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## **Mating Success of Hybrid Trials Between Two Mud Crab Species, *Scylla tranquebarica* and *Scylla olivacea***

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### **ABSTRACT**

*Scylla olivacea* and *Scylla tranquebarica* are two mud crabs species that live associated in the wild. This study was done to investigate the mating success between the two species by determination of the duration and recording the process of mating activity. Wild mud crab samples were collected from Terengganu coastal water, Malaysia. Limb autotomy was subjected to female crabs to induce molting before the introduction of male crab for mating trials. Ten pairs of each mating trial were observed. Mating success of control trials of *S. olivacea* (T1) and *S. tranquebarica* (T2) is 60 and is 50%, consecutively. Mating success of hybrid trials for male *S. olivacea* with female *S. tranquebarica* (T3) is 40% and male *S. tranquebarica* with female *S. olivacea* (T4) is 30%. The highest mean duration of pre-copulatory guarding is T3 (12,240 min±1,859.0) while the lowest is T2 (8,064 min±1,287.9). As for copulation, the highest mean duration is T4 (59.3 min±18.0) while the lowest is T1 (59.3 min±18.0). Meanwhile, the highest mean duration of post-copulatory guarding is T2 (312.0 min±50.2) and the lowest is T3 (82.5 min±28.7). Longer duration of pre-copulatory guarding and copulation were observed on hybrid trials (T3, T4) compare to the controls (T1, T2). It has been observed that all successful mating trials in the present study show general mating activity, involving pre-copulatory guarding, copulation and post-copulatory guarding. Result of the present study shows that hybridization can occur in captivity and there are also possibilities of hybridization between the two mud crab species to occur in the wild.

**Key words:** Mating success, hybridization, mud crab, *Scylla olivacea*, *Scylla tranquebarica*

### **INTRODUCTION**

Mud crab genus *Scylla* is a native species that inhabit the muddy area of mangrove forest. (Ikhwanuddin *et al.*, 2010a, b, 2011; Keenan, 1999). Since the first commercial mud crab aquaculture operations began in Malaysia in 1991, the mud crab has gradually entered local markets and become a main component of the local crab fisheries. However, the crab aquaculture production relies on wild-caught stock which may lead to overexploitation and recruitment failure (Ronquillo *et al.*, 1998; Quintio *et al.*, 2001) due to the high demand and ease of capture (Le Vay, 2001). This combined with the loss of habitat resulted in reduction in both landings and maximum size captured (Keenan, 1999).

Although mud crab culture is gaining more attention, the advancement of this mud crab industry has been quite slow compared to other cultured species (Trino *et al.*, 1988) due to limited seed supply (Keenan, 1999). Therefore, the development of seed production technique is important

in order to maintain reliable seed supply for the industry. A few studies have been done on size at maturity (Ikhwanuddin *et al.*, 2010b, 2011; Robertson and Kruger, 1994) and size at mating of mud crab (Ikhwanuddin *et al.*, 2011). Mating of mud crab occurs when the female mud crab is in the soft bodied (pre-molt) condition following molting. *S. tranquebarica* and *S. olivacea* have distributions focused on the South China Sea extending into the Indian Ocean and the western Pacific (Le Vay, 2001) and they are mostly found associated in the same habitat (Ikhwanuddin *et al.*, 2010a, 2011). Hence, possibilities of natural hybrid between the two species are to be expected. In addition, the production of hybrid between different species has great economical importance for aquaculture (Graziana *et al.*, 2003).

Despite the increasing interest in mud crab farming, very little information exists on mud crab mating behavior and mud crab breeding in detailed. This study was done in order to investigate the mating success between two mud crab species commonly found in Malaysia, *S. olivacea* and *S. tranquebarica* which include to determine the duration of mating activities after pre-molt female being introduced to the inter-molt male and to record the process of mating activities.

## MATERIALS AND METHODS

This study was conducted at the hatchery of Institute of Tropical Aquaculture, Universiti Malaysia Terengganu. Broodstock of *S. olivacea* and *S. tranquebarica* was collected from Terengganu coastal water, Malaysia. Identification of species was done according to Keenan *et al.* (1998). A total of 80 mature specimens comprising 20 males and 20 females of each species were used in the study. Carapace Width (CW) and Body Weight (BW) were measured by using vernier caliper and digital balance before transferred into experimental tanks. CW was measured as the distance between the 9th tips of anterolateral spines. The crab size was range of from 7-11 cm CW and 100-150 g BW.

Crabs were acclimated for 2-3 days to the experimental rearing condition and reared together in fibreglass culture tank of 3.0 m long×1.0 m depth×1.0 m width. The tank was provided with a 20 cm thick layer of sand as bottom substrate and several pieces of PVC tubes (3.0 inch diameter, 20 cm long) to serve as shelter in order to reduce cannibalism during molting. Raw seawater was pumped from the coastal water and passes through 50 µm cotton filter bag into the fiber glass tank to a depth of 60 cm and aerated. Salinity was maintained at 30±1 ppt and temperature at 28±1°C with 100% daily water exchange. Crabs were fed with fresh cockle, *Anadara granosa* at 5% biomass feeding rate.

All crabs were put into culture tank separately according to their sexes. The female crabs was subjected to limb autotomy technique to induce their molting, in which three swimming leg and one pair of it pincher was cut off. As the female crab enter the pre-molt condition where the limb buds start to appear, they were transferred into the culture tank with the inter-molt males. The experiment were design for mating trials in four different tanks (10 pairs per tank) which is T1, *S. olivacea* (male, ♂) with *S. olivacea* (female, ♀); T2, *S. tranquebarica* (♂) with *S. tranquebarica* (♀); T3, *S. olivacea* (♂) with *S. tranquebarica* (♀) and T4, *S. tranquebarica* (♂) with *S. olivacea* (♀).

Mating behavior of the crabs was observed and data on the successful mating were recorded. Duration of male approaching female after the first introduction, pre-copulatory guarding, copulation and post-copulation guarding for different mating trials were also recorded. Percentage of mating success and mating duration were presented by plotting bar chart. Data were analyzed using Microsoft Excel for Mean±Standard deviation.

**RESULTS**

Figure 1a shows the percentages of successful mating, starting with pre-copulatory guarding, followed by copulation and completed with post-copulation. The results show that, the higher numbers of mating success of breeding within the same species which is 60 and 50% for T1 and T2 consecutively. On the other side, the percentages for mating success of the hybrid are 40 and 30% for T3 and T4, respectively. Figure 1b shows the mean duration of male crab approach pre-molt female after the first introduction which are 15 min±4.08, 13 min±3.21, 40 min±5.00 followed by 50 min±4.79 for T1, T2, T3 and T4, respectively. Figure 1c-e, shows the mean duration (min) of pre-copulatory guarding, copulation and post-copulatory guarding for each trial. The mean

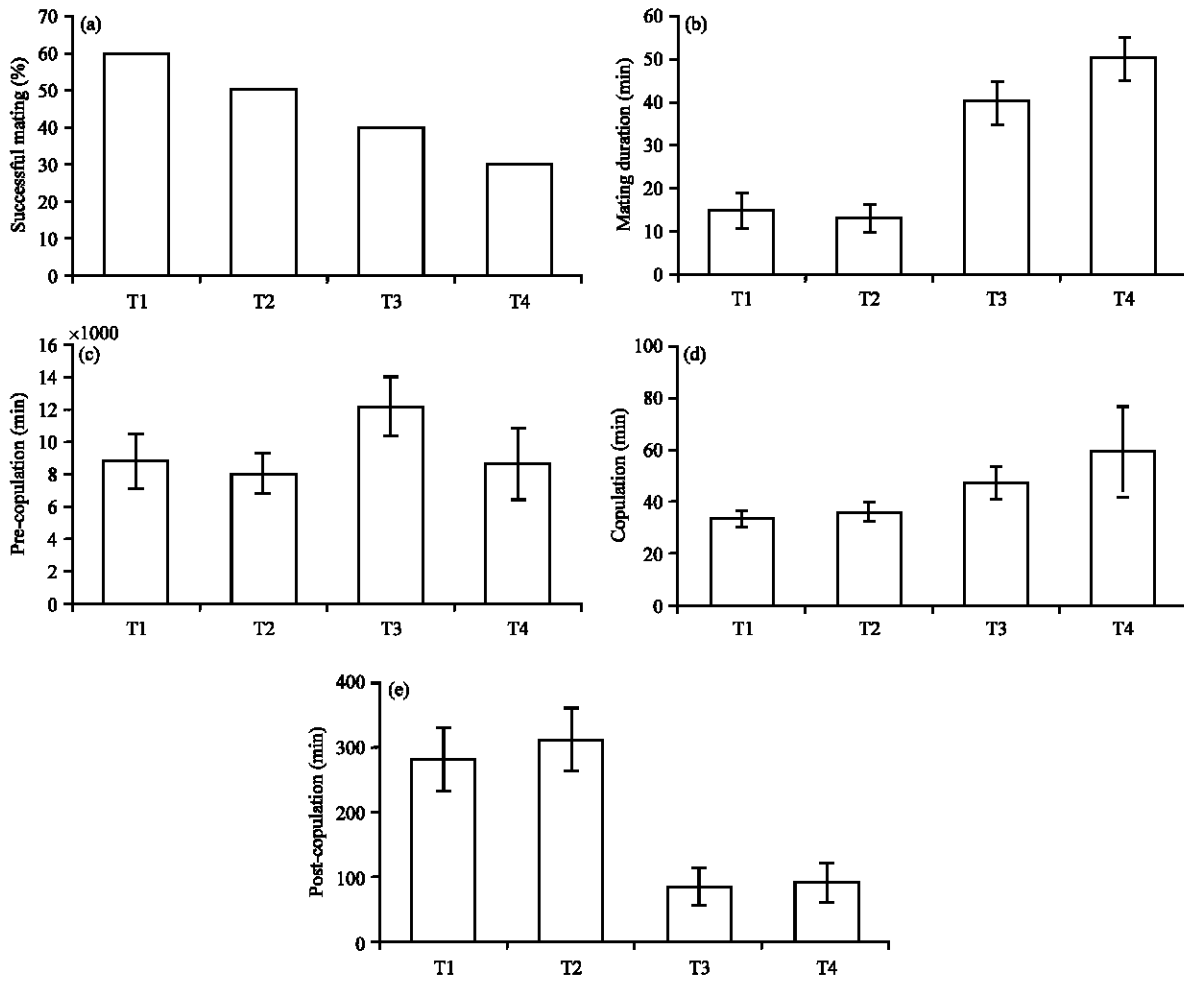


Fig.1(a-e): Bar chart shows the (a) Percentage of mating success, (b) Mean of mating duration-male approaching female after the first introduction, min, (c) Mean duration of pre-copulatory guarding, min, (d) Mean duration of copulation, min and (e) Mean duration of post-copulation, min; for different mating trials. T1, *S. olivacea* (♂)×*S. olivacea* (♀); T2, *S. tranquebarica* (♂)×*S. tranquebarica* (♀); T3, *S. olivacea* (♂)×*S. tranquebarica* (♀) and T4, *S. tranquebarica* (♂)×*S. olivacea* (♀). ♂ = Male, ♀ = Female. n = 10 pairs for each mating trial



Fig. 2(a-d): Mating activities of mud crab, genus *Scylla*. (a) Pre-copulatory guarding, (b) Copulation, top view, (c) Post-copulatory guarding and (d) Copulation, lateral view. ♂ = male, ♀ = female

duration for pre-copulatory guarding of T1, T2, T3 and T4 is 8,840 min $\pm$ 43,653.2 (6.14 days), 8,064 min $\pm$ 1,287.9 (5.60 days), 12,240 min $\pm$ 1,859.0 (8.5 days) and 8,666.7 min $\pm$ 2,199.6 (6.02 days) correspondingly. T3 shows the longest duration of pre-copulatory guarding. As for mean duration for copulation, it is 33.3 min $\pm$ 3.0, 36.0 min $\pm$ 3.8, 47.5 min $\pm$ 6.5 and 59.3 min $\pm$ 18.0 for T1, T2 T3 and T4, respectively. Mating of the two different species takes longer duration of copulation compared to those within the same species. Following that, the mean duration of post-copulatory guarding is 280.0 min $\pm$ 49.0, 312.0 min $\pm$ 50.2, 82.5 min $\pm$ 28.7 and 90.0 min $\pm$ 30, respectively. In contrast of mean duration of copulation, post-copulatory guarding within the same species takes longer time.

Mating activities were including pre-copulatory guarding, copulation and post-copulation. Pre-copulatory guarding is where the male crab clasp and carries the female underneath him using three pairs of his walking legs for a period of time (Fig. 2a). Copulation will follow after the female molt. Male crab will turn her upside down in a manner that ventral sides of both male and female are in contact (Fig. 2b, 2d), where the spermatophore will be transferred into the female spermatechae. Present study shows that all females copulated soon after molting. As the copulation complete, female crab was guarded by the male as in position of pre-copulatory guarding until her shell is harden which is called post-copulatory guarding (Fig. 2c). It has been observed that the mating activities were different between these trials. Different species showed long courtship, with

a longer time for cleaning up and manipulation of the female. On the contrary, mating activities between the same species show a short courtship time in which the males demonstrate less aggressive behavior towards females of the same species.

## DISCUSSION

The potential of the two species will undergoes hybridization in the wild was high as both *S. olivacea* and *S. tranquebarica* are common mud crab species in the South China Sea easily found in association (Keenan *et al.*, 1998). It is proven in the present study that 40 and 30% of hybrid success occurred between male *S. olivacea* and female *S. tranquebarica* and conversely. Very few people have seen mud crabs mating in the wild due to the male can only inseminate the female while her shell is in soft condition which is in a period of about an hour after molting, as been noted in the present study. The male crab will notice when the female is about to molt, thus approaching her to mate.

As described by Kamio *et al.* (2003) and Marshall *et al.* (2005), general features of soft female mating with prolonged pre-copulatory mate guarding, copulation shortly after female molting and followed by post-copulatory guarding has been observed in this study. It shows that soft shell mating is the usual situation in mud crab, *Scylla* spp. (Phelan and Grubert, 2007). It would benefit for a male to mate with newly molted female, as he would have to guard her for several days to ensure involving an investment of time and resources. From the viewpoint of the female, being guarded for an extended time will disrupt normal activities such as feeding, so it is required to develop a mechanism which limits the duration of pre-copulatory guarding. Kamio *et al.* (2003) reported that the abdominal flap of males remained opened during the copulation while the flap of females remained closed as it did not prevent the intromission of the male gonopods as the gonopore is not covered by the flap. However, the present study, the female extends her hinged abdomen (Fig. 2b), exposing two genital pores known as gonopore as the male inserts his gonopods into the genital pores and transfers seminal fluid which contains sperm sacs, called spermatophores, to the female where the sperm is stored for many months.

A total of 22 out of 40 pairs of the present study that are failed to mate are because of cannibalism factors where female crabs was eaten by male crabs, failure of females to molt and sexual competition. In particular, since size variation, distinct molting periods and utilization of refuges are attributes common to all crustaceans, these factors are likely to be important in cannibalistic encounters. Transition stages are at great risk of being cannibalized. Most mortalities in this study involved newly molted females. Cannibalism associated with molting is also observed in other species of crustaceans such as the giant freshwater prawn, *Macrobrachium rosenbergii* (Graziana *et al.*, 2003). During and after molting, individuals are particularly vulnerable as they are either immobile or soft and virtually defenseless (Marshall *et al.*, 2005). Besides, the mortality can also due to sexual competition as described by Rondeau and Sainte-Marie (2001) which is the number of sexually active males relative to the number of fertilizable females at a given site and time. Sexual competition is often more intense to enhance their reproductive success, males may express flexible behaviors, including mate guarding.

Observation of mating behaviors is necessary to estimate the functional maturity, actual size at which males participate in mating in nature. Information on this mating activity is important in fisheries management and seed production of mud crab species.

## CONCLUSION

The present study described the details on the duration of mating activities between *S. olivacea* and *S. tranquebarica*, as well as the process of mating activities. The present study also revealed that there are possibilities of hybridization between the two mud crab species, *S. olivacea* and *S. tranquebarica* to occur in the wild.

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