Sperms deposition examination.

MATERIALS AND METHODS
A total of 40 pairs of S. olivacea were collected from Matang Mangrove Forest, Perak, Malaysia (4°50'12"N;100°37'34"E) and transported to the hatchery of Institute of Tropical Aquaculture, Universiti Malaysia Terengganu (UMT). Body Weight (BW) of the samples collected was approximately 90 to100 g.

The brood stock were stocked in 2 m×1 m×1 m fibreglass rearing tank. Four different water salinities were used in this study which is 15, 20, 25 and 30 ppt. Three units of these fibreglass rearing tanks with 10 pairs of crabs were prepared for each salinity treatment. Water was filled about two third of the tanks height and 100% water exchanges were done at the minimum of once a week to minimize disturbance. Continuous aervations were provided. PVC pipes were placed in the tanks to prevent cannibalism. Prior to stocking, crabs were labelled for identification purpose. Crabs were fed daily with squids, Loligo sp. at 5% of the crab body weight.

Daily monitoring was done within 30 days study period to check for any pair of crabs in different mating stages of pre-copulation, copulation and post-copulation. Each pre-copulated pair was isolated from other crabs to prevent possible disturbance from other crab. The time and dates for the pre-copulated crab pairs were recorded during the observation. The pairs were maintained till the copulation occurred. The males then were isolated from the females after the pair released. While the post copulated females were maintained until further examination for sperms deposition in the spermathecae.

Copulated and post-copulated females were dissected for examination of deposited sperms in their spermathecae, which is whitish in colour. The sperms were examined under Nikon Measuring Microscope MM-800. The data was analysed by one way ANOVA and presented in Mean±Standard deviation.

RESULTS
Table 1 shows the details of mating attempts and duration (hour) for each mating stages of S. olivacea at different salinity, which are pre-copulation (Fig. 1a), copulation (Fig. 1b) and post-copulation. Of all treatment, there were no post-copulation stage were observed. However, laboratory examination shows the presence of white spermatophore in the spermathecae of females (Fig. 2) in the copulated females. The spermathecae is fragile due to thin wall, connected to the ovary.
Table 1: Mating attempts and duration (hour) for each mating stages and sperm deposition of S. ohursday at different salinity

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*na: Not available (no observation), sd: Sample dead, /: Present

Figure 3 shows the number of pairs formed in every mating stages of different salinity. Number of pair crabs involved in overall mating attempts was the highest in 25 ppt followed by 20, 15 and 30 ppt, which is nine, eight, seven and six pairs, consecutively. The other remaining crabs show no sign of mating (pre-copulate) until the end of 30 days study period. About two third of the pre-copulated crab pairs however did not make it to the copulation stage. In addition, half of them were dead during the treatment (Table 1). Pre-copulated crab pairs that successfully advanced to the copulation stage survived until the end of the culture period except for two pair of crabs in 20 and 30 ppt salinity where they ended up in mortality after copulation (Table 1). Number of success
Fig. 1(a-b): Mating stages of *S. olivacea*, (a) Pre-copulate stage, male crab (above) embrace the female crab (below) until the female molt and (b) Copulate mating stage, male crab (above) turn the female crab (below) upside down after the female molt, m: Male, f: Female

Fig. 2: Sperm deposited in the spermathecae (arrow) of the copulated female *S. olivacea*, sp: Spermathecae, ov: Ovary

Fig. 3: No. of crab pairs formed in every mating stage of *S. olivacea* at different salinity treatment, *n* = 10 pairs per salinity treatment.

mating crabs was the highest in 25 ppt followed by 15, 20 and 30 ppt where sperm was present in all copulated female crabs, which indicates as successful mating. The number of pairs for all salinity treatment was decrease sharply from pre-copulation stage to copulation stage.
Fig. 4: Mean duration (hours) of pre-copulation and copulation of *S. olivacea* at different salinity treatment, *n* = 10 pairs per salinity treatment

There was no significance difference can be seen between salinities and the periods of time taken for pre-copulation and copulation stages (Fig. 4). Mean duration for pre-copulation stage of paired crabs is 21.1±19.8, 12.9±12.1, 40.0±40.5 and 15.5±20.4 h for salinity of 15, 20, 25 and 30 ppt, respectively. The time taken during the stage varied greatly from 3-144 h for all the salinity groups. The range was even large within each salinity group. The pattern for mean duration of pre-copulation did not correlate with the mean duration of copulation period of all salinity groups. The mean duration for copulation of crab pairs were 3.6±5.9, 2.8±4.5, 4.3±5.1 and 0.9±2.8 h for 15, 20, 25 and 30 ppt, respectively. The values however did not show significant relationship between the copulation period and salinity. The mean duration of the copulation period was the highest in 15 ppt followed by 20, 30 and 25 ppt. The longest copulation period was 15 h in 15 ppt and the shortest period was 4 h in 25 ppt.

DISCUSSION

Results from this study show the presence of spermatophore inside the female spermathecae as successful mating, instead of extrusion of fertilized egg because berried females usually spawning offshore (Robertson and Kruger, 1994). *S. olivacea* was able to successfully mate in all tested salinities as mud crabs have powerful osmoregulatory physiology (Davenport and Wong, 1987). It was another reflection of the nature of the species to tolerate salinities range in its natural environment. Mud crabs are known for their tolerance to survive in low salinity of freshwater in limited period of time and hypersaline condition at extended period (Shelley and Lovatelli, 2011). Salinity studies on mud crab can be focused to develop hatchery and nursery based techniques to rear the species for commercial production. Recent studies however focused on the culture of immature and lower juveniles' stage of mud crab (Baylon et al., 2001).

Salinity treatment of 25 ppt shows the highest number of mating success. Meanwhile, the numbers of mating success in 15 and 20 ppt were lower than 25 ppt but higher than 30 ppt. The pattern can be seen where the 25 ppt seems to be the optimum salinity to induce mating in captivity. The 30 ppt salinity was the least preferable condition for the broodstock as adult mud crabs are associated with the mangrove ecosystem (Webley et al., 2009), which the water salinity frequently less than 30 ppt. Larval and juveniles of mud crab tend to migrate from high salinity to estuarine as they prefer the brackish water habitat (Srinivasagam et al., 2000). Srinivasagam et al. (2000) also has developed methods of culturing broodstock of *S. tranquubarica* and *S. serrata* using 30-35 ppt filtered seawater. Besides that, the preference of salinity level for *Scylla* spp. in mating attempts could be differs between mud crab species.
Srinivasagam et al. (2000) stated that pre-copulation embraces between males and female crabs took about two to three days. However, in this study it only takes about one to two days period. Meanwhile, the mean for the total copulation period of *S. olivacea* from this present study take less than 24 h. This result was supported by the previous study in which the copulation periods of *S. tranquebarica* and *S. serrata* ranges from 5-52 h (Srinivasagam et al., 2000). The time for the pre-copulation and copulation in captivity can be influenced by other factors. Interference and disturbance towards the pre-copulated crab pairs from other crab pairs can sometimes cause them to be separated from each other as they were threatened. Physical disturbances by observers and other crabs contributed to many incomplete mating.

CONCLUSION

All the tested salinities treatments of 15, 20, 25 and 30 ppt were able to produce copulated female crabs of *S. olivacea* in captivity. The 25 ppt salinity was the optimum salinity preferable by crab pairs to achieve successful mating, in which sperm were deposited. While the 30 ppt salinity yields the lowest mating success. The presence of mortality indicated that the mating process of crab pairs was influenced by other factors as well. The mating success of *S. olivacea* in captivity showed the potential of developing hatchery-based culture technique of *S. olivacea* in the future.

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